Micropatterning Biomacromolecules on Aldehyde-Enriched Polyester Surfaces by a Microtransfer Technique

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A modified microtransfer technique was introduced to create micropatterns of biomacromolecules on activated polyester surfaces. Briefly, a recessed poly(dimethylsiloxane) stamp was first coated with a biomacromolecule solution. After drying the solution and selectively removing the biomacromolecules on the ridges, the stamp was stored at low temperature and high humidity to form a condensed water layer. The stamp was subsequently pressed onto the activated polyester surfaces to yield the biomacromolecular patterns. The notable feature of this method is that the reaction between the biomacromolecules and the polymer surface could be performed at a comparable speed to that in solution. Using this method, albumin and chitosan were micropatterned onto aldehyde-enriched polycaprolactone or poly(L-lactic acid) surfaces. Observations under confocal laser scanning microscopy confirmed that the albumin or chitosan patterns formed by this microtransfer technique had higher contrast and good stability. The amount of water condensed on the stamp surface had significant influence on the quality of the resultant patterns. Deficient water produced rimming patterns, while excessive water caused the contamination of the patterns because of the overflow of the condensed water. This technique will be especially useful to those systems with lower reaction activity in the absence of solvent and hence can be applied widely to create patterns of biomacromolecules on polymer surfaces.

Introduction

The ability to generate patterns of biomolecules on material surfaces is important for biosensor technology,[1−3] tissue engineering,[4,5] and fundamental studies of cell biology.[6,7] There are several ways to pattern proteins and other biomolecules onto material surfaces, such as photolithography,[8,9] soft lithography,[10−12] and spotting techniques.[13] Whitesides and co-workers[14] have developed a reactive microcontact printing technique to pattern biological ligands onto reactive self-assembled monolayers (SAMs) on gold. Motivated by potential applications of biomaterials patterned with biomolecules, Chilkoti et al.[15] developed this method in the polymer field. Although it is a simple and flexible method for covalently coupling biomolecules onto polymer surfaces, it generally needs very high activity for reaction, because no or little solvent is involved in this procedure. Compared with most grafting reactions occurring in solution, most derivatized polymer surfaces are unreactive under the conditions of the microcontact printing technique.

Recently, Chilkoti et al.[16] developed a technique designated "wellpat" to pattern biomolecules onto polymer surfaces. The microwells in a poly(dimethylsiloxane) (PDMS) stamp surface were selectively filled with biomolecule solutions such as biotin or peptide by using the difference of surface hydrophilicity/hydrophobicity between the microwells and the ridges. Then the stamp was pressed onto carboxylic acid-containing and NHS/EDAC (*N*-hydroxysuccinimide/1-ethyl-3-(dimethylamino)-propylcarbodiimide)-activated poly(ethylene terephthalate) film. Covalent patterning of biomolecules on a polymer surface was thus fabricated. This is rather

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important because patterning can be achieved from aqueous solution and the grafting reaction can proceed easily.

Herein covalently patterning biomacromolecules on biodegradable polyesters, e.g. polycaprolactone (PCL) and poly(L-lactic acid) (PLLA) was realized by using a reactive microtransfer technique with a novel process. Bovine serum albumin (BSA) and chitosan were covalently grafted onto aldehyde-enriched polyester surfaces with dot patterns. Confocal laser scanning microscopy (CLSM) was used to follow the patterning process by employing fluorescein isothiocyanate-labeled BSA (FITC−BSA) or rhodamine B isothiocyanate-labeled chitosan (Rd-chitosan) as fluorescent probes.

Experimental Section

Materials. FITC−BSA (Mn 60 000), PCL (Mn 80 000), and 3-aminopropyltrimethoxysilane (3-APS) were purchased from Aldrich. Poly(dimethylsiloxane) (PDMS) elastomer kits (Sylgard 184) were obtained from Dow Corning (Midland, MI). PLLA (Mn = 200 000, Mw = 400 000) was synthesized from L-lactide under the catalysis of stannum octanic acid.[17] Chitosan with medium molecular weight was obtained from Sigma and labeled by rhodamine B isothiocyanate (Aldrich).[18]

PCL/1,4-dioxane (100 mg/mL) or PLLA/1,4-dioxane (60 mg/mL) solution was cast onto a glass plate and dried under vacuum to yield polymer films with a thickness of ∼200 μm. To obtain an aldehyde-enriched surface, the PCL films were first aminolyzed in 10% (w/v) 1,6-hexanediamine (HDA)/2-propanol solution at 37 °C for 10 min[19] and then were treated with 1.0% glutaraldehyde (GA) solution at room temperature for 30 min;[20] the PLLA films were aminolyzed in 6% (w/v) HDA/*n*-propanol solution for 2 min and then treated with 1.0% GA solution for 15 min.[21] All the films were thoroughly rinsed in deionized water and dried under nitrogen.

The PDMS stamp was fabricated using the method described by Jackman.[22] Briefly, an array of posts formed in photoresist on a glass substrate was used as a "master". The stamp was fabricated by molding PDMS against this master to obtain microwells each measuring approx 45 μm in diameter, 15 μm in width, and 4 μm in depth. The amino-silanized glass slides for selective removal of BSA or chitosan were produced by 3-APS treatment.[23]

Microtransfer Patterning. The microtransfer patterning technique described here is a multistep procedure, as being exemplified by patterning BSA on PCL film. First, The PDMS stamp was stored in deionized water for ≥24 h in order to enhance its hydrophilicity. A small drop of FITC−BSA solution (1.0 mg/mL) in phosphate-buffered saline (PBS) was added to the topologically defined face of the stamp. Then a glass stick was gently dragged back and forth on the stamp surface to make BSA fill the microwells as much as possible. After drying by gentle nitrogen flow, an amino-silanized glass slide was pressed onto the stamp surface with a force of ∼100 g/cm^2 for 12 h to selectively remove the BSA on the ridges.[24] After cooling at −20 °C for a couple of minutes (e.g. 3 min), the stamp was subsequently incubated in an environment with high humidity (relative humidity 60−70%, ∼25 °C) for 0.5−1 min to induce the formation of a thin layer of condensed water. The stamp was then brought into physical contact with the aldehyde-enriched PCL immediately under a force of 100 g/cm^2 (Figure 1). Due to the condensed water in the microwells and the lateral diffusion of water on the ridges, BSA solution and microreactors were formed at the noncontact areas. As a result, reaction could be performed at a comparable speed to that which occurs in solution. Three hours later, the patterned PCL film was carefully removed from the stamp, washed extensively with PBS, rinsed in PBS for 24 h, and then dried by nitrogen flow.

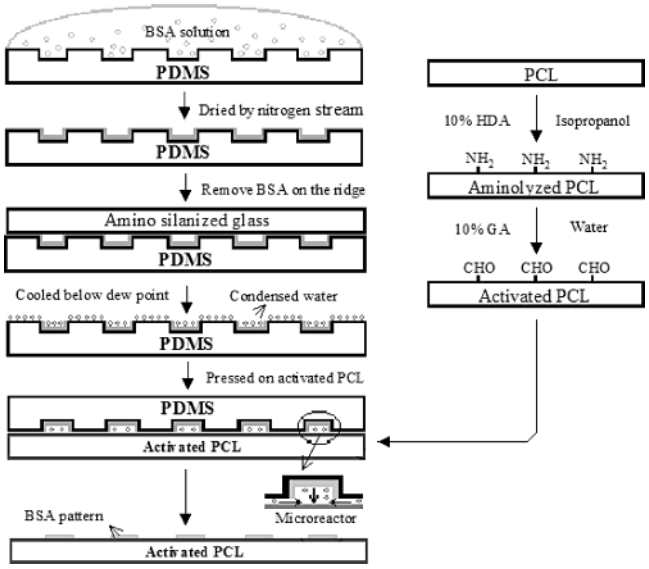

Figure 1. Schematic representation of the modified microtransfer process for patterning biomacromolecules onto activated PCL film (see text).

Characterization. The amino groups on the aminolyzed PCL film have been quantitatively measured by the ninhydrin analysis method.[19] The total amino density on the PCL film was ∼1.6 × 10^{-7} mol/cm^2, supposing that the film surface was an absolute plane and the amino groups were accumulated on one layer. The topographic feature of the stamp surface was measured by atomic force microscopy (AFM, SPI3800N, Seiko Instruments Inc.) in dynamic mode under ambient conditions. The biomacromolecular patterns formed on the activated PCL and PLLA surfaces were visualized on a Bio-Rad Radiance 2100 confocal laser scanning microscope (CLSM). Specimens were excited at 488 nm for FITC−BSA and 543 nm for Rd-chitosan, respectively.

The amount of water condensed on the PDMS stamp surface was evaluated by a gravimetry method. The water per microwell was calculated from the total amount of water and the numbers of the microwells.

Results and Discussion

Figure 2a presents a confocal image of the stamp after selective removal of the BSA on the ridges by an amino-silanized glass slide. It proves that the selective removal of the biomacromolecules can realize the selective filling of BSA into the stamp microwells. This is similar to the results reported by Bernard et al., where they selectively removed lines of BSA from the stamp by contacting the stamp with a patterned, micromachined object.[24] After microtransfer patterning the BSA onto the aldehyde-enriched PCL film, no remaining BSA was detected on the original stamp (Figure 2b). This proves that the BSA in the microwells has completely been transferred.

The confocal image of FITC−BSA patterns on the activated PCL film created by this novel microtransfer

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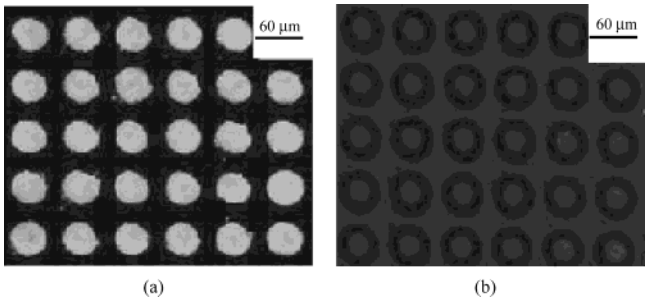

Figure 2. Confocal images of the stamp filled with FITC−BSA solution before (a) and after (b) microtransfer patterning operations.

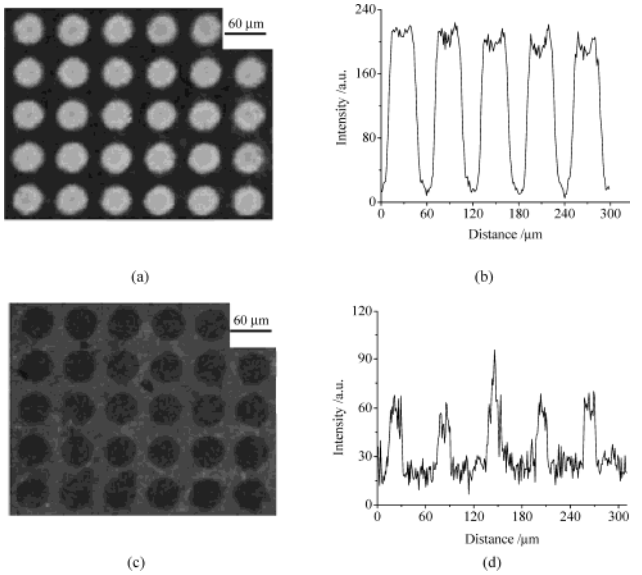

Figure 3. (a) Confocal image of FITC−BSA patterns on the activated PCL surface created by microtransfer patterning technique. (b) The line profile of fluorescence intensity of the FITC−BSA pattern in Figure 3a. (c) Confocal image of FITC−BSA reversed pattern on the activated PCL surface created by the classical μCP technique. (d) The line profile of fluorescence intensity of the FITC−BSA reversed pattern in Figure 3c.

patterning procedure is shown in Figure 3a. It reveals that BSA was spatially localized onto the PCL surface. The average contrast ratio of BSA patterns (Figure 3b) is much higher than that of its reversed patterns (Figure 3c,d) obtained by the classical microcontact printing technique (μCP) using the same stamp but without condensed water, i.e., printing directly after the stamp was dried by soft nitrogen flow. These results demonstrate that the amount of BSA immobilized by the microtransfer patterning is much higher than that by the conventional μCP method supposing other conditions are constant. The patterns are stable after rinsing with deionized water for 24 h, demonstrating that the biomacromolecules should be covalently patterned on the PCL surface. The slightly reduced edge definition of the pattern is attributed to the diffusion of the biomacromolecules along the edges of the microwells after the stamp was pressed onto the activated PCL film. This diffusion, however, is mainly caused by the microwells whose edges are not absolutely sharp as observed under AFM (Figure 4).

In this microtransfer patterning process, the amount of water condensed on the stamp surface has significant

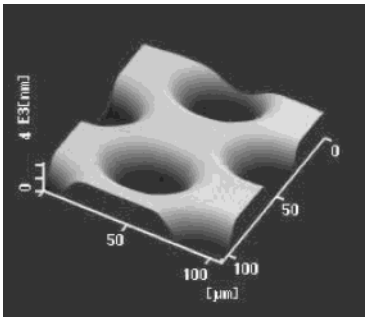

Figure 4. Dynamic-mode AFM image of the PDMS stamp used in the microtransfer patterning procedure showing that the edges of the microwells are not absolutely sharp.

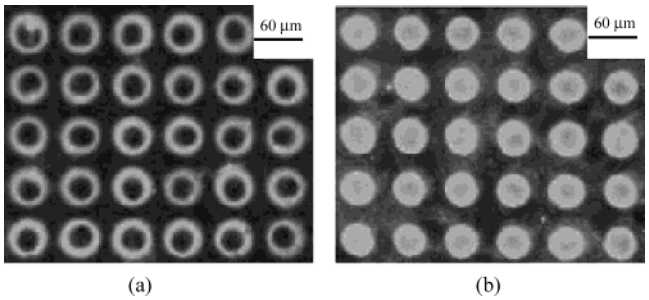

Figure 5. The FITC−BSA patterns created by the microtransfer patterning technique on the activated PCL surface when the amount of water condensed on the stamp surface is not suitable: (a) rimming pattern caused by inadequate water and (b) surface contamination due to excessive water.

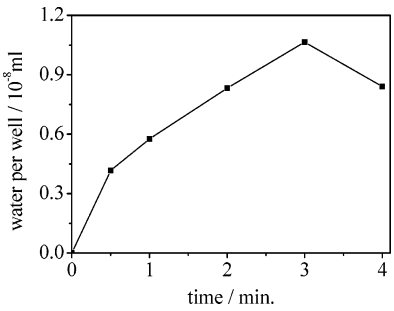

Figure 6. Condensed water per microwell as a function of incubation time. The relative humidity is 60−70%.

influence on the quality of the resultant patterns. Inadequate water often induces rimming patterns, while excessive water can result in contamination, as shown in Figure 5. This can be understood easily from the formation of the microreactors. Deficient water can only bring BSA onto the polymer surface along the inner wall of the microwells, while excessive water will cause the overflow of BSA from the microwells. The amount of condensed water can be controlled by the relative humidity and the incubation time after the stamp has been cooled. At a given relative humidity (60−70%, ∼25 °C), the amount of condensed water increased first and then decreased with the increase of incubation time (Figure 6). Averaging the whole amount of condensed water by the total amount of microwells, one can obtain the condensed water per microwell at different incubation time. According to the dimensions of stamp measured by AFM, the volume of one microwell is $\sim 0.64 \times 10^{-8}$ mL. Incubation of the BSA-filled stamp at a relative humidity of 60−70% for 0.5−1 min is suitable to gain a nice pattern, as shown in Figure 3a. Being

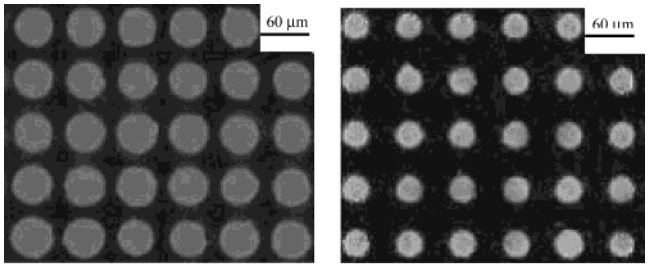

Figure 7. (a) Confocal image of Rd-chitosan patterns on an activated PCL surface created by microtransfer patterning. The PCL film used here was aminolyzed and reacted with 1.0% GA solution for 15 min. (b) Confocal image of FITC−BSA patterns on an activated PLLA surface created by microtransfer patterning. A different stamp was used here to show the diversity.

outside of this range generally resulted in unclear patterns (Figure 5).

This patterning method is not restricted to patterning BSA on PCL surface. Figure 7a shows that Rd-chitosan can also be patterned on the activated PCL surface using the same patterning process. FITC−BSA patterns with high contrast ratio on activated PLLA surface were similarly created (Figure 7b). Therefore, this pattering method can be commonly employed for creating biomacromolecules patterns on polyester surfaces.

To avoid contamination caused by diffusion of the "ink",[25] the stamp used in μCP should be dry or with little solvent prior to its application. For this reason, the reaction between biomolecules and the activated polymer surface is sluggish, especially for those systems with low reaction activity. In the microtransfer patterning procedure, however, a certain amount of water is introduced into the reaction; hence, the covalently patterning can be performed more easily. Moreover, in the μCP process, the amount of BSA adsorbed on the ridges of the stamp is limited. By contrast, larger amount of BSA can be funneled into microwells by the present process. This is another reason for obtaining higher contrast of the biomacromolecule's patterns.

Soft lithography including the μCP technique have achieved big success in patterning SAMs on gold as model organic thin films to realize biomolecule and cell patterning.[12] Patterning biomolecules and cells on tissue-culture polystyrene (TCPS) by direct adsorption has also been reported.[4,5] Recently, Chilkoti et al. have developed reactive μCP and "wellpat" techniques to pattern biofunctional molecules on a polymer surface.[15,16] They have created biotin patterns on poly(ethylene terephthalate) film. The microtransfer patterning technique reported here is also a reactive pattering method, with the difference in filling procedure and surface activation of polymers. A requirement for this technique is the presence of reactive functional groups on the polymer surface. Because the reaction between aldehyde groups and amino groups is nonspecific to a definite molecule pair, it can be predicted that this method is applicable also to other biomacromolecules, except for albumin and chitosan. Other notable features are that the stamp used here does not require chemical pretreatment, and the microwells of the stamp need not be very deep either ($\sim$4 μm).

As a straightforward technique, microtransfer patterning enriches the family of soft lithography methods. By introducing a certain amount of water as solvent, the patterning grafting reaction on polymer surfaces can be performed with relative ease. This reduces the requirements for the high surface activity. In addition, one can also expect other applications of this patterning method, such as creating patterns of nano- and microparticles with reactive groups or electric charge onto a polymer surface with complementary reactive groups or opposite charge.

Conclusion

As a simple and flexible method, the microtransfer patterning technique described here enables covalent patterning of biomolecules onto derivatized biomaterial surface with ease and higher contrast. It will be especially useful for patterning biomacromolecules onto those surfaces with low reaction activity under normal conditions.

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