Engineering protein and cell adhesivity using PEO-terminated triblock polymers

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Abstract: Previous studies on customizing cell culture environments have utilized a variety of microfabrication-based tools to control the spatial localization of adhesive proteins and subsequently mammalian cells. Others have used various methods to immobilize nonadhesive PEO-based polymers on surfaces to inhibit protein absorption and cell adhesion. In this study, we report the application of a well-characterized, commercially available, PEO-terminated triblock polymer (Pluronic™ F108) to create micropatterned nonadhesive domains on a variety of biomaterials that deter cell adhesion for up to 4 weeks in culture. The Pluronic can be applied using microfluidic tools or photolithographic techniques, and can be adsorbed to a variety of common surfaces including tissue culture polystyrene, methylated glass, silicone, and polylactic-*co*-glycolic acid. The effectiveness of the Pluronic in inhibiting cell adhesion in the presence of collagen I is also quantified. Finally, these patterning techniques are generalized to control tissue organization on a variety of common biomaterials. This simple method for micropatterning PEO and, therefore, proteins and cells should prove useful as a tool for biomolecular surface engineering. © 2002 John Wiley & Sons, Inc. J Biomed Mater Res 60: 126–134, 2002

Key words: micropatterning; poly(ethylene oxide); poly(ethylene glycol); microfluidics; cell engineering; cell adhesion

INTRODUCTION

The ability to control cell-surface interactions is of paramount importance in controlling host-biomaterial interactions, in predicting cell behavior in cell engineering, in understanding tissue development, as well as in realizing the potential to tissue engineer solid organs. The control and study of the role of tissue organization with micropatterning tools have recently provided insight in areas as diverse as angiogenesis, hepatocyte differentiation, calcification of bone-derived cells, stratification of keratinocytes in the epidermis, and neuronal growth cone guidance.[1–5]

Previous methods to create micropatterned cultures that control the cellular microenvironment have relied on either regional chemical modification of substrates to promote cell adhesion or physical localization of cells on a chemically uniform surface. Examples of chemical modification include photolithographic patterning of glass and subsequent silane/protein immobilization,[6] microcontact printing to localize hydrophobic alkanethiols/protein,[7] and photoimmobilization of polymers or adhesive peptides.[8,9] Physical methods of localization include microfluidic networks to deliver adhesive proteins or live cells directly.[10–12] Similarly, laser-directed cell writing is another method of physical localization that utilizes a hollow optical fiber coupled with a laser source to direct the placement of individual cells on a target surface.[13]

Another strategy to localize cells, and one of particular interest in regional modification of biomaterials, is the creation of localized nonadhesive domains. In recent years, the mechanism of interaction between a cell and substrate has been attributed to preimmobilized adhesive peptides or proteins or by adsorption of proteins from surrounding medium. PEO has been widely used to resist "biofouling" and subsequent cell adhesion resulting due to protein adsorption to surfaces.[14] Although still an active area of research, the mechanism by which PEO resists protein adsorption is generally attributed to its hydrophilicity, flexibility, chain mobility, and high steric exclusion volume in water.[15] The steric exclusion volume increases with increasing grafting density, and is thought to be due

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primarily to favorable water-PEO interactions and the thermal motion of the PEO chains.[16] There have been several reports in recent years utilizing microfabrication-derived techniques to localize PEO on artificial substrates. These include photoimmobilization of interpenetrated polymer networks,[17,18] microcontact printing of poly(ethylene glycol) (PEG)ylated thiols on gold monolayers,[7] and use of PEGylated silanes and aldehydes to modify Si-based materials.[19] Typically, these techniques require specific chemistries tailored to each application; however, one method has been reported previously that allows coupling of PEO chains to a variety of biocompatible materials solely based on material hydrophobicity. This method reported by Caldwell and coworkers utilizes a commercial triblock polymer $(PEO)_{129}$-$(PPO)_{56}$-$(PEO)_{129}$ (Pluronic™ F108), which spontaneously adsorbs via the hydrophobic PPO domain to hydrophobic surfaces.[20,21] Using this technique, we demonstrate the ability to micropattern growth-competent 3T3 murine fibroblasts in 5% serum and retain cell-free domains for ~4 weeks on polystyrene. Furthermore, we demonstrate strategies to generalize this approach to hydrophilic surfaces (such as glass) by first rendering the surface hydrophobic. Applications of these techniques are many—indeed, our lab has focused on using these tools to control and study the role of the microenvironment around hepatocytes *in vitro*. These techniques have previously allowed us to examine and optimize the role of heterotypic (hepatocyte/fibroblast) cell-cell interactions in hepatocyte function for applications in tissue engineering.[22] Ultimately, these tools may be useful in many areas including cell and tissue engineering, tailoring biomaterial implants, and fundamental studies on signaling in cell-cell and cell-matrix interactions.

MATERIALS AND METHODS

Photolithographic patterning

Detailed procedures for photolithographic patterning of substrates and subsequent modification were previously described,[23] and are depicted schematically in Figure 1(C). Briefly, 2″ diameter × 0.02″ borosilicate glass wafers (Erie Scientific; Portsmouth, NH) were spin coated with positive photoresist (S1813, Shipley). Wafers were baked and then exposed to ultraviolet light in a Bottom Side Mask Aligner (Karl Suss, Waterbury Center, VT) through emulsion masks of the desired dimensions. We utilize emulsion mask as an inexpensive, readily available alternative to chrome/quartz masks. Patterns are drawn in Corel Draw 9.0* and printed using a commercial Linotronic-Hercules 3300 dpi high-resolution line printer. Exposed photoresist was then developed (MF-319 developer, Microchem Corporation, Waltham, MA; developer : water, 1 : 1), baked, and finally cleaned by exposure to oxygen plasma for 10–15 min.

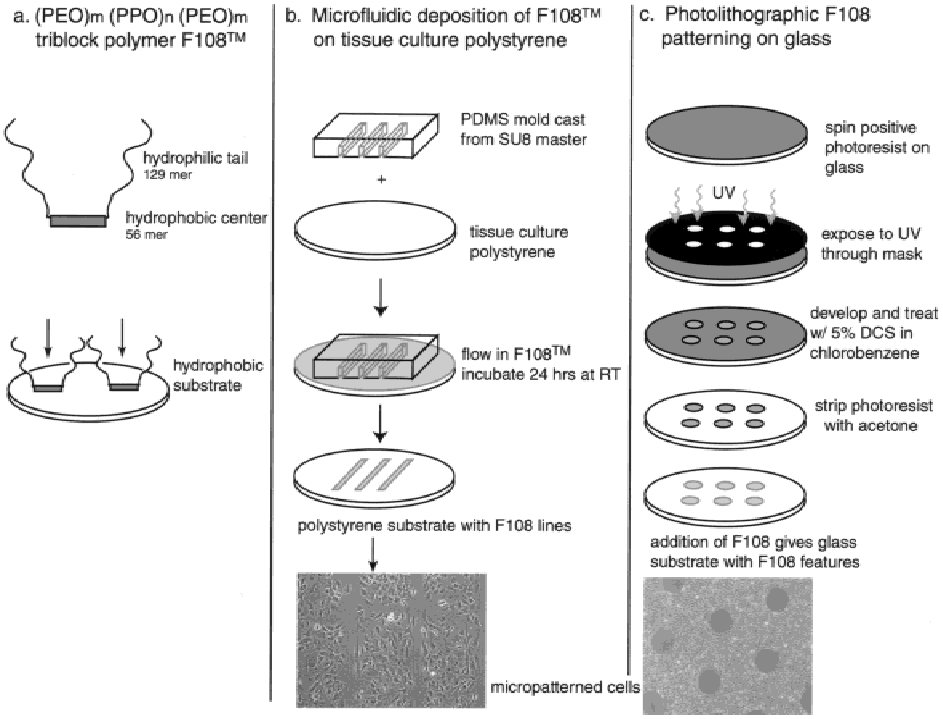


Figure 1. Schematic depiction of two modes of patterning: (A) schematic of triblock (PEO/PPO/PEO) Pluronic™ F108 molecule spontaneously adhering to a hydrophobic surface. (B) Schematic depiction of method to localize Pluronic through microfluidic network on a hydrophobic substrate (such as tissue culture polystyrene). (C) Photolithographic patterning of glass substrates followed by immobilization of "nonadhesive" (PEO) moieties.

Microfluidic patterning

Techniques for microfluidic patterning were adapted from Folch et al.[24] Briefly, a high-aspect ratio photoresist (SU-8, Microchem Corporation, Waltham, MA) (25 μm thick) was spun on silicon wafers (Virginia Semiconductor, Fredricksburg, VA) and exposed to ultraviolet light through an emulsion mask as described above and developed according to manufacturer specifications. This template was used as a mold for casting polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning). PDMS was prepared, degassed under low vacuum, poured over the SU-8 template, and cured at 65 °C for 2 h. The PDMS mold is subsequently removed from the SU-8 template and used as a network of microchannels when superimposed upon a rigid substrate. PDMS forms an aqueous seal with rigid substrates and can, therefore, serve as a vehicle for the localized delivery of adhesive or nonadhesive factors or cells suspended in media.

PEO coupling

Pluronic™ F108 was selected from a family of triblock polymers that are commercially available (BASF, #F-108). This class of polymers have poly(propylene oxide) centers with poly(ethyelene oxide) side chains with the following proportions $(PEO)_{129}$-$(PPO)_{56}$-$(PEO)_{129}$ and a molecular weight of 14,600 g/mol. The poly(propylene oxide) domain adsorbs quasi-irreversibly to hydrophobic surfaces, creating a surface coating of PEO chains; thus, surfaces that are hydrophobic can be modified with PEO regardless of their chemical composition.[14] Although chain length of the PEO domain can vary, Li et al. have previously reported that Pluronic™ F108 was most suitable for deterring protein adsorption within a group of Pluronics with varying PPO and PEO domains. Solutions of 1 or 4% w/w Pluronic™ F108 in water were prepared, injected into microfluidic networks that were opposed to a hydrophobic surface, and allowed to adsorb for 24 h at room temperature. Alternatively, hydrophilic surfaces such as glass were photolithographically patterned, rendered hydrophobic by modification with 5% dimethyltrichlorosilane in chlorobenzene, then stripped of photoresist in acetone, and finally incubated with Pluronic™ F108.

Cell culture

Hepatocytes

Hepatocytes were isolated from 2- to 3-month-old adult female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 180–200 g, by a modified procedure of Seglen.[25] Detailed procedures for isolation and purification of hepatocytes were previously described by Dunn et al.[26] Routinely, 200–300 million cells were isolated with viability between 85 and 95%, as judged by trypan blue exclusion. Nonparenchymal cells, as judged by their size (<10 μm in diameter) and morphology (nonpolygonal or stellate), were less than 1%. Culture medium was Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 0.5 U/mL insulin, 7 ng/mL glucagon, 20 ng/mL epidermal growth factor, 7.5 μg/mL hydrocortisone, 100 U/mL penicillin, and 100 μg/mL streptomycin. Serum-free culture medium was identical except for the exclusion of FBS.

NIH 3T3-J2 fibroblast culture

NIH 3T3-J2 cells were the gift of Howard Green, Harvard Medical School. Cells grown to preconfluence were passaged by trypsinization in 0.01% trypsin (ICN Biomedicals, Costa Mesa, CA)/0.01% EDTA (Boehringer Mannheim, Indianapolis, IN) solution in PBS for 5 min, diluted, and then inoculated into a fresh tissue culture flask. Cells were passaged at preconfluency no more than 10 times. Cells were cultured in 175 cm^2 flasks (Fisher, Springfield, NJ) at 5% CO_2, balance moist air. Culture medium consisted of DMEM (Gibco, Grand Island, NY) with high glucose, supplemented with 5% bovine calf serum (BCS, JRH Biosciences, Lenexa, KS) and 100 U/mL penicillin and 100 μg/mL streptomycin.

Cell adhesion assays

To quantify cell adhesion to Pluronic™ F108-treated surfaces and explore the potential to combine Pluronic™ F108-treated surfaces with specific extracellular matrix (ECM) molecules, cell adhesion to various substrates was determined by light microscopy and image analysis. 125,000 hepatocytes or fibroblasts were initially plated on various substrates in the absence of serum to determine their propensity for mediating cell adhesion. Substrates included: polystyrene (tissue-culture treated) controls, polystyrene + Pluronic™ F108, polystyrene + collagen, polystyrene + Pluronic™ F108 + collagen. After 24 h, unattached cells were removed, and plates were washed with fresh medium and imaged by phase-contrast microscopy. We quantified adhesion using Metamorph Image Analysis software in 12–16 fields per condition. Projected surface areas were measured in Metamorph to determine the area of Pluronic regions that were covered by cells at various time points for 4 weeks.

Microscopy

Specimens were observed and recorded using a Nikon Diaphot microscope equipped with a SPOT digital camera (SPOT Diagnostic Equipment, Software Version 2.2, Sterling Heights, MI), and MetaMorph Image Analysis System (Universal Imaging, Westchester, PA) for digital image acquisition. Fluorescent labels CMFDA (chloromethylfluorescein diacetate, C-2925, Molecular Probes) and CMTMR (chloromethylbenzoylaminotetramethyl rhodamine, C-2927) were utilized to track cells fluorescently. Cells were loaded by incubation in 25 μ*M* dye in media for 45 min, rinsed, and

incubated for 30 min prior to a final rinse. Cells were observed by fluorescence microscopy with ex/em: 492/517 and 541/565 nm.

Statistics and data analysis

Experiments were repeated two to three times with duplicate or triplicate culture plates for each condition. One representative experiment is presented where the same trends were seen in multiple trials. Error bars represent standard error of the mean. Statistical significance was determined using one-way ANOVA (analysis of variance) on Statview with Fisher's PLSD post hoc analysis with $p < 0.05$.

RESULTS

In this study we present methods for micropatterning poly(ethylene oxide) PEO on a variety of materials. Previously, patterns of PEO have been achieved through self-assembled monolayers on gold,[27] photopolymerization of interpenetrated networks [poly (acrylamide-*co*-ethylene glycol)],[28] or silane-based coupling of PEO to Si-based materials.[19] Here we adapt the methods of Neff and coworkers where triblock copolymers (PEO-PPO-PEO) were found to spontaneously adsorb, quasi-irreversibly to hydrophobic biomaterials as seen in Figure 1(A).[14,20,21] The length of PEO chains has been evaluated previously by Neff et al.[20] F108 was found to be superior to other analogues of the triblock copolymer.

Both microfluidic and photolithographic modalities were utilized to localize PEO on biomaterial substrates. Figure 1(B) depicts a patterning scheme for PEO via a fluidic delivery system constructed from a polydimethylsiloxane (PDMS) mold made by soft lithgography. In contrast, Figure 1(C) schematically depicts direct photolithographic patterning of hydrophilic glass substrates with PEO by derivatization of a methyl-terminated silane to the surface. Both primary cells (primary rat hepatocytes) and immortal cell lines (3T3 fibroblasts) were patterned using these surface modifications.

The microfluidic method depicted in Figure 1(B) was utilized to pattern PEO on tissue culture polystyrene. In Figure 2, we demonstrate that patterned PEO domains deterred the attachment of murine 3T3 fibroblasts seeded in the presence of 5% serum.

Previous studies on this family of triblock copolymers have highlighted the potential for serum proteins to elute F108 from the surface. To evaluate the potential for F108 desorption in a conventional cell culture environment, we followed patterned fibroblast cultures in the presence of 5% serum over time. Projected surface area measurements were made over 4 weeks to quantify the cell migration onto the PEO-

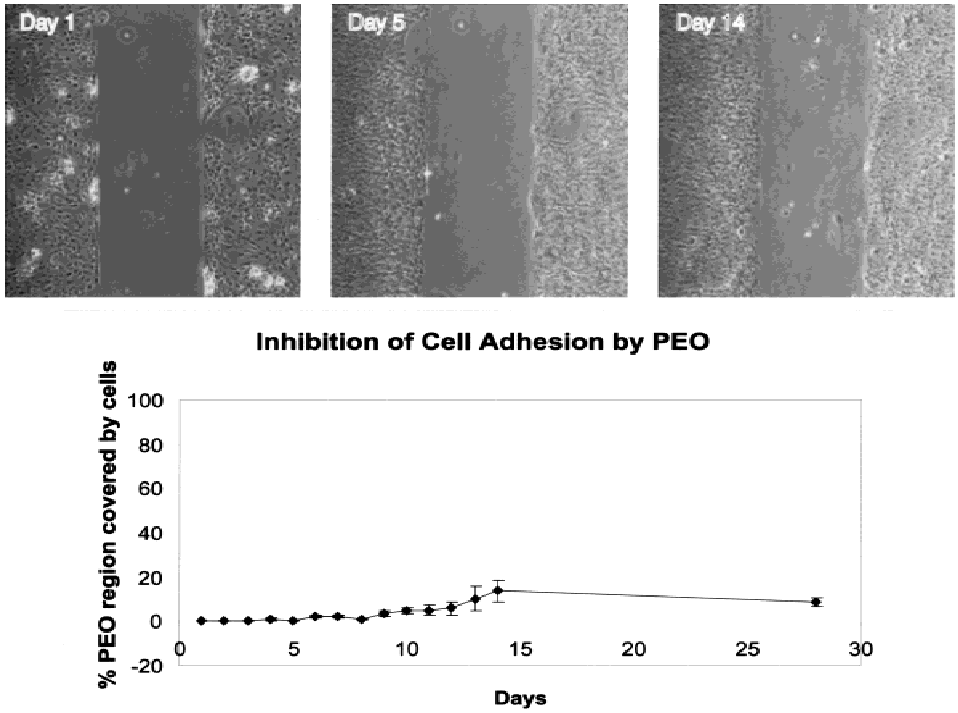
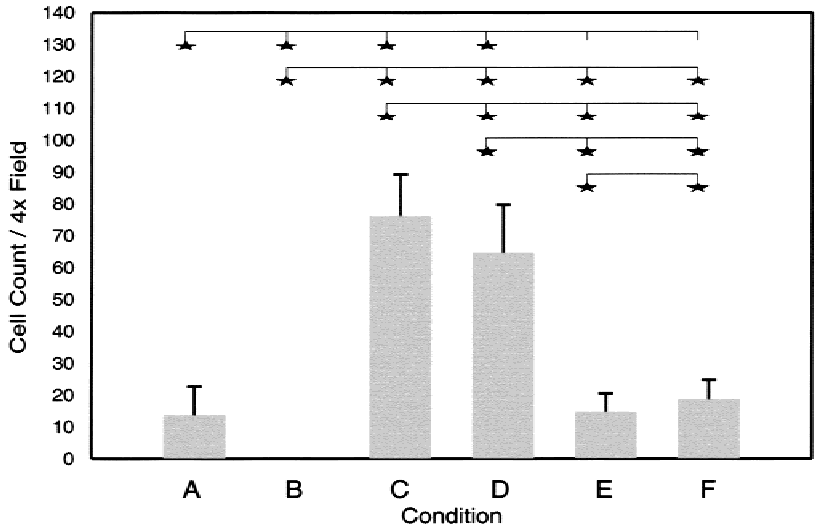


Figure 2. Fluidic localization of PEO adsorption to selectively deter cell adhesion on polystyrene. (A) Repulsion of fibroblasts at days 1, 5, and 14 in the presence of 5% serum in media. (B) Projected surface area measurements of cells in patterned Pluronic regions on polystyrene dishes.

patterned domains. Figure 2 demonstrates that the integrity of the cellular pattern was preserved within 10% of original patterned surface area for 4 weeks. Thus, any desorption of F108 from the surface was not sufficient to promote significant cell adhesion in previously nonadhesive regions. This effect was shown to be cell-type dependent. For example, primary cells (hepatocytes) encroached onto nonadhesive areas much more rapidly (~days rather than weeks), indicating that active cell processes such as ECM production or phagocytosis of F108 may alter its efficacy as a tool to deter cell adhesion.

Because many strategies for biomolecular surface engineering require the presence of serum or extracellular matrix proteins, we also characterized cell adhesion on ECM-coated, F108-treated surfaces. Specifically, we probed a model cell line (murine 3T3 fibroblasts) and a model primary cell (rat hepatocytes) under various conditions (1–100 μg/mL collagen I coating). Figure 3 depicts the result of our studies with primary hepatocytes. Cell adhesion on polystyrene in the absence of serum was minimal (~10 cells per field), and was completely eliminated by treatment with F108. In contrast, pretreatment of polystyrene with 100 μg/mL of collagen for 1 h markedly increased cell adhesion [Fig. 3(C); ~75 cells per field]. Finally, we explored the possibility that F108-passivated surfaces could be rendered adhesive by exposure to high concentrations of adhesive proteins (such as may occur physiologically in serum). Our data indicated that ex-



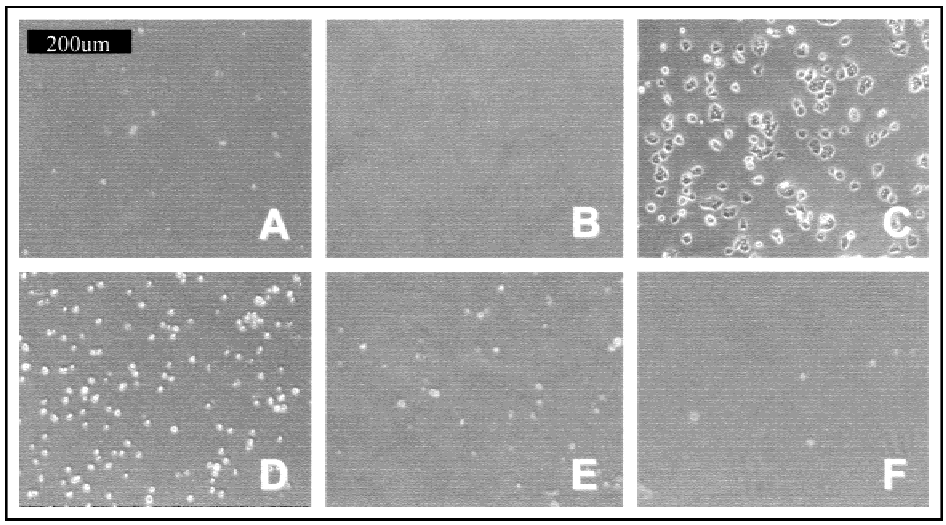


Figure 3. Characterization of the functional interplay between adsorption of extracellular matrix proteins (collagen I) and Pluronic F108 in mediating cell adhesion. Hepatocyte adhesion was assessed on (A) polystyrene control, (B) F108-treated polystyrene, (C) polystyrene coated with 100 μg/mL collagen I and F108-treated polystyrene coated with (D) 100 (E) 10, and (F) 1 μg/mL of collagen I. Adhesion was quantified by image analysis.

posure to 1–10 μg/mL collagen had a modest effect on cell adhesion although substrates were no more adhesive than control polystyrene. In contrast, treatment of F108-passivated surfaces with 100 μg/mL collagen I effectively rendered the surface adhesive (Fig. 3). Similar trends were observed with fibroblasts (data not shown).

The localization of PEO through microfluidic channels in contact with hydrophobic surfaces, although useful and chemically generic, could not be utilized on a common experimental substrate—glass. Indeed, hydrophilic surfaces cannot be modified directly using this adsorptive process. To demonstrate the feasibility of using this technique in conjunction with F108 coupling, borosilicate (glass) wafers with an initial water contact angle of 53° were rendered hydrophobic by coupling of a methyl-terminated silane. The final contact angle with water was 102°. Furthermore, to create discrete PEO islands rather than continuous networks that are easily achieved using microfluidic networks, we explored the use of photolithographic patterning to localize the hydrophobic methyl-terminated silane.

To achieve this, we first photolithographically patterned donuts of 500-μm diameter. These regions were rendered hydrophobic by coupling to a methyl-terminated silane, followed by unmasking of coated glass (known as "lift-off"), and adsorption of F108 to hydrophobic domains. Cell attachment on substrates resulted in nonadhesive donuts as seen in Figure 4(A) [rather than continuous lanes seen in Fig. 4(D)]. To confirm the role of PEO (rather than methylation of glass surface) in deterring cell adhesion on glass, methylated surfaces were compared to methylated surfaces following F108 treatment. Interestingly, methylation itself deterred cell adhesion initially; however, in the presence of media with 10% serum and cells that are known to secrete ECM in the local environment, methylated regions were invaded within 24 h [Fig. 4(B)]. In contrast, methylation followed by F108 exposure retained nonadhesive characteristics similar to those seen in Figure 2 [Fig. 4(C)]. Finally, we explored the resolution and versatility of micropatterned PEO-terminated triblock polymers. Figure 4(D) demonstrates the deposition of Pluronic on a polystyrene surface using the microfluidic technique for features as small as 10 μm. Similarly, cell adhesion was repelled on a commonly used biomaterial such as PLGA [Fig. 4(E)] and silicone (data not shown).

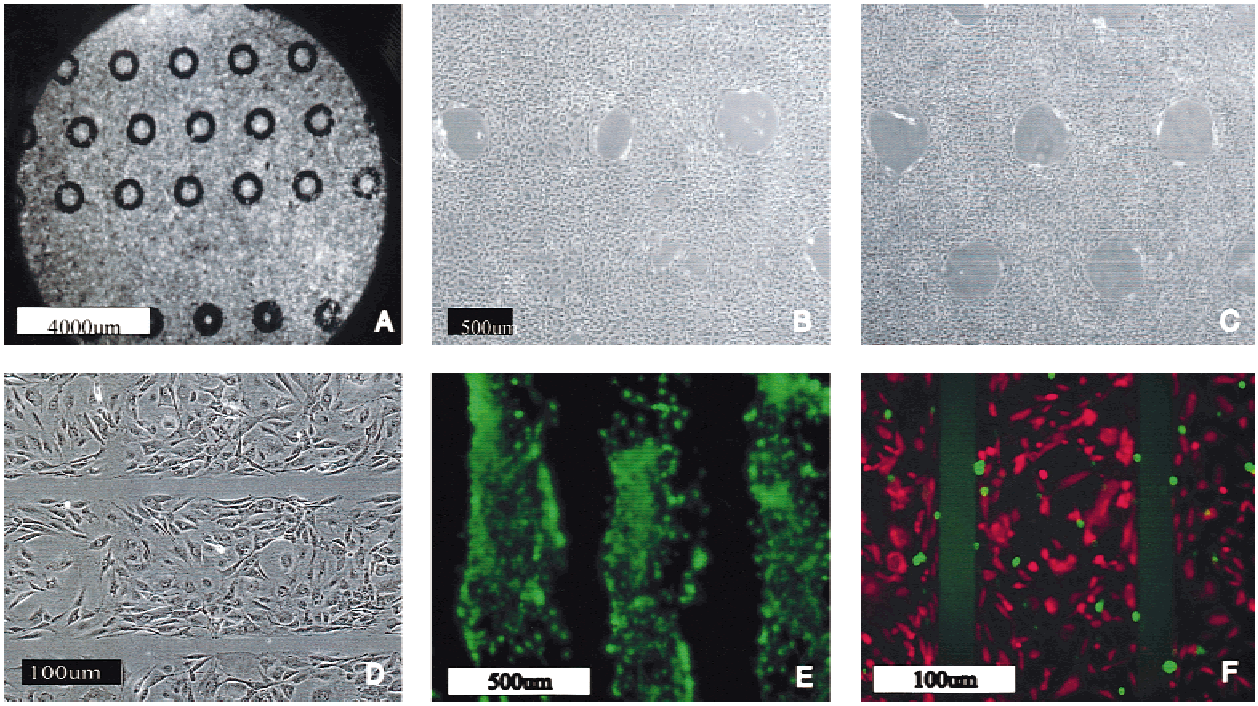


Figure 4. Localization of PEO on a variety of materials including glass 3T3-J2 fibroblasts were seeded on different surfaces that had been patterned with Pluronic. (A) Adsorption of Pluronic F108 to hydrophobic methyl-terminated domains photolithographically patterned on glass deters cell adhesion. (B) Cells patterned on glass via photolithography invade methylated domains on glass within 24 h. (C) Cells patterned on methylated domains followed by Pluronic treatment retain pattern fidelity. (D) Adsorption of Pluronic to hydrophobic tissue culture polystyrene via microfluidic patterning demonstrates resolution of 10 μm. (E) Adsorption of Pluronic to a PLGA film using the microfluidic technique deters cell adhesion. (F) A fluorescein-labeled F108 (Direct-Detect, AllVivo, Birmingham, AL) allows correlation of the PEO pattern (green) on polystyrene with fibroblast localization (red).

DISCUSSION

In this study, we explored the utility of a well-characterized, commercially available, PEO-terminated triblock polymer (F108) to micropattern nonadhesive domains on a variety of biomaterials. These micropatterned domains were shown to deter cell adhesion for up to 4 weeks in culture. F108 is one of a family of triblock polymers that consist of polyethylene oxide tails and polypropylene cores [see Fig. 1(A)]. The hydrophobic polypropylene core mediates adsorption of the polymer to hydrophobic materials, resulting in PEO coated surface by a simple, chemically generic process. This class of polymers has been extensively utilized both in colloidal science as well as biomaterials.[29] Indeed, Neff et al. have demonstrated that the hydrophobic core can be modified with adhesive peptides to generate nonadhesive substrates with well-controlled adhesivities.[21] We have demonstrated that this approach can be combined with microfluidic patterning approaches to localize adsorption on model hydrophobic surfaces such as polystyrene. Furthermore, other hydrophobic biomaterials were similarly modified including PLGA (Poly(DL-lactide-*co*-glycolide), silicone, and polyimide (data not shown). In addition, we generalize this technique to hydrophilic surfaces, such as glass, by first rendering the (patterned) surface hydrophobic using a methyl-terminated silane.

It is widely accepted that deterrence of cell adhesion is due to deterrence of adsorption of adhesive proteins to a surface.[30,31] Li and Caldwell have previously reported that the performance of F108 is superior to other triblocks with regard to resisting protein adsorption; therefore, we selected this triblock polymer for this study.[14] F108 has the advantage of having been shown to be nonimmunogenic, nontoxic, and FDA approved for a wide range of medical applications.[32] The surface concentration, thickness of adsorbed layer, and dynamics of PEO chains have all been shown to be important factors in resisting protein adsorption. Stability of the adsorbed layers has been a point of some debate. While adsorption of F108 is thought to be quasi-irreversible, in fact, F108 can be competitively eluted from a surface. Functionally, however, studies with colloidal polystyrene F108-treated particles indicated that F108 eliminates 90% of plasma protein adsorption from whole human plasma,[14] and our experiments indicate the lack of sufficient protein adsorption in 5% serum-containing culture for cell adhesion for 28 days.

Our studies indicate that the ability to deter cell adhesion *in vitro* was dependent on cell type and culture conditions, as do other techniques of PEO immobilization. Indeed, incubation with high concentrations of collagen I (~100 μg/mL) restored cell adhesion to the cell surface for both fibroblasts and hepatocytes. Whether this is due to elution of F108 from the surface, adsorption of collagen I to exposed polystyrene, modification of cellular activity (e.g., phagocytosis, MMP expression) or a combination is not clear. In contrast, culture with 10% serum, which contains vitronectin, as a likely dominant adhesive protein through binding to $\alpha_v\beta_3$, was not sufficient to render the surface cell adhesive.[33] Thus, the F108 surface is able to resist protein adsorption from culture media and therefore provides a useful in vitro tool for patterning non-adhesive domains. These results, together with *in vivo* data on exposure to whole human plasma, suggests that F108 may provide a valuable *in vivo* tool to control *early* host-biomaterial interactions.[14]

Although surface coverage of F108 was not measured explicitly, our results obtained by adsorption of 1% F108 in water at 37 °C, correlate well with those of Amiji and Park[34] and Neff et al.,[21] who utilized AFM and XPS respectively under similar conditions to characterize surface coverage. The adsorbed F108 density was estimated to lie between 0.17–>0.33 $\mu g/cm^2$ as reported elsewhere. Chain extension is estimated to vary between 52 nm (the length of PEO tail)[35] to 15 nm as reported by Li and Caldwell.[14] In our hands, F108 adsorption (as judged by lack of cell adhesion) produced inconsistent results when adsorbed at 4% w/v in water. We suspect that this is due to occurrence of micelle formation at the critical micelle concentration of 550–3000 μM or 0.7–4.2% (w/v), recently shown more precisely to be 1.4% w/v.[36] Indeed, occasionally a white precipitate reminiscent of micelle formation was observed on the underlying surface. To avoid this regime completely, we are subsequently conducting all experiments at 1.0% w/v, well below the CMC.

In comparison to other previously described techniques for patterning poly(ethylene oxide) domains, the current method of localizing adsorption of triblock polymers via microfluidics or photolithography offers a simple, versatile alternative. The fundamental limitation in patterning a nonadhesive molecule such as PEO is the ineffective adhesion of PEO to the desired surface; therefore, PEO must be derivatized or intermingled with an existing polymer network. For example, Whitesides and coworkers have utilized SH-terminated PEO molecules together with microcontact printing on gold surfaces to create patterned, nonadhesive self-assembled monolayers of PEO.[37] However, this technique has a number of limitations as well. For example, materials must first be coated with metals by evaporation. The metal-material interface, therefore, may deteriorate in certain applications (e.g., *in vivo*). Furthermore, although F108 is commercially available, SH-terminated PEO molecules must typically be synthesized in the laboratory.

Other PEO terminations that have been utilized to pattern PEO include silane-terminated PEO and alde-

hyde-PEO, which are commerically available. These have been utilized to modify photolithographically patterned glass or amine-terminated silanes on patterned glass via a Schiff base reduction.[19] These substrates were characterized by XPS and contact angle measurements and deterred nuerite outgrowth for up to 4 days in serum-free conditions. The resolution of this technique matches that of the photolithographic process, making it ideal for mammalian cells and smaller organisms (bacteria, virus particles); however, it is chemically limited in that silicon-based materials must be utilized to be so terminated. Therefore, this technique would not be easily generalized to polystyrene (tissue culture plastic) or carbon-based biomaterials.

Healy and coworkers have recently reported a method of grafting PEO to surfaces by generation of an interpenetrated network with polyacrylamide.[18,28] Patterned quartz substrates were modified using allyl-terminated silanes. Subsequently, polyacrylamide and PEO are polymerized on the surface by photoinitiation. During the generation of an interpenetrated network, PEO chains must diffuse into the solvent-swollen polyacrylamide network; therefore, the chain length of PEO may be limited by this process (i.e., ~1 kDa as opposed to each PEO chain of ~5.6 kDa used in the current study). Under certain solvent conditions, small PEO chains are poorly interpenetrated, and therefore, diffuse away rapidly and large PEO chains cannot diffuse into the polyacrylamide network.[38,39] Due to the method of polymerization, this technique requires accessibility of all surfaces to light (in this case, indium light source of peak emission at 470 nm). In some cases, materials that have low light transmittance with convoluted surface features or internal conduits may not be suitable to this approach. The functional stability of the interpenetrated PEO layer was demonstrated using bone-derived cells cultured in the presence of 15% serum for as long as 60 days. As with other PEO-immobilization techniques, the ability to deter cell adhesion will likely depend both on cell type and culture conditions.

Finally, other methods of grafting PEO on surfaces in unpatterned (i.e., uniform) surface coatings include gamma-irradiation,[40] and tresyl-terminated linear and star PEO polymers.[16] In the future, these techniques may also be adapted to pattern PEO distribution on material surfaces. We expect that the current technique, which utilizes simple adsorption of triblock polymers, will continue to serve as a simple, chemically generic tool for patterning PEO. This tool for customizing cell culture environments by specifying nonadhesive domains may prove useful for many different cell types rather than specifying adhesive domains with specific integrin-binding ECM molecules. Finally, due to the use of surface hydrophobicity rather than chemistry (gold, silicon) to immobilize PEO, this technique will be useful for a wide range of conventional biomaterials that have carbon backbones. Indeed, Patel et al. recently described the use of microfluidics to render a PLGA template adhesive via modification with adhesive peptides.[41] We propose a similar approach for PEO immobilization. This level of flexibility will broaden the utility of this tool to other fundamental cell and tissue engineering applications.

In the future, we envision that the combination of microfluidic and photolithographic patterning as well as simple adsorption of adhesive (ECM) and nonadhesive (PEO) species may be extended to novel applications such as: modification of the PPO Pluronic core with adhesive peptides to create surfaces with well-defined adhesivity,[20] use of degradable triblocks (PEO-PLGA-PEO) to dynamically modulate adhesivity,[42] and novel substrates such as PEO lipid bilayers[43] and biomaterials (PLGA).[41] Furthermore, the patterning modes utilized may be improved by advances in the contributing disciplines such as recent reports of microcontact printing of proteins[44] and microfluidics with polymer or hydrogel actuation.[45] In summary, techniques to customize cell-surface interactions are increasing our ability to refine both experimental design and also cellular responses to environmental stimuli. These tools may have impact in many diverse fields from cell-based drug discovery to fundamental cell biology to tissue engineering.

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References

1. Healy KE, et al. Kinetics of bone cell organization and mineralization on materials with patterned surface chemistry. Biomaterials 1996;17:195–208.
2. Pins GD, Toner M, Morgan JR. Microfabrication of an analog of the basal lamina: Biocompatible membranes with complex topographies. FASEB J 2000;14:593–602.
3. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276:1425–1428.
4. Bhatia, SN, Balis UJ, Yarmush ML, Toner M. Effect of cell-cell interactions in preservation of cellular phenotype: Cocultivation of hepatocytes and nonparenchymal cells. FASEB J 1999; 13:1883–1900.
5. Hammarback JA, McCarthy JB, Palm SL, Furcht LT, Letourneau PC. Growth cone guidance by substrate-bound laminin pathways is correlated with neuron-to-pathway adhesivity. Dev Biol 1988;126:29–39.
6. Lom B, Healy KE, Hockberger PE. A versatile technique for patterning biomolecules onto glass coverslips. J Neurosci Methods 1993;50:385–397.
7. Singhvi R, Kumar A, Lopez GP, Stephanopoulos GN, Wang DI, Whitesides GM, Ingber DE, Engineering cell shape and function. Science 1994;264:696–698.
8. Clemence JF, Ranieri JP, Aebischer P, Sigrist H. Photoimmobi-

lization of a bioactive laminin fragment and pattern-guided selective neuronal cell attachment. Bioconjug Chem 1995;6: 411–417.
9. Chen G, Ito Y, Imanishi Y, Magnani A, Lamponi S, Barbucci R. Photoimmobilization of sulfated hyaluronic acid for antithrombogenicity. Bioconjug Chem 1997;8:730–734.
10. Chiu DT, et al. Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. Proc Natl Acad Sci USA 2000;97:2408–2413.
11. Folch A, Toner M. Cellular micropatterns on biocompatible materials. Biotechnol Prog 1998;14:388–392.
12. Delamarche E, Benard A, Schmid H, Michel B, Biebuyck H. Patterned delivery of immunoglobulins to surfaces using microfluidic networks. Science 1997;276:779–781.
13. Odde DJ, Renn MJ. Laser-guided direct writing of living cells. Biotechnol Bioeng 2000;67:312–318.
14. Li J, Caldwell K. Plasma protein interactions with Pluronic-treated colloids. Colloids Surfaces B Biointerfaces 1996;7:9–22.
15. Jo S, Park K. Surface modification using silanated poly(ethylene glycol)s. Biomaterials 2000;21:605–616.
16. Irvine DJ, Mayes AM, Satija SK, Barker JG, Sofia-Allgorss, Griffith LG. Comparison of tethered star and linear poly(ethylene oxide) for control of biomaterials surface properties. J Biomed Mater Res 1998;40:498–509.
17. Hubbell, JA. Surface physical intepenetrating networks of poly(ehtylene terephthalate) and poly (ethylene oxide) with biomedical applications. Macromolecules 1992;25:226–232.
18. Bearinger JP, Castner DG, Golledge SL, Rezania A, Hubchak S, Healy KE. P(AAm-co-EG) interpenetrating polymer networks grafted to oxide surfaces: Surface characterization, protein adsorption, and cell detachment studies. Langmuir 1997;13:5175–5183.
19. Saneinejad S, Shoichet MS. Patterned glass surfaces direct cell adhesion and process outgrowth of primary neurons of the central nervous system. J Biomed Mater Res 1998;42:13–19.
20. Neff JA, Caldwell KD, Tresco PA. A novel method for surface modification to promote cell attachment to hydrophobic substrates. J Biomed Mater Res 1998;40:511–519.
21. Neff JA, Tresco PA, Caldwell KD. Surface modification for controlled studies of cell–ligand interactions [In Process Citation]. Biomaterials 1999;20:2377–2393.
22. Bhatia SN, Balis UJ, Yarmush ML, Toner M. Probing heterotypic cell interactions: Hepatocyte function in microfabricated co-cultures. J Biomater Sci Polym Ed 1998;9:1137–1160.
23. Bhatia SN, Yarmush ML, Toner M. Controlling cell interactions by micropatterning in co-cultures: Hepatocytes and 3T3 fibroblasts. J Biomed Mater 1997;34:189–199.
24. Folch A, Hurtado O, Schmidt M, Toner M. Molding of deep polydimethylsiloxane microstructures for microfluidics and biologic applications. J Biomechan Eng 1999;121:28–34.
25. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:29–83.
26. Dunn JC, Yarmush ML, Koebe HG, Tompkins RG. Hepatocyte function and extracellular matrix geometry: Long-term culture in a sandwich configuration [published erratum appears in FASEB J 1989;3:1873]. FASEB J 1989;3:174–177.
27. Prime K, Whitesides G. Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): A model system using self-assembled monolayers. J Am Chem Soc 1993; 115:10714–10721.
28. Thomas CH, Lhoest LB, Castner DG, McFarland CD, Healy KE. Surfaces designed to control the projected area and shape of individual cells. Transactions of the ASME. J Biomechan Eng 1999;121:40–48.
29. Harris JM. Poly(ethylene glycol) chemistry: Biotechnical and biomedical applications. Topics in applid chemistry. New York: Plenum Press; 1992. p xxi, 385.
30. Grinnell F, Feld MK. Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum-containing medium. J Biol Chem 1982;257:4888–4893.
31. Grinnell F. Fibronectin adsorption on material surfaces. Ann NY Acad Sci 1987;516:280–290.
32. BASF. Pluronic and tetronic surfactants. Mount Olive, NJ: BASF; 1990.
33. Thomas CH, McFarland CD, Rezania A, Steele JG, Healy KE. The role of vitronectin in the attachment and spatial distribution of bone-derived cells on materials with patterned surface chemistry. J Biomed Mater Res 1997;37:81–93.
34. Amiji M, Park K. Surface modification by radiation-induced grafting of peo/ppo/peo triblock copolymers. J Colloid Interface Sci 1993;155:231–255.
35. Barnes T, Prestidge C. PEO-PPO-PEO block copolymers at the emulsion droplet-water interface. Langmuir 2000;Published on Web:1–6.
36. McPherson T, Kidane A, Szleifer I, Park K. Prevention of protein adsorption by tethered poly(ethylene oxide) layers: Experiments and single-chain mean-field analysis. Langmuir 1998;14:176–186.
37. Mrksich M, Chen C, Xia Y, Dike LB, Ingber DE, Whitesides GM. Controlling cell attachment on contoured surfaces with self-assembled monolayers of alkanethiolates on gold. Proc Natl Acad Sci USA 1996;93:10775–108778.
38. Klempner D, Sperling L, Utracki L. Interpenetrating polymer networks, vol. IV. New York: American Chemical Society; 1994.
39. Desai NP, Hubbell JA. Surface physical interpenetrating networks of poly(ethylene terephthalate) and poly(ethylene oxide) with biomedical applications. Macromolecules 1992;25: 226–232.
40. Tseng YC, McPherson T, Yuan CS, Park K. Grafting of ethylene glycol–butadiene block copolymers onto dimethyldichlorosilane-coated glass by gamma-irradiation. Biomaterials 1995;16:963–972.
41. Patel N, et al. Spatially controlled cell engineering on biodegradable polymer surfaces. FASEB J 1998;12:1447–1454.
42. Jeong B, Bae YH, Kim SW. In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. J Biomed Mater Res 2000;50:171–177.
43. Dori Y, Bianco-Peled H, Satija SK, Fields GB, McCarthy JB, Tirrell M. Ligand accessibility as means to control cell response to bioactive bilayer membranes. J Biomed Mater Res 2000;50: 75–81.
44. James CD, et al. Patterned protein layers on solid substrates by thin stam microcontact printing. Langmuir 1998;14:741–744.
45. Beebe DJ, et al. Functional hydrogel structures for autonomous flow control inside microfluidic channels. Nature 2000;404: 588–590.