Mussel-Inspired Anchoring for Patterning Cells Using Polydopamine
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ABSTRACT:
This Article introduces a simple method of cell patterning, inspired by the mussel anchoring protein. Polydopamine (PDA), artificial polymers made from self-polymerization of dopamine (a molecule that resembles mussel-adhesive proteins), has recently been studied for its ability to make modifications on surfaces in aqueous solutions. We explored the interfacial interaction between PDA and poly(ethylene glycol) (PEG) using microcontact printing (μCP). We patterned PDA on several substrates such as glass, polystyrene, and poly(dimethylsiloxane) and realized spatially defined anchoring of mammalian cells as well as bacteria. We applied our system in investigating the relationship between areas of mammalian nuclei and that of the cells. The combination of PDA and PEG enables us to make cell patterns on common laboratorial materials in a mild and convenient fashion.

INTRODUCTION
The ability of patterning cells on surfaces provides useful tools for studying cell—cell interactions, constructing in vitro models,1–3 studying the effect of size on cell behaviors like proliferation, migration, and apoptosis,4,5 studying the cell shape on cell division axis orientation,6 for drug screening7 and for cell-based sensors, among others.8,9 Various methods have been developed to pattern cells, such as photolithography, soft lithography, mass spectrometry-assisted lithography, electrochemical activation, and physical constraints.10–17 Among them, microcontact printing (μCP) is still the most convenient way in that μCP can provide mass pattern production with high level of controllability and does not require special skills or complex equipments once masters for molding of stamps are fabricated.18

There are two main types of ink in μCP for cell patterning, molecules that can form self-assembled monolayers (SAMs) and proteins.18,19 Although widely used, the two systems have several disadvantages. Thiols can form well-oriented SAMs on gold easily; however, this system is quite expensive, and sometimes not well adapted for fluorescent microscopy because metallic substrate severely attenuates fluorescence. Silane forms SAMs on relatively inexpensive substrates such as glass, silicon, and poly-(dimethylsiloxane) (PDMS). Yet the process of modification needs careful handling. As for proteins, the transfer of proteins from stamps is driven by physical adsorption, which can be weak as compared to driving force of formation of SAMs. As a result, the printed protein patterns cannot always effectively sustain the adhesion of cells for long-term due to tearing or degradation by cells.19 An additional step of oxidative treatment of PDMS stamps is typically required to promote the wetting of protein solutions.

For a practical technique for cell patterning in common biological laboratories, it should satisfy several criteria: (1) fabrication should be as easy as possible for a biological lab without external help; (2) quality should be highly reproducible; and (3) techniques should be compatible with as many existing experimental systems as possible.19 As glass and polystyrene (PS) comprise common substrates used for cell culture, we aim to develop new techniques that are compatible with these materials, and to achieve convenient and effective cell patterning.

Polydopamine is an underwater polymer that allows multifunctional surface modification.20–22 We have previously reported a straightforward method to pattern cells on oligo(ethylene glycol) self-assembled monolayer (OEG SAM) modified gold by directly printing polydopamine.23 The use of polydopamine to support cell adhesion was inspired by mussels that attach themselves to rocks by secreting adhesive proteins. We have shown that, although OEG SAM can resist protein adsorption and cell adhesion, it could be modified with polydopamine. We modified OEG SAM with polydopamine patterns by microcontact printing and micro-fluidic patterning and demonstrated that polydopamine-patterned...
OEG SAM in cell patterning was as effective as traditional SAM containing printed alkanethiol.

The substrate in our previous work was gold-coated surface, which was expensive and could affect optical characterization as mentioned above. Besides, we do not know whether polydopamine can be printed on surfaces with different surface energies or surfaces presenting PEG through other modification methods. In this Article, we extended our work and further investigated the interaction between polydopamine and other surfaces besides OEG SAM. We employed poly(-lysine)-g-poly(ethylene glycol) (PLL–PEG) or PEG dimethacrylate (PEGDMA) as cell-resistant materials. PLL–PEG is a positively charged copolymer that can adsorb spontaneously onto a variety of negatively charged substrates, including glass and plasma-oxidized PS.24,25 PEGDMA is a UV-curable PEG polymer.26 These two molecules can easily produce surfaces that resist adsorption of proteins or adhesion of cells on a variety of substrates.

We demonstrated that PDA can be transferred onto PLL–PEG-coated substrates (glass, PS, and PDMS) and PEGDMA surfaces. We further investigated the transfer efficiency of PDA on substrates with varied surface energy. We compared PDA and proteins (collagen/fibronectin mixtures) in their ability in supporting cell adhesion. For application in cell patterning, the longest time for confining cells to micropatterns we obtained was 26 days on PS substrates. We showed that bacteria can also be patterned on PLL–PEG coated PS, which may find applications in fabricating microbial microarrays. Using our system, we measured nuclear sizes in patterned single mammalian cells with various areas. Nuclear sizes increase with the increase of size of cell patterns. We believe that our PDA–PEG systems can overcome several shortcomings of existing systems for cell patterning to further broaden the applications of μCP.27,28

![Experimental Section](image)

**Preparation of Stamps.** The fabrication of stamps followed conventional methods in soft lithography. Briefly, we fabricated masters with patterns of photoresists on silicon wafers by photolithography and cast a mixture of prepolymer and curing agent for poly(dimethylsiloxane) (PDMS) with a ratio of 10:1. The mixture was baked at 80 °C for one-half an hour. The cured PDMS elastomers were peeled off the master and cut into the appropriate size for use as stamps.

**Stamp Coating with Polydopamine.** The coating of stamps with polydopamine was achieved according to a published method.20 We immersed PDMS stamps in dopamine solution (Tris-HCl, pH 8.5) at a concentration of 2 mg/mL. Polymerization of dopamine took place, and the polymer coated the PDMS spontaneously. We left the stamps in solution overnight before rinsing them in water and then dried them with an air gun.

**Preparation of Inert Surfaces.** The synthesis of PLL–PEG follows reported protocols.24 Briefly, we added 216 mg of methoxy-poly-(-ethylene glycol) propionic acid (mPEG–SPA, MW 5 kDa, Beijing Kaizheng Biotech Development Co. Ltd.) into an aqueous solution of poly-L-lysine hydrobromide (PLL–HBr, MW = 15–30 kDa, Sigma). The solution was prepared by dissolving 84 mg of PLL–HBr in 1.05 mL of sodium borate buffer solution (50 mM, pH 8.5), filtered under 0.22 μm pore size, Millipore GS). The mixture was left at room temperature for 6 h before dialysis in phosphate buffered saline (PBS), followed by deionized water (molecular weight cutoff size 7 kDa). We dialyzed the solution for 24 h in deionized water and freeze-dried it. The resulting powder was stored at −20 °C before use. The working solution was prepared in a buffer of HEPES [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, pH 7.4] at a concentration of 1 mg/mL.

We used PLL–PEG to modify substrates such as glass, PS, and PDMS. Cover glass was cut into pieces of about 6 × 6 mm. We ultrasonicated the pieces in acetone for 10 min and immersed them in piranha solution with a ratio of concentrated H2SO4 and H2O2 of 3:1. Caution: Piranha solution is highly corrosive and should be handled with care! We fully rinsed glass with deionized water three times and dried it with an air gun. We activated the surfaces by putting them in an air-plasma chamber (155 mA × 622 V) for 3 min and immediately added a PLL–PEG solution (1 mg/mL) onto glass. The adsorption of PLL–PEG lasted for more than 2 h before we rinsed the surfaces with deionized water and dried the surfaces under the air gun.

For polystyrene (PS), we directly put PS Petri dishes in the air-plasma chamber (155 mA × 622 V, 3 min) and left PLL–PEG to incubate in them for 2 h. We washed Petri dishes with deionized water and dried them under the air gun. The modification of PLL–PEG on PS was the same as on glass.

For PDMS, we prepared the polymer by mixing the prepolymer and curing reagent with a ratio of 10:1 and cured the mixture at 80 °C for one-half an hour. The cured polymer film was cut into pieces of appropriate size and oxidized by air plasma (155 mA × 622 V, 3 min). To increase the amount of negative charges in the surface, we deposited six alternating layers of polyelectrolytes (poly(diallyldimethylammonium chloride and poly(acrylic acid), Sigma) before adding PLL–PEG solutions. To ensure thorough adsorption, PDMS pieces were immersed in PLL–PEG solutions overnight before being dried by the air gun.

For PEGDMA, we followed protocols described by Suh et al.26 Briefly, we added 1 wt % of the UV initiator (2,2-dimethoxy-2-phenylacetophenone, Aldrich) into PEG dimethacrylate (PEGDMA, MW = 550, Aldrich). We placed three drops of solutions onto PS Petri dish and allowed the liquid to spread and cover the dish. We polymerized PEGDMA using a UV (365 nm) source for 2 min at an intensity of 90 mW/cm². The cured polymer was added to deionized water and peeled off the PS surface automatically.

**μCP and Imaging.** We placed PDMS stamps on inert surfaces with a weight (4 g) on top of each. For PEGDMA, we dried films in an oven (40 °C, 30 min) and placed them on flat substrates. After 1 min, we peeled off the stamps. PEGDMA films were added with water subsequently to keep them flat.

We used a digital monochrome camera (Leica DFC350 FX) to observe printed polydopamine on various substrates. We took bright-field images of polydopamine patterns.

**Cell Patterning and Staining.** NIH 3T3 cells were chosen because of their high motility. We seeded cells at a density of 5 × 10⁵ to 5 × 10⁶ per milliliter and cultured them in Dulbecco’s modified eagle medium (DMEM) (Invitrogen, US) containing 10% fetal bovine serum (FBS) (Invitrogen, US) and 1% penicillin–streptomycin. We stained live cells with Calcein-AM (C-3099) (0.5%, green, Invitrogen, US) and Hoechst (0.1%, nuclei blue, Invitrogen, US) for 40 min before observation.

**Bacteria Patterning.** Escherichia coli and Staphylococcus Epidermidis were cultivated in nutrient broth at 37 °C overnight. Both of the grown cell solutions were directly loaded onto PLL–PEG coated PS surfaces and further incubated for 4.5 h. We washed PS surfaces with PBS gently three times and visualized them under optical microscopy.

**Measurement of Nuclear Sizes.** We designed a series of patterns with the same shape (rectangles with length:width 2:1) but different areas (from 1250 to 6050 μm²). After we printed polydopamine on a PLL–PEG coated PS Petri dish, we seeded NIH 3T3 cells on the dishes at a density of 5 × 10⁴ per milliliter. We washed the dishes 1.5 h after seeding to remove unattached cells. We cultured cells for 2 days in DMEM supplemented with 0.2% fetal bovine serum (FBS). Cell proliferation was oppressed in such low concentration of serum. We fixed cells and stained cell actin and nuclei using rhodamine-labeled...
phalloidin (Invitrogen) and Hoechst, respectively. We captured single cell patterns and measured the sizes of nuclei using Image-Pro Plus.

RESULTS AND DISCUSSION

We performed μCP of PDA using PDMS stamps. We dried the stamps and substrates by compressed air to carry out the printing in dry conditions. PLL–PEG is a positively charged molecule that can easily modify glass, plasma-treated PS, and PDMS. We examined PEGDMA because this material did not contain PLL moieties and thus was helpful in distinguishing interactions between PDA and PEG. Figure 1 shows the results of printing. PDA patterns are easy to identify by optical microscopy in the bright-field mode and have shapes that correspond to the shapes of the reliefs on the stamps. PDA can be easily transferred onto EG-modified surfaces from PDMS stamps, which meets the basic requirement of μCP. For applications in μCP, inks should easily wet stamps and have more affinity for the substrate than stamps. PDA can be modified on PDMS stamps by immersion in a basic dopamine solution. This is rather convenient as compared to proteins, another type of ink often used in patterning cells, as proteins require hydrophilic treatment of the stamp to wet the surface. By contrast, the PDA coating on PDMS stamp is homogeneous, which is important in producing intact patterns. The PDA patterns on the substrates in our experiments were highly repeatable, which is more advantageous than proteins, for printing proteins on PS cannot always consistently provide satisfactory results.19

As PDA could be an underwater adhesive and can modify almost any type of surfaces,20 we try to examine if it can be transferred or printed onto surfaces with different wettabilities. We prepared hexa(ethyleneglycol) undecanethiol (EG₆OH)/undecanethiol (CH₃) mixed SAMs on gold substrate with different ratios from pure EG₆OH, 1:4, 2:3, 3:2, 4:1, to pure CH₃. Contact angles of water on SAMs increased with the increase of the content of CH₃, which were 32, 61, 76, 87, 94, and 102, respectively (Figure 2). We performed μCP of PDA on each SAM, using grid-like patterns. The printed PDA on SAMs of EG₆OH can be directly observed under optical microscopy, as PDA areas were brighter than the background in the bright-field mode. This result is different from that in Figure 1. From Figure 2, we found that good transfer efficiency of PDA occurred in EG-rich SAMs. For percentage of EG below 80%, PDA patterns were not intact. On pure CH₃—SAM, we observed very low transfer efficiency of PDA. This experiment shows that PDA has low

Figure 1. Patterned polydopamine on glass, polystyrene, poly(dimethylsiloxane), and poly(ethylene glycol) dimethacrylate. PLL–PEG was modified on glass, polystyrene, and poly(dimethylsiloxane) before printing. Poly(ethylene glycol) dimethacrylate was UV-cured polymers that did not need further modification.

Figure 3. Comparison between (a,b) collagen/fibronectin and (c,d) polydopamine in their different abilities in patterning NIH 3T3 cells. (a,c) are cells cultured for 6 h, and (b,d) are cells cultured for 24 h. Cells gradually rolled up and detached from protein-patterned substrates after 24 h; for polydopamine-patterned substrates, cell spread on the patterns.

Figure 2. Printed polydopamine patterns on EG₆OH/CH₃ mixed SAMs with ratios from pure EG₆OH, 1:4, 2:3, 3:2, 4:1, to pure CH₃ (a–f). Transfer efficiency decreased with the decrease of surface energy (from pure EG₆OH to pure CH₃ SAM). Insets show contact angle measurements of each SAM, which are 32, 61, 76, 87, 94, and 102, respectively.
transfer efficiency on hydrophobic surfaces. This result can be compared to the results by Chen et al., where they employed protein as the ink. 

We tested the stability of printed PDA on EG surface by culturing NIH 3T3 cells and compared it to proteins. For protein experiments, we followed the procedure described by Piel et al. 

We printed collagen/fibronectin-mixed solution (50 μg/mL each) on plasma-cleaned glass, and backfilled unprinted areas with PLL–PEG. We found that after 6 h, protein and PDA behaved differently in their patterning qualities (Figure 3). For proteins, the patterned cells rolled up in their predefined areas. By contrast, cells in PDA-patterned areas remained spread. The rolling of cells was caused by underlying proteins that were torn off by cells above. 

Within 24 h, cells on protein-patterned substrates started to detach. The instability of protein patterns most likely originates from weak physisorption of proteins on hydrophilic glass. In this sense, proteins are not good materials to be used as inks. As for PDA, PDA can maintain its stability when supporting cells, indicating that it can sustain ripping forces from cells. We believe that the stability originates from the interaction between PDA and PEG. The interaction was formed by μCP, rather than covalent interaction. 

On the basis of the stability of PDA on EG surfaces, we patterned cells on the substrates described in Figure 1. We used NIH 3T3 cells because fibroblast cells move faster than cells such as HeLa and RPE1 cells. Figure 4 shows cell patterns on various substrates after 2 days of culturing, suggesting that PDA patterns were stable enough on inert surfaces to confine cells. Inert areas in our experiments were effective in resisting cell adhesion for at least 2 days. For all substrates in use, cells may weakly attach to inert areas in the form of spheres (i.e., not spread) shortly after seeding and could be washed away when changing culture medium. Cells sometimes crowded in clusters in patterns, suggesting that cell division took place during the 2 days of culture. In the case of PEGDMA, cell patterns were a bit larger than others, which is most likely caused by swelling of PEGDMA after adding cell culturing medium. Single cell patterns can be achieved by reducing cell densities when seeding cells. It is noteworthy that we used cell culturing medium containing 10% serum in all cell experiments. Low serum-containing medium was often used to slow the invasion of cells and damage to the underlying patterns. However, this was not necessary in our experiments. As for the toxicity of PDA and PLL-PEG, they were demonstrated to be of no harm to fibroblasts. Thus, our system is biocompatible for model cells such as fibroblasts and can maintain the best condition of cells in 10% serum-containing medium.

It is noteworthy that the range of substrates used in our system can be further broadened. PLL–PEG and PEGDMA resist cell adhesion and facilitate transfer of PDA in our experiment, and we believe that substrates having their surface modifications terminated with PEG can be easily patterned with PDA by printing. To achieve similar goals, other approaches for surface modification include atom transfer radical polymerization (ATRP) and plasma-enhanced chemical vapor deposition (PECVD), among others. In comparison, PLL–PEG modification is rather convenient to achieve by a simple process of immersion.

Figure 4. Microscopic images of patterned NIH 3T3 cells on (a,e) PLL–PEG modified glass, (b,f) PLL–PEG modified PS, (c,g) PLL–PEG modified PDMS, and (d,h) PEGDMA. The images were captured 2 days after cell seeding. Cell plasma and nuclei were green and blue, respectively. For (h), only the green channel was used as PEGDMA had blue emission.

Figure 5. Long-term culturing of patterned NIH 3T3 cells on PLL–PEG modified PS. Culture medium was changed every 2 days. We took images on the 7th, 14th, and 21st days after seeding cells.

Figure 6. Escherichia coli (a) and Staphlococcus epidermidis (b) were patterned on polydopamine-patterned PLL–PEG modified PS surface. Bacteria patterns were obtained by incubating PS surface in bacteria solutions for 4.5 h before washing with PBS to remove weakly attached bacteria. Insets: Enlarged images of patterned bacteria.
We found that PS substrates were most effective in keeping cells patterned in the long term. We kept culturing cells and imaging cells that were cultured for 7, 14, and 21 d, respectively (Figure 5). We found that cell patterns could be maintained for as long as over 3 weeks. The capability of long-term patterning of cells indicates that both PDA and PLL—PEG on PS cannot easily be damaged by cells. Other substrates were not as effective as PS; cells migrated out of patterns, formed bridges between patterns, and adhered on previously inert areas. Cell patterns lasted 3 days on glass, 26 days on PS, 4 days on PDMS, and 15 days on PEGDMA (see the Supporting Information). We believe the difference resulted from the different antifouling ability of PLL—PEG on various substrates.\(^\text{38}\)

We next patterned bacteria on PLL—PEG coated PS. The patterning of bacteria is useful in fabricating biosensors based on whole cells on chips, as well as studying quorum sensing and formation of biofilms. PEG were reported to reduce the adhesion of bacteria. We applied our system in measuring nuclear size in different antifouling ability of PLL—PEG on various substrates.\(^\text{38}\)

We developed a straightforward system for microcontact printing by use of the interaction between polydopamine and PEG. Inspired by mussel adhesion, we realized patterning mammalian cells on several common substrates, which could make it easy to pattern cells in common biological laboratories, or directly provide customized products for specialized design of patterns. We also demonstrated that our system was applicable to patterning bacteria. As an application, we measured nuclear sizes in different areas of cell patterns and found a linear dependence between them. In our experiment, we chose mainly PLL—PEG to render substrates to be inert. We anticipate that a versatile choice of substrates could be applied in cell patterning, based on various methods of surface modification.

**CONCLUSION**

We developed a straightforward system for microcontact printing by use of the interaction between polydopamine and PEG. Inspired by mussel adhesion, we realized patterning mammalian cells on several common substrates, which could make it easy to pattern cells in common biological laboratories, or directly provide customized products for specialized design of patterns. We also demonstrated that our system was applicable to patterning bacteria. As an application, we measured nuclear sizes in different areas of cell patterns and found a linear dependence between them. In our experiment, we chose mainly PLL—PEG to render substrates to be inert. We anticipate that a versatile choice of substrates could be applied in cell patterning, based on various methods of surface modification.

**ASSOCIATED CONTENT**

\(\text{Supporting Information.}\) A figure for long-term patterning of NIH 3T3 cells on different substrates. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Figure 7.** Different nuclear sizes in single cell patterns with different areas. NIH 3T3 cells were patterned in rectangular shapes with areas ranging from 1250 to 6050 \(\mu\text{m}^2\). We found a linear dependence between nuclear size and cellular size for different cell areas. For cellular areas (1250, 1800, 2450, 3200, 4050, 5000, 6050 \(\mu\text{m}^2\)), we obtained corresponding nuclear areas (477.63 \(\pm\) 150.65 \(\mu\text{m}^2\), mean \(\pm\) SD, \(n = 40\); 406.62 \(\pm\) 157.99, \(n = 30\); 379.11 \(\pm\) 132.83, \(n = 39\); 316.76 \(\pm\) 134.41, \(n = 29\); 297.63 \(\pm\) 115.02, \(n = 25\); 260.17 \(\pm\) 84.63, \(n = 29\); 222.18 \(\pm\) 56.33, \(n = 26\)). The linear fitting equation was \(y = 160.48 + 0.052x\), and \(R^2\) was 0.9904. Inset: (a) Single cell pattern to an area of 1250 \(\mu\text{m}^2\) and (b) single cell pattern to an area of 6050 \(\mu\text{m}^2\). Scale bar: 25 \(\mu\text{m}\).