Postdiffusion of Oligo-Peptide within Exponential Growth Multilayer Films for Localized Peptide Delivery

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The multilayers of poly(1-lysine) (PLL) and hyaluronic acid (HA) were constructed by alternating deposition of PLL at high pH and HA at low pH. The exponential growth of the multilayer was proved to be amplified by increasing the pH difference between the two deposition solutions. The exponential growth multilayers of PLL/HA assembled at different pH were utilized as reservoirs for loading a trans-activating transcriptional factor (TAT) peptide. The confocal laser scanning microscopy (CLSM) results indicated that the FITC-labeled TAT could diffuse throughout the exponentially growing PLL/HA film. The amount of peptide embedded within multilayer could be adjusted by both multilayer assembly pH and the TAT loading pH. Compared with (PLL/HA 6.5/6.5), multilayer (PLL/HA 9.5/2.9) means that the multilayer film was constructed by using PLL at pH 6.5 and HA at pH 2.9, the (PLL/HA 9.5/2.9) film can be loaded with more TAT peptide at the same loading pH 6.5. The excess of positively charged TAT peptide within (PLL/HA 9.5/2.9) film could not only be ascribed to its extraordinary thickness but also be attributed to its uncompensated negative charge density enhanced by the pH difference between film buildup and peptide loading process. Increasing of the TAT loading pH from 6.5 to 9.5, which increases the pH difference between multilayer assembly and peptide loading process, enhances the uncompensated charge density within (PLL/HA 9.5/2.9) film and elevates the peptide density from 13.8 to 25.0 μg cm⁻². Compared with direct layer-by-layer assembly of TAT and HA, the postdiffusion of TAT into (PLL/HA 9.5/2.9) film was loaded more with peptide. The postdiffusion of peptide into a rapid growth multilayer can be more favorable to load and sustainly release functional oligo-peptide. The cell culture results indicated that the TAT embedded within the film maintained the ability to traverse across the Hep G2 cell membrane. The functionalized (PLL/HA 9.5/2.9) TAT 9.5 film was more efficient than the equivalent amount of free TAT peptide in the TAT uptake test.

The postdiffusion of oligo-peptide within an exponential growth multilayer can serve as an effective approach for localized and sustained peptide delivery.

Introduction

The protein and peptide therapeutics have become a new type of treatment for cardiovascular disease, cancer, inflammation, infection, and other diseases.1 Despite rapid progress in large-scale production of proteins and peptides, the effective delivery systems for the therapeutics remain a major challenge.1-3 Localized and controlled drug delivery from the biomaterial surfaces enhances drug efficiency and reduces toxicity. The control of drug delivery is beneficial for induction of cell responses in close proximity to the implant in tissue engineering and biomedical devices.

Layer-by-layer self-assembly technique is a well-established coating method that can be realized on substrates in different geometries.4 A range of natural biomacromolecules, such as proteins and peptides,5-11 DNA,12-19 and viruses,20 have been successfully incorporated in the multilayer films while maintaining their native structures and bioactivities. Different strategies were explored to incorporate bioactive molecules in the films: deposition of the bioactive molecules during the film buildup process,6,7 postdiffusion of the molecules into the preassembled multilayers,21-24 or precomplex of bioactive molecules with cyclodextrin or polyelectrolyte before self-assembly, etc.25-27

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Polypeptides with high molecular weight have been incorporated into the multilayer films by alternative deposition with oppositely charged polyelectrolytes.\textsuperscript{5,15,17,26} However, the approach was not suitable for embedding oligo-peptides into the film.\textsuperscript{3,8} More recently, a new type of multilayer film that both the mass and the thickness of the film grew exponentially with deposition step has been described.\textsuperscript{28–30} The exponential growth of the film has been ascribed to the ability of polypeptide to “diffuse into and out of” the whole film during deposition process.\textsuperscript{31,32} Although the “in-and-out” diffusion mechanism provides the possibility to load and then release the peptide, few researchers as we known have explored the approach for localized drug delivery. Very recently, we reported a facile method to significantly amplify the exponential growth of multilayers by alternating deposition of polyelectrolyte (PEI) at high pH and poly(acrylic acid) (PAA) at low pH. The alternating pH switches the ionization degrees of the polyelectrolytes in the multilayer, which enhances the diffusion of PEI into and out of the film and hence increases the deposited mass per cycle.\textsuperscript{33} The objective of the paper is to assess the possibility of developing a surface-mediated oligo-peptide delivery system via postdiffusion of oligo-peptide within an exponential growth multilayer films. We hypothesize here the pH tunable charge density within the multilayer will provide new possibility to rapidly construct multilayer with enough thickness and adjust the amount of oligo-peptide embedded within multilayer. Herein, we construct multilayer films by alternating deposition of poly-(l-lysine) (PLL) at high pH and hyaluronic acid (HA) at low pH. A basic peptide, HIV-1 TAT (47–57), is chosen to assess the possibility of oligo-peptide delivery via the “in-and-out” diffusion approach. The pH effects on the peptide loading as well as the translocation behavior of TAT from the PLL/HA films into the cells are further investigated.

Materials and Methods

**Chemicals.** Poly-(l-lysine) (PLL, \(M_w = 83,000\) Da) and propidium iodide (PI) were purchased from Sigma. Hyaluronic acid (HA, sodium salt, from Streptococcus equis sp., \(M_w \approx 700,000\) Da, polydispersity \(\sim 1.3\)) was purchased from Fluka. HIV-1 TAT (47–57) peptide with the sequence YGRKKRRQQRRR (1559 Da) and fluorescein isothiocyanate isothiocyanate conjugated-TAT (TAT-FITC, 1948 Da) were synthesized by Shanghai Science Peptide Biological Technology (Shanghai, China). TAT and TAT-FITC were prepared by solid-state synthesis. FITC was linked to TAT at the N-terminal. FITC labeled PLL (PLL-FITC) was prepared by mixing fluorescein isothiocyanate (10 mg/mL in DMSO solution) in 1 mg/mL PLL solution at 4°C for 2 h. The mixture was dialyzed against distilled water for 4 weeks and then lyophilized. The grafting ratio of FITC to PLL for lysozyme group is 0.00295 examined by UV–vis spectroscopy. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Gibco.

**Preparation of Multilayer Films.** Glass slides (diameter 15 mm) and silicon wafers for multilayer assembly were cleaned by soaking in a Piranha solution (7:3 (vol %) 98% H\(_2\)SO\(_4\):30% H\(_2\)O\(_2\)) for 5 min, then 1:1.5 (vol %) 30% H\(_2\)O\(_2\):25% NH\(_2\)H\(_2\)O mixture at 70°C for 10 min, rinsed thoroughly with water, and dried with a stream of nitrogen.

Multilayer films of PLL and HA were constructed by the layer-by-layer deposition technique. The concentration of PLL solution was 1 mg/mL and HA solution 3 mg/mL. The pH values of polyelectrolyte solutions were adjusted as required with either HCl or NaOH. The substrates were first immersed in PLL solution for 30 min, after being rinsed with water (the pH was the same to that of PLL solution) three times and drying with a stream of nitrogen. The substrates were then immersed alternatively in HA solution and PLL for 15 min each, with intermediate the same rinsing and drying step until the desired bilayer number reached. The pH of the water in each rinsing step was the same as that of the just deposited PLL or HA in the buildup process. Here, the designation (PLL/HA a/b), was used for film with a total bilayer number of \(n\) assembled alternatively from PLL solution at pH \(a\) and HA solution at pH \(b\) with HA as the outmost layer. (PLL/HA 9.5/2.9), PLL 9.5 was referred to the film with a total 5.5 bilayers and PLL was the outmost layer.

**Quartz Crystal Microbalance (QCM).** The PLL/HA film buildup process was monitored with a commercial quartz crystal microbalance (QTZ, Resonance Probe GmbH, Goslar, Germany). A CHI125A quartz crystal with a fundamental frequency of 8 MHz was obtained from CHI. The electrode was fixed in a special apparatus so that only one side of the electrode can be contacted with the solutions (about 0.15 mL). The multilayer films were constructed onto electrodes via the same layer-by-layer deposition as previously described. The resonant frequency decrease, \(\Delta F\), was measured for each deposition step at dry state. The \(\Delta F\) was used to determine the mass adsorbed after each immersion step according to the Sauerbrey’s relation:\textsuperscript{34}

\[
\Delta F = 2F_0^2 A^{-1} (\mu_0 \rho_0)^{-2} \Delta \rho
\]

where \(\Delta \rho\) is the measured resonant frequency decrease (Hz), \(F_0\) is the fundamental crystal frequency (8 MHz), \(A\) is the geometric area of the QCM electrode (0.196 cm\(^2\)), \(\mu_0\) is the shear modulus of quartz (2.947 \times 10\(^{10}\) g cm\(^{-1}\) s\(^{-2}\)), \(\rho_0\) is the density of the crystal (2.648 g cm\(^{-3}\)), and \(\Delta \rho\) is the elastic mass change (g).

In order to verify that the Sauerbrey’s relation was acceptable for the multilayers constructed in our experiments, the buildup process of the film was monitored by QCM with dissipation (QCM-D, Q-Sense, Sweden) in the dry state. In case of (PLL/HA 9.5/2.9), \(\Delta D/\Delta F_0\) are 0.03%, 0.09%, 0.26%, 0.57%, 0.74%, 1.14%, and 1.73% for \(n = 1, 3, 5, 7, 9, 11,\) and 13, respectively. According to the manual provided by Q-sense (“Q-Tools Step by Step” (QCMD, Q-Sense)), Sauerbrey’s relation is applicable to fit QCM data if \(\Delta D\) shifts (in 1 \times 10\(^{-3}\)) are less than 5% of \(\Delta F\) shifts (in Hz). So Sauerbrey’s relation was adopted here to determine the amount change of the film.

**Characterization of the Film Stability.** QCM was used to record the frequency shifts of the PLL/HA film incubated in water at different pH values. At \(t = 0\), the film was just constructed and was in the dry state. (PLL/HA 9.5/2.9), films deposited on QCM crystals were immerged in water with pH 2.9, 6.5, or 9.5 at ambient temperature. At predetermined time intervals, the crystals were moved out and dried with a stream of nitrogen, and the frequency shifts were recorded. The buildup process of PLL/HA film measured by QCM-D in the dry state indicates that Sauerbrey’s relation is applicable to fit the QCM data. The frequency change was not so sensitive to the mechanical properties of the film. The mass change of the film was linear to the frequency change.

The surface morphologies of the films deposited on silicon wafers were monitored by AFM (SPA400, Seiko, Japan) by tapping mode in air. The root-mean-square (rms) roughness of the films was determined from the AFM images.
values were obtained from the software. All measurements were carried out under room temperature.

**TAT Loaded into the Films.** Two methods were used to load TAT within the multilayers. The TAT was used as a structure component in the first method and deposited alternatively with HA as previously described. This kind of functionalized film was designated as PLL 9.5 (HA/TAT 2.9/9.5). The second method involved postdiffusion of TAT into a precursor PLL/HA film at the pH value for 30 min, and the resultant film was nominated as (PLL/HA 9.5) c TAT c. The vertical image of (PLL/HA 9.5/2.9) c TAT-FITC 9.5 film in the wet state was observed by confocal laser scanning microscopy (CLSM, LSM 510, Zeiss) using a 40 objective with 0.62 μm z-section intervals. The amount of TAT in multilayer films was calculated from the QCM data by Sauerbrey’s relation.

**TAT Released from the Film.** The (PLL/HA 9.5/2.9) s films were immersed into the TAT-FITC solutions (pH 9.5) for 30 min, followed by rinsing in water (pH 9.5) for 20 min. TAT released from (PLL/HA 9.5/2.9), TAT-FITC 9.5 film in pH 6.5 and 9.5 water at ambient temperature were monitored by UV–vis spectroscopy (UV-2550, Shimadzu). This experiment was taken at basic pH (pH 9.5) by NaOH adjustment to avoid the difference of FITC absorbance at different pH values. Three measurements were taken and the mean value of the three was used as the final result.

**Cell Culture.** Human hepatocellular carcinoma cell line (Hep G2) was incubated at 37 °C in 5% CO₂ in DMEM supplemented with 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin sulfate (100 U/mL).

The cells were seeded on 24-well plates, at a density of 1.5 × 10⁴ cells/well in 1 mL of complete medium. After 12 h adhesion, PLL/HA films loaded with TAT fabricated on glass slides (diameter 15 mm) were placed on top of the cells. After certain time interval, the slides were removed and the mean fluorescence intensity per cell was determined by flow cytometry (FACSCalibur, Beckton Dickinson). The cells for CLSM imaging were washed with PBS, fixed with 4% paraformaldehyde (PFA) in PBS for 15 min, washed, PI stained for 15 min, and washed thoroughly.

**Results**

**Buildup of Multilayer Films.** Multilayer films of poly-(l-lysine) (PLL) and hyaluronic acid (HA) were constructed by alternating deposition of PLL at high pH and HA at low pH. Three different PLL/HA deposition solution pH of 6.5/6.5, 9.0/3.3, and 9.5/2.9 were chosen to determine the effect of pH difference between the two deposition solutions on film growth. The growth of films was monitored by QCM analysis step by step.

**Stability of the PLL/HA Films.** The stability of the PLL/HA film constructed at 9.5/2.9 with five bilayers was first
investigated by QCM. (PLL/HA 9.5/2.9)₅ films deposited on QCM crystals were immersed in water at pH 2.9, 6.5, or 9.5 at ambient temperature. At predetermined time intervals, the crystals were moved out and dried with a stream of nitrogen, and the frequency shifts were recorded. The relative mass of the films (the frequency shifts to original's) plotted against the time is shown in Figure 3A. Few mass change of (PLL/HA 9.5/2.9)₅ film is observed after incubation of the film in pH 2.9 water for 48 h. When the film was incubated in water at pH 6.5, the mass of (PLL/HA 9.5/2.9)₅ film decreases substantially in the first 6 h and then levels off in the next 42 h at around 81%. The mass loss of (PLL/HA 9.5/2.9)₅ film in pH 9.5 water is similar to that in pH 6.5 water. The mass loss with respect to the outmost HA layer is also plotted against the incubation time (Figure 3B). (PLL/HA 9.5/2.9)₅ film is very stable in the pH 2.9 water, and few mass changes are observed in the whole 48 h incubation time. The mass of (PLL/HA 9.5/2.9)₅ film decreases by 36.4% or 40.0% with respect to the outmost HA layer after incubation in pH 6.5 water or pH 9.5 water for 48 h, respectively. The surface roughness of the original (PLL/HA 9.5/2.9)₅ film is about 465.0 nm, and that decreases to 451.4, 271.6, and 235.3 nm for films incubated in water at pH 2.9, 6.5, and 9.5, respectively. Despite the changes of the films' surfaces, the films are integral (Figure 4). The breakdowns of films are not found throughout the experiments. The mass changes of the (PLL/HA 9.5/2.9)₅ PLL 9.5 film in different pH water (pH 9.5, 6.5, and 2.9) were also observed. The films retain the amount near 94%, 78%, and 73% when immersed for 48 h in water at pH 9.5, 6.5, and 2.9, respectively (Figure 3C). The surface roughness corresponds to 354.3, 308.8, and 272.8 nm, respectively, while that of the original (PLL/HA 9.5/2.9)₅ PLL 9.5 film is around 381.1 nm. The AFM results indicate again that the breakdowns of films are not found throughout the experiments (Figure 4).

**Peptide Loading within and Release from the Multilayers.**

TAT peptide was embedded into the multilayer through postdiffusion of TAT into the preassembled (PLL/HA)₅ multilayer. The kinetics of TAT loading into the PLL/HA films were monitored at first (Figure 5). The amount of TAT in multilayer films was calculated from the QCM data by Sauerbrey's relation. The adsorption of TAT at pH 6.5 onto (PLL/HA 6.5/6.5)₅ multilayer quickly reaches saturation in 20 min and the value is about 0.12 μg/cm². The loading processes of TAT into (PLL/HA 9.5/2.9)₅ film at pH 6.5 and 9.5 were then monitored. A quick increase at the first 10 min is observed for the two loading processes, followed by a gradual increase in TAT amount. It seems that (PLL/HA 9.5/2.9)₅ film could reach an equilibrium state within 30 min. Following TAT postdiffusion into the PLL/HA films were all taken for 30 min.

The (PLL/HA 9.5/2.9)₅ film loaded with FITC-labeled TAT was investigated by CLSM (Figure 6). A green fluorescent band indicates that TAT can diffuse throughout the PLL/HA film. Assuming that the TAT-FITC diffuses in the whole film, the thickness of (PLL/HA 9.5/2.9)₅ TAT-FITC 9.5 film in the wet state is estimated to be ~4 μm.

TAT density in the film can be controlled by preassembled PLL/HA multilayer and the TAT loading pH. TAT density is around 0.2 μg/cm² within PLL/HA 6.5/6.5 multilayer with five bilayers, while the value increases to 13.8 μg/cm² within five bilayers of PLL/HA 9.5/2.9 film at the same TAT loading pH (pH 6.5). The TAT density within (PLL/HA 9.5/2.9)₅ film can further increased by increasing the TAT loading pH, which can reach 25.0 μg/cm² when TAT is loaded at pH 9.5 (Table 1). Correspondingly, the relative amount of TAT in the film is elevated with the TAT loading pH. The ratio of TAT in the (PLL/HA 9.5/2.9)₅ TAT film is 9.0% when TAT loaded at pH 6.5. When the TAT loading pH increases to 9.5, the ratio of TAT in the film is 15.2%.

As a control, TAT was incorporated into the films by alternative deposition of TAT and HA at the same pH as the previously described PLL/HA 9.5/2.9 film. The TAT density within the direct layer-by-layer assembled multilayer of PLL 9.5 (HA/TAT 2.9/9.5)₅ film is 3.4 μg/cm² (Table 1), which is lower than the TAT density of 25.0 μg/cm² in (PLL/HA 9.5/2.9)₅ TAT 9.5 film via postdiffusion of TAT. The postdiffusion of TAT into...
the exponential growth multilayer films is a better way to load oligo-peptide within the multilayers.

TAT release from the (PLL/HA 9.5/2.9)5 TAT 9.5 film was examined by UV–vis spectroscopy, where TAT was labeled with FITC. The characteristic absorbance of TAT-FITC is at 495 nm. Figure 7 shows the release profiles of the TAT from the (PLL/HA 9.5/2.9)5 TAT 9.5 film in pH 6.5 and 9.5 water at ambient temperature. There is an initial burst release of TAT from the film in pH 6.5 water in 40 min, and it is about 63% of the totally loaded amount of the TAT in the film. Comparatively, the TAT release in pH 9.5 is slower and around 40% of the initial loaded TAT is gradually released in 12 h. After the initial release, the films show a sustained release of TAT in the pH 6.5 and 9.5 water. At 48 h, the total release of TAT from the (PLL/HA 9.5/2.9)5 TAT 9.5 film in pH 6.5 and 9.5 water reaches respectively 82% and 46% of the total TAT amount in the film.

TAT Translocation into Cells. To test the ability of TAT peptide translocation from the films into cells, TAT peptide labeled with FITC (TAT-FITC) was used. The PLL/HA films loaded with TAT-FITC were placed directly on top of the cells. The internalization of TAT-FITC by the Hep G2 cells with (PLL/HA 9.5/2.9)5 TAT 9.5 film was imaged by CLSM (Figure 8). The cells were stained red with PI. The internalized FITC-labeled TAT is indicated by the numerous green fluorescent spots punctuated in cells, which appears yellow under CLSM. The TAT can be internalized by more cells with time. After contacting with the functionalized multilayer for 48 h, almost all the cells exhibit strong fluorescent intensity. The TAT embedded within the multilayer can thus be transmitted into cells effectively. The TAT internalization process was further monitored by flow cytometry in Hep G2 cell line. Contacting with the films for predetermined intervals, the cells were digested from the surface, and the mean fluorescence intensity per cell was recorded (Figure 9). The fluorescence intensity of the cells contacted with (PLL/HA 9.5/2.9)5 TAT 9.5 film for 48 h reaches 155, which is much stronger than that obtained with PLL 9.5 (HA/TAT 2.9/9.5)5 multilayer. The exponential growth multilayer loaded with TAT via postdiffusion is more effective than the direct assembled PLL 9.5 (HA/TAT 2.9/9.5)5 multilayer in TAT-FITC translocation into cells. The film embedded with TAT via postdiffusion was
further compared with the free TAT injection methods in TAT uptake test. The TAT density of (PLL/HA 9.5/2.9) TAT 9.5 film is around 25 μg/cm², and the TAT-FITC amount on one-side glass slide (diameter 15 mm) is 55 μg totally. The free TAT-FITC with amount ranging from 60 to 600 μg were tested for the TAT transduction ability. 60 μg of free TAT-FITC in the cell culture medium exhibits comparable TAT uptake amount with PLL 9.5 (HA/TAT 2.9/9.5) multilayer. When the free TAT added into the medium is 5-fold (300 μg) or 10-fold (600 μg) more than the amount in the (PLL/HA 9.5/2.9) TAT 9.5 film, the fluorescence intensity per cell reaches 32 and 81, respectively. The both values are still far lower than the fluorescence intensity per cells for (PLL/HA 9.5/2.9) TAT 9.5 film. The fluorescence intensity for

Table 1. TAT Amount Loaded within the Multilayer Analyzed by QCM

<table>
<thead>
<tr>
<th>multilayer</th>
<th>TAT amount (μg/cm²)</th>
<th>relative amount of TAT in the multilayer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PLL/HA 6.5/6.5) TAT 6.5</td>
<td>0.2 ± 0.1</td>
<td>18.3 ± 9.4</td>
</tr>
<tr>
<td>(PLL/HA 9.5/2.9) TAT 6.5</td>
<td>13.8 ± 2.0</td>
<td>9.0 ± 1.9</td>
</tr>
<tr>
<td>(PLL/HA 9.5/2.9) TAT 8.0</td>
<td>19.0 ± 0.8</td>
<td>11.8 ± 1.0</td>
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<tr>
<td>(PLL/HA 9.5/2.9) TAT 9.0</td>
<td>21.4 ± 1.0</td>
<td>13.2 ± 0.7</td>
</tr>
<tr>
<td>(PLL/HA 9.5/2.9) TAT 9.5</td>
<td>25.0 ± 0.8</td>
<td>15.2 ± 0.6</td>
</tr>
<tr>
<td>PLL 9.5 (HA/TAT 2.9/9.5)</td>
<td>3.4 ± 0.3</td>
<td>32.9 ± 1.9</td>
</tr>
</tbody>
</table>

Average of three measurements.

Discussion

The “in-and-out” diffusion of the exponential growth multilayer is explored here to load and release the oligo-peptide. Poly(1-lysine) (PLL)/hyaluronic acid (HA) film is the mostly investigated exponential-growth polyelectrolyte multilayer, which is attributed to the ability of PLL to diffuse into and out of the film during the deposition process. It has been reported previously that the PLL is a diffusible polyelectrolyte while HA is not able to diffuse in the multilayer film. When the film is brought in contact with PLL, PLL not only adsorbs on the surface of the film but also diffuses into the film. When such a film contacts with HA, the PLL diffuses out of the film and is complexed with HA at the film/solution interface. Because both PLL and HA are weak polyelectrolytes, their charge densities can be controlled by pH value of the solution. The term “pKₐ” has been used to indicate the pH at which 50% of the polyelectrolyte’s functional groups are ionized. pKₐ of PLL provided by Haynie and co-workers is around 10.5. It means at least 50% of the PLL’s functional groups are ionized at relative basic condition (pH 9.0 and 9.5). Our data in Figures 1 and 2 demonstrated that the multilayer of PLL/HA can be fabricated successfully at a high pH of PLL and a low pH of HA. Furthermore, PLL is effectively fully charged at acidic medium (pH 2.9 and 3.3) or neutral medium (pH 6.5). The alternating exposure of the weak polyelectrolyte multilayer to high pH and low pH enhances the concentration of uncompensated polycation/polyanion charges within the multilayer, which will enhance the diffusion of PLL into and


Figure 7. TAT released from (PLL/HA 9.5/2.9) TAT 9.5 film at ambient temperature in pH 6.5 (■) and pH 9.5 (●) water. TAT was labeled with FITC.

Figure 8. Internalization of TAT in Hep G2 cells after contacting with the (PLL/HA 9.5/2.9) TAT 9.5 films at 37 °C for certain time analyzed by confocal laser fluorescence microscopy. TAT was labeled with FITC. The green color corresponds to TAT-FITC and the red color to cells stained with PI.

Figure 9. Flow cytometry analysis of Hep G2 cells after contacting with (PLL/HA 9.5/2.9) TAT 9.5 film (●) and PLL 9.5 (HA/TAT 2.9/9.5) multilayer (○) or treatment with TAT in amount of 60 μg (△), 300 μg (▲), and 600 μg (●). TAT was labeled with FITC. The mean fluorescence intensity per cell (y axis) was plotted against the time (x axis).

(PLL/HA 9.5/2.9) TAT 9.5 film is about twice more than that of free TAT with an amount of 600 μg.

The mean fluorescence intensity per cell for (PLL/HA 9.5/2.9) TAT 9.5 film is about twice more than that of free TAT with an amount of 600 μg.
out of the film and hence increase the deposited mass per cycle (Figure 1). The PLL/HA coatings with high thickness for loading peptide drug can be easily fabricated by increasing pH difference between the two deposition solutions.

The stability of PLL/HA film fabricated from relatively extreme pH was investigated (Figure 3). The slight mass decrease of (PLL/HA 9.5/2.9)₅ films is observed throughout the 48 h of incubation in pH 6.5 or 9.5 water, which may be due to the dissolution of the top HA layer. The uncompensated negative charges can be introduced when (PLL/HA 9.5/2.9)₅ film is incubated in pH 9.5 water, which favors the solubility of the top HA chains. However, the dissolution is not sustained. The total mass loss is about 20% of the whole film and is only 40% of the outmost layer of (PLL/HA 9.5/2.9)₅, film, respectively. The mass data in Figure 3, combined with the AFM images in Figure 4, demonstrate that a gradual dissolution with surface HA layer rather than the breakdown of the whole film happened to (PLL/HA 9.5/2.9)₅ films when the film was incubated in water at pH 9.5. When the (PLL/HA 9.5/2.9)₅ film is incubated in PLL solution at pH 9.5, the uncompensated negative charges can be neutralized by the diffusion of PLL into multilayer, which induces the growth of multilayer (Figures 1 and 2A). When PLL solution is replaced with positive peptide, the pH-dependent uncompensated charge density within the multilayer and the diffusivity of the peptide can be utilized for tunable loading peptide. The loading of TAT took less time to reach equilibrium than PLL deposition at the same pH (pH 9.5) (Figures 2A and 5). It is not surprising to find the amount of peptide embedded within multilayer could be modulated by both preassembled PLL/HA multilayer and the TAT loading pH (Table 1). Compared with (PLL/HA 6.5/6.5)₅ multilayer, the (PLL/HA 9.5/2.9)₅ film can be loaded with more TAT peptide at the same loading pH 6.5 (Table 1). The excess of TAT peptide within (PLL/HA 9.5/2.9)₅ TAT 6.5 film can not only be ascribed to the extraordinary thickness of the film but also be attributed to its uncompensated charge density enhanced by the pH difference between film buildup and peptide loading process. The pH dependence of the uncompensated charge density within multilayer can be utilized to modulate the peptide loading amount. The increasing of the TAT loading pH from 6.5 to 9.5, which increases the pH difference between film buildup and peptide loading process, will enhance the uncompensated charge density within (PLL/HA 9.5/2.9)₅ film and elevate the peptide density from 13.8 to 25.0 μg/cm² (Table 1).

Compared with the postdiffusion of TAT into (PLL/HA 9.5/2.9)₅ film, the PLL 9.5 (HA/TAT 2.9/9.5), multilayer constructed by direct layer-by-layer assembly of PLL and HA, more peptide could be loaded within the film via the postdiffusion of TAT into the pH amplified exponential growth multilayer of PLL/HA. The cell culture results demonstrated that the TAT embedded within the multilayers was proved to be adjusted by the preassembled multilayer and the TAT loading pH. Compared with the direct layer-by-layer assembly of TAT and HA, more peptide could be loaded within the film via the postdiffusion of TAT into the pH amplified exponential growth multilayer of PLL/HA. The cell culture results demonstrated that the TAT embedded within the multilayers maintained the ability to traverse across the Hep G2 cell membrane, which was more efficient than the equivalent amount of free TAT peptide in the TAT uptake. The postdiffusion of oligo-peptide within an exponential growth multilayer can serve as an effective approach for localized transport TAT into cells, which might have great potential in tissue engineering and biomedical implants.

Conclusions

We demonstrated a facile method to rapidly construct multilayer films via the alternate deposition of PLL at high pH and HA at low pH. The exponential growth multilayers of PLL/HA assembled at different pH were utilized as reservoirs for loading TAT peptide. The density of TAT peptide embedded within the multilayers was proved to be adjusted by the preassembled multilayer and the TAT loading pH. Compared with the direct layer-by-layer assembly of TAT and HA, more peptide could be loaded within the film via the postdiffusion of TAT into the pH amplified exponential growth multilayer of PLL/HA. The cell culture results demonstrated that the TAT embedded within the films maintained the ability to traverse across the Hep G2 cell membrane, which was more efficient than the equivalent amount of free TAT peptide in the TAT uptake. The postdiffusion of oligo-peptide within an exponential growth multilayer can serve as an effective approach for localized transport TAT into cells, which might have great potential in tissue engineering and biomedical implants.


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Supporting Information Available: QCM data of TAT loading into PLL/HA films at different pH. This material is available free of charge via the Internet at http://pubs.acs.org.