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Polysaccharide-Modified Synthetic Polymeric Biomaterials

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Abstract

This review presents an overview of polysaccharide-conjugated synthetic polymers and their use in tissue-engineered scaffolds and drug-delivery applications. This topic will be divided into four categories: (1) polymeric materials modified with non-mammalian polysaccharides such as alginate, chitin, and dextran; (2) polymers modified with mammalian polysaccharides such as hyaluronan, chondroitin sulfate, and heparin; (3) multi-polysaccharide-derivatized polymer conjugate systems; and (4) polymers containing polysaccharide-mimetic molecules. Each section will discuss relevant conjugation techniques, analysis, and the impact of these materials as micelles, particles, or hydrogels used in in-vitro and in-vivo biomaterial applications.

Keywords

biomaterial; polysaccharide-modified polymer; polysaccharide conjugates; hydrogel

INTRODUCTION

The use of polymeric materials as biomaterials has evolved over the past several decades, encompassing an expanding synthetic toolbox and many biomimetic approaches. Both synthetic and natural polymers have been used as components for biomaterials, as their unique chemical structures can provide specific functions for desired applications. The integration and widespread use of polymers as biomaterials has significantly expanded owing to advances in the synthesis of polymers with controlled and functional architectures, which has improved the range of materials possible, as well as their biocompatibility.[1,2] Extraction and purification methods have also enabled the use of many natural polysaccharides as biomaterials; such biomacromolecules have found a multitude of uses, especially as drug-delivery vehicles and tissue-engineering scaffolds.[3] Appropriate design of biocompatible polymeric delivery vehicles has afforded controlled release of drugs, such as small molecules, peptides, or proteins, both systemically and locally to a target via molecular recognition.[4] Tissue-engineering scaffolds also often utilize controlled drug delivery, with the added complexity of incorporation of cellular adhesion to the matrix and mimicry of the mechanical properties of target tissues.[5]

The biomaterials used in both drug-delivery and tissue-engineered scaffolds include micelles, particles, or hydrogels depending on the clinical mechanism of action and application. Polymers conjugated to drugs with either affinity-based targeting moieties or cleavage mechanisms have been widely employed as soluble and micellar delivery vehicles,4 and the

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hydrophobic cores of micellar systems are loaded with drugs for local injection or systemic release.[6] Particulate delivery vehicles comprise nano- or micro-scale aggregates of macromolecules (e.g., amphiphilic molecules, hydrophobic molecules, or crosslinked macromolecules) and can be designed to release drugs via local injection or systemic release.[7] Highly hydrated hydrogel materials comprising a continuous network have been produced via both covalent and/or noncovalent mechanisms and can be designed to mimic the mechanical and chemical properties of natural tissue environments.8 Hydrogels are typically modified with drugs for controlled release, as well as with cellular adhesion domains for soft tissue regeneration. Finally, rigid scaffolds—often dehydrated, dense polymeric materials—have been employed in hard tissue-engineering applications.9

The types of synthetic polymers used in biomaterials include hydrophilic and nonhydrolytically degradable materials such as poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and poly (acrylamide) (PAAm). Hydrophobic polymers, such as poly(*n*-butyl acrylate), as well as hydrophobic and hydrolytically susceptible materials such as poly-(α-esters), are also widely employed. Amphiphilic block polymers such as (PEG-*b*-PPO-*b*-PEG) and thermally sensitive polymers such as poly(*N*-isopropylacrylamide) (pNIPAAM) have also been widely employed owing to their lower critical solution temperatures, which afford thermal sensitivity to control microstructure formation, drug delivery, and cell adhesion. The nonhydrolytically degradable polymers are often employed at lower molecular weights (<30–50 kDa) to allow for renal clearance from the body, or are engineered via conjugation techniques to impart points of local hydrolytic or enzymatic degradation.[10–13] Of these polymers, PEG in particular has enjoyed tremendous use as a biomaterial because of its solubility in a range of organic solvents, ease of end-functionalization, low PDI, and reasonable cost.14 Consequently, functionalization with PEG of small molecule drugs, peptides, and proteins has been widely employed, with such benefits as increased circulation lifespan, reduced elimination pathways, and improved efficacy.15

While these biocompatible polymers are useful because they do not specifically interact with biological systems, this has also hindered their use in applications in which interactions are desired to manipulate biological responses such as growth factor binding or enzymatic degradation. To address this need, polysaccharides have been conjugated to synthetic polymers to impart desired bioactivity. Current techniques to conjugate polysaccharides, proteins, or peptides to synthetic polymers include aldehyde, carbodiimide, epoxide, hydrazide, active ester, radical, and addition reactions, some of which are suitable for in-situ conjugation of materials, while others are better suited for ex-situ conjugation and purification.[16] These reactive chemistries provide facile methods of covalent conjugation of functional polymers with peptides and polysaccharides; specific attention has been paid to methods that yield no cytotoxic reactive species and that are active under physiological conditions. Reactions using aldehydes, carbodiimide, and epoxides are typically toxic to cells and are not suitable for in-situ crosslinking of polymers; ex-situ production of conjugates requires extensive purification before their use as a biomaterial.[17] Recently, materials employing mild UV-labile radicals for polymerization have been shown to be well-tolerated for in-vivo and in-vitro use.[18] Addition reactions are also suitable for in-situ conjugation techniques, as they provide mild conditions involving a nucleophilic substituent such as primary amine or thiol and an unsaturated double bond such as vinyl groups, acrylates, or maleimides.[16] These reactions proceed with relatively fast kinetics without the use of harmful catalysts or byproducts and have been used for in-situ crosslinking or ex-situ conjugation techniques.

As mentioned in the abstract, this review will present the methods, purpose, desired outcomes, and responses of synthetic biocompatible polysaccharide–polymer conjugates used in biomaterial applications; four general classifications will be presented: (1) non-mammalian

polysaccharides; (2) mammalian polysaccharides; (3) multipolysaccharide systems; and (4) polysaccharide-mimetic polymer conjugates.

DISCUSSION

Some of the most facile methods of imparting biological activity to materials involve the use of polysaccharides derived from non-mammalian or mammalian sources. Non-mammalian polysaccharides that have been employed in polymeric conjugates include alginate, chitin, and dextran, which possess similar saccharide structure (Figure 1) despite their disparate origins (see below). The relatively simple extraction and purification of these polysaccharides yield large quantities of material at low cost, which, coupled with their low immunogenicity, drives interest in these materials. In addition, their chemical functionality provides ionic charge, which can be employed for noncovalent crosslinking, yields routes for degradation, and is useful for modifications such as crosslinking or grafting. Mammalian polysaccharides, such as the glycosaminoglycans chondroitin sulfate, hyaluronan, and heparin, possess chemical similarities to their non-mammalian counterparts (Figure 1) and thus have similar routes for modification and activity. Their isolation is more difficult than that of the non-mammalian polysaccharides, but they exhibit specific biological functionality, including their specific binding with multiple proteins, which has driven continued interest in their application in biomaterials.

Non-Mammalian Polysaccharide–Polymer Conjugates

Alginate—Alginate is a hydrophilic polysaccharide extracted from marine brown algae such as *Laminaria hyperborea* or soil bacteria such as *Azobacter vinelandii* and consists of blocks of alternating β-D-mannuronic acid and α-L-guluronic acid residues with (1→4)-linkages, displaying carboxylic acid functionality at the C5 residue.[19] The alginates have broad distributions of molecular weights of 10–1000 kDa depending on source and processing.19 The structure of alginate is such that normal enzymatic degradation of the polymeric chains is not possible in mammals,[20,21] although conflicting descriptions of biodegradability have been presented in the literature[22–25]; additionally, cellular adhesion is also not afforded in its unmodified form.26 Nevertheless, alginate has been widely investigated as a biomaterial, dating as far back as the 1940s, because of its rapid formation of ionic complexes with divalent cations such as calcium.[27,28] Coencapsulation of protein growth factors, such as vascular endothelial growth factor (VEGF), is possible by dripping alginate and protein solutions into calcium chloride solutions; such formulation can stabilize the growth factor's activity and delivery over a period of 14 days in solution. The lack of cellular adhesion has been addressed by covalent modification with cellular adhesion peptides.[29] However, alginate hydrogels crosslinked with divalent cations are unstable under conditions in which monovalent cations can be exchanged for the divalent crosslinking cations, leading to varying mechanical strengths and degradation profiles.30 Conjugation of PEG diamine to the carboxylates of alginate, via carbodiimide chemistries, has circumvented these inconsistencies and increased the control over mechanical properties and degradation.[31,32] Responsive hydrogels based on temperature and pH have also been synthesized by conjugating an amine functional pNIPAAm via carbodiimide coupling to alginate with subsequent divalent cation crosslinking.[22,23,33]

Despite its favorable properties, this material has not seen much use as a polymer conjugate perhaps due to the lack of evidence that the high molecular weight alginate species can be enzymatically degraded and cleared in vivo. Current approaches have investigated gamma irradiation to reduce the molecular weight or periodate oxidation treatments to induce hydrolytic susceptibility to the alginate backbone for faster clearance from the body.[33–35] Non-mammalian polysaccharides, chitins and dextrans, have a more clearly defined and studied degradation pathway and have seen broader use as biomaterial conjugates as discussed below.

Chitin—Chitin is a hydrophobic linear polysaccharide derived from many natural sources including the exoskeleton of arthropods and insects and is the second most abundant natural polysaccharide next to cellulose.[36] Chitin comprises a polysaccharide consisting of (1→4)-β-*N*-acetyl-D-glucosamine units, while the modified *N*-deacetylated derivative, chitosan, is a mixture of *N*-acetyl-D-glucosamine and D-glucosamine (Figure 1).[37] Chitin is insoluble in aqueous solutions at neutral pH, but *N*-deacetylation increases the aqueous solubility of the polymer while also providing reactive primary amines for chemical modification. The deacetylation process has been shown to reduce the molecular weight from an average range of 1000–2500 kDa to one of 100–500 kDa, and as for other polysaccharides, the molecular weight distribution is highly dependent on polysaccharide origin and processing methods.37 Dissolving chitosan under acidic conditions and subsequently raising the pH can induce hydrogel-like precipitates.[38] The increased water solubility of chitosan and its amine functionality has prompted its use as a biomaterial polymer conjugate; furthermore, chitosan has also been shown to be degradable by enzymatic hydrolytic cleavage of the β-(1→4) saccharide linkage.[39] Chitosan itself has been investigated for many biomaterial applications including wound-dressing materials, drug-delivery vehicles, and tissue-engineering scaffolds. 40

The improved solubility of chitosan has been exploited in biomaterial conjugates by the grafting of synthetic hydrophobic polymers, which induces amphiphilic pH-sensitive and thermally sensitive properties. Qu et al. initially reported a noncatalyzed condensation reaction of lactic acids with chitosan, forming grafts of poly(lactic acid) (PLA), that induced a physically crosslinked hydrogel by hydrophobic association of the PLA. The formed hydrogel swelled with decreasing pH or ionic concentration.41–43 Tailoring of the extent of chitosan modification and degree of polymerization, with conjugation of other hydrophobic poly(α-esters) such as PLA,44–46 poly(butyrolactone),47 and poly(caprolactone),47–49 has permitted the production of unique self-assembled micellar and nanoparticle structures. Similarly, micelles were formed by conjugating chitosan oligosaccharides with stearic acid and were used as antitumor targeting therapies.50 Further modification of the functionalized chitosan with monofunctionalized PEG-aldehyde reduced the macrophage uptake of the micelle while not impacting cellular uptake by liver tumor cells.51

While incorporation of hydrophobic polymers enables the formation of micellar particles and in some cases hydrogels, chitosan grafted solely with hydrophilic polymers can also show thermoreversible gelation properties. Bhattarai et al. grafted monofunctionalized PEG-aldehyde to the amine groups of chitosan, followed by reduction with sodium cyanoborohydride.[52] Solutions of these materials were free-flowing at room temperature, but were viscous at body temperatures, permitting injection and subsequent localized drug release and cell encapsulation. These materials were consequently shown to have favorable drug-release profiles of bovine serum albumin (BSA) both in this form and when covalently crosslinked with genepin.[53] Similarly, Park et al. functionalized chitosan with monocarboxylated Pluronic® F127, a well studied block copolymer of PEG-*b*-PPO-*b*-PEG, via carbodiimide coupling.[54] These materials also formed thermoresponsive hydrogels that formed a viscous material at room temperature and a viscoelastic material at body temperature. They were thus investigated as an injectable chondrocyte carrier for cartilage regeneration; the materials promoted increased cell viability and supported greater glycosaminoglycan and aggrecan production compared to control alginate hydrogels.

The semiaqueous solubility of chitosan in combination with the grafting of synthetic polymers has provided a wide range of pH-sensitive, ionic, and thermoresponsive materials. The useful biocompatibility of the scaffolds has also prompted the exploration of chitosan in tissue-engineering scaffolds, with cell adhesion imparted by photocrosslinking with monofunctional acroyl-PEG-RGD.[55] Chitosan has enjoyed much investigation as a biomaterial most likely due

to its biocompatibility and biodegradability combined with its relatively low cost, availability, and ease of functionalization.

Dextran—Like the above polysaccharides, dextran is not found in human tissues. The polysaccharide itself is expressed by bacteria, with most research focusing on dextran expressed from *Leuconostoc mesenteroide*. The structure of dextran consists of linear (1→6)-α-D-glucose, with branches extending mainly from (1→3) and occasionally from (1→4) or (1→2) positions accounting for a 5% degree of branching (Figure 1).[56,57] Dextran is highly water soluble and easily functionalized through its reactive hydroxyl chemistries. Characterization of many types of dextran have indicated that branching, average molecular weight, and molecular weight distributions can vary widely depending on the conditions and strain of bacteria used for expression.[56,57] Dextran was investigated as a blood plasma replacement in the early 1940s[58] and has since become of interest as a biodegradable and biocompatible material. Biodegradation occurs through natural enzymatic splitting of saccharide bonds by dextran-1,6-glucosidase found in spleen, liver, lungs, kidneys, brain, and muscle tissue as well as by dextranases expressed by bacteria in the colon.[59,60] It has also been determined that dextran lacks nonspecific cell binding and resists protein adsorption, which has increased interest in its use as a biomaterial.[61,62]

Like the previously discussed non-mammalian polysaccharides, the relatively low cost and availability of dextran as well as its hydroxyl functionality for chemical modification has increased the utilization of dextran in the field of polysaccharide polymer conjugates for biomaterials. Chu and coworkers have conjugated acrylate-functionalized dextran to polymers such as poly(lactic acid) diacrylate using UV-initiated free-radical polymerization to create hydrogel networks.[63–66] Their research group has also investigated free-radical polymerization of NIPAAm with functionalized dextran[67,68] to impart temperature sensitivity or free-radical crosslinking of PEG-diacrylate with amine and allylisocyanate functionalized dextran to incorporate pH and electrolyte sensitivity.[69] Responsive dextran conjugates have also been produced by others via radical-initiated grafting of methacrylate end-functionalized poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) to methacrylated dextran[70,71] or via radical crosslinking of acrylated PLA with allyl dextran and NIPAAm monomer.[72,73] Thermosensitive and reversible hydrogels have also been produced by grafting D-or L-lactide oligomers (degree of polymerization 8–11) to dextrans, forming elastic hydrogels with stereocomplexation of the D-and L-lactides.[74–76]

In-situ formation of hydrogels is appealing for biomaterial applications and therefore has been employed in the formation of dextran hydrogels via addition reactions. Hiemstra et al. have investigated gelation of vinyl sulfone-esterified dextrans, with degrees of substitution of 2–22, with bifunctional or four-arm PEG-SH.[77–79] These materials have shown rapid gelation times and are also hydrolytically degradable via hydrolysis of the conjugated sulfone. The above dextranconjugated materials have been successfully investigated as controlled release delivery vehicles of indomethacin (a low molecular weight hydrophobic anti-inflammatory drug),[64] bovine serum albumin,[63,69,75,79] lysozyme, and immunoglobulin G.[75,79]

Mammalian Polysaccharide–Polymer Conjugates

As mentioned above, the incorporation of mammalian polysaccharides into biomaterials offers advantages over the incorporation of non-mammalian polysaccharides; in particular, mammalian polysaccharides can elicit specific interactions with mammalian cells or environments. The use of glycosaminoglycans, such as hyaluronan, heparin, and chondroitin sulfate, has been most widely explored and has yielded a range of highly useful biomaterials.

Hyaluronan—Hyaluronan, or hyaluronic acid (HA), is a hydrophilic linear glycosaminoglycan composed of alternating (1→4)-β-D-glucuronic acid and (1→3)-β-*N*-acetyl-D-glucosamine (Figure 1), a backbone containing both hydroxyl and carboxylic acid functionalities.[80] HA was initially discovered, in the vitreous body of cattle eye, by Meter et al. in 1934 and was later found to be distributed throughout the body especially in the extracellular matrix (ECM) and synovial fluids.[81,82] The molecular weight of HA has a broad distribution depending on its origin and can exhibit high degrees of polymerization of up to 10,000 kDa.83 Although HA can be isolated by extraction from living tissues, it is produced mainly via microbial fermentation, due to the reduced risk of crossspecies viruses, infection, and contamination.80,[84] HA has been indicated to impact cell–cell and cell–substrate adhesion, cell migration, and proliferation, to aid in the organization of proteoglycans and to bind collagen and fibrin. Importantly, HA has also been shown to promote angiogenesis and aid in wound healing.[85] This range of activities has motivated the widespread use of HA in vocal fold repair, wound repair, anti-inflammatory materials, drug delivery, and void-filling models, as well as in cosmetics and food industry products.[86–100]

The usefulness of HA as a long-term implant biomaterial has been slightly hindered by the rapid enzymatic degradation in the body; for instance, in humans, HA is degraded and synthesized at a rate of up to 5 g of HA daily, of the normal 15 g in the body.[101] To slow and control the degradation, synthetic polymers have often been conjugated to HA. The conjugation of synthetic polymers can also increase the mechanical strength of the materials. Prestwich et al. have extensively used modified HA as a basis for tissue-engineering scaffolds. Their early work investigated the formation of hydrogels by crosslinking HA via carbodiimide coupling of small molecule hydrazides, PEG diamine, or PEG dihydrazide.[86] Eventually, more biologically friendly gelation chemistries were investigated and applied to this system; HA was functionalized with free thiols via carbodiimide coupling and subsequently crosslinked by Michael addition with PEG diacrylate.[95,102,103] These materials have been shown to release anti-inflammatory drugs,[88] increase re-epithelialization of wounds,[104] stimulate localized microvessel growth by cytokine release,[94] mimic adipose tissue,[102] and act as a cleavable cell growth scaffold.[103] The trend of incorporating HA into PEG hydrogel scaffolds using biocompatible crosslinking methods has also been explored by other groups. For example, acrylated HA has been crosslinked by four-arm star thiolated PEG[99] and by photocrosslinking using mild UV-initiated radical reagents.[91,93,97,105,106]

Conjugation of HA to other synthetic polymers has led to the formation of assembled structures; for instance, Lee et al. have grafted HA with poly(lactic-*co*-glycolic acid) (PLGA), inducing the formation of nanoparticles that have been employed to deliver anticancer drugs.[107] They have also investigated the conjugation of Pluronic® F127 di-acrylate with methacrylated HA via photocrosslinking to produce thermosensitive hydrogels as described above for chitosan hydrogels.[90,96] These hydrogels deswelled rapidly with increasing temperature due to the hydrophobic collapse of the Pluronic® polymer, shown to controllably release human growth hormone over a period of 13 days and plasmid DNA over 10 days inducing in-vitro transfection. More recently, they have grafted amine-functionalized Pluronic® F127 to methacrylated HA by carbodiimide coupling, with subsequent photocrosslinking with acrylated cell-adhesion domains, to create hydrogels. Encapsulation of chondrocytes in these constructs resulted in increased production of ECM proteins (Collagen II and aggrecan) over that produced by cells in two-dimensional cell culture.[100]

Chondroitin Sulfate—Chondroitin sulfate (CS) is composed of a linear polysaccharide chain consisting of (1→3)-β-*N*-acetyl-D-galactosamine alternating with (1→4)-β-glucuronic acid presenting sulfates and hydroxyl and carboxylic acid functionalities (Figure 1).[108] CS was discovered in 1861 by Fischer and Boedecker, with the structure eventually elucidated in the early 1900s.[109,110] Various forms of chondroitin sulfate have been discovered and are

designated by the site of sulfation of *N*-acetylgalactosamine in the 4-O and 6-O positions and subsequent other sulfation or epimerization of glucuronic acid.[108] CS is mainly found attached to proteoglycans in connective tissue matrices, functioning as a structural component, or on cell surface and basement membranes, functioning as a receptor.[111] Commercially available CS is obtained via extraction and purification from many sources including shark and whale cartilage, and bovine or porcine tissues.[108] An important function of CS is in articular cartilage, where on the order of one hundred chains of CS are conjugated per protein, as in aggrecan.[112] The high negative charge of CS, coupled with the high density of CS chains on aggrecan, creates a charge gradient that swells the cartilage and enhances the ability of the tissue to absorb load.[113] This natural role of CS has directed its use in tissue-engineering scaffolds for cartilage repair, as explored by Elisseeff and coworkers.[114–119] Specifically, CS modified by reaction with glycidyl methacrylate and photopolymerization with PEG diacrylate has been used as adhesive cartilage repair scaffolds and as an encapsulation medium for mesenchymal stem cells for chondrogenic differentiation.[116,117] Modification of CS with aldehyde or succinimidyl succinate, followed by crosslinking with poly(vinlyalcohol-*co*-vinylamine) or PEG amine, respectively,[118,119] has yielded CS-based adhesives for sealing corneal incisions; these materials show minimal inflammatory responses and scar tissue formation while maintaining high burst pressures. The use of such materials in wound-healing applications has also been investigated; CS-conjugated PEG hydrogel films have been implanted into exterior wounds on mice or internal wounds of the mucosa of maxillary sinus in rabbits; in both cases, the CS-based materials show accelerated healing over controls.[104,120] Bryant et al. have also investigated the encapsulation of chondrocytes using similar techniques employing photopolymerization of methacrylated CS with PVA[121] or PEG[122] methacrylates. Other modifications of CS, for example, by ring-opening polymerization with L-lactide-created amphiphilic CS-*graft*-PLA macromolecules, has permitted the formation of micelles for cellular endocytosis,[123] films for chondrocyte encapsulation,[124] and nanoparticles for protein drug delivery.[125]

Heparin—Heparin is a highly sulfated and heterogeneous linear glycosaminoglycan composed of α or β (1→4) linked uronic acid (90% α-L-iduronic acid, 10% β-D-glucuronic acid) and α-D-glucosamine residues, containing a heterogeneous mixture of carboxylic acids, hydroxyls, sulfates, and amine functional groups (Figure 1). Heparin was first discovered in 1916 by McLean and has been used clinically as an anticoagulant since 1935.[126–128] Heparin is synthesized as a proteoglycan component in mast cells (molecular weight 60–100 kDa) and is cleaved into smaller nonuniform fractions by endoglycosidases (molecular weight 5–25 kDa).[129] The uronic acid saccharides exist as two anomers and epi-mers of the form of α-L-iduronic or β-D-glucuronic acid, both of which may be 2-O-sulfated. The glucosamine has more diversity as it may be N-sulfated, N-acetylated, and 6-O-sulfated, which gives rise to extreme heterogeneity.[126,127] Commercial production of heparin involves complicated extraction and purification processes from tissues such as porcine or bovine intestinal mucosa, which results in additional heterogeneity in chemical structure and molecular weight after purification.[129]

The high negative charge of heparin (−75 for a 15-kDa heparin, averaging −2.7 sulfate groups per disaccharide) gives rise to many ionic interactions with proteins, such as growth factors, proteases, and chemokines, that have been intensely studied.[127] Important to drug delivery and tissue engineering, it has been shown that heparin binding can stabilize growth factors, such as basic fibroblast growth factor (bFGF) and VEGF, from denaturation while increasing the affinity of the complex to cell receptors.[130,131] For these reasons, conjugation of heparin to biomaterials such as collagen, gelatin, fibrin, and HA has been highly attractive, as such conjugation sequesters GFs and prevents their bulk release.[132–139] More recently, heparin has been conjugated with synthetic polymers to provide increased control over mechanical and chemical properties of the resulting materials.[140–155] Ishihara et al. first conjugated periodate-cleaved heparin to styrene monomers to create heparin-conjugated polystyrene plates.[156,157]

These surfaces retained and increased bioactivity of VEGF and FGF-2. Following suit, others have incorporated heparin into synthetic polymers with successful controlled release and increased activity of growth factors.[140,142,143,145,149,150,158] Heparin conjugated to PEG hydrogels has also been used to probe differentiation and phenotype response of mesenchymal stem cells and valvular interstitial cells (VICs) by sequestering of growth factors and other heparin-binding proteins.[140,141,155,159] In the latter case, it was found that covalent conjugation of heparin to a PEG hydrogel network to sequester FGF2 prompted the expression by VICs of myofibroblast phenotype markers, indicating that immobilized heparin can modulate cellular fate through binding of growth factors at the cell/gel interface.[155]

Heparin's binding to many proteins has spurred an interest in utilizing heparin to control the assembly of hydrogels through noncovalent interactions. Initial investigations by the Panitch research group showed that four-arm star PEG functionalized with heparin-binding peptides (HBPs) formed viscoelastic materials upon association with free heparin (Figure 2).[146] The use of high molecular weight heparin permitted the association with multiple HBPs, yielding a physical network whose mechanical properties and degradation could be tailored by adjusting the heparin-binding affinity of the HBPs. The mechanical properties of such hydrogels were augmented by the addition of covalent and enzymatically degradable [matrix metalloproteinase (MMP)-degradable] crosslinks in the hydrogels, yielding materials of greater mechanical strength with properties tunable by manipulation of both the covalent and noncovalent crosslinks (Figure 2).[147,148]

In a similar light, our investigations of heparinized polymeric biomaterials have led to the introduction of soluble PEG-heparin conjugates that are competent for noncovalent hydrogel assembly with multiple heparin-binding partners (Figure 2) and that can be covalently crosslinked into hydrogel networks.[144,145,151–154] Soluble four-arm PEG-LMWH (low molecular weight heparin) has been produced by addition reaction of thiol-terminated four-arm PEG with mono-maleimide-functionalized LMWH. PEG-HBP (heparin-binding peptide) conjugates were formed via an addition reaction of cysteine-containing HBPs to vinyl-sulfone-functionalized four-arm PEG.[151,152,154] While solutions of each conjugate were free-flowing, their mixtures immediately formed a self-supporting hydrogel that undergoes reversible shear thinning with quick recovery of mechanical properties.[151,152,154] The gelation and mechanical properties of these scaffolds were dependent on the binding of LMWH to HBPs, whereby adjusting the stoichiometry between LMWH and HBPs resulted in hydrogels of various elastic moduli. Consequently, a small stoichiometric excess of the PEG-LMWH over the PEG-HBP prevented bulk diffusional release of growth factors such as bFGF while maintaining the hydrogel elastic modulus. The controlled growth factor delivery rates and erosion of the noncovalent networks were sustained over time periods relevant for neovascularization, suggesting the potential use of these materials as injectable scaffolds for delivering bioactive proteins.[152,154]

The noncovalent assembly of PEG-LMWH with multiple HBPs suggested the feasibility of forming responsive hydrogels noncovalently crosslinked with proteins carrying multiple heparin-binding domains, such as VEGF. Each VEGF is equipped with two heparin-binding domains that bind a single HMWH in vivo[160]; however, restriction of the length of the heparin chain (via the use of LMWH in PEG-LMWH) permits VEGF to bind to two individual LMWH, thus creating a mechanically active crosslink. Accordingly, viscoelastic hydrogels comprising four-arm PEG-LMWH and VEGF have been produced (Figure 2); the increase in elastic behavior over control solutions of PEG-LMWH and BSA was confirmed via laser tweezer microrheology.[153] The erosion of these VEGF-crosslinked hydrogels was selectively triggered by VEGFR-2-coated poly(stryrene) particles, indicating that the VEGF crosslinks can be selectively removed by VEGF receptors, and thus suggesting the utility of these approaches in

the targeted delivery of drugs from such hydrogels. Verification of this supposition in cell culture has been demonstrated and in-vivo studies are planned.

Although these studies clearly indicate that the interaction of heparin-binding GFs with PEG-LMWH yield cell-receptor-responsive networks, given the low elastic moduli of these noncovalently assembled materials (<10 Pa), we have also explored the joint covalent and noncovalent crosslinking of PEG-LMWH networks to fashion cell-responsive materials of greater mechanical strength. Thiol-functionalized linear PEGs have thus been used to crosslink multifunctional maleimide-functionalized heparin, yielding heparinized materials capable of controlled release of growth factors. Functionalization of heparin, and thus mechanical properties of the resulting gels, could be controlled through manipulation of solution pH.[145] These hydrogels form rapidly at physiological pH and degrade through the hydrolytic scission of PEG ester bonds. In addition, the versatility of esterification of the PEG has permitted facile control of hydrogel formation and degradation rates via modulation of the hydrophobicity of the ester environment as well as manipulation of thiol nucleophilicity (not shown). These materials have shown promise as injectable vehicles for controlled drug delivery in animal models (not shown). They are also easily modified with cell-adhesion ligands and have been investigated as substrates for controlling cell adhesion and proliferation. Specifically, manipulation of the mechanical properties of the hydrogels has permitted selective control of the adhesion and proliferation of distinct cardiovascular cell types (aortic adventitial fibroblasts, human umbilical vein endothelial cells, and smooth muscle cells),144 affording new scaffolds for fabrication of complex multicellular constructs.

Multipolysaccharide–Polymer Conjugates

Polymeric networks containing multiple polysaccharides may better mimic the complicated environment of the ECM, and a vast number of materials have been investigated. Complex-extracted scaffolding material such as Matrigel™, a commercially available extract from Engelbreth–Holm–Swarm mouse sarcoma, containing laminin, collagen, heparan sulfate proteoglycan, and entactin, has been used in numerous studies, although batch-to-batch variation, the possibility of pathogen transmission, and immunogenicity complicate the general use of these materials.[161,162] In efforts to mimic these complicated matrices, materials comprising multiple natural polysaccharides and/or proteins, including heparin, alginate,[132, 136] chitosan,[133] fibrin,[135] and collagen,[137–139] have been investigated. A more recent trend has been to use multiple types of functionalized polysaccharides and proteins to crosslink synthetic polymers into hydrogel scaffolds. Kirker et al., in concert with Peattie and coworkers, have incorporated heparin, gelatin, HA, and CS into various scaffolds (Figure 3).[104,162–167] The incorporation of a range of functional polysaccharide components was found to provide a useful ECM-mimetic environment, both in cell culture and in vivo, compared to natural extracted matrices, with less concern over batch-to-batch variation, pathogen transmission, and immunogenicity.[162] Studies of these scaffolds indicated that modulation of heparin content exerted dramatic effects on the controlled release of growth factors,[163,166] neovascularization, [166,167] and vessel maturity in dual growth factor release systems.[165]

Polysaccharide-Mimetic Molecules

Conjugation of polysaccharides, such as heparin, to polymeric biomaterials has proven beneficial, as important growth factors can be stabilized, sequestered, and activated. The heterogeneity of heparin, coupled with its possible contamination after isolation from mammalian sources, however, has complicated its use; therefore, identification of a homogeneous synthetic alternative to heparin would provide useful avenues for biomaterial modification. [168–170] To this end, Maynard and Hubbell identified a sulfated tyrosine sequence, from a combinatorial peptide library, that bound VEGF with a dissociation constant on the same order as that of the VEGF–heparin interaction.[171] We have explored the use of this

sulfated sequence and have evaluated the peptide NH_2-GGGG $SY_{SO3}DY_{SO3}$ GGGG-OH (SPa), along with other sulfated peptides, for association with multiple heparin-binding peptides. These studies predicted the potential for the SPa to act as a surrogate for heparin not only in association with VEGF but also in association with other heparin-binding peptides, for the formation of noncovalently crosslinked hydrogels (such as those in Figure 2).[172] Drug-delivery scaffolds were also created by conjugating this sulfated peptide to PEG hydrogel networks.[173] The heparin-mimetic peptide improved the sequestration of VEGF by SPa-modified, PEG-based hydrogels, controlling the release of VEGF while also protecting the VEGF against denaturation. The incorporation of these molecules in drug-delivery and tissue-engineering scaffolds offers opportunities for reducing the immunogenicity and side effects associated with isolating polysaccharides such as heparin from mammalian sources.

CONCLUSIONS AND OUTLOOK

The many examples discussed above indicate the broad range of biomaterial conjugates available by combining polymers with polysaccharides and describe how such combinations dictate the structures and functions of the bioconjugate. The properties of the synthetic polymers, such as hydrophilicity; hydrolytic susceptibility; reactive group placement; and sensitivity to temperature, pH, and ionic concentration, have all been used to tailor the bulk properties of the conjugates. Bio-friendly conjugation techniques, such as use of mild addition reaction chemistries or UV-labile radical initiators, have become mainstream in conjugating synthetic polymers with polysaccharides with the aim of creating implantable biomaterials. The properties of the synthetic polymers have provided necessary versatility to tune the structure and function of a given polysaccharide–polymer conjugate for desired applications including thermally responsive gelation, growth factor sequestration and delivery, and cell-directed remodeling.

The widespread use of polysaccharides in polymeric conjugates arises in part from their ease of mass production, low cost, simple conjugation and purification methods, and the addition of biological recognition to otherwise benign synthetic polymers. Incorporation of non-mammalian polysaccharides has enabled the design of many robust systems maintaining the low cost and low immunogenicity of the material, while conjugates comprising mammalian polysaccharides have been shown to elicit specific receptor responses and cell stimulation. Unfortunately, an obvious disadvantage that plagues all polysaccharides, given their sources (e.g., algae, insects, bacterial expression, and/or mammalian tissues), is their purity and pathogen content; a few different approaches have been developed to address these concerns. For example, the microbial fermentation of hyaluronic acid has increased the acceptance of hyaluronicacid-based polymeric materials and reduced concerns over the viral and prion-based contamination observed for the mammalian extracted form. The application of simple peptides as mimics of polysaccharides, as in the case of heparin, circumvents issues of heterogeneity and pathogen contamination. Newer technologies, such as total chemical or enzymatic synthesis of polysaccharides, offer real promise in the production of well-defined saccharide-based materials, but have not yet been regularly applied in the generation of polymer conjugates.[174]

The production of mammalian-based polysaccharide–polymer conjugates for improved biofunctionality has also been paralleled by investigations of the conjugation of proteins and peptides to synthetic polymeric materials. Conjugation of proteins and peptides to polymers, relative to the conjugation of polysaccharides, offers improved homogeneity and generally reduced risk of contamination. Monodisperse proteins and peptides can be conjugated with polymers via versatile and efficient chemical protocols to provide biologically active conjugates that can be recognized by enzymes or cell receptors.[175] The precise amino-acid sequence and structural conformations of these peptides and proteins dictate high affinity

binding constants and efficient assembly compared to those of heterogeneous polysaccharides. While this homogeneity can lead to more specific control over biomaterial design and properties, it can also limit the biological versatility of materials, and the use of proteins and peptides runs the potential risk of eliciting immunological responses.

Taken together, the development of polysaccharide–polymer conjugates has permitted the production of increasingly diverse and useful materials with tailored biological responses and chemical and mechanical properties, to better mimic the properties of the natural extracellular matrix. This has led to important new classes of environmentally sensitive materials that respond to the biological environment, including cell-demanded growth factor release or degradation that can further increase the efficacy of materials as tissue replacements. The future of polysaccharide and polymer conjugation will witness continued tailoring of the polysaccharides, and thus their properties, via expansion of chemical and biological methods of polysaccharide synthesis that will permit improved sequence control. Advances in these areas will facilitate new generations of polysaccharide-derivatized materials with controlled function, offering expanded options for guiding cellular fate for applications in tissue replacement and drug delivery.[175]

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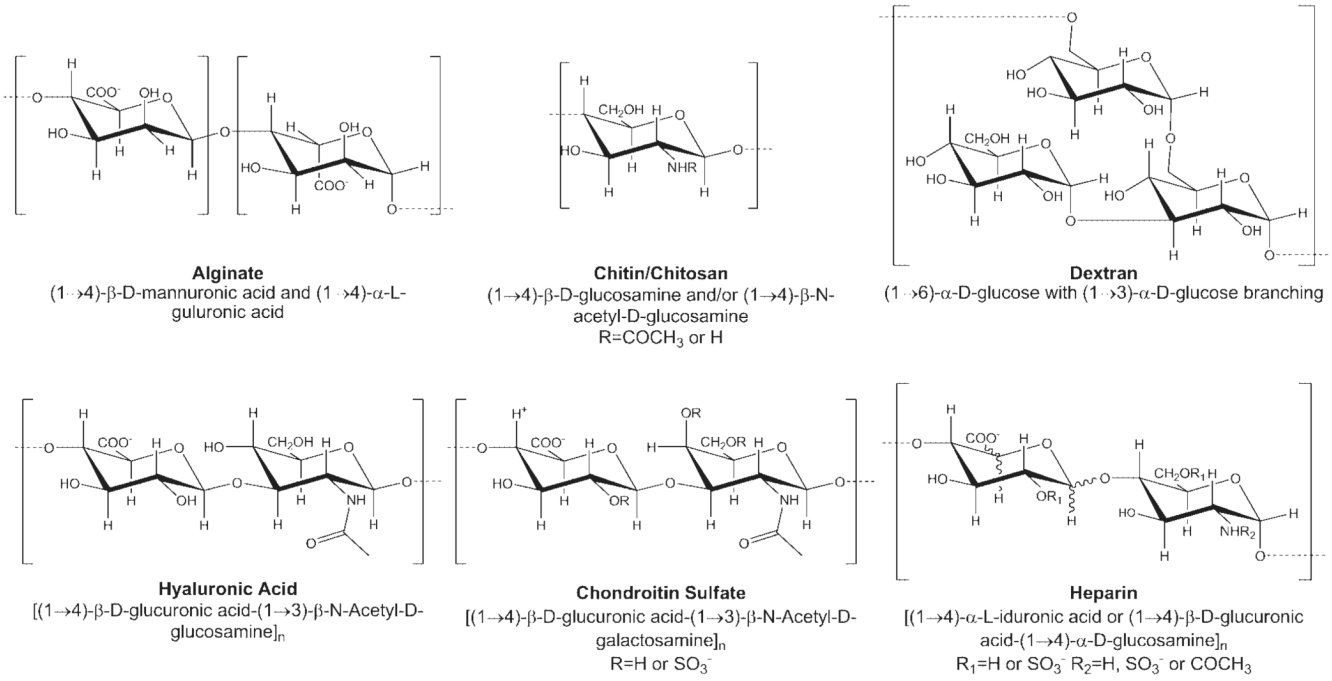

FIGURE 1.

Polysaccharides employed widely in biomaterials applications. Top row: non-mammalian polysaccharides; bottom row: mammalian polysaccharides.

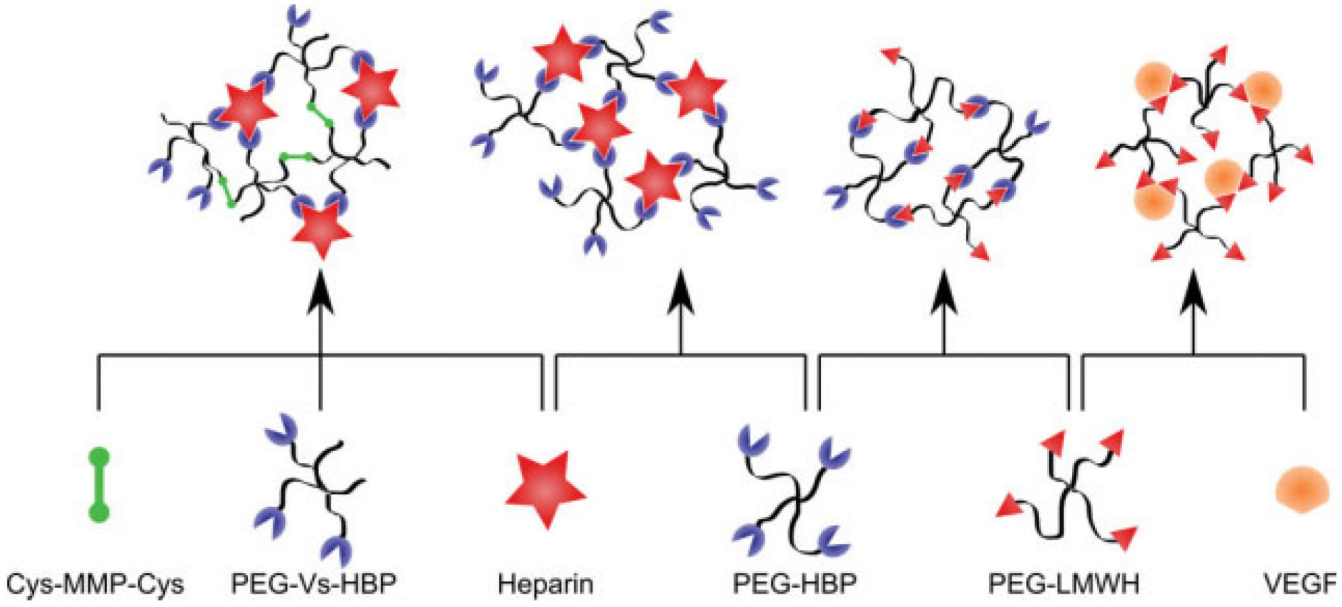

FIGURE 2.

Methods of noncovalent assembly for environmentally sensitive heparin-containing hydrogels. Cys-MMP-Cys: bifunctional cysteine-containing matrix metalloproteinase-cleavable peptide; PEG-Vs-HBP: star PEG modified with vinyl sulfone and heparin-binding peptides; PEG-HBP: star PEG modified with heparin-binding peptides; PEG-LMWH: star PEG modified with low molecular weight heparin; VEGF: vascular endothelial growth factor.

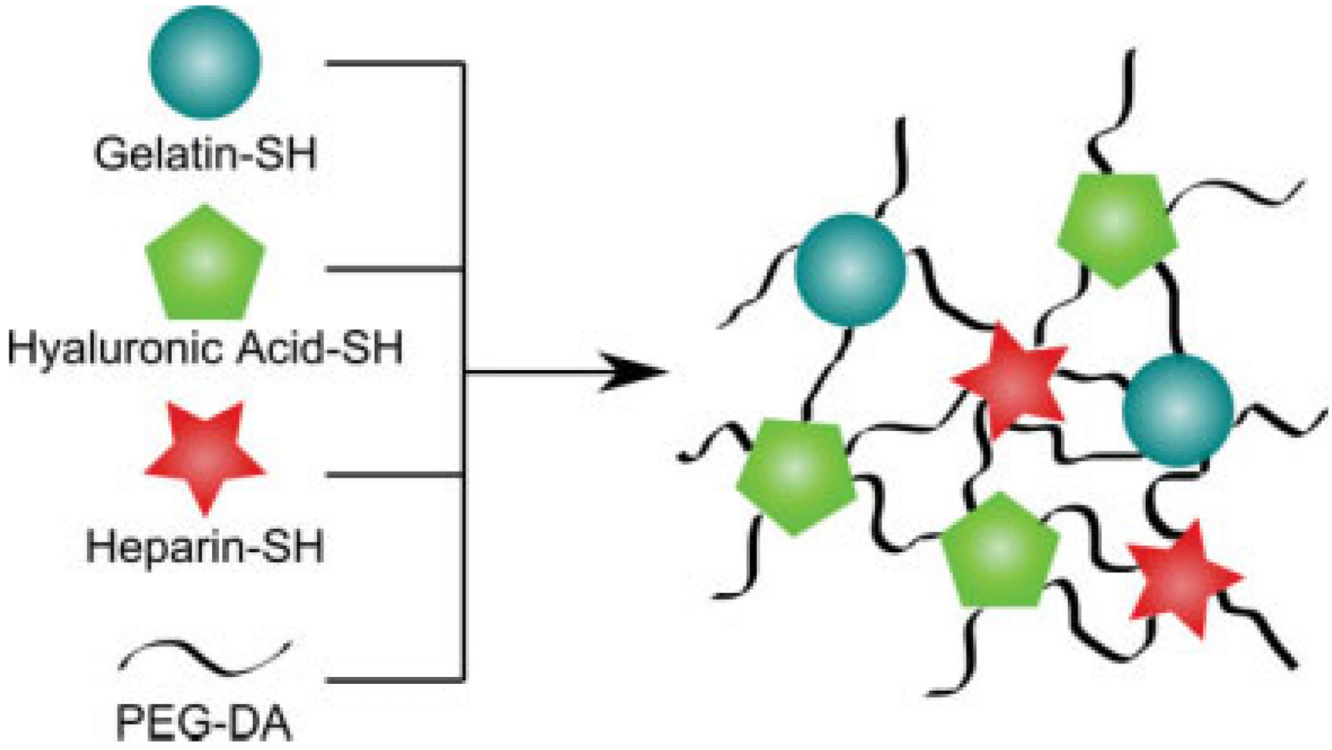

FIGURE 3.
An example of multipolysaccharide–polymer hydrogel conjugates crosslinked by the addition reaction of multifunctional thiolated substituents to PEG diacrylate.[165,166]