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Covalent immobilization of RGDS on hydrogel surfaces to direct cell alignment and migration

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Abstract

This study extends the capability for directing cell behavior using PEG-based hydrogels in tissue-engineering applications to include control over the spatial distribution of the adhesive peptide, RGDS. A continuous linear gradient was formed by simultaneously using a gradient maker to combine precursor solutions and using photopolymerization to lock the RGDS gradient in place. Hydrogels containing entrapped gradients of bovine serum albumin (BSA) were characterized using Coomassie brilliant blue stain, which indicated that BSA concentration increases along the hydrogel's length and that the steepness of the gradient's slope can be varied by changing the relative BSA concentrations in the precursor solutions. Human dermal fibroblasts responded to covalently immobilized RGDS gradients by changing their morphology to align in the direction of increasing RGDS concentration. After 24 h, ~46% of fibroblasts were aligned with the RGDS-gradient axis. This proportion of cells further increased to ~53% ($p < 0.05$) and ~58% after 48 and 96 h, respectively. Also, fibroblasts migrated differentially depending on the concentration of RGDS. Fibroblasts migrated ~48% further going up the concentration gradient (0 to 6 μmol/ml PEG–RGDS) than going down the concentration gradient. Migration up the concentration gradient was also ~33% greater than migration on control surfaces with a constant concentration of RGDS (2 μmol/ml), while migration down the gradient was reduced ~12% relative to the control surface. In addition, directed migration was further enhanced by increasing the RGDS gradient's slope. This hydrogel system is expected to be useful for directing cell migration to enhance the formation of engineered tissues.
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1. Introduction

The spatial distribution of proteins can play an important role in the organization of tissues. Protein gradients, for example, can provide necessary biochemical cues that direct the organized formation of tissue [1]. This role impacts embryogenesis, capillary sprouting, and wound healing. In wound healing, for example, chemotactic factors released by platelets and macrophages can recruit fibroblasts into the wound where the fibroblasts in part repopulate the wound and deposit connective tissue [2]. Protein gradients may

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also be useful for optimizing engineered tissue formation by enhancing migration and the recruitment of cells into scaffolds. Researchers have developed a number of techniques that enable one to control the spatial distribution of proteins. However, these methods are limited to two-dimensional applications or are very short term in their protein gradient presentation.

Photolithography techniques, for example, have made it possible to restrict cell adhesion to specific areas of a biomaterial surface. These techniques often involve the use of an elastomeric stamp to form patterns of protein-resistant and protein-adsorptive regions, which have been shown to lead to changes in both cell spreading and cell proliferation [3–5]. Although this technique is relatively simple to apply and flexible in providing a range of shapes and sizes that can be dictated including protein gradients, it is difficult to apply this method to three-dimensional constructs [4,5]. In addition, select proteins can be displaced by alternative serum proteins that can alter cell behavior in undesirable ways.

Alternatively, methods exist that enable one to study chemotaxis in vitro in response to soluble protein gradients [6–9]. These methods depend on the free-diffusion of protein from one region of a material into another region. When combined with continuous monitoring and recording of cell tracks, it is possible to observe three-dimensional chemotaxis in such materials as fibrin and collagen gels [8,9]. Unfortunately, a stable concentration gradient fades quickly (within a few hours) as it diffuses away. Gradient effects on tissue formation will require longer exposure times not possible with this system. Additionally, this system cannot be used in combination with mechanical forces or in vivo as the gradient can be easily disrupted by agitation. Finally, there are also biochemical cues intrinsic to the fibrin gel that can influence cell behavior and migration making it difficult to control the presentation of bioactive factors. Sequences in fibrin, for example, can interact with cells and extracellular matrix proteins, which can increase fibroblast and endothelial cell proliferation and promote angiogenesis [10,11].

Thus, several technologies exist that aid in controlling the pattern of bioactive factor presentation. These strategies, however, fall short in enabling the sustained presentation of protein patterns in three-dimensional biomaterials. In contrast, hydrogel scaffolds based on poly(ethylene glycol) (PEG) provide the opportunity to control the identity and concentration of adhesive peptide presentation. For example, fibroblasts, osteoblasts, and vascular smooth muscle cells (SMCs) have all been shown to adhere to and grow on PEG-based hydrogel scaffolds when these scaffolds were modified with adhesion peptides [12–15]. Notably, the incorporation of adhesion peptides was required for cells to interact at any significant level with the hydrogel scaffolds. Cell attachment and spreading were also shown to vary depending on the bulk peptide concentration. These cell-interactive effects are due to PEG's hydrophilicity and the ability to covalently incorporate bioactive factors. In summary, PEG-hydrogel scaffolds provide a blank slate to which cell adhesion peptides can be added in a controlled fashion to enable cell interaction with PEG-based hydrogel scaffolds.

Although these cell adhesion studies focused on two-dimensional cell interaction with nondegradable PEG hydrogels, these scaffolds can be further modified to promote three-dimensional remodeling of hydrogel constructs, allowing cell infiltration and migration through the hydrogel structure. For example, protease-sensitive peptide sequences can be inserted into the backbone of PEG-polymer chains [16]. These sequences can then be cleaved to make room for cell proliferation, extracellular matrix protein production, and migration. Thus, PEG-based hydrogels may provide a solution for controlling the pattern of bioactive factor presentation in a three-dimensional tissue-engineering scaffold.

This study extends the capability for directing cell behavior using PEG-based hydrogels to include control over the spatial distribution of cell adhesion peptides. Gradient hydrogels are formed by adapting a gradient maker normally used to make polyacrylamide gels for electrophoresis. A continuous linear gradient is formed using the gradient maker to control how polymer solutions containing varying concentrations of the fibronectin-derived adhesive peptide, RGDS, are combined and then stabilizing the gradient in place via photopolymerization as it is poured into a mold. The ability to make gradient hydrogels with varying slopes was assessed and the effect of RGD gradients on fibroblast alignment and migration was studied. A concentration gradient may mimic the presentation of bioactive factors found in vivo and

may be useful for eliciting enhanced and directional cell migration, which may be useful for optimizing tissue formation.

2. Materials and methods

2.1. Cell maintenance

Human dermal fibroblasts were obtained from Clonetics (San Diego, CA). They were maintained at 37 °C/5% CO_2 on Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS, BioWhittaker, Walkersville, MD), 2 mM L-glutamine (Sigma, St. Louis, MO), 1000 U/l penicillin (Sigma, St. Louis, MO) and 100 mg/l streptomycin (Sigma, St. Louis, MO). All experiments were conducted using cells at passages 8–12.

2.2. Polymer synthesis

PEG diacrylate was synthesized by dissolving 12 g dry PEG (6000 Da; Fluka, Milwaukee, WI) in 36 ml anhydrous dichloromethane. 0.25 g triethylamine and then 0.43 g acryloyl chloride (Lancaster Synthesis, Windham, NH) were added dropwise, and the mixture was stirred under argon for 48 h. The resulting solution was then washed with 2 M K_2CO_3 and separated into aqueous and dichloromethane phases to remove HCl. The dichloromethane phase was subsequently dried with anhydrous $MgSO_4$ (Fisher Scientific, Pittsburgh, PA), and the PEG diacrylate was then precipitated in diethyl ether, filtered, and dried under vacuum at room temperature overnight. The resultant polymer was dissolved in *N*,*N*-dimethylformamide-d_7 (Sigma, St. Louis, MO) and characterized via proton NMR (Avance 400 MHz; Bruker, Billerica, MA) to determine the degree of acrylation.

A cell adhesive component was prepared by reacting acryloyl–PEG–NHS with the adhesion peptide, RGDS (American Peptide, Sunnyvale, CA), in 50 mM sodium bicarbonate (pH 8.5) at a 1:1 molar ratio for 2 h. The solution was then lyophilized and stored at −20 °C. PEG–peptide conjugates and PEG standards were analyzed using gel permeation chromatography equipped with UV/vis (260 nm) and evaporative light-scattering detectors (Polymer Laboratories, Amherst, MA).

2.3. Detection of entrapped gradients of BSA

As depicted in Fig. 1, hydrogels containing gradients of entrapped BSA were formed using a gradient maker (CBS Scientific Co., Del Mar, CA) to pour the hydrogel precursor solutions prior to photopolymerization. Gradient flow was controlled by a Teflon valve centered between two chambers containing polymer solution (0.1 g/ml PEG diacrylate and 10 μl/ml of 2,2-dimethyl-2-phenyl-acetophenone in *N*-

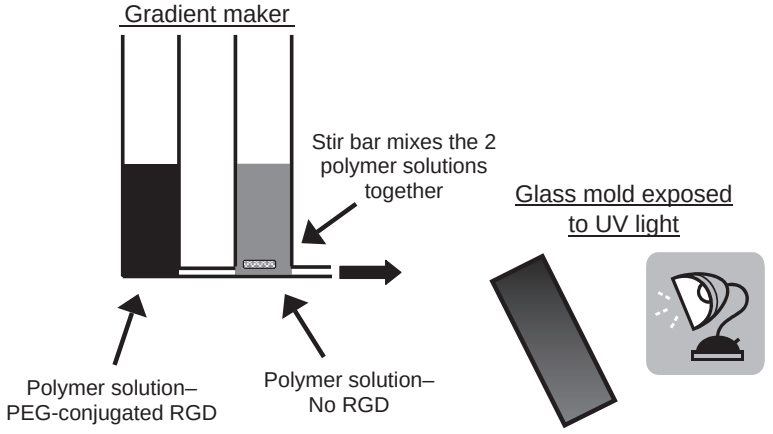

Fig. 1. Gradient hydrogels were formed using a gradient maker as depicted above in combination with a peristaltic pump to control the combination of polymer solutions with and without BSA. The resulting polymer solution is then pumped into a mold where it is exposed to UV light to stabilize the concentration gradient.

vinylpyrrolidone (300 mg/ml)) with or without BSA. The polymer solutions were slowly combined and pumped into a rectangular glass mold ($1.8 \times 5.3 \times 0.2$ cm thickness) and exposed to long wavelength UV light (365 nm, 10 mW/cm^2, 20 min) to form a hydrogel with a gradient of BSA. Coomassie brilliant blue staining solution (BioRad, Hercules, CA) was then used to detect the entrapped BSA gradient. Briefly, hydrogels containing a gradient of 0 to 2 and 0 to 1 mg/ml of BSA were rinsed in PBS (Sigma) for 1 h and subsequently stained with 1.5 ml Coomassie blue staining solution in 50 ml PBS. After 2 h of staining, the gels were scanned, converted to gray-scale, and then assessed for mean staining intensity at 0.5 cm increments using Image J (NIH, http://rsb.info.nih.gov/ij/). In Image J software, 0=black and 255=white, therefore higher concentrations are represented by a low number, while lower concentrations of BSA are represented by a high number.

2.4. Cell alignment on hydrogel surfaces with a gradient of tethered RGDS

A hydrogel with a gradient of tethered RGDS was formed using a gradient maker as described above. In this study, the gradient maker chambers contained polymer solution with and without the PEG–RGDS conjugate. Both polymer solutions contained 0.1 g/ml PEG diacrylate and 10 μl/ml of 2,2-dimethyl-2-phenyl-acetophenone in *N*-vinylpyrrolidone (300 mg/ml). The polymer solutions were slowly combined and pumped into a rectangular glass mold ($1.8 \times 5.8 \times 0.2$ cm thickness) and exposed to long wavelength UV light (365 nm, 10 mW/cm^2, 20 min) to form a hydrogel with tethered RGDS ranging from 0 to 1 μmol/ml (designated as 0–1). A control hydrogel was also formed from a polymer solution containing a constant concentration of the PEG–RGDS conjugate (0.75 μmol/ml). This control hydrogel contained the same concentration of PEG diacrylate and 2,2-dimethyl-2-phenyl-acetophenone in *N*-vinylpyrrolidinone as the hydrogel with a gradient of tethered RGDS. Cells were then seeded on the gels at a density of 10,000 cells/cm^2 and allowed to attach overnight. After 1, 2, and 4 days, hydrogel surfaces were photographed in several areas and assessed for cell alignment under phase contrast microscopy using Image J. Cell alignment was determined for each cell by measuring the cell's angle relative to the direction of the RGDS gradient. Cell angles were determined for at least 100 cells on each hydrogel surface, and then these angles were categorized into nine different groups based on their relative angle to the RGDS gradient. These groups included cells positioned relative to the RGDS gradient within $\pm 0^\circ$ to 10°, 10° to 30°, 30° to 50°, 50° to 70°, or 70° to 90°.

2.5. Cell migration on hydrogel surfaces with a gradient of tethered RGDS

As depicted in Fig. 2, cell migration was assessed on hydrogel surfaces with a gradient of tethered

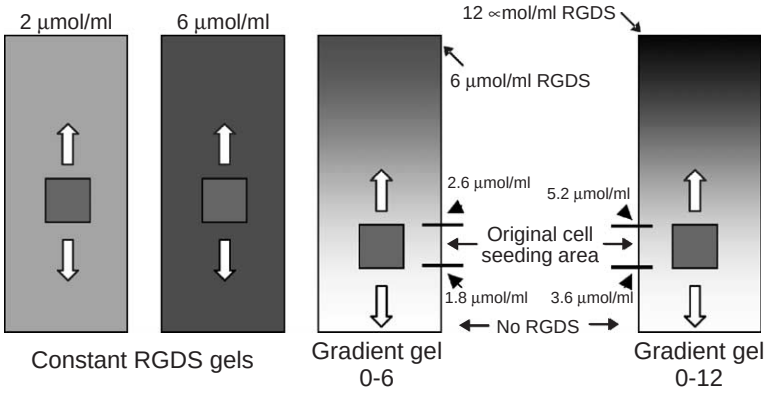

Fig. 2. This schematic depicts the setup for the gradient migration study. Fibroblast migration was monitored in the directions of increasing and decreasing RGD concentrations on each of the RGD-gradient hydrogels containing either 1 to 6 μmol/ml RGD (0–6 gradient) or 1 to 12 μmol/ml RGD (0–12 gradient). This migration was compared to migration on each of the control hydrogel surfaces (2 and 6 μmol/ml RGD).

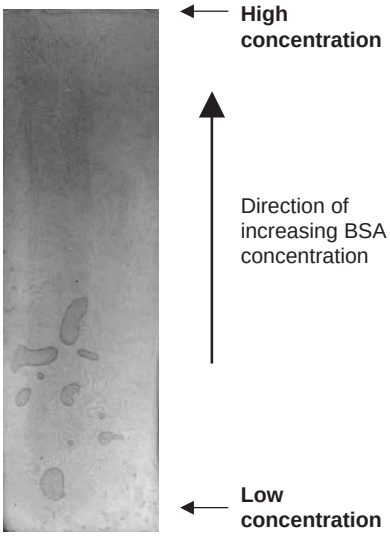

Fig. 3. Representative image of a BSA-gradient hydrogel stained with Coomassie brilliant blue. Staining intensity increased with increasing concentrations of BSA.

RGDS, 0 to 6 μmol/ml (0–6) or 0 to 12 μmol/ml (0–12). These gradient hydrogels were prepared similarly to hydrogels prepared for the cell alignment study. Fibroblasts were seeded within a square stainless steel fence ($7.7 \times 7.7 \times 10.2$ mm), such that the bottom outer edge of this fence ($12.7 \times 12.7 \times 10.2$ mm) was positioned 1.5 cm above the gradient hydrogel's low concentration (bottom) edge. Cell migration on this gradient surface was compared to cell migration on hydrogels with a constant concentration (2 or 6 μmol/ml) of tethered RGDS. For the 0–6 gradient hydrogel, the starting edge concentrations were ~2.6 and ~1.8 μmol/ml, respectively, for the top and bottom edges. For the 0–12 gradient hydrogel, the starting edge concentrations were ~5.2 and ~3.6 μmol/ml, respectively, for the top and bottom edges. These concentrations were determined by using the gradient slope, which depended on the RGD concentration range and hydrogel dimensions (slope (0–6) = 6 μmol/ml/5.8 cm = 1.034 μmol/ml/cm, slope (0–12) = 12 μmol/ml/5.8 cm = 2.07 μmol/ml/cm). Cells were allowed to attach and adhere to the hydrogels. After 27 h, the stainless steel fence creating the initial boundary was removed and the cells at this leading edge were photographed under phase contrast microscopy. Two days later, the cells were photographed again and the distance traveled by the cells was determined by comparing their resulting position to their initial position.

2.6. Statistical analysis

Data sets were compared using Minitab 14 (www.minitab.com) to perform a one-way analysis of variance (ANOVA) followed by post hoc comparisons using Tukey to determine which means were significantly different. In addition, two factors (time and cell angle) were analyzed using the General Linear Model to perform univariate analysis of variance on an unbalanced design. This two-factor analysis was followed by multiple comparisons using Tukey. p-values less than 0.05 were considered statistically significant. All values are reported as the mean and standard deviation of the mean.

3. Results

3.1. Detection of entrapped gradients of BSA

The gradient maker was used to form hydrogel scaffolds containing a gradient of entrapped BSA. Coomassie blue staining made the BSA gradient

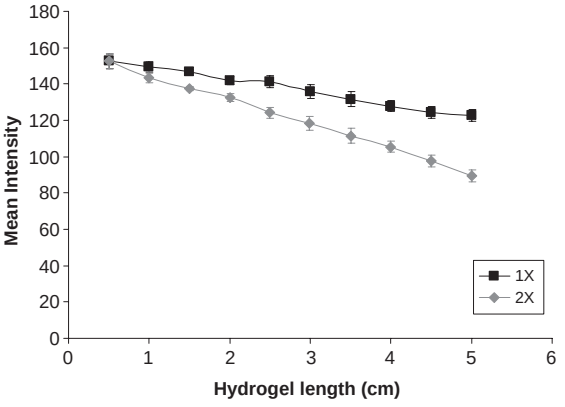

Fig. 4. Hydrogels with varying gradient slopes (0 to 2 and 0 to 1 mg/ml) were stained with Coomassie brilliant blue and then scanned and analyzed for mean stain intensity (in Image J software, 0 = black and 255 = white). The gradient slopes depended on BSA concentration: hydrogels with BSA ranging from 0 to 2 mg/ml had a slope that was ~2 times the slope of the hydrogel with BSA ranging from 0 to 1 mg/ml ($y = -26.63\mathrm{Ln}(x) + 143$, $R^2 = 0.90$ vs. $y = -13.58\mathrm{Ln}(x) + 149$, $R^2 = 0.88$).

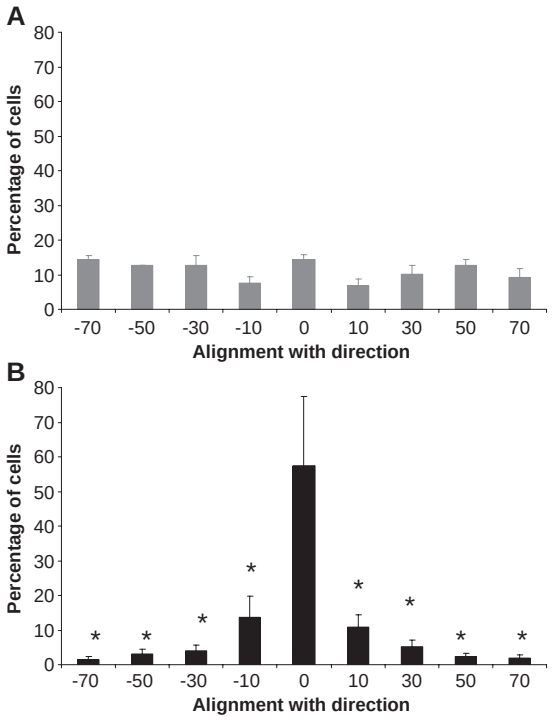

Fig. 5. A 0–1 gradient of tethered RGD influenced cell alignment after 24 h. More fibroblasts were aligned on the gradient hydrogel surface (B) in the direction of increasing RGD concentration than on the control hydrogel surface. Fibroblasts were randomly aligned on the control hydrogel surface, which lacked an RGD gradient (0.75 μmol/ml RGD, A). *$p<0.05$ compared to cells aligned with the gradient axis (within $\pm 10^{\circ}$).

visible so that it could be analyzed using digital image processing. As depicted in Fig. 3, the staining intensity increases in the direction of increasing BSA concentration. This trend was evident for both hydrogel scaffolds examined regardless of BSA concentration range. However, the steepness of the gradient slope depended on the concentration range of incorporated BSA. As shown in Fig. 4, the gradient slope for the hydrogel scaffold, containing BSA concentrations ranging from 0 to 2 mg/ml, was approximately two times steeper ($y=-26.63\mathrm{Ln}(x)+143$, $R^2=0.90$ vs. $y=-13.58\mathrm{Ln}(x)+149$, $R^2=0.88$) than the gradient slope for the other hydrogel scaffold where the BSA concentration ranged from 0 to 1 mg/ml.

3.2. Cell alignment on hydrogel surfaces with a gradient of tethered RGDS

Alignment of human dermal fibroblasts in response to immobilized gradients of the adhesion peptide, RGD, was examined and compared to cell alignment on hydrogels containing a uniform concentration of RGD. As shown in Figs. 5 and 6, fibroblasts aligned on 0–1 gradient hydrogel scaffolds in the direction of increasing RGD concentration. This alignment was evident on all regions of the RGD-gradient hydrogel within 1 day after seeding, but did not occur on control hydrogels with a constant concentration of RGD (0.75 μmol/ml). Further, cell alignment increased with time on the 0–1 gradient hydrogels. Maximum cell alignment, as shown in Fig. 7, was observed after 4 days where ~58% of cells aligned with the RGD-gradient axis ($\pm 10^{\circ}$) compared to ~46% of cells aligned with the gradient axis on day 1 ($p<0.05$). In comparison,

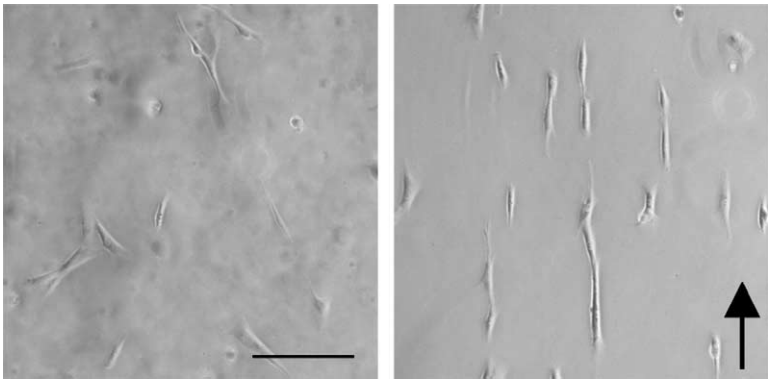

Fig. 6. More fibroblasts were aligned on hydrogel surfaces with a 0–1 gradient of tethered RGD (indicated by arrow) than on hydrogel surfaces containing RGD at a constant concentration (0.75 μmol/ml; bar=100 μm).

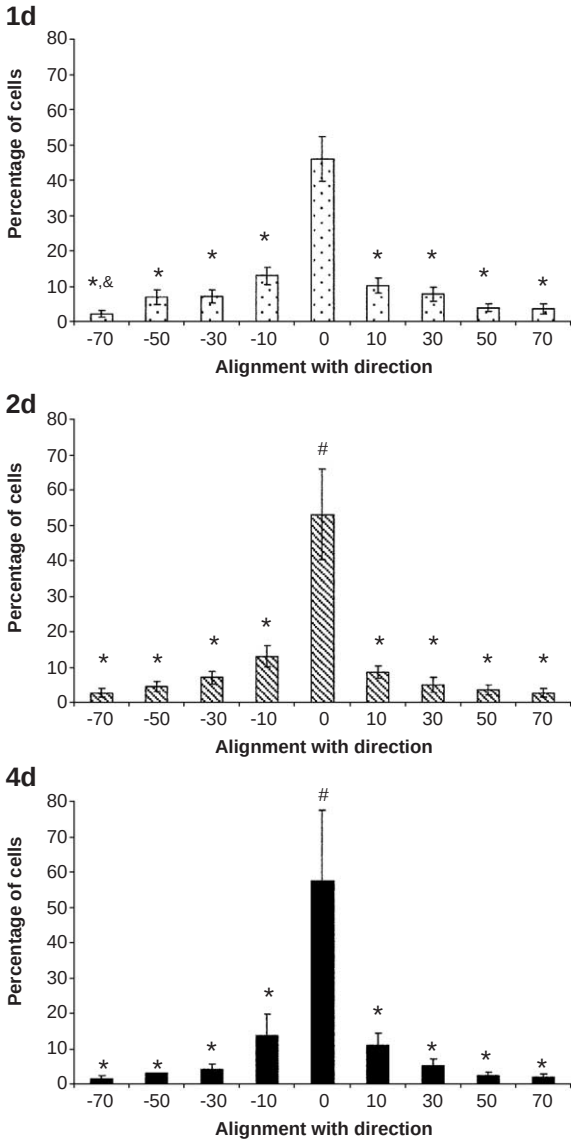

Fig. 7. Cell alignment was evident within 24 h (~46% of cells aligned relative to the gradient axis) and became more apparent with time on the 0–1 RGD-gradient hydrogel surfaces. Maximum cell alignment was observed after 4 days where ~58% of cells were aligned with the RGD-gradient axis. $^{*}p<0.05$ compared to cells aligned with the gradient axis. $^{\#}p<0.05$ compared to cells aligned with the gradient axis at 24 h. $^{\&}p<0.05$ compared to cells at 24 h with angles ranging from -10° to $+10^{\circ}$.

fibroblasts on control hydrogels were randomly distributed with cells showing no significant preference for alignment in any particular direction on these control surfaces ($p<0.52$).

3.3. Cell migration on hydrogel surfaces with a gradient of tethered RGDS

Fibroblast migration depended on the concentration of covalently incorporated RGDS on hydrogel surfaces. Fibroblasts migrated ~52% further on control hydrogel surfaces containing 6 μmol/ml PEG–RGDS than on hydrogel surfaces containing 2 μmol/ml PEG–RGD ($p<0.05$, Fig. 8). Fibroblasts also migrated further up the concentration gradient than down the concentration gradient. This RGDS-gradient effect was evident on both gradient hydrogel surfaces where fibroblasts on the 0–6 gradient surface (0 to 6 μmol/ml RGD) migrated ~48% further going up the concentration gradient than going down and fibroblasts on the 0–12 gradient surface (0 to 12 μmol/ml RGD) migrated ~218% further going up the concentration gradient than going down the concentration gradient. Fibroblasts migrated furthest up the 0–12 gradient hydrogel surface, although this migration distance was not significantly different from the control hydrogel surface with 6 μmol/ml RGDS. Fibroblast migration was reduced going down the 0–12 gradient hydrogel surface and was significantly different from fibroblast migration on all surfaces except for migration down the 0–6 gradient hydrogel

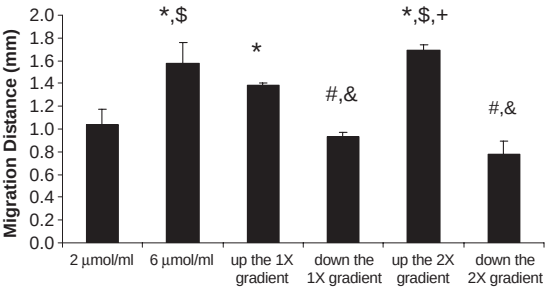

Fig. 8. Fibroblast migration depended on the concentration of RGDS. Fibroblast migration was increased on RGD-gradient hydrogels compared to control hydrogels with a constant concentration of RGD (2 μmol/ml). This migration response was further enhanced by increasing the gradient slope. In contrast, migration down the concentration gradient was decreased. $^{*}p<0.05$ compared to fibroblast migration on the control hydrogel surface containing 2 μmol/ml tethered RGD. $^{\#}p<0.05$ compared to fibroblast migration on the control hydrogel surface containing 6 μmol/ml tethered RGD. $^{\&}p<0.05$ compared to fibroblast migration up the 0–6 RGD-gradient hydrogel (1×). $\$p<0.05$ compared to fibroblast migration down the 0–6 RGD-gradient hydrogel. $^{+}p<0.05$ compared to fibroblast migration down the 0–12 RGD-gradient hydrogel (2×).

surface and migration on the control hydrogel surface containing 2 μmol/ml RGDS.

4. Discussion

PEG-based hydrogels are promising materials for use as tissue-engineering scaffolds. These hydrogels provide the opportunity to more closely control bioactive factor presentation due to PEG's hydrophilicity and the ability to incorporate covalently immobilized adhesion peptides [12,14,17,18]. Previous studies demonstrated that RGD-peptide incorporation enables fibroblasts, osteoblasts, and smooth muscle cells to attach and spread on PEG hydrogels [12–14]. Cell attachment and spreading on hydrogel surfaces depended on the bulk concentration of incorporated RGDS where increasing RGDS concentrations enhanced cell adhesion. Alternative peptide sequences have also been investigated including fibronectin-derived, REDV, and elastin-derived, VAPG, which support cell adhesion on protein resistant substrates [15,19]. Thus, hydrogel scaffolds are useful for controlling both the identity *and* concentration of adhesion peptide presentation. This study extends the capability for directing cell behavior on hydrogel scaffolds to include the spatial distribution of the RGDS-adhesion peptide.

Hydrogels with gradients of entrapped BSA were successfully formed by coordinating the use of a gradient maker to combine polymer solutions containing varying concentrations of BSA with photopolymerization of the resulting polymer solution. This system produced entrapped gradients of BSA that could be visualized by staining with Coomassie brilliant blue. Simply changing the relative BSA concentrations present in each of the gradient maker's chambers made it possible to control the range of BSA concentrations presented and the steepness of the gradient slope. This flexibility should be useful for optimizing the production of hydrogel scaffolds for particular applications where the incorporated peptide is presented at the appropriate concentration range and steepness. Since BSA was simply entrapped rather than covalently immobilized, it may also be possible to apply this system to three-dimensional applications where the hydrogel contains sequences susceptible to proteolytic degradation. That is, soluble proteins could be released from the hydrogel scaffold as cells secrete proteases that break down the hydrogel. Lutolf et al., for example, used a similar system to promote bone formation in critical-sized defects of rat crania [20]. PEG-based scaffolds containing MMP-sensitive linker substrates and entrapped bone morphogenetic protein-2 (BMP-2) recruited cells to infiltrate the scaffold and replace it with bony tissue. Perhaps, bone formation can be further improved in this system by controlling the three-dimensional presentation of the soluble growth factor. Three-dimensional gradients of BMP-2 can make this possible by enhancing cell infiltration into the hydrogel scaffold such that cells migrate up a concentration gradient of BMP-2.

Since staining with Coomassie brilliant blue confirmed the production of entrapped gradients of BSA in PEG-based hydrogel scaffolds, gradient hydrogels with covalently immobilized RGDS were also formed and examined for their effect on cell morphology. Cells responded dramatically to the presentation of an RGDS gradient by aligning with the gradient axis. This alignment was detected as early as 1 day after seeding where ~46% of cells aligned with the gradient axis (within $\pm 10^{\circ}$). Cells also remained aligned for at least 4 days indicating that the RGDS gradient had a sustained effect on cell behavior. In contrast, soluble gradient systems have demonstrated short-term morphological effects—24 h or less, which is too short to impact engineered tissue formation since significant cell migration into a scaffold requires several days [8,9]. An additional disadvantage of these soluble gradient systems is that they cannot be combined with the application of mechanical forces (i.e. shear stress and strain), which may be crucial for the formation of a variety of engineered tissues including cartilage, bone, and blood vessels.

In addition to altering cell morphology, RGDS-gradient hydrogels influenced cell migration. Cells migrated ~48% further up the concentration gradient (0 to 6 μmol/ml PEG–RGDS) than down the concentration gradient. Migration up the concentration gradient was also ~33% greater than migration on control surfaces with a constant concentration of PEG–RGDS (2 μmol/ml), while migration down the gradient was reduced ~12% relative to the control hydrogel surface (2 μmol/ml PEG–RGDS). Thus, immobilized gradients of RGDS can increase cell

migration on PEG-based hydrogel scaffolds. The application of this technology may be useful for directing endothelial cell migration on to vascular graft surfaces, which can result in enhanced endothelialization. Moreover, the incorporation of cell-specific adhesion peptides including REDV, which binds to the integrin $\alpha_4\beta_1$ on the endothelial cell, but not platelets and VAPG, which binds to smooth muscle cells, but not fibroblasts, may help further organize the regrowth of tissues by directing cell migration to specific areas [15,19].

Directed migration on PEG-based hydrogels was further enhanced in this study by increasing the RGDS gradient's slope. Migration up the 0–12 gradient hydrogel (0 to 12 μmol/ml) was ~23% greater than migration up the 0–6 gradient hydrogel (0 to 6 μmol/ml) and ~7% greater than migration on the control hydrogel surface modified with 6 μmol/ml PEG–RGD. Thus, the steepness of the gradient slope can enhance directional migration on PEG hydrogel scaffolds. However, it should be noted that there was no significant difference in migration between cells on the control hydrogel surface (6 μmol/ml PEG–RGDS) and cells moving up the 0–12 gradient surface. Perhaps, the gradient's effect would be more evident at longer time points (i.e. time points greater than 2 days) or on a surface with a steeper gradient. Additionally, there is a certain level of interference from neighboring cells using this experimental setup where migration of a population of cells is monitored. The gradient's effect on cell migration may be more obvious when cells are monitored individually using time-lapse video microscopy. Further studies using covalently immobilized gradients of RGDS can indicate a minimum gradient slope required for directed cell migration. Additional improvements in directed migration may also be possible by further increasing the gradient slope's steepness.

5. Conclusions

This study extended the capability for directing the interaction between cells and PEG-based hydrogel scaffolds to include control over the spatial distribution of RGDS. Covalently immobilized gradients of RGDS were formed by coordinating the use of a gradient maker to combine polymer solutions containing varying RGDS concentrations with photopolymerization of the resulting polymer solution in order to lock in the concentration gradient. Fibroblasts responded to the presentation of RGDS gradients by dramatically changing their morphology to align with the gradient axis and by migrating differentially in the direction of increasing RGDS concentrations. Further, these hydrogel scaffolds provide the flexibility to present different RGDS concentration ranges and present RGDS gradients at varying slopes in order to enhance directed cell migration. Thus, these hydrogel scaffolds may be useful for optimizing engineered tissue formation as these hydrogel scaffolds can potentially lead to improved cellularity and tissue organization via directed cell migration.

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