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Layer-by-layer self-assembly of polyelectrolyte multilayers on cross-section surfaces of multilayer polymer films: A step toward nano-patterning flexible substrates

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Abstract

Polyelectrolyte multilayers (PEMs) of poly(acrylic acid) (PAA)/poly(allylamine hydrochloride) (PAH) were successfully deposited onto novel nano-striped surfaces, which were generated using the cross-section surfaces of multilayer polymer films made from poly(ethylene-*co*-acrylic acid) (EAA) and linear low-density polyethylene (LLDPE). The procedure of PEM patterning consisted of three steps: nano-striped pattern formation, PEM deposition, and chemical crosslinking. It was observed that the PEMs were selectively deposited on the EAA layers and linked through covalent bonds, but those on LLDPE layers were washed off during sonication in water and methanol. In addition, the reactive moieties introduced by PEMs were successfully used to bond covalently with other important molecules, such as amine-terminated fluorescein. This kind of nano-striped surface with alternating wettability could serve as a template for many applications, including biomedical, separations, and electronics.

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1. Introduction

In recent years, a variety of methods have been developed to generate biomolecular patterns on solid substrates, including microcontact printing (μCP) [1,2], dip-pen nanolithography [3], and block copolymer lithography [4]. But multiple steps are normally required to prepare those functionalized patterned surfaces. In our previous study [5], a new approach was introduced to create stripe-patterned surfaces at the submicron scale using multilayer polymer films that contained alternating layers of two different polymers. This method used cross-section surfaces of multilayer polymer films as patterned surfaces, which had greater flexibility than rigid, inorganic substrates that were typically used with other patterning methods [1,3,6]. In addition, this method could generate stripe-patterned surfaces with high repeatability and throughput.

As an example, we used poly(ethylene-*co*-acrylic acid) (EAA) copolymer as a reactive material, which contained 9.5% acrylic acid by weight, and linear low-density polyethylene (LLDPE) as a chemically inert material. By grafting amine-terminated biotin and selectively adsorbing streptavidin on the modified EAA, we successfully formed a striped protein pattern on the film surfaces. However, the reactive groups on the EAA surface are sparse [7] and there are many situations where a higher surface concentration of functional groups is desirable. An EAA with greater AA content can be used, but the rheology of such EAAs renders them more difficult to coextrude.

Researchers have used many methods to conduct surface modification of EAA to introduce desired chemical functionality [7–13]. The modification procedures have typically

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included activation of the carboxylic acids and chemical coupling with other species. In some cases, even after it was successfully modified with small molecules, the EAA surface tended to reconstruct to maintain its hydrophobic nature [7,13]. Others have used polymers like poly(glycidyl methacrylate) (PGMA) or polypropyleneamine dendrimer as anchoring layers to introduce more functionality on EAA surfaces [7]. However, the acrylic acid content formed tiny domains in the EAA copolymer because of hydrogen bonding between the carboxylic acids, leading to incomplete coverage even when anchoring layers were used. Since the carboxylic acids on an EAA surface can be easily deprotonated to yield negative charges ($-COO^-$), we have performed layer-by-layer self-assembly of polyelectrolyte multilayers (PEMs) to modify the EAA surface.

First introduced by Decher [14], electrostatic PEM self-assembly is a well-established method to construct vertically structured multilayer films of polyelectrolytes. This technique is a simple, fast, and economic approach. It involves alternating immersions of a charged substrate in polycation- and polyanion-containing solutions with a rinse between each adsorption step, and has been used for polymer surface modification [15–18]. The great advantage of the PEM approach is that it is possible to incorporate a variety of other materials into the films, such as functional polymers [19], nanoparticles [20,21], proteins, and drugs [22]. Recently, Hammond and Clark [23] and Hammond and coworkers [24] have demonstrated that the PEM films can be directed on patterned structures with surface chemistry. Those patterned PEM films can be potentially used in various applications such as biosensors [25], tissue engineering [26,27], and electronic and photonic devices [28,29].

In this study, poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) were used as the weak polyelectrolytes because carboxyl and amine groups of the PEM films could crosslink to improve their stability [30,31] on the substrates and also provide anchoring sites for functionalization [32]. We have extended our previous work of surface patterning by depositing PAH/PAA films onto the stripe-patterned surfaces to increase the concentration of functional groups on the alternating reactive layers. After chemical crosslinking, the nano-striped surface would have stable hydrophilic regions adjacent to hydrophobic regions. This kind of structured surface with alternating wettability could be used as a template in many applications of selective adsorption [33]. In addition, the hydrophilic PEM films provide moieties that can be used as a functional part of a device, thus offering a means to create inexpensive bio-devices.

2. Experimental

2.1. Materials

Poly(ethylene-*co*-acrylic acid) (EAA, PRIMACOR 1410 from Dow Chemical Co., 9.5% w/w acrylic acid) and linear low-density polyethylene (LLDPE, Dowlex 2517) films were used as received from the Cryovac Division of Sealed Air Corp. (Duncan, SC). Poly(allylamine hydrochloride) (PAH, $Mw = 10{,}000$), sodium chloride (NaCl), hydrochloric acid (HCl), and HPLC water were obtained from VWR. *N*-Hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), poly(acrylic acid) (PAA, $Mw = 5100$) was purchased from Aldrich. Fluorescein cadaverine ($FITC-NH_2$) was obtained from Fisher Scientific and used without purification.

2.2. Surface modification of polymer films

EAA film was first deprotonated in a 1 M aqueous NaOH solution, followed by rinsing with HPLC water and drying with N_2. The resulting film was denoted as EAA–COONa. To deposit PEM films, EAA–COONa films were immersed in a 0.02 M PAH aqueous solution for 15 min at pH 7.5 and then rinsed with HPLC water for 2 min. After drying with N_2, the film was immersed in a 0.02 M PAA aqueous solution for another 15 min at pH 3.5 and then rinsed with HPLC water for 2 min. Consecutive cycles of alternating adsorption of PAH/PAA bilayers was performed for a prescribed number of times. The deposition solutions also contained 0.5 M NaCl and were adjusted to the pH value using 1 M NaOH or 1 M HCl solution. Crosslinking of PAH/PAA films was performed by immersing the PEM-modified EAA film in a solution of NHS/EDC (100 mM/200 mM) for 4 h, and the solution was freshly prepared with a 0.2 M NaCl solution. The film was then rinsed and sonicated with HPLC water three times (5 min for each) and dried with N_2. To check the possibility of grafting other species on the PEM films, $FITC-NH_2$ (1 mg/ml) solution was prepared by dissolving $FITC-NH_2$ in NHS/EDC (100 mM/200 mM) solution; so the crosslinking reaction and attachment of FITC occurred at the same time.

2.3. Preparation of nano-striped surfaces and deposition of PAH/PAA films

The stripe-patterned surfaces were prepared using a multilayer polymer film [5]. A prescribed number of seven-layer films (A/B/A/B/A/B/A where A = EAA and B = LLDPE) were stacked and pressed in a compression molding machine at 130 °C. In this study, a 4032-layer film was prepared and the thickness of the individual layers was about 500 nm. To obtain smooth, nano-striped surfaces, the 4032-layer film was microtomed at −100 °C using a RMC Powertome X with a cryo system equipped with a diamond knife. The smooth surfaces generated by cryo-microtoming were used as the nano-striped template for PEM deposition. After formation of crosslinked PEMs, the modified nano-striped template was sonicated in HPLC water and methanol, respectively, for three times each to remove the PEM films from the LLDPE layers. A Branson Ultrasonic Cleaner (Model 3510) was used with a sonication time of 5 min for each usage.

2.4. Characterization

Attenuated total reflection FTIR (ATR-FTIR) spectroscopy measurements were conducted on the modified polymer films using a Thermo Nicolet Magna 550 single bounce FTIR spectrometer equipped with a Thermo-Spectra-Tech Foundation Series Diamond ATR with deuterated triglycine sulfate (DTGS) detector. Static contact angle measurements were performed on a Krüss G10 instrument. Low voltage scanning electron microscopy (SEM, Hitachi, Model S4700) was utilized to study the nano-striped surfaces that were coated with about 100 Å of platinum before scanning. Atomic force microscopy images were obtained using a Digital Instruments Nanoscope IIIa in tapping mode and the root-mean-square (RMS) roughness was evaluated from the images. Confocal fluorescence images were obtained using a Zeiss LSM510 microscope.

3. Results and discussion

Fig. 1A depicts the procedure of the PEM patterning, including nano-striped pattern formation (as shown in Fig. 1B), PEM deposition, and chemical crosslinking. Using this procedure, the EAA-confined PEM films should form a stable hydrophilic coating, providing multiple functionalities, while PEM films on the LLDPE regions should not bond to the substrate and be easily removed. Since it is difficult to detect the chemical changes on the cross-section surface of these multilayer films, surface characterization of the layer-by-layer (LbL) procedure is first studied on the major surfaces of neat EAA.

The carboxylic acids of EAA film were first deprotonated in a base solution (NaOH 1 M), where the carboxylic acids were converted to carboxylate ions. After rinsing with HPLC water (pH ~ 7), some of the carboxylate ions on the surface may be protonated back to carboxylic acids, but still there should be a substantial fraction of carboxylate ions left on the surface of the EAA film. Based on the literature data [23], the degree of ionization for poly(acrylic acid) (PAA) (pK_a of 4–5.5) is approximately 55–70% at pH 7. Another observation is that the water contact angle of treated EAA film is about 10° lower than that of neat EAA film. After deprotonation of carboxylic acids on EAA film, PAH could be attached on the negatively charged surface (EAA–COONa) through electrostatic interaction since the PAH was highly charged at the selected pH of 7.5. PEMs of PAH/PAA were deposited on the film by alternatively immersing EAA–COONa films in dilute aqueous solutions of PAH at pH 7.5 and PAA at pH 3.5. Rinsing three times with HPLC water followed each deposition to remove weakly bound polyelectrolytes. The formation of PEM films was confirmed by ATR-FTIR and contact angle measurements. From Fig. 2, it was observed that the peak at 1705 cm^{-1} increased with the deposition of alternating PAH/PAA layers due to the carbonyl stretch of uncharged acids in the PAA layers. Another peak appeared at 1650 cm^{-1} when more polyelectrolyte layers were deposited on the EAA film, indicating that a small amount of amide was formed between $-NH_3^+$ and $-COO^-$.

Static water contact angle measurements were performed after each deposition step. As shown in Fig. 3, treatment with base solution to yield $-COO^-$ improved the hydrophilicity of EAA film slightly. After adsorption of PAH at pH 7.5, the water contact angle continued decreasing to about 75°. Subsequent adsorption of PAA at pH 3.5 led to a significant decrease of water contact angle. The value is close to 30°, which is so far the lowest value observed in surface modification studies of EAA film. Surface roughness was also obtained from AFM topography images, and it was found that the roughness increased slowly at the beginning, and then significantly after three depositions of PAH/PAA pairs. When more than 10 layers of PAA/PAH were deposited, a porous structure was observed in AFM phase images (data not

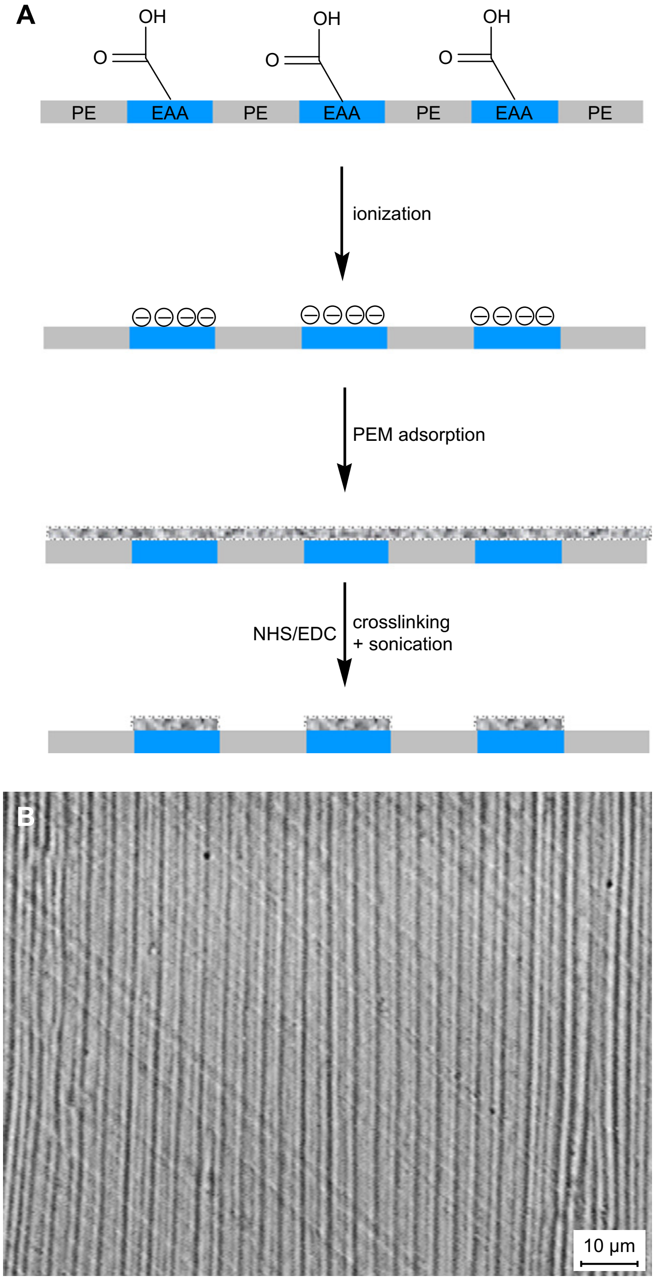

Fig. 1. (A) Illustration of the PAH/PAA multilayer patterning on cross-section surfaces of polymer multilayer films; (B) optical image of the stripe-patterned surface with 4032 layers (all details about the sub-micron structure were described in previous work) [5].

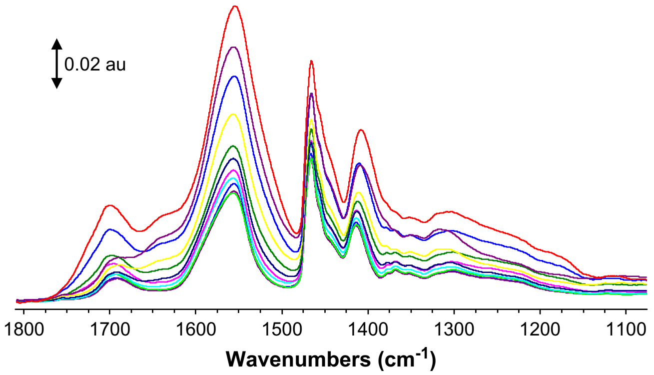

Fig. 2. FTIR spectra of EAA–COONa and PAH/PAA multilayer deposited films up to 10 layers.

shown). A control experiment was done on LLDPE, and it was found that the porous structure existed even for the first two layers of PAA/PAH and a more pronounced porous structure was observed. Therefore, the formation of this porous structure depends significantly on the surface interaction between polyelectrolytes and substrates, especially for weak polyelectrolytes like PAA and PAH. However, polyelectrolyte films on EAA were quite uniform for the first six layers of PAH/PAA deposition, as reflected by the relatively stable and low RMS roughness values.

There are several methods to crosslink the PEM films, such as thermal crosslinking [30,34] and photo-crosslinking [35,36]. Thermal crosslinking generally requires temperatures above 100 °C, which is above the melting temperature (90 °C) of EAA, so it is not suitable for this work. Photo-crosslinking needs a photoinitiator, which limits its application in many polyelectrolyte pairs. An EDC/NHS method was then selected for this phase of study. After deposition on EAA film, the PEMs were crosslinked in aqueous EDC/NHS solution by a carbodiimide coupling reaction between the PAA and PAH chains. The reaction mechanism was discussed in our previous study [7]. Along with the carbodiimide reaction occurring in the PEM layers, the carboxyl groups of EAA also reacted with the amine groups of the first PAH layer. The covalent bonding made the PEMs on EAA film robust and extremely difficult to wash off. The PEM layers were stable even when treated with methanol, 1 M HCl, and 1 M NaOH solutions for three days. The peaks associated with the amide bonds in FITR spectra (data not shown) remained intact after the various treatments. The peak at about 1705 cm^{-1} indicated that there was still a significant number of carboxylic acids existing in the PEM films. A control experiment was conducted for uncrosslinked PEMs and showed that the PEMs disassociated in only a few minutes of immersion in all three solutions: methanol, 1 M HCl, and 1 M NaOH.

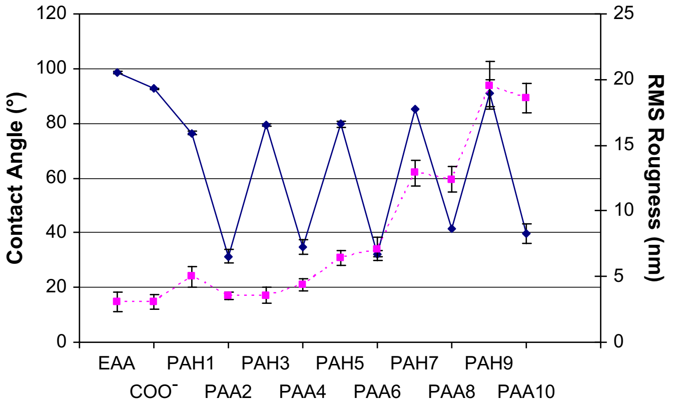

Fig. 3. Water contact angle of PEM deposited EAA film as a function of the number of layers and the relationship with surface RMS roughness.

One main goal of this work was to fabricate PEM patterns on a stripe-patterned surface revealed after microtoming a multilayer polymer film. The nanostructure of cross-sectioned surfaces of a 4032-layer EAA/LLDPE film has been described elsewhere [5]. To generate a hydrophilic coating on the EAA regions, the PEM deposition procedure was conducted on this cross-sectioned, nano-striped surface. Three steps were involved: (i) LbL self-assembly of PAA/PAH; (ii) crosslinking in an aqueous EDC/NHS solution and (iii) sonicating three times in both HPLC water and methanol.

We conducted the crosslinking reaction with amine-terminated FITC, so that the patterned structure could be detected by confocal fluorescence microscopy. Fig. 4 shows the results from several different experiments conducted to see if it is possible to form the hydrophilic PEM on EAA stripes only. By removing the PEM from LLDPE stripes through sonication in HPLC and methanol, we expected to obtain sub-micron-striped PEM patterns. As shown in Fig. 4A, the entire surface showed strong fluorescence without sonication washing, indicating that the PEM covered both the EAA and LLDPE stripes. However, after immersion in HPLC water and methanol with sonication, the striped structure was clearly observed by confocal fluorescence microscopy (Fig. 4B), where the green layers (for interpretation of the references to colour in this figure, the reader is referred to the web version of this article) represent FITC grafted to the PEM on EAA layers and the dark layers represent inert LLDPE layers. Control experiments of FITC grafting without prior PEM deposition were performed on the original microtomed surface, but no fluorescence and contrast was observed (data not shown). Therefore, the PEM layers were deposited and stabilized only on the EAA stripes, and provided many more functional groups for further linkage with amine-terminated FITC. In addition, the 500 nm PEM stripes deposited on EAA were clearly observed by SEM, as shown in Fig. 4C. The light stripes represent EAA regions covered with PEM. This striped structure was not detectable via SEM before PEM deposition because of the compositional similarity between LLDPE and EAA copolymer.

It is interesting to note that the PEMs were removed from the LLDPE stripes. Although the carbodiimide coupling reaction occurred between PAA and PAH layers, we speculate that the crosslink density was relatively low such that the PEMs, which do not have any stable linkage with the LLDPE surface except secondary hydrophobic–hydrophobic interactions, could be removed during the washing steps with sonication. A similar phenomenon was observed by Mallwitz and Laschewsky [30]

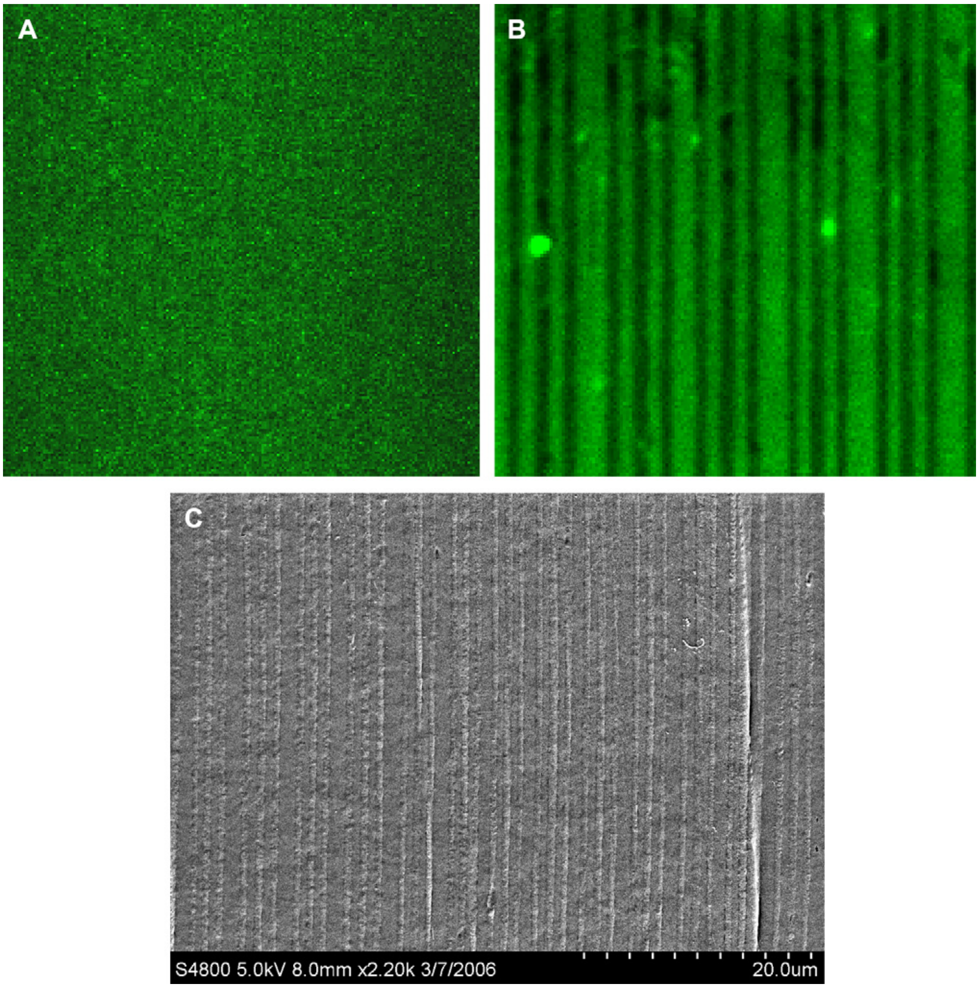

Fig. 4. Fluorescence images (20 × 20 microns) of crosslinked PEM films (three sets of PAA/PAH bilayers) on nano-striped surfaces: (A) before washing and (B) after washing off the crosslinked complex from LLDPE stripes; (C) SEM image of the surface after washing, noting that the nano-stripes were not seen by SEM before washing.

when they prepared freestanding PEMs (PAH/PAA) in the meshes of supports with large pores. They successfully formed freestanding PEMs if there were no rinsing steps between each deposition of polyelectrolyte. The freestanding film was then thermally crosslinked to increase its stability. However, if there were rinsing steps, as we conducted in this study, no freestanding film formed, indicating that the PEMs were not highly crosslinked films spanning the pore openings. In our study, although the PEMs on LLDPE stripes still can be observed by confocal fluorescence microscopy before the washing steps with sonication, there is still no freestanding film formed on the top of LLDPE stripes since the PEMs are easily removed by multiple-step washing with sonication in HPLC water and methanol. The removed PEMs from LLDPE stripes could be polyelectrolytes with partially layered structures with low cross-link density. Meanwhile, the covalent bond between the initial PAH layer and EAA film was strong enough to hold the PEM film, thus forming stable hydrophilic stripes. It is also evident from Fig. 4B that the striped pattern is not defect-free. The edges of the stripes were rougher in some spots than others, while the bright spots are likely agglomerates of FITC that were attached to the stripes during that reaction step. Nevertheless, the stripes were clearly distinguishable, even at this sub-micron length scale.

Finally, with flexible multilayer polymer films (Fig. 5A and B), which could contain hundreds and thousands of nano-layers, it was possible to bend the material into a variety of shapes. To demonstrate the surface-modified template's flexibility, we formed our multilayer EAA–LLDPE film into a spiral and embedded it in wax (Fig. 5C). The fluorescence image of this spiral was clearly observed using fluorescence microscopy (Fig. 5D), where a thick fluorescent region is made up of 4032 layers (every thin layer is 500 nm thick). Therefore, using this approach, flexible patterned surfaces can be formed, with crosslinked PEMs covalently bonded to reactive layers to serve as functional moieties to adsorb or bond with other molecules. Achieving thinner layers is challenging but potentially feasible. Other processes, such as smart blending [37] or layer multiplication [38], can produce layered geometries where the layer thicknesses can be significantly less than 100 nm, and those methods can be explored in the future.

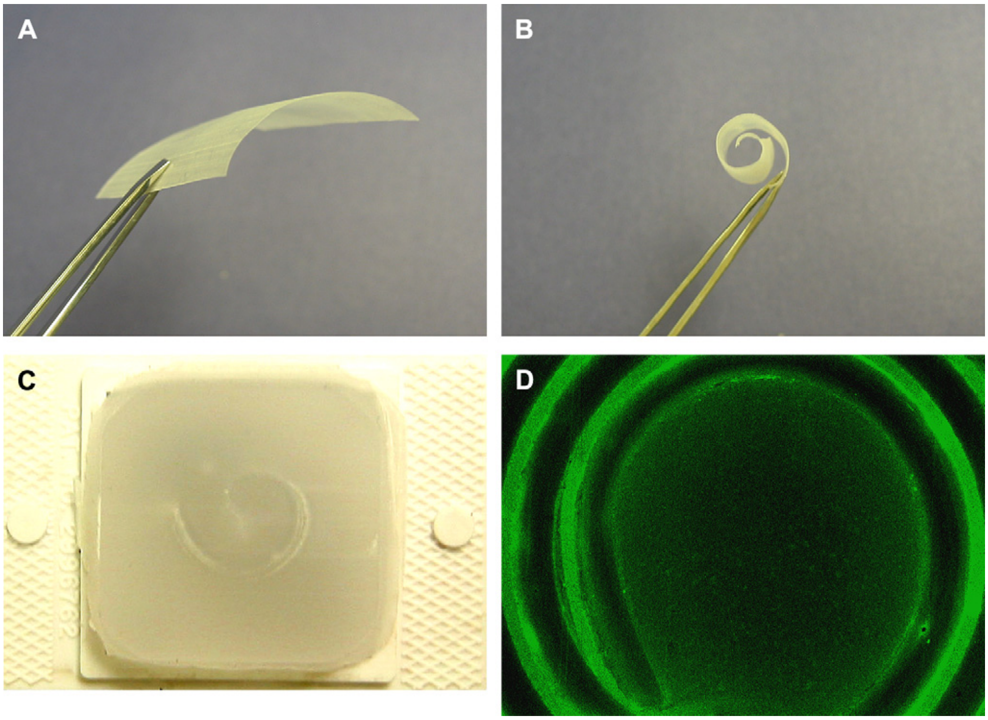

Fig. 5. (A, B) Photograph of the flexible multilayer polymer film (EAA/LLDPE). The multilayer polymer film was cut as a 2 × 2 cm^2 and the film thickness was about 2 mm as measured; (C) multilayer polymer film after embedding in wax, forming a spiral sharp; (D) fluorescence image after hydrophilic PEM coating and FITC grafting, with a single ring having 4032 nano-layers.

4. Conclusions

In summary, we successfully demonstrated that the surface negative charges on EAA could be used to deposit PEMs through the electrostatic interaction between the PEM and the EAA surface. When the PEMs were crosslinked by a carbodiimide coupling reaction in aqueous EDC/NHS solution, the stability was improved significantly. We also extended the PEM assembly technique to nano-striped surfaces, which were generated using the cross-section surfaces of multilayer polymer films made from EAA and LLDPE. The PEMs were deposited on both the EAA and LLDPE regions, but were washed off from the LLDPE with sonication due to the lack of covalent bond between the PEMs and LLDPE. Moreover, the carboxylic acids introduced by the PEM reacted with amine-terminated FITC to yield a nano-striped surface, which was clearly detected by fluorescence microscopy.

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