Chemical and physical modifications to poly(dimethylsiloxane) surfaces affect adhesion of Caco-2 cells

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Abstract: Polydimethylsiloxane (PDMS) silicone elastomer is extensively used in soft lithography processes to fabricate microscale or nano scale systems for microfluidic or cell culture applications. Though PDMS is biocompatible, it is not an ideal material for cell culture due to its poor cell adhesion properties. In this study, PDMS surfaces were modified to promote intestinal cell adhesion, in the interest of testing feasibility of using microfabricated PDMS systems for high throughput drug screening. Modification techniques included changing chemical composition of PDMS (i.e., varying curing to mixing agent ratio, and oxidization of PDMS surface by oxygen plasma), surface treatment of PDMS by coating with charged molecules (i.e., poly-D-lysine, L-α-phosphatidylcholine, and a layer by layer coating), and deposition of extracellular matrix (ECM) proteins (i.e., laminin, fibronectin, and collagen). The influence of these modifications on PDMS properties, including elastic modulus and surface properties (wettability, chemical composition, topography, and protein adsorption) were characterized. Modification techniques were all found to change PDMS properties and influence the attachment and proliferation of Caco-2 cells over three days of culture to varying degrees. Generally, Caco-2 cells preferred to attach on collagen-coated, fibronectin-coated, and fibronectin-coated oxygen-plasma treated PDMS. The results highlight the importance of considering multiple physical and chemical factors that may be influenced by biomaterial modification and result in altered cell attachment to microfabricated systems, including surface hydrophobicity, chemical composition, stiffness, and topography. This study provides a foundation for further miniaturization, utilizing soft lithography techniques, of Caco-2 cell-based system for high-throughput screening of drug intestinal absorption during lead optimization in drug discovery. The understanding of different surface modifications on adjusting cell adhesion on PDMS allows systemic design of Biomicroelectromechanical Systems (BioMEMS) with tunable cell adhesion properties. © 2009 Wiley Periodicals, Inc.

Key words: Caco-2 cells; Polydimethylsiloxane (PDMS); surface modification; cell attachment

INTRODUCTION

Microfabrication techniques, which were initially developed for the semiconductor industry to fabricate integrated circuits, have been modified to manufacture a large variety of tools for biological research.1–3 Microtechnology enables the production of miniature structures in nanometer to millimeter scales with a nanoscale resolution. As most cells and cellular features are of the nanometer to micrometer scale, microfabrication technologies and microfabricated devices are ideal for studies of cellular phenomena at single or multicell levels.4 Using microfabrication tools, researchers are able to precisely design the microenvironment surrounding cells, such as the biochemical composition and topology of the cell culture substrate, the medium composition, and the type of neighboring cells.2,4,5 A wide range of microfabrication techniques has been utilized in fabricating cell-based assay systems and microscale tissue engineered constructs.5 However, photolithography, soft lithography, and etching are the most popular techniques. Soft lithography techniques are based on the replica molding of microstructures in elastomer, most typically poly(dimethylsiloxane) (PDMS).7 Compared with other microfabrication methods, soft lithography technique is preferred for many biological applications, due to the fact that PDMS is biocompatible, transparent, inexpensive, permeable to gases, and amenable to surface.
Modification. Moreover, PDMS was reported to have mechanical properties similar to those of human tissue. For example, the elastic modulus of PDMS is \(\sim 2\) MPa, while the elastic moduli of most human tissues fall into the range of several MPa. Recently, PDMS-based microfluidic systems have been developed as biocompatible and rapidly prototyped systems for microscale-cell culture.6

The human intestinal Caco-2 cell line has many characteristics of normal intestinal epithelial cells and thus has been extensively used as an in vitro drug absorption model in studies aimed at the elucidation of drug permeability and absorption mechanisms over the last twenty years.11,12 Drug transport experiments utilizing Caco-2 cells are usually carried out in diffusion chamber systems in which two compartments, the apical and basal compartments, are separated by a filter membrane. Caco-2 cells are cultured on the filter support for 21 days, and drug is dosed to the apical compartment. Drug concentration in the basal compartment is measured at various time intervals, and the acquired data are used to calculate drug permeability across the Caco-2 monolayer as one of the critical factors determining drug bioavailability.13 Caco-2 drug permeability experiments are often carried out in a high throughput fashion, and the surface area of cell culture filters ranges from 4.7 to 0.14 cm\(^2\), which corresponds to 6-well and 96-well Caco-2 cell-based drug transport systems, respectively. Miniaturization of this system could provide a vehicle for better high throughput drug absorption screening that would increase drug screening efficiency, and also enable single as well as multicell drug absorption mechanism studies. These enhanced capabilities associated with miniaturization could be highly beneficial to drug discovery and development processes. Soft lithography technique is one way to scale down the size of these systems to the milli- or micrometer range. However, the compatibility of PDMS as a cell culture substrate for Caco-2 cells has not yet been investigated. This study on Caco-2 behavior on PDMS substrates will provide a foundation for further miniaturization of Caco-2 cell-based diffusion cell systems.

Poly(dimethylsiloxane) is generally considered as a biocompatible polymer; however, it is not ideal for the adhesion of cells due to the low surface energy between it and water (about 20 dynes/cm). Surface energy, which is directly related to surface wettability, thermodynamically dictates the adhesion forces of cells from a liquid solution onto a solid substrate.14 Neither substrates with low surface energy nor substrates with high surface energy are favorable for cell adhesion. Surfaces with intermediate surface energies (about 57 dynes/cm) were found to best support the adhesion of human skin cells,15 and surfaces with moderate hydrophilicity (20–40 degree water contact angle) promoted the highest levels of NIH 3T3 fibroblast cell adhesion.16

Due to PDMS’ poor cell adhesion properties, it is rather difficult to maintain long-term culture of cells on PDMS; cell detachment has been observed when cell layers reach confluence.17 To improve cell adhesion on PDMS, Kidambi et al. deposited multiple polyelectrolyte layers to increase PDMS wettability.18 Modification of PDMS stiffness by varying the base to curing agent ratio was also proven to affect long-term growth of vascular smooth muscle cells (VSMC) on PDMS.19 Lee et al. found that physisorption of extracellular matrix (ECM) proteins, such as fibronectin, could increase mammalian cell attachment on PDMS. In this study, PDMS surfaces were modified by varying chemical composition (i.e., varying base to curing agent ratio, oxidizing PDMS using oxygen plasma), changing surface charge by coating with charged molecules (e.g. poly-d-lysine, lecithin, layer-by-layer coating), or depositing ECM proteins (e.g. laminin, fibronectin, collagen, Matrigel™). To enhance fundamental understanding of how these modification techniques alter material properties and of relationships between cell attachment and material properties, their influence on material properties was studied. Specifically, amount of protein adsorption, mechanical properties, surface chemical composition, surface topography, and surface wettability were studied and related to intestinal epithelial cell (Caco-2) adhesion. Caco-2 cells were also cultured on Matrigel™ basement membrane matrix and collagen membranes for comparison.

**MATERIALS AND METHODS**

Fabrication and modification of PDMS substrates

The most common form of PDMS used in the microfabrication literature, Sylgard 184 (Dow Corning, Midland, MI), was used in this study. Sylgard 184 contains two parts: a ‘base’ and ‘curing agent’ that are typically mixed in a 10:1 w:w ratio and undergo a hydrosilylation reaction upon cross-linking.9,19 PDMS surfaces were prepared by mixing certain ratios of base and curing agent. 1.5 mL of PDMS mixture was added into each well of 6-well plates. The mixture was degassed in a vacuum desiccator for 30 min, and then baked in an oven at 70 °C for 2 h. All PDMS substrates were sterilized in 70% ethanol overnight and then washed with PBS 3 times before further coating treatment and cell culture.

To fabricate PDMS substrates with different surface energies, the substrates were either prepared by mixing base and curing agent at various ratios or by oxidizing PDMS surfaces using oxygen plasma. Three PDMS substrates having three different base to curing agent ratios (w/w): 5:1, 10:1, and 20:1, were prepared in this study.
The plasma oxidation of 10:1 PDMS substrates was conducted in a custom-built plasma CVD chamber. The oxygen flow rate was 30 standard cubic centimeters per minute (scm) and the pressure was 150 m Torr. The substrates were exposed to oxygen plasma for 2 min. The oxidized surfaces were protected from air by immersion in ethanol immediately after oxygen plasma treatment. This step was included to prevent reorganization of surface functional groups which may occur in the presence of air. All of the above substrates were sterilized by immersion in 70% w/v ethanol solution overnight and washing with sterilized phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO), and used in cell culture immediately.

To prepare 10:1 PDMS substrates with surface charges, the substrates were coated with Poly-D-lysine (PDL, Sigma-Aldrich), l-α-phosphatidylcholine (LAP, Sigma-Alrdrich) or a polystyrene sulfonate (MW 75 k, Alfa Aesar) and polyethyleneimine (branched, MW 25 k, Sigma-Aldrich) layer by layer (LBL) coating. PDL and LAP coated substrates were prepared by flooding sterilizing PDMS substrates with 0.1 mg/mL PDL in PBS for several hours or 0.1 mg/mL LAP in PBS for 15 min. The LBL coating was performed according to the method described by Ai et al. and Brown et al. Briefly, sterilized PDMS substrate was repeatedly flooded with alternating solutions of the positively charged 0.1% w/v polyethylenimine solution in PBS and the negatively charged 0.3% w/v polystyrene sulfonate solution in PBS at room temperature. The adsorption time for each layer was 30 min, and the PDMS substrates were in total coated with six layers (three layers for each solution). After substrates were coated with PDL, LAP, or LBL, they were rinsed in sterile PBS three times, air dried for 30 min, and used in cell culture immediately.

To prepare the 10:1 PDMS substrates with extracellular matrix (ECM) component coating, the substrates were coated with fibronectin from bovine plasma (FN, Sigma-Aldrich), laminin from Engelbreth-Holm-Swarm (EHS) murine sarcoma (LN, Sigma-Aldrich), or collagen type I from calf skin (C, Sigma-aldrich) respectively. Sterilized PDMS substrates were immersed in 50 μg/mL fibronectin in PBS, 5 μg/mL laminin in PBS or 50 μg/mL collagen in 0.02M acetic acid (Sigma-Alrdrich) for 1 h at room temperature. The treated substrates were then washed three times in PBS and used in cell culture immediately.

Fabrication of collagen and matrigel substrates

Pure collagen substrates or collagen glycosaminoglycan (GAG) substrates were fabricated according to Pins et al.. Briefly, 5 mg/ml type I collagen from calf skin in 0.05M acetic acid was blended with or without 0.18 mg/mL shark cartilage chondroitin 6-sulfate (Sigma-Alrdrich) and then poured onto a PDMS coated Petri dish (~330 μL/cm²). The collagen or collagen-GAG solution was air-dried overnight at room temperature in a laminar flow hood, and the dried membranes were then heat cross-linked at 105°C for 24 h. The resulting collagen or collagen-GAG membranes were washed exhaustedly in PBS and sterilized in 70% ethanol prior to cell culture experiments.

To prepare Matrigel™ substrate, high concentration (protein concentration 18–22 mg/mL) phenol red free Matrigel basement membrane matrix (BD Biosciences) was thawed overnight at 4°C on ice. The thawed Matrigel™ was diluted in 1 part ice-cold PBS and dispensed directly into a 6-well cell culture plate. The Matrigel™ solidified after warming up to room temperature and was used in cell culture.

Substrate characterization

Advancing static water contact angles were measured using a SEO Contact Angle Analyzer (Phoenix300 Plus). Briefly, after the surface treatments described earlier, samples were rinsed with PBS and then air dried prior to contact angle measurement. Contact angles at five different locations on each sample were measured.

The elastic modulus was determined using a tensile test method with a small-scale mechanical testing machine (BOSE Endura ELF 3100). Briefly, PDMS sheets (15 mm × 6 mm, length × width) were clamped on both ends. Samples were gradually stretched 2, 4, 6, 8, and 10% of their original length, and the corresponding force for each stretch step was recorded. The elastic modulus was calculated from the slope of the plot of strain (F/A) versus stress (DL/L), where F is applied force, A is unstrained cross-sectional area, L is original length and DL is change in length.

The surface topology and roughness of samples was examined by Atomic Force Microscopy (AFM). AFM characterization was performed using an Ambios Technology 2SAAV0 USPM under wave mode (noncontact/ tapping) with a cantilever frequency of 188 ± 1 kHz. The scan resolution of 512 × 512 pixels and a scan frequency of 1 Hz was used for data collection. The surface chemical composition (specifically, the atomic concentration of three major chemical components of the PDMS, O, Si, and C; as well as S, the component typically associated with protein) of samples was examined using Energy Dispersive X-ray Spectroscopy (EDS) with a 15 keV electron beam. Briefly, samples were subject to different treatments as described earlier and then rinsed with PBS to remove loosely bound molecules. Samples were then air dried and coated with carbon before insertion in EDS.

Quantification of surface bound protein

The surfaces coated with different protein solutions were washed three times with PBS. Surface bound protein was then measured by micro-BCA colorimetry assay (Pierce) according to the manufacturer’s protocol. Bovine serum albumin was used as a standard for cell culture medium coating and known concentrations of fibronectin, laminin, and collagen were used as standards for fibronectin, laminin, and collagen coating, respectively.

Cell culture and reagents

PDMS substrates, glass coverslips (used as a control) as well as collagen and collagen GAG substrates were attached to the bottom of wells of 6-well plates using
PDMS as an adhesive. Four samples of each type of surface were fabricated for each day, over a series of 3 days. Caco-2 cells were grown in Eagle’s minimum essential medium containing 20% fetal bovine serum (American Type Culture Collection) and 1% antibiotic-antimycotic solution (10,000 units penicillin, 10 mg streptomycin, and 25 μg amphotericin B per mL, Sigma-aldrich). Caco-2 cells were maintained at 37°C in a water-jacketed incubator with 5% carbon dioxide (CO2). The cell culture medium was exchanged every 3 days. Confluent cell layers were treated with 0.25% (w/v) Trypsin in 0.53 mM EDTA solution (Sigma-aldrich) and incubated at 37°C until cells fully detached. Cells were then resuspended in the cell culture medium at a density of 6.3×10⁴ cells/mL. A 3 mL suspension was added to each well containing a test substrate as well as to wells containing no substrate [cell culture treated polystyrene (control)], which resulted in a seeding density of approximately 2×10⁴ cells/cm². The cells were allowed to attach and grow for 1–3 days in the cell culture incubator.

Cell attachment

The nuclei of the Caco-2 cells were fluorescently labeled with Hoechst 33,258 nucleic acid stain (Molecular Probes). A 10 μL aliquot of 10 mg/mL Hoechst 33,258 dye was added to each well. The medium was removed and the substrates were rinsed with three washes of PBS solution. The cells were fixed with 4% (w/v) paraformaldehyde (Sigma-Aldrich) solution in PBS for 5–10 min, and rinsed with three washes of PBS; then the cells were incubated with dye at 37°C for 10 min. Images of fluorescently stained Caco-2 cells were acquired with an Olympus digital camera (DP70) using DP Manager software. Cells were counted in each image using Image J software (http://rsb.info.nih.gov/ij/). Each day, nuclei of cells from each treatment group were labeled. Cell growth on each type of modified PDMS surface was determined by counting the number of stained nuclei in a 2.3 mm² area (10× magnification). Cells from 5 fields/well and four different wells were counted, and the cell numbers were averaged and normalized by the cell number on conventional polystyrene (PS) cell culture surfaces 24 h after initially seeded. The data thus represent the average number of cells from 20 images at each condition, and the error bars represent the standard error of the mean.

Cell spreading (actin staining)

The actin structures were labeled using phalloidin (Molecular Probes). Caco-2 cells were fixed in 3.7% formaldehyde in PBS for 10 min at room temperature, then washed twice with PBS and permeabilized by 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 5 min. After two washes with PBS, cells were incubated with 0.16 μM phalloidin in 1% BSA/PBS for 20 min at room temperature. Cells were counterstained with 5 μg/mL Hoechst 33,258 in PBS for another 20 min at room temperature. Cell cytoskeleton was observed using a fluorescence microscope (Olympus X51).

Statistical analysis

A two-sample t-test assuming unequal variance was used as a statistical test. Results are expressed as means ± standard error (SE), and were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Characterization of modified PDMS physical and chemical properties

The physical and chemical characteristics of materials, such as their stiffness, surface energy (wettability), topography, and chemistry, play an essential role in cell adhesion to substrates. In order to investigate the effect of changing base to curing agent mixing ratio on substrate stiffness, elastic moduli of PDMS specimens were measured by tensile test [Fig. 1(A)]. The tensile test results suggested that PDMS with 20:1 mixing ratio has much lower modulus (0.35 MPa) than PDMS with 10:1 mixing ratio (1.63 MPa), while PDMS with 5:1 mixing ratio has a similar modulus (1.56 MPa) to that of PDMS with 10:1 mixing ratio. Generally, a low base to curing agent mixing ratio means a high degree of cross-linking in the polymer, resulting in a high modulus. However, if mixing ratio is lower than the stoichiometry prescribed by the manufacturer (10:1), there will be an excess of cross-linker in the system, such that the degree of cross-linking in the polymer may no longer increase with an increase in curing agent, which could lead to a plateau in modulus below a 10:1 mixing ratio. AFM images suggested that changing mixing ratio also affected surface topography (Fig. 3). Surfaces with 5:1 and 10:1 mixing ratio displayed 20 nm scale surface peak-to-valley “bump-like” features, while the surface with 20:1 mixing ratio had “spike-like” features several nanometers in scale covering the previously observed “bump” topography.

The surface energy of a biomaterial, often measured by contact angle method, has been reported to affect both protein adsorption and cell function. Hydrophobic substrates, which have surface energies <40 mJ/cm, do not promote endothelial cell adhesion; however, extremely hydrophilic substrates do not promote adhesion either. The results of contact angle analysis (Fig. 2) suggest that PDMS is a hydrophobic material, with a contact angle of ~103 degrees, whereas cell culture treated PS and glass surfaces have contact angles of 70 and 56 degrees, respectively. Although the substrate with 20:1 mixing ratio had a slightly smaller contact angle (100 degrees), changing mixing ratio generally had little effect on wettability of PDMS. Precoating PDMS
with charged molecules, such as poly-D-lysine, α-phosphatidylcholine, or layer-by-layer treatment, did not improve wettability. Precoating PDMS with soluble ECM protein (i.e., fibronectin, laminin) affected wettability, however, decreasing the contact angles to 72 and 77 degrees, respectively. Exposure to oxygen plasma dramatically increased the wettability of PDMS; the contact angle decreased from 103 to 14 degrees. Adsorption of fibronectin on plasma treated PDMS slightly increased contact angle to 20 degrees. Type I collagen based membranes were hydrophobic, with a contact angle around 95 degrees. The oxygen plasma reaction converts surface methyl groups into hydroxyl groups, resulting in a more hydrophilic surface which is better able to support cell growth because of improved substrate adhesiveness. The AFM observation also indicated a dramatic change in surface topography after exposure to oxygen plasma [Fig. 3(D)], resulting in a surface dominated by smooth 50 μm deep, 2 μm wide wavy structures.

The chemical composition of PDMS substrates with different modifications was examined using EDS as part of an SEM system (Table I). As expected, chemical composition of biologically derived collagen based substrates (i.e., CM, CAG) is markedly different from PDMS based substrates. Some of the surface modification techniques, such as coating with LAP, LBL coating, and LN coating, had little effect on EDS measured chemical composition of PDMS based substrates. However, some of the surface modification techniques, such as decreasing mixing ratio; exposure to O₂ plasma; and coating with FN, PDL, and type I collagen, had a strong effect on chemical composition as assessed by EDS. Elemental ratio of O/Si for 20:1 and 10:1 PDMS are similar, with an average value of 0.86. O/Si for 5:1 PDMS is 1.09, and for O₂ plasma treated PDMS is 1.98, suggesting that both decreasing base to curing agent mixing ratio and exposure to O₂ plasma significantly affected chemical composition of PDMS.

The amount of protein (i.e., fibronectin, laminin, and cell growth medium proteins) adsorbed onto PDMS surfaces was measured by micro-BCA assay, and the morphology of surfaces with adsorbed

![Figure 1](https://www.interscience.wiley.com)

**Figure 1.** Elastic moduli for (A) and Caco-2 attachment on (B) PDMS prepared with different base to curing agent mixing ratio. (*) significantly different from PS surface for the groups of the same day (p < 0.05), (#) significantly different from PDMS10:1 surface for the groups of the same day (p < 0.05). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

![Figure 2](https://www.interscience.wiley.com)

**Figure 2.** Contact angle of water on different surfaces used for Caco-2 cell culture. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
protein was imaged by AFM. The results suggested that there were significant amounts of protein adsorbed on PDMS surfaces, and the quantity and morphology of adsorbed protein depended on the type of the protein, as well as the surface chemistry of PDMS (Fig. 4). It was found that less fibronectin adsorbed on oxygen plasma treated PDMS than 10:1 PDMS (1.65 μg/cm² vs. 1.89 μg/cm²). The surface coverage of laminin on PDMS is 0.89 μg/cm². Compared to fibronectin, there was more collagen and growth medium related protein adsorbed on PDMS surfaces, the surface coverage is 3.35 μg/cm² and 4.97 μg/cm², respectively (Fig. 4(A)). The AFM analysis suggested that laminin formed irregular \( \sqrt[8]{50 \text{ nm}} \).

**Figure 3.** Tapping mode AFM 10 \( \mu \text{m} \times 10 \mu \text{m} \) images of PDMS at different base to curing agent mixing ratio [i.e., 5:1 (A), 10:1 (B), 20:1 (C)], and PDMS10:1 surface treated with oxygen plasma (D). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

<table>
<thead>
<tr>
<th>Types of Substrates</th>
<th>C (At%)</th>
<th>Si (At%)</th>
<th>O (At%)</th>
<th>S (At%)</th>
<th>Other (Na, Cl, P) (At%)</th>
<th>C:Si:O</th>
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<tr>
<td>20:1 PDMS</td>
<td>61.71 ± 0.27</td>
<td>20.77 ± 0.3</td>
<td>18.06 ± 0.09</td>
<td>0.99 ± 0.05</td>
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<td>10:1 PDMS</td>
<td>60.64 ± 0.36</td>
<td>21.18 ± 0.15</td>
<td>18.18 ± 0.26</td>
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<td>2.86:1:0.86</td>
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<td>5:1 PDMS</td>
<td>60.20 ± 0.87</td>
<td>19.06 ± 2.49</td>
<td>20.74 ± 1.73</td>
<td>0.99 ± 0.05</td>
<td>3.15:1:1.09</td>
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<td>P/O</td>
<td>64.68 ± 0.64</td>
<td>11.84 ± 0.28</td>
<td>23.48 ± 0.36</td>
<td>0.99 ± 0.05</td>
<td>5.46:1:1.94</td>
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<td>P/O/FN</td>
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<td>11.48 ± 0.48</td>
<td>24.44 ± 0.86</td>
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<td>P/PDL</td>
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<td>24.85 ± 0.39</td>
<td>0.01 ± 0.01</td>
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<td>P/LAP</td>
<td>61.72 ± 0.97</td>
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<td>P/LBL</td>
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<td>P/FN</td>
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<td>P/LN</td>
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<td>P/C</td>
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<td>20.74 ± 0.39</td>
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<td>CM</td>
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<td>21.71 ± 0.61</td>
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<td>C/CAG M</td>
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<td>19.64 ± 1.21</td>
<td>0.43 ± 0.08</td>
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to 300 nm diameter aggregates on the surface of PDMS [Fig. 4(B)], while fibronectin formed branch-like aggregates [Fig. 4(C)]. Fibronectin adsorbed on oxygen plasma treated surfaces tended to aggregate into microscale structures [Fig. 4(D)].

**Caco-2 attachment on PDMS with modified surface energy**

To investigate the effect of changing PDMS surface energy on Caco-2 cell attachment, PDMS surface properties were modulated by varying the base to curing agent ratio. Results indicated that PDMS with a common 10–1 base to curing agent mixing ratio did not support attachment of Caco-2 intestinal epithelial cells to a similar extent as traditional cell culture treated PS substrates [Fig. 1(B)]. The amount of cells attached on 10:1 PDMS is significantly lower than on PS. Modifying the PDMS surface free energy by changing the base to curing agent ratio did not result in significant improvement in cell adhesion [Fig. 1(B)]. The PDMS surfaces with 20:1 base to curing agent ratio had a slightly but not significantly \( (p < 0.05) \) higher level of attached cells (0.60, 0.70, and 0.73 relative cell attachment compared to PS for days 1, 2, and 3, respectively) than 10:1 PDMS surfaces (0.44, 0.69, and 0.51 relative cell attachment compared to PS for days 1, 2, and 3, respectively). Decreasing base to curing agent ratio from 10:1 to 5:1, however, was found to significantly decrease cell attachment (0.9, 0.17, and 0.18 relative cell attachment compared to PS for days 1, 2, and 3, respectively).

As described earlier, surface characterization studies suggested that changes in the base to curing agent ratio influenced surface chemistry, topography, and stiffness, but had little effect on surface wettability. As trends in cell attachment for different base to curing agent ratios most closely followed trends in surface chemical composition (i.e., there were no significant differences in Caco-2 cell attachment between 20:1 PDMS and 10:1 PDMS), results indicate that surface chemical composition plays a
more important role in affecting Caco-2 adhesion on PDMS compared to surface topography and stiffness.

PDMS surface energy was also modified by oxygen plasma treatment (Fig. 5). Plasma treatment alone resulted in an insignificant ($p < 0.05$) increase in cell adhesion on 10:1 PDMS. The relative cell attachment (compared to PS) on oxygen plasma treated PDMS was 0.62, 0.73, and 0.88 at day 1, 2, and 3 of culture, respectively, compared with 0.32, 0.49, and 0.45 for 10:1 PDMS. Oxygen plasma treated PDMS was also subsequently incubated with cell growth medium (CGM) containing 20% FBS overnight before cell seeding, or coated with fibronectin. It was found that preincubation with CGM significantly increased cell attachment to an extent similar to that observed on cell culture treated PS surfaces (0.99, 1.48, and 1.32 in relative cell attachment for days 1, 2, and 3, respectively). On day 3, the amount of attached cells on the fibronectin coated, oxygen plasma treated PDMS surface was twice as high as that on the PS surface.

The significant promotion of Caco-2 adhesion on fibronectin preadsorbed plasma treated PDMS may be related to fibronectin orientation. Oxygen plasma treatment alters PDMS surface chemistry by generating more hydroxyl groups; this is demonstrated in EDS results by the Si:O ratio that is significantly higher in oxygen plasma treated PDMS compared to 10:1 PDMS (i.e., 1:1.94 versus 1:0.86) (Table I). AFM results also indicated that fibronectin formed different patterns of aggregation on oxygen plasma treated and 10:1 PDMS surfaces [Fig. 4(C,D)]. These results suggest that surface chemistry might affect the orientation of fibronectin, consequently altering its ability to support cell adhesion, even though there is slightly less fibronectin adsorption on oxygen plasma treated PDMS [Fig. 4(A)]. Similar results were reported for fibronectin adsorbed on gold surfaces. It was found that hydroxyl headgroup chemistry increased cell adhesion compared to surfaces dominated by methyl groups.26 In addition, AFM analysis demonstrated that oxygen plasma treated PDMS had different surface topography than 10:1 PDMS [Fig. 3(B,D)]. The synergistic effect of surface chemistry and topography might facilitate exposure of the cell-binding domain of physiosorbed fibronectin towards cell integrins, thus enhancing Caco-2 adhesion.

Caco-2 attachment on PDMS surface with physiosorbed charged molecules

Another possible way to treat PDMS surfaces is to coat surfaces with molecules charged at physiological pH. The charged molecules l-α-phosphatidylcholine (LAP), poly-d-lysine (molecular weight range of 70–150 kDa, PDL), as well as polystyrene sulfonate and polyethyleneimine layer by layer coating (LBL) were chosen to coat PDMS surfaces. Pphysiosorption of...
poly-D-lysine enhanced Caco-2 attachment on 10:1 PDMS surfaces (Fig. 6). The amount of attached cells on poly-D-lysine coated surfaces (0.51, 0.62, and 0.96 relative cell attachment for days 1, 2, and 3, respectively) was significantly higher than that on untreated 10:1 PDMS surfaces (0.20, 0.26, and 0.52 relative cell attachment for days 1, 2, and 3, respectively). After attaching and proliferating on poly-D-lysine coated PDMS substrates for three days, Caco-2 cells were present at numbers comparable to those observed for traditional cell culture PS surfaces (relative cell attachment of 1.1). However, physisorption of both negatively and negatively/positively charged molecules (i.e., 1-α-phosphatidylcholine and layer-by-layer) did not appear to improve Caco-2 adhesion on PDMS surfaces. The 1-α-phosphatidylcholine coating even appeared to prevent Caco-2 proliferation on PDMS. As shown in Figure 6, the number of attached Caco-2 cells on LAP adsorbed surfaces decreased with time. After 3 days, the number was even lower than that on the untreated PDMS surface. In the case of LBL coating, Caco-2 adhesion and proliferation demonstrated the same trends as cells seeded on untreated PDMS surfaces; no significant improvement was observed. The surface characterization showed that 1-α-phosphatidylcholine, LBL, and poly-D-lysine coating alternated PDMS surface chemistry (Table I), however, had little effect on surface wettability (Fig. 2). In general, results suggested that Caco-2 cells preferred positively charged surfaces to surfaces with negative or with both positive and negative charges, such as 1-α-phosphatidylcholine and LBL coated substrates, in addition, the surface chemistry might also play a role in affecting Caco-2 adhesion.

As most mammalian cells are negatively charged, a physisorbed charged monolayer is able to enhance the electrostatic interaction between cells and substrates, subsequently improving cell attachment. In the case of Caco-2 adhesion on 1-α-phosphatidylcholine coated PDMS, the results are in contrast with the hypothesis that a charged surface in general will enhance cell attachment, but are in agreement with the findings of Andersson et al., who found that epithelial cells were not able to adhere or proliferate on phospholipid coated SiO2 surface. For the poly-D-lysine coating, the amino group on the end of each lysine develops a net positive charge in culture medium which makes it hydrophilic and may enhance electrostatic interaction between negatively-charged ions of the cell membrane. This could be the underlying cause for the observed increase in Caco-2 attachment on poly-D-lysine coated PDMS substrates. The layer by layer modification involves alternatively depositing negatively charged polystyrene sulfonate and positively charged polyethyleneimine onto the PDMS surface. This method was proven to enhance the growth and adhesion of endothelial cells as well as vascular smooth muscle cells on PDMS. However, LBL did not improve Caco-2 cell adhesion on PDMS. The conflicting results may be due to differences in cell type related reaction to the surface chemistry.

Caco-2 attachment on PDMS surfaces coated with extracellular matrix protein

To increase their cell adhesion properties, PDMS substrates were exposed to solutions of fibrous ECM proteins, i.e., laminin, type I collagen, and fibronectin, to enable physical adsorption. 10:1 PDMS precoated with fibronectin or collagen was found to support attachment and proliferation of Caco-2 cells to a similar extent as traditional cell culture PS (Fig. 7). It was also found that Caco-2 cells spread [Fig. 7(C)] to the same extent as cells cultured on PS surfaces [Fig. 7(B)] after 1 day in culture, suggesting that a simple step of predesorbing fibronectin on PDMS surfaces is able to tune cell attachment properties of PDMS close to those of cell culture treated PS. Laminin enhanced Caco-2 initial attachment on 10:1 PDMS surfaces; however, it appeared to suppress Caco-2 proliferation, as total cell population had not increased after 3 days of culture (Fig. 7).

In the native environment, small intestinal epithelial cells rest and attach on extracellular matrix, which is important in controlling cell behavior, such as migration, proliferation, morphology, development and function. The ECM is primarily comprised of glycosaminoglycans and fibrous proteins. Fibrous proteins, such as laminin, collagen (mainly type I and type IV collagen), fibronectin and elastin, have both structural and adhesive functions. Fibronectin, in particular, is known to help cells attach to ECM. The inhibition of cell adhesion and proliferation by physisorption of laminin was in accordance with previous studies which showed that laminin also decreased the proliferation rate of the medullary thyroid carcinoma CA-77 cell line. Laminin and fibronectin differ in the ability to enhance cell adhesion. It has been proposed that fibronectin is relatively nonspecific in promoting cell adhesion, while laminin shows more restricted specificity towards cell type. For example, laminin was not able to promote the attachment of rat kidney cells, whereas fibronectin enhanced their attachment and spreading. In addition, the AFM analysis demonstrated that fibronectin coated PDMS had different surface topography than laminin coated surface [Fig. 4(B,C)], suggesting that fibronectin might have different orientation and conformation than laminin.
on PDMS surface which caused different enhancements on Caco-2 adhesion.

Caco-2 attachment on collagen membrane and Matrigel™

To investigate the influence of collagen density, the function of glycosaminoglycans (GAG), and ECM as a whole mixture, a collagen membrane (having higher collagen concentration relative to physisorbed collagen), a collagen-GAG membrane, and a Matrigel™ substrate were tested. The results (Fig. 8) suggested that the collagen membrane has better cell adhesion properties than the 10:1 PDMS surface (1.45, 2.78, and 1.86 relative cell attachment for days 1, 2, and 3, respectively). Caco-2 cells adhered slightly better on a collagen membrane (Fig. 8) than on the collagen coated 10:1 PDMS surface [Fig. 7(A)]. The addition of GAG to the collagen membrane did not significantly improve cell attachment and growth over collagen alone. Few Caco-2 cells were found to adhere on the Matrigel™ surface. Some cells were observed to be migrating into the Matrigel™. Thus, the cells located inside Matrigel™ were barely stained with fluorescent dye and were not able to be counted. The tendency of cells to not attach to a Matrigel™ surface was reported by others using both Caco-235 and Madin Darby Canine Kidney cells.36 It was also shown that Caco-2 cell attachment decreased with the increase in Matrigel™ concentration, and that they preferred to grow on a dry film form instead of gel form of
MatrigelTM. These findings suggested that in the case of MatrigelTM coating, the stiffness of MatrigelTM and the concentration of ECM protein play important roles in Caco-2 adhesion. The poor attachment of Caco-2 cells on MatrigelTM substrates in our studies might be due to the use of the gel form of MatrigelTM with relatively high protein concentration (9–11 mg/ml).

CONCLUSION

This study explores the ability of PDMS, a material widely used in soft-lithography processes amenable to microscale drug testing, to support the attachment and growth of intestinal epithelial cells. A variety of surface modification techniques demonstrated to enhance attachment of cells to surfaces were investigated. For a PDMS surface, Caco-2 cell adhesion appears to be highly dependent on surface treatment. The influences of chemical composition of PDMS, surface treatment of PDMS by coating with charged molecules (i.e., poly-D-lysine, l-α-phosphatidylycholine and layer-by-layer), and deposition of ECM proteins (i.e., laminin, fibronectin, collagen, matrigel) on the attachment and growth of Caco-2 cells, as well as on PDMS surface characteristics were examined. Varying base to curing agent ratio or treating with oxygen plasma was found altering PDMS surface properties, such as elastic modulus, wettability, chemical composition, and topography, however, not being able to enhance Caco-2 cell adhesion. However, further coating of fibronectin on oxygen plasma treated PDMS greatly enhanced Caco-2 attachment. Surface characterization suggested that synergistic effect of surface chemical composition and topography might facilitate exposure of the cell-binding domain of physisorbed fibronectin towards cell integrins, thus enhancing Caco-2 adhesion. The response of Caco-2 cells to surface charge indicated favorable attachment on positively charged surfaces. Differential response to ECM proteins was also observed. In general, physisorbing ECM protein (i.e., type I collagen or fibronectin) on PDMS subsurface promoted enhanced Caco-2 cell adhesion on PDMS. However, laminin, also an ECM protein, did not increase cell attachment on PDMS. This study characterizes the change of PDMS surface properties after surface modifications, demonstrates the ability to tune Caco-2 adhesion and proliferation on PDMS substrates by surface modification. A simple presorption of fibronectin is sufficient to promote Caco-2 adhesion on PDMS to a level equivalent to cell culture treated PS. These results provide a foundation for the development of a PDMS based miniaturized system for high throughput drug absorption studies.

References
