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Communications

Colloidal Particle Assembly Using Micro-Patterning onto Morphology-Controlled Anti-Fouling Polyelectrolyte Multilayer Films

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Introduction

Micro-contact patterning is a method for generating micro-patterned material, wherein the material was initially adsorbed on an elastomeric stamp and it is transferred to the surface by pressurized contact.[1] Poly(dimethyl siloxane) (PDMS) is usually used as the stamp. This has been widely used for various purposes, as it is an easy and non-destructive method for creating patterned arrays on biosensor chips or selective tissue culture platforms.[2] In early usage of this technique, low molecular weight organic compounds having several ethylene units and functionalized endgroups were used to create self-assembled monolayers.[3-5] However, more recently, polymeric materials are used for micro-contact patterning, as they exhibit more versatile, active, and stable performance in micro-patterning.[6-8] Polymer-microcontact patterning wherein polyelectrolyte is used as ink for patterning on polyelectrolyte multilayer films is an especially promising technique[9] because it delivers multifunctional groups on the surface as well as employing various secondary bonding, such as electrostatic and H-bonding interactions, between the polyelectrolyte ink and the polyelectrolytes of the film to secure the pattern onto the patterned surface area.

Polyelectrolyte multilayer (PEM) deposition, also known as the layer-by-layer (LbL) process, may be one of the most versatile techniques to create conformal surface coating on various types and shapes of substrates with nano-scale control of the film thickness and fine tuning of the surface properties.[10-12] With these advantageous characteristics, PEM films have been introduced in various high-tech applications including electrical,[13-15] optical,[16,17] and biomedical[6,18-21] applications. Furthermore, the chemical functional groups of polyelectrolytes within the PEM coating were found to be readily available for additional chemical modifications.[9,12] Polyelectrolyte micro-contact patterning on PEM films is one of the most efficient and powerful method for further chemical modifications and may provide the opportunity to study very complex and delicate biological system, for example, cellular adhesions on nano-materials. Studies carried out to date with nanomaterials have shown that surface morphology,[22-24] chemical composition[6,9] and wettability[18,20] might play important roles in controlling the cellular responses to materials, and therefore, using multi-functionality driven by polyelectrolytes can be one of the few feasible methods to satisfy the control of these multiple factors.

In this study, we report a simple and robust micro-contact patterning technique for colloidal particles on the surface of the polyelectrolyte multilayer film that has anti-fouling (resistance toward biofilm formation) properties. Controlling the shape, size and surface charge of the stamp-transferred polymer pattern was readily achieved, and the oppositely charged magnetic colloidal particles were well aligned on top of the patterns. These patterned particles can be further chemically modified as needed; therefore, have a great potential in many applications including catalytic chemical processes and biological multiplexed sensing.

Results and Discussion

The micro-patterning of colloidal particles using a polyelectrolyte ink, which may interact with the colloids, patterned onto a polyelectrolyte multilayer (PEM) film surface is illustrated in Figure 1. The PEM films were prepared with poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), and the pH conditions for dipping polymer solutions were adjusted to 8.5 for PAH and 3.5 for PAA. The PAH/PAA films prepared at this pH 8.5/3.5 showed very distinctive surface charge depending on the outermost layer.[22]

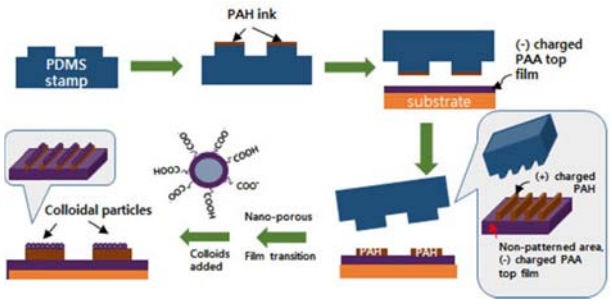

Figure 1. Schematic presentation of the paramagnetic colloidal micro-contact patterning on PEM films.

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Whether the final deposition step was PAH or PAA, the surface charge became either highly positive-charged or negative-charged. This means the surface was dominantly covered by the polyelectrolyte as the last step dipping solution possessed instead underwent rearrangement of deposited polyelectrolytes to form highly blended mixture. This distinctive surface charge of the PAH/PAA (8.5/3.5) films may be useful to make stable polyelectrolyte micropatterns due to the strong electrostatic interactions between oppositely charged polyelectrolyte ink and the film surface. Besides, the PAH/PAA system has a very exclusive property, which is micro-phase separation creating nano-porous structure triggered by the treatment of acidic water of the dried PAH/PAA film. This was revealed in our previous study, and the nano-porous PAH/PAA film showed excellent anti-fouling effect that might be important in biological application.[22]

For this reason, we decided to utilize this PAH/PAA (8.5/3.5) film system for creating micro-patterns of aligned colloidal particles on the surfaces. We chose carboxylated paramagnetic polystyrene (PS) colloids (diameter ~1.02 μm) purchased from Aldrich chemical for micropatterns, because they had a narrow polydispersity with controllable surface charge and magnetism. We used the particles with 1 micron in diameter, which is the smallest commercially available surface-modified product, wanting to reduce the surface roughness that might be caused by colloids. Therefore, we might focus on the charge effect and porosity of the materials during the micropatterning process rather than any other physical properties that could be raised from the colloids.

Since the colloids have negatively charged surfaces, we used positively charged PAH patterns to immobilize the colloids on the surface (Figure 1). If the surface charge of the colloid was positive, the ink in our scheme would be changed. Material descriptions and general synthetic procedure of PEM film followed from our previously reported works.[22,24] The

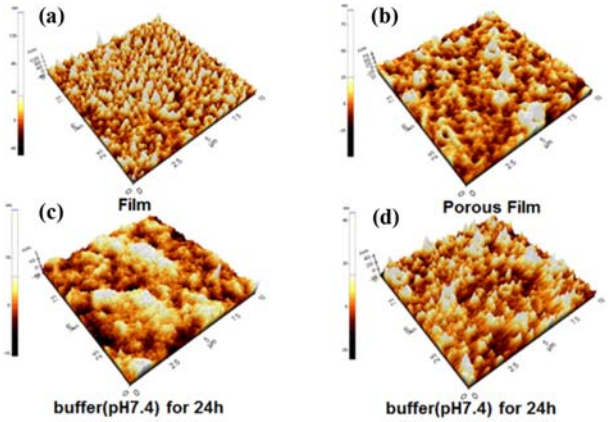

Figure 2. Surface morphology of the PAH/PAA films (a) as assembled, (b) after porous transition, (c) film (b) exposed in PBS solution without further treatment for 24 h, (d) film (b) was thermally treated for crosslinking and then immersed in PBS for a day. All films are $(PAH/PAA)_{6.5}$ and measured by AFM.

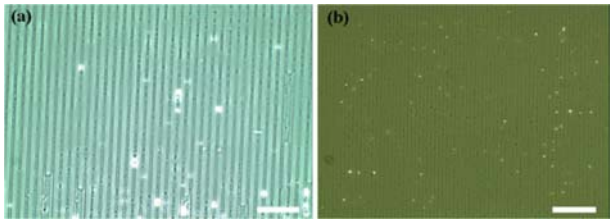

Figure 3. The optical microscopic images of the $(PAH/PAA)_6$ films having the micro-patterned PAH top layer, (a) with 20 micron-line and (b) 2 micron-line patterns (Scale bar, 200 μm).

number of bilayers of PAH/PAA film are designated hereafter as $(PAH/PAA)_n$.

Prior to make microcontact printing, we studied which surface morphology would be the most suitable for making stable colloidal micropatterns. As shown in Figure 2, the surface of the assembled PAH/PAA with a PAH top layer exhibits sharp mound features (Figure 2(a)). This was changed when the film was immersed in an acidic solution (pH~2.0) for a minute to a nano-porous film (Figure 2(b)). The porous structure was not stable with large pH changes, especially under high pH conditions (Figure 2(c)); however, applying a partial crosslinking of the film strengthened the micro-porous structure stable enough at pH 7.4 (phosphate buffered saline (PBS), Figure 2(d)).

Achieving stability of the porous morphology at physiological conditions allowed us exploring to create micro-patterns. As shown in Figure 3, a micro-patterned PAH layer was applied on the PEM film having PAA top layers with 2~20 micron-width stripe patterns by the polydimethylsiloxane (PDMS) micro-stamping process. The patterns showed well-defined, isolated forms in the full range of the film. Surface roughness induced by the porous structure appeared to make no significant defect in micro-contact patterning.

In our previous study of this nano-porous film, water contact angles dramatically decreased due to the highly hydrophilic regime.[22,23] The reason for the wettability increase is probably owing to the capillary force created from the interconnected pores within the film.[22] This can be controlled by the level of crosslinking generated between amine and carboxylic acid groups by thermal treatment.[12]

Next, we performed colloid alignment on the micro-patterned films. A suspended solution of carboxylated paramagnetic polystyrene (PS) colloids (COOH-mag-PS) was applied on the micro-patterned film. The slightly rough surface created by porous structure seemed to be more suitable than the flat surface for stabilizing spherical colloidal particles. It is probable that the mounds on the surface serve as wells to prevent the colloids from slipping. Figure 4 shows the results of colloidal patterning on the PAH/PAA multilayer film. In the case of PAH/PAA film without micro-patterns, COOH-mag-PS colloids were observed over the entire surface to be spread-out, while the colloids were only assembled on the top of the PAH line-patterns.

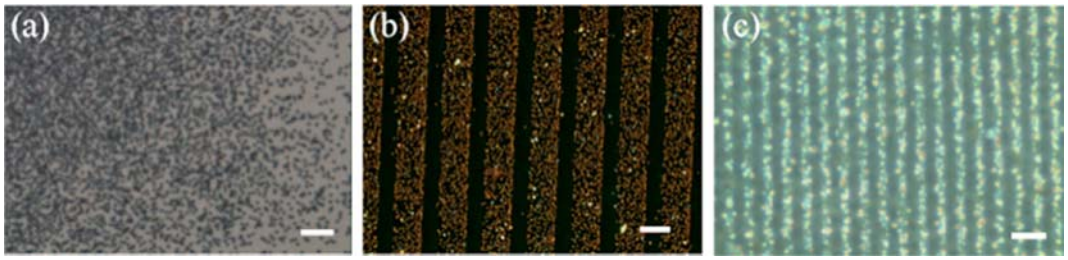

Figure 4. The optical microscopic images of the COOH-mag-PS particles on the surface of $(PAH/PAA)_6$ films having the micro-patterned PAH top layer, (b) with 50 micron-line and (c) 2 micron-line patterns, compared with (a) the film without pattern as a control (Scale bar, 20 μm).

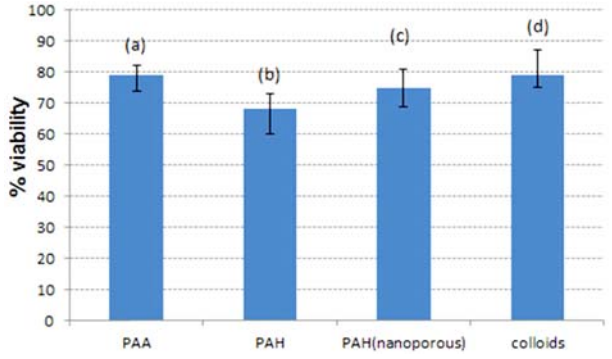

Figure 5. Cell viability study of the (PAH/PAA) films and colloid-patterned film measured by MTT assay of 293 epithelial cells. (a) PAA-top layered, (b) PAH outermost layered film, (c) PAH outermost layer after nanoporous transition, and (d) COOH-mag-PS colloids immobilized on the top of the film (c). The measurements were normalized to the data obtained from tissue culture grade polystyrene plate. Data were taken 1 day-post cell seeding.

Finally, we examined the cytotoxicity of the colloid-patterned PAH/PAA film for prospective biological applications, especially cell-based applications. 293 human embryonic kidney (HEK) cells were cultured on the PAH/PAA and colloid-patterned films for a day, and tested with methylethylthiazolyldiphenyl-tetrazolium (MTT) assay. Prior to the viability study, all the samples were immersed in PBS (buffer solution) for a day to check the stability. To ensure the stability of the colloids in aqueous solution for an extended time, the colloids on the PEM film were heat-treated at 80 °C for 12 h for thermal crosslinking between the carboxylic acids of the colloids and the amine groups of the PAH pattern.

As results, PAH/PAA films are relatively non-toxic to cells and especially nano-porous PAH/PAA film exhibits increased cell biocompatibility than the non-porous film. Colloid-patterned nano-porous films also exhibit good biocompatibility (Figure 5). This is a very promising result for us to pursue the further study of using this colloid-patterned coating for cell culture in the near future.

In conclusion, we have studied colloidal particle assembly using micropatterning onto the nano-porous polyelectrolyte multilayer (PEM) film that shows good biocompatibility. Results show that the surface morphology as well as the colloidal adsorption on the micropatterns was well controlled and further chemical and biological modification proved to be readily possible. Cell viability test results of the colloid-patterned PEM film as well as the PEM film look promising for using these systems for the future cell-interactive study. Due to the limitation of commercially available colloids with desirable size and magnetic property, this research will be extended with the synthesis of functionalized magnetic particles in the future.

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