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Polyelectrolyte multilayer films containing silver as antibacterial coatings

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ABSTRACT

A facile approach to fabrication of transparent antimicrobial coatings based on polyelectrolyte multilayers (PEMs) is presented. Counterions existing in PEMs were utilized via ion exchange and in situ reduction to incorporate into the films silver ions and nanoparticles, and the antibacterial efficacy of the films against *E. coli* was assessed by the Kirby–Bauer method. The PEMs containing silver in the ionic form exhibited high activities in short terms, and the antibacterial effects depended on the ionic strength in the polyelectrolyte solutions used for the PEM fabrication. The PEMs loaded with silver nanoparticles showed lower initial bactericidal effects, but remained active after long periods of time, and the antimicrobial performance can be improved by increasing the silver loading through repeating the ion-exchange/reduction cycle for multiple times. The films were transparent in the visible region. Coatings containing multiple antimicrobial agents for possible synergistic effects can be fabricated in a single process using this method.

1. Introduction

Antibacterial coating is significant not only to environment pollution prevention but also to human health. Metal salts and compounds are often used as antimicrobial agents in medicine and biocides in hospital and other human settings. In fact, antibacterial effects of silver salts have been known since ancient times [1]. Today, silver is used to control bacterial growth in various applications, such as dental work [2], catheters [3], and burn wounds [4–6]. Although the mechanism of silver's effects on the killing of bacteria is not completely clear, its multilevel antimicrobial mode has been well known [7,8], which ensures that silver has a very broad antibacterial spectrum even at low concentrations [1]. However, silver does not have similar cytotoxic effects on eukaryotic cells which show higher structural and functional redundancy compared to prokaryotic cells [9]. Compared to other metal ions, silver has the highest toxicity to microoganisms and is least toxic to animal cells [1,10–13]. Silver's wide antibacterial spectrum and safety to humans guarantee its wide application and have inspired great research interest for centuries.

Many methods have been developed to incorporate silver ions or particles into films for antibacterial coating purpose, depending on the preparation process of the matrix films. For example, silver nanoparticles have been incorporated into surface sol–gel films by ion-exchange/reduction process [14,15]. The binding ability of secondary amines to silver ions has been utilized for the introduction of silver nanoparticles into poly(amidoamine) dendrimers [16] and poly(ethylene imine) (PEI) based hydrogel crosslinked network films [17]. Fibers loaded with silver nanoparticles are also fabricated by electro spinning a polymer solution doped with silver ions and then in situ reduction of the silver ions in the fibers [18,19]. However, these methods depend on complicated processes and/or specific matrix polymers.

In recent years layer-by-layer (LbL) assembly has become a popular technique for thin film fabrication because of its simplicity, robustness, and versatility [20,21]. It is particularly suitable for creating conformal and robust coatings with precise thickness control on substrates of various shapes and dimensions. In addition, as opposed to other polymer matrix films, polyelectrolyte multilayers (PEMs) in general exhibit higher water permeation and diffusion characteristics, which are important for the antimicrobial performance of the silver/polymer composites [7]. The technique has been used to prepare silver-containing thin films, and two approaches to incorporation of silver into PEMs for antibacterial purpose have been reported [22–31]. In one method, a complex of a polyelectrolyte with silver ions [22–26] or prefabricated silver nanoparticles [27,28] is prepared, which is then assembled with another polyelectrolyte with an opposite charge. In the other approach, first a PEM is assembled from weak polyelectrolytes, such as poly(acrylic acid) and poly(allylamine hydrochloride), and then the dissociation equilibrium of the weak electrolytes is capitalized to create ion-binding sites in the PEM by pH manipulation, which are used to bind silver ions via ion exchange, and the silver ions can then be reduced in situ to produce silver nanoparticles [29–31]. Because PEMs are easy to fabricate and can be coated on a wide variety of polymer substrates [32], and the silver-loaded PEMs exhibit excellent performance against microorganisms due to the high delivery rate of silver ions [22,26], they have become very promising candidates for antimicrobial coatings. In

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addition, silver nanoparticles exhibit sustained antimicrobial activity, which is dependent on the silver content in the multilayer, which in turn can be increased via multiple loading and reduction cycles [12].

More recently, we demonstrated that by utilizing the excess charged groups naturally present in all PEMs, the ion-exchange/reduction approach can be extended to incorporate nanoparticles into PEMs fabricated from strong polyelectrolytes, or in principle any polyelectrolyte, without the need of pH manipulation [33,34]. In the present study, antibacterial efficacy of these composite thin films has been evaluated by the zone of inhibition (ZOI) data using standard Kirby–Bauer test, and we show that using our newly developed nanoparticle loading protocol, transparent coatings can be facilely prepared and the same sustained antibacterial performance as demonstrated previously by Rubner et al. [12] can be conveniently realized.

2. Materials and methods

2.1. Materials

Sodium chloride (NaCl), silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were of analytical grade and purchased from Beijing Chemical Reagents Company. Poly(styrene sulfonate) (PSS, MW ~ 70 000) and poly(diallyldimethylammonium chloride) (PDDA, 20 wt.% in water, MW ~ 200 000–350 000) were purchased from Aldrich and used as received. Polished silicon wafers were purchased from Shanghai Wafer Works Corporation. Water used for rinsing and preparing all the solutions was purified with a Millipore Simplicity 185 purification unit (18.2 MΩ cm).

2.2. Preparation of PDDA/PSS films

Quartz and silicon wafers were immersed in a boiling piranha solution (3:1 mixture of 98% H_2SO_4 and 30% H_2O_2) for 20 min and then rinsed with copious amounts of water. PDDA/PSS multilayers were assembled on cleaned wafers following a procedure reported previously [35]. In brief, multilayer films were assembled by sequential dipping of a substrate in PDDA (1.0 mg/mL) and PSS (1.0 mg/mL) aqueous solutions for 30 min each with water rinsing in between each deposition step until the desired number of layers was obtained. The number of bilayers was 7 for specimens for antibacterial and UV-vis tests, and 10 for transmission electron microscopy (TEM) specimens. NaCl was added to the polyelectrolyte solutions in order to control the silver content in the PEMs [33,34], and the PEMs assembled at 0.75, 2.0, and 3.0 M NaCl concentrations were denoted PEM-1, PEM-2, and PEM-3, respectively.

2.3. Incorporation of silver ion and silver nanoparticle into PEMs

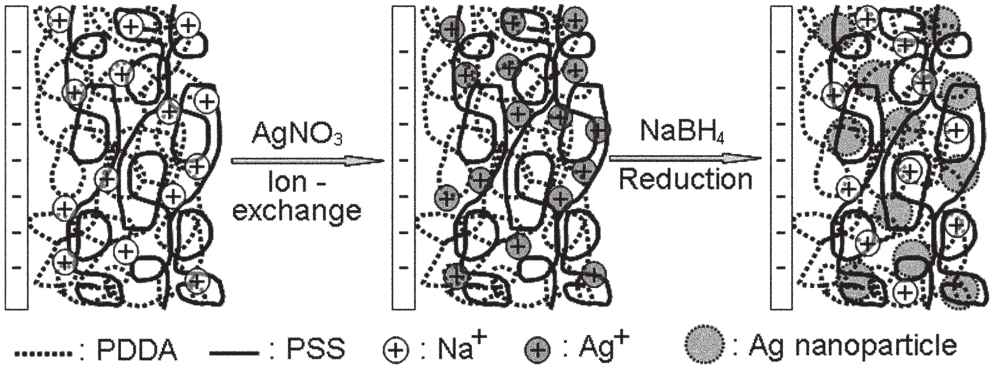
Silver ions and silver nanoparticles were introduced into the PEMs following a procedure described in our previous report [33,34] and is illustrated in Scheme 1. In brief, a PDDA/PSS multilayer film was immersed in an $AgNO_3$ solution (0.010 M) for 5 min to introduce silver ions into the PEM, which were converted into silver nanoparticles in situ by treating with a freshly prepared $NaBH_4$ (0.010 M). Copious water was used for rinsing in between each loading and reduction step. These films containing silver ions and nanoparticles are denoted PEM-n/Ag(I) and PEM-n/Ag(0), respectively, where n is 1, 2, and 3, respectively as described above. The ion-exchange/reduction cycle was repeated for 3 times for PEM-3/Ag(0) to increase the silver loading in the film, which is denoted PEM-3/$[Ag(0)]_3$.

2.4. Film characterization

The Kirby–Bauer test was used to evaluate the zone of inhibition (ZOI) around a 1.5×1.5 cm^2 silicon wafer coated with an antimicrobial film. The sample disks were incubated at 37 °C in a gelatinous agar growth medium. This test was performed following a literature procedure [22] using plates swabbed with solutions of *E. coli* containing approximately 3×10^6 cell forming units (CFUs) per milliliter. The ZOI value for *E. coli* for each composition was reported based on the average of at least three specimens. UV-vis spectra of the multilayers deposited on quartz slides were collected on a Shimadzu UV-2450 spectrophotometer. TEM observations were carried out on a JEM-2010 microscope operating at 100.0 kV. A small piece of the PEM loaded with silver nanoparticles was peeled off from the substrate in hydrofluoric acid, and then floated in water and transferred to carbon-coated copper grids for TEM observation. To examine the cross section, the PEM film floating in water was collected on a small piece of hardened epoxy, and the epoxy piece with the film was embedded in liquid epoxy which was then cured in three steps, lasting 12 h each, at progressively higher temperatures of 35, 45, and 55 °C; The ultrathin sections, of ca. 70 nm thickness, were microtomed at room temperature using a LEICA Ultracut R microtome and a glass knife; slices were floated on a water surface and retrieved with carbon-coated copper grids.

3. Results and discussion

As we described in previous reports [33,34], silver ions can be facilely introduced into PEMs by a rapid ion-exchange process, which can then be converted into silver nanoparticles to create composite thin films, and the size and content of the nanoparticles in the film can be tuned by adjusting the ionic strength in the polyelectrolyte solutions used for the assembly. In addition, after the reduction of the Ag^+, the styrene sulfonate groups of the PSS are again paired with Na^+, which are then available for further ion-exchange/reduction to load more silver into the same film, and the process can be cycled [33,34]. This provides a convenient route to the fabrication of silver-containing PEMs with controllable silver loading. Using the Kirby–Bauer technique, the antibacterial activities of the PEM coatings prepared at various NaCl



Scheme 1. Schematic of the procedure for fabricating PEMs containing silver ions or silver nanoparticles.

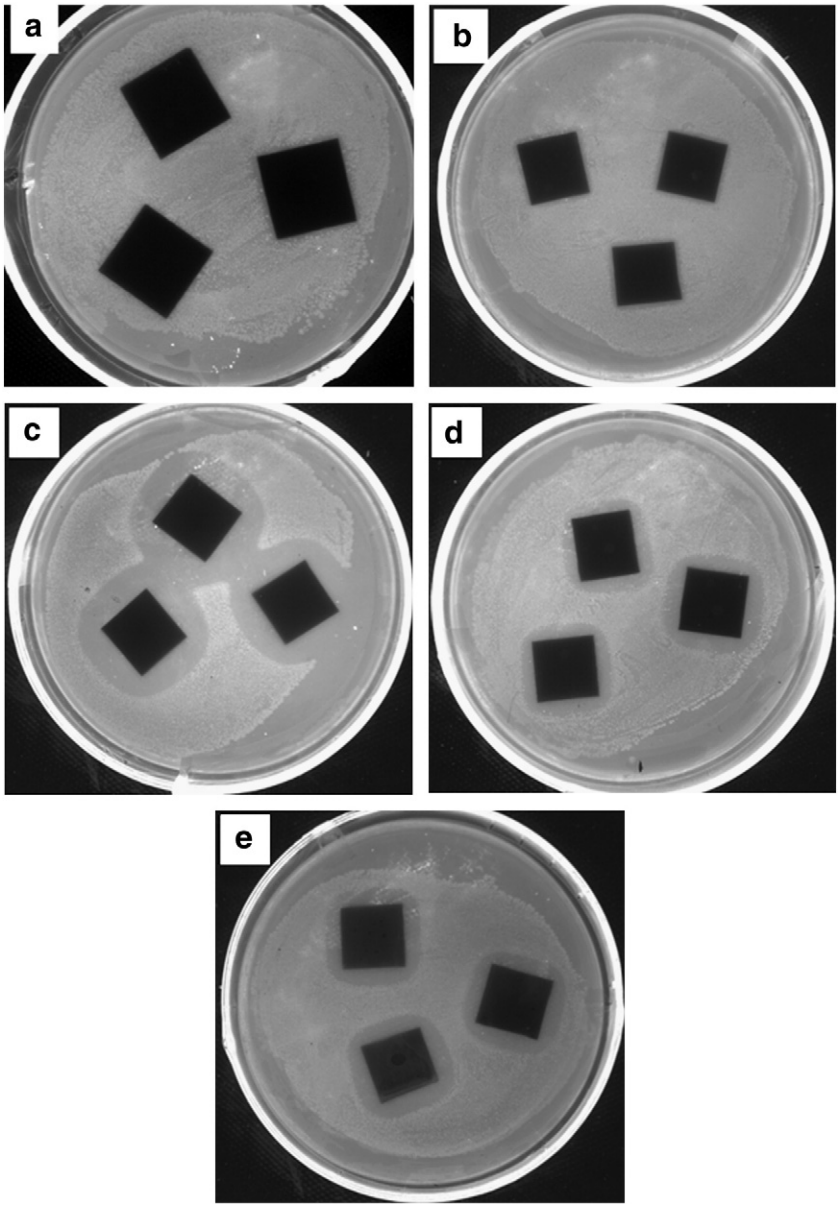


Fig. 1. Photographs of the Kirby–Bauer plates for (a) PEM-3 (control), (b) PEM-1/Ag(I), (c) PEM-3/Ag(I), (d) PEM-3/Ag(0), and (e) PEM-3/$[Ag(0)]_3$. The ZOI numbers are listed in Table 1.

concentrations and containing silver ions or silver nanoparticles were measured. The images of 5 Kirby–Bauer plates are shown in Fig. 1 as examples, and the ZOI results for all samples at different incubation time are summarized in Table 1. After 24 h, the ZOI was observed clearly for all except the PEM control sample, indicating that the bactericidal action is caused by the silver ions or silver nanoparticles embedded in the PEMs, as expected, and the PDDA/PSS multilayer itself has no detectable activity against the *E. coli*. For the PEMs containing silver in the ionic form, PEM/Ag(I), it can be seen that either initially or at extended periods of time, the antibacterial activity increases with the salt concentration in the polyelectrolyte solutions used to assemble the PEM. More specifically, at 24 h incubation time, the ZOI was 1.0, 6.6, and 7.3 mm for the PEM assembled at 0.75, 2.0, and 3.0 M NaCl, respectively. After 3 days, while the ZOI for PEM-1/Ag(I) completely disappeared, the antibacterial activities of PEM-2/Ag(I) and PEM-3/Ag(I) only decreased slightly. After 15 days, the ZOI for the latter two samples have reduced significantly. These results can be correlated to the silver content in the PEM. In the LbL process, when the polyelectrolyte is deposited at higher salt concentrations, much more charged groups are not compensated by the oppositely charged polyelectrolyte in the PEM and are available for binding Ag^+ ions in the ion-exchange step, resulting in higher silver contents in the PEM [33,34]. Therefore the silver content in the PEM increases with the salt concentration in the polyelectrolyte solution, resulting in a higher release rate of silver and a bigger ZOI, and a more sustained antimicrobial activity.

Table 1
The antibacterial activities of the PEMs.

Coating	Zone of inhibition (mm)		
	1 day	3 day	15 day
PEM-3	0	0	0
PEM-1/Ag(I)	1.03 ± 0.03	0	0
PEM-2/Ag(I)	6.63 ± 0.18	6.04 ± 0.34	1.23 ± 0.05
PEM-3/Ag(I)	7.26 ± 0.37	6.54 ± 0.46	1.65 ± 0.08
PEM-3/Ag(0)	3.76 ± 0.33	3.33 ± 0.42	2.82 ± 0.36
PEM-3/$[Ag(0)]_3$	4.14 ± 0.10	4.00 ± 0.23	3.94 ± 0.22

The PEMs containing silver nanoparticles exhibited different antimicrobial behavior. PEM-3/Ag(0), which was prepared from PEM-3/Ag(I) by in situ reduction of the silver ions and in principle should have the same silver content, exhibited a moderate killing effect against *E. coli*. Its ZOI was 3.76 mm at 24 h incubation time compared to 7.26 mm for PEM-3/Ag(I). However, after 15 days, while the ZOI for the PEM containing ionic silver decreased to 1.65 mm, 23% of that observed after one day, the ZOI for PEM-3/Ag(0) was 2.82 mm, which was 75% of the initial value. That is, while the PEMs containing ionic silver exhibit stronger bactericidal effects initially, the performance decreases over time relatively quickly. The PEMs loaded with silver nanoparticles have lower initial antimicrobial properties which decrease over time much slower and last longer. It is known that silver ions are the active species that kills the microbes, while metallic silver is considered to be nonreactive material [3]. The silvers ions directly present in the PEM/Ag(I) films can diffuse out of the film quickly and kill the bacteria around, showing high antibacterial efficacy, but over time the silver ions in the PEM are depleted, and the bactericidal effects diminish and disappear eventually. For the PEM loaded with silver nanoparticles, ionic silver, the effective antimicrobial species, is produced via the oxidation of the zerovalence silver, the concentration of which is therefore relatively low [3]. However, the nanoparticles provide a reservoir of silver that can supply silver ions in stable concentrations, which are constantly being replenished after absorption by the bacteria. Therefore the silver nanoparticles act as a stable supply of silver ions over long periods of time, resulting in sustained antibacterial performance of the PEM.

It is obvious that the antimicrobial efficacy of the PEM containing silver nanoparticles can be further improved by increasing the silver content in the film. This can be realized by repeating the ion-exchange/reduction cycle [33,34]. Fig. 2 shows the TEM cross-sectional images of the PEM-3/Ag(0) and PEM-3/$[Ag(0)]_3$, which were fabricated by loading the PEM-3 with silver in 1 and 3 ion-exchange/reduction cycles, respectively. It can be seen that the average thickness of each bilayer is approximately 48 nm. The large thickness of the layers is attributed to the high concentration of added salt in the polyelectrolyte solution and the exponential growth mode under such condition [36,37]. It is clear that the silver nanoparticles distribute in the whole film evenly, and the PEM-3/$[Ag(0)]_3$ contains not only more silver nanoparticles than the PEM-3/Ag(0), but also bigger ones. As indicated by the ZOI numbers listed in Table 1, the PEM-3/$[Ag(0)]_3$ film exhibited higher activity against *E. coli* than PEM-3/Ag(0) and better long-term performance. After 15 days, its ZOI only reduced by <5%. This indicates that the antimicrobial properties of the silver nanoparticles in the multilayer film can maintain for several days without obvious changes in aqueous solution, which is in line with previous results in the literature [12,13]. Furthermore, if the last in situ reduction step is skipped when loading a PEM with silver via multiple ion-exchange/reduction cycles, i.e. the last batch of silver ions introduced into the PEM via ion exchange are not reduced, the PEM would contain high contents of both silver nanoparticles and silver ions, and thus should have high initial antimicrobial efficacy (due to the ionic silver) as well as long-lasting performance (from the nanoparticles), as suggested by Rubner and coworkers [12]. Alternatively, after the PEM is loaded with silver nanoparticles,

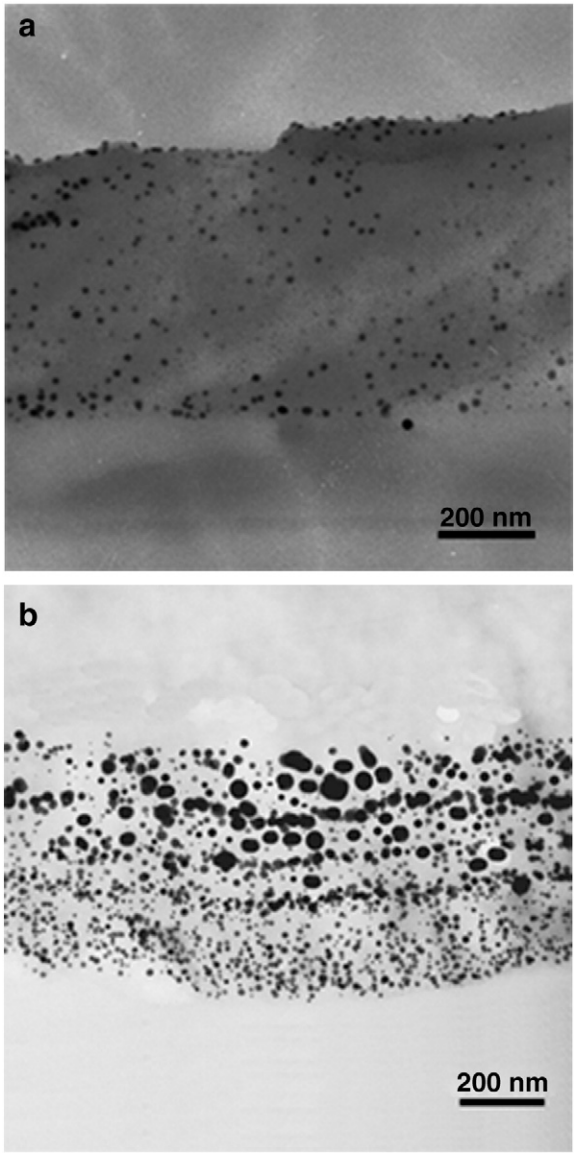


Fig. 2. The cross-sectional images of (a) PEM-3/Ag(0) and (b) PEM-3/$[Ag(0)]_3$.

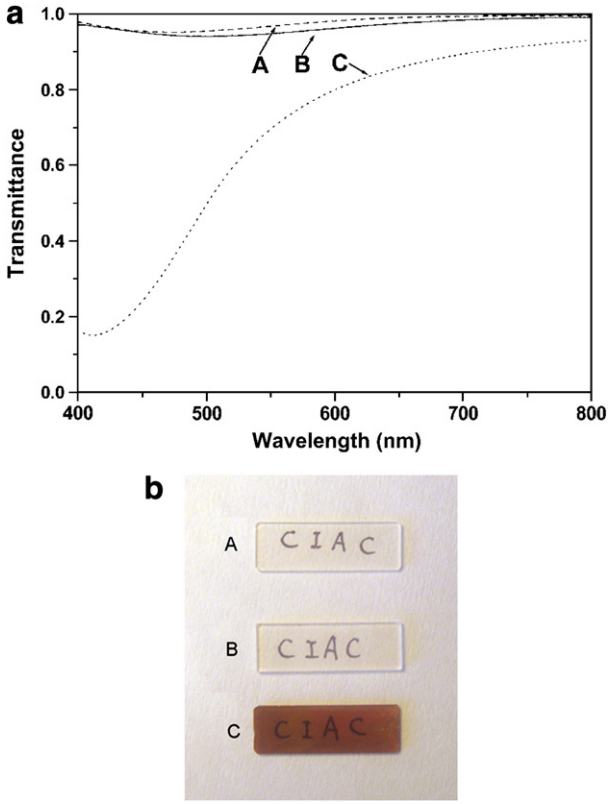


Fig. 3. The UV-vis transmission spectra (a) and the visual transparency (b) of PEMs deposited on quartz. A, B, and C are for PEM-3, PEM-3/Ag(I), and PEM-3/Ag(0), respectively.

other bactericidal agents in ionic forms, such as quaternary ammonium compounds [22,31], can be incorporated into the film via ion exchange as well. This flexibility of combining different kinds of antimicrobial agents in the same coating for possible synergistic effects via the same simple process is an unique advantage afforded by the current method.

An important application of antimicrobial coatings is for wound dressing, where the transparency of the coating is required for observing the wound directly [22]. Visible light (400–800 nm) transmittance for the PEMs we fabricated is shown in Fig. 3a. It can be observed that the PEM-3/Ag(I) displays transmittance values greater than 95% throughout the entire visible spectrum. These large transmission values are due to the small film thickness (480 nm) and the lack of microstructure features that would absorb or scatter light. Although the PEM-3/Ag(0) exhibits much lower transmission at below 550 nm than the PEM-3/Ag(I) and the PEM-3 control due to the surface plasma resonance of silver nanoparticles, it still shows >80% transmittance at higher wavelengths in the visible region due to the small size of the silver nanoparticles. Fig. 3b highlights the visual difference between films containing silver ions and silver nanoparticles. Generally, these films have high transparency and we can see the words covered clearly.

4. Conclusions

Taking advantage of the counterions existing in electrostatic assemblies, silver ions and nanoparticles were incorporated into PEMs to produce highly effective antimicrobial coatings with satisfactory transparency. The silver content in the films can be conveniently manipulated by changing the salt concentration in the polyelectrolyte solutions used in the assembly. Films containing silver in the ionic form exhibited higher initial bactericidal activity than the ones containing silver nanoparticles, while the latter showed better long-term antimicrobial effects. The antibacterial performance of the PEMs loaded with silver nanoparticles can be enhanced by increasing the silver content via repeating the ion-exchange/reduction cycle, and by leaving the silver ions exchanged into the PEM in the last cycle in the ionic form, PEMs containing high contents of both ionic silver and silver nanoparticles can be obtained, which should have high initial antimicrobial efficacy as well as long-term performance. This facile approach can be used to incorporate in the single process multiple antimicrobial agents into the same coating. By combining the versatility of the LbL technique and the simplicity and flexibility of the current modification approach, this provides a very promising route to fabrication of antimicrobial coatings.

Acknowledgments

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