

Heparin functionalized PEG gels that modulate protein adsorption for hMSC adhesion and differentiation

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Abstract

Heparin was modified with methacrylate groups, copolymerized with dimethacrylated poly(ethylene glycol), and analyzed as a localized delivery vehicle for bFGF and synthetic extracellular matrix for the differentiation of hMSCs. By deriving cues from molecules normally present in the extracellular matrix (ECM), a complex network of collagens, laminin, fibronectin, glycosaminoglycans, and growth factors, synthetic cell scaffolds can be designed that actively sequester important bioactive signals. Among the glycosaminoglycans, heparin binds reversibly with many proteins, therefore, poly(ethylene glycol) based biomaterials, normally resistant to cell adhesion, functionalized with heparin in order to sequester important proteins, can actively and selectively stimulate desired cell functions. Results demonstrate that methacrylate-modified heparin retained its ability to bind heparin-binding proteins both in solution and when copolymerized with dimethacrylated PEG in a hydrogel. In addition, the heparin functionalized gels can deliver biologically active bFGF for up to 5 weeks. Finally, the gels were examined as a potential scaffold for hMSC culture and were found to promote adhesion, proliferation, and osteogenic differentiation.

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1. Introduction

Biomaterials selected for their bulk properties (e.g., modulus, biodegradability, water content) may not necessarily possess qualities conducive to cell adhesion, proliferation, and tissue evolution. Consequently, adsorption or immobilization of proteins is an attractive method to improve biomaterial–cell interactions. Fibronectin has been coupled to the surfaces of poly(vinyl alcohol) gels [1,2] and shown to regulate endothelial cell ingrowth and attachment [3]; however, modification of biomaterials with full proteins can be very complex. Pro-

tein coupling to biomaterials requires mild reaction conditions, as the proteins are subject to both denaturation and degradation. As an alternative to inclusion of full proteins, significant interest has emerged in the design of cell scaffolds that actively sequester proteins and other important bioactive signals. Many of these cues are derived from molecules normally present in the extracellular matrix (ECM). The extracellular matrix (ECM) is complex, composed of collagens, laminin, fibronectin, and glycosaminoglycans. In native tissues, ECM molecules have been shown to influence strongly proliferation, differentiation, migration, and particularly important to tissue engineering, regeneration of damaged tissues [4].

The ECM is also known to function as a reservoir of endogenous growth factors [5,6], serving to localize growth factor activity, prevent growth factor

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degradation, and in some cases, enhance binding to cell surface receptors [7]. Growth factors contribute to tissue regeneration at various stages of cell proliferation and differentiation [8,9]. Among the numerous growth factors, basic fibroblast growth factor (bFGF) is well characterized [8]. It is a potent modulator of cell proliferation, motility, differentiation, and survival, as well as playing an important role in normal regenerative processes in vivo including embryonic development [10], angiogenesis [11], osteogenesis [12,13], chondrogenesis [14], and wound repair [15]. bFGF is known to be stored in various sites of the body, interacting with glycosaminoglycans, such as heparin [16].

Polymeric growth factor delivery systems based on heparin can store growth factors in a manner similar to the native ECM. Based on this in vivo storage mechanism, controlled release of growth factors has been described from heparin-sepharose conjugates [17], heparin-carrying polystyrene-bound collagen substrata [18], acid gelatin hydrogels [19], alginate gels containing heparin [20], photocrosslinkable chitosan hydrogels [21], and a fibrin-based system incorporating a heparin-binding peptide [22]. Also, heparin has been studied previously as a means to control the orientation of adsorbed fibronectin [23] and to impart anticoagulant properties to stainless steel by deposition of polyethylenimine and heparin layer-by-layer films [24]. In addition, evidence exists that heparin can also promote cell adhesion [25]. Interestingly, heparin is capable of interacting with numerous proteins associated with human mesenchymal stem cell (hMSC) adhesion (e.g., fibronectin, vitronectin), proliferation (e.g., bFGF), and osteogenic differentiation (e.g., bone morphogenetic proteins, pleiotrophin) [26].

Exploiting the knowledge of cell interactions with natural ECM molecules can be beneficial in the development of biomaterials that actively and selectively stimulate desired cellular functions important for tissue growth and healing. In this regard, poly(ethylene glycol) based biomaterials provide an especially useful platform from which to initiate fundamental studies. PEG-based materials are resistant to non-specific protein adsorption, and thus, PEG-based scaffolds can be used to introduce systematically selected cell signaling epitopes and study how this influences cell attachment and subsequent cellular functions.

Towards the end of designing a PEG-based gel that specifically sequesters growth factors that influence cell function, macromolecular heparin monomers were utilized in a novel growth factor sequestering biomaterial. Many methods have been explored to deliver growth factors, including microparticles [27] and chemical tethering [28]. However, these methods have problems such as growth factor damage or degradation, maintaining biological activity upon release, and the reliance on chemical degradation for the release of the molecule.

Hence, taking advantage of the natural binding mechanism of heparin for the design of a bioactive matrix is very promising. In the present study, macromolecular heparin monomers were synthesized and copolymerized with dimethacrylated PEG monomers to yield hydrogels of varying composition. These gels were analyzed as a possible delivery vehicle for bFGF and synthetic extracellular matrix for osteogenic differentiation of hMSCs.

2. Materials and methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

2.1. Synthesis of poly(ethylene glycol) dimethacrylate and methacrylated heparin

Poly(ethylene glycol) dimethacrylate (PEGDM) was synthesized by dissolving PEG (MW ~ 4600) in methylene chloride. Triethylamine (TEA) at 20% molar excess was added dropwise, and the solution was mixed under argon for 5 min. Methacryloyl chloride at 20% molar excess was mixed with methylene chloride and added dropwise to the PEG/TEA solution, and the final mixture was stirred overnight. The product was precipitated in ice-cold diethyl ether, filtered, and dried in a desiccator. After drying, the PEGDM was redissolved in diH₂O and dialyzed (Spectrum, 1000 MW cutoff) over 24 h with two distilled water exchanges. ¹H NMR analysis (in chloroform-d, Cambridge Isotopes) on the PEGDM revealed an average of 88% methacrylation.

Heparin (sodium salt from porcine intestinal mucosa, MW ~ 16 kDa) was methacrylated following a previous method [29]. Briefly, a 2% (w/v) solution of heparin in diH₂O was prepared and reacted with 5-fold excess of methacrylic anhydride. The pH of the reaction mixture was adjusted to 8.5 using 5 N NaOH, and the reaction was allowed to proceed overnight at 4 °C. The product, methacrylated heparin, was precipitated once in 95% ethanol, dried, and dialyzed (Spectrum, 1000 MW cutoff) for 48 h against diH₂O. ¹H NMR analysis (in D₂O, Cambridge Isotopes) revealed an average of 6% and 22% methacrylation, respectively, for two separate reactions. The percentage of methacrylation refers to the number of methacrylate groups per heparin disaccharide unit. With exception to the native PAGE (see Section 2.3), the 22% methacrylated heparin was utilized for all experiments herein. Structures of PEGDM, heparin, and methacrylated heparin are shown in Fig. 1.

2.2. Interactions of the modified heparin monomer with heparin-binding proteins

Methacrylated heparin with different degrees of methacrylate modification and nonmethacrylated hepa-

of gross increases in cell number, was monitored. At day 1, 4, and 7, cells were rinsed with PBS and trypsinized. The cell number was quantified with a Multisizer 3 Coulter Counter (Beckman Coulter, Inc.).

2.6. Adhesion and spreading of hMSCs on PEG:heparin copolymer hydrogels

hMSCs were trypsinized from culture plates, counted, centrifuged, resuspended, and seeded onto sterile hydrogel disks at a density of 2×10^4 cells/cm². After 4 h, the disks were rinsed with PBS and cells were fixed in 4% paraformaldehyde in PBS for 10 min. Phase contrast images (Nikon Eclipse TE300) were taken of the fixed hMSCs. Attached cells were counted on a minimum of three random fields (0.003 cm²) per disk ($n = 3$ per composition). Cell spreading was quantified by measuring the area of the cells. Using ImageJ software, the perimeter of the cell was outlined, and the area of cell attachment was quantified (~ 7.5 pixels/ μm^2).

2.7. hMSC proliferation on PEG:heparin copolymer hydrogels

Total DNA content, which was used as a measure of cell proliferation, was analyzed using the PicoGreen assay (Molecular Probes). hMSCs were trypsinized from culture plates, counted, centrifuged, resuspended, and seeded onto sterile hydrogel disks at a density of 5×10^3 cells/cm². After 2 days, 1 week, and 2 weeks, gels with attached cells were removed from culture; the gels were rinsed three times with PBS; 0.5 ml of PBS was added; and the samples were sonicated (Model W-380, Misonix, Inc.) for 1 min. The resulting solution was assayed for DNA content based on the manufacturer's instructions.

2.8. hMSC gene expression on PEG:heparin copolymer hydrogels

hMSC gene expression was analyzed using reverse transcription polymerase chain reaction. Cells were trypsinized from culture plates, counted, centrifuged, resuspended, and seeded onto sterile hydrogel disks at a density of 5×10^3 cells/cm². After 2 days, 1 week, and 2 weeks, gels with attached cells were removed from culture and rinsed three times with PBS. Total RNA was isolated using a guanidinium thiocyanate/phenol reagent (TRI reagent, Sigma) and standard manufacturer's protocols. After allowing the RNA pellet to dry, it was resuspended in nuclease-free water, and any residual genomic DNA in the samples was digested (DNase I, Invitrogen). RNA was then quantified using the RiboGreen assay (Molecular Probes) based on the manufacturer's instructions.

Reverse transcription was performed using the iScript cDNA Synthesis Kit (Bio-Rad). A 15 ng total RNA sam-

ple was used for the single strand cDNA synthesis. The reverse transcription reaction was incubated at 25 °C for 5 min, 42 °C for 30 min, and terminated at 85 °C for 5 min. PCR was conducted using the iCycler Real-Time PCR machine (Bio-Rad), and primers and probes were designed using the Beacon Designer primer design program (Table 1). Primers (Invitrogen) and probes (Integrated DNA Technologies) for alkaline phosphatase (ALP), osteopontin (OPN), collagen type I (COL I), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used in a multiplex format. The following PCR parameters were utilized: 95 °C for 90 s followed by 45 cycles of 95 °C for 30 s and 55 °C for 60 s. Threshold cycle (C_T) analysis was used to quantify PCR products, normalized to GAPDH and relative to expression of hMSCs seeded on the homopolymer PEG gels.

2.9. Statistical analysis

Statistical analysis was performed using a two-tailed Student's *t*-test. *P* values less than 0.05 were considered statistically significant. Data are presented as mean \pm standard deviation.

3. Results

Macromolecular heparin monomers were synthesized and copolymerized with dimethacrylated poly(ethylene glycol) monomers to form copolymer hydrogels and investigated as a possible delivery vehicle for bFGF and as a synthetic ECM material to promote osteogenic differentiation of hMSCs. Heparin interacts reversibly with hundreds of proteins. This quality can be exploited for tissue engineering applications by creating heparin functionalized gels that combine a synthetic component to modulate bulk properties with a native ECM component to sequester proteins to which cells will interact and alter their function. By exploring a specific interaction with bFGF, a known heparin-binding growth factor, we demonstrate a significant biological effect based on altering the relative gel composition; when cultured in the presence of bFGF, hMSC proliferation increases substantially [31].

3.1. Interactions of the modified heparin monomer with heparin-binding proteins

Heparin possesses specific binding interactions with many proteins, of which bFGF is one, and this property was retained after modification with methacrylate groups and after copolymerizing methacrylated heparin with dimethacrylated PEG. When bFGF is combined with heparin or methacrylated heparin, decreased staining intensity for bFGF on native PAGE gels indicates bFGF binding to heparin or methacrylated heparin.

Table 1
Primer and probe sequences designed by Beacon Designer software and utilized for real-time PCR

Gene	Sense primer	Anti-sense primer	Probe
Glycerinaldehyde 3-phosphate dehydrogenase (GAPDH)	5'-GCAAGAGCACAAAGAGGAAGAG-3'	5'-AAGGGGTCTACATGGCAACT-3'	5'-ACCCTCACTGCTGGGGAGTCC-3'
Collagen type I (Col I)	5'-GGGCAAGACAGTGATTGAAATACA-3'	5'-GGATGGAGGGAGTTTACAGGAA-3'	5'-CCAAGTCTCCCGCCTGGCCATC-3'
Alkaline phosphatase (ALP)	5'-GTGGAGTATGAGATGACGAGAA-3'	5'-AGATGAAAGTGGGAGTGTGTAT-3'	5'-CCTGGACCTCGTTGACACCTGGGAAG-3'
Osteopontin (OPN)	5'-ATTCTGGAGGGCTTGGTTG-3'	5'-TCTGGTCCCGACGATGCT-3'	5'-CTCTGCCTCCTCCTGCTGCTGTG-3'

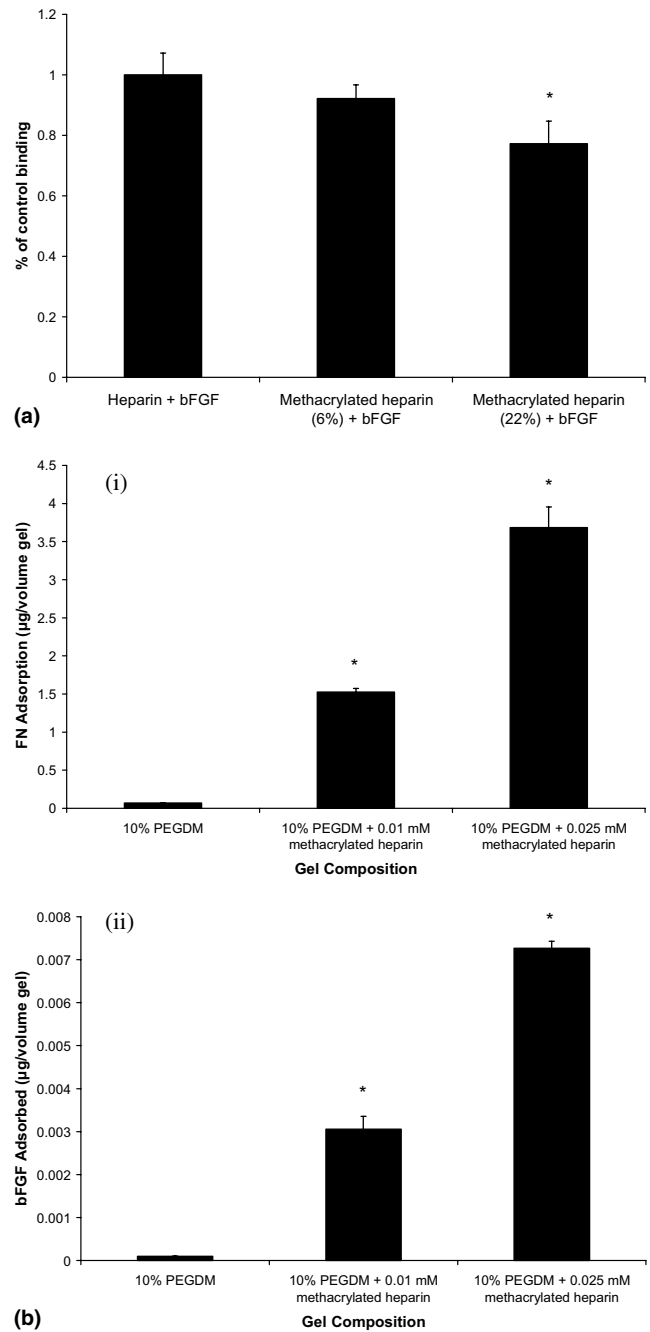


Fig. 2. Methacrylated heparin retained its ability to specifically bind to heparin-binding proteins. A native PAGE analysis of heparin (a) demonstrated that bFGF binding to heparin decreased with increasing extent of methacrylation (% methacrylation shown in parentheses). * $p < 0.05$, $n = 3$ samples per condition. Methacrylated heparin copolymerized with PEGDM into hydrogels retained its ability to specifically bind to heparin-binding proteins fibronectin (FN) (b, i) and basic fibroblastic growth factor (bFGF) (b, ii) in a heparin dose-dependent manner. * $p < 0.05$ of sample versus control (10% PEGDM), $n = 10$ samples per condition.

The results in Fig. 2a are reported as a percentage of the control binding. The degree of bFGF association with methacrylated heparin was dependent on the extent of methacrylation of heparin, with a higher degree

of modification corresponding to a decreased bFGF binding.

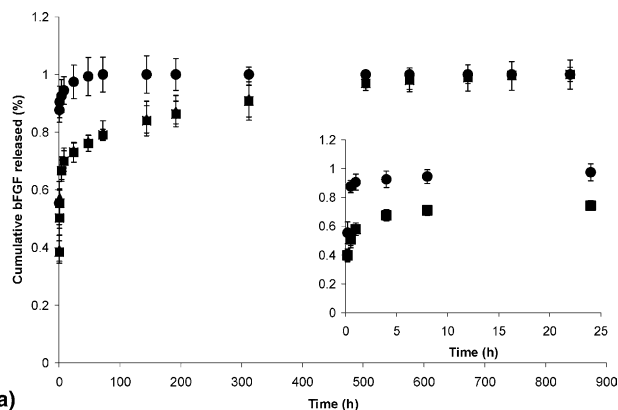
Copolymerized methacrylated heparin and dimethacrylated PEG gels were also shown to specifically retain noncovalently bound proteins, bFGF and FN, as evidenced by the radioactivity of gels swelled with ^{125}I -protein solutions (Fig. 2b). To demonstrate that the proteins were binding specifically to heparin contained in the gels, negative control gels (PEG only) were also assayed and showed no significant protein adsorption. These results indicate that proteins, including bFGF and FN, are still able to associate and bind with methacrylated heparin that has been copolymerized with dimethacrylated PEG to form a hydrogel.

3.2. Analysis of heparin incorporation into copolymer gels

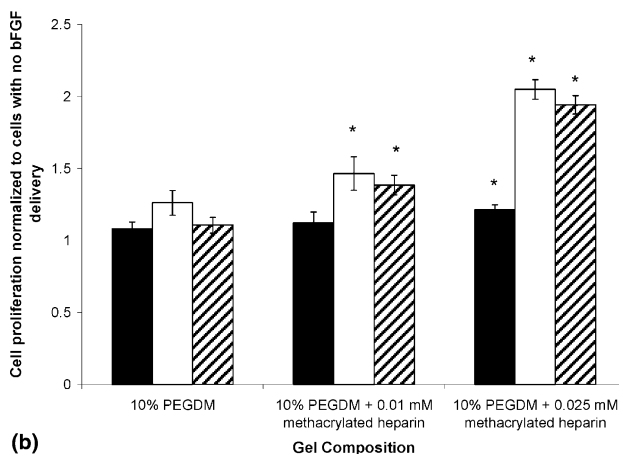
Hydrogel disks were fabricated by polymerizing a solution containing 10 wt.% of the PEGDM monomer and 0.01 mM or 0.025 mM of methacrylated heparin in PBS. The incorporation of heparin was analyzed via an indirect sol fraction analysis. Gels were fabricated and swelled overnight in PBS. As a control, gels were made that encapsulated unmodified heparin. The resulting supernatant was analyzed for concentration of heparin using the DMMB assay. Incorporation of heparin was determined using a mass balance and results of this analysis are shown in Table 2. Gels fabricated from methacrylated heparin incorporated nearly all of the initial heparin (95% and 92% for initial copolymerization of 0.01 mM and 0.025 mM heparin, respectively) while gels encapsulating heparin retained almost no heparin (0.2% and -0.3% for initial encapsulation of 0.01 mM and 0.025 mM heparin, respectively).

3.3. Sequestering of bFGF using heparin gels for localized delivery

The release of bFGF from heparin/PEG copolymerized gels into PBS was measured over time as shown in Fig. 3a. The release of bFGF from unmodified PEG gels was very rapid, releasing all bFGF after 8 h. The release from 0.01 mM and 0.025 mM methacrylated heparin gels was initially rapid, but then continued releasing slowly over a 5-week time period, following zero order release kinetics.



(a)



(b)

Fig. 3. (a) Release of bFGF adsorbed in 10% PEGDM gels (●), 10% PEGDM + 0.01 mM methacrylated heparin gels (▲), and 10% PEGDM + 0.025 mM methacrylated heparin gels (■) at 37 °C. (b) Effect of adsorbed bFGF release on hMSC proliferation normalized to cells with no bFGF delivery after 1 days (black), 4 days (white), and 7 days (striped). Cells were seeded in the bottom of each well of a 12-well transwell plate with bFGF-releasing gels in the upper chamber. Data are expressed as mean \pm standard deviation ($n = 5$). * $p < 0.05$ of sample versus control (10% PEGDM) at that timepoint.

The growth of hMSCs is stimulated by the addition of bFGF in free form [22], and enhanced stimulation was observed by the addition of bFGF incorporated into the PEG:heparin copolymer hydrogels in a heparin dose-dependent manner. Cell number increased by 12%, 46%, and 40% over cells with no bFGF by the addition of 0.01 mM heparin/PEG copolymerized gels containing approximately 3 ng of bFGF, after day 2, 4, and 7, respectively (Fig. 3b). Also, with gels incorporating 0.025 mM heparin and thus ~ 7.5 ng bFGF, cell number

Table 2

Per cent heparin incorporation when polymerized with or encapsulated in 10% PEGDM hydrogels as determined by the DMMB assay and mass balance

	% Heparin incorporation of initial loading	
	Methacrylated heparin	Encapsulated heparin
10% PEGDM + 0.01 mM heparin	95.0 \pm 0.3%	0.2 \pm 0.03%
10% PEGDM + 0.025 mM heparin	92.1 \pm 1.5%	-0.3 \pm 0.02%

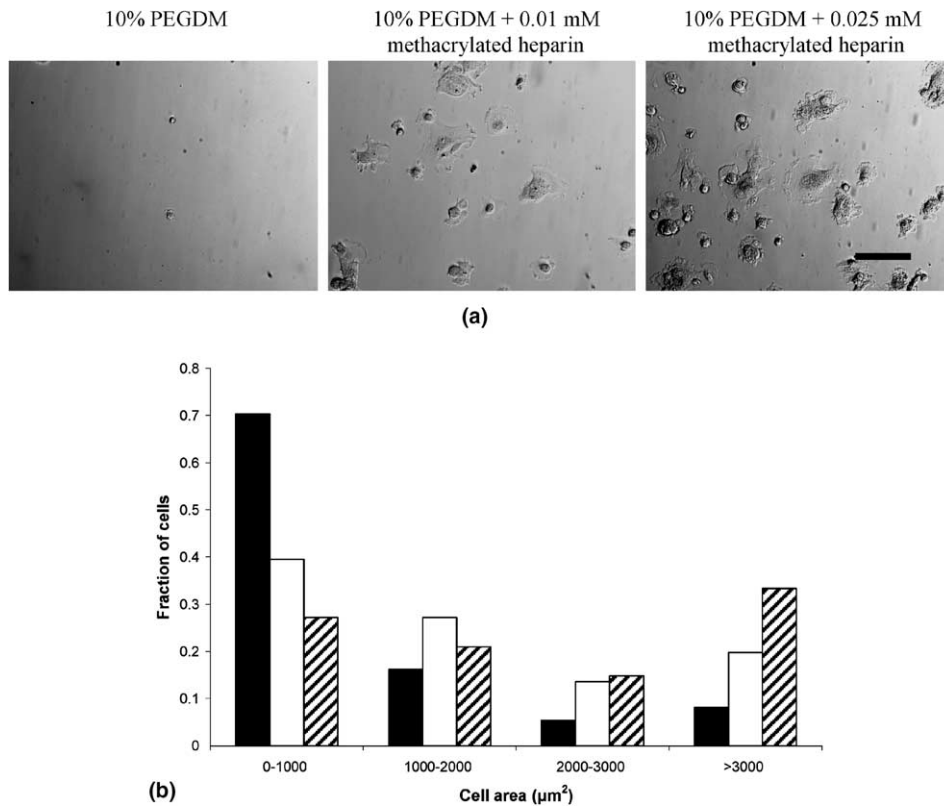


Fig. 4. (a) Light micrographs of hMSCs attached to surfaces of varying composition, bar = 100 μm . (b) Degree of cell spreading as a function of surface modification. 10% PEGDM—black, 10% PEGDM + 0.01 mM methacrylated heparin—white, and 10% PEGDM + 0.025 mM methacrylated heparin—striped. Values are reported as the fraction of attached cells within a range of cell areas for 12 random fields analyzed on a minimum of three hydrogels per composition.

increased by 21%, 104%, and 94% over cells with no bFGF exposure after day 2, 4, and 7, respectively. In contrast, the addition of PEG gels swelled in bFGF solutions only slightly stimulated cell growth (8%, 26%, and 10% after day 2, 4, and 7, respectively).

3.4. Adhesion and spreading of hMSCs on PEG:heparin copolymer hydrogels

Heparin, due to its ability to bind proteins, including extracellular matrix proteins such as fibronectin and collagen, was methacrylated and copolymerized with dimethacrylated PEG. Due to its hydrophilic and uncharged nature, poly(ethylene) glycol (PEG)-based hydrogels are inherently non cell-adhesive. hMSCs, adhesion-dependent cells, were seeded on PEG:heparin copolymer hydrogels. Fig. 4a shows dose-dependent improvement of attachment of hMSCs to hydrogels incorporating heparin over hydrogels with no modification. The spreading of attached hMSCs was also significantly higher on the heparin modified surfaces than on PEG alone. A histogram that reports the surface area of attached hMSCs on the hydrogel surfaces is shown in Fig. 4b (classifications: 0–1000 μm^2 (least spread), 1000–2000 μm^2 , 2000–3000 μm^2 , and >3000 μm^2 (most

spread)). The histogram indicates that most of the cells (~70%) on the unmodified PEG surfaces were rounded and spread very little. In contrast, on the heparin modified gels, cell spreading was significantly enhanced and increased with ~20% and ~33% of the cells categorized in the highest spreading classification (>3000 μm^2), respectively, on the 0.01 mM and 0.025 mM methacrylated heparin modified surfaces. Furthermore, these surfaces had the fewest rounded cells, ~39% and ~27%, respectively, in the 0–1000 μm^2 category.

3.5. hMSC proliferation on PEG:heparin copolymer hydrogels

Proliferation, as determined by total DNA, of attached hMSCs on hydrogel surfaces was determined after 2 days, 1 week, and 2 weeks of culture, and the quantified results are reported in Fig. 5. Proliferation was statistically higher for cells seeded on the heparin-containing gels versus unmodified PEG gels at all time points. Furthermore, proliferation increased over the 2-week study for cells on the heparin-containing gels while the unmodified PEG gels exhibited no statistically significant proliferation. These results suggest that heparin promotes hMSC proliferation.

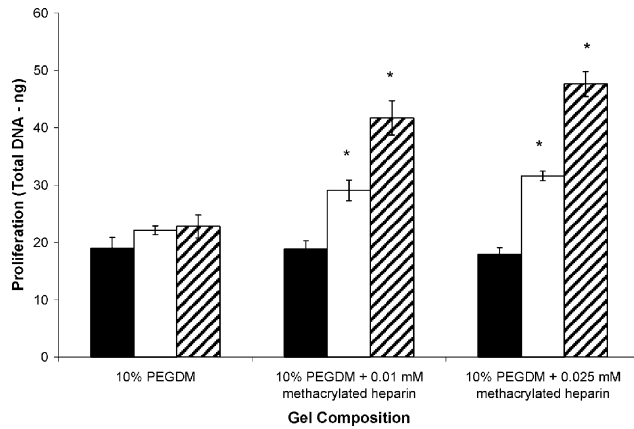


Fig. 5. Proliferation of hMSCs seeded on various gel surfaces after 2 days (black), 1 week (white), and 2 weeks (striped) (error bars designate standard deviation). * $p < 0.05$ of sample versus control (10% PEGDM) at that timepoint, $n = 4$ samples per condition.

3.6. hMSC gene expression on PEG:heparin copolymer hydrogels

Gene expression profiles for the cultured hMSCs, normalized to GAPDH and relative to expression of cells cultured on PEG alone, are quantified in Fig. 6. An initial (day 2) decrease (2-fold compared to 10% PEGDM for both concentrations of heparin), followed by a dramatic increase over unmodified PEG gels at days 7 (1.05-fold and 1.4-fold for 0.01 mM and 0.025 mM heparin) and 14 (2.4-fold and 2.4-fold for 0.01 mM and 0.025 mM heparin) in Col I expression. Similar trends are found for ALP expression, with an initial decrease at day 2 for heparin-containing gels, followed by an increase at days 7 (1.1-fold and 1.4-fold for 0.01 mM and 0.025 mM heparin) and 14 (1.7-fold and 1.9-fold for 0.01 mM and 0.025 mM heparin) over unmodified PEG gels. The expression of Col I and ALP was greatest at day 14, increasing over time with the study. The expression of OPN by hMSCs on heparin-containing gels again showed an initial decrease at day 2 (3-fold reduction compared to 10% PEGDM for both concentrations of heparin) followed by an increase at days 7 (6.7-fold and 8.7-fold for 0.01 mM and 0.025 mM heparin) and 14 (3.1-fold and 4.5-fold for 0.01 mM and 0.025 mM heparin) versus unmodified PEG gels. The trend, however, was slightly different where the greatest expression was at day 7 and reduced marginally at day 14. Gene expression profiles support the notion that heparin-containing hydrogels promote osteogenic differentiation of hMSCs.

4. Discussion

A common objective in regenerative medicine is to design biomaterial niches that actively direct cell func-

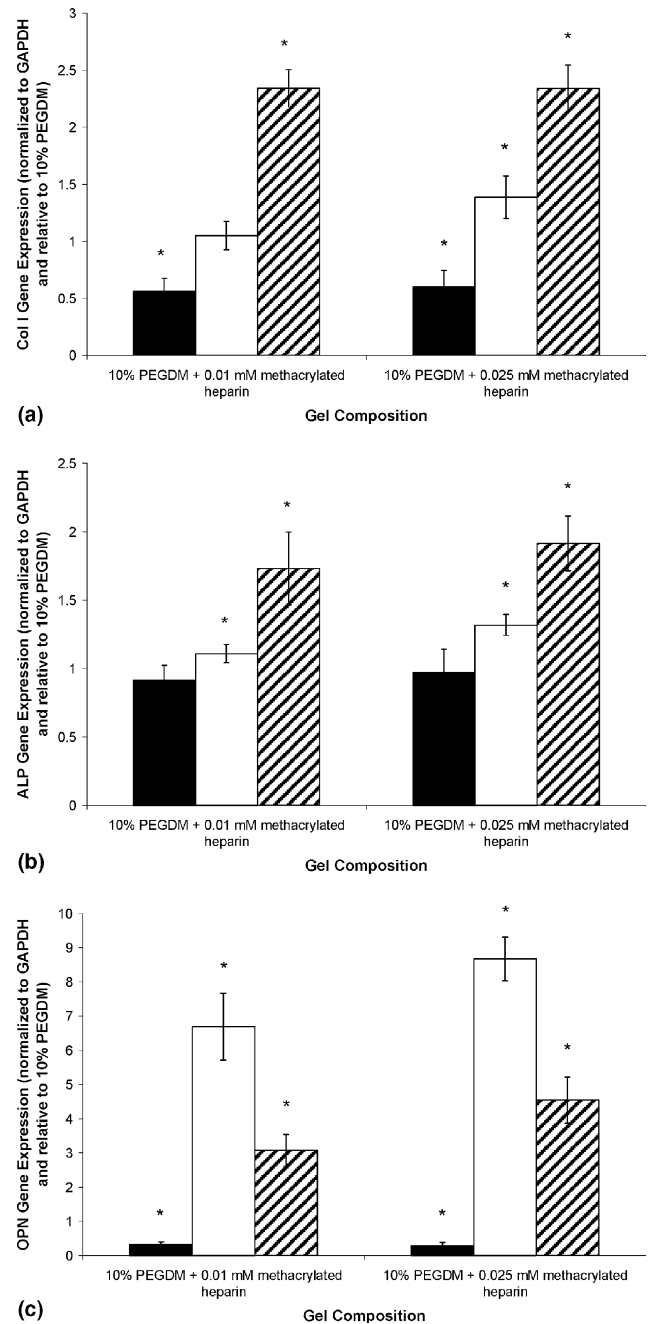


Fig. 6. (a) Collagen type I (Col I), (b) alkaline phosphatase (ALP), and (c) osteopontin (OPN) gene expression normalized to levels of GAPDH of hMSCs cultured on 10% PEGDM (control), 10% PEGDM + 0.01 mM methacrylated heparin, and 10% PEGDM + 0.025 mM methacrylated heparin hydrogels at 2 days (black), 1 week (white), and 2 weeks (striped) (error bars designate standard deviation). * $p < 0.05$ of sample versus control (10% PEGDM) at that timepoint, $n = 4$ samples per condition.

tions, especially adhesion, proliferation, and differentiation. An important area of research includes strategies that control the density, clustering, and orientation of cell signaling epitopes, such as proteins and growth factors, to provide avenues for outside-in signaling. Hepa-

rin, a protein-binding glycosaminoglycan, possesses numerous attractive qualities with respect to functionalizing scaffolds for regenerative medicine applications. In this study, we explored heparin, modified it with methacrylate groups, copolymerized it with dimethacrylated PEG, and found, in both circumstances, it retained the ability to bind proteins. This is in agreement with work done by Masters et al. [32] where methacrylated hyaluronic acid retained the ability to bind fibronectin in solution and when polymerized to form a hydrogel.

The PEG:heparin copolymerized system was explored as a sequestering and delivery system for bFGF. Others have investigated this capacity for heparin containing systems [10–16], and the release of bFGF in our system is very similar to that of a hyaluronate and heparin crosslinked system [33], where bFGF release was initially rapid and then slowly continued for approximately 2 weeks. Similarly, in an alginate and heparin crosslinked matrix [20], there was a bulk release during the first 2 h and then the release concluded after 7 days.

In our work, the heparin-modified gels promoted hMSC adhesion, spreading, and proliferation. It has been previously reported [25] that a chitosan/heparin complex surface showed enhanced fibroblast adhesion. In comparison to RGD-modified PEG hydrogels at similar concentrations, PEG:heparin hydrogels showed ~10% less adhesion and spreading of hMSCs (data not shown). Although Rapraeger et al. [34] found heparin was required for bFGF-mediated fibroblast proliferation [26], heparin may not be solely responsible for increased proliferation in this work. It is possible that the heparin acts as a sequestering and/or stabilizing vehicle to increase the surface concentration of protein, stimulating proliferation. Additionally, in our seeding studies, bFGF was not directly delivered in the heparin containing hydrogels but media used in studies described here contained bFGF at a concentration of ~3 pg/ml. Thus, bFGF was available for sequestering and stimulation of proliferation.

PEG:heparin copolymerized hydrogels were also examined as a scaffold to promote osteogenic differentiation of hMSCs. The importance of sulfated glycosaminoglycans for bone formation is inherent to their ability to bind most of the growth factors involved in the regulation of cells of the osteoblast lineage (e.g., FGFs, TGF- β 1, BMP2 and 4, IGF-II) [34]. In vitro cultures of hMSCs on heparin-containing hydrogels follow the general osteogenic differentiation process with enhancement of various differentiation markers (e.g., Col I, ALP, and OPN) after day 2. However, data at day 2 show a decreased gene expression of Col I and OPN, two important ECM proteins. It is theorized that the hMSCs produced ECM and the heparin functionalized surfaces adsorbed the matrix

proteins, initiating feedback inhibition. Because unmodified PEG hydrogels are inherently protein resistant, the absence of matrix caused hMSCs to increase production of these molecules. At later timepoints, the hMSCs are differentiating and, in general, differentiation begins with an increase in cell density, continues with augmented protein levels, a cascade that starts with an increase in ALP and OPN, followed by a heightened production of Col I which continues at a high level until mineralization proceeds [35]. Shibata et al. [36] found that soluble heparin stimulates collagen synthesis in mineralized cultures of the osteoblast cell line, MC3T3-E1, and Saos-2 cells, an osteoblast-like cell line, exhibited increased ECM deposition in the presence of soluble heparin [37]. Recently, it has been shown that heparin and bFGF act synergistically in modulating fetal rat calvarial cell synthesis of type I collagen [38] and myoblast differentiation [33]. Gupta et al. [39] explored bone marrow stroma, the native hMSC niche, and found that it is the structural specificity of heparin that determines the selective colocalization of cytokines and ECM components that orchestrates their controlled growth and differentiation. Thus, by careful design of hydrogel cell carriers with rationally targeted modification and incorporation of signaling molecules, niches can be synthesized that actively promote cell function such as adhesion, proliferation, and differentiation.

5. Conclusions

Heparin was modified with methacrylate groups, copolymerized with dimethacrylated poly(ethylene glycol), and analyzed as a delivery vehicle for bFGF and synthetic extracellular matrix for the osteogenic differentiation of hMSCs. The methacrylate-modified heparin retained its ability to bind heparin-binding proteins in solution. In addition, the heparin-modified gels can sequester and deliver biologically active bFGF in a controlled dose over a 5-week timeperiod. Finally, the gels were examined as a potential scaffold and were found to promote adhesion, proliferation, and osteogenic differentiation of hMSCs.

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