BioMEMs Materials and Fabrication Technology:  

Control of Mammalian Cell and Bacteria Adhesion on Substrates Micropatterned with Poly(ethylene glycol) Hydrogels

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Abstract. A simple method for controlling the spatial positioning of mammalian cells and bacteria on substrates using patterned poly(ethylene glycol) (PEG) hydrogel microstructures is described. These microstructures were fabricated using photolithography on silicon, glass or poly (dimethylsiloxane) (PDMS) surfaces modified with a 3-(trichlorosilyl) propyl methacrylate (TPM) monolayer. During the photogelation reaction, the resulting hydrogel microstructures were covalently bound to the substrate via the TPM monolayer and did not detach from the substrate upon hydration. For mammalian cell patterning, microwell arrays of different dimensions were fabricated. These microwells were composed of hydrophilic PEG hydrogel walls surrounding hydrophobic TPM floors inside the microwells. Marine 3T3 fibroblasts and transformed hepatocytes were shown to selectively adhere to the TPM monolayer inside the microwells, maintaining their viability, while adherent cells were not present on the hydrogel walls. The number of cells inside one microwell could be controlled by changing the lateral dimension of the microwells, thus allowing only a single cell per microwell if desired. In the case of 30 × 30 μm microwells, as many as 400 microwells were fabricated in 1 mm2. In addition, PEG hydrogel microstructures were also shown to effectively resist the adhesion of bacteria such as Escherichia coli.

Key Words. poly(ethylene glycol) hydrogel microstructures, photolithography, cell patterning, microwells

1. Introduction

Many mammalian cell-based bioassays for the screening of large libraries of potential pharmaceutical agents or the detection of toxic compounds and pathogens in the environment have been developed over the years and some are in use today. Although most of cell-based assays are still performed in 96 well plates, many researches are moving toward 384 well and higher density plate formats to improve the performance and throughput of these assays. With further assay miniaturization in mind, a number of researchers have explored the patterned deposition of cells in microsystems through the control of cellular adhesion (Bousse, 1996; Makohliso et al., 1999; Sundberg, 2000; Kane et al., 1999; Jung et al., 2001; Chen et al., 1997, 1998; Lahiri et al., 1999; Lopez et al., 1993; Mrksich et al., 1997; Singhi et al., 1994; Takayama et al., 1999; Whitesides et al., 1994; Zhang et al., 1999; Turner et al., 1999; John et al., 1998; Bhatia et al., 1997, 1998; Folch et al., 1998; den Braber and Toner, 1996).

Similarly, the control of bacterial adhesion may be a valuable tool toward developing cell-based sensor arrays based on genetically engineered bacteria. Early studies of bacterial adhesion have mostly focused on reducing bacterial adhesion on implanted biomaterials, the critical event in the pathogenesis of foreign body infections (An and Friedman, 1998; Park et al., 1998). However, because analyze specificity can be readily modified by genetic engineering and because of the relatively robust nature of these micro-organisms as compared to mammalian cells, bacterial cells have been studied extensively for sensing applications (Rainina et al., 1996a, 1996b; Larsen et al., 1997; Belkin et al., 1997; Liao et al, 2001). The ability to selectively attach bacteria to micropatterned substrates could be used to create sensor arrays for applications such as rapid screening for infectious diseases or to detect toxic compounds (Cowan et al., 2001; Ostuni et al., 2001a).

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A number of studies reported that cellular adhesion is controlled by the chemical and physical characteristics of surfaces such as hydrophobicity and hydrophilicity, surface charge, and surface roughness (Jung et al., 2001; Folch and Toner, 2000; Ito, 1999). These factors can be readily manipulated to control the spatial distribution of cells on a substrate. The rapid development of microfabrication and surface engineering techniques have stimulated the development of novel methods that may be used to control cell adhesion and spreading with micrometer-scale resolution. The most common approaches to defining and controlling regions that promote or resist the adhesion of cells on this length scale is to control the surface tension and wetting properties by patterning hydrophilic and hydrophobic regions on the surface using photolithography (Matsuda and Sugawara, 1995; Thomas et al., 1997; Dewez et al., 1998) or microcontact printing (μ-CP) patterns of self-assembly monolayers (SAM) on the surfaces (Kane et al., 1999; Chen et al., 1998; Amirpour et al., 2001; Ghosh et al., 1999; Franko et al., 2000; Mrksich et al., 1996; Singhi et al., 1994a; Whitesides et al., 2001; Matsuda and Sugawara, 1995).

Here we controlled cell adhesion using hydrogels of poly (ethylene glycol) (PEG), a non-biodegradable and hydrophilic polymer that has been previously used to minimize protein adsorption and mammalian cell adhesion onto biomaterial surfaces (Zhang et al., 1998; Tziampfazis et al., 2000; Lu et al., 2001). PEG grafting to surfaces has been also used to render a variety of surfaces resistant to bacterial adhesion (Park et al., 1998; Ostuni et al., 2001b; Razatos et al., 2000; Cunliffe et al., 1999). The groups of Inger and Whitesides demonstrated that SAMs containing hydrophilic ethylene glycol oligomers as the terminal segment resist cell adhesion while hydrophobic SAMs promote cell adhesion. These regions can be confined to 10–100 μm wide areas by patterning these SAMs using μ-CP on a gold surface (Singhvi et al., 1994b).

In this study we described the control of cell adhesion and the spatial confinement of cell proliferation by means of micrometer scale PEG hydrogel structures on silicon, glass or poly (dimethylsiloxane) surfaces modified with a chlorosilane coupling agent. Acrylate or methacrylate derivatives of PEG can be cross-linked into hydrogels and photolithographically patterned on silicon, glass, and plastic surfaces (Sirkar and Pishko, 1998; Ward et al., 2001; Revinz et al., 2001). Using these patterned substrates, we demonstrated the ability to capture and confine mammalian cells (fibroblasts and hepatocytes) inside arrays of microwells fabricated from hydrogels of varying dimensions. In addition, we demonstrated the partial resistance of PEG hydrogel microstructures to the adhesion of a model bacterium, Escherichia coli.

2. Materials and Methods

2.1. Materials

Poly(ethylene glycol) diacrylate (PEG-DA, MW 575), 2,2'-dimethoxy-2-phenyl-acetophenone (DMPA), anhydrous carbon tetrachloride, n-heptane and perfluoro-octane were purchased from Aldrich Chemical Co. (Milwaukee, WI). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), antibiotic/antimycotic solution, trypsin, ethylenediaminetetra-acetate (EDTA), sodium chloride, sodium phosphate and potassium phosphate monobasic were purchased from Sigma Chemical Co. (St. Louis, MO). 3-(trichlorosilyl) propyl methacrylate (TPM) was purchased from Fluka Chemicals (Milwaukee, WI). Hydrogen peroxide was purchased from EM Science (Gibbstown, NJ). Sulfuric acid was purchased from Fisher Scientific (Fair Lawn, NJ). E. coli bacteria (DHS) were obtained from Novagen Inc. (Madison, WI). Bacto-tryptone and yeast extract were obtained from Becton & Dickinson (Franklin Lakes, NJ). Gram Safranin stain was purchased from Fisher Diagnostics (Pittsburgh, PA). Murine 3T3 fibroblasts and murine hepatocytes (SV40 transformed) were obtained from American Type Culture Collection (Manassas, VA). Live/Dead Viability/Cytotoxicity Kit (L-7013) was obtained from Molecular Probes (Eugene, OR). Phosphate buffered saline (PBS, pH 7.4) solution consisted of 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate, and 0.15 M NaCl in 18 MΩ·cm deionized water (E-pure, Barnstead, Dubuque, IW). Luria-Bertani (LB) broth for bacterial growth consisted of 10 g of bacto-tryptone, 5 g of yeast extract and 10 g of sodium chloride in 1 L of DI water (pH 7). Silicon wafers were obtained from Wafer World Inc. (West Palm Beach, FL). Poly(dimethyl siloxane) (PDMS) prepolymer and curing agent (Sylgard 184) were obtained from Dow Corning (Midland, MI).

2.2. Equipment

Photogelation reactions were performed using a 365 nm, 300 mW/cm² light source (EFOS Ultracure 100s Plus, UV spot lamp, Mississauga, Ontario). Scanning electron microscopy (SEM) was performed with a JEOL T330A at 15 kV (JEOL, Ltd., Peabody, MA). Before SEM characterization, substrates were attached aluminum mounts with carbon tape and sputter-coated with gold to a thickness of 200 Å. Bright field optical microscopy was performed with a Zeiss Axiophot 135 microscope (Carl Zeiss Inc., Thornwood, NY). For the Live/Dead Viability assay, the same microscope was used, but in the fluorescence mode. The spin-coater used in these studies was obtained from Integrated Technologies (Acushnet,
MA). The plasma chamber was purchased from Harrick Scientific Co. (Ossining, NY). Contact angles were measured with a Rame Hart Model 100 goniometer (Mountain Lakes, NJ) using doubly distilled water. Chrome coated soda lime photomasks used for patterning of microwells were obtained from Advance Reproductions (Andover, MA). USAF negative test target mask was purchased from Melles Griot (Irvine, CA).

2.3. Functionalization of substrates

With the goal of improving hydrogel adhesion on substrate surfaces, all substrates are modified with a methacrylate-functionalized trichlorosilane. Silicon and glass surfaces were silanized as described previously (Rezvin et al., 2001). Briefly, silicon wafers were cleaned in “piranha” solution consisting of 3:1 ratio of 30% w/v H₂O₂ and H₂SO₄ and subsequently immersed in 1 mM solution of 3-(trichlorosilyl) propyl methacrylate (TPM) in 80%/20% mixture of heptane/carbon tetrachloride. PDMS functionalization was performed according to a published protocol (Silver et al., 1999). In brief, the PDMS surface was first treated in an oxygen plasma for 5 minutes. and then immersed in the silanizing solution containing 10–20 μg TPM per gram of perfluoroocetane. Formation of TPM monolayers on the substrates was confirmed by ellipsometry and contact angle measurements.

2.4. Preparation of micropatterned surfaces

PEG hydrogel micropatterns were fabricated from PEG-DA (MW 575) by proximity photolithography. The gel precursor solution was prepared by dissolving 10 mg DMPA (an UV light photoinitiator) per 1 mL of PEG-DA solution. This precursor solution was spin coated at 3,000 rpm for 20 seconds onto the pretreated substrate, forming a uniform layer. A photomask with the desired pattern was brought into proximity of the gel precursor film and illuminated with 365 nm UV light for 0.5 seconds. After UV photopolymerization, only exposed regions underwent free radical induced cross-linking and became insoluble in common PEG solvents such as water. As a result, desired patterns were obtained by washing away unreacted regions with water so that only the projection of the mask remained on the substrate surface. SEM was used to observe pattern morphology.

2.5. Cell culture and the seeding of substrates

Mammalian cells were incubated at 37 °C in 5% CO₂ and 95% air. Murine fibroblasts were cultured in DMEM with 4.5 g/L glucose and 10% FBS. Murine hepatocytes were cultured in DMEM containing 1.0 g/L glucose, 200 mM dexamethasone and 4% FBS. Both phenotypes were cultured to confluence in 75 cm² polystyrene tissue culture flasks and confluent cells were subcultured every 2 to 3 days by trypsinization with 0.25% (w/v) trypsin and 0.13% (w/v) EDTA.

To seed patterned substrates, cells were trypsinized from routine culture and centrifuged at 1,000 rpm at 25 °C for 5 minutes. The supernatant was removed, cells were resuspended in fresh culture medium containing serum, and an aliquot was obtained for cell counting in a hemocytometer to adjust seeding density. The cells then were seeded at a density of 1.0 × 10³ cells/mL on micropatterned substrates which was sterilized by exposure to 365 nm UV light overnight before seeding. The seeded substrates were then cultured under conditions described earlier. Twenty four hours after seeding, the substrates were rinsed with PBS in order to remove non-adherent cells. Adherent cells were either fixed and imaged by bright-field optical microscopy or assayed for cell viability.

Substrates patterned with bacteria were also created. Isolated colonies of E. coli were used to inoculate 5 mL of LB broth and cultured at 37 °C in roller tubes for 18 hours. Patterned substrates were then immersed in 5 mL of LB broth containing 100 μL of cultured E. coli. Hydrogel patterned substrates were then incubated for 6 hours and rinsed with fresh LB media and stained with Gram Safranin for imaging of adherent cells.

2.6. Cell viability

A live/dead viability/cytotoxicity fluorescence assay was used to investigate the viability of adherent cells on micropatterned substrates. This assay uses SYTO 10 and Dead Red as fluorophores to distinguish living cells and dead cells. SYTO 10 stains live cells green, while Dead Red stains dead cells red. For this assay, 2 μL of the two fluorophores were added to 1 mL HEPES-buffered saline (HBS) to make the staining solution. After 24 hours of incubation, the substrates were washed with HBS, the staining solution was placed on the substrates and incubated for 15 minutes in darkness at room temperature. Substrates were washed with HBS again and fixed with 4% glutaraldehyde in HBS for 60 minutes. The fixative was then removed and samples were imaged using fluorescence microscopy.

3. Results and Discussions

3.1. Functionalization of substrates

Surface modification was found to be essential for the fabrication of well-defined, hydrogel micropatterns with good adhesion on silicon, glass and polymer substrates. Self-assembly of trichlorosilanes on substrates was used to create surface-tethered methacrylate groups capable of covalent bonding with PEG-DA during free radical induced photogelation. In order to create such an
adhesion-promoting layer, silicon or glass substrates were first treated with “piranha” solution. This pretreatment step was needed to hydroxylate the substrate surface. Introducing hydroxylated surfaces into the TPM solution resulted in the formation of a dense network of Si–O–Si bonds on the substrate surface and pendant methacrylate functionalities at the substrate/solution interface. This surface modification was easily visualized by the increase in water contact angle associated with hydrophobic methacrylated alkyilsilanes on hydrophilic silicon/glass surfaces (88 ± 1°). Ellipsometry measurements of modified silicon surfaces indicated that the organosilane films was 14 ± 3 Å, indicating the presence of a monolayer of TPM on the substrate surfaces. Previous studies using time of flight secondary ion mass spectroscopy (TOF SIMS) also confirmed the presence of the monolayer (Revzin et al., 2001).

Because of their potential advantages over silicon or glass substrates regarding flexibility and hemo-/biocompatibility, PDMS surfaces were also functionalized with TPM monolayers. Following a similar procedure to that used for glass and silicon, we first treated PDMS in an oxygen plasma to hydroxylate the surface. The oxygen plasma introduced silanol groups (Si–OH) on the surface by the oxidation of methyl groups (Si–CH₃) of PDMS at the plasma/polymer interface (McDonald et al., 2000). This oxygen plasma treatment changed the native hydrophobic PDMS surface to one that was more hydrophilic. The contact angle of water on PDMS oxidized for 5 minutes is almost zero, compare to 90 ± 5° for untreated PDMS. Oxidized PDMS was then treated with TPM in perfluoroctane to produce pendant methacrylate functionalities on the surface. After silanization, the contact angle for PDMS increased to 64 ± 5°. Such glow discharge oxidation followed by silanization will likely be useful for attaching microstructures to other plastics besides PDMS.

3.2. Fabrication of micropatterned substrates
Micropatterned substrates were fabricated with PEG hydrogel microstructures on the functionalized surfaces. The formation of the PEG hydrogels was based on the UV initiated free-radical polymerization of acrylate or methacrylate end groups on PEG derivatives. That is, when exposed to UV light in the presence of a photoinitiator, (meth)acrylate groups formed reactive free radical sites which reacted with each other, thus resulting in the formation of polyacrylate or polymethacrylate networks highly cross-linked with PEG (Mellott et al., 2001). The ability of PEG-DA to gel upon exposure to UV light was used to create negative patterns on substrates using photolithography. Using this technique, we fabricated various geometries of 3-dimensional hydrogel microstructures by changing the design of the mask and encapsulated sensing agents such as living cells or fluorophore labeled proteins inside these microstructures (Revzin et al., 2001; Koh et al., 2002).

To prepare hydrogel microstructures on substrates, a chrome-coated soda-lime photomask containing two different arrays of wells with individual lateral dimensions of 60 × 60 μm and 30 × 30 μm arranged in 20 × 20 arrays were designed and fabricated. Each microwell was surrounded by hydrogel walls which were 20 μm wide and the height of the hydrogel wall was approximately 10 μm as measured by profilometry. The volume of the microwells may be easily controlled by changing their depth and the area of the well, the former controlled by the spin-coating speed which dictates the height of the hydrogel microstructure and the latter by the feature size of the mask. The photomask allowed PEG hydrogel walls to be polymerized upon exposure to UV light while residual unreacted macromer was removed elsewhere by developing the pattern in water. Thus, patterning created a clear contrast between adhesion resistant, hydrophilic hydrogel walls of the microwells and adhesion promoting, hydrophobic methacrylated surfaces inside the microwells. Microstructures were firmly anchored to the surface by TPM monolayers and did not delaminate during prolonged exposure to aqueous environments for several weeks. Figures 1(a) and 1(b) show micropatterned silicon substrates with two different sizes of microwells. Clearly defined 3-dimensional hydrogel patterns without any residual polymer inside microwells were observed with the PEG hydrogel forming the walls of the microwells. Even though the height of the hydrogel wall increased by nearly 30% by water-induced swelling, noticeable lateral swelling did not occur because of covalent bonding between hydrogel structures and substrates.

While inorganic or metal substrates have been used extensively for micropatterning, polymeric substrates present a very attractive and a less explored alternative due to their inexpensive, chemically versatile, and sometimes elastic or biodegradable characteristics. Recently, several research efforts have been directed toward creating a patterned polymeric template (Ward et al., 2001; Hyun and Chilkoti, 2001; Ostuni, 2001b). Most of these efforts used PDMS and were a direct extension of micro-contact printing ("soft lithography") and micro-molding methods developed by Whitesides and coworkers (Whitesides et al., 2001). In order to demonstrate the flexibility of the proposed hydrogel patterning methodology and to enable its future application in combination with soft lithography techniques, we functionalized PDMS surfaces with TPM and fabricated arrays of hydrogel microwells on this substrate. As
shown in Figure 1(c), high quality patterns of hydrogel microstructures could be generated on PDMS substrates. These micropatterns did not delaminate upon hydration or detach when these elastomeric PDMS substrates were flexed.

3.3. Cell adhesion on micropatterned substrates

The patterning of PEG-based hydrogels on a TPM treated surface created hydrophobic and hydrophilic domains which interacted differently with adsorbing proteins and cells. To investigate the adhesion of cells on PEG hydrogels and TPM monolayers, relatively large hydrogel structures were fabricated on silicon and glass substrates functionalized with TPM, immersed in a suspension of murine fibroblasts, and cultured overnight. Figure 2 clearly shows that minimal cell adhesion occurred on the PEG hydrogel microstructures (walls) while large numbers of cells adhered, spread, and proliferated on the hydrophobic TPM monolayer on the surface of the glass substrate. The exclusionary effects of PEG hydrogels against cells or proteins may be attributed to good conformational flexibility, high polymer chain mobility and the hydrophilic nature of PEG (Sofia and Merrill, 1997). This difference in cell adhesion between TPM monolayers (the base of a well) and PEG hydrogel walls was expected to allow the spatial control of cell spreading in microwells (Figure 2). Figures 3(a) and 4(a) show fibroblasts immobilized on micropatterned substrates with individual microwells with lateral dimensions of 60 × 60 μm and 30 × 30 μm respectively, and a 20 μm wide hydrogel wall in both cases. As expected, for both dimensions, cells attached and spread only on the hydrophobic interior of the microwells with hydrogel walls serving as effective barriers to cell adhesion, proliferation and cross-over. Previous studies demonstrated that dimensions of the cell resistant barrier

![Image](image1.png)

**(a)**

![Image](image2.png)

**(b)**

![Image](image3.png)

**(c)**

**Fig. 1.** Scanning electron micrographs of a micropatterned substrate consisting of an array of microwells: (a) microwells having lateral dimensions of 60 × 60 μm on the silicon; (b) lateral dimension of 30 × 30 μm on the silicon; (c) hydrogel microstructures on PDMS.

![Image](image4.png)

**Fig. 2.** Optical micrograph of fibroblasts seeded onto a patterned glass substrate with PEG hydrogel microstructure walls and a hydrophobic TPM base.
are important in containing and confining cells (Chen et al., 1998). Here we demonstrated that a 20 μm hydrogel barrier was sufficient to segregate cells incubated for 24 hours and we anticipate that the relatively high height of these hydrogel walls will be a more reliable method of confining cells inside microwells than other cell patterning technique based on SAMs.

If the area of the cell-adherent region is equal to or less than area of maximum spreading of cells, the phenotypic shape and viability of adherent cells is thought to be compromised (Kane et al., 1999). Previous studies also demonstrated that endothelial cells underwent a high rate of apoptosis within 24 hours when the area of the cell-adhesive region was below 500 μm², while cells confined to larger areas did not undergo apoptosis (Chen et al., 1998). Here we observed nearly identical morphologies for fibroblasts in both sizes of microwells (3,600 μm² and 900 μm²) after 24 hours of incubation (see Figures 3(a) and 4(a)). The viability of cells confined within both corrals was also investigated using a fluorescent live/dead assay that stains live cells green and dead cells red by the difference in membrane permeability between living and dead cells after 24 hours of incubation. Figures 3(b) and 4(b) show fluorescent micrographs of stained cells which adhered inside both sizes of microwells. As is evident by the green emitted light, cells remained viable in both geometries. We also clearly observed that while large microwells contained as many as two or three cells in one microwell, we were able to spatially control cell placement at the single-cell level by reducing the size of the microwells. Based on these results, these microwells were large enough for cells to spread and remain viable, and the number of adherent cells could be controlled, to the single cell level, by changing the size of microwells. Murine SV-40

![Image](a)

**Fig. 3.** Fibroblasts on hydrogel patterned silicon substrates: (a) optical micrograph and (b) live/dead fluorescent viability assay of cells cultured inside microwells having lateral dimensions of 60 × 60 μm.

![Image](b)

**Fig. 4.** Fibroblasts cultured inside microwells (silicon substrate) having lateral dimensions of 30 × 30 μm: (a) optical micrograph; (b) live/dead fluorescent viability assay.
transformed hepatocytes were also seeded onto identical substrates and similar results were observed (Figures 5(a) and 5(b)).

3.4. Patterning of bacteria on surfaces
We also explored the possibility of patterning bacteria on substrates using PEG hydrogel microstructures fabricated on silicon. Patterned silicon substrates were incubated in LB broth containing suspended \textit{E. coli} for 6 hours. This contact time was previously reported as sufficient for the relatively rapid phenomenon of bacterial adhesion to occur (Sommer et al., 1999). After incubation, patterned substrates with adherent bacteria were removed and stained with Gram Safranin for imaging using optical microscopy or substrates were imaged directly using electron microscopy. An ESEM micrograph of \textit{E. coli} anchored to a hydrophobic TPM-modified silicon surface is shown in Figure 6(a). Most of the surface adherent cells are rod-shaped, the phenotype normally associated with \textit{E. coli}. Figure 6(b) demonstrates the adhesion of bacterial cells presented with a choice of TPM-modified silicon and hydrogel surfaces. Hydrogel microstructures were much more resistant to bacterial adhesion than TPM-modified silicon as was evident by the difference in adherent cell density on the two surfaces. Here \textit{E. coli} preferentially adhered to a silicon surface inside 3-dimensional hydrogel trenches. These bacteria patterning results are preliminary, with further work being necessary to quantify colony-forming units and optimize the hydrogel formulation for maximum adhesion resistance. However, these results clearly demonstrate the potential for creating substrates for selective bacterial adhesion which could be used in designing novel, cell-based biosensor arrays based on engineered bacteria in a microwell format.
4. Conclusion

Here we demonstrated a simple and general method using photolithography to fabricate patterned hydrogel microstructures and the ability of these structures to control the adhesion and proliferation of mammalian cells on various substrates. Micropatterned substrates were prepared by the photogelation of PEG macromers onto surfaces functionalized with a 3-(trichlorosilyl)-propyl methacrylate monolayer. These functionalized surfaces not only ensured the attachment of the hydrogel to the substrate but also allowed cell adhesion in areas absent of hydrogel because of their hydrophobicity. Using photomasks, we created arrays of microwells and demonstrated that fibroblasts and hepatocytes adhered and spread only on the TPM monolayer without losing their viability. By changing the size of microwells, we could control the number of cells in one microwell. In the case of 30 × 30 μm microwells, as many as 400 microwells were fabricated in 1 mm² and more than 3 million microwells can be fabricated in the same area as a 96 well plate. Mammalian cells placed in high density microwell plates described here could potentially be used in high content drug screening and may be combined with microfluidic devices to create multianalyte cell-based biosensors. Initial results demonstrating the ability of PEG hydrogel microstructures to control bacterial adhesion were also shown, opening the possibility to create genetically engineering cell-based biosensor arrays for the detection of a variety of analytes including pesticides and neurotoxins.

Acknowledgments

We gratefully acknowledge financial support from the National Aeronautics and Space Administration (NAG 9 1277) and the Texas Advanced Technology Program. We also thank Prof. Richard Crooks (Department of Chemistry, Texas A&M University) for the use of the contact angle instrument. MVP wishes to thank the Alfred P. Sloan Foundation for its support through a research fellowship.

References


