

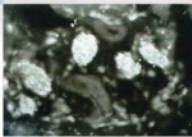
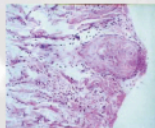
Rong Xiang Xu

Burns Regenerative Medicine and Therapy

Editor **Xia Sun**

Co-Editor **Bradford S. Weeks**

Collaboration of **Mo Xiao · Xiangqing Zhang ·
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Contents

VII	Preface
1	Brief Introduction to the History of Burns Medical Science
5	Introduction
5	Consideration of Scientific Paradigms and Research Reasoning from the Viewpoint of Foundation and Development of Medical Science Systems
7	Research Status of Stem Cell and Regenerative Medicine and Therapy from a Holistic Philosophy
8	Discussion of the Future of Regenerative Medicine and Therapy Based on the Results of Multi-Organ Regeneration Research
13	Rationale Foci of Local Treatment of Burns Medicine and Therapy
13	Pathogenesis Focus of Burns Wounds
14	Pathological Focus of Burns Wounds
16	Therapeutics Focus
19	Evaluation and Classification of Burn Severity
19	Clinical Assessment of Burn Area
20	Clinical Evaluation on Depth of the Burns Wound
23	Clinical Classification of Burns Severity
27	Clinical Principles of Burns Regenerative Medicine and Therapy
27	Standardized Local Treatment of the Burns Wound
27	Background Information of Standardized Local Treatment and Sources
28	Standardized Local Treatment of Burns Wounds
34	Indications and Diagnostic Principles of Burns Regenerative Medicine and Therapy
34	Diagnostic Principles of Burns Medical Therapy
34	Burns Regenerative Medicine and Therapy (BRT with MEBT/MEBO)
35	Burns Surgical Therapy with Excision Followed by Skin Grafting or Cultured Composite Autografting Technique
36	Intensive Description of Burns Regenerative Therapy with MEBT/MEBO
36	Concept and Principle of BRT with MEBT/MEBO
37	Therapeutic Effects of Moist-Exposed Burns Ointment (MEBO)
37	Clinical Application of BRT with MEBT/MEBO
37	Clinical Treatment
40	Systemic Comprehensive Treatment with BRT with MEBT/MEBO

45	Experimental and Clinical Study on Burns Regenerative Medicine and Therapy with MEBT/MEBO
47	Systemic Antishock Effect of Local Treatment with BRT with MEBT/MEBO
47	A Comparative Study on the Antishock Effect between BRT with MEBT/MEBO and Conventional, Dry-Exposed Burn Therapy Using a Rabbit Model
50	Experimental Study on Maintaining Physiological Moist Effect of BRT with MEBT/MEBO on Treating Burns Wounds
53	Clinical Study on Invisible Water Loss of Burns Wounds Treated with BRT with MEBT/MEBO
55	Experimental Study of Moist-Exposed Burn Ointment on Improving Wound Microcirculation of the Zone of Stasis in the Early Stages after Burns
57	Clinical Study of Moist-Exposed Burns Ointment on Improving Microcirculation of Burns Wounds
60	Experimental Study of the Effect of BRT with MEBT/MEBO on Hematological Parameters in the Treatment of Burned Rabbits
63	Studies on the Anti-Infection Effect of BRT with MEBT/MEBO
63	Effect of BRT with MEBT/MEBO on the Immunity of Burns Patients
68	Study on the Bacterial Count of Viable Tissue of Burns Wounds Treated with BRT with MEBT/MEBO
70	Comparative Study of the Effects of Moist-Exposed Burn Ointment, Silver Sulfadiazine and Hot Dry-Exposed Therapy on Controlling Burn Wound Infection with <i>Pseudomonas aeruginosa</i>
74	Experimental Research on the Mechanism of the Anti-Infection Effect of BRT with MEBT/MEBO
77	Primary Exploration on the Mechanism of the Anti-Infection Effect of BRT with MEBT/MEBO
82	Experimental Research on the Anti-Anaerobic and Anti-Fungal Effect of MEBO
88	Studies on the Effects of BRT with MEBT/MEBO on Regeneration and Healing of Burns Wounds
88	A Comparative Study of Fibronectin and Moist-Exposed Burns Ointment (MEBO) in the Treatment of Experimental Corneal Alkali Burns in Rabbits
89	A Comparative Study of the Effects of Moist-Exposed Burns Ointment (MEBO) and Other Drugs on the Healing Rate of Corneal Epithelial Defect in Rabbits
92	Exploration of Pathological Changes and Mechanism of Experimentally Burned Rabbits after Treatment with Moist-Exposed Burns Ointment
96	Electron-Microscopic Observation of One Case of Skin Burns Wounds Treated with MEBO
99	Pathomorphological Changes of Deep Burns Wounds Treated with MEBO
104	Observation of Microcirculation in Nail Folds at the Recovery Stage of Burns Wounds Treated with BRT with MEBT/MEBO
106	Physiological Healing Procedure and Histological Observation on Deep Second-Degree Burns Treated with BRT with MEBT/MEBO
111	Clinical Procedure and Histological Observation of Full-Thickness Burns Treated with BRT with MEBT/MEBO: A Case Report
114	Effect of BRT with MEBT/MEBO on the Expression and Regeneration of Epidermal Regenerative Stem Cells
119	Clinical Reports of Burns Regenerative Medicine and Therapy (MEBT/MEBO)
119	Clinical Trial Report of Burns Regenerative Medicine and Therapy (MEBT/MEBO): Multicenter Study
129	Clinical Demonstrations of Burns Regenerative Medicine and Therapy (MEBT/MEBO) on Successful Treatment of Extensive Burns
130	Extensive Burns Cases with Most Wounds of Superficial Partial-Thickness
131	Extensive Burns Cases with Most Wounds of Deep Partial-Thickness
134	Extensive Burns Cases with Most Wounds of Full-Thickness
141	Clinical Results of Surgical Excision and Skin Grafting Therapy in the Treatment of Extensive Burns Patients
145	A Commentary on Burns Medical and Regenerative Therapy
149	Conclusion

Preface

This book, which you now hold in your hands, will change how medicine is practiced around the world. It is an extraordinary book written by an extraordinary medical doctor who is also a pioneering scientist in the best sense of the word. Prof. Rong Xiang Xu has a very rare spirit, for he is a man with a compassionate heart who observed the terrible suffering of his burns patients and rather than simply accepting conventional treatments (which do little to correct the burns trauma), this doctor created, with much diligence and hard work, the new standard of care for burns treatment.

I first learned of Dr. Xu's work through reading the burns literature and learning of his research efforts in China. After analyzing his published research in the late 1980s, I determined to meet and question this man whose research was so daring and innovative. In 1991, I brought a group of American doctors to China to study Dr. Xu's MEBT/MEBO protocols. What I saw in Dr. Xu's burns clinics astounded me.

I trained at major American teaching hospitals such as Harvard's Massachusetts General Hospital, University of Vermont Medical Center and Dartmouth Hitchcock Medical Center, each of which offered what we believed to be the best burns treatments in the world. We were confident in the 1980s that no one took better care of burns patients than we did. Our burns patients were treated in technologically endowed surgical suites, given potent double antibiotic intravenous protocols along with topical silver-impregnated cold cream, all this administered under utterly sterile conditions in isolation suites and, of course, costing enormous sums of money. Our goals were, in retrospect, quite humble: keep the patients alive, reduce their pain, control their infection, and perform any surgery necessary to maximize their cosmetic and functional recovery. Typically, the majority of our patients left our burns units horribly scarred yet appreciative of our efforts.

Today, I know that the burns treatment protocols offered in the best American hospitals are obsolete and despite our best intentions, scientifically irresponsible. We must not be satisfied with clinical results which leave our patients so disabled and in such pain. That is a provocative statement and I offer it with the earnest hope that you, dear reader, will determine for yourself whether it is a valid statement. The book you hold in your hand with its many references describes a new way of treating burns

patients and, while you may question its scientific rationale, you must, at the end of the day, behold its superior clinical results. Dr. Xu offers intriguing opinions about regenerative medicine and therapy which may or may not be validated in the future. He raises, once again, the ancient dichotomy between Vitalism and Materialism which we, in our infatuation with quantitative scientific methodology, have turned away from as we split atoms into leptons, quarks and neutrinos. Today as we wade into genetic analysis, we are not inclined to step back and see the vital context within which the genetic process operates. We see the trees but not the forest. But again, as clinicians who have taken the oath to serve our patients, I suggest that once you have done your due diligence and investigated Dr. Xu's clinical results, then you will no longer be able to practice conventional dry burns therapy again. Therefore, like all revolutionary books, this one is somewhat disconcerting. My sympathies are with you!

It is my honor to add a few preface words and I see my challenge as helping introduce the reader to these innovative ideas in a manner most conducive to enhancing collegial and collaborative discussion. Therefore, I want to address our human need for certainty and our aversion to new ideas in general. Without intending to evoke defensiveness in the reader, I am reminded of a story of a woman who traveled far and wide to find the right doctor for her problem. Finally, she selected a very famous and talented doctor and during their first consultation, she exclaimed, 'Oh doctor, I am so pleased that you will care for me. I do hope that you can treat what is wrong with me!' whereupon the doctor responded: 'My dear lady, it is my hope that you have what I treat!' We doctors tend to be better practitioners than students of science and we are all guilty at times of being slow to learn new approaches to familiar problems. Innovation is not an easy path for a doctor to follow as lives are at stake and somehow we are encouraged to 'let someone else do the research.' In the old days, the doctor always observed his patient and considered various factors that impacted the progress of the treatment. The doctor was always an innovator and always felt responsible for doing his part in pushing back the frontiers of knowledge. Today, however, things have changed for most doctors and very few of us continue scientific work after beginning to practice. That does not have to be so, but to innovate as a doctor is not without peril.

There is a saying in America that you can determine which is the pioneer in a crowd of men by looking at their backs, for the pioneer is the one with the most knives in his back. All people, scientists and doctors included, are uncomfortable with change and the innovator is often unfairly criticized as he tends to 'rock the boat'. It is part of human nature to be wary of change, especially if someone tries to improve what we ourselves are offering to our patients. In medicine, where unscientific practices can kill people, we all should be cautious before embracing new ideas. I know from experience that most of the medical practitioners are well-intended and we do our heart-felt best to advance science for the benefit of our trusting and long-suffering patients. So why do we resist change? Why are innovations met with distrust and resistance? Consider what a professor might feel if he were to learn that what he taught other doctors and what he published as recommended treatment protocols now no longer were the optimum protocol. That would feel very uncomfortable. That might be, depending upon the character of the professor, almost unbearable, for to the degree we offer out-dated treatments, to that same degree we are exacerbating rather than ameliorate the suffering of our patients.

Therefore, despite ourselves, doctors are slow to study innovative ideas, choosing instead to focus our effort on improving only that which we currently practice, not learning something new and different. The scientists among us know that economics and politics interfere too often in the scientific world and so I urge you, dear reader, to put aside prejudices and comfortable paradigms and to remember the last time you listened to a dressing being changed for a burns patient. Listen in your mind's memory to the screams of pain as the dried scabs are pulled away from living tissue beneath in order to cleanse the burns wound. Remember the look of anguish on the faces of both patient and nurse as the blood flows anew before a new layer of Silvadene[®] is applied. In my clinical experience, no nursing task is more heart-breaking than the dressing change of a burns patient. Now, remember if you will, the last time you shook hands with a 'successfully treated' burns patient upon discharge from the hospital as she returned home, scarred almost beyond recognition and still suffering from restricted movement due surgical procedures and consequent deep-tissue scarring. You know you did your best as her doctor, but what a horrible outcome. She remains scarred for life.

Now, comes the 'what if'? What if, dear reader, a burns treatment protocol exists that takes away severe pain, that requires *no* horrendous dressing changes, that features a self-cleaning circulation within the wound that removes dead cells and bacterial debris and delivers regenerative nutrients to the living tissue at the base of the burns wound? What if this burns treatment protocol works in accordance with the natural laws of tissue regeneration so that minimal antibiotic use is required and so that burns

wounds heal faster and with practically no scarring compared to the burns treatments offered today in the finest hospitals around the world? 'What if' indeed!

As you read ahead, please remember two things:

First, please remember that Dr. Xu is offering his scientific experience to anyone interested in learning about his innovative burns treatment protocol. He has founded research institutions, sponsored international symposia, published scientific journals and been recognized by his government as the inventor of one of the most significant technologies in China today. Dr. Xu is seeking colleagues to continue this research and writes this book now as an invitation for other dedicated scientist to investigate this new paradigm. Dr. Xu has done his research and has published his findings on burns regenerative therapy. Now it is our turn. As his medical colleagues worldwide, it is up to us now to accept the responsibility to determine for ourselves whether there is merit in his claims. He now welcomes medical colleagues from around the world to come and learn what he has to teach. The world can no longer ignore his gift. These medical claims, though they sound fantastic to western ears, are indeed supported by rigorous and controlled scientific studies – both *in vitro* and *in vivo*.

Secondly, remember if you will, that I myself took time off from my practice and went to China on my own expense to determine whether Dr. Xu really was able to treat burns patients with MEBO/MEBT so that his patients were in minimal pain and upon discharge, walked away happy to look in a mirror – not scarred in any significant way. What I saw in Dr. Xu's burns hospital beds and through his microscopes at his research centers has inspired me to treat my burns patients with MEBO/MEBT. He has also inspired me to renew my commitment to practice, first and foremost, scientific medicine so as to always be open to learning innovative ways of offering the best care possible for my patients. He himself is an excellent example of this work ethic.

Burns regenerative therapy with moist-exposed burns ointment is the new standard of care for burns treatment. In the pages ahead, you will learn how Dr. Xu, in cooperation with natural laws inherent in living tissue, founded the new science of regenerative medicine for the benefit of burns patients in particular, and all mankind in general. Let us work together to silence forever the screams of pain during burns dressing changes which haunt too many of us in the field of burns treatment. Great suffering can serve to inspire heroic efforts. Today we can begin a historic collaboration together in the field of regenerative medicine and therapy, thanks to the pioneering effort of Prof. Rong Xiang Xu.

Bradford S. Weeks, MD

The Weeks Clinic Recipient:

International Orthomolecular Physician of the Year, 2003

Brief Introduction to the History of Burns Medical Science

Fire was, perhaps, man's first double-edged sword, for, throughout history, it has both served and destroyed mankind. While fire served to keep wild animals at bay in the night and warm people chilled by the winter air, it also turned on its master. From time to unfortunate time, fire leapt out at man and caused what remains today one of the most painful of human experiences, the burn.

Burns injuries were first described in the Ebers papyrus (1500 B.C.) which tells the reader that a delicate mixture of cattle dung and black mud was 'just what the doctor ordered' for a burn. Through centuries that followed any physician worthy of note had a favorite remedy for the relief of burns pain and suffering. Dupuytren, the famous 19th century French surgeon who first described the contracture that bears his name wrote: 'Burns had been the object of one of the most bizarre treatment methods'. Fabricius Hildanus, a 15th century German physician, was the first to classify burns into three degrees and debates raged well into the 20th century about how best to treat the burns – to cool or to not cool, to moisten and drain or to dry and seal for sterility. Finally, consensus was reached after the First World War that the best treatment for burns was surgical skin transplantation with subsequent scar reduction and pain control medications as needed. In the early 1950s, spurred on by thermal injuries during the Korean War, the US government established the original Surgical Research Unit (The US Army Burn Center) at Brooke Army Hospital in San Antonio, Tex., USA where skin grafting became the preferred treatment for 30% total body surface area (TBSA) burns. Survival was now the expected prognosis and one counted oneself lucky to survive.

Since the 1950s and 1960s, many medical experts from other countries threw themselves into the research work of burns medical science and contributed a great amount of experimental data which advanced the field of burns treatment. By now, patients with more than 90% TBSA burns can expect a fighting chance for survival when of-

fered treatment from a protocol involving surgical burns therapy consisting of localized treatment and systemic medical management. Once established in academic teaching centers, this two-pronged approach was quickly practiced around the world. The localized treatment of the 1960s was typified by a drying of the burned skin which enabled a crust (deep, partial-thickness) or eschar (full-thickness) to develop over the burned tissue. This crusting was accompanied by surgical excision of necrotic skin tissues and of viable dermis (tangential excision of crust). In addition, whole subcutaneous tissue (fascial debridement of eschar) was also an all too frequent aspect of the treatment. After this debridement was achieved, autografts or cultured epithelial autografts were placed on top of the lesion to close the wound from exogenous infectious agents. In the case of small, deep burns, initial excision and immediate autografts were recommended in the early stage after an injury. The systemic treatment, based upon what was then known about burns pathophysiology, was practiced in accordance with conventional surgical wounds management. This combined therapy consisted of medical management to avoid shock syndrome as well as to avoid infection while at the same time offering local and systemic nutrition support for tissue and whole body physiology, respectively. A great many protocol formulas were championed by leading scientists and doctors and these were offered with qualified success worldwide. This treatment became the 'standard of care' and became known collectively as 'conventional surgical burns therapy' or 'surgical excision and skin grafting burns therapy'. Its theories and treatment measures were compiled in medical textbooks worldwide prior to being introduced into China in the late 1950s. A recent improvement of this conventional surgical therapy was the innovation by American doctors who successfully treated patients with extensive, deep burns by using cultured composite autografts. This represented an important advance in the autograft technique.

In the 1980s, burns specialists began to look deeper into the physiology of traumatic burns wounds responding to conventional therapies. To their chagrin, these burns specialists discovered that these 'state-of-the-art' clinical treatment protocols, while representing a life-saving improvement compared to the primitive pre-1940s protocols, nonetheless remained a merely destructive therapy as far as the localized tissue was concerned. These burns specialists noted that conventional therapies neither rehabilitate the burned tissue itself, nor do they cooperate with the natural physiological repair mechanisms of burned tissue. Therefore, the feasibility and reasonableness of conventional surgical therapy, characterized as it is by dryness, excision and grafting, was evaluated and found lacking both in theory and methodology. Although Western researchers conducted massive experimental studies that addressed concerns of desiccation, excision and skin grafting, little progress was attained and ultimately the clinician was left with a suboptimal medical result – the disfiguring scar. This arena of painful dressing changes, rampant infection, devitalized tissue and residual scarring was the frustrating stage upon which the burns therapist pleaded for innovation but upon which no champions advanced until recently.

During World War II, an alert and observant Army surgeon, Joseph E. Murray (born April 1, 1919), had noted that skin grafts were only compatible between identical twins. From this observation, Murray then postulated that transplantation of internal organs might also be fraught with rejection and he began the experimentation, initially with canine and later with human kidneys, which ultimately resulted in his sharing the 1990 Nobel Prize for Physiology or Medicine with E. Donnall Thomas. Murray's work in organ- and tissue-transplant techniques set the tone for burns therapies for the rest of the 20th century. Consistent with the reductionistic genius of the American mind, an ill patient was seen as a collection of parts – some functioning better than others. In the case of the burns patient, the therapeutic goal became to surgically remove the burned parts before transplanting thereupon some unburned parts. It was no surprise that, prior to Murray and Thomas, the host system rejected the graft tissue since a living being is far more than the sum of its parts. Today, potent immunosuppressive pharmaceutical agents are required for successful transplantation protocols in burns. Though life-saving, these drugs, true to their name, hobble the native host immune system of the surviving burns patient. Frequently, the doctor is chagrined at the trade-off whereby his patient survives – but at the expense of his immune system. As in most areas of medicine and surgery, burns specialists suffered along with their patients for they knew that there must be a better way to help those burned patients.

Nonetheless, despite the frustrating situation where the best the burns specialist could offer would be a life hobbled by chronic pain and disfiguring and motion-restriction scarring topped by systemic immunosuppression, no one was 'thinking outside of the box'. Beneath this consensus that transplantation surgery was the treatment of choice, we can now discover another unspoken consensus, i.e. that burns are a disease of the skin and therefore ought to be treated dermatologically rather than systemically or holistically. Everyone saw that the burned part was the problem and that it should be replaced.

In the 1970s, in China, Professor Xu Rong Xiang alone was thinking outside of the box where he boldly established an entirely new theory of burns physiology upon which he then built a dramatically effective burns treatment which he called 'Burns Regenerative Therapy' (BRT). This innovation, which integrates moist-exposed burns treatment (MEBT) and moist-exposed burns ointment (MEBO), was a balm to the struggling burns therapy industry. The therapeutic essence of MEBT/MEBO is to maintain the burns wound in an optimum physiologically moist environment through the use of a specially designed ointment – MEBO. Rather than surgically excising the burned tissue and its underlying dermis, the goal became to heal the burned tissue and stack the cards in favor of tissue regeneration – an unimagined goal. MEBO, the patented topical remedy, is composed of natural plant extracts dissolved in a sterile and refined sesame-oil base with beeswax as a preservative. When applied topically, MEBO promotes burns tissue repair in an astonishingly effective manner. Initially, MEBO cleans the burned tissue by stimulating the discharge and removal of debris (liquefaction of necrotic tissues). As a complementary healing benefit, MEBO also enhances the regeneration and repair of the residual viable tissue at the base and periphery of the burn in order to anchor vitality within the wound-healing process. Coincident with the application of MEBO, a systemic comprehensive treatment is initiated based on the natural pathophysiology of burned tissue. Accordingly, BRT and MEBT/MEBO is distinguished from conventional surgical therapy in that dryness, excision, skin grafting and scarring as well as the excruciating pain associated with dressing changes is no longer a necessary component of burns care.

The history of MEBT/MEBO is quite auspicious and parallels the ascendancy of China in the marketplace of modern times. Today, the West embraces China as one of the three countries in the history of mankind which were able to safely send a man into space. Equally so, Western doctors who have observed the miracle regenerative cures of MEBT/MEBO embrace Dr. Xu and his team as pioneers in burns therapies. The West first learned about MEBT/MEBO on August 16, 1988 via a Chinese press release that declared the clinical success of this newly dis-

covered burns treatment theory and its uniquely efficacious therapy. Bolstered not only by clinical success (both in China and abroad) but also supported by copious scientific research, MEBT/MEBO immediately altered the direction of academic research in burns treatment worldwide.

Dr. Xu is one of the bright lights in the firmament of scientists alive today. Yet he too stands above the shoulders of scientists who came before him. The treatment philosophies of traditional Chinese medicine urge the pursuit of regeneration as opposed to replacement of burned or diseased tissues as have a precious few Western doctors who sought to apply agents to improve and accelerate the wound-healing process. Ambroise Pare (1510–1590) postulated that a surgeon's goal in wound management was to create an environment where the healing process could proceed in an optimal fashion. Pare demonstrated the beneficial effect of the application of hot oil to fresh open wounds. Since then and over the centuries many publications have pointed out that a moist environment enhances epithelialization in the wound-healing process. Controlled experimental and clinical data have in recent times supported the suggestion that a moist environment enhances wound healing in the form of an occlusive dressing compared with a dry environment. Xu has developed MEBT – a therapeutic procedure based on the moist environment of the wound, using an ointment that enhances epithelial repair, and in particular that of partial-thickness burns wounds. MEBO consists only of natural ingredients including – apart from honey and sesame oil – 17 amino acids, 14 fatty acids, and 4 polysaccharides. The ointment's main active substance is considered to be β -sitosterol at a concentration of 0.25%. Clinical and experimental investigations by Chuanji, Yunying and Xu have indicated that MEBO has the following therapeutic effects:

- 1 *Analgesic*: MEBO reduces pain in partial-thickness burns wounds.
- 2 *Anti-shock*: MEBO reduces evaporation of water from the burns wound surface and improves microcirculation by decreasing peripheral and systemic capillary exudation.
- 3 *Anti-bacterial*: MEBO changes the biological behavior of bacteria, inducing a decrease in bacterial toxicity and invasive capacity, as well as sensitivity to antibiotics; it also increases the wound's local and systemic immunity.

- 4 *MEBO promotes epithelial repair*; it also reduces healing time in partial-thickness burns.

- 5 *MEBO improves and reduces scar formation* and contributes to the formation of a smooth, thin, and aesthetically acceptable scar, thus preventing the formation of hypertrophic scars.

In 1989, Americans finally learned that the paradigm had shifted in burn care when *Newsweek* published a report subtitled: 'Could a new medication from China change the world's approach to treating burn injuries?' This caught many US doctors unawares and even today, 14 years later, 90% of US burns specialists are unaware that this BRT and MEBT/MEBO has been validated in hundreds of experimental studies and clinical practices around the world. These results substantiate the claim that the theory and practice of BRT and MEBT/MEBO comprise a successful revolution in burns care by offering a patently superior methodology of burns treatment when compared to the desiccation, excision and grafting required by conventional therapy. In addition, BRT and MEBT/MEBO also offered the first sophisticated and accurate characterization of natural burns pathogenesis, allowing scientists around the world to finally understand the principles of effective therapeutic burns treatment. MEBT/MEBO therefore attained the rarified status of a truly revolutionary and beneficial clinical success story. With this new therapy, which heralds an advancement into a new field of burns medical science, patients sustaining partial-thickness or full-thickness dermis burns cannot only survive what once were life-threatening burns injuries, but can now do so without inordinate pain, immune-depleting surgical excision or the disfiguring scars from the now obsolete surgical technique of skin grafting. Today, the history of burns therapy has advanced into a bright and promising future. Professor Xu is teaching the world to work with the regenerative forces of nature. In the pages that follow, Professor Xu welcomes collaboration as we surge forward together committed to reducing the pain, disfigurement and suffering of burns patients the world over. Let us strive together for this noble and finally attainable goal.

Bradford S. Weeks, MD

Introduction

Regenerative medicine and therapy is an innovative concept described through a new research field and represents a unique approach towards the goal of regenerating functional tissues and organs. On the occasion of the publishing of *Burns Regenerative Medicine and Therapy*, I would like to share with readers the insights into the genesis, current research status and exciting advances in this critically important realm of health sciences – regenerative medicine.

Consideration of Scientific Paradigms and Research Reasoning from the Viewpoint of Foundation and Development of Medical Science Systems

Medical historians today are fortunate to be able to scan, across thousands of years, the extensive research focusing on human health problems and related therapies which have evolved today into the modern disciplines of life science and medicine.

During the development of these modern disciplines, certain questions have consistently arisen in the minds of generations of researchers including: ‘What are the advantages and disadvantages of a current medical system?’, ‘What medical practice will be adopted in the future that is most advantageous for human physiology and health?’, and ‘Is it possible for the average human being to attain one hundred years of age and still be in good health?’ The question as to what the future of medicine will reveal has always teased men and women in the health sciences. As early as 2,000 years ago, both eastern and western medicine originally arose from an apprenticeship with nature and natural phenomena. Everyone attempted to harness nature’s secrets to solve the health problems of their time. The first written documentation on traditional Chinese medicine is the Huang-Di Nei-Jing or Yellow Emperor’s

Cannon of Internal Medicine (<http://www.hungkuen.net/tcm-history.htm>) that was finished during the Spring and Autumn Warring States Period (between 800 and 200 BC). This documentation represents the development of medicine away from sorcery and en route to being used as the foundation of Chinese medicine. Shen Nong (3493 BC), hailed as the ‘Divine Cultivator’, tested myriad herbs and in so doing gave birth to the art of medicine. Hua Tuo (110–207 AD) was the most famous doctor in ancient China who developed the use of Mafei San (surgical anesthesia) a good 1,600–1,700 years before western doctors learned about ether and other chemical or pharmacological anesthetic agents. These and other great achievements supported the foundation of Chinese medicine with its comprehensive and systematic gifts which include modern day’s internal medicine and surgery.

Ancient Greece and Rome dominated the empiricism of the ancient west. At around 6 BC, Alcaemon (<http://emuseum.mankato.msus.edu/prehistory/aegean/culture/greekmedicine.html>), from ancient Greece, performed human autopsies and concluded that the brain was the organ of thought and sense. By the 5th century BC, Hippocrates, father of modern western medicine, after studying the conditions of dying patients (<http://www.cpus.gov.cn/kxrw/index.asp?rw=419&jiang=0>), articulated the elaborate general doctrine that all of the Four Humors, phlegm, blood, yellow bile and black bile, had to be in correct proportion to one another for good health to result (<http://www.med.virginia.edu/hs-library/historical/antiqua/textn.htm>). At almost the same time, Aristotle (<http://www-groups.dcs.st-and.ac.uk/~history/Mathematicians/Aristotle.html>), the student of Plato, pushed back the frontiers of knowledge and superceded his teacher by proposing that the earth was composed of the four elements: earth, water, air and fire (<http://galileo.imss.firenze.it/museo/b/earisto.html>). With about 2,500 years of development, there came into being two academic systems: eastern and western medicine. Eastern medicine,

which originated from ancient Chinese medicine, has brought tons of benefits and contributions to human health by providing treatments based on plain philosophy and holism, while western medicine experienced two periods: one during the warring period of ancient Egypt and ancient Rome when the massive wounded were treated, which brought morphologic research from anatomy to applied surgery, and the other during the Renaissance when medicinal chemistry was developed based on alchemy, thereby resulting in the rudiments of modern western medicine and surgery.

Historically, both eastern and western medicine have continuously integrated modern scientific discoveries into their medical treatments and thus continued to develop. However, historians might also question what kind of significant benefits, whether in Chinese or western medicine, these discoveries have played in promoting human health and in effectively treating diseases. Let me share with you an image that concerns me. Imagine a modern, well-educated medical doctor holding a knife in his left hand and a pharmaceutical drug, a cellular poison, in his right. Now he suggests to the patient: 'I will use the knife to excise your injured organ to cure disease and save your life and then I will use the "poison" to cure the disease. Is that OK?' You see, combating poison with poison, is the paradigm which we were taught by the older generations of doctors. And because no one offered a more reasonable option, western drugs today are made primarily of chemical toxins which are incompatible with life and which, not surprisingly, when applied to diseased human beings, inevitably have deleterious side effects on health. Therefore, it is not an unjust comparison to liken western drugs to poison when seen in the context of the rule of life or vitality.

For many centuries, medical professionals the world over have sought to reduce drug toxicity as much as possible while many governments have set up national drug-control administrations to ensure drug safety for humans. However, no substantial and meaningful changes have been made to the traditional medical system due to the inflexible concept of 'poison' and, until now, due to the lack of effective nontoxic options for the treatment of disease. Where is the new medical system that conforms to the principles of human vitality? In which direction should the practice of human medicine go? Herein, I would like to share with devoted readers the exciting story of the establishment of regenerative medicine and therapy as well as our compelling research which supports this new paradigm shift towards a medicine which is in accordance with the laws of human health and wellness.

We inaugurated the research into the secrets of regenerative medicine and therapy in early 1980. Although many difficult challenges fell before us since 1987 (the year we established our Research Center), our published research results demonstrate that we are presently

amongst the leaders in this field. Back in 1989, I published research demonstrating the heretofore unthinkable result of scar-free healing of burns through the application of regenerative cells. The clinical results were impressive and the pictures demonstrating irrefutable clinical effects (no scars) are available for the interested reader in *The Chinese Journal of Burns, Wounds and Surface Ulcers*.

Subsequently, the work done by Dr. James A. Thomson and his colleagues from Wisconsin University in 1989 revealed that when cells were isolated directly from the inner cell mass of human embryos at the blastocyst stage and then cultured in vitro to produce a pluripotent stem cell line, they would then transform into many types of cells. Thomson's group believe that any cell from a fertilized egg, termed as 'totipotent stem cells', if placed into a woman's uterus, has the potential to develop into a fetus and then to form an entire viable organism. Meanwhile, Dr. John Gearhart and his colleagues isolated pluripotent stem cells from fetal tissue of terminated pregnancies and confirmed Dr. Thomson's results. Their work was published in *Science* and saluted as 'the first breakthrough out of the ten big achievements in 1999'.

This technological achievement triggered a burst of stem cell research and a whirlwind of ethical debate followed immediately by a drive for commercialization, some of which was quite unscrupulous. For example, a certain laboratory announced that they had created a human ear on the dorsum of rats. More stir! Not surprisingly though, on closer inspection, we learned that their statement was not actually true. In fact, the scientists in that laboratory did something different though not entirely insignificant. They managed to first make a human ear model scaffold using polyglycolic acid (macromolecule chemical material) and then, after placing this structure beneath the rat subcutis, cartilage cells cultured and proliferated within the said scaffold creating something that looked like an ear but was not one at all. Like a shadow perpetually attached to its master, commercialization is never far from the frontiers of science.

Imagination, while an important component of science, is only a distraction unless the rigor of the scientific method is also employed. No trickery is allowed. Unfortunately, such tricky performances – such as human ears on the backs of mice – disturb the current field of stem cell research. Traditionally, Chinese scientists and doctors prefer to investigate principles from experimental results and holistic concepts in order to discover tri-dimensional development modes en route to comprehensive conclusions. In contrast, westerners are adept at imaging from scantling phenomenon, then designing several research directions for further exploration before finally attaining an answer. The Western mode of research necessarily requires adequate funding which seems to not be in short supply. For example, a result that might require ten thou-

sand dollars in China might require, in the West, a price of ten million dollars. Nonetheless, despite funding discrepancies, we are pleased to reveal that, though relatively underfunded, Chinese researchers have accomplished the clinical application of regenerative medicine while Western researchers are still formulating strategies. This difference in degree of clinical success validates the eastern way of thinking about research, which produces empirically superior clinical results in an expeditious manner.

Our focus in this book will be to reveal that the clinical results springing from the research on burns wherein data suggest that most dry wounds heal with scar formation whereas most moist wounds heal with less scarring. While probing the mechanism of this superior healing over many years, we discovered one type of unknown cell that has a regenerative capacity which may play a significant role in this process. After years of basic research and clinical study, we found that the cells with regenerative potential turned out to be keratin 19 positive expressed epidermis stem cells which appear to be the primitive cells at the start of human embryonal development. Coincidentally, this understanding shed a great light on the mystery of optimal physiological healing of deep burns by regeneration. Using wound repair as a model, we dynamically demonstrated that the process of skin regeneration and development can resemble embryonic tissue development. Based on the discovered skin regenerative law, we conducted experimental studies on the regeneration and repair of tissues and organs of mammals by creating a vital environment. I am now pleased to report that up to the present, we have had consistent success in repairing and regenerating 55 types of tissues and organs.

Research Status of Stem Cell and Regenerative Medicine and Therapy from a Holistic Philosophy

The ‘healing’ process can be observed to result in one or the other of two major sequelae – scarred and scar-free healing. Healing with scar formation is the result of aberrant and suboptimal physiological processes while scar-free healing is the result of healthy and appropriate physiological processes working in conjunction with the forces of regeneration. Mankind has always known this to be true but until now has failed to discover the dynamics behind the variable healing results. Certainly, if one could comprehend and reveal this mystery in order to apply it to medical fields, then the health of people the world over would be astonishingly enhanced. Such a goal is worthy and, accordingly, that has been my focus and aspiration since I pioneered the science of regenerative medicine and therapy many years ago.

Let’s begin with definitions. The term ‘regeneration’ implies that the human body can be stimulated to regenerate by itself through the use of its own potential but this stimulation requires both an appropriate trigger or promotion factor as well as an appropriate physiological environment. In fact, each tissue or organ, including epidermis, epithelium mucosa, vascular endotheliocyte as well as blood cell in human body is engaged in exactly this process all the time. Disease, therefore, can be understood to occur when the speed of repair is slower than the speed of injury. Until the present, a lot of pathological and physiological mechanisms remain obscure to those using the conventional paradigm. Therefore, in order to uncover the mystery of regeneration in human body, we must avoid the thoughts of traditional medical thought and instead utilize a new body of thought which we can apply to the observation and study of human physiology. This new body of science has led us to the field of regenerative medicine.

Our whole framework of regenerative medicine has epoch-making significance – diseases will be cured and the people’s health will be improved by the potentials whirling unharnessed within each human cell, tissue and organ.

In 1989, I announced the embryonic form of regenerative medicine. Today, 13 years later, American scientists are offering similar concepts, which they call ‘treatment of future regeneration’. Although they use the crude transplantation approach to accomplish the renaissance of organs, nonetheless, they do make use of the human body’s regenerative potential. Our schematic thoughts of regenerative medicine focused on the in vivo and in situ organ regeneration, it’s the life regeneration combined with human physiological activities. While already bearing clinical fruit, I believe our system of regenerative medicine will continue to develop and mature as we complete our research. Until now, our ideas are the most advanced and, to our knowledge, are the only ones whose efficacy is confirmed by clinic practice. Because of this, we submit our proof of regenerative medicine as a scientific conclusion, not a hypothesis.

On February 26th 2002, we attended the Stem Cells Regenerative Medicine Conference held in New Jersey. Participants had intense debates focusing on areas of stem cell research which we had already finished and where we had a lot of great achievements.

Though some scientists announced their success in reconstituting ‘bone’ or ‘heart’, experts and investors alike declared that they only wanted to see some real results. This is in accordance with the principles of science where results are what counts. Results are more important than theories. Accomplished research which springs from the solid foundation of truthful thought is the path to progress and innovation.

Physiological tissue repair and functional organ regeneration through cultivation in deep burns management

has been demonstrated in our research results. The repair and promotion of mucosal tissue regeneration in the gastrointestinal tract is of interest but will not be detailed at this time. Stem cell research, which is widely known to the public, mainly refers to conventional hematopoietic stem cells. However, great debates are continuing over whether hematopoietic stem cells are the appropriate ones to use because these cells are immature. What is a stem cell? A stem cell is an undifferentiated or partly differentiated cell with the capacity of transforming into 'mediate cells' with the structure and function of tissue and organs. Stem cells are similar to tumor cells as regards their proliferative capacity, but the former constitute normal tissue and organs ultimately, while the latter form tumors. The unique characteristic of stem cells is that they can develop into fully functional organs.

In the February conference, I presented our research results. Comparisons were made to current American advances in this field. Though we found that histiocytes of each tissue and organ have the potential to regenerate, the challenging problem to doctors and researchers is how to maintain and induce the regeneration of these cells. In our burns treatment, we have worked out a great success. We use moist-exposed burns ointment (MEBO) to treat deep second-degree burns and by creating a physiological environment and adding life-regenerative substances, we facilitate healing without scar formation. Information about regenerating skin subsequent to second- and third-degree burns wounds will be discussed later. This innovative burns medical therapy (MEBT) is not only applicable to treating burns injuries, but also to the replacement and reformation of human skin – an innovation from which everyone may benefit.

Entering into the 21st century, almost every doctor may question which innovative therapy is most promising for modern medicine. Many life scientists and physicians have turned their attention to stem cell research. There are various research approaches to the study of stem cell potential. Foremost of these is embryonic stem cells, hematopoietic stem cells and adult stem cells. No matter which kind of stem cell, the dream of renewing the human body's physiological function lies in stem cell research both in vivo and in situ. The law of in situ regeneration is the only one with any value for medical application.

Discussion of the Future of Regenerative Medicine and Therapy Based on the Results of Multi-Organ Regeneration Research

Despite continuous progresses in science and technology, few attempts have been made to successfully develop functional tissue or organs from human cells. The excep-

tion is our embryology study and our work on the adult stem cells in vivo and in situ. Almost one decade ago, American researchers tried to establish a new life science system using various approaches and electronic technologies, but ended up only describing an ideal blueprint for the human genome. However, without sufficient understanding about cells, the genomic research that only focuses on life substance within the cell is of little applicable value since genes play their roles under the assistance of the function of cells. It is true that genomic research is very important in the life sciences, but such research will accomplish nothing if it is removed from cellular biochemistry and cytology. While an important approach to life science research, gene technology proves inadequate to solve any health problem or to cure any disease unless combined with the appropriate use of cytology focused on harnessing the function of the cell, life's smallest unit.

Stem cell research and its application is another hot topic in life science apart from genomics. According to current reports from over the world, the most advanced stem cell research is the isolation and culture of stem cell in vitro before transplanting 'tissue' which has been engineered (e.g. epithelium tissues and cartilage transplanted into the patients). However, a challenging problem that remains unsolved is how to maintain continuous proliferation of stem cells in vitro. It is well known that the environment in vitro does not completely meet the actual physiological requirements as that in vivo and in situ. The inadequate transmission of information and suboptimal regulation between histiocytes results in an inadequate physiological linkage and constitution. This failing is magnified when the scale increases to commensurate with the macro-physiological function of tissue or human organs. Our research focused on the adult stem cells* in vivo and in situ and revealed that the damaged tissues and organs are able to repair themselves only if the adult cells can be transformed into stem cells with the potential of reconstituting tissue and organs. Until now, we have accomplished physiological tissue repair and functional organ regeneration in situ by cultivating skin stem cells in deep burns management. The following is the briefing of our current research status and achievements.

* Adult stem cell: Now we named these special cells 'potential regenerative cells' (PRCs), which means that the special differentiated histiocyte has the potential ability to regenerate to a functional tissue similar to a stem cell but normally exists in tissue as a histiocyte. It can also be called the special differentiated histiocyte in all types of organs in the organism coming from proliferating cells during different development stages.

The major difference between PRC and adult stem cell (SC) is: PRC is a mature differentiated tissue cell, while SC normally refers to the undifferentiated cell. Some SCs can be identified by some special markers and, in skin regeneration, SCs are the proliferating form of PRCs. SCs can repair injured and defective skin by restructuring and regenerating new skin according to the original skin physiological structure.

Gastrointestinal Mucosa Regeneration

One paper published in *Science* in the December 7, 2001 issue evoked great responses in the field of cell and tissue research. The authors collected small intestine tissues from embryonic mice and identified the types of cells by a special staining approach. The tissue slices from 17-day-old mouse embryo showed that the intestinal epithelium derived from four principal cell types. The report is an experimental study describing in detail that intestinal mucosa villas are composed of many types of cells.

We herein compared their reports to our results in cloning villas of small intestine with cells. We cultured gastric and intestinal wall tissue from mouse embryos in vitro, using a tissue culture composition called GIC that can promote the proliferation of stem cells*. The results showed that in the culture of gastric tissues, GIC stimulated the cells cluster beneath the gastric wall mucosa to persist in division and to form new tissue by proliferation. In the culture of intestinal tissues, GIC initiated the cells adjacent to the intestinal wall mucosa to become stem cells with the potential of proliferation. They ultimately differentiated into brush-border mucosa with absorptive function, or into endocrine cells in intestinal tract that proliferated until forming new intestinal tissues. The intestinal tissue section worked upon by American researchers is identical to our cultured intestinal tissue section. As a thought for a further step forward, we have reliable results in many functional assays. The cloning process of our gastrointestinal tissues in vitro can be visible during the months of culture but this itself is only attainable through the development of stem cells.

This is the first time in the history of the life sciences that tissue or organs can develop in vitro. To ensure the novelty of our achievement, we have conducted a worldwide search of the published literature on this subject. The search by a subsidiary of the National Science and Technology Ministry did not reveal any report of similar results. The website of www.stemcellresearchnews.com in the United States covered our results as the headline news on the issue of December 23, 2001.

These results offer proof that we have successfully cloned two different types of organ, stomach and intestine, in vitro. GIC, as the necessary substance for cells, serves as the nutritive culture medium and protector. It is regarded as the only agent currently available for initiating cells to proliferate in order to repair tissue. The research of the role on GIC in promoting the growth of mucosal stem cells in the gastrointestinal tract has great clinical value. In the treatment of gastric diseases, GIC can protect the gastric wall and also repair ulcerative tissues. GIC can repair injured intestinal mucosa, and ensure the intestinal mucosal cells' ability to absorb nutrients. Using a mouse model featuring acute mucosal

ulcers, we found that a 3-day treatment with GIC repaired the ulcers without scarring and resulted in recovery of full function. GIC is suggested as the first priority before surgery for any gastrointestinal disease.

Nerve Regeneration

Sciatic nerves from white mice were sampled, cut in two and cultured in two different culture media in vitro with one containing GIC and the other with normal tissue culture medium without GIC. The results showed new nerve which had regenerated from the residual nerve cultured in GIC. Of note, the nerve in the control group shrank. Thus, we demonstrate that regenerative technology makes it possible to physiologically regenerate the defective nerve, thus advancing the tissue and organ regeneration from cytology to histology.

Kidney Regeneration

Failure of renal function is a very tough issue in medical practice. Because of pathological changes to the glomerulus and the renal tubules once deprived of filtering and reabsorption, a lot of patients need dialysis therapy. Our studies suggest a hope of regenerating glomerulus and renal tubules using regenerative technology. Cortical cells were taken from kidney and transformed to stem cells in culture. Glomerulus and renal tubules were formed by the cloning and constitution of stem cells. Regeneration in situ results are the same as the in vitro results, which begin when a regenerative substance is injected into a kidney with function failure. Animal experiments are now in process.

Marrow Regeneration

In this study, we took progenitor cells from marrow and cultured them in specific regenerative substances in vitro to form new marrow. Marrow transplantation is known as the best way to treat colony growth factors and the best method for promoting the formation of marrow progenitor cells. In our research, the regenerative potential of the progenitor was activated. One progenitor can develop into marrow consisting of various hematopoietic stem cells. The regeneration of human marrow tissue, once achieved in vivo and in situ, may lead to the possible cure of various blood disease.

Pancreas Regeneration

In histology and cytology, the function of the pancreas is as follows: The intestinal mucosa is stimulated by the food such as sugar or starch, then the intestinal mucosa sends the signal to the acinar cells to release amylopsim.

After amylopsin enters into the intestine, the starch, after turning into glucose, is absorbed. Meanwhile, the acinar cell also informs its neighbor, the islet cell, to release insulin. At this point, the glucose is converted into energy by insulin after entering into blood. This whole process is controlled by endocrine and nerve functions. The two types of cell in the pancreas coexist and are codependent, each of them having its own secretory role.

Diabetes is the result of a disorder of growth and function of the acinar and islet cells of the pancreas. The disorder may result in excessive hyperplasia of the acinar cells (type II diabetes) or atrophy of the islet cells (type I diabetes). There is no physiologically effective therapy available to treat diabetes until now. It is necessary to understand how acinar cells grow and coexist with islet cells in terms of histological and cytological regulation. Some researchers only isolated and cultured islet cells from embryonic pancreas tissue *in vitro*, which destroyed the integrity of the pancreas. On the other hand, traditional Chinese medicine, working in conjunction with the laws of balance, suggests that both strengthening body resistance and consolidating the constitution are equally important therapeutic goals.

In the experiment, we found that all pancreas cells died after culturing in media only containing regular MEM media for 8 days. In contrast, in the other group, after coexisting for 65 days, acinar and islet cells established a harmonious proliferation when cultured in MEM medium containing additional 'life substance'. On day 80, acinar and islet cells showed the tightest linkage until forming a new pancreas on day 92. Function examination on the nascent pancreas showed that before tissue necrosis in the control group, the amylopsin levels were remarkably different in the two groups. In the control group, it was several times higher than normal; but it was normal in the experimental group. Also, the pH value in the experimental group was normal while that in the control group was much higher. Determination of insulin showed that both the nascent and the mature pancreas is capable of producing abundant insulin while no insulin was produced in the control group because of the death of islet cells. These results indicated that normal pancreas tissue has been successfully cultured *in vitro*. Within 1 or 2 years, such results will be commercialized for therapeutic purposes and diabetic patients will be greatly relieved.

Skin Regeneration

Skin is the largest organ of the human body. The commonly observed skin regeneration occurs as regeneration of epidermis, which is easily achieved as long as basal cells are available. In fact, skin regeneration is not as simple as the regeneration of cells, but involves the physiological

adhesion, assembly and regeneration of multiple cells and multiple tissues with the final formation of functional full-thickness skin as a result. Full-thickness skin should include the combination of three germinal layers, physiological conjunction with subcutaneous tissues and coexistence with the host body. Therefore, it is inappropriate to define skin regeneration as the regeneration of any individual tissue or cell. Last year, in an international conference on stem cell research held in Singapore, French scientists, claiming to be 'Fathers of Skin', announced that they fulfilled skin regeneration *in vitro*. I questioned the French scientists whether the 'skin' that they cultured was composed of epidermis, dermis and appendages, and whether the dermis further involved blood vessel, lymph, nerve, sebaceous gland, follicle and sweat gland. Their faces turned red. Therefore, a quotation mark should be added to their cultured 'skin' as they, in fact, only cultured epidermis.

Skin histiocytes are derived from three germinal layers: ectoderm, mesoderm and endoderm. Skin regeneration requires the regeneration of all skin tissue, such as muscle in the endoderm, connective tissue in the mesoderm and epithelia in the ectoderm. Currently, we alone in the world of scientists have been able to accomplish the regeneration of skin. This book will cover our techniques in detail and demonstrate how these techniques have been widely used in clinics as the dominant modality of burns therapy.

Surgical therapy has been the dominant approach in burns therapy all over the world for decades. However, almost all surgeons admit that they adopted surgical skin grafting not because it is the best therapy, but because quite simply it was the only choice. Surgery treats burns wounds by excising the burned skin and converts burns wounds into surgical wounds in preparation for skin grafting. This technique only treats complications of burns, instead of curing burns tissue. I was a surgeon for many years and I still remember when, as a student in medical school, teachers had such an expression that nobody would be willing to perform surgery as a burns treatment if skin regeneration were possible. Another instance, as textbooks indicate, second-degree burns healed below the scab by epithelial growth and covering the wound along the area below the scab, which indeed is the surgical way to heal the wound. Therefore, it is important to distinguish between the two different medical conceptions.

As early as before 1989, we have matured burns skin regeneration therapy that was derived from successful burns treatment in clinic practice. Subsequent to burns, the human body has an instinct to initiate the regenerative potential of stem cells *in vivo* and *in situ*. However, the typical use of disinfectants and antibacterial agents on burns wounds makes it impossible to create a physiological environment sufficient to initiate and activate stem

cell activation in burns wounds. The goal was to maintain and promote stem cells in order that they might proliferate and differentiate to further repair and clone organs.

In the 1980s, I put forward an innovative conception on burns management, keeping the burn physiologically moist in order to promote repair and regeneration. This innovation finally led to the establishment of Burns Regenerative Medicine and Therapy (Moist-Exposed Burns Therapy, 'MEBT') and the discovery of Moist-Exposed Burns Ointment (MEBO), a topical drug used for maintaining a physiological environment for burns wounds. MEBO should be used under the technical criteria of burns regenerative therapy (BRT) in order to fulfill the therapeutic potential. Years of clinical practice have testified that this treatment can heal deep second-degree burns



Fig. 1. Schematic illustrations of burns regenerative therapy.

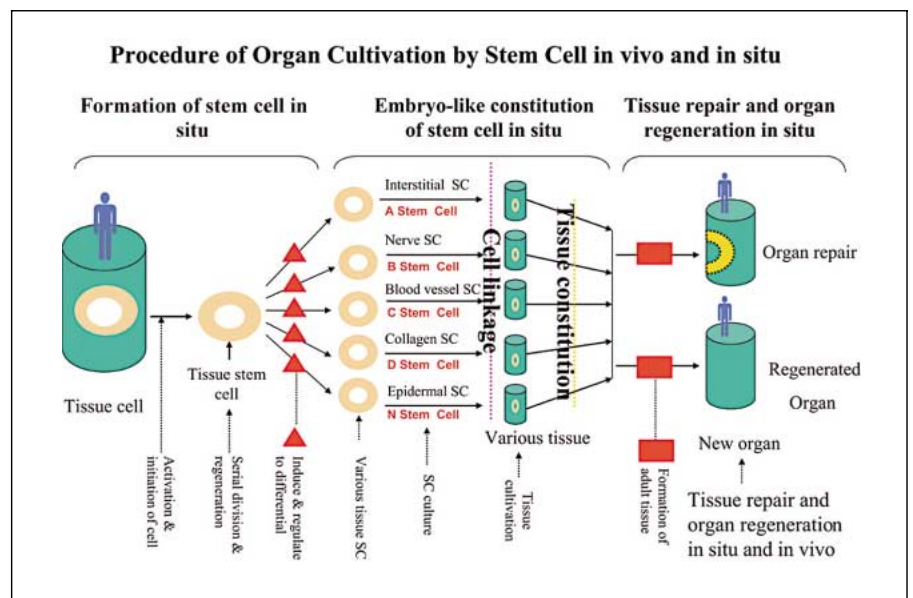


Fig. 2. Procedure of organ cultivation by stem cell in vivo and in situ.

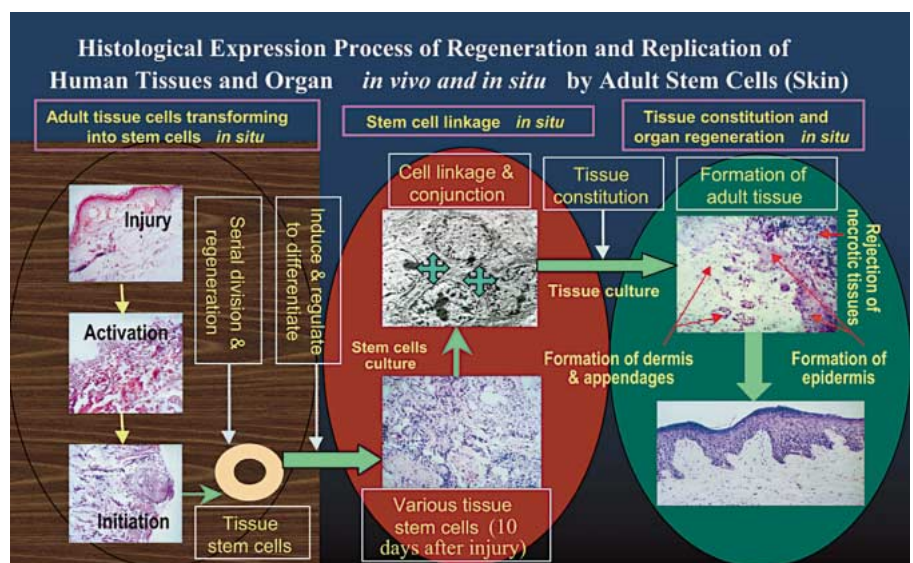


Fig. 3. Histological expression process of regeneration and duplication of human skin tissue and organs in vivo and in situ by adult stem cells after burns.

without scarring and also to spontaneously heal superficial third-degree burns. Numerous successes of clinical practice encouraged me to further explore the mechanisms of wound repair. Eight years of basic research disclosed that the mystery of physiological regeneration of burned skin lay in tissue stem cells. Based on this discovery, burns skin regenerative medicine was established and through physiologically repairing and regenerating skin, we were able to culture stem cells in vivo and in situ.

The principal part of BRT is MEBT and MEBO that consist of two procedures and eight techniques. Two procedures refer to liquefaction and discharge of necrotic tissues without causing secondary injuries, and maximum regeneration of skin tissue over the basal layer of viable tissue on wounds. Eight technologies include: initiation and regulation of stem cells; culture of stem cells in vivo and in situ; discharging necrotic tissues by liquefaction without causing further injury; exogenous tissue culture medium (MEBO) for skin regeneration; physiologically controlling bacteria and toxin infection by non-bactericidal mode; creation of a physiologically moist environment for skin regeneration; micro-isolation of skin wound for regeneration, as well as supply of oxygen and nutrients required for skin regeneration (fig. 1).

BRT is the only technology currently available to successfully repair and clone organs by the culture of stem cells in vivo and in situ. The cloning process of other organs will soon be identified subsequent to the success of cloning skin. On May 28th, 2002, we disclosed one of our research results 'Mapping process of regenerating and cloning human tissues and organs' which has been submitted for patent application. The website www.stemcellresearchnews.com in the United States made a full coverage on this significant event. The mapping objectively demonstrated that evolvement of cells in repairing injured tissue is indeed a process of differentiation and integration. Firstly, when the body is injured, the viable cells in situ are initiated and transformed into adult stem cells. Secondly, adult stem cells are further induced and directionally differentiated into various tissue stem cells that will commit to tissue repair in the late stage. Thirdly, the nascent tissues come into being and the newly regenerated tissue stem cells further link with the nascent tissue. Finally, various nascent tissues integrate into the injured organ to form new functional tissue and organs and therefore fulfill the repair and regeneration of targeted tissues and organs in vivo and in situ (fig. 2).

This research result is a milestone in the human life sciences. It confirmed the assumption of the following:

(1) injured tissues have potentials to repair with full recovery of function in vivo and in situ; (2) activity of cells plays the principal role in this repairing process, and (3) it is within our ability to initiate the stem cells, regulate the directional differentiation, repair tissues and regenerate organs in vivo and in situ.

The mapping process of regeneration and duplication of human tissues and organs in vivo and in situ represents a general and typical process. While each different tissue or organ has its own mapping, we will publish them shortly. Figure 3 shows the histological expression process of regeneration and duplication of human skin tissue and organs in vivo and in situ by adult stem cells after burns.

Burns Regenerative Medicine and Therapy presents the basic research and clinical results in repairing burns wounds by the culture of epidermal stem cells in vivo and in situ, which is only a small proportion of our scientific research results. Achievements on the repair and regeneration of other organs including stomach, intestine, marrow, pancreas, liver, kidney, heart as well as nerve will be published in separate volumes of *Regenerative Medicine and Therapy*. Regenerative medicine, while a dream in the west, is happily a clinical reality in China. We are pleased to be publishing volumes of *Regenerative Medicine and Therapy* in order to contribute to the knowledge base of scientists and doctors the world over who will be challenged and themselves stimulated by our advances in stem cell research.

Above, we present the basic concept of regenerative medicine. Future research in this field requires deliberative and cooperative efforts between scientists and doctors from every country. Embryonic stem cell research is one of the approaches, but it has little value of clinical application as the tissue and organs of humans are more appropriately and advantageously considered as a whole system. Available successful clinical results from traditional Chinese medicine should be considered as the basis of regenerative medicine research in 'total' practice. Traditional Chinese medicine offers a valuable philosophy that should be further expanded through the incorporation of concrete scientific methodology, innovative research approaches, well-established scientific thoughts, rational analysis, and rigorous conclusion. This is our common course as we establish a systemic academic college of world-class scientists. We believe that significant achievements in regenerative research will be ultimately obtained through our collaborative efforts and we welcome all who in a like manner will apply themselves to this noble cause.

Rationale Foci of Local Treatment of Burns Medicine and Therapy

Burns are systemic complex injuries following skin exposure to thermal energy. In this chapter, we focus on the local pathogenesis of burns when and after thermal injuries occur to disclose the pathogenic basis and rationale of local therapeutics.

Pathogenesis Focus of Burns Wounds

Following thermal injury, skin undergoes three injury phases in pathogenic order: physical injury, biochemical injury, and rejection response of necrotic tissue.

Physical Injury Phase

This includes direct and indirect physical injury. Immediately following skin surface exposure to thermal source, the resultant direct injury leads to necrosis of interface skin, which is called 'direct physical thermal injury'. Although the thermal source causing the direct injury is removed, the heat does not dissipate from the skin immediately. The residual heat continues to produce a cumulative thermal effect which causes secondary thermal injury to the skin. This secondary trauma usually persists for 6–12 h. This is called the 'indirect physical injury phase'.

Biochemical Injury Phase

Local biochemical injury begins within 1 h of the thermal insult and lasts for up to approximately 72 h postburn. This persists through the thermal biochemical reaction phase and the biochemical inflammatory reaction phase on the time order. At 1–2 h postburn, there is a significant increase in capillary permeability occurring in the injured, though still viable tissues, contiguous with the necrosis caused by direct thermal injury. This results in

exudation of intravascular fluid toward the wound surface and interstitial space while tissue ischemia is occurring. Simultaneously, the injured but viable tissues and cells in the area of lesion develop edema due to metabolic disorder. At this time, the permeable capillaries release plenty of chemical substances which not only locally aggravate the injury itself and damage the peripheral uninjured areas, but also may subsequently result in systemic injury. Although it is not quite clear what these chemical substances are, they appear to include histamine, 5-HT, hydrogen ion, kinin and bradykinin, etc. This phase is called the 'thermal biochemical reaction phase'. About 2 h later, the thermal biochemical reaction continues to affect the viable tissues in the injured area to cause a series of inflammatory reactions. The initiation of such an inflammatory pathological reaction in the injured area may result in the full spectrum of pathological injuries. For instance, inflammatory reaction activates the blood coagulation system to induce progressive thrombosis of the microcirculation, which may cause necrosis of the injured but viable tissues and may also result in ischemic and anoxic necrosis of the surrounding uninjured tissues. This process may last for 72 h postburn and is called the 'biochemical inflammatory reaction phase'.

Reject Reaction of Necrotic Tissues

At 72 h postburn, the wound tissue comes into a phase of rejection reaction, which is a response of the viable tissues due to disintegration of necrotic tissue and cells in the interface of the lesion area. Usually mixed and extensive, this reaction process primarily includes three pathogeneses: (1) the disintegration of necrotic histiocytes in the injury interface; (2) the regeneration of viable histiocytes in the interface of the lesion area; (3) microbial infection in the injury interface. Besides an inflammatory reaction, disintegration of necrotic histiocytes may induce cell liquefaction in the injury interface and, more impor-

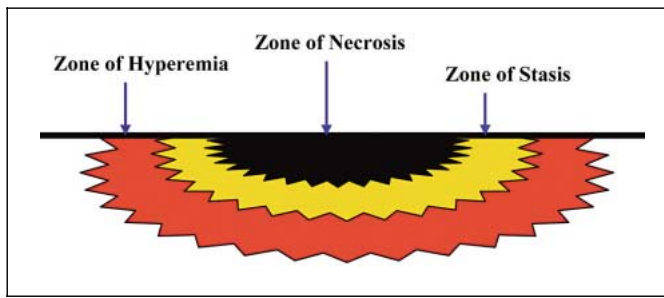


Fig. 4. Illustration of the pathomorphological characteristic of burns wounds.

tantly, the accumulation of cell liquefied products continues the aggravation of the injured tissue. Meanwhile, the residual viable tissues in the injury interface begin instinctive regeneration when disintegrated tissues develop into a destructive substance unfavorable to the environment of cell regeneration, thereby inducing serious inflammation. The combination of the two above pathogeneses disturbs flora residing in the skin and causes the destruction of microbes in the injured area, both of which further aggravate damage and may result in systemic injury at any time. This process is called ‘rejection injury of necrotic tissues’, and is the last primary injury of a burns wound.

Pathological Focus of Burns Wounds

Pathological changes after burns consist of injury pathology, repair pathology and physiology according to the changes of local wound. The injury pathological focus mainly refers to pathomorphological changes following thermal injury of skin while repair pathology and physiology refers to auto-repairing pathological and physiological changes of injured skin.

Characteristic of Morphological Changes of Injury Pathology

The injury area of burns skin is divided into necrotic and reaction layers from superficial to interior. The former results from physical injury, the latter from chemical reactions following thermal injury. In accordance with pathogenesis characteristic of burns, the tissue in the thermal chemical reaction layer is gradually transformed to the progressive necrosis and inflammatory reaction layers, thus the unique morphological appearance of local pathology following burns injury is formed. There are three concentric zones of thermal injury from superficial

to internal which exist in burns wounds (excepting first-degree burns) (see fig. 4). The central ‘zone of necrosis’ is directly injured by the heat source, causing immediate cell death. Outside this zone is the ‘zone of stasis’ which is due to indirect thermal injury and chemical injury resulting from the circulatory stasis and tissue degeneration caused by progressive microcirculatory thrombosis. The outermost zone is the ‘zone of hyperemia’ where skin tissue experiences an inflammatory reaction caused by local thermal and chemical injury. This zone is characterized by a series of fully reversible pathomorphological changes including tissue edema, hyperemia, anoxia and exudation.

The pathological injury changes within the three zones reveal the most complicated biodynamics of all traumatic wounds. Apart from the natural changes among the three zones, their changes are also closely related to the administration of different clinical therapies. The application of a therapy which causes further injury to local wound may worsen the viability of all three zones. If no secondary injury is caused, the three zones may resolve in a natural process. However, if one uses a therapy which is protective and therapeutically effective to the tissue beneath the necrotic tissues, the progressive injury of the tissue in the zone of stasis may be prevented or reversed. Though the necrotic layer of the burns wound surface is impossible to rescue, the management of necrotic tissue of burns wounds affects viable tissue in the deep layer. If the necrotic layer is left alone, a nonphysiological pressure exerted upon the underlying tissue results due to tissue dehydration and lack of normal skin elasticity. The pressure and increased microcirculatory blood concentration may lead to pressure ischemia with consequent anoxia thus aggravating the progressive necrosis of the underlying tissue. Application of therapy characterized by dry, coagulation, formation of crust or eschar will cause lethal injury to stasis and hyperemia tissues, and thereby cause extension of the depth of the burn wound even to full-thickness necrosis. However, if measures for losing the necrotic layer or preventing pressure to the underlying tissue are adopted, this full-thickness necrosis can be prevented and reversible changes of underlying stasis and hyperemia tissue may be attained.

Besides the aforementioned indirect factors, treatment of the zone of stasis is also affected by various direct factors. For example, the application method of crust/eschar formation characterized by drying, dehydration and protein coagulation, or maceration method may speed up the microcirculatory progressive thrombosis. Alternatively, options exist for protecting the deep tissue which optimize the recovery of the tissue.

Repeated observation has taught the astute observer that the zone of hyperemia may recover naturally if no further injury occurs to the stasis tissues. Unfortunately,

most typical burns treatments inadvertently allow progression of burn to necrotic tissue due to serious ischemia, anoxia and cell death.

Characteristic of Repairing Pathological and Physiological Changes

A revolutionary concept for the thorough repair of the aforementioned pathomorphological changes is put forward by the author after years of study of skin regeneration. The data derived from previous studies worldwide is marginally useful as it involved tissues treated by the standard treatment model of conventional burns surgery and burns care. Of note is that this treatment itself prevented people from understanding the natural repair mechanisms of burns wounds healing. A case in point is Dr. Jeckson who stated that he had never had a chance to observe how burns wounds heal in spite of his several decades of experience in the research and treatment of burns. What he had observed, admittedly, was either the burns wound covered by crust/eschar and thick dressing, reactive granulation tissue, or the absence of burn tissue due to surgical excision. His admission suggests that conventional burns therapy worldwide is limited to surgical excision and skin grafting therapy. Confirmation of that unfortunate fact is offered by the famous burn surgeon and chairman of the American Burn Association Dr. Deitch who stated in 1988: 'Burn surgeons only know how to excise and graft skin instead of how to regenerate skin.' These remarks pinpoint the importance of evaluating innovations in burns regenerative medicine and therapy.

Following the separation, rejection or discharge of necrotic tissues, the residual viable skin tissue or information tissue (isogenous tissues and cells residing in subcutaneous tissue related to dermis and epidermis) remains in the injured area. The pathological change of natural burns repairing begins as follows:

- 1 Superficial second-degree burns involve only the epidermis, so the repairing takes place in the epidermis tissue. The wound itself heals spontaneously without leaving a trace of scar whatever therapy is used since epidermis is formed by the layer-by-layer changes of basal cell layers.
- 2 Deep second-degree burns involve part or most of the necrotic dermis. The pathology of repairing varies when different therapeutic techniques are applied. When treatment of dry and crust formation is applied, necrotic tissues are promoted to form a crust that is rejected from the underlying viable tissues along with the zone of leukocyte infiltration. If no infection and suppurative pathological change occur in the sub-crust, then the epithelial cells in residual dermis may grow along the zone of sub-crustal leukocyte infiltration. This then covers the wound under which dermis col-

lagenous fibers and blood vessels proliferate in a disorderly manner. The wound closes pathologically via this epithelization and scar formation follows the shedding of crust. If subcrustal infection and suppurative pathological changes occur, the wound may be further injured and deep second-degree burns may progress into third-degree trauma followed by a full-thickness necrosis resulting in granulation of the wound. The wound resolves with permanent pathological healing even if it had a chance to close by skin grafting. However, suppose the necrotic tissues were to be discharged from the wound without causing any injury to the wound. Suppose also that the residual viable tissues were retained to the degree that a physiological environment is established sufficient to promote spontaneous residual tissue repair. In this case, we would witness wound healing without scar formation. By managing environment and local substances to optimize endogenous repair and regeneration, we facilitate healing of deep second-degree burns resulting in scar-free healing and recovery to normal tissue anatomy and physiology.

- 3 Third-degree burns are equivalent to full-thickness burns and involve tissue beneath the dermis. They are defined according to the concept of skin burns. In terms of anatomy or histology or cytology, the skin consists of two layers: the epidermis derived from ectoderm, and dermis (corium) derived from mesoblast. Full-thickness refers to the combination of epidermis and dermis. As the conjunction area between the underlayer of dermis and subcutaneous tissue is an area like a rugged highland instead of a plane, full-thickness projects deep into the surface layer of subcutaneous tissue. In other words, full-thickness or third-degree burns involve tissue as deep as the surface layer of the subcutaneous tissue. Burn injuries involving most of the subcutaneous tissue and muscle layer extend beyond and should be excluded from the conception of skin burns. Diagnosis should be made in accordance with the injured tissue. For example, burns involving partial or major subcutaneous tissue should be termed subcutaneous tissue burns, burns involving full subcutaneous tissue and muscle layer should be termed muscle burns, burns involving full muscle layer and bone should be termed bone burns. It is same with the diagnosis of electric injury: burns caused by electricity are the ordinary skin burns while burns caused by electric current involve skin, subcutaneous, muscle, bone as well as other tissues which electric current penetrates. For a better and simpler understanding, the author has tried to classify third-degree burns into third superficial and third deep burns, of which the latter refers to non-skin burns involving the tissue under the subcutaneous layer. Thus, we might differentiate between skin and non-skin burns.

The pathological repairing of third-degree burns is characterized by the repairing of granulation tissue. There is no epithelial cell in subcutaneous tissue for closing the wound due to the full-thickness necrosis. It is conventionally recognized that a wound with a diameter of around 2 cm may close by migration of epithelial cells from the wound margin and heal spontaneously, while the larger wound should only be closed and healed by surgical skin grafting. Remarkably, despite this conventional wisdom, the author's studies proved that third-degree burns wounds therapy is possible through direct pathological or physiological healing without surgical intervention. The results of these studies indicated that: (1) Subsequent to burns, the adult tissue cells in residual viable subcutaneous and/or fat layer may be converted into adult skin stem cells. (2) Adult stem cells have the potential to regenerate and duplicate the organ of full-thickness skin. (3) The aforementioned regeneration and duplication was accomplished by the collaborative efforts of endogenous human regenerative potentials and control of localized tissue environmental conditions. (See relevant information below.)

Therapeutics Focus

Burns regenerative medicine and therapy refers to the medical management up to the complex pathogenesis of burns. Emphasis in this volume is made on the therapeutics focus of local burns wounds, an especially conclusive description. Considering the management of the burns wound environment, two techniques are currently available worldwide for local burns treatment. One option is based upon the perceived benefit of maintaining the wound in a dry and dehydrated state while the other strives to maintain the wound in a physiologically moist state. Research clearly demonstrates that the former compromises while the latter encourages tissue regeneration. Simply stated, one is pathological and the other physiological as regards tissue repair. In clinical treatment, careful consideration is needed for choosing the appropriate burn therapy according to the depth of the burns wound. For superficial burns, as long as pain is relieved and further injury is prevented, any burns therapy may achieve successful results. For deep second-degree and/or third-degree burns, the choice of therapy is more critical since pathological healing may result in disability and lifelong distress for the patient.

Due to differences of cultures and academic ideologies in the medical circles, two categories of burns therapy predominate in treating deep burns wounds. These are: (1) 'surgical excision and skin grafting therapy', and (2) 'conservative repairing therapy (burns regenerative

medicine and therapy)'. The former is symbolized by the therapy established in the 1930s, with the characteristic of excision and skin grafting (a variety of autografts) for wound closure. As the main stream in the western medical circles, this therapy has been adopted in hospitals all over the world. The latter, burns regenerative medicine and therapy, involves two modalities: moist-exposed burns treatment (MEBT) and moist-exposed burns ointment (MEBO). This innovative and impressive modality was established by Dr. Rongxiang Xu in the late 20th century. It features the discharge of necrotic tissue by liquefaction in a manner that does not cause further secondary injury and also supports the establishment of a physiological environment sufficient to repair residual viable tissues while regenerating skin tissue. This therapy has been successfully exported to 48 countries and enjoys wide clinical application while attaining the predominant status for burns care in eastern medical circles. Herein to follow are the main points of the two categories of the burn therapies.

Therapeutics Focus of Surgical Excision and Skin Grafting Therapy

Surgical excision and skin grafting therapy is established upon the premise that no effective method is available for treating a series of postburn illness. It is considered that the tissue in the zone of stasis of deep second-degree burns is doomed to a complicated and dangerous progressive necrosis. Additionally, it is assumed that wound with necrosis of full-thickness dermis is unlikely to heal spontaneously. During the procedure of conservative repairing treatment for deep burns wounds, infection, inflammation and other serious complications may develop and become life-threatening, and the treatment result will be pathological. Based upon the above consideration, a therapy was established: First transform the burns wound to a traumatic wound via surgical intervention and then perform the conventional burns treatment in an attempt to increase survival rate. In the clinic setting, the whole burned necrotic tissue together with some viable dermis or subcutaneous tissue are removed, creating a surgical wound of muscle layer over which a variety of autografts are placed to close the wound. Admittedly, this therapeutic option anticipates a compromised and suboptimal result while striving mostly to save the patient's life. This therapy is a purely surgical technique and functions with disregard to burns physiology. As a treatment, it resembles the treatment of a gastric ulcer by surgical intervention – subtotal gastrectomy. Therefore, this therapy does not treat burns tissue itself but constitutes simply a surgical therapy for treating muscle or deeper burns rather than skin burns.

Therapeutics Focus of Burns Regenerative Medicine and Therapy (MEBT/MEBO)

MEBT was invented on the basis of a series of burns natural pathogeneses, appreciating each aspects of burns tissue's physiological response including physical, chemical and biochemical reactions. Additionally, it incorporates an understanding of necrotic tissue rejection as well as principles of physiological repair and regeneration. The main therapeutic focus is manifested in the following aspects: (1) alleviation of wound pain by microprotection of injured nerve ending and by relief of hair arrectores pilorum spasm; (2) prevention or resolution of continuous physical thermal injury by the application of an ointment which draws away the residual heat from the wound through a specially designed frame structure dosage; (3) discharge of necrotic tissues by liquefaction without causing further secondary injury while allowing the residual viable tissues to continue an endogenous process of regeneration; (4) creation of a physiologically moist environment to ensure the physiological repair of residual skin tissues; (5) realization of skin regeneration in compliance with the principles of endogenous histological and cytological regeneration; (6) control of microbial concentration and toxicity at the wound site so as to prevent and control pathogenic infection through continuous active

drainage of the wound as well as by other mechanisms; (7) regulation of the physiological repair of burns wounds with the comprehensive active ingredients of the MEBO ointment.

Burns regenerative medicine and therapy (MEBT/MEBO) was established in the context of a worldwide consensus that surgical burns therapy comprised a suboptimal therapy. It arose in a therapeutic vacuum where no substantial innovations had been offered for modern burns treatment. MEBT/MEBO has basically realized the treatment of burns tissue itself, and become the mainstream medical therapy for skin burns. However, even MEBT/MEBO has its limitations, for presently it also is not suitable for treating burns involving muscle or deeper layers. Unfortunately, current research has made no progress in regenerating new skin from muscle tissue. For burns with a diameter less than 20 cm involving the muscle layer, the wound may heal with MEBT/MEBO by the migrating of epithelial cells from the wound margin transversely to regenerate skin and then close the wound. With the assistance of a surgical technique, electric burns and local burns involving bones may be treated with satisfactory results (data attached below). Happily, burns replacement therapy offers a breakthrough therapeutic benefit in that it may enable larger muscle layer burns to heal spontaneously.

Evaluation and Classification of Burn Severity

Clinical Assessment of Burn Area

In 1961, Wellace advanced the generally accepted ‘rule of nines’ which, while simple and practical, is not very accurate. The ‘hand method’, which uses the patient’s hand as a standard for measurement (i.e. the surface area covered by the patient’s hand with fingers closed being roughly 1% of the whole body surface) is very useful for measurement of small or multiple scattered areas.

The head and neck is 9% of the area of the whole body, two upper limbs is 18% (2×9), two lower limbs (including buttock) is 46% ($5 \times 9 + 1$), the front and the back of the trunk (including perineum) is 27% (3×9). This pro-

portion may be different according to the patient’s age and gender. Therefore, some more accurate methods have been put forward. In 1970, ‘Chinese rule of nines’ was named, this is based on the actual proportion of body surface area of Chinese people, and is now popularly applied (table 1). In China, there is another rule named the ‘rule of tens’ which considers the head and the neck area as 10%, upper limbs $2 \times 10\%$, trunk (including perineum and buttock) $3 \times 10\%$ and lower limbs $4 \times 10\%$. This method is also very simple and easy to apply.

Table 1. The ‘Chinese rule of nines’ for determination of burn area

Position		% of adult body surface area	% of child body surface area
Head and neck	hair	3	9+ (aged below 12)
	face	3 (9 in total)	
	neck	3	
Both upper limbs	upper arms	7	2×9
	forearms	6 (2×9 in total)	
	hands	5	
Trunk	front	13	3×9
	back	13 (3×9 in total)	
	perineum	1	
Both lower limbs	buttocks	5*	$5 \times 9 + 1$ – (aged below 12)
	thighs	21	
	shanks	13 ($5 \times 9 + 1$ in total)	
	feet	7*	

* For adult females, buttocks and feet account for 6%.

Clinical Evaluation on Depth of the Burns Wound

Since burns wounds are conventionally treated with dry-exposed therapy or bandaging method, the resultant obscuring of the wound by scar or dressing prevents inspection of the wound the next day. People can only see the crust and the dressings, thus it becomes very difficult to estimate wound depth accurately. Though some instruments have been invented for diagnosis of burn depth, their accuracy typically proves to be less than visual estimates by experienced doctors. One advantage of burns regenerative medicine and therapy (MEBT/MEBO) is that burns wounds can be inspected in the whole treatment process by the naked eye. This allows doctors to accurately observe the changes in the wound over time, thus allowing for simple determination of wound depth. At this point, thanks to accurate observation of the pathological changes in the burn area, the clinical characteristics of burns wounds and the clinical diagnosis of burn depth can be summarized as follows.

First-Degree Burns

Diagnosis of first-degree burns is the same as the conventional surgical standard. The burns wound is erythematous in appearance, has no blister, may be painful and have slight swelling. These wounds typically heal spontaneously by 3 days postburn with varying degrees of epithelial exfoliation. The injured area should not be included when estimating the total extent of the burn since, pathologically speaking, the skin was only thermally irritated and not burned, as there was no structure injury to epidermal basal layer and no formation of zone of stasis. MEBO can accelerate the recovery of first-degree burns with resolution of erythematous skin within 24 h and immediate cessation of pain. The injured epithelia in the superficial layer of epidermis can be exfoliated and removed together with the MEBO and no systemic reaction occurs.

Second-Degree Burns

Worldwide, the incidence of second-degree burns is clinically the highest. The pathological changes of second-degree burns are very complicated and, until now, quite difficult to manage. Second-degree burn is often painful and sensitive to pin-prick. The microcirculation in the injured tissue is damaged. Congestion and exudation occur, and a zone of stasis may exist in the dermis. When progressive necrosis of the dermal tissue occurs due to microthrombosis formation, this worsens the clinical picture. Second-degree burns destroy the skin barrier and

result in a serial systemic reaction and infection. Without application of BRT, as epithelial tissue is seriously injured, the wounds healed with disfiguring and painful scar formation, dooming the patient to a lifetime of suffering.

The diagnosis of second-degree burns is not difficult. However, with conventional burns treatments, it is difficult to differentiate between superficial and deep second-degree burns because the wounds cannot be inspected directly and clearly. Therefore, the diagnosis is based only on the doctor's conception of the process and not on direct experience. However, when burns regenerative medicine and therapy (MEBT) is applied, there is direct and adequate evidence for establishing the correct diagnosis.

Superficial Second-Degree Burns

Scald Wound. Within 2 h post-scald, obvious color change occurs in the dermis and blisters of different size appear. After blister exfoliation, the skin tissue looks moist, slightly red with exudation and has good elasticity. Fine hair stands straight and is sensitive to pain when pulled out. The wound is smooth. Two hours later, wound exudate increases with bright red color, and wound swelling (especially wounds in the face) can be observed. 48 h later, swelling subsides and exudate decreases gradually. Four days later, epidermis of the wound thickens and the wound heals in about 6 days.

Burns Wound. The wounds may have different appearances according to different causes, e.g. epidermal layer of the wound burned by a gas fire flame has a dark color. Compared with scald wounds, the wound surface caused by flame is drier and after removal of the blisters, the wound looks dark red, because flame results in serious dehydration. Pathological changes in burns wounds caused by flame are the same as in scald wounds.

Deep Second-Degree Burns

Deep second-degree burns may be further differentiated into two subtypes: (1) superficial subtype of deep second-degree burns with thermal injuries reaching the dermal papilla layer; (2) deep subtype of deep second-degree burns with injuries reaching the dermal reticular layer. The key points to distinguishing the deep second-degree burn from the superficial second-degree burn at the early stage are that in the former, the necrotic layer and the zone of stasis do exist in the dermis of the wound, while in the latter (superficial second-degree burn), there is no formation of either the necrotic layer or the zone of stasis. Therefore, in the treating procedure, superficial second-degree burns wounds do not have

liquefied products of necrotic tissue, while deep second-degree burns wounds produce copious amounts of liquefied products from necrotic tissue before the wound healing.

The Superficial Subtype of Deep Second-Degree Burns Wounds. The appearance of this type of wound is similar to superficial second-degree burns wounds. Scald wounds have blisters of different size and after blister exfoliation, the dermal tissue looks light red or pale with much exudate. The wound has good elasticity. 24 h later, numerous red pin-spots appear in the pale wound while the superficial layer of the dermis becomes semi-transparent, sensitive to pain, and gives a positive reaction when the hair is pulled out. After the exudation stage, the wound looks red, superficial layer of the dermis begins to liquefy, the base of the wound is in a position lower than skin surface. In about 10 days, liquefaction products decrease, the wound grows up to the skin surface. Healing occurs within about 15 days without scar formation. Hyperpigmentation may appear temporarily. After 1 month, the skin color recovers to normal. The burns wounds appear dark or brown in the dermis at the early stage and blisters of different size may exist. After removal of the blisters, the dermis looks red alternating with white. Exudate occurs but fine hair disappears. The wound is sensitive to pain, gives positive reaction to needle stab test. After the exudation stage, the wound looks red much as the scald wound does.

The Deep Subtype of Deep Second-Degree Burns Wounds. At the early stage postburn, no blister appears, or small blisters may appear after a while. Scald wounds with no blister have epidermis separated and adhered to the dermis. Flame burns wound is dry with black carbonized substances on the epidermis. The wound is obtuse to pain. Some patients even have no pain sensation. If treated inappropriately, loss of pain sensation will continue, which is an unnecessary and unfortunate consequence. After removal of dead epidermis, the wound looks pale and leathery. In the deep region, dark red spots may be seen. The wound surface is dry with less exudate. After the exudation stage, the necrotic layer begins to liquefy. At this point, the wound base is obviously recessed relative to the skin surface. The wound heals to the skin surface in about 25–28 days. Generally, scar formation is not obvious. Often the final skin color is not uniform as some patients retain a disfiguring hypopigmentation.

Third-Degree Burns

Diagnosis of third-degree burns is easier than second-degree burns. The scald third-degree wound is pale with no blood supply and almost no exudate. Pain sensation is lost or reduced. Flame burns wound has a carbonized epi-

dermis which can be adherent to the dermis. The wound surface is lower than surrounding normal skin surface, there is a loss of pain sensation, and no exudate appears at the exudation stage. After treatment with MEBT/MEBO, sparse exudate can be seen. After the exudation stage, the necrotic tissue begins to liquefy, but this occurs much slower than that of deep second-degree burns wounds. Liquefaction completes in 15–20 days and granulation tissue appears in the wound. The superficial third-degree burn with thermal injury reaching the subcutaneous layer may heal by skin regeneration. A small area of deep third-degree burns wound (diameter <18 cm) with thermal injury reaching the muscle layer may heal without skin grafting but with scar formation. Large deep third-degree burns wound needs skin grafting. Pediatric third-degree burns wound is usually dark and red in color. When conventional surgical burns therapy is applied, it is difficult to estimate burn depth accurately, especially to distinguish deep second-degree and third-degree burns.

Pathological Basis of ‘Three Degree Four Division Method’ for Diagnosis of Burns Depth

First-Degree Burns

Only the epidermal layer is injured without blister. The blood vessels are dilated and the microcirculation is congested. The injured area is red shortly postburn. This area is sensitive to pain, because the sensory nerve endings are still there and endogenous chemicals such as kinin are released from thermal injured tissues, irritating the nerves. First-degree burns wounds heal without scar formation. When treated with conventional therapy, hyperpigmentation may be caused temporarily, but this typically vanishes in a few months. The skin recovers normally. When treated with MEBO, the skin recovers to normal appearance in about half a month.

Second-Degree Burns

Thermal injury reaches epidermal and dermal layers. The injured area is moist, with blister formation and epidermis exfoliation. Congestion is obvious. If injury reaches the dermal layer, swelling in the dermis and deep tissues may press the blood capillaries and obstruct the blood flow which causes the wound to look pale. The injured area is very sensitive to pain as is the first-degree burn. Second-degree burns can be classified as superficial second-degree burns or as deep second-degree burns.

Superficial Second-Degree Burns Wounds. Some epidermis extended to papilla, so they are not injured. The basal layer of the epidermis, i.e. regenerative layer, survives. The basal cells can divide and regenerate to cover the wound, thus the wound could be completely epidermalized.

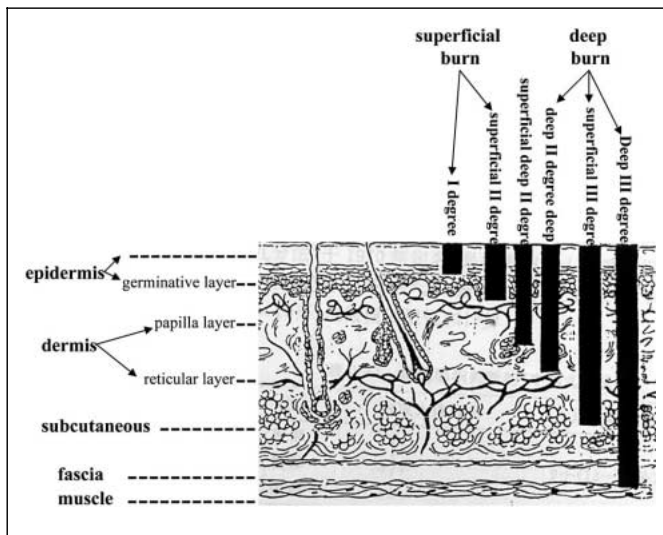


Fig. 5. Diagram of skin structure illustrating burns depth.

Deep Second-Degree Burns Wounds. Epidermis is completely destroyed, only parts of the skin appendages survive, i.e. basal cells of follicles and sweat glands which are located in the deep part of the dermis. The wound can also be covered and healed by division and regeneration of these surviving cells in the appendages. When conventional surgical therapy is applied, in most cases the wound results in infection and worsens, ultimately healing with epithelialization. Conversely, when MEBT/MEBO is applied, epidermidalization can occur, the full thickness of skin can be completely recovered. Changes in skin pigmentation may occur to different extent.

Third-Degree Burns

Progressive stasis zone necrosis and coagulation necrosis of epidermis occur, dermis and deep layer tissues of dermis are completely destroyed by thermal injury. No blood flow can be found in the necrotic area, the wound is pale. When treated with dry-exposed therapy, a black crust may form and even carbonization may occur. In children, wounds are red and black, because children have more water in the tissue and the skin is thinner. In the third-degree burns area, one can see some blood vessels in a thrombotic state. The lesion is not sensitive to pain, because sensory nerve endings are destroyed. The interface of the basal part of the injured area and normal tissue has reduced pain sensation. Small third-degree burns wounds can be covered by wound edge surviving epithelial cell proliferation and regeneration. But for large third-degree burns wounds, skin grafting or epithelial cell transplantation is required. Recently, it was found that sweat gland epithelia in the subcutaneous fatty layer can regenerate to cover the wounds. Accordingly, third-degree

wound injury reaching the fatty layer is capable of spontaneous healing.

Burns due to different causes may have different depths, so for diagnosis of burn depth, the doctor needs to clearly establish the cause of burn and understand the varied characteristics. For example, burns wounds with carbonized surface may not necessarily be third-degree burns. A moist scald wound with blisters must be a second-degree scald. An acid burns wound with crust is much shallower than a non-crust alkali burns wound.

When applying MEBT/MEBO, the wounds are exposed to the naked eye's inspection in the whole course, which provides an advantageous condition for clinical diagnosis.

'Three Degree Six Division Method'

People usually classify burn depth according to the penetration of anatomical structure. i.e. superficial, partial and full-thickness burns. Burns are also classified into three degrees by numbers: first-degree burns, only superficial cell layer is involved; second-degree burns involve basal layer of the epidermis and superficial layer of the dermis, while third-degree burns involve subcutaneous tissue. According to wound manifestation and healing, deep burns wounds can be divided in an even more detailed manner. Second-degree burns can be divided into three grades: Superficial second-degree burns refers to burn injury reaching the basal layer of epidermis with some of the basal layer still surviving. Superficial deep second-degree burns refers to burn injury reaching the dermal papillary layer while deep second-degree deep burns refers to burn injury reaching the dermal reticular layer. The theoretical basis is that in superficial second-degree burns wounds, the skin microcirculation has been injured but there is no stasis zone and most of the skin structure is retained. In deep second-degree burns wounds, the stasis zone occurs in the dermal papillary layer but epidermal structure is lost while most of the skin appendages are still retained. In deep second-degree deep burns wounds, a microcirculation stasis zone is formed in the dermal reticular layer and only a small part of the appendages is retained.

Third-degree burns can be divided into two grades. Superficial third-degree burns refers to necrosis of the dermis, but subcutaneous tissues still have vitality and a small part of the epithelial tissue of the sweat glands in deep subcutaneous areas survives. Deep third-degree burns refers to muscular layer thermal injury. Bone burns refers to bone injury (fig. 5).

In clinical practice, people often find that some burns wounds contain an obvious granulation while, around the lesion, there are a few scattered skin island tissues. This is called mixed-degree burn.

Clinical Classification of Burns Severity

The classification of burns patients according to severity is very helpful as it aids in triage (the organization of rescue and transfer of the patients to medical facilities and the rational allocation of manpower and material resources). With clear classification, care can be delivered in an orderly manner according to relative importance and urgency.

Classification Criteria Applied in China

Slight Burns

Second-degree burns with a total area below 10% body surface area (BSA).

Moderate Burns

Total burn area 11–30% BSA, or third-degree burn less than 10% BSA.

Severe Burns

Total burn area 31–50% BSA of third-degree burns area 11–20% BSA; and total burn area less than 31% BSA, but having one of the following conditions: (1) severe systemic condition or shock; (2) compound injury or complicated injury; (3) moderate or severe inhalation injury.

Extraordinarily Severe Burns

Total burn area above 51% BSA or third-degree burns area above 21% BSA.

Attention Points in Estimation of Burns Severity

- 1 To estimate the severity of burns, in addition to burn area and depth, consideration should include patient's age and health condition, complications, intoxication, etc.
- 2 The purpose of classification is to help organization of rescue, transfer of patients and allocation of manpower and material resources, but not for grading the treatment level. Patients with slight burns may deteriorate into a serious situation so that they also need careful and high level treatment.
- 3 Classification should be done in a proper way and requires confirmation.
- 4 Accurate and detailed notation of the estimation and classification decisions should be recorded to assure quality control.

Clinical Principles of Burns Regenerative Medicine and Therapy



Clinical Principles of Burns Regenerative Medicine and Therapy

Standardized Local Treatment of the Burns Wound

Background Information of Standardized Local Treatment and Sources

In clinical burns treatment, as in all areas of medicine, there is a 'voltage drop' between the rarified academic environment and the trenches of clinical practice. The clinician often cannot keep abreast of academic advances in treatment techniques. Many experienced doctors may disregard innovations preferring to stay with the 'tried and true'. In some cases, fidelity to past protocols and maintenance of their dignity and reputation is more important than the actual therapeutic results experienced by their patients. Thus we see in medicine, as in all arenas of human commerce, an unfortunate phenomenon whereby the innovator must promote an improvement in the status quo to a temperamentally unresponsive professional audience. Rather than being accepted on their own merits, innovations are typically greeted with a cold shoulder and an unfortunate degree of suffering is visited upon patients until the paradigm shift is accomplished. Rare is the doctor who seeks out and consults an inventor about proposed improvements in clinical protocols. Even in today's information age where theories and practices can be easily investigated, many doctors remain unable or unwilling to consider proposed improvements to conventional and outdated treatment techniques.

In order to meet this challenge and to demonstrate to medical professionals and the public the benefits of an innovation in burns treatments, this chapter will present a comparison of two groups of clinical pictures of burns patients treated either by the contemporary methods or by the burns regenerative medicine and therapy (BRT) protocols (MEBO/MEBT). These pictures compel the viewer to rise above petty loyalties to different schools of

thought and to rely instead upon the desire to offer the best possible care to those suffering from burn injury. These pages invite burns doctors around the world to join the collaborative effort and further this exciting area of research and clinical care.

The author has restrained himself from commenting on the relative therapeutic effects pictured below, choosing instead for the reader to experience their merit for him/herself.

Sources of Representative Cases

Case of extensive deep burns treated by conventional surgical dry therapy (excision and skin grafting, abbr. dry therapy): A case of 71% third-degree burns, source from a burns center standing for the international level of burns surgery. Another case with 81% third-degree burns treated with cultured composite autograft (CCA) technology, and the data from the international journal *Burns* [vol. 25, No. 8, 1999].

Extensive deep burns treated by BRT (MEBT/MEBO): A case of 85% third-degree extensive burns treated by a burns team led by Professor Rongxiang Xu who is the inventor of this therapy, data from *The Chinese Journal of Surface Burns, Wounds and Ulcers*, No. 3, 1997.

Severity of Burns of Three Cases

In accordance with the international classifications and standards of burn severity, 3 cases were significantly comparable. Though there are remarkable differences in medical conditions, the results revealed many more differences in therapeutic effects (table 2).

Table 2. Comparison of severity of burns and medical conditions among three cases

Sex	Age	Sign on admission	Cause of burn	Third-degree BSA	Inhalation injury	Hospital level	Complicated injury	Ward condition
<i>Dry therapy</i>								
M	23	shock	flame	71%	tracheotomy	first class	no	sterilization and isolation
<i>Moist therapy</i>								
M	35	shock	flame and hot cement	85 %	tracheotomy	secondary class	open multiple metatarso-phalangeal fractures	ordinary ward
<i>Composite autograft therapy</i>								
F	12	shock	flame	81%	tracheotomy	advanced hospital in USA	open left tibia and fibula fractures	sterilization and isolation

Compared Parts and Burn Depths

To accurately and objectively demonstrate the clinical treatment, anterior chest and face with comparable third-degree burns wounds in each case were selected for comparison. The case of composite autograft therapy serves as a reference to show the common ground and contemporary development of surgical excision and skin grafting therapy. Autografts are widely used in the standard surgical burns management and cultured composite autografts (CCA) have recently been used in the United States for skin grafting.

Standardized Local Treatment of Burns Wounds

To help facilitate the understanding of a variety of burn treatment techniques, 3 cases were compared at the following three clinical procedures: treatment of burnt skin, healing and closure of wound, and need for reconstruction after wound healing.

Case 1: Surgical Excision and Skin Grafting Burns Therapy

Background Information

A 23-year-old male was burned when fire burned his cotton clothes ignited by steel residue at his workplace. Immediate antishock management was administrated at the factory clinic. At 4 h postburn, the patient was transferred and arrived at the hospital 7 h 20 min later. Upon

arrival, initial assessment revealed that the patient suffered severe burns, including face and both auricles, anterior neck, both hands, chest, abdomen, left thigh and both legs; wound showing leather-like; dendritic vascular embolism. His vital signs included: T: 35.9°C, P: 44/min, R: 32/min and BP: unmeasurably low. The patient developed hypovolemic shock postburn which was complicated by inhalation injury.

On admission, rapid fluid resuscitation was started to correct shock and tracheotomy was performed to improve ventilation. Escharectomy was then performed on the third-degree wounds of the left forearm and both legs to relieve pressure and improve blood circulation at the extremities. Superior vena cava puncturing and right cardiac floating catheterization were performed to monitor heart function. On day 2 postburn, surgical eschar excision to the underlying fascia and micro-particle autografting was performed on the extremities. On day 6, the patient received eschar excisions on the chest and abdomen, on which evenly holed allograft sheets were applied. Four days after the operation (day 10 postburn) small pieces of split-thickness autografts were placed on these wounds through the openings of the allograft. The patient developed severe *Pseudomonas* septicemia, and became comatose with low body temperature for 1 week. Septicemia was well controlled after intensive care. After that, repeated skin grafting was performed 9 times and most of the wounds healed. On day 43 postburn, corneal ulcer in the left eye occurred and was treated with eye drops and retrobulbar injection. Corresponding measures were taken to prevent stress ulcer, control infection and prevent pulmonary complications. The length of hospitalization was 70 days.

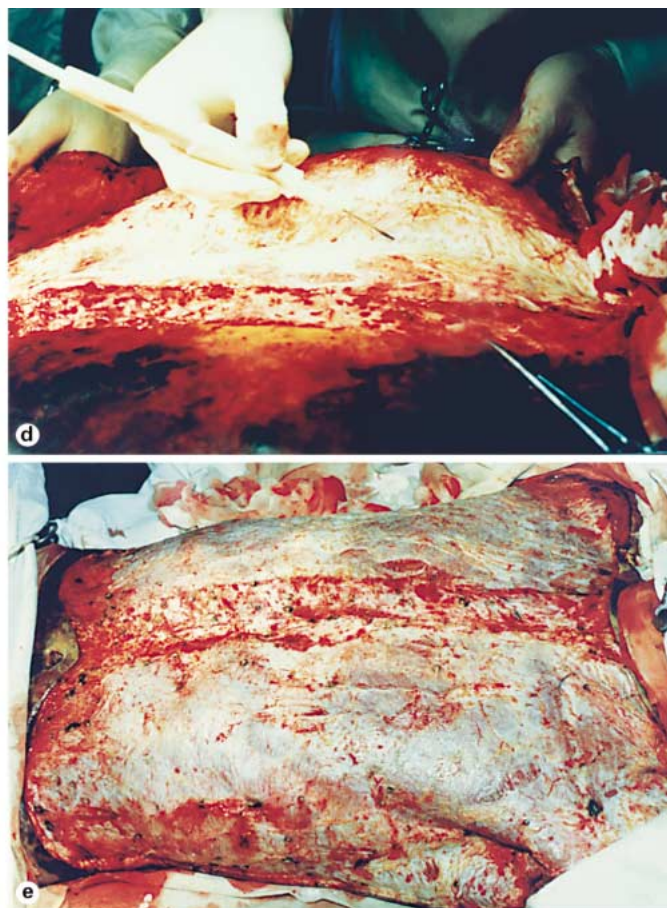


Fig. 6. **a** Before treatment. **b** Exposure and dryness of burned skin. **c** Adopting various methods to enable dryness, dehydration and eschar formation of burned skin. **d** Excision with electric knife and removal of dead burned skin, subcutaneous tissue together with viable fat layer down to the underlying fascia. **e** Muscle layer covered by viable deep fascia appeared after excision.

Procedure and Results (fig. 6, 7)

First Step: Treatment of Burned Skin

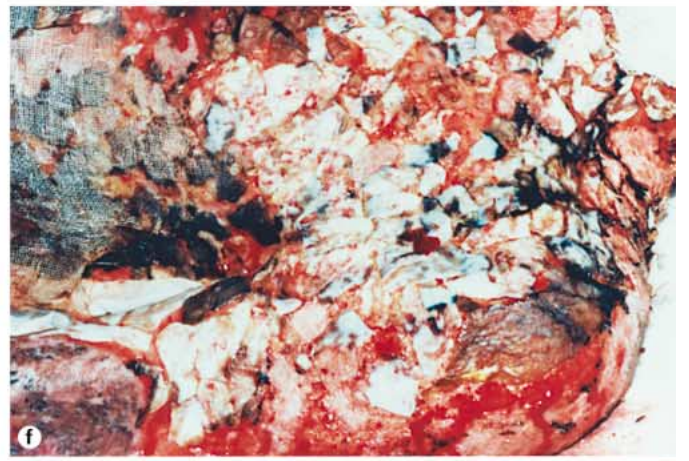
Dryness and debridement of eschar replaced the burn wound by a surgically induced traumatic wound with neither burnt tissue nor skin tissue.

Second Step: Healing and Closure of Wound

Third Step: Reconstruction after Wound Healing

During a period of 14 months, nine surgical reconstructive operations were performed. However, disablement and disfigurement still presented.

Fig. 7. **a** Punching holes evenly on prepared allograft sheet. **b** Stretching the graft as mesh and covering the wound. Four days later, small pieces of split-thickness autografts were placed on the wound through the allograft openings. **c** Bandaged with adequate dressings. **d** After 20 days, the allograft was rejected. Autografts survived partially. **e** Re-autografting of areas where the previous grafting failed. **f** Even monkey skin was grafted (on day 47 postburn). **g** Gradual wound healing after multiple grafts. **h** On the 74th day after injury, the wound was healed but the patient was disabled.



7

(For legend see page 29.)

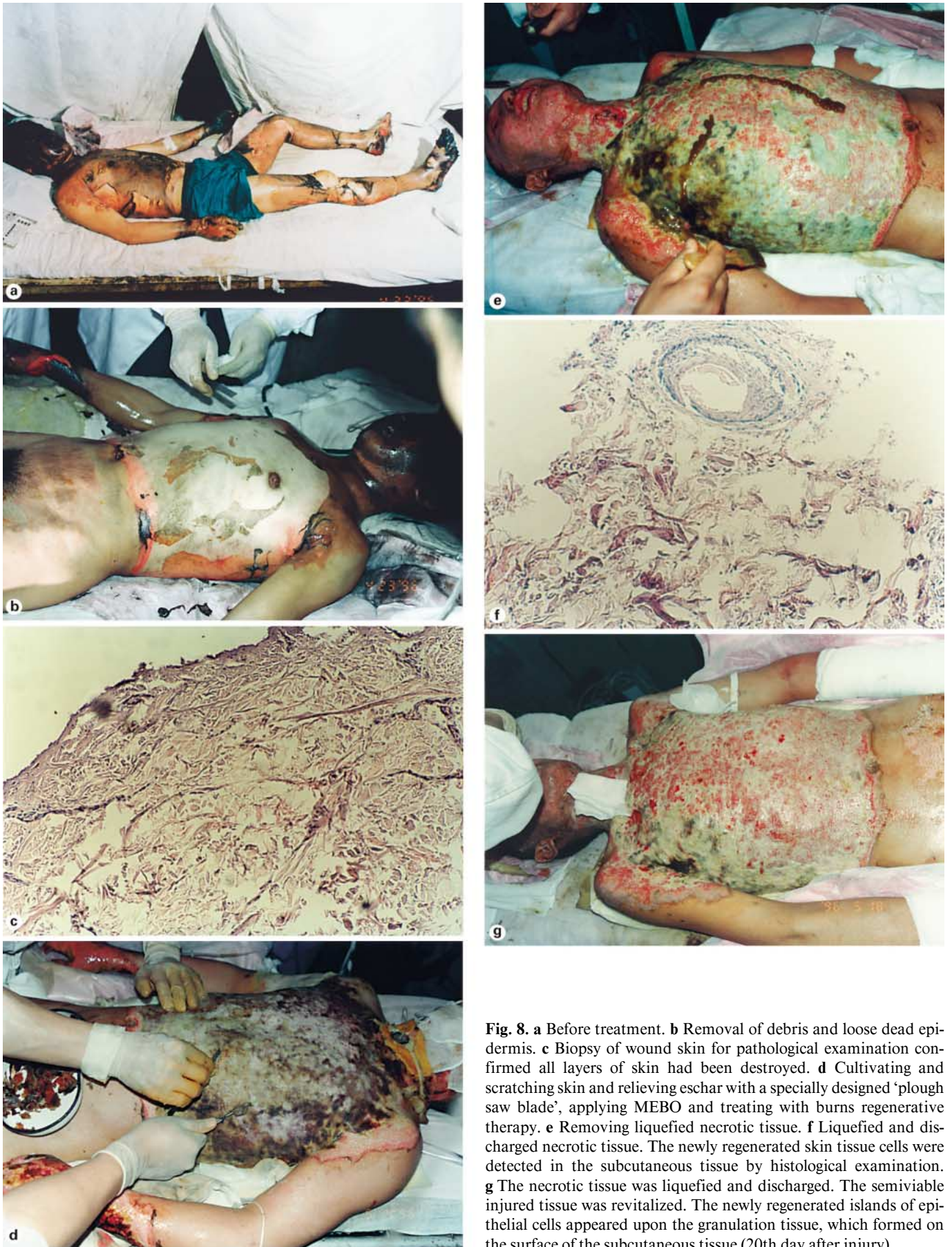
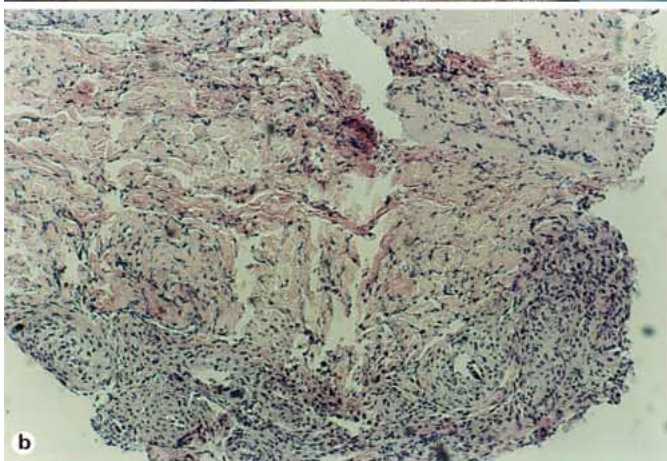
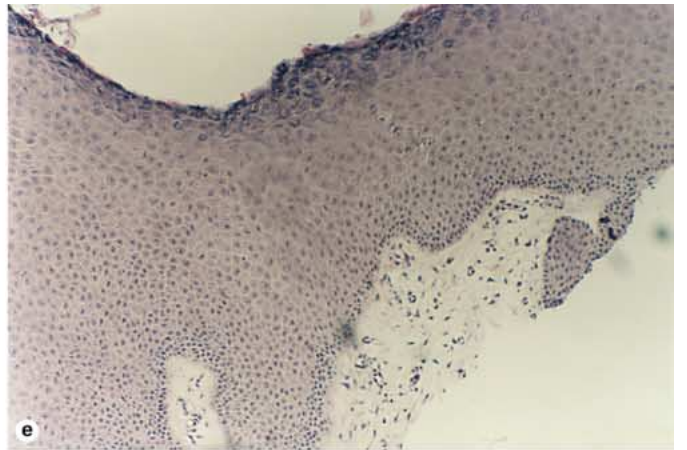


Fig. 8. **a** Before treatment. **b** Removal of debris and loose dead epidermis. **c** Biopsy of wound skin for pathological examination confirmed all layers of skin had been destroyed. **d** Cultivating and scratching skin and relieving eschar with a specially designed 'plough saw blade', applying MEBO and treating with burns regenerative therapy. **e** Removing liquefied necrotic tissue. **f** Liquefied and discharged necrotic tissue. The newly regenerated skin tissue cells were detected in the subcutaneous tissue by histological examination. **g** The necrotic tissue was liquefied and discharged. The semiviable injured tissue was revitalized. The newly regenerated islands of epithelial cells appeared upon the granulation tissue, which formed on the surface of the subcutaneous tissue (20th day after injury).



9

Background Information

A 35-year-old male sustained scalds by 1,000°C hot cement and flame burns secondary to a cement kiln collapse accident at 8:30 p.m. on April 12, 1996. He was admitted to the hospital 4.5 h after injury. Initial assessment showed: (1) burn-blast combined injury; (2) extensive deep burns (85% TBSA); (3) severe inhalation injury; (4) shock; (5) open multiple fractures on both feet.

On admission, the patient was in a critical state and in shock. The extensive deep burns wounds were covered by cement powder. He had inhaled cement and his nasal hairs were singed. He suffered from respiratory abnormalities and hoarseness. Tracheotomy was performed immediately. BRT with MEBT/MEBO treatment and cultivating technique was started on the wound and systemic comprehensive management begun. Histological examination of the wound skin showed third-degree burns. On day 30 postburn, liquefaction and discharge of wound necrotic tissues were finished. On day 49 postburn, newly regenerated skin was present on the wounds. Ten days later, large sections of regenerated skin appeared on the wounds and all wounds had healed completely on day 72 postburn. One year later, follow-up showed the patient free of disablement, capable of independent viability and no need for reconstruction.

Fig. 9. **a** Continuous treatment with BRT with MEBT/MEBO and protection of the wound with the ointment (MEBO). Pathological examination showed regeneration of skin tissue, and there were some islands of epithelial cells distributed on the surface of the granulation tissue. **b** Histological examination revealed that those islands were masses of regenerating skin tissue from subcutaneous tissue composed of capillaries, collagen, epithelial cells, etc. **c** The epithelial islands expanded gradually and started to integrate. **d** The regenerated skin islands connected to form a larger piece. **e** On the 49th day after injury, histological examination confirmed that the newly regenerated skin was of physiological full-thickness. **f** On some parts of already integrating skin, there was a physiological anatomic structure of large blood vessels in the subcutaneous tissue. **g** All areas of the wound were covered by regenerating skin, either closing the wound, or developing new skin of a similar structure, appearance and function to that of normal skin. **h** 72nd day; appearance of healed wound with regeneration of full-thickness skin.

Procedure and Results (fig. 8, 9)

First Step: Treatment of Burned Skin

Second Step: Healing and Closure of the Wound

Third Step: Reconstruction after Wound Healing

No need.

Ed. note: In the spirit of brevity, the author has offered photographic documentation of 2 cases only. However, the author has documented hundreds of similar cases and for those who would appreciate reviewing that extensive photographic library, we refer you either to the literature or to www.mebo.com.

Case 3: Surgical Excision and Cultured Composite Autograft Therapy

Background Information

Cultured epithelial autografts (CEA) have been used as an adjunct in the surgical management of extensive thermal burns. Unfortunately, the lack of a dermal matrix makes CEA susceptible to infection, shearing forces and limits their incorporation into the burn wound. A cultured composite autograft (CCA) has been developed recently in which autologous keratinocytes and fibroblasts are surgically harvested from the burns patient's normal skin. These components are proliferated and then combined to form an epidermal and dermal matrix which grows into confluence and is then applied to the lesion.

Standard wound coverage techniques as well as CCA technology were utilized for successful wound closure in a 12-year-old female with an 81% third-degree burn. After fascial excision and allograft coverage, autografts were placed on her posterior burns and then 7,500 cm² of CCA was placed onto her anterior thorax, abdomen and lower extremities. Sixty percent of the burns was covered with CCA resulting in a success rate of 40%. No evidence of infection was noted, even in areas where CCA failed, although in those areas random epithelialization appeared to occur which then seemed to facilitate autograft placement. Early debridement and allografting followed by conventional autografts and CCA placement may provide an effective skin coverage strategy in patients with extensive deep burns.

Procedure and Results

Disablement and disfigurement. Reconstruction was required. Pictures of the treatment procedure are not available here as copyright is concerned. See *Burns* 1999;25:771–779 for details.

Indications and Diagnostic Principles of Burns Regenerative Medicine and Therapy

It is concluded from a comparison of the descriptions in the previous section that burn injuries involving skin only should be treated with BRT with MEBT/MEBO rather than with surgical excision and skin grafting therapy. The latter is only appropriate in the treatment of burns wounds with full-thickness necrosis of subcutaneous tissue together with muscle or deeper burns. To facilitate the clinical performance, the diagnostic principles and clinical indications of various therapies are standardized below.

Diagnostic Principles of Burns Medical Therapy

Many textbooks describe the method of diagnosis of burn depth. It is based on naked eye observation and the doctor's own experience; therefore, it is often difficult to differentiate between full-thickness burns and deep partial-thickness injury. Understandably, therefore, wounds should not be excised since the result is the removal of all skin tissues and superficial fascia. After surgical excision, we see that the prognosis is worse and the mortality and disablement rates are elevated.

In order to standardize the diagnosis of burn depth, the following principles should be followed.

Principle of Clinical Diagnosis

First of all, it is necessary to determine whether the burn wound requires surgical excision or not. If the wound demonstrates surviving skin tissue in the deep layer with appearance of exudate within 6 h after injury, then the subcutaneous tissues are viable with functional microcirculation and surgical excision is not required. After treatment with this BRT, white exudates will appear on the wound surface. One notices that the more the exudate, the more superficial the wound. If the wound has no exudate 3 days postburn, surgical operation should be considered. If the wound reveals no hemorrhage of subcutaneous tissue after the fasciotomy, it can be excised. However, this does not apply to the wound where the exudate disappeared after treatment with dry therapy. If such cases occur, there are mistakes in the treatment.

Pathological Diagnosis

Pathological diagnosis is used to diagnose the depth of burns wounds without exudate and to determine whether the wounds need to be excised. Wounds with exudate do

not need pathological diagnosis. Pathological diagnosis is easy and painless. If there is misdiagnosis of one biopsy sample of a small piece of skin including subcutaneous tissue from the wound, histological examination of the section is performed. If most of the subcutaneous tissue is necrotic, the wound can be excised and treated with skin grafting. If the subcutaneous tissue is still structurally vital, then the wound should not be excised and BRT (MEBT/MEBO) should be applied. Accurate pathological diagnosis based upon scientific investigation is feasible and, when performed correctly, can afford the patient correct diagnosis and optimum prognosis. It is no longer acceptable for the physician to rely upon the naked eye as too many treatment errors could result.

Burns Regenerative Medicine and Therapy (BRT with MEBT/MEBO)

Indications

BRT with MEBT/MEBO is an independent method:

- 1 For treating superficial second-degree and deep second-degree burns and scald wounds of various causes and in different areas.
- 2 In coordination with cultivating and relieving techniques, BRT can be used for treating full-thickness dermis burns and scald wounds, provided viable subcutaneous tissue of various causes and different areas are present.
- 3 For treating burns wounds deep in the muscular layer with diameters of less than 20 cm.
- 4 For treating wounds at the skin donor site.
- 5 For treating granulation wounds deep in the muscular layer, for promoting regeneration of granulation tissue in burned bone after debridement, and to create a physiological environment at the receiving site for skin grafting.
- 6 For treating all kinds of surface wounds.
- 7 For treating other skin lesions including hemorrhoids, leg ulcers, bedsores, chronic ulcers, infected wounds, chilblains, etc.

Clinical Application

Direct application of MEBO – a specially developed topical drug for BRT with MEBT/MEBO – onto the wound surface to a thickness of 0.5–1.0 mm every 4–6 h. Detailed clinical treatment is recommended as follows:

- 1 For first aid at home (especially in the kitchen): Immediately apply MEBO on the wound to relieve pain, stop bleeding, alleviate injuries and prevent infection in cases of scalds and burns by hot oil, boiling water, or friction burns. The sooner, the better. The consequent treatment should be conducted according to the following specific cases.
- 2 Treatment for first-degree burns or scalds: Directly smear MEBO onto the wound 2–3 times daily.
- 3 Treatment for superficial second-degree burns or scalds: Directly smear MEBO onto the wound to a thickness of 0.5–1.0 mm. Renew the ointment every 4–6 h; before doing so gently wipe off any residual ointment and exudates. It usually takes 6–7 days to heal. Blisters, if present, should be punctured and discharged while blister skin should be kept intact in the early stage. No disinfectant, saline or water is required or in fact even allowed except in the case where exogenous toxins remain at the site such as might be the case with chemical burns or other dirty wounds. Patients sustaining moderate or extensive burns should be sent to hospital or a clinic experienced with the BRT treatment protocols.
- 4 Treatment for deep second-degree burns: Treatment in the early stage is the same as that for superficial second-degree burns. Remove the blister skin on day 5–6 after injury. As the dermis tissues are damaged and white in color, the application of MEBO should be continued on the wound to a thickness of 0.5–1.0 mm every 4 h. White metabolic products resulting from liquefaction of necrotic tissue by the ointment will appear on the wounds (do not misdiagnose this cleansing process as infection). Be sure that the residual ointment and white liquefied products are wiped off gently (do not irritate or debride the tissue) before reapplying MEBO. Allow another 6–7 days for the necrotic tissue to be liquefied and discharged completely, then continue the above treatment using less dosage of MEBO until the wound heals. In the event that the wound is still not healed after 25 days postburn, the diagnosis should be changed to full-thickness degree. In brief, the venerable medical principle of ‘primum non nocere’ (first do no harm) and of ‘no secondary injuries’ should be honored during the whole treatment procedure. We accomplish that by: (1) protection of the treated wound in the early stage from further injuries (avoid any measures which may irritate, debride or exacerbate wounds); (2) liquefaction and removal of the necrotic tissue without causing secondary injuries; (3) regeneration and skin repair without causing secondary injuries (any method which may irritate or damage the wounds is not allowed). Patients sustaining moderate and extensive burns should be sent to hospital or a clinic with experience of BRT and MEBT for appropriate treatment.
- 5 Treatment for second-degree burns: For the small-area burn wound, we recommend cultivating tissue and then preparing the lesion for application of MEBO through gentle loosening of necrotic tissues by scratching with a specially designed device – ‘plough saw blade’ is the appropriate treatment for the deep second-degree burns wounds. For larger burns wounds, the aforementioned method is adopted if the patient’s systemic condition is stable. The principle of ‘no secondary injuries’ should be followed strictly during the treatment. Patient sustaining third-degree burns must be hospitalized at clinics offering care from clinicians experienced in BRT with MEBT/MEBO.
- 6 In the treatment of small burns wounds occurring in inconveniently exposed body parts, bandaging is recommended. However, dressing changes and renewal of MEBO ointment at a thickness of 2–3 mm every 12 h is recommended. Contrary to the typical dressing change protocol, however, rather than debride the wound beneath the bandage, we recommend that the bandage be gently removed leaving the residual ointment and metabolic products to continue their cleansing activity.
- 7 Treatment for other superficial trauma wounds including abrasion, friction burns, skin cracking, and stasis ulcers: Treat the ulcer wounds according to the instructions for either superficial or deep second-degree burns, or dress the wounds with MEBO in accordance with the surgical methods. However, any disinfectant, antiseptic or saline is contraindicated as they are both unnecessary and deleterious to wound health.
- 8 Treatment for hemorrhoids: Directly apply MEBO onto the affected area every morning and evening, or smear MEBO onto the postoperative wound to relieve pain and promote healing.

Burns Surgical Therapy with Excision Followed by Skin Grafting or Cultured Composite Autografting Technique

Indications and Application

- 1 Full-thickness degree burns wounds reaching the lower layer of the subcutaneous tissue of different areas and of different causes.
- 2 Skin grafting technique is used for treating granulation tissue wounds without epithelial regeneration and for plastic surgery.
- 3 The hospitals should be qualified to conduct surgery and the operation should be conducted by surgeons specialized in BRT with MEBT/MEBO and/or burns surgery.

Intensive Description of Burns Regenerative Therapy with MEBT/MEBO

BRT with MEBT/MEBO is an entirely new burns treatment technique that operates in compliance with the law of life. BRT was invented on the basis of academic thoughts according to the pathogenesis of burns. This new therapy comprises a complete set of theories and techniques for the local and systemic treatment of burns. The profile of this therapy is that through liquefaction and removal of the necrotic tissue, culture and regeneration of residual viable skin, and through repair and replication, burns wounds are finally healed. Wounds are not kept in a dry environment as required in conventional surgical burns therapy, but in a physiological moist environment.

For local treatment, BRT with MEBT/MEBO is associated with MEBO. For systemic treatment, it forms an independent system in compliance with the law of life, including its theory, methodology and therapeutic results. In local treatment, BRT resolved the problems of wound pain and complete regenerative healing of deep second-degree wounds. Through reducing bacterial toxicity by variation, and promoting local resistance to infections, BRT with MEBT/MEBO effectively prevented and controlled wound infections. Through creating a physiologically moist environment and good nutrition supply, BRT with MEBT/MEBO promoted the culture and differentiation of stem cells from the epithelia and relevant tissues in the residual fat layer and finally healed the wounds to full-thickness. Furthermore, exposed bone wounds from burns can also be healed by drilling on the bone in combination with MEBO application, culturing granulation tissue to cover bone and heal the wounds. In systemic treatment, measures of strengthening cardiac function and removing the obstacles in blood supply of renal parenchymal blood vessels are adopted, and then blood volume replacement and comprehensive antishock measures are taken. According to the severity of the burn case, effective broad-spectrum antibiotics are applied to control infections at an early stage, but the antibiotics are stopped in order to protect the function of the organs at days 7–10 postburn.

Due to the remarkable effect of this therapy, it has been introduced in Syria, the United Arab Emirates, Thailand, the Republic of Korea, Singapore, etc., and has achieved great clinical success in these countries. Now BRT with MEBT/MEBO is spreading its academic thought as well as its technology to the United States and European countries. At the Congress of the Pan-Arab Association for Burns and Plastic Surgery held in the United Arab Emirates on February 22, 2000, specialists from dozens of countries gave presentations on their research on BRT with MEBT/MEBO.

Concept and Principle of BRT with MEBT/MEBO

Concept

BRT with MEBT/MEBO is a medical treatment which promotes the cell regeneration of residual skin tissue and wound healing by keeping burns wounds in a three-dimensional physiologically moist environment and facilitating the liquefaction and discharge of necrotic skin tissue.

Principle

MEBO, specially invented for BRT with MEBT/MEBO, is an ointment whose base includes a frame structure composed of beeswax which musters a refined botanical oil containing active ingredients.

When applied onto the wounds, the oil sequestered in the beeswax frame is warmed up in contact with body temperature and thus penetrates into the wounds. Four biochemical reactions between MEBO's active constituents and the burn tissue take place while the nutritional substances are supplied continuously onto the surviving cells in the wounds. The oil then loses its lipophilic nature, seeps out of the drug layer and is discharged from the wound. Fresh MEBO continues to penetrate into the tissue, so that liquefied necrotic skin is removed from the superficial to the interior planes without causing further injury. Simultaneously, this mechanism of action also features a replenishment of the drug in continuous cycles. These cycles ensure active drainage of metabolic waste products including pathogenic bacteria from the injured viable tissue, hence the caveat to not apply topical sterilizing agents. Tissue fluid in the wound supplies the requisite physiologically moist environment. The cycle also ensures continuous supply of indispensable nutritional substrates and enzymes to the recuperating wound tissue. Furthermore, the frame structure of MEBO base effectively insulates and isolates the vulnerable wound from the external environment by forming a viscous dressing. A remarkable characteristic of this 'dressing' is that it prevents foreign external contaminants from penetrating while allowing for the exodus of metabolic debris products resulting from the regeneration of the wounded tissue. In a metabolic sense, the MEBO allows the wound to 'breathe' in a manner very similar to that of normal skin.

Therapeutic Effects of Moist-Exposed Burns Ointment (MEBO)

Under the direction of qualified BRT with MEBT/MEBO therapists, MEBO has the following therapeutic effects:

- 1 Variation of pathogenic microorganism and reduction in bacterial toxicity.
- 2 Effectively killing pain by protecting nerve endings and relaxing pilorum arrectors.
- 3 Anti-inflammatory by the effects of β -sitosterol and other ingredients.
- 4 Made of nutrient food, MEBO may protect cells by increasing the tension in the cell membrane and help dying cells convert into vigorous normal ones.
- 5 With the co-ordination of BRT with MEBT/MEBO, MEBO develops a physiologically moist environment, favorable to the regeneration and repair of tissue structures. Thus, it is effective for reducing scar formation, enhancing the power of self-repairing of wounds and promoting the regeneration and differentiation of stem cells from residual epithelial tissue, vascular plexus and fibrous tissue in the fat layer to regenerate skin.

Clinical Application of BRT with MEBT/MEBO

Treatment Conditions

Strictly sterilized conditions are not emphasized. Debridement using any disinfectant, saline or water is forbidden. Small burns can be dealt with at home with MEBO. Moderate and minor burns encountered in the battlefield can also be treated with BRT with MEBT/MEBO. For treating large burns, the room should be kept at a temperature of 30–34°C and first-aid apparatuses or devices should be equipped with it.

General Application

Directly smear MEBO onto the wounds with a thickness of 1 mm. At the beginning, no debridement is required except for chemical burns or dirty wounds. Renew MEBO every 3–4 h, before which wiping off the residual ointment and liquefaction products with gauze or tissue paper (gentle and careful renewal is demanded to avoid pains and bleeding). For wounds with blisters, be sure to preserve the blister skin, directly apply MEBO until the blister skin is removed 5 days later. For deep second-degree burns, after applying MEBO, dermal tissue in the necrotic layer begins to liquefy on day 7 postburn. Renew MEBO and wipe off the liquefaction product timely. After

the complete discharge of necrotic tissue, apply less MEBO and renew every 4–6 h till the wounds heal. For third-degree burns, treatment with a special debridement technique can be applied coordinately.

Special Application

For treating not easily exposed small burns wounds, apply MEBO with a thickness of 2–3 mm, then apply a decompression bandage using dry gauze. Before changing the dressing every 12 h, gently remove the drug sediment and liquefied necrotic tissue. For treating traumatic, ulcerative and operative wounds, 1–2 layers of gauze impregnated with MEBO also could be used.

Principle of Systemic Treatment

Burn is a systemic disease caused by thermal injury. The changes in topical treatment directly affect the systemic pathophysiological status. BRT with MEBT/MEBO systemic treatment is essentially different from conventional surgical dry therapy. While using BRT with MEBT/MEBO, the protocol of surgical dry therapy is forbidden. Two principles should be followed in this treatment on extensive deep burns: (1) In the early stage, a comprehensive antishock treatment principle is applied, which involves enhancing cardiac function, protecting renal function and supplementing effective blood volume according to the vital signs. (2) In the middle and later stages, expectant treatment is applied, with a protocol of keeping a water-electrolyte balance, nutritional support and maintaining a comprehensive balance. In the anti-infection treatment, a large dose of strong and powerful broad-spectrum antibiotics should be used in the early stage for 7–10 days and then withdrawn immediately, in order to protect and enhance the anti-infective function of the internal organs. For nutritional supporting treatment, a protocol of oral administration is desired. Others are dealt with according to the case.

Clinical Treatment

Treatment for First-Degree Burns

The clinical signs of first-degree burns include skin redness, slight swelling and pain. Immediate application of MEBO may relieve the pain. The erythema gradually diminishes as MEBO is warmed in situ and absorbed through the skin. At 12 h postburn, the skin may return to normal. For burns with edema, the epidermis is partially destroyed, the pain may be relieved more slowly and the wounds heal in 2–4 days when the superficial stratum corneum exfoliates.

Treatment for Superficial Second-Degree Burns

According to the pathogenic process of superficial second-degree burns, the treatment can be carried out in two stages.

First Stage. Treatment in the early stage – the period from emergency treatment postburn to the end of shock period (within 3–4 days after injury). The clinical signs in this stage include pain, edema, blisters, and a great amount of blood plasma exudated from the site where blister skin exfoliates. According to the principle of BRT with MEBT/MEBO, apply MEBO directly all over the wound, puncture the blister (if present) on the lower part to discharge liquid. Do not remove the blister skin, directly apply MEBO on the blister skin 3–4 times daily. With the application of MEBO, a layer of thin soft membrane forms upon the wound free of blister skin, the membrane still allows the exudates to ooze through, and then it gradually thickens. Do not remove the soft membrane, since it can substitute the skin role of fulfilling breathing and protection. Continue MEBO application directed by BRT.

Second Stage. It is the wound-repairing period when the shock stage ends and the residual viable epidermis tissue begins to regenerate and recover, usually lasting 3–4 days. In this period, the basal cells in the epidermis recover to form a granular layer and thus promote wound healing. In clinical treatment of BRT with MEBT/MEBO, after the edema period, the blister skin loosens and exfoliates, and the thin soft membrane formed on the wounds also loosens and exfoliates. Simply cleaning away the blister skin and soft membrane is first desired, then continue the application of MEBO to protect the regenerated wounds till healing. During the whole treatment, neither pain nor further injury to wounds is allowed. The correct application method helps the wounds heal without causing any infection, pain, scar formation or hyperpigmentation. Generally, superficial second-degree wounds treated with BRT with MEBT/MEBO heal within 6–8 days and the skin recovers completely to its normal physiological status within 3 months.

Treatment for Deep Second-Degree Burns

According to pathological and clinical manifestations, deep second-degree burns can be divided into injury on the dermal papillary layer and injury on the reticular layer, or simply referred to as deep second-degree superficial (DIIS) and deep second-degree deep (DIID) burns.

DIIS

Clinical signs include wound pain, extensive blisters, wound without blister skin becoming red and white, the superficial dermal tissue is necrotic and turns white, the surviving deep dermis tissue is red, while under pressure

it turns white and soon returns to red after release of pressure (DIID burns wounds respond more slowly). The exudates of the wounds are only less than those of superficial second-degree burns.

BRT with MEBT/MEBO treatment and clinical manifestation: It is a four-period process: firstly, treatment in the early stage (shock period), same as that for superficial second-degree burns; secondly, liquefaction and rejection period of necrotic dermal tissue (rejection period); thirdly, regeneration and recovery period, and, fourthly, rehabilitation period of skin physiological function after wound healing.

First Period. The treatment of the first period is the same as that for superficial second-degree burns, emphasizing on wound protection. A thin layer of soft membrane may appear on the wound free of putrid or blistered skin. The next treatment period starts on day 4–5 postburn when the wound edema diminishes gradually.

Second Period. Clean away the putrid skin, blistered skin or thin soft membrane in the same way as dealing with superficial second-degree burns. Continue the application of MEBO. Gradually, the necrotic layer of wound surface begins to liquefy from the superficialities to the interior and produces white liquefied products floating over the wound surface. Usually at 3–4 h after application of MEBO, the wound is totally covered with whitish liquefied products, indicating that MEBO is consumed completely. The liquefaction products must be cleaned away before the renewal of MEBO. Another 3–4 h later, the renewed MEBO is consumed again when the liquefaction products float over the wound. Clean the liquefaction products, renew MEBO again and keep the clean-renew-clean process going until the necrotic tissues are entirely liquefied and discharged. This process generally occurs 5–15 days postburn. Patients with large-area burns are urged to turn over during drug renewal.

Third Period. After the second period treatment, the chestnut-like residual dermis tissues, millet-sized, are exposed on the basal layer of the wound. Continue MEBO with less thickness directed under BRT with MEBT/MEBO and renew every 4–5 h (every 6–8 h at night). Covered and protected by MEBO, the residual dermal tissue reconstructs and regenerates. Once dermis tissue regenerates to smooth skin, less irritation to the wound is allowed. Again reduce dosage and renewing times as long as the wound is not dry, but crust formation is forbidden, i.e. not only prevent wounds from being macerated by MEBO, but also avoid the wounds becoming dry and getting covered by a crust. Duly keep the normal skin around the wound clean. For large-area burn patients, do as in the second period by helping them turn over regularly on the basis of drug change intervals. The pressured parts of the body still need MEBO protection till the wounds heal. This period occurs 15–20 days postburn.

Fourth Period. Although the wound heals after the third stage, the functions of the newly healed skin still need to rehabilitate completely. The epidermis requires further physiological adjustment and metabolism; the sebaceous glands need compensatory metabolism; the excretory duct of the sweat gland is not yet clear; the functions of pigment cells are unable to meet the requirements of normal skin. Under these circumstances, MEBO is still necessary to be used as a skin-care oil for another 10–15 days. Or apply newly developed MEBO series products – MEBO Cleansing Cream to promote quicker recovery of skin function, or apply MEBO Itch Relieving Cream to stop itching.

DIID

The clinical signs of deep second-degree burns are similar to those of deep second-degree superficial burns, except that DIID has more serious injuries and more serious response during the liquefying period and therefore the reconstruction and regeneration of wounds become more complicated. The clinical treatment for deep second-degree burns also can be divided into four periods.

First Period. Clinical signs – no extensive blisters, epidermis entirely destroyed and adherent to injured dermis, the wounds free of putrid skin are no longer sensitive to pain, the wounds are white, with little exudate. Some of the wounds may be red alternating with white, but the color-changing response to pressure is very slow. This period begins from the first day of burn through the 7th day postburn. During this period, simply apply MEBO to protect the wound.

Second Period. From day 7 to day 20 postburn, clear away all adhering substances to expose necrotic dermis and apply BRT with MEBO/MEBO immediately. For small wounds, simply use this treatment to liquefy and discharge the necrotic layer. For large burns, simple debridement should be used coordinately. The necrotic layer is so deep that the wound liquefying may be incomplete and cause lumps exfoliation of necrotic tissues, which need to be cut with surgical scissors and removed. Attention should be paid to keep a certain distance between the surviving viable tissue and the cutting. Any further damage to the surviving viable tissue such as bleeding (which may cause infection) should be absolutely avoided. Simple debridement can be adopted according to the condition of the wounds. When the necrotic layer is almost completely liquefied, clean away the liquefaction products in time to ensure that the non-smooth survival tissue is kept in a MEBO environment, but not in an environment filled with liquefaction products.

Third Period. The period of reconstruction and regeneration of residual dermis tissue. As little residual dermis tissue is left and the dermis frame is fundamentally destroyed, correct BRT with MEBO/MEBO is quite vital

in this period. The reconstruction of dermis tissue involves three parts: (a) the reconstruction of vascular tree; (b) the reconstruction of fibrous tissue dependent on vascular tree; (c) the regeneration of skin appendages, gland tissue, formation of excretory ducts, and formation of skin tissue. Any careless injuries and pressure to the wound are forbidden. This period usually happens on days 20–28 postburn.

Fourth Period. Aiming at helping the healed skin return to normal, the treatment in this period varies according to burns severity and skill in the treatment. The severe injuries to skin and the factors affecting the skin during reconstruction make the newly healed skin quite different from normal skin in structure, appearance and function. So the rehabilitation is very important, including two aspects: ‘protective therapy for healed skin’ and ‘functional exercise’. The former is accomplished by adjusting the structure of newly regeneration skin tissue with the application of MEBO Scar Lotion, by adjusting the function with MEBO Cleansing Cream, and by killing itch with MEBO Itching Reliever just after the wounds heal.

Treatment for Third-Degree Burns

Third-degree burns are also an indication for BRT with MEBO/MEBO. As the epidermis and dermis of third-degree burn wound are totally destroyed, it is quite difficult to cure third-degree burns. The conventional medical science for burns is convinced that third-degree wounds cannot heal spontaneously, and the only solution is to use surgical skin grafting to close them. The clinical study and administration of BRT with MEBO/MEBO for curing third-degree burns wounds will be described in detail thereafter. The following is just a brief description of the principle and method of this treatment.

Principle. Decompression of the deep tissues to relieve any pressure caused by the necrotic layer is of critical importance; protect the necrotic full-thickness skin; promote stem cells containing the skin information in subcutaneous tissue to regenerate and differentiate to form a skin island; culture the newly regenerated skin island while liquefying and discharging the necrotic dermis; promote the skin island to spread and cover subcutaneous tissue to form new skin; and help third-degree burns wounds heal spontaneously. For third-degree wounds injured down to the muscle layer, excise most of the necrotic tissue by surgical operation, liquefy and discharge the rest of the necrotic layer with BRT with MEBO/MEBO, upon which culture granulation tissue, then plant skin cells till the wound closes and heals. For wounds with bone exposed, clean away the exposed outer soft tissue, drill holes on the bone surface with a bone drill at intervals of 0.5–1 cm, deep into medullary cavity of bone until bleeding. Apply MEBO to cover the wounds, and culture and support the growth of granula-

tion tissue from the holes. When the granulation tissue spreads to cover the bone surface, skin grafting can be performed to close the wound, or the wound heals by migration of epithelial cells from the wound edges.

The necrotic tissue of third-degree burns should be decompressed by cutting both horizontally and vertically at a 1 mm tooth distance and depth with a specially designed method: 'plough saw blade'. Then apply MEBO for protection and clean away the exfoliated or liquefied tissue.

Systemic Comprehensive Treatment with BRT with MEBT/MEBO

Principles of Initial Treatment

Principles of First Aid

- 1 Keep the patient in a horizontal supine position with slight elevation of the head. Turning over is contraindicated. Expectant administration.
- 2 Application of any topical drug that may be harmful or irritative to burns wounds is contraindicated. Adopt measures to relieve pain and protect burns wounds as soon as possible.
- 3 Avoid changes of patient body temperature which consume vital energy striving instead to keep the patient warm as much as possible.
- 4 If appropriate and well-tolerated, cardiotoxic and sedative medications may be given through intramuscular injection or intravenous infusion.
- 5 If the wound is deemed appropriate for treatment with BRT with MEBT/MEBO, smear MEBO directly onto the wound. Once the wound is thus protected, cover the wound with adequate dressing and transfer the patient immediately to the nearest hospital for further treatment.

Principles of Emergent Treatment and Nursing

- 1 Treatment condition: a clean or sterile (which is not absolutely necessary) environment is required. Temperature around the wound surface should be kept at 34–38°C.
- 2 Early wound care: any feculency and dirt should be cleared away. Do not use any method or topical drug that may cause further injury to the wound or promote tissue hydrolysis.
- 3 Principle of initial nursing: do not turn the patient over. Alternately lie on one side or alternately change pressure at various body parts.
- 4 If BRT with MEBT/MEBO treatment is adopted, wound debridement with MEBO and topical application of MEBO should be performed for wound care.

Wound Debridement with MEBO. Cover the wound with MEBO immediately after injury regardless of the presence of dirt or chemicals. Two or three hours later, gently clear away the feculency and dirt together with residual MEBO before the renewal of MEBO. This method is applicable for first-aid treatment as well as wound debridement after hospitalization when daily cleansing is appropriate.

Topical Application of MEBO. Smear MEBO onto the wound at a thickness of 0.5–1 mm immediately after wound cleansing. Gently wipe off liquefied products before renewing MEBO every 4–6 h. Renewing intervals could be increased to every 6–8 h during the wound repair period.

Antishock Therapy

The author considers that in the antishock therapy postburn, it is more important to protect and recover the functions and structures of internal organs than to supplement blood volume only. The principles of the treatment are as follows:

Protection and Enhancement of Cardiac Function

We propose that a lot of protein degradation products released from burned skin tissue could be absorbed into the blood circulation, and could further inhibit and decrease cardiac function, thus inducing cardiogenic shock. Therefore, severely burned patients (TBSA >50% and/or third-degree >10%) should be routinely injected intravenously with cedilanid (lanatoside C) 0.2 mg in 25–50% GS 50 ml q.d. after injury or admission. Then, the amount and frequency of cedilanid should be regulated according to the changes in heart rate and peripheral circulation. 48 h postinjury, the administration of cedilanid should be stopped unless the patient is still suffering from abnormal cardiac function, in which case cedilanid should be applied until the symptoms disappear. If symptoms of heart failure arise during the course of treatment, the patient should be treated with 0.2–0.4 mg cedilanid immediately. One treatment is frequently sufficient.

Protection of Renal Function

After massive burns, one of the main complications in the shock stage is renal dysfunction that is caused firstly by microvascular spasm of the renal parenchyma and renal ischemia. It is also the major etiology of renal failure. Therefore, treatment of renal function is the crux of antishock and comprehensive treatment to relieve the microvessels in the renal parenchyma. This needs to be addressed as early as possible. The principles of renal treatment are follows: After injury or immediately upon admission, severely burned patients routinely require an intravenous drip with 1% procaine 100 ml, caffeine

sodiobenzoate 0.5 g, vitamin C 1.0 g, 25% GS 100–200 ml, q.d. or b.i.d.–t.i.d. depending upon the degree of shock and the amount that urine production is reduced. This intravenous drip should be continued in patients with anuria until urination is recovered. The routine treatment plan may be maintained until wound healing.

Supplement Blood Volume

After massive burns, a great deal of intravascular fluid exudes toward the wound surface and tissue space, which leads to the reduction in effective blood volume resulting in hypovolumic shock. Therefore, during the above treatment course, the blood volume should be monitored and replenished as needed. In particular, attention must be paid to avoid massive intravenous infusion blindly without precise attention being paid to cardiac and renal functions, as well as other excretory functions. The principle is as follows:

Compositions of Fluid Infusion. The ratio of crystalloid solution (normal saline or 5% GNS) to colloid solution should be 1:1. The colloid solution should be composed of 3/4 parts of plasma and 1/4 part of whole blood when the condition allows, otherwise 1/2 part of plasma and 1/2 part of plasma substitute can be used.

Amount of Fluid Infusion. According to the basic principles of surgery, the amount of fluid infusion should be equal to the amount of body deficiency. In the shock stage of massive burns patients (during 48–72 h after injury), we offer a more detailed formula:

$$\begin{aligned} \text{Total amount of fluid infusion (ml/day)} = & \\ & \text{physiological water needs (5\% GS 2,000–2,500 ml) +} \\ & \frac{[1 \text{ (ml/kg)} \times \text{TBSA\% (2nd to 3rd degree)} \\ & \quad \times \text{body weight (kg)} \times 100\%]}{\text{hourly urine volume (ml)/body weight (kg)} \times 1 \text{ (ml/kg)}} \end{aligned}$$

Speed of Fluid Infusion. After extensive burns, the trauma stresses the heart, kidney and brain tissue, making their functions vulnerable. During the first 24 h postburn, 1/2 of total fluid amount should be infused in the first 8 h, another 1/2 should be infused over the next 16 h evenly again, with regard to cardiac and renal functions. During the second 24 h postburn, all of the fluid should be infused at a uniform speed. During the third 24 h after injury, the amount and speed of fluid infusion must be determined strictly in the light of the symptoms of shock and the amount of urine. When the symptoms of shock are improved markedly or disappeared and the amount of urine is >1 ml/h·kg, the speed of fluid infusion should be decreased and the fluid amount should be reduced by 1/3.

Nursing Care in Shock Stage

After severe burns, the onset of shock would be related to thermal injury as well as adequate nursing care. The burns patient can hardly withstand any further stress due

to the already severely compromised condition of all internal organs. Thus, nursing care constitutes a critical service in supporting as stress-free a recovery period as possible. Nurses should:

- a Directly apply MEBO on the wound surface immediately, isolate the wound from contacting with air, relieve wound pain, protect the wound from any irritative damage, resist the tendency to debride the wound.
- b Apply air conditioner or bedstead and sheeting to maintain room temperature at 30–34°C, and prevent fluctuation in room temperature.
- c Smooth out the bed sheet and dressing, protect the wound from any compression, change dressing and MEBO every 12 h gently, while keeping the patient in the horizontal supine position. Again, turning the patient over is contraindicated.
- d Control the speed of fluid infusion such that it flows at a constant rate remembering that rapid fluctuation of infusion speed is forbidden.

Anti-Infection Therapy

We have observed that there are two pathogenic types of postburn infection. One has an endogenous pathogenesis, while the other is exogenous. The endogenous infection is similar, but different from the primary infection typically noted by surgical burns therapists. This consists of subclinical infection in that the possibility of postburn infection always remains a potential reaction to burns. The latter infection consists of postburn infection caused by all exogenous sources and factors including iatrogenic burns.

Principles of Anti-Infection Treatment

Principled Scheme of Routine Treatment. Burned patients with TBSA <30% generally do not need to be treated with systemic antibiotics. All the burned patients with TBSA >30% (TBSA >10% in children) must be treated with systemic anti-infection drugs routinely whether infection occurred or not. The principle is: (1) To apply one or more powerful broad-spectrum i.v. or i.m. antibiotic as early as possible after injury until the 5th to 7th day for massive deep second-degree burns and the 7th to 10th day for massive third-degree burns. (2) The more extensive TBSA and deeper the wounds, the more powerful and broad-spectrum antibiotics are required. (3) Regardless of the patient's condition, stop applying all antibiotics at the aforementioned time.

Principle of Expectant Anti-Infection Treatment. In order to prevent and treat secondary and routine infection, a prophylactic antibiotic protocol should be administered. However, it is very important to rule out inflammatory and noninfectious etiologies initially as antibiotics would be contraindicated if infection is not problematic.

Remember that some patients with fever and increased heart rate may not be infected. After postburn routine treatment, the burned body struggles to repair and re-equilibrate a myriad of physiological functions and unnecessary antibiotic administration at this time would interfere with native resistance functions. Indications for antibiotic use: Three clinical manifestations must occur simultaneously: body temperature $>39.5^{\circ}\text{C}$ or $<36.0^{\circ}\text{C}$; heart rate $>140/\text{min}$; toxic granules in neutrophil leukocytes. Clinical vigilance is required.

Profile of Expectant Anti-Infection Treatment

One single dose of one or more powerful broad-spectrum renal-sparing antibiotics should be applied. It may be repeated until examination reveals that the neutrophilic toxic granules are no longer present. The patients should be examined for secondary infection sites and treated appropriately. The failure to control infections is commonly due to occult foci of infection within internal organs or under the wound surface. The patient recovering from general asthenia should be offered fresh blood infusions to aid in the regulation of internal balance. Abuse of antibiotics without indications of infection is contraindicated.

Balance-Regulating Treatment at the Stage of Wound Liquefaction

After shock stage is addressed, the burn wound enters the rejection stage. For deep second-degree burns wounds, injured and necrotic tissue starts to be rejected from residual viable tissue at approximately the 5th day postburn. This rejection reaction continues until all the necrotic tissue is discharged. The role of the physician and nursing staff at this time is to not interfere with this natural cleansing and regenerative process. During this stage, internal organs and many physiologic systems which had been stressed are particularly vulnerable to rejection which can then lead to single or multiple organ failure. Therefore, this is the most critical stage of the BRT (MEBT/MEBO) and requires extreme vigilance. Based on our clinical experience of many years, we consider that the key to treatment in this stage is to enhance and restore the systemic vitality and comprehensive balance, without which any monotherapy would only have a suboptimal chance of success. This therapeutic measure is termed 'balance-regulating treatment', and its protocol includes the following.

Wound Drainage

During the stage of wound liquefaction, as necrotic skin tissue is liquefied from the superficial to the deeper layer (under the effect of MEBO), it is very important to clean up liquefied materials prior to successive applications. Remember, there are differences between clearance

of MEBT/MEBO liquefied materials and surgical debridement. After treatment with MEBO, the changes in burns wounds should be supervised continuously. When MEBO on the wound surface completely changes into a whitish liquefied material, this layer should be wiped off or cleaned with a soft dry absorbent gauze or tissue paper at once. When the necrotic skin tissue separates into pieces without liquefying completely, it should be cut away gently from the wound (not debridement) and then MEBO ointment should be reapplied immediately. Unlike debridement, the RBT patient typically feels no pain during the cleaning process. If he does, it is an indication that the cleaning is too aggressive. Any harmful tissue stimulation should be strictly prohibited. In order to ensure for correct clinical practice, there are six operative rules for this treatment, i.e. the burn wound must not hurt, there should be no fresh bleeding, no maceration, no desiccation, no liquefied materials, and no lack of applied MEBO.

Treatment of Body Fluid Equilibrium

After extensive burns, large amounts of body fluid exude toward the wound surface and evaporate. We know that this body fluid plays a vital role in systemic reactions that result from traumatic stress. Therefore, it is an important procedure of the comprehensive treatment to maintain body fluid equilibrium. The principles of this treatment are as follows: The amount of fluid infusion for the burns patients suffering from TBSA $>50\%$ should be initially b.i.d. in response to physiologic demand. Subsequently, the amount of fluid infusion should be modified in response to changes of urine volume and shock symptoms. The amount of peroral fluid should be calculated together with intake volume per day. The range of increase or decrease in fluid infusion should not be greater than 10% of total volume. The compositions of fluid infusion and the regulations of water-electrolyte balance conform with the basic principles of surgical treatment. In this treatment stage, the fluid amount of nutritional support treatment should be included into the total fluid volume. Note that after fluid infusion, the quantitative and qualitative changes of urine should be carefully monitored and treated prophylactically.

Regulation of Body Temperature

During the stage of wound liquefaction, the basal metabolism rate upregulates as an adaptive mechanism including an increase in catabolism to supply energy for regenerative needs. At the same time, the burns patients frequently show hyperpyrexia because of an interference in feedback regulation of burned skin to the thermoregulatory center. The clinical treatment is as follows: firstly, make the diagnosis of adaptive hyperthermia clear and, secondly, do not misdiagnose high fever as infection.

Treat accordingly. The diagnostic indexes of this regenerative imbalance of body temperature are as follows: (1) body temperature $>39.5^{\circ}\text{C}$ and which fluctuates irregularly suggests no indication of infection; (2) no relationship between symptoms and high fever (body temperature is high, but the patient feels as 'usual') suggests no infection; (3) no abnormal signs in the wound suggests no localized infection. Rather than inappropriately relying upon the antipyretic effect of antibiotics, the physician should avail himself of simple physical cooling (for example, fanning the patient and the wound surface), as well as clearing away the liquefied materials, thereby facilitating heat release from the wound. If physical cooling produces little effect, especially in pediatric burns, a small dose of glucocorticoid should be applied, being cautious to prevent hemorrhage of digestive tract ulceration.

Trilogy Syndrome of Heart Rate, Respiration and Body Temperature

After a massive burn and during the stage of wound liquefaction, we see an adaptive increase in heart rate of $>120/\text{min}$, respiratory rate of $>30/\text{min}$, and body temperature of $>39.5^{\circ}\text{C}$. The symptoms are similar to sepsis in many ways, e.g. shortness of breath, confusion, marked hypoxia, and a murky gray or brown discoloration of the wound. This trilogy syndrome of heart rate, respiration and body temperature is often due to tiredness, mental stress and insomnia. Most of the patients have a history of the syndrome and are in a calm state of before the onset of the syndrome. It is considered preliminarily that the mechanism of this syndrome is myocardial strain, and that the reaction of heart failure resulted from serious insomnia and mental fatigue. The principle of treatment is immediate enhancement of cardiac function and intravenous injection of lanatoside C (0.2–0.4 mg in 25–50% GS 50–100 ml). If the trilogy is accurately identified, the symptoms should disappear immediately upon treatment. The possibility of concurrent infection should be entertained if the above-mentioned treatment was not very effective. In clinical practice, many patients suffering from this trilogy syndrome are misdiagnosed with sepsis, and treated inappropriately with massive antibiotic intravenous infusion. This is unfortunate and contraindicated as the window of opportunity for optimizing regeneration has been lost, and these patients die of cardiac failure though the cause of death would be mistakenly attributed to sepsis.

Protective Treatment of Multiple Organs' Function

In the stage of wound liquefaction, heart, lungs, kidneys, liver, brain, gastrointestinal tract and other organs are experiencing posttraumatic stress, global hypofunction and setting the stage for their individualized restoration. Any treatment increasing the metabolic burden on

these organs constitutes an additional stress. Therefore, it is necessary to create a favorable physiological environment for the organ's recovery. The methods for creating this environment are exactly the principles of protective treatment of multiple organs' functions: (1) The consequences of all treatment protocols on internal organs in the shock stage should be re-examined. (2) Stop applying any drug that is harmful to the healthy function of heart, lungs, kidneys, liver, digestive tract and other organs. (3) Stop applying any drug that is detrimental to the synthesis of protein. (4) To ensure an adequate energy supplement, reduce all factors predisposing to catabolism. (5) To apply some drugs temporarily which can protect the functions of liver, kidneys, digestive tract and other organs.

Nutritional Support Treatment

Extensive burns patients must be treated continually with nutritional support from the transition from shock stage until full rehabilitation. The principles of nutritional support treatment with BRT (MEBT/MEBO) are basically the same as the principle of supporting treatment of traumatic surgery. However, the supplementary amounts of total energy and protein for the former are significantly higher and of longer duration as compared with that of general traumatic patients. In clinical practice, we recommend nutritional support from the 4th to the 8th day after injury since protein is constantly required for repair and rehabilitation. After the shock stage, it is optimal to take food by mouth as soon as possible as nutrition supply through the digestive tract is encouraged. The principles of treatment are as follows:

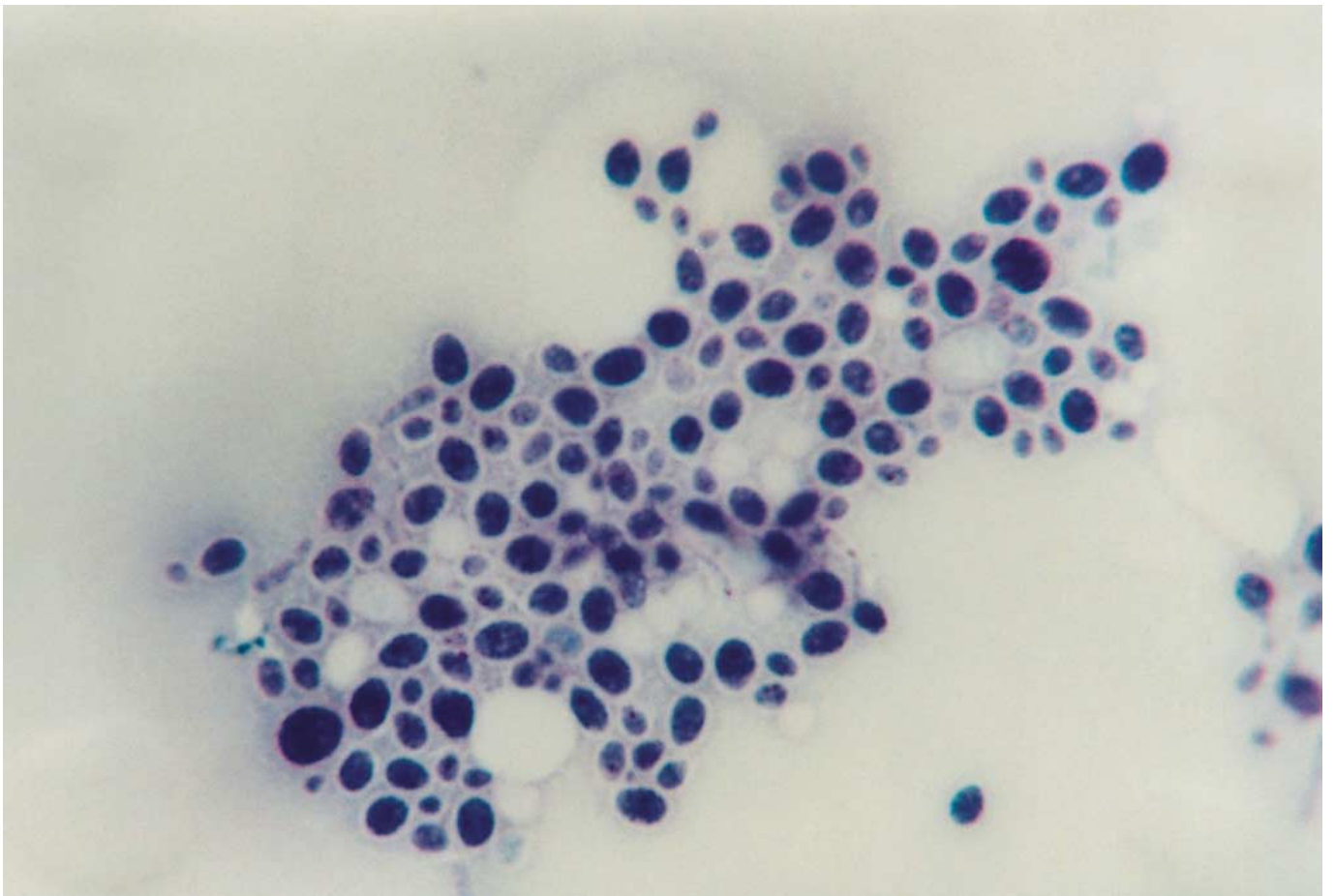
- 1 Daily caloric requirement of burned patient (kcal) = $24 (\text{kcal}/\text{kg}) \times \text{body weight (kg)} + 40 (\text{kcal}) \times \text{TBSA}\%$.
- 2 Glucose should provide between 55 and 60% of the total calories, fat between 20 and 30%, and protein between 15 and 20%.
- 3 The ratio of nitrogen to calorie should be 1:150–200.

In addition to the above supplementation, burned patients should take protein-rich foods and vegetables as liberally as possible.

Comprehensive Expectant Therapy

For extensive burns patients, the comprehensive treatment should include attention to multiple sites, e.g. cardiovascular system, respiratory system, digestive system, urogenital system, nervous system, endocrine system, and not only attend to the healing of local wounds. In clinical practice, there are no fixed models and schemes of comprehensive expectant treatment. The doctors must observe and analyze the changes of burned patients' conditions carefully and work out a medical scheme individually.

Experimental and Clinical Study on Burns Regenerative Medicine and Therapy with MEBT/MEBO



Experimental and Clinical Study on Burns Regenerative Medicine and Therapy with MEBT/MEBO

Systemic Antishock Effect of Local Treatment with BRT with MEBT/MEBO

A Comparative Study on the Antishock Effect between BRT with MEBT/MEBO and Conventional, Dry-Exposed Burn Therapy Using a Rabbit Model

Introduction

Many clinical reports have shown that treatment with BRT with MEBT/MEBO reduces the volume of fluid loss, thereby minimizing the shock effect in the treatment of extensive burns. Using an 8-hour postscald rabbit model, the authors designed a comparative study between this therapy and conventional dry-exposed burns therapy in order to evaluate the antishock effect of BRT with MEBT/MEBO.

Materials and Methods

18 healthy adult male and female rabbits weighing 2.0–2.6 kg were divided randomly into group A (treatment with MEBO, n = 9) and group B (dry exposure, n = 9). The animals were anesthetized with 3% pentobarbital sodium 30 mg/kg intravenously via ear vein, intubated and then catheterized (left common carotid artery). The cannulae were connected with a multi-channel physiological recorder so that the respiratory rate (RR), heart rate (HR), arterial blood pressure (ABP) and electrocardiogram (ECG) were able to be monitored continuously. At 30 min after operation, when the above-noted vital signs were stable, the anesthetized animals' backs were scalded with 75 °C water for 20 s in order to produce consistent deep second-degree burns of 40% BSA. The degree of tissue burn trauma was then verified by pathological examination. Next, the wounds of both groups were put under a 100 W incandescent light bulb at a distance of 50 cm. The wounds of group A were treated with MEBO applied at a thickness of 1 mm, and renewed every 2 h. The wounds of group B were washed with 0.1% bromogeramine and normal saline. Additionally, both groups received the following total

amount of fluid infusion in the first 24 h after injury (ml) = 40 × body weight (kg) × 2 (ml) plasma substitute + 40 × body weight (kg) × 2 (ml) balanced salt solution + 400 ml 5% glucose solution. Half of the total amount was infused at a corrective rate during the first 8 h after injury.

The RR, HR, ABP and ECG were measured at 5 min and at the end of each hour until the 8th hour after injury. Through the carotid artery catheter, blood samples were taken from 6 rabbits in each group at 5 min and 8 h, respectively. A type NXE-1 viscometer was used to measure blood viscosity at different shear rates. The wound tissues were sampled for pathological examination at the end of the experiment. The statistical analysis was derived from auto-control t test.

Results

Before injury and at 5 min after injury, RR, HR and ABP were not significantly different between the two groups. In group B, RR and HR increased, ABP decreased progressively postinjury and blood viscosity was raised markedly after treatment compared with that before treatment. In group A, the MEBO-treated group, the RR, HR and ABP of all animals gradually recovered to the preinjury levels after treatment while blood viscosity decreased significantly (tables 3, 4).

By contrast, 6 animals in group B developed ventricular premature beat at 4 h postinjury. One of them appeared apneic, required the rescue effort of artificial respiration and closed cardiac massage, but died 1 h later despite this. Another animal died at 8 h postinjury. These two deaths were the only fatalities in the study and both were from the non-MEBO-treated group. In group A, 2 animals developed occasional ventricular premature beats, but no death resulted. Upon gross inspection, wounds in group A were moist and supple with a pale red color and normal microcirculation. In group B, wounds

Table 3. Changes in respiratory rate, heart rate and arterial blood pressure (mean \pm SE)

Time	Group A	Group B
<i>Respiratory rate, times/min</i>		
Before injury	48.125 \pm 6.578	47.625 \pm 6.391
0 h postburn	77.375 \pm 9.999***	79.750 \pm 12.033***
1 h postburn	58.500 \pm 10.337*	71.750 \pm 12.256**
2 h postburn	52.125 \pm 6.289	79.375 \pm 11.999***
3 h postburn	50.500 \pm 6.302	89.500 \pm 12.047***
4 h postburn	52.500 \pm 8.502	93.750 \pm 9.823***
5 h postburn	44.250 \pm 10.250	95.750 \pm 7.959***
6 h postburn	44.500 \pm 10.610	97.250 \pm 7.778***
7 h postburn	49.125 \pm 15.179	97.250 \pm 7.790**
<i>Heart rate, times/min</i>		
Before injury	146.25 \pm 5.1755	145.00 \pm 10.6905
0 h postburn	163.50 \pm 8.9921**	166.88 \pm 15.7973**
1 h postburn	141.25 \pm 11.2599	173.75 \pm 9.1613***
2 h postburn	131.75 \pm 13.5831	178.75 \pm 8.3452***
3 h postburn	133.75 \pm 13.0247	187.50 \pm 13.8873***
4 h postburn	134.64 \pm 20.7571	195.50 \pm 28.4076***
5 h postburn	126.62 \pm 17.4105	210.00 \pm 24.6740**
6 h postburn	138.75 \pm 14.3487	218.00 \pm 24.9280***
7 h postburn	137.45 \pm 15.2993	223.00 \pm 33.7004***
<i>Arterial blood pressure, mm Hg</i>		
Before injury	104.5838 \pm 13.31	112.2075 \pm 11.94
0 h postburn	77.7925 \pm 19.89**	79.9530 \pm 19.24**
1 h postburn	88.2500 \pm 11.20*	72.2075 \pm 21.64**
2 h postburn	90.5012 \pm 8.59*	73.2088 \pm 20.27**
3 h postburn	89.7913 \pm 9.69	69.3775 \pm 19.58***
4 h postburn	92.9163 \pm 9.50	56.0838 \pm 11.57***
5 h postburn	94.3750 \pm 14.55	56.8750 \pm 12.16***
6 h postburn	93.8324 \pm 12.15	48.4587 \pm 15.70***
7 h postburn	94.9579 \pm 13.22	41.3750 \pm 15.76***

Compared with before injury: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

appeared wizened with a layer of eschar at the surface, fluid congestion under the crust membrane and compromise in the microcirculation as seen by stasis and dark red flaking lesions. Pathomorphological examination showed epidermis defects and residual degenerative necrotic tissues in dermis surface only in group B. Furthermore, dramatic congestion and edema of the intradermis and a significant tortuosity and thrombosis of the capillaries were also observed. By contrast, pathomorphological examination of group A showed swelling of the dermis, mild congestion with telangiectasia, infiltration of the small lymphocytes and a total absence of thrombosis.

Conclusion

Burns regenerative medicine and therapy (BRT) has antishock effects by the proposed mechanism of reduction of water evaporation from the wound surface, maintenance of a sufficient blood volume, decreasing blood viscosity, improving the local and systemic microcirculation, and reducing the absorption of burn toxins.

Discussion

MEBO Is Superior to Dry-Exposed Burns Therapy in Antishock Effect

The authors used a rabbit model with the same area of second-degree scalds to be treated with the same antishock fluid resuscitation but different wound care management, i.e. MEBT/MEBO in group A and conventional dry-exposed therapy for group B. Measurement of HR, RR and ABP were made and the same statistic analyses indicated that animals in group A

Table 4. Change of whole blood viscosity (mPa) (mean \pm SE)

Shear rate s^{-1}	Group A		Group B	
	before treatment	8 h after treatment	before treatment	8 h after treatment
5.75	8.87 \pm 3.82	6.56 \pm 3.28**	8.28 \pm 2.75	16.02 \pm 2.31**
3.60	6.94 \pm 3.24	5.24 \pm 2.77*	6.57 \pm 2.16	9.04 \pm 2.07**
15.30	5.78 \pm 2.85	4.13 \pm 2.35**	5.80 \pm 1.87	8.74 \pm 1.08**
30.72	4.82 \pm 2.15	3.32 \pm 1.93**	5.59 \pm 1.56	7.76 \pm 1.25*
38.40	4.55 \pm 2.08	3.08 \pm 1.78*	5.38 \pm 1.32	7.08 \pm 1.16*
115.0	3.47 \pm 1.30	2.74 \pm 1.12*	4.75 \pm 0.69	6.02 \pm 1.68*
230.0	3.31 \pm 1.14	2.03 \pm 0.99*	4.50 \pm 0.62	5.62 \pm 0.86*
307.2	2.86 \pm 1.05	2.09 \pm 0.95*	4.34 \pm 0.61	5.07 \pm 0.69*

Compared with before treatment: * $p < 0.05$, ** $p < 0.01$.

experienced a smoother shock period than those in group B. The ECG showed that 2 rabbits in group A developed ventricular premature beat (22.2%) and the death rate was 0, compared to 6 rabbits with ventricular premature beat (66.6%) and two deaths with a mortality of 77% in group B. The results showed that treatment in group A was superior to that in group B with respect to antishock effect. In the management of extensive burns, BRT with MEBT/MEBO has proven itself to be the treatment of choice though questions remain regarding the precise protocols of fluid infusion in order to afford subjects the optimal volume for protection against shock.

Mechanism of Antishock Effect of BRT with MEBT/MEBO

Maintenance of Effective Blood Volume. For extensive burns, the permeability of local capillaries increases significantly with the majority of exudation of plasma-like fluid occurring in the first 2 h postinjury. Another increase in exudation then occurs during the 6–8 h postinjury. Furthermore, the sympathetic upregulation due to pain and nervousness may increase the water loss from evaporation through the respiratory tract. All of the above factors conspire to increase blood concentration, reduce effective circulating volume, and dispose the physiology toward hypovolemic shock. The base of MEBO includes sesame oil and beeswax. Sesame oil has a minimal molecular weight and high lipophilic tendency that utilizes certain properties of its co-ingredient, beeswax, to resist rancidification and saponification. Therapeutic aspects of this sesame/beeswax base conspire to prevent evaporation of water from the wound site while isolating wound tissue from the outside environment. In addition, the application of MEBO to the wound has an immediate and much welcomed analgesic effect. Remembering that pain-induced stress or anxiety and since many clinic reports have verified the effectiveness of MEBO as a potent analgesic, it is reasonable to assume that MEBO also reduces water evaporation from the respiratory tract. This study can be interpreted as confirmatory in that rabbits in group A received BRT with MEBT/MEBO treatment within 8 h postinjury and suffered less water evaporation than those in group B, indicating that an effective blood volume was maintained, thereby protecting them from hypovolemic shock.

To Improve the Microcirculation. Shock is a clinical syndrome mainly characterized by acute dysfunction of the microcirculation. In the clinical setting, symptoms include microvascular contraction, enhanced capillary permeability, endothelial cells injury and functional/morphological changes of blood cells, which may lead to thrombosis. Efforts should be made to reverse microcirculation dysfunction in the period 1–4 h postinjury through

aggressive antishock therapy. Failure to do so can prove lethal. In this study, gross and micropathological examinations revealed unfortunate dryness of the wound, flaking of ecchymosis under the eschar, and tortuosity with thrombosis of capillaries in group B.

The presence of thrombosis postburn was associated with relatively sluggish blood flow, varied vascular structure, altered blood concentration and aggregation of blood cells. Excessive water evaporation from the wound sites magnified blood concentration and deteriorated microcirculation stasis, finally leading to dry necrosis. This cascade contributed to the obvious shock in group B animals. In contrast, wounds in group A appeared rubicund and moist with an absence of capillary microthrombi, and a normal blood circulation without coagulopathy. Accordingly, there were only slight symptoms of shock in group A. These differences may be attributable to the effect of BRT with MEBT/MEBO in improving microcirculation. By preventing water from excessive evaporation, this BRT method maintained an effective blood volume. This relatively fast blood flow prevented the microcirculation from developing stasis and enforced effective perfusion. Additionally, as wound tissue remained moist, cell dehydration was minimized and the physiology resisted tendencies toward shock.

To Reduce Whole Blood Viscosity. Viscosity refers to the degree of friction of blood elements during fluid flow. Viscosity of the blood may be increased by the sympexis resulting from slow blood flow and/or aggregation of erythrocytes, and by increases of macromolecule proteins such as fibrinogen. Trappeiner was the first to propose that burns could cause a concentration of blood. Baxter also reported an increased viscosity of the blood in burn patients. It was observed that following a burn injury, plasma exudation and high concentration of blood might result in the above changes and therefore increase blood viscosity, as burn shock is aggravated by an excessive blood viscosity. The results of this study showed a significantly decreased viscosity of the blood at 8 h postinjury in group A compared to group B. This suggests that BRT with MEBT/MEBO reduces plasma exudation, minimizes aggregation of erythrocytes, improves blood viscosity and flow, and reduces sympexis, all of which significantly minimizes the risk of shock as compared to dry-exposed burns therapy.

To Reduce the Resorption of Burn Toxins. When skin is burned, many new compounds called burn toxins are produced and absorbed into the circulation. Hunter and Rosenthal isolated a 12,000–14,000-dalton protein from burn exudates which contained peptide, polynucleotide, hexose and pentose. This protein inhibited cardiac muscle function and so they named it myocardial depressant factor (MDF). Mitsuyoshi found an increase of serum lipid peroxide (LPO) in burn patients which he proposed

could cross-link with lipoprotein in serum to produce toxins. Mombnhko intravenously administered the rat purified burn toxin extracted via immunoabsorption. At 2 h postinjection, arterial blood pressure decreased from 125 ± 7 to 74 ± 8 mm Hg, suggesting that burn toxins reduced blood pressure to aggravate shock by inhibiting cardiac function. The results of this study showed an obvious decrease in blood pressure, 66.6% of rabbits developing ventricular premature beats and 2 deaths in group B, which suggested a remarkable shock resulting from burn toxins. In group A, shock was minor with less burn toxins. One of three possible solutions put forward to counter burn toxins was the early excision and removal of burned tissue so as to prevent burn toxins from absorption, and therefore alleviate shock.

MEBO was applied to animals in group A at a standard dosage at room temperature. Two layers appeared on the wound surface subsequent to application, an ointment layer and a liquid layer. Warmed by the body temperature of the wound, the ointment layer becomes less viscous and attaining a relatively liquid form it mobilizes the necrotic tissue with the wound. MEBO then mixes with exudates, liquefies necrotic tissue and metabolic products to produce a conglomerate of lipophobic, whitish material which is propelled away from the wound. Thus, by converting the lipophilic necrotic debris into a lipophobic MEBO complex, the wound is cleansed via an automatic drainage process while neither irritating nor distressing the viable wound tissues. This innovative process is superior to the excision of necrotic tissues and results in the advantage of a reduced absorption of burn toxins. In this way, MEBO reduces shock potential as it causes no decrease in blood pressure, no inhibition of cardiac muscle and no damage to the microcirculation. In contrast, conventional therapy involving dry-exposed burns therapy results in the drying of necrotic tissue, and an unhealthy absorption of burn toxins which increases the odds of shock and even death.

Experimental Study on Maintaining Physiological Moist Effect of BRT with MEBT/MEBO on Treating Burns Wounds

Introduction

The essential therapeutic principle of BRT with MEBT/MEBO is to keep the burn wound in a physiological (moist) environment in order to promote the graceful discharge and removal of necrotic tissue as well as the regeneration of healthy tissue from the wound. Only BRT with MEBT/MEBO offers this therapeutic advantage. No other burns protocol offers what is unique to BRT – the

ability of maintaining a moist physiologic wound environment while at the same time optimizing efficient drainage.

Materials and Methods

Forty-two healthy adult rabbits of both sexes (including nonpregnant females) weighing 2.1–3.0 kg were used in this study. Animals had their backs depilated via application of 8% sodium sulfide solution. Anesthesia was achieved by intraperitoneal injection of pentobarbital sodium (40 mg/kg). On each side of the back, one deep second-degree burn wound was formed by igniting a heated cake of copper, 3 cm in diameter copper plate at 100°C for 4 s. The depth and tissue characteristics of the wound were verified by pathological examination.

Vaseline was produced by Yangzizhou Pharmaceutical Factory, Nanchang City, PR China. Model EPIC evaporimeter (Servomed) was used for measuring wound evaporation.

Experiment 1

BRT with MEBT/MEBO treatment with auto-control: Ten rabbits were used in this experiment. The water evaporation value of normal skin was measured before injury. Then the animal was burned on both sides of the back. The wound on one side was treated with MEBO, while the wound on the other side was allowed to dry expose spontaneously as auto-control. MEBO was applied at a thickness of 0.5–1 mm and reapplied at an interval of 6–8 h. Measurement of wound water evaporation was done at 0.5, 6, 24, 48, 72 and 168 h postburn. The healing time of each wound was recorded.

Experiment 2

BRT with MEBT/MEBO treatment with dry-exposed therapy control: Twenty-four rabbits were randomly divided into 2 groups, MEBO and dry-exposed therapy groups, 12 in each. The treatment method and observations were done as described in experiment 1. Pathological examinations were conducted at the same time.

Experiment 3

Vaseline treatment: Eight rabbits with deep second-degree burns wounds on both sides of the back were treated with Vaseline. The treatment method and observations were done as described in experiment 1. The results were compared with the MEBO-treated group.

The ambient temperature was $25\text{--}28^{\circ}\text{C}$ and humidity $50 \pm 5\%$. The data were presented as mean value \pm SD and analyzed statistically using the t test.

Results

Wound Water Evaporation

Compared with normal skin, the water evaporation values of burns wounds treated with dry-exposed therapy in both experiments 1 and 2 increased dramatically and reached their peaks at 6 h postinjury, and then tended to decrease slowly. Conversely, the water evaporation of wounds treated with MEBO was much lower ($p < 0.01$; table 5a, b). The water evaporation of wounds in the Vaseline-treated group was always kept at a low level, significantly different from that of normal skin as well as MEBO treated wounds ($p < 0.01$) (table 5c).

Table 5. Comparisons of water evaporation

Wound	Before injury	Time after injury					
		0.5 h	6 h	24 h	48 h	72 h	168 h
a Between MEBO-treated wounds and dry-exposed wounds in the auto-control group ($\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, mean \pm SE) (data from experiment 1)							
Dry exposed (n = 10)	4.48 \pm 0.81	83.70 \pm 11.12 ⁺⁺	87.00 \pm 10.38 ⁺⁺	81.60 \pm 10.57 ⁺⁺	73.60 \pm 10.46 ⁺⁺	69.40 \pm 6.97 ⁺⁺	61.70 \pm 6.11 ⁺⁺
MEBO treatment (n = 10)	4.48 \pm 0.81	5.69 \pm 1.21 ^{+,**}	5.74 \pm 1.35 ^{+,**}	5.44 \pm 0.84 ^{+,**}	5.54 \pm 0.93 ^{+,**}	5.29 \pm 0.77 ^{+,**}	5.69 \pm 1.36 ^{+,**}
b Between MEBO-treated and dry-exposed groups ($\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, mean \pm SE) (data from experiment 2)							
Dry exposed (n = 24)	4.48 \pm 0.67	82.38 \pm 7.54 ⁺⁺	85.92 \pm 5.11 ⁺⁺	79.58 \pm 7.06 ⁺⁺	74.41 \pm 5.04 ⁺⁺	69.54 \pm 5.14 ⁺⁺	56.62 \pm 5.60 ⁺⁺
MEBO treatment (n = 24)	4.67 \pm 0.75	5.32 \pm 1.09 ^{+,**}	5.37 \pm 1.04 ^{+,**}	5.29 \pm 0.97 ^{+,**}	5.16 \pm 0.82 ^{+,**}	5.18 \pm 0.84 ^{+,**}	5.30 \pm 0.93 ^{+,**}
c Between MEBO-treated and Vaseline-treated wounds ($\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, mean \pm SE) (data from experiment 3)							
Vaseline treatment (n = 16)	4.68 \pm 0.87	1.60 \pm 0.35 ⁺⁺	1.76 \pm 0.51 ⁺⁺	1.64 \pm 0.34 ⁺⁺	1.78 \pm 0.55 ⁺⁺	1.77 \pm 0.50 ⁺⁺	1.79 \pm 0.32 ⁺⁺
MEBO treatment (n = 24)	4.67 \pm 0.75	5.32 \pm 1.09 ^{+,#}	5.37 \pm 1.04 ^{+,#}	5.29 \pm 0.97 ^{+,#}	5.16 \pm 0.82 ^{+,#}	5.18 \pm 0.84 ^{+,#}	5.30 \pm 0.93 ^{+,#}

Compared with the value before burns: + p < 0.05, ++ p < 0.01; compared with dry-exposed control: ** p < 0.01; compared with Vaseline treatment: # p < 0.01.

Gross Observation

After injury, the wounds in all three experimental groups appeared pale or red-white, with inflammation and swelling above the level of the wound edge. Wounds treated with dry-exposed therapy gradually dried and sunk below the level of the wound edge. Wounds treated with MEBO remained moist and swellings subsided within 48 h postburn. Necrotic tissues began to liquefy from superficial to interior and were removed. In experiment 1, MEBO-treated wounds healed more quickly than the dry-exposed auto-control wounds. The difference was statistically very significant ($p < 0.01$) (table 6). Wounds treated with Vaseline became macerated after 6 h and were getting worse with serious and persistent tissue edema. The eschar dissolved earlier than wounds treated with MEBO.

Pathological Examination

Paraffin-embedded sections of the wound tissues in the 3 experimental groups were stained with hematoxylin and eosin staining, and then examined under a light microscope. All of them proved to have pathological changes of deep second-degree burn injury and the pathological changes were almost the same at half an hour postinjury. After 6 h, the injured tissue became loose and there was vacuolar degeneration of cells and attenuation of fiber tissues. These changes were most serious in wounds treated with Vaseline. Micro blood vessels were dilated and had

Table 6. Comparison of healing time of different wounds in the auto-control group (mean \pm SE) (data from experiment 1)

Wound	Number of wounds	Healing time days	p value
Dry exposed	10	19.80 \pm 2.61	<0.01
MEBO	10	15.00 \pm 1.16	

embolism and infiltration of a few white blood cells. After 24 h in dry-exposed treated wounds, the zone of necrosis expanded and deepened continuously. The microcirculation was further blocked with embolism and infiltration of a large amount of inflammatory cells (mainly neutrophils) occurred. After 48 h, a zone of leukocyte infiltration was formed around the junction of necrotic and surviving viable tissues. This phenomenon became increasingly obvious as time passed. In MEBO-treated wounds, tissue in the zone of stasis recovered rapidly. Micro blood vessels showed some dilation and congestion. Infiltration of inflammatory cells was scattered in the micro blood vessels. After 48 h, the cells concentrated on the junction of necrotic tissue and where MEBO was applied to form a dense area. In the Vaseline-treated wounds, pathological changes were quite different from those in the MEBO-treated ones. Vacuolar degeneration of tissues was more serious and the inflammatory reaction was diffused.

Conclusion

This research revealed that burns wounds treated with MEBO had less water evaporation than those treated with conventional dry-exposed therapy. The rate of evaporation was approximate to that of normal skin. Unlike Vaseline, MEBO had excellent permeability allowing active drainage of excreta. MEBO demonstrated that its moist physiological environment is favorable to wound healing.

Discussion

Burn injury destroys the body surface barrier. Injured skin loses its ability to prevent evaporation of water from the body. This results in an excessive loss of body fluid from the lesion area which itself is a stress against healing since a burn wound that is allowed to dehydrate will be further injured by dehydration and necrosis. For deep second-degree burns wounds, the injured tissue in the zone of stasis can be rescued, but excessive drying will cause irreversible damage. This tendency to dehydration is a main disadvantage of conventional dry-exposed therapy. Studies of pathophysiology teach that a moist environment is favorable for the regeneration and repair of injured tissue. The specially designed MEBO ointment alone succeeds in creating an environment in which burns wounds can remain exposed while avoiding the ravages of dehydration and maceration.

The key to the maintaining of water in burns wounds is to prevent evaporation. The results of this study revealed that burns wounds treated with MEBO had significantly less evaporation than those given dry-exposed therapy. MEBO is different from other medical lubricants or ointments such as Vaseline in that MEBO retains wound moisture while keeping the wound surface permeability. For example, excessive water can permeate through the MEBO ointment layer and be removed easily. The difference between water evaporation of MEBO-treated and Vaseline-treated wounds was very significant ($p < 0.01$). This was proved by the test of skin sweat excretion [1]. What attracts our great attention was that although the water evaporation of burns wounds treated with MEBO was significantly different from that of normal skin ($p < 0.05$), the difference between the mean values was less impressive. MEBO's water retention abilities have been compared to sebum. MEBO maintains wound moisture without compromising drainage, therefore comprising a moist environment that is optimal for wound repair and healing [2].

It has been reported that there is a zone of thermal-injured tissue with viability in the deep part and the surroundings of coagulated tissue postburn. This is called the zone of stasis [3]. Blood flow of micro blood vessels in this zone diminishes progressively after burns, and remains

very susceptible to further injury. Dehydration or mechanical injury will tip the scale toward tissue necrosis in this zone of stasis. Therefore, it is really difficult to distinguish this zone from the initially coagulated tissue that presents after 5 days postburn. This tendency to allow the zone of stasis (a recussible wound state) to deteriorate into a non-recussible death state is consistent with conventional dry-exposed therapy. By comparison, MEBO inhibited the deterioration of this process by avoiding secondary injuries such as dehydration. Therefore, MEBO promotes the recovery of tissues within the zone of stasis and thus proves favorable to wound tissue regeneration and repair. Burns wounds treated with MEBO healed more quickly than those treated with dry-exposed therapy ($p < 0.01$). This result could not be attributed to the moisture maintaining effect of MEBO alone, but without a moist environment, it is hard for any medical treatment to achieve [2]. Vaseline treatment, though it also kept the wound moist but macerated, resulted in suboptimal changes in wound tissues as opposed to the healthy tissue resulting from MEBO treatment. Based on our results, MEBO provided a physiologically moist environment uniquely favorable to wound tissue regeneration and repair.

The unique dosage form and pharmacokinetic features of MEBO are responsible for its moisture-maintaining effect. The following three aspects may account for the mechanism of its action:

- 1 MEBO has its unique dosage form with frame structure, which, when applied on the wound, warms and morphs into an equilibrium of two phases, liquid and semisolid. It isolates the wound from external irritation and prevents excessive water evaporation from the wound surface.
- 2 MEBO has an active drainage effect whereby excessive water, liquefaction products and excreta are passively and automatically removed through the drug layer [2, 4].
- 3 MEBO base contains hydrophilic and lipophilic groups and has high surface activity. It has higher affinity to the wound tissue than water, which allows for the formation of a strong adsorptive film on the wound which protects it from either maceration or dehydration.

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Clinical Study on Invisible Water Loss of Burns Wounds Treated with BRT with MEBT/MEBO

Introduction

It has been confirmed that treatment with BRT with MEBT/MEBO offers a unique ability to inhibit excessive loss of water evaporation through the burn wound surface [1–4]. However, there has not been adequate quantitative clinical research at this time. In this study, the authors observed minimal dehydration of burns wounds treated with BRT with MEBT/MEBO in 25 burn patients with different area and different depths compared to the water evaporation value of wound to that of normal skin.

Materials and Methods

Twenty-five acute burns patients, 21 males and 4 females, were hospitalized within 6 h after injury. Burn area ranged from 4% total body surface area (TBSA) to 95% TBSA. After simple debridement, all patients were treated with BRT with MEBT/MEBO [5]. Observation at 6, 24, 48 and 72 h postburn measured the water evaporation capacity of superficial second-degree, deep second- and third-degree wounds, compared to normal skin as a control. In 4 cases with burn area over 50% TBSA, their daily amounts of water evaporation were determined based on wound evaporation and body surface area. During the liquefaction period, 12 cases with deep second- and third-degree wounds received semi-exposed MEBO treatment, and their wound water evaporation was compared with those receiving exposed MEBO treatment.

A Model EPIC evaporimeter (Servomed) was used to measure wound water evaporation [6]. Measurement was done at 2 h after application of MEBO, in a room sheltered from the wind. Room temperature was kept at 32–36 °C with a relative humidity of 45–62%.

Results

Normal skin water evaporation of the 25 burns patients had an average value of $18.48 \pm 5.02 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$. Wounds were classified according to their depths and one typical wound from each degree was selected randomly. Blister skin (necrotic epidermis) of every selected typical wound was divided in half with one half remaining intact and the other half having its protective skin removed. Then the evaporation of both halves of the wound was measured, respectively. The results revealed that water evaporation in wounds with preserved epidermis had a very significantly lower evaporation capacity than those with removed epidermis ($p < 0.01$). Water evaporation reached its peak at 6 h postburn and then decreased, but still remained markedly higher than that of the normal skin ($p < 0.05$). For very deep wounds, water evaporation of wounds with removed epidermis tended to decrease ($p < 0.05$), while evaporation in wounds with preserved epidermis did not decrease significantly ($p > 0.05$) (table 7).

In 4 extensive burns patients with burn area over 50% TBSA, their total body surface and burns areas were measured. Daily water evaporations of the wounds with different depths were calculated, respectively. Then invisible dehydration per hour per 1% of second- and third-degree wounds can be obtained. The average value was $0.71 \text{ ml}\cdot\text{h}^{-1}\cdot\% \text{ TBSA}^{-1}$ (table 8).

During the liquefaction period, deep wounds were treated with exposed or semi-exposed method, the two methods did not result in a significant difference in wound water evaporation ($p > 0.05$) (table 9).

Table 7. Evaporation of wounds with different depth at different intervals ($\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, mean \pm SE)

Depth of wound	Blister skin	Number of wounds	Time after injury			
			6 h	24 h	48 h	72 h
Superficial second-degree	removed	22	$57.45 \pm 6.40^{**}$	$43.86 \pm 5.88^{**}$	$35.73 \pm 4.23^{**}$	$32.68 \pm 3.70^{**}$
	preserved	22	$28.68 \pm 7.58^{**}$	$24.23 \pm 4.62^{**}$	$23.09 \pm 4.56^{**}$	$22.86 \pm 2.88^{**}$
Deep second-degree	removed	25	$44.84 \pm 4.66^{**}$	$37.40 \pm 4.94^{**}$	$32.36 \pm 4.53^{**}$	$30.56 \pm 3.62^{**}$
	preserved	25	$26.40 \pm 4.80^{**}$	$24.68 \pm 3.44^{**}$	$23.24 \pm 4.05^{**}$	$22.20 \pm 4.80^{**}$
Third-degree	removed	15	$42.53 \pm 5.30^{**}$	$35.93 \pm 4.06^{**}$	$32.07 \pm 3.84^{**}$	$29.07 \pm 40.53^{**}$
	preserved	15	$25.13 \pm 2.13^{**}$	$23.67 \pm 2.58^{**}$	$22.33 \pm 4.25^*$	$21.93 \pm 2.85^*$

Compared with normal skin: * $p < 0.05$, ** $p < 0.01$.

Comparison between blister skin removed and preserved, all the differences were very significant: $p < 0.01$.

Table 8. Invisible dehydration of 4 extensive burns patients

Case No.	Burn area of different depth, % TBSA				Wound evaporation, g·m ⁻² ·h ⁻¹			Daily invisible dehydration ml	Invisible dehydration ml·h ⁻¹ ·% TBSA ⁻¹
	TBSA	superficial second-degree	deep second-degree	third degree	superficial second-degree	deep second-degree	third degree		
1	53	23	30		61	44		1,097	0.86
2	73	6	21	46	49	45	26	888	0.50
3	94		40	54		53	39	1,598	0.71
4	95	3	67	25	60	42	36	1,746	0.77

Table 9. Comparison of water evaporation between the exposed and semi-exposed wounds (mean ± SE)

Wound	Number of wounds	Evaporation g·m ⁻² ·h ⁻¹	p value
Exposed	13	42.58 ± 7.89	>0.05
Semi-exposed	12	43.92 ± 8.43	

Conclusion

- 1 BRT with MEBT/MEBO effectively inhibited water evaporation from wounds and decreased total body fluid loss. Additionally, preservation of necrotic epidermis, including blister skin, also helped to decrease water loss.
- 2 The fluid injection of burns patients in the early stage postburn follows the formula: 0.4–1.2 ml/(h × % BSA), with adjustment according to depth of the wound and exfoliation of the epidermis.
- 3 During the liquefaction period, MEBO could be applied in either the exposed or the semi-exposed mode.

Discussion

Skin is the surface barrier of the human body. As such, it plays an important role in maintaining body fluid status. Burn injury causes damage of this barrier and increases vascular permeability, causing a significant rate of increase of dehydration. Loss of body fluid through burns wounds is proportional to the burn area and depth as well as being related to burn intervals postburn [7]. The results of this study showed that water loss through the wound in the first few days postburn was much greater than through normal skin ($p < 0.05$) and reached its peak at 6 h postburn, then tended to decline and gradually decrease along with the deepening of wound. Preservation of necrotic epidermis, including blistered skin, also helped to decrease water loss. Previous studies gave similar results [8, 9]. However, our degree of local wound water loss was

much less than that of those previous reports due to the use of the BRT protocol (MEBT/MEBO) to treat the wounds. We conclude that BRT reduced wound exudation and inhibited evaporation.

Increased capillary permeability causes exudation of body fluid into the interstitial space, thus forming ‘burn edema’. Another pathway of water loss is invisible dehydration through the burn wound. In this study with 4 cases having burn area over 50% TBSA, the average water loss per hour per 1% of second-degree and third-degree wound water loss was 0.71 ml. It would be helpful to evaluate the volume of fluid resuscitation during the shock stage postburn. Based on this study, the fluid injection of burns patients in the early stage postburn can follow the formula: 0.4–1.2 ml/(h × % BSA), also adjust according to the depth of the wound and the exfoliation of the epidermis.

MEBT suggests application of MEBO ointment with the exposed method, but in some cases, the semi-exposed method may be also advisable. The efficacy of the semi-exposed method was comparable to that of the exposed method. Our limited experiment proved that there was no significant difference in wound water evaporation between the two methods during the liquefaction period ($p > 0.05$), indicating that application of MEBO with the exposed or semi-exposed methods had a similar efficacy on inhibiting water loss from the wound surface. It is interesting to note that wound water evaporation measured at the liquefaction period was greater than during the edema resorption period. This might be due to the requirement of more water for the liquefied reaction between MEBO and necrotic tissues as well as the production of liquefaction products.

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Experimental Study of Moist-Exposed Burn Ointment on Improving Wound Microcirculation of the Zone of Stasis in the Early Stages after Burns

Introduction

After sustaining a cutaneous burn, typically three concentric circles develop around the wound. Seen from the interior to the exterior, these three zones are termed the zone of necrosis, the zone of stasis, and the zone of hyperemia. During the initial postburn period, coagulation necrosis of the tissue occurs in the zone of necrosis of burns wounds. Meanwhile, progressive injury occurs in the zone of stasis, especially within 24–48 h postburn. Pathological changes in the stasis zone include the dilation of capillaries and small veins, the swelling and hyperpermeability of vascular endothelial cells, a loosely arranged pattern of endothelial cells with increased intercellular gapping. Lysis products of erythrocytes accumulate in blood vessels and microthrombosis and necrosis occur. The more microthrombi are formed, the more necrotic the tissue [1, 2]. BRT with MEBT/MEBO aims to rescue tissues in the stasis zone by improving microcirculation and avoiding massive formation of microthrombi. We have shown that

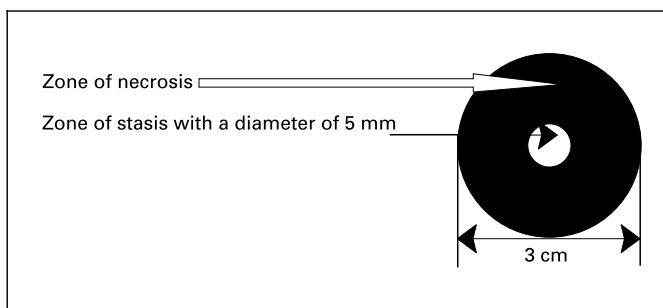


Fig. 10. Illustration of the burn wound area.

progressive necrosis of the tissues in the stasis zone can be prevented [3]. This experiment was designed to investigate the efficacy of BRT with MEBT/MEBO in improving microcirculation of burns wounds through a comparison of the blood circulation volume in zone of stasis in burns wounds treated with BRT with MEBT/MEBO compared to that of conventional dry-exposed therapy.

Materials and Methods

Ninety-six healthy male SD rats, weighing 250 ± 30 g, were used and divided randomly into 2 groups, 48 in each. The animals were depilated on the backs by applying 8% barium sulfide solution and anesthetized by intraperitoneal injection of 3% pentobarbital sodium at a dose of 30 mg/kg. A hollow cylindrical bronze rod with an outside diameter of 3 cm and a bore diameter of 5 mm, weighing about 1 kg, was put into boiling water for 15 min and wiped dry. Then, the hot end of the rod was applied onto one side of the depilated back of the rat for 12 s to form a third-degree burn wound (determined by pathological examination), as shown in figure 10. A preliminary experiment had determined that in the central uninjured area, the microcirculation blood volume decreased quickly postburn and remained low for a long time. This area is considered as the zone of stasis.

In both groups, 8 animals were used to compare the blood circulation volume in the stasis zone and the area of necrotic tissue. The remaining rats (40 in each group) were used to observe pathological changes in the tissue at the intervals before burn and at 4, 24, 48 and 72 h postburn, respectively (8 animals observed at each interval). Rats in the BRT with MEBT/MEBO groups were treated with MEBO by applying the ointment onto the wounds at a thickness of 1 mm, once a day. Rats in the dry-exposed therapy group were treated with 1% silver sulfadiazine (SD-Ag) cold cream once a day. No drug was applied onto the zone of stasis in both groups.

Blood flow volume of microcirculation in the zone of stasis was measured by a Doppler laser blood flow monitor before burn, and at 5 min, 30 min, and 1, 2, 4, 8, 12, 18, 24, 48 and 72 h postburn, respectively. The necrotic area in the zone of stasis was measured using a Leitz automatic image pattern analyzer at 14 days postburn. Tissue water volume in the zone of stasis was determined before burn, and at 4, 24, 48 and 72 h postburn. Before burn and at 4, 24, 48 and 72 h postburn, full-thickness skin tissue samples were taken from the normal skin and the zone of stasis. Each sample (200 mg) was homogenized, centrifuged and the supernatant fluid was used for determination of MDA content.

The data were expressed as mean \pm SE. Student's *t* test was used for statistical analysis.

Results

As shown in figure 11, microcirculation blood flow volume in the zone of stasis decreased rapidly postburn in both groups and reached the lowest value at 2 h postburn. However the reduction of blood flow volume in the dry-exposed therapy group was significantly greater than that in the BRT with MEBT/MEBO group, at all time intervals ($p < 0.01$).

As shown in table 10, the MDA content of tissues in the zone of stasis increased significantly at 4 h postburn in both groups. In the BRT with MEBT/MEBO group, MDA

content turned to the level of pre-injury at 24 h postburn, while in the dry-exposed therapy group, the MDA level rose continuously and was significantly higher than that in the BRT with MEBT/MEBO group, at different time intervals ($p < 0.01$).

As shown in table 11, water volume of tissues in the zone of stasis increased at 4 h postburn in both groups. In

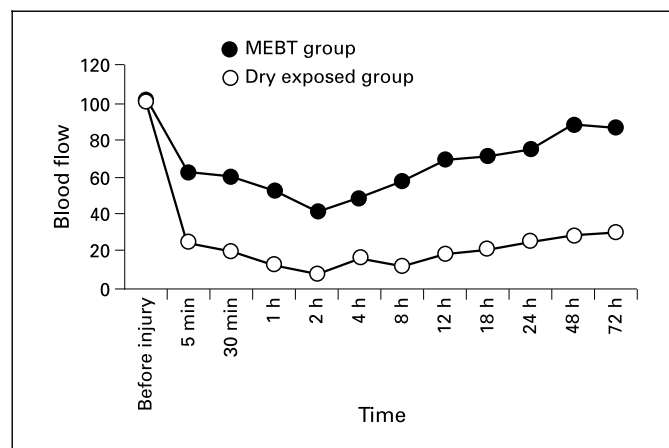


Fig. 11. Dynamic changes of microcirculation blood flow volume in the zone of stasis postburn.

the dry-exposed therapy group, water volume increased significantly at 4 and 48 h postburn and returned to the normal level at 72 h postburn, while in BRT with MEBT/MEBO group, water volume turned to the normal level at 48 h postburn ($p < 0.05$).

In the dry-exposed therapy group, necrotic tissue area in the zone of stasis at 14 h postburn was $20.96 \pm 3.51 \text{ mm}^2$, it was significantly larger than $8.38 \pm 1.78 \text{ mm}^2$ in the MEBT/MEBO group ($p < 0.01$).

Conclusion

Treatment with MEBT/MEBO in the early stages after burns could improve the wound microcirculation in the zone of stasis, lessen further injuries to the support tissues of the burn wound, then increase the recovery of injured tissue at the zone of stasis.

Discussion

Microthrombosis and vascular hyperpermeability are major changes in the wound microcirculation system after burns. In the zone of stasis, microthrombosis is often obvious [1, 2]. BRT involves covering the burn wound with MEBO because this specially designed ointment is effective for improving wound microcirculation and pre-

Table 10. MDA levels in the zone of stasis between the two groups (nmol/mg protein, mean \pm SE)

Group	Before injury	Time after injury			
		4 h	24 h	48 h	72 h
MEBT/MEBO	0.065 ± 0.07	$0.147 \pm 0.047^*$	$0.078 \pm 0.024^*$	0.069 ± 0.019	0.063 ± 0.022
Dry-exposed	0.065 ± 0.021	$0.240 \pm 0.078^{*,+}$	$0.303 \pm 0.097^{*,+}$	$0.363 \pm 0.099^{*,+}$	$0.378 \pm 0.114^{*,+}$

Compared with before injury: * $p < 0.01$; compared with group 1: + $p < 0.01$.

Table 11. Comparison of water volume in the zone of stasis between the two groups (% , mean \pm SE)

Group	Before injury	Time after injury			
		4 h	24 h	48 h	72 h
MEBT/MEBO	66.2 ± 2.5	$77.3 \pm 3.1^*$	$76.5 \pm 2.64^*$	67.1 ± 2.7	65.9 ± 2.4
Dry-exposed	66.2 ± 2.5	$89.9 \pm 4.3^{**,+}$	$86.4 \pm 3.9^{**,+}$	$77.2 \pm 4.0^*$	68.9 ± 3.3

Compared with before injury: * $p < 0.05$, ** $p < 0.01$; compared with MEBT/MEBO group: + $p < 0.05$.

venting progressive necrosis of tissues in the zone of stasis [3]. The zone of stasis is developing progressively at the early stages postburn, so its area is difficult to measure. Our experiment provided a unique model which helped to solve this problem.

In the dry-exposed therapy group, the necrotic area of tissue in the zone of stasis was $20.96 \pm 3.51 \text{ mm}^2$ at 14 days postburn. This value was significantly higher than the $8.38 \pm 1.78 \text{ mm}^2$ of the MEBT/MEBO group ($p < 0.01$). This proved that MEBO is effective for improving microcirculation and preventing further coagulation necrosis of the injured tissue with viability in the zone of stasis. According to the results of this study, we concluded that BRT with MEBT/MEBO has the following advantages:

- 1 Improving microcirculation in the zone of stasis, preventing massive microthrombosis, increasing blood flow volume, lessening the degree and shortening the duration of both ischemia and hypoxia relative to tissues in the zone of stasis, and thus protecting the vitality of the viable tissues.
- 2 Reducing capillary permeability, preventing massive exudation through burns wounds and tissue edema. Exudation and edema will cause ischemia and necrosis in the surrounding tissues of burns wounds or even hypovolemic shock.
- 3 Reducing the production of oxygen free radicals and inhibiting injury due to lipid peroxidation. MDA is a product of lipid peroxidation, its content reflects the degree of the reaction. In this study, MEBO quickly decreased the MDA content of tissues in the zone of stasis, while in the dry-exposed therapy group, the MDA level rose continuously.

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Clinical Study of Moist-Exposed Burns Ointment on Improving Microcirculation of Burns Wounds

Introduction

The typical changes in microcirculation immediately following burn trauma include cellular and gross anatomical changes. At the cellular level, we see an increase of

vascular permeability and an increased risk of microthrombus. At the macro level, we see three concentric circles developing around the wound after cutaneous burns, ranging from interior to exterior and termed the zone of necrosis, the zone of stasis and the zone of hyperemia [1]. The microcirculation in the zone of stasis is characterized by the formation of progressive microthrombus. The location of the zone of stasis varies according to the depth of the burns. Burns affecting the dermis are termed second-degree burns while third-degree burns involve the full-thickness of skin as well as subcutaneous tissues. The ultimate burn depth is determined by the development of the stasis zone, which may extend full-thickness, arresting blood circulation and causing tissue necrosis resulting in a third-degree burn. However, if there is some improvement in the microcirculation in the zone of stasis, deep second-degree burns will rarely deteriorate into third-degree burns. BRT was designed to deal with the zone of stasis by improving the microcirculation, avoiding the formation of microthrombus and thereby arresting progressive necrosis.

Recent research suggests that significant release of vascular active factors in plasma, endothelin (ET) and nitric oxide (NO), has a close correlation with the deterioration of vulnerable tissue within the zone of stasis during the early postburn stage [2–4]. ET and NO released from the burn wound become the primary source of ET and NO in plasma. The studies during the previous decade have paid great attention to ET and NO known as 'star molecules' because they are the most powerful vascular contraction and dilation factors known to man. Under normal non-traumatic situations, vascular endothelial cells maintain a relatively stable ratio of ET/NO to ensure the normal capacity for vasoconstriction and dilation within the microcirculation of the skin. However, certain pathological conditions such as stress, ischemia, hypoxia and acidosis may stimulate the massive increase in the synthesis and release of ET and NO. These challenges upset the original balance of ET and NO, causing a cascade of reactive pathological damages. This study investigates the relative impact on plasma ET/NO ratio by BRT (MEBT) and conventional therapies and gives a further investigation on the mechanism of MEBO for improving the microcirculation of burns wounds.

Materials and Methods

The study involved 34 adult patients who suffered 30–40% TBSA burns with more than 20% TBSA of deep second-degree wounds, all of whom were admitted to the hospital at 4–5 h after injury. All subjects were randomized to receive either BRT with MEBT/MEBO or conventional dry-exposed therapy. The MEBO group included 18 patients (11 male, 7 female) with a mean age of 26 ± 10.9 years while the dry-exposed group comprised 16 patients (10 male, 6 female) with a mean age of 28 ± 12.0 years.

Table 12. Dynamic changes in plasma postburn ET (pg/ml, mean ± SE)

	8 h	1 day	2 days	3 days	5 days	7 days	14 days
<i>ET, pg/ml</i>							
MEBT/MEBO	87.7 ± 23.5**	78.0 ± 15.2**	70.2 ± 20.9**	69.3 ± 14.6*	41.2 ± 13.5	46.9 ± 14.8	40.7 ± 12.1
Dry-exposed	169.6 ± 45.9**	228.1 ± 53.8**	183.2 ± 44.7**	117.8 ± 41.9**	103.4 ± 31.6**	98.7 ± 26.3**	88.1 ± 29.2**
<i>NO, μmol/l</i>							
MEBT/MEBO	21.7 ± 5.4*	18.0 ± 6.2*	20.3 ± 3.9*	16.73 ± 4.0*	3.7 ± 3.9	15.6 ± 4.2	14.7 ± 3.4
Dry-exposed	23.6 ± 6.9**	28.1 ± 7.8**	31.2 ± 9.4**	27.6 ± 7.8**	19.3 ± 7.4	14.7 ± 6.9	13.7 ± 7.0

Compared with normal level: * p < 0.05, ** p < 0.01.

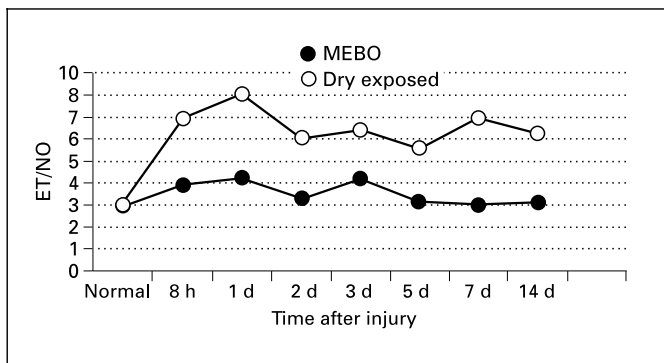


Fig. 12. Changes of the ET/NO ratio in both groups after injury.

All patients were treated with the standard of care as regards fluid supplement, anti-infection and anti-inflammation. For the BRT with MEBT/MEBO group, MEBO ointment was spread at a thickness of 1 mm guided by the following principles: replace the ointment every 4–6 h during the exudation and repair periods; during the liquefaction period, the intervals of reapplying MEBO is determined by the amount of liquefied products on the wound. The typical interval calls for reapplication every 4 h. The patients receiving the dry-exposed therapy received topical debridement and were treated with 1% silver sulfadiazine (SD-Ag) daily.

Venous blood was sampled, respectively, from burn patients in both groups at 8 h, 1, 2, 3, 5, 7, and 14 days postburn, and also from 5 healthy control males and females. NO was determined by the Griess method. ET was measured with radioimmunoassay (RIA). Both ET and NO kits were purchased from East Asia Immunity Technology Institute, General Hospital of CPLA.

Results

The normal level of ET in plasma was 42.8 ± 12.2 pg/ml, NO: 14.5 ± 3.6 μmol/l, the ratio of ET/NO 2.95. Table 12 shows that ET in both groups markedly increased after injury, but we see that the increment in the BRT with MEBT/MEBO group was much lower than that in the dry-exposed group. The MEBO group returned to normal level on the 5th day postburn as compared to the dry-exposed group's value which remained at a high level

even at day 14. Table 12 shows an obvious increase of NO in both groups at 8 h. In the BRT with MEBT/MEBO group, the NO gradually decreased to normal by day 3 compared to day 7 for the dry-exposed group. Figure 12 shows that the ratio of ET/NO in the BRT with MEBT/MEBO group decreased to 2.95 after 1 day, while that in the dry-exposed group remained much higher than 2.95 at all phases.

The MEBO group required 5.6 ± 2.5 days to heal the superficial second-degree burns wounds and required 15.6 ± 4.7 days for the resolution of deep second-degree wounds as evidenced by good skin elasticity and no scar formation. In the dry-exposed group, 8.7 ± 4.6 days were required for healing superficial second-degree burns wounds, and 21.6 ± 6.4 days were required for the completion of healing in deep second-degree wounds. However, note that the end result of this protocol, as contrasted with the MEBO protocol, was suboptimal as it resulted in scar formation and poor elasticity.

Conclusion

We have demonstrated that BRT with MEBT/MEBO improves the microcirculation of burns wounds, shortens healing time, and promotes deep second-degree wounds to heal without scar formation by means which include the optimization of the ratio of ET/NO.

Discussion

The formation of progressive microthrombus in the zone of stasis may deepen and extend the area of burns wounds, thereby directly worsening both the rate of wound healing and the clinical prognosis. Therefore, an important question must be weighed up by the world's burns therapists: How can one best alleviate tissue damage, promote wound healing and reduce the possibility of scar formation? Current research suggests that BRT (MEBT) offers the best answer to these questions.

This study was designed to investigate the effect of MEBO on plasma ET/NO so as to further discover the mechanisms of MEBT therapy. ET, separated from supernatant fluid of cultured pig aorta endothelial cell by Yanagisawa in 1988, is mainly synthesized in the vascular endothelial cell and is known to be the strongest vasoconstrictive peptide in addition to other biological actions. However, the excessive release of ET may cause micro blood vessels to contract and spasm for an extended period of time, resulting in microthrombus formation. NO is a potent gaseous free radical and is synthesized in the cytoplasm of vascular endothelial cells, vascular smooth muscle cells, macrophages, platelets, etc. This reaction occurs when nitrogen monoxide synthetase (NOS) catalyzes the guanidin-end nitrogen of L-Arg to combine with oxygen. There are two types of NOS: constitutional NOS (cNOS) and inducible NOS (iNOS). NO has a very short half-life as it may react with circulating oxyhemoglobin, deoxyhemoglobin, superoxide anion or free oxygen to create stable products such as nitrite and nitrate. These are metabolized mainly through the kidney.

NO expresses its effects in two ways. On the one hand, NO is antagonistic with ET in dilating micro blood vessels, forming a protective layer in the tunica intima, preventing platelets and neutrophils from adhering to the vessel wall, and inhibiting the formation of microthrombus. On the other hand, the excessive release of NO may result in severe wound exudation and edema in the early stages postburn, thus increasing damage to tissue and cells. Under the normal situation, NO and ET maintain a certain dynamic equilibrium as an increase of ET promotes an elevation of NO synthesis while NO inhibits the synthesis of ET. A physiologic ratio of ET/NO represents a healthy balance.

According to the studies in recent years, the iNOS and ET mRNA genes located in burns wounds, heart, lung, kidney, liver and gastrointestinal tract express an increasing activity postburn, which leads to a massive release of ET and NO, especially from the wound. The significant increase of ET and NO in the circulation and the imbalance of the ET/NO ratio have a close correlation with shock, acute renal failure, acute respiratory failure, stress ulcer and cerebral edema after severe burns. Great care must be paid to the role of ET/NO if one is to prevent progressive damage of the wound tissues in the zone of stasis at the early stages postburn [2]:

- 1 NO and ET, known as the most potent vasoconstriction and vasodilatory factors, should maintain a healthy balance during the critical postburn period.
- 2 The increment of NO and ET in blood plasma postburn has a positive relationship with the increase of the capillary permeability, leading to massive exudation and hyperedema [3, 4].

- 3 The increase of ET in plasma, the contraction and spasm of the micro blood vessels, the thrombus formation around the wound and underlying tissue, and edema pressure may cause secondary ischemia and necrosis to the adjacent tissues.
- 4 The exudates, being rich in protein, provide a good culture medium for bacteria growth.

It was found that the application of a non-selective ET receptor antagonist, AK-044, may alleviate the secondary damage to burns wounds resulting from the increase of ET [5]. In this study, the increment of ET and NO in the BRT with MEBT/MEBO group was obviously lower than that in the dry-exposed group. In the former group, the ET/NO ratio in plasma decreased after 1 day toward a normal level of 2.95, representing a timely improvement in the tissue microcirculation status particularly in the zone of stasis. This prevented the occurrence of progressive microthrombosis and prevented the progressive necrosis of tissue by optimizing the wound environment. In the dry-exposed group, ET and NO increased significantly at all phases and the ET/NO ratio in plasma remained much higher than normal (2.95), usually at around 6. ET might play a dominant role in this change by keeping the micro blood vessel contracted and spasming for a long time resulting in microthrombosis, low vitality of wound tissues, and poorer healing process compared to that in the BRT with MEBT/MEBO group. The results suggest that the BRT with MEBT/MEBO group is superior to the dry-exposed group in wound healing time and outcome. However, further study is yet to be conducted for the specific mechanism on how MEBO controls the adequate release of ET and NO from the burns wounds, and on how to promote the return of the ET/NO ratio to normal as rapidly as possible.

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Experimental Study of the Effect of BRT with MEBT/MEBO on Hematological Parameters in the Treatment of Burned Rabbits

Introduction

Many researchers found that treatment with BRT with MEBT/MEBO improved the microcirculation in burns wounds. However, the effect of BRT with MEBT/MEBO on systemic postburn situations has not been established. The authors used a rabbit model for the following experimental study on this subject.

Materials and Methods

Seventy-two healthy adult rabbits of either sex eating a uniform diet and weighing 2.0 ± 0.5 kg were divided randomly into three groups: group 1 (normal control, $n = 12$), group 2 (treated with MEBO, $n = 30$), and group 3 (dry exposure, $n = 30$). Without anesthesia, all animals were shaved on the waist with barium sulfide. Animals in group 1 received no treatment and cardiac blood was sampled for measuring normal hematological parameters. Animals in groups 2 and 3 sustained deep second-degree burns of 10% BSA via 100°C water applied for 5 s (confirmed by pathological examination). Animals in group 2 were then treated with BRT with MEBT/MEBO renewed every 6 h. Animals in group 3 were offered no treatment with wounds remaining dry and exposed. No fluid infusion was administered. Blood samples were taken from the left ventricle of the heart for determining hematological values at 4, 24, 48, 72 h and 6 days postinjury. The parameters tested included apparent blood viscosity at different shear rates, plasma viscosity, hematocrit (HCT), erythrocyte agglutinative index (EAI) and erythrocyte transformation kinetics (ETK). To standardize the experimental conditions and instruments, blood sampling was performed in accordance with requirements for determination of blood flow viscosity established by the International Council for Standardization in Haematology (ICSH) in 1988. These laboratory determinations were made by a

specially assigned technologist at room temperature using a Type N E-1 viscometer (manufactured by Chengdu Instruments, China) [1].

Apparent blood viscosity at the shear rates of 230, 115, 46, 11.5, and 5.75 s^{-1} were determined, respectively, using 1.3 ml blood. Plasma viscosity is the viscosity at the shear rate of 115 s^{-1} . Hematocrit (HCT) was determined by a Wintrobe tube, type LXJ-64-01 centrifuge, at 3,000 rpm for 30 min [2]. Erythrocyte agglutinative index (EAI) was the ratio of apparent blood viscosity obtained at low and high shear rates (5.75 and 230 s^{-1}). Erythrocyte transformation kinetics (TK) was calculated according to the formula given in Chen [3].

Results

Owing to the minor difference of sex and strain in the hematological parameters of the rabbits, it was feasible to work out a uniform range [1–4]. Randomized division kept animals in a normal control group, allowing the MEBO-treated and the dry-exposed groups to live under the same conditions. Therefore, no repeated blood sampling was allowed and the data were significantly valuable. Apparent blood viscosity and plasma viscosity at different shear rates in group 3 were higher than those in group 2. Table 13 shows that the parameters in group 3 began to increase at 4 h post-injury, peaked at 24 h, and remained higher at 48 h, 72 h and 6 days than those in group 2. There were significant statistical differences (table 14; $p < 0.01$). Compared to the normal group, parameters including apparent blood viscosity, plasma viscosity and others in the MEBO-treated group began to increase at 4 h postinjury, peaked at 24 h, decreased at 48 h, further decreased at 72 h and nearly returned to normal at 6 days (table 15). But statistical analysis showed that hematological parameters did not change significantly ($p > 0.05$), except at 24 h postinjury when the blood viscosity at shear rates of 11.5 s^{-1} ($t = 2.4696$, $p < 0.05$) and 5.75 s^{-1} ($t = 2.700$, $p < 0.05$) increased markedly (table 16).

Table 13. Changes of hemorrheological parameters in group 3 (dry group) (mean \pm SE)

Parameters	4 h postinjury	24 h postinjury	48 h postinjury	72 h postinjury	6 days postinjury
Blood viscosity					
230 s^{-1}	5.45 ± 0.54	8.66 ± 0.59	6.24 ± 0.188	4.57 ± 0.588	5.61 ± 0.43
115 s^{-1}	5.92 ± 0.66	8.86 ± 1.37	6.55 ± 0.28	5.27 ± 1.13	6.57 ± 0.46
46 s^{-1}	6.49 ± 0.71	10.87 ± 1.42	7.99 ± 0.48	5.98 ± 0.44	7.14 ± 0.41
23 s^{-1}	7.26 ± 0.43	12.71 ± 1.43	9.32 ± 0.33	8.03 ± 0.46	8.84 ± 1.59
11.5 s^{-1}	12.89 ± 1.32	21.13 ± 2.65	13.12 ± 0.34	10.74 ± 1.52	10.88 ± 0.73
5.75 s^{-1}	18.12 ± 3.42	25.22 ± 0.96	19.99 ± 4.32	14.39 ± 1.74	12.39 ± 0.12
Plasma viscosity (115 s^{-1})	2.71 ± 0.08	2.64 ± 0.32	2.33 ± 0.123	2.15 ± 0.116	2.30 ± 0.095
HCT	0.42 ± 0.051	0.43 ± 0	0.4 ± 0.026	0.36 ± 0.04	0.036 ± 0.015
EAI	3.29 ± 0.27	2.86 ± 0.12	3.17 ± 0.68	3.14 ± 0.023	2.21 ± 0.255
TK	0.64 ± 0.086	0.96 ± 0.098	0.84 ± 0.093	0.82 ± 0.21	0.95 ± 0.144

Table 14. Comparison of hemorrheological parameters between groups 2 and 3

Parameters	4 h postinjury		24 h postinjury		48 h postinjury		72 h postinjury		6 days postinjury	
	t	p	t	p	t	p	t	p	t	p
Blood viscosity										
230 s ⁻¹	4.888	<0.001	10.74	<0.001	5.716	<0.001	1.412	>0.05	3.558	<0.01
115 s ⁻¹	3.776	<0.01	8.212	<0.001	3.364	<0.01	2.765	<0.05	3.289	<0.01
46 s ⁻¹	1.838	>0.05	8.031	<0.001	4.528	<0.001	4.125	<0.01	2.691	<0.05
23 s ⁻¹	0.861	>0.05	7.014	<0.001	4.435	<0.01	3.502	<0.01	3.909	<0.01
11.5 s ⁻¹	4.611	<0.001	116.15	<0.001	6.572	<0.001	4.985	<0.001	3.261	<0.01
5.75 s ⁻¹	4.564	<0.001	10.47	<0.001	23.12	<0.001	6.031	<0.001	2.043	>0.05
Plasma viscosity	0.634	>0.05	0.125	>0.01	1.188	>0.05	1.426	>0.01	0.254	>0.05
HCT	1.866	>0.05	1.237	>0.05	0.563	>0.05	0.574	>0.05	0.563	>0.05
EAI	2.038	>0.05	0.121	>0.05	1.075	>0.05	9.306	<0.001	0.547	>0.05
TK	2.648	<0.05	1.748	>0.05	6.106	<0.001	3.276	<0.01	5.048	>0.001

Table 15. Hemorrheological parameters in group 1 and changes in group 2 (mean ± SD)

Parameters	Group 1 (normal control)	Group 2				
		4 h postinjury	24 h postinjury	48 h postinjury	72 h postinjury	6 days postinjury
Blood viscosity						
230 s ⁻¹	4.20±0.05	4.13±0.52	4.48±0.7	4.01±0.65	4.00±0.72	4.12±0.72
115 s ⁻¹	0.69±0.71	4.54±0.68	5.67±0.91	4.58±0.98	4.27±0.8	4.42±1.10
46 s ⁻¹	5.53±0.99	5.44±1.00	6.97±1.03	5.12±1.07	4.88±0.86	5.11±1.26
23 s ⁻¹	6.79±1.32	6.59±1.30	8.49±1.20	6.00±1.18	5.59±1.17	6.04±1.40
11.5 s ⁻¹	7.61±1.69	8.5±1.69	9.55±1.26	7.34±1.46	6.69±1.52	7.41±1.81
5.75 s ⁻¹	9.33±2.58	12.21±2.7	13.45±1.9	9.92±1.86	8.33±1.88	9.57±2.28
Plasma-viscosity (115 s ⁻¹)	2.05±0.39	2.48±0.6	2.60±0.55	2.60±0.38	2.28±0.16	2.27±0.20
HCT	0.36±0.05	0.37±0.05	0.40±0.04	0.38±0.06	0.34±0.06	0.34±0.06
EAI	2.19±0.58	2.73±0.47	0.82±0.55	2.65±0.86	2.07±0.19	2.32±0.34
TK	0.73±0.08	0.75±0.08	0.82±0.34	0.75±0.05	0.64±0.04	0.72±0.09

Table 16. Comparison of hemorrheological parameters between groups 1 and 2

Parameters	4 h postinjury		24 h postinjury		48 h postinjury		72 h postinjury		6 days postinjury	
	t	p	t	p	t	p	t	p	t	p
Blood viscosity										
230 s ⁻¹	0.1490	>0.05	0.6514	>0.05	0.4635	>0.05	0.1826	>0.05	0.1437	>0.05
115 s ⁻¹	0.3052	>0.05	1.6984	>0.05	0.1818	>0.05	0.2855	>0.05	0.4152	>0.05
46 s ⁻¹	0.0128	>0.05	2.0160	>0.05	0.5630	>0.05	0.9918	>0.05	0.5243	>0.05
23 s ⁻¹	0.3239	>0.05	1.8600	>0.05	0.3276	>0.05	0.7940	>0.05	0.2600	>0.05
11.5 s ⁻¹	0.7449	>0.05	2.4696	<0.05	0.2430	>0.05	0.8100	>0.05	0.1632	>0.05
5.75 s ⁻¹	1.5470	>0.05	2.7000	<0.05	0.3907	>0.05	0.6635	>0.05	0.1456	>0.05
Plasma viscosity	1.2020	>0.05	1.6320	>0.05	2.0210	>0.05	1.0440	>0.05	1.0040	>0.05
HCT	0.2916	>0.05	1.2964	>0.05	0.5249	>0.05	0.5249	>0.05	0.5249	>0.05
EAI	1.4471	>0.05	1.5768	>0.05	0.8872	>0.05	0.3239	>0.05	0.4260	>0.05
TK	0.3536	>0.05	1.7166	>0.05	2.4241	>0.05	2.0142	>0.05	0.1661	>0.05

Conclusion

Treatment with MEBO after burns could ameliorate total body stress reaction, reduce water evaporation from the wound surface, lessen local and systemic capillary exudation and thus improve the hemorrheological characteristics of microcirculation. It also suggested that when the minor or moderately burned patient was treated with MEBO at an early stage, fluid infusion and/or blood transfusion would not be necessary, whereas for severely burned patients, the amount of fluid infusion could be reduced as tolerated.

Discussion

Hemorrhagic change is considered to be one of the pathophysiological changes following burns and serves as a basis of microcirculation disorder. Subsequent to extensive burns, microvascular permeability increases and copious intravascular plasma exudes toward the wound surface and tissue space leading to localized hemoconcentration, reduction of effective blood volume, decreased plasticity of red blood cells and increased blood viscosity. These hemorrhagic changes comprise the pathophysiological basis of burn shock and contribute to the deleterious stress reaction immediately following the trauma of burns. For instance, adrenaline, 5-HT, and prostaglandin may all increase the activation of platelets to erythrocytes, thereby changing the localized electrical potential. The increased secretion of catecholamines due to stress reaction directly promotes platelet adhesiveness. The injured sub-microstructure of the vascular wall elaborates an adhesion protein on platelets and erythrocytes causing significant intravascular platelet aggregation and contributing to adhesion and aggregation of platelets and erythrocytes. This, of course, precipitates thrombotic events [5]. In this study, we compared the blood viscosity of a MEBO-treated group with controls and demonstrate that the viscosity of the MEBO group approximated that of the normal controls. As animals in the MEBO-treated group were treated only with MEBO (they received neither fluid replacement nor special feeding), such changes verified

that MEBO alleviated body stress reaction following burns and ensured body recovery.

Despite becoming a systemic disease, burn injuries begin with a wound on one region of the body surface. It has been reported that BRT with MEBT/MEBO could have both local and systemic therapeutic effects on burn management [6]. We are pleased to report that rabbits in the MEBO-treated group were as active as normal rabbits and fed freely. Blood viscosity did not change significantly ($p > 0.05$), except at shear rates of 11.5 and 5.75 s^{-1} 24 h postinjury.

The blood viscosity value of normal rabbits in this study was slightly lower than the mean values as indicated in many domestic and international reports. This difference is probably explained by the fact that venous blood from rabbits (weighing 2.5 kg) was sampled in those reports instead of cardiac blood from rabbits (weighing 2.0 ± 0.5 kg) which we used in our study. Different dietary factors as well as geographical differences may contribute as well. Blood viscosity is a comprehensive marker as it indicates aggregation, deformability and the rheological properties of platelets, RBCs and WBCs. In this study, blood viscosity and plasma viscosity of rabbits were compared to and found to be lower than those of human beings. It remains to be further discussed whether this lower viscosity is associated with low HCT, difficulty of RBC aggregation, or whether it is simply some biological characteristic associated with 'herbivores'.

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Studies on the Anti-Infection Effect of BRT with MEBT/MEBO

Effect of BRT with MEBT/MEBO on the Immunity of Burns Patients

Introduction

The antibiotic and wound-healing properties of MEBO have been proven in clinical practice. There are different opinions about the mechanism of this antibiotic effect, so research on this subject is very important. During the period from January 1993 through December 1995, we conducted clinical observations on the effect of MEBO on burns patients' immunity and demonstrated that MEBO enhanced patients' immunity as part of its antibiotic and wound-healing effects.

Materials and Methods

Clinical Data

One hundred and twenty burns patients were divided randomly into two groups. Sixty patients in the MEBO group, including 40 males and 20 females, aged 6–65 (35.5 ± 14.8) years. Course of disease: 1–36 h (18.5 ± 8.8 h) before administration of MEBO. Cause of burns: direct flame, 30 cases; scald, 16 cases, and chemical burn, 14 cases. Burn position: craniofacial, 17 cases; neck, 17 cases; trunk, 18 cases, and limbs, 23 cases. Burns depth: superficial second-degree burns, 26 cases; deep second-degree burns, 24 cases; third-degree burns, 10 cases. Burn area: 1–25% ($13 \pm 6\%$) total body surface area (TBSA). By contrast, there were 60 cases in the control group including 41 males and 19 females, aged from 7 to 65 years (36 ± 14.5 years). Course of disease: 1–35 h (18 ± 8.5 h) before administration. Cause of burns: direct flame, 29 cases; scald, 17 cases, and chemical burns, 14 cases. Burn position: craniofacial, 13 cases; neck, 7 cases; trunk, 18 cases, and limbs, 22 cases. Burn depth: superficial second-degree burns, 24 cases; deep second-degree burns, 26 cases, and third-degree burns, 10 cases. Burn area: 1–26% ($13.5 \pm 6.3\%$) TBSA. The data of the two groups were similar and comparable ($p > 0.05$).

Treatment and Examination

All patients were subjected to debridement before collecting samples of skin tissue. Patients in the MEBO group were treated with

burns regenerative therapy (MEBT/MEBO) [1, 2], and skin tissues were taken twice from the original sites before the treatment began and after the wounds healed, respectively. Patients in the control group were treated with another traditional Chinese burns ointment – Jing Wan Hong –, and skin tissue was taken at the same time phase as in the MEBO group. Patients in both groups were observed closely, and their wound healing time and incidence of wound infection were compared.

Five pieces of skin tissues taken from the edge of the burns wounds using a pair of biopsy forceps were immersed in 10% formalin and kept in separate ice bottles, respectively. Four pieces of skin tissue were also taken from the normal (non-burned) skin of the same patient and treated identically as the other tissue. In addition, normal skin tissues from 60 surgical cases were taken during subdermal cyst operations and treated identically to both burns tissues. The levels of IgA-, IgG-, and IgM-producing cells and C_3 were determined using the frozen section immunohistochemical method. The first antibody was supplied from Vector and the second from DAKO. Venous blood samples of burns patients and healthy persons were taken in the morning before breakfast. Peripheral blood immunoglobulin (Ig) levels were determined using the agar diffusion method.

Classification Standard of Antibody Producing Cell and C_3

The classification standard was as follows: – = no positive cells or particles were found or only occasionally found in the whole slide; + = positive cells accounted for less than 30% of the total number of interstitial cells in the lamina propria; ++ = positive cells accounted for 31–70% of the total number of interstitial cells in the lamina propria; +++ = positive cells accounted for more than 71% of the total number of interstitial cells in the lamina propria. Histological diagnosis was based on the criteria stipulated at the China National Pathology Research Group Conference held in Zhengzhou in 1978.

Results

Clinical Efficacy Assessment

In the MEBO group, burns wounds with different depths had shorter healing time than those in the control group ($p < 0.01$; table 17). Only one case (1.7%) in the MEBO group had wound infection, compared to 9 cases (15%) in the control group. The difference between the

Table 17. Comparison of healing time of wounds with different depth in two groups (day, mean \pm SE)

Group	Superficial second-degree	Deep second-degree	Third-degree	Average healing time
MEBO	9.5 \pm 2.3 (5–14)	25.5 \pm 2.3 (21–30)	36.5 \pm 2.8 (31–43)	23.5 \pm 9.3 (5–42)
Control	13 \pm 3 (7–19)	29.5 \pm 2.8 (24–35)	42 \pm 3.5 (35–49)	28 \pm 10.5 (7–49)**
t value	4.65	5.49	3.88	2.49

Compared with MEBO group: ** $p < 0.01$.

Table 18. Comparison of staining intensity of immune factors in burns and non-burns areas of the patients (%)

Area	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
Burns area	120	60 (50.0)	60 (50.0)	59 (49.2)	61 (50.8)	57 (47.5)	63 (52.3)	58 (48.3)	62 (51.7)
Non-burns area	120	80 (66.7)	40 (33.3)	79 (65.8)	41 (34.2)	77 (64.2)	43 (35.8)	82 (68.3)	38 (31.7)
Normal person	60	44 (73.0)	16 (28.0)	42 (70.0)	18 (30.0)	39 (65.0)	21 (35.0)	42 (70.0)	18 (30.0)

Table 19. Staining intensity of local immune factors and depth of burns wound (%)

Wound	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
Superficial second-degree	50	28 (56.0)	22 (44.0)	29 (58.0)	21 (42.0)	27 (54.0)	23 (46.0)	28 (56.0)	22 (44.0)
Deep second-degree	50	26 (52.0)	24 (48.0)	24 (48.0)	26 (52.0)	25 (50.0)	25 (50.0)	24 (48.0)	26 (52.0)
Third-degree	50	6 (30.0)	14 (70.0)	6 (30.0)	14 (70.0)	5 (25.0)	15 (75.0)	6 (30.0)	14 (70.0)

Table 20. Staining intensity of local immune factors and burn area (%)

TBSA	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
≤ 5%	52	29 (55.8)	23 (44.2)	29 (55.8)	23 (44.2)	30 (55.7)	22 (42.3)	30 (57.2)	22 (42.3)
6–15%	50	26 (52.0)	24 (48.0)	25 (50.0)	25 (50.0)	22 (44.0)	28 (56.0)	23 (46.0)	37 (54.0)
≥ 16%	20	5 (27.8)	13 (72.2)	5 (27.8)	13 (72.2)	5 (27.8)	13 (72.2)	5 (27.8)	13 (72.2)

two groups was significant ($\chi^2 = 5.35$, $p < 0.05$). MEBO was proven to have infection-controlling and healing-promoting effects.

Experimental Results

IgA-, IgG-, and IgM-producing cells and C₃ in the burns area had higher staining intensity compared to those of the non-burns area and to those of normal persons ($p < 0.01$). The immunity of the local area changed postburn. Immunologic function and reaction were enhanced. In the non-burns area, the immunity was similar to that of normal persons. The difference was not marked ($p > 0.05$; table 18).

Local immunity was closely related to burn depth. IgA-, IgG-, and IgM-producing cells and C₃ in the local area of third-degree burns wounds had stronger staining intensity than those in superficial second-degree burns wounds ($p < 0.05$). The deeper the wound, the stronger the staining intensity (table 19).

Burn area was positively related to local immune factor staining intensity. IgA-, IgG-, and IgM-producing cells

and C₃ of patients with burn area $\geq 16\%$ TBSA had stronger staining intensity than patients with burn area $\leq 5\%$ TBSA ($p < 0.05$). The larger the burn area of TBSA, the stronger the staining intensity of the immune factors (table 20).

In burns wounds after treatment with MEBO, the local IgA-, IgG-, and IgM-producing cells and C₃ had stronger staining intensity than before treatment and than those in the control group ($p < 0.05$). MEBO significantly enhanced the staining intensity of local IgA-, IgG-, and IgM-producing cells and C₃, while the Jing Wan Hong ointment treatment group did not show a significant effect ($p > 0.05$; table 21).

There was no significant correlation between staining intensity of local IgA-, IgG-, and IgM-producing cells and C₃, and the duration of MEBO treatment ($p > 0.05$). The immunity of the patients was enhanced, irrespective of the duration of the treatment (table 22).

There was no positive correlation between the Jing Wan Hong ointment treatment and the staining intensity of local IgA-, IgG-, and IgM-producing cells and C₃ ($p > 0.05$).

Table 21. Staining intensity of local immune factors of patients in the two groups, before and after treatment (%)

Group	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
<i>MEBO treatment</i>									
Before	60	31 (51.7)	29 (48.3)	29 (48.3)	31 (51.7)	28 (46.7)	32 (53.3)	30 (50.0)	30 (50.0)
After	60	18 (30.0)	42 (70.0)	17 (28.3)	43 (71.7)	16 (30.0)	44 (70.0)	17 (28.4)	43 (71.7)
<i>Control treatment</i>									
Before	60	29 (48.3)	31 (51.7)	30 (50.0)	30 (50.0)	29 (48.3)	31 (51.7)	28 (46.7)	32 (53.3)
After	60	29 (48.3)	31 (51.7)	28 (46.7)	32 (53.3)	28 (46.7)	32 (53.2)	28 (46.7)	32 (53.3)

Table 22. Staining intensity of local immune factors and the course of MEBO treatment (%)

Course days	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
≤ 10	19	6 (31.6)	13 (68.4)	6 (31.6)	13 (68.4)	5 (26.3)	14 (73.7)	6 (31.6)	13 (68.4)
10–20	10	3 (30.0)	7 (70.0)	3 (30.0)	7 (70.0)	3 (30.0)	7 (70.0)	3 (30.0)	7 (70.0)
21–30	13	4 (30.8)	9 (69.2)	3 (23.1)	10 (76.9)	4 (30.8)	9 (69.2)	4 (30.8)	9 (69.2)
≥ 31	18	5 (27.8)	13 (72.2)	5 (27.8)	13 (72.2)	4 (22.2)	14 (77.8)	4 (22.2)	14 (77.8)

Table 23. Staining intensity of local immune factors and the course of Jing Wan Hong ointment treatment (%)

Course days	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
≤ 10	18	9 (50.0)	9 (50.0)	8 (44.4)	10 (55.6)	8 (44.4)	10 (55.6)	9 (50.0)	9 (50.0)
10–20	11	6 (54.5)	5 (45.5)	5 (45.5)	6 (54.5)	5 (45.5)	6 (54.5)	4 (36.4)	7 (63.6)
21–30	12	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	5 (41.7)	7 (58.3)
≥ 31	19	8 (42.1)	11 (57.9)	9 (47.4)	10 (52.6)	9 (47.4)	10 (52.6)	10 (52.6)	9 (47.4)

Table 24. Staining intensity of local immune factors and depth of burns wounds treated with MEBO (%)

Wound	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
Superficial second-degree	26	9 (34.6)	17 (65.4)	8 (30.8)	18 (69.2)	8 (30.8)	18 (69.2)	7 (26.9)	19 (73.1)
Deep second-degree	24	7 (29.2)	17 (71.8)	7 (29.2)	17 (71.8)	6 (25.0)	18 (75.0)	7 (29.2)	17 (71.8)
Third-degree	16	2 (20.0)	8 (80.0)	2 (20.0)	8 (80.0)	2 (20.0)	8 (80.0)	3 (30.0)	7 (70.0)

Changes in staining intensity of immune factors were not attributed to Jing Wan Hong ointment (table 23).

After treatment with MEBO, the deeper the burn wound, the stronger the staining intensity of local IgA-, IgG-, and IgM-producing cells and C₃, but no statistical

difference ($p > 0.05$). MEBO enhanced the local immunity of different depths of burns wounds (table 24).

After treatment with MEBO, the larger the burn wound, the stronger the staining intensity of local IgA-, IgG-, and IgM-producing cells and C₃, but no statistical

Table 25. Staining intensity of local immune factors and area of burns wounds treated with MEBO (%)

Area (TBSA)	Cases	IgA		IgG		IgM		C ₃	
		≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)	≤ (+)	≥ (++)
≤ 5%	24	9 (37.5)	15 (62.5)	8 (33.3)	16 (66.7)	8 (33.3)	16 (66.7)	8 (33.3)	16 (66.7)
6–15%	25	7 (28.0)	18 (72.0)	6 (24.0)	19 (76.0)	6 (24.0)	19 (76.0)	7 (28.0)	18 (72.0)
≥ 16%	11	2 (18.2)	9 (81.8)	3 (27.0)	8 (73.0)	2 (18.2)	9 (81.8)	2 (18.2)	9 (81.8)

Table 26. Changes in peripheral blood and serum immunoglobulin level of patients in two groups before and after treatment (g/l, mean ± SE)

Group	Cases	Blood IgA	Blood IgG	Blood IgM	Serum IgA
<i>MEBO treatment</i>					
Before	60	2.43±0.71	12.50±1.51	1.56±0.36	2.68±0.64
After	60	2.72±0.72	13.42±1.55	1.88±0.38	2.93±0.65
<i>Control treatment</i>					
Before	60	2.41±0.70	12.48±1.50	1.64±0.35	2.68±0.63
After	60	2.42±0.72	12.51±1.27	1.66±0.35	2.67±0.62
Normal persons	60	2.41±0.70	12.50±1.50	1.65±0.25	2.65±0.65

Table 27. Peripheral blood and serum immunoglobulin level and depth of burns wounds treated with MEBO (g/l, mean ± SE)

Wound	Cases	Blood IgA	Blood IgG	Blood IgM	Serum IgA
Superficial second-degree	26	2.57±0.70	12.76±1.48	1.76±0.35	2.82±0.62
Deep second-degree	24	2.67±0.72	13.49±1.56	1.84±0.40	2.89±0.65
Third-degree	10	2.92±0.74	14.01±1.58	2.04±0.40	3.08±0.68

Table 28. Peripheral blood and serum immunoglobulin level and area of burns wounds treated with MEBO (g/l, mean ± SE)

Area	Cases	Blood IgA	Blood IgG	Blood IgM	Serum IgA
≤ 5%	24	2.57±0.70	12.78±1.51	1.78±0.34	2.79±0.63
6–15%	25	2.69±0.71	13.50±1.56	1.86±0.37	2.80±0.64
≥ 16%	11	2.90±0.75	13.98±1.58	2.00±0.42	3.20±0.68

difference ($p > 0.05$). MEBO enhanced the local immunity of burns wounds with different areas (table 25).

There was no significant difference in peripheral blood IgA, IgG, and IgM and serum IgA levels between burns patients and normal persons ($p > 0.05$). After treatment with MEBO, peripheral blood IgA, IgG, and IgM and serum IgA levels were significantly higher than those before treatment and those in the control group and normal persons ($p < 0.05$), while in the control group, the before and after treatment difference was not significant ($p > 0.05$). MEBO significantly raised the levels of peripheral blood and serum immunoglobulin (table 26).

The deeper the burns wounds treated with MEBO, the higher the level of peripheral blood and serum Ig, but no statistical difference ($p > 0.05$). MEBO enhanced the immunity of peripheral blood and serum of patients with different burn depths (table 27).

The larger the burns wounds treated with MEBO, the higher the level of peripheral blood and serum Ig, but no statistical difference ($p > 0.05$). MEBO enhanced the immunity of peripheral blood and serum of patients with different burns area (table 28). Statistical analysis is shown in tables 29 and 30.

Table 29. Checklist of statistical analysis about the data of local immunity (χ^2 test)

Table	Comparing parameters	IgA		IgG		IgM		C ₃	
		χ^2	p	χ^2	p	χ^2	p	χ^2	p
Table 18	Burns area:non-burns area	6.86	<0.01	6.82	<0.01	6.76	<0.01	9.87	<0.01
	Burns area:normal person	8.93	<0.01	7.05	<0.01	4.92	<0.05	7.61	<0.01
	Non-burns area:normal person	0.83	>0.05	0.32	>0.05	0.01	>0.05	0.05	>0.05
Table 19	Superficial second-degree:deep second-degree	0.16	>0.05	1.00	>0.05	0.16	>0.05	0.64	>0.05
	Superficial second-degree:third-degree	3.87	<0.05	4.48	<0.05	4.84	<0.05	3.87	<0.05
	Deep second-degree:third degree	2.79	>0.05	1.87	>0.05	3.65	>0.05	1.89	>0.05
Table 20	$\leq 5\%:6-15\%$	0.15	>0.05	0.34	>0.05	1.91	>0.05	1.40	>0.05
	$\leq 5\%: \leq 16\%$	4.19	<0.05	4.19	<0.05	4.79	<0.05	4.79	<0.05
	$6-15\%: \geq 16\%$	2.87	>0.05	2.65	>0.05	1.45	>0.05	1.31	>0.05
Table 21	Before:after treatment	5.84	<0.05	5.08	<0.05	5.16	<0.05	5.91	<0.05
	Before control:after control	0	>0.05	0.13	>0.05	0.04	>0.05	0	>0.05
	After MEBO treatment:after control treatment	4.23	<0.05	4.30	<0.05	5.16	<0.05	4.30	<0.05

Table 30. Checklist of statistical analysis about the data of blood and serum immunity (t test)

Table	Comparing parameters	Blood IgA		Blood IgG		Blood IgM		Serum IgA	
		t	p	t	p	t	p	t	p
Table 26	Before MEBO treatment:normal person	0.16	>0.05	0	>0.05	0.11	>0.05	0.25	>0.05
	Before MEBO treatment:after MEBO treatment	2.22	<0.05	1.99	<0.05	2.11	<0.05	2.12	<0.05
	After MEBO treatment:normal person	2.39	<0.05	2.00	<0.05	2.43	<0.05	2.37	<0.05
	Before control treatment:after control treatment	0.08	>0.05	0.07	>0.05	0.20	>0.05	0.18	>0.05
	After MEBO treatment:after control treatment	2.28	<0.05	2.06	<0.05	2.13	<0.05	2.24	<0.05

Conclusion

The results revealed:

- 1 Staining intensity of immune factors at the burn site was significantly higher than at the non-burn site and in healthy people ($p < 0.01$).
- 2 Burns depth and area had a positive relationship with the local immune factor staining intensity. The deeper and the larger the burns wounds, the higher the staining intensity of the local immune factor and the higher the immunity ($p < 0.05$).
- 3 MEBO shortened the healing time of burns wounds of different degrees. Compared with the non-MEBO control, the difference was very significant ($p < 0.01$). MEBO promoted healing.
- 4 Patients treated with MEBO had higher local immune factor staining intensities than patients treated with the non-MEBO method ($p < 0.05$).
- 5 Patients treated with MEBO had higher peripheral blood and serum immunoglobulin levels than before MEBO treatment and also higher levels than patients treated with the non-MEBO method ($p < 0.05$). MEBO significantly promoted immunity.

- 6 The wound infection rate of the patients treated with MEBO was significantly lower than that of patients treated with the non-MEBO method ($p < 0.05$). MEBO had an antibiotic effect.

The relationship between changes of local and systemic immunity and the depth and the area of the burns was also investigated. Through the enhancement of native immunity of burns patients, MEBO was capable of controlling burn wound infection by promoting the endogenous defense to bacteria, virus and toxin invasion as well as through the immune enhancement of burns patients. MEBO shortened the healing time of the wounds.

Discussion

Burns is a severe injury which both destroys the skin barrier and lowers the body's native defense against bacterial and viral invasions. At the ACCP and SCCM held in August 1991 in the United States, a new definition of infection was advanced [3]. Invasions of exogenous bacteria and virus cause local infection of the burn wound which can progress to systemic infection. Meanwhile, host

defenses also induce an inflammatory immunologic reaction. Thus, systemic inflammatory reaction syndrome (SIRS) may result [4]. MEBO itself does not have a direct bactericidal effect in vitro. Some researchers considered that MEBO serves as an immunologic barrier in burn wound surface thereby protecting the injured skin. MEBO may create an environment (temperature, humidity, nutrition, oxygen supply, metabolism, etc.) suitable for residual skin tissue repair. In effect, it creates an ideal isolated 'aseptic ward'. In addition, MEBO is capable of altering the toxic potential of bacteria and virus in burn wounds therefore lowering the infection rate [5].

This study investigated the effect of MEBO on the local and systemic immunity of burn patients, and proved that MEBO, through regulating human immunity, protected burn wounds from infection at the same time as it promoted wound healing. After MEBO treatment, the incidence of wound infection was reduced to 1.7%, significantly lower than that in the control group ($p < 0.05$). The average wound healing time in the MEBO treatment group was 23.5 ± 9.3 days, representing a significant course of treatment as compared with the control group ($p < 0.01$). A vast amount of clinical data proved that MEBO significantly lowered the infection rate of burn wounds when compared with other methods [1]. MEBO is applied directly onto the wound surface and is therefore easily absorbed into the local tissue fluid circulation en route to participation in systemic metabolism. MEBO stimulates the immune system via enhancement of the immunoglobulin level and strengthens body resistance against infections. This paper reports the results of local and peripheral blood and serum immunity of patients with different depths and different areas of burns. It proved that MEBO enhanced local and systemic immunologic function as well as enhancing resistance to infection for burn patients.

Determination of local and peripheral blood and serum immunoglobulin level of burn patients is an important criterion for evaluating the effect of MEBO on human immunologic function. IgA, IgG, and IgM are important proteins with anti-bacterial, anti-viral and anti-toxin activities. They also activate complement C_3 to achieve bacteriolysis, phagocytosis and neutralization of toxins. C_3 takes part in nonspecific and specific immune reactions and is a factor of body defense. It helps produce immunoglobulin IgA, IgG and IgM. With appropriate regulation of the neurohumoral system, the human body can enjoy enhanced resistance to infectious factors. MEBO contains polysaccharides, lipids and proteins, which in combination and when applied to burn wounds can bind with bacteria and toxins to form protein complexes. These complexes, in turn, stimulate the human immune system, and induce a variation of the bacteria which reduces their toxicity. Therefore, MEBO should not be

used together with other topical drugs which may lessen its efficacy. When applying MEBO to the burn wound, the thickness of the ointment should be appropriate since the combination of MEBO with the proteins will be hindered if MEBO is smeared too thickly or too thinly. For the same reason, the time interval of MEBO application should be appropriate.

Before treatment, it was found that the local immunity of the burn patients was higher than in the non-burn area and normal persons. Burn patients with large area and deep wounds had higher local immunity than small area and superficial burn patients. However, the systemic immunity of these burn patients was almost the same as compared with that of normal persons. After MEBO treatment, local and systemic immunity of the burn patients increased significantly more than before treatment and in controls. Therefore, we see that MEBO enhanced local and systemic immunologic function of patients suffering with burns of different depths, different areas and with different courses of treatment. Our results may provide a basis for further research and clinical application of MEBO.

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Study on the Bacterial Count of Viable Tissue of Burns Wounds Treated with BRT with MEBO/MEBO

Introduction

In order to verify the ability of BRT with MEBO/MEBO to inhibit localized infections, we conducted a study on the bacterial number on viable tissue of burn wounds. The results showed that MEBO therapy effectively controlled bacterial number to less than 10^4 per gram viable tissue during the whole treatment procedure. This result suggests a strong capacity for the prevention of wound invasive infection.

Materials and Methods

The backs of 28 adult healthy guinea pigs of either sex were depilated and scalded on both sides by a hot test tube to obtain full-thickness necrotic wounds with a diameter of 3 cm. One wound on each side of every animal randomly served as a blank exposed group (control group) while the other side served as the BRT with MEBT/MEBO treatment group (test group). Wounds in the control group were kept clean and allowed to heal spontaneously, while wounds in the test group received BRT in the typical manner. Twenty-eight animals were harvested seven at a time at four different time intervals (days 3, 6, 10 and 20 postinjury). Viable tissue underlying the wounds was sampled for bacterial count and compared with normal subcutaneous tissue.

Viable Tissue Sampling and Bacterial Counting

The wound surface was sterilized with iodine and alcohol after each animal was sacrificed. Sterilized tissue scissors were used to cut tissues from each wound without touching the deep fascia and muscle tissue. The sampled wound tissues were spread flat in sterilized cloth with the subcutaneous tissue exposed. Sterilized ophthalmic scissors were then used to excise viable subcutaneous tissues (excluding necrotic tissues). The sampled viable tissues were weighed, triturated and diluted. Diluents were inoculated into agar culture medium at a specific concentration and cultured for 48 h (at a constant temperature of 37°C). Bacterial colony count was performed from which total bacterial count and bacteria per gram of viable tissue were obtained.

Results

The bacterial number in viable tissues of burns wounds was less than 10^4 /g in both groups, indicating there was no wound invasive infection during the whole test period. However, the bacterial number in wound viable tissue of the test group was significantly less than that in the control group at each observation time (table 31).

Discussion

There are several general clinical methods for determining wound bacterial determination, including (1) swab culture of wound surface; (2) quantifying bacterial number of full-thickness burns wound; (3) bacterial counting of sub-eschar viable tissues. The first method is easy to perform, but fails to provide appropriate proof for the existence of invasive infection on burns wounds. Clinical application and lack of comparability limit the second method. The third method is usually adopted in clinics to predict the possible success or failure of skin grafting and is regarded as an ideal method for detecting the existence of wound invasive infection. Many authors used this method for early diagnosis of wound sepsis.

In 1983, Bharadulj reported that more than 10^5 bacteria per gram sub-eschar viable tissue is diagnostic for wound sepsis. He also found that patients who died from systemic infection bore more than 10^8 bacteria per gram viable tissue. Other authors agree [Robson et al.]. Therefore, we too adopted this method for the purposes of this

Table 31. Bacterial number per gram of viable tissue

Groups	Test group	Control group	p
Day 3	$2.61 \pm 1.14 \times 10^3$	$4.43 \pm 2.09 \times 10^3$	<0.05
Day 6	$3.81 \pm 0.27 \times 10^3$	$3.24 \pm 0.73 \times 10^4$	<0.001
Day 10	$3.57 \pm 0.64 \times 10^3$	$1.08 \pm 0.10 \times 10^5$	<0.001
Day 20	$2.82 \pm 1.16 \times 10^3$	$7.02 \pm 0.43 \times 10^3$	<0.05
Normal skin	$2.10 \pm 0.52 \times 10^3$	$2.2 \pm 0.63 \times 10^3$	>0.05

study. The results demonstrate that in different stages postburn, no more than 10^4 /g was detected in sub-eschar viable tissues taken from the BRT with MEBT/MEBO group. We also noted that there was no obvious difference throughout all stages although the peak occurred during the period of wound rejection reaction. In the control group, although no infection symptoms were observed on wounds, bacterial number per gram sub-eschar viable tissue increased progressively until a peak of 10^5 /g during the wound-rejecting reaction period. A significant difference occurred between the two groups. Based upon bacterial number, we see that MEBO had a unique effect in controlling wound infection compared to normal tissue.

There are four dominant sources for burn wound infection: (1) parasitic bacteria in underlying wound viable tissue; (2) burned tissues; (3) external contamination; (4) hematogenous infection. It has been believed for a long while that a dry, clean or sterilized wound environment may lead to a lower incidence of infection than would a damp environment. Some experiments have been conducted while maintaining the wound moist, but the results suggested that a dry wound status was preferable. Therefore, a therapeutic principle of drying the wound was established to prevent infection and dampness was itself regarded as a risk factor for infection. Interestingly, our research reached a contrary conclusion.

A wet environment, according to the general rule, was understood to provide a favorable surrounding for bacterial growth despite the realization that tissue necrosis, which results from a dry wound status, provides a much more nutrient substrate for microbial proliferation. Accordingly, we advocated that a physiologically moist environment, assuming appropriately regulated humidity, was favorable for tissue recovery as well as enhancement of endogenous resistance to infection with its resultant reduction of infection. Furthermore, it is well understood that burn wound infections are caused by a variety of factors and that each of these should be accounted for along with a clean wound environment. For example, blocked sub-eschar drainage resulted from dryness of wound and eschar formation would support bacterial colonization, thereby increasing the possibility of wound infection. If

wounds were kept wet without taking other effective measures, infection would be likely. We invented BRT with MEBT/MEBO in order to maintain the wound in a physiologically moist environment, which, while causing no infection, also allowed for dramatically less bacterial number in sub-eschar viable tissue than in wounds treated by dry therapy. Why? According to the designing theory of BRT with MEBT/MEBO, we had the following analysis.

Moist Environment for Wound

In our study, a 'moist' instead of 'wet' environment was emphasized. The special formulation of MEBO developed on the basis of Chinese traditional philosophy ensured an appropriate 'moist' wound environment. Composed of beeswax and non-water edible plant oil, the dosage form of MEBO has a strong affinity to skin and wound tissues. Its unique structure protects burn tissues from direct immersion by exuded endogenous fluid, prevents bacteria in water from contacting tissues and therefore keeps wounds moist, but not macerated. The physical change of the ointment from semisolid to liquid allows a circulation of ointment across the wound, ensuring effective drug concentration and addressing the requirements of tissue repair. Prior to the invention of MEBO, exposure therapy with the application of other topical drugs was considered as the predominant measure to monitor wound condition. To its credit, wound exposure allowed better assessment of the need for drug renewal as well as for timely drainage and manual discharge of exudation.

Unobstructed Drainage and Isolation

BRT with MEBT/MEBO features an automatic drainage system that enables the timely drainage and discharge of exudation and liquefied products from the wound surface. This mechanism of action destroys the bacterial growth environment, interrupts bacterial nutriment supply, reduces bacterial concentration and therefore effectively arrests bacterial proliferation and invasion. The unique dosage form of MEBO effectively isolates the wound from bacterial contamination originating from the surrounding environment by forming a barrier that actually provides the wound with a clean and relatively 'sterile' environment.

Drug Ingredients and Other Claims

MEBO also claims to increase local blood flow, promote recovery of the microcirculation, and encourage wound healing. These three factors all enhance the ability of local tissue to resist infection.

Comparative Study of the Effects of Moist-Exposed Burn Ointment, Silver Sulfadiazine and Hot Dry-Exposed Therapy on Controlling Burn Wound Infection with *Pseudomonas aeruginosa*

Introduction

Since 1964 when Teplitz et al. [1] successfully established a representative animal model, burn wound invasive infection has been regarded as one of the main causes of burns-related death. Therefore, topical use of antibacterial agents has played an important role in the control of burn wound infection. In the 1960s, antibacterial agents were developed including sulfamylon [2], silver nitrate [3] and silver sulfadiazine (SD-Ag) [4]. Although SD-Ag has been widely used historically because of its risk/benefit ratio, we now are discouraged by its tendency to enhance drug resistance [5, 6]. Researchers have addressed this weakness in SD-Ag therapy by developing other antimicrobial agents to fight against burn wound infection caused by *Pseudomonas aeruginosa* and other bacteria. These newer agents include a variety of other topical agents such as silver pipram [7, 8], silver norfloxacin [9, 10] and MEBO [11]. We designed a comparative study to verify the effects of MEBO, SD-Ag and hot dry-exposed therapy on controlling *P. aeruginosa* invasive infection of burns wounds.

Materials and Methods

Pathogenic *P. aeruginosa* was collected from burns wounds of invasive infection, cultured for 16–24 h, then produced into a 4×10^8 suspension using normal saline.

One hundred and twenty healthy adult Wistar rats of either sex weighing 100–200 g were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg), shaved of dorsum hair, and scalded on the back with 100 °C water for 10 s to each form a full-thickness burn wound with 20% BSA (determined by pathological examination) [1]. A 1-ml suspension containing 4×10^8 *P. aeruginosa* was smeared evenly onto the wound surfaces to achieve contamination and infection. The animals were kept in separate cages, and divided randomly into 4 groups as follows (30 in each). Group 1 (control group) received no treatment. Group 2 (MEBO group) was treated with MEBO according to the method of BRT with MEBT/MEBO which kept the wound moisturized and covered by MEBO throughout the duration of the study. Group 3 (SD-Ag group) was treated with 1% SD-Ag cold cream, which was applied once a day. Prior to each administration of the SD-Ag, the residual cream and necrotic tissue was wiped off according to the SD-Ag protocol. Group 4 was treated with continuous hot, dry-exposed therapy using a heat-controlled air fan to keep the wound dry.

Six animals in each group were killed under aseptic manipulation at the 1st, 3rd, 5th, 7th and 9th days after treatment. A sample of heart blood was collected and cultured and a specimen of wound skin tissue was taken by sterile scalpel, as deep as the muscular fascia [12], and then cut into two parts. One part was used for bacterial count in sub-eschar viable tissue. The other was fixed in formalin for patho-

logical examination. Sections were observed under a light microscope and the extent of bacterial invasion was classified according to three grades: '0' referring to absence of bacterium, 'I' to invasion of bacteria to necrotic tissues, and 'II' to invasion of bacteria to viable tissues.

Results

Bacterial Count of Sub-Eschar Viable Tissues

Table 32 shows the mean logarithmic values of bacterial count of sub-eschar viable tissues in each group. The results indicated the mean values in groups 2 and 3 were significantly lower than those in groups 1 and 4 ($p < 0.01$). No marked difference of this value was noted between groups 2 and 3 ($p > 0.05$) or between groups 1 and 4 ($p > 0.05$).

Correlation between Bacterial Count of Sub-Eschar Viable Tissue and Clinical Course

As figure 13 shows, the bacterial count of sub-eschar viable tissues in groups 1 and 4 increased progressively during the whole course of the disease. The bacterial count in groups 2 and 3 remained at low levels, less than $10^5/g$ throughout, and even declined, indicating that both these topical drugs were effective in controlling the proliferation of *P. aeruginosa*.

Results of Blood Culture

The incidence of positive blood cultures in groups 2 and 3 was markedly lower than in groups 1 and 4 ($p < 0.005$), as is shown in table 33. There was no significant difference of positive rates between groups 1 and 4 ($p > 0.50$), or between groups 2 and 3 ($p > 0.75$).

Pathological Examination

In the study, grades of '0' and 'I' in the pathological examination were referred to as negative while grade 'II' was referred to as positive [13] (table 33).

As can be seen in table 33, positive rates of pathological examination in groups 2 and 3 were significantly lower than those in groups 1 and 4 ($p < 0.005$). There was no significant difference of positive rates either between groups 1 and 4 ($p > 0.50$), or between groups 2 and 3 ($p > 0.50$).

Comparison of Incidence of Invasive Infection of Burns Wounds

According to the data that bacterial invasion to viable tissue of wound and/or bloodstream in the circulation were indicative of invasive infection of burns wounds [13], table 34 shows the incidence of invasive infection in each group. It was found that the incidences of invasive infection in groups 2 and 3 were dramatically lower than those in groups 1 and 4 ($p < 0.005$). There was no significant difference of positive rates either between groups 1 and 4 ($p > 0.25$) or between groups 2 and 3 ($p > 0.50$).

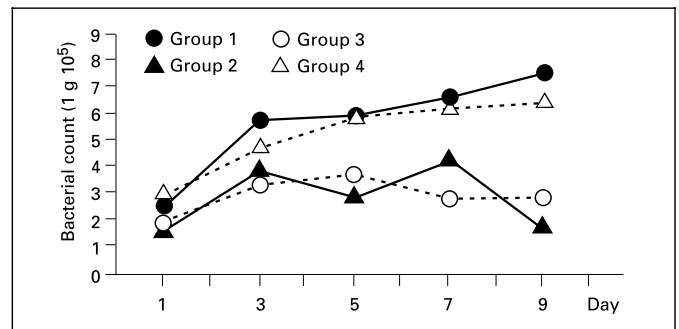


Fig. 13. Illustration of the correlation between bacterial count in sub-eschar viable tissues and the clinical course in each group.

Table 32. Mean logarithmic values of bacterial count of sub-eschar viable tissues (mean \pm SE)

Group	Bacterial count (logarithmic value)
1 (control)	5.8 \pm 2.6
2 (MEBO)	3.8 \pm 2.0
3 (SD-Ag)	3.1 \pm 3.1
4 (hot dry-exposed)	5.4 \pm 2.0

Table 33. Results of blood culture and pathological examination

Group	Positive number	Negative number	Positive rate, %
<i>Blood culture</i>			
1 (control)	25	5	88.33
2 (MEBO)	7	23	23.33
3 (SD-Ag)	8	22	26.67
4 (hot dry-exposed)	19	11	63.33
Total	59	61	49.17
<i>Pathological examination</i>			
1 (control)	21	9	70.0
2 (MEBO)	11	19	36.67
3 (SD-Ag)	12	18	40.0
4 (hot dry-exposed)	20	10	66.67
Total	64	56	53.33

Table 34. Incidence of wound invasive infection

Group	Positive number	Negative number	Positive rate, %
1 (control)	26	4	86.67
2 (MEBO)	12	18	40.0
3 (SD-Ag)	11	19	36.67
4 (hot dry-exposed)	23	7	76.67
Total	72	48	60.0

Table 35. Results of bacterial count of sub-eschar viable tissues and pathological examination

Bacterial count	Pathological examination		
	positive number	negative number	total
<10 ²	3	19	22
10 ²	3	12	15
10 ³	10	4	14
10 ⁴	2	5	3
10 ⁵	8	5	13
10 ⁶	11	1	12
10 ⁷	10	5	15
>10 ⁸	17	5	22
Total	72	48	60

Table 36. Results of positive bacterial count and positive pathological examination

Bacterial count ≥ 10 ⁵	Pathological examination		
	positive number	negative number	total
Positive number	46	16	62
Negative number	18	40	58
Total	64	56	120
Coincidence rate	86/120 (71.67%)		
Non-coincidence rate	34/120 (28.33%)		

Comparison of Bacterial Count of Sub-Eschar Viable Tissue and Pathological Examination for Diagnosis of Burn Wound Infection

According to table 35, there was a direct correlation between the positive rates of the bacterial count of sub-eschar viable tissue and the pathological examination ($r = 0.808$, $p < 0.005$). The positive rate of pathological examination increased as did the bacterial count.

In further analysis, we took a bacterial number of 10⁵/g sub-eschar viable tissue, the level defining invasive infection, as the boundary for positive and negative [14, 15]. It was found by comparison that positive rates of pathological examination of tissue specimens that yielded counts of 10⁵/g sub-eschar viable tissue or more reached 74.2%. The negative rate of pathological examination of those yielding counts lower than 10⁵/g reached 69%. The coincidence and noncoincidence rates of two diagnostic methods were 71.67 and 28.33%, respectively (table 36). Statistical analysis showed an obvious relationship between both methods ($\chi^2 = 17.62$, $p < 0.005$) and there was no significant difference between both methods in the diagnosis of burn wound infection ($\chi^2 = 0.031$, $p > 0.75$).

Conclusion

- 1 MEBO has a similar effect to SD-Ag in controlling burn wound invasive infection by *P. aeruginosa*.
- 2 Hot dry-exposed burns therapy has no effect on controlling third-degree burn wound invasive infection by *P. aeruginosa*.
- 3 The bacterial count of sub-eschar viable tissue can still be used as one of the feasible methods in the early diagnosis of burn wound invasive infection.

Discussion

Evaluation of Antibacterial Effect of Hot Dry-Exposed Therapy

In 1949, Wallace introduced the concept of dry-exposed burns therapy [16, 17] in which the wound was directly exposed to air at a certain temperature. He believed that direct exposure of the wound might allow the formation of a layer of dry eschar/crust by exudation and necrotic tissue on the wound surface, which served as a barrier against bacterial contamination. Our study showed that comparing the hot dry-exposed therapy group to the untreated control group, there was no obvious difference with respect to bacterial count of sub-eschar viable tissue, positive rate of blood culture and positive rate of pathological examination. We now suggest that the hot, dry-exposed therapy has no significant effect on controlling invasive infection with *P. aeruginosa* of third-degree burns wounds.

Moist-Exposed Burn Ointment (MEBO) – New Topical Drug for Burns

Infection is the leading cause of death due to burns complications, and burn wound infection is of great clinical concern as it can result in burn wound sepsis and septicemia. An enormous amount of research has been conducted in this field which has produced many advances in burn infection treatment. However, the very existence of necrotic tissue in deep burns provides culture medium conducive to the growth of pathological micro-organisms. Furthermore, the blocked local blood circulation hinders the delivery of anti-bacterial and immune-enhancing peptides which are integral to host-defense competency.

Mafenide (Sulfamylon), silver nitrate and SD-Ag were developed in the 1960s [2–4]. Sulfamylon is a useful antimicrobial agent which penetrates into eschar but has the disadvantage of inhibiting carbonic anhydrase. Therefore, absorption of topical Sulfamylon may result in metabolic acidosis that limits its use in larger burn areas. Silver nitrate was the initial topical agent but its tendency to stain discouraged widespread use. SD-Ag has a strong antimicrobial effect which, despite its poor penetration into eschar, made it the topical agent of choice against burn infection. Our study gave good proof for this.

In order to improve the antimicrobial effect of a topical agent while reducing deleterious side effects, researchers developed other metal sulfonamides such as zinc, ammonium, cerium and erbium for topical therapy [18–21]. However, a comparison of relative antimicrobial effects showed SD-Ag to be the best of the lot so it remained the agent of choice against *P. aeruginosa*. This remained the case despite its worrisome profile of creating multidrug resistance [5, 6]. Great efforts have been made to deal with *P. aeruginosa* resistance to SD-Ag. In the 1970s, on the basis of nalidixic acid, great improvements were attained in the research of pyridine, pefloxacin and its derivative in the prevention and treatment of burn infection [7, 8, 22]. Recently, silver norfloxacin has emerged, which was found to be effective in the treatment of *P. aeruginosa* with drug resistance to SD-Ag [9, 10].

In 1988, a new topical drug for burns wounds was invented, called moist-exposed burn ointment (MEBO) [11]. This innovation has become widely accepted in clinical use [23–25]. In this study, animals with infection of third-degree burns wounds by *P. aeruginosa* were used, and the comparison showed that MEBO was effective in controlling burn wound *P. aeruginosa* infection. MEBO had a similar effect to SD-Ag in reducing the bacterial concentration of sub-eschar viable tissues, positive rate of blood culture and incidence of invasion infection. In addition to its ability to kill *P. aeruginosa*, the other advantages of MEBO are as follows: easy to apply; non-painful, no need for excruciating debridement between applications, and easy assessment of healing progression. It suggested that MEBO was a useful alternative topical drug for burn treatment. Further investigation is needed in order to find whether MEBO controls the infection of other bacteria and micro-organisms as well as the mechanism of MEBO against *P. aeruginosa*.

Roles of Bacterial Count of Sub-Eschar Viable Tissue and Pathological Examination in the Diagnosis of Burn Wound Infection

Infection has long been one of major life-threatening causes of burn victims. The extent of infection depends on the invasiveness of the pathogenic micro-organism and the power of host resistance [12]. Micro-organism invasiveness has a close correlation to the strains, toxicity and quantity. Therefore, a variety of methods for determining the bacterial count on burns wounds have been developed.

As early as 1964, Teplitz et al. [1] put forward the concept of burn wound invasive infection. They defined wound invasive infection as occurring when bacterial count exceeded 10^5 organisms per gram sub-eschar viable tissue with bacteria penetrating into the underlying tissue and blood vessels. Many researches agree that a bacterial count of 10^5 /g sub-eschar viable tissue was a pivotal level

with wounds containing more than 10^5 /g being predisposed to invasive infection [14, 15]. Therefore, the value of 10^5 /g of viable tissue is used as one indicator to predict and diagnose burn wound invasive infection. However, in a comparative study between bacterial count of sub-eschar viable tissue and pathological examination of 200 cases, McManus and Kim [26] found that only 35.7% of tissue specimen with $\geq 10^5$ /g eschar viable tissue demonstrated invasive infection by pathological examination. They concluded that the bacterial density level of 10^5 or more organisms per gram sub-eschar viable tissue was not a sufficient indicator for the diagnosis of burn wound invasion.

In our study, we made a bacterial count on the sub-eschar viable tissues and performed pathological examination of 120 animals with the results showing a linear correlation between bacterial count and positive rate of pathological examination. The positive rate of pathological examination increased as bacterial density did, and there was a positive relationship ($p < 0.005$). Among 62 specimens showing bacterial count $\geq 10^5$ /g, 46 were found positive in pathological examination with a rate of 74.2%. Of 58 specimens showing bacterial count $< 10^5$ /g, 40 were found to be negative in pathological examination with a rate of 69%. The coincidence rate of both diagnostic methods was 71.67%. The statistical data demonstrated that if a bacterial count $\geq 10^5$ /g was used as the critical level in the diagnosis of burn wound invasive infection, there was a significant relationship between the two methods. Therefore, the results of this study suggested that the bacterial count on sub-eschar viable tissue remained one of the feasible methods for the prediction and diagnosis of burn wound invasive infection. Although it directly reveals the invasive extent of burn wound invasive infection, pathological examination can give false-negative results due to the impact of many factors including sampling, section and staining techniques. Thus, we must keep in mind that pathological examination for the diagnosis of burn wound invasive infection has its limitations and should not be the sole criterion. Similarly, blood culture has its limitations in that it may result in a low positive rate and delay appearance [27]. Therefore, we conclude that bacterial count of sub-eschar viable tissue can still be used as one of the feasible methods in the early diagnosis of burn wound invasive infection.

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Experimental Research on the Mechanism of the Anti-Infection Effect of BRT with MEBT/MEBO

Introduction

Many basic studies and clinical research have proved that BRT with MEBT/MEBO has beneficial effects of anti-infection, promoting burn wound healing and reducing scar formation to name but a few of its attributes. However, the mechanisms of these effects of MEBO remain a bit mysterious. The following study was designed to elucidate the anti-infection effect mechanism of BRT with MEBT/MEBO.

Materials and Method

Apparatus and Reagents

Fully automatic enzyme labeling analyzer, Multiskan, MS; AC-920 hemocyte analyzer; MEBO; RPMI-1640, Gibco, USA; Con A, supplied from Guangzhou Medical Institute; MTT supplied from Fluka; Pathogenic O₁₁₁B₄ of *Escherichia coli* supplied from Binzhou Medical College.

Animals and Methods

Thirty-two BALB/C mice, 8 weeks old, females, weighing 17–19 g, were supplied from Beijing Medical University. Twenty-eight Kunming mice of both sexes weighing 21–24 g, 18 rabbits weighing 2–2.5 kg, and 4 guinea pigs weighing 250–300 g were supplied from Binzhou Medical College.

The animals were divided randomly into a control group and a MEBO treatment group. In the MEBO treatment group, mice were depilated (2 × 2 cm) on their backs and smeared with MEBO. Guinea pigs were depilated (3 × 2.5 cm) on their backs and smeared with MEBO. MEBO was applied 2 times a day. Biopsy of skin tissue was done on day 9 to determine the activity of interleukin-1 (IL-1), and was stained with hematoxylin and eosin (HE) for histopathological observation.

Examination Indexes

Morphological Variation of Pathogenic *E. coli* (O₁₁₁B₄). The bacteria were cultivated on culture medium containing a given concentration of MEBO [1]. Each generation of the bacteria was stained using the G staining method. Staining reaction and morphological variation of the bacteria were observed. The results were compared with that of the *E. coli communis* cultured on the same kind of medium containing MEBO.

Determination of IL-1 Level. MTT was used as a substrate. Since living cells containing active succinate dehydrogenase can reduce yellow-colored MTT to form violet or blue-colored formazan particles, the particles were dissolved by adding isopropyl alcohol hydrochloride [2]. The OD value was determined by colorimetry. The amount of formazan was proportional to the number of living cells. Determination was performed according to the modified methods described previously [2]. Mice were killed under sterile conditions, the thymus was removed, cut and screened. After being centrifuged, a suspension with 10⁷/ml of cells containing 2 μg/ml of Con A was prepared. On a 24-pore plate, we added to each pore 0.5 ml of this suspension and then added mice skin tissue suspension and blood plasma at 0.5 ml/pore. Two more control groups, one with 1640-nutrient fluid (thymocytes plus nutrients) and the other with Con A plus thymocytes were set. The 24-pore plate was placed into an incubator at 37 °C, 5% CO₂ for 68 h. From each pore, 0.5 ml of the supernatant was drawn away, then 0.1 ml of 5 μg/ml MTT solution was added to each pore and incubated again for 4 h. 0.5 ml of isopropyl alcohol hydrochloride was added to each pore and then the plate was shaken to dissolve the particles. Supernatant from each pore was drawn and added to a 96-pore plate, 0.2 ml/pore. The plate was placed into a fully automatic enzyme-labeling analyzer to determine the OD value, reflecting the level of IL-1 in skin tissue cells and blood plasma.

Effect of MEBO on Body Temperature of the Rabbit. The body temperature of the rabbit was taken prior to application of MEBO. Then, an area of 6.0 × 6.5 cm was depilated on the back of the animal and MEBO was applied twice a day while body temperature was recorded simultaneously. This was done for a total of six measurements. Changes in body temperature were recorded.

Effect of MEBO on the Classification of Mice Leukocytes. Every mouse in the MEBO and control groups had a 0.2-ml blood sample taken by enucleation of the eyeball. The blood was added to 10 ml of

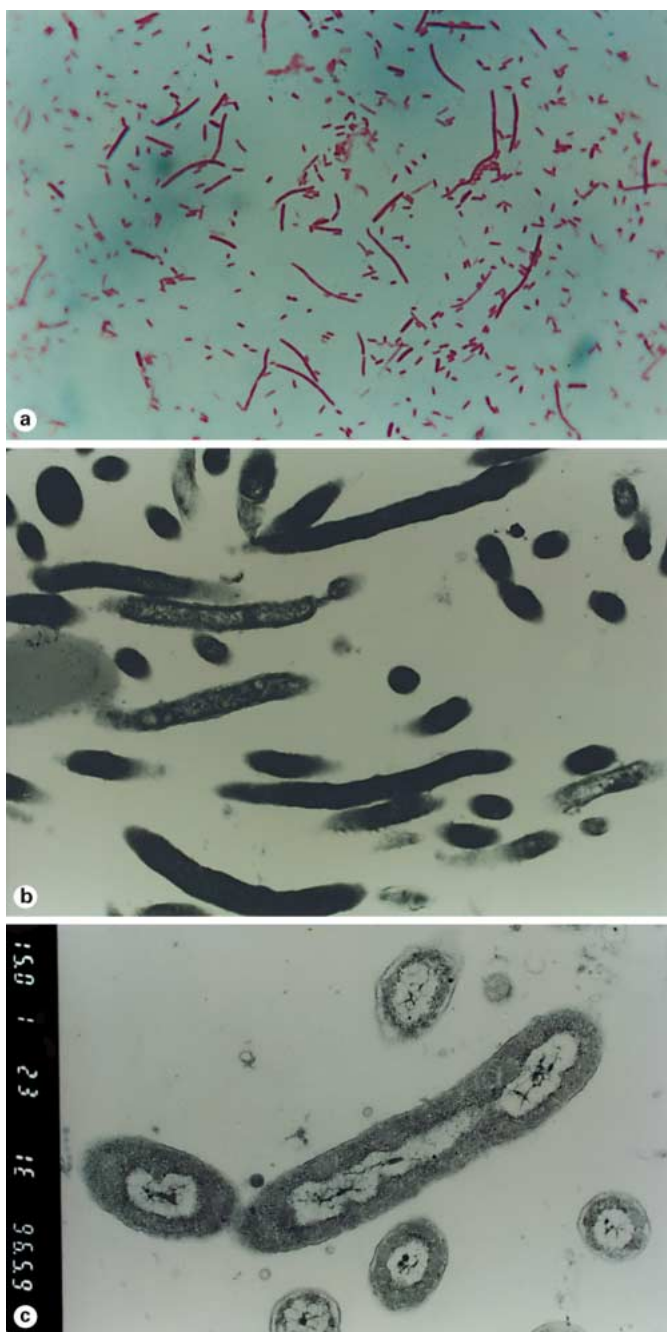


Fig. 14. a *Bacillus proteus*: Morphological variation of long rod. Light microscope. $\times 1,000$. **b** *Bacillus proteus*: Morphological variation of long rod. Electron microscope. $\times 6,500$. **c** The same as **b** but under the electron microscope, nucleoplasm. $\times 13,000$.

the reagent solution and then examined using an AC-920 hemocyte analyzer.

Assay of Humoral Immunologic Function. A quantitative hemolysis spectrophotometry (QHS) method was used to determine the amount of hemoglobin released after hemolysis of RBC mediated by antibody-forming cells [3]. This amount (expressed as OD value) reflected the amount of antibody-forming cells in mice, thus indicating the humoral immunologic function of the mice.

Table 37. Morphological variation of *E. coli communis* and pathogenic *E. coli*

	<i>E. coli communis</i>			Pathogenic <i>E. coli</i>		
Generation (50% MEBO)	1-6	7-9	10-12	1-3	4-6	7-10
Variation	+	++	+++	+	+++	++++

Form of G⁻ bacillus basically normal '+'; a little longer (like diplobacillus) '++'; became larger like round ball '+++'; shape was normal, but had deep particles in the bacteria '++++'.

Table 38. Effect of MEBO on classification of leukocytes (mean \pm SE)

Group	Animal number	Lymphocytes, %	Neutrophils, %
Control	8	69.94 \pm 3.35	30.06 \pm 3.35
MEBO	8	49.59 \pm 4.50	50.41 \pm 4.50
p value		<0.01	

Assay of Cellular Immunologic Function. Blood samples were taken from mice tails in the MEBO and the control groups, respectively, then smeared and stained according to the α -naphthalene acetate esterase (ANAE) method. These were then examined under the microscope. 100 lymphocytes were observed randomly. The percentage of ANAE-positive lymphocytes reflects the cellular immunologic function of the body.

Histological Changes of Mice Skin after Treatment with MEBO. 0.5-cm² skin tissue samples of mice taken from depilated areas of normal skin and skin treated with MEBO were fixed, embedded, stained with hematoxylin and eosin and then observed under the light microscope.

Results

Anti-Infection Effect of MEBO

Morphological Variation of Bacteria. The results of morphological variation of *E. coli communis* and pathogenic *E. coli* cultured in medium with MEBO are shown in figure 14 and table 37. These reveal that MEBO acts to induce the variation of both *E. coli communis* (which is common in burns wounds), and pathogenic *E. coli*. However, the variation of different bacteria might occur at different times.

Effect of MEBO on the Classification of Circulating Leukocytes. Table 38 shows that the amount of neutrophil in blood increased after MEBO treatment.

Table 39. Effect of MEBO on rabbit body temperature (°C, n = 6, average)

	Days after MEBO treatment				
	0	1	3	5	7
Body temperature, °C	38.63	39.05	39.31	39.43	39.47
Average elevation of body temperature, °C	0.84				

Table 40. Effect of MEBO on the production of IL-1 in mouse skin tissue cells (mean ± SE)

Group	IL-1 in skin tissue cells		IL-1 in blood plasma	
	animals	OD	animals	OD
Control	8	0.043 ± 0.019	4	0.429 ± 0.171
MEBO	8	0.142 ± 0.039	4	0.733 ± 0.105
p value	<0.01		<0.05	

Effect of MEBO on Rabbit Body Temperature. 75% of the rabbits had an increase in body temperature after MEBO treatment. On day 7, the average elevation of body temperature was 0.84°C (table 39).

Effects of MEBO on Wound Healing and Scar Formation

Effect of MEBO on the Production of IL-1 in Mouse Skin Tissue Cells. It was found that MEBO was effective in inducing synthesis of IL-1 from IL-1-delivering cells of the skin. IL-1 is capable of promoting the proliferation of thymocytes and has a synergistic action with Con A. The IL-1 levels in both skin tissue and blood plasma of the MEBO treatment group were significantly higher than those of the control group (table 40).

MEBO Promoted Proliferation of Skin Cells and Cells at the Margin of the Sebaceous Gland. In this study, we found that the number of skin basal cells in the division phase was increased, and the number of juvenile flat cells at the margin of the sebaceous gland was also increased in the MEBO group. This finding indicates that the metabolism of the cells was vigorous.

Effect of MEBO on the Specific Immunologic Function of Mice

Table 41 shows that MEBO did not affect the cellular and humoral immunologic functions.

Table 41. Effect of MEBO on specific immunologic function of mice (mean ± SE)

Group	ANA positive rate, % (n = 6)	QHS (n = 8)
Control	58.83 ± 10.61	1.296 ± 0.021
MEBO	54.17 ± 10.23	1.317 ± 0.027
p value	>0.05	

Conclusion

The results suggested that: (1) MEBO can prevent infection; (2) the wound-healing benefit and the reduction of scar formation in the MEBO group were related to its effect of increasing the production of IL-1 by skin cells.

Discussion

Clinical practice has strongly proved that MEBO has anti-infection, pain-killing, wound-healing-promoting and scar-forming-reducing effects. Based on our study, we discussed the mechanisms of its actions as the following.

Anti-Infection Effect of MEBO

MEBO has potent ability to control wound infection and keep the wound moist but not macerated. The active ingredients in MEBO ointment and its unique dosage delivery system create an environment hostile to bacterial growth. In culture medium containing MEBO, morphological structural and physiological variations of the bacteria occurred. MEBO affected the synthesis of the components for formation of a bacterial wall and inhibited related enzymes. Also, the synthesis of DNA was inhibited and the bacteria proliferation rate was decreased. Deep pigments were found in the bacteria, this indicated that the bacteria were in a stable phase of proliferation, during which a high level of glycogen, lipid, etc. was stored in the bacteria.

MEBO induced morphological and physiological variations in bacteria while influencing the production of plasma coagulase of *Staphylococcus aureus* [4]. Bacterial pathogenicity is related to the bacterial wall component and thus MEBO reduced the pathogenicity of the bacteria. The bacterial variation characteristics were varied in different species of bacteria and at different MEBO concentrations. The initiation time of the variation was not standard.

In clinical care, we found that after treatment with MEBO, the body temperature of burn patients might rise

by 1–1.5°C during the initial stage. At 3 h after application of MEBO, the temperature of burn patients with superficial second-degree wounds rose. In this study, the body temperature of rabbits increased after application of MEBO, and rose by 0.84°C on day 7. Furthermore, MEBO induced the production of IL-1, from IL-1-delivering skin cells, such as epidemic cells, keratinocyte, and Langerhans cells. In the early 1940s, people recognized that certain extracts from acute inflammatory foci could cause fever after injection into the body. This type of inflammatory substance is called endogenous pyrogen (EP). In 1979, purified EP was first proved to have IL-1 activity, and EP and IL-1 were considered the same molecule [5]. MEBO stimulates local skin cells to produce IL-1, which in turn was absorbed into the systemic circulation thereby affecting the temperature-regulating center leading to elevated body temperature.

The effect of fever on body resistance of mammals is not clear yet. Some researchers think that fever may promote the immunity of the host. When body temperature is raised, but still <41°C, the phagocytic power of most phagocytes is enhanced. We also found that MEBO can promote phagocytic power of abdominal cavity macrophages in mice [6]. In this study, the quantity of neutrophils in blood circulation was significantly increased after treated with MEBO. Bone marrow stimulated by IL-1 may account for this interesting finding.

MEBO Promotes Wound Healing and Reduces Scar Formation

Clinical data revealed that after treatment with MEBO, patients with superficial or deep second-degree burns wounds healed with full epithelization; superficial third-degree burns wounds healed with a mild scarring that appeared smooth and soft. In this study, we observed that the production of IL-1 was increased in mice skin and subdermal tissues after treatment with MEBO. The difference between the MEBO group and the control group was very significant. Besides macrophages, many other tissue cells when stimulated can produce IL-1 in 1 h [9]. IL-1, IL-8 and tumor necrosis factor (TNF) are cellular factors which are capable of activating and inducing differentiation of T and B lymphocytes, enhancing the activities of monocytes, NK cells and killer cells, thus stimulating lysosomal enzyme activity and phagocytic activity of neutrophils.

Recently, both animal experiments and clinical practice proved that IL-1 does induce a series of pathophysiological changes. These changes are similar to the host's response to infection [6], indicating that IL-1 is an important regulatory factor of the body as regards inflammation and immunologic reaction. We must differentiate between the local effect of IL-1 and the effect of high levels of IL-1 systemically. These are two totally different concepts [6]. In this study, the level of IL-1 in mouse skin

and extracellular subdermal tissues in the MEBO treatment group were significantly different from those of the control group, as well as the IL-1 level in blood plasma. IL-1 is closely related to wound healing and we know that wound exudate typically contains IL-1. IL-1 promotes proliferation of fibroblast and secretion of collagenase [6]. The effect of IL-1 is complicated. It induces inflammation and fever while at the same time promoting wound healing. Inflammation induced by IL-1 is understood to be a kind of host defense reaction [7]. After mice were treated with MEBO, their skin basal cell in division stage increased. Juvenile cells observed around the sebaceous gland were very metabolically active. This proves that MEBO promotes wound healing.

Effect of MEBO on Specific Immune Function

In this study, MEBO could increase the quantity of neutrophils in blood, while relatively decreasing the number of lymphocytes. In addition, MEBO did not affect the cellular and humoral immunologic function.

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Primary Exploration on the Mechanism of the Anti-Infection Effect of BRT with MEBO/MEBO

Introduction

Clinical practice has revealed that MEBO has a remarkable anti-infection effect [1]. During the period May to June 1992, fourteen burns cases were treated in the Burns Department, Affiliated Hospital of this College, and bacteria isolated from the burns wounds were examined. We found that *Bacillus proteus* had the morphological Hauch-ohne Hauch (H-O) variation, and the plasma coagulation ability of *S. aureus* decreased. In order to investigate the mechanism of the anti-infection effect of MEBO, we studied the biological variability of some common bacteria, such as *Bacillus proteus*, *P. aeruginosa*,

E. coli and *S. aureus*, cultured in medium containing a certain amount of MEBO. The effect of MEBO on non-specific immunity in vivo was also observed.

Materials and Methods

Clinical Data

During the period from May to June 1992, 14 cases of burns were treated with MEBO (hospitalized for 4–20 days). Swab samples were taken from the upper and lower (contact with wound) layers of the MEBO ointment, before changing the dressing. Bacteria were isolated and cultured.

Reagents, Bacterium Species and Culture

Antibiotic sensitivity test paper and nutrient agar were purchased from Shanghai Medical Chemistry Institute. *B. proteus*, *P. aeruginosa*, *E. coli* and *S. aureus* were prepared in our department.

The four above bacteria were cultured on ordinary culture medium and medium containing different concentrations of MEBO, respectively, and continuously transferred to 10–15 generations. Each generation of the bacteria was checked. The biological characteristic and drug sensitivity of bacteria were examined.

Animal Experiment

Forty healthy adult mice of both sexes weighing 20–24 g were randomly divided into 3 groups, i.e. blank control group (group 1), liquid paraffin control group (group 2) and MEBO group (group 3). Animals in groups 2 and 3 were depilated (2.5 × 2.5 cm) on their backs and liquid paraffin or MEBO was applied on the depilated area. The frequency was twice a day for 10 successive days. On the 11th day, the mice were sacrificed and abdominal cavity fluids were sampled 30 min after intraperitoneal injection of 0.5 ml of 2% sheep erythrocytes.

Observation Indexes

Bacteria Variation. The four bacteria were cultured and transformed, 18–24 h as one generation. The dynamics of every generation of the bacteria was observed under a dark-field microscope. The bacteria were stained with the G method to observe the staining reac-

tion and morphological characteristic. The colony features, biochemical reactions, and the ability of plasma coagulation were also examined.

Nucleoplasm Staining. The 7th generation of *B. proteus* was smeared, fixed with the vapor of 1% molybdic acid solution, hydrolyzed DNA with 1% hydrochloric acid for 15 min, then stained with Löffler's methylene blue and observed.

Antibiotic Drug Sensitivity Test. The four bacteria were cultured on ordinary agar medium and medium containing 20% of MEBO, respectively. Drug sensitivity test paper of eight kinds of antibiotics, i.e. gentamicin, neomycin, erythromycin, carbenicillin, ampicillin, kanamycin, chloromycetin and polymyxin, were stuck on the medium, respectively, and observed after 24 h. The diameter of the bacterial inhibition zone was measured [2].

Determination of Phagocytic Function of the Intraperitoneal Phagocytes. The abdominal cavity fluids were smeared and stained with Wright's method. The percentage of phagocytes was determined [3].

Determination of Lysozyme Activity in Abdominal Cavity Fluid and Serum. The agar plate method was used: 2 ml of *Micrococcus* solution (9×10^{10} /ml) were added to 1% agar at 70°C, then after mixing well, this was poured into a Petri dish. After cooling, holes (3 mm in diameter) were bored into the substance. Mouse serum from 3 groups was added to the holes in one set of Petri dishes and abdominal cavity fluid was added to the holes in another set of Petri dishes. These were incubated at 37°C for 24 h. The diameter of the bacteriolytic ring was measured [4].

Total Number and Classification of Leukocytes in Peripheral Blood. Blood samples from the tails were taken from 3 groups of mice. Leukocytes were counted, stained with Wright's method and classification was determined.

Results

Species and Variation of Bacteria Isolated from Burns Wounds

Table 42 shows that after treatment with MEBO, *B. proteus* in the wounds had H-O morphological variation. *S. aureus* in the wounds turned from positive plasma co-

Table 42. Species and variation of bacteria isolated from burns wounds¹

Case No.	Bacteria from upper layer of MEBO	Bacteria from lower layer of MEBO
1	<i>S. aureus</i> ; <i>P. aeruginosa</i>	<i>P. aeruginosa</i>
2	<i>Staphylococcus albus</i> ; <i>E. coli</i>	<i>E. coli</i>
3	<i>S. albus</i> (negative coagulase)	<i>S. albus</i> (negative coagulase)
4	<i>S. aureus</i> (positive coagulase)	<i>S. aureus</i> (coagulating ability decreased)
5	<i>B. proteus</i>	<i>B. proteus</i> (H-O morphological variation)
6	<i>S. aureus</i> (positive coagulase)	<i>S. aureus</i> (negative coagulase)
7	<i>S. aureus</i> ; <i>E. coli</i>	<i>E. coli</i>
8	<i>S. aureus</i> (positive coagulase)	<i>S. aureus</i> (positive coagulase)
9	<i>B. amotile</i>	<i>B. amotile</i>
10	<i>B. amotile</i>	<i>B. amotile</i>
11	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
12	<i>S. albus</i>	<i>S. albus</i>
13	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
14	G ⁺ diplococcus	G ⁺ diplococcus

¹ Samples collected on days 4–20 after MEBO treatment.

agulase to negative, or the plasma coagulating ability decreased.

Bacteriostasis of MEBO

Eight species of bacteria, i.e. *S. aureus*, *S. albus*, *E. coli*, *B. proteus*, *P. aeruginosa*, *B. typhosus*, *B. paratyphoid A* and *B. dysenteriae*, were cultured in simple agar dishes. The scraps of MEBO filter paper were pasted on the bacterial surface of streak plating. The results showed that MEBO had no direct bacteriostasis or bactericidal action.

Effect of MEBO on Bacterial Biological Characteristics

Effect of MEBO on *B. proteus* Biological Features. *B. proteus* was cultured for several generations on medium containing certain amounts of MEBO. We noted that the motility of the bacteria gradually decreased before finally vanishing. Also, we noted that H-O variation occurred. The 7th generation of the bacteria became long and filamentous. In culture medium containing 25% MEBO, 90% of the bacteria became long filamentous or long rod, and then became small bacillus. Dark pigments appeared, colonies became small and the bacteria grew very slowly. The decomposition activity of the bacteria to glucose and lactic acid was retarded (table 43; fig. 15). The effect of MEBO on H antigen of *B. proteus* is shown in table 44.

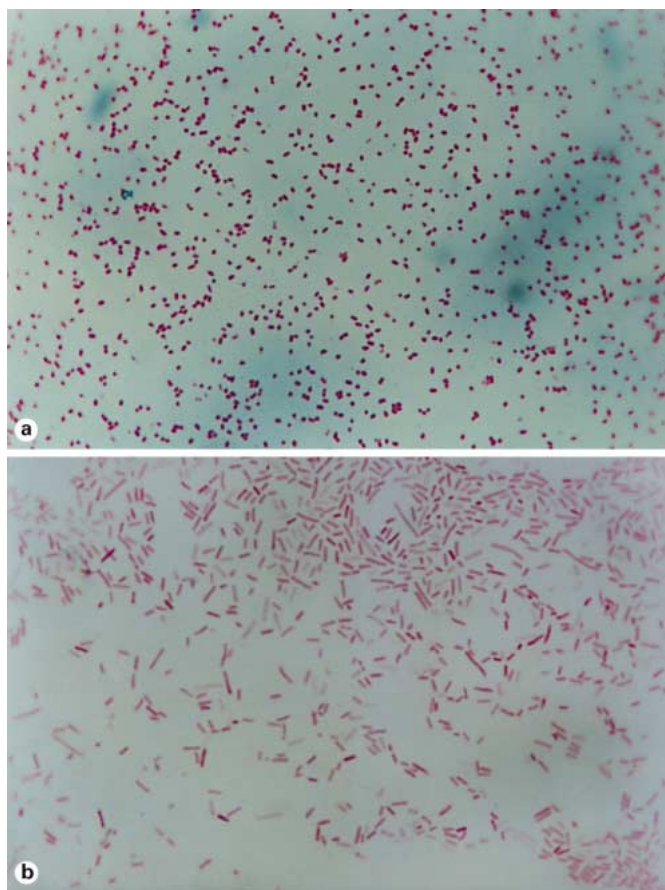


Fig. 15. a Normal appearance of *E. coli*. b Appearance of variant *E. coli* cultured in medium containing MEBO for 6 generations.

Table 43. Effect of MEBO on *B. proteus* biological features

Medium	Motility				Morphology				H-O variation				H ₂ S test	
	G 1	G 2	G 3	G 4-10	G 1-2	G 3-4	G 5-8	G 9-10	G 1	G 2	G 3-4	G 5-10	G 1-7	G 8-10
50% MEBO	+	±	-	-	+	+	++	+++	+	+	-	-	+	retarded decomposition
25% MEBO	+	±	±	-	+	++	++ *	+++	+	+	+	-	+	retarded decomposition

+ = Motile; ± = weak motility; - = no motility.

+ = Typical G⁻ bacillus; ++ = long rod or filament; +++ = G⁻ with dark pigment.

+ Colonial migration: + = 1-3 cm; - = no migration.

G = Generation; * 90% of the 7th generation of the bacteria became long filamentous.

Table 44. Serological test of *B. proteus* cultured in medium containing MEBO

	Original bacteria	Medium containing MEBO, 12th generation		
		10% MEBO	25% MEBO	50% MEBO
<i>B. proteus</i>	1:1,280	1:640	1:640	1:320
H antiserum	++	++	++	++

Effect of MEBO on the Biological Characteristic of P. aeruginosa. It was found that *P. aeruginosa* cultured in MEBO-containing medium started to decrease its motility from the 5th generation, and motility vanished in the 10th generation. Variations of morphology and colonization features of the bacteria were also found (table 45).

Effect of MEBO on the Biological Characteristics of E. coli. From table 46, we can see that after proliferation to the 10th generation while cultured in MEBO containing medium, *E. coli* changed as follows: it lost motility, became sphere shaped, colonies became smaller, dry and flat (fig. 16). The decomposition activity of the bacteria to glucose and lactose was retarded (after 32 h).

Table 45. The effect of MEBO on the biological characteristic of *P. aeruginosa*

Medium	Motility			Morphology			Colonial feature			Oxidase test
	G 1-4	G 5-9	G 10-12	G 1-4	G 5-9	G 10-12	G 1-5	G 6-9	G 10-12	G 1-12
MEBO 50%	+	±	-	+	++	+++	+	++	+++ ^x	+
MEBO 25%	+	±	-	+	++	+++	+	+	++	+

+ = Typical G⁻ bacillus; ++ = a few became long rod or diplococcus; +++ = deep pigment appeared.
 + = Colony moderate size and smooth; ++ = colony smaller and dry; +++^x = pigment formed but not good.
 G = Generation.

Table 46. Effect of MEBO on the biological characteristic of *E. coli*

Medium	Motility			Morphology			Colony feature			Fermentation test			
										glucose		lactose	
	G 1-3	G 4-9	G 10-12	G 1-6	G 7-9	G 10-12	G 1-3	G 4-8	G 9-12	G 1-9	G 10-12	G 1-9	G 10-12
MEBO 50%	+	±	-	+	++	+++	+	++	+++	+	retarded decomposition	+	retarded decomposition
MEBO 25%	+	±	-	+	++	+++	+	++	+++	+	retarded decomposition	+	retarded decomposition

+ = Typical G⁻ bacillus; ++ = long rod (like diplococcus); +++ = bacteria swelling (sphere shaped).
 + = Colony medium size and smooth; ++ = colony medium size, a little flat; +++ = colony small, rough and flat.
 G = Generation.

Table 47. Effect of MEBO on the biological characteristic of *S. aureus*

Medium	Morphology			Colony feature			Plasma coagulase test			Manicol test	
	G 1-7	G 8-11	G 12-15	G 1-7	G 8-11	G 12-15	G 1-7	G 8-12	G 13-15	G 1-11	G 12-15
MEBO 50%	+	++	+++	+	++	+++	++	+	±	+	retarded decomposition
MEBO 25%	+	++	++	+	++	++	++	+	+	+	+

+ = G⁺ arranged in grape shape; ++ = part of the bacteria became diplococcus-like or arranged in short chain; +++ = piled up in grape shape and had scattered diplococcus-like and short chain arrangement.
 + = Colony medium size and smooth; ++ = colony small and slightly flat; +++ = colony smaller, flat and dry.
 Plasma coagulase test: ++ = Fluid was clear and obviously coagulated; + = fluid turbid and small coagulate; ± = fluid turbid, small and few coagulate.
 G = Generation.

Effect of MEBO on the Biological Characteristics of S. aureus. We found that after 10 generations of *S. aureus* cultured in medium containing MEBO, bacteria were piled up in grape shape and had scattered diplococcus-like and short chains. Colonies became smaller, flat and dry. The decomposition activity of the bacteria to mannitol was retarded (after 32 h), and the variation of plasma coagulation ability was very significant (table 47) [3].

Nucleoplasm Staining of B. proteus. Cultured in medium containing 25% MEBO and proliferated to the 7th generation, *B. proteus* appeared as a long filamentous variant. Nucleoplasm staining was done and examined. As the RNA in cytoplasm was hydrolyzed, the nucleoplasm was stained blue. The bacteria became long rod or long filament in the course of binary division, because the formation of cell wall was slower than the division of the nucleoplasm. This result proved that bacteria proliferation was retarded when cultured in medium containing MEBO.

Synergistic Effect of MEBO and Antibiotics on Bacteriostasis. When cultured in medium containing 20% MEBO, *P. aeruginosa* resistant to carbenicillin and chloromycetin became moderately sensitive and *B. proteus* resistant to chloromycetin and ampicillin became sensitive. Both carbenicillin and kanamycin had a synergistic effect with MEBO against *E. coli*. Carbenicillin, ampicillin, kanamycin and erythromycin had a synergistic effect with MEBO against *S. aureus* (table 48).

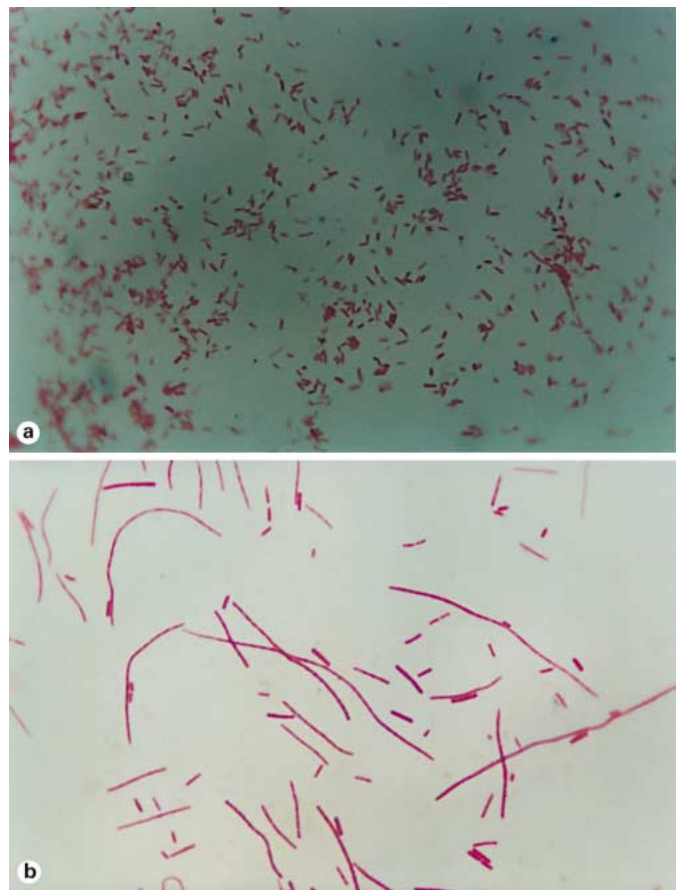


Fig. 16. **a** Normal appearance of *B. proteus*. **b** Appearance of variant *B. proteus* cultured in medium containing MEBO for 2 generations.

Table 48. Synergistic bacteriostasis of MEBO and antibiotics

Culture medium	Carbenicillin			Ampicillin		Kanamycin		Erythromycin	Chloramphenicol		
	DR	MS	S	DR	S	MS	S	S	DR	MS	S
Ordinary											
<i>S. aureus</i>			30		21	17		20			
<i>P. aeruginosa</i>	16									12	
<i>E. coli</i>	12						21				
<i>B. proteus</i>				5						12	
Containing 20% MEBO											
<i>S. aureus</i>			38		30		20	25			
<i>P. aeruginosa</i>		20								16	
<i>E. coli</i>		22					25				
<i>B. proteus</i>					20						20

DR = Drug-resistant; MS = moderate sensitivity; S = sensitivity.

Table 49. Effect of MEBO on peripheral blood leukocytes (mean \pm SE)

Group	Animals	WRC, 10 ⁹ /l	PMN, %
Blank control	8	6.87 \pm 0.85	26.0 \pm 1.53
Vaseline control	8	6.93 \pm 1.22	28.3 \pm 3.86
MEBO	8	8.75 \pm 0.91	48.5 \pm 2.56
p value		<0.01	<0.01

Table 50. Effect of MEBO on phagocytic function and lysozyme activity (mean \pm SE)

Group	Animals	Phagocytosis %	Lysozyme diameter, cm	
			abdominal cavity fluid	serum
Blank control	8	54.23 \pm 6.20	1.70 \pm 0.16	2.08 \pm 0.17
MEBO	8	65.50 \pm 4.18	2.33 \pm 0.38	2.10 \pm 0.11
p value		<0.05	<0.05	>0.05

Effect of MEBO on Nonspecific Immunity

Effect on Peripheral Blood Leukocytes. MEBO significantly increased the peripheral blood count of white blood cells and polymorphonuclear leukocytes (PMN%) in mice (table 49).

Effect on Phagocytic Function and Lysozyme Activity. MEBO significantly promoted the phagocytic function of phagocytes and increased the lysozyme activity in the abdominal cavity fluid (table 50).

Conclusion

MEBO can increase the number of WBC in the peripheral blood of mice. It can also enhance the function of phagocytes in the abdominal cavity of mice. MEBO can induce a variation of bacteria and improve nonspecific immunity.

Discussion

Bacterial inhibitory test proved that MEBO does not have a direct bacteriostatic or bacteriocidal effect. This finding may be understood in part due to the oily texture of MEBO making it very difficult to infiltrate and diffuse into a watery culture medium. After continuous culture in medium containing MEBO, many species of bacteria showed variations in morphological structure and biological characteristics that are closely related with the growth environment. The morphological variation of bacteria may cause changes in its biochemical characteristics, antigenicity and toxicity. *B. proteus* and *P. aeruginosa* had deep pigmented particles and *E. coli* became sphere-shaped after culture in medium containing MEBO.

These variations are non-genetic. MEBO had a synergistic bacteriostatic effect with antibiotics. This is beneficial to the control of local and systemic infections secondary to severe burns. MEBO promoted the phagocytic function of abdominal cavity phagocytes and release of lysozymes, and increased the leukocyte and neutrophil counts in the peripheral blood. This is very important for clearing out the bacteria and toxins both locally and systemically. In summary, the mechanism of the anti-infective effect of MEBO includes inducing variation of the bacteria, decreasing their proliferation rate, reducing bacterial pathogenicity and promoting nonspecific immunity of the body.

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Experimental Research on the Anti-Anaerobic and Anti-Fungal Effect of MEBO

Introduction

Clinical research data of BRT with MEBO revealed that MEBO has a strong ability to retard wound infection. Its mechanism is myriad [1]. This paper reports the effect of MEBO on the morphological structure, colo-

ny character and pathogenicity of anaerobic spore bearing bacillus (*Bacillus tetani*), anaerobic non-spore-bearing bacillus (*Bacteroides fragilis*, *Propionibacterium acnes*) and fungi (*Candida albicans*). MEBO has been proven to possess strong broad-spectrum antibacterial effects. MEBO also creates an environment for preserving the residual surviving cells in the burns area and to promote their proliferation [2]. Thus, MEBO offers a dual regulatory effect.

Materials and Method

Materials

Aquarium Type B224 was designed by the laboratory of the Affiliated Hospital of Binzhou Medical College. Culture medium was supplied from Shanghai Biological Preparation Institute. *Bacteroides fragilis* and *Propionibacterium acnes* were purchased from Shanghai Medical University; *Bacillus tetani* and *Candida albicans* from the laboratory of the Affiliated Hospital of Binzhou Medical College. These bacteria were cultured separately in anaerobic agar medium for use.

Method

MEBO Group. The above-stated four species of bacteria were inoculated separately into the medium containing a certain amount of MEBO. Anaerobic bacteria were incubated at 37 °C for 48–72 h as one generation. *Candida albicans* was incubated at 37 °C for 24–48 h as one generation. After 4–6 successive generations, they were treated with Gram stain and their staining reaction, morphology and colony characteristics were observed.

Control Group. The above-stated original bacteria were observed before being inoculated into the medium containing MEBO.

Examination Indexes

Variation of the Bacteria. (1) *Bacteroides fragilis*, *Propionibacterium acnes* and *Bacillus tetani* were inoculated separately into the medium containing MEBO and cultured for multiple generations during which morphological and colony variations at each generation were observed. (2) Variations of *Candida albicans* were observed after culture in MEBO-containing media.

Spore Tube Test. Original *Candida albicans* and the 1st, 2nd, 5th and 6th generations after being cultured in MEBO-containing media were inoculated into 0.5 ml human serum medium and cultured at 37 °C for 3 h, the fungi were smeared, stained and 500 counts of the fungi were observed to determine the spore tube producing rates.

Effect of MEBO on Bacterial Growth

Equal amounts of the colonies of the 10th generation of *Staphylococcus aureus*, and *Bacillus pyocyaneus* cultured in MEBO-containing media and the original bacteria of the two species were ground and placed into 0.1 ml of saline, respectively, then 1 ml of saline was added and the counts of the bacteria were compared.

Effect of MEBO on the Invasive Power of *Bacillus pyocyaneus*

The 10th generation of *B. pyocyaneus* cultured in MEBO-containing media and the original *B. pyocyaneus* cultured in ordinary media were taken and diluted separately to 3×10^6 /ml. 0.1 ml of the bacteria solutions were injected into mice intracutaneously. After 20 h the mice were killed. A block of rectangular skin tissue to the muscular layer was taken from the injection site of each mouse. The tissue blocks were made into sections and stained with HE and observed.

Results

The effect of MEBO on anaerobic bacteria is given in table 51 and depicted in figures 17–19. The effect of MEBO on *Candida albicans* is given in table 52 and depicted in figure 20. The effect of MEBO on the proliferation rates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is given in table 53. The effect of MEBO on the invasiveness of *Pseudomonas aeruginosa* is shown in table 54.

Table 51. Effect of MEBO on anaerobic bacteria

<i>(1) Bacillus tetani</i>	
Control group:	G positive, slender bacillus, spores could be seen occasionally on the top, bacteria in the shape of a group of drumsticks, colonies grown in films (fig. 17a)
MEBO group:	1, 2 generations: most of the bacteria were in a shape of long rod or long filament ¹ , a few had spores, colonies were flat, rough and dry, none grown in films (fig. 17b) 3, 4 generations: most of the bacteria were bacillus of various length, many of them had spores. The bacteria were in a shape of drumstick, a few were long rods or long filaments, colonies were flat, rough and dry (fig. 17c)
<i>(2) Propionibacterium acne</i>	
Control group:	G positive, non-spore-bearing bacillus, straight or slightly crooked, colonies were small and round with smooth surface (fig. 18b)
MEBO group:	1, 2 generations: basically the same as the control 3, 4 generations: bacillus of different length appeared (like diplobacillus) with aggregation and confluence, colonies were small, slightly flat, rough and dry (fig. 18c) 5, 6 generations: most of them were small coccobacillus with deep colored particulates, colonies were small, slightly flat, rough and dry (fig. 18d)
<i>(3) Bacteroides fragilis</i>	
Control group:	G positive, non-spore-bearing moderate bacillus, with obtuse ends, colonies were a little convex with smooth surface (fig. 18e)
MEBO group:	1, 2 generations: basically the same as the control 3, 4 generations: long bacillus (like diplobacillus) and some coccobacillus appeared, colonies a little flat with dry and rough surface (fig. 18f) 5, 6 generations: small coccus and coccobacillus appeared; colonies aggregated to confluence to form irregular round bodies; colonies were flat, dry and rough (fig. 18g)

¹ Cultured in MEBO-containing media, when MEBO concentration was 25%, the variation percentage of *Bacillus tetani* to form long filaments was higher than that cultured in media containing higher concentration of MEBO.

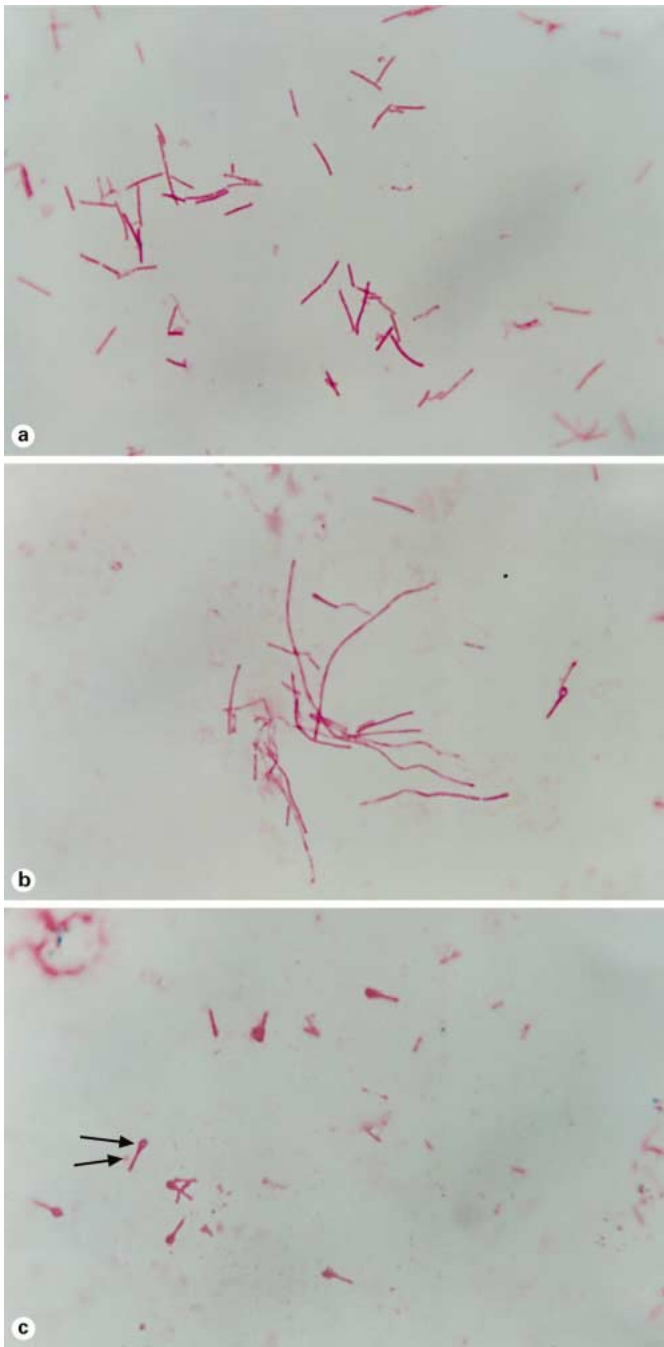


Fig. 17. a The normal *Bacillus tetani* showing slender rod-like shape. **b** The 1–2 generations of *Bacillus tetani* in culture medium with MEBO showing as a shape of long rod or long filament. **c** The 3–4 generations of *Bacillus tetani* in culture medium with MEBO showing in various lengths, many of them having spores. The bacteria were drumstick shaped, a few were long rods or long filaments.

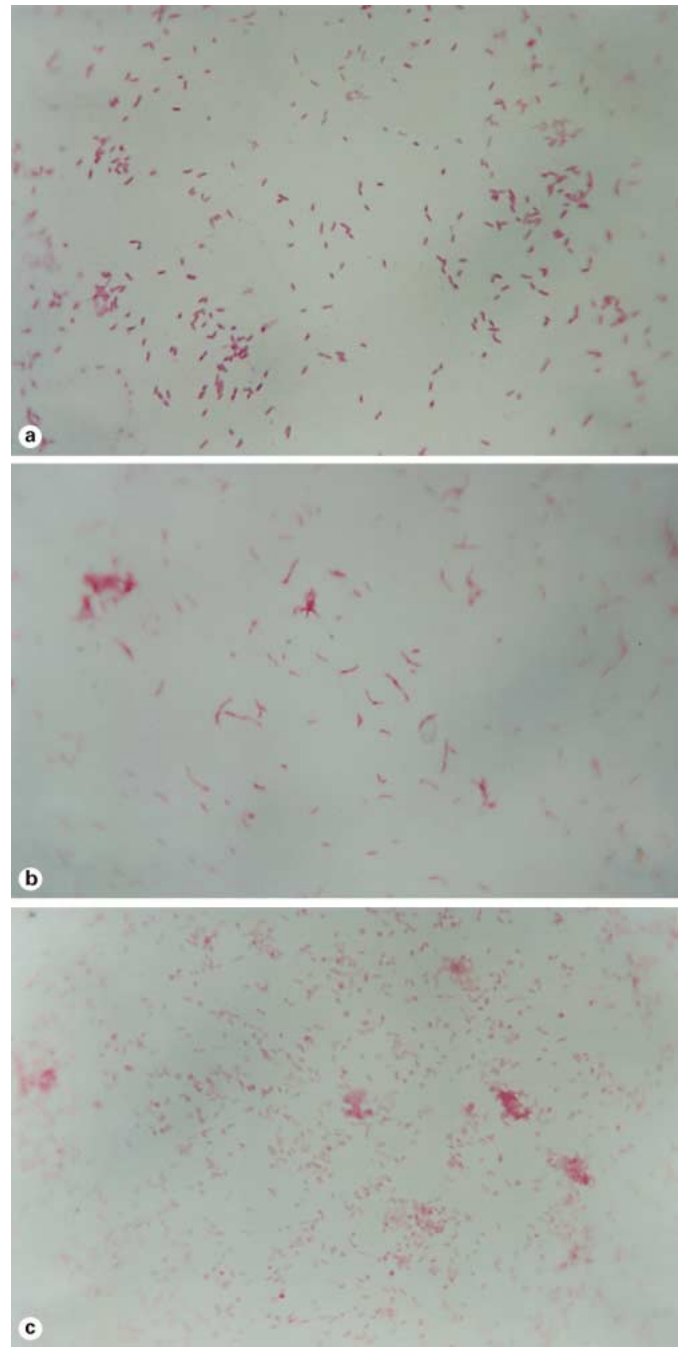


Fig. 18. a The normal form of *Bacteroides fragilis* showing moderate size. **b** The 3–4 generations of *Bacteroides fragilis* varied in length (look like diplobacillus) and bacterial colonies fused together. **c** The 5–6 generations of *Bacteroides fragilis* in culture medium with MEBO were sphere or egg shaped. Many bacterial colonies fused to form irregular spheres.

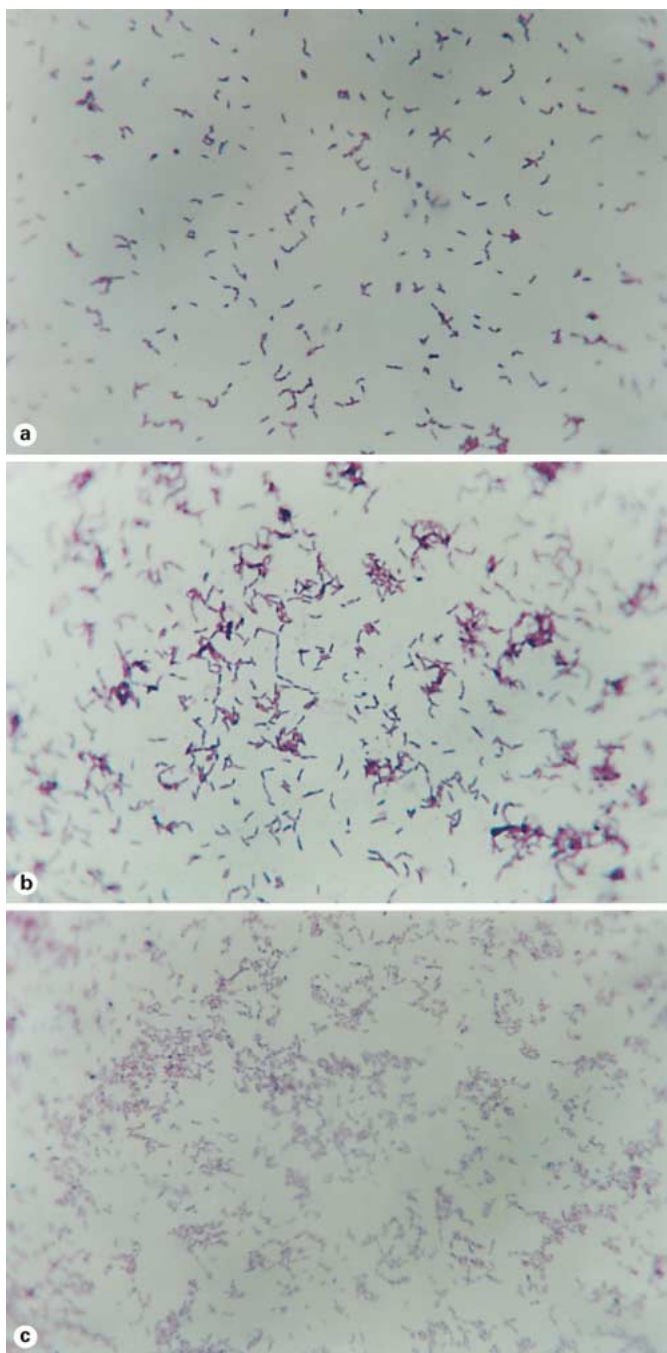


Fig. 19. **a** The normal shape of *Propionibacterium acne* showing G⁺ (some straight or slightly crooked). **b** The 3–4 generations of *Propionibacterium acne* in culture medium with MEBO showing varied rod length. **c** The 5–6 generations of *Propionibacterium acne* were small coccobacilli with deep-colored particulates.

Table 52. Effect of MEBO on *C. albicans*

Control

The fungi were oval with spores and pseudohypha, colonies were milky with smooth and moisturized surface (fig. 19a)

Spore tube test: spore tube producing rate was 90% (fig. 19b)

MEBO

1, 2 generations: the same as the control, colonies were a little more flat and small, compared to the control; spore tube producing rate was about 85% (fig. 19c)

3, 4 generations: some of the fungi were sphere or oval of different sizes, pseudohypha appeared; colonies were flat, dry and rough (fig. 19d)

5, 6 generations: the fungi were sphere or oval, filaments with stick or long rod shape and different length appeared; only few spores; colonies were flat, dry and hard (fig. 19e)

Spore tube rate was about 0.5–2%¹ (fig. 19f)

¹ Cultured in media with different concentrations of MEBO, their spore-producing rates were different. In media containing 25% of MEBO, the spore tube-producing rate was 2%, a little higher than in that containing higher concentration of MEBO.

Table 53. Effect of MEBO on the proliferation of *S. aureus* and *P. aeruginosa*

Bacteria	Primary bacteria cultured counts/ml	10th generation after cultured in MEBO containing media counts/ml
<i>Staphylococcus aureus</i>	1.4 × 10 ⁸	1.5 × 10 ⁶
<i>Pseudomonas aeruginosa</i>	2 × 10 ⁸	6.5 × 10 ⁶

Table 54. Effect of MEBO on the invasiveness of *P. aeruginosa* – pathological examination

Control group

In subcutaneous tissue, there was congestion and edema, infiltration of inflammatory cells and a suppurative zone

MEBO group

In subcutaneous tissue and striated muscles, there was infiltration of a few inflammatory cells without suppurative phenomenon

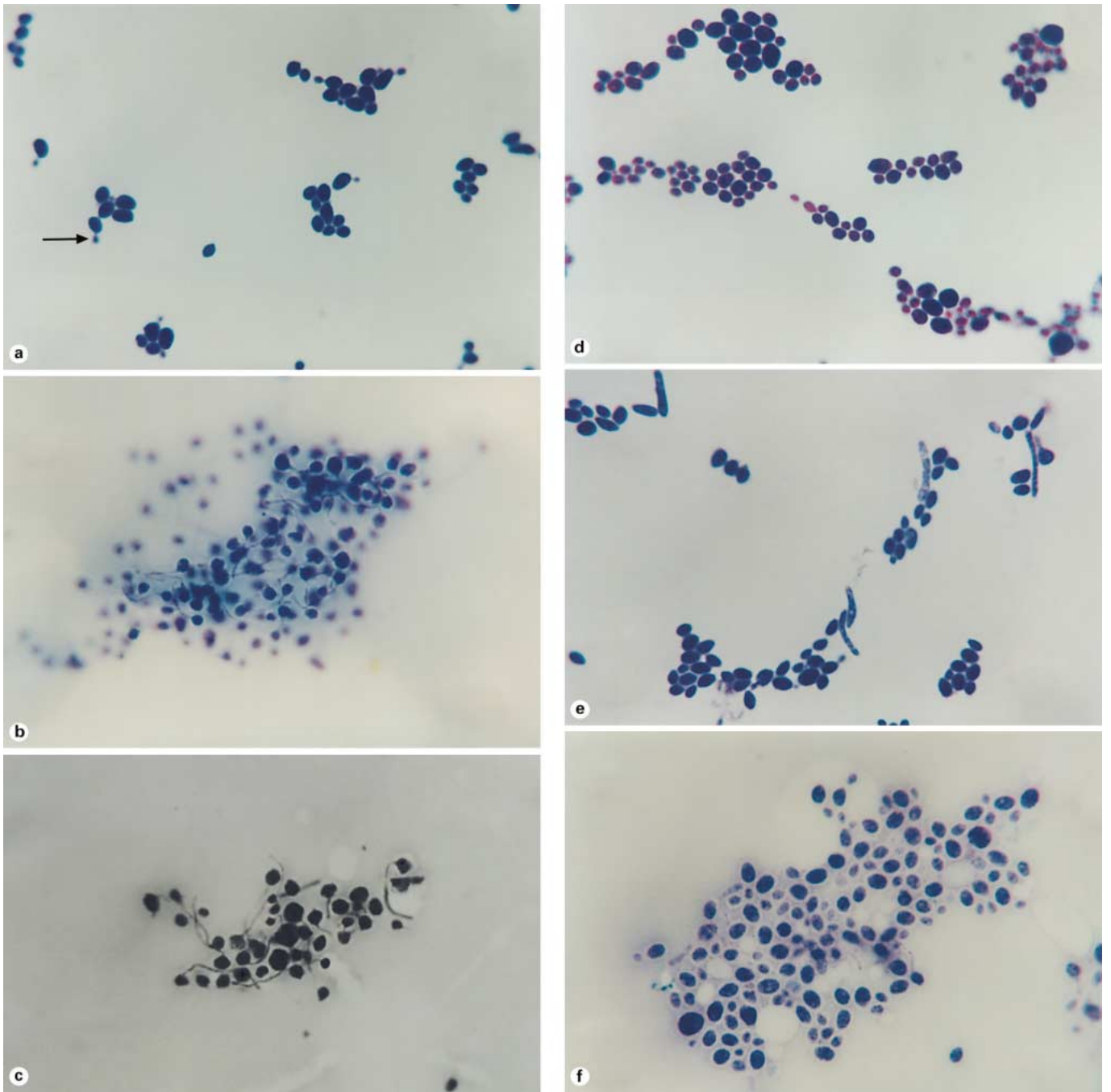


Fig. 20. **a** The normal shape of *Candida albicans* showing egg shape with many blastospores. **b** The germ tube tests on normal *Candida albicans* gave a producing rate of 90%. **c** The germ tube producing rate of 1–2 generations of *Candida albicans* in culture medium with MEBO was 85%. **d** The 3–4 generations of *Candida albicans* in culture medium with 25% MEBO showing large oral or few sphere-

shaped and stick- or filament-shaped ones being observed occasionally. **e** The 5–6 generations of *Candida albicans* showing in shapes of stick or long rod. Bacterial filaments in various length and few blastospores being observed. **f** The germ tube producing rate of 5–6 generations of *Candida albicans* in cultured medium with MEBO was 0.5–2%.

Discussion

Micro-organisms proliferate rapidly and are accordingly susceptible to unfavorable factors in their growing environment. These changes in their environment may result in variations of their characteristics including morphological structure, culture feature, toxicity, antigenicity and drug resistance, etc. Morphological structure variations are directly related to cell division. Cell division is a complicated process and is more susceptible to unfavorable factors in the environment than to protein and DNA syntheses. For example, *Bacillus tetani* and *Bacteroides fragilis* may have polymorphic variations such as polynuclear filaments or spheres because, in an unfavorable environment, cell membrane synthesis is delayed and cell division cannot proceed in a timely manner, while metabolism proceeds normally. Variation is closely related to the effect of the strength and the duration of action of the unfavorable factors. In our experiment, we found that the same bacteria demonstrated variation in morphological features when cultured in media containing different concentrations of MEBO (data not shown).

In this experiment, we observed the antibacterial effect of MEBO on *Bacillus tetani*, *Propionibacterium acnes* and *Candida albicans*. These micro-organisms are normal flora and opportunistic pathogens in the human body. Under certain conditions they become pathogenic and, in most clinical cases, the infections are endogenous in etiology. Burns patients often receive large doses of antibiotics, which always create an imbalance in native flora thereby facilitating the overgrowth of opportunistic pathogens such as *Candida albicans*. Aerobic bacteria infections such as *Staphylococcus aureus*, *E. coli* and *B. pyogenes* predominate in burns wounds though anaerobic bacteria infections may also occur since burns wounds may become ischemic and necrotic. Therefore, bacterial examination of wounds should always consider both aerobic and anaerobic bacteria. In 1983, Wang Dewang reported a positive anaerobic bacteria detection

rate of 23.9% in 34 cases of burns and most of them had mixed infections.

Bacillus tetani is an anaerobic spore-bearing bacteria. After being cultured in MEBO-containing media, long filament variants appeared, and in 3 generations many of them formed only one spore. This spore is not the vegetative form of the bacteria as it cannot proliferate under normal conditions. Spore keeps the bacteria alive though dormant. Only under favorable conditions can the spore proliferate and produce exotoxin to cause disease. After culture in MEBO-containing media for 6 generations, in *Candida albicans* the number of blastospores was significantly reduced, the spore tube producing rate was only 0.5–2%, while the normal fungi had a spore tube producing rate of 90%. The above facts prove that MEBO has an inhibitory effect on the proliferation and pathogenicity of *Bacillus tetani* and *Candida albicans*.

From table 53, we can see the effect on *Bacillus pyocyaneus*. After this bacteria was cultured in MEBO-containing media for 10 generations, we again cultured the 10th generation for another 20 h and discovered that the bacteria count/ml was greatly reduced to only 1/30 of that of the original. The invasiveness of this organism to mice was also greatly decreased. This proved that under an unfavorable environment, not only morphological structures but also physiological features of the bacteria are subject to variations.

Our data as well as many experimental and clinical data reported by other workers proved that MEBO has a broad-spectrum antibacterial effect and can also promote wound healing. Therefore, MEBO is a drug with a dual regulatory effect.

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Studies on the Effects of BRT with MEBT/MEBO on Regeneration and Healing of Burns Wounds

A Comparative Study of Fibronectin and Moist-Exposed Burns Ointment (MEBO) in the Treatment of Experimental Corneal Alkali Burns in Rabbits

Introduction

Worldwide reports regarding the basic science and clinical applications of fibronectin's (FN) contribution to the healing of trauma and burns wounds have shown that FN can enhance the epithelial healing rate by promoting the adhesion and migration of epithelial cells at the wound site [1–3]. Clinical application of eye drops made of FN in treating burned cornea has obtained satisfactory results. However, we are still years from the widespread use of FN because the extraction and production of FN eye drops are time-consuming, expensive and offer specific preservation challenges. We have used MEBO since 1990 in the treatment of burned cornea and we have achieved satisfactory results in terms of improving corneal nutrition, promoting wound healing, ameliorating pain, relieving irritation and reducing the incidence of corneal ulceration. Therefore, we embarked on an experimental study to verify the effect of MEBO as compared with FN in the management of corneal burns in rabbits.

Materials and Methods

MEBO, developed by Beijing Guangming Chinese Medicine Institute for Burns, Wounds and Ulcers, is an ointment containing sesame oil, beeswax and other active ingredients derived from plants such as *Cortex phellodendri* and *Radix scutellariae*. FN produced by the Shanghai Institute of Biological Products was diluted to a solution containing 400 µg/ml with sterile normal saline.

Five healthy New Zealand white rabbits (body weight 2–3 kg) without eye disease were anesthetized intramuscularly with 20 mg/kg sodium thiopental and eye solution of 0.4% Novesine two drops in each eye. 8-mm filter papers, previously soaked completely in 0.5 N NaOH, were laid on the middle of both corneas, respectively. One minute later, the paper was removed and the eyes were rinsed immediately with sterile normal saline. Then eye drops made of FN were administered to the right eyes, four times daily and MEBO was applied on the left eyes, three times daily. Both courses lasted 2 weeks.

At 6, 24, 36 and 48 h postinjury, the corneal fluorescent staining zones were photographed with a DF Haiou camera (China) at fixed focus. After developing the film, we drew the fluorescent staining zones, analyzed and measured the areas at different hours using a computer image pattern analyzer, and then calculated the epithelial healing rate (mm²/h) by linear regression. Observations were made twice daily on days 3–14 postinjury and fluorescent staining zones were regarded as a positive indicator. Epithelial damage rate = (fluorescent staining positive number)/(number of eyes examined) × 100%.

rescent staining positive number)/(number of eyes examined) × 100%.

Besides corneal fluorescent staining zone, conjunctival congestion and corneal transparency were also observed. (1) Conjunctival congestion: + = palpebral conjunctival congestion; ++ = palpebral conjunctival and partial bulbar conjunctival congestion; +++ = whole palpebral and bulbar conjunctival congestion. (2) Corneal transparency: + = slightly opacity with distinct structure of underlying iridial texture; ++ = moderate opacity with blurred structure of iridial texture; +++ = severe corneal opacity with indistinct structure of iridial texture.

Results

In the early stage (at 6 h postinjury) epithelial healing was slow and at 6–48 h the healing rate remained constant. The use of FN on eyes shows an epithelial healing rate of 1.279 ± 0.317 mm²/h compared to 1.285 ± 0.128 mm²/h with the MEBO treatment. There is no significant difference between the two groups although in the MEBO group treatment efficacy is a little faster.

Repeated corneal damage occurred from the early healing stage until 2 weeks postinjury and there is a significant difference in damage rate in both groups: 58.3% in the FN group and 33.3% in the MEBO group ($u = 5.56$, $p < 0.01$).

The cornea showed disk opacity a few minutes following burn. Conjunctival congestion with white secretion was observed at 3 h postinjury. On day 2 postinjury, eye irritation became obvious with increased secretion and corneal edema was noted until 1 week postinjury when edema was still observed in epithelium and matrix. At 2 weeks postinjury, the above symptoms were further relieved. The advantages of MEBO treatment as compared to FN in the management of burns eyes were demonstrated both in terms of corneal epithelial exfoliation and regarding conjunctival congestion and opacity.

Conclusion

Some advantages of MEBO include inexpensive cost, convenient application, safety and product stability. The effect of MEBO in the treatment of alkali-burned corneas compared favorably to that achieved with FN. MEBO proved more effective than FN in reducing the epithelial damage rate and was far easier to use given a widespread application. We suggest that MEBO treatment is remarkably advantageous in reducing the repeated epithelial damage rate of alkali burns eyes compared to FN treatment.

Discussion

It has been reported that FN has the effect of promoting epithelial healing rate on corneal defects [4]. This study verified no significant difference in promoting corneal epithelial healing between the two groups. However, MEBO treatment is remarkably advantageous in reducing the repeated epithelial damage rate of alkali burns eyes compared to FN treatment. The repair of relative integrity of the corneal epithelium is beneficial to the stability of the corneal parenchyma and endothelium. The cornea in the MEBO group showed slight transparency with slight congestion. Subsequent to alkali burns, the integrity of basement membrane underlying corneal epithelium was damaged. The appropriate replacement of exogenous FN may reduce the possibility of epithelium damage, as FN is needed for the repair of basement membrane. This study demonstrated that MEBO is superior to FN with regard to firm adherence of corneal epithelium and maintenance and basement membrane integrity. In addition, MEBO supplies rich nutrients necessary for repairing and regeneration of alkali burns corneas.

FN is a kind of macromolecular glucoprotein which operates at the wound surface. The surge of enzymes released subsequent to alkali burn causes the degradation of FN at the wound surface. The paucity of FN resulting from enzymatic degradation can be compensated with exogenous FN, which reduces the epithelial damage rate by promoting epithelium adhesion. Many combining sites in FN molecular structure served as a bridge between epithelial cells and basement membrane, thereby improving epithelium adhesion. Affinity between FN and intracellular actin may cause the change of epithelial intracellular actin from sphere to fibriform and then induce cell migration.

As avascular tissue, the cornea receives the nutrition and oxygen necessary for metabolism mainly from diffusion of the vascular net in the corneal limbus, from tears and from aqueous humor. Glucose is the main source of energy for the oxygenic metabolism of corneal epithelium and for intraparenchymatous anaerobic metabolism [5]. MEBO contains abundant glucose, organic acid, a variety of vitamins, proteins and enzymes as well, all of which directly provide energy and nutrition for alkali-burned corneal tissue. In this manner, MEBO serves to promote metabolism of the cornea, to accelerate the prompt removal of necrotic tissue and to facilitate swift growth of new epithelium. MEBO also has anti-inflammatory properties, bacterial inhibition, repercussive and analgesic effects [6]. By relieving local congestion and corneal irritation, blepharospasm due to pains and nictitation were alleviated, which reduced the friction to corneal surface and also improved local resistance.

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A Comparative Study of the Effects of Moist-Exposed Burns Ointment (MEBO) and Other Drugs on the Healing Rate of Corneal Epithelial Defect in Rabbits

Introduction

Corneal epithelium consists of three types of cells at three different levels. They are basal cells in the deep layer, wing cells in the middle layer and squamous cells at the corneal surface. Microvillus in the superficial layer absorbs tears to form a tear membrane. Basement membrane behind the deep layer is located in the corneal Bowman membrane (anterior limiting lamina). Corneal epithelial layer is a natural barrier against micro-organism invasion. Once injured, it is prone to infection and shedding. Some corneal diseases such as dendritic keratitis, neuroparalytic keratitis, exfoliation of recurrent corneal epithelium, alkali burns, vesicular keratitis, etc., and surgeries such as scraping the corneal epithelium for intraocular examination and operation when performing cutting of the vitreous and repair for retinal detachment, may injure corneal epithelium. Rapid and complete repair of corneal epithelial defects plays a very crucial role for the recovery of corneal physiological function and for good eyesight. Corneal physiological function contributes greatly to the promotion of deep corneal wound healing and formation of collagenous fiber [1]. In this study, rabbits with corneal epithelial defects of the same size were used and treated with MEBO, homologous serum, 0.5% dexamethasone, 25,000 U/ml vitamin A. In addition, the rabbits wore soft corneal contact lenses to observe the regenerating rate of corneal epithelia and to assess the therapeutic effects of different measures.

Materials and Methods

Eighty-two healthy adult rabbits of either sex weighting 2–3 kg were anesthetized by 35% urethane 2.5 ml/kg intravenously and, if required, by inhalation of ether. Then 1% dicaine was dropped twice for surface anesthesia into the palpebral fissure which was expanded with eyelid retractor. The cornea center was constrictively marked at

Table 55. Average healing rate of corneal epithelium after injury (mm²/h, mean ± SE)

Groups	Number of eyes	Average healing rate
Group 1 (MEBO)	10	1.184 ± 0.106
Group 2 (dexamethasone)	11	1.087 ± 0.087 ^a
Group 3 (vitamin A)	11	1.065 ± 0.066 ^b
Group 4 (homologous serum)	10	1.114 ± 0.038 ^c
Group 5 (saline)	10	1.037 ± 0.059 ^{b, c, f}
Group 6 (contact lens)	10	0.770 ± 0.016 ^{b, d, e, f, g}

^a p < 0.05, ^b p < 0.01 compared with group 1; ^c p < 0.05, ^d p < 0.01 compared with group 2; ^e p < 0.05 compared with group 3; ^f p < 0.01 compared with group 4; ^g p < 0.05 compared with group 5.

surface using an 8-mm corneal trephine and was stained with 1% fluorescein followed by rinsing with normal saline. Subsequent to the appearance of a circular mark, an 8-mm stained disk area of epithelial defect was created with a lancet scraping off the whole epithelium within cycle and by fluorescein staining.

The animals were then divided randomly into 6 groups, 12 in each. In group 1, the animals were treated with MEBO every 6 h. In group 2 to group 5, the animals were given eye drops with 0.5% dexamethasone, 25,000 U/ml vitamin A, homologous serum and normal saline, respectively, every 2 h. In group 6, the animals wore soft corneal contact lens without any eye drops, whereby 6 eyes was removed of lens every 6 h for fluorescein staining and other 6 eyes stained 24 h later. In addition, 10 rabbits had their secretions cleaned away with cotton sticks soaked with normal saline and no eye drops were applied. Corneas were removed at 0, 2, 4, 6, 12, 18, 24, 30, 36 and 48 h for microscopic observation on corneal epithelial healing [2].

Results

The observation record showed the following mean healing time: 48.6 h in the normal saline group, 47.36 h in the vitamin A group, 45.18 h in the homologous serum group, 42.78 h in the MEBO group, 46.5 h in the dexamethasone group and 65.3 h in contact lens group. The results revealed that there was no significant difference of average healing rate between groups 1 and 4, as well as groups 2 and 4, groups 2 and 3, groups 3 and 5. However, there were statistical differences between other each two groups. MEBO, homologous serum, vitamin A, and dexamethasone had superior healing effects compared with normal saline, while wearing of contact lens retarded the healing of the defect. Beside homologous serum, MEBO is remarkably superior to other drugs in promoting corneal epithelial healing rate (table 55).

Discussion

Cornea has sources of nutrition from the vascular net in corneal limbus, tears and aqueous humor. Corneal epithelium is a functional barrier between tear membrane

and intraocular tissues through which the fluid output from the stroma is regulated in order to keep the stroma in a normal hydration. A corneal epithelial defect caused by injury or scraping off can repair rapidly in two stages. The latent period comes first, with a mean time of 5.5 ± 0.3 h during which extensive cellular and subcellular changes at the wound edges are expressed by desquamation of superficial cells, by loss of columnar appearance of basal cells, by damage on hemidesmosome link of the basement membrane as well as by formation of a cell process, indicating that residual viable epithelial cells are transforming into functional cells. A healing period is followed afterwards when epithelial cells around the wound migrate towards the center at a constant pace without mitochysis. This process starts from the uninjured epithelial cells adjacent to wound edge. We see basal cells in particular being enlarged and flatted with pseudopodium migrating towards the center and becoming the migratory edge of monolayer cells, which is followed by two or more layers of epithelial cells. The migration stops as wounds completely close and a firm hemidesmosome adhesion to the basement membrane is re-formed. At this point, mitochysis begins and mature corneal stratified epithelium is finalized.

The experiment revealed us the two periods for epithelial healing. At 2 h postsurgery, the defects did not decrease, instead, some increased from 8 to 8.1–8.5 mm, which may have resulted from the contraction of tissues at the wound edge and the shedding of injured cells. It was not until 6 h postsurgery that epithelial defects began to diminish progressively until complete wound healing was achieved. Systemic or local administration and either intra- or extra-ocular agents may be factors contributive to the successful repairing process of corneal epithelial defects from the latent period till the end of the healing period. By means of smearing, eye drops or wearing of corneal contact lenses, the study investigated various factors relative to epithelial healing in order to verify the therapeutic effects of methods and drugs.

MEBO is an ointment containing sesame oil, beeswax and other active ingredients extracted from plants such as *Cortex phellodendri* or *Radix scutellariae*. It is highly effective in the treatment of burns, wounds and ulcers and its advantages include superior analgesic effects, anti-inflammation, infection prevention and minimal scar formation post healing [3]. Although recent reports prove MEBO is significantly superior to other therapies in treating various burns, wounds and ulcers [4], there is no comparative study on the management of eye injury reported. Therefore, we conducted a preliminary comparative study on fibronectin (FN) and MEBO in the treatment of experimental corneal alkali burns in rabbits, which verified that MEBO was remarkably effective in the treatment of burns on avascular corneal tissue [2, 5]. Further,

we performed this study to investigate the effect of MEBO on repairing and healing of corneal defect and the roles of other drugs as well. The results verified that MEBO is obviously superior to other medications except for homologous serum in promoting wound healing while no obvious corneal macula was formed posthealing under slit-lamp microscopic examination.

The pharmacological mechanisms of MEBO with respect to its role of promoting healing of corneal wounds also became understood as follows: (1) Composed of a macromolecular sticky base, MEBO has an affinity to tissue protein at the wound site. The application of MEBO to the wound may serve as a bridge to directly stimulate and induce cell division and migration in an orderly manner that promotes wound healing. (2) MEBO contains various nutrients necessary for wound healing. For example, glucose is an obvious energy source. Vitamins and organic acids, which are related to the maintenance of tissue metabolism and proliferation of connective tissues, may directly support local nutritional needs, thereby preventing scar formation [6]. Zinc and enzymes may accelerate epithelial repair [7]. Protein as the basic element of the cell membrane may support the growth, differentiation and regulation of cells. (3) MEBO promotes the formation of a unique, integrated automatic drainage circulation system which corrects for local dysfunctional metabolism and circulation resulting from injury. The base ingredients contained in MEBO absorb metabolic products from the wound and then transport them to the outer layer of the ointment. Meanwhile, active ingredients of the ointment continuously penetrate into the wound to renew the supply of ingredients necessary for tissue repair. The automatic microparticle transportation and processing of emulsification and dispersion are considered as the main measure for the treatment of injured avascular tissue [8]. (4) Obaculactone contained in MEBO offers properties of anti-inflammation, detumescence, analgesic effects, and enhancing local immunity and controlling infection.

Many reports demonstrate the presence of FN in plasma. FN is a macromolecular glycoprotein that is the conjunctive medium between cell and extracellular fibers and matrix. It adheres to collagen, polysaccharide protein, and receptors on the cell surface and serves as an intercellular bridge of epithelial cells. It has some correlation with cytoskeleton structures, e.g. microfilament, actin, to induce the migration of the cell. FN plays an important role in the firm adhesion between migrating epithelial cells and wound surface of corneal epithelial defect. Eye chemical burns can be treated with eye drops or subconjunctival injection composed of autoblood. Autoblood is beneficial because it contains macroglobulins that inhibit collagenase, release fibrinolysin (which may reduce symblepharon, thereby promoting the recovery of the blood ves-

sel net around the cornea as well as restoring sensation in the injured cornea), improve corneal nutrition and promote tissue regeneration. Our previous study has verified that FN can speed up the migration of corneal epithelial cells [2]. Observation on the rabbit corneal epithelial healing rate after application of homologous serum in this study has also suggested an obvious effect of serum in promoting the migration of corneal epithelial cells. However, even serum offers less benefit than does the application of MEBO.

Various reports can be found about the role of corticosteroids in corneal wound healing. It is believed that long-term administration of corticosteroids at high concentration may retard epithelial regeneration and that stromal wound healing though the mechanism remains unclear. On the other hand, the use of corticosteroids immediately after corneal burns may have good anti-inflammatory effects and reduce occurrence of ulcers and neogenetic vascularization [9]. Others report that corticosteroids may inhibit conjunctival cells from migrating towards the corneal surface without impairing the reformation of corneal epithelium [10]. In this study, eye drops of 0.5% dexamethasone did not inhibit the migration of corneal epithelial cells and wound healing. On the contrary, it significantly promoted the healing. Therefore, it is feasible to drop 0.5% dexamethasone on simple corneal epithelial defects to control inflammation and promote healing, though even this modality, due to adverse effects, is far inferior to the effects achieved by MEBO.

Vitamin A plays a role in promoting epithelial growth and maintaining epithelial normal functions. Many reports have confirmed vitamin A contributing to promote wound healing, but some researchers reported that vitamin A failed in promoting epithelial regeneration. The use of 25,000 U/ml vitamin A in this study revealed little effect on corneal epithelial healing.

Corneal epithelium, when wearing corneal contact lenses made of polymethyl methacrylate (PMMA), is provided with nutrients and oxygen necessary for metabolism which derive primarily from tears penetrating between the lens and cornea. Eyelid pressure when eyes are open produces a positive pressure behind the lens to discharge tears and the negative pressure produced behind the lens after winking allows the entrance of the tears to float the lens. Such a process goes in cycles to achieve the exchange and renewal of tears. Subsequent to corneal epithelial injury, the ability of local metabolism decreases and wearing lenses further impairs oxygen absorption of the cornea, which is unfavorable to wound healing. Though some reports suggested that wearing corneal contact lenses might be used for treating corneal ulcer, the observation in this study verified that such management impaired the corneal wound-healing process and healing rate was much lower than those in other groups. We con-

clude that careful attention should be taken to avoid harming patients through the application of corneal contact lenses in the treatment of corneal injury.

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Exploration of Pathological Changes and Mechanism of Experimentally Burned Rabbits after Treatment with Moist-Exposed Burns Ointment

Introduction

BRT with MEBT/MEBO, available over the past 10 years, is a remarkable innovation for the management of burns, wounds and ulcers. Satisfactory results were obtained using this treatment to deal with profound problems in conventional surgical treatment such as pain, infection, healing with scar formation, and progressive necrosis of tissues in the zone of stasis. Clinical applications worldwide demonstrated that BRT with MEBT/MEBO is superior to all other therapies and represents the clear standard of care in burns treatment. To verify the therapeutic effect of MEBO in treating burns wounds, we studied a rabbit model of deep second-degree burns treated with MEBO and with Vaseline, respectively. Serial histological sections were performed during the treatment in order to observe the pathomorphological changes, progression and mechanism of repair. This study provides references for the prevention and research of burns, wounds and ulcers.

Material and Method

Thirty healthy adult rabbits of either sex weighing 1.5–2.0 kg were used in this study. The dorsal hair of each animal was depilated using 20% sodium sulfide. Rabbits were restrained in a self-made soaking support frame, and two 4 × 4 cm deep second-degree wounds of zygomorphic skin on the back were created via scalding with 100°C water for 5 s (lesions were determined by pathological examination). The wounds were then contaminated with 1 ml suspension containing 3.0×10^8 cfu *Staphylococcus aureus*. At this point, the animals were divided randomly into two groups, 15 animals offering 30 wounds in each group. Animals in the control group were treated with Vaseline ointment, while animals in the experimental group were treated with MEBO ointment. Both ointments were applied once every 3 h. The rabbits were caged separately and freely fed. At six different time phases (days 3–6, 7–9, 10–12, 13–15, 16–18 and 19–22 postburn), five full-thickness wound tissues (0.5 × 0.5 cm) were taken from each group. All samples were fixed with 10% formaldehyde solution, embedded with paraffin, stained with hematoxylin and eosin, and studied by the light microscope for pathomorphological changes.

Results

Normal rabbit skin demonstrated an absence of dermal papilla, but revealed abundant structures of skin hair and appendages (fig. 21a). Deep second-degree burns wounds on rabbit back skin involved deep dermis causing necrosis of full epidermis and partial dermis. Fibers in the dermis reticular layer and the subcutaneous layer appeared to be thick and sparse with partially survived skin appendages (fig. 21b). The results of pathomorphological examinations of two groups at different phases are shown in table 56.

Conclusion

BRT with MEBT/MEBO treatment can make injured tissue regenerate in a relatively physiological environment that conforms to the natural law of tissue regeneration. As a result, scar formation is reduced to the maximum extent. These experimental results were in accordance with clinical observations.

Discussion

Histologically, the regenerative capacity of skin tissue has a close correlation with tissue repair. Skin cells can be classified into two categories according to the different capability of regeneration: (1) Constantly changing cells, i.e. epidermal cells that have the ability to divide for indefinite periods under a normal state and proliferate to compensate shed and consumed cells. (2) Stable cells as epithelium in the skin body of a gland that cease proliferation when the organs mature, but have a continuous potential for division which is activated after injury to regenerate [1]. Tissue repair is also achieved by two

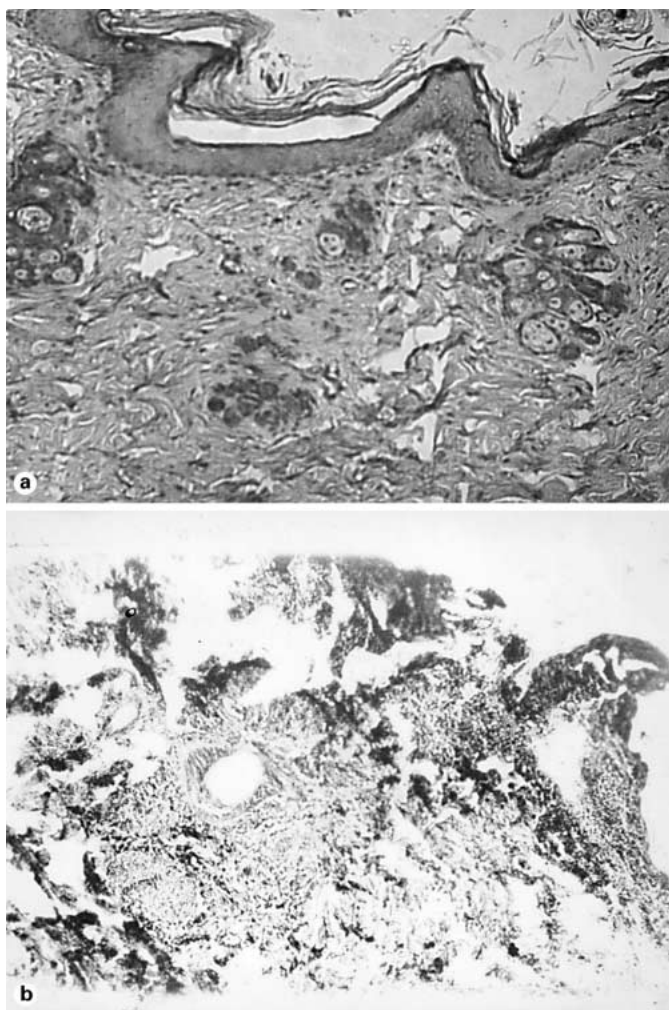


Fig. 21. **a** Normal rabbit skin. HE. $\times 100$. **b** Deep second-degree burns wound on rabbit back skin with partially survived skin appendages, epidermis exfoliated. HE. $\times 100$.

approaches. First, by the regeneration of tissues similar both in structure and function – the structure and function of repaired tissue can be entirely identical to those of the original [2]. Take, for example, the tissue repair of superficial second-degree burns wounds. On days 3–4 postburn, epithelia began to grow, and continued thusly on days 5–8 and was mostly completed on days 8–10 postburn. Secondly, repair of the damaged tissues can be achieved through the formation of fibrous tissue, beginning with formation of granular tissues and ending with scar formation. Microscopic examinations of the tissue repair of deep second- and third-degree burns wounds showed an intertexture mainly comprised of fibroblasts and neof ormative blood capillaries. Together with infiltration of plasmocytes (such as neutrophil, lymphocyte, plasma cell, macrophage), we noted neof ormative granu-

Table 56. Summary of pathomorphological changes at different time phases postburn in both the Vaseline and the MEBO groups

Days postburn	Pathomorphological characteristics
<i>Control group (Vaseline)</i>	
3–6	Diffusion inflammation, visible infiltration of many inflammatory cells aggregating beneath epidermis layer (fig. 22a)
7–12	Massive infiltration of inflammatory cells, few macrophages, few new blood capillary formations, narrow lumen, poor wound healing with proliferation of fibrous tissue (fig. 22b)
13–18	Scattered colonies, tissue necrosis patency tending to worsening (fig. 22c)
19–22	Infiltration of inflammatory cells, wound tissue edema, disruption of collagen fibers, marked proliferation of fibrous tissue, progressing to form hyperplastic scars (fig. 22d)
<i>Experimental group (MEBO)</i>	
3–6	New epithelial regeneration on wound, increase of blood capillaries, active regeneration of granulation tissue, inconspicuous proliferation of collagen fiber (fig. 23a)
7–9	Active regeneration of epithelia, increased large basal layer cells, new skin regenerating in varied thickness (fig. 23b)
10–12	Epidermis regenerating from residual skin appendages, visible transition from skin appendages to regenerated epithelia (fig. 23c)
13–15	Gradual decrease of necrotic tissues, contracting to wound surface (fig. 23d)
16–18	Presence of a lot of macrophages in neof ormative granulation tissues, limited inflammatory cells (fig. 23e)
19–22	Wounds covered by squamous epithelia and presence of a few skin appendages in the dermis (fig. 23f)

lation tissues that were then replaced by a great quantity of closely aligned collagenous intercellular fibers. Subsequent to the decrease of fibroblasts, showing a long and narrow shape, and of blood capillaries, these tissues eventually developed into scars.

According to our experimental results, in the control group there was sparse blood capillary formation with narrow lumen. The tissue was swollen with fiber proliferation and massive infiltration of inflammatory cells. The disruption of collagen fiber and absence of regenerated epidermis to cover wounds eventually resulted in wound healing by eschar (showed in fig. 22d). In the experimental group treated with MEBO, residual skin appendages regenerated into epidermis with multilayers and large nuclei that progressed and covered the wounds (fig. 23b–d). Granulation tissue was promoted into regenerative tis-

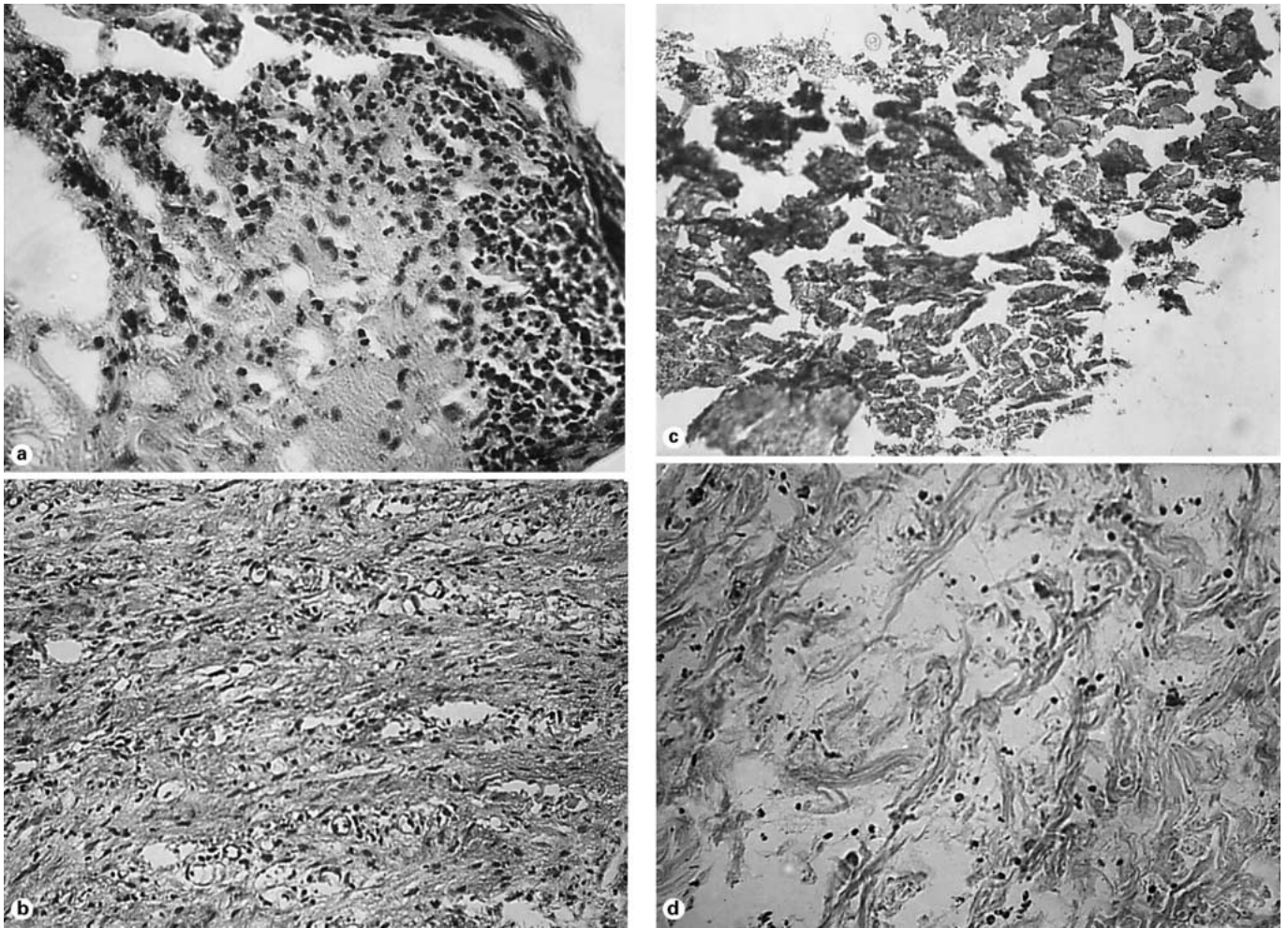
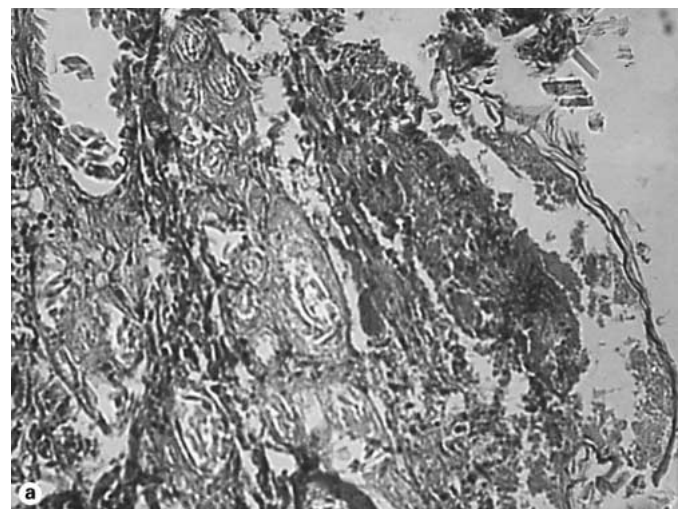


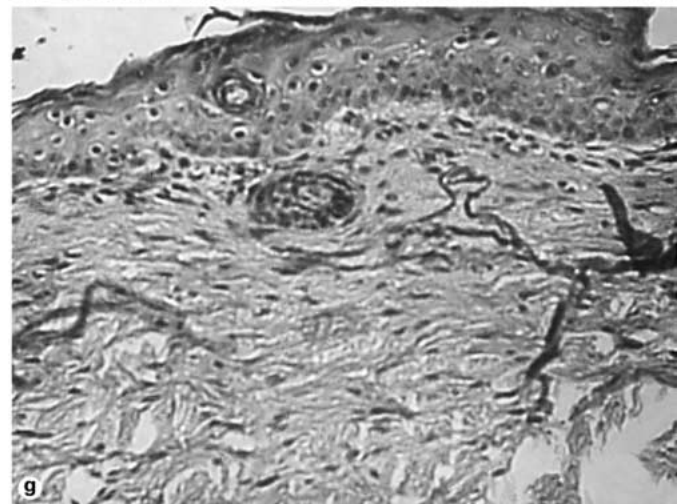
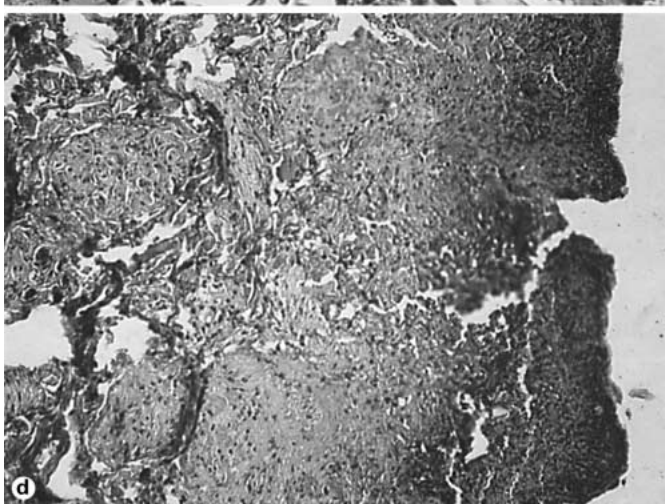
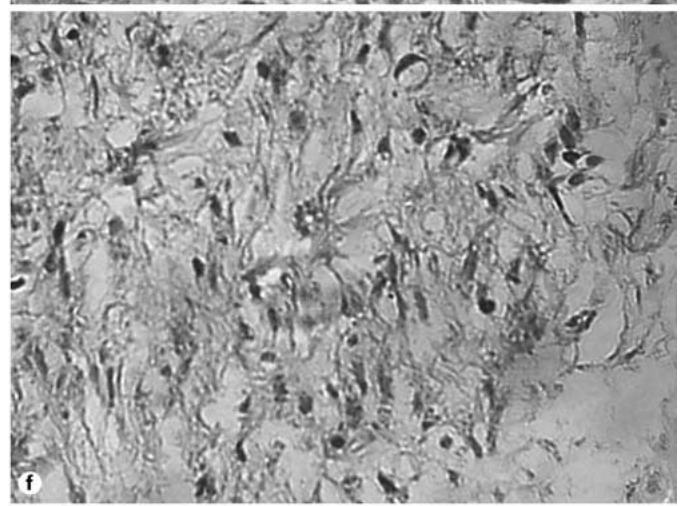
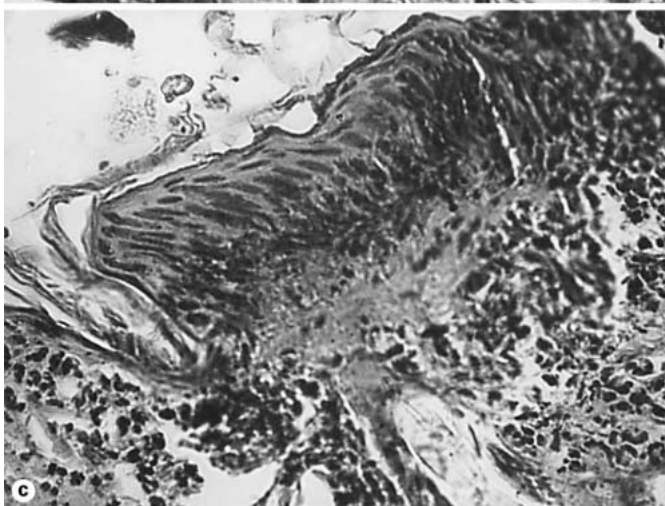
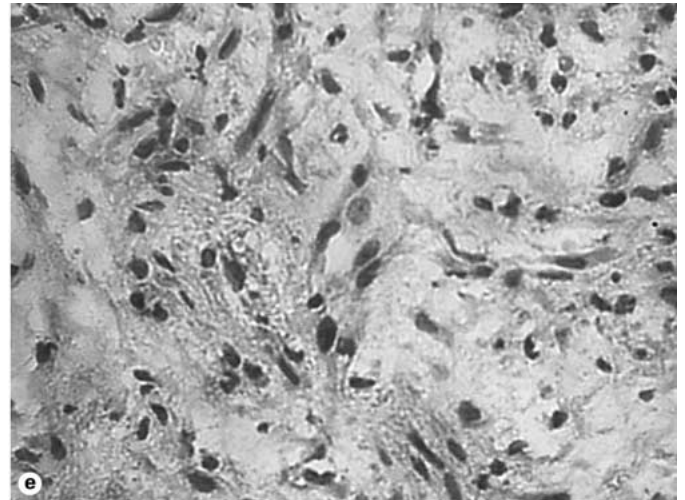
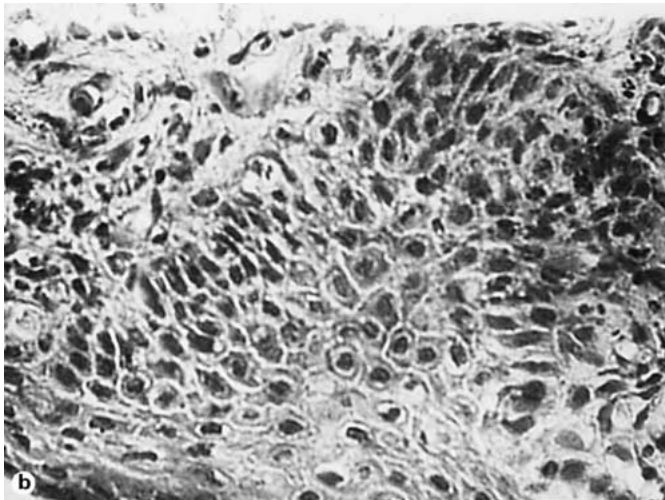
Fig. 22. **a** In the control group 3–6 days postburn, the wound showed diffusion inflammation, visible infiltration of much inflammatory cells aggregating beneath epidermis layer. HE. $\times 200$. **b** In the control group 7–12 days postburn, there was some new blood capillary formation, narrow lumen, poor wound healing with proliferation of fibrous tissue. HE. $\times 100$. **c** In the control group 13–18 days postburn, colonies were scattered, tissues obviously necrosed. HE. $\times 100$. **d** In the control group 19–22 days postburn, marked proliferation of fibrous tissue and progressing hyperplastic scars is seen. HE. $\times 100$.

Fig. 23. **a** In the MEBO group 3–6 days postburn, new epithelial regeneration on the wound, increase of blood capillaries, and active regeneration of granulation tissue can be seen. HE. $\times 100$. **b** In the MEBO group 7–9 days postburn, epithelia regenerated actively, basal layer cells became larger, and new skin regenerated in varied thickness. HE. $\times 400$. **c** In the MEBO group 10–12 days postburn, epidermis regenerated from residual skin appendages to regenerated epithelia. HE. $\times 400$. **d** In the MEBO group 13–15 days postburn, necrotic tissues gradually decrease and contracted to wound surface. HE. $\times 100$. **e, f** In the MEBO group 3 weeks postburn, there were a lot of macrophages in neoformative granulation tissues, limited inflammatory cells. HE. $\times 400$. **g** In the MEBO group 4 weeks postburn, wounds were covered by squamous epithelia and there were a few skin appendages in dermis. HE. $\times 200$.



sue; neoformative blood capillaries were enhanced more than in the control group with larger lumen and richer blood supply, both of which facilitate an enhanced metabolism (fig. 23a). Finally, wounds in the experimental group were covered by squamous epithelia and healed without scarring (fig. 23g). We noted that when burns

wounds were treated by BRT with MEBT/MEBO, the perpetually changing cells began to divide and proliferate toward the wound center along the wound edges or the basal part of residual epithelia, whereas after burns injury, the stable cells residing in skin appendages and granular epithelium were activated to divide and regenerate into



epidermic tissue that in turn migrated toward and finally closed the wound. Pathological examination on days 10–12 postburn showed a transitional migration from skin appendages to regenerated epithelia. When epithelia proliferated and divided, wounds were covered by stratified squamous epithelium. The macroscopic appearance of healed wounds was initially red or pink and progressively became normal in color.

In clinical management, we observed the elevation of residual hair follicles and skin islands over the wound surface when deep second-degree burns wounds were treated with MEBO. Epithelial tissue was the first to achieve the same height as the wound, followed by skin islands or connective tissues among the hair follicles. Eventually, we noted wounds healed by epithelialization with mild or no scarring. We suggested, according to clinical observation and pathological examination, that if the dermis network and Leydig cells (interstitial cells of Leydig) are kept intact, then as regards the treatment of deep burns, epithelia regenerated from skin appendages might grow along the network and eventually recover the normal dermal architecture without scar formation. However, if the dermis network, Leydig cells and granular epithelia were damaged, then the residual granular epithelia might form a cell mass with a disordered structure and fail to recover the normal structure and function of the skin architecture [3]. This is the case in sweat gland epithelia in adipose tissue that regenerated and divided into nonsecretory epithelial tissues to close and heal wounds.

Wounds in the control group (Vaseline) showed slow repairing, obvious proliferation of fibrous tissue and healed with hyperplastic scars. By comparison, wounds in the experimental group (MEBO) expressed rapid repairing, active growth of neof ormative epidermis, inconspicuous proliferation of fibrous tissue and eventually healed without scarring. These results demonstrate that MEBO retains optimal wound moisture, while tissue is not immersed. MEBO created a drug membrane that protected and isolated wound tissue from outer contaminants, allowing native histocytes to propagate in a relatively physiological environment in accordance with the nature regenerative law of skin. Local microcirculation was also improved and pathological changes of three zones of burns wounds (necrosis zone, stasis zone and hyperemia zone) were reversed. These conditions were favorable to the recovery of tissue in the stasis zone. Therefore, MEBO was believed to promote epithelial regeneration, control the increased speed of connective tissue, and keep epithelia and connective tissue in an almost normal rate of proliferation so as to heal deep burns wounds with less or minimal scarring.

In the experiment, wounds were contaminated by *S. aureus*. Microscopic observation showed massive infiltration and aggregation of inflammatory cells in the Vase-

line group with few macrophages and scattered colonies without boundaries (fig. 22c). All wounds were visibly infected within 1 week. In the MEBO group, inflammatory cells were large in quantity with enhanced capacity of anti-infection (fig. 23e). Gross observation revealed that wounds in this group repaired rapidly with absence of inflammatory response such as red swelling. It was believed that MEBO demonstrated efficacy in promoting the blood circulation by removing blood stasis, clearing away heat and toxic material, relieving inflammation and removing the necrotic tissue while promoting granulation. The experiment also demonstrated that MEBO might inhibit or kill the growth of *S. aureus*.

In this study, the rabbit burns model was kept stable with zero mortality. Light-microscopic examination revealed a distinct process of histocyte repair. The results showed that the application of BRT with MEBT/MEBO in burns management could prevent and control infection, promote wound repair, minimize scar formation, shorten healing time, avoid complications and relieve pain as well. BRT with MEBT/MEBO also has the advantages of facilitating the observation of wound repair and easy application. BRT with MEBT/MEBO is now irrefutably considered to be the standard of care for burns management worldwide.

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Electron-Microscopic Observation of One Case of Skin Burns Wounds Treated with MEBO

Introduction

To further investigate the mechanism of deep burns wounds healing without hyperplastic scar formation after treatment with BRT with MEBT/MEBO, we took a biopsy from a deep wound site of a severely burned child before and after treatment in order to observe it via light and transmission electron microscopy. The aim of the study was to find the histological evidence of scar-free healing.

Case Report

A 12-year-old boy was admitted (PID. 172650) on November 4th 1989 after suffering direct gas flame burns on the face, trunk and extremities. Clinical assessment indicated a total burns surface area (TBSA) of 75%, including 45% second-degree and 30% third-degree wounds. The condition of the patient remained stable during anti-shock therapy, but he developed sepsis on day 6 postburn. Serial blood cultures $\times 3$ were negative. On day 10 postburn, escharectomy and microskin grafting were performed on the left upper and right lower extremities. On day 20, excision and microskin grafting were performed on the back again. On day 30, burns wounds of the right leg and dorsum pedis with mixed second- and third-degree as well as deep second-degree burns on the back were treated with BRT with MEBT/MEBO. Wound tissue biopsy was taken from the right leg before and after treatment, then pathological examinations were carried out light and transmission electron microscopically.

Result

Pathological examination revealed satisfactory healing of the burns wounds treated with MEBO without formation of obvious hyperplastic scar tissue.

Light Microscopy

Before treatment, the infiltration of inflammatory cells was visible around sweat glands and hair follicles, some having formed local foci (fig. 24a, b). After treatment, skin recovered to normal structure with regenerative capillaries and fibroblasts in dermis (fig. 24c).

Transmission Electron-Microscopic Observation

Before treatment, a lot of circular vacuoles were present in the surrounding nucleus that showed irregular nuclear membrane with disappearance of nucleolus. Elastic fibers in the dermis varied in thickness and had a disorderly arrangement with deposits in the lumen (fig. 25c). After treatment, cells in the stratum spinosum became regular, showing distinct nucleus, clear nucleolus and uniform distribution of nuclear chromatin. Desmosomes of the intercellular bridge recovered to normal (fig. 26a–c).

Conclusion

The results proved that after using MEBO, the burns wounds healed without formation of macroscopic hyperplastic scar. The ultrastructure of the healing burns wound was similar to that of an ordinary traumatic wound.

Discussion

In dermis of normal skin, the dominant cell relating to traumatic repair and proliferative inflammation is the fibroblast. It is located adjacent to a collagenous fiber bundle, showing as fusiform, stellar or polygonal shapes, and having thick and short cell process. The fibroblast

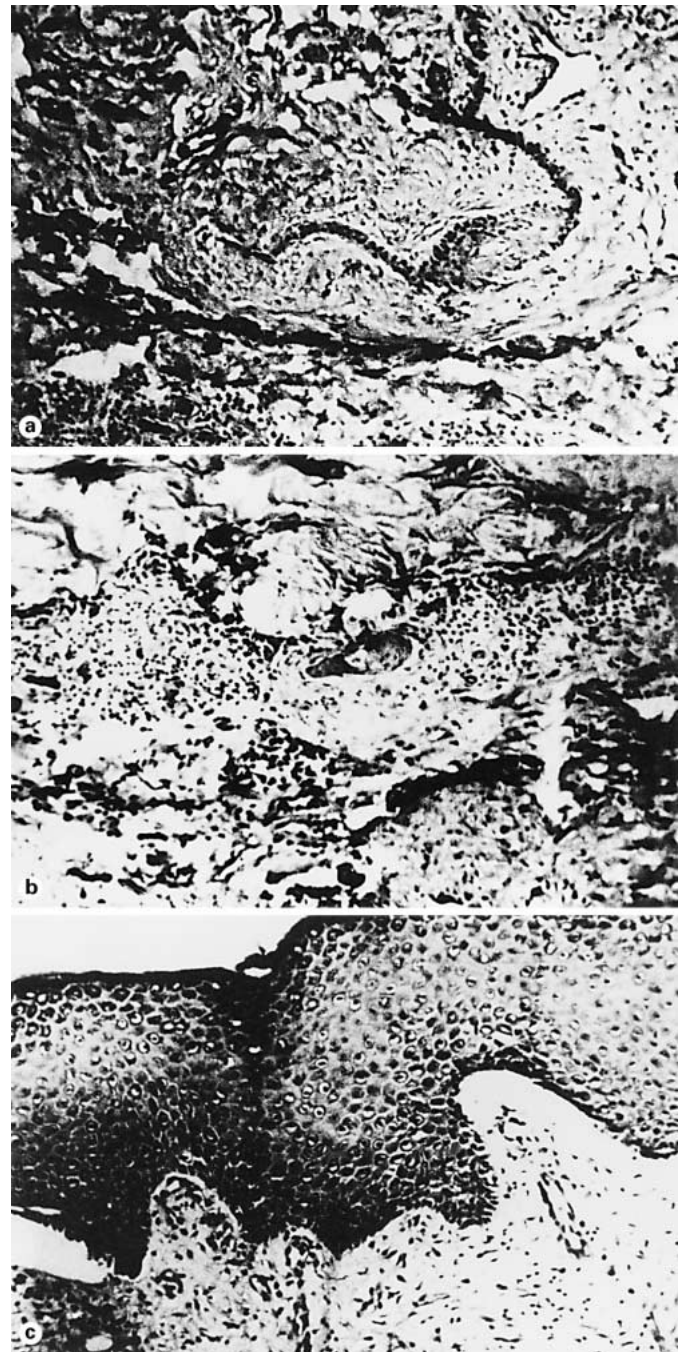


Fig. 24. **a** Before MEBO treatment, the infiltration of many neutrophils was visible around subcutaneous hair follicles, some having formed local foci. HE. $\times 200$. **b** Before treatment, infiltration of inflammatory cells was visible around sweat glands and hair follicles. HE. $\times 200$. **c** After MEBO treatment, epidermis recovered to normal structure and there was the presence of regenerative capillaries and fibroblasts in the dermis. HE. $\times 200$.

contains an oval nucleus which occupies one third of whole cell. It also reveals an obvious nuclear membrane and one or two nucleoli. There is expanded lumen of intracytoplasmic rough endoplasmic reticulum (RER). There are four major types of cell junctions between epi-

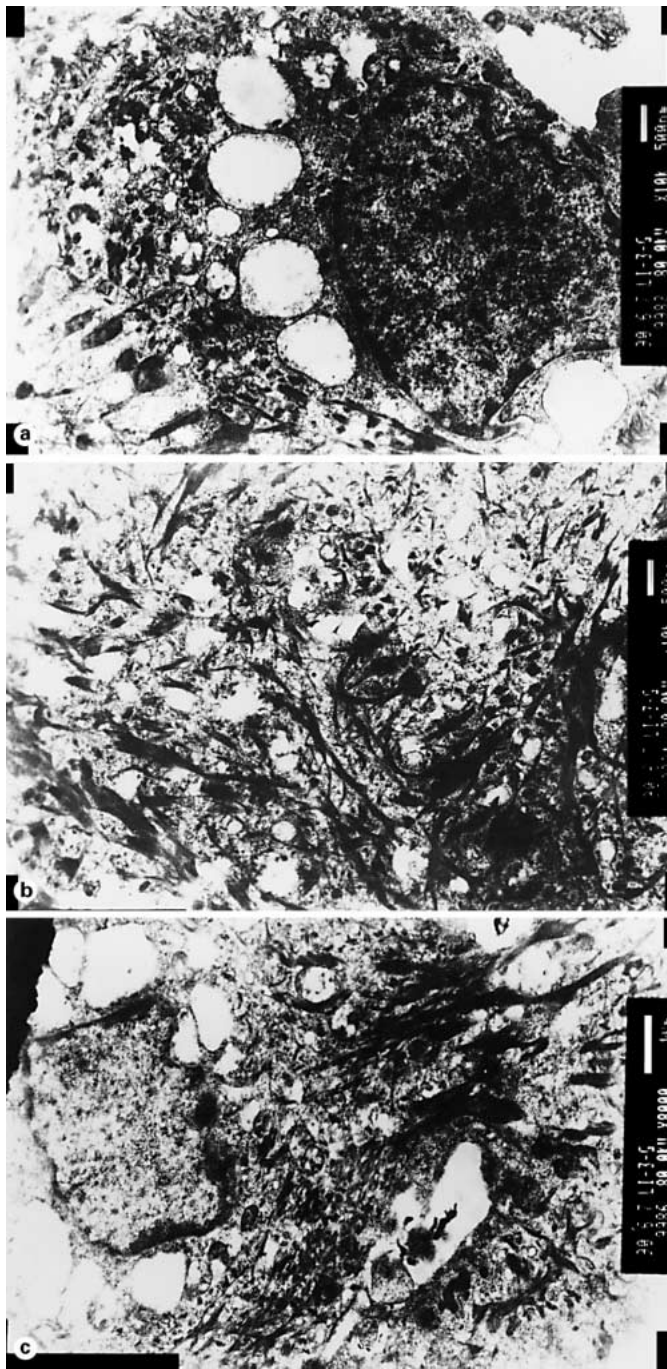


Fig. 25. a Before MEBO treatment, many circular vacuoles were present in the surrounding nucleus that showed irregular nuclear membrane with disappearance of nucleolus. TEM. $\times 10,000$. **b** Before treatment, elastic fibers in dermis were in varied thickness and disorderly arrangement with vacuolar degeneration. TEM. $\times 10,000$. **c** Before MEBO treatment, appearance of irregular nucleus, presence of perinuclear vacuoles and disordered elastic fibers with deposit. TEM. $\times 8,000$.

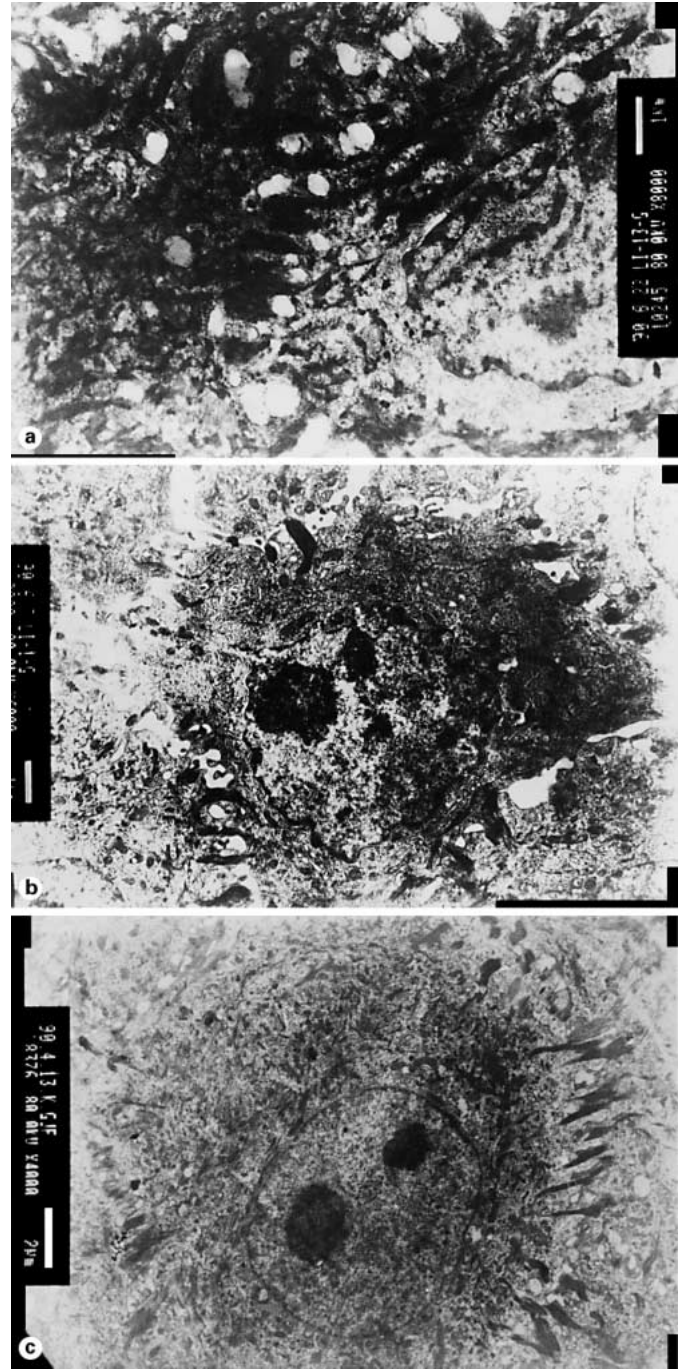


Fig. 26. a After MEBO treatment, intercellular bridge of cells in stratum spinosum recovered to normal with distinct nucleus and clear nucleoli. TEM. $\times 4,000$. **b** After treatment, desmosome of cell junction almost recovered to normal with clear shape of cell, regular nucleus and uniform distribution of euchromatin. TEM. $\times 6,000$. **c** After MEBO treatment, structure of desmosome recovered to normal with uniform distribution of nuclear chromatin and regular cell shape with nucleolus in center. TEM. $\times 5,000$.

thelial cells, i.e. tight junction, intermediate junction, gap junction and desmosome.

This study showed that perinuclear vacuoles, disordered elastic and collagenous fibers were presented before MEBO treatment, comparing to desmosome of intercellular bridge recovering to normal structure after MEBO treatment. When in the hyperfunction stage, the intracytoplasmic RER appeared as small fragmental vesiculi-form, and when in vigorous synthesization, it appeared tight with flocculation within the cisternae. The main function of the RER is to synthesize protein. Smooth endoplasmic reticulum (SER) has functions correlating with the synthesis of lipoids and steroids.

Stephen [1] reported the presence of myofibroblasts in hypertrophic scar tissue according to electron-microscopic observation. Myofibroblast contained incomplete nuclear membrane with developed RER. As it has both the characteristics of smooth muscle cells and the shape of fibroblasts, it is also termed 'modified myofibroblast'. We have reported the ultrastructure of scar resulting from burns injuries in 1985 [2]. Comparison of the previous and present studies indicates that no macroscopic hypertrophic scar was formed on burns wounds treated with BRT with MEBT/MEBO, and the ultrastructure of the healing burns wound appeared no different from the ordinary traumatic wound. Though we have previously reported the clinical experience of applying MEBO for treating burns wounds of varying degrees [3], this was our first presentation of light-microscopic and transmission electron-microscopic observations regarding burns wounds. We would like to disclose our research achievements here in order to stimulate further studies.

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Pathomorphological Changes of Deep Burns Wounds Treated with MEBO

Introduction

BRT with MEBT/MEBO has been in wide use for many years domestically and internationally [1, 2]. Although many clinical practices have confirmed its advanced and scientific results in burns management, the

mechanism involved in the healing of deep burns wounds without hypertrophic scar is not yet clear. From March to November 1994, the authors treated 12 patients sustaining deep burns with MEBO and performed light- and electron-microscopic observations on wounds before and after MEBO treatment. The aim was to explore the therapeutic effectiveness of MEBO on the healing of deep burns wounds.

Materials and Methods

Twelve patients sustained 2-98% total body surface areas (TBSA), including 2-82% third-degree burns. Most patients had burns mainly on the face and extremities and one patient was complicated with inhalation injury. Some patients suffered extremely severe burns covering the whole body skin. Areas which received MEBO treatment included chest, back, upper arm, thigh, leg, and instep. MEBO was applied on burns wounds in accordance with the standardized MEBO protocol.

All of these patients were initially treated by conventional surgical therapy in other hospitals. Therefore, this study focused on deep second-degree and superficial third-degree burns wounds with intermediate and late granulation tissue on days 3-42 postburn that were treated with MEBO. The ointment application lasted as long as 5-50 days and the wound healed on days 30-92 postburn. Macroscopic observation of healed wounds showed the coverage of soft, flat and smooth epithelium or soft and flat scars, without the appearance of hypertrophic or contractive scars.

Consent to receive MEBO treatment was obtained from patients or their guardians. Two wound tissue samples 1-2 mm³ in size were taken from each patient before and after treatment. One of the samples was fixed with 75% alcohol before being transferred to the pathology department for hematoxylin and eosin staining and light-microscopic examination (Olympus). The other was fixed with 2.5% glutaraldehyde and 1% osmic acid, stained with uranium acetate and lead citrate, and gradient dehydrated with ethanol and acetone. The ultrathin sections were examined under a transmission electron microscope (CM 10, Philips).

Results

Light Microscope

The third-degree burns wounds penetrated to beneath the dermis and subcutaneous tissue that appeared as uniform pink necrosis. Some of the muscular tissue was also involved, where cross-striation of striated muscle disappeared and had the appearance of the pink color of coagulated necrosis. Infiltration of inflammatory cells presented around the sweat glands and hair follicles. During the course of treatment, collagenous fibers were found to proliferate severely, showing thick and disordered arrangement at the beginning. These progressed to moderate proliferation with thin fasciculi, and finally had the appearance of being delicate and orderly. After healing, the epidermis recovered to normal, and neoregenerated blood capillaries and fibrocytes appeared in the dermis (table 57).

Electron Microscope

Before treatment, fibroblasts showed disrupted karyomorphism, contracted nucleoli, expanded perinuclear space and paranuclear vacuolar degeneration. A faint staining area was observed in the paranuclear margin of mussels arranged fibrocytes. Collagenous fibers varied in thickness with breaking and dissolution. After MEBO

treatment, the nucleoli and nuclei of fibroblasts recovered to normal and collagenous fibers appeared to be uniform in thickness and orderly in arrangement. Master cells were occasionally visible after treatment in a few cases. Fibrocytes recovered to normal with orderly arrangement and intracytoplasmic rough endoplasmic reticulum appeared (table 57).

Table 57. Profile of granulation tissues present on deep burns wounds of 12 cases treated with MEBO

No.	PID	Sex/age (years)	TBSA/ third-degree, %	Site and depth of MEBO application	Days post-burn for first biopsy	Duration of MEBO treatment	Days post-burn for second biopsy	Appearance of healed wounds
1	245563	M/28	98/82, inhalation injury	beneath clavicle in left chest, deep second-degree	42	50	92	flat, soft texture
2	255409	M/37	9/2	anklebone of left foot, deep second-degree	3	33	36	flat and smooth, no disablement (fig. 27a, b)
3	254161	F/6	65/18	left thigh and chest, deep second-degree	35	31	66	flat and thin scarring (fig. 28a, b)
4	255970	M/38	59/13	left forearm, deep second-degree	30	15	45	flat and smooth, soft scar (fig. 29a, b)
5	259202	M/21	70/6	right thigh, deep second-degree	28	5	33	thin scar in soft texture (fig. 30a, b)
6	259203	M/23	10/1.5	left lower extremity, deep second-degree	28	22	50	thin scar in soft texture (fig. 31a, b)
7	258466	M/35	68/4	left upper arm, superficial third-degree	18	21	39	flat, smooth with slightly hard scar (fig. 32a, b)
8	257211	M/27	10/0	right upper arm, deep second-degree	10	20	30	flat (fig. 33a, b)
9	154082	M/28	92/80	back, superficial third-degree	36	18	54	thin scar in soft texture
10	172650	M/12	75/30	instep of right foot, mixed degree	30	15	45	flat, smooth and soft (fig. 34a, b)
11	263623	M/20	2/0	both feet, superficial third-degree	5	31	36	flat and smooth
12	261873	M/62	2/0	both feet, superficial third-degree	30	14	44	flat and smooth (fig. 35a, b, 36a, b)

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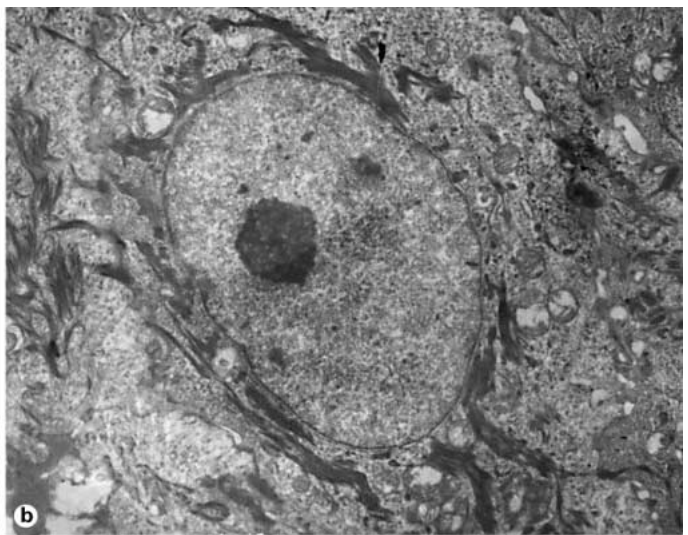
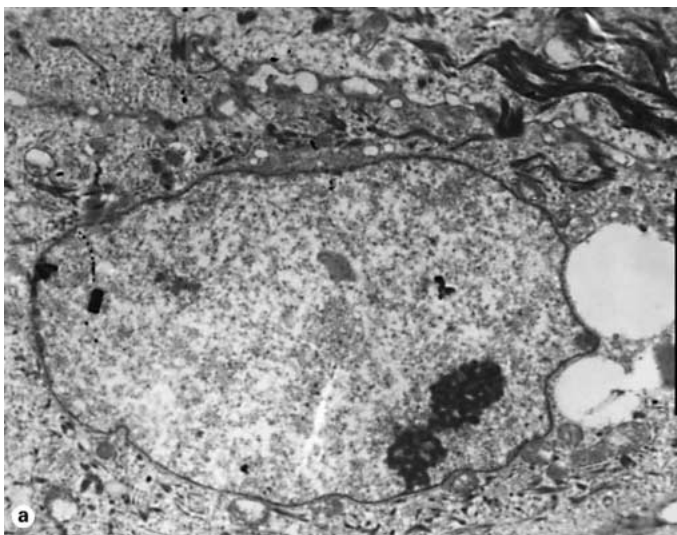
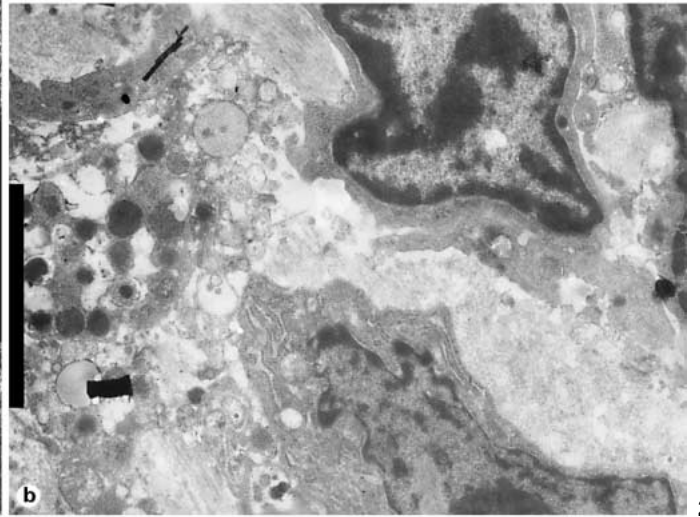
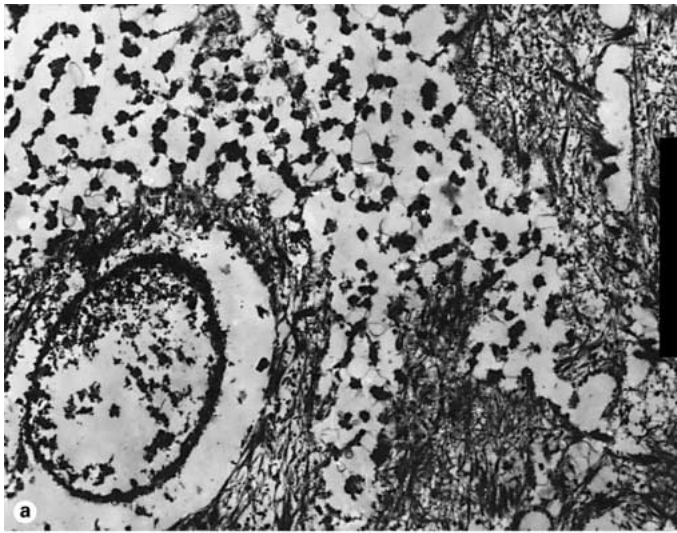
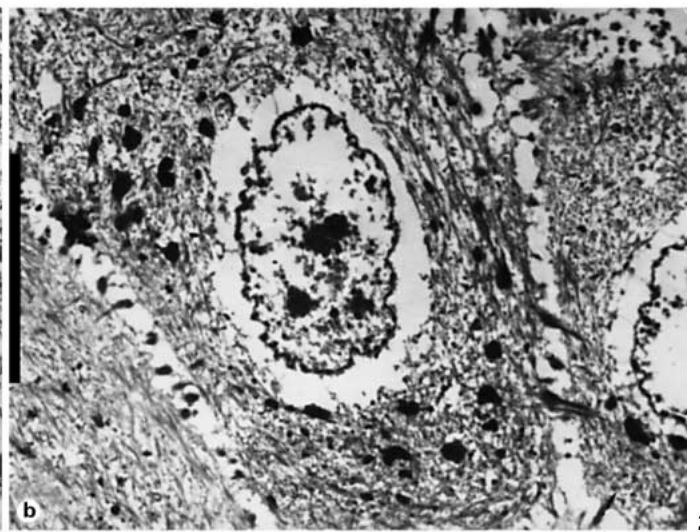
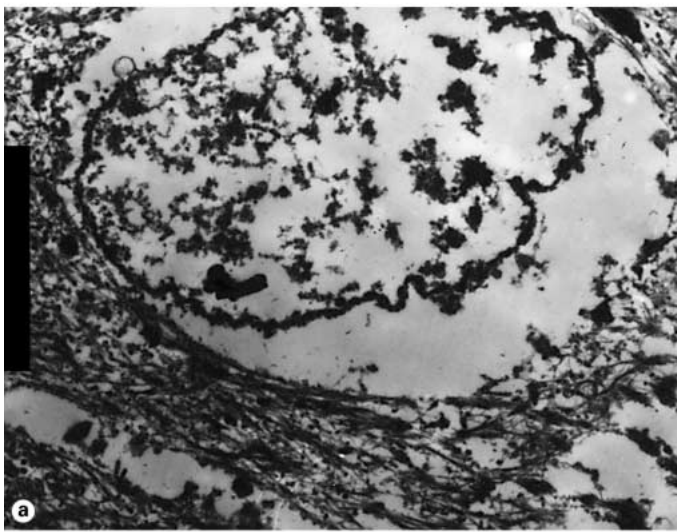


Fig. 27. Case 2. **a** Before treatment, fibroblast showed disrupted nuclear membrane, contracted nucleolus and intracytoplasmic vacuoles. **b** After treatment, fibroblast recovered to normal with central nucleolus. Collagenous fibers were orderly arranged.

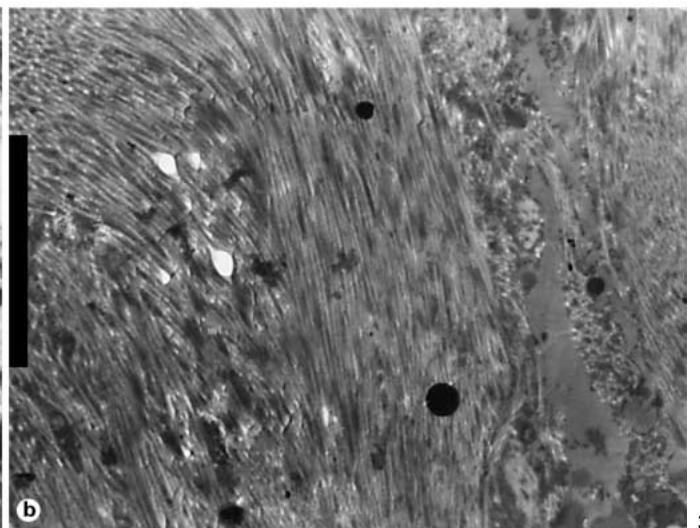
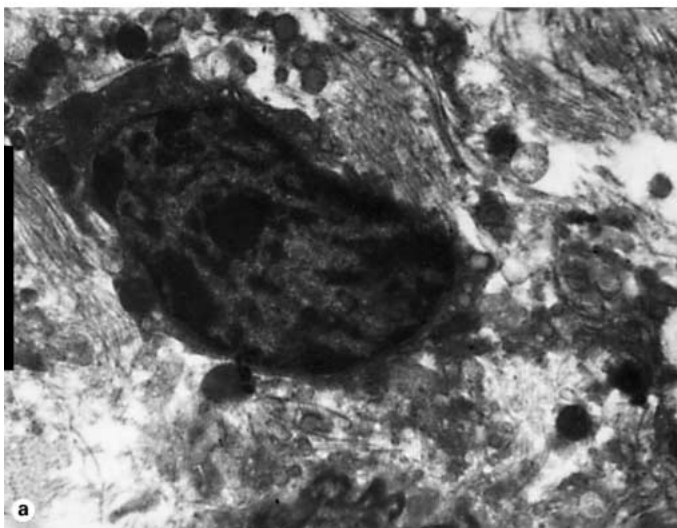
Fig. 28. Case 3. **a** Before MEBO treatment, appearance of fibroblast with expanded perinuclear space, paranuclear light staining areas and space. Intracytoplasmic collagenous fibers were dissolved and necrosis. **b** After MEBO treatment, presence of special granule in mast cells in small quantity.



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Fig. 29. Case 4. **a** Before treatment, fibroblast showed disrupted karyomorphism with loose chromatin. Paranuclear light staining appeared. Intracytoplasmic collagenous fibers were disrupted and dissolved. **b** After treatment, karyomorphism almost recovered to normal and intracytoplasmic collagenous fibers were uniform in thickness.

Fig. 30. Case 5. **a** Before treatment, intracytoplasmic collagenous fibers varied in thickness and were disorderly arranged. **b** After treatment, collagenous fibers were uniform in thickness and aligned in order.

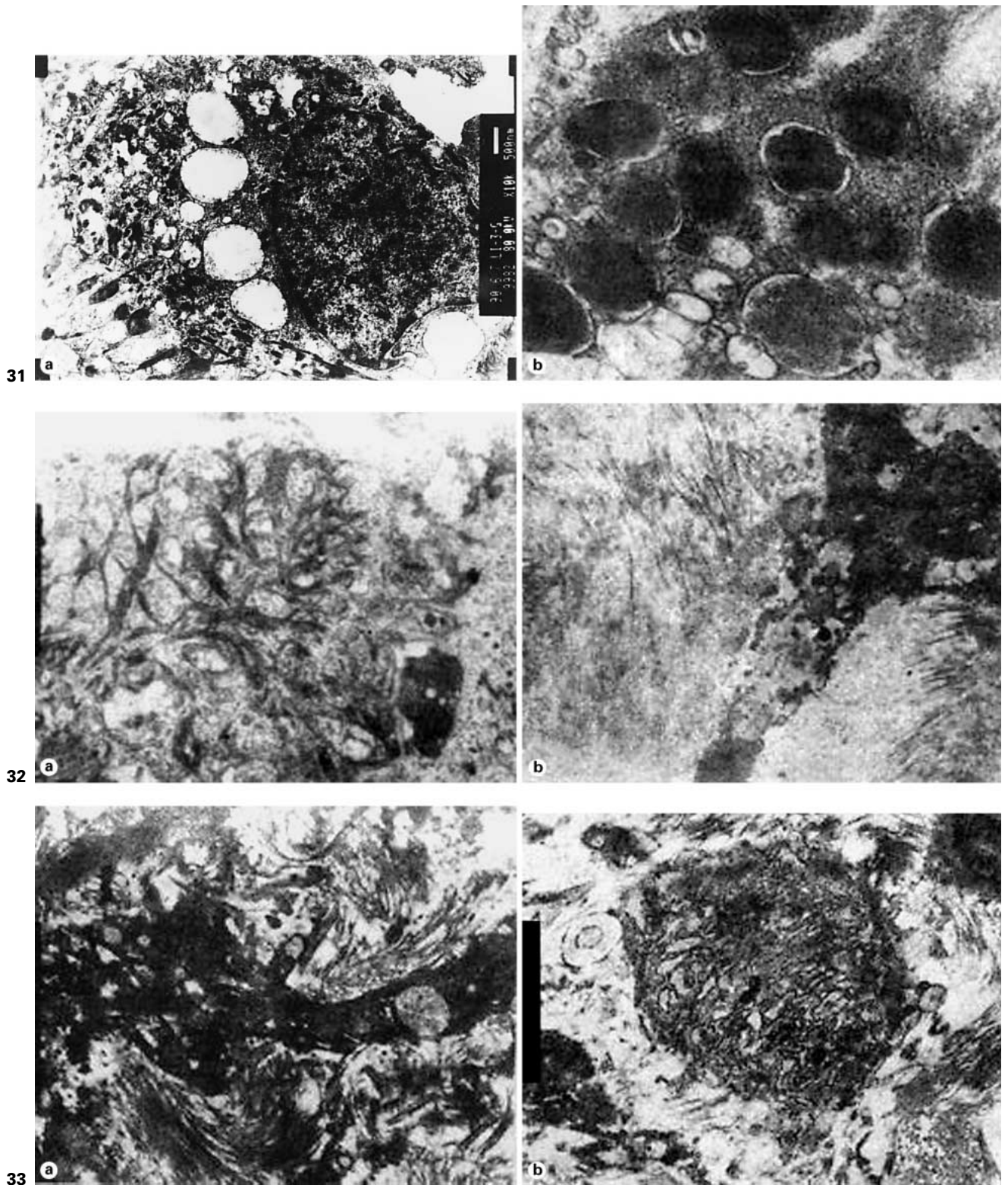


Fig. 31. Case 6. **a** Before treatment, presence of much paranuclear vacuolar with absence of nucleus. **b** After treatment, presence of mast cell with much special granules in a shape of wheel and digitiform arrangement.

Fig. 32. Case 7. **a** Before treatment, collagenous fibers varied in thickness and were disorderly arranged. **b** After treatment, intracytoplasmic rough endoplasmic reticulum recovered, collagenous fibers were uniform in thickness with orderly arrangement.

Fig. 33. Case 8. **a** Before treatment, collagenous fibers varied in thickness and were disorderly arranged. **b** After treatment, intracytoplasmic rough endoplasmic reticulum recovered, collagenous fibers were uniform in thickness.

Fig. 34. Case 10. **a** Before treatment, elastic fibers in dermis were disorderly arranged with varied thickness, intracytoplasmic structure showed vacuolar degeneration. **b** After treatment, the intercellular bridge of stratum spinosum epidermidis recovered to normal with clear nucleus.

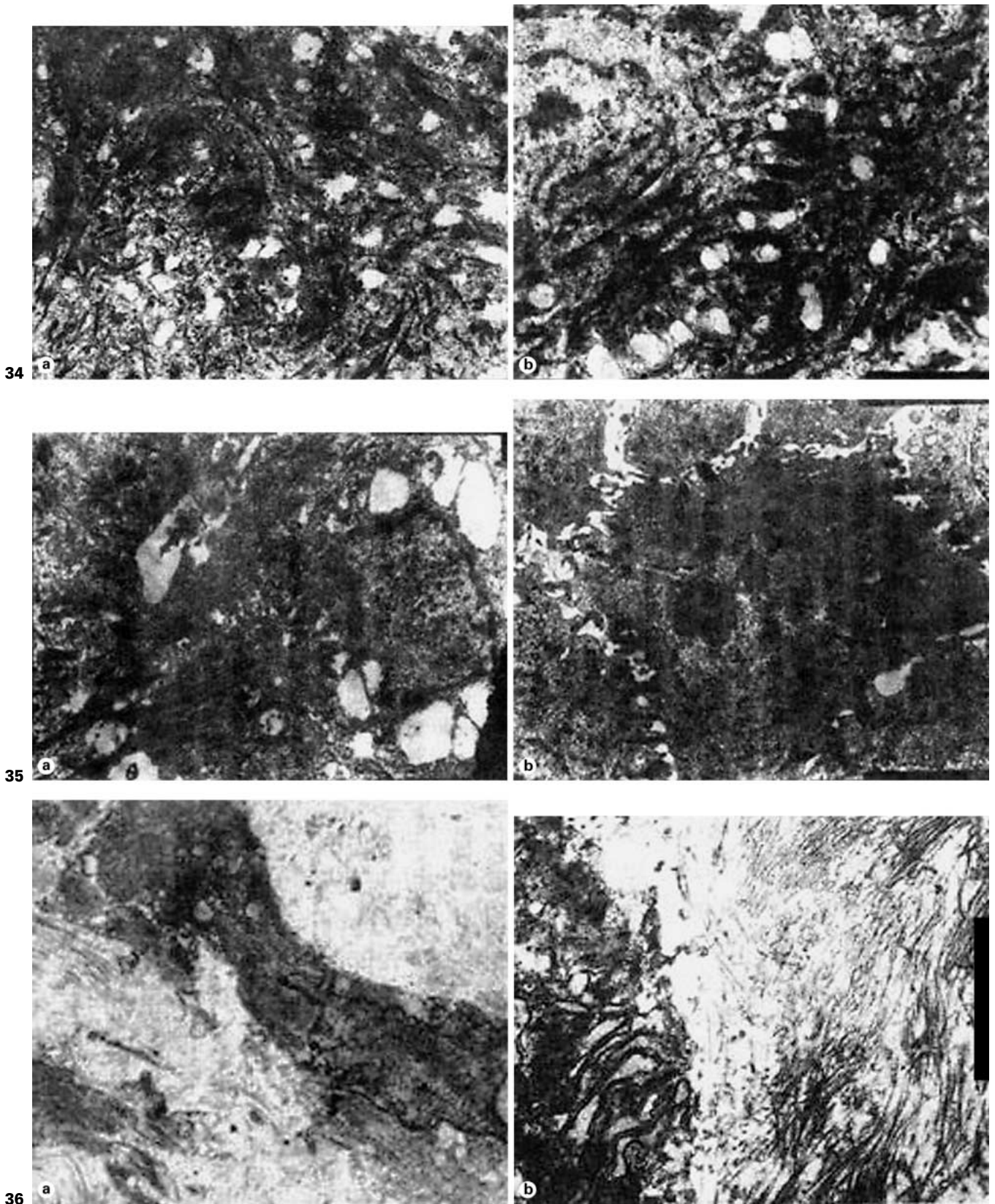


Fig. 35. Case 12. **a** Before treatment, irregular nucleus, perinuclear space vacuolar, and disrupted elastic fibers with deposit were shown. **b** After treatment with MEBO distinct form of cells, existence of desmosome and uniform staining are seen.

Fig. 36. Case 12. **a** Before treatment, fibroblasts were in irregular karyomorphism, intracytoplasmic collagenous fibers varied in thickness and were disorderly arranged. **b** After treatment, rough endoplasmic reticulum recovered, collagenous fibers showed consistent thickness and orderly arrangement.

Conclusion

Light- and electron-microscopic observations of 12 cases of deep burns wounds with granulation tissues demonstrated the dynamic comparison of histopathological alterations before and after MEBO treatment. We noted especially the changes in fibroblasts, collagenous fibers and mast cells. No uniform histological changes were observed such as are reported in the scientific literature where burns wounds were treated with conventional surgical therapy resulting in hypertrophic scarring. In our study, short-term macroscopic observation revealed that healed burns wounds formed no scars which were elevated from skin surface. This suggests that MEBO offers excellent prognosis for the treatment of deep second-degree and superficial third-degree burns wounds without scarring.

Discussion

Many factors are involved in the wound healing process from trauma in general and burns wounds in particular. Among these factors, the importance of those inherent to the cell itself has been especially recognized lately by scholars [3–6]. However, when conducting research to explore the mechanism of wound healing free of hypertrophic scarring, one should attend to histopathology, in other words, try to understand the alterations of collagenous fibers, fibroblasts and mast cells [7].

In 1973 and 1974, Linares used light and electron microscopes for observation and found that hypertrophic scarring as a result of postburn granulation tissue appeared whirlpool-like or in a nodosity arrangement. In the early stage after wound healing, there were a paucity of elastic fibers initially but eventually over years a structure of lamellar or plexiform shape could be established. Mast cells took on varied shapes: round, oval or claviform with deep granular staining. These mast cells released histamine to stimulate the synthesis of collagen and also secreted many active substances to promote scar proliferation. The greater the number of mast cells in granulation tissues, the greater the likelihood of healing with hypertrophic scarring.

In 1971, using the electron microscope, Gabbiani's group noted the presence of fibroblast-like cells in granulation tissues which correlated to wound contraction. In 1980, Rudolph demonstrated that these cells had the same ultrastructure as fibroblasts and smooth muscle cells. They named these cells myofibroblasts, the presence of which in granulation tissues predicted the possibility of hypertrophic scar formation [7, 8].

In the present study, the authors found absence of whirlpool-like or nodosity-arranged collagenous fibers and myofibroblast after MEBO treatment. Small numbers

of mast cells were observed in 2 cases which are consistent with the number expected in normal skin. Research conducted by He [7] showed the existence of many more mast cells in hypertrophic scars than in normal skin. Therefore, the light- and transmission electron-microscopic observations as well as our pictures after wound healing revealed wounds free of hypertrophic scars after MEBO treatment. Long-term effects remain to be investigated histologically as these were short-term observations.

Acknowledgments

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Observation of Microcirculation in Nail Folds at the Recovery Stage of Burns Wounds Treated with BRT with MEBO

Introduction

From May 1992 to May 1994, we treated 13 cases of people with nail fold burns and observed the repair of the microcirculation in the nail folds. The results revealed that after repair, the microcirculation was normal in superficial second-degree burns wounds, almost normal in deep third-degree burns wound and moderately abnormal in mixed second- and third-degree wounds.

Materials and Methods

Object of Observation. 13 young male patients with nail fold burns were treated with BRT with MEBT/MEBO. As soon as the burns wounds began healing, the morphology of the microvessels, blood flow status and repair of the vascular loop surroundings in the nail fold were observed.

Apparatus. WX-6 multiple position microcirculation photomicroscope was manufactured by Xuzhou Factory of Medical Optical Instruments and supplied by the Department of Pathophysiology of Binzhou Medical College.

Method of Observation. At a fixed time in the morning, the finger temperature of the patient was taken using an electron thermometer at room temperature. The patient was kept in the sitting position with the arms at the level of the heart. The observation was done under a mercury lamp light at an angle of 45° from the back. Photographs were taken synchronously and were visualized using a closed-circuit TV system. If the patient had injured only one hand, the other hand served as the normal control.

Observation Index. Appearance: vascular clearness, loop number, input branch, output branch, loop top diameter, loop length, percentages of crossed and deformed loops. Status of blood flow: flow rate, vasomotion, erythrocyte aggregation, leukocyte number, whitish micro-thrombus and blood color. Condition at the surroundings of the loops: exudation, bleeding, sub-papillary venous plexus, papillae and sweat duct.

Results and Discussion

The microcirculation status was scored and classified according to the standard for quantitative analysis of nail fold microcirculation established by Tian [1] (table 58). Abnormal results were observed in 13 patients (7 cases with superficial second-degree burns and 6 cases with deep second-degree burns), and are listed in table 59.

At the initial healing of the wound, the comprehensive scores of 7 patients with superficial second-degree burns were in the normal range. Some of them had newly generated microvessels slightly shorter and smaller than normal and had exudate in the surroundings. Some individuals had more leukocytes and their papillae were not clear. The vessels in the recovery stage demonstrated exudate and, therefore, papillae were not easy to observe.

Changes in the appearance of the microcirculation of 6 patients with deep second-degree burns wounds were significant. Most of the loops were shorter. Two cases had 100% crossed loops. One case had a deformed loop exceeding the normal ratio. As a whole, parts of the microvessels in the nail fold resembled those in the superficial layer of the skin. Some cases had slower flow rate, which may be due to the changes of the loop appearance, such as crossed and deformed loops. One case had remote hemorrhage. Three cases had flat papillae, which may be related to the damage of deep tissue.

This study revealed that the microcirculation was repaired after the treatment of burns wounds. This repair can be attributed to local application of BRT with MEBT/MEBO and its systemic benefit. MEBO protected the

Table 58. Repair of microcirculation in nail folds of 13 patients

Normal	Almost normal	Slightly abnormal	Moderately abnormal	Severely abnormal
8	1	3	1	0

Table 59. Cases with abnormalities of microcirculation observed at the recovery stage

	Superficial second-degree wound (case No.)	Deep second-degree wound (case No.)
Clearness	0	0
Loop number	0	2 cases with 5 loops
Input branch	2	0
Output branch	3 cases with slightly small	0
Loop top	4 cases	0
Loop length	4 cases with slightly short	4 cases with short
Crossed loop	0	2
Deformed loop	0	1
Flow rate	0	3
Vasomotion	0	0
Erythrocyte aggregation	0	1 case slight
Leukocyte number	2 cases >30	2 cases 0–50
Whitish microthrombus	0	0
Blood color	0	1 case with crimson
Exudation	3	0
Bleeding	0	1 case with remote hemorrhage
Sub-papillary venous plexus	0	0
Papillae	2 cases without clear	3 cases with flat
Sweat duct	0	0

burns wound from dehydration and helped avoid bandage pressure. This was favorable to the repair of the microvessels.

The microcirculation in the normal hands of these burns patients was examined and nothing abnormal was found (data not shown). The effect of other diseases could be excluded.

Reference

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Physiological Healing Procedure and Histological Observation on Deep Second-Degree Burns Treated with BRT with MEBT/MEBO

Introduction

Moist-exposed burn therapy is a local burns management by which burned tissue is exposed to a three-dimensional physiologically moist environment to reject, regenerate and repair [1]. We herein present a report on the dynamic pathomorphological changes of deep second-degree burns wounds. Studies in this subject are seldom performed and seldom available.

Materials and Methods

Clinical Data

Six cases were enrolled throughout the study, 5 males and 1 female, aged from 21 to 36 years. Total burns surface area varied from 12 to 45%, with an average of $24.33 \pm 12.37\%$. All patients were admitted within 6 h postburn. After admission, patients received fluid resuscitation and antibacterial therapy on the basis of burn severity. Burns wounds were applied with MEBO at a thickness of 1 mm, which was renewed every 4–6 h till wound healing.

Pathological Observation

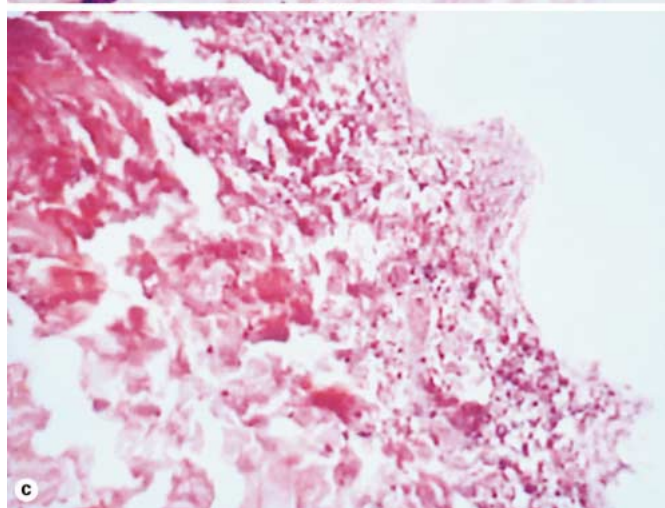
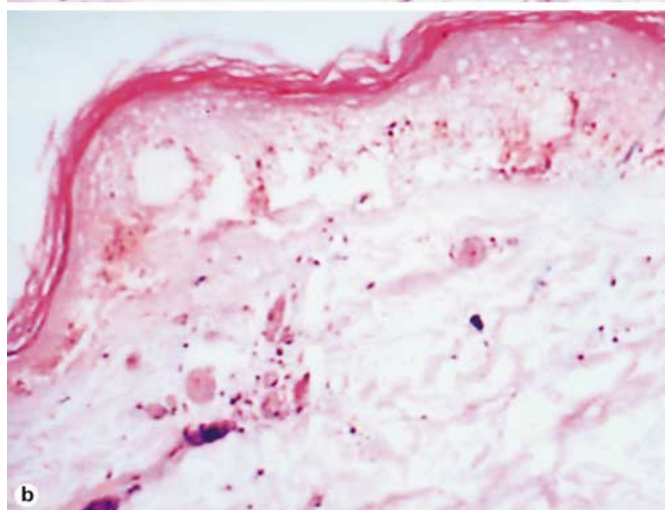
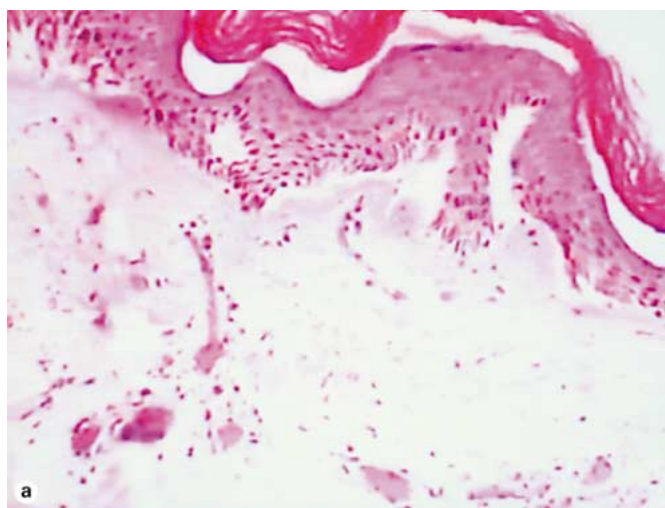
Tissue specimens were collected on days 1, 3, 5, 7, 10, 15, and 20 postburn and after healing from wound sites including trunk, upper limb and both lower limbs. All specimens were allocated into two parts, one immobilized with 10% formalin, embedded in routine paraffin, sliced up and HE stained for light-microscopic observation; the other was immobilized with 2.5% glutaraldehyde solution, prepared on routine basis for observation using TEM 1200EZX-model electron transmission microscope.

Results

Light-Microscopic Observation

Under the light microscope, all burns wounds were noted to involve deep dermis and the pathological changes of burns wounds at different time phases were almost the same.

On day 1 postburn, epidermis became necrotic due to coagulation and necrotic basal layer cells were seen arranged in a column-fencing formation. Edema was present between epidermis and dermis. Collagenous fibers in the superficial layer of dermis were denatured, and swollen and loosely arranged while epithelia of the dermal adnexal epithelia were also denatured. We observed contracted nucleoli of the capillary endothelium, blood clot or stasis in lumen with little infiltration of neutrophil (fig. 37a). Only collagenous fibers and dermal appendages in deep layer of dermis were approximately normal. On day 3 postburn, necrotic epidermal cells progressed to vacuolation with mild edema between the epidermal and



37

dermal layers. Collagenous fibers in the superficial dermis had hyaline degeneration while in the deep tissue, the structure was loose, and blood vessels were slightly swollen with congestion. Scattered inflammatory cells (mainly neutrophils) infiltrated the tissue (fig. 37b). On day 5

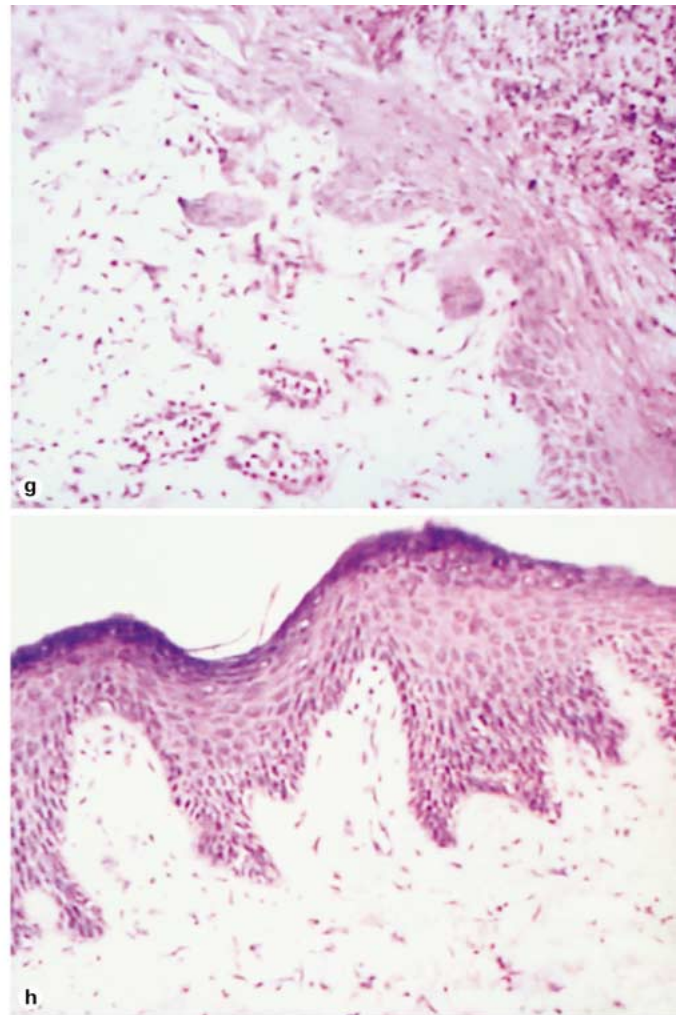
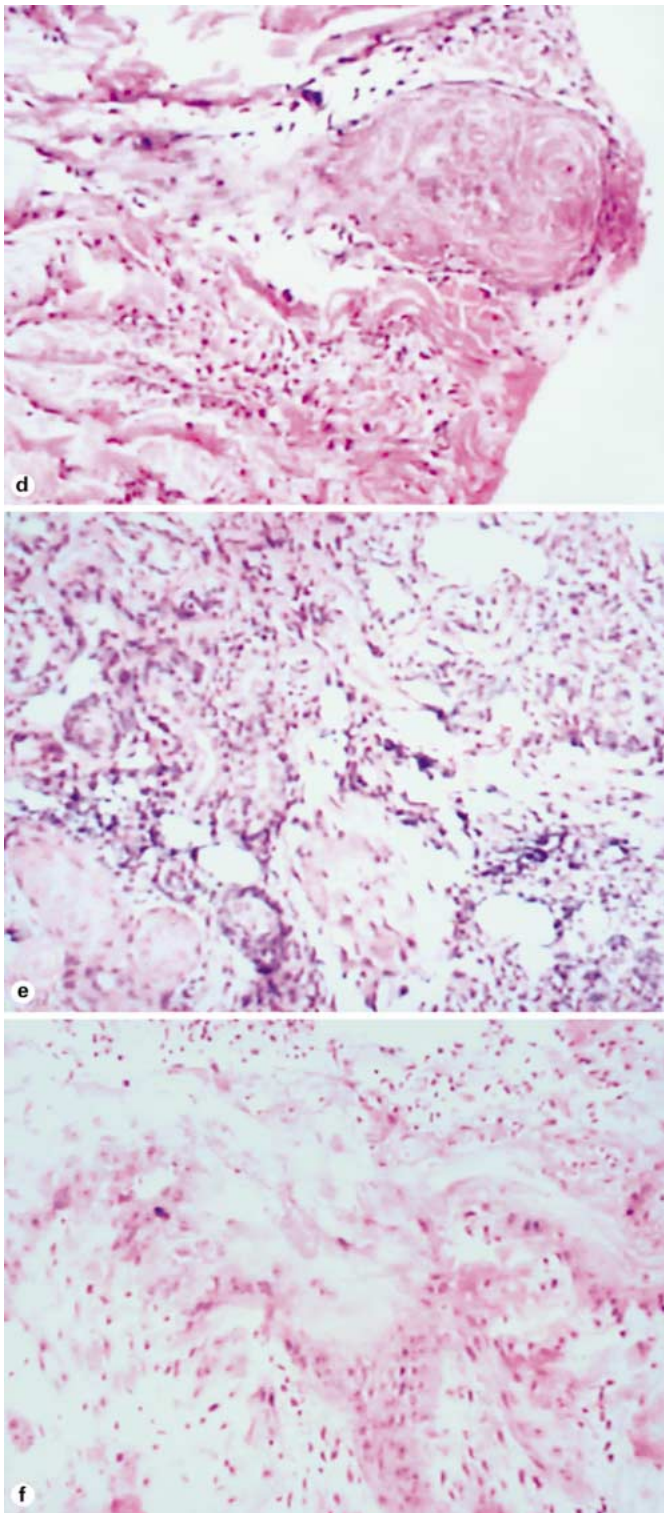


Fig. 37. **a** Appearance of deep second-degree burns wound on day 1 postburn. HE. $\times 100$. **b** On day 3 postburn, epidermic cells progressed to vacuolation, collagenous fibers in superficial dermis had hyaline degeneration and scattered inflammatory cells infiltrated in dermis. HE. $\times 100$. **c** On day 5 postburn, superficial tissues in dermis were liquefactively necrotic and exfoliated. Inflammatory cells, mainly neutrophil and occasionally with few macrophages, infiltrated. HE. $\times 100$. **d** On day 10 postburn, the appearance of epithelia islands was seen. HE. $\times 100$. **e** On day 10 postburn, sweat glands in dermis proliferated with the infiltration of inflammatory cells all round, mainly neutrophils, as well as many lymphocytes and mononuclear macrophages. HE. $\times 100$. **f** On day 15 postburn, the adnexal epithelia in deep dermis grew vertically from the deep, then migrated toward and covered the wounds. HE. $\times 100$. **g** Most wounds were covered with stratified squamous epithelium. Actively proliferated fibroblasts and neoformative capillaries were visible in the dermis, accompanied by the infiltration of lymphocytes. HE. $\times 100$. **h** Neoformative skin has an approximately normal structure. HE. $\times 100$.

when the necrotic epidermis exfoliated, superficial tissue in the dermis liquefied and loosened due to necrosis and neutrophilic infiltration (occasional lymphocyte and mononuclear macrophages) (fig. 37c). On day 7 postinjury, the aforementioned pathological changes became

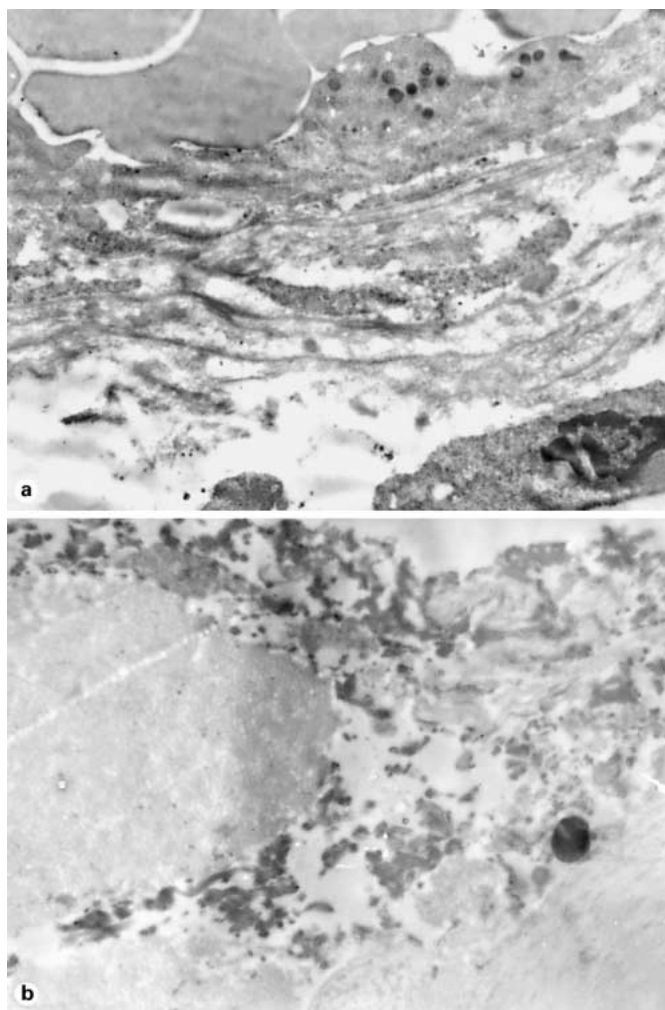
more notable. On day 10, the superficial necrotic tissue in the dermis liquefied, discharged and thinned. A large amount of neutrophils infiltrated into the liquefied necrotic tissue, interwoven with few mononuclear macrophages. The dermal adnexal epithelia in the underlying

deep tissue revealed squamous metaplasia with enlarged cells, deep karyon, rich and red cytoplasm that clustered to form 'epithelium islands' (fig. 37d). Among these islands, fibroblasts proliferated and were characterized by large volume of cells, conspicuous nucleolus (binucleolate was frequently present) and basophilic cytoplasm. In addition, infiltration of many inflammatory cells including neutrophils (mainly), lymphocytes and mononuclear macrophages was visible in wounds (fig. 37e). On days 15 and 20 postburn, an inflammatory exudation layer replaced the necrotic layer whose liquefaction was now accomplished. The underlying residual adnexal epithelia, fibroblasts and endothelia showed active proliferation. The regenerated 'epithelial islands' grew vertically, then migrated toward and covered the wounds (fig. 37f, g). Neoformative capillaries were noticed and granulation tissues were formed. The infiltration of inflammatory cells (mainly lymphocytes) in the dermis was still present, especially in the periphery of the regenerated skin appendages. After healing, regenerated skin appeared to be almost normal in structure and the majority of skin appendages were restored completely. Infiltration of certain inflammatory cells and few macrophages in dermis persisted (fig. 37h).

Electron-Microscopic Observation

Ultrastructural alterations of the superficial dermis layer were observed on days 1, 3 and 5 postburn, while those of full-thickness skin were done on days 7, 10, 15 and 20 postburn and after healing.

On day 1 postburn, electron-microscopic observation indicated loose and disorderly arranged collagenous fibers in the superficial dermis, and a distinct and recognizable light-dark zone of the fasciculus. Fibroblasts which had a cytomembrane profile which remained the same although being deprived of the cell organ. Nuclear membrane loomed with increscent nucleopore to cause the interpolation of nuclein and chromatin, leading to lumpish chromatin. Microsanguial endothelia had similar changes as did the fibroblasts. The vascular walls appeared as a lamellar membranous structure. In the lumen, we noted hemagglutination, frequently joined with blood platelet adherence to the vascular walls (fig. 38a). On day 3 postburn, collagenous fiber bundles in the superficial dermis varied in thickness and appeared disorderly with faint or absent light-dark zones. The cell ultrastructure interwoven between fibers disappeared. On day 5 postburn, the superficial collagenous fibers merged to produce floccules with high electron density surrounded by scattered electron-dense organelle-like structures and necrotic cell fragments as well as occasionally by varied lower electron-dense lipid droplets without coating of the limiting membrane (fig. 38b). On day 7, when the ultrastructural change of the superficial dermis was similar to that on



38

day 5, deep collagenous fibers and the structure of dermal adnexal epithelia nearly returned to normal, except for a slight broadening of the intercellular space. On day 10 postburn, fibroblasts in the dermis showed condensed nuclear chromatin, hypertrophic and shift aside nucleoli. Rough endoplasmic reticulum (RER) proliferated showing flat vesicular and vesicular-like expansion, while residual dermal adnexal epithelia were distributed in clusters containing a high amount of tonofibrils (fig. 38c). On days 15 and 20 postburn, collagenous fiber bundles of dermis varied in thickness and appeared disorderly; fibroblasts were binucleated and showed obvious nucleoli with the presence of karyosome; perinuclear pool demonstrated beading swelling and the RER proliferated (fig. 38d). In dermal adnexal epithelia, nucleoli were distinct and increased numbers of karyosomes were frequently observed.

The chondriosome was rich and intracytoplasmic tonofibril showed an increase in cytoplasmic presence. Also, we observed a gradual establishment and improvement of the intercellular desmosomal junction. After healing, neo-

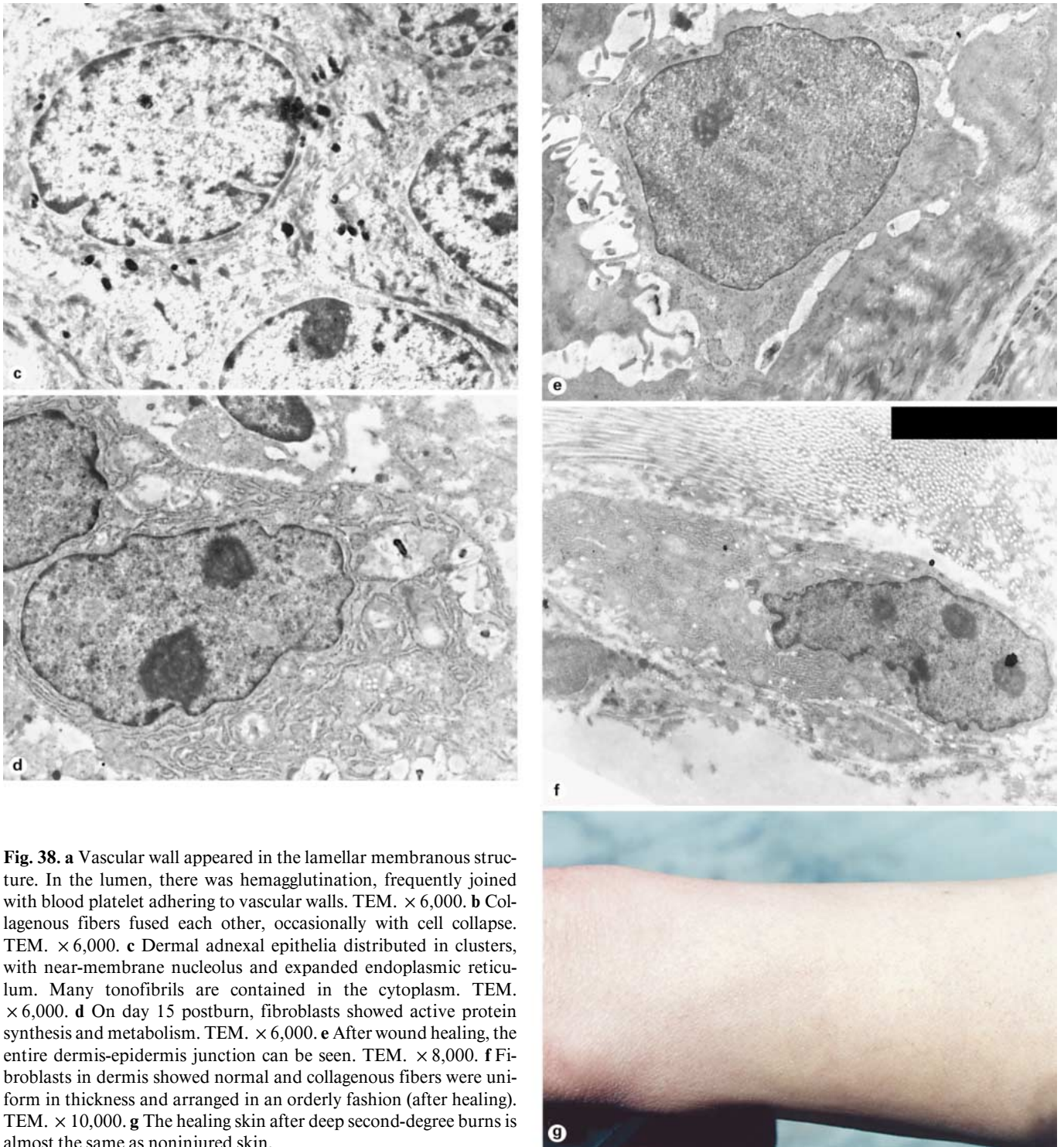


Fig. 38. **a** Vascular wall appeared in the lamellar membranous structure. In the lumen, there was hemagglutination, frequently joined with blood platelet adhering to vascular walls. TEM. $\times 6,000$. **b** Collagenous fibers fused each other, occasionally with cell collapse. TEM. $\times 6,000$. **c** Dermal adnexal epithelia distributed in clusters, with near-membrane nucleolus and expanded endoplasmic reticulum. Many tonofibrils are contained in the cytoplasm. TEM. $\times 6,000$. **d** On day 15 postburn, fibroblasts showed active protein synthesis and metabolism. TEM. $\times 6,000$. **e** After wound healing, the entire dermis-epidermis junction can be seen. TEM. $\times 8,000$. **f** Fibroblasts in dermis showed normal and collagenous fibers were uniform in thickness and arranged in an orderly fashion (after healing). TEM. $\times 10,000$. **g** The healing skin after deep second-degree burns is almost the same as noninjured skin.

formative epidermis cells and intercellular junction recovered almost to normal and the integrated dermis-epidermis junction reappeared (fig. 38e). The majority fibroblasts in the dermis appeared as a long strip rich in cytoplasm with well-developed organelles. However, no addi-

tional active metabolism of the same was observed. Fibroblasts were uniform in thickness, fasciculated and well orientated (fig. 38f). The healing skin of deep second-degree wounds became almost identical to that of noninjured skin (fig. 38g).

Conclusion

Pathological changes of the deep second-degree burn wounds treated with BRT with MEBT/MEBO can be divided into three stages: (1) denaturation and necrosis, (2) liquefaction, and (3) restoration. These stages may overlap. Burns wounds healed by complete physiological regeneration.

Discussion

Among the available literature regarding the pathological changes of burns wounds treated with BRT with MEBT/MEBO, the majority report the light microscopic observation before and after treatment with MEBT [2–4]. There are relatively few monographs or systemic study reports on the ultrathin pathological processes during BRT with MEBT/MEBO.

Our study demonstrated that the pathological changes of burns wounds treated with BRT with MEBT/MEBO were totally different from pathological processes when treated conventionally [5, 6]. On days 1–3 postburn, the so-called ‘acute inflammatory response period’ with its typical denaturation and necrosis occurred in the burned epidermis and superficial dermis, revealing a slight inflammatory response, but without signs of a ‘leukocyte infiltration zone’. Then the necrotic tissue began to liquefy and discharge increasingly as the disease course progressed. Such changes climaxed around day 10 when dermal adnexal epithelial cells, fibroblasts as well as other repaired cells showed signs of regeneration. Microscopic observation indicated large cell bodies of fibroblasts, increased basophil of the cytoplasm, hypertrophic nucleoli, and RER which proliferated, demonstrating the typical flat vesicular and vesicular-like expansion. At this time, the proliferation and migration of residual dermal adnexal epithelia and proliferation of granulation tissues became dominant. The most spectacular observation was that the ‘epithelia island’ (formed by the regenerated adnexal epithelia) initially grew vertically, then migrated toward and covered the wound as granulation tissues proliferated. This resulted in a flat skin surface. During this period, either residual adnexal epithelia or fibroblasts displayed ‘active protein synthesis and metabolism’ [7]. The prominent characteristic changes involved significant proliferation of RER and the appearance of increased karyosomes. Finally, the wounds were noted to be completely covered by regenerated squamous epithelia as healing was accomplished.

We observed that inflammatory cells demonstrated a series of responses during wound healing. On day 1 postburn, we observed infiltration of neutrophils into the dermis followed by lymphocytes and mononuclear macrophages. This infiltration increased on day 3. Neutrophils were the predominant infiltrating cells in necrotic tissue,

and their numbers grew or declined depending upon the length of liquefaction time. Meanwhile, lymphocytes and mononuclear macrophages, mainly located in the residual dermis, dramatically increased during the period of time that necrotic tissue was being rejected and wound repair was initiated. These cells remained the dominant cells impacting wound repair subsequent to the liquefaction period. This study does not resolve questions as regards the significance of the space-time distribution of these cells nor does it answer definitively questions about sequential changes. These questions merit further study. However, we do note the remarkable truth that, during wound healing, the function and activity of the above cells directly or indirectly, alone or cooperatively, participated in and regulated wound healing.

The study results indicated a staged pathological change of burns wounds when treated with BRT with MEBT/MEBO. Wounds changed in three stages according to the different time phase postburn: (1) denaturation and necrosis stage, (2) liquefaction stage, and (3) stage of repair by regeneration, which may overlap during wound healing.

The first stage began subsequent to burn injury till day 3 and the pathomorphological change was characterized by the denaturation and necrosis of burns tissue from which we derived the name ‘denaturation and necrosis stage’. We postulated that these changes were the result of direct thermal exposure, local microcirculation blockage and other secondary injuries. The minor inflammatory response at this stage had a close correlation with the effects of MEBT/MEBO. On day 5 postburn, wound liquefaction became conspicuous and climaxed on day 10. After this time, liquefaction diminished and we noted less necrotic tissue. The inflammatory response, however, remained correlated with the liquefaction of necrotic tissue. Repair by regeneration was noted on day 10 postburn and was manifested mainly by active proliferation of residual dermal adnexal epithelia. This stimulated the proliferation of peripheral fibroblasts and endothelia, and further formed granulation tissues, leading to final healing by epithelization. After experiencing further differentiation and reformation, the regenerated skin finally attained the structure of normal skin. This study demonstrates that the effect of BRT with MEBT/MEBO delivered similar pathological changes for both deep second-degree and third-degree burns wounds. The only difference lay in the ultimate healing modes.

Remarkably, this study revealed that the healed wounds appeared as flat fully regenerated skin, featuring restored, viable hair, almost normal skin elasticity and no scar formation. Histomorphological observation confirmed that the epidermal cells of neoformative skin had the equivalent structure as normal epidermis and that the epidermis had good joints with dermis papilla. The healed

skin demonstrated a fully functional and integrated dermis-epidermis junction. The initially infantile and active fibroblasts gradually grew into stable fibrocytes. Arterioles and venules with thick walls and integrated structure replaced the neoformative capillaries. Collagenous fibers were uniform in thickness and orientated fascicularly, without the presence of whirl-like and nodular arrangement. Follicles, sweat glands and sebaceous glands as well as other dermal accessory organs also regenerated completely. Therefore, we concluded that treatment of deep second-degree burns wounds with BRT with MEBO/MEBO results in complete physiological regeneration with minimal scar formation.

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Clinical Procedure and Histological Observation of Full-Thickness Burns Treated with BRT with MEBO/MEBO: A Case Report

Introduction

A 20-year-old female patient sustained a 35% TBSA burn secondary to exposure to gasoline fire (15% deep partial-thickness and 20% full-thickness loss). The patient was hospitalized at 12 h postburn and was diagnosed as suffering with full-thickness loss (third-degree) burns on both lower extremities. The epidermis was necrotic and detached and the dermal layer was degenerated and necrotic with a waxy yellow and waxy white appearance (fig. 39a). The pathological section examination of the sampled local wound tissues revealed necrosis of full-thickness epidermis and dermis, degeneration and structural disturbance of collagenous fibers in dermis, and microcirculation stasis (fig. 39b).

Results

After admission, the patient was treated initially with MEBO burns ointment to protect burn tissue before we performed skin cultivation and relief as per our BRT protocol. At 48 h postburn, the wounds began to liquefy and the liquefaction was complete by day 4 (fig. 40). The liquefied products were gently removed from the wound surface before MEBO was reapplied every 3-4 h.

Repeat biopsy at the same burned location was performed for pathological examination. The results showed massive granular tissues among the necrotic epithelial tissue, a proliferation of newly regenerated epithelial cells with collagenous fibers, as well as the typical skin embryonic base (EB) (fig. 41a, b). After a 10-day application of MEBO, the comparably primitive epithelial tissues were observed under pathological examination of epithelial tissues sampled from the wound edge.

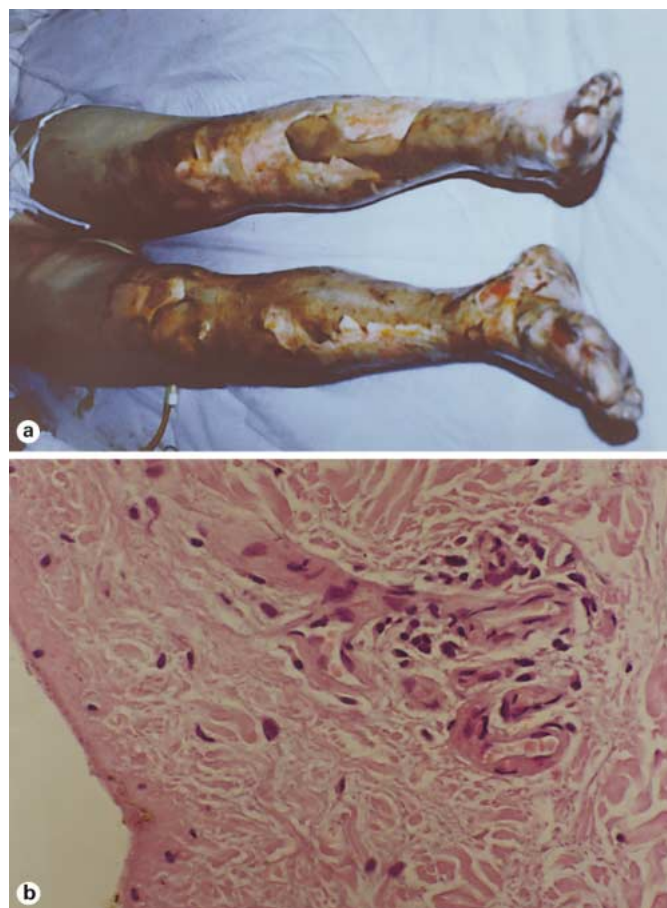


Fig. 39. **a** A 20-year-old female patient sustained a 35% TBSA burn secondary to exposure to gasoline fire (15% deep partial-thickness and 20% full-thickness loss). **b** Necrosis of full-thickness epidermis and dermis, degeneration and structural disturbance of collagenous fibers in dermis, and microcirculation stasis. HE. $\times 20$.



Fig. 40. On day 4, the wounds began to liquefy under the action of MEBO, and the wound skin began to regenerate.

At 20 days post-treatment with MEBO, the pathological examination of deep burns wounds tissue showed the presence of the newly formed intact stratified squamous epithelium. The epithelial cells of the superficial layer appeared normal. Appearance of the collagenocytes and microangium in the dermis layer was typical. On day 30 post-treatment, epithelial tissues showed a remarkable degree of regeneration (fig. 42a), and skin structure was almost normal (fig. 42b).

Immunohistochemical Examination

Twenty days post-treatment with BRT with MEBT/MEBO, wound tissue was examined and the results showed the clear appearance of collagenous fibers in epithelial tissue and subcutaneous tissue. Argentaffin stain

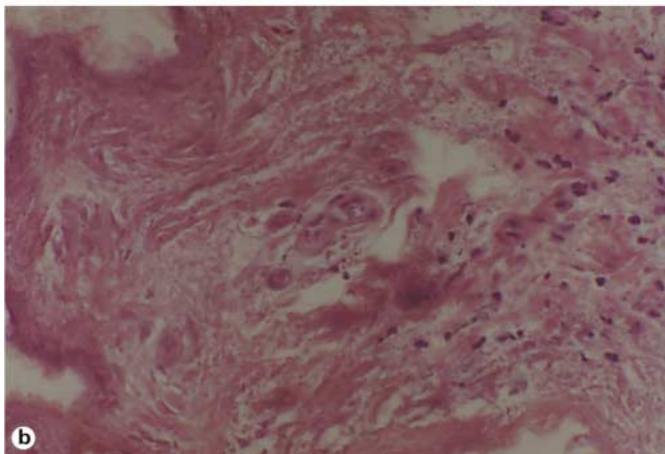
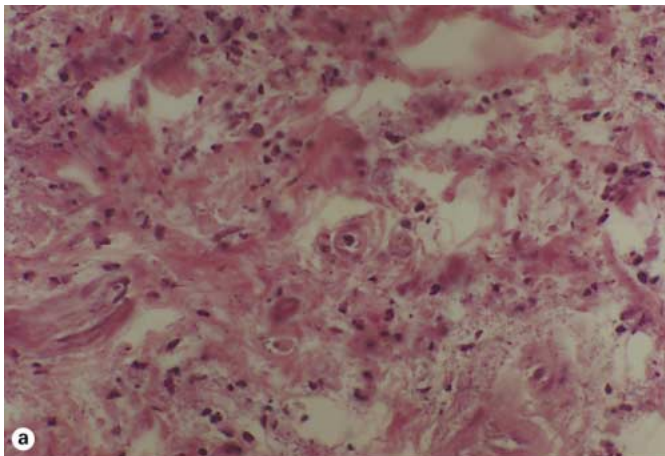


Fig. 41. a Group proliferation of newly regenerated epithelial tissues and collagenous fibers as well as typical skin embryonic base (EB). **b** The skin EB formed by microangium, collagenous fibers and epithelium (stem cell) in newly regenerated epithelial tissues. HE. $\times 40$.

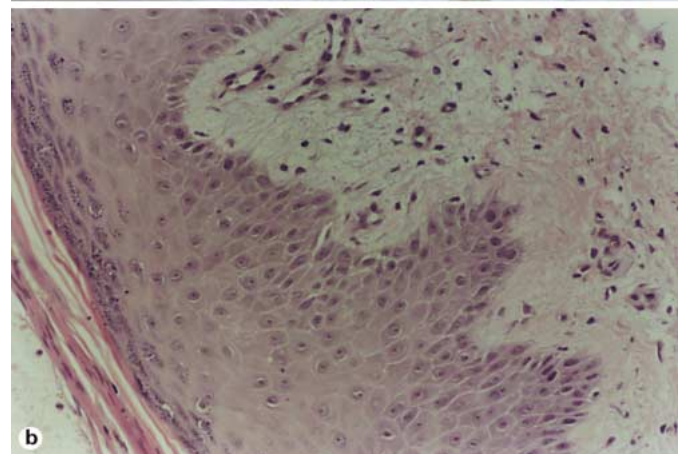


Fig. 42. a On day 30 post-treatment, wound tissue showed good process of regeneration. **b** Day 30 post-treatment, epidermis and dermis showed physiological structure.

indicated active regeneration of reticular fibers. The reticular fibers around the small blood vessels of subcutaneous tissue appeared relatively normal. The basal cells of epidermis regenerated actively (fig. 43a, b).

Thirty days after BRT with MEBT/MEBO treatment, the regenerated and repaired epithelial tissue was AE3 stained. The pictures showed positive proteins of squamous epithelium, brown-stained cytoplasm and blue-stained nucleus of granular cells in epidermis (fig. 43c, d).

AE1 stain showed negative proteins of glandular epithelium, which is representative of glandular epithelium already transformed into squamous epithelium (fig. 43e).

Electron-Microscopic Observation

On the day of injury, the epithelium showed necrotic degeneration, and the monocytes showed a clear nuclear shift with karyopyknosis and even phagocytosis (fig. 44).

Five days post-treatment with MEBO, we noted active growth of fibrocytes and fibroblasts (fig. 45).

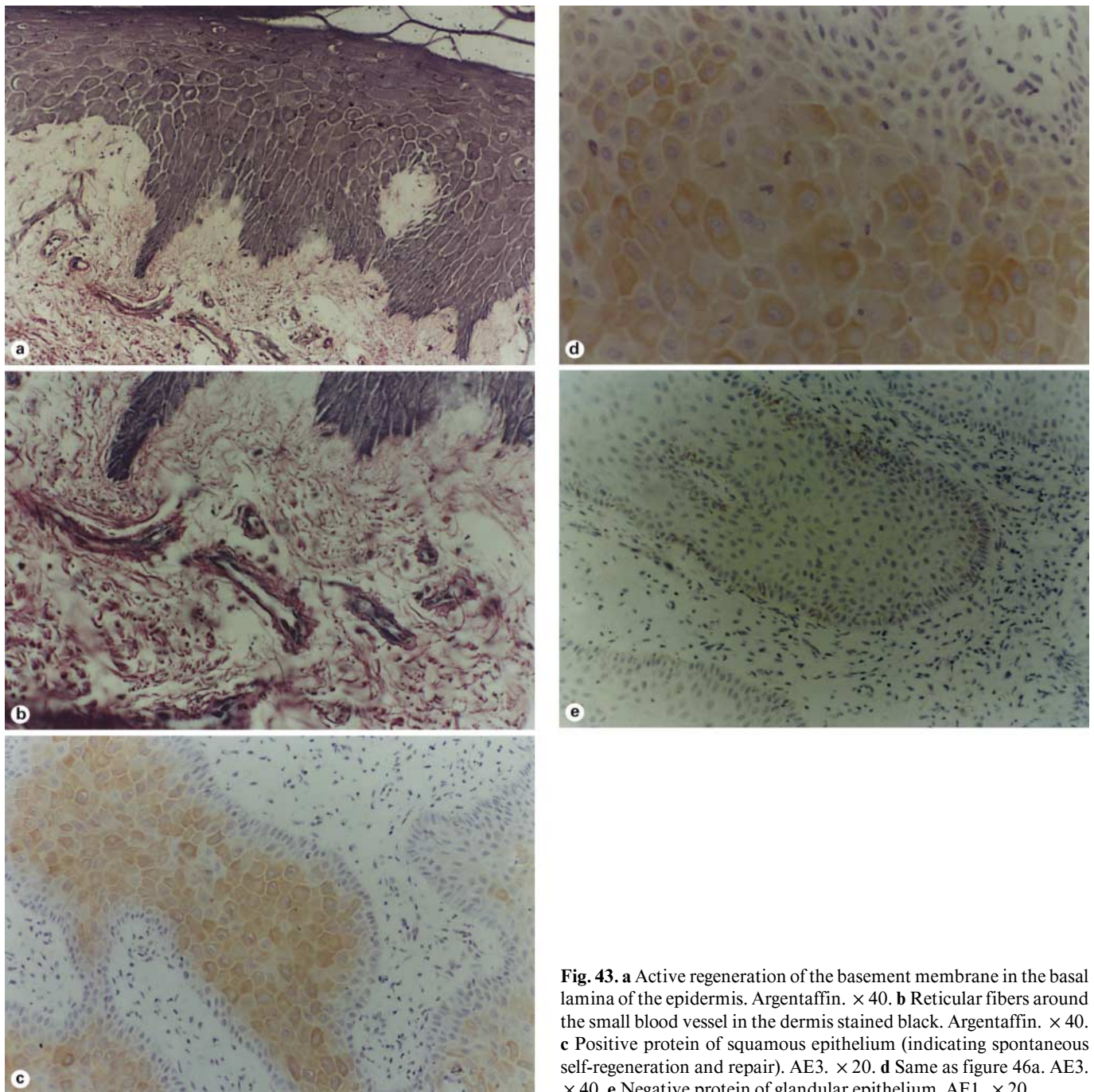


Fig. 43. a Active regeneration of the basement membrane in the basal lamina of the epidermis. Argentaffin. $\times 40$. b Reticular fibers around the small blood vessel in the dermis stained black. Argentaffin. $\times 40$. c Positive protein of squamous epithelium (indicating spontaneous self-regeneration and repair). AE3. $\times 20$. d Same as figure 46a. AE3. $\times 40$. e Negative protein of glandular epithelium. AE1. $\times 20$.

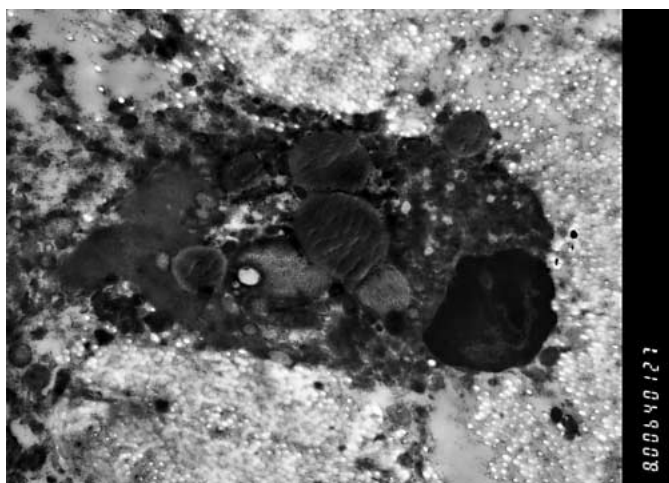


Fig. 44. On the day of injury, nuclear shift, karyopyknosis and phagocytosis of monocyte is seen. TEM. $\times 8,000$.



Fig. 45. Five days post-treatment with BRT, active growth of fibroblast. TEM. $\times 2,000$.

Ten days post-treatment with BRT with MEBT/MEBO, we noted the appearance in epidermis of echinocytes and desmosome in the stratum spinosum (fig. 46a, b), granular cells in stratum granulosum, and melanosomes in the basal cell layer (fig. 46c, d). We also observed phagocytes active around the small vessels, indicating recovery of function (fig. 46e).

Twenty days post-treatment, we noted the appearance of the hemidesmosome junction between the basal cell layer and epithelia. Active mitochondria and RER in fibroblasts also appeared (fig. 47a–c).

Thirty days post-treatment with BRT with MEBT/MEBO, with the regeneration and repairing of epithelium almost complete, collagenous fibers were mature with a

diameter of 0.1–0.5 μm and arranged in an orderly fashion (fig. 48). Light and dark periodic cross-striation (64 nm) was also observed. No pathomorphological changes of collagenous fibers such as distortion, helicoid (whirlpool) or cauliflower-like were observed.

After wound healing, functional exercises and physiotherapy of the lower extremities were required. MEBO was continued as ordinary skin oil. The patient healed and was discharged home on day 45 postburn.

Effect of BRT with MEBT/MEBO on the Expression and Regeneration of Epidermal Regenerative Stem Cells

Introduction

As an innovative therapeutic system in burns therapy, BRT with MEBT/MEBO has enjoyed wide clinical acceptance as part of a protocol including the topical drug ointment MEBO. This therapeutic system successfully solved four major clinical problems: pain, wound infection, progressive necrosis, and scarring in deep second-degree burns wounds. Recently, a new landmark innovation, the regeneration and replication of skin tissue in the subcutaneous fat tissue of full-thickness burns wounds, has been accomplished by this innovative protocol. We know that no stem cells remain in the basal layer of epidermis of deep second-degree and superficial third-degree burns wounds. Therefore, we investigated the source of the regenerative epidermal stem cells which makes the fatty layer burns wounds repair spontaneously. This study was designed to observe dynamic changes in the regenerative epidermal stem cells of deep burns wounds tissues using the immunocytochemistry method.

Materials and Methods

Tissue samples were taken from both normal skin and burns wounds of the following 2 burns patients who received BRT with MEBT/MEBO treatment as first aid immediately after the burns incident.

Case 1. A 6-year-old boy was scalded by hot water on his back and both lower limbs with an area of 33% TBSA and depth of deep second-degree.

Case 2. A 24-year-old male sustained flame burns on his four extremities with an area of 25% TBSA, and depth of deep second-degree and superficial third-degree.

Tissue samples of the injured areas were taken from wounds of the 2 patients at 24 h and on 4, 7, 14, 21 and 28 days postburn. The samples were placed in plastic tubes and frozen immediately in liquid nitrogen, then embedded in Tissue-Tek OCT Compound and frozen in liquid nitrogen. Sections of 10 μm thickness were made in a constant freezing microtome.

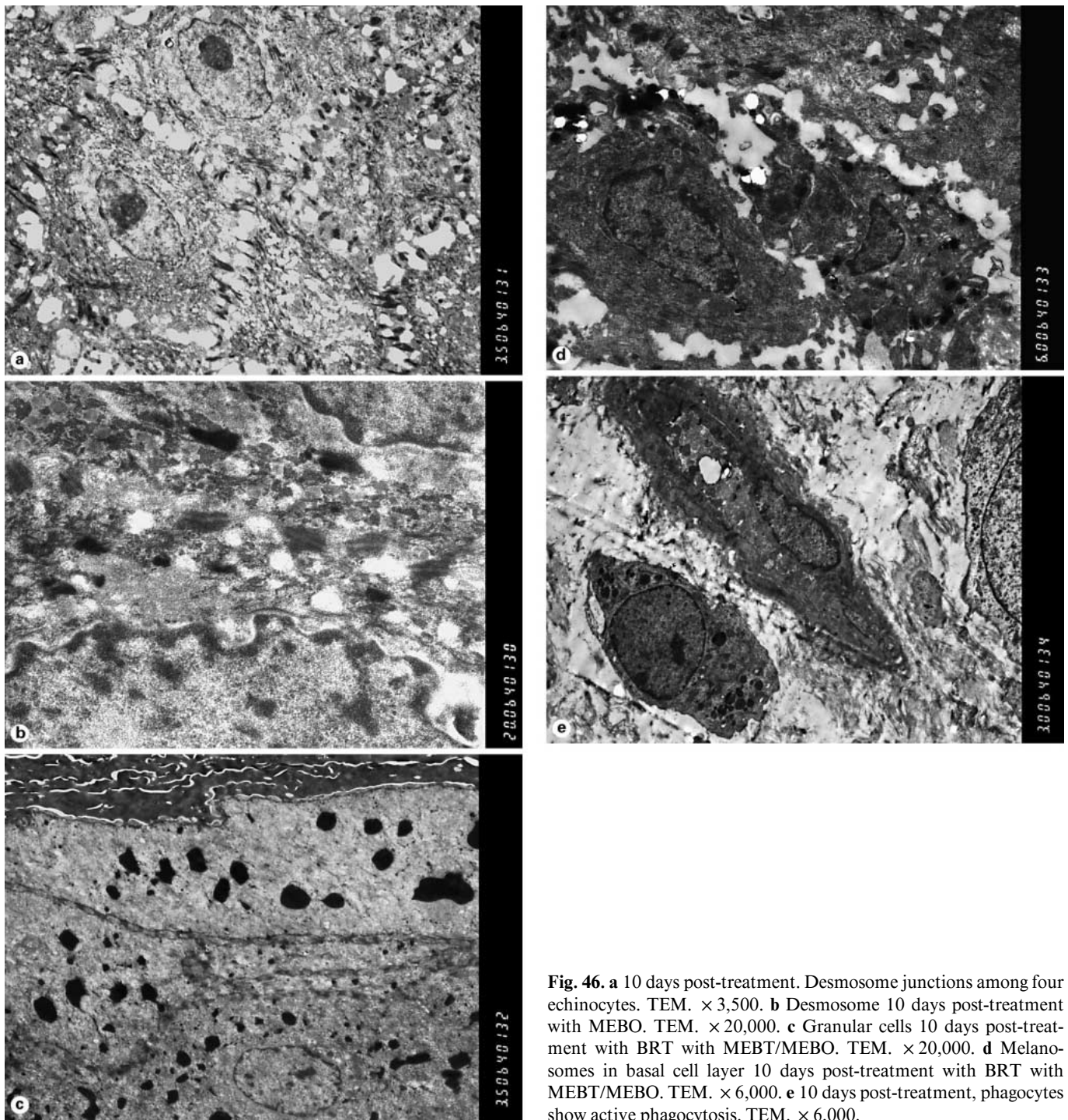


Fig. 46. **a** 10 days post-treatment. Desmosome junctions among four echinocytes. TEM. $\times 3,500$. **b** Desmosome 10 days post-treatment with MEBO. TEM. $\times 20,000$. **c** Granular cells 10 days post-treatment with BRT with MEBT/MEBO. TEM. $\times 20,000$. **d** Melanosomes in basal cell layer 10 days post-treatment with BRT with MEBT/MEBO. TEM. $\times 6,000$. **e** 10 days post-treatment, phagocytes show active phagocytosis. TEM. $\times 6,000$.

Indirect immunofluorescence staining with a biotin-avidin DCS system was performed. The frozen section was incubated with 10% horse serum at 4°C for 20 min, then a diluted (1:20) solution of mouse anti-human keratin type 19 monoclonal antibody (the 1st antibody) was added and the mix was again incubated overnight at 4°C. Subsequent to washing with phosphate buffer solution, the section was added to 7.5 $\mu\text{g}/\text{ml}$ of biotinized horse anti-mouse IgG antibody (the 2nd antibody, Vector Laboratories, Burlingame, Calif.,

USA) and incubated at 4°C for 1 h. After another washing, 10 $\mu\text{g}/\text{ml}$ of biotin-avidin DCS (Vector Laboratories) was added and incubated at 4°C for 1 h. The section was rinsed and then mounted with glycerin containing 10% PBS and 1% *p*-phenylenediamine. A control section of normal skin was stained in the same way, but without adding the 1st antibody. All specimens were observed under an Olympus reflecting fluorescence microscope (Japan) and photos were taken using ASA400 KODAK films.

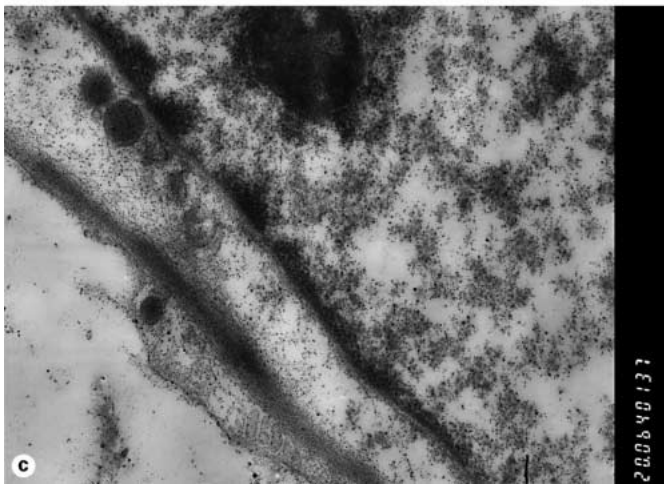
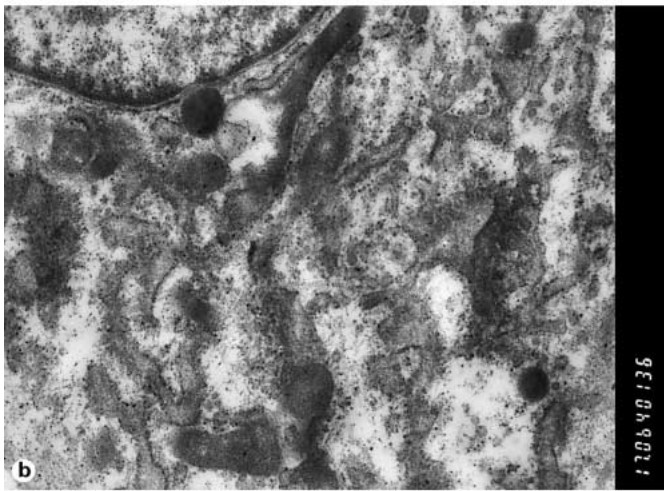
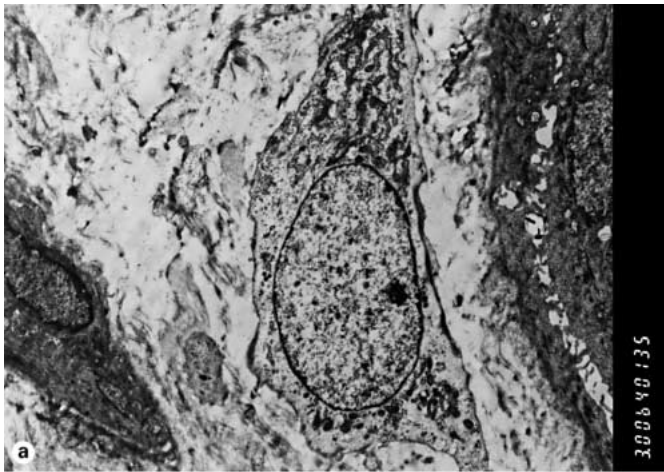


Fig. 47. **a** 20 days post-treatment, the epithelia adhered with hemidesmosome and fibroblast in the basal cell layer. TEM. $\times 3,000$. **b** 20 days post-treatment, active mitochondrion and rough endoplasmic reticulum in the fibroblast. TEM. $\times 17,000$. **c** Same as figure 47b. TEM. $\times 20,000$.

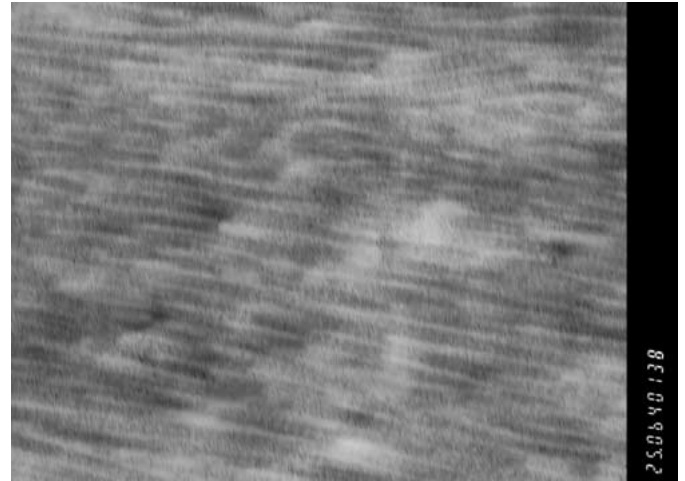


Fig. 48. 30 days post-treatment with BRT with MEBT/MEBO, the collagenous fibers are in orderly arrangement with a diameter of 0.1–0.5 μm and have light and dark periodic cross-striation (64 nm). TEM. $\times 20,000$.

Results

Immunocytochemical examinations were made on normal skin and burns wounds tissue sections treated with specific mouse anti-human keratin type 19 monoclonal antibody. The results revealed that in the normal controls of both cases, there were few positive numbers of epidermal stem cells with keratin type 19 (fig. 49a). Wound tissue at 24 h postburn showed a moderate amount of positive epidermal regenerative stem cells (fig. 49b) and on day 4 postburn, the number of positive epidermal stem cells around the sweat gland, capillaries and hair follicles increased (fig. 49c). On days 7 (fig. 49d) and 14 (fig. 49e), epidermal stem cells containing human keratin type 19 continually increased and exceeded the level attained at day 4 postburn, before gradually reaching a peak level. Prior to days 21 (fig. 49f) and 28 (fig. 49g) postburn, the number of positive regenerative stem cells decreased to a certain level as the burns wounds progressed to healing. The observation showed that, after treatment with BRT with MEBT/MEBO, the proliferation status of the potential regenerative stem cell of the burns patients changed at a regular rate. We propose that regenerative stem cells may be the source of epidermal regenerative stem cells. The glowing fluorescent cells found observed under the microscope represented the potential regenerative stem cells in the wound tissues of deep second- and superficial third-degree burns. After treatment with MEBO, these stem cells may aid the deep partial thickness burns wounds to heal without scar formation and aid the superficial full-thickness burns wound to regenerate skin while healing spontaneously.

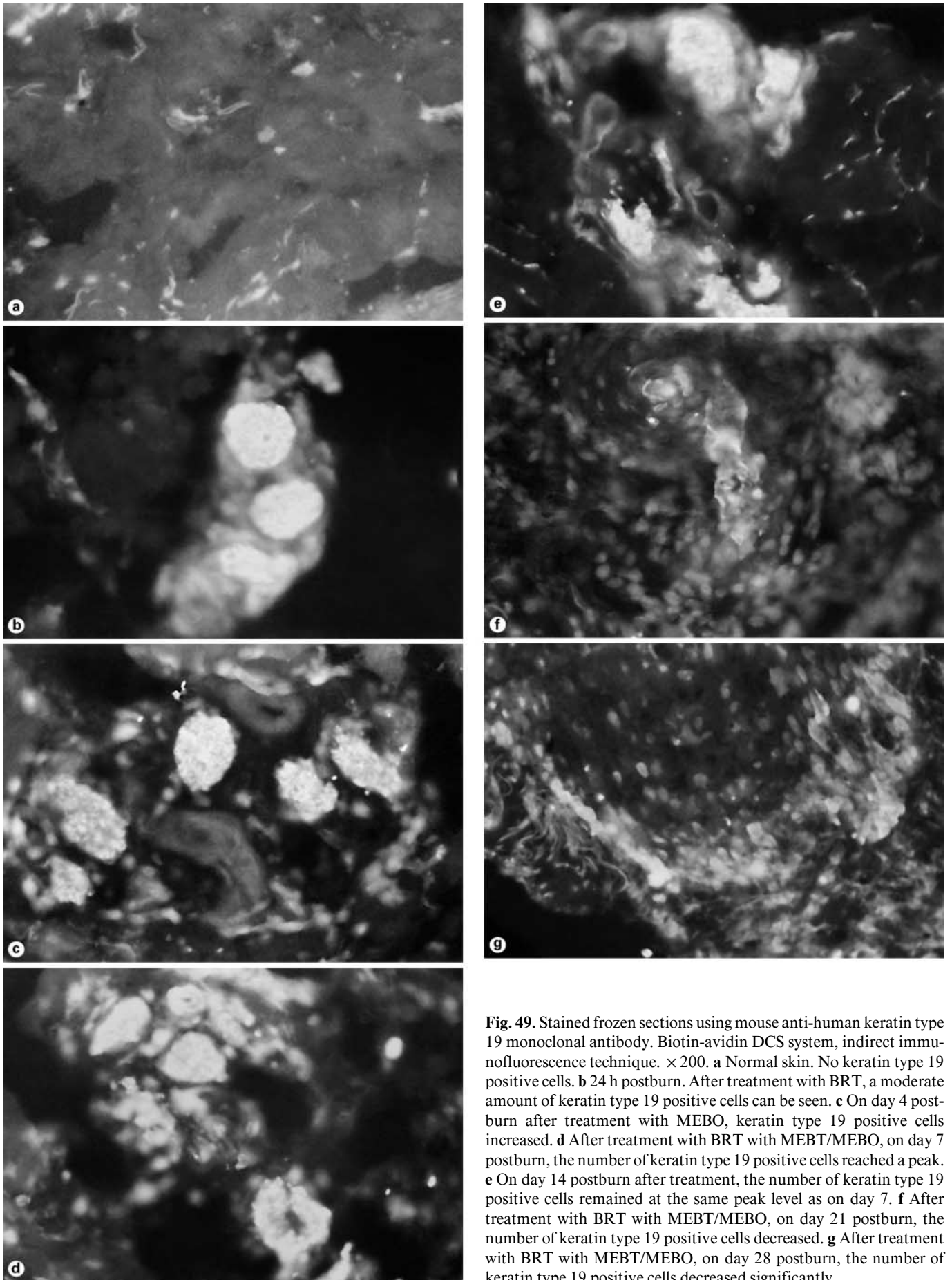


Fig. 49. Stained frozen sections using mouse anti-human keratin type 19 monoclonal antibody. Biotin-avidin DCS system, indirect immunofluorescence technique. $\times 200$. **a** Normal skin. No keratin type 19 positive cells. **b** 24 h postburn. After treatment with BRT, a moderate amount of keratin type 19 positive cells can be seen. **c** On day 4 postburn after treatment with MEBO, keratin type 19 positive cells increased. **d** After treatment with BRT with MEBT/MEBO, on day 7 postburn, the number of keratin type 19 positive cells reached a peak. **e** On day 14 postburn after treatment, the number of keratin type 19 positive cells remained at the same peak level as on day 7. **f** After treatment with BRT with MEBT/MEBO, on day 21 postburn, the number of keratin type 19 positive cells decreased. **g** After treatment with BRT with MEBT/MEBO, on day 28 postburn, the number of keratin type 19 positive cells decreased significantly.

Conclusion

BRT with MEBT/MEBO promotes the activation and proliferation of epidermal regenerative stem cells in the residual viable tissue of superficial full-thickness burns wounds, and these stem cells play a unique role in the spontaneous wound healing of deep burns.

Discussion

Research on the cell cycle has revealed that cell division is closely related to physiological regeneration and wound repair [1–11]. Some cells stay at phase G_0 or G_1 for a long time and will not proliferate unless the condition becomes favorable. But some cells can undergo continuous division yielding daughters destined to differentiate to mature cells, while others retain their ability to continuously proliferate. These are termed ‘stem cells’. Stem cells in the basal layer of the epidermis are capable of proliferating continuously. Newly proliferated cells move upwards until reaching the deep area of the stratum spinosum layer, where they replicate two or three times before losing their proliferative ability.

In deep second- and third-degree burns wounds, the whole epidermis and deep part of dermis are damaged so all resident stem cells in the basal layer of epidermis are destroyed. The residual viable mesenchymal cells around the hair follicles, sweat glands and capillaries in subcutaneous tissue may provide a source of available regenerative epidermal stem cells. These stem cells can synthesize specific cellular keratin type 19, and therefore can be identified immunocytochemically. In this study, anti-human keratin type 19 monoclonal antibody was used. With the biotin-avidin DCS system and the indirect immunofluorescence technique, specific and exact detection of residual epidermal regenerative stem cells in the subcutaneous tissue of deep second- and superficial third-degree burns wounds is accomplished. We observed the number of potential regenerative stem cells with the positive label of human keratin type 19 reaching the peak level on 7–14 days postinjury in burns wounds treated with BRT with MEBT/MEBO. The immunofluorescent cells were epidermal stem cells, which induced the fatty layer burns wound to repair spontaneously. Therefore, we conclude that BRT with MEBT/MEBO treatment activates the dormant potential epidermal regenerative stem cell to proliferate, thereby ensuring the spontaneous repair and healing of deep burns wounds without scar formation. BRT with MEBT/MEBO treatment eliminates the lifetime of pain caused by the hyperplastic scars of patients with deep burns.

After transformation and differentiation, the epidermal regenerative stem cells can yield cells capable of synthesizing other types of keratin, i.e. keratin types 9 and 16.

These cells still have the ability of transformation and can transform into the cells capable of synthesizing harder keratin – types 1 and 10. These are the typical types of keratin contained in mature epidermal cells. This finding proved that MEBO has the effect of promoting the activation, proliferation and transformation of epidermal stem cells.

It has been reported that when the cells are awoken from their dormant phase, the first activated protein is cyclin D. We know that cyclin D can only be expressed after being stimulated by growth factors. For eukaryotes, cells at phase G_1 can either enter into the proliferation state or withdraw from the cell cycle. The main regulator of the phase G_1 limiting point is the cyclin D_1 /CDK₄ complex. In order to further investigate the mechanisms of the effect of BRT with MEBT/MEBO, we will continue our research on the gene regulation for the proliferation cycle of epidermal regenerative stem cells.

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Clinical Reports of Burns Regenerative Medicine and Therapy (MEBT/MEBO)

Clinical Trial Report of Burns Regenerative Medicine and Therapy (MEBT/MEBO): Multicenter Study

Introduction

In order to further examine and evaluate the therapeutic effects, main indications and possible side effects of BRT (MEBT/MEBO) in burns treatment, this multicenter study was designed to conduct a MEBO phase III clinical trial. In this trial, MEBO was used for treating vast numbers of burns patients of both sexes and varied ages, all suffering burns from different causes, occurring at different sites, encompassing varied areas and penetrating to varied depths.

The phase III trial was carried out at five branch centers of the China National Science and Technology Center for Burns, Wounds and Ulcers. These centers are the departments of five general hospitals located, respectively, in the cities of Changsha, Taishan, Dalian, and Nanjang, China. The trial period spanned January 1 1996 to June 30 1999.

Materials and Methods

Clinical Data

General Information

Five hundred and eight hospitalized burns patients were observed. They were divided randomly into two groups: treatment group (MEBO group) and control group (silver sulfadiazine (SD-Ag) group). There were 363 patients in the treatment group, 282 males and 81 females (male:female = 3.5:1), aged 10 days to 73 years (average 28.4 ± 15.5 years), who were treated with MEBO. 145 patients were in the control group, 122 males and 23 females (male:female = 5.3:1), aged 8 months to 72 years (average 29.2 ± 12.1 years).

Causes of Burns

The most frequent causes of burns injury in this trial were flame and hot liquid – 211 cases (41.5%) and 202 cases (39.8%), respectively. 58 burns were caused by chemicals (11.4%), and among these chemical burns, 28, 26, and 4 cases were injured by acid (5.5%), alkali (5.1%), and phosphorus (0.8%), respectively. 22 cases had electric burns, accounting for 4.3%. The remaining 15 cases (3.0%) were accidental scalding by hot solid metal. The burn causes of both the treatment and the control groups were comparable.

Anatomical Regions of Burns

The anatomical regions of burns are shown in table 60. In both groups, the burns often involved the extremities, hands or the head and neck regions of the body. The predilection for these smaller anatomic areas sustaining the majority of the burns may be understood since there are: (1) the most easily exposed at the time of burn, (2) the upright nature of the human being, and (3) the use of these

extremities in attempts to extinguish the fire or remove scalding clothing.

Patient Assessment

Extent and Depth of Burns Wounds (table 61). The largest burns area was 94% TBSA, and the largest third-degree wound was 82% TBSA. In 508 cases of this trial, there were 106 (20.9%) extensive burns patients with TBSA > 50%, 75 cases in the treatment group and 31 cases in the control group. The wounds show no significant differences between the two groups ($p > 0.05$).

Burn Severity. According to the national standard of classification, the burn severity of the patients is shown in table 62. There are no obvious differences between the two groups ($p > 0.05$).

Burn Management

Local Wound Treatment

Generally, exposure therapy was adopted in both groups. The occlusive method was used unless the individual requirement contraindicated it. In both groups, the superficial and deep partial-thickness wounds were permitted to heal spontaneously. Skin grafting could be performed in order to repair full-thickness burns wounds at the appropriate time.

In the treatment group, patients received standard burns regenerative therapy (MEBT) throughout the treatment procedure, with MEBO ointment being applied to the wounds at a thickness of 0.5–1 mm every 4–6 h. No conventional debridement was required.

In the control group, the wounds were treated with 1% silver sulfadiazine (SD-Ag) cream or paste according to the typical SD-Ag protocols after debridement.

Systemic Treatment

Systemic treatment was delivered as required by the patients' conditions. These systemic treatments included anti-shock, anti-infection, and nutritional support.

In the control group, the anti-shock regime was the standard resuscitation protocol of burns surgery.

In the treatment group, the following formula was recommended as a guideline for estimating daily fluid requirements during 48–72 h after injury:

Total amount of fluid infusion (ml) =

Physiological water needs + $1 \text{ ml/kg} \times 1\% \text{ TBSA}$
(second and third degree) $\times 1 \text{ kg body weight}$

$\frac{\text{Amount of hourly urine output (ml)/}}{1 \text{ kg body weight} \times (\text{ml/kg})}$

In both adults and children, the desirable denominator should be monitored, adjusted and maintained at a value of 1 ml.

Clinical Observation Index

Healing Process and Wound Appearance

Any changes in the wound during the whole treatment process were observed and recorded. We noted the presence or absence of infection, regeneration and growth of epithelium, need for skin grafting as opposed to spontaneous healing, duration of healing time, and formation and characteristic of scar, etc.

Standard of Wound Healing. Complete healing of all wounds in patients with total body surface area (TBSA) < 50% burned; residual wound healing is less than 5% in patients with TBSA > 50% burned.

Table 60. Frequency of anatomical regions burned in 508 patients of this trial

	Head/ face	Neck	Left arm	Right arm	Left leg	Right Leg	Left Hand	Right hand	Left foot	Right foot	Trunk	Perineum/ genital
<i>Treatment group</i>												
n	304	293	310	317	243	263	360	373	127	130	135	57
%	59.8	57.8	61.0	62.2	47.2	52.7	70.8	73.4	25.0	25.6	26.6	11.2
<i>Control group</i>												
n	92	83	97	84	96	98	90	81	62	54	98	22
%	63.4	57.2	66.9	57.3	66.2	67.6	62.1	55.9	42.8	37.2	67.6	15.1

Table 61. Extent and depth of burns wounds

Group (n)	TBSA % (n)	Superficial second-degree % (n)	Deep second-degree % (n)	Third-degree % (n)
Treatment group (363)	29.9 ± 18.1 (363)	9.2 ± 8.2 (310)	17.7 ± 13.7 (285)	11.9 ± 11.7 (150)
Control group (145)	31.3 ± 16.4 (145)	10.4 ± 7.3 (135)	16.9 ± 1.8 (128)	12.8 ± 7.4 (56)

Table 62. Classification of burns severity

Group (n)	Mild		Moderate		Severe		Extraordinarily severe	
	n	%	n	%	n	%	n	%
Treatment group (363)	96	26.4	110	30.3	83	22.6	75	20.7
Control group (145)	48	33.1	42	29.0	24	16.6	31	21.3

Assessment of Pain during Treatment and Changing Dressing

Pain sensation during treatment was classified by the following four levels:

- 0: No pain, infant and young patients remaining quiet.
- I: Mild pain, infant and young patients remaining quiet.
- II: Moderate pain but bearable in adults, infant and young patients crying, wounds bleeding.
- III: Unbearable severe pain, wounds bleeding, analgesic required.

Evaluation of the Analgesic Effect

The analgesic effect was classified as the following five levels:

- Excellent: Relief of wound pain within 10 min after application of MEBO burns ointment.
- Good: Alleviation of wound pain within 10 min and relief within 30 min after application of MEBO.
- Fair: Alleviation of wound pain within 30 min after application, with no analgesic required.
- Poor: Wound pain not alleviated within 30 min after application, with analgesic required.
- Very poor: Aggravation of wound pain after application, with analgesic required.

Complications

Local Complications. These were observed mostly if wound infection occurred during treatment, such as serious inflammation of normal tissue surrounding a wound, wound cellulitis, sub-crustal/sub-

eschar empyema, bleeding and necrosis of newly regeneration tissues resulting in inactive growth, etc.

Systemic Complications. These were observed with the incidence of any systemic complication such as hypovolemic shock, cardiac failure, renal failure, respiratory failure and adult respiratory distress syndrome, hemorrhage of upper digestive tract, sepsis or septicemia, etc.

Toxicity and Side Effects of the Topical Medicines

Hematological, biochemical and serological tests were carried out to ascertain the degree of toxicity and side effects of the topical medicines. The potential for localized skin or systemic allergic reactions to the topical medicines used in this trial was also monitored.

Results

Wound Healing Mode

Superficial Second-Degree Burns Wounds

The average healing time in the treatment group was determined to be significantly shorter than that of the control group. Remarkably, whereas no scarring occurred in the treatment group, by comparison, scar formation of superficial second-degree wounds did occur in the control group, which was treated with SD-Ag (table 63).

Table 63. Comparison of healing mode of superficial second-degree wound

Group (n)	Area, %	Spontaneous healing time days	Scar formation	
			n	%
Treatment group (310)	9.7 ± 8.2	9.1 ± 3.6	0	0
Control group (135)	10.4 ± 7.3	13.5 ± 2.7 *	5	3.7 **

Compared with treatment group: * $t = 9.582$, $p < 0.01$; ** $\chi^2 = 11.612$, $p < 0.01$.

Table 64. Comparison of healing mode of deep second-degree wounds

Group (n)	Area, %	Spontaneous healing time days	Skin grafting		Scar formation	
			n	%	n	%
Treatment group (285)	17.7 ± 13.7	20.3 ± 3.1	2	0.7	38	20.4
Control group (128)	16.9 ± 11.8	27.2 ± 3.4*	11	8.5**	81	63.3***

Compared with treatment group: * $t = 11.977$, $p < 0.01$; ** $\chi^2 = 17.609$, $p < 0.01$; *** $\chi^2 = 72.906$, $p < 0.01$.

Table 65. Comparison of healing mode of third-degree wounds

Group (n)	Area, %	Healing time by skin grafting, days	Skin grafting		Scar formation	
			n	%	n	%
Treatment group (150)	11.9 ± 11.7	38.1 ± 7.1	65	43.3	72	48.0
Control group (56)	12.8 ± 7.4	42.9 ± 5.5	47	83.9*	51	91.1**

Compared with treatment group: * $\chi^2 = 27.086$, $p < 0.01$; ** $\chi^2 = 31.440$, $p < 0.01$.

Deep Second-Degree Burns Wounds

Most deep second-degree wounds in the treatment group healed spontaneously. The spontaneous healing time in the treatment group was significant less than that of the control group, as were the incidences which required skin grafting and which resulted in scar formation (table 64). These results indicate that treatment with MEBO promotes superior wound healing.

Third-Degree Burns Wounds

The majority of third-degree burns wounds in the control group were healed by skin grafting, in contrast to 43.3% in the treatment group. The rate of scar formation in the control group was markedly higher than that in the treatment group (table 65).

Analgesic Effect and Alleviation of Pain Sensation during Treatment

Regarding both the analgesic effect and severity of pain sensation during treatment and changing dressing, pa-

tients in the treatment group were all dramatically superior to the control group (tables 66, 67).

Wound Infection

The incidence rate of wound infection in the control group was significantly higher than that of the treatment group (table 68). This finding confirmed that treatment with MEBO controls wound infection effectively.

Toxicity and Side Effects

In this trial, no toxicity, deleterious side effects, local or systemic allergic reactions were found in either group.

Effects on Extensive Burns Patients

In both groups, the patients suffering TBSA <50% burns were discharged with complete healing. In each group, 106 patients were extensively burned. Each of the 75 extensive burns patients (TBSA >50%) in the treatment group was healed. The cure rate for extensive burns in the treatment group was 100%. There were no deleteri-

Table 66. Comparison of analgesic effect after application of topical medicines

Group (n)	Excellent		Good		Fair		Poor		Very poor	
	n	%	n	%	n	%	n	%	n	%
Treat group (363)	204	56.2	142	39.1	17	4.7	0	0	0	0
Control group (145)	10	6.9*	29	20.0*	65	44.8*	31	21.4*	10	6.9*

Compared with treatment group: * $\chi^2 > 50$, $p < 0.01$.

Table 67. Comparison of pain sensation during the treatment and dressing changes

Group (n)	0		I		II		III	
	n	%	n	%	n	%	n	%
Treatment group (363)	176	48.5	165	45.5	22	6.0	0	0
Control group (145)	5	7.4*	31	21.4*	70	48.3*	39	26.9*

Compared with treatment group: * $\chi^2 > 50$, $p < 0.01$.

ous side effects noted in the treatment group. On the other hand, 2 of the 31 extensive burns patients in the control group died and 18 of them were discharged with some residual wounds. The total cure rate for the control group was 73.7%, while the rate of improvement was 24% and mortality rate 2.3%. We conclude from these results that it is very safe to use burns regenerative medicine and therapy (MEBT/MEBO) for the treatment of patients with massive burns.

Discussion

Purpose of This Trial

In 1988, the Public Health Ministry of China approved MEBO as a 'national grade new topical medicine for burns wound treatment'. Since then, millions of domestic and overseas burns patients have been treated with MEBT/MEBO, and the total cure rate remains more than 99%. In 1991, according to the 10-year plan of the Public Health Ministry of China, MEBT/MEBO was listed as one of the first ten significant medical achievements which merited announcement across the country. However, as the changes in pathology and pathophysiology after burns are very complicated, many questions are not quite clearly answered as of this printing. For example, it is asserted by proponents of conventional burns surgery that deep partial-thickness burns wounds cannot be healed without scar formation. In our opinion, that assertion is not substantiated. We also suggest a more open-minded investigation prior to agreeing unquestioningly with another assertion that full-thickness burns wounds

Table 68. Comparison of wound infection between the two groups

Group (n)	Wound infection	
	n	%
Treatment group (363)	28	15.98
Control group (145)	57	39.31**

Compared with treatment group: ** $\chi^2 > 50$, $p < 0.01$.

must be healed by skin grafting. We also query whether full-thickness burns wounds might be better treated so that a spontaneous healing mode might be facilitated. How safe and effective would MEBT/MEBO be is offered throughout the entire treatment procedure in a patient with full-thickness burns wounds? Would there be any deleterious side effects? In order to answer these questions, we designed a multicenter study on an MEBO phase III clinical trial.

Reason for Selecting SD-Ag as a Topical Medicine for Control

It is well known that since the 1960s, SD-Ag has been considered the optimal topical medicine for the treatment of burns wounds. Accordingly, SD-Ag is usually selected as a classical control to examine and compare new topical medicines. However, more and more physicians have recognized that SD-Ag offers only a mild topical antibiotic benefit. The physiological consequence of SD-Ag exposed application to a burns wound will promote desiccation

and scarring of the wound. While SD-Ag offers a degree of prevention of wound infection, it does so at the expense of wound regeneration and tissue repair because SD-Ag inhibits these healthy recovery processes. In an effort to reduce the negative consequences of SD-Ag on tissue regeneration, a modification of SD-Ag application was developed whereby more frequent applications in an occlusive manner strove to enhance the retention of tissue moisture. However, when SD-Ag cream has been used at an increased frequency to avoid wound desiccation, many disadvantages occur including toxicity, and other side effects of using SD-Ag have been reported. These include: transient leukopenia; sulfonamide hypersensitivity and kernicterus; argyria; local reactions of burning, itching and skin rash; delayed separation of burn eschar and regeneration of epithelial cells resulting in an increase of scar formation; increase of bacterial resistance, etc. The pharmacological mechanism of MEBO to treat burns wounds is completely different from SD-Ag. MEBO can promote the regeneration of survival viable skin tissue and cells, then accelerate wound healing by keeping burns wounds in a three-dimensional physiological moist environment while simultaneously facilitating the liquefaction and discharge of necrotic tissues without causing further injury to viable tissue. MEBO achieves its infection control by means of enhancing the resistance of local tissue to infection in the context of an environment which is no longer conducive to bacterial colonization and proliferation. Rather than exercising a direct bactericidal action, MEBO changes the biological characteristics and decreases the toxicity and invasive activity of bacteria. Therefore, we selected SD-Ag as a control to clarify the irrefutably superior therapeutic effects of MEBO as compared to the industry's standards.

Effects of MEBO on Burns Wounds Management

According to the results of this trial, MEBO promotes burns wound healing for each depth of wound. Remarkably, all of the deep partial-thickness burns wounds healed spontaneously after treatment with MEBO, thereby requiring no skin grafting. The rate of scar formation was also markedly reduced compared to that of the control group. Furthermore, in this trial, more than half of the clinically diagnosed full-thickness burns wounds could be healed spontaneously by treating with MEBO throughout the treatment procedure without requiring any skin grafting. Treatment with MEBO provided burns wounds an optimum physiological environment for regeneration and repair. Subsequent to application of MEBO, the wound would heal spontaneously without further damage to viable tissue. Therefore, the rates of scar formation, deformity and disability were all significantly decreased compared to those of the control group. Meanwhile, it was found that in the MEBO treatment group, the incidence

rate of wound infection was significantly reduced compared to that in the control group, indicating that MEBO is capable of preventing wound infection.

Analgesic Effect and Alleviation of Pain of MEBO

It was determined in this trial that throughout the treatment and during dressing changes, the analgesic effect and alleviation of pain in the MEBO treatment group was dramatically superior to that of the SD-Ag control group. Unlike SD-Ag which required painful peeling away of crusted and dried dressing from fragile tissue, treatment with MEBO neither aggravated the suffering and pain sensation during the treatment and changing of dressing, nor did it fail to offer a satisfactory analgesic effect. The need for an analgesic agent was rare in the MEBO-treated group.

Feasibility of MEBO for Treating Extensive Burns Patients

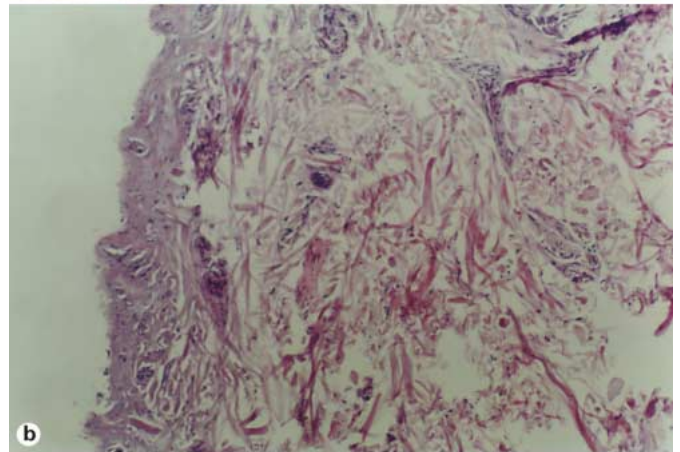
In this trial, 75 extensive burns patients with TBSA >50% were treated by BRT with MEBO/MEBO which resulted in a 100% success rate whereby all patients were completely healed when discharged from the hospital. No toxicity, side effects and local or systemic allergic reactions were found. In contrast, 2 of 31 extensive burns patients in the SD-Ag control group died and 18 of them were discharged with some residual wounds. Meanwhile, almost half of the extensive burns patients in the SD-Ag control group who were failing treatment due to complications of wound infection, bleeding, severe painful or delayed healing were switched into the MEBO group for ethical reasons, and, despite the relatively late access to MEBO, these patients achieved the same satisfactory results. Therefore, it was concluded that treatment with MEBO throughout the clinical procedure for extensive burns patients was both feasible and safe. In addition, it indicated that when the treatment with SD-Ag for extensive burns patients appeared to be unsatisfactory, switching them into a MEBO protocol was reasonable and appropriate. MEBO seemed to promote spontaneous healing in the delayed-healing wound and was able to prevent or decrease the need for skin grafting.

In conclusion, a clinical display of MEBO/MEBO on treating superficial and deep partial-thickness burns is shown in figure 50. In figure 51, the clinical procedure and display of MEBO/MEBO on treating facial full-thickness burns can be seen. Figure 52 is a clinical graphic report of the granulation tissue regenerating from burned bone wounds treated with MEBO/MEBO. Finally, a case report on the treatment of electrical injury with MEBO/MEBO is shown in figure 53.



Fig. 50. **a** Burns wounds of superficial and deep partial thickness degrees on the 1st day after injury. **b** Applying MEBO directly, treatment with MEBT. **c** On the 3rd day after burn. **d** On the 4th day after injury, the wound of superficial partial thickness degree has started to heal. The wound of deep partial thickness degree has started to be liquefied. **e** On the 8th day after burn, the wound of superficial partial thickness degree has healed completely. The wound of deep partial

thickness degree has liquefied. **f** On the 8th day after burn, the wound of deep partial thickness degree has liquefied. **g** On the 12th day after injury, most of the necrotic tissue has liquefied and discharged and the wound of deep partial thickness degree has started to heal. **h** On the 15th day after burn, most of the deep partial-thickness wounds is healed. **i** On the 25th day after burn, the wound of deep partial thickness degree has healed completely.



50

Fig. 51. **a** Patient with facial burns after exposure to flame. No pain sensation, no exudate is observed. **b** Histological examination of burns wounds showing full-thickness burns. **c** Perform 'skin cultivating and tension relieving' with unique blade knife on burns wounds.

(For fig. 51d–i see next page.)



Fig. 51. **d** After cultivating, directly apply MEBO according to the procedure of BRT. **e** Day 2 postburn. After cleaning the burns wounds covered with MEBO, perform secondary 'skin cultivating and tension relieving' according to MEBT. **f** An excellent visual example of the process of wound liquefaction on day 12 postburn. **g** On day 18 postburn, the necrotic tissues were almost liquefied and

painlessly discharged while subcutaneous tissues were allowed to regenerate new skin. **h** On day 27 postburn, some burns wounds healed with complete regeneration while other burn lesions were in the process of healing. **i** On day 50 postburn, burns wounds healed and skin regenerated physiologically. Note the symmetrical smile and the lack of scarring. Full sensation has returned.

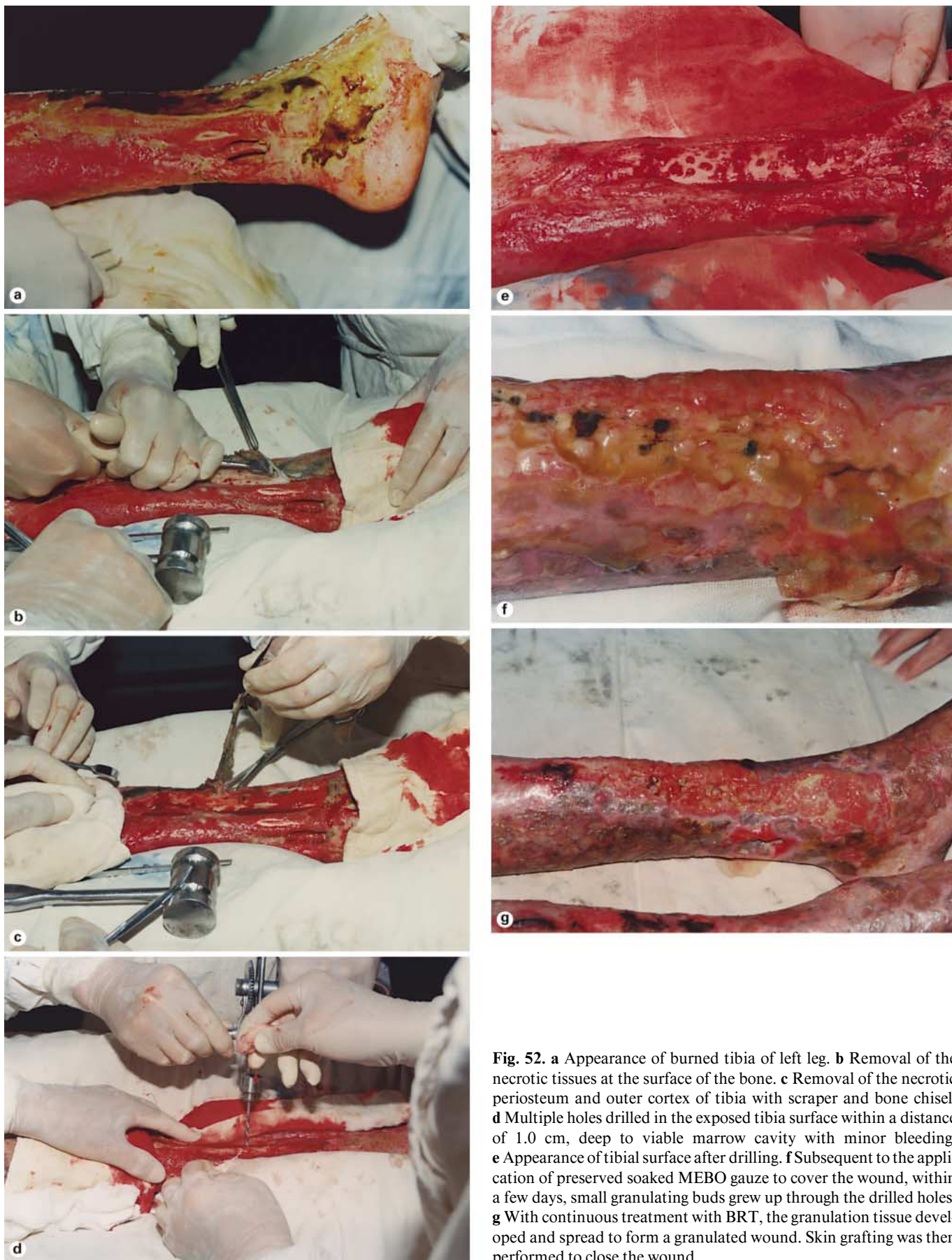


Fig. 52. **a** Appearance of burned tibia of left leg. **b** Removal of the necrotic tissues at the surface of the bone. **c** Removal of the necrotic periosteum and outer cortex of tibia with scraper and bone chisel. **d** Multiple holes drilled in the exposed tibia surface within a distance of 1.0 cm, deep to viable marrow cavity with minor bleeding. **e** Appearance of tibial surface after drilling. **f** Subsequent to the application of preserved soaked MEBO gauze to cover the wound, within a few days, small granulating buds grew up through the drilled holes. **g** With continuous treatment with BRT, the granulation tissue developed and spread to form a granulated wound. Skin grafting was then performed to close the wound.



Fig. 53. **a** This case involved sustained electrical injury to wrist and forearm. The picture shows necrosis of anterior forearm muscles, radial and ulnar arteries. The interosseous artery was viable. The pressure pain point of the upper extremity was at the elbow. **b** After 55 days of treatment with BRT, the necrotic tissues were liquefied and discharged while the wound on anterior wrist at the site of defective muscles was filled with granulation tissue. The wound is now healing from its margins by epithelial migration. **c** In another case, a 9-year-old patient was injured by a 150,00-volt electric impulse on the head. The injury went deep into the skull. **d** Two days after the burn, the wound was cleaned and the necrotic tissue removed, then multiple holes were drilled into the skull followed by treatment with bone BRT. MEBO was applied directly onto the wound. **e** Fifteen days after treatment with MEBT/MEBO, small granulating buds grew up through the holes.

f Twenty-two days of treatment with BRT and MEBT/MEBO, granulating tissue developed and spread to form a granulated wound and the new epithelial skin island appeared. **g** Follow-up picture 2 years later. The wound was healed and the skull was completely covered.

Clinical Demonstrations of Burns Regenerative Medicine and Therapy (MEBT/MEBO) on Successful Treatment of Extensive Burns



Remarkably, rigorous sterile conditions are not required if burns regenerative medicine and therapy (MEBT/MEBO) is used in the care of extensive burns patients. Basic equipment and general surgical conditions typically suffice. However, ambient room temperature of 32–36°C and careful, well-trained, intelligent nursing care are required so that removing liquefied product in a timely manner is accomplished. In general, surgical principles of systemic comprehensive treatment should be followed. Figure 54a–e shows Prof. Rong Xiang Xu, inventor of burns regenerative medicine and therapy (MEBT/MEBO), as he assesses and directs the treatment for a burns patient with deep burns of TBSA 100%.

Fig. 54. a TBSA 100%, deep second-degree 6%, third-degree 94%, treatment with MEBT/MEBO. b Prof. Rong Xiang Xu evaluating and directing BRT treatment. c Posterior view of wounds during treatment. d The healing image (posterior). e The healing image (anterior).

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Extensive Burns Cases with Most Wounds of Superficial Partial-Thickness

Case 1 (fig. 55a, b)



Fig. 55. Anterior (above) and posterior (below) views. **a** A 2.5-year-old baby patient, TBSA 70%. **b** Discharged with complete healing after treatment with MEBT/MEBO for only 14 days.

Extensive Burns Cases with Most Wounds of Deep Partial-Thickness

Case 2 (fig. 56a–f)



Fig. 56. a A 21-year-old man, TBSA 98%. b 24 h after treatment with MEBT/MEBO. c, d Regeneration of skin tissue and wound healing on the 30th day after treatment with MEBT/MEBO.

(For fig. 56e–f see next page.)



Fig. 56. **e** At the present time. **f** Three years post-treatment, the form, structure and function of the new skin is identical to normal skin (chest and abdomen).

Case 3 (fig. 57a–d)





Fig. 57. a A 28-year-old patient. TBSA 82%. **b** During the treatment with MEBT/MEBO. **c** Wound repair and physiological healing on the 32nd day after treatment with MEBT/MEBO. Anterior and posterior views. **d** At the present time.

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Extensive Burns Cases with Most Wounds of Full-Thickness

Case 4 (fig. 58a, b)



Fig. 58. a TBSA 94%. Before treatment. **b** Wound healing on the 40th day after treatment with MEBT/MEBO.

Case 5 (fig. 59a-c)

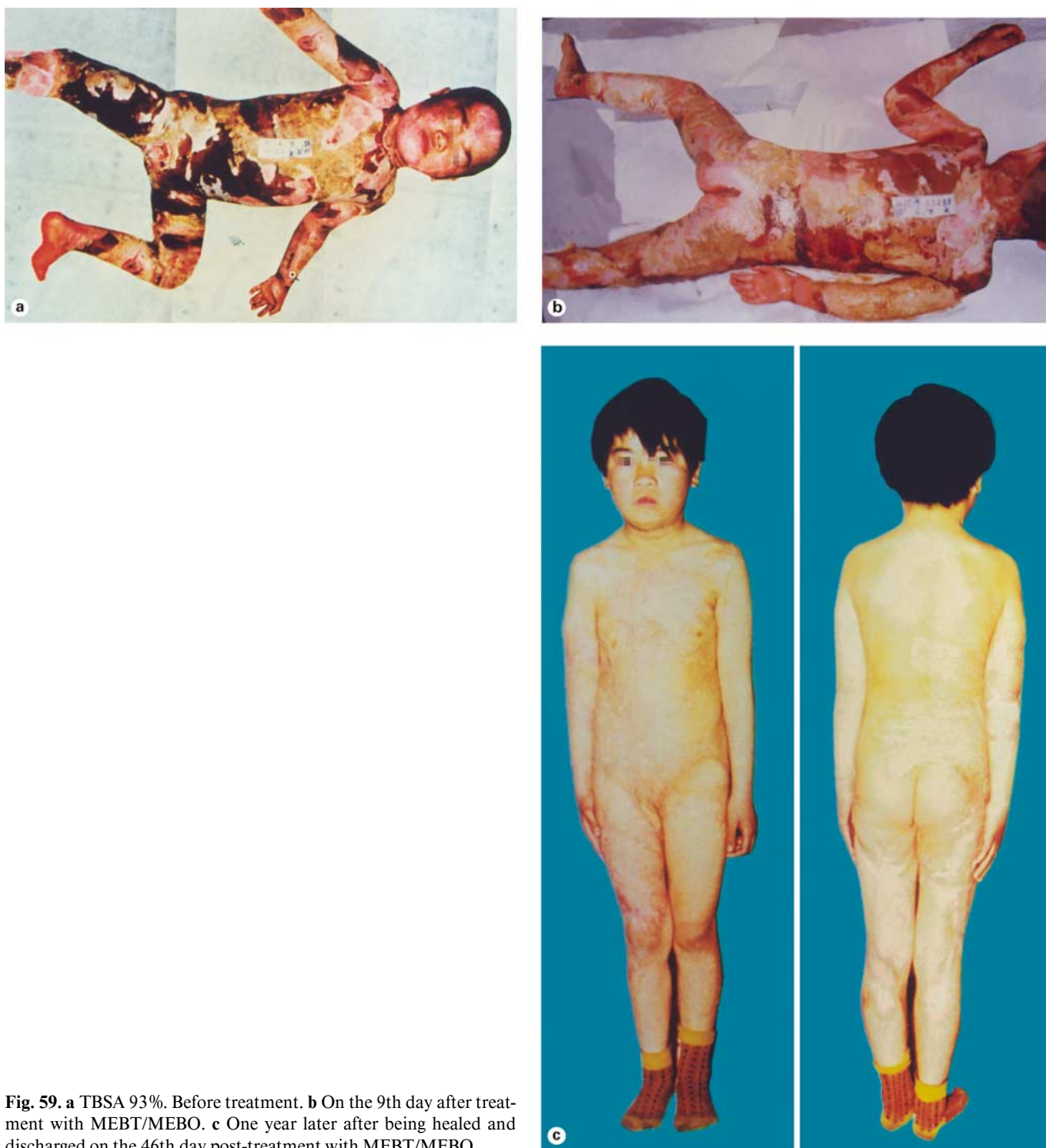


Fig. 59. a TBSA 93%. Before treatment. b On the 9th day after treatment with MEBT/MEBO. c One year later after being healed and discharged on the 46th day post-treatment with MEBT/MEBO.

Case 6 (fig. 60a-c)



Fig. 60. a Upper and lower: TBSA 90%. Before treatment. b Upper (anterior) and lower (posterior) views: All wounds healed with skin regeneration after 51 days of MEBT/MEBO treatment. c At the present time.

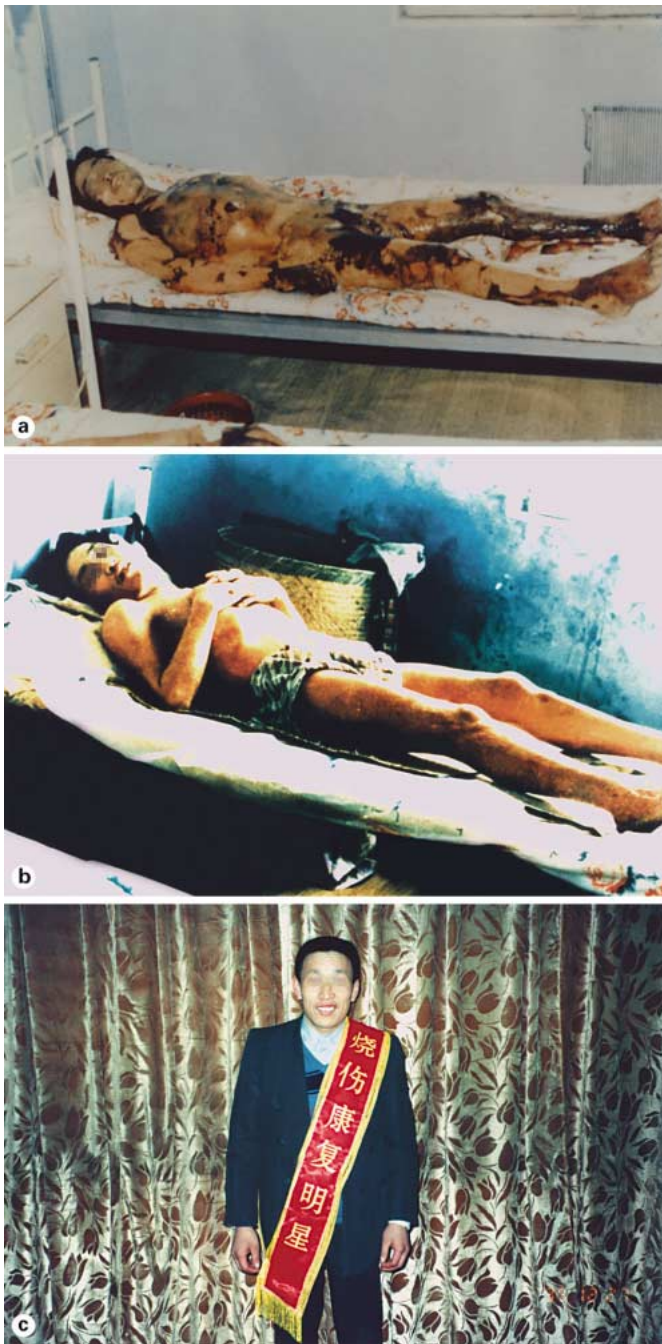


Fig. 61. **a** TBSA 90%. Before treatment. **b** The wound healed on the 43rd day after treatment with MEBT/MEBO. **c** At the present time.



Fig. 62. Three years later. **a** The skin healed spontaneously from deep second-degree wounds (chest and abdomen) and had identical appearance as normal skin in structure and function. Note the lack of scar tissue. **b** The skin healed spontaneously from wounds mixed of deep second- and superficial third-degree burns (inside of left upper arm) and recovered to normal structure and function. **c** The skin healed spontaneously from superficial third-degree wounds (back) and almost recovered to normal in structure without obvious scars.

(For fig. 62d-e see next page.)



Fig. 62. **d** The skin healed spontaneously from superficial third-degree wounds (outside of right thigh and knee). There are few smooth and soft scars with slight hypo-pigmentation but good elasticity and no hyperplasia or dysfunction. **e** The scars healed spontaneously from deep third-degree wounds (inside of right thigh and knee). Tissue appeared smooth and soft without contracture or deformity.

Case 8 (fig. 63a-c, 64a-e)





Fig. 63. a TBSA 95%. Before treatment. b On the 55th day after treatment with MEBT/MEBO, the skin tissue regenerated and the wound healed. c At the present time.



Fig. 64. Three years later. **a** The skin healed spontaneously from deep second-degree wounds (dorsal surface of right wrist). The tissue appears completely identical to normal skin in structure and function. **b** Most skin healed spontaneously from wounds of mixed deep second- and superficial third-degree burns (inside of right forearm and wrist). Tissue recovered normal structure with little hypopigmentation. **c** The skin healed spontaneously from superficial third-degree wounds (right cheek) with almost normal function (hair growth and secretion of sweat glands). **d** The skin healed spontaneously from superficial third-degree wounds (chest and abdomen) appearing normal in structure without obvious scars. **e** Few scars upon deep third-degree wounds (right shoulder) appeared smooth and soft without contracture or dysfunction.

Clinical Results of Surgical Excision and Skin Grafting Therapy in the Treatment of Extensive Burns Patients

Case 1: Male, 23 Years Old. Admission No. 212911
(fig. 65a–c)

Final Diagnosis

- (1) Direct flame burns with 92% TBSA (superficial second-degree 2%, deep second-degree 19%, third-degree 71%).
- (2) Inhalation injury (mild).
- (3) Hypovolemic shock postburn.
- (4) Septicemia (*Pseudomonas aeruginosa*); corneal ulcer (*Pseudomonas*, left eye).



Fig. 65. a Before treatment. b Wound healed by multiple skin grafting at 74 days after injury. c 14 months later, the appearance after plastic and reconstructive operations.

Case 2: Female, 28 Years Old. Admission No. 212918
(fig. 66a, b)

Final Diagnosis

- (1) Direct flame burns with 95% TBSA, third-degree 90%.
- (2) Inhalation injury.



Fig. 66. a Before treatment (left). **b** Appearance after the wounds healed and plastic operations (right).

Case 3 (fig. 67a, b)

Final Diagnosis

- (1) Direct flame burns with 95% TBSA, third-degree 91%.
- (2) Inhalation injury.



Fig. 67. a Before treatment. **b** Most wounds closed after microparticle autografting. Residual granulation wounds on his chest, back, hands and feet were still left for skin grafting later.

A Commentary on Burns Medical and Regenerative Therapy

A Commentary on Surgical Excision and Skin-Grafting Therapy

Burns therapy with surgical excision and skin grafting is a surgical technique in that it treats the burns wounds with a surgical method. Surgical technique, in essence, treats disease through a destructive means while prioritizing the survival of the patient about the importance of the appearance and function of the burned limb. Before BRT with MEBT/MEBO was invented, surgical burns therapy had become a major method of burns treatment. However, subsequent to the invention of burns regenerative medicine and therapy helpful comparisons have been made between both modalities. Impartial investigators have learned that deep second-degree burns wounds should no longer be treated with surgical therapy because burns regenerative medicine and therapy is objectively superior to the surgical approach. One remaining indication for the use of surgical excision and skin grafting for the treatment of burns may involve third-degree burns with surviving subcutaneous tissues. This, however, must only be done after prudent consideration. The indication of surgical burns therapy should now be defined as: severe large-area burns reaching the lower layer of superficial fascia. Surgical burns therapy should no longer be the major method of burns treatment.

This book also introduces the latest technique of skin grafting using cultured composite autografts after surgical excision. This new technique aims at overcoming the difficulty of the incorporation of the cultured epithelial autograft into the burns wound. This technique can effectively prevent 'autograft exfoliation' and secondary ulceration. The doctors of the laboratory of Culture Technology, Inc., Sherman Oaks, Calif., USA, harvested two components of the skin, autologous keratinocytes and fibroblasts from burns patients and cultured them to enhance proliferation, and then combined them to form epidermal and dermal matrix. Once grown to confluence, the composite autografts are ready for application to the burn wound.

These results were published in *Burns* 1999;25:771-779. This technique had been successfully applied in the treatment of large-area burns after surgical excision in the Burn Center in Arizona State. While this is a significant step forward, we must acknowledge that its treating principle is the same as that of surgical burns therapy. It protects the autograft but cannot avoid the damage or disablement caused by excision. Another comparable disadvantage to this technique is its expense. Therefore, indication for this technique should be third-degree burns and burns in the muscle layer. This skin grafting using cultured composite autografts after surgical excision should not be considered a major method of burns treatment.

A Commentary on Moist-Exposed Burns Therapy

BRT with MEBT/MEBO is a comprehensive therapeutic technique aiming at treating burns tissue in compliance with the law of burns pathogenesis. Compared with surgical burns therapy, BRT with MEBT/MEBO is a technique treating the burns wound in the skin, while surgical burns therapy is a technique treating wounds in the muscle. Together, these two approaches, when used appropriately, form a complementary therapeutic system. BRT with MEBT/MEBO can be applied for the treatment of skin burns while surgical burns therapy can be best applied to the treatment of muscle burns. Briefly, BRT with MEBT/MEBO offers unique therapeutic breakthroughs in treating skin burns as follows:

A BRT with MEBT/MEBO removes the necrotic skin without causing any damage. Removal of necrotic skin layer is the first step of burns treatment. Doctors found no way to remove the necrotic tissue during the past century, except the destructive method which cut away the injured wound tissue together with the surrounding surviving tissues and resulted in further traumatic injuries. Taking the advantages of the relevant biochemical principles, BRT with MEBT/MEBO can spontane-

ously remove the necrotic tissue through liquefaction and drainage without causing further injury to the surrounding surviving tissue. It alone has successfully resolved this difficult problem.

- B** BRT with MEBT/MEBO preserves the surviving tissue to the greatest extent currently possible. Burns wound surface is not smooth and a surgical knife cannot distinguish between injured tissue and surviving tissue. Surgeons always excise the surviving tissue together with dead tissue and this is a very serious attack on the patient – at times it can be more serious than burns injury itself. Moreover, after excision, the body surface typically never recovers the loss of subcutaneous surviving tissue. However, studies demonstrate that, if not excised, this recovery can occur. BRT with MEBT/MEBO takes advantage of the frame structure of the nutritive base of the drug and the principle of biochemistry therewith successfully preserving the surviving tissue.
- C** BRT with MEBT/MEBO demonstrates that the dream of skin regeneration has come true. For about a century, scientists made great efforts to achieve the regeneration of injured skin. In the early 20th century, doctors discovered that the subcutaneous tissues survive after full thickness third-degree burns and may be capable of regeneration. However, they did not find an adequate measure to achieve this survival and therefore they pursued research on in vitro skin cell culture and transplantation of the cultured autograft. By utilizing the regeneration gene for skin information in the subcutaneous tissue, in concert with the creation of a favorable nurturing environment (one favorable to physiological regeneration of the skin), BRT with MEBT/MEBO successfully achieves the skin regeneration within large areas of deep burns wounds. This achievement greatly decreased the disablement rate, and increased the survival rate of large-area burns by 50–80% (compared with the data published in 1997 and 1994).
- D** BRT with MEBT/MEBO resolves the problem of pain in second-degree burns patients. As any person who has cared for burns patients knows all too well, burns wound pain is the worst aspect of the suffering of superficial burns patients. Surgical treatment aims at saving the life without considering the problem of pain. Surgical operations typically make the pain more serious and many patients with large-area superficial burns die because their cases worsen after operation. Severe pain causes shock and wound stress regulation disturbance which can tip the scales toward multiple system organ failure and death. That is why large-area as well as small-area burns are described as life-threatening in the burns care textbooks. Pain remains one of the main causes of burns-related death in all countries. BRT with MEBT/MEBO takes the advantage of the drug MEBO with

a unique frame structure base to eliminate pain almost immediately upon application. MEBO covers the wound surface, protects the wound from irritations and relieves the pain. This unique effect of MEBO finally resolved the problem of burns wound pain.

- E** BRT with MEBT/MEBO opens up a new approach to the prevention and treatment of infection. Local and systemic infection is a difficult problem of burns treatment and today in the era of multidrug resistant pathogens, we are scarcely further ahead than we were years ago. Many antibiotics have been applied but the efficacy proves unsatisfactory. BRT with MEBT/MEBO resolves this problem by treating the local area in compliance with the pathogenesis of the infection of burns wound. This treatment controls infection of burns wound by changing the ecological environment. Concurrently, by applying BRT with MEBT/MEBO to the large-area burns, in accordance with the law of systematic pathogenesis of infections, we discover that BRT with MEBT/MEBO is capable of mobilizing and coordinating the potential physiological energy of the systemic wound stress reaction. This alone has successfully advanced a systematic anti-infection principle for treating large-area burns. To be more specific: At the shock stage, when wound stress reaction is on the upsurge, we recommend the systemic application of broad spectrum antibiotics with no adverse effect on the kidney. After this stage, when synthetic metabolism of protein begins, we recommend that one stop the application of *any* antibiotics. In the whole course of treatment, if systemic infection occurs occasionally, a single large dose of broad-spectrum antibiotic (one with no side effects on kidney) is applied. In this fashion, BRT with MEBT/MEBO offers a systematic scheme for removing the focus of infection and minimizes the dependence upon antibiotics.
- F** BRT with MEBT/MEBO allows one to create a new antishock scheme. It is a common understanding that shock is a serious disease of burns. For a long time, no matter what treating method is adopted, the same standardized fluid infusion antishock scheme is applied. BRT with MEBT/MEBO considers that there should be different antishock schemes for different treating methods and different cases. Surgical operation always makes shock more serious and therefore, fluid infused to replenish the blood volume is of paramount importance. Remarkably, BRT with MEBT/MEBO does not produce any new injury. On the contrary, it helps to develop spontaneous resuscitation. Antishock measures mainly aim at protecting and strengthening the cardiac and renal function. Blood volume replenishment is required only according to the principle of general traumatic surgery. Shock, the greatest killer of burns patients, is finally tamed.

G BRT with MEBT/MEBO relieves the economic and mental load of the burns victims. Textbooks over-emphasize that surgical operation is the only method for treating burns, so people are frightened of the sufferings during the operation and the high cost of the treatment. In the US, it typically costs a burns patient USD 150,000 to be treated in the hospital and this does not include the expense of subsequent plastic surgery. Because surgical operation requires strictly sterile and isolated wards, such wards are very expensive to build and maintain. In western countries, treating burns victims with burns area over 50% BSA is considered to have no economic value, because most of the patients will become disabled. BRT with MEBT/MEBO is very revolutionary in this matter as it does not require strictly sterile conditions nor does it require isolation. On the contrary, large-area burns patients can be treated in ordinary hospital wards or even in battlefield hospitals and they will recover to become healthy and normal people. The cost is extremely low by comparison and small area burns patients, if treated with BRT with MEBT/MEBO, do not require hospitalization.

BRT with MEBT/MEBO can cure burns of different causes and different areas, including superficial second-degree, deep second-degree, and full-thickness third-degree burns. It is also an ideal technique for granulation tissue regeneration and repair of burns in muscular layer and bone. BRT with MEBT/MEBO is the major method of burns treatment.

To sum up, what is described above is not speculation. This is clinically demonstrated and despite the skepticism of the reader, responsible investigation into these claims will convince all that burns therapy has now developed into a new historic stage. In the past, only surgical excision and skin grafting were the standard of care and offered great benefit to those whose lives were threatened. Today, however, with the invention of BRT with MEBT/MEBO a major method for burns treatment is available. Either alone or in combination with surgical care, we now offer an elevation in the standard of care for the treatment of burns. As we move together into the 21st century, burns therapy will continue to develop along the lines of BRT with MEBT/MEBO.

Conclusion

An Inevitable Outcome of Scientific Research

This book provides an introduction to the existing therapeutic techniques of local treatment of burns wounds and discusses two therapeutic techniques for treating burns wounds as regards their historical, scientific and technological development. This volume aims at aiding burns medicine researchers and clinicians in developing a correct idea regarding the relative indications of the two therapeutic techniques. It is further hoped that this volume will assist in the elimination of prejudice between different schools as well as to improve the level of the comprehensive burns treatment.

Today, there are only two categories of therapeutic techniques for burns treatment worldwide. One is BRT with MEBT/MEBO, which treats skin burns wounds in such a manner as to achieve both repair and regeneration by creating a wound environment that optimizes the potential of remaining viable tissue. Compared to other treatment protocols, this therapy reduces the rates of scar formation and disability as well as pain suffered and economic burden more than the other technique, which is a surgical skin grafting technique. This second treatment protocol, which aims at treating burns involving whole thickness of the skin and subcutaneous tissues without leaving any viable wound tissue in place, is well known to extract a great physiological price in terms of pain, scarring and residual suffering. It is also far more expensive than BRT with MEBT/MEBO. Prior to the invention of BRT with MEBT/MEBO, widespread utilization of the surgical technique in the treatment of muscle burns and skin burns was acceptable. However, now that scientific studies have demonstrated that BRT with MEBT/MEBO is superior in every way, surgical technique is only a reasonable standard of care in the treatment of skin burns. This conclusion is now a consensus within academic circles worldwide. Surgical burns therapy was born when no other technique could be applied to treat skin burns. Medical researchers and clinicians had been working hard to

find a technique for treating skin burns. The emergence of BRT with MEBT/MEBO represents a blessing to everyone suffering from burns trauma – both the patients and their loyal caregivers. Anyone who has ever cared for burns patients will be relieved and grateful to use BRT with MEBT/MEBO immediately. BRT with MEBT/MEBO is the realization of the dream of all the medical workers and is welcome news to burns patients around the world.

An Inevitable Outcome of the Development of Medical Science

Life science research spans a history of more than 2,500 years. Medical science is only one part of the life science. Medical workers and doctors of successive Chinese dynasties took infinite pains in searching for the key to the door of life science and with it, the ideal methods for controlling diseases. In ancient Greece, Hippocrates established anatomy and surgery, and laid the foundations of modern surgery. Since then, medical workers of the east and the west started using plants and herbs for treating diseases. After the Renaissance, human biochemistry was established, which laid the foundations for the modern treatment strategy of antagonist chemotherapy. Medical sciences developed along with the development of human civilization but the paradigms of medical science were slow to change. In the east, the thinking developed along macro lines of ‘chi’ and patterns of energy flow. Whole plant extracts and consideration of diet predominated in the east whereas in the west, the focus was on more narrow and abstract ideas such as active principles of plants, essential elements and ultimately genetic dynamics.

The development of medical treatment methods and drugs lagged behind the development of human civilization. Despite progress in other areas of human endeavor, the methods and materials for enhancing health and sav-

ing human life are still limited to two categories, i.e. surgery and internal medicine. The former, while saving lives, compromises viable tissue as well as future anatomical function. The latter, availing itself primarily of chemicals unfavorable to human physiology (e.g. chemotherapy), remains fundamentally antagonistic to tissue health and vitality. Neither modality cooperates with the endogenous vital forces and, therefore, medical workers are ill-equipped to facilitate regeneration of tissues and maintenance of health. Appreciating the present limitations of medical science, all health professionals are encouraged to remain open to new trains of thought, to new methods, techniques and materials, especially those that purport to be working in harmony with the principles of human physiology. The inevitable progress of medical science lies in this direction.

BRT with MEBT/MEBO treats in accordance with the principles of human physiology. The techniques and materials of burns regeneration therapy, by being in compliance with the law of human life, and aiming as it does at maintaining normal physiological functions and physiological activities, becomes a new paradigm for healing. In this book, we publish the theory of this technique, research results and examples of its clinical application. All the data prove that BRT with MEBT/MEBO, with new methodology and a new drug (MEBO), points to a new direction in burns medicine research. The success of BRT with MEBT/MEBO is not only limited to the field of burns medicine but also sheds light upon the nascent research of life medicine itself. Burns wounds are typical traumatic wounds and innovations in burns care are generally applicable in the treatment of traumatic wounds of all kinds. In this book, our data proved that after loss of epidermal tissue, viable epithelial cells in the sweat glands of the subcutaneous tissues can, given the appropriate environment, transform into epithelial stem cells and that these stem cells can, in turn, form new epidermal tissues. This regeneration of epidermal tissue accomplished the first cloning of human tissues and organs in the 21st century. We have demonstrated the inexpensive and potent cloning of a 'tissue stem cell'. Therefore, the establishment of BRT with MEBT/MEBO provides the basis for further research in one intriguing aspect of life science – cloning.

The Formation of Burns Medical Therapy in the 21st Century

The history of burns therapy as a specialized field of research is less than a hundred years old. While humans have burned themselves since the dawn of time, no systemic intelligent protocols have been established to serve mankind in this regard. Hippocrates did record burns treatment but his methods were not described with any scientific basis. Subsequently, no specialists in burns medicine were formed until after 1930. Research into burns pathogenesis had not produced any impressive methods or materials for treating burns wounds along physiologic lines. Surgical methods saved lives but left patients disfigured and disabled. Surgery, while representing a big step forward, treats the patients while doing nothing to encourage regeneration of the skin. Finally, at the end of the 20th century, Chinese doctors invented burns regenerative medicine and therapy, which offered an entirely new therapy operating in compliance with principles of human physiology. Now, as we enter a new century and while we pause on that threshold, we all share the opportunity to cooperate and combine the best of all approaches from the east and the west. As described above, the technology of the east, i.e. BRT with MEBT/MEBO, should be applied for all burns including muscle burns; while for treating burns in the muscle layer, the technology of the west, i.e. surgical excision and skin grafting therapy, should be applied. As a whole, it can be called integrated east and west burns therapy. In the era of information, any new technology, thinking, method and material when produced, will immediately be known all over the world and the information be shared. New technology is no longer a legendary tree of a doctor that sheds coins when shaken, and the backward technique will no longer be applied to produce tragedy. We believe that in the 21st century, people will make greater progress in the field of burns medicine and the problems will be completely solved as people succeed in the cloning of organs.

Subject Index

- Antibiotics, infection prophylaxis after burn injury 41, 42
- Antimicrobial effects, MEBT/MEBO
- anaerobic bacteria infection inhibition 82–87
 - bacterial counts in viable tissue 68–70
 - bacteriostasis 77–82
 - fungal infection inhibition 82–87
 - mechanisms 74–82
 - Pseudomonas aeruginosa* infection 70–73
- Blood viscosity, MEBT/MEBO effects 60, 62
- Bone marrow, regenerative medicine 9
- Burn area assessment, Chinese rule of nines 19
- Burn depth
- clinical evaluation 20–22
 - standardization of diagnosis 34
- Burn pathogenesis
- biochemical injury phase 13
 - burns regenerative therapy rationale 17
 - physical injury phase 13
 - reject reaction of necrotic tissues 13, 14
- Burn pathology
- burns regenerative therapy rationale 17
 - MEBO response 92–96
 - morphological changes 14, 15
 - repair changes
 - deep second-degree burns 15
 - superficial second-degree burns 15
 - third-degree burns 15, 16
 - standardization of burn depth diagnosis 34
 - three degree four division method of diagnosis 21, 22
- Burn severity, classification 23
- Burns regenerative therapy, *see also* MEBT/MEBO
- historical perspective 2, 3, 6, 11, 12
 - overview 12, 13
 - rationale 17
- Burns therapy, historical perspective 1–3
- Chinese medicine, historical perspective 5, 6
- Chinese rule of nines, burn area assessment 19
- Cultured composite autograft, skin graft outcomes 33, 145
- Electron microscopy, studies of MEBO response in burn injury
- microcirculation 108, 109
 - scarring 96–104
 - wound healing 113, 114
- Endothelin (ET), MEBT/MEBO effects 61
- Erythrocyte agglutinative index, MEBT/MEBO effects 60
- Erythrocyte transformation kinetics, MEBT/MEBO effects 60
- Extensive burns
- MEBT/MEBO outcomes 129–140
 - surgical excision and skin grafting outcomes 141–143
- Extraordinarily severe burns, burn severity classification 23
- Fibronectin, MEBO comparative studies in corneal burn model 88–92
- First aid, MEBT/MEBO 40
- First-degree burns
- diagnosis 20
 - MEBO application guidelines 35
 - MEBT/MEBO therapy 37
 - pathology of three degree four division method of diagnosis 21
 - three degree six division method of diagnosis 22
- Gastrointestinal mucosa, regenerative medicine 9
- Hematocrit, MEBT/MEBO effects 60, 62
- Hemorrhoids, MEBO application guidelines 35
- Histology, MEBO response studies in burn injury 92–96, 111–114
- Immunoglobulin response, MEBT/MEBO effects 63–68
- Kidney
- protection in shock 40, 41
 - regenerative medicine 9
- MEBT/MEBO
- antimicrobial effects
 - anaerobic bacteria infection inhibition 82–87
 - bacterial counts in viable tissue 68–70
 - bacteriostasis 77–82
 - fungal infection inhibition 82–87
 - mechanisms 74–82
 - Pseudomonas aeruginosa* infection 70–73
 - antishock mechanisms 49, 50
 - clinical trial outcome in China 119–128
 - extensive burns outcomes 129–140
 - fibronectin comparative studies in corneal burn model 88–92
 - hematologic parameter effects 60–62
 - historical perspective 2, 3, 11, 12
 - immunoglobulin response 63–68
 - indications 34
 - limitations 17
 - microcirculation promotion 55–59, 104–111

- MEBT/MEBO (cont.)
- ointment
 - application guidelines 34, 35, 37, 40
 - components 3
 - therapeutic effects 3, 36, 37
 - tissue interactions 36
 - wound debridement 40
 - popularity of use 3, 16, 36
 - procedures and techniques 12
 - prospects 149, 150
 - rationale 17, 145–147
 - standardized local treatment
 - treatment comparison with MEBT/MEBO 27–29, 33, 40–43, 149
 - stem cell differentiation promotion 36, 114–118
 - systemic treatment 37
 - wound liquefaction, *see* Wound liquefaction
 - wound water evaporation prevention 50–54
- Microcirculation, MEBT/MEBO promotion 55–59, 104–111
- Moderate burns, burn severity classification 23
- Moist-exposed burns treatment and moist-exposed burns ointment, *see* MEBT/MEBO
- Nerve, regenerative medicine 9
- Neutrophil, MEBT/MEBO response 77
- Nitric oxide, MEBT/MEBO effects 61
- Nutritional support, MEBT/MEBO 43
- Pancreas, regenerative medicine 9, 10
- Phagocyte, MEBT/MEBO response 82
- Pseudomonas aeruginosa*, MEBT/MEBO effects 70–73
- Regenerative medicine
 - bone marrow regeneration 9
 - concept 6
 - funding discrepancy with Western medicine 6, 7
 - gastrointestinal mucosa regeneration 9
 - kidney regeneration 9
 - nerve regeneration 9
 - pancreas regeneration 9, 10
 - prospects 8–12
 - regenerative stem cells 6–8
 - scar-free healing 7
 - skin regeneration 10–12
- Scarring
 - electron microscopy studies of MEBO response in burn injury 96–104
 - regenerative medicine and scar-free healing 7
- Second-degree burns
 - diagnosis
 - deep burn subtypes 20–22
 - superficial burn subtypes 20, 21
 - MEBO application guidelines
 - deep burns 35
 - small burns 35
 - superficial burns 35
 - MEBT/MEBO therapy stages
 - deep deep burns 39
 - deep superficial burns 38, 39
 - superficial burns 38
 - pathology of three degree four division method of diagnosis 21, 22
 - repair changes
 - deep second-degree burns 15
 - superficial second-degree burns 15
 - three degree six division method of diagnosis 22
- Severe burns, burn severity classification 23
- Shock
 - management in burn injury
 - cardiac function enhancement 40
 - fluid therapy 41
 - nursing care 41
 - renal function protection 40, 41
 - MEBT/MEBO antishock effect and mechanisms in rabbit burn model 47–50
- β -Sitosterol, MEBO composition 3
- Skin, regenerative medicine 10–12
- Skin graft
 - cultured composite autograft 33, 145
 - extensive burns outcomes 141–143
 - historical perspective 1, 2, 16
 - indications 35
 - rationale 16
 - rejection 2
 - standardized local treatment
 - treatment comparison with MEBT/MEBO 27–29, 33, 149
- Skin ulceration, MEBO application guidelines 35
- Slight burns, burn severity classification 23
- Stem cell
 - MEBT/MEBO differentiation promotion 36, 114–118
 - organ cultivation 12
 - regenerative medicine 6–8
- Surgical burns therapy
 - extensive burns outcomes 141–143
 - historical perspective 1, 2, 16
 - indications 35
 - rationale 16
 - standardized local treatment
 - treatment comparison with MEBT/MEBO 27–29, 33, 149
- Third-degree burns
 - diagnosis 21
 - MEBT/MEBO therapy 39, 40
 - pathology of three degree four division method of diagnosis 21
 - repair changes 15, 16
 - three degree six division method of diagnosis 22
- Western medicine, historical perspective 5, 6
- Wound liquefaction
 - body temperature regulation 42, 43
 - drainage of wound 42
 - fluid therapy 42
 - organ protection 43
 - trilogy syndrome management 43
- Wound water evaporation, MEBT/MEBO prevention 50–54