Poly(dimethylsiloxane) Elastomers with Tethered Peptide Ligands for Cell Adhesion Studies

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Abstract

Poly(dimethylsiloxane) (PDMS) is the choice of material for a wide range of bio- and non-biological applications because of its chemical inertness, non-toxicity, ease of handling, and commercial availability. However, PDMS exhibits uncontrolled protein adsorption and cell adhesion, and it has proven difficult to functionalize to present bioactive ligands. We present a facile strategy for functional surface modification of PDMS using commercial reagents to engineer polymer brushes of oligo(ethylene glycol) methacrylate that prevent cell adhesion and can be functionalized to display bioadhesive ligands. The polymer brushes resist biofouling and prevent cell adhesion, and bioadhesive peptides can be tethered either uniformly or constrained to micropatterned domains using standard peptide chemistry approaches. This approach is relevant to various biomedical and biotechnological applications.

Keywords
defeformable substrate; fibronectin; cell mechanics; focal adhesion

Introduction

Mechanochemical interactions of cells with their surrounding matrix provide forces and signaling cues regulating cell fate and processes such as survival, cell cycle progression and the expression of differentiated phenotypes \cite{1–3}. Cell adhesion to extracellular matrix components, including fibronectin (FN), collagen, and laminin, is primarily mediated by the integrin family of transmembrane receptors \cite{4}. Following ligand binding, integrins cluster together and promote the assembly of supramolecular complexes containing signaling and structural components that coordinate mechanotransduction pathways \cite{5}. Adhesive interactions have been exploited in various biomedical and biotechnological applications to control cell and tissue responses \cite{6–8}. These strategies have principally focused on presenting
bioadhesive proteins or oligopeptides derived from extracellular matrix proteins to target integrin receptors in order to direct cell adhesive responses. Moreover, recent evidence indicates that the mechanical properties of the surrounding matrix (e.g., elastic modulus) significantly contribute to mechanotransduction events in diverse cellular processes including stem cell commitment, cell differentiation, and transformation [9–12].

Synthetic and natural materials, including poly(acrylamide) and poly(ethylene glycol) gels, alginate, and agarose, have been used to engineer substrates with defined mechanical properties that are functionalized with bioadhesive ligands to direct adhesion [13–20]. Nevertheless, these materials present limitations related to processability, range of mechanical properties (modulus, strain to failure, duty cycle), and compatibility with other materials that hinder their broad applicability in other fields such as MEMs and microfluidics. In contrast, poly(dimethylsiloxane) (PDMS) is the choice of material for a wide range of bio- and non-biological applications because of its chemical inertness, non-toxicity, ease of handling, and commercial availability [21–23]. However, PDMS exhibits uncontrolled interactions with biological components (proteins, cells) and it has proven difficult to functionalize to present bioactive ligands. To address these limitations, we developed a facile strategy for functional surface modification of PDMS using commercial reagents to generate an initiator-integrated PDMS (iPDMS) which is amenable to surface-initiated polymerization [24]. In the present work, we engineered polymer brushes of oligo(ethylene glycol) methacrylate that prevent cell adhesion and can be functionalized to display bioadhesive ligands.

Materials and Methods

Reagents and Cells

The vinyl-terminated initiator, undec-10-enyl 2-bromo-2-methylpropanoate (H₂C=CH(CH₂)₉OCOC(CH₃)₂Br), was purchased from HRBio (Beijing, China). Sylgard 184 was obtained from Dow Corning. Oligo(ethylene glycol) methacrylate (Mₙ = 526, OEGMA526) and other chemicals were purchased from Aldrich and used as received. Human plasma FN, cell culture reagents, rhodamine-phalloidin, and AlexaFluor-conjugated antibodies were purchased from Invitrogen (Carlsbad, CA). Monoclonal antibody against vinculin (clone V284) was obtained from Millipore (Billerica, MA). NIH3T3 fibroblasts (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, UT) and penicillin-streptomycin. Cells were sub-cultured every two-three days using standard techniques.

Preparation of iPDMS and Polymer Brushes

Sylgard 184 was used as the model elastomer. To prepare regular PDMS substrates, the viscous base (component A) and the curing agent (component B) were mixed well (10:1 ratio by weight) and cured at 80 °C for 2 h. To prepare iPDMS, a third component (component C), the vinyl terminated initiator, was mixed well with the base and curing agent, and cured as described for PDMS. The component C reacts with hydrosilane hydrogens in the presence of Pt catalyst, and is covalently integrated into the highly cross-linked three-dimensional network [24]. PDMS or iPDMS substrates were rinsed in hexane for 24 hours (8h × 3 times) to extract low molecular weight components.

Surface-initiated atom transfer radical polymerization with OEGMA526 was carried out as previously described [24]. Briefly, the reaction mixture was prepared by thoroughly mixing two parts. Part 1 was a transparent, pale-blue solution, prepared by adding a specified amount of CuCl₂/2,2'-bipyridine (Bipy) (1:2 mole ratio) and a fixed amount of monomer to 5 mL MilliQ-water. Part 2 was a colorless solution, prepared by adding a specified amount of ascorbic acid to 5 mL MilliQ-water. After both solutions were deoxygenated, two parts were mixed.
together under nitrogen. The mixture was further deoxygenated and the resulting mixture was red in color due to the reduction of deactivator Cu(II)/Bipy complex to activator Cu(I)/Bipy complex. The mixture had a molar ratio of OEGMA526/HEMA/CuCl$_2$/Bipy/ascorbic acid =20/200/1/2/1, with a feed of 2.76 mM CuCl$_2$. This mixture was then transferred to cover the iPDMS sample under nitrogen atmosphere and surface-initiated polymerization was initiated and continued for 30 min at 25 °C. The polymerization was stopped when iPDMS was removed from the solution. The iPDMS sheets after surface-initiated polymerization were first incubated in an aqueous solution of 1 M bromoacetic acid and 2 M sodium hydroxide for overnight to generate terminal carboxyl groups [31]. Samples were thoroughly rinsed with methanol, milliQ-water, and dried under flowing nitrogen before further treatment.

Polymer brushes were characterized by goniometry and X-ray photoelectron spectroscopy (XPS). Static water contact angles were measured on a Dataphysics OCA20 contact angle system (Filderstadt, Germany) at room temperature. XPS was carried out using monochromatic Al Kα X-rays (1486.7 eV) in an AXIS Ultra instrument (Kratos Analytical, UK). The X-ray source was 2 mm nominal X-ray spot size operating at 15 kV and 12 mA for both survey and high-resolution spectra. Survey spectra (0–1100 eV) were recorded at 160 eV pass energy with an energy step of 1.0 eV, and a dwell time of 200 ms. High-resolution spectra were recorded at 40 eV pass energy with an energy step of 0.1 eV and a dwell time of 500 ms with a typical average of 3 scans. All peaks were referenced to C1s (CHx) at 285 eV in the survey scan spectra and C1s (CHx) at 284.8 eV in the deconvoluted high resolution C1s spectra. All data were collected and analyzed using software provided by the manufacturer.

The Young’s modulus of PDMS and iPDMS were obtained using a TriboIndenter (Hysitron) equipped with a Berkovich tip (50 nm). The indenter was loaded and unloaded at a rate of 2 μN/s, the load was held constant for a period of 2 s at the peak value from 10 μN to 45 μN.

**Biofunctionalization and Cell Adhesion Studies**

Human plasma FN was tethered to polymer brushes using standard peptide chemistry as previously described [25]. Briefly, following extensive washing in 70% ethanol and ultrapure H$_2$O, samples were incubated in 2.0 mM EDC and 5.0 mM NHS in 0.1 M 2-(N-morpho)ethanesulfonic acid and 0.5 M NaCl (pH 6.0). FN solution (20 μg/mL in PBS) was then incubated on the activated supports for 30 min and unreacted surface NHS esters then quenched in 20 mM glycine. For micropatterning experiments, PDMS stamps with desired features were inked with FN solution (100 μg/mL) and stamped onto activated substrates for 30 sec.

Cells were detached (0.05% trypsin) from culture dishes, resuspended in serum-containing media, and plated onto PDMS samples. Cells were allowed to adhere and spread in the presence of 10% serum. On the next day, cells were permeabilized in cytoskeleton buffer with protease inhibitors (0.5% Triton X-100 in 50 mM NaCl, 150 mM sucrose, 3 mM MgCl$_2$, 20 μg/mL aprotinin, 1 μg/mL leupeptin, 1 mM PMSF, 50 mM Tris, pH 6) for 10 min and fixed in 3.7% formaldehyde for 5 min. After blocking in 5% FBS, samples were incubated in primary antibodies against vinculin, washed, incubated in AlexaFluor488-conjugated antibodies, rhodamine-phalloidin, and Hoechst dye. Images of adherent cells were obtained using a 60X objective using a Nikon TE-300 microscope (Melville, NY) equipped with a Spot RT camera (Diagnostic Instruments, Sterling Heights, MI).

**Results**

In the present study, we expand on a facile strategy for functional surface modification of PDMS using commercial reagents to engineer polymer brushes of oligo(ethylene glycol) methacrylate that prevent cell adhesion and can be functionalized to display bioadhesive ligands. This strategy is outlined in Figure 1.
Commercially available PDMS typically consists of two parts: a viscous base and a curing agent, which are mixed at a 10:1 ratio by weight to form PDMS elastomer via a curing process. The main component of the base is a poly(dimethyl-methylvinylsiloxane) prepolymer (component A). It also contains a small amount of platinum (Pt) metal complex as catalyst dissolved in the main component. The curing agent contains a mixture of vinyl-endcapped PDMS precursors and poly(dimethyl-methylhydrogenosiloxane) precursors as cross-linkers (component B). Upon mixing together base and curing agents, the vinyl groups and the hydrosilane hydrogens undergo a hydrosilylation reaction in the presence of Pt catalyst, which results in highly cross-linked three-dimensional networks. The mechanical properties of the elastomer can be altered by varying the A:B ratio. We previously showed that a vinyl-terminated initiator (component C) could compete with vinyl-endcapped PDMS precursors in the hydrosilylation reaction during the curing process, and thus the vinyl-terminated initiator could be integrated in situ into the PDMS networks to generate iPDMS (step 1, Fig. 1) [24].

We examined different A:B:C ratios and settled on 10:1:0.5 as the mixture cured as regular PDMS and exhibited no significant differences in hydrophobicity (water contact angle (average ± S.E., n=6) 112 ± 1° (PDMS) vs. 114 ± 1°). However, there were differences in mechanical properties (Young’s modulus (average ± S.E., n=15), 3.82 ± 0.19 MPa (PDMS) vs. 1.26 ± 0.16 MPa (iPDMS)).

We carried out surface-initiated polymerization of OEGMA brushes on iPDMS as previously described [24] (step 2, Fig. 1). XPS analysis confirmed successful polymerization on iPDMS as demonstrated by the disappearance of the Br peak and characteristic spectra for OEGMA at the C1s peak [26] (Figure 2). Significant reductions in water contact angle (114° (pre) vs. 57° (post)) following polymerization also showed successful surface modification.

We next functionalized iPDMS substrates presenting polymer brushes with human plasma fibronectin as a model bioadhesive ligand. Fibronectin was tethered onto the brushes using standard EDC/NHS chemistry (step 3, Fig. 1) and the resulting bioactivity was analyzed via cell adhesion and spreading studies (step 4, Fig. 1). Figure 3A presents representative images of cells cultured overnight on substrates in the presence of 10% serum. As expected, unfunctionalized brushes (iPDMS-OEGMA) supported extremely low levels of cell adhesion and the few cells that attached remained round. iPDMS substrates presenting polymer brushes that were exposed to fibronectin without EDC/NHS activation also exhibited minimal levels of cell adhesion and spreading. These results confirm the non-fouling character of the OEGMA brushes, even in the presence of high concentrations of fibronectin. In contrast, substrates that were incubated to fibronectin following EDC/NHS activation supported high levels of cell adhesion and spreading. Furthermore, cells adhering to these functionalized support displayed prominent actin fibers and vinculin-containing focal adhesions (Figure 3B). Control substrates that were activated in EDC/NHS but incubated in non-adhesive serum albumin instead of fibronectin exhibited minimal cell adhesion and spreading.

As a final demonstration of our ability to functionalize PDMS elastomers with bioadhesive ligands, lanes of fibronectin were microcontact printed onto PDMS samples presenting EDC/NHS-activated polymer brushes (Figure 4). Cells adhered and spread on the micropatterned lanes of fibronectin but remained constrained to the micropatterned domains. Varying the width (5 vs. 50 μm) of the fibronectin stamp resulted in differences in the number of cells, extent of spreading and focal adhesion assembly per fibronectin lane. These results demonstrate spatial control over the presentation of bioadhesive ligands on non-fouling PDMS elastomers.

Discussion

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and can be functionalized to display bioadhesive ligands. The polymer brushes resist biofouling and prevent cell adhesion, and bioadhesive peptides can be tethered either uniformly or constrained to micropatterned domains using standard peptide chemistry approaches. We expect that this technique will be relevant to numerous bio-MEMS and microfluidics applications as well as fundamental studies of mechanotransduction.

Cell adhesion studies demonstrated that the tethered fibronectin retained biological activity as evidenced by cell spreading, robust actin cytoskeleton and focal adhesion assembly. Because this approach uses the standard EDC/NHS tethering chemistry, we expect that this technique will be applicable to other peptides and proteins, including growth factors, as well as other molecules presenting primary amines. Whereas the density of tethered ligand was not determined in the present study, we have previously shown that this parameter can be easily controlled by varying the solution concentration of the biomolecule [25,27]. Finally, we only examined cell adhesion and spreading at one day post-seeding. Further studies are necessary to establish the long-term stability of these engineered surfaces.

Recent efforts have focused on tethering bioactive factors onto PDMS substrata and demonstrated successful coupling and bioactivity [28–30]. These approaches rely on generating co-polymers with PDMS or multi-step modifications of plasma-treated PDMS. In contrast, the present work shows a more facile and flexible approach that does not alter the mechanical properties of the underlying PDMS support. Furthermore, because there are no intermediate functionalization steps for the biomolecules, the current tethering approach has the flexibility to tether multiple ligands either simultaneously or sequentially without purification steps [27].

Conclusion

We validate a strategy for functional surface modification of PDMS using commercial reagents to engineer polymer brushes of oligo(ethylene glycol) methacrylate that prevent cell adhesion and can be functionalized to display bioadhesive ligands. The polymer brushes resist biofouling and prevent cell adhesion, and bioadhesive peptides can be tethered either uniformly or constrained to micropatterned domains using standard peptide chemistry approaches. As such, this approach is relevant to various biomedical and biotechnological applications.

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References


Figure 1.
Figure 2.
XPS spectra of functionalized PDMS. A. Spectra for PDMS (black) and iPDMS (gray). Inset shows Br 3d narrow spectrum for iPDMS. B. Spectrum for iPDMS following surface-initiated polymerization showing characteristic bands for OEGMA. Inset shows C1s narrow scan.
Figure 3.
Cell adhesion to functionalized PDMS in the presence of 10% serum. Immunofluorescence staining for vinculin (green), actin (red) and DNA (blue). A. PDMS presenting polymer brushes functionalized with FN support cell adhesion and spreading (iPDMS-OEGMA-FN) whereas as unfunctionalized brushes (iPDMS-OEGMA) or brushes exposed to FN in the absence of tethering reagents (iPDMS-OEGMA + FN) display minimal cell adhesion. Scale bar 50 μm. B. Cells on PDMS presenting polymer brushes functionalized with FN (iPDMS-OEGMA-FN) exhibit prominent actin stress fibers and vinculin-containing focal adhesions (white arrow heads). Scale bar 20 μm.
Figure 4.
Cell patterning on PDMS presenting polymer brushes functionalized with microcontact printed FN for 5 and 50 μm width lanes. Both images are at the same magnification; scale bar 50 μm.