

Three-dimensional chemical structures by protein functionalized micron-sized beads bound to polylysine-coated silicone surfaces

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Abstract: A novel method is described here that allows three-dimensional (3D) control of both chemistry and morphology by a series of wet chemical steps: the attachment of protein functionalized micron-sized beads onto a flat silicone surface that has been functionalized with a distinct chemical modification. Bovine serum albumin (BSA), laminin, or polylysine is covalently bound to 6.5- μm -diameter spherical beads. A chemical method is then used to bind these beads to a flat silicone surface that is subsequently functionalized with polylysine. This process leads to a non-specific cell adhesive background on the flat surface (polylysine) with the option of differing chemistry on the third-dimension due to the protein BSA or laminin on the bead

protruding from the surface. The beads do not detach during cyclic stretching *in vitro*. Neo-natal rat cardiac fibroblasts are cultured on the beaded surfaces and compared with fibroblasts cultured on nonbeaded, flat polylysine surfaces. Fibroblast plating density, integrin, and physical responses are examined as a function of varying the ligands on the beads. © 2005 Wiley Periodicals, Inc. *J Biomed Mater Res* 00: 000–000, 2005

Key words: protein functionalized micron-sized beads; bovine serum albumin (BAS); laminin; polylysine; silicone surfaces

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INTRODUCTION

Cell growth, adhesion, migration, and secretion and gene expression are influenced by both the chemistry and three-dimensional (3D) organization of the extracellular matrix and neighboring cells in their local environment.^{1,2} Artificial constructs have attempted to make this 3D organization for embryonic regeneration,³ muscle cell organization in heart tissue,^{4–7} and other applications.^{2,8} The ability to produce chemical, physical, or mechanical cues on a cell culturing substrate is of importance not only for therapeutic applications, but also to understand cellular processes.

Various studies have employed synthetic micron and nanometer scale structures on surfaces to guide cellular behavior.^{2,9} There are several well-known strategies to synthetically produce micron scale two-dimensional (2D) features, including photolithography and soft lithography such as microcontact print-

ing,¹⁰ laminar flows,¹¹ and microstamping on an activated polymer surface.¹² 2D chemical patterns, such as cell adhesive proteins on a non-cell adhesive background,^{12,13} are effective models for studying cellular behavior. 3D projections have also been used to guide cell growth. Polydimethylsiloxane (PDMS or silicone) polymer surfaces with micron-sized 3D projections produced by photolithography were found to guide myocyte growth.^{4,5} However, improved understanding of cellular behavior from *in vitro* cell culturing systems requires both cell responsive, micron-sized, 3D features, and spatially localized cell-adhesive chemistry.

A novel method is described here that allows 3D control of both chemistry and morphology by a series of wet chemical steps: the attachment of protein functionalized micron-sized beads onto a flat silicone surface that has been functionalized with a distinct chemical modification. This method can attach a wide variety of proteins or other ligands to the bead, whose diameter can be readily selected to accommodate different cell dimensions. These functionalized beads can be attached to silicone, other polymers, or other surfaces that are commonly used as cell culture surfaces. The method is demonstrated here by first attaching

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polylysine, bovine serum albumin (BSA), or laminin onto 6.5- μm -diameter spherical beads. A chemical method is then used to bind these beads to a flat silicone surface that is subsequently functionalized with polylysine. This process leads to a nonspecific cell adhesive background on the flat surface with differing chemistry on the third-dimension due to the protein on the bead protruding from the surface. The beads do not detach during cyclic stretching *in vitro*. Neonatal rat cardiac fibroblasts are cultured on the beaded surfaces and compared with fibroblasts cultured on nonbeaded, flat polylysine surfaces. Fibroblast plating density, integrin, and physical responses are examined as a function of varying the ligands on the beads.

MATERIALS AND METHODS

Functionalization of beads with proteins and polylysine

Carboxy polystyrene beads, 0.5 mL of 6.7- μm -diameter (5% w/v, Spherotech, Libertyville, IL) are centrifuged to remove sodium azide. 3 mg of EDC (Pierce Biotechnology, Rockford, IL) and 6 mg of NHSS (Pierce Biotechnology) at pH 5.5 (MES buffer with 0.15 M NaCl) are added to the beads, shaken for 15 min, and then centrifuged. BSA (4 mg), laminin (0.5 mg, from basement membrane of Engelgreth-Holm-Swarm mouse sarcoma, Sigma-Aldrich), or polylysine (4 mg, >70,000 GMW) in a total of 40 mL of pH 7.4 borate-borax buffer is added to the bead suspension, shaken, and then centrifuged to remove unbound proteins, as verified by a TNBSO test (see below). The presence of protein on the beads is verified by a TNBSO test and an amino acid analysis. The resultant suspension of protein bound beads is rediluted to 5% w/v and stored at 4°C until use.

TNBSO method and amino acid analysis

Protein-coated beads, 100 μL , are centrifuged and suspended in 200 μL of DMF, to which 25 μL of 10% DIPEA is added, followed by 25 μL of 5% TNBSO (Pierce). The yellow color of the centrifuged pellet indicates the presence of amine groups,¹⁴ as verified by amine-coated beads used as positive controls and carboxy beads as negative controls. Protein-coated beads are also analyzed by PICO-tag amino acid analysis (Waters Corporation, Milford, MA) at the UIC Research Resources Center.

Modification of silicone to attach protein beads

Silicone is coated onto tissue culture polystyrene or coated onto Bioflex dishes (Flexcell International Corp., McKeesport, PA), and then surface amine groups are formed by

reaction with 3-aminopropyltriethoxysilane (APTES) in 95% ethanol, as described previously.⁷ The only change in this procedure is that the initial silicone surface oxidation is accomplished here by treatment with concentrated HCl rather than the water plasma previously employed. One percent glutaraldehyde is prepared in 3.3 mM KOH, allowed to polymerize for 1 h, sonicated, and then applied to the amine functionalized silicone surface for 1.7 h. After repeated rinsing in water, an appropriate bead aliquot in 3.3 mM KOH solution is added to the glutaraldehyde surfaces, followed again by water rinsing. Finally, 1 mg/mL of polylysine in carbonate-bicarbonate buffer (pH 9.5) is added to the beaded surfaces and incubated at 4°C for 2 h. The final beaded surfaces are washed in deionized water to remove loosely bound polylysine, dried, and then stored at 4°C until use.

Labeling Protein bound beads with FITC

Protein-coated beads are labeled with FITC (Isomer 1, Aldrich) to verify that the functionality on the beads does not change during fabrication. FITC, 0.9 mg, in borate buffer (pH 9.2) with 0.05% Tween 80 is added to 0.25 mL of bead suspension W is incubated at room temperature for 18 h and then separated from unreacted FITC by centrifugation.

Bead density and bead-to-bead distance

Bead density is measured by placing a 234 \times 234- μm square on the beaded surface to facilitate bead counting. Beads within the grid are averaged and standard error is calculated from 15 grid pictures taken from 8 surfaces prepared on at least 2 different days. Bead-to-bead distance is measured as center-to-center distance between each bead and its nearest neighbors using Adobe Photoshop. Eight surfaces from different days are averaged for each different type of beaded surface.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is used to monitor the surface chemical variation between glutaraldehyde and polylysine, as represented by variation in the nitrogen and silicon peak intensities. The monochromatic XPS instrument was described previously.¹⁵ Approximately 7-eV electron charge neutralization is used to prevent charge buildup on these \sim 1-mm-thick silicone membranes during XPS data collection. Photoemission angle is kept normal to the polymer surface, pass energy of 44 eV is used, peaks are referenced to the aliphatic C 1-s peak at 285.0 eV, and both Shirley background and (47:53) Lorentzian:Gaussian product function are used for peak fitting. Atomic percentages are determined from the peak areas by using the transmission function of the electron energy analyzer (VSW) and sensitivity factors.¹⁶

In vitro evaluation: flexing, cell culture, and fluorescence

Silicone is stretched to determine whether the beads remain attached on the silicone surface after repeated stretching of the underlying silicone. Cyclic mechanical deformation is produced with a strain unit (Model FX-4000, Flexcell International), as described previously.^{7,17} Briefly, the strain unit consists of four six-well plates that radially and cyclically stretch the silicone membranes to a user-defined percentage of elongation (% strain).

Primary neonatal heart fibroblast cells are isolated from neonatal rat hearts by collagenase digestion and cultured as described previously.^{7,18} Culturing of fibroblasts is carried out for 24 h on both beaded and nonbeaded, nonflexing silicone surfaces in serum media to determine response to the chemical functionality on the bead. After culture, the surfaces are rinsed and fixed in preparation for fluorescence labeling as described earlier.⁴ Briefly, cells are fixed in 4% paraformaldehyde (pH 7.4) for 10 min and washed in phosphate-buffered saline (PBS). After washing, cells are incubated with paxillin antibody (BD Transduction Laboratories, San Diego, CA) at 1:1000 dilution for 1 h. Cells are then rinsed three times with PBS and subsequently placed in a blocking solution of 0.1% BSA in PBS for 1 h. After blocking, rhodamine-conjugated goat anti-mouse secondary antibody (Molecular Probes, Eugene, OR) in blocking buffer is used at 1:500 dilution for 1 h to fluorescently label paxillin antibody. Cells are then washed in PBS three times for 10 min each, and membranes are mounted on glass slides using permount adhesive (Fisher Scientific, Hampton, NH). Nuclei are stained by addition of mounting medium for fluorescence with DAPI (Vectorshield, Vector Laboratories Inc, Burlingame, CA). Fluorescently labeled cells are then examined under confocal microscopy (Model LSM 510, Carl Zeiss MicroImaging Inc., Thornwood, NY) and digital images are captured. Images demonstrate that fluorescence labeled paxillin exhibits a distribution of focal adhesions and is indicative of overall cellular response to the beads.

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Statistical analysis

All data are presented as the means \pm SEM (standard error of the mean), unless otherwise specified. Results are evaluated to test significance by analysis of variance (ANOVA) with the Neuman-Keuls test.

RESULTS AND DISCUSSION

Preparation and analysis of beaded surfaces

The functionalization and binding of the beads to the silicone surfaces shown schematically in Figure 1 are verified experimentally by x-ray photoelectron spectroscopy (XPS), wet chemical methods, and fluorescence. The amine layer bound to the silicone sur-

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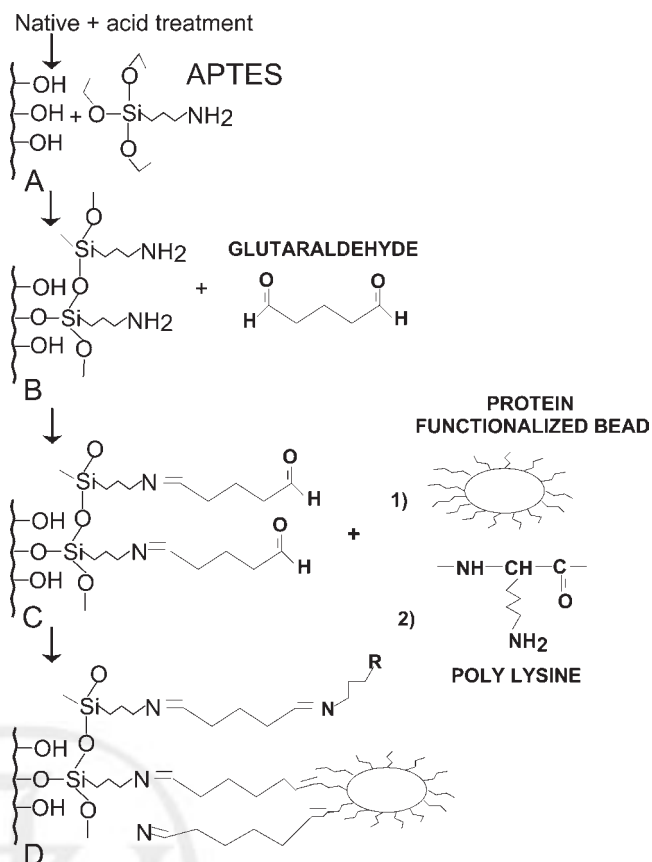


Figure 1. Surface modification steps in the formation of beaded surface. (A) Acid-oxidized silicone; (B) amine surface; (C) glutaraldehyde surface; (D) beaded surface with polylysine background.

face via the aminosilane reaction is monitored by the SDTB wet chemical method,⁷ which indicates that 0.5 ± 0.1 nmol amine/cm² or approximately equivalent to one monolayer are present on the surface, although multilayers of aminosilane likely form here. Nitrogen present in the upper ~ 10 nm of the glutaraldehyde- and polylysine-coated surfaces are also monitored by XPS and quantified as atomic percentages (Table I), where the polylysine surfaces for XPS analysis are prepared by maintaining the same reaction conditions but omitting the beads. Glutaraldehyde (GA) binding to the amine-silicone surface followed by polylysine binding displays an increase in nitrogen content by XPS and a decrease in silicon content (see Table I), indicating a build up of amine-containing glutaraldehyde and polylysine layers as the silicone is covered. The presence of primary amine groups of the polylysine surface is verified by SDTB, which gives a value of 0.2 ± 0.03 nmol amine/cm².

Figure 2(a) shows phase contrast micrographs of polylysine beads attached to silicone surfaces also bound with polylysine. Similar micrographs are also seen for other protein-coated beads (data not shown), indicating the method successfully binds these mi-

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TABLE I
Atomic Percentage of C, O, Si and N from X-ray Photoelectron Spectroscopy (XPS) for Silicone Surfaces Modified with Glutaraldehyde and Polylysine Surface (without beads)

Surface	C (%)	O (%)	N (%)	Si (%)
Glutaraldehyde	28 ± 1	25 ± 1	1 ± 1	47 ± 2
Polylysine	35 ± 1	24 ± 1	3 ± 1	40 ± 1

cron-sized beads to silicone. Protein coating of the beads is verified by amino acid analysis and the TNBSO method. The amino acid analysis of the beaded surface is similar to that for the native protein in solution (not bead bound), verifying the binding event. TNBSO method gives the expected yellow color when reacted with the available amine groups on the outer surface of the beads, whereas the unmodified carboxyl-terminated beads used as a control give a reddish color. Proteins bound to the beads are labeled with FITC and then bound to the surface in the same manner as the protein-only coated beads. These FITC-protein bound beads fluoresce brightly with high optical contrast compared with the flat silicone surface onto which they are attached. The bead fluorescence indicates that proteins remain bound to the beads throughout the fabrication process and remain intact despite the use here of concentrated basic solution. There is a low surface density of FITC-labeled beads here, because the outer amine groups on the protein-coated beads react with FITC leaving only a few amine groups to react with aldehyde.

The protein-coated beads display some agglomeration in suspension when viewed under an optical microscope, probably because of electrostatic interaction, so efforts must be made to minimize this agglomeration upon surface adsorption. Before incubating with the aldehyde surface, the bead suspension is subjected to sonication for a short time to disrupt bead-bead interaction. This technique helps reduce agglomeration and results in an even distribution of beads as seen in Figure 2(a). However, there are some areas on the surface where agglomeration of two to four beads still occurs (data not shown).

Water plasma treatment was previously employed to oxidize the surface of silicone surfaces before reaction with the aminosilane.⁷ HCl is instead used here to oxidize the silicone surface¹⁹ and is found to produce a more resilient silicone surface upon even repeated stretching *in vitro*, as demonstrated by cell counts before and after stretching (data not shown).

Controlling bead surface density

Figure 2 shows the difference in polylysine bead density distribution as a function of the fraction of

beads in the initial suspension. Bead fraction is varied from 0.005, 0.010, and 0.025% during the incubation step over the glutaraldehyde surface. Figure 2 shows that the surface bead density increases with the input fraction of beads in the suspension as it is varied from 0.005 to 0.025%. Figure 3 shows surface bead density differences on polylysine, laminin, and BSA beaded surface (* indicates a significant difference of $p < 0.05$, $N = 3$). Total bead density of the various protein functionalized beads is in the range of 100–300 beads/mm². Only BSA beads show a significant difference ($p < 0.05$) for different input fractions and then, only for the 0.005% and 0.010% bead fractions (indicated by “*” in Fig. 3). No change in the surface bead density with input bead fraction is observed for the laminin or polylysine beads. Photographs taken at random sites in the dish show that there is a local change in the

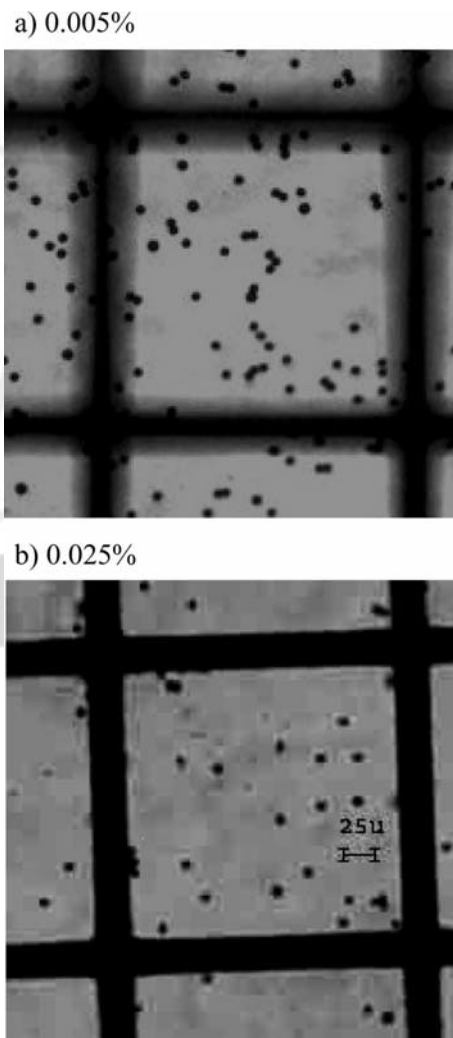


Figure 2. Phase contrast micrograph of surface bound beads prepared with initial suspension fractions of (a) 0.005% and (b) 0.025% of polylysine-coated beads. The external grid is placed on top of the beaded surface to assist in counting. Internal grid dimension, 234 μm; bead diameter, 6.5 μm.

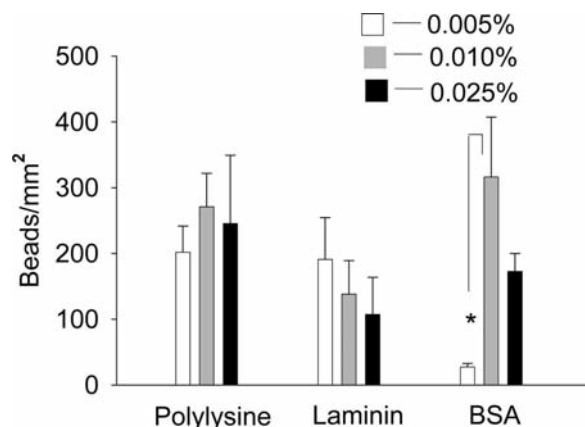


Figure 3. Densities of polylysine-, laminin-, and BSA-beaded surfaces at 0.005, 0.01, and 0.025% initial fractions of beads, respectively. *Significant difference of $p < 0.05$, $N = 3$ for sets paired by brackets.

polylysine beaded surface (see Fig. 2), but this change is not significant when averaged over many sites at a given fraction. It thus appears that there are changes in polylysine bead surface distribution with the fraction of beads in the initial suspension, but that overall averages are insufficient to characterize these changes.

The bead surface binding can be further quantified by measuring average bead-to-bead distance, as shown in Figure 4 for polylysine, laminin, and BSA beaded surface for different bead suspension fractions. There is a significant difference for polylysine beads ($p < 0.05$) between 0.005 vs. 0.025%, whereas there is no significant difference between the other types of beads and bead suspension fractions. BSA and laminin surface bead density reaches saturation at 0.005%, whereas polylysine beads tend to reach saturation at a higher input fraction because of higher density of amine groups on the outer surface. Conversely, it is hypothesized that if the number of amine groups on the outer surface of the laminin and BSA beads can be increased to that of polylysine beads, then the bead surface density on the former can also be increased. The bead-to-bead distance varies from 20 to 60 μm , sufficient for making *in vitro* cell culture systems that mimic *in vivo* conditions because typical cells are in this size range.²⁰ If the beads are closer than this distance, then cells might not recognize the underlying flat surface and its chemical contrast.

The beads are bound to the silicone surface via the aldehyde group of glutaraldehyde, which undergoes aldol condensation at high pH. Glutaraldehyde crosslinks in the presence of amine groups at high pH, maintaining its polymeric form.^{21,22} However, glutaraldehyde maintains its monomeric form at low pH and reacts as such in the presence of amine. Glutaraldehyde is therefore employed here in a high pH solution so that it can polymerize before reacting with amine. Experimental evidence indicates that without

preincubation, there is a marked difference in bead attachment that is apparently due to a variation in the density of surface aldehyde groups (data not shown). Varying the glutaraldehyde concentration in the preparation solution from 1 to 10% also affects the surface bead density somewhat.

Resilience of bead-silicone binding *in vitro*

The ability of beads to remain attached to silicone in response to mechanical disturbance and exposure to growth media is a central requirement, because bead detachment causes loss of the desired substrate dimensionality *in vitro* and detached beads may induce foreign body responses *in vivo*. The attachment of beads under such conditions is verified by repeated stretching of the underlying silicone surface, performed in serum media and cyclically stretched for 48 h in a cell culture chamber. Figure 5 (polylysine columns) indicates that there is no significant removal of the beads upon repeated stretching, with the total bead fraction remaining constant near ~ 200 beads/ mm^2 . Similar behavior is observed upon stretching of laminin beaded surfaces (data not shown).

Glutaraldehyde and its interaction with amine form a noncovalent bond referred as a Schiff base,²² which may either hydrolyze and eventually remove the beads or remain strongly attached because of multiple Schiff base formation. To show that these multiple noncovalent bonds can retain beads similar to multiple covalent bonds, the reducing agent sodium cyanoborohydride (in carbonate-bicarbonate buffer at pH 9.5) is used to form multiple covalent bead-surface bonds. The reducing agent converts Schiff base to covalent amide bond between the aldehyde and amine

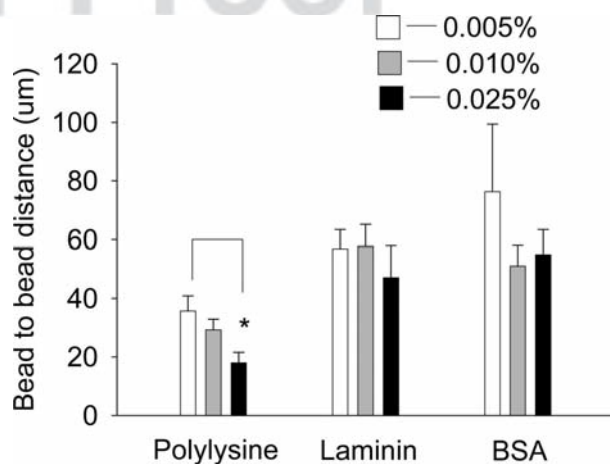


Figure 4. Bead-to-bead distance on polylysine-, laminin-, and BSA-beaded surfaces for different input bead fractions. *Significant difference of $p < 0.05$, $N = 3$ for sets paired by brackets.

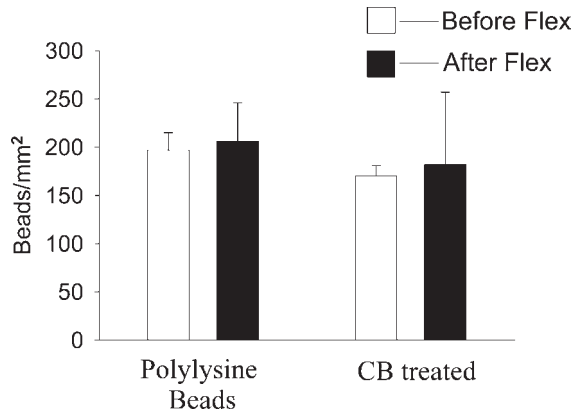


Figure 5. Effect of flexing for 48 h in serum media on density of polylysine beads attached to silicone surfaces. Polylysine beads refers to standard binding method, CB treated refers to a covalent binding method performed for comparison (see text). No significant change in bead density occurs after flexing.

groups. No significant difference is found for *in vitro* stretching of these covalently bound beads (Fig. 5, CB columns) and those bound by the standard method (Fig. 5, polylysine columns), indicating that the reaction between glutaraldehyde and the beads is strong enough to prevent beads detachment during multiple stretches. Because the standard method involves strong binding of the beads, cells and beads both can respond to mechanical forces without being detached or rolled by the cells.

Cell culture on beaded surfaces

Neonatal rat fibroblast cell response is assessed by cell physiology as revealed in confocal images of the beaded surfaces and the percentage of various protein functionalized beads to which cells terminate. Figure 6 shows fluorescent micrographs of fibroblast cell culture for 24 h on (a) BSA, (b) laminin, and (c) polylysine-beaded surfaces. The red color indicates paxillin staining, and the blue color indicates nuclear staining. Cells ignore the BSA beads but respond positively to polylysine or laminin beads by either terminating at or wrapping around the beads. The images show that there is no movement of the nuclei within the cell in the direction of the BSA beads, whereas cells on laminin beads do show nuclear movement toward the beads. Cell shape on laminin-coated beads is more elongated, whereas it is rounder on the polylysine and BSA surfaces. The focal adhesion protein paxillin is an indicator of focal adhesion sites and is seen on all beaded surface in the flat regions. Cells showed adhesion sites near the polylysine and laminin beads, but not near the BSA beads, indicating that polylysine and laminin on the beads are recognized by the cells.

The number of cells responding to the beads is assessed by the percentage of beads terminating in cells. Figure 7(a) shows the percentage of beads attached to the cells after 24-h growth on BSA, laminin, and polylysine-beaded surface. There is a significant difference ($p < 0.05$) between the total numbers of BSA

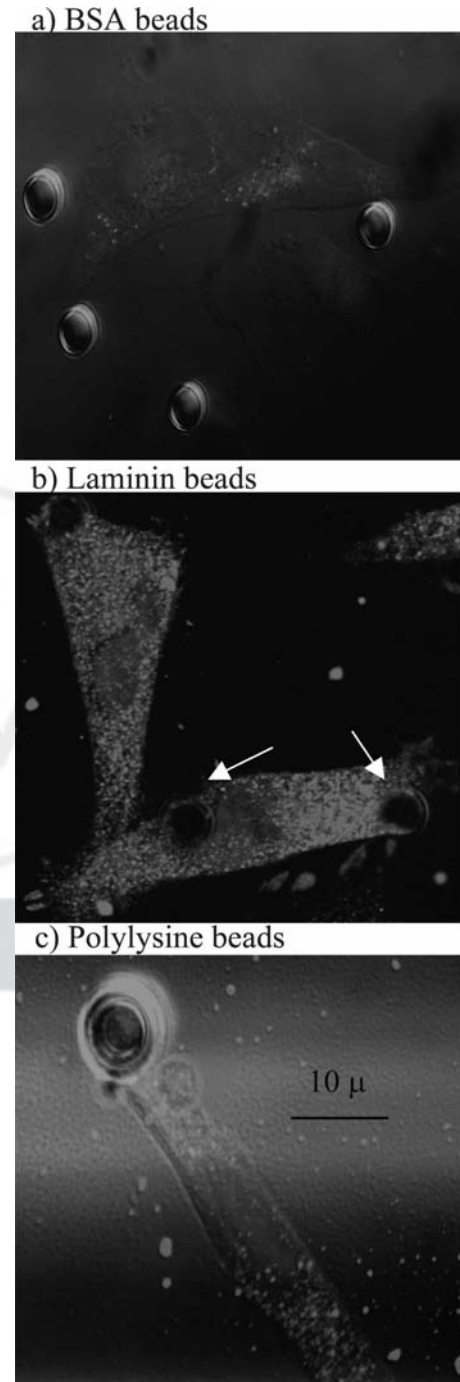


Figure 6. Micrographs of cardiac fibroblasts cultivated for 24 h on surface attached beads coated with (a) BSA, (b) laminin, or (c) polylysine. Scale bar = 10 μm. Arrows indicate cells interacting with beads. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

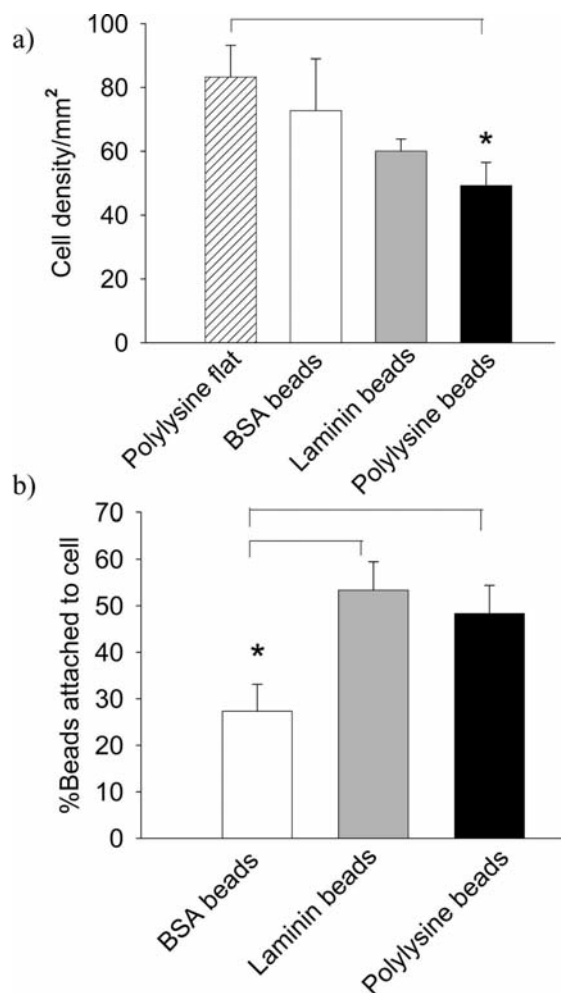


Figure 7. (a) Percentage of beads attached to fibroblasts after 24 h of culture on BSA-, laminin-, or polylysine-beaded surfaces. *Significant difference of $p < 0.05$, $N = 3$ between sets marked with brackets. (b) Cell plating density after 24 h of culture with polylysine flat surfaces without beads or with BSA-, laminin-, and polylysine-beaded surfaces. *Significant difference of $p < 0.05$, $N = 3$ for sets paired by brackets. Note significant decline in cell proliferation compared with flat polystyrene.

beads ending with cells compared with the laminin and polylysine beads. There is no significant difference between polylysine and laminin beads. Figure 7(b) shows the cell density after 24 h on a flat polylysine surface without beads as well as the BSA-, laminin-, and polylysine-beaded surfaces. Comparison of total cell attachment on the various surfaces show that there is reduced ($p < 0.05$) attachment of fibroblasts on the polylysine-beaded surface compared with flat polylysine-coated surfaces. These results show that cells responded to the third dimension by undergoing contact inhibition, leading to decreased proliferation. This is similar to what is seen *in vivo*. Some of the authors have shown recently that cardiac fibroblasts cultured on microtextured, 10- μm -high pegged surfaces also undergo contact inhibition.⁵

Unsuccessful methods of binding beads to silicone surfaces

Other physical and chemical methods were also attempted to attach micron-sized beads on the silicone polymer, but with less success. Adding beads at various intervals of time during the curing process of silicone lead to beads that either sank too deeply into the viscous uncured silicone or were too loosely bound and easily washed away by saline solution. Heat-induced attachment of beads onto polystyrene dishes has been shown previously by some of the authors.⁵ However, heating polystyrene beads to $\sim 95^\circ\text{C}$, near their glass transition temperature, caused them to melt into the silicone surface. Applying a heated solution of beads to the silicone surface led to the unwanted formation of large bead clumps on the surface, rather than the dispersed beads required for cell growth. Adsorption of protein-coated beads directly onto amine surfaces, without the glutaraldehyde treatment, also showed agglomeration. Finally, many of these methods did not retain beads on the surface when subjected to cell culture conditions.

CONCLUSIONS

A method is shown here that describes how 3D projections can be fabricated on flat silicone polymer surface by the strong binding of chemically distinct micron-sized beads. This method provides the ability to change the chemical functionality in the third dimension while maintaining distinct chemistry on the intervening flat surface. The beads are strongly adherent to the surface and do not detach with repeated stretching of the underlying silicone. These surfaces have bead-to-bead distances that vary from 20 to 60 μm and are appropriate for culturing cardiac fibroblasts, cardiac myocytes, and various other cells that fall within this size range.

Neonatal rat fibroblasts, a typical example of mammalian cells, show selective response to the beaded surface by forming focal adhesion sites near and around the beads. The microfabrication strategy used here allows the production of surfaces for *in vitro* cell culture that mimic *in vivo* conditions for any soft surface onto which amine films can be formed. The method may also work on hard surfaces because the binding event is chemical in nature. These bead bound surface may also be used for mechanobiology studies.

Several researchers have previously used nanometer and micron-sized beads to study cellular behavior. When cell adhesion molecules are bound to micron-sized beads, which are then placed on top of cells, the cells respond positively to the chemical functionality on the beads.²³ Micron-sized beads adsorbed onto flat

surfaces have been found to activate cells.²⁴ Gold nanoparticles have been randomly attached to mercaptan-terminated silane layers, and then thiol terminated peptides were specifically bound to the gold particles via specific gold-thiol reactions and the remaining flat mercaptan-terminated silane surface was passivated with BSA for subsequent cell culture.²⁵ Streptavidin-functionalized beads were also immobilized to gold-plated surfaces with various surface density promoted by electrical fields applied between two electrodes. Biotin-linked fibronectin was subsequently attached to the beads, and the resultant beaded surfaces were found to promote cell attachment on top of the beads.²⁰

The method described in this article displays several advantages compared with these other bead-based methods. The former does require use of a gold surface, the application of an electric field, or the availability of specific groups such as thiol or biotin. Some of these previously described bead methods do not form strong bead-surface bonds and are therefore expected to permit undesirable bead detachment *in vitro*. The method described here forms a covalent bond between the bead and the surface that is shown to survive *in vitro* conditions. Furthermore, the use here of aminosilane films on the flat substrate and reactions with amine groups on the bead-targeted biomolecule renders this method applicable to a very wide range of materials and ligands.

References

- Kleinman HK, Edds LL, Cannon FW, Sephel GC. Use of extracellular matrix components for cell culture. *Anal Biochem* 1987;166:1-13.
- Folch A, Tomer M. Microengineering of cellular interactions. *Annu Rev Biomed Eng*, 2000;2:227-256.
- Clark P, Connolly P, Curtis ASG, Dow JAT, Wilkinson CDW. Cell guidance by ultrafine topography *in vitro*. *J Cell Sci* 1991; 99:73-77.
- Motlagh D, Senyo SE, Desai TA, Russell B. Microtextured substrata alter gene expression, protein localization and the shape of cardiac myocytes. *Biomaterials* 2003;24:2463-2476.
- Boateng SY, Hartman TJ, Ahluwalia N, Vidula H, Desai TJ, Russell B. Inhibition of fibroblast proliferation in cardiac myocytes cultures by surface microtopography. *Am J Physiol Cell Physiol* 2003;285:C171-C182.
- Boateng S, Lateef SS, Crot CA, Motlagh D, Desai T, Samarel AM, Russell B, Hanley L. Peptides bound to silicone membranes and 3D microfabrication for cardiac cell culture. *Adv Mater* 2002;14:461-463.
- Lateef SS, Boateng S, Hartman TJ, Crot CA, Russell B, Hanley L. GRGDSP peptide bound silicone membranes withstand mechanical flexing *in vitro* and display enhanced fibroblast adhesion. *Biomaterials* 2002;23:3159-3168.
- Curtis A, Wilkinson C. Topographical control of cells. *Biomaterials* 1997;18:1573-1583.
- Flemming RG, Murphy CJ, Abrams GA, Goodman SL, Nealey PF. Effects of synthetic micro- and nano-structured surfaces on cell behavior. *Biomaterials* 1999;20:573-588.
- Kane RS, Takayama S, Ostuni E, Ingber DE, Whitesides GM. Patterning proteins and cells using soft lithography. *Biomaterials* 1999;20:2363-2376.
- Takayama S, Ostuni E, Qian X, McDonald JC, Jiang X, LeDuc P, Wu MH, Ingber DE, Whitesides GM. Topographical micropatterning of poly(dimethylsiloxane) using laminar flows of liquids in capillaries. *Adv Mater* 2001;13:570-574.
- Yang Z, Belu AM, Liebmman-Vinson A, Sugg H, Chilkoti A. Molecular imaging of a micropatterned biological ligand on an activated polymer surface. *Langmuir* 2000;16:7482-7492.
- Branch DW, Corey JM, Weyhenmeyer JA, Brewer GJ, Wheeler BC. Microstamp patterns of biomolecules for high-resolution neuronal networks. *Med Biol Eng Comput* 1998;36:135-141.
- Satake K, Okuyama T, Ohashi M, Shinoda T. The spectrophotometric determination of amine, amino acid and peptide with 2,4,6-trinitrobenzene 1-sulfonic acid. *Biochemistry* 1960;47: 654-660.
- Wijesundara MBJ, Ji Y, Ni B, Sinnott SB, Hanley L. Quantifying the effect of polyatomic ion structure on thin-film growth: experiments and molecular dynamics simulations. *J Appl Phys* 2000;88:5004-5016.
- Scofield JH. Hartree-Slater subshell photoionization cross-sections at 1254 and 1487 eV. *J Elec Spect Rel Phenom* 1976;8:129-137.
- Cadre BM, Qi M, Eble DM, Shannon TR, Bers DM, Samarel AM. Cyclic stretch down-regulates calcium transporter gene expression in neonatal rat ventricular myocytes. *J Mol Cell Cardiol* 1998;30:2247-2259.
- Boateng SY, Lateef SS, Mosley W, Hartman TJ, Hanley L, Russell B. RGD, YIGSR synthetic peptides facilitate identical cellular adhesion as laminin and fibronectin but alter the physiology of neonatal cardiac myocytes. *Am J Physiol Cell Physiol* 2004. Forthcoming.
- Perutz S, Kramer EJ, Baney J, Hui CY. Adhesion between hydrolyzed surfaces of poly(dimethylsiloxane) networks. *Macromolecules* 1997;30:7964-7969.
- Gleason NJ, Nodes CJ, Higham EM, Guckert N, Aksay IA, Schwarzbauer JE, Carbeck JD. Patterning proteins and cells using two-dimensional arrays of colloids. *Langmuir* 2003;19: 513-518.
- Monsan P. Optimization of glutaraldehyde activation of a support for enzyme immobilization. *J Mol Catal* 1977/1978;3:371-384.
- Richards FM, Knowles JR. Glutaraldehyde as a protein cross-linking reagent. *J Mol Biol* 1968;37:231-233.
- Curtis A, Quinn R, Mcgrath M. Activation of neuron adhesion and neurite extension by tethered neutrophins and adhesion molecules. *Exp Biol Online* 1996;1:1.
- Miyaki M, Fujimoto K, Kawaguchi H. Cell response to micropatterned surfaces produced with polymeric microspheres. *Coll Surf A* 1999;153:603-608.
- Abdelghani-Jacquin C, Abdelghani A, Chmel G, Kantlehner M, Sackmann E. Decorated surfaces by biofunctionalized gold beads: application to cell adhesion studies. *Eur Biophys J Biophys* 2002;31:102-110.