
Cellular alignment by grafted adhesion peptide surface density gradients

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Received 8 December 2003; revised 28 May 2004; accepted 14 June 2004

Published online 12 October 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30137

Abstract: The extracellular matrix and extracellular matrix-associated proteins play a major role in growth and differentiation of tissues and organs. To date, few methods have been developed that allow researchers to examine the affect of surface density gradients of adhesion molecules in a controlled manner. Fibroblasts cultured on surfaces with a surface density gradient of RGD peptide aligned parallel to the gradient while fibroblasts on constant density RGD surfaces spread but did not align as has been shown in numerous earlier studies. Not only did fibroblasts align on the gradient surfaces, but they also showed significantly greater elongation than on constant density peptide surfaces or on control surfaces. This type of method is easy to replicate and

can be used by laboratories interested in investigating alignment of various cell types without mechanical force or other stimulation, and without cell–cell interaction or for investigation of affects of surface density gradients of molecules on cellular biochemistry and biophysics. This method also has potential applications for developing scaffolds for tissue engineering applications where cellular alignment is necessary. © 2004 Wiley Periodicals, Inc. *J Biomed Mater Res* 71A: 403–411, 2004

Key words: fibroblast; surface interaction; tissue engineering; polymer; haptotaxis

INTRODUCTION

The interaction of cells with materials is related to the properties of the surface on which the cell is grown.¹ Control of the location of cellular growth² and cellular response^{3,4} can be accomplished by adjusting surface chemistry. Polymeric coating of surfaces has become accepted as a method of creating passive surfaces with little protein adhesion, and thus little cellular adhesion if proper polymers are chosen.^{5,6} This allows specific cell adhesion for the analysis of specific proteins. To investigate the interaction between cells and their environment, the extracellular matrix (ECM) selective addition of cell binding molecules can be added to polymer-grafted surfaces.^{7,8}

The ECM plays a powerful part in the life cycle of the cell, providing cell anchorage, guidance during development, cues for cell morphology, as well as

transmitting environmental signals to the cell. Cells interface with the ECM through a variety of cell adhesions via ligand–receptor binding.^{9,10} The majority of these adhesions are mediated by a class of receptors known as integrins, which bind to many ECM components, including fibronectin, vitronectin, laminin, and various collagens.¹¹ The integrin subunits create adhesion sites only on specific peptide sequences within proteins. For example, the tripeptide arginine-glycine-aspartate (RGD) derived from fibronectin and other proteins is a primary recognition site for cell adhesion and attachment.¹²

Previous studies have shown that covalently bound matrix peptides can enhance cell attachment and motility.⁸ Specific spacing of adhesion peptides, as well as peptide surface density, has been shown to greatly influence the adhesion of cells on surfaces.^{7,13} Other studies have shown that patterning these molecules may allow researchers to control cellular behavior and localization.^{14–18} Each of these studies has shown that the intracellular biochemistry is affected by the adhesion of cells to the surface. The change in morphology of the cells is one of the most significant effects in the binding of cells to adhesion molecules. The typical morphologic responses have been uniform spreading or migration of cells on the surface.¹⁹ It is anticipated

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Contract grant sponsor: Rockefeller Brothers Fund, Charles E. Culpepper Biomedical Pilot Initiative (awarded to R.A.G.)

Contract grant sponsor: Sigma Xi, Grant-in-aid (to R.A.G. and C.E.K.)

that cellular attachment to materials may not always be in a uniform manner, depending upon the surface distribution of adhesion molecules.

Gradients of surface chemistry have been widely investigated,^{20,21} but gradient patterns of biologic molecules have not been well described. Gradient patterns of biologic molecules are suggested to be the directing force for migrating cells *in vivo* and also may play a role in attachment. The exact roles of surface density gradients of adhesion molecules are unknown due to a lack of efficient and well-defined methods for producing gradients. Methods for producing surface bound gradients of proteins and peptides have been few in the literature. Of these, micropatterning has been conducted using UV irradiation,^{21,22} electrochemistry,²³ plasma polymerization,^{24–26} and microfluidic systems.^{27,28} These systems have been successful on the small scale, however, larger scale studies are not possible with these methods. Other methods for examining surface density gradients of molecules have been identified, but these methods have not been widely accepted.^{29–34} For these reasons, alternate methods for the production of well-defined and reproducible surface density gradients of adhesion molecules are desired. In this article, a novel method for reproducibly forming immobilized surface density gradients on polymer surfaces is presented. The morphology of NIH/3T3 fibroblasts on substrates with surface density gradients of grafted adhesion peptides was analyzed and compared to equivalent surfaces with fixed peptide density and cell adhesive materials. Further research in this area is ongoing and will be applied to various biologic systems of interest. This work suggests that this method can be used to reproducibly examine the response of various cell types to any surface bound molecule. The methods can also be easily developed for examining interactions of cells with three-dimensional materials and gradients of molecules in three dimensions.

MATERIALS AND METHODS

Preparation of gradient materials

All chemicals were purchased from Fisher Scientific unless otherwise noted. Glass microscope slides were cleaned and oxidized in chromic/sulfuric acid for 1 h, then serially rinsed three times each in double distilled water, ethanol, and, finally, chloroform. Each slide was then dipped in a solution of 3% 3-acryloxypropyl trichlorosilane (AcPTS, United Chemical Industries Inc., Bristol, PA) in chloroform. The chemistry of AcPTS modification is presented in Figure 1.³⁵ The slides were serially rinsed in chloroform and ethanol before being cured overnight in a vacuum oven at 25°C. Polymerization chambers were produced with a silicone gasket (0.79 mm) placed between a cleaned and rinsed mi-

croscope slide and a silane-treated slide with the silane-treated glass slide placed farthest from the UV source.

Peptides were produced by solid phase synthesis at the University of Illinois Protein Research Laboratory. Acryloyl functionality was incorporated in peptides at the N-terminus using acryloyl succinimide during the solid phase synthesis, the peptide used in these studies being acryloyl-YRGDK(fluorescein)G (aRGD) (Fig. 2). All peptides were analyzed immediately after synthesis by mass spectroscopy and high pressure liquid chromatography (HPLC) to determine purity of at least 95% (data not shown). An aqueous solution of 30% acrylic acid with a dimethyl sulfoxide solution of 2,2-dimethoxy-2-phenylacetophenone (DMPA) was prepared and mixed to make a solution containing 0.1 mol % of DMPA to acrylic acid. An identical solution was made with one additional ingredient: aRGD. Therefore, each solution had equivalent molar concentrations of the initiator, DMPA, monomer; one solution contained aRGD at a concentration of 37.5 μM .

A gradient of monomers was formed using a precision gradient pump (Hitachi) which fractionally mixed two solutions, acrylic acid and acrylic acid/aRGD, while filling the polymerization chamber at a flow rate of 1.0 mL/min. Surface density gradients were formed by programming the pump to mix the two solutions at prescribed ratios as is typical with gradient elution for HPLC. Uniform surface density surfaces were produced by programming the pump to fill the polymerization chamber with only the aRGD/acrylic acid solution. As the monomer solutions filled the polymerization chamber, a 365-nm lamp (≈ 2.5 mW) initiated the polymerization of the graft polymers (Fig. 2). The UV lamp was filtered to exclude emission above 500 nm and below 300 nm. Polymer formed between the slides was washed thoroughly with 0.22 μm filtered phosphate buffered saline (PBS) for approximately 3 d, changed at least twice daily. Only polymer that was grafted to the surface of the glass slide was retained following the wash.

To confirm all modification steps, X-ray photoelectron spectroscopy (XPS) was utilized. Samples were formed on silica wafers that possessed a 10 Å silica-oxide layer. Silica was washed repeatedly in methylene chloride. All modifications were conducted as described with glass slides after being cut to the appropriate size. Samples were rinsed thoroughly in distilled water and dried *in vacuo* prior to examination (Kratos Axis Ultra XPS) in survey and high-resolution scans.

Assessment of gradient profile

Modified surfaces were produced as described above with minor modifications. A crosslinker, ethidium bromide-*N,N*-bismethacrylamide (EtBrBis), was added to aRGD/acrylic acid monomer solutions and the crosslinker, *N,N*-methylene-bisacrylamide (BIS), was added to the acrylic acid solution. In these studies, methyl trichlorosilane coated glass was chosen instead of AcPTS coated glass to allow hydrogels to be easily separated from the glass surfaces. These gels were then allowed to swell to maximum size in 0.22 μm filtered PBS with repeated changes for at least 3 d. Fluorescence detection along the entire gel was performed at an

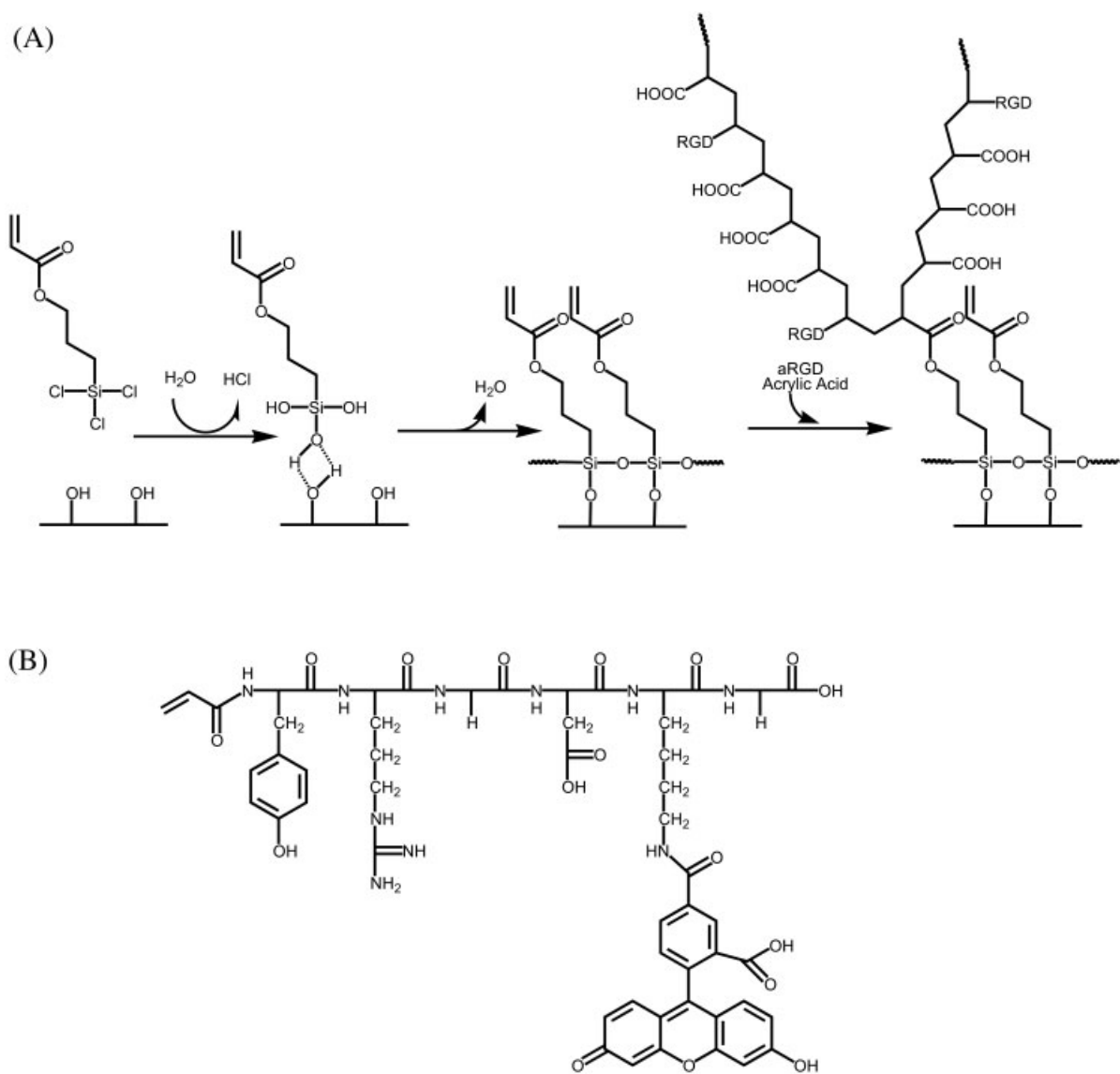


Figure 1. Schematic representation of silane coating on glass slides (A). Silicon oxide reacts with the 3-acryloxypropyl trichlorosilane to form a surface that contains polymerizable acryloyl groups. The graft polymer may be in the form of a single polymer chain initiated at the surface or two chains derived from a solution-initiated polymer that interacts with the functionalized surface. The abbreviation RGD in the polymer indicates peptide acryloxy-YRGD(fluorescein)G. (B) The structure of the functional peptide utilized in these studies, aRGD.

excitation wavelength of 635 nm with an emission wavelength of 650 nm using Storm 860 Phosphorimager (Molecular Dynamics). Data was analyzed using Image Quant software.

Cell culture

Several control surfaces were used to compare cellular response to the surface density gradient of the adhesion molecule. AcPTS-coated surfaces were used as cell adhesive surfaces. Due to the design of the polymerization chamber, a section of the AcPTS coated glass slide was not grafted with poly(acrylic acid) or poly(acrylic acid-co-aRGD) (Fig. 2). This area of AcPTS could be used as an internal positive

adhesion control on each slide. To identify how the grafted polymer affects cellular spreading or adhesion, poly(acrylic acid) was grafted on silane-coated slides with no peptide present. Also, slides with a constant density of grafted aRGD were used to confirm that specific cellular response was indeed due to the concentration gradient. Only the maximum density aRGD content surfaces were examined in these studies.

Each of these modified glass surfaces was secured to sterile tissue culture dishes using high vacuum grease. NIH/3T3 fibroblast cells were then plated on these surfaces at 1×10^3 cells/mL under aseptic conditions in Dulbecco's modified Eagle's medium supplemented with 10% calf bovine serum, 20 IU/mL penicillin, and 20 μ g/mL streptomycin. The cells were then incubated at 37°C and 5% CO₂ for 72 h. Substrates were rinsed with sterile Dulbecco's PBS before obtaining images at

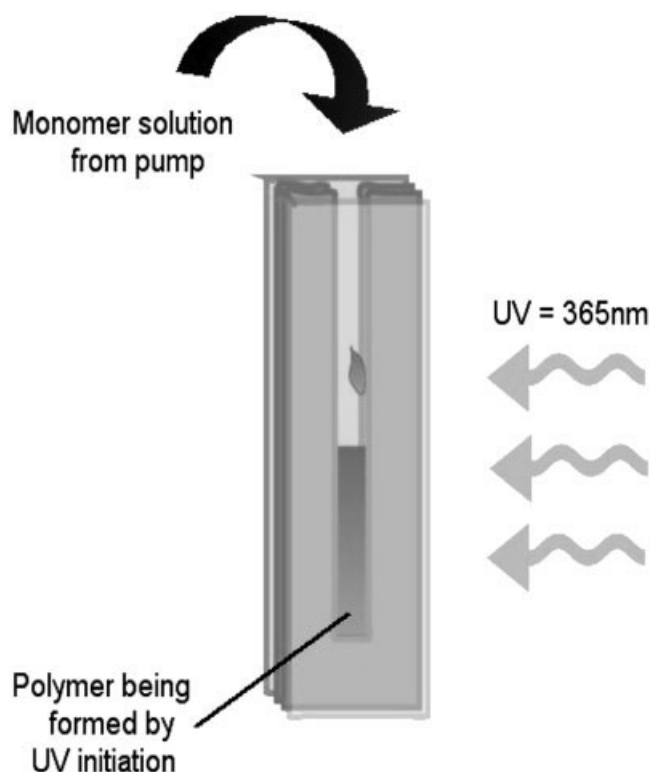


Figure 2. Schematic representation of the polymerization apparatus. A gradient of monomer solution was pumped into the polymerization apparatus while a constant (≈ 2.5 mW) light source was used to polymerize the polymer.

24 and 48 h. Morphology of adherent cells was determined using an Olympus IX-70 inverted microscope and captured using a Retiga 1300 CCD camera.

To ensure that cellular spreading was not due to intercellular interactions, cells in contact with other cells were not included in the analysis. The angle of the cells relative to the axis of the gradient was measured, as well as cell length for eligible cells. Cell length was measured as the long axis of an ellipsoid that would completely encompass the cell. Cells that appeared spherical with no protrusions were designated to be not responding to the surface, and were not measured but counted. Image analysis was performed using Scion Image software.

Statistical analysis

Statistical analysis was performed for both the cell length and cell angle using ANOVA with Tukey's posthoc test. All experiments were replicated at least three times and at least 200 cell measurements were conducted for all samples ($n > 200$).

RESULTS AND DISCUSSION

Assessment of gradient profile

Each level of surface modification was assessed using XPS. The original silica wafers possessed a minor

carbon peak due to surface contaminations (Fig. 3). Following silane modification, substantial carbon and oxygen peaks are confirmed. Poly(acrylic acid) is present on the surface following the polymerization process. In the dry state, the poly(acrylic acid) layer is on the order of 10 nm as confirmed by a significant decrease, but not complete removal, of the silicon signal. A significant increase in nitrogen is observed for surfaces containing peptides. These data confirm that all surface modifications are as described in our model (Fig. 1). Although this data does not confirm the configuration of our polymer, earlier studies using acryloxysilane confirmed that the acryloyl groups are predominantly incorporated in the polymer randomly.³⁵

The gradient profiles derived from fluid elution indicate that a linear concentration gradient was achieved and was reproducible. The gradient of fluorescent peptide or crosslinker was simple to evaluate, and even visual observation was sufficient to determine that the gradient was maintained following photopolymerization. There was some difficulty with obtaining consistent data when incorporating fluorescent peptides. For this reason, the fluorescein in the peptide was only used for qualitative assessment. Ethidium bromide dimethacrylate was therefore used because it exhibited little photobleaching when incorporated

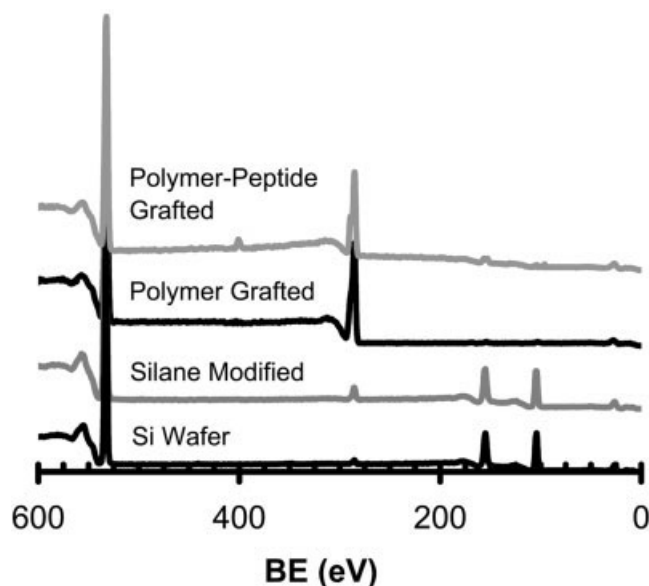


Figure 3. X-ray photoelectron spectroscopy survey scans of a clean silica wafer, acryloxy propyl silane modified wafer, poly(acrylic acid)-acryloxy propyl silane modified wafer, and acryloxy propyl silane-poly(acrylic acid)-acryloyl RGD peptide modified wafer. The relative intensity to the largest peak demonstrates the surface is silica oxide before modification and very little contamination. An increase in the carbon peak (≈ 286 eV) is noted after acryloxy propyl silane treatment. After polymerization, a thick layer comprised of only carbon and oxygen is generated; silicon is no longer detected due to the polymer on the surface.

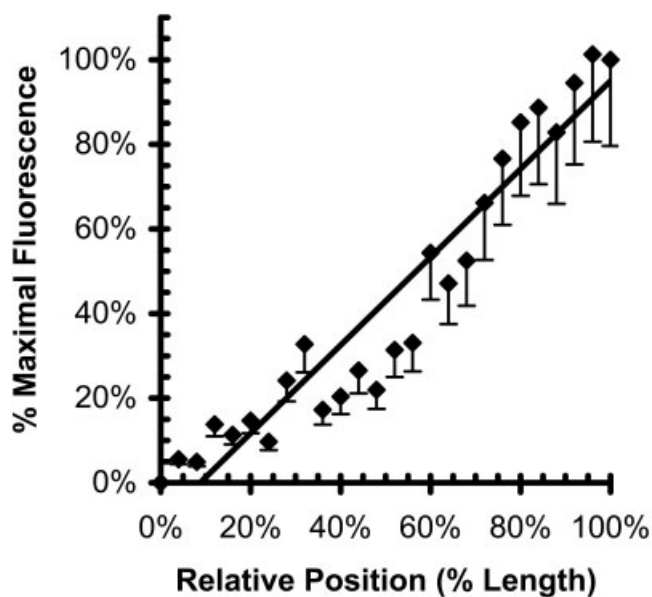


Figure 4. Gradient composition of the surface formed as found by incorporation of ethidium bromide dimethacrylate into hydrogels formed by the gradient method. The relative composition of the surface (hydrogel) increases linearly with feed composition of fluorescent dye. Fluorescein incorporated into the peptide was not used to assess incorporation due to photobleaching during polymerization. Ethidium bromide was not significantly bleached during the polymerization.

into a hydrogel (Fig. 4). The swollen size of the hydrogels was much greater than the original surface; thus, the relative position in the hydrogel was used. Some diffusional broadening of the gradient was expected and did occur; however, the diffusion of the molecules did not influence the linearity of the gradient. In fact, the diffusional process is equivalent to that in typical HPLC, but the length of the flow is greatly reduced when compared to typical HPLC since no detectors or columns were used and minimal tubing with a small radius was employed. Diffusion did not affect the lowest and highest concentrations on the surface because the active gradient range was completed within the length of the polymerization chamber. The viscosities of the monomer solution was only slightly greater than that of water (≈ 1 cP) prior to polymerization, but upon UV irradiation, the viscosity increased to more than 75,000 cP within 30 s of irradiation. This allowed formation of a short, defined segment of no grafted RGD peptide at one end of the surface, and a short, defined segment containing the highest density of grafted RGD at the other end of the surface.

In several studies, the polymer was formed with a crosslinking agent to produce a three-dimensional hydrogel. In these studies, it was determined that greater than 80% of aRGD was incorporated into the polymer and the gradient was maintained. Hydrogels formed with EtBrBis maintained an identical profile to that

shown in Figure 4, which was maintained for days following production, even when placed in buffered saline. This simple method for producing gradients of small peptides or small molecules can be accomplished in many laboratories because the apparatus is readily accessible to many laboratories. The polymerization of the hydrogel is well established; however, the monomers chosen for this study may not be appropriate for cellular incorporation studies. The monomers, acrylic acid and aRGD, were chosen to allow a random incorporation of high density aRGD. Acrylic acid monomer is toxic at a relatively low concentration for many cells, thus the focus of these studies was surface cell growth. Current studies are focusing on appropriate polymers that would allow entrapment of cells and determination of three-dimensional effects of the gradients on different cell types. These gels can easily be produced as described above, and cells could be incorporated in the monomer mixture if appropriate monomers and macromers are chosen.^{36,37}

Cell culture

Microscopic evaluation of cells on all surfaces revealed substrate-dependent cell morphologies. Fibroblasts exhibited typical morphology, polygonal bodies, when cultured on glass microscope slides or on AcPTS surfaces with little or no difference in growth rate or shape [Fig. 5(a), above dashed line]. The attachment of fibroblasts to glass and AcPTS is through adsorption of serum proteins. It is possible that serum proteins also play a role in attachment to aRGD and gradient aRGD surfaces; however, the end result of cell alignment has not been described in systems that use surface adsorption of serum proteins. As expected, fibroblasts cultured on poly (acrylic acid) grafted surfaces were characteristically spherical with few or no protrusions, indicating little attachment to the surface [Fig. 5(a), below line]. Cells appeared to be predominantly dead on these substrates. Fibroblasts cultured on acrylic acid polymerized surface that did not have the AcPTS modification responded in a manner identical to cells cultured on the unmodified glass slides, suggesting that the glass had not been grafted with poly(acrylic acid) and that all poly(acrylic acid) had been washed from the surface. Fibroblasts cultured on surfaces grafted with an aRGD density gradient exhibited preferential alignment parallel to the axis of the concentration gradient. Particularly noticeable on these substrates, cells typically had two long, thin projections, each in opposite directions from an elongated cell body [Fig. 5(c)]. Conversely, fibroblasts on substrates with constant peptide concentration had multiple protrusions that were shorter, and cells did

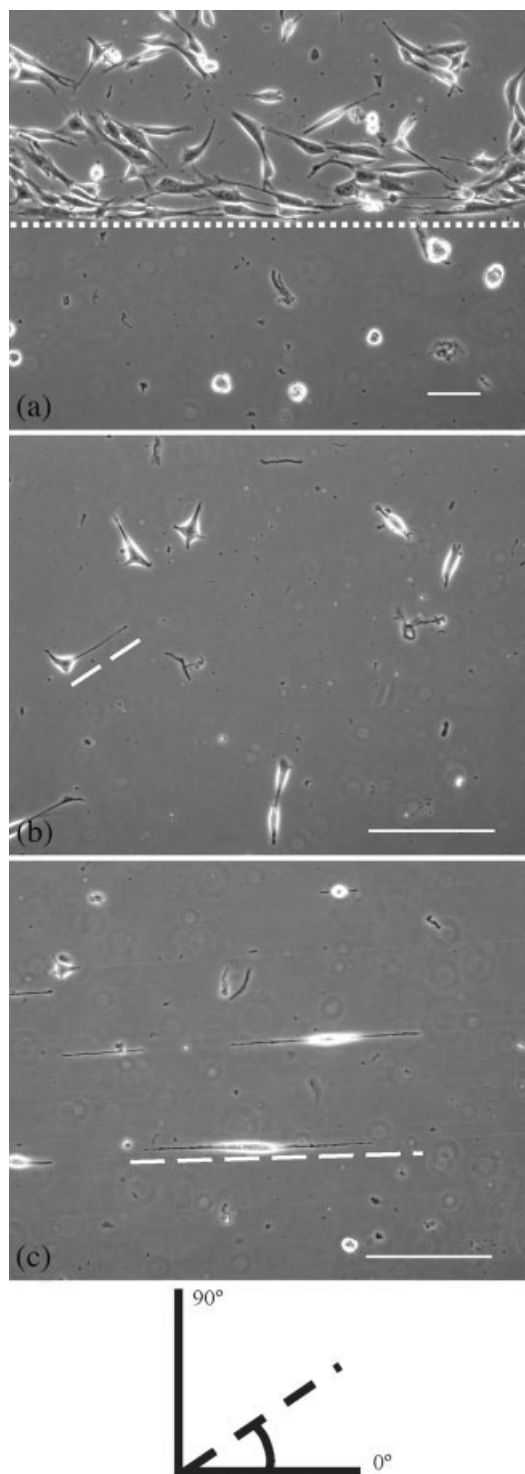


Figure 5. Light micrographs of NIH/3T3 fibroblasts cultured for 24 h on (a) acrylic acid modified surface below the line and silane treated but not acrylic acid grafted surface above the line, (b) constant RGD density surface, and (c) linear RGD gradient surface. A schematic of the angle that was used to determine angle of orientation of the cells. The dashed white line in (b) and (c) are representative lines for the constant RGD density and linear RGD gradient measurements.

not demonstrate a directional preference, as shown in Figure 5(b). The orientation of fibroblast spreading was found to be aRGD density dependent.

These results indicate that directional cellular spreading seen on the substrates with a surface density gradient is not mediated by the polymer, or simply by the presence of an adhesion peptide. In fact, no other system using gradient of biologic molecules has identified this spreading phenomena despite reports of gradients of hydrophobicity^{38,39} or adhesion peptides or proteins.^{40,41} The peptide gradient appears to influence cells to preferentially spread along the gradient. In these studies, cellular morphology is defined as spreading; however, it is possible that the cells are in fact migrating or exhibiting haptotaxis. This possibility is unlikely because data presented is from 24 h after initial culture.⁴² This system would be an excellent method for examining haptotaxis of any cell type in a two-dimensional system and with slight modifications in three dimensions. Most current methods for examining haptotaxis utilize one of several methods for producing a solution gradient of molecules.^{43,44} Because in these previous studies the molecule of interest is, in fact, in solution, the chemotactic response cannot be separated from the haptotactic response. The methods proposed here could be used to assess the pure surface density component of migration independent of or in combination with solution concentration.

In other studies, a similar method was used to produce gradients of molecules on hydrogel surfaces. A similar molar concentration of RGD groups were examined; however, no elongation of cells used was observed even with a similarly derived fibroblast cell line.³² This could be due to the fact that the cells were examined after 3 to 6 days following seeding. This could also suggest that cell movement may still be playing a role in cell morphology after 1 or 2 days but this is not expected. The comonomer in the previous studies was acrylamide, while in our experiments acrylic acid was used. The cellular response to these copolymers may affect morphology, but this has not been confirmed. The only other difference between these studies was the substrate for cellular growth. In the studies by Schnaar and colleagues, a true hydrogel was used, while we utilized grafted hydrophilic polymers.³¹⁻³⁴ The mechanical forces exerted by different surfaces on the cells plays an important role in morphology. By creating a more rigid surface, our results may be greatly influenced by the mechanical forces on cells.^{45,46} The difference in cell morphology between these studies needs further investigation, but the difference in morphology is clear and reproducible.

The alignment of fibroblasts on the grafted gradient surfaces was significant when compared to constant density surfaces (Fig. 6). When a gradient of aRGD is present, greater than 55% of fibroblasts are aligned

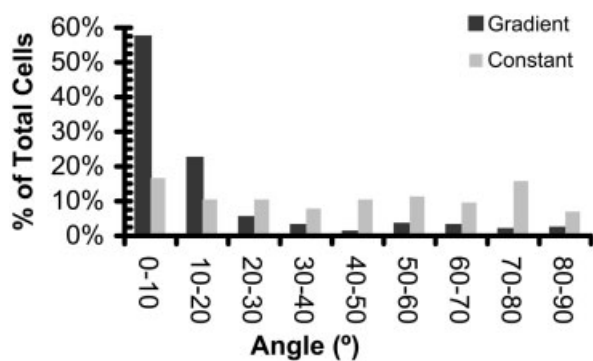


Figure 6. Angular distribution of NIH/3T3 fibroblasts orientation on constant RGD surfaces (white bars) and linear RGD gradient surfaces (black bars). All cells from all experiments are included, so no standard deviation is present.

within 10° of the gradient and approximately 80% are aligned within 20° . It is impossible to determine a “front” or “back” to the cells, so all cells were between 0° and 90° to the gradient. Fibroblasts cultured on a constant aRGD surface density had nearly equivalent proportions of cells oriented in any direction, and it was significantly difficult in many cases to determine a longest dimension because the cells had equally long projections in nearly every direction, so the major or long axis of an ellipse encompassing the entire cell was used to determine length and orientation. Nearly every cell cultured on the grafted RGD density gradient had a distinct elongated morphology that made identification of angles simple [Fig. 5(c)]. There was a small population of fibroblasts that did not orient to the gradient, but this fraction was small and may include several cells that were not adherent or were dead because our assay did not eliminate dead, elongated cells. Cells were washed from the surface by gentle aspiration with media; therefore, the number of dead or nonadherent cells should be minimal.

Alignment of cells, fibroblasts in particular, by haptotaxis or chemotaxis may play some role in several situations. One such situation is the development and organization of ligament.⁴⁷ The primary events in alignment in developing ligament and tendon is cellular alignment after cells have migrated into the developing tissues.⁴⁸ Cell alignment is followed by collagen production and alignment without any significant mechanical force exerted on the tissue.⁴⁹ In gingival ligaments, fibroblasts align during ligament development and contribute to the stability of the tissue. To date, no method for surface alignment of fibroblasts, without mechanical force,⁴⁵ has been proposed. If properly designed, this type of system could be applied to production of ligament repair mechanisms that would allow seeding low densities of fibroblasts that would spontaneously align, prior to implantation without mechanical force. Although this is somewhat speculative at this point, the applicability of

this method of aligning fibroblasts and other cells should not be overlooked.

Not only are the fibroblasts aligned, but the cells are typically longer than typical adherent fibroblasts. The long axis of the cell, used to determine the length, was measured from micrographs. Fibroblasts on glass, AcPTS treated surfaces, and constant aRGD density had projections that occasionally extended to nearly $20\ \mu\text{m}$, but the average length of the cells was $8.7 \pm 3.9\ \mu\text{m}$. Fibroblasts cultured on the aRGD surface density gradient were shown to extend to more than $40\ \mu\text{m}$, and the average cell length was $12.6 \pm 6.2\ \mu\text{m}$ (Fig. 7). This result is not in perfect agreement with a chemotactic/haptotactic assessment of fibroblasts grown on two-dimensional surfaces that restricted growth to one dimension.⁵⁰ The difference in observations could be due to the inability of the previous studies to separate the chemotactic from the haptotactic response of the cells. A second possibility for the inconsistency in observation could be cell type. In the previously reported studies, fibroblasts lines AGO1523 and M19 were compared to epitheliocytes. These two fibroblast lines may have different surface interactions. The most significant difference was that the fibroblasts in these previous studies did not experience a surface density gradient of adhesive molecules. It is possible that the localization of integrin adhesion may orient the actin-myosin network of the cell in a way that allows extension. Unfortunately at this point, this idea cannot be confirmed and more investigation is warranted. It is also possible that the cells are orienting in more than one direction; the third dimension of height was not explored in these studies, or in earlier studies.

Not surprisingly, the length of the fibroblast correlated well with the alignment of cells on the density gradient substrates (Fig. 8). Fibroblasts over $20\ \mu\text{m}$ in length, some as long as $40\ \mu\text{m}$, were oriented within 10° of the gradient. Fibroblasts that did not extend on the surface density gradient surface still had a higher orientation tendency, but the trend was not as significant for cells that had not elongated. Fibroblasts cultured on the constant aRGD density surfaces did not exhibit any correlation between the length and the angle of orientation. Because greater than 50% of the cells exhibit an orientation when cultured on the RGD density gradient surfaces, these surfaces can be considered to be an excellent model for assessment of cellular response to surface density gradients of adhesion molecules. It is possible to examine other small molecules in this way, as long as the chemical modifications do not alter the activity of the molecules. This method could also be utilized for more complex molecules, such as proteins, but the relatively harsh polymerization technique may alter the tertiary structure of macromolecules. Also, the chemical modification of proteins may interfere with activity. Small peptides are known to undergo little alteration by chemical

modification, but proteins are significantly more unstable and sensitive. Care must be taken if this method is advanced for protein–cell interactions.

CONCLUSIONS

A precise, controllable method for the production of a surface bound adhesion factor density gradient is presented. These studies suggest that surface bound concentration gradients play a significant role in controlling cellular spreading and morphology of fibroblasts. This method is reproducible, relatively inexpensive, and can be replicated in nearly any laboratory. Although these *in vitro* studies may not be indicative of the exact content of the extracellular matrix, this method is significantly better than many methods of assessing cellular morphology changes due to a density gradient of surface molecules. These surfaces can be used for biochemical and biophysical studies on any cell type to determine the effect of two-dimensional gradients. Although preliminary in nature, the EtBrBis hydrogels indicate that three-dimensional scaffolds can be designed using this method and could potentially be developed for analysis of cellular response in three dimensions.

In the present studies, the focus was primarily on development of a cellular assay system. This type of system could also be utilized for orienting cells in a tissue engineering approach. The orientation of fibroblasts in the tendon was mentioned as a possibility, although several other systems are also being considered. Development of complex tissues is dictated by controlling the three-dimensional density of ECM components and soluble factors and the control of both soluble and insoluble factors will allow us to more effectively design materials that can be used to regenerate tissues and organs that are functionally equivalent to the native tissue.

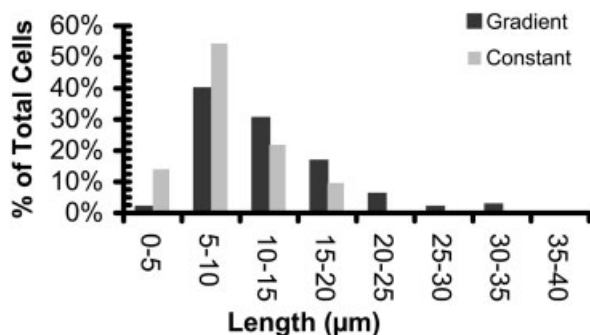


Figure 7. Length distribution of NIH/3T3 fibroblasts that spread on constant RGD surfaces (white bars) and linear RGD gradient surfaces (black bars). All cells from all experiments are included, so no standard deviation is present.

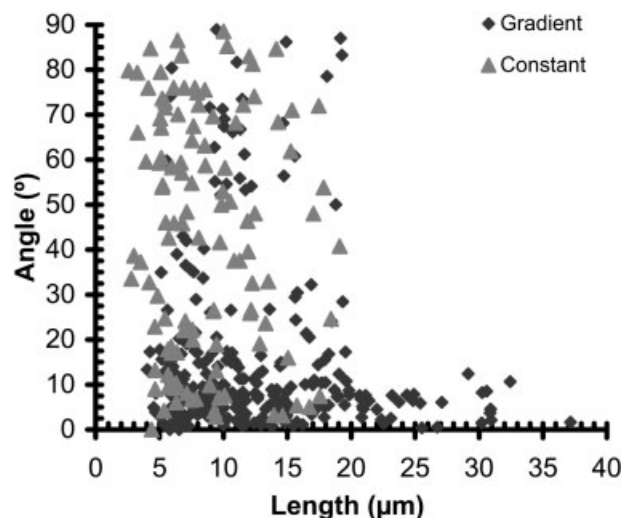


Figure 8. Relationship between the orientation (°) and length (μm) of NIH 3T3 fibroblasts on a constant RGD surface (▲) and a linear RGD gradient surface (◆).

Our thanks to Samir Shah for technical assistance and Richard Haasch, PhD, for assistance with XPS analysis. XPS was carried out in the Center for Microanalysis of Materials, University of Illinois, which is partially supported by the US Department of Energy under grant DEFG02-91-ER45439.

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