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# HEPARINOID-BASED BIOMATERIALS AND THEIR APPLICATIONS

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## ABSTRACT

Heparin, heparan sulfate (HS), heparin-like molecules from animal or plant origins, and synthetic derivatives of sulfated polysaccharides are referred to heparinoids. The various biological activities of heparinoids are generally ascribed to their specific interaction with heparin-binding proteins such as growth factors (GFs) and extracellular matrix (ECM) biomolecules, mediated by specific domains with distinct saccharide sequences. The specific interactions of heparinoids with GFs or ECM biomolecules require different combinations of sulfated groups, and thus, require different highly sulfated saccharide sequences. Multivalent and cluster effects of heparinoids are also important factors that control their interactions and biological activities. In this review, we describe heparinoid-based biomaterials to offer novel means to engineer functional protein delivery systems for biomedical applications.

**KEYWORDS:** **Heparinoid, Heparinoid-Based Biomaterials, Heparin-Binding Protein, Heparinoid-Carrying Polystyrene, Polyelectrolyte Complexes.**

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## INTRODUCTION

Heparinoids are members of the glycosaminoglycan (GAG) group of complex polysaccharides. GAGs include other carbohydrates such as chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS), all bearing negative charges that vary in density and position within thosepolysaccharides.[1-3] GAGs are normally present in the form of proteoglycans (PGs), in which multiple GAGs are covalently attached to a core protein.[1,4,5] Heparin is isolated on a commercial basis from animal tissues (pig or bovine intestinal mucosa, bovine lung, etc.) and has clinical use as an antithrombotic drug. In intact tissue, it is confined to mast cells, where it is stored in cytoplasmic granules.[6,7] In contrast, heparan sulfate (HS) is ubiquitously distributed on the cell surface and in the extracellular matrix (ECM).[8,9]Aside from their well-known anticoagulant action, heparinoids are associated with the various biological processes of growth factors (GFs) and cytokines, as well as being implicated in cell adhesion, recognition, migration, and regulation of various enzymatic activities.[10-13] Most of their biological functions depend upon the binding of various functional proteins to the polysaccharide chains, mediated by specific domains with distinct saccharide sequences.[8,14] For example, interactions of heparinoids with fibroblast growth factor (FGF)-1 and FGF-2 require different combinations of sulfate groups, and thus, require different saccharide sequences.[15-18] GF activity is localized within the natural ECM by specific non-covalent interactions with ECM biomolecules such as proteins and PG.[19] Those interactions have inspired to develop biomaterials that can regulate GF activities for applications.[20-22] For example, biomaterials covalently or non-covalently modified with heparinoids can augment their stability, controls of release, and activation. In addition, assemblies of heparinoids and other polyelectrolytes retain GFs at cell- material interface via specific interactions.[23,24] In this review article, the structures, biological activities, and therapeutic potential of those heparinoids are detailed to describe the various bioinspired strategies. They function to localize and to control GF activity together with various heparinoid-based biomaterials such as hydrogels and micro/nanoparticles. In addition, we will highlight our studies that have used these heparinoid-based biomaterials in functional protein delivery systems, wound repair, and tissue engineering strategies *in vivo*.

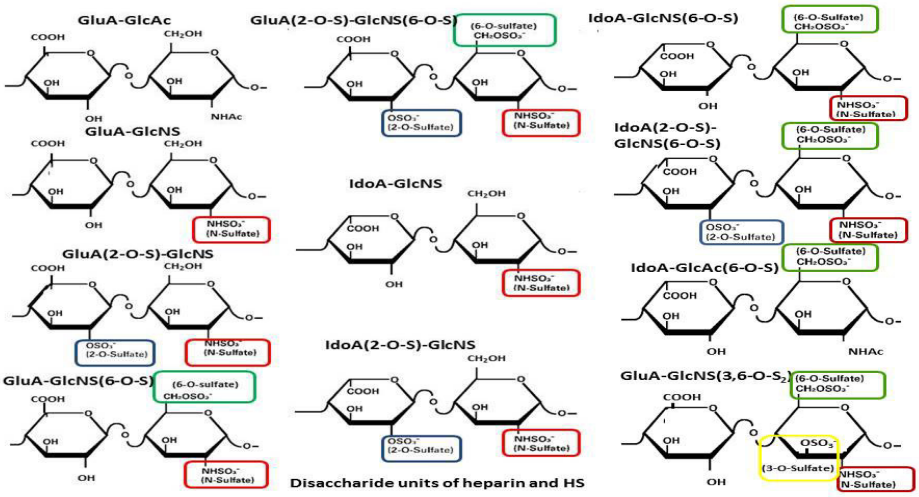
### *1. Molecular structures of heparinoids*

Heparin and HS are synthesized as PGs, which consist of polysaccharide chains covalently bound to a protein core. A single protein, serglycin, has been identified as the protein constituent of heparin-PGs, whereas a variety of proteins provide the core structures of heparan sulfate proteoglycan (HSPG).[8,25] Both heparin and HS fundamentally consist of a disaccharide repeat of (1→4 linked) D-glucosamine and hexuronate in which the glucosamine residues may be either *N*-acetylated (GlcNAc) or *N*-sulfated (GlcNS), and the hexuronate residues are present as either D-glucuronate (GlcA) or the C-5 epimer, L-iduronate (IdoA). Ester *O*-sulfations, principally at the C-2 position of IdoA and the C-6 position of the glucosamine residues, but also rarely at the C-2 position of GlcA and the C-3 position of glucosamine residues, add notable charge density and structural complexity to the polysaccharide chains (Figure 1).[26] There are structural differences between heparin and HS in IdoA content and *N*- and *O*-sulfate contents. Heparin is extensively *N*-sulfated, and is rich in the IdoA and *O*-sulfate groups, whereas HS contains more *N*-acetylated regions.[6,27] In general, approximately 80% of the glucosamine residues in typical commercial heparin are *N*-sulfated and the content of *O*-sulfate is higher than that of *N*-sulfate. In addition, approximately 70% of the hexuronate in the heparin is IdoA. In contrast, although there are large differences in HS produced by various cell types, typically fewer than 50% of glucosamine residues in HS are *N*-sulfated, and the content of *O*-sulfate is even fewer than that of *N*-sulfate. However, the distinction only serves to define the two families of polysaccharide composed of the same

repeating disaccharide units, and is not entirely evident (Figure 1).[8,26] The molecular design of HS appears to be well adapted for playing a fundamental role in various cellular activities. HS is an ordered polymeric structure in which sulfated sugar residues are clustered in a series of short domains widely separated by relatively long regions with low sulfate content.[6,14,27] The glucosamine residues in the highly sulfated clusters are highly *N*-sulfated, and most of the various *O*-sulfates and IdoA residues are present in these domains. The domain organization is a characteristic feature of HS that distinguishes it from heparin (Figure 1).

**Figure 1**

***Typical structures of heparin and HS (above) and disaccharide units (below).***




***2. Naturally occurring heparinoids***

A major group of heparinoids found in animal tissues except heparin and HS are the GAGs including CS, DS, and KS. CS and DS is composed of a disaccharide repeat of (1→3 linked)*N*-acetyl-D-galactosamine (GalNAc) and hexuronate (GlcA or IdoA) residues in which the galactosamine residues may be *O*-sulfated at the C-4 and/or C-6 position and at the C-2 positions of IdoA residues.[1-3] Fucoidan is isolated from marine plants, and is a highly sulfated polysaccharide (30－40%) like heparin, but fucoidan contains neither *N*-acetylated nor *N*-sulfated groups. The polysaccharide is primarily composed of 4-sulfated 1,2-linked α-L-fucose with branching or a sulfate group at C-3, and has been reported to have anti-thrombotic, anti-infective, and anti-inflammatory activities.[28,29]

***3. Synthetic derivatives of sulfated polysaccharides***

Chitin is the major organic component of the exoskeleton of crabs, shrimps, and insects, and is a (1→4 linked) co-polymer of *N*-acetyl-glucosamine units. Chitosan is a product obtained from the de-*N*-acetylation of chitinin the presence of hot alkali.[30] Chitosan interacts with FGF-2 and protects it from inactivation.[31] A chemically sulfonated chitosan has structural and functional similarities to heparin.[32] Chemically sulfonated dextran (dextran sulfate) has low anticoagulant activity with high lipoprotein-releasing activity.[33] Treatment of capsular K5 polysaccharide from *Eschericia coli* with mild acid to remove branches affords a (1→4 linked) copolymer of GlcNAc and GlcA. Re-*N*-acetylation, *N*- and *O*-sulfonations, and enzymatic C-5 epimerization affords heparin-like polysaccharides.[34,35]

***4. Interaction of heparinoids with proteins***

The biological activities of heparin result from its binding to proteins and modulation of their activities. These interactions are often very specific: for example, heparin's anticoagulant activity primarily results from binding antithrombin III at a discrete pentasaccharide sequence containing 3-*O*-sulfated glucosamine residue (Figure 1 and 4).[36] Although most ligands do not require the 3-*O*-sulfation, early studies of heparin and its interaction with antithrombin III have guided much of our

thinking about similar specific interactions between heparin-binding proteins and HS chains. The large number of heparin-binding proteins can be classified into a small number of groups (Table 1). Most of the functional properties of heparin and HS are ascribed to interactions between the polysaccharides and the various heparin-binding proteins. Those interactions generally depend upon the presence of specific highly sulfated regions in HS chains[37]. Examples of GFs include the FGF family (such as FGF-1, FGF-2, and FGF-4),[18,38,39] platelet-derived growth factor,[40] platelet factor-4,[41,42] hepatocyte growth factor (HGF),[43,44] vascular endothelial growth factor (VEGF),[45,46] and stem cell factor.[47] The interactions of heparinoids with heparin-binding proteins generally involve both ionic and hydrogen bonding interaction.[48]. Arginine and lysine in the proteins are positively charged (basic) amino acids, and hydrogen bonding interactions can involve basic and other polar amino acids (Asn, Gln, Ser, etc.). Typically, ionic and hydrogen bonding residues lie in a spatially tight array on the surface or in a shallow binding pocket of a heparin-binding proteins.[11,49,50]

**Table 1**

***Classes and examples of heparin-binding proteins***

| Class | Examples |
|---|---|
| Enzymes | glycosaminoglycan biosynthetic enzymes, thrombin and coagulation factors (proteases), complement proteins (esterases), extracellular superoxide dismutase, topoisomerase |
| Enzyme inhibitors | antithrombin III, heparin cofactor II, secretory leukocyte proteinase inhibitor, C1-esterase inhibitor |
| Cell adhesion proteins | P-selectin, L-selectin, some integrins |
| Extracellular matrix proteins | laminin, fibronectin, collagens, thrombospondin, vitronectin, tenascin |
| Chemokines | platelet factor IV, γ-interferon, interleukins |
| Growth factors | fibroblast growth factors（FGFs）, hepatocyte growth factor, vascular endothelial growth factor, platelet-derived growth factor, insulin-like growth factor–binding proteins, TGF-β-binding proteins, stem cell factor |
| Morphogens | hedgehogs, TGF-β family members |
| Tyrosine-kinase growth factor receptors | fibroblast growth factor receptors, vascular endothelium growth factor receptor |
| Lipid-binding proteins | apolipoproteins E and B, lipoprotein lipase, hepatic lipase, annexins |
| Plaque proteins | prion proteins, amyloid protein |
| Nuclear proteins | histones, transcription factors |
| Pathogen surface proteins | malaria cirumsporozoite protein |
| Viral envelope proteins | herpes simplex virus, dengue virus, human immunodeficiency virus, hepatitis C virus |

### *5. Heparin-based structurally modified sulfated polysaccharides*

The isolation of a sequence from HS responsible for a specific biological activity is a way to establish relationships between structure and function. An alternative approach is to prepare a series of structurally modified oligo-heparinoids and to determine the effects of these structural changes on biological activity. All of the sulfate groups in the heparinoids can be modified to introduce structural changes. Several such studies of heparin molecules have included procedures such as *N*-, 2-*O*-, and 6-*O*-desulfation,[50-53] *N*-deacetylation/sulfation, *O*-sulfation,[54-57] and carboxyl reduction.[58] These modification procedures have been useful for obtaining heparinoids with altered biological properties. Furthermore, studies on the binding of the modified heparinoids to various heparin-binding proteins have revealed some of the structural features involved in the binding.

*N*-sulfate groups of heparin can be selectively removed by solvolysis. The solvolysis has been performed by heating the pyridium salt of heparin in dimethyl sulfoxide containing a small amount of water.[54,55] When the reaction is performed at 50°C for a short period of time, almost all of the *N*-sulfate groups are removed, but none of the other structural features

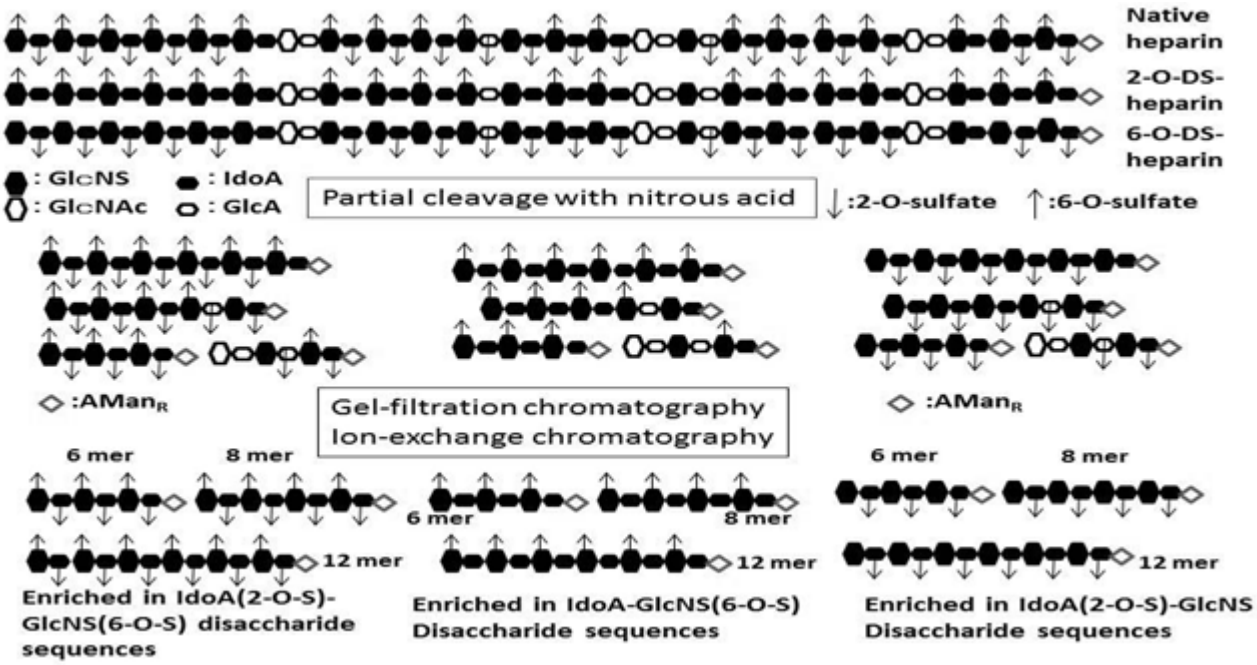
are modified. The modified solvolytic procedure used for the *N*-desulfation of heparin can also be applied to 6-*O*-desulfation. When heparin is heated in dimethyl sulfoxide containing a small amount of water at 90°C, the rates of desulfation decrease in the following order:*N*-sulfate >6-*O*-sulfate > 2-*O*-sulfate.[54,55] Because 6-*O*-desulfation occurs more rapidly than 2-*O*-desulfation, most of the 6-*O*-sulfates can be removed while a high proportion of the 2-*O*-sulfates remains. Following the reaction, the intermediates can be converted into 6-*O*-desulfated heparin by the re-*N*-sulfation of *N*-desulfated glucosamine residues, involving treatment with trimethylamine-sulfur trioxide complex in alkaline (pH 9) aqueous media.[17] Another method for specific 6-*O*-desulfation has been achieved by treatment of heparin (pyridiniumsalts) with *N*-methyltrimethylsilyl- trifluoroacetamide causes specific 6-*O*-desulfation without detectable depolymerization or other chemical changes.[51,53] Similarly, complete drying of heparin with various concentrations of NaOH by lyophilization cause specific 2-*O*-desulfation of hexuronate residues.[52] The degree of conversion in these *N*- and *O*-desulfation may be controlled, permitting the preparation of a range of partially modified heparinoids. The conversion can be controlled by limiting the reaction time or the amounts of reactants consumed in the reaction, or by modifying the reaction conditions.[51,52] These specific and controlled desulfation reactions result in the formation of unique heparinoid structures that may offer further possibilities for polymer modification.

### *6. Size- and structure-defined heparin-based oligosaccharides and their affinities and activation for FGF*

The structural variability of heparinoids makes it difficult to identify the protein-binding domains of a heparinoid without converting the polymeric heparinoid to oligosaccharides. Heparinoids can be partially cleaved by nitrous acid, heparin lysate, or byother methods.[48] All of the cleavage methods yield mixtures containing various oligosaccharide species that vary in both size and structure.[59] It is desirable to perform an initial experiment to identify the cleavage method that gives the maximal yield of the desired oligosaccharides.A library of size- and structure-defined oligosaccharides was prepared from intact heparin, 2-*O*-desulfated heparin, and 6-*O*-desulfated heparin by partial depolymerization with nitrous acid at pH 3 for 10 min (Figure 2). Resulting oligosaccharides were separated according to sizes by gel-filtration. Those size-defined oligosaccharides were then fractionated by ion-exchange chromatography to separate them based on their charges. Those 6-mers, 8-mers, 10-mers, and 12-mers enriched in IdoA (2-*O*-S)-GlcNS (6-*O*-S), IdoA-GlcNS (6-*O*-S), and IdoA (2-*O*-S)-GlcNS disaccharide sequences (≥80%) were finally prepared. Those oligosaccharides were then evaluated for binding affinities to FGFs and their abilities to promote their biological activities (Figure 2).[14,59]

**Figure 2**

***Preparation of size- and structure-defined oligosaccharides from native, 2-O-desulfate, and 6-O-desulfate heparins.***

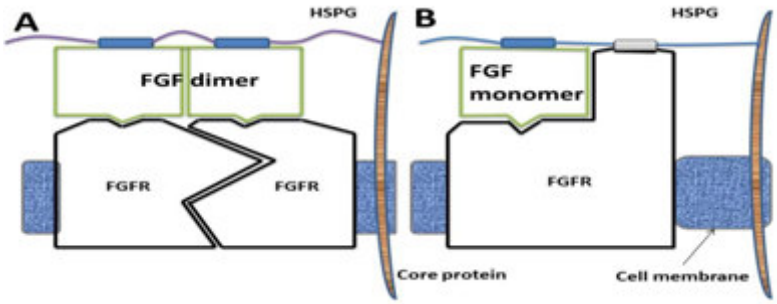



Oligosaccharides derived from chemically modified heparins bind to both FGF-1 and FGF-2 with different affinities. Our structural studies using selectively 2-*O*- and 6-*O*-desulfated heparins to various extents suggested that structural requirements in heparin and HS for promoting FGF-1 are different from those for FGF-2.[18,39] In the case of chlorate-treated A31 cells, which do not produce endogenous sulfated HSPG, intact heparin can restore the mitogenic activities of both FGF-1 and FGF-2. Partial 2-*O*-desulfation of heparin resulted in a decrease in the ability to restore the mitogenic activities of both FGF-1 and FGF-2, and 75% or higher 2-*O*-desulfation resulted in the complete loss of ability.[52] Similarly, partial 6-*O*-desulfation of heparin resulted in a decreased ability to restore the mitogenic activity of FGF-1, and 62.2% or higher of 6-*O*-desulfation resulted in the complete loss of ability.[51] In contrast, the ability to restore FGF-2 activity was not significantly decreased by partial 6-*O*-desulfation up to 66.8%. Thus, a high content of 6-*O*-sulfate groups in heparin and HS, in addition to a high content of 2-*O*-sulfate and N-sulfate, is required for the activation of FGF-1, but it is not necessary for FGF-2.[51,52] Selectively *O*-desulfated heparin wasapplied to affinity column-immobilized FGF-1 and FGF-2, and eluted using a discontinuous gradient of NaCl. The salt concentration required for complete elution from both the columns was dependent upon the size and the specific structure.[18,53,59] In general, smaller oligosaccharides (2-mers and 4-mers) from the modified heparins do not show even a small affinity for FGF-1 and FGF-2. In each 6-mers, 8-mers, 10-mers, and 12-mers, the binding affinities for FGF-1 and FGF-2 were dependent on the specific structure. The 10-mers and 12-mers enriched in IdoA (2-*O*-S)-GlcNS (6-*O*-S) disaccharide sequences exhibited high affinities and activations for both FGF-1 and FGF-2, whereas the same-sized oligosaccharides enriched in IdoA-GlcNS (6-*O*-S) disaccharide sequences did not influence them.[18,59] It should be pointed out that the 6-*O*-sulfate groups of GlcNS residues of the large oligosaccharides (10-mers or 12-mers) strongly influence the interaction with FGF-1, but not FGF-2.Mitogenic activities of FGF-1 and FGF-2 are exerted via the formation of ternary complexes with heparinoid, FGF, and FGF-receptors (FGFR).[12,60,61] In these complexes, heparinoid

oligosaccharides keep together the GF and its receptor, allowing functional contacts that promote signaling (Figure 3A). In contrast, many proteins, such as FGF-1 and FGF-2, exist or self-assemble into homodimers or multimers in their active states, and these structures are often required for protein activity (Figure 3B).[62,63] Using the library of heparin-derived oligosaccharides, the common binding motifs required for binding to FGF-1 and FGF-2 have been shown to be IdoA (2-*O*-S)-GlcNS (6-*O*-S) and IdoA-GlcNS (6-*O*-S) disaccharide sequences, respectively.[38,59,64] The 6-mersand 8-mers in size were found to be sufficient for binding FGF-1 and FGF-2, but 10-mers or larger oligosaccharides are required for their biological activities.[12,18,59,65] Because the 6-mers and 8-merscan display sulfate groups on only one FGF molecule, it may not be able to promote FGF dimerization.

**Figure 3**

***Interactions of FGF and highly sulfated regions of HS***



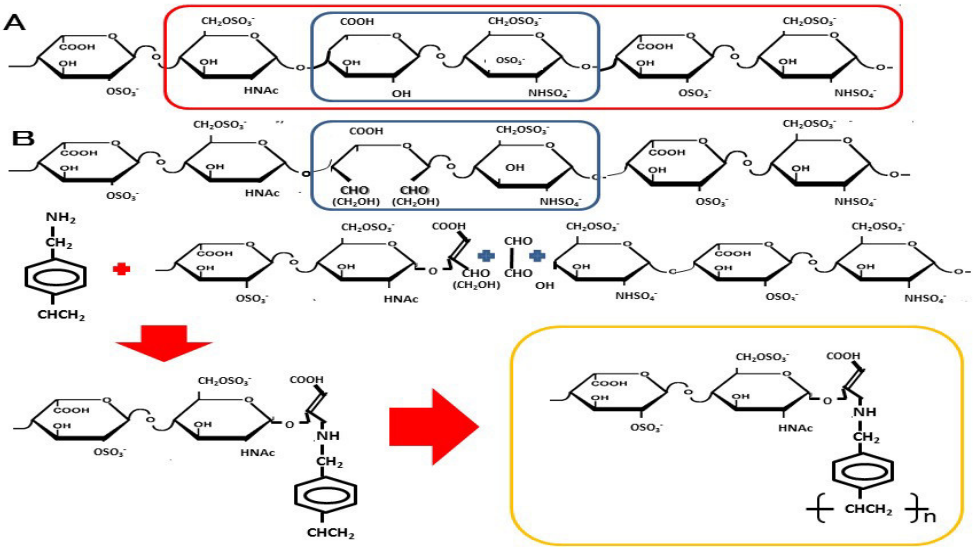

***Panel A dipicts self-assembly into homodimers or multimers, Panel Bdepicts a formation of ternary complexes with heparinoid, FGF, and FGFR.***

### *7. Non-anticoagulant (NAC-) heparin and its application*

Heparinoids specifically interact with functional proteins with high affinity including GFs, cytokines, ECM components, and adhesion molecules.[8,48] In fact, heparin is a therapeutic agent in various pathological conditions that involve functional proteins; however, high-dose heparin cannot beused because of the excessive risk of bleeding.[26,66] NAC-heparin can be obtained by removing a specific sequence [GlcNAc (6-*O*-S)-GlcA-GlcNS (3,6-di*O*-S)-IdoA (2-*O*-S)-GlcNS (6-*O*-S)] from unfractionated (native) heparin.[36] This removalcan be achieved by removing with specific modifications such as *N*-desulfation/acetylation, 2-*O*-desulfation, and 6-*O*-desulfation. However, those modifications substantially reduce various physiological activitiesas well as anticoagulant activity of native heparin. A modification of the procedure[67] was used for the preparation of periodate-oxidized ($IO_4$-), alkaline-degraded ($IO_4$-LMW-) heparin as NAC-heparin (Figure 4).[67,68] The reduced $IO_4$- and $IO_4$-LMW-heparin lost unsulfated hexauronate (GlcA or IdoA)-containing structures and were composed of trisulfated disaccharide units [> 85% UA (2-*O*-S)-GlcNS (6-*O*-S)]. They could be mixed with chitosan for controlled the release of FGF-2 as a drug delivery carrier to control angiogenesis.[69]

**Figure 4**

***Structure of antithrombin III binding sites (panel A), preparation of periodate-oxidized ($IO_4$-), alkaline-degraded ($IO_4$-LMW-) heparin as NAC-heparin (panel B), and $IO_4$-LMW-heparin-carrying styrene monomer and polystyrene (NAC-HCPS)***




***The specific sequence (A) could remove by the modification procedure to $IO_4$-LMW-heparin.***

The loading of heparinoid-based drug delivery systems primarily occurs through electrostatic mechanism between negatively charged heparinoids and positively charged molecular cargo. Besides the electrostatic mechanism, negatively or non-charged cargo molecules can be loaded via specific interactions between heparinoids and cargo molecules.[70,71] Biodegradable heparinoid-based hydrogels containing GFs as such cargo molecules may provide a valuablesystem.[72]Water-soluble chitosan molecules (CH-LA) at neutral pH-values have been prepared by the introduction of lactose. The material is a viscous solution and readily gels upon mixing with heparinoid solution, resulting in an injectable hydrogel owing to the polyelectrolytic interaction between heparinoids (negatively charged) including reduced NAC-heparin,[73,74] 6-*O*-desulfated heparin,[75] andfucoidan,[76] and CH-LA (positively charged).When the FGF-2/NAC-heparin/CH-LA was subcutaneously injected into the backs of mice or rats, marked neovascularization and fibrous tissue formation were induced near the injected sites. Furthermore, controlled release of biologically active FGF-2 from FGF-2/NAC-heparin/CH-LA led to the induction of angiogenesis and, possibly, collateral circulation.[73,74]

### *8. NAC-heparin carrying polystyrene and its applications*

$IO_4$-LMW-heparincarrying polystyrene (NAC-HCPS) is a synthetic glycoconjugate that is soluble in water. NAC-HCPS has a molecular weight of approximately 80 – 120 kDa and comprises approximately ten $IO_4$-LMW-heparins linked to a polystyrene core (Figure 4).[77,78] The hydrophobic polystyrene core of NAC-HCPS in water may be buried inside the large molecule to form a hydrophobic core hidden from water.[78] The NAC-HCPS showed a markedly reduced anticoagulant activity relative to native heparin and an enhanced ability to interact with various heparin-binding GFs such as FGF, VEGF, and HGF. However, NAC-HCPS strongly inhibited heparin-binding GF-induced endothelial cell proliferation*in vitro*. NAC-HCPS contains a high density of trisulfated disaccharide [IdoA (2-*O*-S)-GlcNS (6-*O*-S)] enriched $IO_4$-LMW-heparin

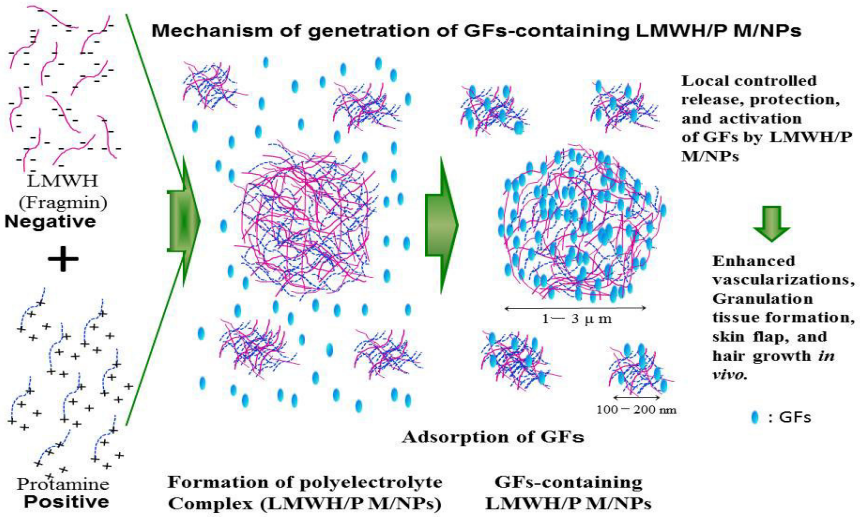
chains oriented towards the solution. The hydrophilic $IO_4$-LMW-heparin chains tend to be oriented toward the outside of the polymer, resulting in a higher concentration of carbohydrates on the polymer surface. The ability of cell surface receptors to recognize the target is greatly enhanced by an increase in the density of carbohydrate chains.[69] Similarly, enhanced biological activities due to the carbohydrate-clustering effect and immobilization of carbohydrate-clustered PGs have been reported and ascribed to the presence of multiple GAG chains in the core protein.[69,78] It is possible that the excessively high density and clustering structure of $IO_4$-LMW-heparin chains does not support the overall interaction of heparin-binding GFs with their receptors to induce mitogenic activities, although NAC-HCPS interacts more strongly with GFs than $IO_4$-LMW-heparins. In fact, NAC-HCPS inhibited angiogenesis *in vivo* and inhibited subcutaneous induced tumor growth and metastasis *in vivo.*[69] NAC-HCPS also inhibited neointimal proliferation of balloon-injured arteries.[79] NAC-HCPS is efficiently adsorbed onto plastic surfaces such as those of tissue culture plates, and heparin-binding GFs are immobilized on the surface of NAC-HSPS-coated plates.[77] Mouse adipose tissue-derived stromal cells (ATSCs) grew well in low serum and maintained their multilineage potential for differentiation on NAC-HSPS-coated plates with FGF-2.[80] Thus, the NAC-HSPS-coated plates with FGF-2 in low-serum media may be used for autologous ATSC expansion in clinical cell therapy.

### *9. Heparinoid-based micro/nanoparticles and their applications.*

Heparinoids, conjugated to micro/nano-materials,have recently been investigated for their chemical and biological properties and applications.[20] Heparinoid has been conjugated to the surface of micro/nanoparticles (M/NPs), such as magnetic[81] and metallic nanoparticles,[82] systemic polymers,[83] and biopolymers.[84] When biological molecules such as functional proteins and DNA are incorporated on or within M/NPs, they provide novel and enhanced activities with potential applications in therapeutics, biosensors, imaging, and drug delivery.[20,85] Those biomolecules can be passivated on M/NPs, improving their biocompability. One class of molecules of interest for their ability to cloak foreign materials is a family of polysaccharides including heparinoids, which naturally cover the surfaces of all eukaryotic cells. Polyelectrolyte complexes (PECs) are generated by electrostatic interactions between oppositely charged polyelectrolytes, such a slow-molecular-weight heparin (LMWH) (MW: approximately 5000 Da) and protamine (P). When this interaction occurs in non-equivalent ratios, nonstoichiometric PECs are produced, causing each PEC particle to carry an excess charge.[22,23,86,87] Proteins interact with both synthetic and natural PECs.[24]Heparin is useful as a therapeutic agent in various pathological conditions that involve functional proteins. However, high-dose heparin cannot be used because of the excessive risk of bleeding.[66] In contrast, LMWH offers pharmacological and practical advantages compared with heparin. The lower protein-binding activity of LMWH produces a low, stable, and predictable anticoagulant response, thereby bypassing the need for laboratory monitoring of drug levels to adjust the dosage.[66] In addition, one or two subcutaneous injections per day are sufficient to maintain therapeutic concentrations because of its longer plasma half-life.[66] In contrast, P, a purified mixture of proteins obtained from fish sperm, neutralizes heparin and LMWH by forming a stable complex that lacks anticoagulant activity.[88] P is also employed clinically to reverse the anticoagulant activity of heparin following cardiopulmonary bypass as well as in cases of heparin-induced bleeding.[89]Low-molecular-weight heparin and protamine (LMWH/P)N/MPs have previously been prepared as PECs(Figure 5).[21,22] LMWH/P N/MPs are specifically bound to FGF-2,[90,91] HGF,[92] and other GFs secreted from platelet-rich plasma (PRP).[93]

**Figure 5**

***LMWH/P N/MPs were prepared as PECs.***




***LMWH/P N/MPs are specifically bound to FGF-2, HGF, and other secreted GFs***

LMWH/P N/MPs can be retained on cell surfaces and matrix in various tissues *in vivo* to control its release, and can protect and activate GFs. GF-containing LMWH/P N/MPs induced vascularization and fibrous tissue formations by stabilizing, activating, and gradually releasing GFs from GF-containing LMWH/P N/MPs.[93] LMWH/P N/MPs bind to various adhesive cell surfaces, including adipose-derived stromal cells and bone marrow-derived mesenchymal stem cells (BMSCs) through specific interactions between LMWH/P N/MPs and cell surface heparin-binding proteins such as integrins.[22] Interaction of the cells with LMWH/P N/MPs resulted in cells and LMWH/P N/MP-aggregate formation within a few hours. Those aggregates increased cellular viability *in vitro*. Injection of the aggregates induced vascularization and fibrous tissue formation *in vivo*.[94] Thus, LMWH/P N/MPs as cell carriers can enhance cell viability.

***10. Toxicities of heparinoid-based biomaterials.***

Heparin is as one of the oldest drugs currently in widespread clinical use as an anticoagulant, first natural product, and one of the few carbohydrate drugs. However, high-dose heparin cannot be used because of the excessive risk of bleeding.[66] For this reason, it was necessary to use NAC-heparin ($IO_4$- or LMW-heparin) when applying as biomaterials. In 2008, certain heparin lots were associated with anaphylactoid-type reactions, Oversulfated chondoroitin sulfate (OSCS), a semi-synthetic GAG, was identified as a contaminant, It has been considered that the OSCS was toxic leading to the anaphylatoid-tyle reaction.[95] Thus, native heparin, $IO_4$-, and LMW-heparin have little toxicity, However, chemically over-sulfated polysaccharides or synthetic non carbohydrate-derived heparinoids are generally toxic.[48,96] Sufficient data are not available on the complete toxicity profile of NAC-HSPS. Although NAC-HSPS inhibited angiogenesis *in vivo*, subcutaneous induced tumor growth and metastasis *in vivo*,[69] NAC-HCPS, and neointimal proliferation of balloon-injured arteries,[79] we observed weight loss in the animal. A standard toxicologic study should be performed in advance of clinical application. LMWH/P N/MPs have so far been observed no toxic effect. In fact, the two components for LMWH/P N/MPs, LMWH and P, are already clinical use, their clinical safety is ensured.

## OVERVIEW

Haparinoids could be incorporated into drug delivery systems in tissue engineering and biotechnology. In addition to their well-known anticoagulant action, they associated with various biological processes of GFs and cytokines, as well as being implicated in cell adhesion, recognition, migration, and regulation of enzymatic activities. The use of heparinoids in medical and biotechnological fields often requires adequate chemical modifications to the polymers that can change their properties and influence their affinities for functional proteins such as GFs. As described in this review, knowledge of the interaction of native and modified heparinoids with functional proteins may result in the production of valuable heparinoid-based biomaterials for controlled protein delivery. Many studies have identified specific binding sequences for different heparinoid-binding proteins, and some biochemical processes, such as anticoagulation and FGF signaling, proceed only with specific binding sequences, as described in this review. $IO_4$-LMW-heparin that is simply modified NAC-heparin, composed of more than 85% of trisulfated disaccharide units (IdoA (2-*O*-S)-GlcNS (6-*O*-S)) can interact with almost functional protein and can affect their biological activities. However, NAC-heparin often has only weak biological activities *in vivo*. Engineering approaches are important for further reinforcement of chemically modified heparinoids to improve their *in vivo* applicability as biomaterials. Covalent or non-covalent modification of biomaterials with heparinoids can augment their stability, localization, control of release, and activation. In addition, assemblies of heparinoids and other polyelectrolytes retain GFs at local cell－material interfaces via specific non-covalent interactions. In the present review, heparinoid-based biomaterials such as NAC-HCPS, hydrogel, and M/NPs and their functions were discussed with respect to their applications as versatile biomaterials. Heparinoid-based materials such as membrane, hydrogel,[73,74] and M/NPs[90,93] have been shown to be safe and efficacious *in vivo* for the delivery of a variety of heparin-binding molecules.

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## CONFLICT OF INTEREST

There are no conflicts of interest to reports.

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