Polysaccharide-Based Materials

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Polysaccharide-based materials created by physical processes have received considerable attention for biomedical applications.

growth factors scaffolds antimicrobial coatings

1. Introduction

Polysaccharides have hydrophilic functional groups (charged groups, as well as hydrogen bond donors and acceptors) that can stabilize macromolecular assemblies. Polysaccharide assembly can also be achieved via electrostatic crosslinking using small-molecule or metal counterions, and through cooling and freezing–thawing of polysaccharide-based mixtures. These assemblies include polyelectrolyte complexes (PECs), polyelectrolyte multilayers (PEMs), coacervates, and hydrogels. PECs are assemblies mainly formed from the electrostatic complexation in solution of oppositely charged polyelectrolytes. The resulting complexes may remain highly hydrated, and are therefore often characterized as hydrogels or coacervates as well. Coacervates are the result of a liquid–liquid phase separation, resulting in a polysaccharide-rich (liquid) phase that remains hydrated and is suspended in an (aqueous) solution. Coacervates and PECs often have polydisperse size distributions. Hydrogels are hydrophilic condensed (solid) networks of macromolecules, which are capable of absorbing large amounts of water (greater than 90% by weight). Whether formed by coacervation or gelation, the result is often three-dimensionally structured nano- or microparticles. Polysaccharides can also be assembled through various film-forming, fiber-spinning, and phase-separation methods. Films are often obtained by solvent evaporation method or through the layer-by-layer assembly of PEMs [1][2][3]. The formation of polysaccharide PECs, coacervates, hydrogels, fibers, and films is generally achieved at relatively mild conditions; however, processing conditions used for any of these assembly methods (e.g., solution pH, concentration, temperature, and ionic strength) can greatly influence the resulting material structure and properties.

Polysaccharide-based materials have been used as wound dressings, drug delivery systems (DDSs), scaffolds, and coatings for tissue-engineering purposes [4][5]. Polysaccharides are attractive materials for these applications due to their cytocompatibility, biodegradability, high bioavailability, and natural abundance [5]. Many polysaccharides also exhibit antimicrobial, antimycotic, anti-adhesive, anticoagulant or procoagulant, and wound-healing properties. They have hydrophilic groups (carboxylic acids, amino, hydroxyl, and sulfate groups) in their structures that support bio-adhesion through non-covalent bonds toward biological tissues and growth factors (GFs) [6]. Some polysaccharides naturally occur in the extracellular matrix, and play important roles in binding proteins, cells, and tissues.

2. Principal Polysaccharides Used for Biomedical Materials

Polysaccharides can be chemically stable, pH-responsive, and thermosensitive. These properties, combined with their chemical and biochemical functionality, gelling properties, and structural similarity to extracellular matrix components make them excellent candidate materials for use in biological systems. Here, we highlight the properties of glycosaminoglycans (GAGs) [7], alginate [8], chitosan [9], carrageenans, ulvan, fucoidan [7], and polysaccharide derivatives (especially sulfated materials [10]). Polyanionic polysaccharides (GAGs and marine polysaccharides) have often been used to develop DDSs for cationic GFs [11] and surface coatings. Cationic polysaccharides (chitosan and its derivatives) comprise DDSs for anionic GFs [12], surface coatings, wound dressings, and scaffolds with antimicrobial properties. Our principal focus is on charged polyelectrolytes (polyanionic and polycationic polysaccharides) because these can mainly interact through electrostatic interactions, forming durable assemblies (physical materials) for biomedical applications. Moreover, we focus on cellulose (a neutral polysaccharide), because it is the most abundant polysaccharide in the world. It provides nanocrystalline structures that improve the mechanical properties of polysaccharide-based materials, and bacterial cellulose is attracting significant attention for biomedical applications.

2.1. Glycosaminoglycans (GAGs)

GAGs are linear anionic polysaccharides mainly composed of disaccharide units containing a hexuronic acid (glucuronic acid or iduronic acid) and a hexosamine (glucosamine, or galactosamine). GAGs comprise complicated chemical structures, distinguished by their specific disaccharide repeat sequences, glycosidic bonds, and substituents (*O*-sulfates, *N*-sulfonates, and *N*-acetyl groups). They are present in many human and animal tissues, and are obtained commercially from the tissues of pigs, poultry, sharks, and reptiles. GAGs molecular masses mainly depend on the extraction method and source. They include sulfated polymers, such as heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate [7][13][14] (Figure 1). Hyaluronic acid (often called hyaluronan) is the only non-sulfated GAG.

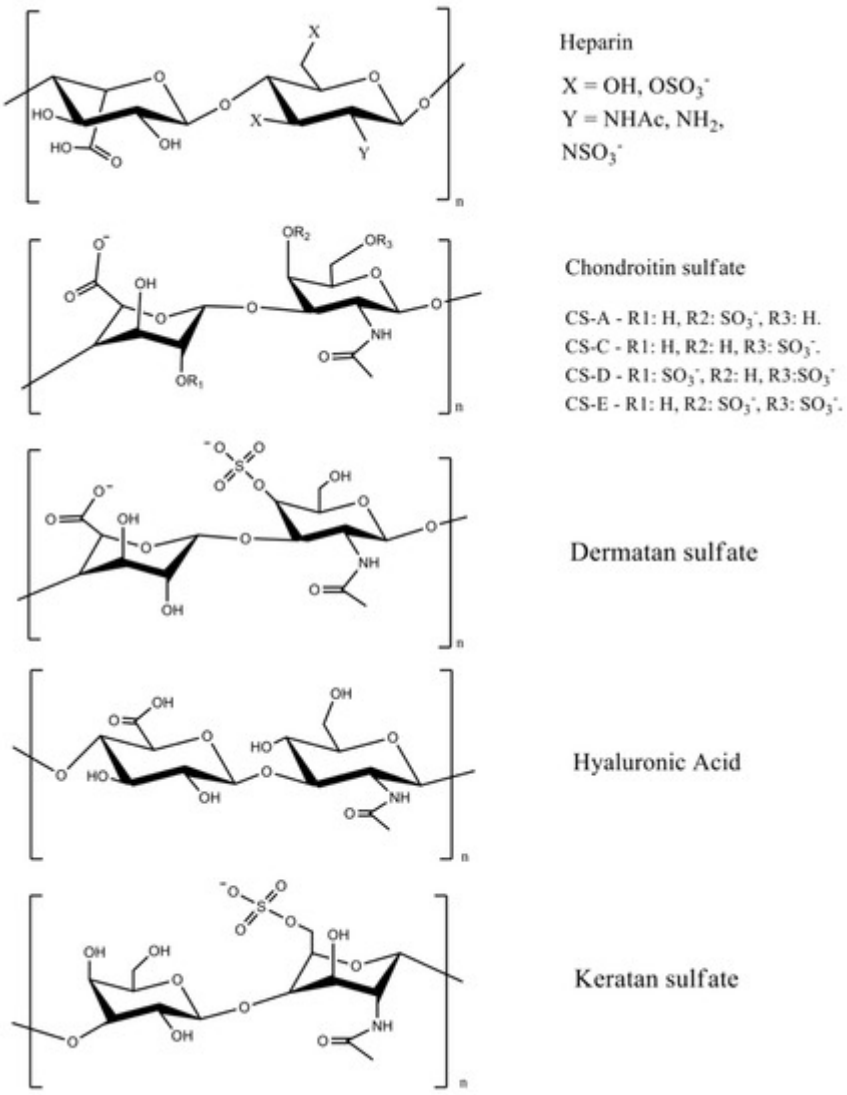

Figure 1. GAG chemical structures. Adapted with permission from [7][15]; published by Elsevier 2019 and Wiley, 2020. CS = chondroitin sulfate, and the letters A, B, C, D, and E represent different types of chondroitin sulfates.

The most important GAGs for biomedical applications are heparin, chondroitin sulfate, and hyaluronic acid, because they are abundant extracellular membrane components. Heparin has a linear chain consisting of an alternating sulfated uronic acid and d-glucosamine units linked by α- and β bonds (1→4). The uronic acid can be l-iduronic or d-glucuronic acid, while the d-glucosamine is *N*-sulfated or *N*-acetylated. The l-iduronic acid is sulfated at the C2 position, and the d-glucosamine unit is *N*- and 6-*O* sulfated. l-Iduronic acid corresponds to approximately 85% of the uronic acid content, and d-glucuronic acid comprises 15% [7][16].

Chondroitin sulfate is composed of repeating β-1,3-linked *N*-acetyl galactosamine and β-1,4-linked d-glucuronic acid disaccharide units [14][17]. The chemical structure depends on the sulfate groups' positions on the pyranose ring and sulfation degree. It is often classified as chondroitin sulfate A, C, D, and E (Figure 1). Hyaluronic acid has the highest molecular mass among the GAGs, and it is composed of β-1,4-d-glucuronic acid and β-1,3-*N*-acetyl-d-glucosamine disaccharide units (Figure 1) [18]. The high molecular mass of hyaluronan imparts viscoelastic and bio-

adhesive properties to materials [19][20]. It is a major component of the extracellular matrix of many tissues, including skin [21].

Sulfated disaccharides on GAGs containing sulfate and carboxylic groups have pK_a values between 2.0 and 4.0 [16][22][23]; therefore, all the sulfated GAGs are ionized in water and biological fluids. The ionized sulfates are hydrophilic, making them water-soluble. Sulfated GAGs can strongly bind positively charged proteins [24]. The physicochemical and biochemical properties of GAGs (hydrophilicity, biocompatibility, biodegradability, and chemical cues that regulate major biological processes, including cell growth and differentiation) rely on their chemical structure, specific architecture, sulfation degree, molecular mass, and conformation in solution [21][25][26]. Low-molecular-weight heparin has anti-inflammatory properties and anticoagulant effects, whereas unfractionated heparin has less predictable and controllable biological properties [27].

The sulfated GAGs occur covalently end-grafted to proteins, forming three-dimensional bottlebrush structures called proteoglycans [28]. Proteoglycans are complex macromolecules found in cell membranes, the extracellular milieu, and intracellular granules. These structures can have different amino acid sequences, lengths, and different types and numbers of GAGs attached to their backbones [29]. Proteoglycans and their constituent GAGs are responsible for many biochemical functions of the extracellular matrix and cell membranes, including organizing the nano- and microstructure; enhancing tribological and mechanical properties; regulating the transport of oxygen and nutrients; restoring the structure and function of damaged tissues; providing microenvironments for cell survival [30]; and binding, stabilizing, and activating GFs to control signaling [31].

2.2. Chitin and Chitosan

Chitin is a linear polysaccharide mainly composed of β (1→4) units linked to *N*-acetyl-2-amino-2-deoxy-d-glucose residues found in fungi cell walls (*Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium notatum*, and others), and in the exoskeletons of crustaceous (shrimps, lobster, krill, goose barnacle, and crabs), insects (cockroach, ladybird, butterfly, and others), algae (Phaeophyceae, Chlorophyceae, and others), and mollusks (cuttlefish, octopus, and squids) [32][33]. It occurs in different polymorphic forms (α, β, and γ-chitin). The α-chitin arranged in anti-parallel strands is the most stable and abundant form [34]. Chitin is biodegradable and mainly extracted from crustacean wastes that are byproducts of the food fishing industries, comprising an acetylated polymer with aqueous insolubility [33][35].

Chitin can be formulated into materials (films, beads, hydrogels, and fibers). However, these materials are mainly prepared in volatile organic solvents, ionic liquids, and NaOH/urea mixtures [36]. Residual traces of these solvents are potentially toxic for biomedical applications. Chitin deacetylation in aqueous alkaline solutions at 60–80 °C creates the partially acetylated chitin derivatives [32][35]. Deacetylation degrees higher than 50% are referred to as chitosan, which are random copolymers of *N*-acetyl d-glucosamine and 2-amino-2-deoxy-β-d-glucosamine residues (Figure 2).

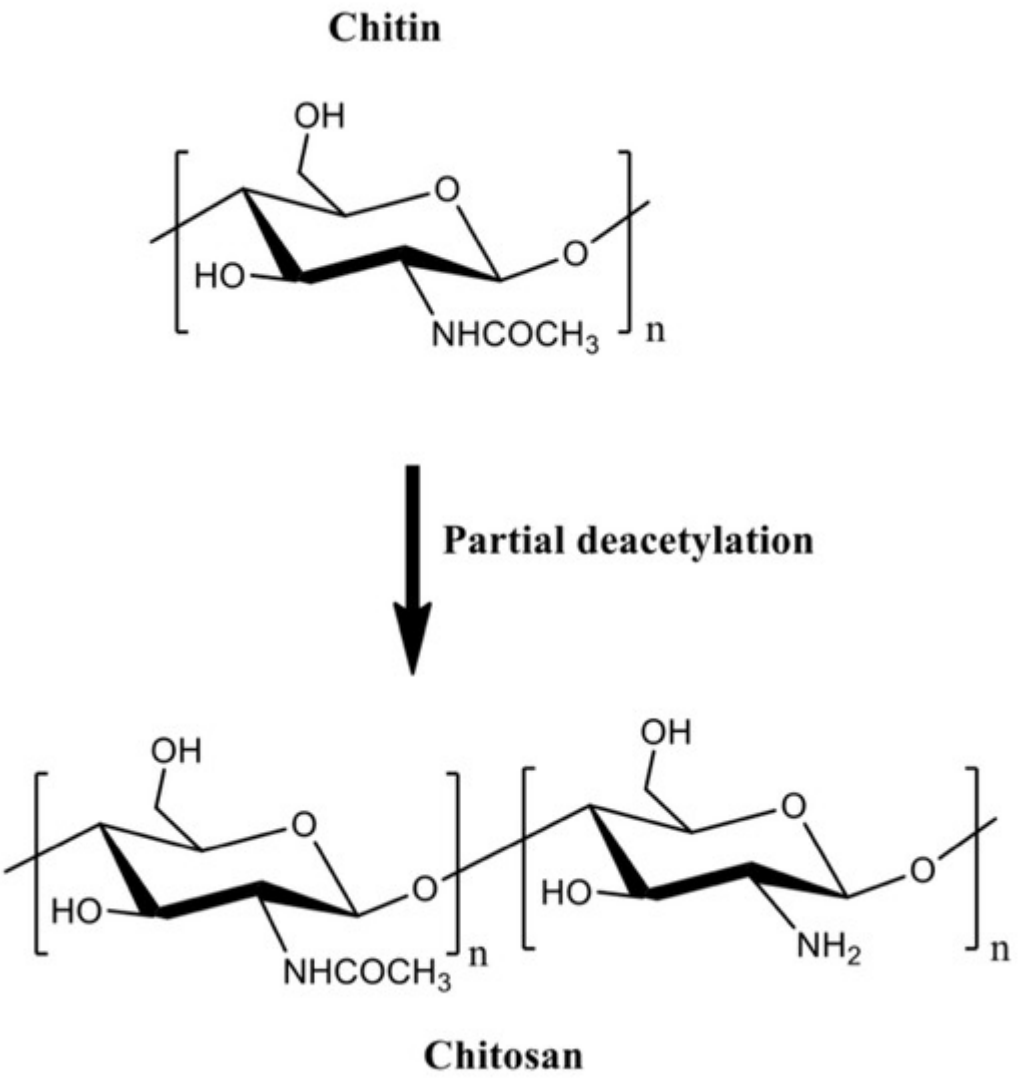

Figure 2. The partial chitin deacetylation produces chitosans with deacetylation degrees higher than 50%.

Chitosan is a linear cationic polysaccharide with pendent amine groups. The amine moieties on chitosan are protonated at low pH, making chitosan soluble in dilute acidic aqueous solutions [37]. Chitosan advantages over chitin include enzymatic degradation [38], gelling properties [39][40], pH-responsiveness [41], mucoadhesion, ability to open epithelial tight junctions (due to its cationic behavior that enhances interactions with mucous membrane [42]), and antimicrobial activities [43]. Molar mass and acetylation degree significantly influence the processing of chitosan-based materials [32]. These properties affect the chitosan hydrophobicity, solubility, viscosity, rheological, and gelling features. Chitosan gelation temperature decreases from 75 to 30 and 25 °C when the deacetylation degree is 83, 94, and 96%, respectively. Higher loss modulus (G'') indicates that the gelation of chitosan solutions forms weak structures, and high deacetylation degrees support stiffer networks due to the effective H-bonds and polymer entanglements [44].

The protonated amino groups on chitosan interact with anionic materials at suitable pH [45]. Chitosan complexes with anions and polyanions have been used to encapsulate proteins. Their pH responsiveness can be used to modulate the release of proteins while protecting them against degradation [46][47][48][49]. The anionic materials

commonly used to form complexes with chitosan include alginate [50][51], collagen [52][53], gelatin [9][54][55][56], poly(γ-glutamic acid) [57], β-glycerophosphate [58][59][60], and tripolyphosphate [47][61]. Chitosan can associate with synthetic polymers (poly(vinyl alcohol) [56][62], polyethylene glycol [63], and poly(lactic-*co*-glycolic acid) [64]) and other materials (including clays [65] and graphene oxide [52]) for producing DDSs with enhanced mechanical properties and hydrophilic–hydrophobic balance.

Chitosan generally has low solubility in biological fluids. It can easily be modified to overcome this disadvantage. Chitosan has a reactive amino group at carbon C2 and hydroxyl groups at carbons C3 and C6, in the deacetylated residues. Many reports of chemical modification of chitosan have been published. These chitosan derivatives include graphitized copolymers with poly(ε-caprolactone) [66][67], and polyethylene glycol [67][68][69], carboxymethyl chitosan [70][71][72], *N*-succinyl-chitosan [73][74], hydroxyphenyl acetamide chitosan [73], *N*,*N*,*N*-trimethyl chitosan [75], and chitosan conjugated with mesoporous silica nanoparticles [12][76] (Figure 3). These chitosan-based materials have been used in biomedical applications.

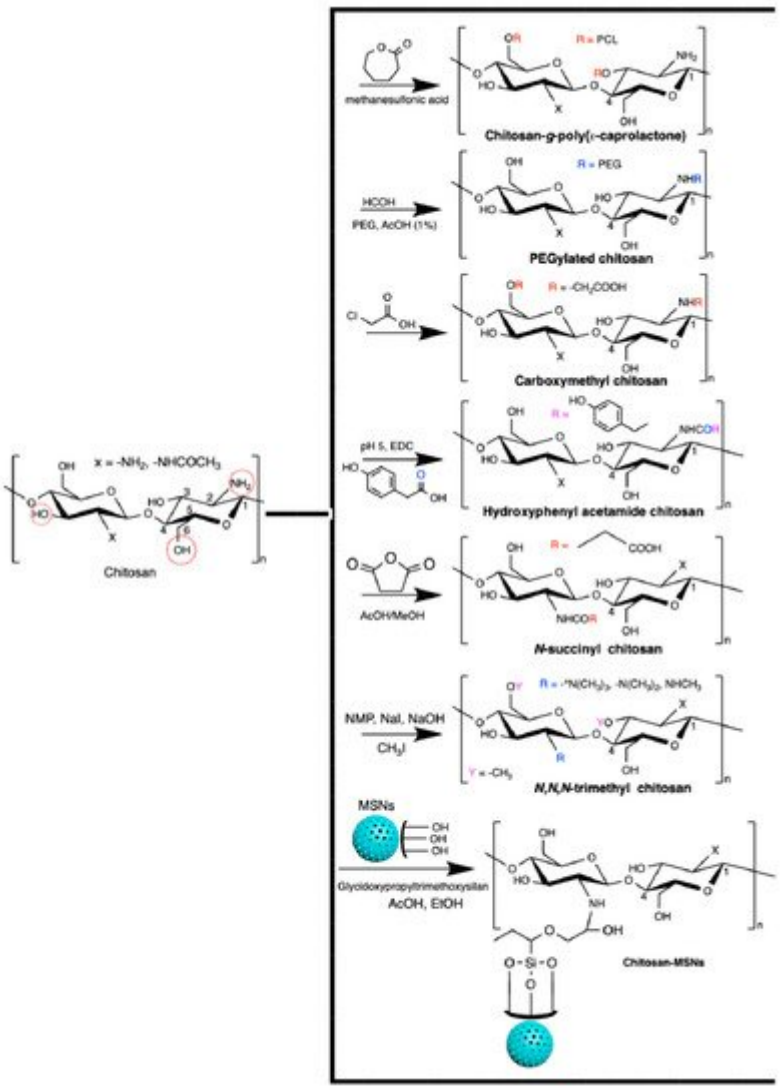

Figure 3. Chitosan-based materials used in biomedical-engineering applications.

One of the most reported types of chitosan derivatives is carboxymethyl chitosans. These can be prepared from different synthetic pathways [77]. *O*-Carboxymethyl chitosan is prepared by suspending chitosan in an isopropanol/NaOH mixture and dropping (slowly) monochloroacetic acid in isopropanol in the suspended chitosan at 55 °C. This synthesis occurs using a NaOH excess to prevent the *N*-carboxymethylation. *N*-Carboxymethyl chitosan is obtained through the reaction between the free amines on chitosan with glyoxylic acid and sodium borohydride at pH between 3.2 and 4 (60 °C). *N*,*O*-Carboxymethyl chitosan is prepared by dissolving chitosan in an isopropanol/sodium hydroxide/chloroacetic acid mixture in a low NaOH concentration at 50 °C. *N*,*N*-dicarboxymethyl chitosan is prepared by tuning the chitosan, water, acetic acid, glyoxylic acid, and sodium borohydride contents at pH between 2 and 3. The ratio between amine and glyoxylic moieties should be 1:9. These chitosan derivatives are also used in biomedical applications [77].

2.3. Alginates

Alginates are natural polyuronates that have been used to engineer injectable drug delivery devices because of their low-cost of production, cytocompatibility, gelling, mucoadhesive, and pH-responsive properties [78][79][80][81][82]. Alginates are marine polysaccharides and comprise linear anionic polymers extracted from brown algae (*Phaeophyceae*, including *Laminaria hyperborean*, *Laminaria japonica*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Macrocystis pyrifera*) [83]. Alginate hydrogels established by divalent cations (magnesium, calcium, barium, and strontium) naturally occur in the *Phaeophyceae* extracellular matrix. The seawater equilibrium influences the counter-ion types found in alginates [84].

The alginate repeat units are composed of (1,4)-linked β-d-mannuronic acid (M) and α-l-guluronic acid (G) residues. The structure of alginate is characterized by homopolymer blocks (MMMM or GGGG) or alternating copolymer blocks (MGMG) [85] (Figure 4A). The relative percentages of M, G, and MG blocks depend upon the seaweed algae source and extraction method, that generally involves (i) acid extraction, (ii) filtration and washing steps, and (iii) filtrate solubilization in an aqueous NaOH solution to create sodium alginate. Further steps of floatation, centrifugation, filtration (to remove impurities and insoluble particles), precipitation (in alcohol), and extraction with barium ions are also carried out. Barium ions have a high affinity to bind to the anionic moieties on alginates, separating them from cytotoxic impurities. Alginates are recovered by precipitation, forming sodium alginates for biomedical materials [83][84]. These procedures provide purified and water-soluble alginates that are stable alginate gels in mildly acidic conditions [86]. On the other hand, they have instability in alkaline medium. The water solubility depends on the pH, ionic strength and presence of metallic cations in the aqueous solutions [85][87].

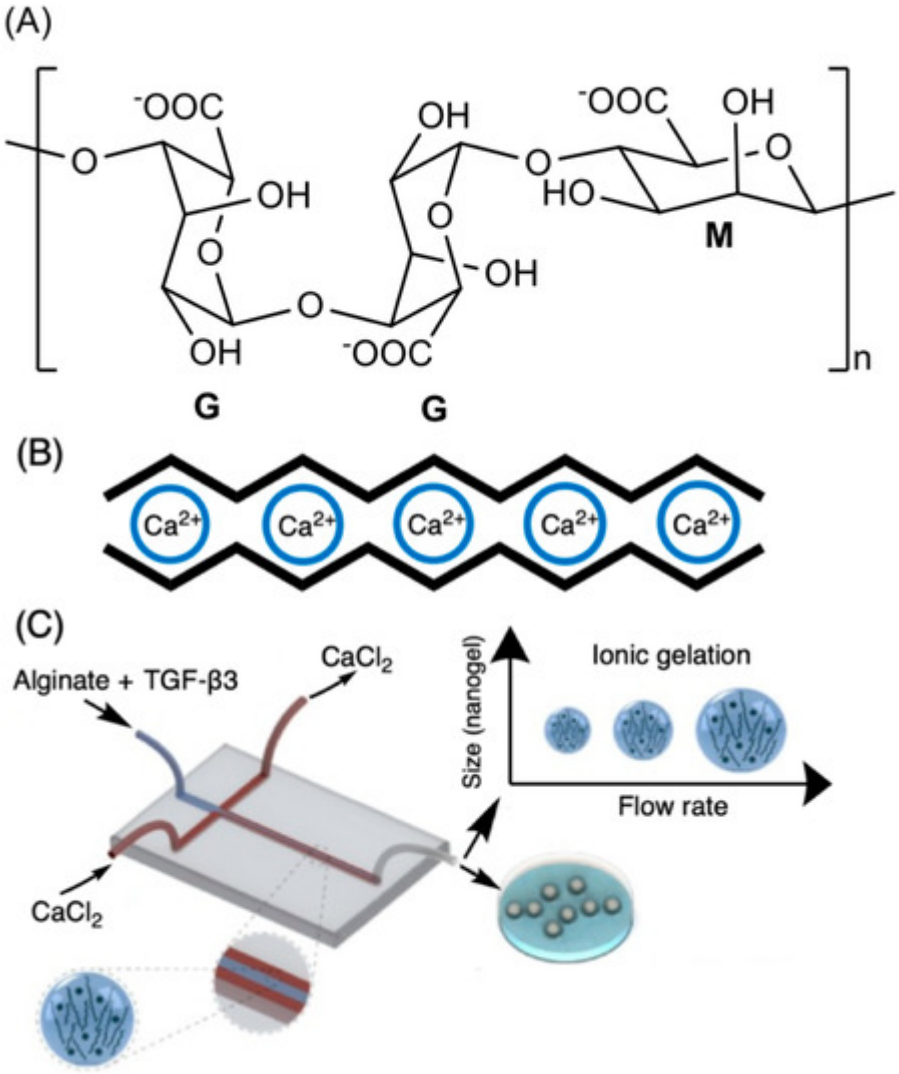

Figure 4. Chemical alginate structure (**A**), well-established egg-box gelation of alginate with calcium ions (**B**), and schematic illustration of a microfluidic device for hydrodynamic flow-focusing consisting of one inlet for focusing (core) flow and two separate inlets for the sheath (side) flows. (**C**) Adapted with permission from [88]; published by Elsevier, 2020.

Higher amounts of G blocks provide stiff alginate-based materials due to axial links and desirable chain conformation to form well-established egg-box structures with metallic cations (especially with calcium ions, Figure 4B) [80][89]. Both G and GM blocks participate in the egg-box gelation mechanism with divalent cations. High contents of alternating GM sequences increase the aqueous alginate solubility, while the gelation features mainly rely on the alginate molecular mass, G/M ratio, and pH. A higher molecular mass improves the gelling properties of aqueous alginate solutions due to the increase of polymer viscosity, supporting polymer entanglements, and thereby elastic and durable alginate-based materials [90][91][92]. The G/M ratio plays an essential role in the gelation process. At low pH (pH < 2.5), alginates are protonated, making water-insoluble alginic acids [85].

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