Saurabh Bhatia

Systems for Drug Delivery

Safety, Animal, and Microbial Polysaccharides

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Contents

Chapter 1
Mammalian Polysaccharides and Its Nanomaterials

Abstract Mammalian polysaccharides based nanomaterials emerged as potential drug delivery and diagnostic candidates for their wide applications in therapeutic world. Recently a variety of mammalian polysaccharides have been explored with their diverse derivatives and their role in drug delivery as nanomaterials. Recent research has explored various mammalian polysaccharides such as hyaluronan, chondroitin sulfate and heparin and their wide applications in biomedical research. This chapter emphasized on the nanoapplications of mammalian polysaccharides based nanomaterials with their applications in biomedical research.

Keywords Nanoparticles • Mammalian polysaccharides • Drug delivery • Heparin • Hyaluronic acid

1.1 Introduction

In general polysaccharides are the polymers of monosaccharides. In natural world, polysaccharides have a range of resources from microbial origin (e.g. dextran, xanthan gum), plant origin (e.g. pectin, guar gum), algal origin (e.g. alginate), and animal origin (chitosan, chondroitin). Polysaccharides have variety of reactive groups, broad rangeof molecular weight, unstable chemical composition, which supply to their multiplicity in structure and in property. From the standpoint of polyelectrolyte, polysaccharides can be classified into polyelectrolytes and nonpolyelectrolytes, the previous can be further categorized into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, pectin, etc.). Owing to the occurrence of different derivable groups on molecular chains, polysaccharides can be effortlessly modified chemically and biochemically, lead to various types of polysaccharide derivatives. Since natural biomaterials, polysaccharides are safe, highly stable, hydrophilic, non-toxic and biodegradable. Moreover, polysaccharides have rich resources in environment and low cost in their processing. Mainly, majority of natural polysaccharides have hydrophilic groups e.g. carboxyl, hydroxyl, and amino groups, which could form non-covalent bonds with biological tissues, forming bioadhesion. For an instance,

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alginate, chitosan, starch, and so on is excellent bioadhesive materials. Nanoparticle carriers made of bioadhesive polysaccharides could extend the residence time and thus enhance the absorbance of loaded drugs. All these merits provide polysaccharides a capable prospect as biomaterials. For the utilizing these naturally occurring polysaccharides as drug carriers, concerns of toxicity, safety and availability are really simplified. Recently, array of investigations have been performed on polysaccharides and their derivatives for their potential utilization as nanoparticle drug delivery systems.

1.1.1 Polysaccharide-Based Nanoparticles

Since for polysaccharide-based nanoparticles, previous researchers have ever made outstanding reviews in 2001 and 2005, correspondingly, spotting on the fabrication and application of chitosan nanoparticle carriers. As time goes on, more polysaccharide based nanoparticles appear which significantly augment the adaptability of nanoparticle carriers in terms of category and function. According to structural characteristics, these nanoparticles are fabricated mainly by four different mechanisms, specifically covalent crosslinking, ionic crosslinking, polyelectrolyte complexation, and self-assembly of hydrophobically modified polysaccharides.

1.2 Hydrophobically Modified Hyaluronic Acid

Hyaluronic acid (or hyaluronan, HA) (Fig. 1.1) is a linear, nonsulphated glycosaminoglycan composed of β-1,4-linked disaccharide units of β-1,3-linked glucuronic acid and N-acetyl-D-glucosamine. HA is one of the main components of the extracellular matrix (ECM) and is present at high concentrations in all connective tissues where it executes a rheological/structural function. In addition, owing to its capacity to interact with some cell receptors, HA plays a significant role in processes such as

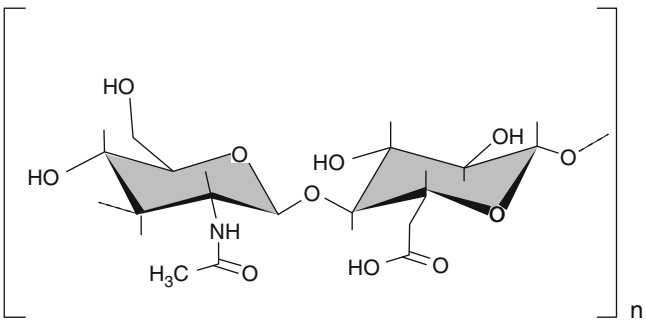

Fig. 1.1 Structure of hyaluronic acid

cell migration, proliferation, and differentiation [1]. In the earlier period HA was derived by extraction from rooster combs, however currently it is preferably derived as product with superior features however with some impurities by fermentation of Streptococcus strain. Recently, commercial HA has been obtained by recombinant Bacillus subtilis sp. that is identified as a GRAS (safe) microorganism [2]. The features of HA can be improved and altered in various ways inorder to derive materials with novelphysico-chemical and biologicalfeatures (hydrophobicity, amphiphilicity & particular biological activities). Currently various HA derivatives are synthesized and developed for different delivery system (Fig. 1.2)

The most commonly adopted chemical modifications of HA target three functional groups, specifically the glucuronic acid group, the primary and secondary hydroxyl groups, and the amine group (after deacetylation of N-acetyl group) (Table 1.1).

Particularly, carboxylates are commonly altered by esterification and amidation reactions typically recognized using carbodiimide assisted coupling reactions. In addition tobis-epoxide and divinylsulfone crosslinking, hydroxyl groups have been altered by etherification and esterification reactions, resulting in linear and crosslinked HA-based products, respectively [3–5]. Owing to the outstanding biocompatibility and biodegradability, HA is one of the most commonly used biopolymers used in the biomedical field and industry. In fact, numerous HA linear or cross-

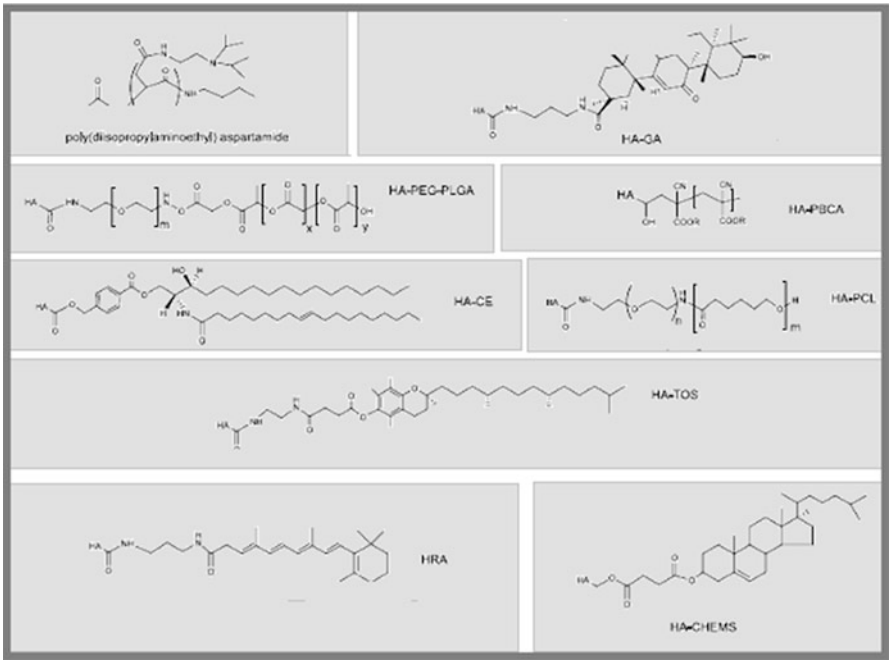

Fig. 1.2 Hyaluronic acid derivatives

Table 1.1 Modifications of hyaluronic acid

Polysaccharides	Modification approaches	Description of reactions or products	Potential applications
Hyaluronic acid	Esterification	Esterification of HA by alkylation using alkyl halides (chlorides, iodides, bromide), by using diazomethane, and by using epoxides	Cell carrier for skin wounds, drug carrier
	Amidation	Amidation of HA in water or of HA's TBA salt in organic solvent with coupling agents, e.g. EDC, NHS	HA–drug conjugates for controlling release, target specific delivery of biomolecules
	Ugi condensation	Formation of diamide linkage between polysaccharides chains by using formaldehyde, cyclohexylisocyanide and diamine	Controlled drug delivery

linked derivatives have been fabricated that are utilized for tissue repair, treatment of joint diseases, wound healing, anticancer drug delivery, and as scaffolds for tissue engineering. HA structure, elucidated by Karl Meyer [4] and revealed on Fig. 1.3, consists of the reappearance of a disaccharide unit of an N-acetyl-glucosamine and a β-glucuronic acid. Its molecular weight is relatively high, above a million. Its most significant physicochemical features are its capability to retain water, a very high hydration ratio, and its viscoelasticity, these two features being interdependent. Combined with its negative charge, HA plays a significant role in the regulation of tissue hydration, permeability to small or large molecules and the physicochemical features of tissues, as well as in several signaling pathways.

1.3 Chemically Crosslinked Hyaluronic Acid Semi-IPN

Earlier researchers [6] prepared a semi-IPN composed of HA and a network of poly(2-hydroxy ethyl methacrylate-co-2-methacryloxyethyl trimethyl ammonium) (p(HEMA-co-METAC)) crosslinked by ethylene glycol dimethacrylate (EGDMA). Owing to the incomplete neutralization of the positive charges of the synthetic networks by HA, the water uptake of this IPN declined within rising weight fraction of the polysaccharide in the matrix. This event was even stronger by substituting HA with chondroitin sulphate, a polysaccharide with a higher charge density due to the occurrence of sulphate groups. The p(HEMA-co-METAC)/HA semi-IPN demonstrated excellent cytocompatibility with mouse fibroblasts and the net positive charge of the IPN gels developed the cell adhesion in contrast to that of gels composed of only HA.A semi-IPN system appropriate

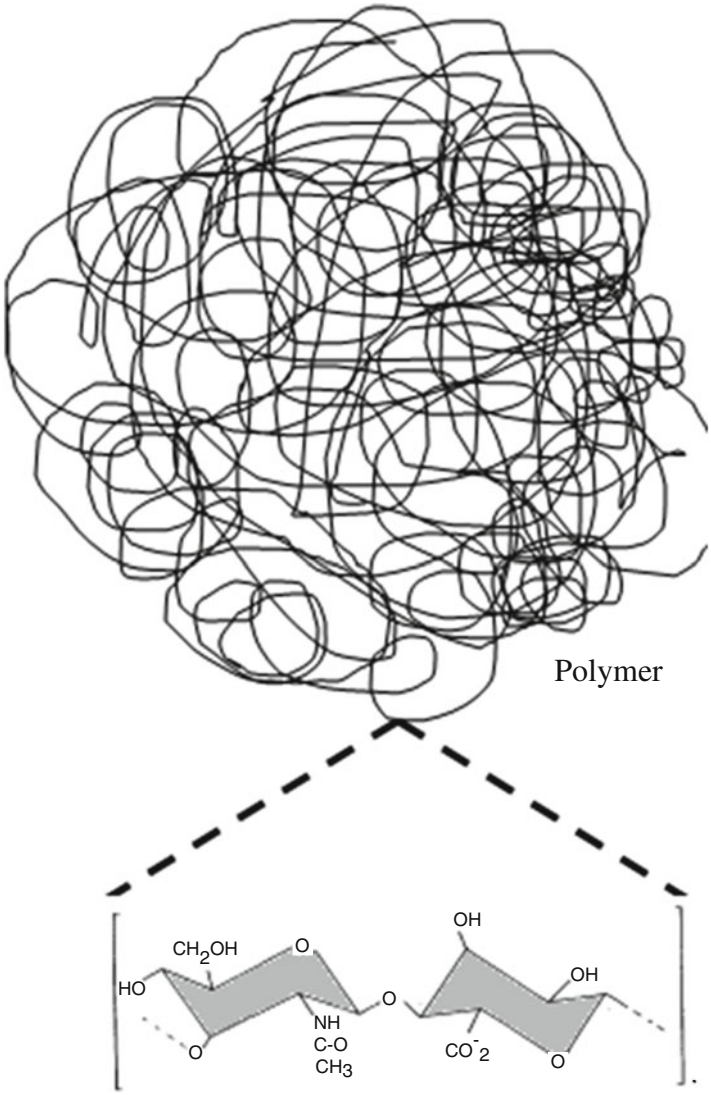

Fig. 1.3 Repeating disaccharide unit of HA and plan illustration presenting its space filling and expanded configuration

for bioprinting was prepared by previous researchers [7] by means of a photopolymerizable dextran derivative, dex-HEMA (hydroxyethyl-methacrylate-derivatized dextran) as crosslinkable component and high molecular weight HA. Dex-HEMA dissolved in an aqueous solution of Alg was crosslinked upon UV exposure by means of Irgacure 2959 as photoinitiator. Mechanical characterization of these semi-IPN hydrogels with variable HA contents were carried out proofing, specially, that the crosslinking kinetics were approximately

instantaneous, as revealed by the sudden augment of the storage modulus G′ after 10 s of UV exposition. The system demonstrated excellent capability of chondrocytes after 3 days of incubation. Bioprinting [8] was approved by using a bioscaffolder pneumatic dispensing system. The polymer solution was extruded via needle on a stationary platform subsequent to layer-by-layer deposition procedure, and stabilized by photocuring. The outcome demonstrated that the acquired 3D construct had a high porosity with well-defined strand spacing and that the overall architecture could be simply tuned by regulating the procedure parameters, e.g. fiber spacing and orientation, representing the appropriateness of the HA/dex-HEMA systems for bioprinting applications in tissue engineering.

1.4 Photopolymerized Hyaluronic Acid IPNS

The commercial attention for HA-based semi-IPNs and IPNs is established by a world patent dated 1994 filed by the Italian Industry Fidia Farmaceutici SpA, that demonstrates IPN biomaterials based on native HA or semi-synthetic HA derivatives and a non-carcinogenic, non-toxic synthetic polymer as second IPN component. The patent also declares HA derivatives with pharmacologically vigorous molecules for IPN applications in a broad variety of sanitary fields, from urology, dermatology, orthopaedics up to plastic and cardiovascular surgeries, in the form of films, hydrogels, membranes, sponges, non-woven tissues, etc. [9]. The majority of significant chemically modified HA polymers for the IPN formation are the methacrylated or acrylated derivatives, due to the mild conditions require for their synthesis [10, 11]. Methacrylic moieties can be conveniently incorporated on the polysaccharide chains by exploiting the reactivity of carboxyl or hydroxyl groups of HA, and the properties of the obtained networks can be suitably modified by altering the polysaccharide derivatization degree [12].

1.5 Hydrophobically Modified Hyaluronic Acid

In the previous work, hyaluronic acid was chemically bonded to dioleoylphosphatidylethanolamine (DOPE) in the presence of EDC chloride as a coupling agent for 24 h at 37 8C. Ultrafiltration removes the coupling agent and the unreacted DOPE [13]. The ensuing product was used in the fabrication of cationic liposomes to yield lipoplexes used in gene therapy [14, 15].

1.6 Hydrophobically Modified Heparin

Heparin is a natural sulfated polysaccharide containing units of sulfonated glucuronic acid and glucosamine derivatives (Fig. 1.4). From decades, heparin is used as an anticoagulant and is also being studied as a potential agent to control complement activity and inflammation. In addition, heparins can intervene with the activity of growth factors e.g. beta fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), ensuing in the obstruction in angiogenesis and tumor development. Obviously these features, spherical and monodisperse heparin-based nanoparticles that are chemically modified with deoxycholic acid were improved with different DS (6.2, 8 and 10 %) [16]. Deoxycholic acid-bearing heparin nanoparticles were enclosed with negatively charged heparin shells, demonstrating zeta potentials by 56 mV. Partition equilibrium constants for pyrene in the nanoparticles showed that rising DS improved the hydrophobicity of the nanoparticle core. The mean aggregation number of deoxycholic acid per hydrophobic microdomain, evaluated by the fluorescence quenching methods by means of cetylpyridinium chloride, showed that five to nine amphiphilic heparin chains contains a hydrophobic domain in the conjugates [16].

1.7 Chondroitin Sulfate, Heparin and Hyaluronic Acid: pH/Ion-Responsive Networks

These three animals-from polysaccharides are getting reticently growing consideration as elements of responsive crosslinked networks for the release of small drugs and proteins. The acidic character of chondroitin sulfate makes it appropriate for intermingling with positively charged molecules, including chitosan, and for being used as polyanions in layer-by-layer (LbL) assemblies. Microcapsules of chitosan–chondroitin sulfate crosslinked with glutaraldehyde are potentially useful for parenteral delivery of low molecular weight heparin [17] and oral administration of 5-fluorouracil [18]. Correspondingly, tablets of chitosan–chondroitin sulfate exhibiting pH-responsive release of indomethacin can be appropriate for colonic administration [19]. Microspheres of complexes of heparin and albumin crosslinked with

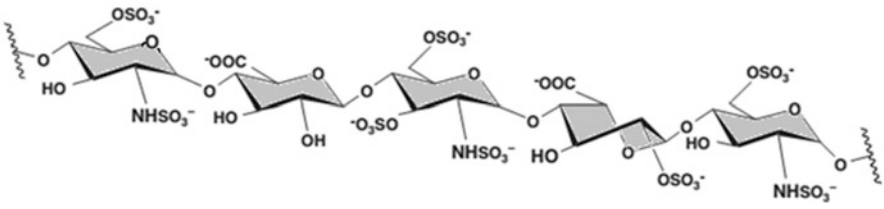

Fig. 1.4 Hydrophobically modified heparin

glutaraldehyde have also revealed pH- and ionic strength-dependent swelling, because of the ionic groups present in both albumin and heparin [20, 21]. The pH dependent performance of hyaluronic acid hydrogels has been examined for photo-crosslinked networks of a polymerizable derivative of hyaluronic acid. After loading with thrombin, the release occurs faster at pH 7 than at pH 1 [22].

1.8 Chondroitin Sulfate and Hyaluronic Acid: Electrical Field-Responsive Network

1.8.1 Chondroitin Sulfate and Hyaluronic Acid

The release level of negatively charged macromolecules from hyaluronic acid hydrogels crosslinked with EGDE was presented to decrease when an electric field was switched on. Hyaluronic acid hydrogels speedily swell in water; however the swelling is restricted in the presence of ions. Likewise, applying an electrical field dramatically minimized the swelling and, therefore, the release rate of poly(styrene sulfonic acid) and poly(glutamic acid, tyrosine) sodium salts. When the electric field was removed, the release level augmented again. Therefore, these hydrogels showed pulsate on–off release as the electric field was switched off–on [23]. Chondroitin sulfate hydrogels crosslinked with EGDE have been revealed as appropriate for electro-responsive administration of peptides and proteins, including vasopressin, aprotinin, lysozyme and albumin. Chondroitin sulfate and albumin are negatively charged at physiological pH, while vasopressin, aprotinin and lysozyme have positive charges. The release of aprotinin and lysozyme could be regulated by means of the voltage, while albumin and vasopressin were flaccidly released. The performance of aprotinin and lysozyme could be explained owing to the fact that positive charged macromolecules under electrical field incline to move from the hydrogel to the cathode. While the similar performance was predictable for vasopressin, it did not ensue perhaps because its smaller size and low charge make its passive diffusion easier. As albumin is negatively charged, variances in the release rate owing to fluctuations in the electrical field are not anticipated [24]. These initial outcomes propose that chondroitin sulfate hydrogels may be suitable for the development of electro-responsive implantable DDSs.

1.9 Heparin & Hyaluronic Acid: Anti-adhesive Surfaces

Prevention of bacterial adhesion on surfaces via anti-adhesive coatings is one of the easiest, possibly cost-effective alternatives to ignore biofilm formation. Bacterial adhesion is a complex process which is influences by various factors involving — as stated above — the physical and chemical features of material surface, nevertheless also

bacterial cell properties and environmental causes e.g. the bulk medium composition (ionic strength, presence of organic substances) and flow conditions. Adhesion of bacteria to negatively charged surfaces under physiological pH environment may be influenced by electrostatic repulsion forces as the net electrostatic charge of maximum bacterial cell walls is negative at neutral pH [25]. It has also been frequently reported that hydrophilic, low surface energy materials are less vulnerable to bacterial adhesion than hydrophobic ones, however opposing outcomes do exist. It is usually accepted that hydrophilic surfaces in connection with media encompassing organic molecules e.g. proteins oppose the development of a conditioning film sheltering adhesion sites for bacteria — restricting specific adhesion/attachment of bacteria and following biofilm development. Anionic polysaccharides with hydrophilic features have been subsequently acknowledged potential candidates to explain anti-adhesive surfaces.

1.9.1 Hyaluronic Acid

The most studied polysaccharides as a biofilm repelling coating is hyaluronic acid [26–28]. In 1999, Morra and Cassineli [26] recognized non-fouling features of glass surfaces modified with hyaluronic acid covalently bound to a first layer of poly(ethyleneimine). Presenting hydrophilic features, this coating minimize adhesion of *S. epidermidis* and *E. coli* by some orders of extent, in contrast to the unmodified glass slide. Harris and Richards [27] studied *S. aureus* adhesion on titanium surfaces, displaying differences in and grafted or not with hyaluronic acid. Viewing no clear dependence on surface roughness, bacterial adhesion was considerably minimized by the coating. In the similar approach, adhesion of *S. aureus* on Ti foils functionalized with hyaluronic acid-catechol was lesser than on pristine substrates [28]. The bacteria-repelling features of hyaluronic acid have been presently demonstrated by a decline in adhesion of *S. aureus* cells to hyaluronic acid-coated Ti surfaces [29] and poly(methyl methacrylate) intraocular lenses [30] in contrast to untreated surfaces. A graft copolymer derivative of hyaluronic acid bearing amino and carboxyl groups showed better prevention of *S. aureus* adhesion on Ti disks than the pristine hyaluronic acid hydrogel [29]. However, many commercial hyaluronic acid-based coatings currently available.

1.9.2 Heparin

Heparin is another natural polysaccharide of animal origin whose anti-adhesive features have been widely studied. Heparin is usually used as an antithrombotic coating in implanted devices that are in contact with blood, in specific catheters and stents. The Bioline Coating® from Maquet Cardiopulmonary GmbH, Rastatt, Germany-a subsidiary of Getinge AB, Göteborg, Sweden, the Bioactive Surface CBAS® from Carmeda AB, Upplands Väsby, Sweden – a subsidiary of W.L. Gore

and Associates, Inc., Newark, Del., and the Trillium® biosurface from Medtronic, Inc., Minneapolis, Minn., are some HP-based antithrombotic coatings available on the market. This negatively charged, linear polysaccharide has been immobilized on material surfaces via numerous physical or chemical approaches comprising electrostatic deposition, layer-by-layer self-assembly and covalent attachment [31]. Bacterial adherence to heparinized commercial devices, e.g., ureteral [32, 33] and biliary [34] stents, central vein [35] and dialysis [36] catheters, has been assessed *in vitro* or *in vivo*. Majority of the investigations demonstrated anti-adhesive effects of heparin coatings though Lange et al. noted no important variation in the number of bacteria adhered to heparin-coated stents and non-coated controls [37].

1.10 Hyaluronic Acid and Chondroitin Sulfate (Polysaccharides of Human Origin): Biodegradable Polymers as Biomaterials

1.10.1 Hyaluronic Acid

Hyaluronic acid (HA) was initially separated in 1934 from the vitreous humor of the eye by Meyer and Palmer [38]. This biopolymer has gradually raised attention as a exclusive biomaterial since its discovery. Hyaluronic acid is a member of the glycosaminoglycan family, which are linear polysaccharides consisting of alternating units of N-acetyl-D-glucosamine and glucuronic acid, and are found in virtually every tissue in vertebrates. HA can be considered to be the major glycosaminoglycan having molecular weights up to numerous millions. In contrast with other members of the glycosaminoglycan family existing in the human body, e.g. dermatan sulfate, keratin sulfate, chondroitin sulfate, and heparin sulfate, HA is not covalently bond to proteins. HA is water-soluble and forms extremely viscous solutions with distinctive viscoelastic features. HA can form 3-D structures in solution with widespread intramolecular hydrogen bonding. It has been reported to be present at high concentrations in synovial fluid and vitreous humor and considerably contributes to the viscoelastic features of these tissues. Additionally, HA plays a significant structural role in a variety of tissues including articular cartilage, the nucleus pulposus, skin, the cervis, and the glycocalyx of endothelial cells. Reports have demonstrated that within the cells, HA is manufactured on the cytosol surface of the plasma membrane under the direction of three glycosyltransferases: hyaluronan synthase-1 (Has-1, Has-2and Has-3 [39]. Between these, Has-2 is the main enzyme accountable for HA production while embryogenesis; nevertheless, particular roles played by Has1 and Has3 are not yet apparent [40]. The traditional sources for HA isolations are rooster combs and bovine vitreous humor. Nevertheless, utilizing bioprocess methodologies for HA fabrication is gaining attention and numerous bacterial fermentation procedures are presently under progress. HA can experience

degradation within the body by free radicals e.g. nitric oxide and MMPs found in the extracellular matrix, trailed by endocytosis. It can also experience digestion by lysosomal enzymes to form mono and disaccharides, which can be subsequently transformed into ammonia, carbon dioxide and water via the Krebs cycle [41]. In previous investigations, HA was considered to be a passive structural component of connective tissues; nevertheless, later investigations shown it to be energetically elaborate various biological procedures e.g. modulating cell migration and differentiation during embryogenesis, regulating extra cellular matrix organization and metabolism, in addition playing significant roles in wound healing, metastasis, and inflammation [42]. Since HA is synthesized by cells while initial wound healing, this polymer has been widely studied for wound dressing applications. Additional distinctive features of HA include its capability to encourage angiogenesis, to control wound site inflammation by acting as a free radical scavenger, and to be identified by receptors on a diversity of cells related with tissue repair. Owing to the high functionality and charge density of HA, it can be cross-linked by a different physical and chemical methods [43]. Improved HA, e.g. esterified derivatives like ethyl/benzyl esters (HYAFFs) and cross-linked hyaluronic acid gels have been broadly studied for wound dressing application. These chemical alterations have also been found to significantly minimize the degradation rate of the polymer. The benzyl esters (HYAFFs) experience hydrolytic degradation via ester bonds in the absence of enzymatic activity with degradation time's varying from 1–2 weeks to 2–3 months, depending on the degree of esterification. The de-esterified polymers are more hydrated and soluble and resemble native HA [44, 45]. HA also plays an important role in tissue repair by encouraging mesenchymal and epithelial cell migration and differentiation, thus improving collagen deposition and angiogenesis. This character, in addition to its immunoneutrality makes HA an ideal biomaterial for tissue engineering and drug delivery applications. Its aqueous solubility permits HA to be synthesized into various kinds of porous and three-dimensional structures for these applications. Therefore a viscous formulation of HA containing fibroblast growth factor (OSSIGELs) is experiencing late stage clinical trial as a synthetic bone graft to hasten bone fracture healing. Likewise HYAFFs 11 is presently been utilized as a carrier vehicle for a different growth factors and morphogens as well as bone marrow stromal cells. In an investigation that associated HYAFFs 11 with an absorbable collagen sponge as a carrier vehicle for osteoinductive protein, recombinant human bone morphogenetic protein-2 (rhBMP-2) shown a well healing response with HYAFFs11 carrier than collagen [46]. HA-based materials have also replaced collagen-based materials as injectable soft tissue fillers [47]. High molecular weight viscous HA solutions (AMVISCs and AMVISCs PLUS) are being used as a vitreous humor substitute as well as to shield the sensitive eye tissue through cataract extraction, corneal transplantation and glaucoma surgery. Viscous HA solutions (SYNVISCs, ORTHOVISCs) are clinically utilized as a synovial fluid substitute to relieve pain and improve join mobility in osteoarthritis patients [48]. A recent animal investigation established the merits of exogenous HA in treating vascular diseases [49].

1.10.2 Chondroitin Sulfate

Reports have shown that a significant phase of wound healing includes the secretion of glycosaminoglycans by fibroblast cells to form a hydrophilic matrix appropriate for remodeling while healing. A current investigation expending rat embryonic fibroblast cells showed that the most of the glycosaminoglycan chains produced were chondroitin sulfate, signifying the implication of this natural polymer for its utilization in biomedical applications [50]. Chondroitin sulfate is the main component of aggrecan, the most plentiful glycosaminoglycan found in the proteoglycans of articular cartilage. Reports have revealed that CS can trigger the metabolic response of cartilage tissue and has antiinflammatory features [51]. It is also elaborate cell recognition, intracellular signaling, and the connection of extracellular matrix components to cell-surface glycoproteins [52]. Chondroitin sulfate entails repeating unit formed by N-acetyl galactosamine (GalNAc) and glucuronic acid (GlcA) modified by sulfation, where the location of sulfation differs with the kind of CS [53]. In mammals chondroitin sulfate disaccharides have been found to be monosulfated in the fourth or sixth position of the GalNAc residue or disulfated in the second and sixth position of the GlcA and GalNAcor in the four and six positions of GalNAc residue [54]. The enzymes accountable for these alterations are chondroitin sulfotransferases. Owing to its biocompatibility, non-immunogenicity and pliability, CS hydrogels have been broadly studied for wound dressing applications [55]. Alike to HA, numerous physical and chemical crosslinking techniques have been established for CS to form hydrogels for biomedical applications [56]. As CS plays a significant role in controlling the expression of the chondrocyte phenotype, it has been broadly studied as a scaffolding material for cartilage tissue engineering. This is mainly significant since investigations have revealed that effective cartilage regeneration can be attained via the use of a tissue engineered implant, simply if the seeded cells experience normal proliferation and phenotype development within the biodegradable scaffold together with the fabrication of a novel cartilage-specific extracellular matrix. Numerous investigations have examined the efficiency of utilizing composite scaffolds composed of CS and other biopolymers, e.g. collagen or synthetic biodegradable polymers, as scaffolds for cartilage tissue engineering. These investigations have explored a strong correlation between the use of CS and the bioactivity of the seeded chondrocytes [57]. Additional natural bioactive polysaccharides that are being acknowledged as potential biomaterials for different biomedical applications comprise heparin sulfate, keratin sulfate and dermatan sulfate.

1.11 Natural–Origin Polymers as Carriers and Scaffolds for Biomolecules and Cell Delivery in Tissue Engineering Applications

1.11.1 Hyaluronan

Hyaluronic acid is most often called as hyaluronan owing to the information that it exists in vivo as a polyanion and not in the protonated acid form [58]. Hyaluronan is a naturally occurring non-sulfated glycosaminoglycan and a main macromolecular component of the intercellular matrix of most connective tissues e.g. cartilage, vitreous of the human eye, umbilical cord and synovial fluid [58]. Hyaluronic acid is a linear polysaccharide that comprised of alternating disaccharide units of α-1,4-Dglucuronic acid and β-1,3-N-acetyl-D-glucosamine, connected by β(1→3) bonds [59]. Hyaluronan and its linked networks have various physiological functions that comprise tissue and matrix water regulation, structural and space-filling properties, lubrication, and a number of macromolecular functions [58]. Particularly for its enhanced viscoelastic features, hyaluronan function as a lubricant and shock absorber in synovial fluid. Hyaluronan has been extensively investigated for drug delivery, for dermal, nasal, pulmonary, parenteral, liposome-modified, implantable delivery devices and for gene delivery (reviewed in Liao et al. [58]). Hyaluronan for tissue engineering has been intensive on cartilage, bone and osteochondral applications, most probable owing to the information that it is a major macromolecular component of the extracellular matrix. Industrially available hyaluronan is derived from various sources, chiefly by isolation from rooster comb, umbilical cord, synovial fluid, or vitreous humor. In addition, hyaluronic acid can be simply and controllably fabricated in large scales via microbial fermentation, from strains of bacteria such as Streptococci [58], enabling the scale-up of derived products and avoiding the risk of animal-derived pathogens. Hyaluronan is accessible for numerous applications, for lubrication and mechanical support for the joints in osteoarthritis (Artz® from Seikagaku Corporation in Japan; Hyalgan® and Hyalubrix® from Fidia in Italy) as a viscoelastic gel for surgery and wound healing (Jossalind® from Hexal in Germany; Bionect® from CSC Pharmaceutical in USA), for implantation of artificial intraocular lens (Healon® from OVD from Advanced Medical Optics in USA, Opegan R® from Seikagaku in Japan, Opelead® from Shiseido in Japan, Orthovisc® from Anika in USA) and as culture media for use in in vitro fertilization (EmbryoGlue® from Vitrolife, USA) [58]. Hyaff® commercialized by Fidia in Italy has been extensively employed as a biomaterial for biomedical applications. From a chemical viewpoint, Hyaff® is a benzyl ester of hyaluronic acid and its key features are that HYAFF® preserves the biological features of the natural molecule from which it originates, the natural degradation of Hyaff® releases hyaluronic acid, which is then degraded via well-known metabolic pathways and that depends on the extent of esterification, it is likely to obtain polymers with various levels of hydrophobicity.

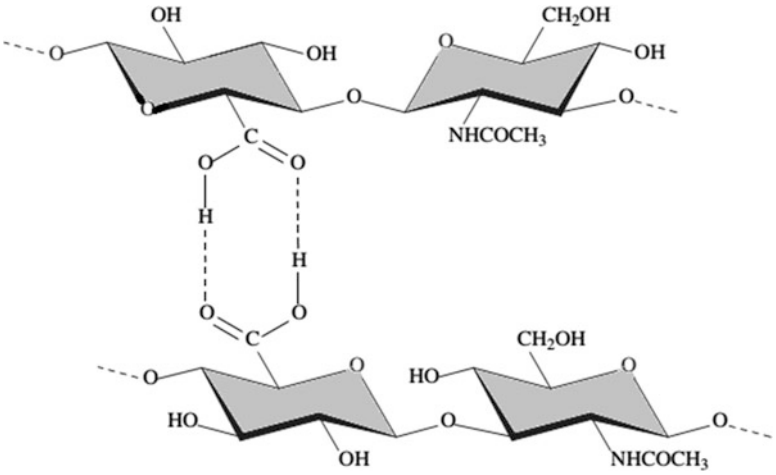

Fig. 1.5 HA cryogels and hydrogen bonding between –COOH groups

The word hydrogel explains 3-D network structures derived from a class of synthetic and/or natural polymers which can absorb and retain considerable amount of water or biological fluids (Fig. 1.5). Polysaccharides that are employed to produce physical cryogels: carboxymethylated cellulose, xanthan, hyaluronan, carboxymethylated curdlan, starch (amylose, amylopectin and their mixtures), β-glucan, locust bean gum, maltodextrins and agarose. A variety of physically crosslinked cryogels from polysaccharides with tunable mechanical, structural, biological features as well as numerous applications is considered and the studies of the fabrication mechanism for these cryogels are also explored. The accurate forming method of HA cryogel has not been completely understood. The complication in gel formation method of HA cryogel might be primarily obtained from its chemical structure, which includes not only massive –OH groups as in PVA and galactomannan, but also –COO and –NHCH3 groups along with potential hydrophobic regions. The intermolecular and intramolecular hydrogen bonding induced from –COOH in HA chains may play a significant role in respect to the network formation and stabilization of HA gel, and the probable example is revealed in Fig. 1.5.

1.11.2 Chondroitin Sulphate

Extracellular matrix components are appreciated building elements for the fabrication of biomaterials involved in tissue engineering, particularly if their biological, chemical and physical features can be regulated. An instance is chondroitin sulfate, one of the best physiologically vital glycosaminoglycans. Glycosaminoglycans (GAGs) are present in the lubricating fluid of the joints and as components of

cartilage, synovial fluid, bone, and heart valves. With the exception of hyaluronan, these polysaccharides are covalently connected to a protein core, thus creating proteoglycans [60]. Bio-characteristics of GAGs comprise the binding and modulation of growth factors and cytokines, proteases inhibition, and the participation in adhesion, migration, proliferation and entiation of cells [61]. Additionally, GAGs are virtually non-immunogenic and degrade to non-toxic oligosaccharides. These features together with their well-defined physical and chemical properties make them very fascinating materials for tissue engineering. Owing to its GAG nature, chondroitin sulfate is a smart natural–origin polymer applied fundamentally in cartilage tissue engineering. However, and owing to its biological characteristics, is frequently used in other tissue engineering applications to valorize other polymers so as to interact with cells and proteins modifying cell behavior of the developed materials. Chondroitin sulfate comprised of repeating disaccharide units of D-glucuronic acid and N-acetyl galactosamine sulfated at either 4- or 6-positions [62]. Chondroitin sulfate can conjugate with core protein to harvest highly absorbent aggregan, which is a chief structure inside cartilage and functions as a shock absorber or it can offer syndecan, which is a cell receptor which can interact with adhesion proteins, cells and the extracellular matrix (ECM) [62]. In vitro studies suggest that chondroitin sulfate is also able to advance matrix component production by human chondrocytes [63]. Additionally, chondroitin sulfate proteoglycans have a serious role in renewal and plasticity in the central nervous system as suggested by Galtrey and Fawcett [64]. However, the readily water-soluble behavior of chondroitin sulfate restricts its application as a solid-state drug delivery vehicle. Accordingly, it is usual to carry out a crosslinking behavior to tailor the properties of chondroitin sulfate as examined in various researches [65] or to syndicate it with other polymers, e.g. chitosan, gelatin and hyaluronan, collagen, poly(vinyl alcohol) or poly-(lacticco-glycolic acid) so as to harvest more stable materials. Additionally, and meanwhile chondroitin sulfate in negatively charged, interaction with positively charged molecules e.g. polymers or growth factors is expected being an important concern to enable the design of delivery systems. For examples this is employed to harvest chondroitin sulfate–chitosan sponges as delivery systems for platelet-derived growth factor BB (PDGF-BB) for bone regeneration as evidenced by JeongPark et al. [66] where this communication revealed to induce more sustained release of the growth factor. As earlier mentioned, owing to its biofeatures, chondroitin sulfate has been used in some extent in the tissue engineering field, chiefly in cartilage applications.

1.12 Rationale for the Use of HA in Drug Delivery

For biomedical functions, HA is chiefly produced by microbial fermentation; it can also be isolated from rooster combs and umbilical cords [67]. HA depolymerization can be attained in batch cultures via either by enzymatic reaction or physical or chemical degradations [68–70]. HA can be related chemically to drugs or to drug carriers. The formation of HA drug conjugates, or the relationship of HA to

colloidal carriers such as micelles, or to nanotechnology-derived particles, give several advantages. The most significant merit is the simplicity of associating drugs with the polysaccharide, either directly or through a drug carrier, consequently solving any solubility issues. A second merit based concerns HA's biopharmaceutical features: it has been recommended that, in various events, HA might improve a drug's blood plasma half-life, reducing the clearance mechanism, and thus contributing a alike role to polyethylene glycol (PEG) [71]. Thirdly, regarding anticancer therapy, the opportunity of tumor targeting is an important merit. Considering their improved pharmacokinetic properties, some HA-conjugates or HA-drug carriers may encounter the well-known improved permeation and retention (EPR) effect, resulting in improved drug distribution in tumor tissues [72, 73]. Additionally, as CD44 is overexpressed in tumor cells and, mainly, in cancer stem or circulating cells, drug selectivity versus target cells may be enhanced [74]. The chance of overcoming the multidrug resistance (MDR) effect, which is occasionally linked to over expression of the efflux transmembrane Phospho-glycoprotein (P-gp), has also been assessed [75]. At high concentrations in solution, HMW-HA can form viscoelastic intertwined molecular networks called as hydrogels, in which drugs can be loaded either by association or via covalent linkage [5]. These hydrogels can be employed for local delivery of antitumor drugs. Nevertheless, solutions of HA do not have long-lasting mechanical integrity, particularly in physiological conditions [76]: HA hydrogels can swell by water absorption, or shrink on degradation. Covalent cross-linking is therefore essential to incorporate stability and improve functionality. Recognitions to the versatility of HA, a variability of chemically-modified forms of this polysaccharide have been developed, for use as tissue repair and regeneration materials, and also for the delivery of anticipated molecules in therapeutics; more specifically, this final concerns anticancer agents. The reactivity of HA, and the main chemical techniques employed in developing drug delivery systems, will now be shortly demonstrated. The carboxylic groups and the mainly hydroxyl groups offer suitable sites for conjugation, and are the most extensively used groups for chemical modification. Comprehensive reviews by Schanté et al. [77] and Collins et al. [78] provide a full description of the variety of chemical modification methods and synthetic routes to obtain HA derivatives. The carboxylic groups are involved in amidation and esterification reactions, and the primary hydroxyl residues in ester or ether bond formation. The acetyl group might be enzymatically removed from the *N*-D-acetylglucosamine, creating it a possible site for conjugation [79]. When carboxylate and hydroxyl groups are altered, multiple connections take place, and the groups are casually linked to the polysaccharide chain, whether they are drugs, lipids, or polymers. Specifically when the carboxylate group is designated as connecting point, it is significant to regulate the degree of substitution (DS) so as to maintain HA's overall charge and targeting features: it has been examined that a DS ratio above 25 % reduced HA's capability to target CD44 receptors [80]. Amidation in water with carbodiimides is one of the most extensively applied methodologies for HA modification; the most extensively used carbodiimide is 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide, owing to its water solubility. The active intermediate, obtained at acidic pH values, does not simply react with amines.

Substituting reacting amines by hydrazides, which have much lower pKa values, higher coupling degrees can be attained: one of the most extensively used reactants is adipic acid dihydrazide. To obtain more stable and more hydrolysis-resistant intermediates, *N*-hydroxysuccinimide or 1-hydroxybenzotriazole are also often used. The acquired active esters display excellent reactivity against the amines. The hydroxyl groups of HA are usually transformed into ester derivatives, by reacting them with the conforming anhydride. Otherwise, acyl-chloride-activated carboxylate compounds can be grafted via ester bonds. The terminal reducing end of HA, which can react as an aldehyde group, may be involved so as to achieve a 1:1 stoichiometric ratio between polymer and reacting molecule. This style entails the reductive amination reaction, typically using sodium cyanoborohydride as reducing agent, with an amino group of the reacting molecule. Additionally aldehyde groups may be attained by reaction with sodium periodate, which oxidizes the hydroxyl groups of the glucuronic acid moiety of HA to dialdehydes, thus opening the sugar ring. Nevertheless, this reaction results in substantial decline of HA's molecular weight. Recognitions to the high hydrophilicity of HA, chemical modification can be achieved in water; nevertheless, in the aqueous phase, several reactions necessitate acidic or alkaline environment that might encourage significant HA chain hydrolysis, or entail the use of reagents sensitive to hydrolysis. Otherwise, organic solvents, e.g. dimethylsulfoxide or dimethylformamide can be but, in this case, the HA sodium salt must be converted to its acidic form, or to a tetrabutylammonium salt, to make it soluble in organic solvents. The use of dimethoxy-polyethylene glycol to solubilize HA in dimethylsulfoxide has also been described.

1.13 Chondroitin Sulfate-Based Nanocarriers for Drug/Gene Delivery

Chondroitin sulfate (ChS), one kind of glycosaminoglycans, repeating disaccharide units of b-1,3-linked N-acetyl galactosamine (GalNAc) and b-1,4-linked d-glucuronic acid (GlcA) with certain position(s) sulfated. According to different points of sulfation, ChS is characteristically recognized with various letters: chondroitin-4-sulfate. Chemical structures of ChS, including ChS-A, ChS-C, ChS-D, ChS-E.A.), chondroitin-6-sulfate, chondroitin2,6-sulfate, and chondroitin-4,6-sulfate (chondroitin sulfate E). It was stated that ChS distributed in animal tissues had an average molecular weight of 20 kDa, which signifies over 100 individual sugar units which might be sulfated at various sites in a chondroitin chain. In this approach, industrially offered ChS are most likely not structurally homogenous substances. ChS-A is naturally isolated from bovine, porcine cartilage, while ChS-C typically from shark cartilage. ChS-E was isolated from squid cartilage for the first time. As a naturally occurring anionic mucopolysaccharide, ChS is a biomaterial found in mammals chiefly abundant in bone, cartilage, skin, ECM, nerve tissue and blood vessels. In biomedical field area, ChS is used in the therapy of osteoarthritis

as a type of nutraceuticals due to its antiinflammatory activity. Moreover, ChS exhibits some biological functions, e.g. antioxidation, anti-atherosclerosis, anti-coagulation, anti-thrombosis, minor immunogenicity, etc. Moreover, it was reported that ChS played a dynamic role in the development of central nervous system, signal transduction, and regulation of cell division and morphogenesis. Additionally, since the electrostatic repulsion produced from the tightly packed and highly charged sulfate groups of ChS that offers much of the resistance of cartilage to compression, ChS has been used in the therapy of osteoarthritis and other cartilage damage diseases. Xiao et al. (2014) synthesized-linolenic acid (-LNA)–ChS conjugates with the effort to advance the bioavailability of low molecular weight ChS. The synthesized amphiphilic -LNA–ChS conjugates were reported to be with low cytotoxicity and high bioavailability, which established that -LNA–ChS might be a possible substitute for CS in clinical use. In addition, Avachat and Kotwal [81] developed an oral controlled release tablet of diclofenac sodium and ChS for concomitant administration in the management of arthritis, and the chitosan–hyaluronan/nano ChS ternary composite sponge was stated to be prepared and designated to be a potential candidate for wound dressing as well. Instead, owing to its biocompatible and biodegradable merits, an growing studies of ChS as a component of drug/gene delivery systems has been elevated. Particularly, ChS can be freely hydrophobically modified because of the presence of a diversity of derivable groups, like carbolic groups and hydroxyl groups, on molecular chains. Then the brush-like graft amphiphilic copolymers are proficient of self-assembling into nano-sized carriers. More significantly, specific features of ChS bestow it with the capability of site-specific drug/gene delivery. For example, ChS can be degraded by colonic micro flora, so ChS has been studied as a matrix material for colon-specific drug delivery systems. ChS was also delivered with the potential of tumor homing by binding with CD44 which over expresses on the surfaces of various tumor cells and the capacity of retention in articular cartilage which is mainly possessing to fact that ChS fits to a part of ECM of cartilage (Fig. 1.6).

The inherent excellent features, biocompatibility, biodegradability, non-immunogenicity, etc., make ChS tremendously popular in terms of a novel type material functional in drug/gene delivery systems. As mentioned above, different nanocarriers for drug/gene delivery based on ChS have been fabricated and assessed in terms of their physicochemical features, drug-loading capacity, in vitro toxicity, and a slice of reasonably simple in vivo examinations. Owing to the large quantity of reactive groups, ChS could be hydrophobically modified to derive a manifold of brush-like grafted amphiphilic copolymers which can self-assemble into nano-sized carriers when dispersed in aqueous medium. Predominantly, ChS is also adept to alter formulated nano-vehicles to present them with special properties, such as more stability, longevity, and targetability etc. There are also some additional significant nanocarriers based on ChS in order to improve the pharmacokinetic behaviors and therapy effect of loaded drug/gene(s). Nevertheless, linked with some other members of glycosaminoglycan family heparin e.g. HA and CS, ChS is still in its infancy as carriers for drug/gene

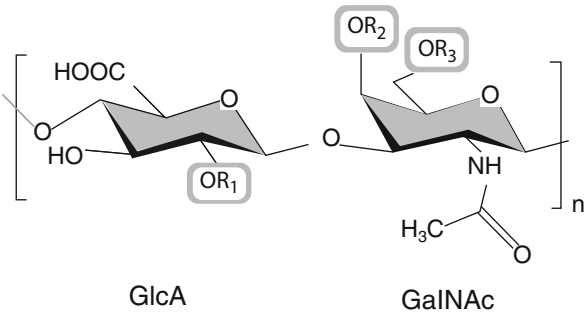

Fig. 1.6 Chemical structures of chondroitin sulfate

ChS-D: R_2=H,R_1=R_3=SO_3H;
ChS-A: R_1=R_3=H,R_2=SO_3H;
ChS-C: R_1=R_2=H,R_3=SO_3H;
ChS-E: R_1=H,R_2=R_3=SO_3H;

delivery. Consequently, it can be predictable that more nanometric delivery systems based on ChS and a increasing number of ChS derivatives will appear in the near future. Additional capable advantages of ChS will be exploited and utilized as a potential carrier for drug/gene delivery. Moreover, there is a crucial prerequisite for a description of mechanism issues, entailing the disposal process of ChS in human body, the specific interaction of ChS with human organs, tissues, cells or even biomolecules.

1.14 Chondroitin Sulphate: Colon-Specific Drug Delivery

Chondroitin sulphate is a muco-polysaccharide found in animal connective tissues especially in cartilage. Chemically, it consists of D-glucuronic acid linked to *N*-acetyl-D-galactosamine which is sulphated at C-6. Chondroitin sulphate is degraded by the anaerobic bacteria of the large intestine mainly by *Bacteroides thetaiotaomicron* and *B. oatus*. Such a degradation profile suggests the use of chondroitin sulphate as a drug carrier to deliver drugs especially to the large intestine where *bacteroides* are found in abundance. However, the high water solubility of chondroitin sulphate is disadvantageous. There was 100 % release of indomethacin within 1 h of dissolution test using chondroitin sulphate alone as a carrier. To overcome this difficulty, cross-linked chondroitin was developed as a drug carrier for colon-specific delivery. Chondroitin sulphate was cross-linked with 1,12-diaminododecane via dicyclohexyl carbodiimide activation. Cross-linked chondroitin sulphate was used to form a matrix tablet with indomethacin. Release of indomethacin from this tablet was studied in the presence of rat cecal contents as compared to release in phosphate buffer saline. A significant difference in drug release was

observed after 14 h in the two dissolution media. Also, different degree of cross-linked chondroitin sulphate was used to study their effect on drug release from the matrices. The cumulative percent release of indomethacin from cross-linked chondroitin matrix tablet showed that release was increased in the presence of rat cecal contents. Studies on rat cecal contents with various cross-linked chondroitin sulphate showed greater cumulative drug release when cross-link. Structure of inulin was less and as cross-linking increased the cumulative release decreased i.e. a linear relationship was found between the degree of cross-linking of polymer and the amount of drug released in rat cecal content. This suggests that the drug release in the colon can be controlled by adjusting the relative amount of different cross-linked chondroitin sulphate in the matrices.

1.15 Hyaluronan and Its Medical and Esthetic Applications

Hyaluronan (HA) is one of the most ubiquitous linear polysaccharides widely distributed throughout evolution in a large number of animal species [82, 83]. It appeared relatively early during evolution, during the silurian period of the Paleozoic era, possibly from its most probable precursor, chondroitin, of much earlier origin (about 540 millions) [84]. Its structure, elucidated by Karl Meyer [85], consists of the repetition of a disaccharide unit of an N-acetyl-glucosamine and a b-glucuronic acid. Its molecular weight is quite high, above a million. Its most important physicochemical properties are its capacity to retain water, a very high hydration ratio, and its viscoelasticity, these two properties being interdependent. Combined with its negative charge, HA plays an important role in the control of tissue hydration, permeability to small or large molecules and the physicochemical properties of tissues, as well as in several signaling pathways. One example is the high HA content of the umbilical cord, protecting its vessels against mechanical compression during fetal development in utero. Similar role was attributed to hyaluronan in semen, the protection of spermatozoids during their risky travel through the uterus to the Fallopian tubes [86]. Another tissue relatively rich in hyaluronan is the skin, where hyaluronan, with its high hydration largely contributes to the "youthful" appearance of the skin. Because of its abovementioned physicochemical properties, hyaluronan controls molecular traffic through tissues. This function could be controlled and experimentally demonstrated by its degradation with hyaluronidase preparations as those obtained with testicular extracts. An early and convincing demonstration by Duran-Reynals of this property consisted in determining the tissue distribution of the vaccinia virus after subcutaneous injection in rabbits [87–90]. This process was considerably accelerated by co-injecting with the virus a testicular extract rich in hyaluronidase. Although this early experiment clearly demonstrated the accelerated diffusion of virus particles through tissues by this "spreading factor", shown later to be hyaluronidase. The study of hyaluronan started seriously after the elucidation of its structure followed by experiments carried out by a rapidly increasing number of investigators, as nicely analyzed by Balazs and Denlinger [91]. Several cell types

were shown to synthesize hyaluronan, among them fibroblasts, the most important cell type because of their large number in the skin, the most voluminous tissue of the body. Besides fibroblasts, several other cell types were shown to synthesize hyaluronan, even some micro-organisms. A Streptococcus strain acquired this ability, probably by horizontal gene transfer [92]. Hyaluronan is rapidly degraded by endoglycosidases called hyaluronidases, such as those in testicular extracts. Several other hyaluronidases have been isolated from a variety of tissues and cells [93]. HA is also very sensitive to degradation by free radicals [94]. This reaction is also of great biological significance, because of the generation of ROS capable of degrading HA in tissues during a number of pathological processes as for example inflammatory reactions. Advanced glycation end products (AGE-s) generated by the Maillard reaction, were also shown to induce free radical mediated degradation of hyaluronan [95]. Breakdown products, oligo- and polysaccharides resulting from hyaluronan degradation were shown to possess several important biological properties, among them the stimulation of hyaluronan-resynthesis [96]. Another important physicochemical property of HA resides in its stereochemical structure. The HA polysaccharide chain exhibits an asymmetric distribution of its hydrophilic and hydrophobic side chains. On one side the polysaccharide chain is hydrophobic, on its other side hydrophilic [97, 98]. This property was shown to play an important role in its biological behavior, and also in its medical applications, especially in ophthalmology.

1.15.1 Aging and Hyaluronan

The biosynthesis and turnover of HA were shown to decrease with age. This decrease is of major importance for the age related increase of several tissue and organ modifications as for instance in osteoarthritis, because of lack of protection against frictional erosion of articular cartilage and also retinal detachment due to the degradation of HA in the joints and the vitreous body in the eye. Wrinkling of the aging skin is also one of its consequences. The precise cellular nature of this age-dependent decline of HA biosynthesis remains to be more deeply investigated.

1.16 Polysaccharides Based Composites

1.16.1 Heparin-Based Composites

A new heparin- and cellulose-based biocomposite at 7/100(w/w) ratio is produced by developing the increased dissolution of polysaccharides in room temperature ionic liquids (RTILs) [99]. This signifies the principal published instance of utilizing a novel class of solvents, RTILs, to prepare blood-compatible biomaterials. Employing this strategy, it is likely to fabricate the biomaterials in any form, e.g.,

film or membranes, fibers and spheres (nanometer- or micron-sized), or any shape using templates. Surface morphological investigations on the biocomposite film demonstrated the homogeneously distributed presence of heparin via cellulose matrix. Activated partial thromboplastin time and thromboelastography establish that this composite is greater to other exiting heparinized biomaterials in averting clot formation in human blood plasma and in human whole blood. Membranes made of these composites permit the path of urea though retaining albumin, signifying a most promising blood-compatible biomaterial for renal dialysis, with a possibility of eliminating the systematic administration of heparin to the patients experiencing renal dialysis. An electrospinning processing was representing by utilizing10% cellulose solution in 1-butyl-3-methylimidazolium chloride or 2 % (w/w) heparin in 1-ethyl-3-methylimidazolium benzoate. The solutions were collected together and mixed by using vortex for 2 min to give a clear cellulose-heparin solution. Both cellulose and heparin-cellulose solution were exposed to electrospinning [99]. A 1 mL sample of polysaccharide RTIL solution was shifted to a syringe attached to a syringe pump. A voltage of 15–20 kV was applied to a needle of the syringe, with a ground charge, in the form of an aluminum sheet placed beneath the ethanol collector. The nozzle-to-grounded-target distance was fixed at 15 cm. The flow rate of the syringe pump (0.03–0.05 mL/min) was attuned in tandem with the applied voltage giving fiber formation. Both of the RTILs selected for the investigation, are entirely miscible in ethanol, while neither of the polysaccharides are ethanol soluble. Therefore as the fibers prepared, the ethanol extractively removed the RTIL solvents, giving pure polysaccharide fibers [99]. The fibers in the form of a twisted web were washed with additional ethanol and then dried in vacuum to eliminate the residual ethanol. Heparinized cellulose matrices (H-CM) were used as affinity substrates for binding of basic fibroblast growth factor, a heparin-binding peptide, to facilitate cellular proliferation and substrate-mediated transgene delivery. It was revealed that H-CM was a welcoming substrate for cellular adhesion using HT-1080 fibroblasts and Saos-2 osteoblasts. It is likely that inexpensive polysaccharides will be used for APCs fabrication with features close to heparin and heparin containing APCs [99].

1.16.2 Hyaluronan-Based Composites

Oxidized hyaluronic acid was coupled with chitosan to form porous scaffolds after freeze drying. The proportion of porosity of the freeze-dried chitosan–hyaluronic acid dialdehyde composite (CHDA) gels enhanced with augmentation in oxidation. Fibroblast cells seeded onto CHDA porous scaffold adhered, proliferated and offered extracellular matrix components on the scaffold [99]. Chondrocytes encapsulated in CHDA gels retained their viability and specific phenotypic features. The gel material is therefore projected as a scaffold and encapsulated material for tissue engineering applications. Films of hyaluronan (HA) and a phosphoryl choline-modified chitosan (PC-CH) were constructed by the electrolyte multilayer (PEM)

statement technique [99]. The HA/PC-CH films were constant over a broad pH range (3.0–12.0), displaying a stronger resistance against alkaline environment in contrast to HA/CH films. The fluid gel-like features of HA/PC-CH multilayers were recognized to their high water content (50 wt%), which was projected by associating the surface coverage values derived from SPR and QCM measurements. Assumed the versatility of the PEM methodology, HA/PC-CH films are attractive tools for developing biocompatible surface coatings of controlled mechanical features. Heparin-conjugated hyaluronan microgels with dissimilar heparin content, namely 1 %,5 %, and 10 %(w/w), were produced for the controlled release of bone morphogenetic protein-2. Hyaluronan microgels presented a smooth surface and dense network, while HA-Hp microgels showed a rough surface with holes and concaves, and a looser internal structure with increasing the heparin content as an alternative [99]. Nevertheless, the major microgel size of about 3 m was independent of the heparin amount. Between the samples, HA-Hp-10 % microgels occurred the utmost equilibrium swelling ratio of 11.8 due to its least crosslinking network. A advanced BMP-2 loading efficiency and a microgels was in favor of BMP-2 binding and the sustained delivery maybe credited to the electrostatic interaction between heparin andBMP-2. By means of crosslinking of HA with various polysaccharides new opportunities are exposed in medical applications and also fabrication of HA derivatives from various polysaccharides gives new standpoints for APCs [99].

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Chapter 2
Microbial Polysaccharides as Advance Nanomaterials

Abstract The microorganisms offer great amounts of polysaccharides in the presence of additional carbon source. Certain polysaccharides serve as storage compounds. The polysaccharides excreted by the cells, called as exopolysaccharides, are of industrial importance. The exopolysaccharides may be reported in association with the cells or may remain in the medium. The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups such as carboxyl, which can function as polyelectrolytes, are commercially more important. These emerging microbial polysaccharides are recently explored as nano-materials for diverse biomedical applications. This chapter emphasize on nano-applications of microbial polysaccharides in diverse discipline of biomedical science.

Keywords Microbial • Polysaccharides • Nanoparticles • Drug delivery

2.1 Introduction

Polysaccharides are non-toxic, natural, and biodegradable polymers that envelop the surface of most cells and play significant functions in a variety of biological mechanisms e.g. immune response, adhesion, infection, and signal transduction. Studies on the optional treatments applied by diverse cultures all the way through the history exposed the fact that the utilized plants and fungi were rich in bioactive polysaccharides with established immune-modulatory activity and health encouraging effects in the treatment of inflammatory diseases and cancer. Therefore significant research has been directed on illuminating the biological activity mechanism of these polysaccharides by structure-function analysis. In addition to the attention on their applications in the health and bio-nanotechnology sectors, polysaccharides are also employed as stabilizers, thickeners, bioadhesives, probiotic, and as emulsifier, and gelling agents in food and cosmetic industries, biosorbent and bioflocculant in the environmental sector. Polysaccharides are either isolated from biomass capital like algae and higher order plants or derived from the fermentation broth of bacterial or fungal cultures. For economical and sustainable production of bioactive polysaccharides at commercial scale, in spite of plants and algae, microbial sources are favored because they facilitate fast and high yielding production procedures under

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Table 2.1 Classification of polysaccharides

Polysaccharides	Complete class
Microbial Polysaccharides	**Bacterial polysaccharide:** bacterial cellulose, dextran, bacterial hyaluronic acid, xanthan, emulsan, β-d glucans, curdlan, alginate, gellan and pullulan, scleroglucan and schizophyllan. bacterial hyaluronic acid, kefiran, exopolysaccharide, xanthan gum, dextran, welan gum, gellan gum, diutan gum and pullulan
	Fungal polysaccharides: Chitin, scleroglucan, lentinan, schizophyllan krestin, galactofuranose
	Yeast polysaccharide: Zymosan, glucans, glycogen, mannan
Mammalian Polysaccharides	Glycosaminoglycans (hyaluronic acid or hyaluronan, Chondroitin sulphate), gelatin and heparin sulfate, chitin and chitosan
Others	B-1,3-glucans derived from a variety of natural sources (such as yeasts, grain, mushroom or seaweed), poly-gamma-glutamate (amino acid polymer)

completely controlled fermentation conditions. Microbial production is attained within days and weeks in contrast to plants where production takes 3–6 months and highly experiences from geographical or seasonal differences and ever growing issues about the sustainable utilization of agricultural lands. In addition, production is not only independent of solar energy which is indispensable for production from microalgae but also favorable for employing various organic resources as fermentation substrates. In relation to recent reports, the global hydrocolloid market dominated by algal and plant polysaccharides like starch, carrageenan, galactomannans, pectin, and alginate is predictable to arrive at 3.9 billion US dollars by 2012. Intervening these traditionally used plant and algal gums by their microbial counterparts entails new strategies and significant development has been made in discovering and developing new microbial extracellular polysaccharides (exopolysaccharides, EPSs) that enjoy novel industrial importance. Recent review explored four EPSs, namely, xanthan, pullulan, curdlan, and levan, as biopolymers with exceptional potential for a variety of industrial sectors. Nevertheless, when evaluated with the synthetic polymers, natural origin polymers still symbolize only a small portion of the current polymer market, typically owing to their costly production processes. Thus, a lot of inputs have been devoted to the progress of cost-effective and eco-friendly production processes e.g. studying the possible use of cheaper fermentation substrates. Tables 2.1 and 2.2 demonstrate complete class of microbial polysaccharides (Fig. 2.1).

The microorganisms can offer great quantity of polysaccharides in the existence of surplus carbon source. A number of these polysaccharides serve as storage compounds. The polysaccharides excreted by the cells, known as exopolysaccharides, are of great commercial importance. The exopolysaccharides may be originate in association with the cells or may stay in the medium. The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups e.g. carboxyl, which can utilize as polyelectrolytes, are commercially more significant.

Table 2.2 Commercially significant microbial polysaccharides and its applications

Polysaccharide	Source(s)	Description	Application(s)
Dextrans	Leuconostoc mesenteroides, Acetobacter Sp., Streptococcus mutans	Dextrans are among the oldest known complex bacterial polysaccharide made of many glucose molecules, composed of chains of varying lengths (3–2000 kda)	Blood plasma expander Used in the prevention of thrombosis (as adsorbent). In the laboratory for chromatographic and other techniques involved in purification, widely used in foods, cosmetics and biotechnology, wound dressing
Xanthan	Xanthan gum is a polysaccharide secreted by the bacterium Xanthomonas campestris	It is composed of pentasaccharide repeat units, comprising glucose, mannose, and glucuronic acid in the molar ratio 2:2:1	In food industry for stabilization and gelling and viscosity control, in oil industry to enhance oil recovery, in the fabrication of tooth pastes and paints
Pullulan	Pullulan is a polysaccharide polymer produced from starch by the fungus Aureobasidium pullulans	It consisting of three maltotriose units, also known as α-1,4-;α-1,6-glucan, connected by an α-1,4 glycosidic bond	Biodegradable polysaccharide used in food packing and coating
Gellan	Gellan gum produced by the bacterium Sphingomonas elodea (formerly Pseudomonas elodea)	Gellan gum is a water-soluble anionic polysaccharide. It is composed of repeating unit of the polymer is a tetrasaccharide, which consists of two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid	In food industry as thickener and solidifying agent
Recombinant hyaluronan	rDNA technology	It is an anionic, nonsulfated glycosaminoglycan distributed in nature	Clinical significance in cancer, wound repair, inflammation, granulation and organization of the granulation tissue matrix, cell migration, skin healing, fetal wound healing and scarring, for cosmetic uses
Curdlan	Curdlan is produced by non-pathogenic bacteria such as Agrobacterium biobar. The production of curdlan by Alcaligenes faecalis is being developed to be used in gel production as well	Curdlan is a linear beta-1,3-glucan, a high-molecular-weight polymer of glucose. Curdlan consists of β-(1,3)-linked glucose residues and forms elastic gels upon heating in aqueous suspension	As a gelling agent in cooked foods and form strong gel above 55 °C, for immobilization of enzymes

(continued)

Table 2.2 (continued)

Polysaccharide	Source(s)	Description	Application(s)
Scleroglucan	Scleroglucan is produced by fermentation of the filamentous fungus *Sclerotium rolfsii*	Scleroglucan is a water soluble, nature-derived polysaccharide	Used for stabilizing latex paints, printing inks and drilling muds
Bacterial Alginate	The bacteria Pseudomonas aeruginosa and Azotobacter vinelandii have been shown to secrete exocellular polysaccharides similar to the alginic acid from algae	Alginic acid, also called algin or alginate, is an anionic polysaccharide distributed widely in the cell walls of brown algae and several bacterial strains where through binding with water it forms a viscous gum	In food industry as thickening and gelling agent, used as ion exchange agent, and used for the immobilization of cells and enzymes
β-Glucans	Naturally occurring in the cell walls of cereals, yeast, bacteria, and fungi	Comprised of a group of β-D-glucose polysaccharides with considerably varying physicochemical properties dependent on source. Typically, β-glucans form a linear backbone with 1–3 β-glycosidic bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals	Used in various nutraceutical and cosmetic products, as texturing agents, and as soluble fiber supplements, but can be problematic in the process of brewing.
Levan	Levans are a group of fructans; polymers of fructose forming a non-structural carbohydrate, which in the case of levans can themselves link together to form super-molecules comprising even hundreds of thousands	Synthesized by levansucrase from Pseudomonas syringae	Approach for food supplements to provide safe and efficient delivery of microelements
Emulsan	Produced by Acinetobacter calcoaceticus	Emulsan is a polyanionic heteropolysaccharide bioemulsifier	In oil industry to enhance oil recovery and in cleaning of oil spills

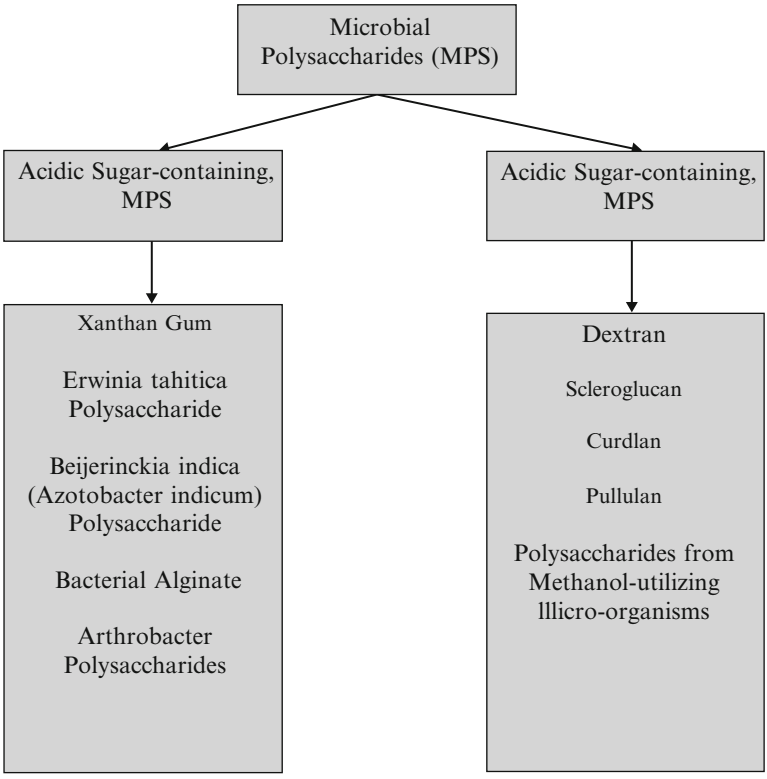

Fig. 2.1 Microbial polysaccharides

2.2 Microbial Polysaccharides: General Applications

Microbial polysaccharides have great commercial significance. They are engaged in the stabilization of foods, and development of various industrial and pharmaceutical compounds. The commercial importance of a polysaccharide depends on its potential to modify the flow characteristics of solutions (technically known as rheology). Polysaccharides can enhance the viscosity and, are therefore useful as thickening and gelling agents. Microbial polysaccharides are of immense significance in oil industry. Via conventional methodologies, only 50 % of the oil can be extracted. And the rest is either trapped in the rock or too viscous to be forced out. It is now likely to recover such oils also by a procedure known as microbial enhanced oil recovery (MEOR). This can be achieved by means of injecting surfactants and viscosity decreasing biological agents (i.e. the microbial polysaccharides e.g. xanthan and emulsan).

2.3 Microbial Polysaccharides Production

The production of polysaccharides positively occurs in the surplus amount of carbon substrate in the growth medium while limiting nitrogen supply. A carbon/nitrogen ratio of around 10: 1 is acknowledged to be positive for optimal polysaccharide production. The production process is usually carried out by batch culture fermentation. Via manipulating the nutrient flow, differential production of polysaccharides can be attained. By means of limiting nitrogen flow in the medium, typically neutral polysaccharides are produced. When amount metal ions are inadequate, acidic polysaccharides are principally synthesized. Molecular oxygen supply of approximately 90 % saturation is perfect for excellent growth and polysaccharide synthesis.

2.4 Biosynthesis of Polysaccharides

Microorganisms are proficient in synthesizing a large number of polysaccharides. The pathways for their biosynthesis are similar to the procedures that take place for the formation bacterial cell wall. It is anticipated that there are well over 100 enzymatic reactions, directly or indirectly involved in the synthesis of polysaccharides. Initially with glucose, suitable sugars (by transforming glucose to others) are included in the formation of polysaccharides.

2.5 Polysaccharides Recovery

Since the polysaccharide production enhances, there arises a noticeable increase in viscosity of the culture broth. The polysaccharides can be precipitated by acids, salts, or organic solvents, and recovered via utilizing appropriate techniques.

2.6 Microbial Polysaccharides vs Plant Polysaccharides

Owing to immense competition between microbial and plant polysaccharides for industrial applications, various advance techniques and methodologies have been explored by several researchers to explore their structural backbone and their associated biological functions. Development of plant polysaccharides is comparatively cheap, while it is uncontrolled and takes place for a short span in a year. On the contrary, microbial polysaccharides production is well regulated and can be sustained throughout the year. Nevertheless, fermentation procedures for fabrication of cheap (from plant sources) polysaccharides are not advisable.

2.7 Microbial Polysaccharides: General Features

Among the various microbial polysaccharides, approximately 20 are of industrial significance. As previously mentioned, the commercial worth of a polysaccharide is usually based on its rheological features specifically its capacity to modify the flow properties of solutions. A preferred record of commercially significant polysaccharides, the microorganisms employed for their synthesis, and their applications are mentioned in the Table 2.2. A number of the significant characteristics of individual microbial polysaccharides are shortly explained hereunder.

2.7.1 Xanthan

Xanthan or more often known as xanthan gum was the foremost polysaccharide presented commercially. It is a well investigated and most extensively used hexopolysaccharide (Fig. 2.2).

It has molecular weight in the range of $2–15 \times 10^4$ Da. The central repeating unit of xanthan is a pentasaccharide containing mannose (Man), glucose (Glc) and glucuronic acid (GIcA) with acetate (Ac) and pyruvate (Pyr) as represented below. Fundamentally, xanthan is a branched polymer with β $(1 \rightarrow 4)$ linked glucan (glucose polymer) backbone bound to a trisaccharide (Man, GIcA, Man) side chain on alternate glucose residues. The mannose has either acetate or pyruvate groups. The number of acetate or pyruvate molecules in xanthan is variable and is based on the bacterial strain used. The culture environment and the recovery procedures also affect the amount of pyruvate and acetate residues. It is assumed that the viscosity of xanthan gum is affected by the contents of pyruvate and acetate. Xanthan gum is employed as a food additive for the fabrication of soft foods. It is also employed in oil industry for increasing oil recovery. In addition, xanthan is functional for the fabrication of tooth pastes and water based paints. For xanthan biosynthesis, the monomers are linked to a carrier lipid molecule and then moved to an increasing polymer chain. The triggered monosaccharide nucleotides flow energy for the configuration of glycosidic bonds between neighboring units. The biosynthesis of other exopolysaccharides is similar with that of xanthan. Dextran production

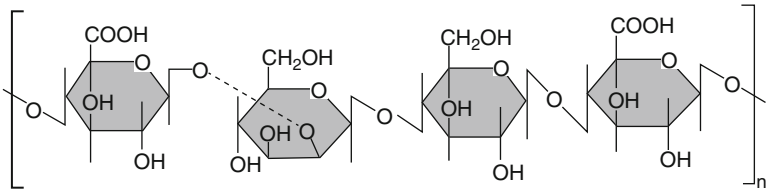

Fig. 2.2 Xanthan gum

Table 2.3 Drug delivery applications of xanthan gum

Polysaccharide involved	Size	Application	Ref
Xanthan gum	Nanoparticles	Lysozyme delivery (protein/ polysaccharide NP)	[1]
Xanthan gum, locust bean gum	Nanoparticles	Silicon dioxide Delivery	[2]
Locust bean gum, xanthan gum	Microparticles	Celecoxib drug delivery	[3]
Xanthan gum	Microparticles, NPs	Sustained release of antiseptic agent (Chlorhexidine)	[4]
Chitosan–Xanthan Gum starch–xanthan gum galactomannan from Schizolobium parahybae and xanthan	Liposomes	Pulmonary Delivery of Rifampicin for controlled drug delivery	[5]
	Hydrogels		[6]
	Hydrogels	Curcumin delivery	[7]

however is much simpler as described later. Xanthan is commercially developed from the Gram-negative bacterium, Xanthomonas campestris. The culture medium generally consists of 4–5 % carbohydrate (glucose, sucrose, corn starch hydrolysate), 0.05–0.1 % nitrogen (ammonium nitrate, urea, yeast extract) and salts. The pH is maintained approximately at 7.0, and the fermentation is take place by batch culture for 2–3 days. Precipitation of xanthan in the culture broth is usually achieved by isopropanol or methanol and these agents also kill the microorganisms. The precipitated xanthan can be dried and employed for commercial reasons. Table 2.3 describes drug delivery applications of xanthan gum.

2.7.1.1 Microwave Irradiation in the Grafting Modification of the Xanthan Gum

Xanthan gum is a polysaccharide synthesized by the fermentation of the bacterium Xanthomonas campestris [8]. This organism is reported in nature on the leaf surfaces of green vegetables, particularly the cabbage family. The gum is employed as a food additive and rheology modifier. The %G of poly(acrylamide) on xanthan gum under microwave-initiated grafting has been reported to be much higher in contrast to ceric-induced conventional grafting (62.87 %G) [8]. The grafting yield was found directly proportional to the microwave power and exposure time. The swelling of the xanthan gum was reported to differ contrariwise with the %G, while erosion varied directly with the %G. The microwave-assisted graft co-polymerization was employed as an effective tool to alter the release features of xanthan gum by the grafting of acrylamide on xanthan gum.

2.7.2 Dextrans

Dextrans are glucans (polymers of glucose) chemically containing $1 \rightarrow 6$ glycosidic linkages, having molecular weights range between 15,000 and 500,000 kDa (Fig. 2.3). Currently dextrans are used as blood plasma expanders, for the prevention of thrombosis and in wound dressing.

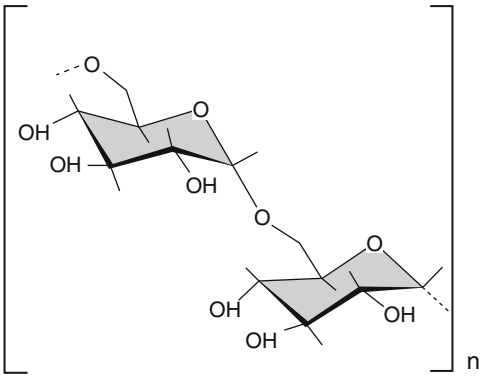

Fig. 2.3 Structure of Dextran

Moreover, dextrans are also functional in the laboratory analytical techniques for purification of biomolecules. Dextrans can be produced by a extensive variety of Gram-positive and Gram-negative bacteria. For an example *Leuconostoc mesenteroides* and Streptococcus mutans are utilized for the synthesis of dextran. On the contrary to other exopolysaccharides (which are synthesized within the cells), dextrans are synthesized by extracellular enzyme in the medium. The enzyme which is responsible for this synthesis is dextransucrase (a transglucosidase) which take action on sucrose and carried about polymerisation of glucose residues, and concurrently releases free fructose into the medium. The commercial production is achieved by means of lactic acid bacterium, *L. mesenteroides* by a batch fermentation process. In addition to sucrose, the culture medium comprised of organic nitrogen source and inorganic phosphate. The crude dextran formed is precipitated by alcohol and then subjected to acid hydrolysis. Recently, the alcohol precipitated polymeric dextran is directed to enzymatic hydrolysis by using exo- or endo-a dextranases to get dextrans of desired molecular weight. The resulting dextrans can be fractionated and dried. It is also feasible to employ a cell free system for the synthesis of dextrans. The extracellular enzyme dextransucrase can convert sucrose into dextran in a cell-free nutrient solution. This reaction is optimum at pH 5.0–5.5 and temperature 25–30 °C. Table describes drug delivery applications of dextrans.

Cationic polysaccharides are extensively investigated in various areas such as water treatment, food, cosmetic, papermaking, chemical, and petroleum industries. The combination of cationic polysaccharides with anionic polymers can results in interpolyelectrolyte complexes with hydrogel-like structures further expanding the application of the former. Incomplete oxidation of dextrans with periodate, to form a dialdehyde in the oxidized sugar unit and subsequent reductive amination employing diverse polyamines of interest such as spermine or quaternary monoammonium derivatives; these products were employed for gene delivery. A corresponding synthesis is found for schizophyllan, a β-glucan. Utilizing alike approach, the hydroxyl in the 6 position of a β-glucan from oat was oxidized with paraformaldehyde and then underwent a reductive amination with ammonium acetate and $NaBH_4CN$ (Fig. 2.4). The kinetic of the reaction of polysaccharides, with epichlorohydrin and various tertiary amines was investigated, by quantification of reagents

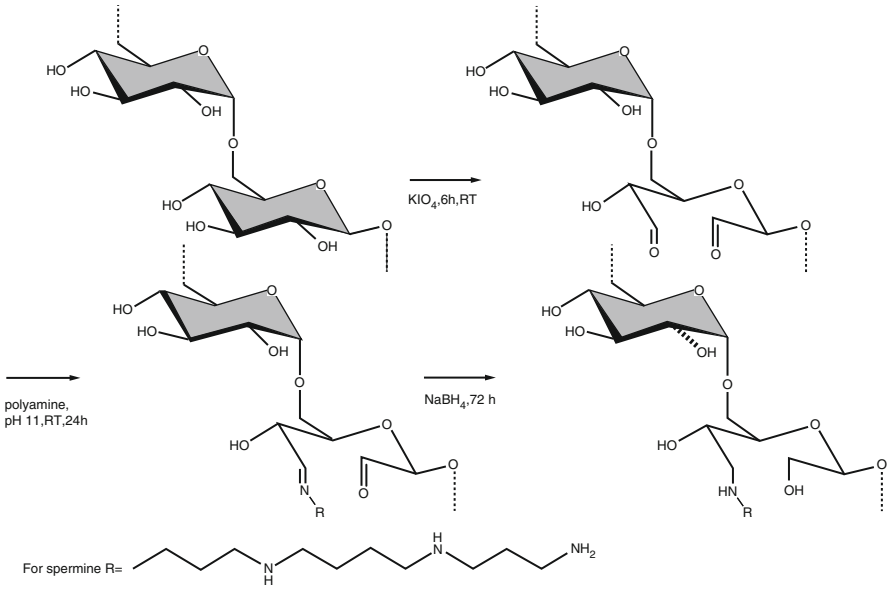

Fig. 2.4 Periodate partial oxidation and subsequent reductive amination of dextran

and products in the reaction mixture with or without the presence the polysaccharide, at steady intervals. They investigated that the use of cyclic amines such as 1-methyl-imidazol or 1,4-diazabicyclo[2,2,2]octan as "catalysts" together with another tertiary amine, used for the substitution, was not necessary and led to a cationic polysaccharide with mixed and uncontrolled chemical composition. After 30 min of reaction at 40 °C, 80 % of epichlorohydrin had reacted with the amine, reaching a 94 % after 3 h. Initially, the original epoxy groups remained unchanged, but they started to decline at 24 h (Fig. 2.4). The paramount solvent for this reaction was water and an equimolar amine: epichlorohydrin ratio contributed satisfactory results.

2.7.2.1 Hydrophobically Modified Dextran

In the report by Nichifor et al. [9], dextran molecular weight close to 30,000 g/mol was covalently bound to bile acids (cholic and deoxycholic acids) through ester linkages. Bile acids are natural products consisting of a facially amphiphilic steroid nucleus with a hydrophobic b-side and a hydrophilic a-side [9, 10]. When these compounds are chemically bound to a water-soluble polymer the resulting amphiphilic polymer might exhibit a better compatibility with biological systems and interact favorably with proteins, enzymes or lipids [11].

2.7.2.2 Dextrans: Colon-Specific Drug Delivery

Dextrans are polysaccharides with a linear polymer backbone with principally 1,6--D-glucopyranosidic linkages. They are derived from bacterial cultures of Leuconostoc mesenteroides. These glycosidic linkages are hydrolysed by moulds, bacteria and also by the mammalian cells. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Dextranases activity of the colon is revealed by anaerobic gram-negative intestinal bacteria especially the Bacteroides. Dextran has also been reported to be degraded in human feces owing to bacterial action. Numerous drug-dextran prodrugs in which the drug molecule is associated to the polar dextran macromolecule remain intact and unabsorbed from the stomach and the small intestine but when the prodrug arrive into the colonic microflora containing as much as 1011 Bacteroides per gram it is acted upon by dextranases which cleave the dextran chain randomly and at the terminal linkages releasing the drug, free into the colon. Growing attention is being focused on dextran prodrugs. Major attempt was carried out by Harboe et al. [12] who conjugated naproxen to dextran by ester linkage. Dextran ester prodrugs of ketoprofen and naproxen using dextran with molecular weight (MW) 10,000–500,000 were reported for their releasing the drug specifically in the colon region of pig. The release of naproxen was up to 17 times higher in the cecum and colon homogenates of pig than in control medium or homogenates of SI. A series of prodrugs, naproxen-dextran, ketoprofen-dextran and ibuprofen-dextran have been tested in vitro and in vivo in pigs. They suggested this prodrug system as a active system for site specific delivery presenting high bioavailability of the drug but still no absorption of the prodrug into the circulation. Also, this system could offer protection to the drug in the upper GIT and selective regeneration in the cecum/colon. This method delivers drug particularly to the colon and can be employed for colon targeting. There are various additional nano-applications of dextran explored (Table 2.4).

2.7.2.3 Cationization of Dextrans

There are various modified forms of dextran available (Fig. 2.5). Nichifor et al. have widely reported about the fabrication of amphiphilic cationic dextrans, formerly crosslinked with epichlorohydrin or not, by replacing them with groups alike to the mentioned ones however in which one of the quaternary ammonium substituent's was an alkyl chain between C2 and C16 instead of methyl [24]. In this circumstance the reagent active for cationization was a 2,3-epoxypropyl alkyl dimethyl ammonium chloride derived from epichlorohydrin and a dimethyl alkylamine [24]. Products with a DS between 0.18 and 0.94 were derived. The kinetic of the reaction of polysaccharides, with epichlorohydrin and various tertiary amines was investigated [24], by quantification of reagents and products in the reaction mixture with or without the presence the polysaccharide, at regular intervals. They reported that the use of cyclic amines e.g. 1-methyl-imidazol or 1,4-diazabicyclo [24] octan as "catalysts" together with another tertiary amine, used for the substitution, was not

Table 2.4 Drug delivery applications of dextrans

Polysaccharide involved	Size	Application	Ref
Dextran sulphate	Silver nanoparticles	Antimicrobial activity	[13]
Dextran	Zwitterionic pH/redox nanoparticles	For enhancing tumor intercellular uptake of doxorubicin	[14]
Chondroitin sulfate and dextran sulfate	Nanoparticles	Delivery of chloramphenicol to treat intracellular Salmonella infections	[15]
Chitosan–dextran sulfate	Nanoparticles	For controlled delivery of bioactive molecules and cells in bone regeneration	[16]
IgA-Loaded Chitosan–Dextran Sulfate	Nanoparticles	Enhanced Immune Response	[17]
Dextran	Nanoparticles	Delivery of doxorubicin	[18]
Dextran sulfate	Superparamagnetic iron oxide nanoparticles	As a contrast agent for atherosclerosis	[19]
Dextran	Graphene oxide gold nanoparticles	For sensitive detection of concanavalin A	[20]
Dextran	Nanoparticles	Targeted delivery of cisplatin for breast cancer growth and metastasis	[21]
dextran/chitosan shell	Nanoparticles	BSA/chitosan core—Doxorubicin loading and delivery	[22]
Poly(DL-lactide-co-glycolide)-grafted dextran	Nanoparticles	To enhance antitumor effect of adriamycin	[23]

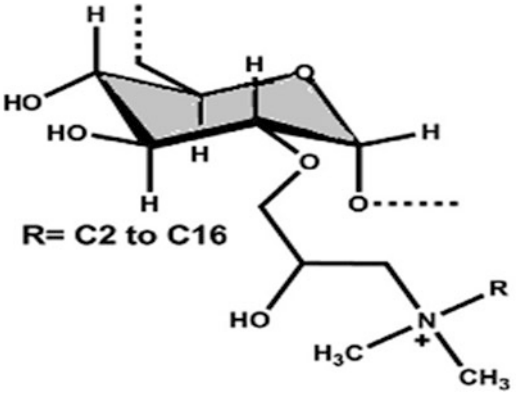

Fig. 2.5 Dextran modification via cationic substitution which present alkyl chains of varying length

essential, and led to a cationic polysaccharide with mixed and uncontrolled chemical composition. After 30 min of reaction at 40 °C, 80 % of epichlorohydrin had reacted with the amine, reaching a 94 % after 3 h. Originally, the original epoxy groups remain unchanged, nevertheless they started to decline at 24 h. The finest solvent for this reaction was water and an equimolar amine: epichlorohydrin ratio furnished satisfactory results.

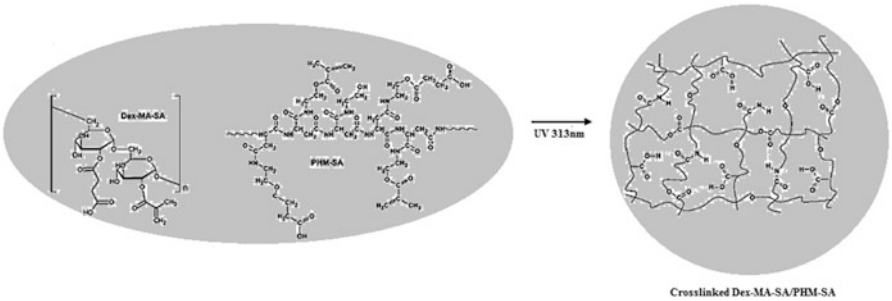

Fig. 2.6 Cross-linking, succinic and methacrylated derivative of dextran with a methacrylated and succinic derivative of poly(N-2-hydroxyethyl)-dl-aspartamide (PHEA), PHM-SA

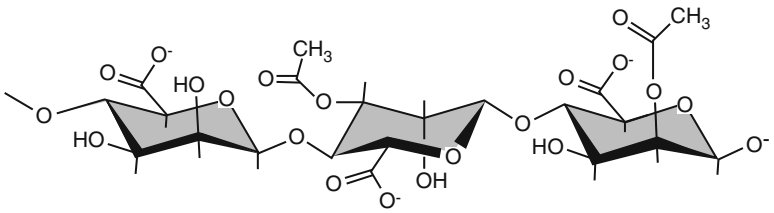

Fig. 2.7 Structure of alginate

There are various derivatives explored by using several cross linking agents. In earlier studies methacrylated and succinic derivative of dextran with a methacrylated and succinic derivative of poly(N-2-hydroxyethyl)-dl-aspartamide (PHEA), PHM-SA were prepared as mentioned in Fig. 2.6. These and many alike methodologies have revolutionize the dextran applications in diverse areas of biomedical science.

2.7.3 Bacterial Alginate

Alginate is a linear polymer composed of mannuronic acid and glucuronic acid (both of them being uronic acids) in a proportion ranging from 4: 1 to 20: 1. Some of the mannuronic acid residues are acetylated (Fig. 2.7).

Alginate is commercially produced by Gram-negative bacteria, Pseudomonas aeruginosa and Azotobacter vinelandii. The type of organism used and the culture conditions determine the relative proportion of mannuronic acid and glucuronic acid residues and the degree of acetylation in alginate. Alginates with high contents of mannuronic acid are elastic in nature while those with high concentration of glucuronic acid are strong and brittle. Algal (seaweed) alginates are also polymers of mannuronic acid and glucuronic acid, and comparable in structure with bacterial alginates. However, algal alginates lack acetylation. For commercial purposes, sea-

Table 2.5 Drug delivery applications of bacterial alginate

Polysaccharide involved	Size	Application	Ref.
phenylalanine ethyl ester-alginate conjugate	Nanoparticles	Vitamin B2 delivery	[25]
Alginate	Gold nanoparticle	To encourage cellular interactions	[26]
Alginate	Silk sericin loaded nanoparticles	To promote anti-inflammatory efficacy	[27]
Polyvinyl alcohol/sodium alginate	Nanoparticles	Antibacterial activity	[28]
biodegradable graft copolymer sodium alginate-g-poly (N,N-dimethylacrylamide-co-acrylic acid)	Gold nanoparticles	Anti micro bacterial application	[29]
Chitosan/alginate	pH-sensitive core-shell nanoparticles	For efficient and safe oral insulin delivery	[30]
Alginate	Calcium carbonate hybrid nanoparticles	For combination chemotherapy	[31]
Polyvinyl alcohol/sodium alginate	Silver nanoparticles	Antibacterial	[28]
Nanoparticles of chitosan-alginate	Chitosan-alginate	Improvement of crocin stability	[32]

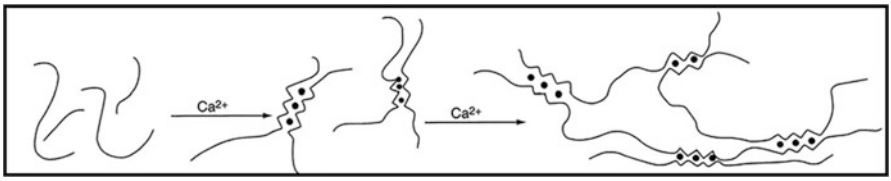

Fig. 2.8 Divalent mediated sodium alginate beads formation

weed alginates are more commonly used than bacterial alginates. This is mainly because bacterial alginates are relatively unstable and get easily degraded. Alginates are useful as thickening agents in food industry, and for immobilization of cells and enzymes. Table 2.5 describes drug delivery applications of bacterial alginate. Alginate in the presence of divalent ion like Ca^{2+} form gel which explore its application in various immobilization experiment (Fig. 2.8).

2.7.4 Scleroglucan

Scleroglucan is a glucose polymer (glucomer). It is a neutral polysaccharide with β 1 → 3 glucan backbone and single glucose (Glc) residue branches (β 1 → 6 linkage) (Fig. 2.9).

The branching occurs at a regular sequence at every third glucose unit in the polymer backbone chain. Scleroglucan is a fungal hexopolysaccharide. It is commercially produced by Sclerotium glucanicum, S. rolfsii and S. delphinii. Scleroglucan is useful

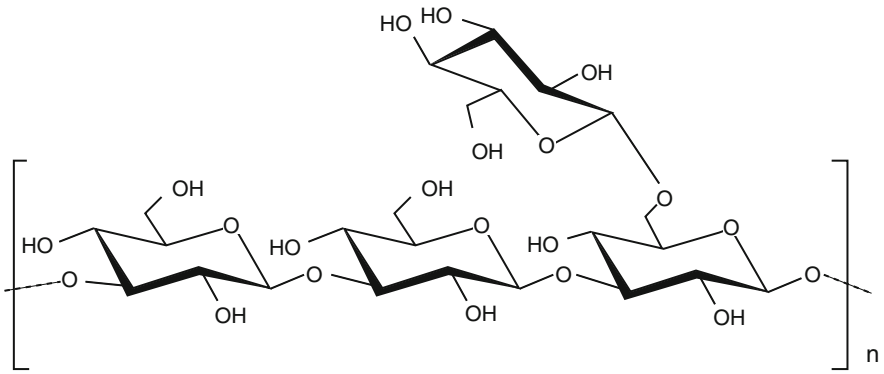

Fig. 2.9 Structure of Scleroglucan

for stabilizing latex paints, printing inks and drilling muds. Scleroglucan is recently explored as biocompatible material by incorporating an aqueous ferrofluid in poly(vinyl alcohol) and scleroglucan (SCL) hydrogels, loaded with theophylline as model drug for release studies. Rheological results showed that higher storage modulus and a more compact structure are obtained by incorporating the ferrofluid into the hydrogels. Schizophyllan is a natural b-(1-3)-D-glucan polysaccharide produced by the fungus Schizophyllum. Modified schizophyllan forms stable complexes with antisense oligonucleotides, and when studied in different melanoma and leukaemia cell lines, the cytotoxicity was found to be negligible [33]. Schizophyllan is a new potential candidate for an antisense oligonucleotides carrier [34].

2.7.5 Gellan

Gellan is a linear heteropolysaccharide. The repeating unit of gellan is composed of two glucose, one glucuronic acid and one rhamnose molecules (Fig. 2.10). Gellan is produced by Pseudomonas elodea. A deacetylated gellan which forms firm and brittle gels under the trade name Celrite has been developed by a reputed company in USA (Kalco Inc). Gellan is used in food industry. Even at a low concentration, it is a thicker. Table 2.6 describes drug delivery applications of gellan.

2.7.6 Pullulan

Pullulan is an α-glucose polymer (α-glucan) with α $1 \rightarrow 4$, and a few α, $1 \rightarrow 6$ glycosidic bonds. Pullulan is produced by using the fungus, Aureobasidium pullulans (Fig. 2.11). Hydrophobically modified pullulan (Fig. 2.12): Various cholesterol-bearing pullulans with different molecular weights from the parent pullulan and

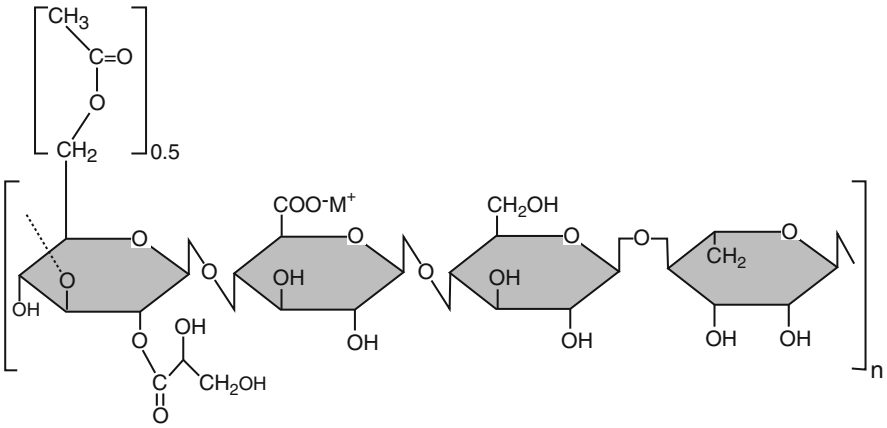

Fig. 2.10 Structure of gellan

Table 2.6 Drug delivery applications of gellan

Polysaccharide involved	Size	Application	Ref.
Gellan	Gum/titanium dioxide nanoparticle	For the cleaning and disinfection of parchment	[35, 36]
P123/TPGS mixed micelles and gellan gum	In situ gel systems	For ophthalmic delivery of curcumin	[37]
Hexadecyl gellan amphiphilic	Nanoparticles	In vivo lipid-lowering potential	[38]
Gellan gum methacrylate and laponite	Nanocomposite hydrogel	Biomedical applications	[39]
Gellan	Gel beads containing magnetic nanoparticles	Containing magnetic nanoparticles	[40]
Gellan gum blended PEI nanocomposites as gene delivery agents:	PEI nanocomposites	As gene delivery	[41]
Gellan gum	Aqueous dispersions/its nanohybrids	Reducing and stabilizing agent and	[42]
Quaternized gellan gum	Nanoparticles	Antibacterial activity (for controlled release of ciprofloxacin with potential dermal applications)	[43]

different DS from the cholesteryl moiety were synthesized by Akiyoshi et al. [44] and formed stable and monodisperse self-aggregates (20–30 nm) by intra- and/or inter-molecular self-aggregation in a diluted aqueous solution [45]. The cholesterol-bearing pullulan self-aggregates are regarded as a hydrogel nanoparticle, in which pullulan chains are cross-linked noncovalently by associating cholesteryl moieties. The sizes of the self-aggregates decreased with an increase in the DS of the

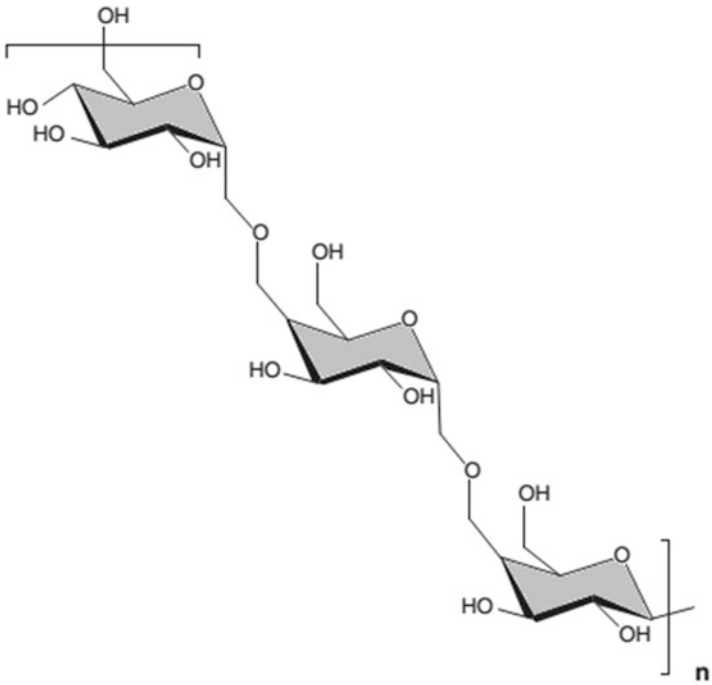

Fig. 2.11 Structure of pullulan

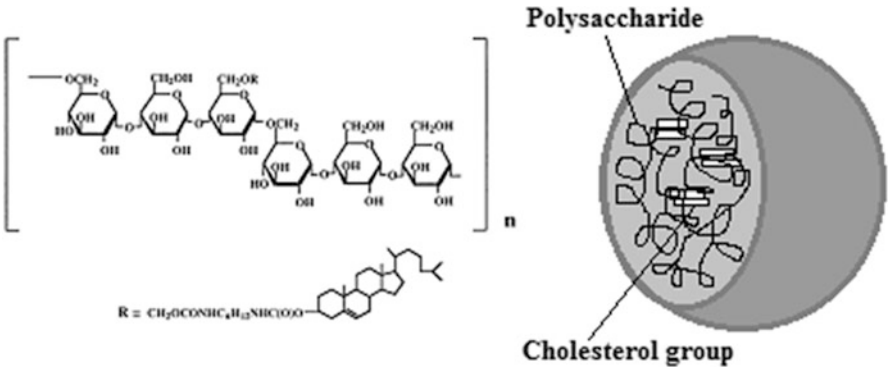

Fig. 2.12 Structural features of cholesterol modified pullulan and nanogels formed by self-assembly

cholesteryl moiety, whereas the aggregation number of cholesterol-bearing pullulans in one nanoparticle was almost independent of the DS [46].

It is estimated that about 70 % of glucose (the substrate) is converted to pullulan during fermentation, although the time taken is rather long (5–7 days). Pullulan is mainly used in food coating and packaging. Table 2.7 describes drug delivery applications of pullulan.

Table 2.7 Drug delivery applications of pullulan

Polysaccharide involved	Size	Application	Ref.
Pullulan–spermine	Magnetic nanoparticles	Plasmid EGFP-p53 delivery	[47]
Pullulan	pH-responsive nanoparticles & charge-reversible pullulan-based shells	Carriers of anticancer drugs for combination therapy	[48]
Pullulan	Nanoparticles	For transmucosal protein delivery	[49]
Pullulan	Silver nanoparticles	Antimicrobial activities	[50]
Pullulan stabilized gold nanoparticles	Pullulan stabilized gold nanoparticles	For cancer targeted drug delivery	[51]
Pullulan	pH-sensitive nanoparticle	Carrier for adriamycin to overcome drug-resistance of cancer cells	[52]
Pullulan	pH-sensitive pullulan-based nanoparticle	Carrier of methotrexate and combretastatin A4 for the combination therapy against hepatocellular carcinoma	[53]

2.7.6.1 Cationic Modified Pullulan

Pullulan is a natural water-soluble polysaccharide with a repeated unit of maltotriose condensed through the α-1,6 linkage and is non-toxic, non-mutagenic non-immunogenic, and noncarcinogenic in nature [54]. Stable complexes were produced from cationic modified pullulan/DNA as temperature-sensitive gene carriers [55]. Thomsen et al. [56] used cationic non-viral gene carriers prepared from pullulan and spermine for conjugation with P-DNA and to transfect rat brain endothelial cells (RBE4s) and human brain microvascular endothelial cells (HBMECs). The HBMECs and RBE4s were successfully transfected with the fluorescent reporter gene pHcRed1-C1, with good transfection efficiency and low cytotoxicity. Secretion of hGH1 protein was detected after in vitro transfection of HBMECs with pullulan–spermine complexed with pCMV6 Entry GH1. Thus, the pullulan–spermine delivery system may be used as a method to deliver DNA to brain endothelial cells and to use these cells as factories for protein secretion. Similarly, complexes were also studied for liver targeting gene expression. Thakor et al. [57] studied in vitro transfection using conjugated pullulan–spermine/pDNA anionic complexes in rat sensory neurons. Complexes were found to be stable for 1 week and to protect the DNA from degradation. In vitro transfection of rat sensory neurons occurred at different spermine nitrogen: DNA phosphate ratios, but the efficiency was highest for anionic complexes (anioplexes). Anioplexes did not exhibit any measurable cytotoxicity up to 20 lg ml^{-1} DNA. The transfection efficiency was also maintained in the presence of serum and antibiotics. This suggests that pullulan–spermine/DNA anioplexes are an effective gene delivery technology, particularly for neurons. Cationic pullulan, dextran and mannan complexed with pDNA were also tested in cellular models and in vivo mice models. The cationized pullulan is reported to a promising non-viral

carrier of pDNA for mesenchymal stem cells. Similarly, cationic pullulan was also reported for gene delivery applications targeted at liver cells. Cationic groups were introduced by reacting various amounts of glycidyl trimethyl ammonium chloride with pullulan. The cationic derivatives readily formed polyionic complexes with DNA. The nanoplexes were taken up by the liver cells in a time dependent manner. They were found to have excellent blood compatibility, and in vitro transfection on HepG2 cells demonstrated good transfection efficiency. The high solubility and chain flexibility of pullulan may be contributory factors to its blood compatibility [58]. PEI-conjugated pullulans were also developed and investigated for possible use in gene delivery applications. The pullulan–PEI conjugate seems to be a promising gene delivery vector that shows good hemocompatibility and low toxicity without compromising the transfection efficacy of PEI [59].

2.7.6.2 Cholesterol-Bearing Pullulans

Various cholesterol-bearing pullulans (Fig. 2.12) with different molecular weights from the parent pullulan and different DS from the cholesteryl moiety were synthesized by Akiyoshi et al. [55–57] and formed stable and monodisperse self-aggregates (20–30 nm) by intra- and/or inter-molecular self-aggregation in a diluted aqueous solution [55]. The cholesterol-bearing pullulan self-aggregates are regarded as a hydrogel nanoparticle, in which pullulan chains are cross-linked noncovalently by associating cholesteryl moieties. The sizes of the self-aggregates decreased with an increase in the DS of the cholesteryl moiety, whereas the aggregation number of cholesterol-bearing pullulans in one nanoparticle was almost independent of the DS [57].

2.7.7 Curdlan

Curdlan is a β-glucose polymer (β-glucan). The glucose residues are held together by β 1 → 3 glycosidic bonds (Fig. 2.13).

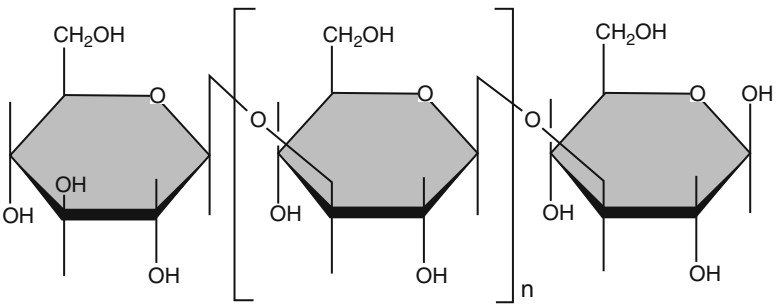

Fig. 2.13 Structure of curdlan

Table 2.8 Drug delivery applications of Curdlan

Polysaccharide involved	Size	Application	Ref
Curdlan	Nanoparticles	Cell Type-Specific Delivery of RNAi	[60]
Curdlan-conjugated PLGA	Nanoparticles	Enhancement of macrophage stimulant activity and drug delivery capabilities	[61]
Curdlan-capped gold	Nanoparticles	To enhance interaction with protein	[62]
Carboxylic curdlan-deoxycholic acid	Self-aggregated nanoparticles	Carrier of doxorubicin.	[63]
Curdlan	Nanoparticles	For intracellular siRNA delivery	[64]
Carboxymethyl curdlan-capped silver nanoparticles		Application in surface enhanced Raman scattering	[65]
Cholesterol-conjugated carboxymethyl Curdlan	Self-assembled nanoparticles	Carrier for epirubicin.	[66]
Curdlan derivatives	Self-assembled hydrogel nanoparticles	Anti-cancer drug delivery	[67]

The exopolysaccharide curdlan is commercially produced by employing Alcaligenes faecalis. Curdlan-like polysaccharides are also produced by other microorganisms such as Agrobacterium rhizogenes and Rhizobium trifolii. Curdlan forms strong gels when heated to above 55°C. Therefore, it is used as a gelling agent for cooked foods. In addition, curdlan is also employed for immobilization of enzymes. Table 2.8 describes drug delivery applications of curdlan.

2.7.8 Levan Polysaccharides

Levans are a group of fructans; polymers of fructose forming a non-structural carbohydrate, which in the case of levans can themselves link together to form super-molecules comprising even hundreds of thousands (Fig. 2.14).

Levans are synthesized in approximately all bacterial versions of fructan production, as well as being possible to produce by fracturing soybean mucilage. Levan, fructose-composed biopolymer of bacterial origin, has potential in biotechnology due to its prebiotic and immunostimulatory properties. It was suggested that the combination of levan and nutritionally important microelements in the form of NPs serves as a first step towards a novel "2 in 1" approach for food supplements to provide safe and efficient delivery of microelements for humans and support beneficial gut microbiota with nutritional oligosaccharides [68].

2.7.9 Bacterial Polysaccharides

Recently, considerable development has been made in exploring new bacterial polysaccharides that exhibit novel and highly functional properties, e.g. the association between the exclusive properties of xanthan gum (the foremost microbial

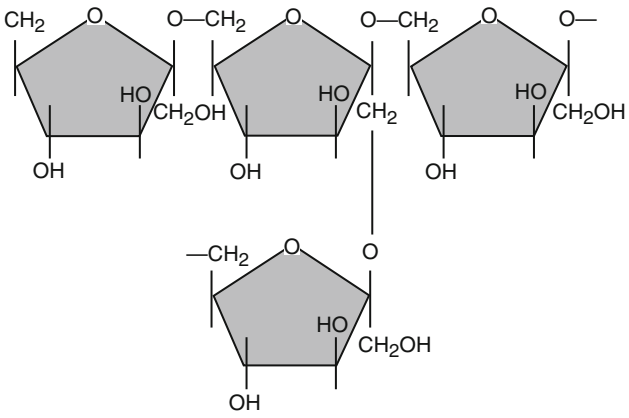

Fig. 2.14 Structure of levan

polysaccharide of commercial significance) and its use in major food, industrial, and oil field applications is conferred. Moreover, gellan gum (the extracellular polysaccharide produced by *Pseudomonas elodea*) can be employed in microbiological media and in gelled and structured food products [69]. Three other industrially useful bacterial polysaccharides have been explored:

- S–130, the extracellular, high viscosity polysaccharide produced by a strain of *Alcaligenes*, has excellent suspending and heat stability features functional in oil field drilling, work over and completion, and enhanced oil recovery fluids.
- S–194 has outstanding suspending features and remarkable compatibility with salts, making it significant in agricultural applications, specifically flowable pesticides and liquid fertilizers.
- S–198 has exceptional stability to shear and has potential application in the growing market of water–based lubricants.

2.7.10 Gellan, Guar and Xanthan Gums

Gellan gum, a linear anionic polysaccharide comprising tetrasaccharide glucose:glucuronic acid: rhamnose. The industrial polysaccharide is deacetylated with alkali, which facilitates the presence of free carboxylic groups. The initial heating of concentrated solutions followed by the cooling facilitates alteration in the chain conformation from coil to helix, results in ordered junction zones, and therefore to temperature-reversible gels [70]. Much stronger physical hydrogels that can be derived in presence of di and trivalent ions, are the foundation of in situ gelling ophthalmic and oral formulations. The mechanism of gelation encompasses the formation of double helical junction zones, followed by the aggregation of double helical segments to form a 3D network by complexation with cations and hydrogen bonding with water. The subsequent gels are pH and ionic strength sensitive [70–72]. Beads of gellan ionically crosslinked with Al^{3+} only or co-crosslinked

with glutaraldehydes welled more and released faster glipizide at pH 7.4 than at pH 1.3. Enhanced release at pH 7.4 is triggered not only since the ionization of carboxylic acid groups, nevertheless also owing to reduction of aluminum ions by ion exchange with sodium ions of the medium. This concluding mechanism did not ensue in the case of chemically crosslinked beads, which displayed more sustained release at pH 7.4. Related release pattern was reported for beads of gellan crosslinked with calcium ions. Networks with other polysaccharides have been fabricated taking advantage of the prospect of using gellan as cross-linker. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) has been used to activate the carboxylic groups of gellan, so as to react with the hydroxyl groups of scleroglucan. The hydrogels presented pH and ionic strength sensitivities, owing to the protonation state of the carboxylic groups of gellan and scleroglucan and the screening role of the ions to the COO−. Investigations of the outcome of Na^+, Ca^{2+} and pH on the release rate of theophylline revealed that high Na^+ or Ca^{2+}/COO− ratio and low pH results in the release rate, owing to the cross-linking effect of the ions that stretches a further strength to the complete network, or to the protonation of the COOH that allows the co-cross-linking, correspondingly [73]. Alike outcomes were examined for scleroglucan crosslinked with 1,6-hexanedibromide. The presence of the salt in the medium results in shrinking of the network and, though the interaction between the COO− groups of scleroglucan and theophylline became weaker, a substantial reduction in the drug release was reported [74, 75]. The nonionic polysaccharide guar gum that can be altered with anionic and cationic substituent's to attain pH-sensitive release [76, 77]. Carboxymethyl derivatives crosslinked with barium cations can shield proteins from the acidic pH of the stomach and regulate the release in simulated intestinal conditions [78]. If the microbeads are crosslinked with trivalent ions, such as Al^{3+} or Fe^{3+}, instead of divalent ions e.g. Ba^{2+}, Ca^{2+} or Cu^{2+}, a slower drug release can be achieved at pH 7.4, achieving around 100 % released in 20 h. Conversely, cationic guar gums crosslinked with EGDE have high attraction for oppositely charged drugs, mainly at acid pH. Nevertheless, at alkaline pH or in the presence of salts the interactions are broken, and the drug is released. Hydrogels of xanthan gum have also shown to be pH and ionic sensitive. Crosslinked xanthan networks have been fabricated using two strategies. In the first one, cyclic trisodium trimetaphosphate was employed in an alkaline medium for sustaining the coil conformation [79]. In the next approach, adipic acid dihydrazide and a soluble carbodiimide in acidic conditions were employed. Both hydrogels performed decidedly different each other. Release of methylene blue from the hydrogels crosslinked with cyclic trisodium trimetaphosphate depended on the swelling of the network; namely, higher amounts of methylene blue were released in serum-mimicking than in acid medium. This performance is recognized to the ionization of the phosphate groups at alkaline pH, which results in electrostatic repulsive forces that in turn cause a rise in the swelling ratio. At acid pH, the protons screen the most of the phosphate anions, which results in reduction of the repulsive interactions and of the swelling. The modification in the conformations also plays a role in the reduction of the swelling at acidic pH. Via distinction, networks crosslinked with adipic acid dihydrazide released methylene blue faster in acidic pH than in the

saline solution. This is because of the low amount of carboxylic groups free in the helix conformation after the cross-linking, which also hinders the helix to coil transition. Examining for a different approach to control drug release, sodium dodecyl sulfate (SDS) was added to co-networks of alginate and xanthan. Sodium dodecyl sulfate is a well-known anionic surfactant, chiefly used to enable the wetting and to enhance the apparent solubility of drug. Nevertheless, after ionotropic crosslinking SDS is supposed to organize to the crosslinker cation, becoming more hydrophobic.

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Chapter 3
Chitosan Based Nanomaterials and Its Applications

Abstract Chitosan-based nanomaterials with good biodegradability, biocompatibility, and low toxicity display immense potential as carriers for controlled delivery of various therapeutic agents. Chitosan is a linear polysaccharide composed of randomly distributed b-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. It is one of the major cationic polymers and the second most abundant polysaccharides in nature. It is extensively used to the biomedical and the industrial fields. Particularly, chitosan has been investigated much in the field of gene therapy during the last decade for its biocompatibility and non-cytotoxicity. Nevertheless, it has a number of problems such as solubility, low transfection efficiency, and low specialty on targeted disease. To circumvent these issues, different approaches have been reported to enhance them.

3.1 Introduction

Chitosan and its derivatives are natural polysaccharides derived from chitin. They have been established to be nontoxic in both animal models and humans. Moreover, chitosan has a special feature of adhering to mucosal surfaces and is capable of penetrating the tight junctions between epithelial cells, which make it ideal material for drug delivery. Thus, chitosan and its derivatives have attracted important interests as carriers for drug or gene (delivery due to their excellent biocompatibility, biodegradability, biological activities, and adsorption properties. There is no doubt that chitosan is a very promising material for drug delivery. Presently, a variety of chitosan-based drug delivery materials in the forms of gels, tablets, films and particle have been developed and studied chemical or ionic gelation, coacervation/precipitation and spray-drying are the commonly used methods to prepare chitosan carriers. Nevertheless, the challenges involving the use of high cost materials, large amount of chemical agents, and the tedious and time-consuming process avert the real world large scale applications of chitosan drug carriers. Current advances in chitosan research have exposed the potential of chitosan-based materials in drug delivery. Like most other polysaccharides, chitosan has excellent biodegradability, biocompatibility and non-immunogenicity. As the only natural positive polysaccharide, chitosan is mainly beneficial in forming stable complex with negative compounds, which makes chitosan an excellent candidate for the drug encapsulation

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and controlled release. Moreover, chitosan has mucoadhesive and absorption-enhancing properties. It had been found that chitosan can interact with mucus and epithelial cells resulting in opening of cellular tight junctions. These features make chitosan also a perfect candidate for the delivery of macromolecular therapeutics, like protein and peptides. Molecular weight, degree of deacetylation, genetic materials concentration, and serum stability of chitosan and the a variety of alterations (targeting ligand, thiol group, and amino acid, hydrophilic group, hydrophobic group, cationic group,) are very significant factors in preparing chitosan derivatives to improve the efficiency of gene therapy.

3.2 Chitin

Chitin is the polysaccharide derivative comprising amino and acetyl groups and are the most abundant organic component in the skeletal material of the invertebrates. It is usually found in annelids, arthropods, mollusks, and also as a component of the mycelia and spores of various fungi. It is also reported as a derivative of cellulose, in which the hydroxyl groups of the second carbon of each glucose unit have been replaced with acetamido group. The novel polyelectrolyte complex gel beads based on phosphorylated chitosan were fabricated for controlled release of ibuprofen in oral administration. The phosphorylated chitosan gel beads were usually fabricated from soluble phosphorylated chitosan by using an ionotropic gelation with counter polyanion, tripolyphosphate, at pH 4.0. Approximately 90 % of Ibuprofen was highly loaded in the phosphorylated chitosan gel beads. The %age of release of ibuprofen from phosphorylated chitosan gel beads was found to be triggered as the pH of dissolution medium enhanced. Chitosan and their derivatives are safe and effective absorption enhancers to advance mucosal delivery of hydrophilic macromolecules e.g. peptide and protein drugs and heparins. This absorption increasing effect of chitosan is produced by the opening of the intercellular tight junctions, thus favouring the paracellular delivery of macromolecular drugs. Chitosan nano- and micro particles are also appropriate for controlled drug release. Relationship of vaccines to some of these particulate systems has proven to improve the antigen uptake by mucosal lymphoid tissues, thus persuading strong systematic and mucosal immune responses against the antigens. The a specific adjuvant activity of chitosans appears to be reliant on the degree of deacetylation and the type of formulation. From the investigations it is observed that chitosan and chitosan derivatives are capable polymeric excipients for mucosal drug and vaccine delivery.

3.3 Chitosan and Chitooligosaccharides

Chitosan is a naturally occurring biopolymer isolated by the N-deacetylation of chitin. It is the main constituent of the exoskeletons of marine crustaceans, including shrimp and crab. Recently, Chitosan is of a special interest for applications in the

chemical, nutraceutical, and pharmaceutical industries. Chitooligosaccharides are the depolymerization products of chitin or Chitosan by enzymatic and acidic hydrolysis methods. These methods have attracted special interest because of their ease of control and safety. Several enzymes have been used to prepare chitooligosaccharides. They are highly water soluble and nontoxic, good biocompatibility, excellent biodegradability, and low cost. Chitosan and chitooligosaccharides have a great promise in recent years because of their biomedical applications, namely antimicrobial activity, tissue engineering, wound healing, drug delivery, antitumor effects, and hypocholesterolemic effects.

3.4 Chitin Nanoparticles

Chitin (Fig. 3.1) is also a biopolymer that is abundant in nature. It is isolated from crab, shrimp, and lobster shells as a byproduct of the seafood industry. Worldwide, several million tons of chitin are generated annually as waste by the seafood industry. Chitin has been successfully prepared by several methods such as enzymatic methods, methods using hydrolytic conditions of boiling HCl and vigorous stirring, and methods using chitin whiskers of slender parallelepiped rods. Previous research has reported the preparation of presumably functional chitin nanoparticles and their

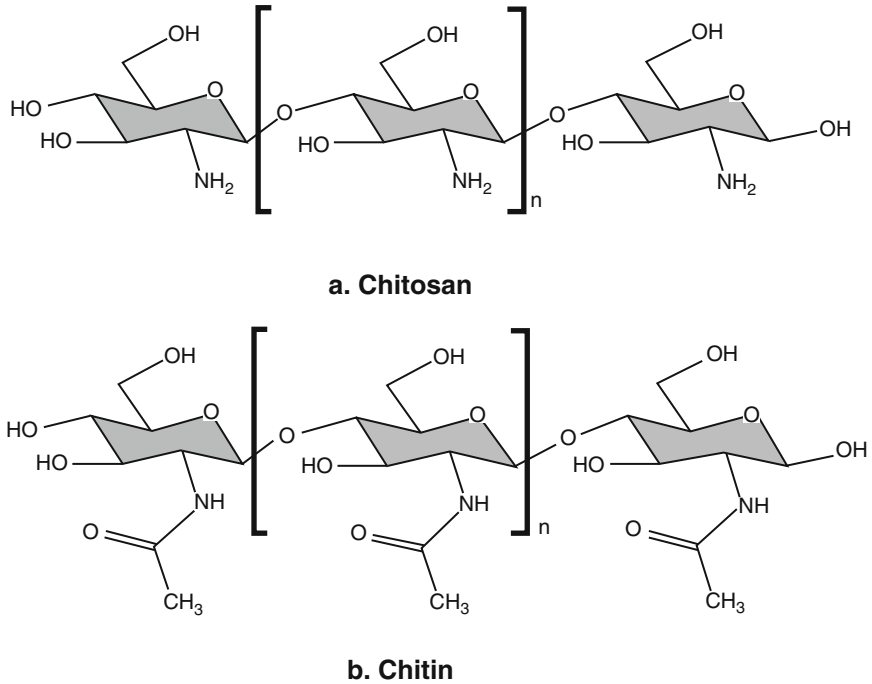

Fig. 3.1 Structure of chitosan and chitin

application as reinforcing fillers in polymeric matrices. Carboxymethyl chitin is a nontoxic, water-soluble anionic derivative of chitin containing carboxyl groups. It is used as a constituent of wound-healing dressings, exhibits good biocompatibility and can be synthesized from chitin. Previous researcher prepared chitin nanoparticles using the cross-linking approach with $FeCl_3$ and $CaCl_2$ that was used for controlled drug delivery applications. These nanoparticles exhibited in vitro cytotoxic activity against mouse L929 cell lines. The nanoparticles showed strong antibacterial activity against Staphylococcus. Previous researcher demonstrated novel amorphous chitin nanoparticles that were widely used for colon cancer drug delivery. Paclitaxel was used as the main chemotherapeutic agent, and the drug was loaded into amorphous chitin nanoparticles through ionic cross-linking approach using pentasodium tripolyphosphate. The paclitaxel-loaded amorphous chitin nanoparticles had an average size of 200 ± 50 nm. These nanoparticles were confirmed to be hemocompatible and in vitro drug release exhibited a sustained release of Paclitaxel. Drug delivery into intracellular compartments is a big challenge in the present treatment systems. To deliver drugs into the intracellular compartments, numerous drugs encapsulated in nanoparticles and microparticles were prepared, which showed enhanced efficacy in delivering the drug into the intracellular compartments [1]. Earlier researcher, designed rifampicin-loaded amorphous chitin nanoparticles for intracellular delivery of rifampicin inside polymorphonuclear leukocytes. The rifampicin molecules entrapped in the amorphous chitin polymer remained in the bioactive form, and the antibacterial activity was retained upon their release. Furthermore, sustained drug delivery could reduce dosing frequency, lower toxicity, enable long-term treatment, and prevent potential side effects related to the free drug. Rifampicin-loaded amorphous chitin nanoparticles could be used for an effective delivery of rifampicin into the intracellular compartments of the polymorphonuclear leukocytes to resolve serious bacterial infections faster and more effectively.

3.5 Chitosan Nanoparticles

Chitosan (Fig. 3.1) is a naturally occurring linear polysaccharide composed of glucosamine and N-acetylglucosamine units. It's randomly or block-spread linked throughout the polymer chain, based on the extraction procedures to obtain chitosan from chitin. The degree of deacetylation is well defined as the molar ratio of glucosamine to N-acetyl glucosamine. This degree of deacetylation is significant factor in evaluating its physicochemical properties and commercial applications. Next to deacetylation process, chitosan is able to dissolve in an acidic medium and develop as the only sulfated polysaccharide that holds a high density of positive charges, owing to the protonation of amino groups on its backbone. Moreover, chitosan has been found to have various other intrinsic characteristics, particularly good biocompatibility, biodegradability and non-toxicity. Current research has explored various chitosan derivatives for number of applications (Figs. 3.2 and 3.3).

Presently, chitosan has involved significant scientific and nanotechnological interest in the biomedical sector for various applications, containing tissue engineering,

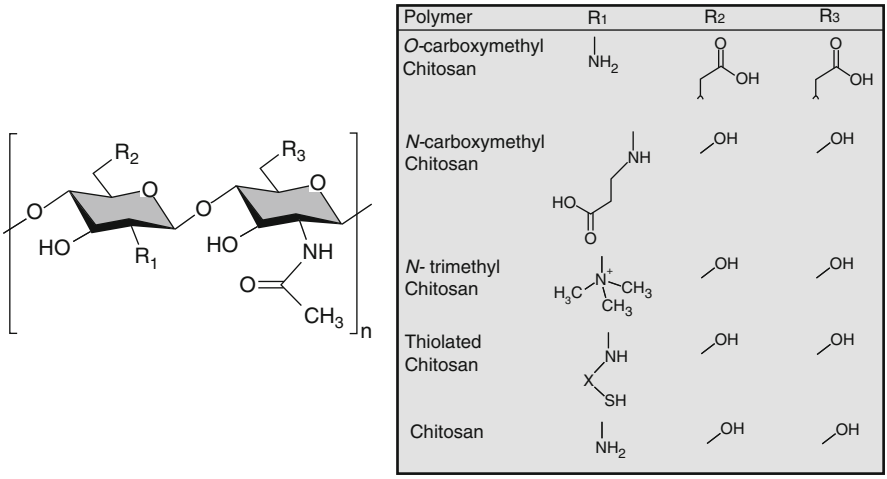

Fig. 3.2 Chitosan and its derivatives-I

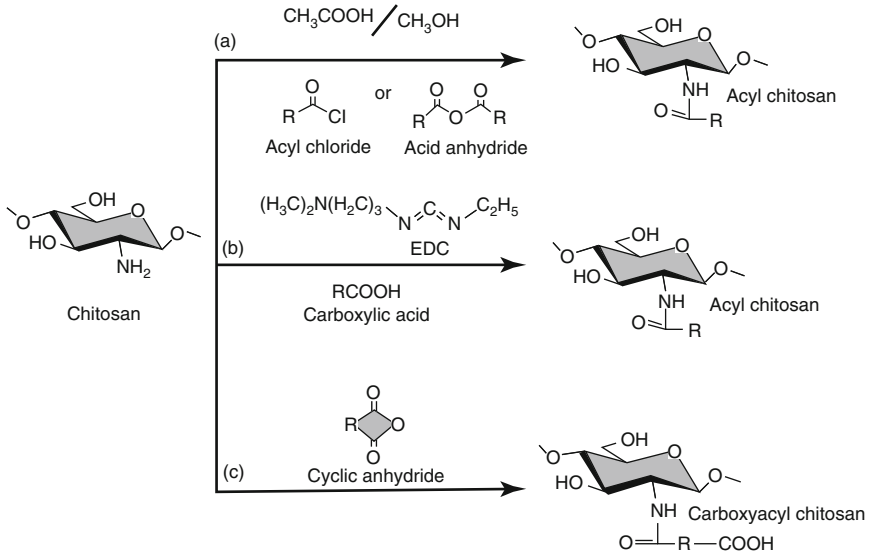

Fig. 3.3 Chitosan and its derivatives-II

drug delivery, and nutrition. Earlier researcher has reported the chitosan and chitosan gold nanoconjugates of salmon luteinizing hormone releasing hormone of desired dispersity, size, and zeta potential were prepared and examined at half the dose rate against full dose of bare luteinizing hormone releasing hormone for their reproductive efficiency in female fish, Cyprinus carpio. Since the reaction contains complex formation between oppositely charged species chitosan experienced ionic gelation and precipitates to yield spherical particles. Janes et al. investigated the possibility of chitosan nanoparticles as a delivery system for doxorubicin. Assessment of the cytotoxic activity

of doxorubicin-loaded cell lines demonstrated that accumulation of dextran sulfate which allowed the conservation of cytotoxic activity, which is comparative to free doxorubicin, while doxorubicin complexed with chitosan before nanoparticle development attained somewhat reduced activity. Mitra et al. synthesized dextran- doxorubicin encapsulated in chitosan nanoparticles using reverse microemulsion and investigated their antitumor activity in murine tumor models. DeCampos et al. [2] studied the possible use of chitosan nanoparticles as an innovative carrier for the targeted delivery of drugs to the ocular mucosa, via the immunosuppressive peptide cyclosporin A as a model drug. Chitosan nanoparticles cross-linked with glutaraldehyde has been fabricated in AOT/n hexane reverse micellar system. The particle size of these chitosan nanoparticles was chiefly affected by the degree of cross-linking. The particle size of these chitosan nanoparticles at infinite dilutions was 30 nm, when 10 % of the amine group's in the polymeric chains were cross-linked. Further it shoots up to 110 nm when all the amine groups were cross-linked [3]. Qi et al. [4] displayed the in vitro antibacterial activity of chitosan nanoparticles and copper-loaded nanoparticles against pathogenic microorganisms. These nanoparticles presented antibacterial activity against *E. coli*, *Salmonella choleraesuis*, *Salmonella typhimurium*, and *Staphylococcus aureus*. These outcomes suggested that chitosan nanoparticles and copper-loaded nanoparticles could prevent the growth of pathogenic bacteria. Gan et al. [5] investigated polyanion-initiated gelation process in fabricating chitosan-tripolyphosphate nanoparticles. This was studied in the size range from 100 to 250 nm, planned to be used as carriers for the delivery of gene or protein macromolecules. Katas and Alpar [6] fabricated chitosan nanoparticles as a delivery system for double-stranded small interfering RNA (siRNA). In vitro investigations in two various types of cell lines such as CHO K1 and HEK 293 have uncovered that the fabrication technique of siRNA association to chitosan plays a main part on the silencing affect. Chitosan–tripolyphosphate nanoparticles with entrapped siRNA were proven as good vehicles for siRNA delivery in contrast with CS–siRNA complexes, perhaps because of their admirable binding capacity and high loading efficiency. Min et al. presented that hydrophobically modified glycol chitosan nanoparticles encapsulated camptothecin with a high loading efficiency and camptothecin-hydrophobically modified glycol chitosan nanoparticles representing sustained camptothecin release preserved the stability of the active lactone form of camptothecin by shielding the lactone from hydrolysis. In contrast with free camptothecin, camptothecin-hydrophobically modified glycol chitosan nanoparticles showed a longer circulation time in the blood, an improved targeting of the drug to tumor tissue and a considerable improvement of antitumor activity. Ali et al. demonstrated the synthesis and characterization of chitosan and silver-loaded chitosan nanoparticles for antimicrobial textile applications. Silver loading on the fabricated chitosan nanoparticles showed synergistic antibacterial activity against S. aureus. These outcomes recommended that the fabrication of novel chitosan nanoparticles for antimicrobial textile utilization as chitosan in nano form is extremely active owing to their high surface area and very low concentration. Lima et al. presented the assessment of the hemagglutination activity of chitosan nanoparticles by means of human erythrocytes. Chitosan nanoparticles offered hemolytic activity varying from 186.20 % to 223.12 %.

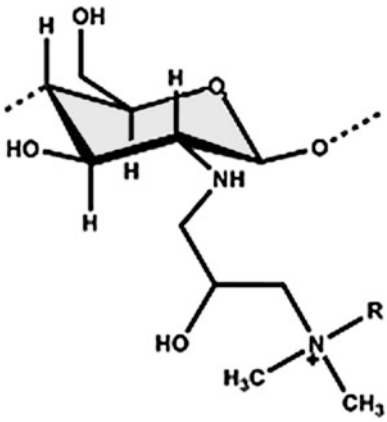

Fig. 3.4 Cationized chitosan

Cationized chitosan: Chitosan has a linear structure, formed by b-(1?4)-2-amino-2-deoxy-D-glucose units. One disadvantage of chitosan is its insolubility at pHs above 6.5 by deprotonation of its amino group. As a result its known antimicrobial activity limits to acid pHs. Cationized chitosan presents better properties of hygroscopicity, moisture retention, antibacterial activity, mucoadhesivity and as a dermal permeation enhancer of drugs. The most obvious cationization of chitosan: the permethylation of the amino group in C-2 generates an expensive product due to the costs of reagents such as sodium borohydride and methyl iodide. Substitution is reported to occur only in the amine group of chitosan (Fig. 3.4) since in the conditions employed hydroxyl groups are not sufficiently nucleophilic to induce EPTAC ring opening. Cationized chitosan has been employed as a biomedical material for a broad range of applications, including wound

3.6 Chitooligosaccharide Nanoparticles

Chitooligosaccharide, a low molecular weight depolymerization product of chitosan, has attracted increasing attention in pharmaceutical and biomedical applications because it not only is water-soluble, biocompatible, biodegradable, and nontoxic in nature, but also demonstrates unique biological activities such as antimicrobial, immune-enhancing, and antitumor activities. Chitooligosaccharide is particularly suitable for developing polymer-drug conjugate because of its availability for coupling with the primary amino groups and hydroxyl groups of each polymer subunit and the cationic nature that allows ionic crosslinking. Hydrophobically modified amphiphilic chitooligosaccharide derivatives can fabricate self-assembled polymeric nanoparticles and therefore facilitate drug delivery in cancer therapy by improving the solubility of insoluble drugs, drug targeting, and absorption. Lopez-Cruz et al. prepared magnetic nanoparticles consisting of iron oxide cores modified with covalently linked chitooligosaccharide that was colloidally stable in water and buffers.

The enhanced colloidal stability of these chitooligosaccharide-coated nanoparticle, owing to the covalent linking of chitooligosaccharide to the nanoparticle, makes them attractive for biomedical applications. Bae et al. prepared chitosan oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes as an effective heat nanomediator for cancer hyperthermia. Chitosan oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes showed superior magnetic heating efficiency with a high specific loss powervalue (2614 W/g) compared with commercial superparamagnetic Feridex nanoparticles (83 W/g). They exhibited strong antitumor activity on an animal tumor model without any severe toxicity. Liu et al. investigated a novel multidentate dithiolane lipoic acid and phosphorylcholine conjugated chitosan oligosaccharide derivative that can serve as a ligand to effectively stabilize gold nanoparticles. These nanoparticles could be widely used for biological applications. Lin et al. prepared the chitooligosaccharide nanoparticles by the formation of polyelectrolyte complexes. These nanoparticles exhibited strong inhibition of the proliferation of HeLa and B16 melanoma cells. Lu et al. prepared the synthesis of chitooligosaccharide-based multidentate ligand for ultrastable, cytotoxicity and biocompatible nanoparticles.

3.7 Chitosan Applications

3.7.1 Thermosensitive Gels

Chitosan made thermosensitive gels are liquid at room temperature however it gels rapidly when heated at 37 °C which can further allows the controlled release of macromolecules over a short to long duration period. Sustained release in these gels representing macromolecules are governed by gelation rate, molecular weight and degree of deacetylation on chitosan. These properties with favourable structural modification make them suitable for cell encapsulation, drug delivery or tissue-engineered scaffolds. Various chitosan based thermosensitive mixtures such as Chitosan/Pluronic hydrogels for gene therapy, methoxy poly(ethylene glycol)-grafted chitosan nanoparticles in a local minimally invasive drug delivery system, chitosan-silica thermosensitive nanoparticles for vaccine delivery, chitosan/glycerophosphate (GP) thermosensitive gel for sustain delivery of macromolecules are recently explored in nanoforms [7–12].

3.7.2 Chitosan Nanoparticles and Gene Therapy: Chitosan-DNA Conjugated

Gene therapy is based on the concept that involves the treatment of defective gene in specific cell by either replacement or supplementing the genetic material in order to correct the defective one. Transfection efficacy covers many drawbacks in gene therapy. Viral or non-viral gene delivery systems are the two main types of vectors

used in gene therapy. The viral gene delivery system shows a high transfection yield but it has many disadvantages, such as oncogenic effects and immunogenicity. This problem is resolved by the cationic polymers like chitosan, which is having tremendous potential in DNA complexation and may be useful as non-viral vectors for gene therapy applications. The use of nanoparticles for gene therapy is gaining more and more interest for medical applications. Chitosan is a non-toxic, mucoadhesive, biodegradable and biocompatible cationic polysaccharide with low immunogenicity and chosen as best suitable candidate for nucleic acid condensation, nano-complexation and protection against nuclease degradation. The chitosan and their nanoparticles have potential to form polyelectrolyte complex with DNA and it is useful for non-viral vectors for gene therapy applications with its reasonable transfection efficiency combined with a minimal level of cytotoxicity. Chitosan-DNA polyelectrolyte complex does not require sonication and solvation for its preparation. This prevents the possible DNA damage and form more stable complex which may improve the transfection efficacy for non-viral vectors for gene therapy applications. However its poor water solubility and low transfection efficacy have impeded its use as an NA carrier. In order to overcome such limitations, a multitude of strategies for chitosan modification and formulation have been proposed.. Strategies such as Copolymerization, Functional group modification, hydrophilic and hydrophobic modification, pH-sensitive modification, temperature-sensitive modification and specific ligand modification (Galactose, Transferrin, Folate and Mannose ligand), of these complexes are helpful in improving the transfection efficacy more. Factors like Cell type dependent transfection efficacy (higher in HEK 293 Cells and lower in primary and differentiated cell), optimum Serum content, pH, Molecular weight of chitosan, charge ratio of chitosan's nitrogen and DNA's phosphate (optimum charge ratio: 3–5) degree of deacetylation (increases the transfection and DNA binding efficacy) Influence of additives, Plasmid concentration, Chitosan salt form, Stability against polyanions. Preparation techniques of chitosan particles, Influence of route of application, Chitosan salt form greatly affects the transfection efficacy and delivery of chitosan mediated DNA delivery of complex. Furthermore derivatization of chitosan leads to furnish various novel non viral vectors such as Deoxycholic acid modified-chitosan vector, Dodecylated chitosan vector, Quaternized chitosan vectors, Galactosylated chitosan vector, Lactosylated chitosan, Transferrin–KNOB protein conjugated chitosan vector. Some of the recent advances such as intercalated chitosan in to clay were able to deliver DNA to human cells with reduced efficacy compared to chitosan/DNA nanoparticles which was studied in relation with chain length variation and glycosylation in chitosan [13]. 2Chitosan-modified poly(D,L-lactide-co-glycolide) nanospheres for plasmid DNA delivery and HBV gene-silencing could provide an effective pDNA delivery system in vitro, proven that such an approach could be useful in the treatment of viral diseases in vivo [14].

For corneal gene therapy recently oligomeric chitosan-DNA nanoparticles as was selected as a promising approach for the treatment of acquired and inherited corneal diseases inside and outside the cornea that otherwise lead to blindness [15]. Gene therapy success depends on efficient delivery of DNA which is further depends

on different level of cellular uptake and the distinct endocytic pathways. Tailoring of chitosan to optimize it for transfection in form of self-branched and trisaccharide-substituted chitosan oligomers (SBTCO) show superior transfection efficacy then linear chitosan (LCO). It has been proven that these polyplexes were taken up by the majority of the cells, but the uptake of LCO was lower than SBTCO polyplexes [16]. To reduce the cytotoxicity several methylated chitosan derivatives were synthesized and their transfection efficacy was tested. It was found that among all methylated form, methylated N-(4-pyridinylmethyl) chitosan chloride showed high transfection efficacy with low cytotoxicity reaction [17]. Conventional high molecular weight chitosans mediated Nonviral gene delivery systems are efficient as DNA vaccine delivery system, but have poor physical properties. Moreover, shorter oligomers of Chitosan lesser than 14 monomers units found to form weak complexes with DNA, resulting in physically unstable polyplexes. Thus it was studied that covalently attached nanoplex of low molecular weight but average molecular mass chitosans and gold nanoparticles (GNPs), resulted in 10 times more potency than naked DNA vaccine [18]. The potential of gene transfer of chitosan was successfully studied by examining the ideal molecular weight and degree of deacetylation. Certain N:P ratio and the pH depend combinations of deacetylated and ideal molecular weighted chitosan showed maximum expression although similar expression levels could be achieved by simultaneously lowering MW and increasing DDA or lowering DDA and increasing MW, suggesting a predominant role of particle stability, through co-operative electrostatic binding, in determining transfection efficiency [19]. Apart from various established advantages chitosan still shows difficulties in DNA release once arrived at the site of action. For this gamma poly(gamma-glutamic acid) was incorporated in Chitosan (CS)/DNA complex nanoparticles. This incorporation not only enhances transfection efficiency but also significantly increases the cellular uptake [20]. Chitosan (CH)–gadolinium (Gd) diethylenetriaminepentaacetic acid (DTPA) nano conjugates were proven as potential agents for magnetic resonance imaging [21]. Chitosan-DNA-fibronectin molecules were proven as a promising targeted carrier for gene delivery for the lung diseases such as cystic fibrosis and lung cancer [22]. Chitosan particles are potential vectors for the transfer of DNA into mammalian cells. Cellular transfection by the chitosan-pGL3-Control particles showed a sustained expression of the luciferase gene for about 10 days. This Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles lays a new path way in gene therapy for sustained release products [23, 24]. Before studying gene therapy using chitosan-DNA nanoparticles in vivo it's very essential to study their interaction with cells and their behavior. As macrophages play an important role in inflammatory processes, Chellat F et al. studied that studied the Metalloproteinase and cytokine production by THP-1 macrophages following exposure to chitosan-DNA nanoparticles [25]. Immunopotentiator effect of microg CpG oligodeoxynucleotide (ODN) in alginate coated chitosan nanoparticles for nasal delivery of hepatitis B surface antigen to generate humoral mucosal immune response was also studied [26]. Mesenchymal stem cells, MG63 and HEK293 transfection using chitosan-DNA nanoparticles suggested that chitosan-DNA nanoparticles have favorable characteristics for non-viral

gene delivery, are cell type dependent and not cytotoxic [27]. Cationically modified poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles have recently been introduced as novel carriers for DNA/RNA delivery. It has been proved that the efficacy of chitosan-coated PLGA nanoparticles as a flexible and efficient delivery system for antisense oligonucleotides to lung cancer cells [28]. Under one more success in vaccine delivery Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice were proved to be efficient delivery mode of immunization [29, 30]. Chitosan nanoparticles are the suitable gene carriers though its transfection efficacy is dependent on several factors. These various factors such as charge ratio, molecular weight, cell type, pH, temperature, concentration of DNA, protection against PEG degradation, PEG conjugation, effect of serum concentration were studied by Mao et al. [31]. DNA vaccines encapsulated with chitosan nanoparticles increases the immunogenicity by increasing the IFN-gamma secretion against tuberculosis in pulmonary delivery mediated immunization when compared to intramuscular immunization [32]. For targeting in gene therapy chitosan-DNA are sometime complexed with folic acid because mechanism of folic acid uptake by cells to promote targeting and internalization could improve transfection rates. And it has been studied that FA-nanoparticles have lower cytotoxicity, good DNA condensation, positive zeta potential and particle size around 118 nm, which makes them a promising candidate as a non-viral gene vector [33]. Chitosan-DNA complex as a oral vaccine is one of the most promising way to target the intestinal enterocytes and M-cell. It has been demonstrated that chitosan-DNA nanoparticles are more stable in the upper regions of the small intestine suggests that higher uptake rates may occur in the duodenum compared to the ileum and the colon [34]. It was well suggested that DNA vaccine encapsulated in chitosan nanoparticles are immunologically effective against swine influenza. These nanoparticles are having good morphology, high encapsulation rate and high stability, good bioactivity, prolonged release rate of the plasmid DNA [35]. Fibronectin attachment protein of mycobacterium bovis (FAP-B) which is responsible for the attachment of many mycobacteria on the fibronectin molecule of epithelial cell membrane can be considered as a new targeting ligand and can improve transfection rates in epithelial cells. In this study, chitosan-DNA nanoparticles were prepared using coacervation process. Chitosan-DNA-FAP-B nanoparticles showed good transfection efficiency without cell toxicity. They have small particle size around 279 nm which make them a promising candidate as a novel non-viral gene vector for gene delivery to lung epithelial cells [36].

3.7.3 Chitosan in Gene Therapy: Bio-Conjugated Nano Applications

Nasal delivered antigenic mucoadhesive nanoparticles, made up of N-trimethyl chitosan crosslinked (TMC) with tripolyphosphate (TPP) has been confirmed to overcome certain obstacles faced during nasal delivery such as poor absorption and

nasal tolerance. However in contrary antigen loaded TMC/TPP nasally administered nanoparticles induce a strong humoral response, antibody subtyping indicates a Th2 bias. This can be prevented by replacing TPP with physical crosslinker in ovalbumin. On the side unmethylated CpG dinucleotides are added deliberately to DNA as adjuvant to enhance the Th1 immune response. Thus overall OVA loaded TMC nanoparticles, containing CpG as adjuvant and crosslinker, are capable of provoking strong humoral as well as Th1 type cellular immune responses after nasal vaccination [37, 38]. In another study DNA vaccine delivered by chitosan/ plasmid (pCR-X8-HBc-CETP) nanoparticles against atherosclerosis could significantly attenuate the progression of atherosclerosis by intranasal immunization [39]. It was reported that stearic acid (SA) grafted chitosan oligosaccharide (CSO) (CSO-SA), low cytotoxic biodegradable micelles and could be used as an effective DNA condensation carrier for gene delivery system [40]. Zheng et al. reported three types of chitosan nanoparticles [quaternized chitosan –60 % trimethylated chitosan oligomer (TMCO-60 %), C(43–45 KDa, 87 %), and C(230 KDa, 90 %)] were used to encapsulate plasmid DNA (pDNA) encoding green fluorescent protein (GFP) with an objective to investigate the in vitro and in vivo transfection efficiency of chitosan nanoparticles used as vectors for gene therapy. TMCO-60 % having optimal chitosan/pDNA ratio were proved to be the most efficient non-viral vector with high transfection efficacy and minimal toxicity which made it a desirable non-viral vector for gene therapy via oral administration. In vivo study showed most prominent GPF expression in the gastric and upper intestinal mucosa [41]. Yu et al. reported the genipin-crosslinked carboxymethyl chitosan-chitosan/poly(γ-glutamic acid conjugate loaded with bovine serum albumin with fluorescence emissions as stimuli-responsive materials under hyper-gluconic acid condition so that they release bovine serum albumin under that conditions. This material is hence proved as a stimuli-responsive material for optical sensing and protein delivery purposes [42]. Gan et al. reported Chitosan-BSA-TPP nanoparticle as good protein delivery carrier in spite of difficulty in controlling initial burst effect in releasing large quantities of protein molecules [43]. Wang et al. explored the N-phosphorylcholine-chitosan derivative (N-PCCs) with biomembrane-like phosphorylcholine (PC) group with biomembrane-like phosphorylcholine (PC) group [44]. In another study chitosan nanoparticles coated with zein have been newly demonstrated as a promising encapsulation and delivery system for hydrophilic nutrient such as α-tocopherol with enhanced bioactivities in our previous study. In contrast with zein nanoparticles, zein/CS complex provided better protection of TOC release against gastrointestinal conditions, due to CS coatings. Zein/CS complex is believed to be a promising delivery system for supplementation or treatment of hydrophobic nutrients or drugs [45]. In one more study surface coated poly-(ε-caprolactone) (PCL) nanoparticles with chitosan (CS) were developed as a carrier system for nasal immunization using recombinant Influenza A virus (A/California/07/2009) H1N1 hemagglutinin (HA) protein, for the induction of humoral, cellular and mucosal immunity. And it was also demonstrated that high potential of CS-PCL nanoparticles can be used for as a carrier adjuvant for nasal administered influenza antigens [46, 47]. Bal et al. demonstrated the potential of N-trimethyl chitosan nanoparticles to induce dendritic cells maturation and

enhance immune responses after intradermal injection which demonstrates that N-trimethyl chitosan functions as an immune potentiator for antigens delivered via the skin [48]. Zhao et al. reported chitosan and epoxy propyl trimethyl ammonium chloride (EPTAC) which were used to prepare the water-soluble N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC). HTCC and sodium tripolyphosphate (TPP) were mixed to form HTCC nanoparticles. Parathyroid hormone-related protein 1-34 (PTHrP1-34) was incorporated into the HTCC nanoparticles. These studies showed that HTCC/PTHrP1-34 nanoparticles are suitable for the treatment of osteoporosis, because of their slow-continuous-release properties [49, 50]. Xu et al. reported the N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) which is a water-soluble derivative of chitosan having good encapsulation efficacy. Its efficacy was studied against increase in bovine serum albumin and polyethylene glycol concentration encapsulation efficacy decreases in contrary addition of sodium tripolyphosphate and alginate, led to increase in encapsulation efficacy [51]. Sayın et al. demonstrated the potential role of N-trimethyl chitosan (TMC, polycationic) and mono-N-carboxymethyl chitosan (MCC, polyampholytic) in inducing both the mucosal and systemic immune response indicating that this newly developed system has potential for mucosal administration of vaccines [52]. Li et al., has reported the non cytotoxic interaction of bovine serum albumin with self-assembled nanoparticles of 6-O-cholesterol modified chitosan and complex stability is governed by many factors such as hydrogen bonding, hydrophobic and electrostatic interactions. Some other effects on addition of O-CHCS NPs led to the decrease of α-helical content of BSA and the increase of β-strand content. Furthermore the size and the zeta potential of the complex increased with the increasing concentration of O-CHCS NPs [53]. Xue et al., fabricated Fe3O4-CTS-SEA immunomagnetic beads. These beads displayed a high adsorption capacity and recognition specificity for SEA, (Staphylococcus aureus enterotoxin A (SEA)) and the adsorption quantity could reach $6.48 \times 10 - 3$ μmol/g. The specificity evaluation results showed that the Fe3O4-CTS-SEA immunomagnetic beads had high enrichment and affinity property for SEA compared to SEB (Staphylococcus aureusenterotoxin B) and SPA (Staphylococcus aureus protein A) [54]. Zhang et al., synthesized O-carboxymethyl-chitosan which is a kind of water-soluble chitosan derivative and also has good biocompatibility. O-Carboxymethyl-chitosan-organosilica hybrid nanoparticles could protect DNA against DNase I and serum degradation and could be used as efficient and safe vectors for gene delivery [55]. In another study Vongchan et al., have used N,N,N-Trimethyl chitosan chloride to form nanocomplexes with protein through ionotropic gelation. A monoclonal antibody, raised against human liver heparan sulfate proteoglycan and specifically inhibiting hepatocellular carcinoma in vitro, was prepared in nanocomplexes of this modified chitosan. The results demonstrated that the nanocomplexes could enter cells and were retained for a longer period of time in cancer cells where they exhibited greater toxicity. These nanocomplexes appear safe and could potentially enhance the half-life of added antibodies [56]. Kumar et al., has demonstrated the potential of chitosan as a polycationic gene carrier for oral administration. The potential efficacy of DNA vaccine against Vibrio anguillarum in Asian sea bass through oral

route using chitosan nanoparticles encapsulation was explored in this study [57]. Bal et al., reported the role of N-trimethyl chitosan nanoparticles that shown to increase the immunogenicity of subunit antigens after nasal and intradermal administration. Second generation of N-trimethyl chitosan nanoparticles containing ovalbumin as a model antigen and an additional immunopotentiator had shown site-dependent and adjuvant immunogenicity in mice [58]. Chae et al. have fabricated self assembled amphiphilic nanoparticles of deoxycholic acid-conjugated chitosan oligosaccharide for their efficient gene delivery and low cytotoxic effects [59]. Cui et al. explored the topical application of chitosan-based nanoparticles containing plasmid DNA (pDNA) as a potential approach for genetic immunization [60]. Bowman et al. reported factor VIII-chitosan nanoparticles mediated gene transfer to hemophilia A as a novel and attractive strategy for therapeutic gene transfer [61]. Growth factors are essential in cellular signaling for migration, proliferation, differentiation and maturation. Chitosan mediated growth factors delivery in sustained form was reported as a potential approach in tissue engineering applications [62]. In another study porcine interleukin-2 gene encapsulated in chitosan nanoparticles were reported to enhance immune response of mice to piglet paratyphoid vaccine [63]. Chen et al. explored alginate modified TMC nanoparticles as potential carriers for oral protein and vaccine delivery [64]. Inter macromolecular complexation between chitosan and sodium caseinate was investigated by Anal et al., as stable complex which gets solubilized in the pH range 4.8–6.0 whereas further increase in pH lead to phase separation [65]. In another study chitosan-based nanoparticles for delivering virus structures like particle antigens, using recombinant hepatitis B surface antigen were proved as promising adjuvant for vaccine delivery of subunit antigens [66, 67]. Recent breakthroughs in nanotechnology have shown the great advantages of nanoparticles (NPs) in protein encapsulation and controlled release. α-Galactosidase and chitosan (CS) were chosen as models for food enzymes and coating materials, respectively [68]. Amidi et al., explored the potential of N-trimethyl chitosan nanoparticles as a carrier system for the nasal delivery of a monovalent influenza subunit vaccine and it was reported that TMC nanoparticles act as potent new delivery system for i.n. administered influenza antigens [69]. Earlier study demonstrated the potential benefit of using chitosan modification technology as a cytokine delivery system for the successful prevention of colorectal cancer liver metastasis by exploiting liver immunity [70]. Earlier results suggested that chitosan-DNA nanoparticles have favorable characteristics for non-viral gene delivery to primary chondrocytes, and have the potential to deliver therapeutic genes directly into joint [71]. Chitosan has been reported for its efficient nanoparticulate delivery to overcome the poor immunogenicity of nasally administered soluble antigens by studying the physicochemical differences to their adjuvant effect of various nanoparticles, using ovalbumin (OVA)-loaded poly(lactic-co-glycolic acid) nanoparticles (PLGA NP), N-trimethyl chitosan (TMC) based NP (TMC NP) and TMC-coated PLGA NP (PLGA/TMC NP) [72–76]. It was reported that TMC nanoparticles are a potential new delivery system for transport of proteins through the nasal mucosa [77].

3.7.4 Chitosan Based Amino Acid Polymer Conjugate

Moon et al. suggested chitosan conjugation with amino acid for mucosal immunization with recombinant influenza hemagglutinin protein. In this study these prepared poly gamma-glutamate/chitosan nanoparticles were induced protection against highly pathogenic influenza A virus [78]. Hence it was proved that chitosan based amino acid polymer conjugate can act as potential carrier for recombinant influenza hemagglutinin protein for providing protection against highly pathogenic influenza A virus [78].

3.7.5 Chitosan Based Quantum Dots

Chitosan forms ultrafine, stable and biocompatible nanoparticles, encapsulating optically active quantum dots having wide biomedical applications. Recent research explored that chitosan can also be utilized for development of acetylcholinesterase biosensor based on CdTe quantum dots/gold nanoparticles. These NPs were modified from chitosan microspheres interface [79, 80].

3.7.6 Chitosan Based Ceramic Glass Nanoparticles

According to previous report ceramic modification of N-acylated chitosan stabilized gold nanoparticles triggered its biological potential. Development of bioactive and biodegradable chitosan-based injectable systems containing bioactive glass nanoparticles with enhanced in vivo applications allows their more stable delivery in biological fluids under physiological environment. Chitosan based metallic NPs e.g. Selenite-loaded chitosan/TPP nanoparticles with or without zein coating could give new applications to and enhance their biological effects. Chitosan based Metallic nanoparticles are having huge potential in nanotechnology. Chitosan Copper(II) nano complexation is helpful in controlling microbial growth [81–84].

3.7.7 Chitosan Based Metallic Nanoparticles

It has been reported that chitin–chitosan/nano TiO2-composite scaffolds has wide applications in tissue engineering field [85]. Current research has developed a novel immunoassay strategy based on combination of chitosan and a gold nanoparticle label [86] which explored the unanimous role of chitosan in biochemical research. Further bioinspired mineralization of chitosan-based nanocomplexes encouraged its role in bone tissue engineering applications [87]. Fabrication of chitosan/ZnO

nanoparticle composite membranes promoted its application in biomedical research [1]. Moreover production of silver nanoparticle-loaded chitosan–starch based films and chitosan/Ag/ZnO blend films, chitosan and sago starch impregnated with silver nanoparticles were reported for their improved antimicrobial properties [84–87]. pH sensitive chitosan enclosed mesoporous silica nanoparticles showed their applications in drug delivery at narrow pH range [88]. Sun et al. investigated degradation behavior of chitosan chains in the 'green' synthesis of gold nanoparticles which can further affect the release profile of drug [89]. Further Twu et al. investigated role of chitosan suspensions in the fabrication of silver nanoparticles [90]. Additional report on improved antimicrobial properties with the use of chitosan and silver nanoparticles as pudding with raisins was reported by Rodríguez-Argüelles et al. [91]. Pinto et al., reported the antibacterial activity of optically transparent nanocomposite films based on chitosan or its derivatives and silver nanoparticles [92]. Tiwari et al., has examined the potential role of chemically modified chitosan matrix in stabilisation of silver and copper nanoparticles [2]. Chitosan suspensions were also investigated for fabrication of gold nanopowders and nanoparticles [3]. Advance one pot synthesis of chitosan-induced gold nanoparticles by microwave irradiation was studied by Fan et al. [4]. Later on simple synthesis of Ag and Au nanoparticles utilizing chitosan as a mediator agent was explored [5]. Huang et al. explored the synthesis of gold nanoparticles in carboxymethylated chitosan aqueous solutions [6]. Further fabrication of polyelectrolyte complex using chitosan–sodium alginate microcapsules containing ZnS nanoparticles and its effect on the drug release was studied by Li et al. [93]. Zhang et al. reported that encapsulation of selenium in chitosan nanoparticles improves selenium availability and protects cells from selenium-induced DNA damage response [94]. Chitosan-capped gold nanoparticles and its attenuated effects on LPS-induced toxicity in laboratory rats was also studied [95]. Caseli et al. explored the controlled fabrication of gold nanoparticles biomediated by glucose oxidase immobilized on chitosan layer-by-layer films [96]. Improved uptake and biological effects of chitosan-capped gold nanoparticles on Chinese Hamster Ovary cells was reported [97]. Wei et al. explored role of chitosan as an active support for assembly of metal nanoparticles and application of the resultant bioconjugates in catalysis [98]. Improved antibacterial activity and hydrogenation activity were observed in chitosan and grape polyphenols stabilized palladium nanoparticles [99, 100]. Further improved antibacterial activity of chitosan-based silver nanoparticles were explored by Wei et al. [101]. Better antimicrobial effects of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles were also reported [102]. In addition chitosan tripolyphosphate nanoparticles loaded with various metal ions were proved for their improved antibacterial activity [103]. Biosensor applications of chitosan such as heavy-metal ion sensors using chitosan-capped gold nanoparticles and glucose biosensor based on chitosan–glucose oxidase–gold nanoparticles biocomposite were explored [104, 105]. A simple method to fabricate a chitosan-gold nanoparticles film and its application in glucose biosensor was also studied [106]. Further synthesis of novel chitosan nanofiber/gold nanoparticles composite towards improved performance for a cholesterol sensor was investigated by Gomathi et al. [107]. Chitosan nanoparticles

and its films have wide applications in improving antimicrobial effects of whole formulation. Vimala et al., reported production of porous chitosan films impregnated with silver nanoparticles: A simplistic approach for superior antibacterial application [108]. Chitosan and its derivatives have wide applications in biochemical research. Feng et al., reported the direct electrochemistry and electrocatalysis of heme proteins immobilized on gold nanoparticles stabilized by chitosan [109]. In addition chitosan has shown its improved biological properties in cancer research. For an instance chitosan-coated triangular silver nanoparticles reported as a novel class of biocompatible, highly effective photothermal transducers for in vitro cancer cell therapy [110]. Chitosan derivatives were also proven for their stabilization effects e.g. Pd–Fe nanoparticles stabilized by chitosan derivatives for perchloroethene dechlorination [111]. Chitosan also plays an important role bioremediation process e.g. lead sorption from aqueous solutions on chitosan nanoparticles was reported by Qi and Xu [112]. Effects of chitosan and copper-loaded nanoparticles were compared and investigated for its potential cytotoxic activities against different cell line and it was observed that chitosan NPs showed profound cytotoxic effects [113]. Various chitosan based significant methodologies were explored for the production of different types of metallic nanoparticles e.g. fabrication of silver nanoparticles in the presence of chitosan by electrochemical method, production of chitosan-stabilized gold nanoparticles by atmospheric plasma and a simple method for electrospinning of Ag nanoparticles/poly (vinyl alcohol)/carboxymethyl-chitosan nanofibers [114–116].

3.7.8 Chitosan Based Cationic-Cationic Polymer: Macromolecule Grafted NPs

Chitosan based Grafted NPs plays an important role in enhancing the biological properties of the prepared formulation e.g. in one study Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles [117]. Similarly fabrication of biocompatible chitosan grafted poly(lactic acid) nanoparticles with improved biological property was observed [118]. In another study methotrexate-incorporated polymeric nanoparticles of methoxy poly(ethylene glycol)-grafted chitosan was investigated for drug release and biological functions. It was observed that fabricated nanoparticles showed improved properties [119].

3.7.9 Chitosan Based Functionalized Nanoparticles

Functionalization and derivatization of chitosan is known to be the most reliable alternative for modifying its physico-chemical and biological properties. Recently N,N,N-trimethyl chitosan nanoparticles has explored as a vitamin carrier system whereas N-Acylated chitosan stabilized iron oxide nanoparticles has proven as a novel nano-matrix for ceramic modification [120, 121]. Similarly various other

chitosan functionalized NPs such as thiolated chitosan-coated acrylic, thiolated chitosan coated poly hydroxyethyl methacrylate and O-carboxymethyl and N,O-carboxymethylchitosan nanoparticles have been explored in previous research [120–125]. Even though few fabricated NPs such as oleoyl-chitosan nanoparticles, O-carboxymethyl and N,O-carboxymethylchitosan nanoparticles were proven for their improved antimicrobial and cytotoxic effects [124, 125]. Chitosan functionalization e.g. mono-N-carboxymethyl chitosan (MCC) and N-trimethyl chitosan (TMC) nanoparticles has been reported for non-invasive vaccine delivery [126]. Further fabrication of functionalized rapamycin-loaded chitosan/PLA nanoparticles has reported for immunosuppression in corneal transplantation [127].

3.7.10 Chitosan Based Self Assembled/Amphiphilic NPs

Chitosan based self assembled/amphiphilic NPs plays a vital role in delivery of number of drugs, nucleic acids and metals. Recently, the effects of hydrophobic and hydrophilic modifications on gene delivery of amphiphilic chitosan based nanocarriers investigated and it has been observed that this type of modification allowed the improved gene delivery via amphiphilic nanoparticles [128]. It was reported that the chitosan-based self-assembled nanoparticles can be readily obtained by chemically attaching the hydrophobic moiety to the backbone of chitosan and its derivatives, as shown in Fig. 3.5. These self-assembled nanoparticles can circulate in the

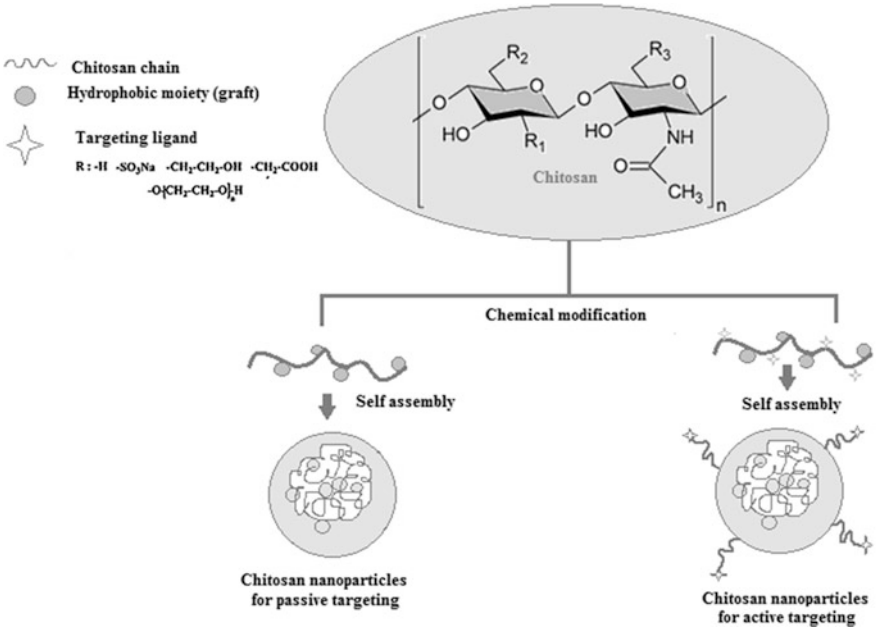

Fig. 3.5 Self assembled chitosan nanoparticles for passive or active cancer targeting [129, 130]

bloodstream for a relatively long time without recognition by phagocytes and can easily accumulate at the leaky vasculature throughout the enhanced permeability and retention effect. One major limitation with passive targeting alone is its inability to achieve a sufficiently high level of drug concentration at the nidus site, which results in lower therapeutic efficacy and eliciting adverse systemic effects (Br To further improve delivery efficiency and cancer specificity, a strong emphasis has been placed on developing PM/NPs with active targeting ability which can be achieved by functionalizing polysaccharides and their derivatives with targeting ligands such as folic acid, antibodies, peptides, hyaluronic acid, biotin, and avidin, which can recognize and bind to specific receptors that are unique to cancer cells.

Multilayer film self-assembled chitosan/heparin NPs has been explored as novel template for in situ synthesis of silver nanoparticles [131]. Amphiphilically modified low molecular weight chitosan potential was investigated for delivery hydrophobic anticancer drug. From this study it was investigated that amphiphilically modified low molecular weight chitosan showed improved delivery of hydrophobic anticancer drug [132]. Wang et al., reported the improved interaction between bovine serum albumin and the self-aggregated nanoparticles of cholesterol-modified O-carboxymethyl chitosan [133]. Yang et al., explored the self-aggregated nanoparticles from methoxy poly(ethylene glycol)-modified chitosan. Improved methotrexate release under in vitro conditions was observed in this study [134]. Moreover improved in vivo biodistribution and anti-tumor activity was reported in self-assembled nanoparticles. These nanoparticles were based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin [135]. Wang et al., explored cholesterol-modified chitosan conjugate based self-aggregated nanoparticles as a novel carrier of epirubicin [136]. Similarly self-assembled nanoparticles based on linoleic-acid modified carboxymethyl-chitosan were also reported as carrier of adriamycin (ADR) [137]. Various self assembled nanoparticles and its applications are highlighted in Table 3.1.

Li et al. explored novel amphiphilic oleoyl-carboxymethyl chitosan self-assembled nanoparticles [147]. Morris et al., investigated the effect of prolonged storage at different temperatures on the particle size distribution of tripolyphosphate (TPP) –chitosan nanoparticles and it was observed that fabricated nanoparticles showed uniform particle size distribution [129]. In one study, novel MPEG–chitosan diblock copolymer and self-assembly of nanoparticles were explored [148]. In another study self-assembled nanoparticles of 6-O-cholesterol-modified chitosan for drug delivery were fabricated [149]. These nanoparticles were reported for their improved delivery.

3.7.11 Chitosan Based Coacervative Nanoparticles

Current research has reported various surface charged chitosan coacervates and its wide applications in biomedical research. Tavares et al. reported various chitosan coacervated nanoparticles and their applications. Similarly various other reports have been explored with its simplistic fabrication methods and its applications in biomedical research especially in drug delivery [150].

Table 3.1 Self-assembled nanoparticles and its applications

Type of nanoparticles	Applications	References
Self-assembled nanoparticles of modified-chitosan conjugates	Sustained release of DL-α-tocopherol	[138]
Self-assembled nanoparticles containing hydrophobically modified glycol chitosan	For gene delivery	[139]
self-assembled chitosan nanoparticles	Oral insulin delivery	[140]
Self-assembled nanoparticles based on hydrophobically modified chitosan as	Carriers for doxorubicin	[141]
Self-assembled nanoparticles conjugated O-carboxymethyl chitosan	Carriers for methotrexate	[142]
Self-assembled nanoparticles based on linoleic-acid modified chitosan	For drug delivery	[143]
Chitosan N-betainates/DNA self-assembly nanoparticles	Carrier for gene delivery	[144]
Self-aggregated nanoparticles from anacardoylated chitosan	Carrier for insulin	[145]
Arginine-chitosan/DNA self-assemble nanoparticles	Carrier for gene delivery	[146]

3.7.12 Chemically Modified Chitosan NPs

Recent research explored various chemically modified conjugates and derivatives to improve its physico-chemical and biological properties. This modification allows significant applications of chitosan in various disciplines of biomedical research. So far various fabrication methods have been employed for the development of chemically modified chitosan e.g. chitosan–poly(acrylic acid) nanoparticles and acylated chitosan nanoparticles have been recently explored to examine their modifications effect on physicochemical properties and blood compatibility [151, 152]. Similarly self-aggregated NPs of cholesterol-modified O-carboxymethyl chitosan conjugates were fabricated to improve the pharmaceutical and biomedical applications of chitosan [153]. Various examples of chitosan and its chemically modified synthetic derivatives are mentioned in Table 3.2.

3.7.13 Chitosan Based NPs for Poorly Soluble Drug

Chitosan act as excellent carrier in acting as carrier for poor soluble drugs. Among the various pharmaceutical applications, chitosan significantly plays an important role in improving the bioavailability and solubility of various drugs e.g. thiolated chitosan-coated pMMA NPs were prepared to improve the bioavailability and solubility of paclitaxelon different normal and cancer cell lines [186].

Table 3.2 Chemically modified chitosan derivatives and its applications

Chemically modified	Applications	References
Hydrotropic oligomer-conjugated glycol chitosan NPs	Carrier for paclitaxel	[154]
N-succinyl-chitosan NPs	For local hydroxycamptothecin delivery	[155]
Folate conjugated chitosan NPs	For cellular uptake of its nanoparticles in HT-29 cells	[156]
Chitosan/PLA NPs	carrier for the delivery of anthraquinone:	[157]
Trimethylated chitosan-conjugated PLGA NPs	For delivery of drugs to the brain	[158]
Folate–modified chitosan NPs	Anti-oxidative applications	[159]
Folate–modified chitosan NPs	To enhance biocompatibility	[160]
Glycol chitosan modified by 5β-cholanic acid NPs	For the sustained release of proteins during murine embryonic limb skeletogenesis	[161]
N-acetyl histidine-conjugated glycol chitosan self-assembled NPs	For intracytoplasmic delivery of drugs	[162]
Folate conjugated carboxymethyl chitosan–manganese doped zinc sulphide NPs	For targeted drug delivery and imaging of cancer cells	[163]
NPs based on the complex of chitosan and polyaspartic acid sodium salt	For 5-fluorouracil delivery	[164]
NPs made of hydrophobically-modified chitosan	For cellular uptake and intracellular trafficking	[165]
Size-controlled self-aggregated N-acyl chitosan NPs	For vitamin C carrier	[166]
Chitosan-TPP NPs	For hepatic tissue after severe bleeding	[167]
Cholesterol-modified chitosan self-aggregated NPs	For delivery of drugs to ocular surface	[168]
Polyelectrolyte NPs based on water-soluble chitosan–poly(L-aspartic acid)–polyethylene glycol	For controlled protein release	[169]
PLGA-based NPs	Chitosan effect in the aggregate stabilization (dielectric relaxation spectroscopy study)	[170]
Chitosan NPs	For methacrylic acid delivery	[171]
Self-aggregated NPs from linoleic acid modified carboxymethyl chitosan	For linoleic acid delivery	[172]
Hydrophobically modified glycol chitosan NPs	For carriers for paclitaxel	[173]
PMAA–chitosan–PEG NPs	For oral drug delivery	[174]
Glycyrrhizin-modified O-carboxymethyl chitosan NPs as drug vehicles	For targeting hepatocellular carcinoma	[175]
N-Succinyl-chitosan NPs	For inducing mitochondria-dependent apoptosis in K562	[176]

(continued)

Table 3.2 (continued)

Chemically modified	Applications	References
Oleoyl-chitosan NPs	As carriers for doxorubicin	[177]
O-Carboxymethyl chitosan NPs	For metformin delivery to pancreatic cancer cells	[178]
Oleoyl-chitosan NPs	Inhibits Escherichia coli and Staphylococcus aureus by damaging the cell membrane and putative binding to extracellular or intracellular targets	[179]
Microspheres containing lipid/chitosan NPs complexes	For pulmonary delivery of therapeutic proteins	[180]
poly(lactic acid)/chitosan nanoparticles	For anti-HIV drug delivery	[181]
Acylated chitosan NPs	For improving physicochemical properties and blood compatibility	[152]
Lactosaminated carboxymethyl chitosan NPs	Tissue distribution in mice	[182]
N,O6-partially acetylated chitosan nanoparticles hydrophobically-modified	For controlled release of steroids and vitamin E	[183]
Chitosan NPs	For delivery of vitamin C	[184]
Chitosan oligosaccharide NPs	For sustained release of ATP encapsulated	[185]

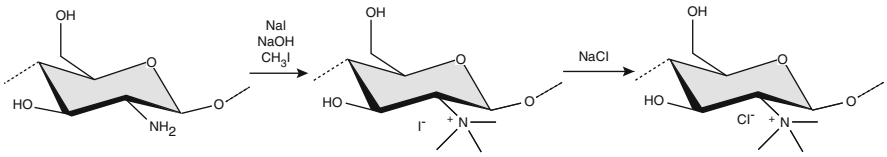

Fig. 3.6 Quaternization of nitrogen of chitosan

3.7.14 Chitosan Based Quaternized Nanoparticles

Chitosan is a linear polysaccharide with good biocompatibility, biodegradability and antimicrobial activity, which makes it potentially valuable for biomedical applications, such as antimicrobial agent either alone or blended with other polymers. Nevertheless, the poor solubility of chitosan in most solvents at neutral or high pH substantially limits its use. Quaternary ammonium chitosan, which was prepared by introducing a quaternary ammonium group on a dissociative hydroxyl group or amino group of the chitosan, exhibited better water solubility and stronger antibacterial activity relative to chitosan over an entire range of pH values; therefore, this quaternary modification increases the potential biomedical applications of chitosan in the field of anti-infection. Quaternized chitosan NPs emerged as most reliable carrier for protein delivery. Trimethyl chitosan was synthesized by various authors. A typical method of N-trimethylation is the dispersion of chitosan in N-methylpyrrolidone that contains sodium iodide and methyl iodide in the presence of NaOH as a base (Fig. 3.6). The iodide counterion of the reaction product is

Table 3.3 Quaternized NPs and its applications

Quaternized NPs	Applications	References
Quaternized chitosan (QCS)/poly (aspartic acid) nanoparticles	For protein drug-delivery	[187]
Chitosan and its quaternized derivatives NPs	For insulin delivery	[188]
Quaternized chitosan–organic rectorite intercalated composites based NPs	For protein controlled release	[189]
N-trimethyl chitosan (TMC) and N-diethylmethylchitosan (DEMC) NPs	For insulin delivery	[190]
NPs of quaternized chitosan derivatives	As a carrier for colon delivery of insulin: Ex vivo and in vivo studies	[191]
N,N,N-Trimethyl chitosan NPs	For controlled intranasal delivery of HBV surface antigen	[192]

interchanged with chloride, to obtain a more stable salt. The varied chemistry of chitosan led to some revisions.

Wang et al., has explored quaternized chitosan (QCS)/poly (aspartic acid) NPs as a most reliable carrier protein drug-delivery system. Similarly various other examples are below mentioned in tabular form (Table 3.3).

3.7.15 Chitosan Based PEGyalated Nanoparticles

PEGylation is an excellent approach to modify the properties of biomaterials and to explore their diverse applications in biomedical science [193]. Various pegylated biomaterials especially PEGylated chitosan derivatives with their significant applications are reported e.g. Casettari et al. explored synthesis, characterizations and pharmaceutical applications of PEGylated chitosan derivatives [193]. Similarly Zhu et al. demonstrated the synthesis and characterization of PEG modified N-trimethylaminoethylmethacrylate chitosan nanoparticles [194].

3.7.16 Chitosan Based Glycosylated Nanoparticles

Chitosan "a natural polymer" has received extensive attention in drug delivery systems owing to its important physicochemical and biological characteristics. Particularly, hydrophobic moiety-conjugated glycol chitosan can form amphiphilic self-assembled glycol chitosan nanoparticles (GCNPs) and simultaneously encapsulate hydrophobic drug molecules inside their hydrophobic core. This self-assembled glycol chitosan nanoparticles-based drug delivery systems display outstanding tumor-homing efficacy, attributed to the long blood circulation and the enhanced permeability and retention effect; this tumor-targeting drug delivery results in improved therapeutic efficiency. Many other applications of self-assembled glycol chitosan nanoparticles are highlighted in Table 3.4.

Table 3.4 Glycosylated nanoparticles and its applications

Glycosylated NPs	Applications	Reference
Glycol chitosan NPs	In real-time and non-invasive optical imaging of tumor in different tumor models	[195]
Photosensitizer loaded and conjugated glycol chitosan NPs	For cancer therapy	[196]
Glycoconjugated chitosan stabilized iron oxide NPs	As a multifunctional nanoprobe	[197]
Photosensitizer-encapsulated glycol chitosan-based NPs	For enhancing tumor specificity and therapeutic efficacy in tumor-bearing mice	[198]
Cisplatin-loaded glycol chitosan NPs	For enhancing antitumor efficacy in tumor-bearing mice	[199]
Hydrophobically modified glycol chitosan NPs -encapsulated camptothecin	For enhancing the drug stability and tumor targeting in cancer therapy	[200]
Glycol chitosan NPs	As carrier for low water soluble drugs	[201]
Hydrophobically modified glycol chitosan NPs	Cellular uptake mechanism and intracellular fate of	[202]
Self-aggregated NPs of cholesterol-modified glycol chitosan conjugate	As drug carrier	[203]

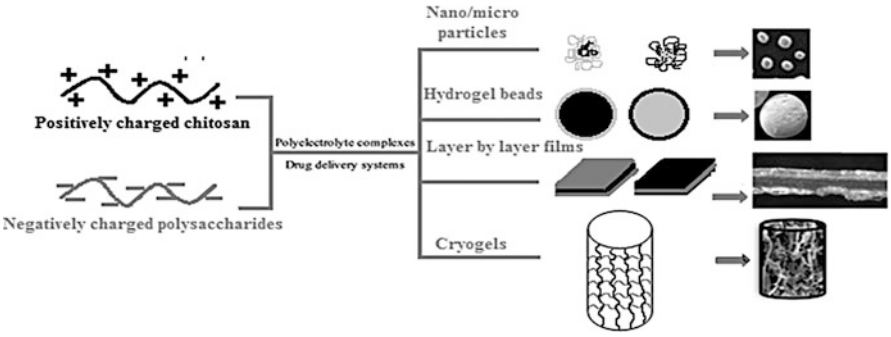

Fig. 3.7 Chitosan based polyelectrolyte complexes as drug delivery systems

3.7.17 Chitosan Based Nanoparticles

Chitosan based magnetic nanoparticles emerged as potential tool for the delivery of various drugs, biosensor and immobilization applications of various macromolecules. Various fabrication methods have been developed. Various chitosan based polyelectrolyte complexes as drug delivery systems are mentioned in Fig. 3.7.

Zang et al. explored the synthesis of carboxymethyl-chitosan-bound magnetic nanoparticles by the spraying co-precipitation method [204]. Similarly Zhi et al. [205] demonstrated the in situ preparation of magnetic chitosan/Fe3O4 composite NPs in tiny pools of water-in-oil microemulsion. In addition Suspension of Fe3O4 nanoparticles

Table 3.5 Magnetic nanoparticles and its applications

Magnetic chitosan NPs	Applications	Ref.
Magnetite chitosan NPs	For Paclitaxel delivery	[209]
Fe3O4–chitosan composite NPs	For localized hyperthermia	[210]
magnetic nanoparticles-chitosan film	For hemoglobin immobilization	[211]
PEG-chitosan-coated iron oxide NPs with high saturated magnetization	As carriers of 10-hydroxycamptothecin	[212]
Superparamagnetic iron oxide NPs encapsulated within chitosan	Biomedical applications	[213]
Magnetic NPs coated with sulfonated chitosan	Delivery of catecholamines	[214]
Glycolic acid-g-chitosan–Pt–Fe3O4 NPs	Nanohybrid scaffold for tissue engineering and drug delivery	[215]
Fe3O4–chitosan NPs	For hyperthermia	[216]
Chitosan-coated magnetic NPs	As carriers of 5-Fluorouracil	[217]
Microcapsules of alginate/chitosan containing magnetic NPs	For controlled release of insulin	[218]
Fe3O4-chitosan NPs	For lipase immobilization	[219]
N-hexanoyl chitosan-stabilized magnetic NPs	Enhancement of adenoviral-mediated gene expression both in vitro and in vivo	[220]
Chitosan based Fe3O4 NPs	BSA separation	[221]
Fe3O4-chitosan NPs	Optimal covalent immobilization of α-chymotrypsin	[222]
Superparamagnetic iron oxide NPs-loaded chitosan-linoleic acid nanoparticles	As an effective hepatocyte-targeted gene delivery system	[223]
Surface-imprinted chitosan-coated magnetic NPs modified multi-walled carbon nanotubes biosensor	For detection of bovine serum albumin	[224]
ethylenediamine-modified magnetic chitosan nanoparticles	Adsorption of acid dyes from aqueous solutions	[225]
Chitosan magnetic NPs	For in situ delivery of tissue plasminogen activator	[226]
Magnetic chitosan NPs	adsorption of Co(II) ions	[227]
Chitosan based NiFe2O4 NPs	Amperometric glucose biosensor	[228]
Magnetic chitosan NPs	Adsorption of bovine serum albumin (BSA)	[229]
Magnetic Fe3O4–chitosan NPs	Adsorption of Saccharomyces cerevisiae mandelated dehydrogenase	[230]
Magnetic carboxymethyl chitosan NPs with immobilized metal ions	For lysozyme adsorption	[231]

stabilized by chitosan and o-carboxymethyl chitosan was fabricated by Zhu et al. [206]. Zhang et al., demonstrated the control synthesis of magnetic Fe3O4–chitosan nanoparticles under UV irradiation in aqueous system [207]. Guo et al. explored the fabrication and characteristics of carboplatin-Fe@C-loaded chitosan nanoparticles with dual physical drug-loaded mechanisms [208]. Various other examples of chitosan based magnetic NPs and its applications are explored in Table 3.5.

Table 3.6 Fluorescent chitosan NPs and its applications

Chitosan based fluorescent system	Applications	Ref.
Chitosan-based systems	For molecular imaging	[232]
Chitosan NPs	For sorption of acid dye	[233]
Fluorescent Chitosan NPs	Act as fluorescent Chitosan NPs Probe	[234]
Chitosan NPs	For sorption of acid dye	[235]
Chitosan NPs	For adsorption properties of chitosan NPs for eosin Y as a model anionic dye	[236]
Chitosan NPs	For tracing transport of chitosan NPs and molecules in Caco-2 cells by fluorescent labeling	[237]
Core–shell silica@chitosan NPs and hollow chitosan nanospheres using silica nanoparticles	As templates: ultrasound bubble application	[238]

3.7.18 Fluorescent Nanoparticles (C Dots or Core-Shell Silica Nanoparticles)

Chitosan based Fluorescent NPs are utilized by current research for various imaging, labeling and sorption. Chitosan based NPs plays a significant role in diagnosis and imaging applications. Guo et al. explored the preparation and characteristics of carboplatin-Fe@C-loaded chitosan nanoparticles with dual physical drug-loaded mechanisms [208]. Various examples and applications are mentioned in Table 3.6.

3.7.19 Crosslinked Chitosan Polymers Based NPs

Crosslinked chitosan polymers emerged as excellent carrier for macromolecules such as protein. Adriana et al. proposed static mixing technique showed good control over the ionic gelation process and 152–376 nm CSNPs were achieved in a continuous and scalable mode. Then they used salicylic acid as a model drug and it was successfully loaded into the synthesized CSNPs during the fabrication process. The CSNPs exhibited a nearly spherical shape and the size was around 200 nm. The ionic gelation technique was a good method adopted to formulate the nanosystems because the approach mainly relies on the simple mixing of oppositely charged aqueous solutions without any organic solvent or covalent crosslinking agent (i.e., glutaraldehyde) as depicted in Figs. 3.8 and 3.9.

Various derivatives have been explored in various studies e.g. in one study three types of cross-linked chitosan polymers were investigated for their adsorption capability for multiple mycotoxins, including aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEN), fumonisin B_1(FB_1), deoxynivalenol (DON) and T-2 toxin (T2). Among these synthetic adsorbents, cross-linked chitosan-glutaraldehyde complex presented the highest adsorption capability for aflatoxin B_1 (73 %), ochratoxin A

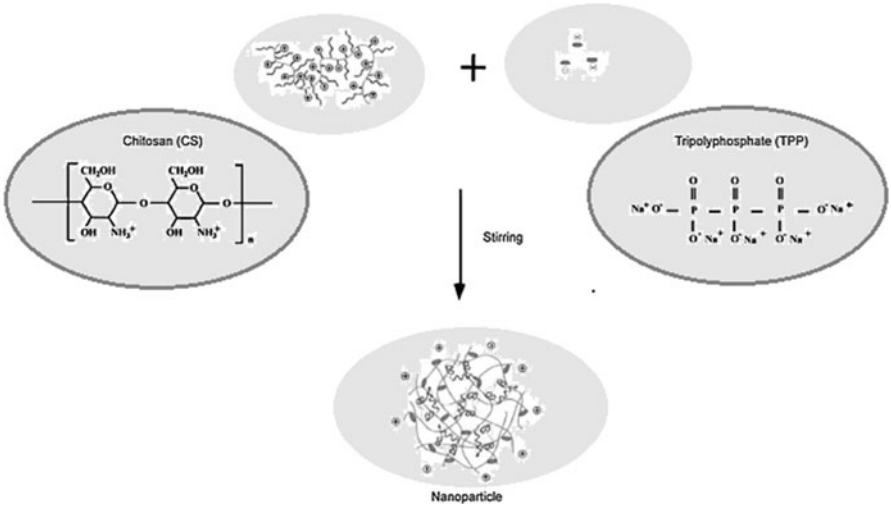

Fig. 3.8 Schematic formulation of CS/TPP NPs

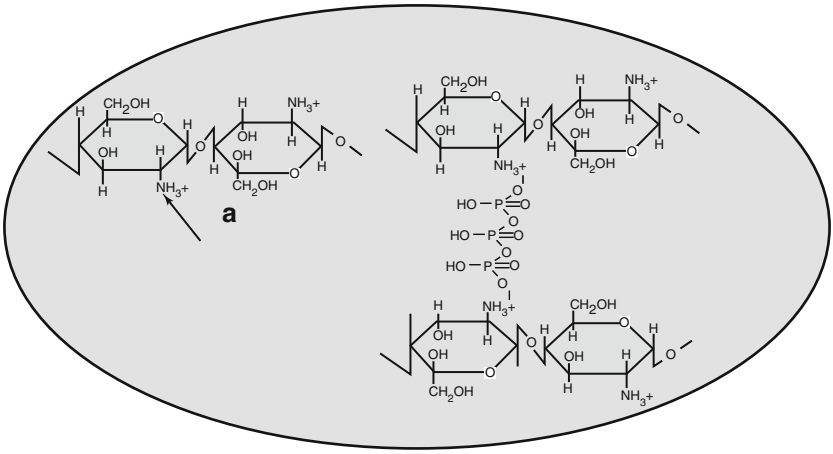

Fig. 3.9 Crosslinking of chitosan by tripolyphosphate

(97 %), zearalenone (94 %) and fumonisin B_1 (99 %), but no obvious adsorption for deoxynivalenol and T-2 toxin (<30 %). The results showed that the coexisted multiple mycotoxins didn't exaggerated the adsorption capability of adsorbent, whereas the adsorption amounts of toxins were declined by some gastrointestinal components. The conclusion of this research proposed that chitosan–glutaraldehyde complex has the potential to be applied as multitoxin adsorbent material for reducing the combined adverse effect of multiple mycotoxins on humans and animals. Additionally these NPs merged as potential carrier for various other applications mentioned in Table 3.7.

Table 3.7 Crosslinked chitosan NPs and its applications

Crosslinked chitosan nanoparticle	Applications	Ref.
Crosslinked chitosan NPs	For delivery from pressurized metered dose inhalers	[239]
Crosslinked-chitosan NPs	Competitive biosorption of azo dyes from aqueous solution	[240]
Chitosan nanoparticles crosslinked by glycidoxy-propyltrimethoxysilane	For pH triggered release of protein	[241]
Ionically crosslinked chitosan/ tripolyphosphate NPs	For oligonucleotide and plasmid DNA delivery	[242]
Chitosan-g-PEG NPs ionically crosslinked with poly(glutamic acid) and tripolyphosphate	As protein delivery systems	[243]
Cross-linked chitosan NPs	Serum production against *Tityus serrulatus* scorpion venom using (chitosan act as immunoadjuvant)	[244]

3.7.20 Solid Lipid Nanoparticles (SLNPs)

Solid lipid nanoparticles are at the vanguard of the rapidly rising field of nanotechnology with a number of potential applications in drug delivery, clinical medicine and research, as well as in other various sciences. Solid lipid nanoparticles (SLN) commenced in 1991 represent an alternative delivery system to traditional colloidal carriers, e.g. liposomes, emulsions and polymeric micro- and nanoparticles. SLN combine merits of the traditional systems however evade some of their major disadvantages. Owing to their unique size-dependent properties, lipid nanoparticles present the option to develop new therapeutics. The capacity to incorporate drugs into nanocarriers presents a new example in drug delivery that could be used for secondary and tertiary levels of drug targeting. Therefore, solid lipid nanoparticles offer guarantee for reaching the goal of controlled and site specific drug delivery and thus have involved wide attention of researchers. Different types of chitosan based SLNPs emerged as potential carrier in drug delivery and for other applications. Sarmento et al. demonstrated the influence of chitosan coating in overcoming the phagocytosis of insulin loaded solid lipid NPs by mononuclear phagocyte system [245]. Ridolfi et al. explored the chitosan SLNPs as carriers for topical delivery of tretinoin [246]. Similarly Fonte et al. explored chitosan-coated SLNPs for insulin delivery [247]. Xiao-Ying et al., demonstrated SLNPs modified with chitosan oligosaccharides for the controlled release of doxorubicin [248].

3.7.21 Synthetic Nanoparticle: Chitosan B-Cyclodextrin NPs

β-cyclodextrin conjugated chitosan NPs emerged as novel carrier system for the delivery of various drugs. Number of fabrication methods has been reported by various researchers. Maestrelli et al. demonstrated a novel drug nanocarrier consisting of chitosan and hydoxypropylcyclodextrin [249]. Similarly Jingou et al. suggested that

Table 3.8 Chitosan β-cyclodextrin NPs and its applications

Chitosan β-cyclodextrin NPs	Applications	Ref.
Cationic β-cyclodextrin polymers from alginate/chitosan NPs	Effective protection and controlled release of insulin	[251]
magnetic β-cyclodextrin–chitosan NPs	As nano-adsorbents for removal of methyl blue	[252]
Fabrication of magnetic chitosan NPs grafted with β-cyclodextrin	As effective adsorbents toward hydroquinol	[253]
Hydroxypropyl-β-cyclodextrin inclusion complexes loaded chitosan NPs	For delivery of ranitidine hydrochloride and furosemide across a Caco-2 cell monolayer	[254]
β-cyclodextrin incorporated multiwalled carbon nanotube and gold NPs-polyamide amine dendrimer nanocomposites combining with water-soluble chitosan derivative	A molecularly imprinted sensor for the detection of chlortetracycline	[255]
Methyl-β-cyclodextrin/poly(isobutyl-cyanoacrylate) NPs coated with thiolated chitosan	Intestinal permeation enhancement of docetaxel	[256]
β-cyclodextrin modified chitosan–poly(acrylic acid) nanoparticles	Use as drug carriers	[257]
Chitosan and chitosan/cyclodextrin NPs	As potential carriers for the oral delivery of small peptides	[258]
Chitosan/cyclodextrin NPs	As macromolecular drug delivery system	[259]
Chitosan/sulfobutylether-β-cyclodextrin NPs	Ocular drug delivery	[260]
Warfarin-β-cyclodextrin loaded chitosan NPs	For transdermal delivery	[261]
Chitosan/cyclodextrin NPs	For efficiently transfection of the airway epithelium in vitro	[262]

fabrication, characterization of hydrophilic and hydrophobic drug in combine loaded chitosan/cyclodextrin nanoparticles [250]. Various applications of chitosan β-cyclodextrin NPs are mentioned in Table 3.8.

3.7.22 Lecithin Polymer Conjugates

Lecithin is a mixture of phospholipids with phosphatidylcholine as a main component (up to 98 % w/w). Egg or soy lecithin as well as purified phospholipids is used for pharmaceutical purposes as components of liposomes, mixed micelles, and submicron emulsions. Aqueous lecithin dispersion (water lecithin-dispersion) is a system obtained by dispersing lecithin in water or in an isotonic aqueous solution with means of extensive mixing at temperature 40–60 °C in order to obtain good hydration of lecithin. Neither special manufacturing procedure nor additional lipids and surfactants are used. Lecithin/chitosan NPs appeared as more reliable targeted delivery system in current research. Sonvico et al. fabrication of self-organized NPs by lecithin/chitosan ionic interaction [263]. Hafner et al. demonstrated melatonin-loaded lecithin/chitosan NPs and their permeability via Caco-2 cell monolayers [264]. Şenyiğit et al. explored the lecithin/chitosan NPs of clobetasol-17-propionate which is capable of accumulation in pig skin [265].

3.7.23 Glycosylated Chitosan Based NPs

Glycosylated chitosan based NPs appeared as therapeutic NPs having more specific, improved and targeted applications e.g. Makhlof et al. reported the role of glycol chitosan NPs and its thiolated derivative in the pulmonary delivery of calcitonin. It was observed that these fabricated NPs significantly improved the release of calcitonin [266]. Similarly Quiñones et al. reported the role of self-assembled NPs of glycol chitosan in controlled release of ergocalciferol succinate conjugate [267].

3.7.24 Galactosylated Chitosan Based NPs

Galactosylated chitosan NPs emerged as potential carrier for gene, protein and other biomolecules. Alex et al. reported spermine grafted galactosylated chitosan for improved NP mediated gene delivery [268]. Similarly Jiang et al. [269] demonstrated the role of galactosylated chitosan/tripolyphosphate NPs as a gene carrier for targeting SMMC7721 cells. Wang et al. synthesized norcantharidin-associated galactosylated chitosan NPs for hepatocyte-targeted delivery [270]. Zheng, et al. explored the role of galactosylated chitosan NPs for hepatocyte-targeted delivery of oridonin [271]. Galactosylated chitosan/DNA nanoparticles were fabricated using water-soluble chitosan as a gene carrier [272].

3.7.25 Phytochemicals Based Chitosan Nanoparticles

Natural products carry enormous therapeutic potential with considerable safety. Chitosan has been recently used for delivery of various phytochemicals. Various forms of chitosan based drug delivery systems have been explored in which chitosan act as carrier and phytochemicals act as model drug. These carrier and model drugs are often utilized in form of nanoparticles for cell, tissue or organ specific delivery. Various examples of other phytochemicals and their applications are mentioned in Table 3.9.

3.7.26 Glycosylated Chitosan Nanoparticles: siRNA Chitosan Conjugate

Chitosan/siRNA NPs have profound application in gene silencing, especially in cancer/malignant disorders. Holzerny et al. investigated the biophysical properties of chitosan/siRNA polyplexes. In this study he has demonstrated the profiling the polymer/siRNA interactions and its bioactivity [283]. Various applications of chitosan/siRNA nanoparticle are highlighted in Table 3.10

Table 3.9 Phyto-chitosan NPs and its applications

Phyto-chitosan NPs	Applications	References
Water soluble O-carboxymethyl chitosan nanocarrier	For the delivery of curcumin to cancer cells	[273]
Carboxymethyl chitosan carrying ricinoleic NPs	As an emulsifier for azadirachtin	[274]
Nano-hybrid carboxymethyl-hexanoyl chitosan modified with (3-aminopropyl)triethoxysilane	For camptothecin delivery	[275]
Curcumin-loaded chitosan/poly(butyl cyanoacrylate)nanoparticles	In vitro/in vivo anti-cancer treatment	[276]
Co-encapsulated doxorubicin and curcumin in chitosan/poly(butyl cyanoacrylate) NPs	Reversion of multidrug resistance	[277]
Chitosan-coated NPs loaded with curcumin	For mucoadhesive applications	[278]
Curcumin loaded dextran sulphate–chitosan NPs	Delivery of curcumin	[279]
Chitosan NPs	Oral delivery of curcumin to cure mice from Plasmodium yoelii	[280]
Encapsulation of curcumin in alginate-chitosan-pluronic composite NPs	For delivery of curcumin to cancer cells	[281]
Curcumin encapsulated in chitosan NPs	Treatment of arsenic toxicity	[282]

Table 3.10 Applications of chitosan/siRNA NPs

Chitosan/siRNA nanoparticle	Applications	Ref.
Chitosan/VEGF-siRNA nanoparticle	For gene silencing	[284]
chitosan/siRNA nanoparticle formulation	Gene silencing	[285]
Oligoarginine-modified chitosan	For siRNA delivery	[286]
Chitosan as a carrier	For targeted delivery of small interfering RNA	[287]
		[283]
Chitosan/DsiRNA nanoparticles Original	Intraperitoneal administration of NPs targeting TNFα prevents radiation-induced fibrosis	[288]
Chitosan nanoparticles	For siRNA delivery	[289]
Chitosan/polyguluronate nanoparticles	For siRNA delivery	[290]
Tumor-homing glycol chitosan/ polyethylenimine nanoparticles	For the systemic delivery of siRNA in tumor-bearing mice	[291]
siRNA chitosan nanoparticles:	Gene silencing	[292]
siRNA/chitosan-g-deoxycholic acid polyplexes loaded within biodegradable polymer nanoparticles	Prolonged gene silencing	[293]

3.7.27 Chitosan Based Microencapsulated NPs

Recent research has explored various chitosan based microencapsulated nanoparticles for circumventing various problems of conventional delivery systems. One beautiful example of chitosan-alginate PEC microcapsules by membrane emulsification method is mentioned in Fig. 3.10.

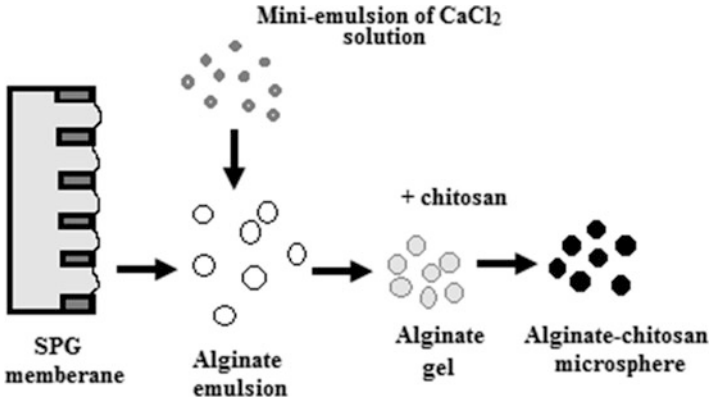

Fig. 3.10 Plan representation of chitosan-alginate PEC microcapsules by membrane emulsification method

Table 3.11 Applications of chitosan based microencapsulated NPs

Microencapsulated chitosan NPs	Applications	Ref
O-carboxymethyl chitosan nanoparticles	Delivery of tetracycline against intracellular infections of Staphylococcus aureus	[294]
Chitosan NPs	Delivery of ascorbyl palmitate	[295]
Chitosan NPs	Delivery of (Inhibitory effects) of trolox on tert-butyl hydroperoxide induced RAW264.7 apoptosis	[296]
Chitosan nanoparticles	For pulmonary protein delivery (insulin-loaded formulations)	[297]
siRNA/chitosan-g-deoxycholic acid polyplexes loaded within biodegradable polymer NPs	Prolonged gene silencing	[293]
Microencapsulated chitosan NPs	For lung protein delivery	[298]
Chitosan NPs and microspheres	For the encapsulation of natural antioxidants extracted from Ilex paraguariensis	[299]

O-carboxymethyl chitosan nanoparticles were employed for the delivery of tetracycline against intracellular infections of Staphylococcus aureus. Similarly various applications have been explored which are mentioned in Table 3.11.

3.7.28 Chitosan Based Monodisperse Nanoparticles

Chitosan based monodisperse nanoparticles appeared as potential NPs in therapeutic research. Various fabrication methods have been explored for producing monodisperse nanoparticles e.g. fabrication of monodisperse chitosan nanoparticles by

ionic gelation method [300] Additionally these NPs are known for their active role in removal of ions e.g. Chang et al. reported the monodisperse chitosan-bound Fe3O4 magnetic nanoparticles for removal of Cu(II) ions [301]. Tran et al. explored antibacterial and antiproliferative activities of monodisperse chitosan-based silver nanoparticles [302]. Similarly Loh et al. studied cytotoxicity of monodispersed chitosan nanoparticles against the Caco-2 cells [303].

3.7.29 Improved Stable Conjugates

Nafee et al. explored significance of the colloidal stability of chitosan/PLGA NPs on their cytotoxicity profile. It has been observed that chitosan/PLGA NPs enhances the colloidal stability and induced potential cytotoxicity effects [304].

3.7.30 Chitosan Based Coreshell Nanoparticles

Several fabrication methods have been explored for the production of chitosan based coreshell nanoparticles. Haidar et al. suggested the protein release kinetics for core–shell hybrid NPs based on the layer-by-layer assembly of alginate and chitosan on liposomes [305]. Silva et al. demonstrated the simple route for the synthesis of core–shell chitosan–gold nanocomposites [306]. Chen et al. reported chitosan/β-lactoglobulin core–shell NPs as nutraceutical carriers [307]. Wen et al. explored the fabrication steps and characterization of quaternized carboxymethyl chitosan/poly(amidoamine) dendrimer core–shell NPs [308]. Fabrication of core–shell chitosan/PCL-PEG triblock copolymer nanoparticles with ABA and BAB morphologies and the influence of intraparticle interactions on physicochemical properties was investigated [309]. Calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core–shell nanoparticles was investigated under in vitro conditions and it was observed that these prepared NPs showed active binding towards calcium ions [310]. Further Inphonlek et al. reported the production of poly(methyl methacrylate) core/chitosan-mixed-polyethyleneimine shell nanoparticles and their significant antibacterial property [311]. Thiolated chitosan-pHEMA core-shell nanoparticles were fabricated and showed significant mucoadhesion and permeation enhancement under in vitro conditions [312]. Further Bravo-Osuna et al. demonstrated the tuning of shell and core characteristics of chitosan-decorated acrylic NPs and mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell NPs [313, 314]. Bravo-Osuna et al. also demonstrated specific permeability modulation of intestinal paracellular pathway by chitosan-poly(isobutylcyanoacrylate) core-shell NPs [315].

3.7.31 Chitosan Based Surface Modified Nanoparticles

Various surface modified chitosan based NPs were reported for their targeted delivery. Lin et al., demonstrated the role of glycyrrhizin surface-modified chitosan NPs for hepatocyte-targeted delivery [316].

3.7.32 Lipid Nanoparticles: Large Molecule Carrier Nanoparticle

Chitosan based lipid NPs act as large molecule carrier especially proteins/DNA. There are various reports available on the delivery of DNA/protein via Chitosan based lipid NPs. Khatri et al., explored plasmid DNA loaded chitosan NPs for nasal mucosal immunization against hepatitis B [317].

3.7.33 Chitosan Based Controlled Release Nanoparticles

Rejinold et al. has developed potential anticancer saponin loaded chitosan based nanoformulation for enhanced and sustain released and their cytotoxicity to cancer cell lines in vitro [318]. Among the vast applications chitosan based nanoparticles were proved to be effective in strengthening HPMC film tensile properties and water vapor permeability. The thermal stability of the films was also increased with addition of nanoparticles as demonstrated by Moura et al. [319]. In vitro and in vivo experiment indicated that the drug-loaded CS-PAsp nanoparticles presented a sustained release of 5FU compared to the 5FU solution and the areas under curve (AUC) were increased by about four times as demonstrated by Zheng et al. [164]. Abreu et al. reported chitosan/cashew gum nanogels for essential oil encapsulation. In conjugation with cashew gum, chitosan nano gel can also be used in sustained delivery and for improving larvicide efficacies of essential oil Lippia sidoides [320]. Among the potential roles of chitosan in peptide drug delivery, the delivery of calcitonin as oral delivery system by surface modified lipid nanoparticles was independent of the surface modification or composition of nanoparticles as suggested by Garcia-Fuentes et al. [321]. Thiolated chitosan-thioglycolic acid (chitosan-TGA) (NPs) developed via ionic gelation with tripolyphosphate (TPP) have shown enhanced bioavailability of leuprolide against unmodified chitosan nanoparticles due to facilitated transport by thiolated NPs rather than improved release [322]. Shahnaz, et al. explored thiolated chitosan nanoparticles for the nasal administration of leuprolide. The green tea catechin (-)-epigallocatechin gallate (EGCG) has attracted significant research interest due to its beneficial therapeutic effects, which include anti-oxidant, neuro-protective and anti-cancer effects. However, the therapeutic potential of EGCG following oral consumption is limited by its poor absorption.

To address this issue, EGCG was encapsulated in chitosan-tripolyphosphate nanoparticles (CS NPs) and it was found that CS NPs may be a useful approach for enhancing oral delivery, and therapeutic application, of EGCG in a number of disease conditions [323].

3.7.34 Chitosan Based Bioadhesive Nanoparticles

Chitosan based bioadhesive nanoparticles emerged as potential tool in drug delivery system for delivery number of drugs or protein at targeted site. These NPs has wide applications in biomedical research. Amphiphilic chitosan derivative (SA-chitosan), water insoluble chitosan modified with salicylic acid (SA), was characterized and investigated for antiplatelet aggregation and adhesion properties. These NPs formed stable nanomicelles based on chitosan derivative and showed significant in vitro antiplatelet aggregation and adhesion properties [324]. Bioadhesive chitosan nanoparticles (CS NPs) were prepared and controlled release of catechin in GIT was studied. Bioadhesive catechin encapsulated chitosan nanoparticles have shown significant adhesion properties and better release profile in vitro conditions [325]. Mucoadhesive re-dispersible nanoparticles made of thiolated quaternary chitosan crosslinked with hyaluronan were reported better formulation for oral use for its fairly stable size and thiol content and showed a significant mucoadhesivity and easily internalised by endothelial progenitor cells in direct relation to their surface charge intensity [326]. There are many ongoing investigations to improve the oral bioavailability of peptide and protein formulations. Bioadhesive polysaccharide chitosan nanoparticles (CS-NPs) would seem to further enhance intestinal absorption of them. it has been proven that a bioadhesive polymer, chitosan have shown the improved intestinal absorption of insulin in vivo [327]. In addition chitosan is an ideal candidate for oral DNA delivery and DNA vaccination due to its mucoadhesive properties. Plapied et al. explored the bioadhesive NPs of fungal chitosan for oral DNA delivery [328]. Anti-HIV microbicide (tenofovir) loaded chitosan based nanoparticles were reported for no cytotoxicity and have shown the better mucoadhesive properties after decreasing the size of nanoparticles [329].

3.8 Targeted Applications

Chitosan has encouraged the movement for the development of safe and effective drug delivery because of its distinctive physicochemical and biological characteristics. Chitosan backbone constitutes primary hydroxyl and amine groups which allow its chemical modification to regulate its physical properties. After hydrophobic substitution it forms the resulting self-assembled amphiphilic nanoparticles that can encapsulate a quantity of drugs and deliver them to a specific site of action. Chemical binding of the drug to the chitosan through the functional linker may

produce useful prodrugs, exhibiting the appropriate biological activity at the target site. Enhancement of its mucoadhesive and absorption properties improves the bioavailability and increases the in vivo residence time of the dosage form in the gastrointestinal tract and drugs. Because of its excellent physicochemical properties chitosan is widely used for targeting of drugs in nano forms. Chitosan act as a excellent nanocarrier for targeting low molecular weight drugs [330]. Chitosan has shown diverse targeting strategies especially in cancerous cells. In recent studies it was well demonstrated that chitosan/polyoxometalates nano complexation will be applied as a novel anti-tumor formulation especially against cancers which are difficult to be treated clinically [331]. Cinnamaldehyde cross-linked chitosan nanoparticles were studied for enhanced delivery of baicalein for inducing apoptosis [332]. Chitosan–tripolyphosphate conjugated chloroquine nanoparticle was studied for the targeted attenuation against Plasmodium berghei infection in Swiss mice 3. Chitosan nanoparticles were also studied in targeting anti-depressant drugs in brain. It was reported that venlafaxine loaded chitosan nanoparticles enhanced the uptake of venlafaxine to brain via intranasal delivery and suggested as better formulation against mental depression 4. For targeting lungs chitosan was studied for its compatibility with respiratory epithelial cells in vitro condition. It was also studied for pulmonary administration of macromolecules through chitosan [333]. Chitosan polymeric nanoparticles efficiently circulate in the bloodstream for a prolonged period of time is often a prerequisite for successful targeted delivery. Paclitaxel loaded chitosan and polyethylene glycol coated PLGA (PLGA-CS-PEG) nanoparticles were formulated and characterized for this purpose. It was reported that a combinational coating of PEG and chitosan may represent a significant step in the development of long-circulating drug delivery carriers for tumor drug delivery [334]. In another study using an ionic cross-linkage process lactosyl-norcantharidin N-Trimethyl chitosan was synthesized for liver targeting in cancer therapy with high targeting efficacy [335]. For targeting tumours Chitosan acts by Electrostatic interaction. Enhanced electrostatic interaction between chitosan-modified paclitaxel-loaded PLGA nanoparticles and acidic microenvironment of tumor cells was studied as underlying mechanism of lung tumor-specific accumulation of paclitaxel from C-NPs-paclitaxel [336]. Conjugating chitosan with nucleoside (gemcitabine) and monoclonal antibody herceptin for targeting pancreatic cancer therapy was studied as superior antiproliferative activity along with an enhanced S-phase arrest, leading to apoptosis in comparison with unconjugated gemcitabine-loaded nanoparticles and free gemcitabine due to higher cellular binding with eventual uptake and prolonged intracellular retention [337]. Grafting studies of oleoyl onto the –NH2 at C-2 in the chitosan explored the new approach as drug carrier system to deliver drugs into the Adenocarcinomic human alveolar basal epithelial cells or human lung carcinoma cell line [338]. A novel folate conjugated carboxymethyl chitosan-ferroferric oxide doped cadmium telluride quantum dot nanoparticles reported as promising candidates for its high drug loading efficiency, low cytotoxicity and favorable cell compatibility, and carboxymethyl chitosan-based targeted drug delivery and cellular imaging [339]. This novel carrier opens new avenues for drug delivery which better meets the needs of anticancer research

3.8.1 Chitosan Bio-Targeted Applications

3.8.1.1 Ocular Delivery

Chitosan is widely used ocular application recently two drugs Dorzo and Prami were studied for their complexation with chitosan to control the release of both drugs. It was observed that Ocular administration of Dorzo in the form of mucoadhesive CS nanoparticles can be expected to increase the time of drug residence on eye surface, increasing drug bioavailability and prolonging the pharmacological effect whereas incorporation of Prami in a controlled release system, such as the CS nanoparticles, could provide for a more efficient control of drug levels in blood, reducing side-effects and, by decreasing the frequency of administration, could improve patient compliance [340].

3.8.1.2 Liver

Glycyrrhetinic acid-modified sulfated chitosan was reported as a good drug carrier and a potential vehicle for liver-cancer targeting [341]. It was also reported that Glycyrrhetinic acid-modified chitosan/poly(ethylene glycol) nanoparticles could effectively inhibit tumor growth in H22 cell-bearing mice and studied as good carrier for liver targeting [342]. Chitosan nanoparticles (CNP), a kind of widely used drug carrier, have shown potent cytotoxic effects on various tumour cell lines in vitro and in vivo. Antitumour effect of CNP on growth of human hepatocellular carcinoma (BEL7402) and the possible mechanisms involved was reported. Results showed a strong antitumour effect of CNP on human hepatoma cell line BEL7402 in vitro and in vivo. These findings suggested that CNP could be a kind of promising agent for further evaluations in the treatment of hepatocellular carcinoma [343]. Chitosan was reported for targeting hepatocytes. Superparamagnetic iron oxide nanocrystals loaded chitosan-linoleic acid magnetic nanoprobe as an MRI contrast agent was successfully studied to target hepatocytes for the diagnosis of hepatic diseases [344]. Despite extensive research into the biomedical and pharmaceutical applications of nanoparticles, and the liver being the main detoxifying organ in the human body, there are limited studies which delineate the hepatotoxicity of nanoparticles. Biological interactions between liver cells and chitosan nanoparticles, which have been widely recognised as biocompatible was studied by using MTT assay and it was studied that human liver cells were shown to tolerate up to 4 h of exposure to 0.5 % w/v of chitosan nanoparticles. At nanoparticle concentrations above 0.5 % w/v, cell membrane integrity was compromised as evidenced by leakage of alanine transaminase into the extracellular milieu, and there was a dose-dependent increase in CYP3A4 enzyme activity. Uptake of chitosan nanoparticles into the cell nucleus was observed after 4 h exposure with 1 % w/v of chitosan nanoparticles. Electron micrographs suggested the cell death, possibly caused by cell membrane damage and resultant enzyme leakage [345].

3.8.1.3 Cancer and Tumour

Hydrophobically modified glycol chitosan nanoparticles were prepared by introducing a hydrophobic molecule, cholanic acid, to water soluble glycol chitosan. These anticancer loaded nano-sized drug carriers were proved as promising nano-sized drug formulation for cancer therapy [346]. The poly (lactide-co-glycolide) (PLGA)-based nanoparticles, coated by the heparin- or chitosan-Pluronic conjugate, were used to improve a relatively low tumor-targeting efficiency of the bare PLGA nanoparticles. Therefore, it has been proven that the surface-functionalization by the chitosan- or heparin-conjugated Pluronic may be an effective approach for the hydrophobic nanoparticle systems aiming for the enhanced tumor imaging and therapy [347]. It was suggested that high molecular weight glycol chitosan nanoparticles remain for longer periods in the blood circulation, leading to increased accumulation at the tumor site. Accordingly it was proposed that enhanced tumor targeting by high molecular weight glycol chitosan nanoparticles is related to better in vivo stability, based on a pharmacokinetic improvement in blood circulation time [130]. Doxorubicin (DXR) commonly used in cancer therapy produces undesirable side effects such as cardiotoxicity. Encapsulation of the drug conjugate in biodegradable, biocompatible long circulating hydrogel nanoparticles, can further improved the therapeutic efficacy of the conjugate. It has been suggested that encapsulation of the dextran conjugated drug with chitosan in nanoparticles not only reduces the side effects, but also improves its therapeutic efficacy in the treatment of solid tumors [348]. Antitumor activity of all-trans retinoic acid (RA)-incorporated glycol chitosan (GC) nanoparticles was investigated and it was reported that RA-incorporated GC nanoparticles could be a promising vehicles for RA delivery to HuCC-T1 cholangiocarcinoma cells [349]. Chitosan nanoparticles have been synthesized as potential anticancer agents, and evaluated, in vitro, against various cancer cell lines. In vivo antitumor activity of chitosan nanoparticles against Sarcoma-180 and mouse hepatoma H22 was investigated earlier and it was observed that chitosan nanoparticles showed significant antitumor activity in vivo. The doses and particle size made a great effect on their efficacy [350]. Glutaraldehyde cross linked chitosan nanoparticles loaded with paclitaxel characterize against blank chitosan nanoparticles for the parameters such as loading efficiency, ph dependent mean diameter, drug release profile and cytotoxicity [351]. Sustained released paclitaxel-loaded chitosan nanoparticles with higher cell toxicity due to sudden arrest of G2-M phase cell cycle have revealed the potential uses of chitosan nanoparticles as anticancer drug carriers with enhanced anticancer effect [352]. N-succinyl-chitosan nanoparticles inhibited the proliferation of K562 by decreasing the zeta potential and disrupting the potential of mitochondrial membrane with increased ROS generation and Ca(2+) concentration. Further it was reported that N-succinyl-chitosan nanoparticles inhibit the K562 cells growth by necrosis and apoptosis induction [353]. Folic acid receptor (FR) is an important anti-cancer therapy target that is applicable to many cancer types. Folic acid conjugated hydroxypropyl-chitosan nanoparticles were prepared and targeted by using antisense oligodeoxy-nucleotides to reduce production of permeability glycoprotein, in order to overcome

tumor drug resistance [354]. Self assembled Glycosylated chitosan nanoparticles with different degrees of hydrophobic substitution were studied for tumor-targeting efficiency. Degree of hydrophobic substitution of self-assembled nanoparticles could determine their stability and deformability. Importantly, they were founded to be the key factors which affect their tumor-targeting efficiency in vivo, and so that these factors should be highly considered during developing nanoparticles for tumor-targeted imaging or drug delivery [355].

3.8.1.4 Brain

CS nanoparticles represent an interesting technological platform for Dopamine brain delivery and, hence, may be useful for Parkinson's disease treatment [356]. Anti-neuroexcitation peptide (ANEP) is a promising candidate for the treatment of neuroexcitation-associated diseases. TMC nanoparticles are potentially useful brain-targeting delivery systems for ANEP. It was reported that the targetability of ANEP to brain was significantly increased by TMC nanoparticles. Absorptive-mediated transcytosis was believed to be the main pathway for the brain-targeting of FITC-ANEP-TMC/NPs [357]. The estradiol(E(2))-loaded chitosan nanoparticles must be directly transported from the nasal cavity into the CSF and shown improved drug targeting index of nasal route for being transported into central nervous system (CNS) [358]. Chitosan was tested for brain targeting in the treatment and prevention of Alzheimer's disease. It was reported that rivastigmine (RHT) loaded chitosan nanoparticles have shown improved bioavailability, enhanced uptake of rivastigmine to the brain, better brain targeting efficiency and a promising approach for brain via intranasal delivery [359].

3.8.1.5 Imaging

Against cancer gadolinium neutron-capture therapy was recently modified by complexing gadolinium with chitosan to retard the growth of solid [360]. Gadolinium loaded chitosan nanoparticles for neutron-capture therapy was successfully reported for in vitro cellular accumulation of gadolinium [361].

3.8.1.6 GIT

Chitosan based oral vaccine specifically targets the follicle-associated epithelium region of Peyer's patch (PP) using M cell-homing peptide selected by phage display technique [362]. To fight against cancer, chitosan was studied for its conjugation with a green tea derived poorly soluble antioxidant compound Epigallocatechin gallate. Chitosan and casein phosphopeptides was assembled for improving the bioavailability of active ingredients, especially for compounds such as EGCG that is soluble in water but having low permeability in small intestine. This antioxidant

compound was targeted with the help of chitosan and a casein derived peptide and it was reported that this type of targeting has increased the intestinal permeability of Epigallocatechin gallate [363].

3.8.1.7 Lungs

Gemcitabine (2', 2'-difluorodeoxycytidine) is a deoxycytidine analog with significant antitumor activity against variety of cancers including non-small cell lung cancer. However, rapid metabolism and shorter half-life of drug mandate higher dose and frequent dosing schedule which subsequently results into higher toxicity. Chitosan/poly(ethylene glycol) is a vector which can reduce the burden of frequent dosing and higher toxicity associated with the use of gemcitabine. This study also reveal its sizeable compatibility, comparatively less organ toxicity and higher antitumor activity in vitro as well as in vivo [364]. Telomerase inhibiting chitosan/PLGA nanoparticles for the delivery of antisense 2'-O-methyl-RNA directed in lung cancer cells were studied as well tolerated, good binding efficiency, complex stability and high uptake when compared to 2OMR alone [365].

3.9 Miscellaneous Applications

3.9.1 Food Industry

Kim et al. has explored the potential application in food industry [366]. Browning has an important economic cost causing deterioration of the value of products in the food market. Enzymatic browning usually takes place in the presence of polyphenol oxidase and catechol oxidase which affects the overall cost of food product. Ascorbyl palmitate, a fat-soluble powerful antioxidant form of vitamin C, is approved for use as a food additive in the EU, USA and responsible for inhibition of polyphenol oxidase to prevent its associated browning effect. Recently it has been observed that Ascorbyl palmitate when nano encapsulated with chitosan showed improved polyphenol oxidase inhibitory activity chitosan when compared with no encapsulation. Mucoadhesive chitosan based films, incorporated with insulin loaded nanoparticles (NPs) made of poly(ethylene glycol)methyl ether-block-polylactide (PEG-b-PLA) has proved for increased encapsulation efficiencies and in vitro release with improved delivery of insulin was developed by Giovino et al. [367]. Hydrophobically modified glycol chitosan (HGC) NP have shown excellent deposition to the tumor site and non-destructive intracellular release. It has been suggested that HGC NP can be successful candidates for use as pulmonary delivery vehicles, owing to their excellent biocompatibility, transiency, and low pulmonary toxicity, and property of rapid elimination without accumulation [368]. Itraconazole was encapsulated into the chitosan:tripolyphosphate nanoparticles. The nanoparticles were spray dried in the presence of lactose, mannitol and/or leucine. Processing of nanoparticles with

mannitol and leucine improved the aerosolization properties of the drug significantly [369]. Gemcitabine is a known cytotoxic agent with a wide spectrum of antitumor activity [370]. It has been employed in therapeutic regimens for various malignancies such as the lung, ovary, breast, and bladder cancers. It also has been used in the treatment of pancreatic cancer, in combination chemotherapy of non-small cell lung cancer (NSCLC) and in leukemia. Its effect results from incorporation into DNA with subsequent inhibition of cell proliferation. Unfortunately, Gemcitabine is rapidly metabolized by the so-called cytidine-deaminase which limits its efficacy. Because of extensive deamination by intestinal cells, its oral administration results in very low bioavailability. Chitosan based oral nanoparticles of the drug have shown improved physicochemical properties such as particle size and shape, loading efficiency and release rate [370]. Eugenol encapsulation chitosan nanoparticles improve its thermal stability which suggested the possible use of eugenol-loaded chitosan nanoparticles as antioxidants in bioactive plastics for food packaging [371]. Catechins found in green tea have received considerable attention due to their favourable biological properties which include cardioprotective, neuroprotective and anti-cancer effects. However, their therapeutic potential is limited by their low oral bioavailability, attributed to poor stability and intestinal absorption. It was found that encapsulated (+)-catechin (C) and (−)-epigallocatechin gallate (EGCg) in chitosan nanoparticles (CS NP) enhances their intestinal absorption and is a promising strategy for improving their bioavailability [372]. Compared with micrometer chitosan (CS) particles, the adsorption performance of many drugs could be greatly improved with nanometer chitosan particles prepared by water-in-oil nanoemulsion system. The adsorption equilibrium of the diuretic furosemide on to nanoparticles was achieved much faster, and the adsorption loading highly increased in comparison with chitosan particles in micrometer size [373]. Carvacrol, or cymophenol is a monoterpenoid phenol which is widely used as food additive, antimicrobial agent, anticancer agent, quickly metabolized and excreted in the body and anti-inflammatory agent. Chitosan has the efficiency to control the release rate of carvacrol which was found to be superior in an acidic medium to either alkaline or neutral media, respectively [374]. Polymeric delivery systems based on nanoparticles (NP) have emerged as a promising approach for peroral insulin delivery. Trimethyl chitosan (TMC) and a PEG-graft-TMC copolymer, polyelectrolyte complexation and nanoparticles can be suggested as a potentially useful technique for generating insulin delivery systems for peroral administration [375]. Paclitaxel-loaded chitosan oligosaccharide (CSO) nanoparticles were prepared by interfacial polyaddition by fixing the molar ratio of ethylene glycol diglycidyl ether to chitosan oligosaccharide which leads to decrease in average size of nanoparticles, which further resulted in increased drug entrapment efficiency and decreased drug release rate efficiency [376]. Chitosan was proved as an excellent carrier for the drug delivery of anthracycline drug. It has been proven that complexation of chitosan with dextran sulphate doubled DOX encapsulation and loading efficient and proved to deliver the drug it into the cells in its active form [377]. Bulmer et al. demonstrated that fabrication of novel chitosan nanoparticles for controlled release of rHu-Erythropoietin [378]. Encapsulation of the glycoprotein recombinant human erythropoietin (rHu-EPO)

with tripolyphosphate chitosan shown controlled release over a long time [378]. In addition chitosan (CS) nanoparticles are promising system for simultaneously delivering hydrophilic drugs such as 5-fluorouracil and leucovorin in treatment of colon cancer [379]. Chitosan is one of the most promising polymers for drug delivery through the mucosal routes because of its polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties. Bile salts are known to interact with lipid membranes, increasing their permeability. The addition of bile salts to chitosan matrices may improve the delivery characteristics of the system, making it suitable for mucosal administration of bioactive substances. Low cytotoxic chitosan nanoparticles using sodium deoxycholate as a counter ion has proven as a new potential vehicle for mucosal delivery of pDNA [380]. Chitosan nanoparticles were investigated as delivery system for tacrine, a drug with potential significance in Alzheimer's disease. The preparation showed optimal pharmacokinetic characteristics in a rat model. Coating of nanoparticles with Polysorbate 80 has shown good drug-loading capacity, a continuous and slow diffusion-controlled release of the drug [381]. Retinol-encapsulated chitosan nanoparticles were prepared for application of cosmetic and pharmaceutical applications. This encapsulation was proved to be efficient and stable encapsulation and it has increased the solubility of retinol more than 1600-fold. Furthermore it was suggested that this type of encapsulation completely reconstituted into aqueous solution as same as original aqueous solution and zeta potential of reconstituted chitosan nanoparticles was similar to their original solution [382]. Aspirin (acetylsalicylic acid, ASA), a hydrophilic drug and probucol (PRO), a hydrophobic drug, are chosen as typical drugs, which are widely used to treat restenosis. Chitosan nanoparticles loaded with two different drugs simultaneously have shown continuous and controlled release with high encapsulation efficacy [383]. Various other applications are mentioned in Table 3.12.

Table 3.12 Controlled released Chitosan NPs and its applications

Controlled released NPs	Applications	Ref.
Levofloxacin-loaded Chitosan NPs	Levofloxacin delivery	[384]
Bone cement impregnated with chitosan NPs	Antibacterial and mechanical properties	[385]
Chitosan loaded NPs	Aspirin and probucol in combinational delivery	[383]
Chitosan nanoparticles loaded with gentamicin and salicylic acid	Combinational delivery gentamicin and salicylic acid	[386]
Tea catechins-loaded NPs prepared from chitosan and an edible polypeptide	Delivery of catechins	[387]
Peroral chitosan–insulin NPs in diabetic rats	Insulin delivery	[388]
Modified chitosan NPs	Oral insulin delivery	[389]
Low-molecular-weight chitosan NPs containing insulin	Insulin delivery	[390]
Alginate/chitosan NPs	Insulin delivery	[391]
Chitosan NPs	Naja naja oxiana snake venom delivery	[392]
Chitosan NPs	Delivery of drugs to the ocular surface	[393]

3.9.2 Immobilization

Chitosan matrix is known to be the superior matrix for immobilization of various biological organisms or its elements. Valerio et al. demonstrated high operational stability of invertase from Saccharomyces cerevisiae immobilized on chitosan nanoparticles [394]. Similarly Luo et al. explored electrochemically deposited chitosan hydrogel for horseradish peroxidase immobilization through gold nanoparticles self-assembly [395].

3.9.3 Chitosan as a Drug

In addition to its various applications in drug delivery or improving the physicochemical/biological property of drug, chitosan itself act as a potential drug. Zhang et al. explored hypolipidemic effects of chitosan NPs in hyperlipidemia rats induced by high fat diet. Similarly various antifungal effects of chitosan were also reported in several studies [396].

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Chapter 4
Advance Polymers and Its Applications

Abstract Recent advancements in polysaccharide chemistry led to the exploration of various potential derivatives which offers outstanding results as carrier in drug delivery system. Recent researches have explored various polysaccharides in different forms such as physically crosslinked hydrogels, smart polymers, auto-associative amphiphilic polysaccharides, supramolecular hydrogels, star polymers, ordered polysaccharides, interpenetrating polymer networks polysaccharide hydrogels, polysaccharide-based antibiofilm surfaces and polymers, and their complexes used as stabilizers for emulsions, having tremendous potential in biomedical research. This chapter covers these potential polysaccharides to augment their future applications in biomedical field.

Keywords Click reaction • Crosslinked hydrogels • Smart polymers • Amphiphilic polysaccharides • Supramolecular hydrogels • Star polymers • Ordered polysaccharides • Interpenetrating polymer networks • Antibiofilm

4.1 Introduction

Polymers, the most multipurpose category of materials, have altered our day-to-day lives over the past some decades. Nevertheless, the difference between temporary and permanent biomedical applications of polymers was made only 30 years ago. Afterward, the combination of polymer science with pharmaceutical sciences led to a quantum jump in terms of 'novelty' in design and development of novel drug-delivery systems (DDSs). Polymeric delivery systems are chiefly projected to complete either a temporal or spatial control of drug delivery. The beginning of the first synthetic polymer-based (polyglycolic acid) drug-delivery systems led to a sharp interest in the design and production of novel biodegradable polymers that obviated the requirement to eliminate the drug-delivery systems, unlike the nondegradable polymeric systems. Identifying that close call between a delivery system and an epithelial cell layer will advance the residence time as well as the efficiency of the drug-delivery systems resulted in the design of bioadhesive polymers. Additional advancements in polymer science result in 'smart' polymeric hydrogel systems that can self-regulate the administration of a therapeutic agent in response to a particular stimulus. The assortment and design of a polymer is a demanding task because of

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the intrinsic variety of structures and involves orderly consideration of the surface and bulk properties of the polymer that can give the desired chemical, interfacial, mechanical and biological functions. The selection of polymer, additionally to its physico-chemical features, is dependent on the requirement for wide biochemical characterization and specific preclinical assessments to establish its safety. Recently, Angelova and Hunkeler have anticipated a flow chart for coherent selection of polymers for biomedical applications. Surface properties like lubricity, smoothness, hydrophilicity and surface energy direct the biocompatibility with tissues and blood, moreover to affecting physical properties e.g. permeability, durability and degradability. The surface characteristics also decide the water sorption ability of the polymers, which experience hydrolytic degradation and swelling (hydrogels). In contrary, materials for long term use should be water-repellent to circumvent degradation or erosion procedures that results changes in modification and loss of mechanical strength. Surface features can be enhanced by chemical, physical and biological means to enhance their biocompatibility. Enzymes, drugs, proteins and antibodies attachment to the polymer surface has results in development of 'polymer therapeutics' for targeting to organs and cells. Mass features that required to be considered for controlled delivery systems consist of molecular weight, adhesion, solubility based on the release mechanism (diffusion- or dissolution-controlled), and its site of action. Bioadhesiveness require to be taken into consideration when drug-delivery systems are targeted to mucosal tissues, while polymers for ocular devices have to be aqueous or lipid-soluble in addition to having good film forming potential and mechanical stability for good retention. Considerable factors such as micromorphology and pore size are important with regard to mass transport into and out of the polymer. When nonbiodegradable matrices are considered, drug release in most events is diffusion-controlled and peptide drugs with low permeability can only be released via the pores and channels created by the dissolved drug phase. In relation to biodegradable polymers, it is important to identify that degradation is a chemical process, while erosion is a physical phenomena based on dissolution. Some of the factors that influenced the biodegradation of polymers:

- Chemical structure and composition
- Physico-chemical factors (ion exchange, ionic strength, pH)
- Physical factors (shape, size, chain defects)
- Morphology (amorphous, semicrystalline, crystalline, microstructure, residual stress)
- Mechanism of degradation (enzymatic, hydrolysis, microbial)
- Molecular-weight distribution
- Processing conditions and sterilization process
- Annealing and storage history
- Route of administration and site of action and diffusion process.

Based on the chemical structure of the polymer backbone, erosion can take place by either surface or bulk erosion. Surface erosion take place when the tempo of erosion surpasses the tempo of water permeation into the bulk of the polymer and is desirable since the kinetics of erosion and rate of drug release are extremely reproducible.

Bulk erosion takes place when water molecules infuse into the bulk of the matrix at a faster velocity than erosion, consequently displaying complex degradation/erosion kinetics. Majority of the biodegradable polymers used in drug delivery undergo bulk erosion. Nevertheless employment of nanoparticle or microparticle formulations acquiring massive surface areas results in bulk- and surface-eroding materials that display similar erosion kinetics.

Additionally, the erosion event can be influenced by altering the surface area of the drug-delivery systems or by encompassing hydrophobic monomer units in the polymer. Microstructural design and chemical profile can be used to acclimatize the structure–property relationship and modify improved polymeric matrices. A variety of polymer architectures and combinations of polymer species either physically mixed or chemically bonded present incredible scope as delivery systems. Though selection of polymers is a main concern, particularly with view to compatibility with the drug, the manufacturing procedure also requires to be considered, as the additives used throughout polymerization perhaps degrade the drug. Natural polymers are generally biodegradable and present admirable biocompatibility, however suffer from batch to batch variation because of difficulties in purification. Alternatively, synthetic polymers are obtainable in an extensive variety of compositions with readily adjustable properties.

4.2 Polymers and Their Physically Crosslinked Hydrogels by Freeze–Thaw Technique

The word hydrogel depicts 3-D network structures obtained from a category of synthetic and/or natural polymers which can take up and hold considerable amount of water or biological fluids [1–3]. The hydrogel structure is formed by the hydrophilic groups or domains present in a polymeric network upon the hydration in an aqueous environment. There has been growing attention in biopolymer hydrogels owing to their intrinsically desirable biocompatibility and degradability, ecological welcoming features and bioactivities. Hydrogels have broad possible applications in the fields of agriculture, biomaterials, water purification, food, etc. Presently, strong effects are contributed to synthesizing novel hydrogels for applications as biomaterials for tissue engineering [4, 5] drug delivery [6, 7], sensors [8], wound dressing [9, 10], purification [11, 12], and catalyst [13]. Based on protocol of crosslinking among the macromolecules in hydrogels, two major types can be divided by whether the crosslinking is chemically or physically based [14, 15]. Chemical crosslinking is confidently a highly adaptable method to produce hydrogels with agreeable and modified mechanical performances [16]. Nevertheless, the crosslinking agents utilized are frequently toxic compounds, which have to be extracted from the gels before used. Additionally, crosslinking agents can offer redundant reactions with the bioactive substances there in the hydrogel matrix. Employment of crosslinking agent or the continuation of reacting by products in ultimate hydrogels cannot be entirely ignored in the process of producing hydrogels by chemical reaction.

These will harm the biocompatibility and endow the hydrogels with threat in both short and long-term applications, particularly in biomedical characteristics [17]. These unpleasant effects are circumvented with the use of physically crosslinked gels. Physical hydrogels, particularly a number of based on natural biopolymers are excellent option and are considered to be capable materials with enormous potential applications in biomedical field as the gel development can be frequently executed under mild conditions and in the absence of organic solvents and toxically crosslinking agents [18]. Among these hydrogels, some hydrogels with significant potential of biomaterials is physical cryogel fabricated by freeze–thaw technique, particularly the gel based on polysaccharides, owing to their well recognized biocompatibility, low or non-toxicity and degradability under physiological conditions either enzymatically or chemically [19–22].

4.3 Smart Polymers: Controlled Delivery of Drugs

Smart polymers have vast potential in various applications. Especially, smart polymeric drug delivery systems have been discovered as "intellectual" delivery systems competent to release, at the suitable time and site of action, entrapped drugs in response to exact physiological causes. These polymers show a non-linear reaction to a small stimulus resulting in macroscopic modification in their structure/properties. The responses differ extensively from swelling/contraction to disintegration. Blend of new polymers and crosslinkers with better biocompatibility and improved biodegradability would augment and improve present applications. The most interesting characteristics of the smart polymers crop up from their multifunctioning and tunable sensitivity. The main considerable limitation of all these external stimuli-sensitive polymers is slow response time. The multi-functioning property of polymer sources and their combinatorial production manage it feasible to alter polymer sensitivity to a specified stimulus within a narrow range. Growth of smart polymer systems might results in more precise and programmable drug delivery. Pharmaceutical and biological therapeutics are frequently restricted by their poor bioavailability, short half-lives, and physical and chemical instability. Physical instability chiefly comprises modification of highly ordered protein structure, resulting in undesirable processes e.g. aggregation, denaturation, and precipitation. Reactions such as deamidation, oxidation, hydrolysis and racemisation contribute to the chemical instability of drugs. Stimuli-responsive polymers present a drug delivery stand that can be utilized to transport drugs at a controlled rate and in a stable and biologically vigorous form. From decades, attention in stimuli-responsive polymers has amplified and great deal of work has been dedicated to synthesizing environmentally sensitive macromolecules that can be moulded into new smart polymers (Fig. 4.1). List of several stimuli and smart polymers that can arbitrate such spectacular behavior mentioned in Table 4.1. Smart polymers are fetching significant applications in the fields of controlled drug delivery, biomedical applications, and tissue engineering, and it is frequently advantageous to utilize polymers

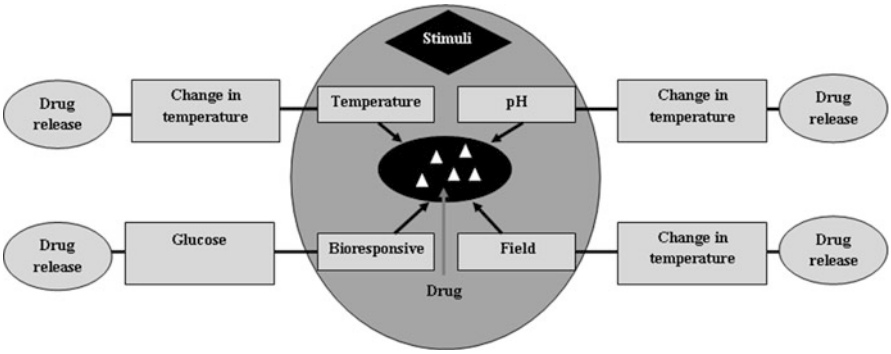

Fig. 4.1 Different stimuli responsible for regulating drug release from smart polymeric drug delivery systems [23]

Table 4.1 Different stimuli and responsive materials [23]

Environmental stimulus responsive material	Responsive material
Ultrasound	Ethylene vinyl acetate
Temperature	Poloxamers
	Poly(N-alkylacrylamide)s
	Poly(N-vinylcaprolactam)s
	Cellulose, xyloglucan
	Chitosan
pH	Poly(methacrylicacid)s
	Poly(vinylpyridine)s
	Poly(vinylimidazole)s
Light	Modified poly(acrylamide)s
Electric field	Sulfonated polystyrenes
	Poly(thiophene)s
	Poly(ethyloxazoline)

that can react to stimulus which are intrinsically present in natural systems. A variety of smart polymeric drug delivery systems are mentioned in Table 4.2.

A stimuli-sensitive or smart polymer experiences an abrupt change in its physical properties against any small environmental stimulus (Fig. 4.1). These polymers are also called as intelligent polymers since small changes takes place in response to an external stimuli until a critical point is attained, and they have the capability to return to their unique shape after elicit is removed. The uniqueness of these polymers present in their nonlinear response elicited by a very small stimulus and which generates a perceptible macroscopic modifications in their structure. Figure 4.1 illustrates different stimuli accountable for controlling drug release from smart polymeric drug delivery systems. These changes are reversible and entail changes in physical state, solvent interactions, shape and solubility, hydrophilic and lipophilic balances and conductivity. The driving forces following these transitions comprise

Table 4.2 Several smart polymeric drug delivery systems [23]

Stimulus	Advantage	Limitation
Ultrasound	Controllable protein release	Specialized equipment for controlling the release
		Surgical implantation required for nonbiodegradable delivery system
Temperature	Ease of incorporation of active moieties	Injectability issues under application conditions. Simple manufacturing and formulation
	Low mechanical strength, biocompatibility issues and instability of thermolabile	
	Drugs	
pH	Suitable for thermolabile drugs	Lack of toxicity data
		Low mechanical strength
Mechanical stress	Possibility to achieve the drug release	Difficulty in controlling the release profile
Light	Ease of controlling the trigger mechanism	Low mechanical strength of gel, chance of leaching out of noncovalently attached
	Accurate control over the stimulus chromophores	
Electric field	Pulsative release with changes in electric current	Surgical implantation required
		Need of an additional equipment for external application of stimulus
		Difficulty in optimising the magnitude of electric current

neutralisation of charged groups by the addition of oppositely charged polymers or by pH shift, and varying in the hydrophilic/lipophilic balance or changes in hydrogen bonding owing to increase or decrease in temperature. The main advantages of smart polymer-based drug delivery systems entails simplicity of preparation, reduced dosing frequency, maintenance of desired therapeutic concentration with single dose, sustained release of incorporated drug, reduced side effects and improved stability.

Blend of several responsivities is significant in following practically only in events where each responsivity straightly orthogonally stimulates the others in attainment the desired consequence or in case they influence each other in a preferred way (Fig. 4.2) [24]. In this fashion, amalgamation/blend inspired by viral capsid blending the pH + reductive + calcium(II)-chelation sensitivity seems to be a leading approach in intracellular active component administration (Fig. 4.2) [24].

4.4 Auto-Associative Amphiphilic Polysaccharides as Drug Delivery Systems

The hydrophilic chains of polysaccharides include various groups of diverse molecular weights and chemical compositions. The character of these groups can distinguish the polysaccharides from a structural point of view and results in different

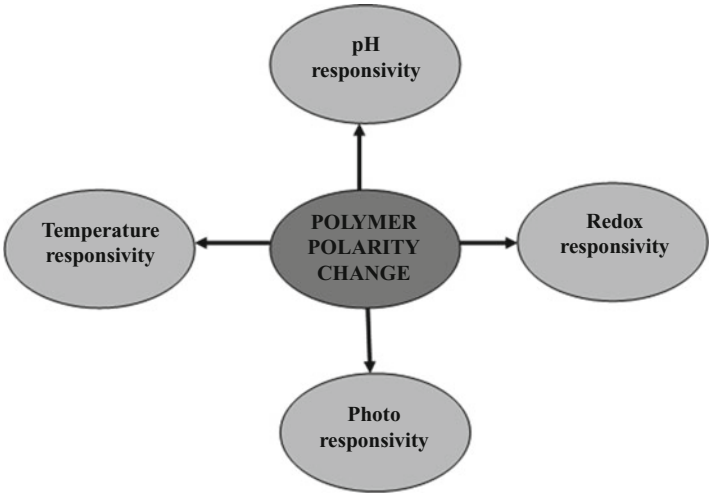

Fig. 4.2 Crosstalk among stimuli-responsivities

physicochemical and biological properties [18, 25]. A number of polysaccharides e.g. dextran and cyclodextrins have a neutral charge, others such as chitosan are positively charged. In conclusion, polysaccharides such as alginate, heparin, hyaluronic acid and pectin are negatively charged (Fig. 4.3). The polysaccharides can be linear, for an instance chitosan, dextran, and hyaluronic acid, or cyclic e.g. cyclodextrins. In recent times, there has been growing attention in the use of nanoparticles containing natural polysaccharides for drug delivery applications [26]. Nevertheless, in majority of the cases the requirement to introduce organic solvents (for emulsion solvent diffusion, emulsion evaporation, nanoprecipitation, interfacial polycondensation combined with spontaneous emulsification methods) and/or highly acidic pH alterations (e.g. for emulsion polymerization of alkyl cyanoacrylates) symbolizes a difficulty from a formulation point of view. To circumvent these limitations, the polysaccharides can be chemically modified by grafting hydrophobic groups. Due to intra and/or inter-molecular hydrophobic interactions, the amphiphilic polysaccharides can self-associate in aqueous solution resulting in diverse types of drug delivery systems e.g. microspheres [27], micelles, nanoparticles, liposomes [28–31] and hydrogels. Fundamentally, the structure of self-assembling polysaccharides can be selected on the basis of physicochemical properties of the drug to be loaded and the essential route of administration. By similarity with the event of micelle formation of small surfactants or lipids, aggregation of amphiphilic polymers is controlled by the balance between the interaction of the hydrophobic groups and the hydrophilic chains. The concentration at which the polymer aggregation initiates is typically known as the critical aggregation concentration (CAC). At relatively high polymer concentrations intermolecular relations of polymers are influence for the duration of the participation of hydrophobic groups, resulting in a astonishing improvement in solution viscosity. As a result, these forms

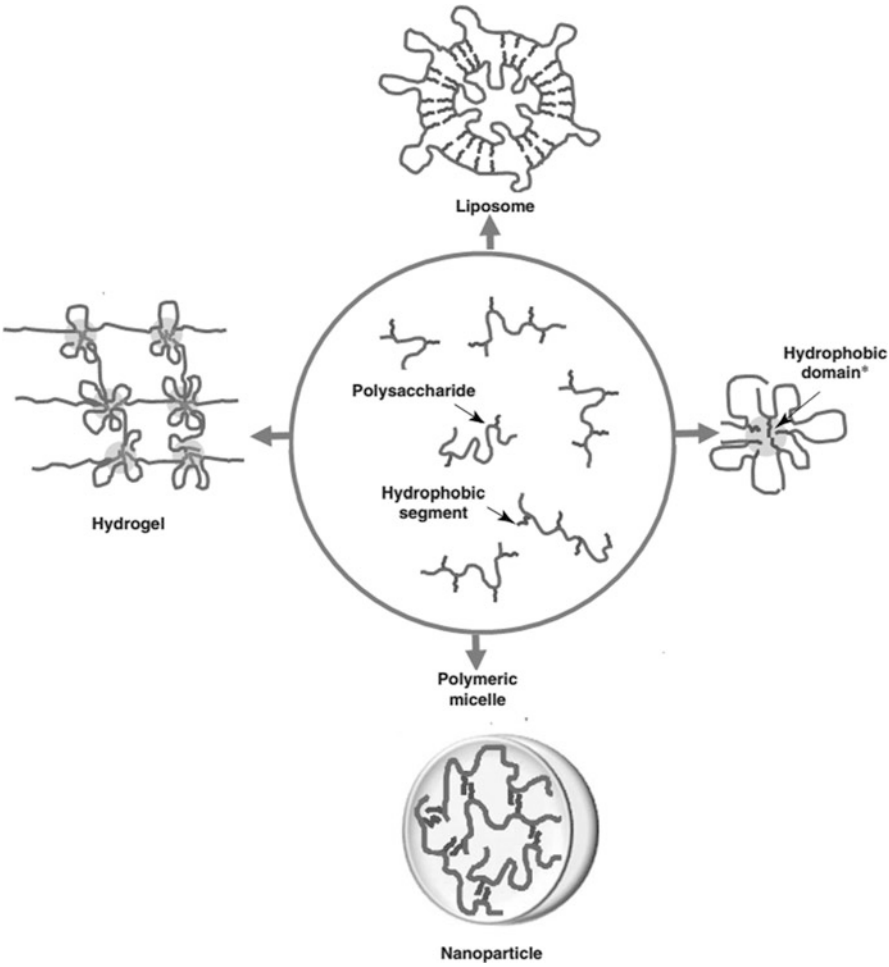

Fig. 4.3 Plan representation of various drug delivery systems formed by self-association of amphiphilic polysaccharides in aqueous solution (*Hydrophobic domain owing to the involvement of hydrophobic groups)

of polymers are called as associating polymers and are employed as thickeners to regulate solution viscosity. Phase separation or gelation can be experiential at elevated polymer concentrations. The hydrophobic core of these structures could be utilized to solubilize and encapsulate active ingredients with low solubility. Inside aqueous media, while the hydrophilic shell would adsorb hydrophilic molecules during non-covalent interactions.

4.5 Supramolecular Hydrogels: Potential Mode of Drug Delivery

Even though a low molecular mass gelator was explored in the early nineteenth century, the supramolecular nature of these materials was weakly understood and they were mainly ignored until the late twentieth century. In the latest history, vast structural diversity molecules, e.g. from the simplest alkanes to the complex phthalocyanines, have been explored to be gelators. Eventually, the exploration of such molecules has been mainly unexpected (normally from a unsuccessful crystallization effort!) [32]. Nevertheless, with the information achieved on the aggregation of gelator molecules while the past decade, efforts are being contributed to 'design' gelators through the integration of structural features e.g. H-bonding motifs such as amides, ureas and saccharides that are recognized to encourage one-dimensional aggregation. Gels of a low molecular mass compound are typically fabricated by heating the gelator in a suitable solvent and cooling the ensuing isotropic supersaturated solution to room temperature [32]. As the hot solution is cooled, the molecules begin to condense and three situations are possible:

- An extremely ordered aggregation giving rise to crystals, i.e., crystallization
- A arbitrary aggregation ensuing in an amorphous precipitate
- An aggregation process transitional between these two, yielding a gel.

The course of gelation encompass self-association of the gelator molecules to yield long, polymer-like fibrous aggregates, which get intertwined while the aggregation process yielding a matrix that traps the solvent primarily by surface tension. This process averts the stream of solvent under gravity and the mass appear like a solid. The matrix structure is assorted and superstructures ranging in size from nanometers to micrometers can be establish as a consequence of the hierarchal aggregation process [32]. At the microscopic level, the structures and morphologies of supramolecular gels have been studied by conventional imaging techniques e.g. TEM, SEM, and AFM, as thermal and mechanical investigations are employed to recognize the interactions between these structures. On the other hand, at the nanoscale, X-ray diffraction, small angle neutron scattering and X-ray scattering are necessary to explain the structures of supramolecular gels. Beside of all these studies, various features of the process by which gelators aggregate to yield gels are unsuccessfully understood and the course of gel formation leftovers as an area of powerful attention [32]. Nevertheless, in spite of the lack of a exhaustive information of the method of aggregation of gelators, or the structures of the aggregates, a extensive range of advanced applications have been predicted for these materials.

4.6 "Click" Reactions in Polysaccharide Modification

Polysaccharides (including cellulose, alginate, chitosan, hyaluronic acid, dextran and others) are amongst the most abundant natural polymers on globe. Polysaccharides and their modified derivatives are under wide study and at present used for applications such as biomedical materials [33–35], drug delivery [36, 37], coatings [38], and owing to the sustainability of biopolymers, the biological roles they exhibit, and also to the fact that the structure and properties of these biopolymers are readily modified. Chemical alteration is one imperative approach to modify polysaccharide structure and properties. By chemical alteration of uniformly dispersed polysaccharide molecules or on the surfaces of polysaccharide materials, derivatives bearing different functional groups and conjugates can be obtained. Alteration of polysaccharides also offers a range of derivatives that are capable of yielding particular architectures e.g. hydrogels [39], nanogels [40], and micelles [41]. From this stand point, chemical modification incorporates preferred features to the polysaccharide materials so that they meet up the necessity of definite applications. Conventional modification strategies usually entail esterification or etherification, captivating the benefit of the straightforwardness of the reactions and the relatively simple contact to many esterification and etherification reagents. Additional modification methods encompassing nucleophilic displacement reactions, oxidation, and (controlled) free radical polymerization have also been usually utilized. Positively these synthetic ways have distended the family of polysaccharide derivatives e.g. esterification of polysaccharides has contribute extraordinarily to cellulose and polysaccharide chemistry in the last few decades owing to the development of latest acylation methods and unconventional solvents [42]. Studies of regioselective reactions and protection/deprotection groups, in contrast, offers alternatives for regioselectively modified polysaccharide derivatives with well-controlled structures, and allows deeper knowledge of structure-property relationships of polysaccharide derivatives [43]. Whereas such methods are very functional and are still contributing to the survival of whole industries, they are limited in scope. In general esterification entails harsh reaction environment (e.g. strongly acidic catalysts) that are incompatible with sensitive functional groups on either polysaccharides or the acylation reagents. In the lack of protecting groups, simple esterification is also incompatible with difunctional reagents e.g. dicarboxylic acids or reagents with both carboxylic acid and hydroxyl groups, which could results in undesired crosslinking or uncontrolled polymerization [44]. The introduction of acyl activation reagents, e.g. N,N dicyclohexylcarbodiimide, has permitted the presentation of esterification under milder conditions, nevertheless this mild esterification is still not functional with difunctional reagents. Etherification usually entails powerfully basic conditions, and so is incompatible with base-sensitive moieties and mostly incompatible with difunctional reagents. Additionally, extended reaction times, dull steps, and modest yields are also at times linked with these conventional methods. The idea of "click chemistry" [45], first coined by Sharpless and his coworkers, has had a enormous influence on the chemistry community [46–48], comprehensive explanation of which is beyond the scope of this section. To meet the requirements as "click" chemistry, a reaction must accomplish most, if not all, of the necessities listed below:

- Reaction must be modular and wide in scope
- Offer very high yields
- Produce only inoffensive and easily removable byproducts
- Reaction must be stereo specific; and
- The process should also accomplish “simple reaction conditions and product isolation, readily available reagents, and the use of no solvent or benign ones”.

The idea of modular, simplistic and economical synthesis of structurally and functionally different molecules has also drawn consideration from polysaccharide chemists, and has brought considerable development to this field. In addition to “click” reactions, however they also can quickly synthesize molecules with diverse functional addition. In this section, we have emphasized on some of the most commonly used “click” reactions in polysaccharide chemistry, encompassing the well known

- Azide–alkyne Huisgen cycloaddition
- Diels–Alder reaction, thiol-ene
- Thiol-Michael reactions
- Theoxime click reaction

As several novel reactions satisfying the criteria of click chemistry have been exposed in the last decade, we also mention the introduction of these new click reactions to the ground of polysaccharide chemistry, encompassing two metal-free [3 + 2] cycloaddition reactions, and the inverse electron-demand Diels–Alder reaction. In this section we emphasized on the chemistry of the reactions, and try to offer to readers a guide for executing such reactions. Simultaneously, the extremely diverse and controllable structures and functionalities of the click reaction products are established. Various types of click reactions involved are illustrated in Fig. 4.4. In addition several examples of alkynes and azides generally used to functionalized polysaccharides are mentioned in Table 4.3.

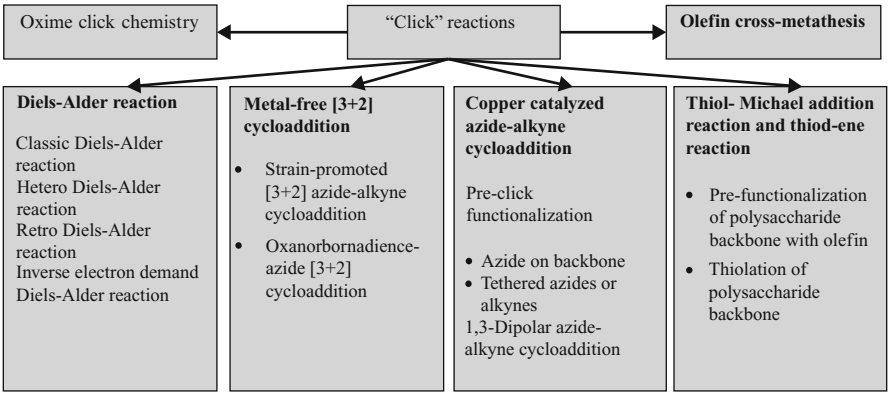

Fig. 4.4 Types of “Click” reactions in polysaccharide modification

Table 4.3 Alkynes and azides commonly used to functionalize polysaccharides

Reagent structure	General reaction condition	Polysaccharides
Propargyl halide	Base (NaH, NaOH), 40–50 °C, 72–96 h	Cellulose [49–51], chitosan [52], starch, [53]
Propargyl amine	80 °C, 24 h in DMSO	Cellulose (6-tosyl, cellulose) [54]
5-Hexynoic acid	(S)-tartaric acid, 110 °C, 6 h	Cellulose [55]
Pent-4-ynoic acid	EDC/NHS, r.t., 16 h	Chitosan [56]
Pent-4-ynoic acid	EDC/NHS, r.t.,	16 h Chitosan [56]
Propargyl alcohol	CDI	Dextran [57]
Propargyl	3-succinate, EDC/HOBta, r.t.,	24 h Chitosan [58]
1-Azido-2,3-epoxypropane	Base (NaOH), 30 °C, ~20 h	Cellulose [59], dextran [60]
4-Azidomethylene benzoic anhydride	TEAb/DMAPc, r.t., overnight	Cellulose surface [61]
6-Azidohexanoic acid	CDI	Dextran [62]
3-Azido-1-propanol	CDI	Dextran [46]

4.7 Star Polymers: Advances in Biomedical Applications

Star polymers have multifaceted macromolecular structural designs with at least three macromolecular chains(arms) radiating from one central core (atom, small molecule, branched macromolecule, nanogel, nanoparticle, etc.) [63–69]. They have been extensively considered because of their distinctive topological structures and smart physical/chemical properties. They also demonstrate exclusive hydrodynamic volumes and encapsulation capabilities owing to their three-dimensional globular structures. In contrast with linear analogs with the same molecular weight, star polymers possess lower solution viscosity in dilute solutions because of fewer arm entanglements [70, 71]. Additionally, their internal and peripheral active groups offer a suitable pathway of commencing various important functionalities. Moreover, star polymers display superior stimuli-responsiveness because of their high density of functionalities. Although equivalent to the 3D globular structure of dendritic polymers, in contrast with the typical branched arms and dense shell of dendritic polymers [72–74], star polymers show linear arms, reducing shell density with growing arm length and leading in differences in features e.g. viscosity and flexibility. Star polymers can be conveniently fabricated because of their comparatively simple linear arms and highest limited arm length, ensuing in comparatively low steric hindrance from the condensed dense shell [75–77]. Consequently, owing to these better physical/chemical properties, researchers have paid attention on star polymers to design different advanced materials for biomedical applications, including diagnosis, drug/gene delivery, antibacterial/antifouling coatings and implanted medical devices [78, 79]. Star polymers are usually categorized into two types:

- Regular star polymer
- Miktoarm star polymer

Regular star polymers have the same arm segments, including homopolymer arms and block copolymer arms, while the miktoarm star polymers can be further subdivided into a number of types depending on their asymmetric architectures involving different topologies, chemical structures, MWs, and functional groups [80]. With the merits of both star topology and block structure, miktoarm star polymers are anticipated to be involved in the fabrication of new morphological nanostructures and supramolecular assemblies for biomedical applications [81–86]. Whereas the advantages of miktoarm star polymers can be explored, it should be noted in the interim that it is not easy to prepare well-defined structures with various accurately designed arms because of the firm necessities of the complex production and purification protocols. The production of star polymers is frequently accomplished by three strategies:

- Core-first
- Coupling-onto
- Arm-firs

In the "core-first" approach, arms are directly developed from a multifunctional initiating core. Additionally, the initiating sites of formerly fabricated star polymers can be additionally used for the synthesis of another segment to form star block copolymers. This approach permits the synthesis of star polymers with a accurate number of arms by modifying the number of functionalities of the initiating core. The "coupling onto" method encompasses preformed linear arms with end functional groups conjugated onto a multifunctional core during efficient coupling reactions e.g. as one of the representative reactions employee in the "coupling-onto" strategy, "click" reactions have merits in the synthesis of miktoarm star polymers with distinct structures and high yield because of the high coupling efficiency. In the "arm-first" approach, the distinct preformed linear arm is produced by end-joining via covalently binding with a convinced amount of cross-linking agent to yield the core. This approach explores the benefit of the accurate control of arm length for the production of miktoarm star polymers. In recent times, Aoshima et al. explored a very remarkable domino synthesis approach to synthesize star polymers in one step by means of monomer-selective living cationic polymerization, which was based on the various reactivities of monomers. Outcomes showed that star polymers could be effectively derived with a very narrow molecular weight distribution. Modern advances in star polymers have involved considerable attention for their extensive variety of applications, especially for biomedical use.

4.8 Ordered Polysaccharides: Stable Drug Carriers

Discovery and development of drug are difficult, laborious and luxurious endeavors. High throughput screening procedures constantly augment a range of novel molecules; nevertheless, most of them not pass as potential drugs owing to, in part, poor pharmacokinetics. If successful, the method by which the drugs are transported to

the target site has a controlling effect on their bioavailability and as a result on the therapeutic effectiveness. Majority of the drugs are administered through oral route. These conventional delivery strategies depend mainly on the dissolution and stability properties inherent to the active pharmaceutical ingredient and alterations possible through the integration of pharmaceutically inactive components, i.e. excipients. Therefore, there is a documented unfilled requirement for the design and exploitation of novel pharmaceutical materials, particularly composites of recognized and reproducible structures made from active pharmaceutical ingredients and functional excipients. Certain drugs have a narrow therapeutic index in which the utmost advantage can be resulting and outside the therapeutic window it is toxic if too high or unsuccessful if too low. Incidentally, there is a increasing attention to formulate delivery systems with predefined systemic contact level, specific rate and time intervals. Such stimulus, united with the complication of recently developed drug molecules and associated elevated costs, requires the design and invention of optimal delivery vehicles. Synthetic and biodegradable polymers voluntarily serve the purpose [87, 88]. However, the potential side effects and toxicity force restrictions on satisfactory dose formulations and necessitates the hunt of novel carrier systems that are safe and cost effective. Bio-macromolecules are viable substitutes [88, 89] but many of them deficient in organized networks and stable molecular architecture, which prevent their extensive deployment. Natural polysaccharides such as cellulose, chitin and starch attain semi-crystalline association during their biosynthesis. On the contrary, polysaccharides consistently engaged in food, pharmaceutical and medicinal applications as thickening and gelling agents [90] do not acquire ordered networks. On the other hand, they can be coaxed, under appropriate experimental conditions, to yield extended fibers having sturdy molecular and packing structures e.g., systematic studies on iota-carrageenan, an anionic sulfated seaweed, has exposed that well-oriented and crystalline fibers can be fabricated by sensibly choosing polysaccharide concentration, salt amount and relative humidity [91–95]. Three-dimensional structure investigation demonstrates that it implements a threefold, parallel and reasonably rigid double helical structure with flexible peripheral sulfate groups. The negative charges prevent iota-carrageenan involvement owing to repulsion but cations encourage inter-helical interactions. This procedure leads to well-orchestrated hexagonal network having 8–15 A ° wide web's instilled with water molecules [91–95]. These structural pockets are intrinsic characteristic of the crystalline iota-carrageenan fibers and are of similar size of various drug molecules, and therefore can be favorably employed as molecular cavities for entrapping molecules of interest, as illustrated in Fig. 4.5. On the whole, approach of this section is regarding inclusion of host drug molecules into the crystalline biopolymer networks, and the ensuing API-biopolymer composites are similar to co-crystals observed in the case of small molecules.

Materials from renewable resources are drawing a increasing interest in a different fields as a way to attain sustainable advancement [96]. Pharmaceutical technology is not an exception and natural source excipients are getting better places against the synthetic materials [97]. The strong progress of organic chemistry and polymer science allow synthetic excipients to swiftly engage an wonderful place in the list

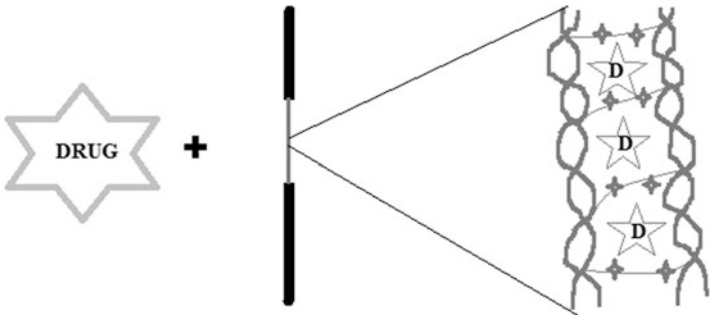

Fig. 4.5 Graphic encapsulation of drug molecules in the polysaccharide fibers. The drug molecules are firmly enclosed between a pair of helices, analyzed normal to helix-axis, and get protection from external factors

of accessible materials. Accurate control of molecular weight and number and distribution of functional groups and the adaptability to create chains of various architecture, and therefore presentation, are the main merits of the production methods. Nevertheless, pollution due to toxic solvents and starting components and surroundings issues associated to the accumulation of non-biodegradable plastics in the soil and the sea have become significant disadvantages [98]. Fascinatingly, the developments in the purification and the characterization techniques applied for the synthetic materials are robustly profiting the standardization of natural products. Additionally, the present state-of-art in biotechnology create it even potential to genetically engineer the sequences of the components of biomacromolecules [99]. All these progresses are particularly prompting the accessibility of optimized and well characterized natural excipients; the two characteristics that had been earlier limited their applications in the pharmaceutical field [100]. It should be observed that inadequate information about the composition and structure limits not only the functionality, but also could compromise the protection and can results undistinguished regulatory difficulties [101]. Possibly one of the materials that are taking more advantage from these developments is the polysaccharide family. Polysaccharides can be derived from various sources including seaweeds, fungi, insects, crustacea, animals, plants, bacteria and also humans, and can be structurally adjusted through genetic engineering [18, 102]. In number of cases, the natural sources can be widely cultivated to extract the polysaccharides. In further cases, the source is involved in the obtaining of other products and the by-products can be employed to extract the polysaccharide. In actual fact polysaccharide-containing materials symbolize an appropriate part of the by-products of the fishery and the agriculture activity [103]. Therefore, the hunt of pharmaceutical and biomedical applications for materials coming from by-products may give an added advantage to the source and contribute to solve the problem of unexploited waste accumulation [104]. The majority polysaccharides are effectively biocompatible since the similarity of their structure with many body components. This information jointly with their process ability using universal pharmaceutical equipment validate their

widespread use as binders, fillers, and thickeners in solid and liquid formulations and as components for site-specific oral delivery systems. In addition, they are voluntarily degradable in soil and water by common microorganisms. From a chemical standpoint, the polysaccharide phrase meet together relatively different large carbohydrates that can be composed of only one type of repeating monosaccharide or created by two or more monomeric units. Polysaccharides can be also categorized as non-polyelectrolites and polyelectrolites, which in turn are divided in positively and negatively charged [25]. The arrangement of the polysaccharides chains is particularly reliant not only on the pH and ionic strength of the medium, predominantly in the case of the polyelectrolytes, however also on the temperature and the concentration of definite molecules e.g. lecithins. Such responsive conformation can be explored to activate phase transitions of isolated and crosslinked chains by the act of chemical or physical stimuli. The stimulus can openly act on the polysaccharide, however also through an agent that transduces the signal [105]. The assortment of stimuli to which the polysaccharides can react creates them predominantly smart as components of smart drug delivery systems. In a different way from non-responsive drug delivery systems envisage to discharge the drug according to a pre-established outline, smart or intelligent drug delivery systems present the possibility of particularly releasing the drug in the precious tissues or cells, and/or to adapt the release profiles to the development of the illness or to certain physiological events. As an extra unique characteristic, a number of polysaccharides may provide nanocarriers with surface properties that control the interactions with the key components they will come in into contact during absorption and biodistribution, specifically blood, mucosa and target cells. Biomimicking the surface of eukaryotic cells, bacteria and viruses, polysaccharides can allow the identification and binding to desirable surfaces, while scaping from opsonization and complement activation [106]. Therefore, addition of the polysaccharide characteristics in nano/micro/macro-hydrogel networks is principally attractive to obtain new responsive, biocompatible and even targetable DDSs, appropriate to be administered via almost any route. Polysaccharide networks can be derived through crosslinking supported by interactions of diverse potency, from weak physical entanglements to irreversible covalent bonds [31, 107].

4.9 Interpenetrating Polymer Networks Polysaccharide Hydrogels for Drug Delivery and Tissue Engineering

Drugs release from a suitable dosage form at predetermined time interval and at prefixed speed, symbolize a most essential challenge for researchers concerned in pharmaceutical studies. And, though important progresses have been finished in recent years in the region of modified drug release, various aims still require to be engage in for treating clinical pathologies. In addition, the swift development of tissue engineering encourages the research for new biocompatible materials suitable for adhesion and proliferation of various types of cells. Among the synthetic and

natural polymers that can be employed for cell culture and for formulations intended to optimize drug targeting and/or release rate, polysaccharides signify a class of macromolecules of specific interest. This is because they are typically abundant, in the majority of the cases obtain from renewable sources and have a large diversity of composition and properties that may permit suitably modified chemical modifications. In addition hydrogels composed of crosslinked polysaccharides and their derivatives have been often investigated for innovative dosage forms [18, 108]. In addition, ecologically sensitive hydrogels have been studied as "smart" delivery systems proficient to release an entrapped drug in response to particular physiological stimulus, at the suitable time and site of action [109]. Recently multicomponent drug delivery systems have been produced for potential therapeutic and diagnostic applications and among these, semi-Interpenetrating Polymeric Networks(semi-IPNs) and Interpenetrating Polymeric Networks (IPNs) have appeared as novel biomaterials for drug delivery and as scaffolds for cell cultures [110]. These networks frequently demonstrate physico-chemical properties that can extraordinarily vary from those of the macromolecular constituents. Notably, the network properties can be modified by the sort of polymer and its concentration, by the functional crosslinking method in addition to the general protocol employed for their preparation. In various studies, polysaccharides are chosen for the development of IPN hydrogel networks, which are either chemically or physically crosslinked. Occasionally both entangled macromolecules are based on polysaccharides, however also mixtures of synthetic polymers together and polysaccharides chains are employed to generate (semi)-IPNs. A reasonably great number of polysaccharides have been studied for the design of (semi)-IPNs for drug delivery and tissue engineering applications. Table 4.4 enlisted structure, source and responsiveness of different ionic polysaccharides for drug delivery. Figure 4.6 demonstrates a scheme for the blend of polysaccharides and cross-linkers that offer neutral and ionic polysaccharide networks.

4.10 Polysaccharide-Based Antibiofilm Surfaces

It is now well identified that bacteria connect to solid supports to shape structured communities called biofilms, also known as biopolymer matrix-enclosed microbial populations adhering to each other and/or surfaces [111]. Biofilms occur on both living and inert supports in all environments [112]. They influence various industrial and domestic areas [113] and are accountable for a broad range of human diseases [111]. In view of the ever growing number of implanted patients, biofilm-linked infections of indwelling medical devices are more predominantly a foremost public health issue. Various examples of implants that can be inflated by biofilm formation are mechanical heart valves, catheters, pacemakers/defibrillators, ventricular assist devices, vascular prostheses, coronary stents, neurosurgical ventricular shunts, cerebrospinal fluid shunts, neurological stimulation implants, ocular prostheses, inflatable penile, cochlear, joint prostheses, fracture-fixation devices, breast, and dental implants and contact lenses, intrauterine contraceptive devices [114–116].

Table 4.4 Structure, source and responsiveness of the ionic polysaccharides for drug delivery

Polysaccharide	Source	Responsiveness
Chitosan	Exoskeleton of crustacean and insects or cell walls of bacteria and fungi	Ions, pH, electrical field (composites with inorganic particles), temperature (grafted with PNIPAAm or PEO-PPO-PEO), redox (if thiolated), magnetic (with Fe3O4), and specific molecules (dynamic Schiff bases)
Alginate	Marine brown seaweeds and microorganisms	Ions, pH, electrical field, surfactants, light (anthracene grafted) temperature (grafted with PNIPAAm), redox (if thiolated), and magnetic (with Fe3O4)
Agar	Seaweeds of genus Gelidium, Euchema, Gracilaria and others	Ions and pH
Carrageenan	Red seaweeds of genus Rhodophyceae	Red seaweeds of genus Rhodophyceae
Chondroitin sulfate	Animals and humans	Ions, pH, and colon enzymes
Cellulose ethers, ionic	Higher plant cell walls, followed by substitution reactions	Ions, pH, and temperature
Gellan gum	Extracellular secretion of Pseudomonas elodea	Ions, pH, and temperature
Guar gum, ionic	Seed of a plant (Cyamopsis tetragonolobus), followed by substitution reactions	Ions, pH, and temperature
Heparin	Animals and humans	Ions, pH, redox (if thiolated)
Hyaluronic acid	Extracellular matrix of higher animals	Ions, pH, electrical field, light (anthracene grafted), temperature (grafted with PNIPAAm), and redox (itself and thiolated)
Pectin	Higher plant cell walls	Ions, pH, and colon enzymes
Scleroglucan	Fungi of the genus Sclerotium	Ions and pH
Xanthan gum	Microbial exopolysaccharide of Xanthomonas campestris	Ions, pH, and temperature

Bacteria usually derived from biofilm-infected implants comprise the gram positive Enterococcus faecalis, Staphylococcus epidermidis Staphylococcus aureus, and Streptococcus mutans, and the gram-negative Escherichia coli, Proteus mirabilis Klebsiella pneumoniae, and Pseudomonas aeruginosa [117, 118]. Biofilm-associated infections are mainly challenging since sessile bacteria are much more resistant to antibiotics and biocides than their planktonic counterparts [119]. Therefore, the behavior of biofilm infections requires high concentrations of disinfectants or antibiotics, which may form the basis of severe environmental compensation and multi-resistance emergence. In this concern, anticipation of biofilm development is really considerable to any post-infection treatment. At the biomaterial surface level, two chief approaches are now proposed to oppose biofilm formation, i.e., the growth of anti-adhesive or bactericidal surfaces (Fig. 4.7) the application of biofilm-degrading

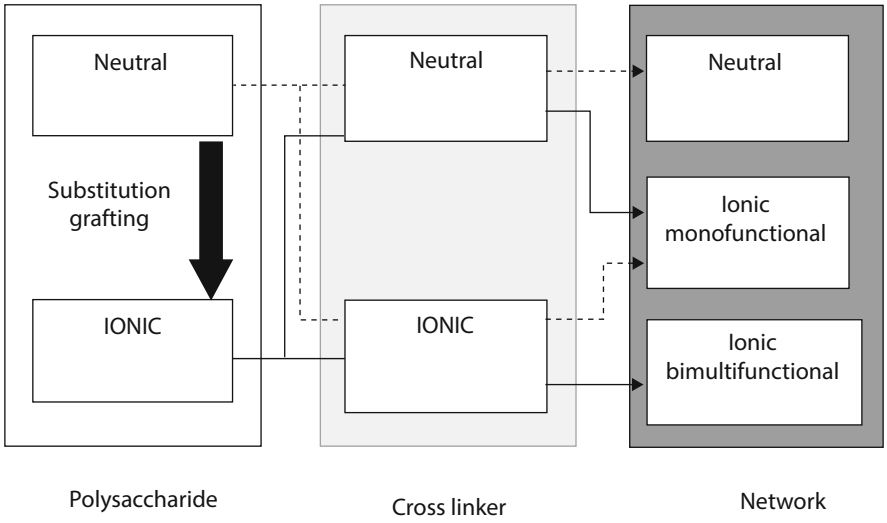

Fig. 4.6 Blend of polysaccharides and cross-linkers that provide neutral and ionic polysaccharide networks

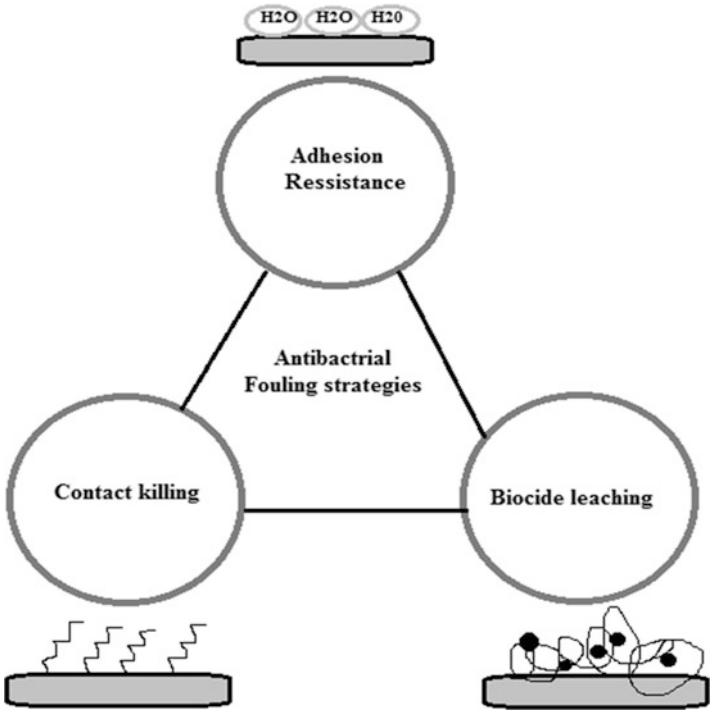

Fig. 4.7 Prominent approaches for antibacterial surface design

agents [120] being still in its development period. Surfaces that are principally repellent are evaluated by a decline in the number but no considerable loss in viability of attached bacteria. Anti-adhesive features of inert materials can be developed by altering surface features known to influence microbial cell adhesion, specifically surface topography(roughness) and physico chemistry (hydrophilic or hydrophobic, surface free energy, cationic or anionic behavior) [121–124]. A physical handling of the surface such as plasma irradiation pursued or not by attachment of anti-adhesive molecules or polymers, is usually functional for that point [125]. Nevertheless, sustained cell adhesion on implanted materials is mandatory for appropriate tissue incorporation of permanent implants e.g. vascular grafts or joint prostheses. Therefore, the characteristics of such implant surfaces must equilibrate between repellency against bacterial cells and adhesiveness for tissue cells, regulating the "race for the surface" [126, 127] between bacteria and tissue cells. Killing effect of the surface against attached and/or suspended bacteria is decorated by a decline in adherent cell viability and/or the number of viable suspended cells. As illustrated in Fig. 4.7, bacterial killing features can be attaining by non-covalent immobilization of an antimicrobial agent via direct integration in the biomaterial bulk or deposition on the surface, resulting in additional release of the drug in the adjoining medium. Alternate approach comprised of covalent binding of an antibacterial compound to the biomaterial surface to develop a contact-killing coating. The primary method has been extensively employed in commercial devices e.g. catheters that are heparinized for thrombo-resistance and loaded with antimicrobials [128]. The covalent methodology offers the merit of circumventing possible noxious effects of classical biocidal compounds and failure in effectiveness owing to a partial reservoir facility of the biomaterial [129]. In addition, both approaches could be assorted to detail infection-resistant biomedical materials with synergic anti-adhesive and bactericidal features. Among all properties of biofilm development is the fabrication of an extracellular matrix composed of 90 % water and 10 % extracellular polymeric substances [130]. The later are principally consisting of polysaccharides and proteins, but also comprise nucleic acids, lipids and other biological macromolecules. Their components mediate cell-to-cell and cell-to-surface interactions that are essential for biofilm production and stabilization [130]. A number of reports also recommend that some bacterial extracellular polysaccharides might hinder and/or destabilize the biofilm [131, 132]. Nevertheless, none of antibiofilm exopolysaccharides recognized to date that demonstrates antibacterial activity. All of them perform as surfactant molecules, altering the physical characteristics of bacterial cells and a biotic surfaces [133]. On the other hand, several bacterial exopolysaccharides have been exposed to present antimicrobial effectiveness [134–137], as have been chitosan, a chitin derivative [138], and some polysaccharides of algal [139, 140], fungal [141] and plant origins. Therefore, modified polysaccharides are being produced as bacteria-repellent and/or –killing coatings for material surfaces exposed to biofilm formation.

4.11 Polymers, and Their Complexes Used as Stabilizers for Emulsions

Emulsions are extensively employed in pharmaceutics for the encapsulation, solubilization, entrapment, and controlled delivery of active ingredients [142]. With the aim to answer the growing demand for clean label excipients, natural polymers can swap the potentially irritative synthetic surfactants employed in emulsion formulation. Certainly, biopolymers are at present employed in the food industry to stabilize emulsions, and they emerge as capable candidates in the pharmaceutical field too. Most of the proteins and a number of polysaccharides are able to adsorb at a globule surface, consequently declining the interfacial tension and increasing the interfacial elasticity [142]. Nevertheless, most polysaccharides stabilize emulsions merely by enhancing the viscosity of the continuous phase. Proteins and polysaccharides may also be related either through covalent bonding or electrostatic interactions. The blend of the features of these biopolymers under suitable environment results in increase in emulsion stability. Substitute layers of oppositely charged biopolymers can also be fashioned around the globules to acquire multi-layered "membranes". These layers can offer electrostatic and steric stabilization consequently enhancing thermal stability and resistance to external treatment. The new biopolymer-stabilized emulsions have a immense prospective in the pharmaceutical field for controlled digestion, encapsulation and targeted release while a number of challenging subject e.g. storage and bacteriological concerns still require consideration [142]. Various destabilization mechanisms for an oil in water emulsion are mention in Fig. 4.8.

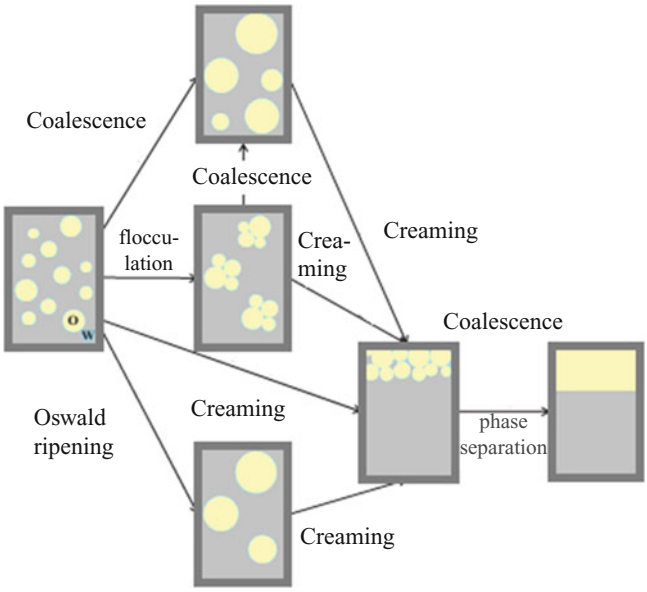

Fig. 4.8 Plan presentation of different destabilization mechanisms for an oil in water emulsion

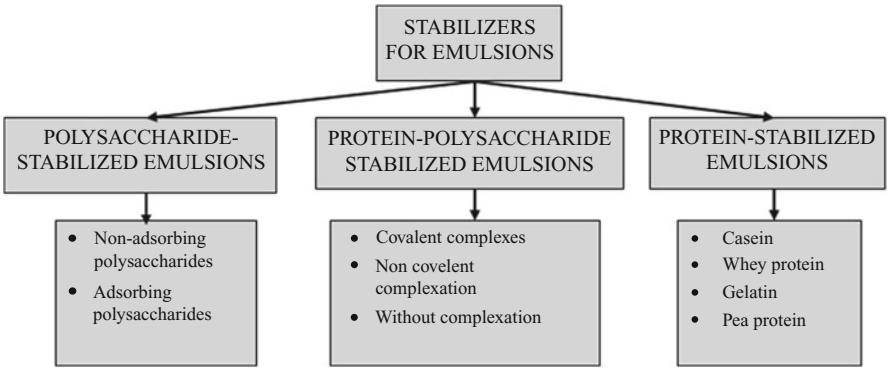

Fig. 4.9 Polysaccharides as stabilizers for emulsions

Polysaccharides are widely recognized for their water-holding and thickening features because of their hydrophilic character and high molecular weight. They can be divided in two groups for their application in stabilizing emulsions droplets. Most frequent polysaccharides do not have a great deal of an affinity to adsorb at fluid interfaces. Non-adsorbing polysaccharides have no or restricted surface activity and augment the emulsion stability by gelling or altering the viscosity of the aqueous continuous phase, which slows down droplet progress [142]. A number of other polysaccharides e.g. naturally occurring galactomannan hydrocolloids (guar gum, fenugreek gum), gum arabic, chemically modified starch or cellulose derivatives, acetylated pectin from sugar beet, etc. exhibit surface/interfacial activity. They foremost stabilize emulsions by adsorption at the oil droplet surface and then avert droplet flocculation and coalescence through electrostatic and/or steric repulsive forces [142]. For gum arabic and galactomannans, the surface action effect generally from the presence of a protein fraction in their structure. It also appears that the protein related with the pectin plays a significant role in stabilizing emulsion. On the other hand, for cellulose derivatives, the surface activity is due to the combination of hydrophobic and hydrophilic groups along the cellulose backbone [142]. Polysaccharides are classified in non-adsorbing polysaccharides if they do not display surface activity and adsorbing ones if they can stabilize emulsions through their adsorption at the oil–water interface by lowering the interfacial tension (Fig. 4.9).

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Chapter 5
Advanced Application of Natural Polysaccharides

Abstract One of major contributing factor in the development of biomedical research are the number of researches carried out for the advancement of polymeric materials with their advance drug delivery systems to explore their potential applications in the similar area. This chapter has covered some of the recent hot topics of polymeric science directing its prospects towards biomedical sector.

Keywords Polysaccharides • Drug delivery • Nanoparticle • Chitosan • Application

5.1 Introduction

Polymers, the most multipurpose class of materials, have altered our day-to-day lives over the past numerous decades. Nevertheless, the dissimilarity between temporary and permanent biomedical applications of polymers was made only 30 years ago. Afterward, the combination of polymer science with pharmaceutical sciences led to a quantum leap in terms of 'novelty' in design and development of novel drug-delivery systems. Polymeric delivery systems are chiefly proposed to attain either a temporal or spatial control of drug delivery. The exploration of the first synthetic polymer-based (polyglycolic acid) drug-delivery systems led to a sharp interest in the design and development of novel biodegradable polymers that obviated the call to eliminate the drug-delivery systems, unlike the nondegradable polymeric systems. Distinguishing that close contact between a delivery system and an epithelial cell layer will advance the abode time as well as the effectiveness of the drug-delivery systems resulted in the design of bioadhesive polymers. Additional advancements in polymer science results in development of 'smart' polymeric hydrogel systems that can self-regulate the delivery of a bioactive agent in response to a specific stimulus.

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5.2 Biodegradable Polymers as Bio-Materials

From last few decades major advances have been made in the development of biodegradable polymeric materials for biomedical applications. Biomaterials especially degradable polymeric are ideal candidates for fabricating therapeutic devices e.g. three-dimensional porous structures as scaffolds for tissue engineering, temporary prostheses and as controlled/sustained release drug delivery vehicles. All of these applications claim materials with definite physical, chemical, biological, biomechanical and degradation properties to offer efficient therapy. As a result, a broad range of natural or synthetic polymers competent of undergoing degradation by hydrolytic or enzymatic route are being studied for biomedical applications. Recent decades saw a standard shift from biostable biomaterials to biodegradable (hydrolytically and enzymatically degradable) biomaterials for medical and related applications [1–3]. Current research forecast in the next few years, various stable prosthetic devices used for provisional therapeutic applications will be replaced by biodegradable devices. This might assist the body to restore and renew the damaged tissues. There are various causes for the positive consideration of biodegradable over biostable materials for biomedical applications. Main strength is the long-term biocompatibility issues with several existing permanent implants. In addition various stages of ethical and technical concerns associated with revision surgeries is also in limelight. Although the biomedical significance of enzymatically degradable natural polymers e.g. collagen dates back thousands of years, the application of synthetic biodegradable polymers started only in the latter half of 1960s [4]. Nevertheless, recently growth of a range of latest generation synthetic biodegradable polymers and related natural polymers particularly developed for biomedical applications have been explored. The attention is partial, owing to the appearance of novel biomedical technologies including: tissue engineering, gene therapy, regenerative medicine, bionanotechnology and controlled drug delivery, majority of which entail biodegradable platform materials to build on. Sluggish evolution in the development of biodegradable biomaterials can be accredited to several unique challenges in developing resorbable clinical materials compared to developing commodity polymers. As far as the definition of biomaterial is concerned a biomaterial can be defined as a material anticipated to edge with biological systems to estimate, treat, augment or replace any tissue, organ or function of the body [5]. The most important requirement to qualify a material as a biomaterial is biocompatibility, which is the potential of a material to achieve with a suitable host response in a specific application. The tissue reaction against implant depends on a numerous factors varying from the chemical, physical and biological characteristics of the materials to the shape and structure of the implant. In study of biodegradable biomaterials, their dynamic biocompatibility must be established over time. The chemical, physical, mechanical and biological features of a biodegradable material will fluctuate with time and degradation products can be formed that have varying intensity of tissue compatibility in contrast with starting parent material. A number of the important features of a biodegradable biomaterial can be reviewed as follows [6]:

- The material should not evoke a sustained inflammatory or toxic response upon implantation in the body.
- The material should have adequate shelf life.
- The degradation time of the material should equivalent the healing or regeneration process.
- The material should have suitable mechanical properties for the specified application and the difference in mechanical properties with degradation should be well-matched with the healing or regeneration process.
- The degradation products should be non-toxic, and proficient to get metabolized and cleared from the body.
- The material should have suitable permeability and processability for the anticipated application.

Numbers of inherent properties of polymeric biomaterials that can have an influence biocompatibility include:

- Water absorption
- Surface energy
- Structure of the implant
- Solubility
- Shape
- Molecular weight
- Material chemistry
- Lubricity
- Hydrophilicity/hydrophobicity
- Erosion mechanism
- Degradation

With known complexity and the diverse applications polymeric biomaterials are presently used, there is not just single polymeric system existing that could be acknowledged as an perfect biomaterial. This emphasizes the requirement for developing a broad variety of biodegradable materials offered for implant fabrication which can properly contest the particular and exclusive necessities of each individual medical application. Present contributions in biodegradable polymer synthesis have been paying attention on custom designing and fabricating polymers with tailored properties for specific applications by:

- Synthesizing novel synthetic polymers with exclusive chemistries to augment the diversity of polymer structure
- Implementing combinatorial and computational approaches in biomaterial design to hasten the innovation of novel resorbable polymers.
- Designing biosynthetic processes to form biomimetic polymer structures and

Biodegradable polymeric materials are being studied in fabricating therapeutic devices e.g. three-dimensional porous structures as scaffolds for tissue engineering, temporary prostheses, and for pharmacological applications, such as drug delivery (both localized and targeting systems). Several current biomedical applications of biodegradable polymeric materials include:

- Small implants, such as staples, sutures and nano- or micro-sized drug delivery vehicles,
- Plain membranes for guided tissue regeneration and
- Multifilament meshes or porous structures for tissue engineering [7].
- Large implants, such as bone screws, bone plates and contraceptive reservoirs,

Best approach in tissue engineering is to employ biodegradable construct to as further assemble cells in 3-D to finally develop into functioning tissue. Polymeric materials with a broad range of exclusive mechanical and degradation properties are constructed to mimic the properties of various tissues. While considering controlled drug delivery, therapeutic agents are entrapped within a biodegradable polymer matrix from which they are released in an erosion- or diffusion-controlled fashion or a combination of both. In vivo or in vitro release profile of the therapeutic agents can be successfully modulated by properly engineering or designing the matrix parameters. Owing to the versatility of polymers, they are frequently replacing other material classes, such as alloys, metals and ceramics for their utilization as biomaterials.

5.2.1 Biodegradable Polymers

Both synthetic polymers and natural polymers have been broadly studied as biodegradable polymeric biomaterials. Polymeric biomaterials based biodegradation entails cleavage of hydrolytically or enzymatically sensitive bonds in the polymer resulting in to polymer erosion [8]. Based on the means of degradation, polymeric biomaterials can be further divided into:

- hydrolytically degradable polymers
- enzymatically degradable polymers

Majority of the biologically derived polymers undergo enzymatic degradation. These biologically derived polymers can be acknowledged as the first biodegradable biomaterials utilized clinically. Frequency of in vivo degradation of enzymatically degradable polymers nevertheless, differs considerably with the site of implantation depending on the availability and concentration of the enzymes. Alternative and the most usual strategy of chemical modification of these polymers also can considerably affect their rate of degradation. Biologically derived polymers exhibit several inherent advantages e.g. ability to present receptor-binding ligands to cells, bioactivity, natural remodeling and susceptibility to cell-triggered proteolytic degradation. The intrinsic therapeutic effectiveness of these natural polymers has its own downsides such as strong immunogenic reaction linked with most of the polymers, the complications associated with their purification and the chances of disease transmission. In contrary, synthetic biomaterials usually biologically inert, they have more expected properties and batch-to-batch consistency and they have the exclusive benefits having tailored property profiles for specific

applications, devoid of many of the drawbacks of natural polymers. Hydrolytically degradable polymers are usually chosen as implants owing to their minimal site to-site and patient-to-patient variations in contrast with enzymatically degradable polymers [8]. The most thriving performance of the foremost synthetic poly(- glycolic acid) based suture system during the late 1960s resulting in to the design and development of a new range of biodegradable polymers as transient implants for orthopaedic and associated medical applications. Broad research has gone since then to practice designing biodegradable polymer systems with expected erosion kinetics as drug/gene delivery vehicles or as scaffolds for tissue engineering. For functions that require materials with a definite level of biological activity, approaches to include biological motifs onto synthetic polymers in the form of hybrid materials have also been developed.

5.2.2 Hydrolytically Degradable Polymers as Biomaterials

As far as the simple definition is concerned hydrolytically degradable polymers are the polymers that have hydrolytically labile chemical bonds in their back bone structure. In these biomaterials the functional groups that are more vulnerable to hydrolysis include esters, orthoesters, anhydrides, amides, urethanes, carbonates, ureas, etc. [9]. In general synthesis are used to develop hydrolytically sensitive polymers for biomedical applications including step (condensation) polymerization and addition (chain) polymerization. This addition (chain) polymerization included ring opening polymerization. Step process is employed to fabricate a variety of hydrolytically sensitive polymer classes, e.g. polyanhydrides, polyurethanes and poly(ortho esters). Ring opening polymerization (ROP) is an broadly studied polymerization means to fabricate hydrolytically sensitive polymers, including the poly(a-esters) and polyphosphazenes. Radical polymerization generally results in the development of non-degradable polymers; nevertheless, current investigations have established the possibility of developing synthetic degradable polymers or cross-linked gels by radical polymerization processes. Moreover, various polymers developed by microbial bioprocess are gaining considerable attention as biodegradable polymers. Illustration (Fig. 5.1) demonstrated most promising hydrolytically sensitive natural and synthetic polymers developed and their biomedical applications.

5.3 Natural Polysaccharides as Carriers and Scaffolds FOR Biomolecules and Cell Delivery in Tissue Engineering Applications

Tissue engineering is the most reliable therapeutic approach that combines cells, biomaterials, and microenvironmental factors to stimulate differentiation signals into surgically transplantable formats and additionally encourage tissue repair

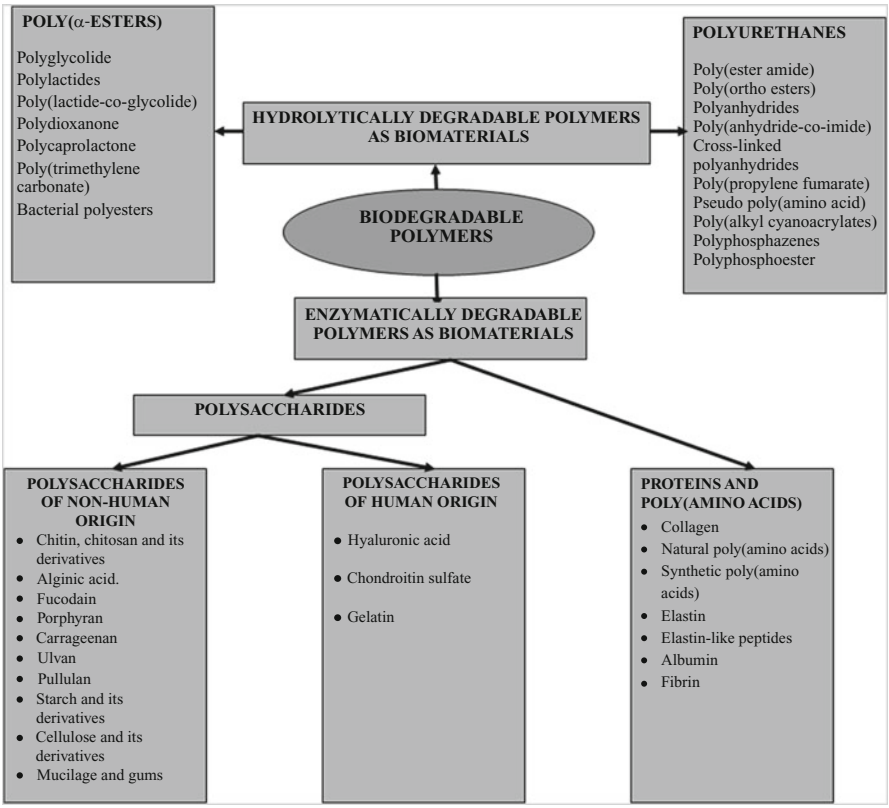

Fig. 5.1 Promising hydrolytically sensitive natural and synthetic polymers

and/or functional restoration. In spite of various advances, tissue engineers/researchers still gone through considerable confronts in repairing or replacing tissues that provide principally biomechanical functions e.g. articular cartilage. One of the major problems can be recognized as the scaffolds play a vital role as the extracellular matrix however they are often incapable to generate the exact/correct microenvironment throughout the engineered tissue development to encourage the perfect in vitro tissue development. The rising and most promising next generation of engineered tissues is dependent on development of scaffolds with an informational function. For an instance material controlling growth factors sequence which allows cell attachment, proliferation and differentiation is superior to non-informational polymers. Utilization of growth factors has been acknowledged as a means to control not only the host healing response at the site of injury to allow the tissue repair, however also to control and improve the in vitro tissue growth to facilitate more biofunctional engineered tissues. Therefore alternate, the approach is to mimic matrix and offer the essential information or signaling for cell attachment, proliferation and differentiation to match the prerequisite of active reciprocity for tissue

engineering. This may explained the significance of drug delivery in tissue engineering applications. Additionally, natural polymers execute a various set of functions in their native surroundings e.g. polysaccharides employed in membranes and intracellular communication and also as storage, and proteins function as structural materials and catalysts [10]. Recent development is to mimic nature and what superior approach than materials from nature to do it? Natural biopolymers demonstrate, as an extraordinary model, how all the properties presented by biological materials and systems are entirely evaluated by the physical–chemical properties of the monomers and their sequence. A distinct molecular structure can present a rich complexity of structure and function on the mesoscale [11]. At this juncture, competing interactions, structural flexibility and functional properties are engineered by the sequential arrangement of monomeric units taken from a relatively limited set. Since macromolecules link the extent of nanometers up to micrometers by excellent feature of their length and flexibility, they facilitate a unique control of hierarchical organization and long-range interactions. In spite of the tissue/organ concerned, there are various models that can be extrapolated from nature and consequently appropriate in the tissue engineering field to repair/regenerate the tissue/organ. In various cases, the matrices and scaffolds would preferably be made of biodegradable polymers whose features strongly look like those of the extracellular matrix

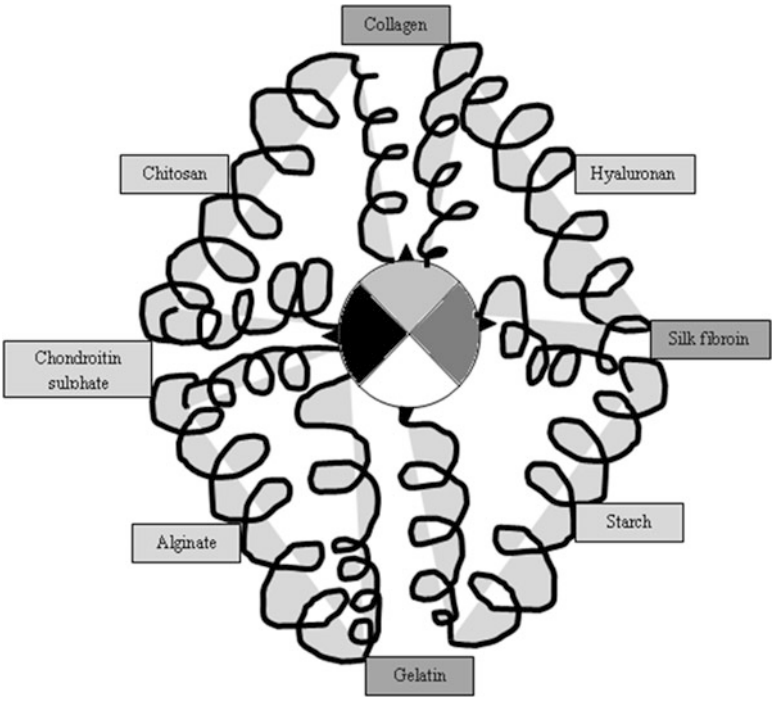

Fig. 5.2 Macromolecules for the encapsulation of cell in tissue engineering application

(ECM), a soft, tough, and elastomeric proteinaceous network that offers the mechanical stability and structural reliability to tissues and organs [12]. If the continuously emerging information on how our body functions and the requirement to achieve repair, there is the call to assemble into a exclusive fabricate both structural support and drug delivery properties. Combining together, current research has considered cell encapsulation as a delivery system, as this is believed to be most reliable therapeutic approach. Encapsulation actually separates a cell mass from an external environment and intends to retain normal cellular physiology within a desired permeability barrier [13]. Usually encapsulation techniques are divided as microencapsulation (involving small spherical vehicles and conformal coated tissues) and macroencapsulation (involving larger flat-sheet and hollow-fiber membranes) [13]. Multiple numbers of polymers in natural and synthetic form are employed to encapsulate cell and other bioactive molecules. As illustrated in Fig. 5.2 various polymers are engineered to mimic the natural matrix to further encourage its compatibility with biological cell, tissue or an organ.

Centre circle: cell, Dark gray boxes: protein origin polysaccharides, Light gray boxes: polysaccharides, coiled lines: polymers, light gray engravings in coiled lines: depending upon the coiling (spring fashion) space facilitate the mobility to cell (more coiling more flexibility) and open free ends of polymer chain present at the interface of cell to interact with cell receptors (dark triangle); different zones in circle demonstrate zone which require utmost polymer exposure according to receptor interaction or any specified organelle or sub organelle targeting.

5.4 Natural and Synthetic Polysaccharides for Wounds and Burns Dressing

In the last decades, health care professionals featured various patients suffering from wounds and burn not easy to treat and heal. In the wound healing process, the dressing protects the injury and furnishes the healing of dermal and epidermal tissues [14]. Since their biocompatibility, biodegradability and resemblance to macromolecules accepted by the human body, a number of natural polymers such as polysaccharides (chitosan, heparin, alginates, chitin, chondroitin), proteoglycans and proteins (collagen, gelatin, fibrin, keratin, silk fibroin, eggshell membrane) are broadly used in wounds and burns treatment [14]. Synthesized by electrospinning technique, some synthetic polymers like biomimetic extracellular matrix micro/nanoscale fibers based on polyglycolic acid, poly-ε-caprolactone, polyvinylpyrrolidone, polylactic acid, polyacrylic acid, polyvinyl alcohol, polyethylene glycol, display in vivo and in vitro wound healing properties and improve re-epithelialization. They offer an optimal microenvironment for cell proliferation, migration and differentiation, owing to their biodegradability, biocompatibility, peculiar structure and good mechanical features [14]. Therefore, synthetic polymers are employed also in regenerative medicine for vascular, nerve, cartilage, bone and ligament repair and restoration. Biocompatible with fibroblasts and keratinocytes, tissue engineered

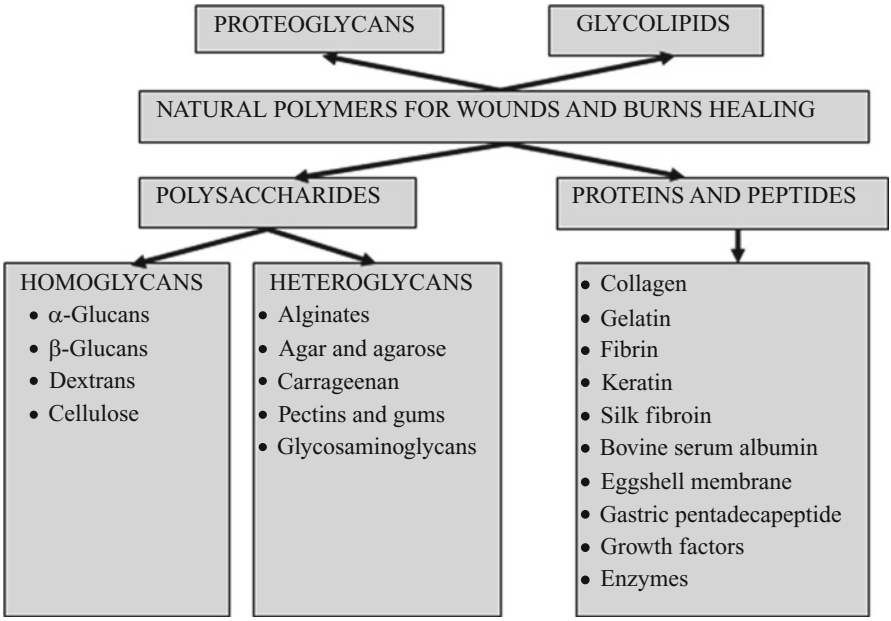

Fig. 5.3 Natural polymers for wounds and burns healing

skin is designate for renewal and remodeling of human epidermis and wound healing improving the management of severe skin defects or partial-thickness burn injuries. Various natural and synthetic polymers for wounds and burns dressing are illustrated in Fig. 5.3.

5.5 Present Research on the Blends of Natural and Synthetic Polymers as New Biomaterials

Polymeric materials are extensively utilized in the biomedical field. While it is much easier to employ synthetic polymers in the biomedical field, natural polymers are also essential owing to their biocompatibility and biodegradability. An additional method of fabrication of polymeric materials for biomedical applications is to mix together synthetic polymers with natural ones. Growing interest in innovative materials based on blends of two or more polymers has been studied through the last three decades. Blends of synthetic and natural polymers can shape a novel class of materials with superior mechanical properties and biocompatibility in comparison those of single components. This emerging class called as bioartificial or biosynthetic polymeric materials [15–18]. Natural polymers are typically biocompatible, while synthetic polymers can include a residue of

initiators and other compounds/impurities that do not permit cell growth [19, 20]. Synthetic polymers have superior mechanical features and thermal stability, even much superior than a number of naturally occurring polymers. There is also a drawback in the presentation of several natural polymers as compared to synthetic polymers. Synthetic polymers can be fabricated into a wide range of shapes, while for natural polymers several shapes are not easily attained; for an instance, high temperatures forced in processing can demolish their native structure. Recently developed polymeric materials based on the blends of natural polymers and man-made ones should be biocompatible whereas, simultaneously, acquire good thermal and mechanical properties for their utilization in biomedical applications. The chief biopolymers used in fabrication of materials for biomedical applications are chitosan, collagen, chitin, silk, keratin and elastin, the entire natural polymers derived from animal body. There is also a group of natural polymers, obtained from plants, e.g. starch, cellulose and pectin. Various synthetic polymers can be mixed together with naturally occurring polymers to synthesize new materials. In addition, the polymeric blends can be reinforced by minerals, which nucleate and develop in a polymeric matrix to offer the appropriate size, shape and distribution of individual crystals, comparable to hard tissue. Natural polymers, e.g. collagen and elastin are typically insoluble in both water and organic solvents, apart from collagen which is extracted from the tissue of young animals and soluble in dilute acetic acid. Furthermore chitosan is soluble in dilute acetic acid solution, but the concentration that can be arrived at rather low and usually depends on the molecular weight of the biopolymer. The solubility of collagen and chitosan in acetic acid offers the prospect to blend them with other water soluble polymers [21–23]. In contrast, polymers like silk, elastin and keratin are extremely insoluble natural polymers and therefore the processing of these biopolymers is challenging, but these may be hydrolysed to support solubility. Blends of collagen with synthetic polymers as well as with other natural polymers have been extensively investigated as biomedical materials [24–28], as have been collagen itself [29–34]. This development has continued in the recent literature [35–38]. Chitosan has been extensively investigated as a potential biomedical material [39–45], as have blends of chitosan with synthetic and/or other natural polymers. Elastin has been reported for biomaterial preparation, however in practice it is rarely utilized for blend preparation [46–52], with mere a small number of papers on blends of elastin with other polymers [46–52]. Silk is an excellent biopolymer for the fabrication of biomaterials [46–52], however soluble forms of silk are not easily derived. There are very not many research papers in scientific literature on the subject of novel materials based on the mixtures of silk with other polymers. Newly keratin has been extensively investigated as a potential material for biomedical applications. Investigations regarding biodegradable starch-based polymers have established that these materials have a variety of characteristics which formulate them appropriate for use in several biomedical applications, varying from bone plates and screws to drug delivery carriers and tissue engineering scaffolds.

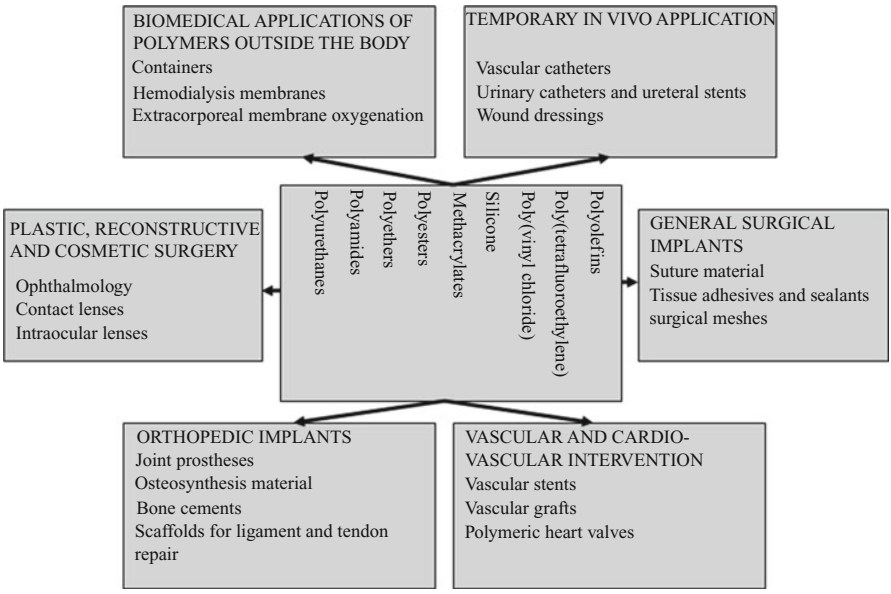

Fig. 5.4 Applications of synthetic polymers in clinical medicine

5.6 Applications of Synthetic Polymers in Clinical Medicine

Numerous biological, synthetic and hybrid polymers are utilized for various medical applications. A broad range of diverse polymers is existing, and they have additional the advantage to be compatible in physical, chemical and biological properties in a wide variety to match the necessities of particular applications. The different polymer classes with tailored formulations like adjusted molecular weight, crosslinking degree, degree of crystallization, co-polymers and blends and additional bioactive surface functionalization facilitate this broad variety of applications [53]. Whereas engineering-related characteristics e.g. stiffness, tensile stability and elasticity are typically most important features for screening a polymer, also toxicity and biocompatibility feature have to be taken into consideration. Biodegradation as a more superior property of some polymers finds application in a various fields from suture materials via orthopedic stabilizing materials to vascular stents, since these devices may perhaps vanish after they fulfilled their role [53]. Receptive degradation of polymers upon defined trigger also facilitates controlled drug release applications. These impressions now present the most vigorous fields of research and products should shortly materialize on the medical device market (Fig. 5.4).

5.7 Current Progress on Gelatin NPS in Drug and Vaccine Delivery

New drug delivery system by means of nanoscale materials with a broad spectrum of applications furnishes a new therapeutic establishment for technological integration and advancement [54]. Nanoparticles are appropriate drug carrier for a variety of routes of administration as well as swift identification by the immune system. Gelatin, the biological macromolecule is a versatile drug/vaccine delivery device in pharmaceutical field owing to its biodegradable, biocompatible, non-antigenicity and low cost with easy accessibility. Surface modification of gelatin nanoparticles with site-specific ligands, cationized with amine derivatives or, coated with polyethyl glycols allows targeted and sustained release drug delivery. In contrast with other colloidal carriers, gelatin nanoparticles are better stable in biological fluids to offer the desired controlled and sustained release of entrapped drug molecules [54]. Current research demonstrated the various formulation aspects of gelatin nanoparticles which may influence the particle characteristics like polydispersity index, zeta potential, entrapment efficacy and drug release properties [54]. In addition considerable emphasis was given on the major applications of gelatin nanoparticles in drug and vaccine delivery, gene delivery to target tissues and nutraceutical delivery for improving the poor bioavailability of bioactive phytonutrients.

5.7.1 Drawbacks and Challenges

Considerable applications of GNPs as drug/vaccine delivery vehicle in different fields have been developed. Nevertheless, there is a still serious problem related with the use of animal origin gelatin which carries the danger of contamination with transmissible spongiform encephalopathy (TSE) [54]. The rigorous fabrication processes such as acid, alkaline and heat treatments are utilized to inactivate TSE agents and minimize TSE risk in drug products. Presently, there are commercial suppliers (e.g. Fibrogen South San Francisco, CA, USA) that offers gelatin by

Table 5.1 Examples of drug loaded gelatin nanoparticles [54]

Drug loaded	Method of preparation
Didanosine	Two-step desolvation
FITC-dextran	One step desolvation
Paclitaxel	Desolvation with sodium sulphate
Pilocarpine	Desolvation with ethanol
Resveratrol	Coacervation
Methotrexate	Emulsion
Cisplatin	Two-step desolvation
Cycloheximide	Two-step desolvation
Rosiglitazone	Two-step desolvation
Amphotericin B	Two-step desolvation

recombinant DNA technology. Recombinant human gelatin (rHG) is nontoxic and functional for developing nanostructures for drug delivery owing to its non-immunogenicity [54]. Commercial gelatin utilized in the pharmaceutical industry is assorted mixtures of different sized proteins obtained from bovine or porcine bones or skins with a broad variety range of molecular weights offering heterogeneous nanoparticle size distribution [54]. An appealing approach to surmount this problem is the use of the two-step desolvation technique or the use of rHG due to its homogeneity in molecular weight and accurately defined properties to form nanoparticles with narrow size distribution (Table 5.1).

5.8 Current advancement of Chitosan-Based Polyelectrolyte Complexes with Natural Polysaccharides for Drug Delivery

Chitosan, as an exclusive positively charged polysaccharide, has been one of the most accepted biopolymers for improvement of drug delivery systems for a variety of applications, owing to its capable properties such as excellent biodegradability, high biocompatibility, low toxicity, as well as low production cost and abundant availability. Since last decade, growing consideration has been fascinated by delivery systems synthesized from natural biopolymer-based polyelectrolyte complexes (PEC), produced by electrostatic interactions between two oppositely charged biopolymers. To facilitate tailor specific applications of chitosan-based PEC drug delivery systems, a range of forms have been fabricated in current years such as nanoparticles, beads, microparticles, tablets, gels, as well as films and membranes. The recent report focuses on the current advances in drug delivery applications of chitosan-based PEC with other natural polysaccharides, including carrageenan, xanthan gum, gellan, alginate, hyaluronic acid, pectin, gum, gum arabic, and carboxymethyl cellulose, etc. With the increasing demands of natural polymer-based drug delivery systems, chitosan-based PEC with natural polysaccharides has established considerable interest year after year (Table 5.2). The functional properties and characteristics of chitosan-based PEC drug delivery systems can be modified for a range of applications. More specifically, the current advances bring in the following possibilities including but not limit to

- diverse forms of PEC can be easily fabricated to optimize the drug deliver efficacy, including beads, gels, nano-/micro- particles, films and membranes;
- diverse drugs can be encapsulated and administered in a controlled manner, including macromolecular drugs, growth factors, small molecular drugs, antimicrobials, and pesticides
- controlled swelling behavior, enhanced mechanical strength, and delayed digestibility to confirm the successful and sustained release of encapsulated drugs.

Table 5.2 Chitosan-based PEC with natural polysaccharides for biomedical applications [55]

Polysaccharides	Preparation method	PEC forms	Biomedical applications
Alginate	Extrusion; one-stage: alginate into chitosan w/ or w/o calcium; two-stage: alginate into calcium followed by chitosan coating	Nanoparticles; microparticles; hydrogel beads; gels; tablets; films/ membranes	Drug delivery; Enzyme immobilization; Tissue engineering
Hyaluronic acid	Extrusion: w/or w/o TPP; layer-by-layer assembly	Nanoparticles; microparticles; films/ membranes	Drug delivery; Tissue engineering
Pectin	Extrusion, w/or w/o calcium	Beads; tablet; films/ membranes	Drug delivery
Carrageenan	Extrusion, w/or w/o TPP; Composite with nanotubes	Nanoparticles; microparticles; hydrogel beads; fibers	Drug delivery; Enzyme immobilization
Xanthan gum	Extrusion; cryogelation	Hydrogel beads; tablets; cryog	Drug delivery; Enzyme immobilization; Tissue engineering
Gellan gum	Extrusion; layer-by-layer assembly	Hydrogel beads; films	Drug delivery; Tissue engineering
Cashew gum	Extrusion method	Nanoparticles; hydrogel beads	Drug delivery
Gum Arabic	Extrusion; composite	Nanoparticles; tablets	Drug delivery
Carboxymethyl cellulose	Extrusion	Nanoparticles; hydrogel	Drug delivery
Konjac glucomannan	Extrusion	Nanoparticles; microparticles	Drug delivery
Gum kondagogu	Extrusion	Nanoparticles	Drug delivery

Ahead additional understanding of chitosan-based PEC network development mechanisms, more and more novel drug delivery systems will be produced with tailored applications, particularly the delivery systems for macromolecular drugs e.g. protein and gene delivery. In addition, the in vivo assessment of chitosan-based PEC delivery systems in animal models will be one of the upcoming directions, so as to entirely know their biological fate and in vivo drug delivery effectiveness.

5.9 Relevance of Chitosan and Chitosan Derivatives as Biomaterials

Chitosan, chitosan derivatives, and chitosan/anionic materials complexes can be tailored by enhancing the physicochemical features for genetic materials transport for gene therapy [56]. The molecular weight, genetic materials concentration, degree of deacetylation, and serum stability of chitosan and the diverse modifications (cationic group, targeting ligand, thiol group, hydrophilic group, hydrophobic group, and

Table 5.3 The classification of various strategies to provide the advantage in chitosan [56]

Modification	Materials	Advantage
Hydrophilic material modification	Trimethyl group	High solubility, low cytotoxicity, high transfection efficiency
Hydrophobic material modification	Alkyl group	High transfection efficiency, alleviate serum
		Inhibition, enhanced protection efficiency, improved cell membrane permeation
5b-cholanic acid	Urocanic acid	Improve cationic density, enhance condensation capability, high transfection efficiency, effectively escape from endosome
Cationic material modification		
Targeting ligand modification	Saccharide	Hepatocyte targeting (Galactose)
Thiol group modification	Thioglycolic acid	Increase extracellular stability, high cellular uptake,
Amino acid and peptide modification		Enhance cell penetrating, Induced thiol group, Induced hydrophilicity

amino acid) are very key features in fabricating chitosan derivatives to trigger the effectiveness of gene therapy. Moreover, anionic materials such as TPP, HA, and PGA also encourage the cellular uptake and transfection efficiency of chitosan/genetic materials [56]. Various reports have been recently focused on the chitosan derivatives, chitosan/anionic materials fabricating techniques and site-specific targeting to advance the chitosan properties for genetic materials delivery. These reasons, alterations, and complexation with anionic materials successfully developed the chitosan properties including solubility in aqueous solution, escapes in endosome, cellular uptake, toxicity in HM chitosan, buffering capacity, genetic material release from chitosan based polyplexes, transfection efficiency, and silencing efficiency [56]. Nevertheless, majority of these outcomes were derived from experiments in vitro. Consequently, further investigation is required on chitosan for gene therapy in vivo. Investigation on chitosan derivatives, chitosan, and chitosan/anionic materials complexes still requires understanding the effects of the characteristics of the gene carriers on intracellular trafficking processes and cellular entry. In addition, majority of the studies used HM chitosan [56]. Investigation on in vivo and LM chitosan is known for the improvement of genetic materials delivery. Table 5.3 describes different set approaches to offer the advantage in chitosan.

5.10 Hyaluronic Acid for Anticancer Drug and Nucleic Acid Delivery

Hyaluronic acid (HA) is the major constituent of the extracellular matrix (ECM) and is universally dispersed in vertebrate tissues. Over half of the total body HA occurs in the skin [57] where it plays a structural position that depends on its exclusive hydrodynamic properties and its dealings with other ECM components. On the

other hand, HA has an instructive, cell signaling role during dynamic cell processes such as wound repair [58], and cancer, morphogenesis [59], inflammation [60], wherein HA–receptor interactions are triggered and collaborate in driving various signaling pathways. HA is a nonsulfated glycosaminoglycan, comprising a comparatively simple linear structure of alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine. This chemical structure is quite standard, with the exception of irregular deacetylated glucosamine residues [61]. It is produced by three transmembrane hyaluronan synthases (HAS1, HAS2, and HAS3) on the inner surface of cell membrane, and secreted in the ECM. These enzymes mediate the transglycosylation of D-glucuronic acid and *N*-acetyl-D-glucosamine, using their activated nucleotide sugars, uridine-5′-diphosphate-D-glucuronic acid, and uridine-5′-diphosphate-*N*-acetyl-glucosamine, as substrates. The degradation of HA is owing to hyaluronidases (HYALs), a class of enzymes that catalyze degradation. The term "hyaluronan" was established to cover the different forms the molecule can take [62]. In mammalian organisms, native HA is present as a linear high-molecular-weight (HMW) polymer (106–107 Da), and this great molar mass provide it its exclusive physicochemical properties, and report for the significant roles it plays in living organisms. HA is extremely hydrophilic, and can absorb water and expand its solid volume by up to 1000 times, yielding a very viscous and elastic gel with a large hydrodynamic volume [63]. The cluster of differentiation (CD) protein CD44 is the main hyaluronan binding receptor [64]. CD44 is accountable for the interaction between HA and the surface of specific cells. This interaction has been investigated in depth, being involved in various cellular functions (both physiological and pathological processes). Particularly, in usual physiology CD44 is implicated in cellular adhesion processes (aggregation and migration), in inflammatory responses, and in repair systems. On the other hand, the CD44 receptor is also related with human cancer, being occupied in tumor invasion and metastasis [65]. For biomedical applications, HA is chiefly formed by microbial fermentation; it can also be isolated from rooster combs and umbilical cords [66]. HA depolymerization can be attained in batch cultures through either by enzymatic reaction or physical or chemical degradations [66]. HA can be associated chemically to drugs or to drug carriers. The arrangement of HA drug conjugates, or the involvement of HA to colloidal carriers such as micelles, or to nanotechnology-derived particles, offer various benefits. The main important benefit is the ease of associating drugs with the polysaccharide, either straight or through a drug carrier, therefore solving any solubility problems. Additional benefit of HA's in association with its biopharmaceutical properties: it has been recommended that, in several cases, HA may improve a drug's blood plasma half-life, slugging the clearance mechanism, and therefore playing a similar role to polyethylene glycol (PEG) [66]. Thirdly, concerning anticancer therapy, the possibility of tumor targeting is a considerable benefit. Whole recognition to their improved pharmacokinetic properties, a number of HA-conjugates or HA-drug carriers may come across the well-known enhanced permeation and retention (EPR) effect, leading to enhanced drug distribution in tumor tissues [66]. Additionally, because CD44 is overexpressed in tumor cells and, mainly, in cancer stem or circulating cells, drug specificity versus target cells may

be enhanced [66]. The opportunity of surmounting the multidrug resistance (MDR) effect, which occasionally linked to overexpression of the efflux transmembrane Phospho-glycoprotein (P-gp), has also been accounted [66]. Nearly at high concentrations in solution, HMW-HA can yield viscoelastic entangled molecular networks called as hydrogels, wherein drugs can be loaded either by association or via covalent linkage [66]. These hydrogels can be used for local delivery of antitumor drugs. Nevertheless, solutions of HA do not have long-lasting mechanical integrity, particularly in physiological conditions [66]: HA hydrogels can swell by water absorption, or shrink on degradation. Covalent crosslinking is therefore essential to pass on stability and improve functionality. Considering the recognition to the versatility of HA, a range of chemically-modified forms of this polysaccharide have been produced, for use as tissue repair and regeneration materials, and also for the delivery of desired molecules in therapeutics; specifically, this latter concerns anticancer agents. The carboxylic groups and the mainly hydroxyl groups offers suitable sites for conjugation, and was widely used groups for chemical modification. Broad reviews by Schanté et al. [66] and Collins et al. [66] offer a complete explanation of the range of chemical modification methods and synthetic routes to obtain HA derivatives. The carboxylic groups are occupied in amidation and esterification reactions, and the primary hydroxyl residues in ester or ether bond formation. The acetyl group perhaps enzymatically eliminated from the *N*-D-acetylglucosamine, building it a possible site for conjugation [66]. When carboxylate and hydroxyl groups are altered, various attachments take place, and the groups are aimlessly linked to the polysaccharide chain, whether they are drugs, lipids, or polymers. Particularly when the carboxylate group is selected as bridging point, it is significant to establish the degree of substitution (DS) to preserve HA's overall charge and targeting properties: it has been found that a DS ratio above 25 % reduces HA's capability to target CD44 receptors [66]. Amidation in water with carbodiimides is one of the most extensively applied methods for HA modification; the most extensively used carbodiimide is 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide (EDC), due to its water solubility. The active intermediate, attain at acidic pH values, does not easily react with amines. Substituting active amines by hydrazides, which have much lower pKa values, higher coupling degrees can be attain: one of the most widely used reactants is adipic acid dihydrazide (ADH) [66]. To achieve more stable and more hydrolysis-resistant intermediates, *N*-hydroxysuccinimide (NHS) or 1-hydroxybenzotriazole (HOBt) are also frequently used. The derived active esters present immense reactivity towards the amines [66]. The hydroxyl groups of HA are usually transformed into ester derivatives, by reacting them with the corresponding anhydride [66]. On the other hand, acyl-chloride-activated carboxylate compounds can be grafted through ester bonds [66]. The terminal reducing end of HA, which can respond as an aldehyde group, may be involved so as to achieve a 1:1 stoichiometric ratio between polymer and reacting molecule. This approach encompasses the reductive amination reaction, typically using sodium cyanoborohydride as reducing agent, with an amino group of the reacting molecule. Additionally aldehyde groups may be derived by reaction with sodium periodate, which oxidizes the hydroxyl groups of the glucuronic acid moiety of HA to

dialdehydes, thus opening the sugar ring. Nevertheless, this response results inconsiderable decrease of HA's molecular weight. Again, appreciation goes to the high hydrophilicity of HA, chemical modification can be executed in water; nevertheless, in the aqueous phase, some reactions need acidic or alkaline conditions that may encourage considerable HA chain hydrolysis, or involve the utilization of reagents sensitive to hydrolysis. On the other hand, organic solvents, e.g. dimethylformamide or dimethylsulfoxide, can be used but, in this case, the HA sodium salt must be converted to its acidic form, or to a tetrabutylammonium salt, to make it soluble in organic solvents.

5.11 Chondroitin Sulfate-Based Nanocarriers for Drug/Gene Delivery

Recently, the naturally existing polysaccharides captured a growing amount of focus in the field of drug/gene delivery systems due to their exceptional tendencies, encompassing biocompatibility, biodegradability, non-immunogenicity, extremely low toxicity, and many more [67]. Moreover, the naturally occurring polysaccharides have different reactive groups like carboxyl, hydroxyl and amino groups, which provide the possibility of multiple modifications to polysaccharides. More prominently, polysaccharides are enormously found in nature, e.g., synovial fluid and extracellular matrix (ECM) are chiefly rich in hyaluronic acid and chondroitin sulfate [67]. Thus, the application of polysaccharides and their derivatives with a broad range of molecular weight, varying chemical structures and properties has been extensively spread. Chondroitin sulfate (ChS), a member of glycosaminoglycan family, consists of repeating disaccharide units of β-1,3-linked N-acetyl galactosamine(GalNAc) and b-1,4-linked d-glucuronic acid (GlcA) with certain position(s) sulfated, which has been extensively functional in nano-sized carriers [67]. The inherent excellent characters, biocompatibility, biodegradability, non-immunogenicity, etc., create ChS tremendously popular in terms of a new type material applied in drug/gene delivery systems. As reported earlier, different nanocarriers for drug/gene delivery based on ChS have been fabricated and assessed in terms of their drug-loading capacity, physicochemical characteristics, in vitro toxicity, and a slice of relatively simple in vivo tests.

Owing to the huge sum of reactive groups, ChS could be hydrophobically tailored to acquire a multiple of brush-like grafted amphiphilic copolymers which can self-assemble into nano-sized carriers when dispersed in aqueous medium. Predominantly, ChS is also competent to change formulated nano-vehicles to provide them with special properties e.g. longevity, more stability, and target ability, etc. [67]. There are also some other significant nanocarriers based on ChS with the purpose to advance the pharmacokinetic behaviors and therapy effect of loaded drug/gene(s). Nevertheless, in contrast with some other members of glycosaminoglycan family e.g. heparin, HA and CS, ChS is still in its infancy as carriers for drug/gene delivery. Therefore, it can be forecasted that more nanometric delivery systems based on ChS and a increasing number of ChS derivatives will appear in the

Table 5.4 Various ChS-based carriers and their applications [67]

Nanoplatforms	Main components	Main applications
Nanoparticles ChS,	Selenium	Therapy of Kashin–Beck disease (KBD) and osteoarthritis
Nanoparticles loaded scaffolds ChS,	CS	A promising candidate for dual protein delivery system for tissue engineering applications
Nanoparticles	ChS, CS	A potential new delivery system for the transport of hydrophilic compounds such as proteins
Nanoparticles	ChS, CS	Improving the oral absorption of bovine serum albumin
Hydrogel	ChS, HA	A biocompatible hydrogel system for skin tissue engineering

CS chitosan, *HA* hyaluronic acid

near future [67]. More and more promising advantages of ChS will be explored and utilized as a potential carrier for drug/gene delivery. Moreover, there is an vital requisite for an details of mechanism concerns, including the elimination process of ChS in human body, the specific interaction of ChS with human organs, tissues, cells or even biomolecules [67]. Additionally, the growth of nanocarriers, the ones based on ChS awaits advance researches as well (Table 5.4).

5.12 Nanoengineering of Vaccines Using Natural Polysaccharides

At present, there are more than 70 licensed vaccines, which avert the pathogenesis of around 30 viruses and bacteria. However, there are still significant challenges in this field, which consist of the development of more active, non-invasive, and thermo-resistant vaccines. Significant biotechnological progresses have result in safer subunit antigens, e.g. proteins, peptides, and nucleic acids [68]. Nevertheless, their inadequate immunogenicity has claimed potent adjuvants that can reinforce the immune response. Particulate nanocarriers clutch a high possibility as adjuvants in vaccination [68]. Owing to their pathogen-like size and structure, they can improve immune responses by mimicking the natural infection process. In addition, they can be modified for non-invasive mucosal administration, and control the delivery of the related antigens to particular site and for sustained time period, making an opportunity for the development of single-dose vaccination (Fig. 5.5). In addition, they facilitate co-association of immunostimulatory molecules to develop the complete adjuvant capacity [68]. Features of polysaccharides e.g. natural and ubiquitous, and their intrinsic immunomodulating properties, their biocompatibility, and biodegradability, rationalize their interest in the engineering of nanovaccines. In 1970s, the effort of Kreuter and Speiser explored the approach for

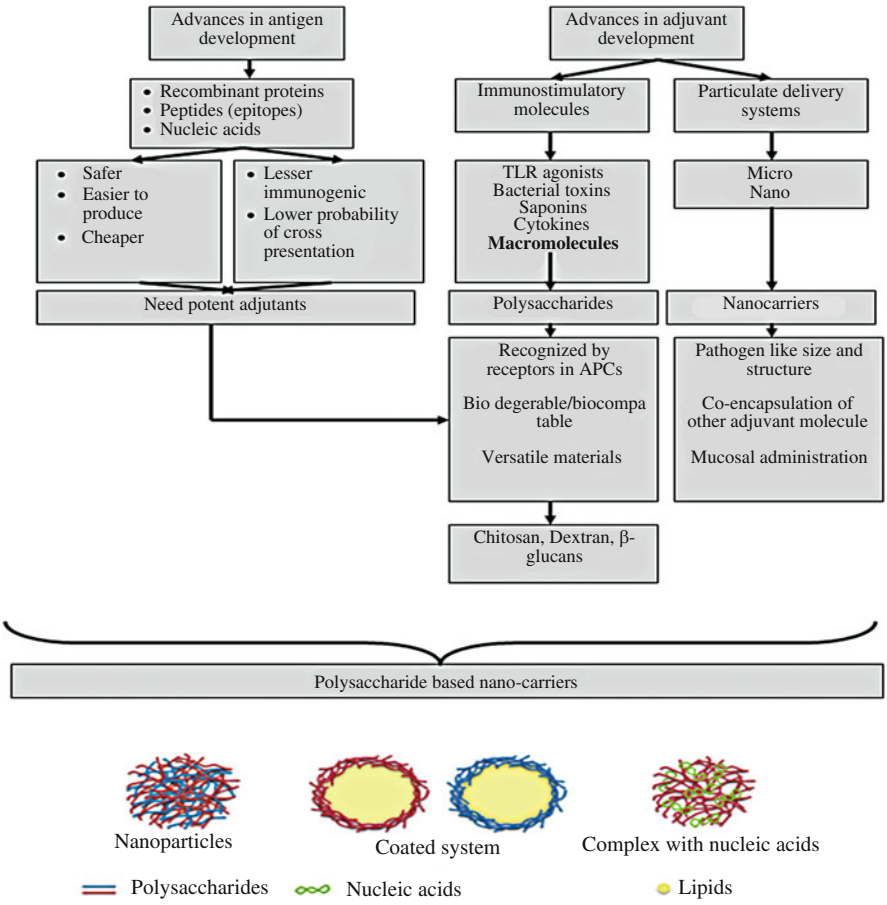

Fig. 5.5 Progresses in biological and microbiological technologies have augmented the information of pathogens and results in the growth of newer and safer subunit antigens. However, these antigens are less efficient in triggering protective immune responses and consequently entail a parallel progress of potent adjuvants e.g. immunomodulating molecules and particulate delivery systems. Among these, polysaccharide-based nanosystems have established potential to be effectively used in vaccine formulations

the specific use of polymers e.g.polymethyl methacrylate, as materials for the production of antigen nanocarriers. Since that period, a considerable number of investigations have put in support of the potential of nanoparticles to augment the immune response against various antigens in a sustained and prolonged way. Recently, encapsulation of model proteins and antigens within poly(lactic-co-glycolic acid) (PLGA) [68] and polylactic acid–polyethylene glycol (PLA–PEG) nanoparticles [68] have been explored which was followed by various researches, whose contributions results in the clinical development of PLGA-based nanovaccines (www.clinicaltrials.gov). From the very beginning this production course, it

became apparent that a major difficulty of this biomaterial was the degradation of the antigen encapsulated in the path of the polymer degradation [68] (Fig. 5.5). While specific formulation approaches were establish to considerably minimize this effect over the encapsulated antigens, on the whole the outcome realized using PLGA based nanoengineering influenced researchers to look for novel biomaterials which might have a gentle interaction with antigens. Naturally occurring polymers, particularly polysaccharides attracted the attention in the mid 1990s as biomaterials for antigen nanoengineering. With this objective, researchers reported the time the production of nanoparticles consisting of assemblies of proteins and chitosan. Following this, various researchers have projected the utilization of polysaccharides, i.e. dextran, mannan and beta glucans for the nanoengineering of vaccines. These final biomaterials are originated from the cell walls of several pathogens such as bacteria or yeast, a feature that offers them with inherent targeting potentials to APCs (acting as PAMPs on the PRRs present in these cells) and, as a result, a normal ability to improve the immune response against the associated antigens [68]. Additional significant characteristics e.g. high biocompatibility and low toxicity create polysaccharides more interesting for pharmaceutical development purposes. Additional merit related to the use of polysaccharide based antigen nanocarriers is associated to the technologies used to produce them [68] (Fig. 5.5). These technologies depend on physicochemical processes such as complexation, ionic gelation, and solvent displacement, among others. These are usually simple techniques, which reduce the utilization of solvents and easy to scale-up, high-energy sources, and significantly, appropriate for the contribution of labile biomolecules. In addition from screening an appropriate technology, other appropriate technical features for the development of nanovaccines, i.e. the stability of the formulation while storage, and the stability of the antigen, in terms of biological activity, are to be acknowledged at early stages of progress. Assortment of raw materials with pharmaceutical quality i.e. produced according to specific criteria that assure their high purity and satisfactory features for use in humans and with superior inter-batch reproducibility, are also key topic to take into concerns in the course of nanovaccine design and manufacturing [68] (Fig. 5.5). However, the possibility of these biomaterials in this area merits a deeper investigation of the available material about polysaccharide-based nanosystems in vaccination.

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Chapter 6
Modern Polysaccharides and Its Current Advancements

Abstract Polysaccharides based nanomaterials have diverse applications in biomedical research. This chapter covers one of the major achievements in modification of polysaccharides using microwave irradiation and cationization methods. Additionally chapter focused on mucoadhesive polysaccharides and its recent advancement in nano drug delivery system. Applications such as gene transfection, bone regeneration and vaccine delivery are also separately discussed.

Keywords Polysaccharides • Drug delivery • Nanoparticle • Mucoadhesive

6.1 Introduction

Natural polysaccharides from various sources have been investigated and extensively utilized in diverse areas, such as food and feed, medicine and pharmaceutics, and in papermaking. Recently, there has been an increased attention in the utilization of polysaccharides, particularly bioactive ones, for various significant applications due to their biocompatibility, biodegradability, non-toxicity, and some specific therapeutic activities. Polysaccharides and their derivatives hold various advantages above the synthetic polymers, since they are non-toxic, biocompatible, biodegradable, and less expensive in comparison to their synthetic counterparts. All these advantages present polysaccharides and their derivatives a broad spectrum of applications in different areas, such as in food, biomedical or pharmaceutical, and cosmetic applications. Currently polysaccharides play significant roles in traditional disease control and health care, in the meantime many new emerging areas are also explored such as in tissue engineering, in wound treatment (both internal and external in drug delivery), diagnosis, in cancer prevention, and therapy, and in treatment of bacterial and viral diseases as already described in the earlier for each polysaccharide and their derivatives following functionalization. Hence, here in this chapter we emphasize the development of bioactive polysaccharides for various biomedical applications as tissue engineering, wound dressing/healing, and drug delivery applications.

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6.2 Polysaccharide Colloidal Particles Delivery Systems

Mucosal delivery of complex molecules especially macromolecules such as proteins, peptides, oligonucleotides, and plasmids is one of the most recent studied subjects. Colloidal carriers made of hydrophilic polysaccharides, i.e. chitosan, have emerged as a promising alternative for improving the delivery of such macromolecules across biological surfaces. Chitosan has been reported to form colloidal particles and entrap macromolecules through various mechanisms such as ionic crosslinking, desolvation, or ionic complexation, nevertheless some of these systems have been appreciated only in conjunction with DNA molecules. An alternative concerning the chemical modification of chitosan has also been valuable for the association of macromolecules to self-assemblies and vesicles [1]. So far, the in vivo efficacy of these chitosan-based colloidal carriers has been investigated for two different applications: while DNA-chitosan hybrid nanospheres were found to be acceptable transfection carriers, ionically crosslinked chitosan nanoparticles appeared to be efficient vehicles for the transport of peptides across the nasal mucosa [1]. Various types of chitosan NPs that are usually employed in the current research for delivery of macromolecules are described in illustration (Fig. 6.1).

6.3 Polysaccharides Scaffolds: for Bone Regeneration

Utilization of natural polymers as structural materials is not new. Nature itself has always used, e.g. chitin as the exoskeleton of several molluscs, keratin for thermoinsulation in hair, cellulose offer the structure of higher plants, silk in spiderwebs

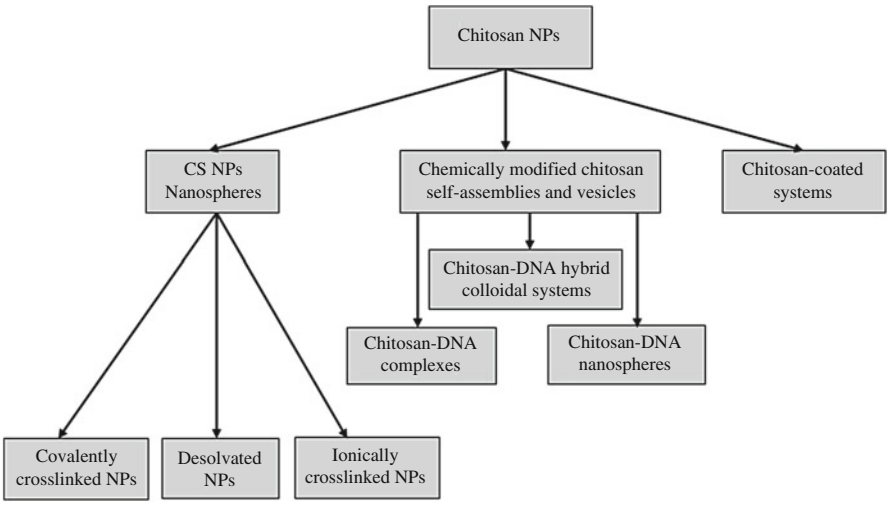

Fig. 6.1 Types of CS-NPs utilized in delivery of macromolecules. *CS* Chitosan, *NPs* nanoparticles

and collagen for mechanical support in connective tissues,. Currently the socioeconomic circumstances of the present world have promoted the interest in these bio-materials. Issues related with environment are playing an important role, contributing to the rising interest in natural polymers due to their biodegradability, low toxicity and low disposal costs. Generally low manufacture costs of biopolymers, associated to their large agricultural availability and renewability, are additional benefits. Additionally, their usefulness of chemical structures and their well-known chemistry facilitate the development of advanced functionalized materials that can match several varied requirements. Moreover, the rapid advancement in understanding of basic biosynthetic pathways through genetic manipulations will offer tailoring of biopolymer structure and function, hence crafting new scopes for these materials [2–4]. In biomedical research, natural polymers degradation under physiological conditions facilitate the production of physiological metabolites which makes them outstanding candidates for a variety of applications, such as drug delivery. Excellent properties of these polysaccharides, which make them the polymer group with the longest and widest medical applications: [5–8]

- Nontoxicity (monomer residues are not hazardous to health),
- Water solubility or
- High swelling ability by simple chemical modification,
- Stability to ph variations
- A broad variety of chemical structures

These versatile features makes these bio-materials able to overcome some disadvantages like proneness to microbial and enzymatic degradation and low mechanical, temperature and chemical stability, which, in some cases, can be used as an benefit.

6.4 Polysaccharides-Based Nanodelivery Systems

Nanoparticle drug delivery systems are nanometeric carriers used to deliver drugs or biomolecules. celles, nanoliposomes, and nanodrugs, etc. [1, 2]. Nanoparticle drug delivery systems have outstanding advantages [1]:

- They can improve the utility of drugs and reduce toxic side effects; etc.
- They can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged;
- They can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lung, spinal cord and lymph
- They could show controlled release properties due to the biodegradability, ph, ion and/or temperature sensibility of materials

Currently, the researches on nanoparticle drug delivery system focus on:

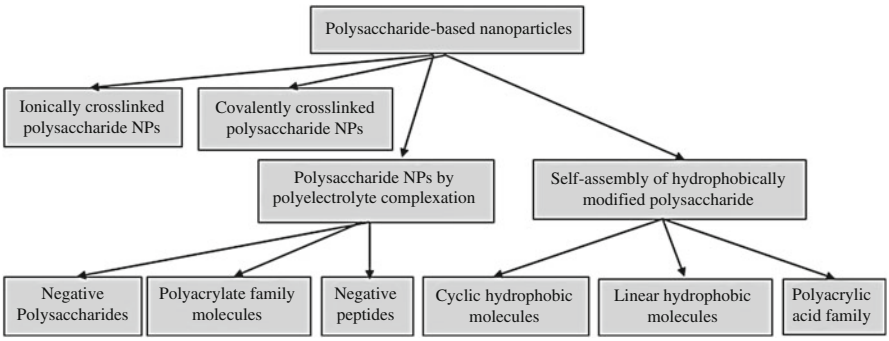

Fig. 6.2 Polysaccharides based nanomaterials

- The investigation of in vivo dynamic process to disclose the interaction of nanoparticles with blood and targeting tissues and organs, etc.
- The optimization of the preparation of nanoparticles to increase their drug delivery capability, their application in clinics and the possibility of industrial production;
- The selectness and combination of carrier materials to obtain suitable drug release speed;
- The surface modification of nanoparticles to improve their targeting ability;

Natural polysaccharides, owing to their exceptional merits, have gained more and more attention in the field of drug delivery systems. Especially, polysaccharides appear to be the most promising materials in the fabrication of nanometeric carriers. Owing to the presence of different derivable groups on molecular chains, polysaccharides can be easily altered chemically and biochemically, ensuing in several types of polysaccharide derivatives. As natural biomaterials, polysaccharides are highly stable, safe, non-toxic, hydrophilic and biodegradable. Moreover, polysaccharides have rich resources in nature and low cost in their processing. Predominantly, majority of natural polysaccharides have hydrophilic groups such as carboxyl, hydroxyl and amino groups, which could form non-covalent bonds with biological tissues, forming bioadhesion [5] e.g. alginate, starch, chitosan and many more are excellent bioadhesive materials. Nanoparticle carriers made of bioadhesive polysaccharides could extend the residence time and consequently enhance the absorbance of loaded drugs. These advantages provide polysaccharides a promising opportunity as biomaterials. For the application of these biomaterials for drug carriers, various concerns of toxicity, safety and availability are greatly simplified. Recently, various reports have been explored on polysaccharides and their derivatives for their potential application as nanoparticle drug delivery systems [4, 6–8]. Polysaccharides based nanomaterials and their main types are illustrated in Fig. 6.2. These natural polysaccharides are having potential applications in modifying the properties of various hydrophobic molecules (Table 6.1). According to structural features, these nanoparticles are fabricated mainly by four different mechanisms, specifically:

Table 6.1 Hydrophobic molecules used to modify polysaccharides

Polysaccharides	Hydrophobic molecules	Ref.
Chitosan	Poly(ethylene glycol)	[9–11]
β-Cyclodextrin	Hexanoic acid	[12]
Hexanoic acid		
Chitosan	Linoleic acid	[13]
Chitosan	Palmitic acid	[14]
Chitosan	Palmitic acid	[14]
Chitosan	Oleic acid	[15]
Dextran chitosan	Poly(ε-caprolactone)	[16]
Heparin, Hyaluronic acid	Pluronic	[17], [18]
Pullulan	Hexadecanol	[19]
Chitosan Carboxymethyl chitosan Pullulan	Cholesterol	[20, 21]
Chitosan heparin Glycol chitosan	Deoxycholic acid	[22–24]
Glycol chitosan	5β-Cholanic acid	[25]
Glycol chitosan	Fluorescein isothiocyanate (FITC)	[26]
Glycol chitosan	Doxorubicin	[26]
Pullulan	Vitamin H	[27]
Glycol chitosan	N-Acetyl histidine	[28]
Heparin Dextran	Poly(methyl methacrylate)	[29]
Chitosan	Poly(isobutyl Cyanoacrylate)	[30–33]
Dextran		
Dextran sulfate		
Thiolated chitosan		
Heparin		
Hyaluronic acid		
Pectin		

- Covalent crosslinking
- Ionic crosslinking
- Polyelectrolyte complexation
- Self-assembly of hydrophobically modified polysaccharides

6.5 Polysaccharides and Its Recent Advances In Delivering

Colon specific delivery gained increasing significance for the management colonic diseases, such as colorectal amebiasis, ulcerative colitis, Crohn's disease and cancer [34]. Various approaches are used for targeting drugs to the colon include enzymatically degradable polymers:

Table 6.2 Colon s targeting polysaccharides

Polysaccride	Sources	Structural units	Ref
Hyaluronic acid	Animal (synovial fluid, vitreous humour of the eye, umbilical tissue; microbial (fermentation Bacillus subtilis)	**β**-glucuronic acid and N-acetyl-**β**-glucosamine (GlcNAc) linked by **α**-(1/3) bond.	[35]
Dextran	Microbial (bacterium Leuconostoc mesenteroides)	**α**-(1/6)-linked d-glucose residues with some degree of branching via **α**-(1/3) linkages.	[36]
Gellan gum as a repeating unit	Microbial (bacterium Sphingomonas elodea)	Tetrasaccharide, (1/4)-Lrhamnose--(1/3)-**β**-glucose--(1/4)-**β**-glucuronic acid--(1/4)-d-glucose	[37]
Pullulan	Microbial (fungus Aureobasidium pullulans)	Maltotriose (-(1/4)-linked) joined by -(1/6) linkages	[38]
Chitin	Animal (crustacean shells, exoskeletons of insects and other arthropods); microbial (fungal cell walls)	**α**-(1/4)-linked N-acetyl-d-glucosamine residues.	[39]

- Osmotically controlled and pressure-controlled drug delivery systems.
- Prodrug based strategy, coating with time or pH-dependent polymers.

Polysaccharides that are accurately triggered by the physiological environment of the colon hold great promise, since they offer better site specificity and meet the preferred therapeutic requirements. The colon specific delivery systems based on a single polysaccharide do not competently allow targeted release [34]. The transit time and pH can differ depending upon the individual and the particular disease state. The conventional strategies present premature drug release [34]. Drug release can be premature or even non-existent in these cases. Therefore combination/chemically modified forms of polysaccharides eliminated the shortcomings linked with the use of single polysaccharide. The industrial scientists are going on with the use of mixtures of polysaccharide and their structurally/chemically modified forms (Table 6.2).

6.6 Unexplored Potentials of Polysaccharide Composites

Composites made exclusively from polysaccharides are typically natural as they can degrade without leaving behind ecologically harmful end products, in comparison with composites which contain synthetic polymers. Here, the subsequent groups of all-polysaccharide composites (APCs) are mentioned:

- An all-cellulose group that includes cotton composites
- Cellulose combined with other polysaccharides
- As well as those based on chitin/chitosan, heparin, hyaluronan, xylan, glucomannan, pectin, xyloglucan, arabinan, starch, carrageenan, alginate, galactan as one of the components in combination with other polysaccharides.

Table 6.3 List of well known polysaccharide composites

Polysaccharide composites
• Alginate-based composites
• All-cellulose and cotton related composites
• Arabinan-based composites
• Chitin/chitosan-based composites
• Composites made by combining cellulose with other polysaccharides
• Glucomannan-based composites
• Heparin-based composites
• Hyaluronan-based composites
• Pectin-based composites
• Starch-based composites
• Xylan-based composites
• Xyloglucan-based composites Galactan-based composites

They can be employed in medical, packing, paper, food, mechanical engineering, textile, electronic and other applications. The composites were evaluated for crystallinity, rheology absorptivity, biodegradability and mechanical, gelling, pasting, film-forming, adhesive, antimicrobial properties, optical, separation, as well as water repellency, dye uptake, water vapor permeability, and fire-retardancy. In addition to food applications, composites based on more than two types of polysaccharides have rarely been used and many possible combinations remain unexplored (Table 6.3).

6.7 Use of Microwave Irradiation in the Grafting Modification of the Polysaccharides

Natural polysaccharides are the renewable supply for fabricating high performance macromolecular materials. The most versatile, accepted, and suitable route to develop polysaccharide based materials is the grafting of synthetic polymers onto natural polysaccharides. In addition to the striking chemical and physical properties of polysaccharide based copolymeric materials, undesired homopolymer formation in the simultaneous opposing reaction decreases the copolymer yield, posing problems in the commercialization of the grafting protocols. This requires modification of polysaccharide which can improve the properties of natural polymers. Modification of polysaccharide materials is often carried out through derivatization of functional groups [40, 41], grafting of polymeric chains [42, 43] and by oxidative [44] or hydrolytic [45] degradation. Additionally, the prerequisite for an inert atmosphere is a further shortcoming for various conventional grafting protocols. Application of microwave irradiation has been exploited from many years to improve these drawbacks in the production of a range of graft modified polysaccharide materials. In fact, elevating interest in clean and green environment

friendly chemistry has encouraged the utilization of microwaves in the polysaccharide grafting modification for various applications. Microwave irradiation considerably minimizes the utilization of toxic solvents and the reaction time for almost all the grafting reactions of interest here. This ensures high yields, product selectivity and clean product formations. In addition, in various examples microwave synthesized polysaccharide copolymers demonstrate improved properties for commercial exploitation than their conventionally synthesized counterparts. The grafting strategy is a technique to manipulate natural polysaccharides, with the precise control over the graft polymer under microwave irradiation. Among various modifications methods this can be a powerful strategy for the development of valuable derivatives with tailor made properties. It has been observed that the properties of microwave synthesized graft polysaccharides are usually better in contrast to the derivatives synthesized conventionally. The method offers many advantages (Fig. 6.3) such as:

- Short reaction times
- Solvent less or aqueous working conditions,
- Fast and homogeneous heating
- The possible high-temperature chemistry with modified selectivity
- It also offers an alternative environment friendly, cleaner, greener approach resulting in greater control
- Higher reproducibility of percentage grafting in the final product,
- Suitable for commercial mass production

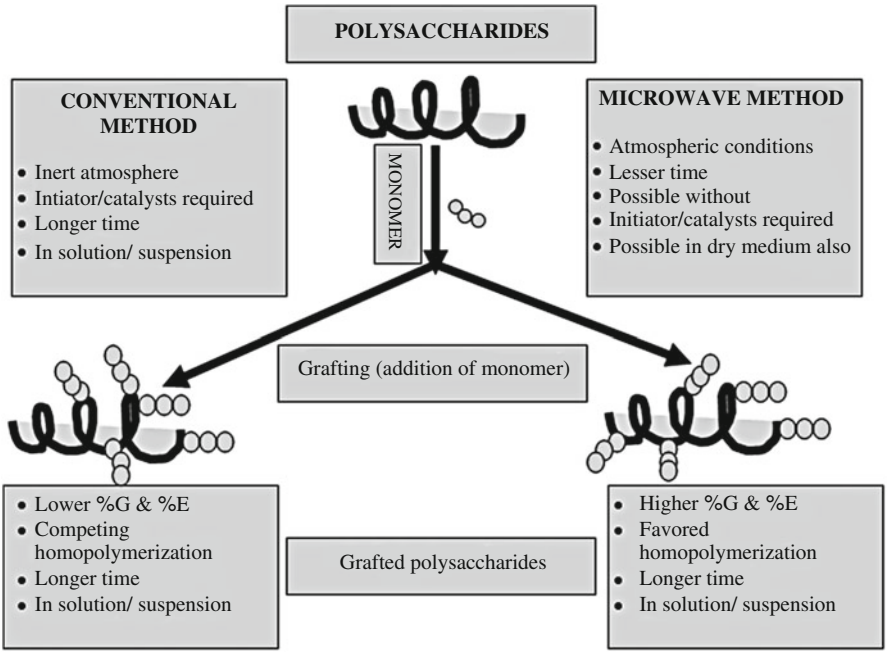

Fig. 6.3 Representation of microwave induced grafting of the polysaccharides

There are some considerable limitations (mentioned below) which require more research in this area, to derive maximum benefits of microwave technology, which is gaining popularity worldwide.

- Require a high degree of control over the properties, though further optimization of the reaction conditions can lead to improved efficiencies.
- Industrial dedicated microwave reactors have an edge over domestic units as the grafting efficiency
- High equipment costs. While prices for dedicated microwave reactors for organic synthesis may have come down considerably in the past decade, the current price range is still many times higher than that of conventional heating equipment,
- One possible difficulty with the commercialization of microwave-assisted synthesis of polymers could be the scale-up, as higher energy input is required for larger quantities.

6.8 Cationization of Polysaccharides for Promoting Greener Derivatives with Many Commercial Applications

Cationic polysaccharides are extensively utilized in various areas e.g. water treatment, chemical, food, papermaking, cosmetic, and petroleum industries [43]. The combination of cationic polysaccharides with anionic polymers can results in production interpolyelectrolyte complexes with hydrogel-like structures which may further expanding the application of the former. Polysaccharides such as dextran, cellulose, hemicellulose, pectin, starch, chitosan and its derivatives, and seaweed polysaccharides are considered. Cationized polysaccharides can be fabricated by reaction with various reagents [43]. Primary objective is on the substitution with dialkylamino hydroxypropyl and trialkylammonium hydroxypropyl ethers. This is the most frequent modifications which involves the introduction of the 2- hydroxy-3-(trimethylammonium)propyl group by reaction of the polysaccharide with 2,3-epoxypropyltrimethylammonium chloride in an alkaline solution. Optional method involves production of the reagent in situ from 3-chloro-2-hydroxypropyltrimethylammonium chloride [43]. Moreover, polysaccharides substituted with other type of cationic groups and amphoteric derivatives are offered. Various toxicological investigations, analysis methods, and applications of the modified polymers are also included. Schematic representation of cationization of polysaccharides is mentioned in Fig. 6.4.

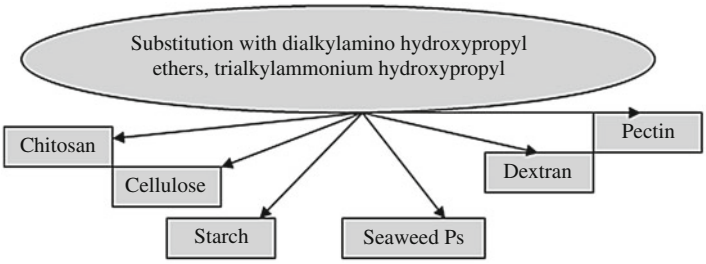

Fig. 6.4 Cationization of polysaccharides

6.9 What Could Be Greener Than Composites Made from Polysaccharides?

Composites fabricated from various polysaccharides offer a new class of environmentally safe materials for applications to explore [44]. Till date synthetic polymers are principally used for composite fabrication earlier to polysaccharides. It is linked to ecological difficulties owing to utilization of organic solvents in the course as well as owing to the crisis of petrochemical industry-related plastics [44]. Materials fabricated by this means are not biodegradable and their production costs are high. If the constituents of composites could be solubilized and chemically bonded than the formed insoluble composite could be fabricated from a single type of polysaccharide. Amalgamation of polysaccharides that are isolated from distinct sources is suggested. Cyclodextrin-based composites are also acknowledged as part of polysaccharide-composite family. There is a requirement to compare characteristics of composites with varying polysaccharide composition to make a best choice. Cost related part also speaks for the alternative of economic polysaccharides to attain similar properties in regard to the more expensive possibilities of composite production. Foams, gels, films, drug components, artificial tissues, building materials or components for civil engineering, medical, paper or food applications, are potential end products without environmental disadvantages [44].

6.10 The Use of Mucoadhesive Polymers in Buccal Drug Delivery

Buccal administration of the preferred drug by means of mucoadhesive polymers has been the subject of interest since the early 1980s. Merits associated with buccal drug delivery have made this route of administration functional for different drugs. Usually, a number of the essential structural features for bioadhesive polymers include strong hydrogen bonding groups, strong anionic or cationic charges, high molecular weight, chain flexibility, and surface energy properties favoring spreading on a mucus layer [45].

6.10.1 New Generation of Mucoadhesive Polymers

Recent report classified bioadhesive polymers as first generation and second generation polymers [45]. Older generation mucoadhesive polymers also known as off-the shelf "polymers as they, lack specificity and targeting capability. These polymers stick to the mucus non-specifically, and undergo short retention times owing to the turnover rate of the mucus. Non-covalent type of interaction takes place between mucoadhesive polymers and the mucus or tissue surfaces. Nevertheless, newer polymers are accomplished in forming covalent bonds with the mucus and the underlying cell layers., thus show improved chemical interactions. On the other side new generation of mucoadhesives (with the exception of thiolated polymers) can adhere directly to the cell surface, rather than to mucus. These mucoadhesives interact with the cell surface by means of specific receptors or covalent bonding instead of non-specific mechanisms (which are characteristic of the older polymers). The examples of most developing and recent bioadhesive polymers are the incorporation of l-cysteine into thiolated polymers and the target-specific, lectinmediated adhesive polymers. These categories of polymers hold promise for the delivery of a extensive variety of new drug molecules, specifically macromolecules, and create new possibilities for more specific drug–receptor interactions and improved targeted drug delivery.

6.10.2 Thiolated Mucoadhesive Polymers

Thiolated mucoadhesive polymers are synthesized by a covalent attachment between a cysteine (Cys) residue and a polymer of choice, such as polycarbophil [46], poly(acrylic acid) [47], and chitosan [48]. These polymers are known as a new generation of mucoadhesive polymers. These moderen class of polymers contain a carbodiimide-mediated thiol bond which exhibit much improved bioadhesive properties.

6.10.3 Target-Specific, Lectin-Mediated Bioadhesive Polymers

Opportunity of synthesizing a bioadhesive polymer to selectively create specific molecular interactions with a specific target (such as a receptor on the cell membrane of a specific tissue) is a very attractive potential for targeted delivery (Fig. 6.5). The potential of a specific receptor–bioadhesive polymer interaction can circumvent the limiting factors of rapid mucus turnover and short residence time. In contrast with general mucoadhesive polymers (which bind to the mucosal surface universally) a specific receptor mediated interaction with the mucosal surface could permit for direct binding to the cell surface, rather than only the mucus

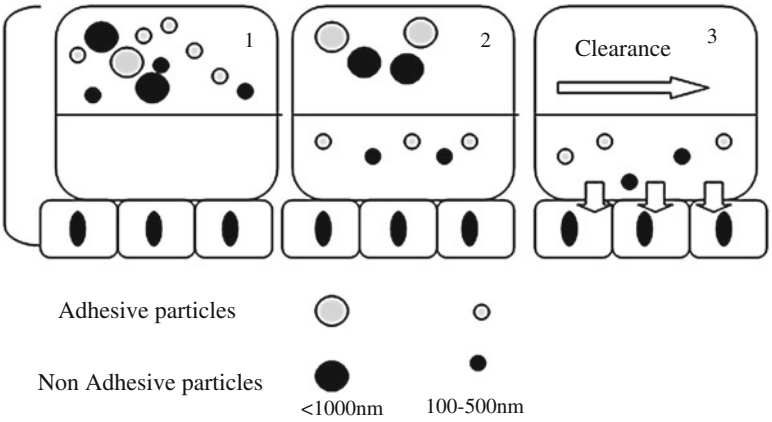

Fig. 6.5 Plan representation of the interactions of various types of particles with the mucus layer. (1) After initial administration/arrival to the mucosal site, particles will interact and diffuse via the mucus in a different way according to their adhesive features. (2) Larger particles are not capable to diffuse via the mucus layer owing to steric hindrance, but can interact with the luminal layers in cases where adhesive bonding with mucin chains (in gray) can be recognized. Since for smaller particles, these can diffuse via the mucus layer depending on adhesive features: diffusion of adhesive NPs is slower as these will be hold on to particularly at the luminal/external layers of mucus due to interaction with mucin; in the case of non-adhesive NPs, systems with diameters around 200–500 nm can diffuse quickly and reach the epithelial lining, while smaller ones experience decreased diffusion rates, most probably because of retention in "dead end" pockets of the mucin mesh. (3) In the lead natural mechanisms of clearance, which are typically felt at the luminal side of mucus, particles are gradually eliminated from the mucosal site while NPs that have arrived at the epithelial cell lining can further experience cell uptake or tissue penetration. Legend: A—mucosal tissue lumen/external environment; B—mucus layer; C—epithelial cell lining

layer. Specific proteins or glycoproteins (which can potentially to bind certain sugars on the cell membrane) can enhance bioadhesion and potentially improve drug delivery via specific binding. In addition these proteins can also increase the residence time of the dosage form and this type of bioadhesion termed as cytoadhesion [49] (Table 6.4).

6.10.4 Mucoadhesive Polssacharides in the Design of Nano-Drug Delivery Systems for Non-Parenteral Administration

The occurrence of a mucus layer that present at the surface of various organs has been exploited to develop mucoadhesive dosage forms. These layers act as administration site to prolong time, and increase the local and/or systemic bioavailability of the administered drug [58]. The appearance of micro and nanotechnologies simultaneously with the execution of non-invasive and painless administration routes has transforms the pharmaceutical market and the management of disease. Intending to minimize the chief limitations of the oral route and

Table 6.4 Mucoadhesive polymers in buccal drug delivery

Active ingredient	Polymers used	Ref
Acyclovir	Chitosan HCl and PAA sodium salt	[50]
Chitosan	Chitosan	[51]
Chlorhexidine digluconate	Chitosan	[52]
Insulin	Gelatin and CP 934P	[53]
Thiocolchicoside	Gelatin and CMC	[54]
Tetracycline	Atelocollagen	[55]
Nifedipine or Propranolol HCl	Chitosan with or without an anionic crosslinking polymer (PC, sodium alginate, gellan gum)	[56]
Insulin	Gelatin and CP 934P	[57]
Chitosan and PVP	Glibenclamide	[49]

to maintain patient acquiescence high, the manufacturing of innovative drug delivery systems administrable by mucosal routes has come to light and gained the attention of the scientific community owing to the possibility to considerably change pharmacokinetics [58]. Moreover, to attain the aim of mucosal drug administration, the production of biomaterials has been refined to fit specific applications. Table 6.5 describes the list of potential biomaterials explored as nano-drug delivery systems for mucosal administration by diverse non-parenteral routes (e.g., oral, inhalatory, etc.).

6.11 Polysaccharide Based Gene Transfection Agents

Gene delivery is an excellent technique that involves in vitro or in vivo introduction of exogenous genes. These genes are introduced into cells for experimental and therapeutic purposes. Optimal gene delivery depends on the development of efficient and safe delivery vectors. There are two prominent delivery systems, viral and non-viral gene carriers, are at present deployed for gene therapy. As majority of present gene therapy clinical trials are based on viral approaches, non-viral gene medicines have also appeared as potentially safe and successful for the treatment of a wide variety of genetic and acquired diseases. Non-viral technologies involve plasmid-based expression systems. This contains a gene linked with the synthetic gene delivery vector. Polysaccharides accumulate a large family of heterogenic sequences of monomers with various applications and various benefits as gene delivery agents. Current research progress in polysaccharide based gene delivery is based on the recent developments of polysaccharide employed for in vitro and in vivo delivery of therapeutically important nucleotides, e.g. plasmid DNA and small interfering RNA. Polysaccharides can also offered a stable drug and gene delivery platform [58]. Cationic polysaccharides are non-toxic, biodegradable and biocompatible materials. They are particularly appropriate for transfection and biological uses as they are water soluble and can be readily transported to cells in vivo. Therefore these polysaccharides act as effective vehicles

Table 6.5 List of mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes [58, 59]

Mucoadhesive polymers
Natural polymers
• Alginate
• Chitosan
• Thiolated chitosan
• O-Carboxymethyl chitosan
• N-trimethyl chitosan
• N-carboxymethyl chitosan
• Guar gum, xanthan gum and pectin
• Galactomannan and glucomannan
• Carrageenan-type II
• Hyaluronic acid and other glycosaminoglycans
• Gelatin
Synthetic polymers
• Poly(ethylene glycol) and poly(ethylene oxide) and its copolymers
• Poly(acrylic acid) and poly(methacrylic acid) derivatives
• Poly(vinyl pyrrolidone)
• Poly(vinyl amine)
• Boronate-containing polymers
Semi-synthetic polymers
• Cellulose derivatives

for delivering agents complexed with them [58]. Most of the cationic polysaccharides employed for gene delivery purposes are either natural or semisynthetic in origin. Semisynthetic cationic polysaccharides are fabricated by the conjugation of different oligoamines to oxidized polysaccharides e.g. polycations of dextran, pullulan and arabinogalactan grafted with oligoamines of 2–4 amino groups were also investigated and were found to be effective in gene delivery [58]. One of the most related features of this type of carrier is that the polysaccharide hydroxyl groups can be easily modified and possibly the presence of sugar-recognition receptors on the cell surface can assist internalization [58] (Fig. 6.6).

6.12 Polymeric Micro/Nanoparticles: Particle Design and Potential Vaccine Delivery Applications

Particle based adjuvant endow hopeful signs on transporting antigen to immune cells and behaving as stimulators to elicit preventive or therapeutic response. Nevertheless, the wide size distribution of existing polymeric particles has so far masked the immunostimulative effects of particle adjuvant, and compromised the development in pharmacological researches [60]. To overcome this obstacle, Yue et al. has conceded out a series of research activities regarding the particulate

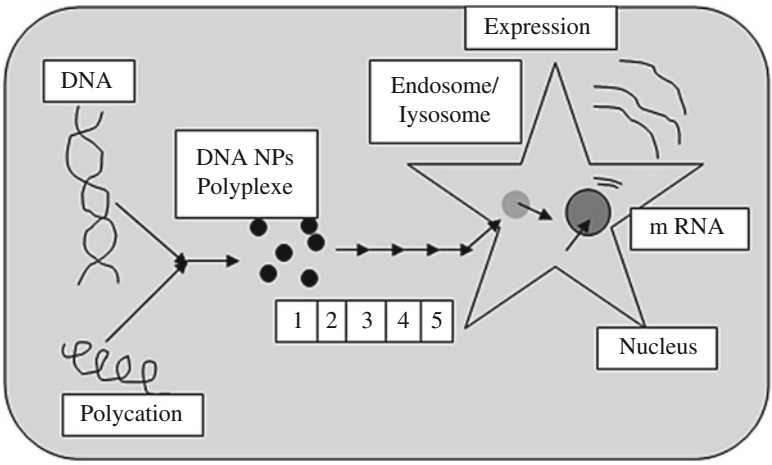

Fig. 6.6 Mechanism for targeted delivery of nucleotides using cationic polymers and different cellular barriers for in vitro gene delivery, 1: interaction of DNA nanoparticle with targeted DNA; 2: entry in to the cell; 3: escape from the endosome; 4: dissociation of DNA nanoparticle; 5: nuclear transport

vaccine, by taking benefit of the successful fabrication of polymeric particles with uniform size. In this investigation Yue et al. demonstrated the insight and practical development focused on the effects of physiochemical property and antigen loading mode on the resultant biological/immunological outcome. With the help of a unique microporous membrane emulsification technique, Yue et al. fabricated particles with uniform and controllable size with good reproducibility. This research offers roadway for further investigation on biological/immunological response. Through these particles, the influence of a single property (e.g. size, charge, shape) can be explained, and the influence of other factors is reduced to guarantee reliable results. Attractive advantages of successful exploration of particle-bio interaction, positive charge, smaller size, hydrophobic surface, rod shape, specific chemical component were implicated in the active immune response. Based on the understanding, particles with high optimized attributes and antigen payload could be designed for expected adjuvant purpose, resulting in the development of high efficient vaccine candidates [60].

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Chapter 7
Toxicity of Nanodrug Delivery Systems

Abstract The study of the potential toxicity of NPs is essential for the safe development of these vehicles. The lack of strategies, regulatory requirements, and validated protocols, along with the particular physical, chemical and biological properties of these materials, makes the evaluation of the preclinical toxicity very complex. Taking into consideration the outcomes of the toxicological investigations reviewed here, it seems that the toxicity of these nanocarriers is quite low. Most of the in vivo toxicological investigations did not demonstrate effects at very high doses whereas in vitro studies showed some toxic effects. However, more in vitro and in vivo toxicity assessments are required to reach a apparent conclusion about the potential toxic effect of these nanocarriers; the progress of validated protocols and the performance of chronic studies are of immense importance.

Keywords Nanoscience • Nano-toxicity • Drug delivery • Nanocarriers

7.1 Introduction

Nanoscience has been variously explained at different books, journals, fora, and the web, however one thing is universal; it engages the investigation of the control of matter on an atomic and molecular scale. Generally, a nanometer is one billionth of a meter and the properties of materials at this atomic or subatomic level vary considerably from properties of the same materials at larger sizes. Nevertheless, the primary features of nanomaterials studied were for its electrical, magnetic, physical, mechanical, chemical and biological applications, recently, focus has been geared towards its pharmaceutical application, particularly in the field of drug delivery. This is due to the confronts with utilization of large size materials in drug delivery, some of which include in vivo stability, poor bioavailability, solubility, intestinal absorption, therapeutic effectiveness, sustained and targeted delivery to site of action, generalized side effects, and plasma fluctuations of drugs. Recently, different workers in nanotechnology science have been

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designed to overcome these confronts through the development and fabrication of nanostructures. It has been studied that, nanoparticles have the capability to shield drugs from the degradation in the gastrointestinal tract. This technology can enables target delivery of drugs to various areas of the body. Additionally this technology allows the delivery of drugs that are poorly water soluble and can provide means of bypassing the liver, thereby preventing the first pass metabolism. Regardless of the immense potentials of nano drug delivery systems in revolutionalizing patient management, its safety in humans is of great concern. It has been investigated that, smaller nanoparticles show increased toxicity due to their increased surface area [1]. For an instance, reports have shown that nanotubes are cytotoxic and induce granulomas in lungs of laboratory animals. Metallic nanoparticles such as cobalt, titanium copper, and silicon and their oxides have also been reported to have inflammatory and toxic effects on cells [1]. Additionally metallic nanoparticles e.g. titanium oxide nanoparticles have been shown to stimulate chromosomal aberrations and DNA damage, whereas hydroxyapatite nanoparticles, a substance closely related to the mineral component of bones and teeth, were reported to induce cell death [1].

7.2 Nanotoxicology

Nanotechnology is one of the most pioneering areas of research, which can find application in approximately all necessary aspects of our life. Therefore, the development of novel nanomaterials is growing considerably and many products based on nanotechnology are already on the market. Nevertheless, the achievements of nanotechnology have not been associated with a parallel progress of sufficient methods to assess and manage the possible risks for humans. Incidentally, it has been criticized that “a novle technology will only be successful if those encouraging it can demonstrate that it is safe, but history is littered with instances of promising technologies that never satisfied the exact potential and/or caused countless damage since initial warnings were ignored” [2]. Earlier it has been extensively demonstrated that through the application of nanotechnology in health care (Nanomedicine) it is possible to develop conventional medical therapies and diagnosis for a variety of pathological conditions. Nevertheless, the same features determining their effectiveness in the host (targeting and controlled release properties) and making NPs so striking in medicine, may contribute to toxicological issues. Therefore, to utilize the complete potential of NPs in nanomedicine, particular consideration must be paid to safety and toxicological issue [3]. In this regard, nanotoxicology has raised as a multidisciplinary field to correctly review the safety of NPs. So far, most nanotoxicological research has concerned a restricted group of engineered NPs, chiefly inorganic nanomaterials. Dendrimers, polymeric NPs, liposomes, and other nanocarriers toxicityhas been studied to a smaller extent, even though they are the most capable devices in medicine. This circumstance is not unexpected since

there is substantial information about the toxicological profile of various "organic" materials (e.g., polymers, proteins, lipids, polysaccharides) utilized to fabricate these nanocarriers that, in various cases, are also utilized in other healthcare and pharmaceutical products. Hence, these "organic" NPs have been considered safe and not much consideration has been paid to their toxicity based on the safety of their bulk material. Nevertheless, the features and behavior at the "nano" level are expected to vary significantly in contrast with the similar material used in a macroscopic dosage form and, therefore, the toxicological way has to be significantly different from the classical approach to address adverse health effects. In this regard, to accurately examine nanoparticle toxicity two concepts should be highlighted:

- NPs are developed for their particular properties in contrast with bulk materials. Since the surface of NPs is in direct contact with the body tissue and thus evaluate its response, the exceptional surface features of NPs require to be examined from a toxicological viewpoint.
- Nano-sized particles have qualitatively different physicochemical characteristics in comparison with micron-sized particles. Owing to this, NPs might demonstrate unforeseen distribution within the body, which can lead to undesirable results e.g. crossing the blood barrier and activating blood coagulation pathways. Considering this fact, both pharmacokinetics and distribution of NPs should be examined carefully. Indeed, associated to NP biodistribution, there is a lack of fundamental knowledge about the biological response of NPs at both organ and cellular levels [4].

Specific consideration should be dedicated to the toxicity of non-drugloaded NPs, mainly in the case of slowly degradable or nondegradable NPs employed for drug delivery. For nanodevices employed in medicine the primary focus in most of the research papers is mostly on the reduction of toxicity of the incorporated drug, while the likely toxicity of the carrier used is not measured. Following the delivery of the cargo, NPs' residual components may accumulate in the body causing possible side effects or toxicities. In addition, not only NPs determination but also their size should be considered as toxicity risk factors. In this concern, Müller et al. have projected a nanotoxicological classification system (NCS) [5], based on the structure of the Biopharmaceutics Classification System (BCS), that would be valuable as a initial approach, because it permits a obvious and basic explanation of likely toxicological risks of NPs used in medicine. Nevertheless, additional physicochemical properties also exert an influence on the overall adverse effects in cells and tissues [5]. Various in vitro/vivo toxicities investigations on polymeric nanoparticles is mentioned in Table 7.1.

Table 7.1 In vitro/vivo toxicities studies on polymeric nanoparticles

Nanoparticles	Therapeutic field/drug	Observations	Reference
In vitro/vivo toxicities studies of polymeric nanoparticles			
CS	Infections/ceftriaxone sodium	2 and 4 h incubation: loaded-CS-NP non-cytotoxic	[6]
		24 h incubation: loaded-CS-NP effect-cytotoxic in	
		Caco2 and in J774.2 (1.8 and 0.72 mg/mL, respectively)	
CS	Diabetes/insulin	Loaded-NP non-cytotoxic (50, 100 and 250 mg/mL)	[7]
		HT-29: Loaded-NP (50, 100 and 250 mg/mL) non-cytotoxic (3 h incubation)	
LSC-CS	Diabetes/insulin	Loaded-LSC2-CS-NP non-cytotoxic	[8]
O-CMC	Infections/tetracycline	In all cell lines and concentration tested: Loaded-NP non-cytotoxic	[9]
		No alteration of cell morphology	
O-CMC	Infections/tetracycline	Mild hemolysis (8 %, 18 % for 45.90 lg/mL respectively)	[10]
		No significant erythrocyte lysis (<5 %)	
TMC-Cys-CS	Diabetes/insulin	Loaded-TMC-Cys-CS-NP (30, 200 and 500 kDa) non-cytotoxic	[11]
Dex/sulfate-CS	Empty NP	Dex/sulfate-CS-NP (ratio 1:5:1) non-cytotoxic in both line cells	[12]
Toxicity results from in vivo studies of polymeric nanoparticles			
CS-c-PGA	Diabetes/insulin	No significant differences in clinical signs and body weight compared to control	[13]
		Normal hematological and biochemical values	
PLGA-CS + PEG	Tuberculosis/anti-tuberculosis drugs	Not significant effect in the immune response	[14]
	Empty NP		

CS chitosan, *HPCD* hydroxy propyl-b-cyclodextrine, *DEX* dextran, *MNBNCs* micronucleated binucleated cells, *LSC-CS* lauryl succinyl chitosan, *NP* nanoparticles, *O-CMCS* O-carboxymethyl chitosan, *PEG* polyethylene glycol, *PLA* poly(lactic acid), *PLGA* poly(lactide-co-glycolide), *PLGA–PEO* poly-lactide-co-glycolic acid–polyethylene oxide copolymer, *TMC-Cys* CS thiolated trimethyl chitosan, *Tmx* tamoxifen. *CS*-c-*PGA* poly-c-glutamic-chitosan

7.3 In Vitro and In Vivo Tests to Assess Oral Nanocarriers Toxicity

Preclinical toxicology assessment is an essential step in the growth of novel pharmaceutical drugs. In vitro and in vivo toxicology examinations facilitate the safety of medicaments before their administration to humans. A series of guidelines to achieve safe medicines developed at the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH; http://www.ich.org/) [15], containing approaches which include and combine in vitro and in vivo assessment, to execute an integral estimation of various toxicological aspects. They propose following the guidelines developed by the Organisation for Economic Co-operation and Development (OECD) to perform the various in vitro and in vivo examinations [16]. The OECD guidelines are developed after a accurate validation of each assay and the validation trials are usually harmonized by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM) and/or similar organizations in different countries. So far, there have been no regulatory necessities to check the preclinical toxicity of nanocarriers that will be administered in corresponding with the drug/medicament and can also exert a harmful effect. Additionally, owing to the particular chemical, physical and biological properties of these materials, it is not clear enough whether the ICH strategies and the OECD guidelines are appropriate to examine their possible hazard. Regulatory bodies, industry and researchers are putting various efforts in rising strategies and protocols to assess the toxicity of nanocarriers in a more consistent way. Prominent setback in the development of guidelines is the reality that different nanocarriers can offer very different physical, chemical and biological properties. Until now, majority of the investigations performed with NPs developed for oral delivery purposes have been concentrated on enhancing the ability of the loaded drug to bypass through the epithelial membrane and, therefore, encourage its efficiency when delivered orally. In various cases, modifications of intestinal membrane permeability that might entail potential accumulation of the nanocarriers in the body, and significant adverse effects, have been underestimated. On the other side, only in vitro tests have been performed to examine the toxicity of the nanocarrier. However, to assess NPs toxicity, in vitro models are not enough to forecast potential hazards to humans and to bring on successful evolution through clinical trials to the markets [17]. Hence, information obtained for in vitro studies need verification from in vivo assays to precisely assess nano toxicity. Currently, toxicologists are applying the conventional in vitro and in vivo tests to achieve the toxicological evaluation of nanocarriers. In vitro and in vivo considerations to evaluate oral nanocarriers toxicity (Table 7.2).

Table 7.2 In vitro and in vivo parameters to assess oral nanocarriers toxicity

Parameters	Description	Ref
Cytotoxicity studies	In vitro tests based on cell culture techniques, though with various restrictions as models for the behavior of cells in an organism, are very functional in the screening of NPs and in mechanistic assays; they are comparatively economical and can have a highthroughput	[19]
Cell models	There are enormous assortments of well-known cell lines derived from different human tissues that keep some of the original characteristics. A number of them even show the possibility of differentiation, by means of specific cell culture conditions, to better exhibit the characteristics of the organ. Additionally, 3D cell cultures or co-cultures have also been developed in effort to mimic the target organ	[19]
Assays	Various in vitro assays, with diverse toxicological endpoints, have been projected to evaluate the adverse effect that NPs may provoke on organs of the human body. Viability assays detect whether cells are dead or alive, generally by evaluating the cells' capability to multiply or to form clones. Cytotoxicity assays examine the consequence of the NPs at different levels within the cell such as membrane integrity (e.g. lactate dehydrogenase (LDH) leakage, or oxidative status (e.g. 2070-dichlorofluorescein diacetate (DCFA) assay to detect reactive oxygen species), trypan blue uptake), metabolic activity (e.g. 3-(4,5-dimethylthia zol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, adenosine triphosphate (ATP) detection assay)	[7, 9, 18]
In vivo assessment of nanoparticle toxicity	Currently, in vitro models, though giving very value data, are inadequate to predict potential hazards to humans and to rationalize transition through clinical trials to the market. In vitro approaches yield incomplete information and they do not symbolize a realistic model of how NPs will interact with a specific organ of the body. For an instance, if the toxic effects of NPs are connected to inflammation, simple in vitro assays may not be sufficient for examining the toxic potential. Hence currently information, obtained for in vitro studies involve verification from in vivo experiments to precisely evaluate nanotoxicity	[17]

7.4 Toxicity of Nanocarriers for Oral Delivery

In contrast to the fact that polymeric nanocarriers may offer a number of distinct advantages over microdevices or macroscopic drug delivery systems, including the capability to reach some specific areas or improve the intracellular delivery of macromolecular drugs, they can also produce toxicological issues. In fact, the same physicochemical parameters determining their fate and efficacy would be involved in the possibility to induce toxicological effects. However, the main problem is that most investigations are concentrated on the efficacy of NPs and toxicity aspects, if studied, are usually restricted to a screening of their cytotoxicity. In this way very little information is known about the genotoxicity and immunogenic potential of

Table 7.3 Toxicity considerations of nanocarriers for oral delivery

Toxicity consideration	Description
Physicochemical properties of polymeric NPs affecting their toxicological profile	Physicochemical parameters of polymeric NPs such as size, material, shape, surface properties, or the presence of ligands may result in different "kinetic" properties when administered orally
Materials	Various polymers, macromolecules and lipids, both synthetic and natural, have been employed in formulating biodegradable nanocarriers. Usually most of these compounds are utilized as excipients for other pharmaceutical applications or they have the consideration of "Generally Recognized as Safe" (GRAS) when administered by the oral route. Nevertheless, as mentioned before, their conversion into nanoparticulate devices opens the door to toxicological concerns
Size and shape	Particle size has obvious effect on the toxicity of nanomaterials. An inverse relation between size and potential toxic effects is usually established; small NPs offer a higher surface area and as a result a higher number of potentially reactive molecules in comparison with larger ones (given equal mass) [20]. Decreasing the size of NPs triggers the potential reactivity of these materials in an exponential way [21]
Surface properties	For an instance CS-derived NPs as a model of negatively and positively charged nanocarriers were investigated in their ability to be taken up by phagocytic cells [22]. Macrophage uptake enhanced as the surface charge (either positive or negative) increased. This outcome would be linked to the concern of electrostatic interactions between particles and phagocytic cells that would allow their internalization [23]. Nevertheless, when the absolute values of zeta potential were alike, positively charged NPs offered a higher phagocytic uptake in contrast to negatively charged ones, irrespective of their composition [22]
Biodegradability	Theoretically nanocarriers capable of "disappearing" in the body conditions and/or of being "inert" when in contact with a living system or tissue would have a lower hazardous effect [23–26]. Nevertheless, the biodegradability of NPs may produce biodegradation products with a different toxicological profile from that of the nanocarrier. In addition, the biodegradation process, which would occur during the interaction of nanocarriers with the biological medium, can alter the physicochemical properties of nanocarriers (e.g. size, shape and surface properties) and therefore the toxicological performance may also be influences throughout this process. In contrast, non-biodegradable materials should present a high toxicity risk linked with their accumulation in the body

nanocarriers capable of translocating and entering into the circulation after their administration by the oral route. Similarly, the study of in vivo toxicity has been pursued only with a small number of polymeric nanocarriers. Various toxicity considerations of nanocarriers for oral delivery is mentioned in Table 7.3.

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