Review

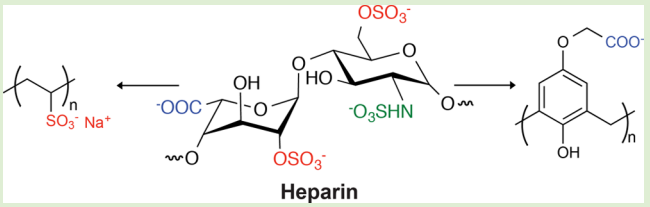
pubs.acs.org/Biomac

Heparin-Mimicking Polymers: Synthesis and Biological Applications

Samantha J. Paluck, Thi H. Nguyen, and Heather D. Maynard*

Department of Chemistry and Biochemistry and the California NanoSystems Institute, University of California−Los Angeles, 607 Charles E. Young Dr East, Los Angeles, California 90095, United States

ABSTRACT: Heparin is a naturally occurring, highly sulfated polysaccharide that plays a critical role in a range of different biological processes. Therapeutically, it is mostly commonly used as an injectable solution as an anticoagulant for a variety of indications, although it has also been employed in other forms such as coatings on various biomedical devices. Due to the diverse functions of this polysaccharide in the body, including anticoagulation, tissue regeneration, anti-inflammation, and protein stabilization, and drawbacks of its use, analogous heparin-mimicking materials are also widely studied for therapeutic applications. This review focuses on one type of these materials, namely, synthetic heparin-mimicking polymers. Utilization of these polymers provides significant benefits compared to heparin, including enhancing therapeutic efficacy and reducing side effects as a result of fine-tuning heparin-binding motifs and other molecular characteristics. The major types of the various polymers are summarized, as well as their applications. Because development of a broader range of heparin-mimicking materials would further expand the impact of these polymers in the treatment of various diseases, future directions are also discussed.



1. INTRODUCTION TO HEPARIN

Heparin is a linear, highly sulfated glycosaminoglycan produced by mast cells. Its chemical structure consists of repeating monomeric disaccharides of uronic acid and glucosamine in a 1,4-linkage (Figure 1), and the three-dimensional structure exists primarily in a helical form.[1] On average, there are 2.7 sulfate groups per disaccharide monomer, which when combined gives heparin a total negative net charge of approximately −75.[2−4] Since heparin has an average molecular weight of 15 kDa, this property gives heparin the highest negative charge density of any known naturally derived biomolecule.[3] The size of heparin varies greatly between tissues with molecular weights ranging from 5−40 kDa with structural variations such as amount of sulfation, epimerization and degree of acetylation. Heparin and heparin sulfate (HS) are similar due to their high degree of sulfation, however heparan sulfate differs from heparin in that it is a proteoglycan presented on the surface of virtually all native cells and is significantly less sulfated than heparin.[1]

Heparin is most well-known for its role in blood clotting, but also plays a role in other cellular functions such as cell adhesion, proliferation, differentiation, migration, and inflammation (Table 1).[5−7] Heparin interacts with proteins primarily through electrostatic interactions between its sulfate and carboxylate groups with clusters of positively charged amino acid residues, such as arginine and lysine, in the heparin binding sites of the biomolecules. In addition to electrostatic interactions, heparin-binding domains also contain amino acids such as asparagine and glutamine that can participate in hydrogen bonding with heparin. These interactions help stabilize proteins, regulate their affinity for cell receptors and aid in extracellular matrix (ECM) assembly.[8]

While there are over 400 heparin binding proteins,[9] research has mainly focused on the serine protease inhibitor antithrombin III (ATIII), as well as proteins including acidic fibroblast growth factor (FGF1), basic fibroblast growth factor (FGF2), vascular endothelial growth factor (VEGF), heparin binding-epidermal growth factor (HB-EGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF).[4,10] Heparin binding motifs, the clusters of positively charged amino acids called heparin binding domains, have been defined on these proteins by molecular modeling and crystallographic studies.[11−13] Other heparin binding proteins include adhesion proteins, chemokines like platelet factor 4 (PF4), and lipid or membrane binding proteins such as apolipoprotein E (apoE).[4]

Heparin itself is currently Federal Drug Administration (FDA) approved for clinical uses, such as for the treatment of deep vein thrombosis and pulmonary embolism, making it an attractive platform for new applications. Heparin-based materials are thus widely studied for use in tissue engineering, wound healing, cell replacement therapies, angiogenic treatments and encapsulation and release of proteins.[14] Since the realization of its importance and necessity as an anticoagulant a century ago, heparin has been exploited most heavily in the clinic for this purpose, yet the only sources of heparin are animal tissues. This raises the concern of the possible risk of virus contamination and adverse effects.[15,16] Heparin also has notable variable patient-dependent dose−response[17−19] Another issue with using heparin in therapeutics is that heparin can be

Received: July 27, 2016
Revised: September 26, 2016
Published: October 14, 2016

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

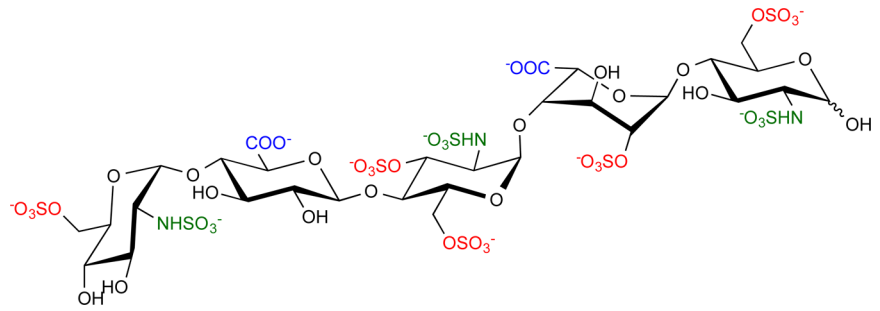


Figure 1. Chemical structure of a heparin pentasaccharide showing various repeats containing sulfate (red), sulfamate (green), and carboxylate (blue) groups.

Table 1. Examples of Some of the Biological Properties of Heparin

Biological Property of Heparin	Interactions Leading to Biological Outcome
Anticoagulant	Binds fibrin, antithrombin, factor Xa, factor IXa, thrombin, heparin cofactor II and protein C inhibitor
Protein Stabilizer	Forms strong electrostatic interactions with many heparin-binding proteins such as FGF1 and FGF2, protecting them from deactivation
Anti-Inflammatory	Binds to selectins on leukocytes inhibiting their interaction with sialyl Lewis X (sLe^x), and thus preventing interactions with endothelial cells
	Binds and neutralizes proteins, enzymes, chemokines and cytokines that activate or are released from inflammatory cells
Alterations in cellular migration and/or proliferation	Binds various growth factors involved in cell growth and migration, such as FGFs, VEGF, and PDGF, thus altering their ability to bind with receptors on the cell surface

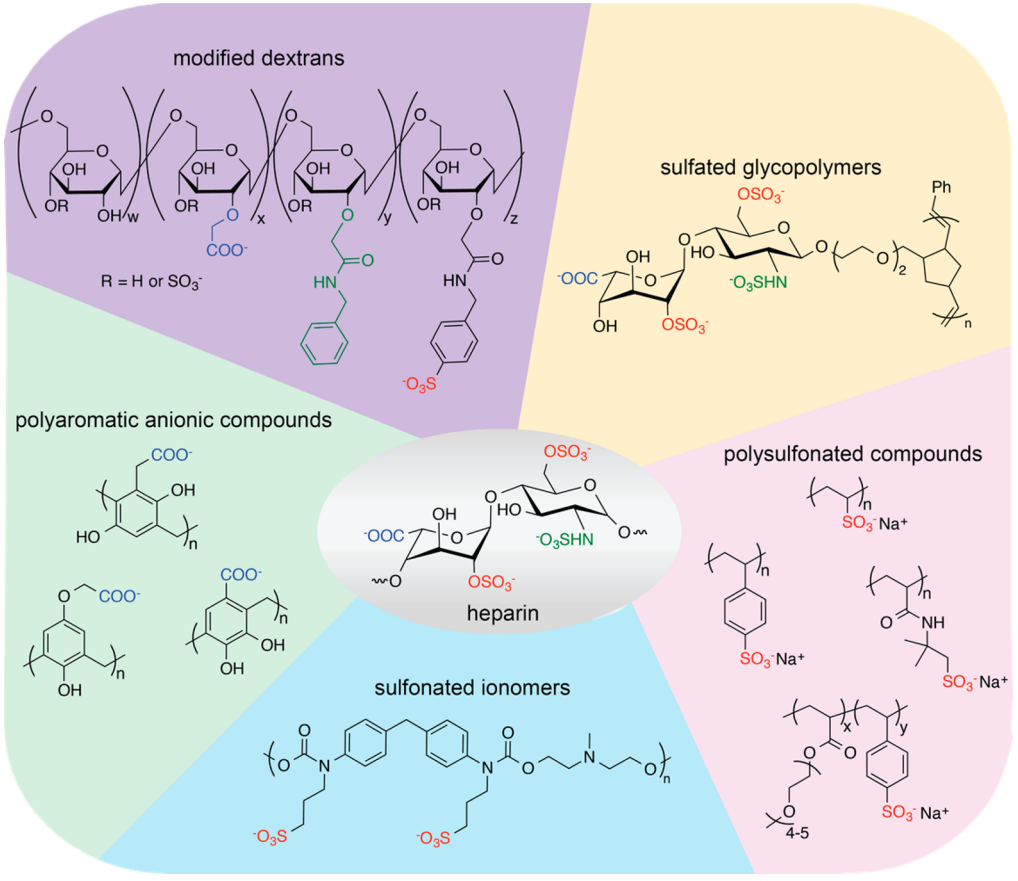


Figure 2. Examples of heparin mimicking polymers discussed in this review, including modified dextrans, sulfated glycopolymers, polysulfonated compounds, sulfonated ionomers, and polyaromatic anionic compounds.

degraded or desulfated in vivo by heparinases and other enzymes, which is not necessarily harmful, but it could result in unwanted loss of bioactivity. Researchers also found that patients prescribed longer-term treatments of unfractionated heparin were at increased risk for negative clinical effects of heparin induced thrombocytopenia (low platelet count).[20] At first, low molecular weight heparins (LMWH) with an average molecular weight of 6 kDa were used because of their more predictable pharmacokinetics as well as their ability to reduce unwanted side effects.[21] Oligomers of heparin, termed ultralow

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

molecular weight heparins (ULMWH), were also utilized and preferred over unfractionated heparin. However, due to the high cost and difficult synthesis of producing ULMWH and LMWH, new synthetic routes had to be explored as alternatives to heparin therapies.[22] In addition, heparin is extremely heterogeneous in structure with different binding motifs, leading to a broad range of biological activities which can lead to side effects.[8] All of the downfalls of heparin and oligoheparins, along with batch-to-batch variability, have inspired researchers to study synthetic alternatives. There are a range of synthetic mimics such as small molecules,[23,24] peptides,[25−28] polysaccharides[29,30] and polymers.[29,31] A few of these are even approved for clinical use, including small molecule heparin mimics Suramin and Carafate, the first of which is an antiparasitic and has also been studied as an anticancer drug; the later is used to treat intestinal ulcers.[32,33] In this review, the main focus will be on synthetic polymer mimics.

2. HEPARIN-MIMICKING POLYMERS

Polymeric heparin-mimics can provide better control over structure, sulfation and purity. It is also possible to obtain narrow molecular weight dispersities. The ability to tune parameters such as molecular weight and sulfation percentage allows for controlled tuning of binding affinity and other factors. This can in turn provide for more specific interactions with receptors and proteins. In addition, heparin-mimicking polymers typically resist degradation/desulfation by heparinases, allowing for increased longevities in the body; this can be a positive or negative depending on the application. Preparing heparin-mimics by synthetic means also permits incorporation of reactive handles, which allows for easier functionalization and conjugation of the polymers. Thus, many groups have focused on the synthesis of polymeric heparin mimics as a way to improve current heparin based therapies. Research has shown that some of these synthetic heparin mimics are superior to heparin in terms of purity and resistance to degradation, while still providing desired effects. This review provides an introduction for newcomers in the field of heparin-mimicking polymers. Heparin mimicking hydrogels have been reported elsewhere.[34] Surfaces and membranes modified with heparin and heparin mimicking polymers have recently been reviewed[29,35−38] and reviews on glycosaminoglycans (GAGs), in general, have published.[29,39] Thus, herein, we highlight an example of semisynthetic polymers and many examples of synthetic polymers (overview provided in Figure 2) focusing mostly on soluble mimics. For the semisynthetic polymers, we focus on one class, derivatives of dextran, which have been the most extensively studied. Semisynthetic polymers based on other naturally occurring polysaccharides such as alginate,[40−42] cellulose,[43,44] and chitosan[45−47] that have been sulfated for use as heparin mimics will not be reviewed here. Applications of the polymers are discussed where appropriate, as well as future directions for heparin mimicking polymers.

2.1. Carboxymethyl Benzylamide Sulfonate Dextrans (CMDBS). The first semisynthetic heparin-mimicking polymers reported were functionalized dextrans, called carboxymethyl benzylamide sulfonate dextrans (CMDBS; Figure 3).[48] Dextran, a complex branched glycan, was chosen as the polymeric base for these mimics for many reasons including its approved used in the clinic as a plasma volume expander, as well as its ease of modification. Mauzac and Jozefonvics reported the modification of dextrans with the addition of

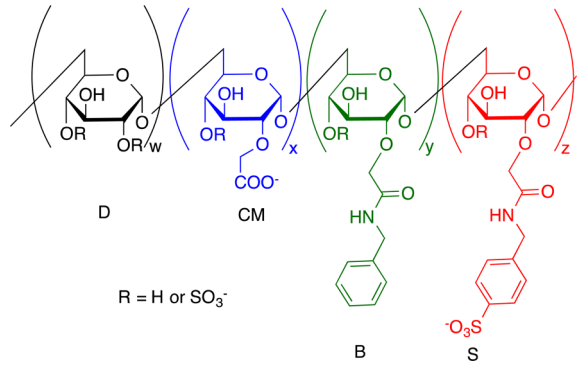


Figure 3. Chemical structure of carboxymethyl benzylamide sulfonate dextrans (CMDBS).

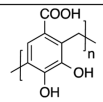
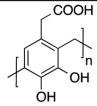
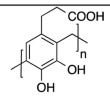
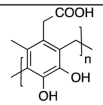
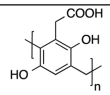
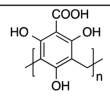
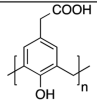
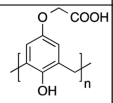
benzyl sulfonates and benzyl amines to form soluble CMDBS polymers.[49] The synthesis of the CMDBS polymers was achieved by first carboxymethylating (CM) the hydroxyl groups on dextran (D). Next, benzylamidation (B) was performed and subsequent sulfonation (S) afforded the CMDBS polymers (Figure 3) in three reaction steps, and varying degrees of modification were achieved by repeating reaction steps multiple times.

Initially, the polymers were studied for their anticoagulant activity by measuring the clotting time of platelet-poor plasma (PPP) in the presence of CMDBS polymers or heparin. The authors found that changes in the overall percentage of carboxylic and benzyl sulfonate groups had an effect on anticoagulant activity; specifically, CM content greater than 40% was required to exhibit anticoagulant activity. When the CM content was maintained at 47.5%, antithrombic activity increased exponentially as S content increased.[49] The effect of molecular weight on anticoagulant activity was tested in CMDBS polymers with molecular weights ranging from 5.5 to 190 kDa. The anticoagulant activity of the dextran derivatives increased with increasing molecular weight up to 40 kDa.[50] Anticoagulation effects of these polymers are likely due to the distribution of carboxylic and sulfonate groups on the dextran backbone. It is important to note that, while the CMDBS polymers were able to induce clotting, they exhibited much lower antithrombic activity than heparin itself.

In addition to its study as an anticoagulant, the CMDBS family also attracted much attention for its potential as a heparin mimicking material in other applications. Biological activities reported include anti-inflammatory activity, antibacterial and antiviral activities, regenerating activity, modulation of vascular cell proliferation, and antiproliferative and antitumoral activity.[31] The capability of the functionalized dextrans to mimic the role of heparin in skin,[51,52] bone,[53−58] colon,[59] cornea,[60] and muscle[61−64] have also gained a lot of attention. CMDBS polymers were named "ReGeneraTing Agents" (RGTA) for their ability to help regenerate various types of tissues. The polymers were found to interact with heparin-binding growth factors; for example, in 1989 Tardieu and colleagues reported that a member of the CMDBS family with 82% CM, 6% B, and 5.6% S potentiated the mitogenic activity of FGF1 to Chinese hamster fibroblast cells similar to heparin when 20× higher concentration of the CMDBS was used.[65] In terms of growth factor protection, a CMDBS polymer with 82% CM, 23% B, and 13% S was shown to protect FGF2 against pH and heat stressors more effectively than heparin.[66] However, the same CMDBS polymer was not as effective at stabilizing

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 2. Structures and Biological Activities of Polyaromatic Anionic Heparin-Mimicking Compounds[a] (Modified with Permission from Ref 81; Copyright 2002 Wiley-Liss, Inc., John Wiley and Sons)

Polymer		Antiproliferative activity of Smooth Muscle Cells stimulated with			Release of ECM-bound FGF2	Heparanase inhibitory activity
		Thrombin 10^{-7} M	FGF2 2 ng/ml	Serum 10%		
RG-13525		++	++	+++	+	++
RG-13527		+++	++	+++	+++	+++
RG-13528		++	++	+	+++	++
RG-14444		+	+	+	+	++++
RG-13576		+++	++	++	+	+++
RG-13530		+++	++	+	+	++
RG-13519		+++	++	+	+++	++
RG-13524		+	++	+	+	+
RG-13577		+++	+++	+++	++	+++

[a]The relative activity of each compound is presented as +, ++, +++, and ++++ representing a low (0−30%), medium (30−60%), high (60−90%), or almost complete (90−100%) inhibition of heparanase activity and SMC proliferation, or stimulated release of ECM-bound FGF2 expressed as percentage of the total ECM-bound FGF2. Note: In most cases, this polymerization will result in polymers with different structures and substitution patterns. Only one possible structure is drawn in each case.

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

FGF1 compared to heparin. This is likely due to the polymer having different binding affinities for each individual protein.

CMDBS polymers have also been tested in vivo for functions in tissue regeneration. Meddahi et al. used RGTA11 polymer comprising of 110% CM, 2.5% B, and 36.5% S to promote rat extensor digitorum longus (EDL) muscle regeneration post crushing via a single systemic administration.[62] Mice receiving an injection of RGTA11 polymer displayed increased muscle regeneration compared to mice receiving no treatment. Furthermore, RGTA11 was found to promote enhanced proliferation and migration in endothelial cells as well as endothelialization of vascular prostheses in combination with FGF2 in vitro when compared to FGF2 alone.[67]

Papy-Garcia and co-workers reported an improved synthesis and characterization of another member of the family, RGTA OTR4120, in 2005.[68] The compound was prepared by carboxymethylation and subsequent *O*-sulfonation of T40 dextran in the presence of an acid scavenger 2-methyl-2-butene to reduce glycosidic bond cleavage. RGTA OTR4120 was shown to enhance VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation and migration in vitro and VEGF-induced angiogenesis in a chick embryo assay.[69] Additionally, the positive dermal effects of RGTA OTR4120 have been demonstrated in various animal models including necrotic skin ulcers in mice,[70] burn wounds in rats,[71] surgical excision wounds in rats,[72,73] dermal ischemia ulcers in rats,[74] and diabetes-impaired wounds in rats.[75] To summarize, RGTAs have been extensively utilized, and additional applications not discussed here have been recently reviewed elsewhere.[76] The polymers have also been employed in the clinic: for example, RGTA OTR4120 is marketed in France under the name CACIPLIQ20 to treat chronic wounds.

Dextran derived CMDBS polymers provided the initial groundwork toward new polymeric heparin mimics and also gained approval for use in humans. They have a wide range of bioactivities similar to heparin and also applications. However, they do not overcome the issue heterogeneity unless fractionated. Also, the dextrans from which CMDBS polymers are derived are isolated from animal tissues or bacteria, and therefore are not purely synthetic heparin mimics. The modification of these materials can also be tedious and can introduce additional heterogeneity. Thus, researchers continued to look for alternative solutions.

2.2. Polyaromatic Anionic Compounds. Regan and colleagues reported a series of nonsulfated heparin mimicking polymers (Table 1).[77] These polyaromatic compounds were synthesized from the acid-catalyzed polymerization of various anionic group-substituted phenols with formaldehyde. Unlike CMDBS polymers, the polyaromatic anionic compounds were not necessarily sulfated and some relied solely on carboxylic acids for their negative charge density. The group tested these polymers for their heparin mimicking ability by using NIH3T3 mouse fibroblast cells transfected with FGF2 conjugated to a signal peptide sequence to afford so-called spFGF2 cells; these cells were studied because of their potential relation to cancer.[78] Specifically, the incorporation of cells with spFGF2 causes FGF2 to be secreted resulting in transformation of the cells in culture and tumorgenicity in animals.[79] But molecules such as heparin that bind FGF2 had been shown to interfere with this process, thus, reverting the phenotype. Among the polymers tested, poly(4-hydroxyphenoxyacetic acid) named RG-13577 (Table 2) was first identified as the most worthy candidate of its class for its ability to revert the FGF2-mediated transformed phenotype of these tumorigenic cells.[78] Furthermore, Benezra and co-workers reported that RG-13577 mimicked heparin in many other aspects including its ability to inhibit proliferation of vascular smooth muscle cells (SMCs) induced by FGF2, efficiently releasing surface-bound FGF2, and inhibiting heparanase activity.[80,81] Additionally, the compound exhibited only 1−10% of the anticoagulant ability of heparin suggesting that the polymer can be utilized for specific biological purposes when anticoagulation is not desired.[77] RG-13577 has a lower molecular weight than heparin, and although polydisperse (2.53 dispersity) is not as heterogeneous in size as heparin (MW = 5−40 kDa).

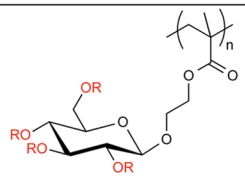
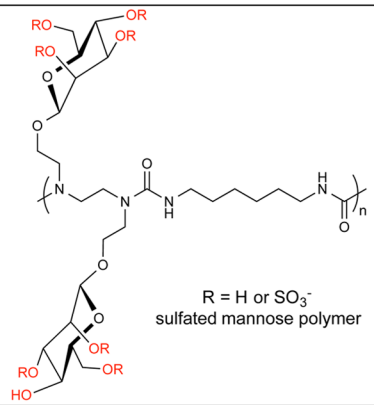
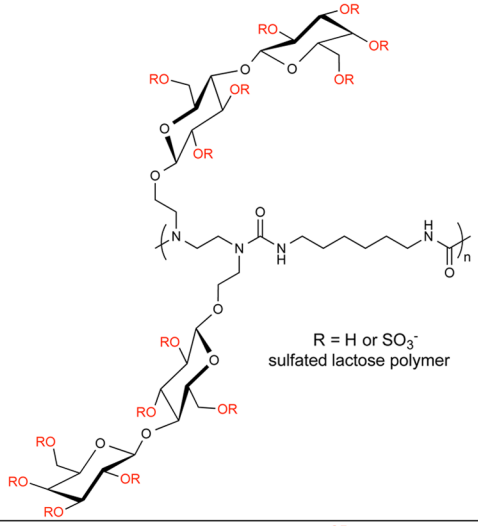
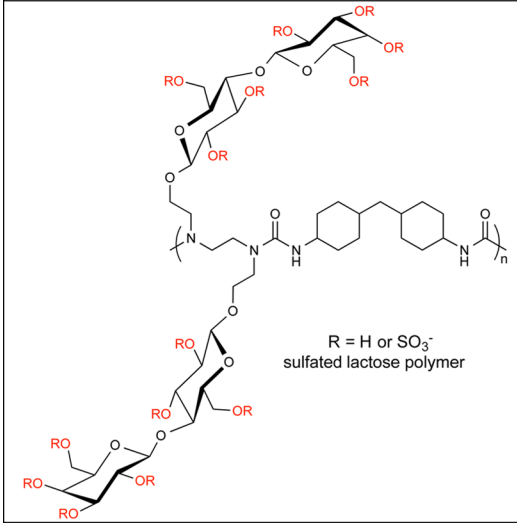
Heparin and HS proteoglycan are well-known cofactors in growth factor-induced angiogenesis, a key event in cancer growth.[82] In contrast, heparin can also act as an antiangiogenic factor depending on the concentration in HS-expressing cell lines by binding to growth factors such as FGF2 and preventing binding to FGFRs, thus, abrogating the vital formation of HS/FGF2/FGFR complex in many cell types.[83−85] Because of this, increasing interest has been focused on developing chemical structures that can turn off the angiogenic-promoting activity of heparin/HS in many diseases, including cancer. Miao et al. found that polyaromatic compounds, including RG-13577, inhibited heparin-mediated dimerization of FGF2 as well as binding of FGF2 to its receptor FGFR1, presumably by competitive binding.[86] RG-13577 completely inhibited FGF2-induced tyrosine phosphorylation of FGFR1 in cells where the heparan sulfate had been removed, compared to only partially in untreated cells, suggesting that the compound competed directly with HS for binding to FGF2. Furthermore, RG-13577 was shown to inhibit proliferation in both HS-expressing and HS-deficient cells in the presence of heparin. Microvessel formation was completely inhibited in the presence of 10−25 μg/mL of RG-13577, and this was reversible. Interestingly, the authors found that when up to 1 μg/mL of RG-13577 was incubated in the presence of 20 ng/mL of heparin, increased binding of FGF2 to its receptor was observed. Even though RG-13577 has received attention for its inhibitory activity in cells that cause angiogenesis, arteriosclerosis, glomerulosclerosis, and spinal chord inflammation,[87−91] this last data at lower concentrations suggested that RG-13577 could be further investigated as candidate for regenerative therapy in combination with heparin.

Despite the many positive features of this class of materials, there are some drawbacks to these nonsulfated polyanionic polymers including lack of control over degree of negative charges. It is well-known that location of negative charges in heparin is important for its function and this kind of control cannot be obtained with these polymers. Additionally, the polymers are often chemically ill defined due to the use of acid catalyzed condensation polymerization, which can allow for functionalization at more than one position on the phenyl ring, and also, the polymers are typically polydisperse in molecular weight. Thus, researchers continued to explore other options.

2.3. Sulfated Synthetic Glycopolymers. The heterogeneous polysaccharide backbone in heparin provides specific structural motifs that have different interactions and bioactivities. Thus, the isolation of these oligosaccharide units is desirable. Total synthesis of these structural motifs is possible, but extremely cumbersome and low yielding.[92] Even though LMWH (average MW 6 kDa) and synthetic ultralow-molecular-weight heparin (average MW 1.5 kDa) have been developed to overcome problems associated with unfraction-

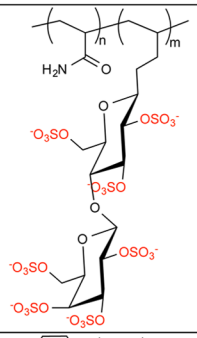
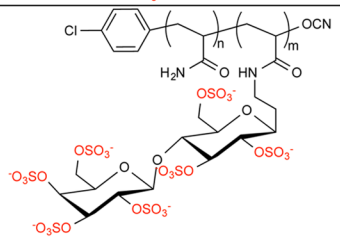
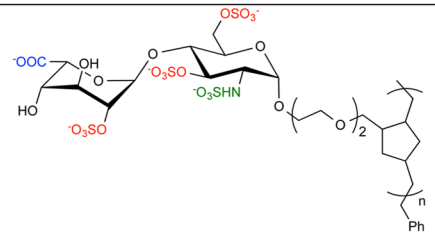
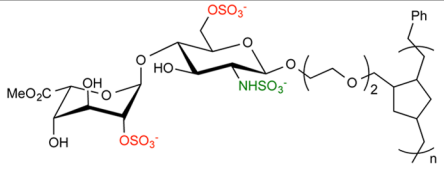
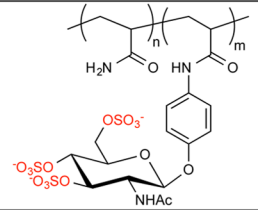
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 3. Heparin Mimicking Glycopolymers and Their Biological Applications[a]

Polymer[1]	Polym. Tech.[2]	Mn (kDa)	Đ	Sulfation	Biological Properties	Ref.
 R = H or SO_3^- poly(GEMA)-sulfate	FRP	18.0	NA	Random sulfation post polymerization with DMF/SO_3^- (1.91-3.75 sulfates per saccharide)	Increases clotting time Polymer forms insoluble complex with fibrinogen, slowing fibrin polymerization	98, 100
 R = H or SO_3^- sulfated mannose polymer	SG	22.7	1.41	Random sulfation post polymerization with SO_3^- /pyridine (3.99 sulfates per saccharide)	Increases anticoagulation time Shows blood compatibility Exhibits anti-IIa activity in heparin's therapeutic range	103, 104
 R = H or SO_3^- sulfated lactose polymer	SG	27.0	1.75	Random sulfation post polymerization with SO_3^- /pyridine (6.88 sulfates per disaccharide)	Increases anticoagulation Prolongs blood clotting Shows blood compatibility	104
 R = H or SO_3^- sulfated lactose polymer	SG	11.0	1.42	Random sulfation post polymerization with SO_3^- /pyridine (5.97 sulfates per disaccharide)	Increases coagulation time Inhibits thrombin in the presence of ATIII	105

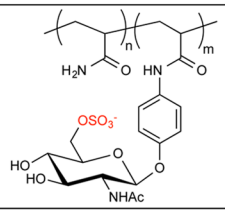
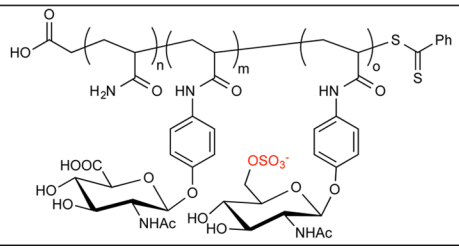
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 3. continued

Polymer[1]	Polym. Tech.[2]	Mn (kDa)	Đ	Sulfation	Biological Properties	Ref.
	FRP	70.5	1.59	Heptasulfated at all available positions	Enhances FGF2 binding to FGFR1 (although not to the same extent as heparin)	110
	CMP	9.30 and 33.4	1.46 and 1.47	Heptasulfated at all available positions	Increases anticoagulant activity (higher than dermatan sulfate, but not as high as heparin) Protects FGF2 from degradation as efficiently as heparin	110, 124
	ROMP	11.2-43.0	1.25-1.41	Tetrasulfated at specific positions	Increases anticoagulant activity Prolong APTT (for polymers ranging from 11.2 kDa – 43.0 kDa) Prolongs prothrombin time (for polymers ranging from 32.7 kDa to 43.0 kDa)	125
	ROMP	27.9	1.22	Trisulfated at specific positions	Binds strongly with RANTES Inhibits migration of L1.2 cells similarly to heparin No anticoagulant activity	126
	FRP	41.4	2.9	Trisulfated (3,4,6-sulfated)	Significantly inhibits BACE-1 activity	121

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 3. continued

Polymer[1]	Polym. Tech.[2]	Mn (kDa)	Đ	Sulfation	Biological Properties	Ref.
	FRP	340-600	1.8-2.0	Monosulfated at the 6 position (11-28% sugar incorporation)	Inhibits amyloid-beta formation	122
	RAFT	7.6	1.4	Monosulfated at the 6 position (17.4% sugar incorporation)	Inhibits amyloid-beta formation	123

[a]Other acronyms: APTT, activated partial thromboplastin time; FGF2, fibroblast growth factor 2; FGFR1, fibroblast growth factor receptor 1; BACE-1, β-site APP cleaving enzyme-1. [1]Location of sulfate is shown in red. If random all positions are highlighted in red. [2]FRP, free radical polymerization; SG, step growth; CMP, cyanoxyl-mediated polymerization; ROMP, ring opening metathesis polymerization; RAFT, reversible addition−fragmentation chain transfer polymerization; NA, not available/not reported.

ated heparin such as heparin-induced thrombocytopenia, their syntheses are also difficult and the structures are still heterogeneous.[20,93] Methods allowing for the assembly of minimal units to form multivalent heparin-mimicking glycopolymers are more straightforward and could be used for similar purposes. In fact, the minimal saccharide sequences required for heparin binding to proteins, such as ATIII, FGF1, and FGF2, are known and could be useful for rationally designing these heparin mimics.[94−96] Glycopolymers provide tunable multivalent interactions with proteins and other biological targets, and thus are a great starting point for GAG mimics. Indeed, glycopolymers with hydrocarbon backbones and pendant sulfated mono- or disaccharide units have been used as heparin mimics. Sulfated glycopolymers have also been employed as mimics of other GAGs such as chondroitin sulfate and dermatan sulfate.[29] Glycopolymers can be synthesized through multiple polymerization methods including free radical polymerization (FRP), reversible addition−fragmentation chain transfer (RAFT) polymerization, ring-opening metathesis polymerization (ROMP), and nitroxide-mediated radical polymerization (NMP); some of these methods allow for specific control over polymer structure, end group, and molecular weight.[39,97] Pendant saccharides can be modified with sulfate groups either before or after polymerization. Other types of heparin mimicking glycopolymers such as dendrimers and branched polymeric glycopolymers have been studied; however, herein we will focus on synthetic and linear sulfated glycopolymers mimicking heparin specifically, as summarized in Table 3.

Initially, heparin-mimicking glycopolymers were synthesized by first polymerizing neutral glycomonomers, followed by postpolymerization functionalization with sulfate. For example, glucosyloxyethyl methacrylate (GEMA) was polymerized via free radical polymerization to form polymers and hydrogels.[98,99] Subsequent sulfation with *N*,*N*-dimethylformamide/sulfur trioxide afforded poly(GEMA)-sulfate with degrees of sulfation ranging from 1.91 to 3.75 out of 4 available hydroxyl groups per monomer depending on the reaction time (Figure 4a).[100] Increasing doses of poly(GEMA)-sulfate and increasing degrees of sulfation resulted in prolonged coagulation of human blood similar or better than heparin. Controls were also studied. Sulfated synthetic polymers poly(styrenesulfonic acid) (pSS) and poly(vinylsulfonic acid) (pVS) were minimally effective at prolonging clotting times, while dextran sulfate (DS) (degree of sulfation = 1.0) was better than sulfated poly(GEMA). The results suggest that sulfated glycopolymers are somewhere in between the anticoagulation ability of nonsugar containing synthetic polymers and sulfated polysaccharides. The results may also bode well for use of pSS and pVS in applications where anticoagulation is not desired (discussion of these types of polymers is the focus of section 2.4). Akashi and co-workers went on to study the mechanism of poly(GEMA)-sulfate anticoagulation and found that inhibition of coagulation was due to the polymer forming an insoluble complex with fibrinogen, thus, slowing fibrin polymerization.[101] This mechanism is different than heparin, which binds to ATIII activating it to bind to thrombin and other proteases, thus, stopping the blood clotting cascade.[9] In subsequent studies, the authors found that the anticoagulation properties of poly(GEMA)-sulfate were also due to the polymer having heparin cofactor II (HCII)-mediated, but not ATIII-mediated, thrombin inhibition.[102] In addition, the polymers inhibited Tenase (Factor IXa, Factor VIIIa, calcium, and phospholipid complex), which is an activator of Factor X similar to dextran sulfate, but to a lesser extent than heparin.

In another example of postpolymerization sulfation, Ayres and co-workers synthesized sulfated glycopolymers through step growth polymerizations.[103] To do this, isopropylidene protected saccharide tetramers were synthesized with secondary amines on each end. The tetramers were then copolymerized with hexamethylene diisocyanate (HDI) via step growth polymerization to yield polymers with saccharide side chains. After polymerization, the isopropylidene protecting groups were removed and the alcohols on the sugar groups were sulfated using SO_3/pyridine to obtain heparin-mimicking polymers with approximately 3.5 sulfates per saccharide (Figure 4b). To study the effects of differing saccharide groups, Ayres and co-workers synthesized the polymers containing sulfated

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

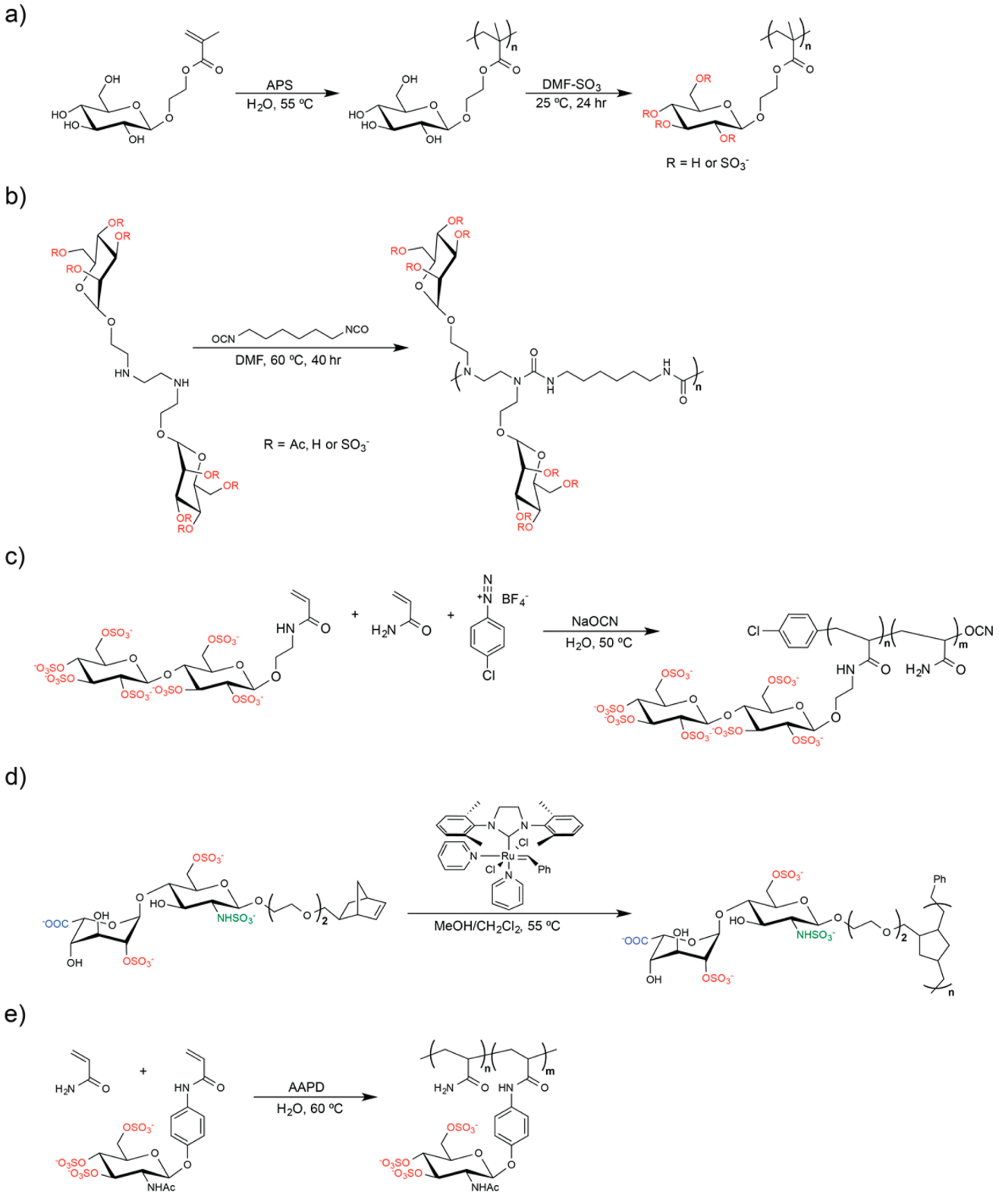


Figure 4. Syntheses of heparin mimicking glycopolymers. (a) Polyglucosyloxyethyl methacrylate-sulfate p(GEMA)-sulfate synthesized by Akashi and co-workers via free radical polymerization.[100] (b) Hexamethylene diisocyanate-based isocyanates polymerized by step growth polymerization by Ayres and co-workers.[103] (c) Heptasulfate lactose-based glycopolymers polymerized by cyanoxal mediated polymerization by Chaikof and co-workers.[111] (d) Heparin mimicking polymers by ROMP to contain pendant disaccharides with three sulfates fabricated by Hsieh-Wilson and co-workers.[118] (e) 3,4,6-Sulfoglucosamines copolymerized by free radical polymerization by Miura and co-workers.[121]

glucose, mannose, lactose, and glucosamine pendant groups. The nonsulfated polymers did not exhibit any prolonged anticoagulation activity; however, the sulfated polymers did, with sulfated mannose and lactose polymers resulting in the longest anticoagulation times, as well as the best blood compatibility.[104] To further study these polymers the authors

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

varied the isocyanate monomer to determine the effects of backbone chemistry on anticoagulation times of lactose polymers and found that backbones made with isophorone diisocyanate (IPDI) and methylene bis(4-cyclohexyl isocyanate) (HMDI) provided better anticoagulation times than polymers made with HDI or toluene 2,4-diisocyanate (TDI).[105] This was hypothesized to be due to a higher degree of flexibility in the polymer backbone of HMDI and IPDI polymers. Furthermore, the authors varied the degree of sulfation on the lactose/HMDI polymers from 3 to 15% and saw a direct correlation between increasing degree of sulfation and increasing anticoagulation times. Yet, all of the polymers were significantly lower in their ability to inhibit thrombin activity than heparin. The authors also showed they could prepare similar glycopolymers via RAFT polymerization, giving more well-defined polymers.[106]

To overcome the postpolymerization modification steps, the Chaikof group used cyanoxyl-mediated polymerization (CMP) of sulfonated glycomonomers to prepare the sulfated polymers directly.[107,108] This synthesis method also had the advantage of allowing control of the molecular weight and provided polymers with good to moderate control over molecular weight dispersity ($Đ$ between 1.1 and 1.6).[107−109] Sulfated 2-acrylamidoethyl β-lactosides were copolymerized with acrylamide yielding heptasulfate lactose-based glycopolymers (Figure 4c).[110,111] These copolymers were shown to have prolonged coagulation time compared to nonsulfated glycopolymers and homopolymers of the sulfated 2-acrylamidoethyl β-lactosides. None of the polymers were as effective as heparin; yet, the authors demonstrated that anticoagulant activity could be tuned to be increasingly heparin like by altering the ratios of acrylamide as a comonomer.[110] The copolymer was later demonstrated as a chaperon for FGF2 in protecting the protein from trypsin, acid, and heat-induced degradation.[111] The sulfated glycopolymer was able to replace heparin in facilitating binding of FGF2 to FGFRs, as well as in dimerization of FGF2 and FGFR, which are key events leading to cell proliferation in HS-deficient cell lines. To utilize these polymers in bioconjugations, Chaikof and co-workers copolymerized the sulfated lactose acrylamide monomers with acrylamide using functionalized arylamines as initiators in cyanoxyl mediated copolymerizations. This resulted in heparin mimicking polymers with varying groups at the α-chain end and cyanate groups at the ω-functionalized chain ends, and the authors demonstrated the possibility of bioconjugation by conjugating biotin-functionalized heparin mimicking polymers to streptavidin.[112] These well-defined polymers have potential applications in proteins conjugations for protein delivery and protein stabilization.[113]

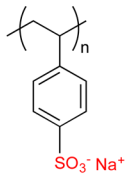
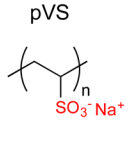
The Kiessling group has devoted much effort to preparing glycopolymers by ROMP and showed that the polymers, even sulfated ones, could be made with control.[114−116] These polymers were very effective at inhibiting L- and P-selectins. The Hsieh-Wilson group also reported the synthesis of glycopolymers via ROMP, in their case of norbornene functionalized tetrasulfated disaccharide of L-iduronic acid and glucosamine to target the anticoagulation property of heparin.[117] In their studies, they aimed to develop new heparinoids that improve upon the commercially available ultra low molecular weight heparin, Arixtra, a heparin pentasaccharide. The disaccharide moiety was rationally designed to include the iduronic acid moiety for flexibility and the glucosamine moiety due to its 3-*O*-sulfation, in order to improve the affinity of the polymer to antithrombin III. The disaccharide monomers were sulfated prior to polymerization, which allowed for homogeneous sulfation of the resulting glycopolymers. The glycopolymers were shown to have potent anticoagulant activity depending on the polymer size. For example, a minimum polymer size of 11.2 kDa (10 repeats of the disaccharide) was required to prolong the activated partial thromboplastin time (APTT) in human blood, whereas a size of at least 32.7 kDa (30 repeats) was required to alter both APTT and the prothrombin time (PT). The results underscore the importance of being able to systematically alter the molecular weight to change the number of repeat units using a synthetic polymer.

In the above-described report, the authors found an interesting effect whereby the glucosaminyl 3-*O*-sulfate was required for anticoagulant activity.[117] In a subsequent report, the same group studied the effects of varying the degree and position of the sulfate groups on the iduronic acid and glucosamine disaccharide polymerized via ROMP on the ability to mimic heparin by binding to the proinflammatory chemokine RANTES (regulated on activation, normal T cell expressed and secreted).[118] Compared to disulfated and nonsulfated disaccharides, the trisulfated epitopes with one sulfate on the iduronic acid moiety and two sulfates on the glucosamine moiety were identified to be necessary to bind to most chemokines. Glycopolymers with these pendant groups were synthesized via ROMP (Figure 4d) and were shown to bind to and inhibit the activity of RANTES similar to heparin. Importantly, these heparin-mimicking glycopolymers did not exhibit anticoagulant activity; therefore, they have potential therapeutic value in treatment of inflammation since RANTES recruits leukocytes to inflammatory sites. Because of the synthetic methodology used, the sulfate groups can be moved around the disaccharide unit and be precisely controlled. The authors noted that this feature could allow for the preparation of other heparin mimics with activity relevant to diseases such atherosclerosis, cancer, and autoimmune disorders. The same group extended this ROMP based approach to synthesize mimics of other GAGs such as chondroitin sulfate.[119,120]

Other researchers investigated both location and number of sulfates, as well as the spacing between sugars as a factor. For example, Miura and co-workers synthesized heparin mimics containing glucosamine saccharides and acrylamide by free radical polymerization to bind to heparin binding proteins such as ß-Secretase (BACE-1) involved in Alzheimer's disease (AD). By varying the sulfation pattern on the pendant glucosamines from 3-sulfo, 4-sulfo, 6-sulfo and 3,4,6-sulfo, the authors found that polymers containing 3,4,6-sulfo pendant sugars significantly inhibited BACE-1 activity (a protease known to be involved in the pathogenesis of AD), while the 6-sulfo modestly inhibited (Figure 4e).[121] It was later shown that polymers containing 6-sulfo pendant sugars reduced amyloid fibril formation.[122] Interestingly, polymers with modest sugar contents inhibited amyloid β fibril formation more effectively than polymers with high sugar contents. This could be due to spacing required in the sulfation pattern to obtain appropriate structure and binding relating to multivalency, and the flexibility of the polymer backbone. Miura and co-workers have also polymerized similar polymers through RAFT[123] polymerization to obtain more well-defined polymers. This allowed the group to study the influence of degree of polymerization (molecular weight) on amyloid β fibril inhibition. Further, the group showed the strongest inhibition

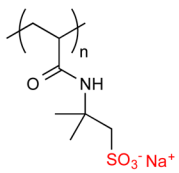
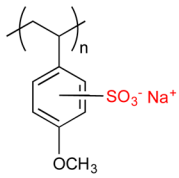
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 4. Heparin Mimicking Polysulfonated Compounds and Their Biological Applications[a]

Polymer	Polym Tech.[1]	Mn (kDa)	Đ	Degree of sulfation	Biological Properties	Ref.
pSS 	FRP	70.0	NA	100% of monomers contain one sulfonate	Inhibits FGF2 binding to FGFRs and HSPGs on endothelial GM 7373 cells Inhibits mitogenic activity of FGF2 in GM 7373 cells Inhibits FGF2 mediated cell-cell adhesion of HSPG-deficient CHO cells as effectively as heparin Protects FGF2 from proteolytic degradation Inhibits FGF2-induced cell proliferation in endothelial cells Angiogenesis inhibitor	127, 128
pVS 	FRP	2.0	NA	100% of monomers contain one sulfonate	Inhibits FGF2 binding to FGFRs and HSPGs on endothelial GM 7373 cells Inhibits FGF2 mediated cell-cell adhesion of HSPG-deficient CHO cells Protects FGF2 from proteolytic cleavage No antiangiogenic activity in a rat aorta-ring assay or CAM assay	127, 128

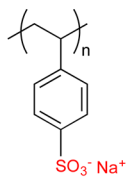
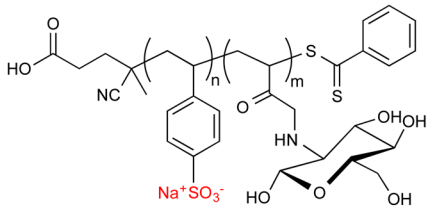
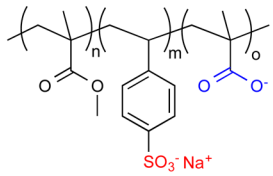
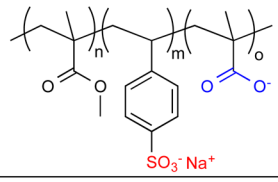
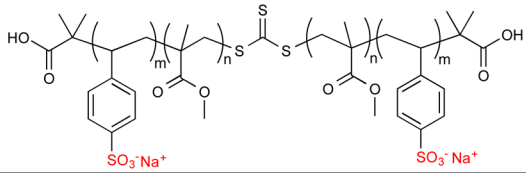
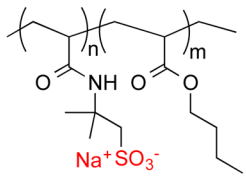
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 4. continued

pAMPS 	FRP	7.0-10.0	NA	100% of monomers contain one sulfonate	Inhibits FGF2 binding to FGFRs and HSPGs on endothelial GM 7373 cells Protects FGF2 from proteolytic degradation more efficiently than other sulfonated polymers Inhibits FGF2 mediated cell-cell adhesion of HSPG-deficient CHO cells Inhibits mitogenic activity of FGF2 in GM 7373 cells Inhibits FGF2-induced cell proliferation in endothelial cells Angiogenesis inhibitor	127, 128
pAPS 	FRP	9.0-11.0	NA	100% of monomers contain one sulfonate	Inhibits FGF2 binding to FGFRs and HSPGs on endothelial GM 7373 cells Inhibits FGF2 mediated cell-cell adhesion of HSPG-deficient CHO cells Protects FGF2 from proteolytic cleavage Inhibits FGF2-induced cell proliferation in endothelial cells Angiogenesis inhibitor	127, 128

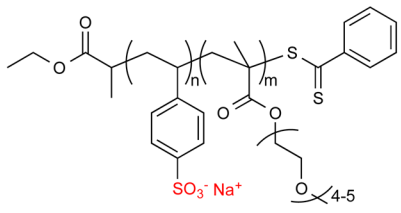
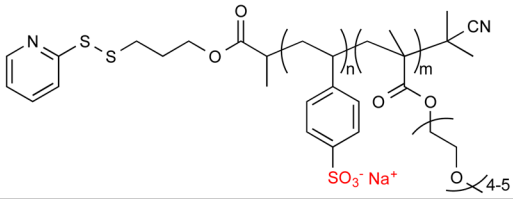
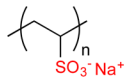
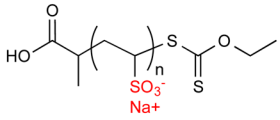
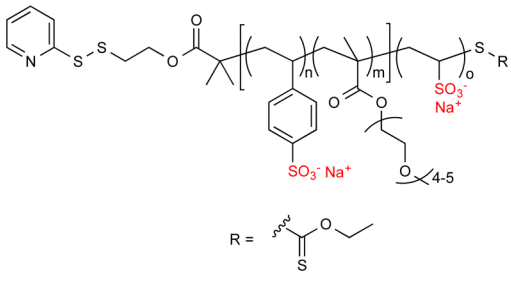
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 4. continued

pSS 	FRP	70.0	3-5	75% of monomers sulfonated	Promotes FGF2 induced myogenic differentiation and myotube formation in muscle progenitor cells	132
p(SS-*co*-MAG) 	RAFT	8.0-9.0	1.17-1.20	35-64%	Promotes fibroblast proliferation better than heparin Stimulates mESC proliferation better than heparin Promotes neural differentiation of mESCs	131
p(MMA-*co*-MA-*co*-SS) 	FRP	NA	NA	2-7%	Interacts with fibronectin to inhibit adhesion of bacteria onto surfaces	133, 134
p(MMA-*co*-MA-*co*-SS) 	FRP	M_v = 75.2	NA	7.5% SO_3^- and 7.5% COO^-	Polymer films absorb fibronectin and inhibit fibroblast adhesion and proliferation	135
p(MMA-*co*-SS) 	RAFT	27.3-31.0	1.69-1.75	20-80% SO_3^-	Increases APTT with increasing $SO3^-$ incorporation	136
p(AMPS-*co*-BA) 	FRP	23.3-72.5	1.6-2.6	25-75%	Inhibits FGF1 induced mitogenic activity of fibroblasts (higher AMPS incorporation leads to greater inhibition at lower concentration)	137

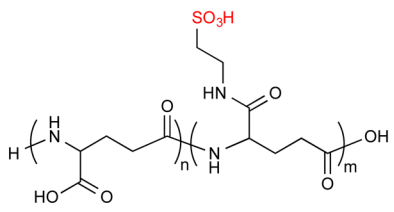
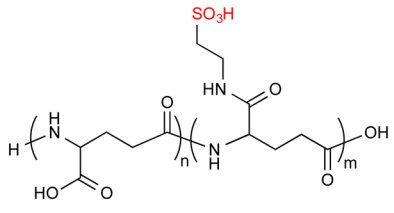
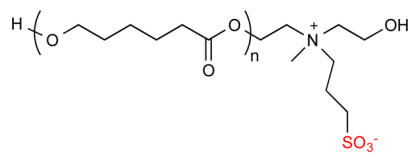
DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 4. continued

p(SS-*co*-PEGMA) 	RAFT	24.0	1.17	69%	Strongly binds FGF2 and VEGF to high salt concentrations Binds FGF2 when immobilized on surfaces allowing endothelial cell spreading	138
p(SS-*co*-PEGMA) 	RAFT	26.1	1.16	68%	When conjugated to FGF2, polymer helps stabilize FGF2 to trypsin degradation, heat, storage and acidic pH	139
pVS 	FRP	6.4	1.21	100%	Facilitates binding of FGF2 to FGFR Promotes FGF2-induced cell proliferation	140
pVS 	RAFT	6.6-80.3	1.14-1.40	100%	Promotes FGF2-induced cell proliferation in HSPG deficient cell lines	140
p(SS-*co*-PEGMA)-*b*-VS 	RAFT	57.2	1.46	86.5%	Facilitates binding of FGF2 to FGFR Conjugate promotes FGF2 induced endothelial cell migration and cord-like structure formation Conjugate stabilizes FGF2 from denaturation upon storage at room temperature or refrigeration	141

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 4. continued

pGA-sulfonate 	NA	1,230	1.20	13-81%	Increases blood coagulation time with increasing degree of sulfonation Degradable at pH 7.4 at 80 °C over 48 hours	142
pGA-sulfonate 	NA	310	1.20	72%	Promotes FGF2 induced fibroblast growth in cells lacking native HSPG (better than exogenous heparin) Slightly stabilizes FGF2 from heat and acidic environments	143
pCL-APS 	ROP	3.02-10.73	1.09-1.43	NA	Increased blood coagulation times Reduces platelet adhesion on surfaces coated with polymer	144

[a]Other acronyms: mESC, mouse embryonic stem cell; VEGF, vascular endothelial growth factor; HSPG, heparin sulfate proteoglycan; CHO cell, Chinese hamster ovarian cell; CAM, chick chorioallantoic membrane; FGF2, fibroblast growth factor-2; FGF1, fibroblast growth factor-1; FGFR, fibroblast growth factor receptor; APTT, activated partial thromboplastin time; VEGF, vascular endothelial growth factor. [1]FRP, free radical polymerization; RAFT, reversible addition−fragmentation chain transfer polymerization; ROP, ring-opening polymerization; NA, not available/not reported.

was obtained from a terpolymer of acrylamide, 6-sulfo-glucosamine monomer, and glucuronic acid.

In many of the above examples, controlled polymerizations were utilized, resulting in polymers with well-defined molecular weight dispersities. This is an important advance allowing one to control and target the molecular weight. The results also nicely showed that by taking components of heparin and controlling the sugar identity and location of sulfates, the heterogeneity in biological activity could be reduced, thereby targeting particular biological paths over others. Great progress has been made in this regard by Hsieh-Wilson, and further use of minimal oligosaccharide sequences known to be required for binding to particular proteins would be advantageous to the field. In addition, the results showed the importance of spacing the sugars for certain applications. However, a drawback of these polymers is the need to prepare sulfated glycomonomers. Sugar chemistry can be tedious and the sulfate groups can still be desulfated under acidic conditions. Thus, some researchers have explored nonsaccharide synthetic polymers that mimic the negative charge of the sulfate, rather than sugar structure itself.

2.4. Polysulfonated Compounds. Polysulfonated polymers are fully synthetic polymers that make up another large class of heparin-mimicking polymers (Table 4). These often rely on the negative charge from sulfonate groups on side chains of polymers for their heparin mimicking properties and do not contain any sugars. Liekens and co-workers studied the salt forms of various sulfonated polymers (Figure 5a) including poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (pAMPS), poly(anetholesulfonic acid) (pAS), poly(4-styrenesulfonic acid) (pSS), and poly(vinylsulfonic acid) (pVS) for their ability to inhibit cell proliferation similar to heparin at higher concentrations.[127] The polymers were prepared by free radical polymerization and, thus, were polydisperse. pAMPS, pAS, and pSS were found to have potent antiproliferative activity in fetal bovine aortic endothelial GM7373 cells by inhibiting binding of FGF2 and antiangiogenic effects in an in vitro rat aorta ring assay.[128] Therefore, the polymers had promising therapeutic value in treating angiogenesis-promoted cancers. All of the polymers protected FGF2 to proteolytic enzymes, but none protected against heat degradation. For the former, the protein was incubated with polymers as excipients in a trypsin digestion assay and, in the presence of sulfonated polymers, showed an increase in undigested FGF2, with pSS and pAMPS performing better than pAS or pVS. To understand the interactions with

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

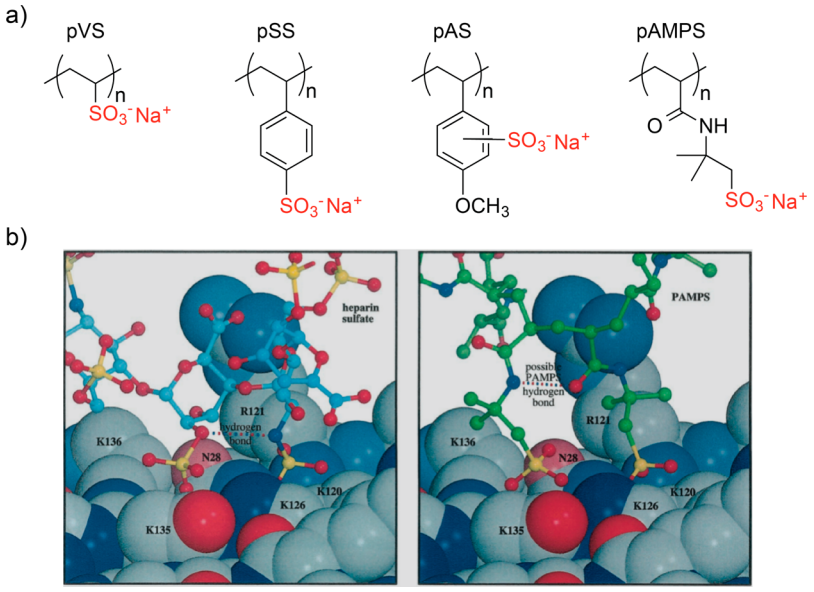


Figure 5. Heparin mimicking sulfonated polymers. (a) Chemical structures of the various sulfonated polymers tested by Liekens et. al and (b) molecular modeling showing heparin and pAMPS interacting with the heparin binding domain of FGF2. Reprinted with permission from ref 127. Copyright 1999 American Society for Pharmacology and Experimental Therapeutics.

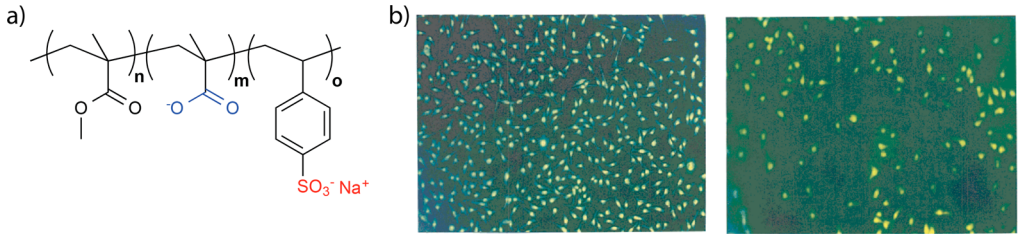


Figure 6. (a) Chemical structure of heparin mimicking terpolymers polymerized by Migonney and co-workers and (b) their ability to inhibit fibroblast adhesion (right) vs control (left). Reprinted with permission from ref 135. Copyright 2002 American Chemical Society.

FGF2, computational molecular modeling was performed and showed that the sulfonate groups in pAMPS are able to adopt a low-energy conformation, the polymer is likely helical, to interact with the heparin-binding domain of FGF2 (Figure 5b).[127] Of the sulfonated polymers that were tested, pSS was found to be the most effective in its ability to inhibit cell−cell adhesion and most potent in stabilization of FGF2. In later studies performed by Varghese and co-workers, pSS was tested for its ability to mimic heparin and effect FGF2 signaling in muscle progenitor cells, where addition of heparin promotes myogenesis. When incubated with muscle progenitor cells, the polymer facilitated an increase in myogenic differentiation and myotube formation similar to heparin.[129] Later studies showed that the hydrophobicity of polyanions also effects the complexation between proteins and sulfated polymers such as pSS.[130]

Due to the promising biological activities of these sulfated/sulfonated polymers, work was done on determining the effect of incorporating these monomers in copolymer systems. Considering what was discussed in the glycopolymer section above (section 2.3), spacing the sulfonate groups may be an important factor. Rather than synthesizing a simple polysulfonated polymer, Chen and co-workers developed a new heparin-mimicking polymer that incorporated both nonsulfated glycomonomers for saccharide incorporation and SS for sulfonate incorporation. SS was copolymerized with 2-methacrylamido glucopyranose (MAG) by RAFT polymerization to yield polymers p(SS-*co*-MAG) between 8 and 9 kDa with SS incorporation ranging from 35 to 64%.[131] The polymers were well-defined, with molecular weight dispersities between 1.17 and 1.20. They found that when cultured with FGF2, copolymers with 50% SS incorporation promoted cell proliferation in fibroblasts better than heparin. Additionally, the copolymer exhibited higher proliferation of mouse embryonic stem cells (mESCs) after 20 days better than either pSS or the MAG homopolymer, suggesting that both components are important for the increase in activity; the mESCs also proliferated better with the copolymer than heparin itself. The authors also looked at the ability of the polymers to promote neural differentiation in mESCs and found that pSS performed the same as heparin, pMAG did not promote neural differentiation, and p(SS-*co*-MAG) performed significantly better than pSS or heparin. These results suggest that there are synergistic effects between the sulfonate units and the sugar units in the copolymer and that both contribute to the high biological activity of this new polymer.

The Migonney group investigated the incorporation of SS into terpolymers for use in materials that require heparin or heparin mimics. They synthesized polymers by FRP using methyl methacrylate (MMA), methacrylic acid (MA) and sodium styrenesulfonate (SS) to obtain terpolymers containing both sulfonate and carboxylate moieties (Figure 6a), thus, incorporating the various components of heparin including hydrophobic domains.[133] Heparin-coated surfaces are known to

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

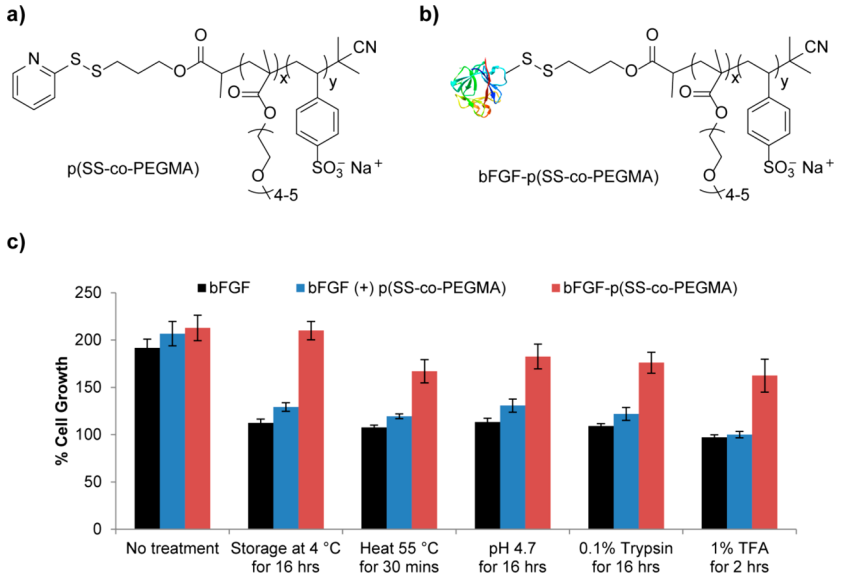


Figure 7. Polysulfonated heparin-mimicking polymer, p(SS-*co*-PEGMA) and the stability profile of its conjugate to FGF2. (a) Chemical structure of the polymer. (b) Structure of the FGF2-p(SS-*co*-PEGMA) conjugate. (c) Stability of the conjugate against various stressors, tested on human dermal fibroblast cells for stimulated cell proliferation. Modified with permission from ref 139. Copyright 2013 Nature Publishing Group.

be antibacterial, and the authors found the terpolymer also inhibited *Staphylococcus aureus* bacterial adhesion.[134] It was found that the ratio of carboxylates to total negative charge mattered, and that values between 0.28 and 0.8 inhibited the bacteria from attaching. To further study the heparin mimicking properties of these polymers, the group studied the ability of the polymers to inhibit fibroblast cell growth on films of the polymers (Figure 6b). They found that polymers containing 15% ionic groups (specifically 7.5% sulfonate and 7.5% carboxylate) had the highest inhibitory effects and that the total number of ionic groups could be altered as long as the number of carboxylates to sulfonates was equal.[135] Zhao and co-workers prepared similar polymers changing methyl acrylic acid for acrylic acid (AA) but varied the comonomers to yield p(SS-*co*-MMA), p(AA-*co*-MMA), and p(SS-*co*-AA-*co*-MMA) via RAFT polymerization. However, instead of polymerizing styrenesulfonate directly, the authors copolymerized with styrene, and then sulfonated the styrene moieties post polymerization with concentrated sulfuric acid. They found that increasing incorporation of AA or SS in the polymers increased coagulation time, and free polymers in solution prolonged coagulation time at 0.5 mg polymer/0.1 mL platelet-poor plasma (PPP) and the solutions were incoagulable at 2 mg/0.1 mL polymer/PPP.[136] The polymers were not directly compared to heparin in this assay.

Copolymers of pAMPS have also been synthesized. Aguilar and co-workers copolymerized AMPS with either vinylpyrrolidone (VP) or butyl acrylate (BA) by FRP to yield p(AMPS-*co*-VP) and p(AMPS-*co*-BA). In this study they looked at the ability of the polymers to inhibit heparin binding growth factor-induced cell mitogenic activity.[137] They found that polymers containing BA inhibited FGF1 stimulated mitogenic activity of mouse fibroblasts (Balb/c 3T3); however, none of the VP containing polymers had an effect on the mitogenic activity. Not surprising, the sulfonated concentration in the copolymers was the important factor with polymers containing a larger amount of AMPS (50% or greater) inhibiting at lower concentrations. The authors wrote that the differences in activity between VP and BA were likely due to the reactivity ratios of the two monomers with AMPS. Specifically, that BA/AMPS copolymers had BA-rich and AMPS-rich sequences allowing for a helical conformation of the pAMPS to interact with the FGF1, while the VP system had an alternating sequence which prevented the helical type structural formation. pAMPS has also been used in the fabrication of core−shell particles by emulsion polymerization of butyl methacrylate (BMA) and studied for retention and release of heparin binding growth factors important in wound healing. Rimmer and co-workers polymerized AMPS by RAFT to give both linear and hyperbranched core−shells and chain extended with BMA during the emulsion polymerization.[145] Interestingly, they found that the release of VEGF from linear-grafted shells was slower in the first 200 h compared to the branched shells; but after 200 h, the release from the branched shells stopped, while release from the linear shells continued out to at least 800 h. For PDGF, the rate of release from the linear shells remained slower than from branched throughout the entire 800 h.

Recently, we reported the synthesis of poly(sodium 4-styrenesulfonate-*co*-poly(ethylene glycol) methyl ether methacrylate) (p(SS-*co*-PEGMA)) via RAFT polymerization (Figure 7a) and showed that the polymer bound to FGF2 to high salt concentrations and in cellular media.[146,147] Furthermore, the polymer immobilized on surfaces was able to present FGF2 in a manner that could be utilized by human endothelial cells, enlarging their area compared to integrin-binding peptide presenting surfaces alone. In a subsequent study, this heparin mimicking polymer was conjugated to FGF2 through a disulfide linkage, resulting in a highly stable protein-heparin-mimicking polymer conjugate, FGF2-p(SS-*co*-PEGMA) (Figure 7b).[139] Heparin is a natural stabilizer for many heparin-binding proteins including FGF2, which is typically very unstable and denatures quickly.[148] FGF2-p(SS-*co*-PEGMA) was demonstrated to be stable to a variety of environmentally and therapeutically relevant stressors such as heat, mild and harsh acidic conditions, storage and proteolytic degradation (Figure 7c). The conjugate also induced proliferation of human dermal fibroblast cells, a critical cell line in wound healing, as effectively

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

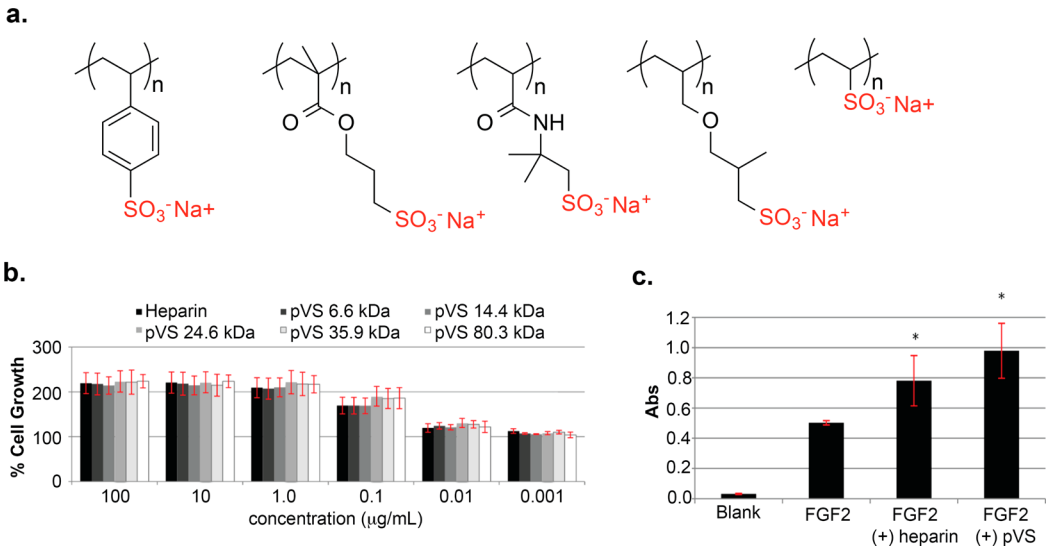


Figure 8. Screening study of various sulfonated polymers. (a) Chemical structure of heparin mimicking polymers polymerization by free radical polymerization. (b) Cell proliferation studies in BaF3-FR1C cells showing the heparin mimicking nature of pVS. (c) FGFR based ELISA assay showing pVS increasing the binding of FGF2 to FGFR compared to heparin. Reprinted with permission from ref 140. Copyright 2015 American Chemical Society.

as the native protein. Interestingly, neither the polymer nor conjugate induced proliferation in cells lacking natural HS, suggesting that, in contrast to heparin, the polymer at the molecular weight explored did not bind to the receptor to help induce receptor mediated signaling.

To search for a new heparin-mimicking polymer that would stabilize FGF2 and activate the cell receptors, we screened a variety of sulfated and sulfonated polymers, including poly-(potassium 3-sulfopropyl methacrylate) (pSPM), poly(sodium 1-allyloxy-2 hydroxypropyl sulfonate) (pAHPS), pSS, pVS, and pAMPS (Figure 8a) using a cell line that lacks native HS.[140] The polymers were added and cell proliferation in the presence of FGF2 was utilized as a readout (compared to cells with no FGF2 or FGF2 and no heparin as controls). In this assay it was found that pVS-activated FGF2-induced cell proliferation at all concentrations tested. Figure 8b shows the results for different molecular weight pVS compared to heparin. The results were verified by an ELISA based receptor assay (Figure 8c). Using these assays, it was determined that pVS enhanced FGF2 receptor binding when added as an excipient to the same extent as heparin, meaning that the polymer facilitated FGF2 binding to its receptor. This effect is likely due to the polymer binding to both the protein and receptor in the active tetrameric complex as does heparin. This was the first fully synthetic polymer reported to be as good as heparin in facilitating FGF2 binding to its receptor and subsequent activation. In a later study it was found that, by combining pVS in a block copolymer with p(SS-*co*-PEGMA), a new block copolymer conjugate was fabricated that both stabilized FGF2 and facilitated receptor binding, thus, leading to an increase in endothelial cell migration and tubulogenesis compared to unmodified FGF2.[141]

The backbone of all the polymers mentioned above are hydrocarbon based and not degradable. Additional backbones were also studied that completely degrade over time, which has advantages for many different biomedical applications of heparin mimics. Akashi and co-workers utilized a poly(glutamic acid) (pGA) polymer backbone for biological degradation to form heparin mimicking polymers.[142] The group functionalized pGA with sulfonate groups by reacting the amine of taurine with the carboxylic acids along the polymer chain (Figure 9a).

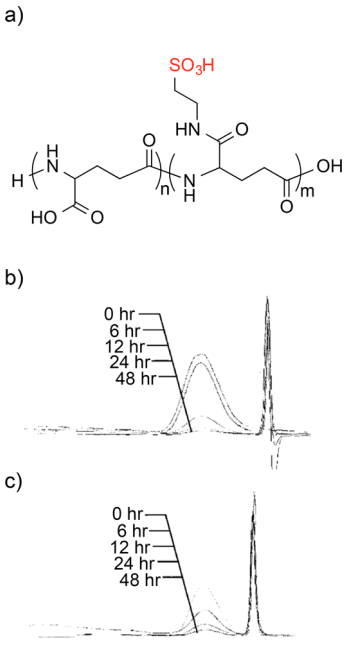
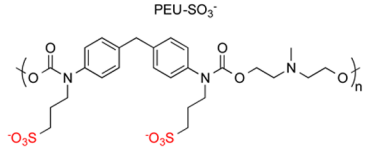
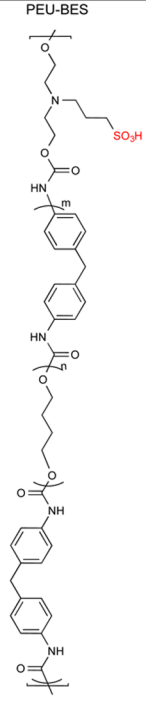


Figure 9. (a) Chemical structure of biodegradable sulfonated poly(glutamic acid) and GPC traces showing degradation of (b) nonsulfonated polyglutamic acid and (c) sulfonated poly(glutamic acid). Reprinted with permission from ref 142. Copyright 2002 American Chemical Society.

They found that increasing polymer concentration and degree of sulfation both impacted blood clotting by increasing coagulation time. While coagulation time was increased when compared to nonsulfonated pGA, the clotting time was significantly less than heparin. pGA-sulfonate was compared to well-known heparin mimics, pVS and pSS, and delayed clotting longer than both of these polymers, while the clotting time was much less than dextran sulfate. The authors also

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

Table 5. Heparin-Mimicking Ionomers and Their Biological Applications

Polymer	Polym Tech.	Mn (kDa)	Đ	Degree of sulfation	Biological Properties	References
PEU-SO_3^- 	Step Growth	NA	NA	15-20%	Increased fibrinogen deposition compared to surfaces not coated with polymer Decreased platelet deposition on surfaces coated with polymer Inhibited thrombin Inhibited fibrin assembly	152,153
PEU-BES 	Step Growth	32.0-36.0	1.3-1.4	NA	Increased fibrinogen adsorption	154,155

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

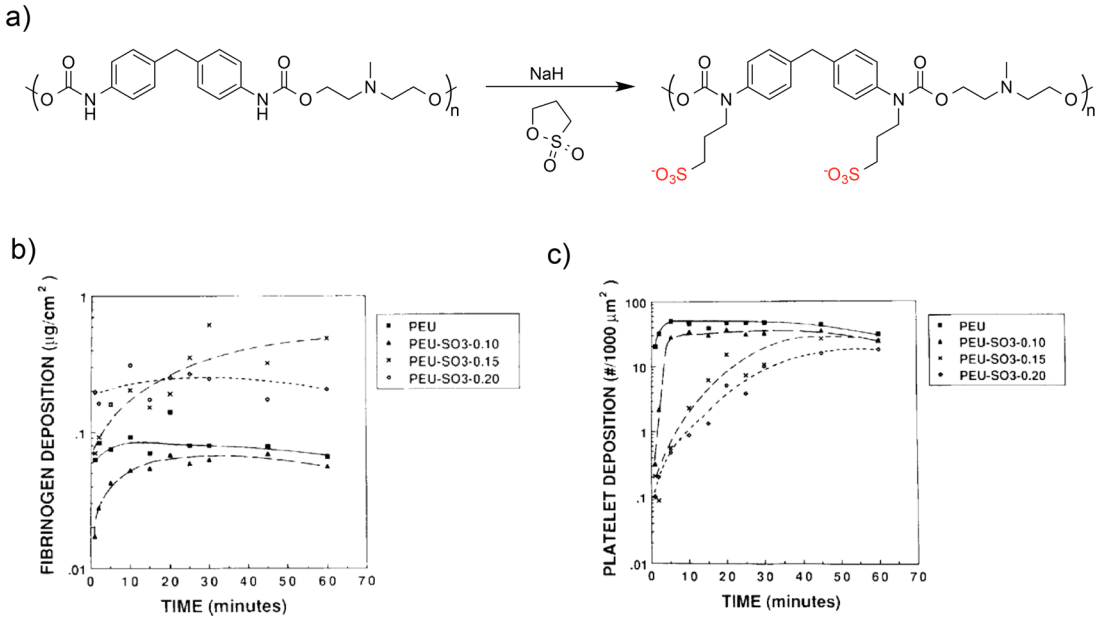


Figure 10. Sulfonated urethane ionomers used in development of new anticoagulants. (a) Chemical structure of polyurethane ionomers. (b) Degree of fibrinogen deposition on modified polyurethane materials showing an increase in fibrinogen accumulation with increasing degree of sulfonation. (c) Degree of platelet deposition on modified polyurethane materials showing a decrease in platelet accumulation with increasing degree of sulfonation. Reprinted with permission from ref 152. Copyright 1989 John Wiley and Sons.

showed that pGA-sulfonate was degradable by studying GPC over 48 h in phosphate buffer at pH 7.4 at 80 °C (Figure 9b,c). In a follow-up study, pGA with varying degrees of sulfonation were analyzed in an FGF-2 dependent mouse fibroblast proliferation assay, and pGA with 72% of the carboxyl groups converted to sulfonates provided the maximal FGF2-induced proliferation, greater cell number compared to higher and lower percent sulfonation.[143] The 72% pGA sulfonate also increased cell proliferation above that of pSS, pVS and heparin itself. Additionally, pGA-sulfonate was able to slightly protect FGF2 from heat and acidic environments, but not to the extent of heparin. Molecular modeling studies showed that pGA-sulfonate with 72% sulfonation provided polymers with sulfonate groups in the right location to bind to the heparin binding site. Akashi and co-workers have gone on to use these polymers in hydrogels for growth factor delivery.[149,150]

Another biodegradable backbone utilized by Luo and co-workers was poly(caprolactone).[144] Polycaprolactone containing *N*,*N*-bis(2-hydroxyethyl) methylamine ammonium propanesulfonate (MDEAPS) was made via ring opening polymerization (ROP) to yield pCL-APS. First 1,3-propane sultone was opened with *N*,*N*-bis(2-hydroxyethyl) methylamine to give MDEAPS. Caprolactone was then polymerized in the presence of MDEAPS to give pCL polymers containing sulfobetaine. Reduced platelet adhesion was observed on surfaces with the pCL-sulfobetaine polymer, and the polymers showed prolonged coagulation times compared to analogous unsulfonated polymers.

In all of the above examples, stable sulfonated polymers were utilized. The polymers showed a wide range of activities including inhibiting and promoting cell proliferation and stabilizing growth factors. By utilizing the synthetic polymers, the amount and presentation of the negative charge could be altered, for example, by exploiting comonomers with different reactivity ratios. The results allow the community to start to understand the differences in biological activity depending on how the sulfonate is presented and what the backbone and side chain linkages are. Another advantage of this approach is that in many instances the monomers are commercially available. Employing controlled radical polymerization with specially designed chain transfer agents also allows for the synthesis of well-defined and near monodisperse polymers with targeted molecular weights. Furthermore, the polymers contain end groups that can easily be modified or covalently attached to proteins, surfaces and other materials. However, a disadvantage of sulfonated synthetic polymers is that in many instances the activities were lower than that of heparin or sulfated polysaccharides such as dextran sulfate or the glycopolymers.

2.5. Polyionomers as Heparin Mimics. Ionomers, which are copolymers consisting of both neutral and ionic monomers with an ionic incorporation of less than 15%, have also been studied for use as heparin mimics. Most of the work done on heparin mimicking ionomers has been focused on polyurethanes. Polyurethanes have been widely used as materials in biomedical devices such as stents and catheters because of their biocompatibility. To improve upon their blood compatibility, research on studying the effects of sulfonating polyurethane has been undertaken. While much work has been done on insoluble polyurethanes,[151] some groups have studied soluble forms to learn more about their heparin like properties. Heparin containing urethanes have been reviewed elsewhere;[151] here we focus on heparin-mimicking polyurethanes that are sulfonated and soluble (Table 5).

Sulfonating polyurethanes can be accomplished by post-polymerization modification of the backbone with sulfonate side chains, or by polymerizing sulfonated segments on active isocyanate end groups after polymerization. For example, Grasel and Cooper synthesized polyurethanes from methylene bis(*p*-phenyl isocyanate) (MDI), poly(tetra-methylene oxide) (PTMO), and 1,4-butanediol.[152] After polymerization, the polyurethanes were reacted with NaH to remove the urethane hydrogen and then subsequent reaction with propane sultone

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

afforded the sulfonated urethane (Figure 10a). The authors used these polymers to coat polyethylene and studied the blood compatibility in a canine ex vivo model. Tubes coated with sulfonated urethane showed a decrease in platelet deposition and an increase in fibrinogen deposition compared to the unsulfonated polyurethane (Figure 10b,c). To further study the properties of these polymers, Cooper and co-workers studied the mechanism by which these soluble polymers prolong blood coagulation.[153] They found that sulfated polyurethanes inhibited thrombin, likely via interaction with antithrombin III as does heparin. It was also found that the polymers directly inhibit fibrin assembly, rather than complexing free calcium or interfering with factor XIIIa.

Sulfonated polyurethanes can also be synthesized by using a sulfonated chain extender after polymerization. Brash and co-workers synthesized sulfonated urethanes by polymerizing MDI and poly(propylene glycol) (PPO) to yield urethanes with isocyanate end groups. The sulfonated segments were then added by reacting with 4,4′-diamino-2,2′-biphenyldisulfonicacid disodium or dipotassium salt (BDDS).[154] They found that thrombin times increased (i.e., plasma coagulation time was delayed) with increasing sulfonate content.[155] Kuo and co-workers also synthesized heparin mimicking polyurethanes by adding a chain extender containing either sulfonate or carboxylate groups.[156] When anions were incorporated into the polyurethanes there was less platelet adhesion than on polyurethane alone; however, the carboxylate chain extenders provided less platelet adhesion than the sulfonated ones.

Another example of heparin mimicking ionomers have been developed by Yui and co-workers consisting of sulfonated polyrotaxanes. To synthesize heparin mimicking polyrotaxanes the authors first fabricated polyrotaxanes consisting of α-cyclodextrin around PEG (in a pluronic triblock copolymer) and then reacted the sodium salt with 1,3-propane sultone to form the sulfonated α-cyclodextrins.[157] They found that the sulfonated polyrotaxanes improved anticoagulation compared to the unsulfated version, which in turn was better than just the pluronic. To further study these polymers, the authors synthesized polyrotaxanes with both sulfonate and carboxylate groups.[158] Carboxyethyl ester groups were conjugated to the α-cyclodextrins followed by taurine to afford mixed sulfonated and carboxylated polyrotaxanes. Importantly, the authors found that lower percentages of threaded α-cyclodextrin were better, likely because of charge spacing; for these polymers there was a maximal SO_3^-/COO^- ratio between 2 and 3. Shorter polymers also gave better anticoagulation properties. In a more recent study Yui and co-workers found that their sulfonated polyrotaxanes increased osteogenic differentiation when incubated with bone morphogenic protein-2 (BMP-2); this is similar to the positive effect of heparin complexed with this protein.[159] This class of sulfonated polyionomers provides new and interesting architectures with easy variability for use as heparin mimics.

The development of polyionomers has advantages in that the polymers are wholly synthetic. In addition, ionomers with low incorporation of sulfonate can retain materials properties of the parent polymer, while imparting heparin-like activity. This means the materials can be used as biomedical devices (for example medical tubing), rather than serving as coatings. Thus, ionomers with post polymerization modification allows for the polymers to be easily functionalized after polymerization with widely varied structures. However, the polymerization technique used does not provide control over polymer molecular weight and post polymerization modification does not allow for easy control over the placement of negative charges.

3. CONCLUSIONS AND FUTURE PERSPECTIVES

Herein, we have summarized the synthesis and application of heparin mimicking polymers and showed that they can be important in a wide range of applications, including protein protection, promoting cell differentiation, inhibiting cell adhesion, and anticoagulant activity. Polymeric synthetic mimics have addressed several disadvantages of heparin, including its heterogeneous structure. Recent advances in heparin-mimicking polymers offer opportunities for development of more structurally defined molecules that can target a specific biological interaction such as anticoagulant activity only. This can be useful when no cross-reactivity and low side reactions in vivo are desired. In addition, several examples have been shown to stabilize important heparin binding proteins to stressors that normally inactivate them. The ability of heparin-mimics to stabilize proteins opens a window for new therapeutics, including new wound dressings produced by various techniques, such as electrospinning. Stabilization would also allow for administration of protein therapeutics through additional avenues and for easier storage of the drugs.

The synthetic polymers have the advantage compared to semisynthetic or heparin itself of being stable, although desulfation of sulfates can still occur in vivo. However, there are many applications where a persistent heparin would not be desirable. While there are many advantages to synthetic heparin mimics, FDA approval is often a lengthy process and extensive testing on safety and efficacy need to be undertaken for human use. Future directions that could improve the outlook for FDA approval include incorporation of degradable moieties in the backbones of heparin-mimicking polymers. A few sulfonated degradable polymers have been synthesized. For those nondegradable sulfated and sulfonated polymers prepared by radical polymerizations, this could be undertaken by incorporating cyclic ketene acetals into the polymerization mixtures, thus, providing points of degradation.[160−165] Use of heparin mimics to even further modulate the activity of native proteins, for example, to produce superagonists, would be significant in the fields of tissue regeneration, cell replacement therapies, and wound healing. Such approaches would be of value in further enhancing therapeutic efficacy and reducing side effects by fine-tuning the heparin binding motif and other molecular characteristics.

The examples thus far have shown that the spacing, sulfation presentation, addition of carboxylates or other chemical moieties, the molecular weight, the comonomers (i.e., reactivity ratios), and the backbone identity with regard to flexibility and degradability are all going to be important factors. Much work has been done to elucidate the minimal saccharide sequences required for heparin binding, which has helped inform researchers on oligosaccharides useful for glycopolymer mimics. However, little is known about the minimum units needed in synthetic heparin mimicking polymers. Despite a lot work in this area, there are still no clear rules or extensive structure−property relationships on how the parameters above relate to resultant biological properties. Further systematic studies on type and presentation of sulfate and sulfonated groups and interactions with proteins coupled with computation and docking studies would be invaluable in this respect. Although this has been done on smaller scale, large-scale studies with

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

many of these variables would be very useful. It could allow one to design a polymer that could interact with a specific domain of a heparin binding protein, thus, targeting a specific interaction/biological pathway. This could lead to further design optimization and target-based study versus empirical testing, which will be important to advance the field. In addition, the development of a broader range of heparin- and heparin-mimicking-based materials, tapping into new developments in polymer synthesis, such as precision control, would certainly further expand impact of these materials in the treatment of various diseases.

AUTHOR INFORMATION

Corresponding Author

*E-mail maynard@chem.ucla.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Health (NIH R01EB013674). S.J.P. thanks the NIH Chemistry Biology Interface Training Fellowship (T32 GM 008496) and UCLA Graduate Division for funding.

REFERENCES

(1) Sasisekharan, R.; Venkataraman, G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 626−631.

(2) Hileman, R. E.; Fromm, J. R.; Weiler, J. M.; Linhardt, R. J. *BioEssays* **1998**, *20*, 156−167.

(3) Hirsh, J.; Warkentin, T. E.; Shaughnessy, S. G.; Anand, S. S.; Halperin, J. L.; Raschke, R.; Granger, C.; Ohman, E. M.; Dalen, J. E. *Chest* **2001**, *119*, 64S−95S.

(4) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 390−412.

(5) Khorana, A. A.; Sahni, A.; Altland, O. D.; Francis, C. W. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 2110−2115.

(6) Quaranta, M.; Erez, O.; Mastrolia, S. A.; Koifman, A.; Leron, E.; Eshkoli, T.; Mazor, M.; Holcberg, G. *PeerJ* **2015**, *3*, 1−20.

(7) Olczyk, P.; Mencner, L.; Komosinska-Vassev, K. *BioMed Res. Int.* **2015**, *2015*, 1−7.

(8) Rosenberg, R. D.; Lam, L. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 1218−1222.

(9) Peysselon, F.; Ricard-Blum, S. *Matrix Biol.* **2014**, *35*, 73−81.

(10) Ornitz, D. M.; Yayon, A.; Flanagan, J. G.; Svahn, C. M.; Levi, E.; Leder, P. *Mol. Cell. Biol.* **1992**, *12*, 240−247.

(11) Li, Y.-C.; Ho, I. H.; Ku, C.-C.; Zhong, Y.-Q.; Hu, Y.-P.; Chen, Z.-G.; Chen, C.-Y.; Lin, W.-C.; Zulueta, M. M. L.; Hung, S.-C.; Lin, M.-G.; Wang, C.-C.; Hsiao, C.-D. *ACS Chem. Biol.* **2014**, *9*, 1712−1717.

(12) Schlessinger, J.; Plotnikov, A. N.; Ibrahimi, O. A.; Eliseenkova, A. V.; Yeh, B. K.; Yayon, A.; Linhardt, R. J.; Mohammadi, M. *Mol. Cell* **2000**, *6*, 743−750.

(13) Pellegrini, L.; Burke, D. F.; von Delft, F.; Mulloy, B.; Blundell, T. L. *Nature* **2000**, *407*, 1029−1034.

(14) Yingkai, L.; Kiick, K. L. *Acta Biomater.* **2014**, *10*, 1588−1600.

(15) Blossom, D. B.; Kallen, A. J.; Patel, P. R.; Elward, A.; Robinson, L.; Gao, G.; Langer, R.; Perkins, K. M.; Jaeger, J. L.; Kurkjian, K. M.; Jones, M.; Schillie, S. F.; Shehab, N.; Ketterer, D.; Venkataraman, G.; Kishimoto, T. K.; Shriver, Z.; McMahon, A. W.; Austen, K. F.; Kozlowski, S.; Srinivasan, A.; Turabelidze, G.; Gould, C. V.; Arduino, M. J.; Sasisekharan, R. *N. Engl. J. Med.* **2008**, *359*, 2674−2684.

(16) Guerrini, M.; Beccati, D.; Shriver, Z.; Naggi, A.; Viswanathan, K.; Bisio, A.; Capila, I.; Lansing, J. C.; Guglieri, S.; Fraser, B.; Al-Hakim, A.; Gunay, N. S.; Zhang, Z. Q.; Robinson, L.; Buhse, L.; Nasr, M.; Woodcock, J.; Langer, R.; Venkataraman, G.; Linhardt, R. J.; Casu, B.; Torri, G.; Sasisekharan, R. *Nat. Biotechnol.* **2008**, *26*, 669−675.

(17) Hirsh, J.; van Aken, W. G.; Gallus, A. S.; Dollery, C. T.; Cade, J. F.; Yung, W. L. *Circulation* **1976**, *53*, 691−695.

(18) Kroon, C.; ten Hove, W. R.; de Boer, A.; Kroon, J. M.; van der Pol, J. M.; Harthoorn-Lasthuizen, E. J.; Schoemaker, H. C.; van der Meer, F. J.; Cohen, A. F. *Circulation* **1992**, *86*, 1370−1375.

(19) Manson, L.; Weitz, J. I.; Podor, T. J.; Hirsh, J.; Young, E. *J. Lab. Clin. Med.* **1997**, *130*, 649−655.

(20) Warkentin, T. E.; Levine, M. N.; Hirsh, J.; Horsewood, P.; Roberts, R. S.; Gent, M.; Kelton, J. G. *N. Engl. J. Med.* **1995**, *332*, 1330−1335.

(21) Merli, G. J.; Groce, J. B. *P T.* **2010**, *35*, 95−105.

(22) Linhardt, R. J.; Liu, J. *Curr. Opin. Pharmacol.* **2012**, *12*, 217−219.

(23) Middaugh, C. R.; Mach, H.; Burke, C. J.; Volkin, D. B.; Dabora, J. M.; Tsai, P. K.; Bruner, M. W.; Ryan, J. A.; Marfia, K. E. *Biochemistry* **1992**, *31*, 9016−9024.

(24) Hassan, H. H. A. M. *Mini-Rev. Med. Chem.* **2007**, *7*, 1206−1235.

(25) Maynard, H. D.; Hubbell, J. A. *Acta Biomater.* **2005**, *1*, 451−459.

(26) Vazquez-Cameos, S.; St Hilaire, P. M.; Damgaard, D.; Meldal, M. *QSAR Comb. Sci.* **2005**, *24*, 923−942.

(27) Mammadov, R.; Mammadov, B.; Guler, M. O.; Tekinay, A. B. *Biomacromolecules* **2012**, *13*, 3311−3319.

(28) Kim, S. H.; Kiick, K. L. *Peptides* **2007**, *28*, 2125−2136.

(29) Miura, Y.; Fukuda, T.; Seto, H.; Hoshino, Y. *Polym. J.* **2016**, *48*, 229−237.

(30) Deirdre, R.; Coombe, W. C. K. *Handb. Exp. Pharmacol.* **2012**, *207*, 1−462.

(31) Logeart-Avramoglou, D.; Jozefonvicz, J. *J. Biomed. Mater. Res.* **1999**, *48*, 578−590.

(32) Alpsoy, E.; Er, H.; Durusoy, C.; Yilmaz, E. *Arch. Dermatol.* **1999**, *135*, 529−532.

(33) Stein, C. A.; Larocca, R. V.; Thomas, R.; McAtee, N.; Myers, C. E. *J. Clin. Oncol.* **1989**, *7*, 499−508.

(34) He, C.; Cheng, C.; Ji, H. F.; Shi, Z. Q.; Ma, L.; Zhou, M.; Zhao, C. S. *Polym. Chem.* **2015**, *6*, 7893−7901.

(35) Cheng, C.; Sun, S.; Zhao, C. *J. Mater. Chem. B* **2014**, *2*, 7649−7672.

(36) Tao Xiang, C. C.; Zhao, C. *J. Membr. Sep. Technol.* **2014**, *3*, 162−177.

(37) Ma, L.; Su, B. H.; Cheng, C.; Yin, Z. H.; Qin, H.; Zhao, J. M.; Sun, S. D.; Zhao, C. S. *J. Membr. Sci.* **2014**, *470*, 90−101.

(38) Deng, J.; Liu, X. Y.; Ma, L.; Cheng, C.; Shi, W. B.; Nie, C. X.; Zhao, C. S. *ACS Appl. Mater. Interfaces* **2014**, *6*, 21603−21614.

(39) Miura, Y. *Polym. J.* **2012**, *44*, 679−689.

(40) Arlov, O.; Aachmann, F. L.; Sundan, A.; Espevik, T.; Skjak-Braek, G. *Biomacromolecules* **2014**, *15*, 2744−2750.

(41) Ruvinov, E.; Freeman, I.; Fredo, R.; Cohen, S. *Nano Lett.* **2016**, *16*, 883−888.

(42) Ronghua, H.; Yumin, D.; Jianhong, Y. *Carbohydr. Polym.* **2003**, *52*, 19−24.

(43) Wang, Z.-M.; Xiao, K.-J.; Li, L.; Wu, J.-Y. *Cellulose* **2010**, *17*, 953−961.

(44) Groth, T.; Wagenknecht, W. *Biomaterials* **2001**, *22*, 2719−2729.

(45) Vikhoreva, G.; Bannikova, G.; Stolbushkina, P.; Panov, A.; Drozd, N.; Makarov, V.; Varlamov, V.; Gal'braikh, L. *Carbohydr. Polym.* **2005**, *62*, 327−332.

(46) Suwan, J.; Zhang, Z.; Li, B.; Vongchan, P.; Meepowpan, P.; Zhang, F.; Mousa, S. A.; Mousa, S.; Premanode, B.; Kongtawelert, P.; Linhardt, R. J. *Carbohydr. Res.* **2009**, *344*, 1190−1196.

(47) Ding, K.; Wang, Y.; Wang, H.; Yuan, L.; Tan, M.; Shi, X.; Lyu, Z.; Liu, Y.; Chen, H. *ACS Appl. Mater. Interfaces* **2014**, *6*, 20043−20050.

(48) Mauzac, M.; Aubert, N.; Jozefonvicz, J. *Biomaterials* **1982**, *3*, 221−224.

(49) Mauzac, M.; Jozefonvicz, J. *Biomaterials* **1984**, *5*, 301−304.

(50) Crepon, B.; Maillet, F.; Kazatchkine, M. D.; Jozefonvicz, J. *Biomaterials* **1987**, *8*, 248−253.

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

(51) Meddahi, A.; Blanquaert, F.; Saffar, J. L.; Colombier, M. L.; Caruelle, J. P.; Josefonvicz, J.; Barritault, D. *Pathol., Res. Pract.* **1994**, *190*, 923−928.
(52) Meddahi, A.; Caruelle, J. P.; Gold, L.; Rosso, Y.; Barritault, D. *Diabetes Metab.* **1996**, *22*, 274−278.
(53) Albo, D.; Long, C.; Jhala, N.; Atkinson, B.; Granick, M. S.; Wang, T.; Meddahi, A.; Barritault, D.; Solomon, M. P. *J. Craniofac. Surg.* **1996**, *7*, 19−22.
(54) Blanquaert, F.; Saffar, J. L.; Colombier, M. L.; Carpentier, G.; Barritault, D.; Caruelle, J. P. *Bone* **1995**, *17*, 499−506.
(55) Lafont, J.; Colombier, M. L.; Barritault, D.; Saffar, J. L. *J. Dent. Res.* **1998**, *77*, 1248−1248.
(56) Blanquaert, F.; Barritault, D.; Caruelle, J. P. *J. Biomed. Mater. Res.* **1999**, *44*, 63−72.
(57) Lafont, J.; Baroukh, B.; Berdal, A.; Colombier, M. L.; Barritault, D.; Caruelle, J. P.; Saffar, J. L. *Growth Factors* **1998**, *16*, 23−28.
(58) Lafont, J.; Blanquaert, F.; Colombier, M. L.; Barritault, D.; Carueelle, J. P.; Saffar, J. L. *Calcif. Tissue Int.* **2004**, *75*, 517−525.
(59) Meddahi, A.; Benoit, J.; Ayoub, N.; Sezeur, A.; Barritault, D. *J. Biomed. Mater. Res.* **1996**, *31*, 293−297.
(60) Fredjreygrobellet, D.; Hristova, D. L.; Ettaiche, M.; Meddahi, A.; Jozefonwicz, J.; Barritault, D. *Ophthalmic Res.* **1994**, *26*, 325−331.
(61) Desgranges, P.; Barbaud, C.; Caruelle, J. P.; Barritault, D.; Gautron, J. *FASEB J.* **1999**, *13*, 761−766.
(62) Meddahi, A.; Bree, F.; Papy-Garcia, D.; Gautron, J.; Barritault, D.; Caruelle, J. P. *J. Biomed. Mater. Res.* **2002**, *62*, 525−531.
(63) Papy-Garcia, D.; Barbosa, I.; Duchesnay, A.; Saadi, S.; Caruelle, J. P.; Barritault, D.; Martelly, I. *J. Biomed. Mater. Res.* **2002**, *62*, 46−55.
(64) Rouet, V.; Meddahi-Pelle, A.; Miao, H. Q.; Vlodavsky, I.; Caruelle, J. P.; Barritault, D. *J. Biomed. Mater. Res., Part A* **2006**, *78A*, 792−797.
(65) Tardieu, M.; Slaoui, F.; Josefonvicz, J.; Courty, J.; Gamby, C.; Barritault, D. *J. Biomater. Sci., Polym. Ed.* **1989**, *1*, 63−70.
(66) Tardieu, M.; Gamby, C.; Avramoglou, T.; Jozefonvicz, J.; Barritault, D. *J. Cell. Physiol.* **1992**, *150*, 194−203.
(67) Desgranges, F.; Barritault, D.; Caruelle, J. P.; Tardieu, M. *Int. J. Artif. Organs* **1997**, *20*, 589−598.
(68) Papy-Garcia, D.; Barbier-Chassefiere, V.; Rouet, V.; Kerros, M. E.; Klochendler, C.; Tournaire, M. C.; Barritault, D.; Caruelle, J. P.; Petit, E. *Macromolecules* **2005**, *38*, 4647−4654.
(69) Rouet, V.; Hamma-Kourbali, Y.; Petit, E.; Panagopoulou, P.; Katsoris, P.; Barritault, D.; Caruelle, J. P.; Courty, J. *J. Biol. Chem.* **2005**, *280*, 32792−32800.
(70) Barbier-Chassefiere, V.; Garcia-Filipe, S.; Yue, X. L.; Kerros, M. E.; Petit, E.; Kern, P.; Saffar, J. L.; Papy-Garcia, D.; Caruelle, J. P.; Barritault, D. *J. Biomed. Mater. Res., Part A* **2009**, *90A*, 641−647.
(71) Garcia-Filipe, S.; Barbier-Chassefiere, V.; Alexakis, C.; Huetl, E.; Ledoux, D.; Kerros, M. E.; Petit, E.; Barritault, D.; Caruelle, J. P.; Kern, P. *J. Biomed. Mater. Res., Part A* **2007**, *80A*, 75−84.
(72) Tong, M.; Tuk, B.; Hekking, I. M.; Vermeij, M.; Barritault, D.; van Neck, J. W. *Wound Repair Regen.* **2009**, *17*, 840−852.
(73) Tong, M.; Zbinden, M. M.; Hekking, I. J. M.; Vermeij, M.; Barritault, D.; van Neck, J. W. *Wound Repair Regen.* **2008**, *16*, 294−299.
(74) Tong, M.; Tuk, B.; Hekking, I. M.; Pleumeekers, M. M.; Boldewijn, M. B.; Hovius, S. E. R.; van Neck, J. W. *Wound Repair Regen.* **2011**, *19*, 505−514.
(75) Tong, M.; Tuk, B.; Shang, P.; Hekking, I. M.; Fijneman, E. M. G.; Guijt, M.; Hovius, S. E. R.; van Neck, J. W. *Diabetes* **2012**, *61*, 2633−2641.
(76) van Neck, J.; Tuk, B.; Barritault, D.; Tong, M. In *Tissue Regeneration - From Basic Biology to Clinical Application*; Davies, J., Ed.; InTech, 2012; pp 1−520.
(77) Regan, J. R.; Bruno, J. G.; Chang, M. N.; Sabatino, R.; Dalisa, R.; Bensasson, S. A.; Eilat, D. *J. Bioact. Compat. Polym.* **1993**, *8*, 317−337.
(78) Benezra, M.; Vlodavsky, I.; Yayon, A.; Bar-Shavit, R.; Regan, J.; Chang, M.; Ben-Sasson, S. *Cancer Res.* **1992**, *52*, 5656−5662.
(79) Rogelj, S.; Weinberg, R. A.; Fanning, P.; Klagsbrun, M. *Nature* **1988**, *331*, 173−175.
(80) Benezra, M.; Bensasson, S. A.; Regan, J.; Chang, M.; Barshavit, R.; Vlodavsky, I. *Arterioscler., Thromb., Vasc. Biol.* **1994**, *14*, 1992−1999.
(81) Benezra, M.; Ishai-Michaeli, R.; Ben-Sasson, S. A.; Vlodavsky, I. *J. Cell. Physiol.* **2002**, *192*, 276−284.
(82) Yayon, A.; Klagsbrun, M.; Esko, J. D.; Leder, P.; Ornitz, D. M. *Cell* **1991**, *64*, 841−848.
(83) Ferrao, A. V.; Mason, R. M. *Biochim. Biophys. Acta, Mol. Basis Dis.* **1993**, *1180*, 225−230.
(84) Ishihara, M.; Tyrrell, D. J.; Stauber, G. B.; Brown, S.; Cousens, L. S.; Stack, R. J. *J. Biol. Chem.* **1993**, *268*, 4675−4683.
(85) Cariou, R.; Harousseau, J. L.; Tobelem, G. *Cell Biol. Int. Rep.* **1988**, *12*, 1037−1047.
(86) Miao, H. Q.; Ornitz, D. M.; Aingorn, E.; BenSasson, S. A.; Vlodavsky, I. *J. Clin. Invest.* **1997**, *99*, 1565−1575.
(87) Katz, A.; Vlodavsky, I.; Davies, M.; Miao, H. Q.; BenSasson, S. A.; Darmon, D.; Hurwitz, H.; Borgel, H.; Benezra, M. *J. Am. Soc. Nephrol.* **1997**, *8*, 1688−1697.
(88) Schmidt, A.; Vlodavsky, I.; Volker, W.; Buddecke, E. *Atherosclerosis* **1999**, *147*, 387−397.
(89) Benezra, M.; Vogel, T.; Ben-Sasson, S. A.; Panet, A.; Sehayek, E.; Al-Haideiri, M.; Decklbaum, R. J.; Vlodavsky, I. *J. Cell. Biochem.* **2001**, *81*, 114−127.
(90) Neuger, L.; Ruge, T.; Makoveichuk, E.; Vlodavsky, I.; Olivecrona, G. *Atherosclerosis* **2001**, *157*, 13−21.
(91) Irony-Tur-Sinai, M.; Vlodavsky, I.; Ben-Sasson, S. A.; Pinto, F.; Sicsic, C.; Brenner, T. *J. Neurol. Sci.* **2003**, *206*, 49−57.
(92) Dulaney, S. B.; Huang, X. F. *Adv. Carbohydr. Chem. Biochem.* **2012**, *67*, 95−136.
(93) Junqueira, D. R. G.; Perini, E.; Penholati, R. R. M.; Carvalho, M. G. *Cochrane Database Syst. Rev.* **2012**, *9*, CD007557−CD007557.
(94) Kusche, M.; Oscarsson, L. G.; Reynertson, R.; Roden, L.; Lindahl, U. *J. Biol. Chem.* **1991**, *266*, 7400−7409.
(95) Faham, S.; Hileman, R. E.; Fromm, J. R.; Linhardt, R. J.; Rees, D. C. *Science* **1996**, *271*, 1116−1120.
(96) Mach, H.; Volkin, D. B.; Burke, C. J.; Middaugh, C. R.; Linhardt, R. J.; Fromm, J. R.; Loganathan, D.; Mattsson, L. *Biochemistry* **1993**, *32*, 5480.
(97) Vazquez-Dorbatt, V.; Lee, J.; Lin, E. W.; Maynard, H. D. *ChemBioChem* **2012**, *13*, 2478.
(98) Kitazawa, S.; Okumura, M.; Kinomura, K.; Sakakibara, T. *Chem. Lett.* **1990**, *19*, 1733−1736.
(99) Fukudome, N.; Suzuki, K.; Yashima, E.; Akashi, M. *J. Appl. Polym. Sci.* **1994**, *52*, 1759−1763.
(100) Akashi, M.; Sakamoto, N.; Suzuki, K.; Kishida, A. *Bioconjugate Chem.* **1996**, *7*, 393−395.
(101) Sakamoto, N.; Kishida, A.; Maruyama, I.; Akashi, M. *J. Biomater. Sci., Polym. Ed.* **1997**, *8*, 545−553.
(102) Onishi, M.; Miyashita, Y.; Motomura, T.; Yamashita, S.; Sakamoto, N.; Akashi, M. *J. Biomater. Sci., Polym. Ed.* **1998**, *9*, 973−984.
(103) Huang, Y.; Taylor, L.; Chen, X.; Ayres, N. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 5230−5238.
(104) Huang, Y.; Shaw, M. A.; Mullins, E. S.; Kirley, T. L.; Ayres, N. *Biomacromolecules* **2014**, *15*, 4455−4466.
(105) Huang, Y.; Shaw, M. A.; Warmin, M. R.; Mullins, E. S.; Ayres, N. *Polym. Chem.* **2016**, *7*, 3897−3905.
(106) Taylor, L.; Chen, X.; Ayres, N. *Polym. Int.* **2014**, *63*, 127−135.
(107) Grande, D.; Baskaran, S.; Baskaran, C.; Gnanou, Y.; Chaikof, E. L. *Macromolecules* **2000**, *33*, 1123−1125.
(108) Grande, D.; Baskaran, S.; Chaikof, E. L. *Macromolecules* **2001**, *34*, 1640−1646.
(109) Baskaran, S.; Grande, D.; Sun, X. L.; Yayon, A.; Chaikof, E. L. *Bioconjugate Chem.* **2002**, *13*, 1309−1313.
(110) Sun, X. L.; Grande, D.; Baskaran, S.; Hanson, S. R.; Chaikof, E. L. *Biomacromolecules* **2002**, *3*, 1065−1070.
(111) Guan, R.; Sun, X. L.; Hou, S. J.; Wu, P. Y.; Chaikof, E. L. *Bioconjugate Chem.* **2004**, *15*, 145−151.

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440

(112) Hou, S. J.; Sun, X. L.; Dong, C. M.; Chaikof, E. L. *Bioconjugate Chem.* **2004**, *15*, 954−959.
(113) Sun, X. L.; Faucher, K. M.; Houston, M.; Grande, D.; Chaikof, E. L. *J. Am. Chem. Soc.* **2002**, *124*, 7258−7259.
(114) Sanders, W. J.; Gordon, E. J.; Dwir, O.; Beck, P. J.; Alon, R.; Kiessling, L. L. *J. Biol. Chem.* **1999**, *274*, 5271−5278.
(115) Manning, D. D.; Strong, L. E.; Hu, X.; Beck, P. J.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 11937−11952.
(116) Manning, D. D.; Hu, X.; Beck, P.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 3161−3162.
(117) Oh, Y. I.; Sheng, G. J.; Chang, S.-K.; Hsieh-Wilson, L. C. *Angew. Chem., Int. Ed.* **2013**, *52*, 11796−11799.
(118) Sheng, G. J.; Oh, Y. I.; Chang, S. K.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2013**, *135*, 10898−10901.
(119) Lee, S.-G.; Brown, J. M.; Rogers, C. J.; Matson, J. B.; Krishnamurthy, C.; Rawat, M.; Hsieh-Wilson, L. C. *Chem. Sci.* **2010**, *1*, 322−325.
(120) Rawat, M.; Gama, C. I.; Matson, J. B.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2008**, *130*, 2959−2961.
(121) Nishimura, Y.; Shudo, H.; Seto, H.; Hoshino, Y.; Miura, Y. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6390−6395.
(122) Miura, Y.; Yasuda, K.; Yamamoto, K.; Koike, M.; Nishida, Y.; Kobayashi, K. *Biomacromolecules* **2007**, *8*, 2129−2134.
(123) Miura, Y.; Mizuno, H. *Bull. Chem. Soc. Jpn.* **2010**, *83*, 1004−1009.
(124) Guan, R.; Sun, X.-L.; Hou, S.; Wu, P.; Chaikof, E. L. *Bioconjugate Chem.* **2004**, *15*, 145−151.
(125) Oh, Y. I.; Sheng, G. J.; Chang, S. K.; Hsieh-Wilson, L. C. *Angew. Chem., Int. Ed.* **2013**, *52*, 11796−11799.
(126) Sheng, G. J.; Oh, Y. I.; Chang, S.-K.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2013**, *135*, 10898−10901.
(127) Liekens, S.; Leali, D.; Neyts, J.; Esnouf, R.; Rusnati, M.; Dell'Era, P.; Maudgal, P. C.; De Clercq, E.; Presta, M. *Mol. Pharmacol.* **1999**, *56*, 204−213.
(128) Liekens, S.; Neyts, J.; Degreve, B.; DeClercq, E. *Oncology Res.* **1997**, *9*, 173−181.
(129) Sangaj, N.; Kyriakakis, P.; Yang, D.; Chang, C. W.; Arya, G.; Varghese, S. *Biomacromolecules* **2010**, *11*, 3294−3300.
(130) Sedlak, E.; Fedunova, D.; Vesela, V.; Sedlakova, D.; Antalik, M. *Biomacromolecules* **2009**, *10*, 2533−2538.
(131) Wang, M.; Lyu, Z.; Chen, G.; Wang, H.; Yuan, Y.; Ding, K.; Yu, Q.; Yuan, L.; Chen, H. *Chem. Commun.* **2015**, *51*, 15434−15437.
(132) Sangaj, N.; Kyriakakis, P.; Yang, D.; Chang, C.-W.; Arya, G.; Varghese, S. *Biomacromolecules* **2010**, *11*, 3294−3300.
(133) Belleney, J.; Helary, G.; Migonney, V. *Eur. Polym. J.* **2002**, *38*, 439−444.
(134) Berlot, S.; Aissaoui, Z.; Pavon-Djavid, G.; Belleney, J.; Jozefowicz, M.; Helary, G.; Migonney, V. *Biomacromolecules* **2002**, *3*, 63−68.
(135) El Khadali, F.; Hélary, G.; Pavon-Djavid, G.; Migonney, V. *Biomacromolecules* **2002**, *3*, 51−56.
(136) Ran, F.; Nie, S.; Yin, Z.; Li, J.; Su, B.; Sun, S.; Zhao, C. *Int. J. Biol. Macromol.* **2013**, *55*, 269−275.
(137) Garcia-Fernandez, L.; Aguilar, M. R.; Fernandez, M. M.; Lozano, R. M.; Gimenez, G.; San Roman, J. *Biomacromolecules* **2010**, *11*, 626−634.
(138) Christman, K. L.; Vázquez-Dorbatt, V.; Schopf, E.; Kolodziej, C. M.; Li, R. C.; Broyer, R. M.; Chen, Y.; Maynard, H. D. *J. Am. Chem. Soc.* **2008**, *130*, 16585−16591.
(139) Nguyen, T. H.; Kim, S.-H.; Decker, C. G.; Wong, D. Y.; Loo, J. A.; Maynard, H. D. *Nat. Chem.* **2013**, *5*, 221−227.
(140) Nguyen, T. H.; Paluck, S. J.; McGahran, A. J.; Maynard, H. D. *Biomacromolecules* **2015**, *16*, 2684−2692.
(141) Paluck, S. J.; Nguyen, T. H.; Lee, J. P.; Maynard, H. D. *Biomacromolecules* **2016**, *17*, 3386−3395.
(142) Matsusaki, M.; Serizawa, T.; Kishida, A.; Endo, T.; Akashi, M. *Bioconjugate Chem.* **2002**, *13*, 23−28.
(143) Matsusaki, M.; Serizawa, T.; Kishida, A.; Akashi, M. *Biomacromolecules* **2005**, *6*, 400−407.
(144) Cao, J.; Chen, Y.-W.; Wang, X.; Luo, X.-L. *J. Biomed. Mater. Res., Part A* **2011**, *97A*, 472−479.
(145) Platt, L.; Kelly, L.; Rimmer, S. *J. Mater. Chem. B* **2014**, *2*, 494−501.
(146) Christman, K. L.; Vazquez-Dorbatt, V.; Schopf, E.; Kolodziej, C. M.; Li, R. C.; Broyer, R. M.; Chen, Y.; Maynard, H. D. *J. Am. Chem. Soc.* **2008**, *130*, 16585−16591.
(147) Kolodziej, C. M.; Kim, S. H.; Broyer, R. M.; Saxer, S. S.; Decker, C. G.; Maynard, H. D. *J. Am. Chem. Soc.* **2012**, *134*, 247−255.
(148) Gospodarowicz, D.; Cheng, J. *J. Cell. Physiol.* **1986**, *128*, 475−484.
(149) Matsusaki, M.; Serizawa, T.; Kishida, A.; Akashi, M. *J. Biomed. Mater. Res., Part A* **2005**, *73A*, 485−491.
(150) Matsusaki, M.; Akashi, M. *Biomacromolecules* **2005**, *6*, 3351−3356.
(151) Zia, F.; Zia, K. M.; Zuber, M.; Tabasum, S.; Rehman, S. *Int. J. Biol. Macromol.* **2016**, *84*, 101−111.
(152) Grasel, T. G.; Cooper, S. L. *J. Biomed. Mater. Res.* **1989**, *23*, 311−338.
(153) Silver, J. H.; Hart, A. P.; Williams, E. C.; Cooper, S. L.; Charef, S.; Labarre, D.; Jozefowicz, M. *Biomaterials* **1992**, *13*, 339−344.
(154) Santerre, J. P.; Brash, J. L. *Macromolecules* **1991**, *24*, 5497−5503.
(155) Santerre, J. P.; Tenhove, P.; Vanderkamp, N. H.; Brash, J. L. *J. Biomed. Mater. Res.* **1992**, *26*, 39−57.
(156) Chen, K. Y.; Kuo, J. F.; Chen, C. Y. *Biomaterials* **2000**, *21*, 161−171.
(157) Dal Park, H.; Lee, W. K.; Ooya, T.; Park, K. D.; Kim, Y. H.; Yui, N. *J. Biomed. Mater. Res.* **2002**, *60*, 186−190.
(158) Joung, Y. K.; Sengoku, Y.; Ooya, T.; Park, K. D.; Yui, N. *Sci. Technol. Adv. Mater.* **2005**, *6*, 484−490.
(159) Terauchi, M.; Ikeda, G.; Nishida, K.; Tamura, A.; Yamaguchi, S.; Harada, K.; Yui, N. *Macromol. Biosci.* **2015**, *15*, 953−964.
(160) Bailey, W. J.; Ni, Z.; Wu, S. R. *Macromolecules* **1982**, *15*, 711−714.
(161) Lutz, J.-F.; Andrieu, J.; Uezguen, S.; Rudolph, C.; Agarwal, S. *Macromolecules* **2007**, *40*, 8540−8543.
(162) Siegwart, D. J.; Bencherif, S. A.; Srinivasan, A.; Hollinger, J. O.; Matyjaszewski, K. *J. Biomed. Mater. Res., Part A* **2008**, *87A*, 345−358.
(163) Delplace, V.; Tardy, A.; Harrisson, S.; Mura, S.; Gigmes, D.; Guillaneuf, Y.; Nicolas, J. *Biomacromolecules* **2013**, *14*, 3769−3779.
(164) Decker, C. G.; Maynard, H. D. *Eur. Polym. J.* **2015**, *65*, 305−312.
(165) Hedir, G. G.; Bell, C. A.; O'Reilly, R. K.; Dove, A. P. *Biomacromolecules* **2015**, *16*, 2049−2058.

DOI: 10.1021/acs.biomac.6b01147
Biomacromolecules 2016, 17, 3417−3440