Published in final edited form as:

Acta Biomater. 2014 April ; 10(4): 1705–1719. doi:10.1016/j.actbio.2013.09.039.

Molecular engineering of glycosaminoglycan chemistry for biomolecule delivery

Tobias Miller¹, Melissa C. Goude¹, Todd C. McDevitt^{1,2}, and Johnna S. Temenoff^{1,2}

¹Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Drive, Atlanta, Georgia 30332, USA

²Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332, USA

Abstract

Glycosaminoglycans (GAGs) are linear, negatively charged polysaccharides that interact with a variety of positively-charged growth factors. In this review article, the effects of engineering GAG chemistry for molecular delivery applications in regenerative medicine are presented. Three major areas of focus at the structure-function-property interface are discussed: 1) macromolecular properties of GAGs, 2) effects of chemical modifications on protein binding, and 3) degradation mechanisms of GAGs. GAG-protein interactions can be based on 1) GAG sulfation pattern, 2) GAG carbohydrate conformation, and 3) GAG polyelectrolyte behavior. Chemical modifications of GAGs, which are commonly performed to engineer molecular delivery systems, affect protein binding and are highly dependent on the site of modification on the GAG molecules. The rate and mode of degradation can determine the release of molecules as well as the length of GAG fragments to which the cargo is electrostatically coupled and eventually released from the delivery system. Overall, GAG-based polymers are a versatile biomaterial platform offering novel means to engineer molecular delivery systems with a high degree of control in order to better treat a range of degenerate or injured tissues.

3 INTRODUCTION

Glycosaminoglycans (GAGs) are a class of linear polysaccharides that are ubiquitous in the human body and possess multiple biological functions essential for life [1]. Such functions consist of 1) osmotically attracting water and therefore maintaining hydrostatic pressure to confer mechanical stability in connective tissues such as cartilage [2–6], 2) covalent attachment to proteoglycans that regulate cell function [7], and 3) acting in conjunction with proteins on cell surfaces via receptors or co-receptors to modulate the local biological environment [8]. Based on their numerous biological functions, GAGs have been extensively explored as biomaterials for controlled protein delivery to improve treatment for a variety of diseases [9–12].

© 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Correspondence to: Johnna S. Temenoff.

9 Conflict of Interest

The authors declare no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Many of their biological functions are conferred by the unique chemical structure of GAGs, consisting of repeating disaccharide units that are specific for each GAG species. Sulfated GAG species such as chondroitin sulfate (CS), heparin, heparan sulfate (HS), dermatan sulfate (DS), and keratan sulfate (KS) bear negative charges that vary in density and position within the disaccharide units [13]. In addition to sulfated GAGs, hyaluronic acid (HA) is non-sulfated and therefore is the GAG with the least net negative charge [14]. Based on this negative net charge, GAGs attract positively-charged proteins, however, these binding processes are very challenging to investigate because they are governed by the complex, inherent chemical properties of GAGs [15–17]. For protein delivery applications, a number of GAG-based approaches have been developed that mimic the interactions that occur naturally between GAGs in the ECM and growth factor binding partners. GAGs can possess specific carbohydrate sequence-specific, electrostatic binding sites for some growth factors, or they can bind growth factors via a non-sequence specific electrostatic mechanism [18].

Although protein- specific binding sites including conformational changes upon binding have been reviewed previously [19–21], this work focuses on reviewing the chemical properties and modifications of GAGs for protein binding and incorporation into complex biomolecule delivery systems. Besides considering the effects on protein binding, chemical modifications affect degradation processes [22, 23], which, in turn, influence molecular release characteristics, and therefore degradation mechanisms are also discussed in detail here. A thorough understanding of the chemical properties of GAGs, both native and modified, and how they relate to protein binding, is a key factor for successful implementation of GAG-based biomaterial strategies in tissue engineering and drug delivery applications. As described throughout this review, a better understanding of GAG chemistry will lead to enhanced predictability of protein uptake and release from GAG-based biomaterials, and thus the ability to design more efficacious strategies for harnessing the unique, innate properties of GAGs for a broad range of regenerative medicine applications.

4 GAG-PROTEIN BINDING: A FUNCTION OF SULFATION PATTERN, 3D CONFORMATION AND POLYELECTROLYTE PROPERTIES

Generally, it is believed that net negative charge is primarily responsible for mediating GAG interactions with oppositely charged proteins, but polyelectrolyte complexation does not fully explain protein affinity to GAGs. The primary structure of GAGs is determined by carbohydrate repeat units with their specific sulfation patterns, which influence complex 3D structures that contribute to the pharmacological activity of GAGs. Moreover, most GAG species do not exist *in vivo* in an isolated state, but, instead, are synthesized in the form of proteoglycans (PGs) or serve as co-receptors by GAG-growth factor complex formation on cell surfaces [24]. GAG attachment to PGs is not directly linked to the sulfation pattern, but to a specific carbohydrate end-group sequence by which GAG chains are linked to the PG core protein. GAG-PG attachment has already been reviewed [7, 25] and, thus, this section focuses on the importance of GAG 3D structure combined with sulfation pattern on growth factor binding for each major representative of the GAG family.

4.1 Carbohydrate structures and nomenclature

For the reader's reference, this section summarizes the most important monosaccharide structures, conformations and configurations of GAG subunits in order to better understand the specific epitopes presented in the following sections. Recommendations on sugar nomenclature rules and determination of conformation were published by the International Union of Pure and Applied Chemistry (IUPAC) [26, 27]. Monosaccharide units of relevance for GAGs are uronic acid and amino sugars (Figure 1). Such monosaccharides can acquire different solution conformations (Figure 2A). Among the most well-known conformations

are chair (*C*), boat (*B*), and envelope (*E*). The chair conformations and intermediate conformation between chair and boat, skew-boat (*S*), plays an important role for antithrombin III (AT III) binding with heparin (Figure 2A). In solution, each carbohydrate is in equilibrium with its different conformations. Furthermore, the α/β - and D,L nomenclature is also used to distinguish between different configurations (Figure 2B).

4.2 Structure of heparin and heparan sulfate

The carbohydrate composition for heparin and heparan sulfate (HS) is similar but differs in monosaccharide ratios and sulfation pattern distribution. The most prominent disaccharide repeat unit in heparin consists of 2-O sulfated L-iduronic acid (IduA2S, α -1,4) and a mixture of either N- and 6-O sulfated (GlcNS6S) or N-acetylated D-glucosamine (GlcNAc, α -1,4). In HS, instead of heparin's IduA2S, the majority of uronic acid residues are D-glucuronic acid (GlcA, β -1,4). These repeat units are connected in a complex pattern including other residues with additional O- and N-sulfated groups: GlcNAc can be additionally 6-O sulfated (GlcNAc6S), or GlcNS less commonly 3-O sulfated (GlcNS3.6S) [28]. Unfractionated heparin has a molar mass between 3 to 30 kDa (15 kDa average) [29], whereas heparan sulfate, e.g. from human liver, was found to have a molar mass around 24 kDa [30]. Heparan sulfate is a key component of PGs secreted into the extracellular matrix, such as perlecan [31], or agrin [32], but HS transmembrane PGs can also serve as receptors or co-receptors (e.g. syndecans) [33]. Consequently, HS is present in many tissues and can serve multiple functions, while the presence of heparin in humans is limited to very few tissues. The best-described occurrence of heparin is in mast cell granules, where its function and evolutionary role is still not fully understood [34, 35].

Besides tissue distribution and function, there are general structural differences between HS and heparin. Specifically, these species differ in the overall charge distributions along the polymeric chain: heparan sulfate exhibits sulfate-rich, or "S-rich," regions [36] that are separated by disaccharide units that contain mainly unsulfated, acetylated glucosamine and GlcA (NA-regions [37]). Interestingly, the number of HS sulfate clusters change during cell differentiation, leading to more sulfated regions with greater differentiation, whereas stem cells exhibit fewer amounts of sulfate clusters [38, 39]. The combination of sulfated and non-sulfated regions in HS leads to a very flexible conformational structure because the alternating structure consisting of regions with high and low sulfation may cause HS to bind and "wrap" around a variety of proteins through non-carbohydrate sequence-specific interactions [40, 41]. Although HS-protein interactions may not always be sequence-specific, different cell types produce HS derivatives with various repeating monosaccharide patterns in the sulfated regions that potentially account for some protein specificity in certain tissues, but the exact physiological role of tissue-dependent HS compositions remains unknown [42].

In contrast to non-sequence specific interactions, carbohydrate sequence-specific GAG-protein interactions have been elucidated for heparin/HS. The most well-investigated example is basic fibroblast growth factor (FGF-2) binding to heparin/HS, for which specificities and effects have been studied since the 1980s [43]. The FGF family consists of 22 distinct isoforms that are sub-divided into 7 sub-families [44]. The transmembrane tyrosine kinase receptor for FGF is activated by heparin or HS as a co-factor, which induces FGF-dimerization and enhances FGF signaling [45]. From a series of studies on this topic, a minimal pentasaccharide sequence [46] from heparin was found to be responsible for FGF-2 pairing. Moreover, a 6-O desulfated (glucosamine residue) heparin was deemed to promote FGF-2 binding and 2-O sulfate groups (iduronic acid residue) were found to be essential for interaction. Besides FGF-2 attraction, 2-O-sulfation of the iduronic acid was found to be essential for binding to proteins such as human glial cell neurotropic factor [47], and

endostatin [48]. However, in other studies, a decasaccharide unit, fully N-sulfated and partially 2-O- (IduA residue) and 6-O- (GlcNS residue) sulfated, was determined to be the minimum binding motif for FGF-1 and FGF-4 [49]. In addition, the carboxyl function of the uronic acid residues (Figure 1) was found to be necessary for binding to FGF-2.

The results described above highlight the inconsistencies in the minimal binding sequence lengths and sulfation patterns thought to be required for FGF binding [50–53]. In order to explain the different results for the FGF superfamily, one important point to consider is the heterogeneity of the FGF protein family, as well as conformational changes of the polysaccharide itself. Conformational aspects are under current investigation using molecular modeling [54] and analytical tools such as NMR spectroscopy [55, 56]. Generally, pyranose sugars (6-membered ring system consisting of 5 ring carbons and 1 oxygen) such as iduronic acid are present in different conformations simultaneously: chair conformations (4C_1 , 1C_4) or skew-boat conformation (2S_0) (Figure 2A). These conformations are in equilibrium to each other when sugars are in solution and the glycosidic bonds confer a small degree of freedom to switch between conformations [57]. In this context, the role of 2-O sulfation (IduA) for binding of antithrombin III AT-III for anti-coagulation purposes is important, as the 2-O-sulfation (IduA) as well as the 3-O-sulfation (GlcNS3.6S) instigate a conformational change to the skew-boat conformation upon binding of heparin to AT-III [58]. Once bound to AT-III, heparin potentiates the anticoagulant activity of AT-III by factor 2000 [59]. However, the AT-III binding region [57, 58, 60, 61] lies outside of the active binding region for FGFs (FGF-1/2) [62], so the relative contribution of GAG conformation to interaction with FGF remains unknown. The carbohydrate conformations and sulfate group activities that are relevant for protein binding are summarized in Figure 3.

Overall, heparin and HS exhibit structural similarities but differ mainly in the distribution and quantity of sulfate groups per polymer chain. The sulfate cluster structure of HS allows for a variety of non-carbohydrate sequence-specific protein interactions, whereas heparin possesses a defined AT-III carbohydrate binding sequence. Moreover, the fact that the polyelectrolyte nature of the GAGs alone might lead to “random” binding based solely on electrostatic interactions is well characterized for HS [18, 41] and can likely be assumed for heparin as well.

4.3 Structure of chondroitin sulfate and dermatan sulfate

Chondroitin sulfate (CS) and dermatan sulfate (DS) are structurally related and often found jointly in PGs. The monosaccharide components of the most prominent repeating disaccharide units in chondroitin sulfate (CS) are D-glucuronic acid (GlcA, β -1,4) and N-acetyl-D-galactosamine (GalNAc, β -1,3) [63]. Epimerization at C-5 (Figure 1) during DS biosynthesis of some D-glucuronic acid residues to L-iduronic acid (IduA) converts CS to DS, which is done *in vivo* by specific epimerases [64, 65]. Generally, the IduA residues can only be found adjacent to GalNAc-4-sulfate (GalNAc4S) [66]. Although molar masses of CS and DS are similar (23 kDa [67] and 25 kDa [68], respectively), the sulfation patterns of both GAGs are very diverse and a separate nomenclature for different CS species has been established. Generally, CS polymers are referred to as CS-A (GalNAc4S), CS-C (GalNAc6S), CS-D (GlcA2S, GalNAc6S) and CS-E (GalNAc4,6S) depending on the combination of sulfate positions. For DS, the most frequent sulfation pattern is the GalNAc4S associated with IduA [69]. Functions of the sulfate groups for CS and DS are summarized in Figure 4.

Interaction of growth factors with CS and DS occurs in a PG-bound state, as neither CS nor DS are available in their free state in blood or tissue. PGs often contain mixtures of CS and DS that are specific for the tissue in which they were synthesized. CS-rich PGs such as

versican (connective tissues) and aggrecan (cartilage) and DS-rich PGs such as decorin and biglycan (both found in connective tissues) [70, 71] are important co-factors for receptor function [72].

For DS, the IduA residue, similar to heparin and heparan sulfate, is able to exist in multiple conformations including the 4C_1 , 1C_4 and 2S_O [63]. In heparin, besides the carbohydrate conformations mentioned in the previous sub-section, a specific pentasaccharide carbohydrate sequence is essential to bind to AT-III and inhibit thrombin (Figure 3A) [58]. Although it does not contain this specific carbohydrate sequence, DS was found to be an alternative anticoagulant [73, 74], whose anticoagulative properties are linked to the content of IduA2S. In contrast to regular DS, highly sulfated DS derivatives with high IduA2S were found to bind heparin cofactor II in a less specific manner than DS with lower sulfation [75] and, in tests of platelet aggregation, was found to bind also to AT-III [76]. A 4,6-disulfated DS is marketed for this purpose [77] with, however, a different mode of action compared to heparin. Heparin anticoagulation effects are mostly caused by the specific interaction with AT-III, whereas DS activates heparin cofactor II which then inhibits thrombin [78, 79]. Similarly, for chemically oversulfated CS [80], a change of the GlcA residue from the 4C_1 to 1C_4 conformation mimics the stereochemistry of the 2-O sulfated IduA of heparin (a part of the specific AT-III binding sequence), making oversulfated CS more active toward heparin cofactor II with similar potential compared to DS.

Carbohydrate sequence and sulfation specificity of growth factor to CS and DS has been researched extensively and, especially for neurotropic growth factors, there is strong evidence for a carbohydrate sequence specificity that triggers protein interactions and amplifies neuronal regeneration [81]. However, for many other growth factors, a clear carbohydrate sequence specificity has not consistently been reported and therefore pure electrostatic interactions between growth factors and CS/DS are possible.

CS and DS have received much attention as components of PGs that can affect neural outgrowth and neural stem cell differentiation [82, 83]. Depending on the presentation of the C4,6S epitope, it was found to either inhibit (as part of a PG mimic) [84] or promote (as part of a tetrasaccharide) [81] neuronal outgrowth. Neuronal growth factors such as midkine and brain-derived neurotrophic factor bind with high affinity to C4,6S, and it has been suggested that C4,6S recognition motifs are present in receptors or co-receptors in the neural environment for such growth factors [85]. Besides effects in neural regeneration and scarring, CS is able to interact with growth factors in other tissues as well. The 6-O sulfate group was deemed to be essential for binding TGF β 1 [86], which is important in cartilaginous tissue formation. In another study [87], CS and sulfated HA were compared to each other in terms of binding affinity to bone morphogenetic protein 4 (BMP-4), which has a variety of targets in regenerating bone and cartilage tissue [88, 89]. CS and HA share one carbohydrate unit, GlcA, and C4S was compared to HA that was sulfated at position 6-O (HA) of the GalNAc. C4S was discovered to have a lower BMP-4 affinity compared to 6-O sulfated HA and it was hypothesized that, besides other conformational differences, the 6-O sulfate in GalNAc promoted binding of BMP-4. However, the dual 4,6-sulfation pattern on CS was found to be important for binding of BMP-4 to stimulate osteoblast differentiation and mineralization [90], which leads to the assumption that C4,6S might be a strong promoter of growth factor activity outside the neuronal environment.

For soluble DS-PGs secreted into wounds, interaction with FGF-2 after injury was found to have a tremendous positive effect on wound healing [91]. When CS/DS mixed polymers were isolated from the proteoglycan decorin, a trisulfated pentasaccharide FGF-2 binding motif within CS/DS was suggested containing two potential sulfation patterns: 2-O sulfated IduA with 4,6-disulfated GalNAc or 2-O sulfated IduA and GlcA combined with 4-O

sulfated GalNAc [92]. In other studies, an octasaccharide binding sequence for FGF-2 and a decasaccharide sequence for FGF-7 was discovered and it was found that 4-O sulfation of GalNAc and the presence of IduA were essential for effective binding [93]. In addition, a decasaccharide sequence containing IduA was elucidated as the minimal binding motif for FGF-10 [94]. The examples above indicate the importance of specific oligosaccharide carbohydrate binding sequences that often demonstrate high activity with a 4,6-sulfation pattern for CS and 4-O sulfation for DS. It also appears that epimerization at C-5 to form IduA from GlcA, the most important structural difference between CS and DS, changes the growth factor selectivity and affinity between CS and DS.

CS and DS are closely related GAGs found in a wide range of PGs. In contrast to the common non-sequence-specific binding of HS to many proteins, there is strong evidence that the 4,6-sulfation epitope of CS enhances growth factor binding and signaling. This has been found to play an important role in the central nervous system by regulating neurite outgrowth [84]. In addition to sulfation pattern, carbohydrate conformation is a key aspect in DS for both growth factor interactions as well as promoting anticoagulant activities.

4.4 Structure of keratan sulfate

Keratan sulfate (KS) primarily consists of the repeating units D-galactose (Gal, β -1,3) and N-acetylglucosamine (GlcNAc) that occur in many combinations within the polymer chain [95]. Both monosaccharides were found to have a sulfate group at position 6-O [96]. KS is an important component of PGs (e.g. aggrecan) where it is attached with CS and DS. A specific nomenclature of different types of KS based on the type of attachment to the PG core protein has developed, referring to KS as type I (N-linked to Asn residue in PG), II (O-linked to Ser/Thr residues in PG), or III (mannose linked to Ser residue in PG) [97]. Typical molar masses of KS range from 7 to 22 kDa [98] and KS PGs are present in high quantities especially in the cornea [99], with lower concentrations in cartilage (e.g. in aggrecan) [100] and many other tissues, such as brain [101] and intervertebral disc [102].

The sulfation pattern of monosaccharides is not equally distributed over the KS chains, leading to regions of high and low sulfation [103]. Generally, unsulfated regions confer a conformational flexibility that can result in the formation of helical structure in solution, as determined by X-ray diffraction data [104], which makes it conformationally similar to CS and DS.

Relatively little is known about the influence of the sulfation pattern of KS on binding to specific proteins. The sulfation pattern of KS was shown to be of importance for the binding affinity to galectin proteins [105], a class of mainly intracellular proteins possessing a carbohydrate-binding motif that can be secreted into the ECM and regulate cell adhesion [106]. By examining KS tri- to pentasaccharides with various sulfation patterns, the unsulfated 6-OH group of the Gal residue was found to be essential for galectin binding, whereas 6-O sulfation of the GlcNAc residue did not increase binding of galectins [105] (Figure 5). Furthermore, desulfated KSPolysaccharides (containing galactose) exhibited binding to galectins whereas the sulfated species did not exhibit any detectable effects, suggesting that for this specific class of proteins, 6-O sulfation of Gal residues inhibits binding and that interactions of galectins with GAGs are primarily mediated by hydrophobic and/or van-der-Waals forces instead of electrostatic interactions [105].

KS is jointly found with CS and DS in PGs, but the effect of protein binding to single KS polymer chains within PGs has not been a focus of current research and therefore knowledge remains limited on this topic. However, what is known about KS-protein binding (with galectins) demonstrates that the unsulfated Gal residue is essential for binding through a non-electrostatic route. This behavior contrasts with what is known about Heparin/HS and

CS/DS, where sulfation primarily determines affinity to growth factors, and suggests that strategies for protein binding may need to be tailored based on the class of GAG to be incorporated in the drug delivery vehicle.

4.5 Structure of hyaluronan

Hyaluronan (HA) is an unusual member of the GAG family compared to the aforementioned species because it is the only one that is not sulfated. HA polymers consist of the disaccharide units D-GlcA (β -1,4) and D-GlcNAc (β -1,3) and are naturally found with polymerization degrees ranging between 2,000 – 25,000 disaccharide units (1–10 MDa) [107] (Figure 6). The size of HA assemblies can increase further by aggregation that occurs in tissues such as cartilage, where HA forms a ternary complex with aggrecan and a link protein [108]. HA can be found in every connective tissue and with high concentrations in the vitreous humor of the eye [109]. Carbohydrate conformation within the polymer can be assumed to be in the 4C_1 conformation which is stabilized intramolecularly by strong hydrogen bonding between the carbohydrates [110]. This hydrogen bonding is in rapid exchange with water molecules which is one explanation of the remarkably high water binding capacity even though HA is unsulfated and therefore has diminished electrostatic interactions with water [109].

HA is known to act intracellularly (e.g. intracellular Rhamm-protein) [111] and extracellularly where it serves as a direct messenger molecule by binding to the cell-surface receptor CD-44 [112]. In addition to CD-44, HA is known to interact with other cell surface receptors, such as extracellular Rhamm [113] and ICAM-1 [114]. The anticipated mode of interaction and binding to proteins/receptors is based on carboxyl-amino group interactions. The interaction of basic amino acids of CD-44 with HA was revealed via scrambling of amino acid sequences of soluble CD-44 [115]. However, in a rather recent study an octasaccharide binding sequence of HA to CD-44 was found where the interactions between both species were mainly governed by hydrogen bonding and less pronounced by electrostatic interactions [116].

HA is able to induce signaling via extracellular receptors (e.g. CD-44, Rhamm) as well as via binding to intracellular proteins to regulate cellular functions. This is in strong contrast to other GAGs that act through growth factor binding to induce signal transduction. However, promotion of growth factor binding to HA can be achieved by chemically sulfating the polymer (see Section 5.1 for further discussion of sulfation methods).

4.6 Paradigm of biological activity upon growth factor binding

Despite the generally-held belief that GAG-growth factor binding enables significant biological activity, there are scenarios that could challenge this paradigm, whereby 1) changes in sulfation pattern of GAGs can lead to binding of growth factors, but the resulting GAG-growth factor complex has attenuated biological activity, and 2) modified GAGs needed as signaling cofactors do not bind to growth factors or target receptors alone, but the growth factor nevertheless leads to activation of cell-surface receptors by forming a growth factor – GAG complex.

A good example of the former scenario was demonstrated with selectively desulfated heparin and FGF-2 [117]. Signaling is generally achieved by formation of a ternary complex of FGF-2, the FGF receptor FGFR-1 and heparin/HS. FGF-2 binding to 2-O and 6-O-desulfated heparin was investigated and subsequent signaling through the FGFR-1 receptor was monitored via proliferation of HS deficient Chinese hamster ovary cells (CHO677). 2-O sulfation was necessary for heparin to bind to FGF-2, whereas both the 2-O and 6-O sulfation motifs were required to bind and activate the receptor. The 6-O desulfated heparin

FGF-2 complex bound with considerably less affinity to its receptor compared to unmodified heparin, thereby preventing receptor signaling as measured by Erk2 kinase activity and DNA synthesis in CHO677 cells. Moreover, in a competition experiment, the stimulatory effect of unmodified heparin could be fully abolished by adding an excess of 6-O desulfated heparin.

The latter scenario where GAGs neither bind to the growth factor nor to the receptor can be illustrated with a study of HS and FGF-1/FGFR-1 binding [118]. Results suggested that 6-O or 3-O sulfated, and N-sulfated HS bound to neither FGFR-1 nor FGF-1 alone, but 6-O or 3-O, N-sulfated HS species were able to induce a ternary complex that led to the anticipated mitogenic activity on FGFR1 expressing BaF3 cells. Importantly, N-acetylated HS, which does not carry a N-sulfate group, independent of its O-sulfation pattern, was not able to bind to growth factor or receptor or induce the ternary complex, thus, only the triple combination of GAG, growth factor, and receptor led to signaling within the cells. This result supported the necessity of the N-sulfate groups to induce signaling through the FGF-1/FGFR-1 complex. In the case of FGF-1, these results also indicate that the investigation of GAG-growth factor binding or GAG-receptor binding may not be predictive for the formation of the required triple complexes known to induce signaling and biological activity in cells.

GAGs have been found to bind to growth factors electrostatically in a carbohydrate sequence-specific or non-sequence-specific manner. The examples discussed above challenge the conventional paradigm that GAG-growth factor binding necessarily leads to signaling in target cells. Therefore, there is an obvious need to measure the activity of growth factors via relevant biological (typically cellular) assays after incorporation of growth factors and GAGs into drug delivery systems, particularly if working with chemically modified GAGs, as discussed in the next section.

5 STRUCTURAL MODIFICATION OF GAGs FOR DEVELOPMENT INTO DRUG DELIVERY SYSTEMS

Prior to utilizing the electrostatic interactions between positively-charged growth factors and GAGs to achieve the desired release kinetics for a particular biomedical application, GAGs need to be incorporated into drug delivery systems that can be efficiently deployed *in vitro* or *in vivo*. Such incorporation methods often consist of crosslinking the GAG into some kind of matrix which requires prior chemical modifications of GAGs. However, as discussed in the previous section, carbohydrate sequence-specific and non-sequence-specific interactions of growth factors with GAGs are in part based on sulfation pattern and solution conformation of the polysaccharides. Thus, in this section, common chemical modifications of GAGs are summarized and related to their potential effects on protein binding and release.

5.1 Adjusting sulfation patterns

Several attempts have been made to modulate GAG sulfation properties chemically through either 1) selective desulfation of natural GAGs, or 2) complete desulfation with selective re-sulfation. Most selective desulfation procedures have been developed for heparin and comprise either solvolytic processes or specific esterification followed by solvolysis. Direct solvolysis can produce selective N-desulfated heparin [119], selective 6-O desulfated [120] and completely desulfated heparin [121]. Depending on the type of solvent mixture used for solvolysis, degradation of the polysaccharides can occur [122] and is more pronounced for chondroitin sulfate than for heparin [123] but the exact reasons for this difference in susceptibility to backbone cleavage remain unknown. Preparation of 2-O and 3-O

desulfated heparin has also been prepared by alkaline lyophilization which is accompanied by depolymerization similar to the processes in above mentioned solvent mixtures [28, 124].

The opposite reaction route, re-sulfation with or without prior desulfation, has been established for all GAG species to prepare either oversulfated GAGs (reaction without prior desulfation) or specifically sulfated GAGs (full desulfation with resulfation). Full desulfation can be achieved simply by acidic methanol treatment over several days, which was originally described for chondroitin sulfate [125]. Following desulfation, introduction of sulfur trioxide complexes lead to re-sulfation and can also be used for oversulfation of partially sulfated GAGs [126, 127]. Using this oversulfation method, for artificially sulfated HA (sHA) polymers, a correlation between increased degree of sulfation of sHA and greater binding of TGF β 1 and BMP-4 was found [86, 87]. Apparently, the chain conformation of the HA polymer is beneficial to interact with some growth factors because, in comparing sHA to CS with similar degrees of sulfation, sHA bound higher amounts of TGF β 1 [86]. For heparin, a temperature dependent, O-selective re-sulfation technique has been described [128]. Through either reaction method, O-selective sulfation patterns can be achieved, with sulfation patterns equally distributed over the polymer chain (no clustering). One of the differences between HS and heparin is the conformational flexibility achieved by the sulfate cluster structure of HS that can lead to enhanced electrostatic interactions with proteins. However, mimicking the S-/NA domain structure found in HS is not currently technically possible with the above-mentioned methods as the preparation of sulfation clusters cannot be achieved by relatively non-specific reactions in solution. If deemed necessary, an option for mimicking this behavior might be the application of carbohydrate ring-opening reactions to heparin (discussed in Section 5.3) which may lead to a local loss of the stiff heparin ring structure.

5.2 Modifications at functional groups

Most published reports focus on GAG modifications at their functional groups, such as 1) the amino groups of the non-acetylated, non-amino sulfated amino sugars, 2) the carboxyl functions of uronic acid residues, and 3) the hydroxyl groups. Especially for heparin where the amount of free, unsulfated amino groups is quite low, functionalization at the amino groups of unsubstituted GlcN has rarely been examined because an N-desulfation step would need to be conducted prior to further modification which is not a straight-forward approach for the preparation of drug delivery systems. However, some reports focus on the preparation of selectively N-desulfated heparin [119, 129] which could be used for mechanistic studies of protein interactions and molecular delivery, because N-desulfated heparin/HS loses a large portion of its anticoagulant activity (3–5 times less than regular heparin) [130]. Crosslinking of N-desulfated heparin with glutaraldehyde under formation of high molar mass polymers or hydrogels was reported as well [131].

Modifications at the carboxyl function occur at the uronic acid residues where carbodiimide chemistry [132], which is able to link primary amino groups to carboxyl groups with reasonable yield and reproducibility, is widely used for GAG-based materials. Heparin-coated collagen matrices for cell culture application were prepared by crosslinking heparin carboxyl groups to collagen primary amines [133, 134]. Moreover, this chemistry has been used to functionalize GAGs with methacrylamide [135, 136], cysteamine for subsequent thiolation [137, 138], or azide-containing compounds for Huisgen cycloaddition reactions [139, 140]. These functionalized materials can then be cross-linked either via radical polymerization or cycloaddition to form particulate or hydrogel drug delivery systems.

Hydroxyl-group modifications are also less common, but the reaction of CS with glycidyl methacrylate can yield a mixture of carboxyl and hydroxyl-modified GAGs [141–143]. Coupling this reagent to GAGs could be a beneficial route to increase crosslinking density

by enhancing the number of potential reactive sites, which might be necessary in the case of low reaction yields from carboxyl group modifications or when a high degree of modification is required for crosslinking.

The location of GAG modifications for delivery system incorporation can alter protein binding compared to most published literature that has focused on GAGs in solution [41, 58, 144, 145]. In one study using different grafting chemistries to modify heparin with biotin derivatives via 1) EDC/NHS chemistry at the carboxyl function, 2) coupling to the rare unsubstituted intrachain amines, and 3) carbohydrate ring opening at the reducing end terminus [146], binding to 4 proteins was screened. For all proteins, binding to the reducing end terminus-modified heparin was higher compared to the two other methods. The lowest binding was found for the carboxyl function-modified heparin. Similarly, reduced binding to the carboxyl function modification was previously reported between carboxymethylated heparin and AT-III [147] and carboxymethylated, sulfated HA and TGF β 1 [86]. A decrease in bFGF interaction was also found with increasing degree of heparin acrylate functionalization in solution [148].

5.3 Modifications with carbohydrate ring opening

The aforementioned results make it clear that GAG modification, particularly at the carboxyl group, and immobilization in drug delivery systems can significantly affect protein binding capacities and subsequent release. Therefore alternative chemistries are needed that allow for similar versatile applicability compared to the EDC/NHS chemistry but with less influence on the important carboxyl function.

One alternative chemistry is the carbohydrate ring opening of GAGs which can occur naturally at the reducing-end terminus of the polymeric chain as reductive sugars are in equilibrium with their aldehyde forms in solution. This equilibrium between open and closed carbohydrate rings can be utilized for labeling with dyes or surface grafting at the reducing end. The general modification procedure at the reducing end involves a reaction with a primary amine under formation of a Schiff's base, followed by reduction to a secondary amine [149]. A similar reaction to label heparan sulfate with sulfo-NHS-biotin could be used to graft the GAG to streptavidin-coated surfaces. However, a disadvantage of the reducing end reaction is that, as there is only one reducing end per chain, such end-group reactions will lead to only one reactive site on the polymer. The low number of reactive positions can be problematic for the formation of most drug delivery systems because the crosslinking density for GAGs would be quite low, which can lead to incomplete incorporation.

However, application of ring-opening chemistry to create multiple binding sites can be done for some GAGs by periodate oxidation (see example in Figure 7). The oxidation with periodate opens the carbohydrate ring between two vicinal hydroxyl groups resulting in two aldehydes and, depending on the number of vicinal hydroxyl groups, the release of formic acid [150]. The resulting aldehyde groups can then be further modified by coupling to amines or hydrazones, forming a Schiff base or hydrazine, respectively. The Schiff's base can then be further reduced to form a stable secondary amine. Such a carbohydrate ring opening strategy was used to couple cysteamine and synthesize a thiolated heparin that could form nanogels via disulfide bonds [151]. In addition, periodate reactions for CS, HA and dextran sulfate, followed by coupling of the aldehyde groups to ethylene diamine has been achieved [152] and the resulting free amino group was then utilized to couple fluorescence dyes to label the GAG at multiple positions within the carbohydrate chains. Using a similar reaction scheme, aldehyde-HA was synthesized by periodation and crosslinked with N-succinyl chitosan of varying ratios [153]. In contrast, in another study, pre-crosslinked HA hydrogels were prepared and treated with periodate to allow further

modification with aldehyde groups [154], which allowed for coupling of a RGD containing peptide to the matrices' surface through the added moieties.

Structural modifications of GAGs of relevance for drug delivery systems focus mainly on sulfation degree adjustment and efficient functionalization for crosslinking GAGs into delivery systems. Several chemical methods are available to adjust the degree of GAG sulfation by either desulfating or re-sulfation/oversulfating existing biopolymers with a certain O-selectivity within the carbohydrate structures. The affinity to positively-charged growth factors can be engineered by tuning the sulfation degree. Prior to crosslinking GAGs with themselves or other biomaterials, functionalization is often required. Carboxyl modifications are popular due to the ease of the chemistry involved, but have been shown to impact protein binding by reducing affinity to the GAG. As an alternative, carbohydrate ring-opening reactions are available, but require more careful investigation regarding any potential interference with protein, enzyme or receptor binding.

6 BIOMOLECULAR LOADING AND DEGRADATION CHARACTERISTICS OF GAG-BASED DRUG DELIVERY SYSTEMS

Due to the intrinsic properties of GAGs such as sulfation patterns, carbohydrate conformations and linkages, and the presence of growth factor binding sequences, the inclusion of GAGs has a strong influence on the loading, biodegradation and release of biomolecular delivery systems. Sulfation patterns and degrees of sulfation dictate the electrostatic affinity between GAGs and growth factors, and therefore a reduction in GAG net charge can result in decreased growth factor loading and increased release rates from the delivery system. Degradation mechanisms of the system are important to consider in addition to loading and release strategies since some chemical modifications of GAGs reduce the degradability by enzymes that are often responsible for *in vitro* and *in vivo* degradation. More specifics on how GAG modification can affect both protein loading and degradation are presented in this section in order to help guide rational design of GAG-containing formulations for controlled release applications.

6.1 Methods of biomolecule loading

Because most GAGs are highly anionic, they are often loaded with molecular cargo that is positively-charged, such as many growth factors and other proteins. Depending on the form of the GAG-based delivery system, the type of drug loading can vary, but can be generally classified into two methods: 1) *in situ* loading during vehicle formation and 2) loading after vehicle formation. For GAG-based micelles or self-assembled films, cargo is first dissolved in appropriate solvents and then added to the monomer solution [155–158]. The cargo becomes incorporated as the delivery system self assembles. For GAG delivery systems in the form of microparticles, nanoparticles or hydrogels that require chemical crosslinking, loading generally occurs after formation. Post-formation loading is performed by adding cargo-containing solution for several hours to pre-swollen hydrogels or lyophilized matrices [159–161].

There are three main molecular mechanisms governing the loading of cargo in GAG-based delivery systems (Figure 8). In the first, GAG-based vehicles are used to sequester positively-charged growth factors or molecules. In this case, cargo is loaded due to the electrostatic interactions with the sulfate groups on the GAGs. For instance, TGF- β 1 has been shown to electrostatically complex to CS microspheres, unlike negatively-charged TNF- α [136]. In a second method, incorporation of anionic molecules is possible via physical incorporation, but this approach generally yields low loading efficiency due to electrostatic repulsion. The loading of bovine serum albumin (BSA, isoelectric point: 4.7) in

CS-poly(ethylene glycol) diglycidyl ether hydrogel only reached 4–8% (w/w) in swollen hydrogels starting from 10% (w/v) BSA solutions with incubation over 2 days [162]. A third mechanism that is found commonly in HA-based delivery systems is based on hydrophobic interactions where hydrophobic domains of HA can interact with cargo of varying degrees of hydrophobicity, as shown for HA hydrogel films with several anti-inflammatory agents [157]. Similarly, doxorubicin has also been successfully integrated into heparin nanoparticles via hydrophobic interactions [163].

6.2 Degradation of GAG-based drug delivery systems

Degradation of GAG molecules can occur by (see Figure 9): 1) chemically-induced degradation, and 2) enzymatically-induced degradation. These methods are similar insofar that they are able to cleave glycosidic bonds within the polysaccharide backbone. However, differences in their cleavage mechanisms lead to varying degradation products.

Chemical degradation mechanisms—Generally, GAGs have been found to be very stable against chemical degradation (i.e. CS and KS were seen to be stable in 2 N NaOH at room temperature for more than 120 h [164]). Chemical degradation reactions were originally explored to uncover the structure of GAGs by breaking the polymer into sugar monomers or oligomers for subsequent downstream analysis. Reaction conditions include harsh treatments with highly concentrated hydroxides [165], nitrous acid treatment [166–168], radiolysis [169], hydrazinolysis [170], hydrogen peroxide treatment [171], treatment with ozone and sunlight [172] and the application of Fenton's reagents ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) [173].

Because GAGs themselves are generally found to be quite chemically stable, if degradation of GAG-based drug delivery systems occurs, it is generally through degradation at functionalization sites introduced prior to loading of the delivery system. In this vein, a rather nonspecific degradation mechanism is hydrolytic cleavage of ester bonds that were previously added on GAG molecules, e.g., by esterification with carbodiimide chemistry. For example, in a recent study [174], photopolymerized, methacrylamide modified alginate-heparin hydrogels degraded in water primarily through this mechanism, showing significant (70%) mass loss over 8 weeks.

Another example of dissociation through reactions at functional groups is the cleavage of disulfide bonds by glutathione (GSH). Disulfide bonds can be formed between modified GAGs, in this case hyaluronan, and another molecule with thiol groups (e.g. thiolated PEG) to form cross-linked hydrogels that can be degraded through the addition of GSH [175]. In the case of GSH treatment, hydrogels were found to undergo bulk degradation whereas treatment with hyaluronidase led to surface degradation.

Overall, chemical degradation of GAG-based delivery systems under physiological conditions result in the release of cargo and full-length modified GAG molecules. Moreover, non-enzyme based degradation occurs through a bulk mechanism that makes cargo release dependent on diffusion length and can therefore be controlled by the geometry of the drug delivery systems (e.g. thickness, shape).

Enzymatic degradation mechanisms—For enzymatic degradation reactions, the varieties of conditions as well as GAG cleavage mechanisms has been thoroughly reviewed [176, 177]. Therefore, in this section we will only briefly describe the enzymatic modes of action and highlight potential effects of GAG chemical derivatization on their digestion by enzymes. Generally, heparin/HS-cleaving enzymes can be categorized into two groups that cleave the glycosidic bond between two sugars, but have different modes of action: lyases (bacterial in origin, prefer heparin) and hydrolases (mammalian, prefer HS) (Figure 10). Lyases are either endo- or exoglycosidases that cleave glycosidic bonds by an eliminative

mechanism involving uronic acid residues by abstracting the proton at C-5 position with formation of a double bond between C-4 and C-5. The carboxyl function of the uronic acid residue is essential for the cleavage mechanism [178]. The group of GAG lyases is comprised of heparinases, chondroitinases, and hyaluronidases, each with varying subtypes requiring individual reaction conditions and exhibiting their own reaction kinetics.

In contrast, hydrolases are endoglycosidases that form an intermediate oxonium by delivering a proton to the glycosidic oxygen followed by water attack (hydroxyl addition removes the double bond). For hydrolases, the resulting cleaved residues are completely saturated. The group of hydrolases consists of heparin hydrolases, keratanases, and hyaluronan hydrolases. Depending on the tissue origin of the hydrolases (e.g. platelets, tumors, fibroblasts), enzyme activity can depend on sulfation or acetylation of carbohydrates and therefore sulfation pattern and adjacent carbohydrate clusters matter in some cases leading to incomplete cleavage regardless of specific GAG chemistry [176].

Comparing these two classes of enzymes, for heparin hydrolases in general, the O-sulfation pattern and glucuronic acid content of the polymer is important for enzymatic cleavage whereas, as previously mentioned, lyases require a carboxyl function and are active against glucuronic and iduronic acid residues adjacent to glucosamino sugars. An additional difference is that lyases are only able to cleave bonds on the non-reductive side of sugars, whereas hydrolases can be specific for both reducing or non-reducing sites [176]. Each heparin lyase and hydrolase enzyme isoform has its own carbohydrate cleaving sequence and therefore we refer the reader to published literature on this topic [176, 179–182].

Chemical GAG modifications, such as the commonly-performed carboxyl modifications of the uronic acid residues of heparin, affect enzymatic digestion. Since lyases require a free carboxyl function for their action, heparin-based drug delivery systems cannot be degraded via this mechanism with high degrees of modifications at this position. On the other hand, hydrolases, which have specific preferences for O-sulfate groups and specific carbohydrates adjacent to them (e.g. glucuronic acid for heparin hydrolases), will be sensitive to sulfation pattern and compositional changes of the GAG molecule.

Generally, lyases and hydrolases degrade GAGs to a different extent: lyases degrade GAGs into oligosaccharides [183], but hydrolases produce smaller, still polymeric GAG fragments of lower molecular weight compared to the starting material. In this context, an example for heparin hydrolases was carried out with PVA/heparin crosslinked hydrogels where platelet extract, a known source of heparin hydrolases, was used for degradation [184]. By analyzing soluble heparin prior to hydrogel incorporation, it was found that heparin was degraded by the enzyme into fragments with approximately half the molar mass of the original molecule (17–19 kDa heparin into 8.6 kDa fragments). As heparin hydrolases represent the enzyme class that is relevant in mammals, these degradation properties are assumed to be relevant to *in vivo* conditions for crosslinked hydrogels. Moreover, this finding highlights the fact that even if *in-vitro* degradation (often tested with lyases) fails, due to different modes of action between lyases and hydrolases, *in-vivo* degradation may still be achieved.

Instead of relying on GAG-degrading enzymes, another approach to achieve enzyme-specific degradable drug delivery systems is via incorporation of specific peptide sequences into the crosslinking chemistry or within the GAG backbone. For example, a peptide-functionalized PEG containing a matrix metalloprotease (MMP) cleavable sequence [185] was crosslinked with heparin and loaded with vascular endothelial growth factor (VEGF), which is known to promote the proliferation of human umbilical vein endothelial cells (HUVECs). A comparison between MMP-degradable and non-degradable hydrogels indicated that the number of HUVECs increased significantly and spread in 3D after one

week only in the MMP-degradable hydrogel group. Thus, it was concluded that VEGF function was supported by the degradability of the hydrogels.

As outlined in this section, loading of GAG-based delivery systems primarily occurs via an electrostatic mechanism between negatively charged GAGs and positively-charged molecular cargo. Besides electrostatic interactions, negatively or non-charged cargo molecules can also be loaded in GAG carriers, but the loading capacity is typically less, generally leading to a faster release based on diffusion. More controllable mechanisms of cargo release due to GAG-based drug delivery system degradation are based on chemical and enzymatic reactions. Chemically degraded gels (hydrolysis) will undergo primarily bulk degradation, whereas for enzymatically degraded hydrogels, surface vs. bulk degradation is dependent on the enzymatic reaction rate and enzyme diffusion rate through the gel [186].

In general, enzymatic degradation of GAG-based drug delivery systems seems to be a beneficial approach over chemical degradation because of the much milder conditions and greater *in-vivo* relevance, but several caveats remain. For example, the commonly used lyases are commercially available but do not represent the class of enzymes present *in vivo* in mammals. This drawback can be circumvented by including peptide-based enzyme-responsive sequences, which may lead to much more controlled degradation profiles in response to enzymes specifically upregulated in diseased tissues. GAG-based drug delivery systems often rely on electrostatic interactions with the cargo and such electrostatic forces typically confer a high binding strength to the GAG-cargo complex, which can make it difficult to disrupt the complexes. Therefore, cargo release is often dependent on GAG release (assuming GAG binding sites exceed cargo concentration). Regardless of chemical vs. enzymatic degradation mechanisms, it is thus very plausible to assume the release of GAG-cargo complexes, which may have additional effects on biological activity of the released cargo [187].

7 Summary, conclusions and future outlook

To harness the beneficial effects of GAGs for tissue engineering applications, they are often incorporated into drug delivery systems. However, GAG incorporation often requires chemical modifications to the polymers that can change the innate properties of GAGs, which affect protein affinities and therefore alter cell and tissue responses. Therefore, as demonstrated throughout this review, more detailed knowledge of how the chemistry of native and modified GAGs affects protein binding and release will allow better engineering of GAG-based biomaterials for controlled protein delivery.

In general, GAG-protein binding is based on 1) polyelectrolyte properties, 2) sulfation patterns, and 3) carbohydrate conformations. While there have been many attempts to elucidate specific binding sequences for different GAG-binding proteins, in some cases the interactions can only be explained by non-carbohydrate sequence-specific electrostatic interactions between oppositely charged macromolecules. It is known that particular biochemical processes (e.g. anticoagulation, FGF-2 signaling) only proceed with very specific binding sequences and carbohydrate conformations (e.g. heparin-AT-III, heparin/HS-FGF-2). However, this high carbohydrate and sulfation pattern specificity is not required in all cases for growth factor binding. Certain modification chemistries (e.g. carbohydrate ring opening reactions) allow for less impact on the solution conformation of GAGs and therefore may present a relatively “native” protein binding site. However, more in-depth experimental analyses should to be conducted before conclusions can be drawn about “preferred” and “unfavorable” GAG modifications.

In terms of drug delivery systems, for the sulfated GAGs, electrostatic interactions with cargo molecules, whether they consist of small molecules, peptides, or proteins, are the most prominent mechanism that presently governs loading and release. The release of GAG-cargo complexes in most cases is suggested from bioactivity data of released cargo and indicates GAGs can be beneficial for cargo stabilization and signaling enhancement.

Current challenges with use of GAGs in drug delivery systems center on three primary issues: 1) understanding the ratio of sequence-driven vs. non-sequence-driven protein binding combined with tuning release and degradation, 2) determining applicability of modification chemistries to *in-vivo* settings, and 3) metabolism and excretion of modified GAGs. Concerning issue 1), carbohydrate sequence specificity for protein affinity can be changed when GAGs undergo chemical modifications. In the case of pure electrostatic, non-sequence-driven interactions between GAGs and proteins, only the sulfation pattern accounts for affinity and therefore GAG-protein interactions are less sensitive to specific modification chemistries. In a mixed-mode interaction scenario where sequence and non-sequence-based interactions occur simultaneously (e.g. protein prefers a specific sulfate group position), chemical modifications of GAGs could easily lead to significant reduction or even complete loss of protein affinity.

In any scenario, the type and amount of GAG-protein interaction will determine the loading capacity of GAG-based drug delivery system. The knowledge of affinity and loading capacity is very important because from both parameters, for any given *in vitro* or *in vivo* scenario, the likelihood can be estimated that the loaded cargo will be released, exogenous proteins from the environment will be sequestered along with the cargo, or exogenous proteins will encourage cargo release due to competition for GAG affinity. Consequently, in a particular biological environment, the GAG delivery system could result in burst release (cargo depletion) or very incomplete release (further sequestration, very high affinity of cargo), depending on relative affinities of the various local biomolecules for the GAG carrier. Thus, specific knowledge of loading and cargo affinity will be required in designing and evaluating GAG-based delivery systems for specific *in vitro* or *in vivo* environments.

Engineering approaches are very helpful for further refinement of chemical modifications to improve *in-vivo* applicability (issues 2 and 3 above). Particularly if crosslinking into delivery systems occurs *in-situ*, catalysts and other by-products of polymerization processes may introduce toxicity concerns, so additional modifications of these reactions may be necessary. Furthermore, maintaining *in-vivo* degradability is an important component. In the future, further studies on the macromolecular properties of GAGs are needed to investigate if GAG-based degradation products of drug delivery systems that were chemically modified prior to encapsulation can be metabolized and excreted from living organisms in a similar manner to their non-modified counterparts.

To date, GAG-based materials have proven safe and efficacious to deliver a variety of molecules, from growth factors to small molecule drugs, as demonstrated by their incorporation in medical devices approved for clinical use (e.g. heparin coated stents [188]). However, more precise control of biomolecule delivery, such as may be required for tissue regeneration applications in the future, requires increasingly sophisticated modifications to the GAG molecules to further tune affinity and release. With additional research further elucidating how to control cargo binding and degradation, GAG-based delivery systems have all potential to be an ideal carrier system for controlled release of therapeutics for a wide range of regenerative medicine applications because of their biodegradability, non-toxicity and versatility for many different cargos.

Acknowledgments

The authors are supported by funding from the NIH (R01 AR062006) and NSF (DMR 1207045).

2 LIST OF ACRONYMS

AT-III	Antithrombin III
BSA	Bovine serum albumin
C4S	Chondroitin-4-sulfate
C6S	Chondroitin-6-sulfate
C4,6S	Chondroitin-4,6-sulfate
CS	Chondroitin sulfate
dCS	Desulfated chondroitin
DS	Dermatan sulfate
EDC	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GAG	Glycosaminoglycan
Gal	Galactose
GalNAc	N-acetyl-galactosamine
GlcA	Glucuronic acid
GlcNAc	N-acetyl-glucosamine
GMA	Glycidylmethacrylate
GSH	Glutathione
HA	Hyaluronic acid
HS	Heparan sulfate
IduA	Iduronic acid
KS	Keratan sulfate
MMP	Matrix metalloprotease
NHS	N-Hydroxysuccinimide
PEG	Polyethylene glycol
PEG-DA	Polyethylene glycol diacrylate
PG	Proteoglycan
SPR	Surface plasmon resonance
TGF	Transforming growth factor

References

1. Stick, RV.; Williams, S. Carbohydrates: The Essential Molecules of Life: The Essential Molecules of Life. Elsevier Science; 2010.
2. Hay ED. Extracellular matrix. J Cell Biol. 1981; 91:205s–23s. [PubMed: 6172429]

3. Robert L, Labat-Robert J. Macromolecules of the extracellular matrix. *Annales de biologie clinique*. 1986; 44:145–51. [PubMed: 3752631]
4. Robert L, Robert AM, Renard G. Biological effects of hyaluronan in connective tissues, eye, skin, venous wall. Role in aging *Pathol Biol*. 2010; 58:187–98.
5. Scott JE. Structure and function in extracellular matrices depend on interactions between anionic glycosaminoglycans. *Pathologie-biologie*. 2001; 49:284–9. [PubMed: 11428163]
6. Urban JP, Maroudas A, Bayliss MT, Dillon J. Swelling pressures of proteoglycans at the concentrations found in cartilaginous tissues. *Biorheology*. 1979; 16:447–64. [PubMed: 534768]
7. Esko, JD.; Kimata, K.; Lindahl, U. Proteoglycans and Sulfated Glycosaminoglycans. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P.; Bertozzi, CR., et al., editors. *Essentials of Glycobiology*. 2. Cold Spring Harbor (NY): 2009.
8. Rapraeger A, Jalkanen M, Endo E, Koda J, Bernfield M. The cell surface proteoglycan from mouse mammary epithelial cells bears chondroitin sulfate and heparan sulfate glycosaminoglycans. *J Biol Chem*. 1985; 260:11046–52. [PubMed: 3161889]
9. Bali JP, Cousse H, Neuzil E. Biochemical basis of the pharmacologic action of chondroitin sulfates on the osteoarticular system. *Semin Arthritis Rheu*. 2001; 31:58–68.
10. Lallam-Laroye C, Escartin Q, Zlowodzki AS, Barritault D, Caruelle JP, Baroukh B, et al. Periodontitis destructions are restored by synthetic glycosaminoglycan mimetic. *J Biomed Mater Res A*. 2006; 79A:675–83. [PubMed: 16832824]
11. Kock L, van Donkelaar CC, Ito K. Tissue engineering of functional articular cartilage: the current status. *Cell Tissue Res*. 2012; 347:613–27. [PubMed: 22030892]
12. Huskisson EC. Glucosamine and Chondroitin for Osteoarthritis. *J Int Med Res*. 2008; 36:1161–79. [PubMed: 19094424]
13. Casu B, Naggi A, Torri G. Chemical derivatization as a strategy to study structure-activity relationships of glycosaminoglycans. *Semin Thromb Hemost Semin Thromb Hemost*. 2002; 28:335–42.
14. Kogan G, Soltes L, Stern R, Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol Lett*. 2007; 29:17–25. [PubMed: 17091377]
15. Gandhi NS, Mancera RL. The Structure of Glycosaminoglycans and their Interactions with Proteins. *Chem Biol Drug Des*. 2008; 72:455–82. [PubMed: 19090915]
16. Gandhi NS, Mancera RL. Prediction of heparin binding sites in bone morphogenetic proteins (BMPs). *Bba-Proteins Proteom*. 2012; 1824:1374–81.
17. Sattelle BM, Shakeri J, Almond A. Does Microsecond Sugar Ring Flexing Encode 3D-Shape and Bioactivity in the Heparanome? *Biomacromolecules*. 2013; 14:1149–59. [PubMed: 23439078]
18. Seyrek E, Dubin P. Glycosaminoglycans as polyelectrolytes. *Adv Colloid Interfac*. 2010; 158:119–29.
19. Capila I, Linhardt RJ. Heparin–Protein Interactions. 2002; 41:390–412.
20. Imberty A, Lortat-Jacob H, Perez S. Structural view of glycosaminoglycan-protein interactions. *Carbohydr Res*. 2007; 342:430–9.
21. Hileman RE, Fromm JR, Weiler JM, Linhardt RJ. Glycosaminoglycan-protein interactions: definition of consensus sites in glycosaminoglycan binding proteins. *Bioessays*. 1998; 20:156–67. [PubMed: 9631661]
22. Segura T, Anderson BC, Chung PH, Webber RE, Shull KR, Shea LD. Crosslinked hyaluronic acid hydrogels: a strategy to functionalize and pattern. *Biomaterials*. 2005; 26:359–71. [PubMed: 15275810]
23. Jeon O, Song SJ, Lee KJ, Park MH, Lee SH, Hahn SK, et al. Mechanical properties and degradation behaviors of hyaluronic acid hydrogels cross-linked at various cross-linking densities. *Carbohydr Polym*. 2007; 70:251–7.
24. Kuo WJ, Digman MA, Lander AD. Heparan Sulfate Acts as a Bone Morphogenetic Protein Coreceptor by Facilitating Ligand-induced Receptor Hetero-oligomerization. *Mol Biol Cell*. 2010; 21:4028–41. [PubMed: 20861306]
25. Hook M, Kjellen L, Johansson S, Robinson J. Cell-Surface Glycosaminoglycans. *Annu Rev Biochem*. 1984; 53:847–69. [PubMed: 6433783]

26. McNaught AD. Nomenclature of Carbohydrates. 2008; 1:2727.
27. Boeyens JC. The conformation of six-membered rings. 1978; 8:317–20.
28. Fryer A, Huang YC, Rao G, Jacoby D, Mancilla E, Whorton R, et al. Selective O-desulfation produces nonanticoagulant heparin that retains pharmacological activity in the lung. *J Pharmacol Exp Ther*. 1997; 282:208–19. [PubMed: 9223556]
29. Hirsh J, Anand SS, Halperin JL, Fuster V. Mechanism of action and pharmacology of unfractionated heparin. *Arterioscl Throm Vas*. 2001; 21:1094–6.
30. Vongchan P, Warda M, Toyoda H, Toida T, Marks RM, Linhardt RJ. Structural characterization of human liver heparan sulfate. *Bba-Gen Subjects*. 2005; 1721:1–8.
31. Knox SM, Whitelock JM. Perlecan: how does one molecule do so many things? *Cell Mol Life Sci*. 2006; 63:2435–45. [PubMed: 16952056]
32. Groffen AJA, Buskens CAF, van Kuppevelt TH, Veerkamp JH, Monnens LAH, van den Heuvel LPWJ. Primary structure and high expression of human agrin in basement membranes of adult lung and kidney. *Eur J Biochem*. 1998; 254:123–8. [PubMed: 9652404]
33. Pap T, Bertrand J. Syndecans in cartilage breakdown and synovial inflammation. *Nat Rev Rheumatol*. 2013; 9:43–55. [PubMed: 23090508]
34. Nader HB, Chavante SF, dos-Santos EA, Oliveira FW, de-Paiva JF, Jeronimo SMB, et al. Heparan sulfates and heparins: similar compounds performing the same functions in vertebrates and invertebrates? *Braz J Med Biol Res*. 1999; 32:529–38. [PubMed: 10412563]
35. Yong LCJ. The mast cell: origin, morphology, distribution, and function. *Exp Toxicol Pathol*. 1997; 49:409–24. [PubMed: 9495641]
36. Merry CLR, Lyon M, Deakin JA, Hopwood JJ, Gallagher JT. Highly sensitive sequencing of the sulfated domains of heparan sulfate. *J Biol Chem*. 1999; 274:18455–62. [PubMed: 10373453]
37. Mobli M, Nilsson M, Almond A. The structural plasticity of heparan sulfate NA-domains and hence their role in mediating multivalent interactions is confirmed by high-accuracy (15)N-NMR relaxation studies. *Glycoconjugate J*. 2008; 25:401–14.
38. Smith RA, Meade K, Pickford CE, Holley RJ, Merry CL. Glycosaminoglycans as regulators of stem cell differentiation. *Biochem Soc Trans*. 2011; 39:383–7. [PubMed: 21265809]
39. Baldwin RJ, ten Dam GB, van Kuppevelt TH, Lacaud G, Gallagher JT, Kouskoff V, et al. A developmentally regulated heparan sulfate epitope defines a subpopulation with increased blood potential during mesodermal differentiation. *Stem cells*. 2008; 26:3108–18. [PubMed: 18787209]
40. Gallagher JT. Heparan sulfate: growth control with a restricted sequence menu. *J Clin Invest*. 2001; 108:357–61. [PubMed: 11489926]
41. Mulloy B, Khan S, Perkins SJ. Molecular architecture of heparin and heparan sulfate: Recent developments in solution structural studies. *Pure Appl Chem*. 2012; 84:65–76.
42. Gallagher JT, Turnbull JE, Lyon M. Patterns of sulphation in heparan sulphate: polymorphism based on a common structural theme. *Int J Biochem*. 1992; 24:553–60. [PubMed: 1516727]
43. Barzu T, Lormeau JC, Petitou M, Michelson S, Choay J. Heparin-derived oligosaccharides: affinity for acidic fibroblast growth factor and effect on its growth-promoting activity for human endothelial cells. *J Cell Physiol*. 1989; 140:538–48. [PubMed: 2550475]
44. Xu R, Ori A, Rudd TR, Uniewicz KA, Ahmed YA, Guimond SE, et al. Diversification of the Structural Determinants of Fibroblast Growth Factor-Heparin Interactions: IMPLICATIONS FOR BINDING SPECIFICITY. *J Biol Chem*. 2012; 287:40061–73. [PubMed: 23019343]
45. Loo BM, Kreuger J, Jalkanen M, Lindahl U, Salmivirta M. Binding of heparin/heparan sulfate to fibroblast growth factor receptor 4. *J Biol Chem*. 2001; 276:16868–76. [PubMed: 11278860]
46. Maccarana M, Casu B, Lindahl U. Minimal sequence in heparin/heparan sulfate required for binding of basic fibroblast growth factor. *J Biol Chem*. 1993; 268:23898–905. [PubMed: 8226930]
47. Rickard SM, Mummery RS, Mulloy B, Rider CC. The binding of human glial cell line-derived neurotrophic factor to heparin and heparan sulfate: importance of 2-O-sulfate groups and effect on its interaction with its receptor, GFR α 1. *Glycobiology*. 2003; 13:419–26. [PubMed: 12626395]

48. Ricard-Blum S, Feraud O, Lortat-Jacob H, Rencurosi A, Fukai N, Dkhisli F, et al. Characterization of endostatin binding to heparin and heparan sulfate by surface plasmon resonance and molecular modeling: role of divalent cations. *J Biol Chem.* 2004; 279:2927–36. [PubMed: 14585835]
49. Ishihara M. Structural requirements in heparin for binding and activation of FGF-1 and FGF-4 are different from that for FGF-2. *Glycobiology.* 1994; 4:817–24. [PubMed: 7537556]
50. Spivak-Kroizman T, Lemmon MA, Dikic I, Ladbury JE, Pinchasi D, Huang J, et al. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell.* 1994; 79:1015–24. [PubMed: 7528103]
51. Mori H, Kanemura Y, Onaya J, Hara M, Miyake J, Yamasaki M, et al. Effects of heparin and its 6-O- and 2-O-desulfated derivatives with low anticoagulant activity on proliferation of human neural stem/progenitor cells. *J Biosci Bioeng.* 2005; 100:54–61. [PubMed: 16233851]
52. Moy FJ, Safran M, Seddon AP, Kitchen D, Bohlen P, Aviezer D, et al. Properly oriented heparin-decasaccharide-induced dimers are the biologically active form of basic fibroblast growth factor. *Biochemistry.* 1997; 36:4782–91. [PubMed: 9125499]
53. Volkin DB, Tsai PK, Dabora JM, Gress JO, Burke CJ, Linhardt RJ, et al. Physical stabilization of acidic fibroblast growth factor by polyanions. *Arch Biochem Biophys.* 1993; 300:30–41. [PubMed: 7678726]
54. Sattelle BM, Almond A. Less is More When Simulating Unsulfated Glycosaminoglycan 3D-Structure: Comparison of GLYCAM06/TIP3P, PM3-CARB1/TIP3P, and SCC-DFTB-D/TIP3P Predictions With Experiment. *J Comput Chem J Comput Chem.* 2010; 31:2932–47.
55. Sattelle BM, Shakeri J, Roberts IS, Almond A. A 3D-structural model of unsulfated chondroitin from high-field NMR: 4-sulfation has little effect on backbone conformation. *Carbohydr Res.* 2010; 345:291–302.
56. Yu F, Wolff JJ, Amster IJ, Prestegard JH. Conformational preferences of chondroitin sulfate oligomers using partially oriented NMR spectroscopy of C-13-labeled acetyl groups. *J Am Chem Soc J Am Chem Soc.* 2007; 129:13288–97.
57. Herrera AJ, Beneitez MT, Amorim L, Canada FJ, Jimenez-Barbero J, Sinay P, et al. Synthesis of a bicyclic analog of L-iduronic acid adopting the biologically relevant 2S0 conformation. *Carbohydr Res.* 2007; 342:1876–87. [PubMed: 17359949]
58. Hricovini M, Guerrini M, Bisio A, Torri G, Naggi A, Casu B. Active conformations of glycosaminoglycans. NMR determination of the conformation of heparin sequences complexed with antithrombin and fibroblast growth factors in solution. *Semin Thromb Hemost.* 2002; 28:325–34. [PubMed: 12244479]
59. Avci FY, Karst NA, Linhardt RJ. Synthetic oligosaccharides as heparin-mimetics displaying anticoagulant properties. *Curr Pharm Design.* 2003; 9:2323–35.
60. Das SK, Mallet JM, Esnault J, Driguez PA, Duchaussoy P, Sizun P, et al. Synthesis of conformationally locked L-iduronic acid derivatives: Direct evidence for a critical role of the skew-boat (2)S(0) conformer in the activation of antithrombin by heparin. *Chem-Eur J.* 2001; 7:4821–34. [PubMed: 11763451]
61. Das SK, Mallet JM, Esnault J, Driguez PA, Duchaussoy P, Sizun P, et al. Synthesis of conformationally locked carbohydrates: A skew-boat conformation of L-iduronic acid governs the antithrombotic activity of heparin. *Angew Chem Int Edit.* 2001; 40:1670–3.
62. Guglier S, Hricovini M, Raman R, Polito L, Torri G, Casu B, et al. Minimum FGF2 binding structural requirements of heparin and heparan sulfate oligosaccharides as determined by NMR spectroscopy. *Biochemistry.* 2008; 47:13862–9. [PubMed: 19117094]
63. Scott JE, Heatley F, Wood B. Comparison of Secondary Structures in Water of Chondroitin-4-Sulfate and Dermatan Sulfate - Implications in the Formation of Tertiary Structures. *Biochemistry.* 1995; 34:15467–74. [PubMed: 7492548]
64. Malmstrom A, Bartolini B, Thelin MA, Pacheco B, Maccarana M. Iduronic acid in chondroitin/dermatan sulfate: biosynthesis and biological function. *J Histochem Cytochem.* 2012; 60:916–25. [PubMed: 22899863]
65. Malmstrom A. Biosynthesis of dermatan sulphate. Loss of C-5 hydrogen during conversion of D-glucuronate to L-iduronate. *Biochem J.* 1981; 198:669–75. [PubMed: 7326032]

66. Fransson, LÅ.; Cheng, F.; Yoshida, K.; Heinegård, D.; Malmström, A.; Schmidtchen, A. Patterns of epimerization and sulphation in dermatan sulfate chains. In: Scott, JE., editor. *Dermatan Sulfate Proteoglycans: Chemistry, Biology, Chemical Pathology*. 1993. p. 11-25.
67. Tanford C, Marler E, Davidson EA, Jury E. Determination of Molecular Weight Distribution of Chondroitin Sulfate B by Sedimentation Equilibrium. *J Biol Chem*. 1964; 239:4034–40. [PubMed: 14247643]
68. Dol F, Petitou M, Lormeau JC, Choay J, Caranobe C, Sie P, et al. Pharmacologic Properties of a Low-Molecular-Weight Dermatan Sulfate - Comparison with Unfractionated Dermatan Sulfate. *J Lab Clin Med*. 1990; 115:43–51. [PubMed: 2299256]
69. Kobayashi M, Sugumaran G, Liu JA, Shworak NW, Silbert JE, Rosenberg RD. Molecular cloning and characterization of a human uronyl 2-sulfotransferase that sulfates iduronyl and glucuronyl residues in dermatan chondroitin sulfate. *J Biol Chem*. 1999; 274:10474–80. [PubMed: 10187838]
70. Malavaki C, Mizumoto S, Karamanos N, Sugahara K. Recent advances in the structural study of functional chondroitin sulfate and dermatan sulfate in health and disease. *Connect Tissue Res*. 2008; 49:133–9. [PubMed: 18661328]
71. Yung S, Chan TM. Glycosaminoglycans and proteoglycans: overlooked entities? *Perit Dial Int*. 2007; 27 (Suppl 2):S104–9. [PubMed: 17556287]
72. Mizumoto S, Sugahara K. Glycosaminoglycans are functional ligands for receptor for advanced glycation end-products in tumors. *FEBS J*. 2013
73. Fernandez F, Van Ryn J, Ofosu FA, Hirsh J, Buchanan MR. The haemorrhagic and antithrombotic effects of dermatan sulphate. *Br J Haematol*. 1986; 64:309–17. [PubMed: 3778825]
74. Bendayan P, Boccalon H, Dupouy D, Boneu B. Dermatan sulfate is a more potent inhibitor of clot-bound thrombin than unfractionated and low molecular weight heparins. *Thromb Haemost*. 1994; 71:576–80. [PubMed: 8091383]
75. Ofosu FA, Modi GJ, Blajchman MA, Buchanan MR, Johnson EA. Increased sulphation improves the anticoagulant activities of heparan sulphate and dermatan sulphate. *Biochem J*. 1987; 248:889–96. [PubMed: 2963622]
76. Maaroufi RM, Giordano P, Triadou P, Tapon-Brethaudiere J, Dautzenberg MD, Fischer AM. Effect of oversulfated dermatan sulfate derivatives on platelet aggregation. *Thromb Res*. 2007; 120:615–21. [PubMed: 17222891]
77. Hong TT, Van Gorp CL, Cardin AD, Lucchesi BR. Intimatan (dermatan 4,6-O-disulfate) prevents rethrombosis after successful thrombolysis in the canine model of deep vessel wall injury. *Thromb Res*. 2006; 117:333–42. [PubMed: 15893368]
78. Hennen JK, Hong TT, Shergill AK, Driscoll EM, Cardin AD, Lucchesi BR. Intimatan prevents arterial and venous thrombosis in a canine model of deep vessel wall injury. *J Pharmacol Exp Ther*. 2002; 301:1151–6. [PubMed: 12023550]
79. O’Keeffe D, Olson ST, Gasiunas N, Gallagher J, Baglin TP, Huntington JA. The heparin binding properties of heparin cofactor II suggest an antithrombin-like activation mechanism. *J Biol Chem*. 2004; 279:50267–73. [PubMed: 15371417]
80. Maruyama T, Toida T, Imanari T, Yu G, Linhardt RJ. Conformational changes and anticoagulant activity of chondroitin sulfate following its O-sulfonation. *Carbohydr Res*. 1998; 306:35–43. [PubMed: 9691438]
81. Gama CI, Tully SE, Sotogaku N, Clark PM, Rawat M, Vaidehi N, et al. Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nat Chem Biol*. 2006; 2:467–73. [PubMed: 16878128]
82. Purushothaman A, Sugahara K, Faissner A. Chondroitin sulfate “wobble motifs” modulate maintenance and differentiation of neural stem cells and their progeny. *J Biol Chem*. 2012; 287:2935–42. [PubMed: 22094467]
83. Sugahara K, Mikami T. Chondroitin/dermatan sulfate in the central nervous system. *Curr Opin Struct Biol*. 2007; 17:536–45. [PubMed: 17928217]
84. Brown JM, Xia J, Zhuang B, Cho KS, Rogers CJ, Gama CI, et al. A sulfated carbohydrate epitope inhibits axon regeneration after injury. *Proc Natl Acad Sci U S A*. 2012; 109:4768–73. [PubMed: 22411830]

85. Deepa SS, Umehara Y, Higashiyama S, Itoh N, Sugahara K. Specific molecular interactions of oversulfated chondroitin sulfate E with various heparin-binding growth factors - Implications as a physiological binding partner in the brain and other tissues. *J Biol Chem.* 2002; 277:43707–16. [PubMed: 12221095]
86. Hintze V, Miron A, Moeller S, Schnabelrauch M, Wiesmann HP, Worch H, et al. Sulfated hyaluronan and chondroitin sulfate derivatives interact differently with human transforming growth factor-beta1 (TGF-beta1). *Acta Biomater.* 2012; 8:2144–52. [PubMed: 22426137]
87. Hintze V, Moeller S, Schnabelrauch M, Bierbaum S, Viola M, Worch H, et al. Modifications of hyaluronan influence the interaction with human bone morphogenetic protein-4 (hBMP-4). *Biomacromolecules.* 2009; 10:3290–7. [PubMed: 19894734]
88. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth factors.* 2004; 22:233–41. [PubMed: 15621726]
89. Miljkovic ND, Cooper GM, Marra KG. Chondrogenesis, bone morphogenetic protein-4 and mesenchymal stem cells. *Osteoarthritis Cartil.* 2008; 16:1121–30. [PubMed: 18406633]
90. Miyazaki T, Miyauchi S, Tawada A, Anada T, Matsuzaka S, Suzuki O. Oversulfated Chondroitin Sulfate-E Binds to BMP-4 and Enhances Osteoblast Differentiation. *J Cell Physiol.* 2008; 217:769–77. [PubMed: 18720384]
91. Penc SF, Pomahac B, Winkler T, Dorschner RA, Eriksson E, Herndon M, et al. Dermatan sulfate released after injury is a potent promoter of fibroblast growth factor-2 function. *J Biol Chem.* 1998; 273:28116–21. [PubMed: 9774430]
92. Zamfir A, Seidler DG, Kresse H, Peter-Katalinic J. Structural investigation of chondroitin/dermatan sulfate oligosaccharides from human skin fibroblast decorin. *Glycobiology.* 2003; 13:733–42. [PubMed: 12799343]
93. Taylor KR, Rudisill JA, Gallo RL. Structural and sequence motifs in dermatan sulfate for promoting fibroblast growth factor-2 (FGF-2) and FGF-7 activity. *J Biol Chem.* 2005; 280:5300–6. [PubMed: 15563459]
94. Radek KA, Taylor KR, Gallo RL. FGF-10 and specific structural elements of dermatan sulfate size and sulfation promote maximal keratinocyte migration and cellular proliferation. *Wound Repair Regen.* 2009; 17:118–26. [PubMed: 19152659]
95. Huckerby TN. The keratan sulphates: structural investigations using NMR spectroscopy. *Prog Nucl Mag Res Sp.* 2002; 40:35–110.
96. Meyer K, Linker A, Davidson EA, Weissmann B. The Mucopolysaccharides of Bovine Cornea. *J Biol Chem.* 1953; 205:611–6. [PubMed: 13129238]
97. Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. *Glycobiology.* 2000; 10:951–8. [PubMed: 11030741]
98. Hopwood JJ, Robinson HC. Molecular-Weight Distribution of Glycosaminoglycans. *Biochem J.* 1973; 135:631–7. [PubMed: 4273184]
99. Quantock AJ, Young RD, Akama TO. Structural and biochemical aspects of keratan sulphate in the cornea. *Cell Mol Life Sci.* 2010; 67:891–906. [PubMed: 20213925]
100. Rodriguez E, Roland SK, Plaas A, Roughley PJ. The glycosaminoglycan attachment regions of human aggrecan. *J Biol Chem.* 2006; 281:18444–50. [PubMed: 16679517]
101. Schwartz NB, Domowicz M, Krueger RC, Li H, Mangoura D. Brain aggrecan. *Perspect Dev Neurobi.* 1996; 3:291.
102. Roughley PJ, Melching LI, Heathfield TF, Pearce RH, Mort JS. The structure and degradation of aggrecan in human intervertebral disc. *Eur Spine J.* 2006; 15:S326–S32. [PubMed: 16736203]
103. Huckerby TN, Tai GH, Nieduszynski IA. Oligosaccharides derived by endo-beta-galactosidase digestion of bovine corneal keratan sulphate - Characterisation of tetrasaccharides with incomplete sulphation and containing unsulphated N-acetylglucosamine residues. *Eur J Biochem.* 1998; 253:499–506. [PubMed: 9654103]
104. Arnott S, Guss JM, Hukins DWL, Dea ICM, Rees DA. Conformation of Keratan Sulfate. *J Mol Biol.* 1974; 88:175. [PubMed: 4280446]
105. Iwaki J, Minamisawa T, Tateno H, Kominami J, Suzuki K, Nishi N, et al. Desulfated galactosaminoglycans are potential ligands for galectins: Evidence from frontal affinity chromatography. *Biochem Biophys Res Commun.* 2008; 373:206–12.

106. Hughes RC. Galectins as modulators of cell adhesion. *Biochimie*. 2001; 83:667–76. [PubMed: 11522396]
107. Toole BP. Hyaluronan: From extracellular glue to pericellular cue. *Nat Rev Cancer*. 2004; 4:528–39. [PubMed: 15229478]
108. Dudhia J. Aggrecan, aging and assembly in articular cartilage. *Cellular and molecular life sciences: CMLS*. 2005; 62:2241–56. [PubMed: 16143826]
109. Almond A. Hyaluronan. *Cell Mol Life Sci*. 2007; 64:1591–6. [PubMed: 17502996]
110. Pogany P, Kovacs A. Conformational properties of the disaccharide building units of hyaluronan. *Carbohydr Res*. 2009; 344:1745–52.
111. Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. *Curr Opin Cell Biol*. 2000; 12:581–6. [PubMed: 10978893]
112. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. Cd44 Is the Principal Cell-Surface Receptor for Hyaluronate. *Cell*. 1990; 61:1303–13. [PubMed: 1694723]
113. Turley EA, Noble PW, Bourguignon LYW. Signaling properties of hyaluronan receptors. *J Biol Chem*. 2002; 277:4589–92. [PubMed: 11717317]
114. Entwistle J, Hall CL, Turley EA. Receptors: Regulators of signalling to the cytoskeleton. *J Cell Biochem*. 1996; 61:569–77. [PubMed: 8806080]
115. Peach RJ, Hollenbaugh D, Stamenkovic I, Aruffo A. Identification of Hyaluronic-Acid Binding-Sites in the Extracellular Domain of Cd44. *J Cell Biol*. 1993; 122:257–64. [PubMed: 8314845]
116. Banerji S, Wright AJ, Noble M, Mahoney DJ, Campbell ID, Day AJ, et al. Structures of the Cd44-hyaluronan complex provide insight into a fundamental carbohydrate-protein interaction. *Nat Struct Mol Biol*. 2007; 14:234–9. [PubMed: 17293874]
117. Lundin L, Larsson H, Kreuger J, Kanda S, Lindahl U, Salmivirta M, et al. Selectively desulfated heparin inhibits fibroblast growth factor-induced mitogenicity and angiogenesis. *J Biol Chem*. 2000; 275:24653–60. [PubMed: 10816596]
118. Wu ZL, Zhang L, Yabe T, Kuberan B, Beeler DL, Love A, et al. The involvement of heparan sulfate (HS) in FGF1/HS/FGFR1 signaling complex. *The Journal of biological chemistry*. 2003; 278:17121–9. [PubMed: 12604602]
119. Inoue Y, Nagasawa K. Selective N-Desulfation of Heparin with Dimethyl-Sulfoxide Containing Water or Methanol. *Carbohydr Res*. 1976; 46:87–95.
120. Baumann H, Scheen M, Huppertz B, Keller R. Novel regio- and stereoselective O-6-desulfation of the glucosamine moiety of heparin with N-methylpyrrolidinone-water or N,N-dimethylformamide-water mixtures. *Carbohydr Res*. 1998; 308:381–8.
121. Nagasawa K, Inoue Y, Kamata T. Solvolytic Desulfation of Glycosaminoglycuronan Sulfates with Dimethyl-Sulfoxide Containing Water or Methanol. *Carbohydr Res*. 1977; 58:47–55.
122. Toida T, Sato K, Sakamoto N, Sakai S, Hosoyama S, Linhardt RJ. Solvolytic depolymerization of chondroitin and dermatan sulfates. *Carbohydr Res*. 2009; 344:888–93.
123. Nagasawa K, Inoue Y, Tokuyasu T. Improved Method for the Preparation of Chondroitin by Solvolytic Desulfation of Chondroitin Sulfates. *J Biochem*. 1979; 86:1323–9. [PubMed: 521436]
124. Jaseja M, Rej RN, Sauriol F, Perlin AS. Novel Regioselective and Stereoselective Modifications of Heparin in Alkaline-Solution - Nuclear Magnetic-Resonance Spectroscopic Evidence. *Can J Chem*. 1989; 67:1449–56.
125. Kantor TG, Schubert M. A Method for the Desulfation of Chondroitin Sulfate. *J Am Chem Soc*. 1957; 79:152–3.
126. Nagasawa K, Uchiyama H, Wajima N. Chemical Sulfation of Preparations of Chondroitin 4-Sulfate and 6-Sulfate, and Dermatan Sulfate - Preparation of Chondroitin Sulfate E-Like Materials from Chondroitin 4-Sulfate. *Carbohydr Res*. 1986; 158:183–90.
127. Li BZ, Suwan J, Martin JG, Zhang FM, Zhang ZQ, Hoppensteadt D, et al. Oversulfated chondroitin sulfate interaction with heparin-binding proteins: New insights into adverse reactions from contaminated heparins. *Biochem Pharmacol*. 2009; 78:292–300. [PubMed: 19389385]
128. Ogamo A, Metori A, Uchiyama H, Nagasawa K. Reactivity toward Chemical Sulfation of Hydroxyl-Groups of Heparin. *Carbohydr Res*. 1989; 193:165–72.

129. Wei Z, Deakin JA, Blaum BS, Uhrin D, Gallagher JT, Lyon M. Preparation of heparin/heparan sulfate oligosaccharides with internal N-unsubstituted glucosamine residues for functional studies. *Glycoconjugate J.* 2011; 28:525–35.
130. Purkerson ML, Tollefson DM, Klahr S. N-Desulfated Acetylated Heparin Ameliorates the Progression of Renal-Disease in Rats with Subtotal Renal Ablation. *J Clin Invest.* 1988; 81:69–74. [PubMed: 3335644]
131. Diancourt F, Braud C, Vert M. Chemical Modifications of Heparin .1. Reaction of Partially N-Desulfated Heparin with Glutaraldehyde. *J Bioact Compat Pol.* 1994; 9:267–81.
132. Ponedel'kina IY, Odinkov VN, Lukina ES, Tyumkina TV, Khalilov LM, Dzhemilev UM. Chemical modification of heparin. *Russ J Bioorg Chem+*. 2006; 32:472–7.
133. van Wachem PB, Plantinga JA, Wissink MJB, Beernink R, Poot AA, Engbers GHM, et al. In vivo biocompatibility of carbodiimide-crosslinked collagen matrices: Effects of crosslink density, heparin immobilization, and bFGF loading. *J Biomed Mater Res.* 2001; 55:368–78. [PubMed: 11255190]
134. Wissink MJB, Beernink R, Pieper JS, Poot AA, Engbers GHM, Beugeling T, et al. Immobilization of heparin to EDC/NHS-crosslinked collagen. Characterization and in vitro evaluation. *Biomaterials.* 2001; 22:151–63. [PubMed: 11101159]
135. Seto SP, Casas ME, Temenoff JS. Differentiation of mesenchymal stem cells in heparin-containing hydrogels via coculture with osteoblasts. *Cell Tissue Res.* 2012; 347:589–601. [PubMed: 22083260]
136. Lim JJ, Hammoudi TM, Bratt-Leal AM, Hamilton SK, Kepple KL, Bloodworth NC, et al. Development of nano- and microscale chondroitin sulfate particles for controlled growth factor delivery. *Acta Biomater.* 2011; 7:986–95. [PubMed: 20965281]
137. Kim M, Shin Y, Hong BH, Kim YJ, Chun JS, Tae G, et al. In Vitro Chondrocyte Culture in a Heparin-Based Hydrogel for Cartilage Regeneration. *Tissue Eng Part C-Me.* 2010; 16:1–10.
138. Tae G, Kim YJ, Choi WI, Kim M, Stayton PS, Hoffman AS. Formation of a novel heparin-based hydrogel in the presence of heparin-binding biomolecules. *Biomacromolecules.* 2007; 8:1979–86. [PubMed: 17511500]
139. Crescenzi V, Cornelio L, Di Meo C, Nardecchia S, Lamanna R. Novel hydrogels via click chemistry: synthesis and potential biomedical applications. *Biomacromolecules.* 2007; 8:1844–50. [PubMed: 17523655]
140. Testa G, Di Meo C, Nardecchia S, Capitani D, Mannina L, Lamanna R, et al. Influence of dialkyne structure on the properties of new click-gels based on hyaluronic acid. *Int J Pharmaceut.* 2009; 378:86–92.
141. Reis AV, Fajardo AR, Schuquel ITA, Guilherme MR, Vidotti GJ, Rubira AF, et al. Reaction of Glycidyl Methacrylate at the Hydroxyl and Carboxylic Groups of Poly(vinyl alcohol) and Poly(acrylic acid): Is This Reaction Mechanism Still Unclear? *J Org Chem.* 2009; 74:3750–7. [PubMed: 19361172]
142. Reis AV, Guilherme MR, de Almeida EAMS, Kunita MH, Muniz EC, Rubira AF, et al. Copolymer Hydrogel Microspheres Consisting of Modified Sulfate Chondroitin-co-Poly(N-isopropylacrylamide). *J Appl Polym Sci.* 2011; 121:2726–33.
143. Li Q, Williams CG, Sun DD, Wang J, Leong K, Elisseeff JH. Photocrosslinkable polysaccharides based on chondroitin sulfate. *J Biomed Mater Res A.* 2004; 68:28–33. [PubMed: 14661246]
144. Rudd TR, Nichols RJ, Yates EA. Selective detection of protein secondary structural changes in solution protein-polysaccharide complexes using vibrational circular dichroism (VCD). *J Am Chem Soc.* 2008; 130:2138. [PubMed: 18225900]
145. Rullo A, Nitz M. Importance of the Spatial Display of Charged Residues in Heparin-Peptide Interactions. *Biopolymers.* 2010; 93:290–8. [PubMed: 19885920]
146. Osmond RIW, Kett WC, Skett SE, Coombe DR. Protein-heparin interactions measured by BIAcore 2000 are affected by the method of heparin immobilization. *Anal Biochem.* 2002; 310:199–207. [PubMed: 12423639]
147. Danishefsky I, Siskovic E. Heparin derivatives prepared by modification of the uronic acid carboxyl groups. *Thromb Res.* 1972; 1:173–81.

148. Benoit DSW, Anseth KS. Heparin functionalized PEG gels that modulate protein adsorption for hMSC adhesion and differentiation. *Acta Biomater.* 2005; 1:461–70. [PubMed: 16701827]
149. Ruhaak LR, Zauner G, Huhn C, Bruggink C, Deelder AM, Wuhrer M. Glycan labeling strategies and their use in identification and quantification. *Anal Bioanal Chem.* 2010; 397:3457–81. [PubMed: 20225063]
150. Robyt, JF. *Essentials of Carbohydrate Chemistry.* Springer Verlag; 1998.
151. Bae KH, Mok H, Park TG. Synthesis, characterization, and intracellular delivery of reducible heparin nanogels for apoptotic cell death. *Biomaterials.* 2008; 29:3376–83. [PubMed: 18474396]
152. Liberda J, Ticha M, Jonakova V. Preparation of fluorescein-labelled and biotinylated derivatives of polysaccharides for lectin-saccharide binding studies. *Biotechnol Tech.* 1997; 11:265–7.
153. Tan HP, Chu CR, Payne KA, Marra KG. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials.* 2009; 30:2499–506. [PubMed: 19167750]
154. Glass JR, Dickerson KT, Stecker K, Polarek JW. Characterization of a hyaluronic acid-Arg-Gly-Asp peptide cell attachment matrix. *Biomaterials.* 1996; 17:1101–8. [PubMed: 8718970]
155. Lee JS, Go DH, Bae JW, Lee SJ, Park KD. Heparin conjugated polymeric micelle for long-term delivery of basic fibroblast growth factor. *J Control Release.* 2007; 117:204–9. [PubMed: 17196698]
156. Jeon O, Kang SW, Lim HW, Hyung Chung J, Kim BS. Long-term and zero-order release of basic fibroblast growth factor from heparin-conjugated poly(L-lactide-co-glycolide) nanospheres and fibrin gel. *Biomaterials.* 2006; 27:1598–607. [PubMed: 16146647]
157. Luo Y, Kirker KR, Prestwich GD. Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. *J Control Release.* 2000; 69:169–84. [PubMed: 11018555]
158. Chen AL, Ni HC, Wang LF, Chen JS. Biodegradable amphiphilic copolymers based on poly(epsilon-caprolactone)-graft chondroitin sulfate as drug carriers. *Biomacromolecules.* 2008; 9:2447–57. [PubMed: 18662028]
159. Jensen M, Birch Hansen P, Murdan S, Frokjaer S, Florence AT. Loading into and electro-stimulated release of peptides and proteins from chondroitin 4-sulphate hydrogels. *Eur J Pharm Sci.* 2002; 15:139–48. [PubMed: 11849910]
160. Oprea AM, Nistor MT, Popa MI, Lupusoru CE, Vasile C. In vitro and in vivo theophylline release from cellulose/chondroitin sulfate hydrogels. *Carbohydr Polym.* 2012; 90:127–33.
161. Lee JS, Bae JW, Joung YK, Lee SJ, Han DK, Park KD. Controlled dual release of basic fibroblast growth factor and indomethacin from heparin-conjugated polymeric micelle. *Int J Pharm.* 2008; 346:57–63. [PubMed: 17689210]
162. Wang SC, Chen BH, Wang LF, Chen JS. Characterization of chondroitin sulfate and its interpenetrating polymer network hydrogels for sustained-drug release. *Int J Pharm.* 2007; 329:103–9. [PubMed: 16996709]
163. Park K, Lee GY, Kim YS, Yu M, Park RW, Kim IS, et al. Heparin-deoxycholic acid chemical conjugate as an anticancer drug carrier and its antitumor activity. *J Control Release.* 2006; 114:300–6. [PubMed: 16884806]
164. Lyons H, Singer JA. Effect of Mucopolysaccharide Linkage Region and Antrachain Alkali Labilities on Chain Weight Determination. *J Biol Chem.* 1971; 246:277. [PubMed: 5541997]
165. Hirano S, Ishigami M, Koga Y. Depolymerization and de-N-acetylation of glycosaminoglycans by the action of alkali in the presence of sodium borohydride. *Connect Tissue Res.* 1975; 3:73–9. [PubMed: 126130]
166. Shively JE, Conrad HE. Formation of anhydrosugars in the chemical depolymerization of heparin. *Biochemistry.* 1976; 15:3932–42. [PubMed: 9127]
167. Bienkowski MJ, Conrad HE. Oligosaccharides formed by treatment of heparin with nitrous acid. *Semin Thromb Hemost.* 1985; 11:86–8. [PubMed: 4035369]
168. Bienkowski MJ, Conrad HE. Structural characterization of the oligosaccharides formed by depolymerization of heparin with nitrous acid. *J Biol Chem.* 1985; 260:356–65. [PubMed: 3965453]
169. Edwards HE, Moore JS, Phillips GO. Effects of ⁶⁰Co gamma-irradiation on chondromucoprotein. *Int J Radiat Biol Relat Stud Phys Chem Med.* 1977; 32:351–9. [PubMed: 302830]

170. Shaklee PN, Conrad HE. Hydrazinolysis of heparin and other glycosaminoglycans. *Biochem J.* 1984; 217:187–97. [PubMed: 6421280]
171. Roberts CR, Mort JS, Roughley PJ. Treatment of cartilage proteoglycan aggregate with hydrogen peroxide. Relationship between observed degradation products and those that occur naturally during aging. *Biochem J.* 1987; 247:349–57. [PubMed: 3122723]
172. Schmut O, Ansari AN, Faulborn J. Degradation of hyaluronate by the concerted action of ozone and sunlight. *Ophthalmic Res.* 1994; 26:340–3. [PubMed: 7715914]
173. Nagasawa K, Uchiyama H, Sato N, Hatano A. Chemical change involved in the oxidative-reductive depolymerization of heparin. *Carbohydr Res.* 1992; 236:165–80. [PubMed: 1337863]
174. Jeon O, Powell C, Solorio LD, Krebs MD, Alsberg E. Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels. *J Control Release.* 2011; 154:258–66. [PubMed: 21745508]
175. Choh SY, Cross D, Wang C. Facile Synthesis and Characterization of Disulfide-Cross-Linked Hyaluronic Acid Hydrogels for Protein Delivery and Cell Encapsulation. *Biomacromolecules.* 2011; 12:1126–36. [PubMed: 21384907]
176. Ernst S, Langer R, Cooney CL, Sasisekharan R. Enzymatic degradation of glycosaminoglycans. *Crit Rev Biochem Mol Biol.* 1995; 30:387–444. [PubMed: 8575190]
177. Casu B, Lindahl U. Structure and biological interactions of heparin and heparan sulfate. *Adv Carbohydr Chem Bi.* 2001; 57:159–206.
178. Linhardt RJ, Galliher PM, Cooney CL. Polysaccharide Lyases. *Appl Biochem Biotech.* 1986; 12:135–76.
179. Vlodavsky I, Ilan N, Naggi A, Casu B. Heparanase: Structure, biological functions, and inhibition by heparin-derived mimetics of heparan sulfate. *Curr Pharm Design.* 2007; 13:2057–73.
180. Baker JR, Pritchard DG. Action pattern and substrate specificity of the hyaluronan lyase from group B streptococci. *Biochem J.* 2000; 348:465–71. [PubMed: 10816443]
181. Pikas DS, Li JP, Vlodavsky I, Lindahl U. Substrate specificity of heparanases from human hepatoma and platelets. *J Biol Chem.* 1998; 273:18770–7. [PubMed: 9668050]
182. Naggi A, Casu B, Perez M, Torri G, Cassinelli G, Penco S, et al. Modulation of the heparanase-inhibiting activity of heparin through selective desulfation, graded N-acetylation, and glycol splitting. *J Biol Chem.* 2005; 280:12103–13. [PubMed: 15647251]
183. Xiao ZP, Tappen BR, Ly M, Zhao WJ, Canova LP, Guan HS, et al. Heparin Mapping Using Heparin Lyases and the Generation of a Novel Low Molecular Weight Heparin. *J Med Chem.* 2011; 54:603–10. [PubMed: 21166465]
184. Nilasaroya A, Martens PJ, Whitelock JM. Enzymatic degradation of heparin-modified hydrogels and its effect on bioactivity. *Biomaterials.* 2012; 33:5534–40. [PubMed: 22575836]
185. Tsurkan MV, Chwalek K, Levental KR, Freudenberg U, Werner C. Modular StarPEG-Heparin Gels with Bifunctional Peptide Linkers. *Macromol Rapid Comm.* 2010; 31:1529–33.
186. Lin CC, Metters AT. Hydrogels in controlled release formulations: network design and mathematical modeling. *Adv Drug Deliv Rev.* 2006; 58:1379–408. [PubMed: 17081649]
187. Chu HH, Gao J, Chen CW, Huard J, Wang YD. Injectable fibroblast growth factor-2 coacervate for persistent angiogenesis. *P Natl Acad Sci USA.* 2011; 108:13444–9.
188. Parkinson RJ, Demers CP, Adel JG, Levy EI, Sauvageau E, Hanel RA, et al. Use of heparin-coated stents in neurovascular interventional procedures: Preliminary experience with 10 patients. *Neurosurgery.* 2006; 59:812–21. [PubMed: 17038945]

Important monosaccharide components of GAGs

Uronic acid residues

Amino sugar residues

Figure 1.

Most prominent monosaccharides present in GAGs. Uronic acid sugars possess a carboxyl function connected to C5 of the ring atom, whereas amino sugars have an amino function at position C2. This amino moiety may exist as a free amine (rare), acetylated (shown above) or sulfated (see Figure 3) within GAG polysaccharides.

A. Selected monosaccharide conformations **B. Definition of C_1/C_5 stereochemistry and D,L configuration** **Figure 2.**

(A.) Carbohydrate conformations are shown as chair (*C*) and skew-boat (*S*). In the chair conformations, the gray, imaginary planes connect ring carbons on parallel sites. Ring carbons which are out-of-plane, determine the conformations. In this nomenclature, the carbon above the plane is noted with a superscript to the *C*, while the carbon below becomes a subscript. For the skew-boat conformation, the gray plane connects carbons 1,3,4,5. Carbons above this plane are indicated with superscripts, while those below become subscripts. As no carbons are below the plane in this conformation, the subscript is O. (B.) Carbohydrate stereochemistry and D,L configuration with glucuronic acid as an example. The α/β nomenclature describes the configuration of the stereo-centers at C1 and C5 whereas D,L nomenclature is related to the substituent position in the Fischer-projection (will not be further explained here – please see [26] for details).

Figure 3.

Conformation of carbohydrates in heparin and heparan sulfate: (A.) pentasaccharide binding sequence of heparin for AT-III, (B.) important binding sites for growth factors to a heparin/HS trisaccharide sample sequence. Position of sulfate groups is labeled with their biological activity. The blue boxes indicate necessity of presence for protein binding.

Figure 4.
Structures of CS (A.) and DS (B.). Blue boxes indicate necessity for protein binding.

Figure 5. Primary sequence of keratan sulfate: 6-O sulfate D-galactosamine and 6-O sulfate N-acetylglucosamine. Blue box indicates necessity for growth factor binding, whereas red box indicates reduction of galectin binding.

Figure 6.
Disaccharide units of HA: D-glucuronic acid and D-N-acetylglucosamine.

Figure 7.

Reaction scheme presenting the concept of carbohydrate ring opening by periodate oxidation. (Note: periodate oxidation occurs at unsulfated IduA or GlcA residues.) The example is given with a primary amine to be coupled in a second step with formation of a Schiff base. Finally the Schiff base is reduced to a secondary amine.

Figure 8.

Four common mechanisms of loading in GAG-based delivery systems. Electrostatic interaction between the cargo and GAG is the most common mechanism, resulting in strong sequestration. Hydrophobic interaction between GAG strands and the cargo is found most commonly in HA vehicles. Physical entrapment, adsorption or absorption further enable loading of uncharged or negatively charged cargo into GAG-based vehicles.

Figure 9.

Degradation principles of GAGs. Chemical degradation focuses on degradation at GAG modification sites as the polymers themselves are chemically quite stable. Enzymatic mechanisms consist of GAG-specific enzymes that cleave glycosidic bonds, whereas peptide sequence-specific bonds can be cleaved by enzymes if a specific sequence is introduced into the drug delivery system.

Figure 10.

Mechanisms of enzymatic heparin/HS degradation. Lyases cause eliminative cleavage whereas hydrolases lead to hydrolytic degradation. Red color indicates the center of action in the scheme.