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Non-adhesive PEG hydrogel nanostructures for self-assembly of highly ordered colloids

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Abstract
In this paper, we report on the effect of patterned non-adhesive hydrogel nanosurfaces on the self-assembly of highly ordered colloids. Polyethylene glycol (PEG) hydrogel is employed as the substrate material in the study, for its desired non-adhesive property, and biocompatibility as well as photopatterning ability. Ultrafine PEG features are photopatterned onto glass substrates with minimal feature resolution of 500 nm using ultraviolet or deep ultraviolet exposure. By simply controlling the colloidal concentration of the nanoassembly solutions and the dimensions of the wells, a range of highly organized nanocolloidal patterns are formed inside the PEG wells. Unlike the traditional surface modification techniques, ours takes advantage of the unique non-adhesive property of PEG hydrogels to achieve extremely high selectivity in the pattern-assisted nanoassembly. Our experiments show that with oxygen plasma treatment, the non-adhesive property of the PEG surface deteriorates significantly, leading to non-selective assembly with complete surface coverage of nanocolloidal beads under the same processing condition. Therefore, benefiting from the unique non-adhesive surface property, the pattern-assisted nanoassembly method enables a highly predictable and robust process for colloidal nanofabrication, and the obtained nanocolloidal arrays with well organized patterns could potentially find applications in photonic crystal fabrication, biological sensing and analytical detection.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Over the past decades, two-dimensional micropatterned surfaces with desired morphology and surface property have attracted considerable attention and led to a wide range of applications in self-assembled biochemical sensors, well-controlled cellular microenvironments, and medical diagnostic microdevices [1–6]. Several approaches have been proposed to prepare patterned surfaces as self-assembly templates [7–14], among which photolithography-based and soft lithography-based techniques have been the most popular choices. Besides the traditional lithographic techniques, the route of using monolayer colloidal crystal as a template shows a promising alternative process recently to fabricate the two-dimensional micropatterned surfaces with different designed morphologies [15, 16].

As one of the best known nonfouling biomaterials with extremely low energy and non-adhesive surfaces [40–42], polyethylene glycol (PEG) hydrogel, has been micromachined using both photolithography and soft lithography methods [43–45]. These patterned PEG surfaces are widely used in cell culture, drug delivery, and biomedical devices [46–48].
The non-adhesive property of PEG hydrogel comes from its high surface resistance to non-specific adsorption of proteins, cells and particles in aqueous environment [49, 50]. In this article, we present the nanoassembly of colloids onto patterned PEG hydrogel surfaces with non-adhesive property, referred to as the pattern-assisted nanoassembly (PAN). Ultrathin PEG features are photopatterned onto glass substrates with minimal feature resolution of 500 nm using ultraviolet (UV) or deep ultraviolet (DUV) exposure. By simply controlling the colloidal concentration of the nanoassembly solutions and the dimensions of the wells, a range of highly organized nanocolloidal patterns can be formed reliably inside the PEG nanowell structures.

2. Experimental section

2.1. Materials

Poly(ethylene glycol) diacrylate (PEG-DA; MW 575), 2-hydroxy-2-methyl-propiophenone, IgG-FITC from human serum, anhydrous toluene, ethanol, the desiccator, and the glovebag were purchased from Aldrich (Milwaukee, WI). Shipley® S1805 photoresist and MF-319 developer were purchased from MicroChem (Newton, MA). Styrene (St), methyl methacrylate (MMA) and acrylic acid (AA) were distilled before use. Sodium dodecyl sulfate (SDS) was purified by recrystallization in ethanol before use. Ammonium persulfate (SDS) was purified by recrystallization in ethanol before use. Ammonium persulfate ((NH₄)₂S₂O₈) and ammonium bicarbonate (NH₄HCO₃), chemical grade reagents, were used as received.

2.2. Silane modification of glass substrates

Glass slides were scrupulously cleaned prior to silane assembly. The slides were immersed for 30 min in the ‘piranha’ solution consisting of a 3:1 ratio of aqueous solutions of 50% (v/v) sulfuric acid and 30% (w/v) hydrogen peroxide. After removal from the ‘piranha’ bath, glass slides were thoroughly rinsed with deionized (DI) water and dried under nitrogen. Clean slides were stored at room temperature in a Class 1000 cleanroom environment. Prior to silane modification, those slides were placed under oxygen plasma (Yes-R3, Yield Engineering systems, Inc., San Jose, CA) for a 5 min treatment at 300 W, providing hydroxyl groups for the following silane self-assembly. Surface modification with silane adhesion promoter was achieved by immersing glass slides into a 20 mM solution of 3-acryloxypropyltrichlorosilane in anhydrous toluene for 60 min. After removing from the silane solution, slides were rinsed in toluene and clean-dried with nitrogen flow. Both the solution preparation and the silane self-assembly reaction were conducted under a nitrogen atmosphere in a glovebag, at room temperature. The silanized glass slides were stored under vacuum in a desiccator [43, 44].

2.3. Fabrication of PEG hydrogel micropatterns

PEG hydrogel patterns were fabricated from the precursor solution of PEG-DA (MW 575) with 1% (w/v) photoinitiator, 2-hydroxy-2-methyl-propiophenone. This solution was spun at 800 rpm for 8 s onto the silane-treated glass surface containing terminal acrylate groups using a spin coater (Machine World, Inc., Redding, CA). The thin layer of the PEG-DA precursor solution on glass was then exposed through a chrome/soda lime photomask to an UV light source of the photointensity of 15 mW cm⁻² at 365 nm (Omnicure 1000, Exfo America Inc., TX) or a DUV mask aligner of 15 mW cm⁻² at 220 nm (ABL 60, ABL Inc., CA). The exposure time was 0.7 s. The regions of PEG-DA exposed to UV light underwent free-radical polymerization and became crosslinked, while unexposed regions were dissolved in DI water after 5 min development. The height of the resultant hydrogel microstructures was about 0.3 µm, as measured with SEM (Philips XL 30, Netherlands) and AFM (Nanoscope IIIa, Veeco Instruments, Santa Barbara, CA). Hydrogel structures (2.2 × 2.2 cm²) for nanocolloidal fabrication consisted of either a nanowell array with dimensions of 500 × 500 nm² and 1500 nm spacing, or a microwell array with dimensions of 1 × 1 µm² and 1 µm spacing. High-resolution images of the hydrogel structures were obtained using SEM operating at 10 kV of accelerating voltage. To reduce charging effects, substrates were sputter-coated with a thin layer of gold (~5 nm) prior to the SEM exam. The same protocol was applied to the SEM imaging of nanocolloidal array on the S1805 photoresist samples as well.

2.4. Fabrication of S1805 micropatterns

The S1805 photoresist was spun at 8000 rpm for 30 s onto the silane-treated glass surface containing terminal acrylate groups using a spin coater (Machine World, Inc., CA). The thin layer of the photoresist on glass was soft baked at 115°C for 60 s, then exposed through a chrome/soda lime photomask at contact mode to an UV light source of the photointensity of 25 mW cm⁻² at 365 nm (ABL 60, ABL Inc., CA). The exposure time was 5 s. After developed in the MF-319 developer for 10 s, the S1805 micropatterns on glass slides were obtained. The height of the resultant microstructures was about 0.3 µm, as measured with SEM (Philips XL 30, Netherlands). S1805 structures (2.2 × 2.2 cm²) for nanocolloidal array fabrication consisted of a microwell array with dimensions of 1 × 1 µm² and 1 µm spacing.

2.5. Synthesis of monodispersed P(St-MMA-AA) colloidal particle

Emulsion polymerization was used to synthesize monodispersed P(St-MMA-AA) colloids of ~190 nm in diameter. A typical procedure was prepared as following: (A) a 120 ml aqueous solution of (NH₄)₂S₂O₈/0.4 g, NH₄HCO₃/0.8 g and SDS/0.01–0.08 g in a funnel, and (B) a 25 ml of monomer mixture consisted of St/MMA/AA (90:5:5 v/v/v) in an another funnel were added at the same time into a 250 ml flask, the mixture was agitated at 70°C in N₂ atmosphere for 5 h to obtain a homogeneous colloidal suspension with the particle
diameter of 190–250 nm, and the colloidal particles were highly monodispersed, as measured by TEM (JEM-100CX, Japan). Prior to use, the colloidal particles were further purified by centrifuge separation at 12 000 rpm, washed and dried at 25 °C, and then, diluted to a definite concentration using deionized water.

2.6. Fabrication of nanocolloidal arrays on PEG wells

Figure 1 illustrated the pattern-assisted nanoassembly method used to fabricate nanocolloidal arrays onto PEG wells. Experimental procedures were described as follow. First, a suspension of the P(St-MMA-AA) colloidal particles was diluted to a defined concentration using deionized water. Then, the glass slide fabricated with PEG hydrogel wells was immersed vertically into the dispersion and lifted up at a constant speed varied from 0.1 µm s⁻¹ to 1 mm s⁻¹, which was accurately controlled by a precision step motor (Micros VT-80, Germany). All the experiments were operated at regular room condition (25°C and 1 atm).

3. Results and discussion

3.1. Fabrication of PEG hydrogel patterns

Coating silane coupling agents onto the glass substrate is critical to the adhesion of PEG hydrogel micro/nanostructures [51–53]. In our study, after the silane modification, the self-assembled 3-acryloxypropyltrichlorosilane layer is covalently bonded to the glass surface through the formation of Si–O bonds. The precursor solution, consisting of both photoinitiator and PEG-DA, is spin-coated onto 75 × 25 mm² glass slides, forming a uniform thin layer. In the following step, the coated slides are exposed to UV or DUV light through a photomask containing the design features in contact mode. UV-initiated reactions among C=C bonds in PEG-DA molecules lead to localized formation of a highly crosslinked hydrogel structure. Meanwhile, the reactions between acrylate groups in the bulk and acrylate moieties of the alkylsilane result in covalent anchoring of the hydrogel micro/nanostructures to the surface [43, 44]. Polymerization of the precursor polymer films containing 1% (w/v) photoinitiator occurs after 0.7 s of exposure to a collimated 365 nm UV or 220 nm DUV source operating at 15 mW cm⁻². Unexposed regions of the precursor solution are developed in water, leaving only surface-anchored hydrogel micro/nanostructures.

Figure 2 shows SEM and AFM images of the fabricated PEG micro/nanopatterns. As can be seen, the feature size of 500 × 500 nm² with 1500 nm spacing, as well as that of 1 × 1 µm² with 1 µm spacing lead to an ultrahigh feature density of 250 000 wells mm⁻² on glass substrates. The depth of the microwell features is about 300 nm as measured by AFM, which aims to form single-layer nanocolloids. Severe charging effect of the SEM images has been observed because of the poor electrical conductivity of PEG hydrogels and glass substrates. To achieve high-resolution PEG photolithography, the contact mode between the PEG substrate and photomask is employed, given the fact that the minimal feature size is proportional to the square root of the separation distance [54]. Additional cleaning procedure of the photomask with acetone is necessary to remove excess uncrosslinked PEG pre-polymer in the post-exposure treatment. The 500 × 500 nm² nanowells define the highest resolution of PEG hydrogel patterns achieved by photolithography method to our best knowledge, compared with the minimal resolution of 20 µm in the previous study using the conventional proximity exposure [43, 44].

3.2. Assembly of nanocolloidal arrays in different sizes of PEG wells

As illustrated in figure 1, the pattern-assisted nanoassembly method is performed on a nanopatterned PEG hydrogel substrate of 500 × 500 nm² nanowells and a micropatterned substrate of 1 × 1 µm² microwells at the pulling speed of 0.1 µm s⁻¹ and colloidal concentration (190 nm) of 1.5 mg ml⁻¹, respectively. As shown in figure 3, the number of nanocolloids assembled in the PEG wells can be controlled by varying the size of the wells, where the arrows indicate the pulling direction of the substrate. In figures 3(a) and (b), P(Si-MMA-AA) nanocolloids are self-assembled, and display repetitive single-bead nanocolloidal patterns inside each PEG nanowell. While at the same pulling speed and nanocolloidal (190 nm) concentration, an ordered 3-bead nanocolloidal arrays is formed in the PEG hydrogel microwells of 1 × 1 µm², as shown in figures 3(c) and (d). Therefore, increment in the well size raises volume of the trapped colloidal suspension, and increases the number of nanocolloids assembled in the PEG wells. As illustrated in the inset of figure 1, under the influence of gravitation, the colloids inside the trapped suspension tend to move downwards in the well during the evaporation. Once the self-assembly process accomplishes, all the beads are uniformly aligned along the bottom edge of the wells.
Figure 2. SEM and AFM images of PEG hydrogel wells on the silane-modified glasses. (a), (b) $500 \times 500$ nm$^2$ nanowells; (c), (d) $1 \times 1 \mu m^2$ microwells.

Figure 3. SEM images of nanocolloidal arrays formed in the (a), (b) $500 \times 500$ nm$^2$ PEG nanowells and (c), (d) $1 \times 1 \mu m^2$ PEG microwells using 1.5 mg ml$^{-1}$ P(St-MMA-AA) colloidal particle concentration and 0.1 $\mu m$ s$^{-1}$ pulling speed (the arrow indicates the pulling direction).
3.3. Assembly of nanocolloidal arrays at different colloidal concentrations

Figure 4 shows varying the concentration of P(St-MMA-AA) colloidal particles (190 nm) in the aqueous suspension can lead to different morphologies of nanocolloid arrays in the PEG microwells, such as the 1–3 bead arrays ((a), (b), (c)) and 1–2 line arrays ((c), (d)), which is of potential use in photonic crystal fabrication. Notably, results in figure 4 also imply that increasing the colloidal concentration of the aqueous suspension increases the number of nanocolloids assembled in the PEG wells, given the other conditions unaltered. Further controlling the pulling speed and inclined angle of the substrate as well as the shape of the PEG patterns during the nanoassembly fabrication are currently under investigation.

3.4. Influence of non-adhesive property to the nanoassembly

In the pattern-assisted nanoassembly, surface property plays an essential role in addition to the topology. The traditional way to achieve high selectivity of assembly, i.e., preventing assembled colloids from adhering to the top surface, is through the control of surface hydrophobicity or wettability [31–39]. For instance, achieving surface super-hydrophobicity would effectively eliminate aqueous suspension in contact with the surface, and therefore, the colloidal assembly is only confined onto the hydrophilic (wettable) patterns. In our case, the PEG hydrogel surface exhibits super-hydrophilicity and high wettability (contact angle of 22°). Therefore, unlike the traditional patterned wettability technique, we take advantage of the unique non-adhesive (or nonfouling) property of PEG hydrogels to achieve the highly selective nanoassembly. As one of the best known biomaterials with an extremely low surface energy [40–42], the non-adhesive property of the PEG hydrogel is attributed to its high surface resistance to non-specific adsorption [49, 50], which is crucial to prevent the P(St-MMA-AA) nanocolloids from sticking to the surface during the pattern-assisted nanoassembly process. To verify this unusual non-adhesion mechanism, oxygen plasma treatment has been carried out to alter the surface property. At 200 W for 20 s, about 100 nm PEG hydrogel on the surface is removed under oxygen plasma, and the contact angle of the PEG hydrogel surface reduces from 22° to 9°, which indicates chemical/physical alternation on the surface due to oxidation. More importantly, the non-adhesive property of the PEG hydrogel surface is largely eliminated since hydroxyl and carboxyl groups are introduced during the modification. In the following step, the plasma modified PEG microwells are used to assemble colloidal arrays under the same conditions as the unmodified ones, and the comparison is illustrated in figure 5 as a result. As expected, figure 5(b) shows that with the oxygen plasma surface modification, nanocolloidal arrays are packed into the microwells, but also adhere onto the modified PEG surfaces unlike the highly selective assembly result in figure 5(a).

In the second study, we confirm the importance of non-adhesive property using an alternative approach. Here, the nanoassembly process is performed onto the identical
micropatterns yet constructed by a different polymer material. Figure 6(a) shows 1 × 1 µm² microwells with a depth of ~300 nm made from Shipley® S1805 photoresist by photolithography method. The S1805 surface possesses 75° contact angle of water. Although the S1805 microwells have the same dimensions as their PEG counterparts, the polymer matrix mainly comprising of cresol novolak resin has higher surface energy than that of the PEG. The photoresist template is then used for the nanoassembly at the same conditions as the previous study, and the result is demonstrated in figure 6(b). In comparison with figure 5, it shows that nanocolloidal arrays occupy the entire surface of the photoresist micropatterns, similar to the oxygen plasma modified PEG surface shown in figure 5(b). Overall, both comparison studies reach the same conclusion that non-adhesive surface of the unmodified PEG hydrogel is of essence to the pattern-assisted self-assembly process.

3.5. Assembly of IgG-FITC labeled nanocolloidal arrays in PEG microwells

After the emulsion polymerization, the P(St-MMA-AA) nanocolloid has carboxy functional groups (from AA) on surface, which can easily be labeled by antigen/antibody, biomolecules and fluorescent agents. For example, IgG-FITC can be coupled onto its surface by using the carbodiimide method described elsewhere [55]. The IgG-FITC labeled P(St-MMA-AA) colloidal particles (~5.0 mg ml⁻¹) are used to fabricate nanocolloidal arrays, and the results are shown in figure 7. The IgG-FITC labeled colloid arrays emitted green light (520 nm) via an excitation wavelength of 490 nm under fluorescence microscope, which could have potential implications for microchip-based drug identification, analyte detection, cell sorting, and biological sensing [56].

3.6. Assembly of binary nanocolloidal arrays in PEG microwells

Figure 8 shows the nanoassembly of two different sizes of colloids (190 and 250 nm) together in the PEG microwells. An interesting phenomenon is noted here that the bigger beads (250 nm) tend to settle down to the bottom inside the microwells compared with the smaller ones (190 nm), as shown in figure 8(b). This could be possibly explained by subtle difference in the Brownian motion between the two sizes of beads during the evaporative process of trapped suspension. Furthermore, using different functionalized blocks in the nanoassembly, binary or polynary nanocolloidal arrays may lead to broader applications in combinatorial chemistry, drug screen, and clinical diagnosis.
Figure 7. (a) SEM and (b) fluorescent microscope images of nanocolloid arrays formed in the 1 × 1 µm² PEG microwells using 5.0 mg ml⁻¹ IgG-FITC labeled P(St-MMA-AA) colloidal particle concentration and 0.1 µm s⁻¹ pulling speed.

Figure 8. SEM images of binary nanocolloidal arrays formed in the 1 × 1 µm² PEG microwells by using a mixture of 1.5 mg ml⁻¹ 250 nm and 3 mg ml⁻¹ 190 nm P(St-MMA-AA) colloidal particles at 0.1 µm s⁻¹ pulling speed (the arrow indicates the pulling direction).

4. Conclusions

Surface property and topology play important roles on self-assembly of nanocolloidal arrays. Based on the non-adhesive property of PEG hydrogels, we demonstrate a novel self-nanoassembly process of monodispersed P(St-MMA-AA) nanocolloids on patterned PEG hydrogel surfaces, referred to as the pattern-assisted nanoassembly (PAN). Using this simple controllable assembly method, various patterns of colloids on PEG microwells can be reliably achieved. Several controlling parameters have been experimentally investigated, including the concentration of the nanocolloids in the aqueous suspension and the dimension of the microwells. In addition, effect of non-adhesive surface property is further investigated. After oxygen plasma treatment, the nonfouling property of the PEG surface deteriorates significantly, leading to complete surface coverage of nanocolloidal beads. Alternatively, microwell surface constructed by a regular photoresist leads to a similar result as the oxygen plasma modification. Therefore, the unique non-adhesive property of PEG hydrogel is found with particular importance in the pattern-assisted nanoassembly process. Benefiting from the PEG hydrogel, the pattern-assisted nanoassembly enables a highly predictable and robust process to achieve well organized nanocolloidal arrays within desirable patterns, which could find its potential applications in photonic crystal fabrication, biological sensing and analytical detection.

References
