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Cell Adhesion on Polyelectrolyte Multilayer Coated Polydimethylsiloxane Surfaces with Varying Topographies

SRIVATSAN KIDAMBI¹, NATASHA UDPA¹, STACEY A. SCHROEDER¹, ROBERT FINDLAN¹, ILSOON LEE¹, and CHRISTINA CHAN^{1,2}

¹Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, Michigan

²Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan

Abstract

This article demonstrates that the micro-topography of the surface with respect to the pattern size and pitch influences cell adhesion and proliferation. Extensive research has shown the dependence of cell proliferation on substrate chemistry, but the influence of substrate topography on cell attachment has only recently been appreciated. To evaluate the effect of substrate physical properties (i.e., periodic microstructures) on cell attachment and morphology, we compared the response of several cell types (fibroblasts, HeLa, and primary hepatocytes) cultured on various polydimethylsiloxane (PDMS) patterns. PDMS has been used as an artificial construct to mimic biological structures. Although PDMS is widely used in biomedical applications, membrane technology, and microlithography, it is difficult to maintain cells on PDMS for long periods, and the polymer has proved to be a relatively inefficient substrate for cell adhesion. To improve adhesion, we built polyelectrolyte multilayers (PEMs) on PDMS surfaces to increase surface wettability, thereby improving attachment and spreading of the cells. Micrographs demonstrate the cellular response to physical parameters, such as pattern size and pitch, and suggest that surface topography, in part, regulates cell adhesion and proliferation. Therefore, varying the surface topography may provide a method to influence cell attachment and proliferation for tissue-engineering applications.

INTRODUCTION

Cell–substratum interactions are important to many biological phenomena. Elucidating these interactions and how they may be controlled is crucial to understanding how to manipulate and design better biological systems and medical devices. Tissue-engineering application is an example where control of these interactions is essential to the creation of functional engineered tissues.^{1–3} The physical (such as surface irregularities, roughness) and chemical properties (such as hydrophilicity, charge) of a substrate affect the attachment and growth of cells.^{4–7} This study aimed to gain a better understanding of the cellular response to micro-

patterns (i.e., periodic microstructures), which is of significance to the design and application of biomaterials. Many studies have demonstrated that different surface chemistries of materials affect cell attachment,^{8–13} but the role of surface topographical features on cell growth has been less well studied. Microtextured surfaces affect how the cells attach, spread, and proliferate on these surfaces.^{14,15} We characterized the attachment and spreading of different types of cells on polydimethylsiloxane (PDMS) surfaces with varying topographies. PDMS is a silicon-based organic polymer that can be used to build constructs with a wide range of micro- and nano-topographies. PDMS has been used extensively to study cell–substrate interactions in medical implants and biomedical devices because of its biocompatibility,^{16–19} low toxicity,^{17,20,21} and high oxidative and thermal stability.^{22,23} PDMS is elastic and optically transparent and has low permeability to water and low electrical conductivity.^{24,25} These properties, in addition to the ease with which it can be fabricated into microstructures using soft-lithography,²⁶ have made this material attractive for use in cell biology studies, including contact guidance, chemotaxis, and mechanotaxis.^{27–29} Despite the many advantages of PDMS, its applications in microfluidics and medicine have been problematic because PDMS is highly hydrophobic. Even when the surface is made hydrophilic, PDMS gradually reverts to its hydrophobic state because of surface rearrangements. As a result, it is difficult to maintain long-term culture of cells on PDMS because of the difficulty in irreversibly modifying PDMS surfaces to have a stable cell-adhesive layer.³⁰

An approach we used to overcome this problem is the ionic layer-by-layer (LbL) self-assembly technique developed by Decher. Building a polyelectrolyte multilayer (PEM) film coating on top of the PDMS surface increases surface wettability and imparts lasting hydrophilicity, thereby improving adhesion and proliferation of cells on PDMS surfaces.^{31–33} This method holds promise because of the ease with which these films can coat PDMS surfaces, and the thickness of the films can easily be controlled. PEM is a strikingly simple method that allows formation of nanoscale structures by alternate adsorption of poly-anions and poly-cations on virtually any substrate.³⁴ PEMs are excellent candidates for biomaterial applications because of their biocompatibility and bioinertness,^{35–38} their ease of incorporating biological molecules, such as proteins;^{39,40} and the ease of control of the film structure and thickness, providing a simpler alternative for constructing complex 3-dimensional surfaces than with photolithography.^{41,42} Rubner and co-workers have examined the effect of surface chemistry on cell attachment and proliferation.^{35,43,44} In contrast, in this study we examined the influence of PDMS surface topography on cell attachment and proliferation.

We demonstrated that PEM-coated PDMS surfaces with different topographies affect the attachment, spreading, and proliferation of 3 types of mammalian cells: transformed 3T3 fibroblasts (3T3s), HeLa (transformed epithelial) cells, and primary hepatocytes. The PEMs were built using LbL assembly of polyelectrolytes poly(diallyldimethylammonium chloride) (PDAC, the poly-cation) and sulfonated poly(styrene) sodium salt (SPS, the poly-anion), as shown in the scheme in Figure 1A. After cell seeding, we observed differences in cell attachment and spreading depending on the grooves and patterns on the PDMS surfaces. Cell morphology and attachment varied depending on the pattern geometries. Using imaging techniques, we show that changes in the surface topographical features alter the attachment

and spreading of cells, suggesting a physical means of controlling the interaction between the cell and its environment.

EXPERIMENTAL DETAILS

Materials

PDAC (Mw ~ 100,000–200,000) as a 20 wt % solution, SPS (Mw ~ 70,000), fluorosilanes, and sodium chloride were purchased from Aldrich (Milwaukee, WI). PDMS from the Sylgard 184 silicone elastomer kit (Dow Corning, Midland, MI) was used as substrates with varying topographies. The PDMS stamps were used for microcontact printing.⁴⁵ Dulbecco's modified Eagle medium (DMEM) with 4.5 g/L glucose, 10× DMEM, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Life Technologies (Gaithersburg, MD). Insulin and glucagon were purchased from Eli Lilly and Co. (Indianapolis, IN) and epidermal growth factor from Sigma Chemical (St. Louis, MO). Adult female Sprague-Dawley rats were obtained from Charles River Laboratories (Boston, MA). Actin cytoskeleton and focal adhesion staining kit was purchased from Chemicon (Temecula, CA).

Preparation of PDMS stamps

An elastomeric stamp was made by curing PDMS on a microfabricated silicon master, which acts as a mold, to allow the surface topology of the stamp to form a negative replica of the master.⁴⁶ The PDMS stamps were made by pouring a 10:1 solution of elastomer and initiator over a prepared silicon master.⁴⁷ The silicon master was pretreated with fluorosilanes to facilitate the removal of the PDMS stamps from the silicon master. The mixture was allowed to cure overnight at 60°C. The masters were prepared in the Microsystems Technology Lab at the Massachusetts Institute of Technology and consisted of circles with varying diameters with pitch distances of 18 μm and pattern heights of 2.5 μm, as illustrated in Figure 1B.

Preparation of PEMs

Figure 2 shows the chemical structure of the polyelectrolytes, namely SPS and PDAC, used to build PEM films. PDAC and SPS polymer solutions were prepared with deionized (DI) water at concentrations of 0.02 M and 0.01 M, respectively (based on the repeating unit molecular weight) with the addition of 0.1 M sodium chlorides. A Carl Zeiss slide stainer equipped with a custom-designed ultrasonic bath was connected to a computer to perform LbL assembly. Polyelectrolyte dipping solutions were prepared with DI water supplied by a Barnstead Nanopure-ultraviolet (UV) 4-stage purifier (Barnstead International Dubuque, IA) equipped with a UV source and final 0.2-μm filter. Solutions were filtered with a 0.45-μm Acrodisc syringe filter (Pall Corporation, Dow Corning, Midland, MI) to remove particulates. PDMS surfaces were subjected to a Harrick plasma cleaner (Harrick Scientific Corporation, Broomfield, NY) for 3 min at 0.15 torr and 50 sccm flow of oxygen in a plasma chamber. To form the first bilayer, the PDMS substrates were immersed for 20 min in a poly-cation solution. After 2 sets of 5-min rinses with agitation, the PDMS substrates were placed in a poly-anion solution and allowed to deposit for 20 min. Afterwards, the PDMS surfaces were rinsed twice for 5 min each. The samples were cleaned for 3 min in an ultrasonic cleaning bath after depositing a layer of poly-cation/poly-anion pairs. The

sonication step removed weakly bounded polyelectrolytes on the substrate, forming uniform bilayers. This process was repeated to build multiple layers. All experiments were performed using 10 bilayers (i.e., 20 layers), with SPS as the topmost surface, thereby keeping the surface chemistry the same for all surfaces irrespective of the surface topography. Spectroscopic ellipsometry (model M-44, J.A. Woollam Co., Lincoln, NE) was performed according to a method previously described by Rubner and co-workers to obtain the thickness of the PEM film on top of the PDMS surface.⁴⁸ The average thickness of a pair of PDAC/SPS films was determined to be approximately 3.7 to 4.0 nm. The PEM-coated PDMS surfaces were dried before seeding the cells.

Cell culture

Hepatocyte isolation—Primary rat hepatocytes were isolated from 2-month-old adult female Sprague-Dawley rats (Charles River Laboratories), using a two-step collagenase perfusion technique described by Seglen⁴⁹ and modified by Dunn.⁵⁰ The liver isolations yielded 150×10^6 to 300×10^6 hepatocytes. Using trypan blue exclusion, the viability ranged from 90% to 98%. Primary hepatocyte culture medium consisted of DMEM supplemented with 10% FBS, 14 ng/mL glucagon, 20 ng/mL epidermal growth factor, 7.5 µg/mL hydrocortisone, 200 µg/mL streptomycin (10,000 µg/mL) –penicillin (10,000 U/mL) solution, and 0.5 U/mL insulin.

Hepatocyte culture—The cells were seeded under sterile tissue culture hoods and maintained at 37°C in a humidified air/carbon dioxide incubator (90/10 vol %). Primary hepatocytes were cultured on PEM-coated 6-well tissue-culture polystyrene surfaces (TCPS). The multilayer coated TCPS plates were sterilized by spraying with 70% ethanol and exposing them to UV light before seeding the cells onto these surfaces. The cell culture experiments were performed on PEM surfaces without adhesive proteins. Collagen-coated TCPS and uncoated TCPS were used as. A collagen gel solution was prepared by mixing 9 parts of the 1.2 mg/ mL collagen suspension in 1 mM hydrochloric acid with 1 part concentrated (10×) DMEM at 4°C. The control wells were coated with 0.5 mL of this collagen gel solution, and the coated plates were incubated at 37°C for 1 h. Freshly isolated hepatocytes were seeded at a concentration of 2×10^5 cells per well, and 2 mL was added to all the surfaces studied. One mL of fresh medium was supplied daily to the cultures after removal of the supernatant. Samples were kept in a temperature- and humidity-controlled incubator.

NIH 3T3, HeLa cell culture—NIH 3T3 fibroblast and HeLa cell lines were purchased from American Tissue Type Collection (Manassas, VA). Cells grown to 70% confluency were trypsinized in 0.01% trypsin (ICN Biomedicals, Costa Mesa, CA) solution in phosphate buffered saline (PBS) for 10 min and re-suspended in 25 mL medium. Approximately 10% of the cells were seeded into a fresh tissue culture flask, and the rest of the cells were used for the co-culture experiments. Fibroblast medium consisted of DMEM with high glucose supplemented with 10% bovine calf serum and 200 U/mL penicillin and 200 µg/mL streptomycin. NIH3T3 and HeLa cells were seeded at a concentration of 2.5×10^4 cells/mL, and 2 mL was added to all the surfaces studied.

Cell immunostaining

Cells were rinsed with PBS, followed by fixation with 4.0% paraformaldehyde in PBS for 20 min, rinsed 3 times in PBS, and then permeabilized with 0.1% Triton X-100 in PBS for 5 min and washed 3 times with PBS before adding the monoclonal antibody for vinculin. The cells were then washed 3 times with PBS and incubated with fluorescein isothiocyanate-conjugated secondary antibody for vinculin and tetramethyl rhodamine iso-thiocyanate-conjugated phalloidin to label the actin filaments for 60 min. The cells were then washed 3 times with PBS and then incubated with 4',6-diamidino-2-phenylindole for nuclei counterstaining for 5 min and washed again 3 times with PBS. A Leica inverted-phase-contrast and fluorescence microscope with Soft RT (Bannockburn, IL) 3.5 software was used to capture the images of the stained cells.

Determination of cell size and number of cells on the projected area

The Soft RT 3.5 software was used on the phase-contrast images of the cells to determine the average area occupied by a HeLa or mouse 3T3 cell. The surface area occupied by a typical cell on TCPS surfaces was measured from 5 different areas and repeated on 3 different substrates and then averaged for each surface. The number of cells on the projected cell area on the different surfaces was measured using Image J (NIH, Bethesda, MD) software. The projected cell area refers to the area occupied by the cells as seen under the microscope. The number of cells per unit projected area was plotted over time for the various surfaces. The surfaces that supported proliferation showed a linear increase in the number of cells attached per unit area over time. The slope of this plot provided the rate of cell proliferation. To determine the amount of time for the cells to reach confluence, we first counted the number of fibroblasts or HeLa cells on the projected area at confluence. Then a theoretical amount of time was calculated for the cells to reach confluence by extrapolating the amount of time to reach the cell number at confluence from the above plot. This was confirmed visually under the microscope by observing when the cells reached confluence. Statistics was performed using the Student t-test. A *p*-value of 0.05 or lower was considered to be significant.

RESULTS AND DISCUSSIONS

Fabrication of PDMS substrate

The dimensions and the topography of the patterns on the PDMS surfaces are shown in Figure 1B, Figure 3, and Table 1. The circle patterns have a pitch distance (center to center) of 18 μm , whereas the diameter of the circle patterns ranges from 1.25 to 9 μm . The height of the patterns was 2.5 μm . The PDMS patterns were coated with PEMs (PDAC/SPS)₁₀, with SPS as the topmost surface. Thus, the variations in cell morphology and orientation on the different substrates were attributed to the surface topography rather than the surface chemistry. Previously, we used PDAC and SPS to build the multilayers and compared the attachment and spreading of primary hepatocytes on PEM films, with PDAC or SPS as the topmost surface, to TCPS. We reported that primary hepatocytes attached and spread better on SPS surfaces than on PDAC surfaces.¹² In the previous study, we observed that variation in cell adhesion depended on surface chemistry. In contrast, here we studied the effect of surface topography on cell attachment while maintaining the same surface chemistry.

Cell attachment on PEM-coated PDMS surfaces

PDMS surfaces are highly hydrophobic, and it is difficult to irreversibly modify these surfaces to have a stable cell-adhesive layer. As shown in Figure 4, the cells did not attach on PDMS surfaces. Coating the PDMS surfaces with (PDAC/SPS)₁₀ with SPS as the topmost layer improved the adhesion of primary hepatocytes, fibroblasts, and HeLa cells on the PDMS surfaces (Fig. 4). To determine whether cells attached preferentially on a particular type of topography on the PEM-coated PDMS surfaces, we evaluated the 3 different cell types on 9 different surface topographies. The physical topographies varied in pitch distances (center to center of 18 μm) and pattern heights of 2.5 μm , and the diameters of the circle patterns were 1.25 μm (P1), 2 μm (P2), 3 μm (P3), 4 μm (P4), 5 μm (P5), 6 μm (P6), 7 μm (P7), 8 μm (P8), and 9 μm (P9). All PDMS surfaces with varying topographies were coated with (PDAC/SPS)₁₀ with SPS as the topmost layer, and adhesive proteins or ligands were not used. PEM-coated PDMS surfaces without any topographical changes and TCPS were used as controls. The cells were allowed to grow for up to 5 days on the different surface topographies. Each day, the cells on the different types of topographies were imaged using optical microscopy. At least 5 images were taken for each substrate, and at least 3 substrates were tested for each type of surface topography.

Primary hepatocytes—The cells display different attachment preferences and morphologies depending on the pattern size and topography, as shown in Figure 5. The difference in the projected cell area for primary hepatocytes on the different topographies is shown in Table 2. There was a general trend of decreasing cell number with increasing diameter of the circular patterns and decreasing distance from a to d. The number of primary hepatocytes that attached on the P1 (211 – 176 cells/mm²), P2 (201 – 164 cells/mm²), and P3 (191 – 155 cells/mm²) surfaces was comparable with the TCPS control (250 – 210 cells/mm²) and the PEM-coated PDMS surfaces (245 – 200 cells/mm²) (Fig. 5 and Table 2). Cells on the P4–P9 surfaces showed more-limited cell attachment, similar to the uncoated PDMS surfaces (98 – 5 cells/mm²) (Table 2). The cells on the (P4–P9) patterns did not spread and started to lift off over time, resulting in a lower density of cells. Previous studies have showed that primary hepatocytes attached and spread onto SPS surfaces without the need for adhesive proteins.¹² Hence, the observed behavior in this study is independent of the surface chemistry of the substrate and is due to the effect of topography and thus provides an alternative approach to modulating the attachment of primary hepatocytes.

3T3 Fibroblast—The observations were similar when these micro-patterned PDMS surface topographies were cultured with fibroblasts. The cells display varying attachment preferences and morphologies depending on the pattern size and topography, as shown in Figures 6 and 8. Fibroblasts showed varying cell adhesion depending on the diameter of the circle patterns and the distance from a to d. On smooth PEM-coated PDMS surfaces, the morphologies and attachment patterns of the cells were similar to those on TCPS surfaces. On patterned PDMS surfaces, the cell attachment varied as the diameter of the circular patterns and the distance from a to d changed. The cells attached better on the smaller-diameter patterns (P1, P2, P3), which had a correspondingly higher distance from a to d, than on the patterns with the larger diameters and smaller distance from a to d. On the P4–P9 surfaces where the cells detached, the cells appeared more rounded. The number of

fibroblast cells that attached on the P1 (66 – 877 cells/mm²), P2 (62 – 865 cells/mm²), and P3 (57 – 841 cells/mm²) surfaces was comparable with the numbers that attached on the TCPS control (72 – 928 cells/mm²) and the PEM-coated PDMS surfaces (69 – 893 cells/mm²) (Fig. 6 and Table 2). Hence, these PDMS topographies can be used to culture 3T3 fibroblasts, whereas PDMS surfaces P4–P9 were cytophobic to the fibroblast. Few cells attached onto the uncoated PDMS surfaces (16 – 7 cells/mm²) (Fig. 6 and Table 2).

HeLa cells—The results for the HeLa cells were similar to those for the fibroblasts. A higher number of HeLa cells attached to the P1 (98 – 1087 cells/mm²), P2 (93 – 1045 cells/mm²), and P3 (90 – 1036 cells/mm²) surfaces than to the P4 through P9 surfaces and was comparable with the TCPS control (101 – 1240 cells/mm²) and the PEM-coated PDMS surfaces (95 – 1170 cells/mm²). Few cells attached to the uncoated PDMS surfaces (15 – 7 cells/mm²) (Fig. 7 and 8 and Table 2). The coated PDMS surfaces were all covered with PEMs, with SPS as the topmost surface; thus, the observed behavior is due to the surface topography.

Rate of proliferation on PEM-coated PDMS surfaces

Fibroblast—The number of fibroblasts on the projected area was plotted against time, as shown in Figure 9. The surfaces that supported proliferation (TCPS, PEM-coated PDMS, P1–P3) showed a linear increase in the number of cells that attached over time. This linear increase was observed for TCPS, PEM-coated PDMS, P1, P2, and P3, but only TCPS, PEM-coated PDMS, and P1 are shown for illustration. The curves for P2 and P3 were similar to the curve for P1. Figure 9 was used to determine the rate of proliferation on the various surfaces. Table 3 compared the rate of cell proliferation on the surface topographies that support proliferation (P1–P3 and P4) with that of those that do not (P5–P9), as well as on TCPS and PEM-coated smooth PDMS. The rates of proliferation of the fibroblast on the P1–P3 surfaces (11–12 cells/mm² per h) were on par with those of the control TCPS and the PEM-coated smooth PDMS surfaces (12–13 cells/mm² per h), as shown in Table 3. The rate of proliferation of fibroblasts on the P4 surface, although linear, was significantly slower than on the control surfaces (4.6 cells/mm² per h) and did not reach confluence by day 5. The proliferation rate was much lower on the P5–P9 surfaces and close to zero for the P9 surface (Fig. 9 and Table 3). Few fibroblasts attached on the uncoated PDMS surfaces, thus, the rate of proliferation was close to zero, as illustrated in Figure 9 and Table 3.

The number of fibroblasts on the projected area when they reached confluence was measured to be 1350 cells/mm². The theoretical amount of time for the fibroblast to reach confluence was calculated by extrapolating the amount of time to reach the cell number at confluence from Figure 9. The amount of time for the fibroblasts to reach confluence was estimated to be between 4 and 5 days (Table 3). It was confirmed visually under the microscope that the fibroblasts cultured on the P1–P3 samples grew to confluence by day 5, similar to the TCPS and the PEM-coated smooth PDMS surfaces.

Fibroblast cells are present in almost all tissue types and organs, and they play a central role in the support and repair of tissues and organs. When a tissue is injured or a device is implanted, the nearby fibroblasts proliferate and migrate into the affected area and produce a

large amount of collagenous matrix, which helps to isolate and repair the affected tissue.⁵¹ On the other hand, overgrowth and overspreading of fibroblasts can cause diseases such as liver cirrhosis and nonfunctional scar tissues.^{52,53} Thus, surfaces that can modulate fibroblast growth and spreading can be useful in preventing conditions such as scar tissue formation associated with implanted medical devices or engineered tissue constructs.

HeLa Cells—The number of HeLa cells on the projected area was plotted against time, as shown in Figure 10. The surfaces that supported proliferation (TCPS, PEM-coated PDMS, P1–P3) showed a linear increase in the number of HeLa cells that attached over time, similar to that observed with fibroblasts. This linear increase was observed for TCPS, PEM-coated PDMS, P1, P2, and P3. Although not shown, the curves for P2 and P3 were similar to the curve for P1. As with the fibroblasts, Figure 10 was used to determine the rate of proliferation of the HeLa cells on the various surfaces. Table 3 compared the rate of cell proliferation on the various topographies. The rates of proliferation of HeLa cells on the P1–P3 surfaces (14–15 cells/mm²/h) were on par with those of the control TCPS and the PEM-coated smooth PDMS surfaces (15–17 cells/mm² per h), as shown in Table 3. The rate of proliferation of the HeLa cells on P4 surfaces, although linear, was significantly slower than that of the control TCPS surfaces (6.4 cells/mm² per h) and did not reach confluence by day 5 despite a faster rate of proliferation than that of the fibroblasts. The proliferation rate was much lower on the P5–P9 surfaces and close to zero for the P9 surface (Fig. 9 and Table 3). Few HeLa cells attached on the uncoated PDMS surfaces; thus, the rate of proliferation was close to zero (Fig. 10 and Table 3).

The number of HeLa cells on the projected area when they reached confluence was measured to be 1650 cells/mm² on day 5. The theoretical amount of time for the HeLa cells to reach confluence was determined (as described above) to be between 4 and 5 days (Table 3). This was confirmed visually under the microscope. The HeLa cells cultured on the P1–P3 samples grew to confluence by day 5, similar to the TCPS and the PEM-coated smooth PDMS surfaces.

HeLa cells are virulent in nature; they invade other cell cultures and result in the change of many continuous human cell lines into HeLa cell lines. These PDMS surfaces with varying topographies can be used to modulate HeLa cell growth and spreading, thereby potentially preventing them from invading other cell cultures.

Potential explanation of the observed effect of topography on cell attachment

Previous studies have shown a similar effect of topography as seen on the PDMS surface topographies; namely, the spacing and diameter of the features was critical to the attachment and spreading of the cells.⁴ As seen from the data, the smaller-diameter (1.25–3 μ m) P1–P3 surfaces appeared to have a higher cell proliferation rate than the larger-diameter (4–9 μ m) P4–P9 surfaces. A possible explanation for the varying attachment and proliferation of the cells on the different topographies (P1–P9 surfaces) may be attributed to the difference in the area between the features. Figure 1B is a schematic illustrating the overlap of a cell and a flat area between the circle patterns (features). Using the Soft RT 3.5 software on the phase-contrast images of the cells, we determined the average area occupied by a HeLa or mouse

3T3 cell (Table 1). We observed that the area between the 6 adjacent circle patterns (features) decreased from $603 \pm 10 \mu\text{m}^2$ for the P1 surface to $324 \pm 10 \mu\text{m}^2$ for the P9 surface. The average area occupied by a HeLa or mouse 3T3 cell was measured to be $521 \pm 15 \mu\text{m}^2$ (Region 2 in Fig. 1B).

The fibroblast and HeLa cells proliferated on the P1–P3 surfaces, where the surface area between any 6 adjacent features (Region 1 in Fig. 1B) ranged from $603 \pm 10 \mu\text{m}^2$ for the P1 surface to $540 \pm 8 \mu\text{m}^2$ for the P3 surface. The P4 surface, on the other hand, has a surface area of $504 \pm 10 \mu\text{m}^2$, which is on par with the size of an average cell. Even though the cells proliferated on the P4 surfaces, they proliferated significantly slower than on the control TCPS surfaces, likely because fewer cells were able to attach initially. The cells did not proliferate extensively on the P5–P9 surfaces, where the surface area ranged from $468 \pm 6 \mu\text{m}^2$ for the P5 surface to $324 \pm 10 \mu\text{m}^2$ for the P9 surface, which is less than the average size of a cell. The cells proliferated on surfaces where the surface area between the features were larger than the size of an average cell (P1–P3), whereas proliferation was slower on surfaces where the area between the features was on par with the size of the cells (P4) and did not proliferate extensively on surfaces where the area between the features was smaller than the average size of a cell (P5–P9). The results suggest that the amount of surface area between the features, as well as the proliferation rate of the cells on the various topographies, may affect the ability of the cells to attach. Therefore, controlling the surface topography provides an alternative approach for modulating cell attachment and proliferation for tissue-engineering applications.

SUMMARY

In this study, we demonstrated that the hydrophobic and cell-resistant PDMS surfaces can be made to be cell-adhesive surfaces by coating with PEM films. We also demonstrated that the addition of topographical features on the PEM-coated surfaces provided an alternative approach to chemistry for controlling the attachment of primary cells (hepatocytes) and the attachment and growth of transformed cells (3T3 fibroblasts and HeLa cells). The attachment and growth characteristics of the PEM-coated PDMS surfaces were similar for the different cell types. We observed that, in general, the rates of growth of the transformed cells on the PEM-coated smooth PDMS surfaces without any topographical features were comparable with the rates of growth on the control TCPS surfaces. The surface topographies, however, altered the attachment and spreading of the cell lines and primary hepatocytes as well as the proliferating ability of the cell lines.

CONCLUSIONS

PDMS is a useful material for cell biology studies because it can be easily manipulated into different sizes, shapes, and dimensions using soft-lithographic techniques. We found that differences in physical environment (e.g., the surface micro-topography on the PDMS surfaces) influenced the attachment and growth of the cells. Therefore, depending on the application requirements, the surface topography may be used as an alternative approach to chemical properties for controlling the attachment and growth of cells. These PDMS surfaces with varying topographies may be used, for example, to modulate fibroblast growth

and spreading, which can be desirable in preventing conditions associated with fibroblast overproduction and overspreading. Overall, there are many advantages to fabricating devices made of PDMS (e.g., their low cost and ease of fabrication and their biocompatibility and permeability to gas). Finally, as demonstrated in this study, PDMS, when appropriately modified, can be a suitable substrate for culturing and controlling the adhesion of various types of mammalian cells.

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FIG. 1.

(A) Schematic diagram illustrates the method for treating the polydimethylsiloxane (PDMS) surfaces with polyelectrolyte multilayers (PEMs) and culturing cells on the surfaces with different topographies. PEMs (poly(diallyldimethylammonium chloride) (PDAC)/sulfonated poly(styrene) (SPS))₁₀ are built on top of the PDMS surface, and cells are then seeded. (B) Illustration of the overlap of a cell and a flat area between the circle patterns. The diameter (d) changes, whereas the center-to-center distance (a) remains constant for the features: a = 18 μm. Region 1 represents the area between any 6 adjacent patterns (features). Region 2 represents a cell attached between the patterns. Region 3 is the cell nucleus.

FIG. 2. Chemical structure of polyelectrolytes used to build the polyelectrolyte multilayers (A) poly(diallyldimethylammonium chloride) and (B) sulfonated poly(styrene).

FIG. 3.

Phase contrast microscope images of circle patterns on polydimethylsiloxane surfaces of varying diameter (**A**) P1, diameter = 1.25 μm ; (**B**) P2, diameter = 2.0 μm ; (**C**) P3, diameter = 3.0 μm ; (**D**) P4, diameter = 4.0 μm ; (**E**) P5, diameter = 5.0 μm ; (**F**) P6, diameter = 6.0 μm ; (**G**) P7, diameter = 7.0 μm ; (**H**) P8, diameter = 8.0 μm ; (**I**) P9, diameter = 9.0 μm . All the patterns have constant pitch distance (center to center) of 18 μm and height of 2.5 μm (scale bar, 50 μm). Color images available online at www.liebertpub.com/ten.

FIG. 4.

Optical micrographs of HeLa, primary hepatocytes, and fibroblast after 3 days in culture on various surfaces. HeLa cells cultured on (A) polydimethylsiloxane (PDMS), (B) polyelectrolyte multilayer (PEM)-coated smooth PDMS, and (C) tissue-culture polystyrene surfaces (TCPS). Primary hepatocytes cultured on (D) PDMS, (E) PEM-coated smooth PDMS, and (F) TCPS. Fibroblasts cultured on (G) PDMS, (H) PEM-coated smooth PDMS, and (I) TCPS (scale bar, 250 μ m).

FIG. 5. Optical micrographs of primary rat hepatocytes after 3 days in culture on various surfaces: (A) tissue-culture polystyrene surfaces, (B) polyelectrolyte multilayer-coated smooth polydimethylsiloxane surfaces, (C) P1, (D) P5, and (E) P9 (scale bar, 50 μm).

FIG. 6. Fluorescence micrographs of focal adhesion and actin cytoskeleton in fibroblasts revealed with triple labeling using tetramethyl rhodamine iso-thiocyanate-conjugated phalloidin (staining F-actin), anti-vinculin(focalcontacts) and 4',6-diamidino-2-phenylindole(nuclei) after 3 days in culture on various surfaces (**A**) tissue-culture polystyrene surfaces, (**B**) polyelectrolyte multilayer-coated smooth polydimethylsiloxane surfaces, (**C**) P1, (**D**) P5, (**E**) P9 (scale bar, 50μm).

FIG. 7.

Fluorescence micrographs of focal adhesion and actin cytoskeleton in HeLa cells revealed with triple labeling using tetramethyl rhodamine iso-thiocyanate-conjugated phalloidin (staining F-actin), anti-vinculin (focal contacts) and 4',6-diamidino-2-phenylindole (nuclei) after 3 days in culture on various surfaces: (A) tissue-culture polystyrene surfaces, (B) polyelectrolyte multilayer-coated smooth polydimethylsiloxane surfaces, (C) P1, (D) P5, (E) P9 (scale bar, 50 μm).

FIG. 8.

Proliferation of cells on various surfaces at 8, 24, and 72 h after cell seeding: (A) fibroblast and (B) HeLa cells. Data represents mean $\pm$ standard error of 3 independent experiments (* $p < 0.05$ compared with control tissue-culture polystyrene surfaces). PEM, polyelectrolyte multilayer; PDMS, polydimethylsiloxane. Color images available online at www.liebertpub.com/ten.

FIG. 9. Fibroblast proliferation on various surfaces (A) tissue-culture polystyrene surfaces, (B) polyelectrolyte multilayer-coated smooth polydimethylsiloxane (PDMS) surfaces, (C) P1, (D) P5, (E) P9, (F) PDMS. Data represent mean $\pm$ standard error of 3 independent experiments.

FIG. 10. HeLa proliferation on various surfaces (A) tissue-culture polystyrene surfaces, (B) polyelectrolyte multilayer-coated smooth polydimethylsiloxane (PDMS) surfaces, (C) P1, (D) P5, (E) P9, and (F) PDMS. Data represent mean $\pm$ standard error of 3 independent experiments.

Table 1

Dimensions of the Different Surface Topographies Used in The Study

Surface	Diameter (d), μm	Pitch Center to Center (a), μm	(a-d), μm	Area between 6 adjacent features (μm^2)
P1	1.25	18	16.75	603 ± 10
P2	2	18	16	576 ± 11
P3	3	18	15	540 ± 8
P4	4	18	14	504 ± 10
P5	5	18	13	468 ± 6
P6	6	18	12	432 ± 9
P7	7	18	11	396 ± 9
P8	8	18	10	360 ± 11
P9	9	18	9	324 ± 10

Table 2

Cell Numbers on the Projected Area on the Different Surfaces used in the Study After 8 H, 24 H, and 3 Days

Surface	Primary Hepatocytes (#cells/mm ²) (5×10 ⁵ , initial concentration)			Fibroblasts (# of cells/mm ²) (5×10 ⁴ , initial concentration)			HeLa (# of cells/mm ²) (5×10 ⁴ , initial concentration)		
	8 h	24 h	3 days	8 h	24 h	3 days	8 h	24 h	3 days
Mean ± Standard Deviation									
TCPS	250 ± 27	230 ± 25	210 ± 22	72 ± 2	292 ± 11	928 ± 25	101 ± 11	401 ± 19	1240 ± 25
PDMS	98 ± 11 ^a	7 ± 1 ^b	5 ± 1 ^c	16 ± 2 ^d	15 ± 2 ^e	7 ± 1 ^f	15 ± 3 ^g	10 ± 3	7 ± 1
Polyelectrolyte multilayer-coated PDMS	245 ± 23	210 ± 18	200 ± 19	69 ± 3	288 ± 13	893 ± 18	95 ± 12	395 ± 22	1170 ± 18
P1	211 ± 19	187 ± 21	176 ± 20	66 ± 1	255 ± 14	877 ± 21	98 ± 11	382 ± 18	1087 ± 21
P2	201 ± 18	170 ± 15	164 ± 18	62 ± 2	252 ± 12	865 ± 15	93 ± 13	371 ± 19	1045 ± 15
P3	191 ± 20	161 ± 14	155 ± 7	57 ± 1	242 ± 15	841 ± 19	90 ± 10	321 ± 26	1036 ± 22
P4	182 ± 14	75 ± 3 ^b	67 ± 6 ^c	48 ± 4 ^d	128 ± 11 ^e	357 ± 15 ^f	77 ± 15	220 ± 25 ^h	520 ± 12 ⁱ
P5	189 ± 11	65 ± 2 ^b	56 ± 6 ^c	35 ± 3 ^d	113 ± 13 ^e	125 ± 14 ^f	62 ± 11 ^g	191 ± 14 ^h	220 ± 12 ⁱ
P6	139 ± 17 ^a	59 ± 4 ^b	48 ± 9 ^c	27 ± 1 ^d	117 ± 18 ^e	79 ± 19 ^f	37 ± 5 ^g	137 ± 15 ^h	165 ± 17 ⁱ
P7	142 ± 19 ^a	45 ± 2 ^b	34 ± 7 ^c	19 ± 2 ^d	69 ± 12 ^e	65 ± 13 ^f	36 ± 8 ^g	76 ± 17 ^h	98 ± 19 ⁱ
P8	110 ± 15 ^a	26 ± 1 ^b	19 ± 4 ^c	15 ± 2 ^d	40 ± 9 ^e	56 ± 10 ^f	22 ± 3 ^g	42 ± 13 ^h	58 ± 10 ⁱ
P9	115 ± 16 ^a	28 ± 2 ^b	17 ± 1 ^c	16 ± 2 ^d	25 ± 8 ^e	19 ± 9 ^f	19 ± 4 ^g	39 ± 14 ^h	47 ± 12 ⁱ

Student *t*-test was used for analyzing the differences between cell adhesion on various surfaces (^a–^c *p* < 0.05 compared with primary hepatocyte adhesion on tissue-culture polystyrene surfaces (TCPS), ^d–^f *p* < 0.05 compared with fibroblast adhesion on TCPS, ^g–ⁱ *p* < 0.05 compared with HeLa cell adhesion on TCPS).

PDMS, polydimethylsiloxane.

Table 3

Rate of Proliferation of Fibroblast and HeLa Cells on Surfaces that Supported Proliferation

Surface	Rate of Proliferation (# of cells on projected area/mm ² /h)	Theoretical number of days to confluency
Fibroblasts		
TCPS	12.63 ± 0.5	4.6 ± 0.1
PEM-coated PDMS	12.13 ± 0.6	4.7 ± 0.2
P1	12.06 ± 0.6	4.8 ± 0.2
P2	11.93 ± 0.7	4.9 ± 0.1
P3	11.65 ± 0.6	5.0 ± 0.2
P4	4.56 ± 0.9 ^a	12.2 ± 1.1
P9	0 (−0.11 ± 0.09)	n.a.
Uncoated PDMS	0 (−0.14 ± 0.08)	n.a.
HeLa cells		
TCPS	16.78 ± 0.7	4.2 ± 0.1
PEM-coated PDMS	15.76 ± 0.6	4.4 ± 0.1
P1	14.46 ± 0.5	4.8 ± 0.2
P2	13.90 ± 0.4	4.9 ± 0.1
P3	14.02 ± 0.4	5.0 ± 0.2
P4	6.41 ± 0.8 ^b	10.5 ± 1.0
P9	0.12 ± 0.05	573 ± 6.0
Uncoated PDMS	0 (−0.13 ± 0.05)	n.a.

^a $p < 0.05$ compared with rate of proliferation of fibroblasts on tissue-culture polystyrene surfaces (TCPS),

^b $p < 0.05$ compared with rate of proliferation of HeLa cells on TCPS.

PEM, polyelectrolyte multilayer; PDMS, polydimethylsiloxane.