Poly(ethylene glycol) micro-patterns as environmentally sensitive template for selective or non-selective adsorption

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Abstract
Poly(ethylene glycol) (PEG), a hydrophilic and repulsive polymer to non-specific adsorption, was stamped onto carboxylic acid-enriched polymer surfaces using the micro-contact printing technique. The patterns are stabilized via hydrogen bonds. Areas printed with PEG were then shown to be non-adsorbed with fluorescein isothiocyanate (FITC)-labeled dextran, while the poly(methacrylic acid) (PMAA) regions could via hydrogen bonding. Due to this contrast, well defined dextran patterns were obtained. Tuned with pH and temperature, the PEG molecules could be detached from the surfaces, erasing the template. Moreover, ionization of PMAA at higher pH induced an abrupt transition to an extended conformation, weakening the interactions between PMAA and dextran. Not only the dextran patterns lose their spatial selectivity, but also the overall adsorption amount is much lower. The pH sensitivity was in a quite narrow range, i.e. around pH 5. As the hydrogen bonds are also temperature sensitive, the attach points of PEG molecules on the surfaces disappeared at higher temperature. For poly(acrylic acid) (PAA) photografted surfaces, the pH sensitivity was more complicated due to the formation of the compact complexes of PEG and PAA molecules.

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1. Introduction
In recent years, patterned organic thin films have attracted great attentions in many fields due to their potential wide applications. The uses as integrated optics, microelectronic devices, sensors and optical memory devices all require precise control on the surface architecture and properties. Conventional lithographic techniques have limitations when applied to curved surfaces and involve multiple processing steps in harsh conditions. Micro-contact printing, introduced by Whitesides and co-workers [1], provides a versatile pathway of fabricating chemically patterned surfaces. In this procedure, a stamp is coated with desired molecules. When the stamp is printed, the molecules residing on the raised regions of the stamp are thus transferred onto the substrates.

Due to the effective resistance to nonspecific adsorption of many kinds of molecules, colloids and even cells [2], poly(ethylene glycol) (PEG) monolayers would act as widely applicable templates for fabrication of patterned surfaces. Typically, PEG has been incorporated onto surfaces by grafting, simple surface treatments based on primary or secondary adsorption and bulk incorporation via crosslinking or block copolymerization [3]. More recently, Kidambi et al. have created patterned PEG monolayers on polyelectrolyte multilayers by sonic interactions to act as resistive templates [4].

While many researches have focused on the improvements of stability [5], motivated by the potential applications of environmentally sensitive patterns, we introduce here hydrogen bonds between PEG molecules and carboxylic acid-enriched surfaces to fabricate environmentally sensitive templates on micron scale. The PEG molecules can be detached from the surfaces when the proton donors on the surfaces disappear at higher pH value. An advantage of such erasable templates is that, after selectively deposition of a species onto the PEG-free regions and erasion of the PEG templates, the PEG pre-occupied regions can be ready for further modifications. It is quite convenient to fabricate heterogeneous surfaces by this strategy. On the other hand, we choose to introduce carboxylic acid groups on the surfaces, because not only they are proton donors but also they are functional groups for convenient
conjugation to a wide variety of molecules, especially biomacromolecules. In addition, hydrogen bonds, which are mediated by many parameters, will provide us with better control on the patterns.

We choose to demonstrate this idea on flexible polymer substrates, as opposed to gold or silicon substrates. Poly-l-lactide (PLLA), a widely used biocompatible polymer [6], and fluorescein isothiocyanate-dextran (FITC-dextran) are adopted as substrates and model adsorbates, respectively, to testify the feasibility of this strategy in the field of biomaterials.

2. Materials and methods

2.1. Materials

The PLLA (Mn = 200,000; Mw = 400,000) was synthesized using the method described by Schindler and Harper [7]. PLLA films were prepared by casting 6 wt% PLLA/1,4-dioxane solution onto stainless steel plates. The acrylic acid monomers were purified by distillation under reduced pressure. PEG (Mw = 2000) and fluorescein isothiocyanate-labeled dextran (Mw = 19,400; FITC content 0.004 mol/mol glucose) were obtained from Sigma–Aldrich. PDMS prepolymers were obtained from Dow Corning, Sylgard 184.

2.2. Photo-grafting

Fig. 1 (a) shows the procedure of creating carboxylic group-enriched PLLA surfaces by photo-grafting. The PLLA films were first immersed into 40 ml hydrogen peroxide solution (30%) under agitation at 30 °C for 120 min under UV irradiation generated from a high-pressure mercury lamp (250 W). Then the photooxidized films were rinsed with deionized water to remove the excess hydrogen peroxide. The films were subsequently immersed into 10 vol% monomer solution in a Pyrex quartz tube purged with nitrogen. Graft polymerization was carried out under UV irradiation at a distance of 12.5 cm for 60 min at 30 °C. The grafted films were sufficiently rinsed with deionized water at 70 °C to remove the homopolymers [8].

2.3. Fabrication of PEG templates and selective adsorption of FITC-dextran

A temporarily hydrophilic poly(dimethyl siloxane) (PDMS) stamp, molded from lithographically prepared masters [1], was achieved through treatment in a room-temperature plasma cleaner/sterilizer for 10 min. Shown in Fig. 1(b), PEG patterns were created as follows. Firstly, the stamp was inked with PEG aqueous solution (10 mg/ml) for 20 min, and then dried with nitrogen stream. Subsequently, the inked stamp was printed on the carboxylic group-enriched PLLA surface for 20 min. After peeling off the stamp, the PEG patterns on the substrate were obtained. FITC-dextran adsorption was accomplished by applying droplets of dextran solution (2 mg/ml) of pH value ranging from 2 to 8 directly on the patterned surfaces for 30 min, followed by rinsing with solution having corresponding pH value.

Fig. 1. Schematic illustration showing the processes of (a) photografting of carboxylic group containing hydrophilic polymers on PLLA films and (b) fabrication of the PEG template via micro-contact printing. For details, please refer the text.
2.4. Characterizations

The confocal laser scanning microscopy (CLSM) images were obtained from a Bio-Rad Radiance 2100 confocal laser scanning microscope. The ADR-IR spectra were obtained on a Bruker Vector 22 machine. To fully dehydrate the films, all the samples were dried under reduced pressure at room temperature for at least 48 h before attenuated diffuse reflectance-infrared (ADR-IR) characterizations. Fluorescence spectra were recorded on a fluorescence spectrophotometer (HITACHI F-4500). The concentration of the FITC-dextran solutions was 10 μg/ml. Zeta potential measurements were carried out on a MALVERN ZetaSizer 3000HS.

3. Results and discussions

The carboxylic acid-enriched surfaces were achieved by UV-induced grafting of vinyl monomers onto polyester surfaces. This is a multi-step procedure. Firstly, photo-oxidation resulted in hydroperoxide groups on the surfaces of the polymer films, which under UV irradiation were decomposed into macromolecular radicals P–O• (where P represents polymer chain) and free hydroxyl radicals OH•. In a subsequent step, the macromolecular radicals would initiate grafting copolymerization [9]. In this study, PLLA films photografted with poly(methacrylic acid) (PMAA) or poly(acrylic acid) (PAA) were adopted as substrates for following patterning. As reported previously, the photographing of the acrylic polymers has been confirmed by X-ray photoelectron spectroscopy and attenuated total reflectance-infrared spectroscopy [9b].

Following the process of micro-contact printing, the PEG molecules were printed on the PLLA-COOH substrate for 20 min, by using a plasma-oxidized PDMS stamp inked with 10 mg/ml PEG pH 4 aqueous solution. The PEG chains are supposed to be attached to the surface via hydrogen bonding, which will be discussed more afterwards. FITC-dextran was used as model molecules to study the environmental sensitivity of the PEG patterned templates.

PLLA-g-PMAA films patterned with PEG were immersed in FITC-dextran solutions with pH values ranging from 2 to 8 for 30 min at 30°C, respectively. CLSM was used to examine the formation of dextran micro-patterns on the PEG patterned films. Fig. 2(a) shows that the patterns’ dimension and quality changed dramatically around pH 5. At pH 4, due to the repulsive effect of the pre-printed PEG chains, clear FITC-dextran patterns could be observed. With increasing pH value, the dextran patterns extended in diameter and the contrast had dropped (Fig. 2(b)). Up to pH 6, no clear patterns could be observed (Fig. 2(c)). The corresponding fluorescence intensity profiles, shown in Fig. 2(d), also demonstrate that the fluorescence contrast between the regions stamped with PEG and PEG-free regions decreases as pH value increases.

Fig. 2. CLSM images to show FITC-dextran patterns adsorbed on PEG templates from solutions of (a) pH 4; (b) pH 5; and (c) pH 6. (d) Line profiles (1); (2); and (3) are used to quantify the fluorescence intensity on the line in (a); (b); and (c), respectively. PDMS stamp, with holes each measuring 30 μm in diameter, 30 μm in space, was utilized to fabricate continuous PEG patterns. The black regions were pre-occupied by the PEG molecules, and the bright regions were taken up by FITC-dextran.

(a) (b) (c)
The fluorescence contrast ratio can be quantitatively measured by the intensity spectra (Fig. 3(a)) of the CLSM images, by dividing the intensity of the PEG-free regions into the intensity of the PEG stamped regions. When the two peaks overlap, the contrast ratio is defined as 1. The high average contrast ratio at lower pH (Fig. 3(b), solid squares) demonstrated the successful localization of the dextran molecules on the PEG unshaded regions, mediated by repelling effects of hydrophilic chains and entropy effects [2]. It is well known that dextran has only a poor affinity to the solid surfaces [10]. Therefore, the high-adsorbed amount at low pH should be attributed to the hydrogen bonding between the carboxylic groups of PMAA and the hydroxyl groups of dextran chains. On the contrary, there are only very weak interactions between PEG and dextran even in concentrated solution [11]. The different properties of these two kinds of regions result in the high contrast of dextran adsorption. With increase of pH value, the contrast ratio decreases dramatically. The transition pH is pH 5.

As reported earlier, PMAA and PEG are presumed to form H-bonded complexes between the COOH groups of PMAA and the ether oxygens of PEG at acidic pHs (e.g., <5.0) [12]. This kind of interaction (H-bonds) could be supposed to anchor the PEG chains by the specific regions (patterns) of the surfaces. Dissociation of the complex would result in the contamination of the patterns. Moreover, the hydrophobic interaction between the methyl groups of PMAA and the ethylene units of PEG can make PMAA····PEG H-bonded complexes strongly stable [13]. This supposition was further confirmed by ADR-IR. PLLA-g-PMAA surfaces patterned with PEG were rinsed at pH values of 4.0 and 6.0, which are below and above the pK_a of PMAA, respectively. The ADR-IR spectra showing alteration of chemical composition on the surfaces are presented in Fig. 4. According to the molecular structure of PLLA, there is no absorption above 3000 cm⁻¹, which is attributed to the stretching vibration of O-H or N-H. Therefore, the emergence of the broad absorption between 3000 and 3700 cm⁻¹, which can be assigned to carboxyl groups in PMAA, proves that the grafting copolymerization had occurred. After rinsing the PEG-patterned PLLA-g-PMAA at pH 4.0, the sharp peak at 1179.2 cm⁻¹, which is assigned to the ether bonds in PEG, was recorded (Fig. 4, upper line). By contrast, this peak disappeared after rinsing at a pH value above the pK_a of PMAA, which can be regarded as an evidence of the dissociation of the PEG chains from the surfaces. Furthermore, peak at 1754.9 cm⁻¹ has shifted to 1708.6 cm⁻¹ after rinsing at pH 6.0. This red shift confirms the deprotonation of the carbonyl groups which certainly destroys the H-bonds (Fig. 4, lower line).

The mechanism was further proved by carrying out the experiments at an elevated temperature, for example, at 40 °C. Investigating the chain dynamics of PMAA····PEG H-bonded complexes by solid state 13C NMR, Miyoshi et al. [12] have observed that at 37 °C and above, PMAA molecules from the PMAA····PEG complexes dissociate and form H-bonded complexes with themselves. This would mean that at the present case the PEG patterns cannot stably exist on the PLLA-g-PMAA surface if they are rinsed at an elevated temperature regardless of the pH value. Thus, the repelling effect created by PEG molecules will be largely weakened, leading to poor localization of the fluorescent probes. Fig. 3(b) (open circles) indeed shows that the contrast ratio of the dextran patterns under acidic condition only reached approximately 1.5. This temperature-sensitive feature of the PEG templates on the PLLA-g-PMAA surface would provide wide foreground for potential applications, especially that the transition temperature is around 37 °C.
Another interesting phenomenon is that the overall fluorescence intensity of the PEG-free regions decreases with the increase of pH value. This observation is more important considering the emission intensity of the free FITC-dextran is elevated at higher pH value (Fig. 5(a), open circles), due to the different forms of FITC groups [14]. This should not be the contribution of electrostatic force between the anion species of FITC and the deprotonated carboxylic groups of PMAA, as the zeta potential of FITC-dextran had very minimal variation along with the increase of pH (Fig. 5, solid squares). There is strong evidence showing that PMAA chains present a compact coil at low pH, with brushlike acid layers outwards [15]. It is possible for dextran to be attached to the PMAA regions via hydrogen bonding, because that COOH$\cdots$HO bonds (including OH$\rightarrow$O$\cdots$COH and HO$\leftarrow$HOOC bonds) are much stronger than intra-COOH bonds between the PMAA chains [16]. At higher pH, the HO$\rightarrow$HOOC hydrogen bonds disappear due to the deprotonation of the carboxylic groups. More importantly, ionization of PMAA induces an abrupt transition to an extended conformation [17]. The interactions among the hydrated PMAA and dextran chains become much weaker due to the existence of water layer [18]. As a result, not only the dextran patterns lose their spatial selectivity, but also the overall adsorption amount is much lower at high pH.

As a comparison, PLLA-g-PAA surfaces patterned with PEG were also studied. As described above, the contrast ratio of the dextran patterns was also utilized to characterize the state of the PEG templates (Fig. 6). As in the case of PMAA, the contrast ratio was similarly decreased along with the increase of pH value. It is well understood that at a higher pH value (>5.0) the H-bonded complexes cannot be formed because of the ionization of the carboxylic groups. Around pH 4.5, the H-bonds form as expected. However, contrast to PMAA, a further decrease of the pH value leads to the formation of more compact hydrophobic complexes between PAA molecules, which make the PEG chains lose the feature of repelling. [19] Below pH 3.5, the complexes start to aggregate and form hydrophobic domains [20]. The original hydrophilic polymer chains retract from the solution phase forming a compact layer on the surfaces (Fig. 6, insets). The PEG molecules are entrapped in the complexes losing the repellent properties. No temperature dependence under 50°C was observed. This is consistent with the results of other groups, where formation of PAA$\cdots$PAA complexes only occurs at rather high temperatures [21].

4. Conclusions

In summary, we have shown here the creation of PEG patterns on PLLA films enriched with carboxylic acid groups through H-bond interaction. These environmentally sensitive templates exhibit selective or non-selective adsorption of macromolecules depending on pH or temperature. Different sensitivity of the PEG patterns at low pH or at elevated temperature on PMAA and PAA enriched surfaces has been demonstrated. The selectivity is attributed to the repellent effect of the PEG chains as well as the hydrogen bonding between the carboxylic-enriched polymers and dextran. For poly(acrylic acid) photografted surfaces, the pH sensitivity is more complicated due to the formation of the compact complexes of PEG and PAA molecules. Mediated by H-bonds, the templates are expected to be erased by environmental variation, which in turn may find wide applications in design of electronic and photonic devices as well as biosensors.

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References

[1] (a) A. Kumar, G. Whitesides, Science 263 (1994) 60; 


