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Electrodeposition to construct free-standing chitosan/layered double hydroxides hydro-membrane for electrically triggered protein release

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ABSTRACT

In this study, we report the electrodeposition of a chitosan/layered double hydroxides (LDHs) hydro-membrane for protein release triggered by an electrical signal. The electrodeposition was performed in a chitosan and insulin loaded LDHs suspension in the absence of salt. A free-standing chitosan/LDHs hydro-membrane was generated on the electrode with improved mechanical properties, which is dramatically different from the weak hydrogel deposited in the presence of salt. The amount of LDHs in the hydro-membrane affects the optical transmittance and multilayered structure of the hybrid membrane. Compared to the weak chitosan/LDHs hydrogel, the hydro-membrane has a higher insulin loading capacity and the release of insulin is relatively slow. By biasing electrical potentials to the hydro-membrane, the release behavior of insulin can be adjusted accordingly. In addition, the chitosan/LDHs hydro-membrane showed no toxicity to cells. Our results provide a facile method to construct a chitosan/LDHs hybrid multilayered hydro-membrane and suggest the great potential of the hydro-membrane in controlled protein release.

1. Introduction

Chitosan, an amino polysaccharide derived from chitin, has been widely studied for drug release systems due to its biocompatibility and biodegradability [1–4]. In particular, chitosan hydrogels, which contains a large amount of water and an inter-connected chitosan network, provides a competitive matrix for protein entrapment and subsequent controlled release [5–7]. By taking the advantages of its pH sensitivity and film-forming properties, chitosan can be electrodeposited on a conducting electrode as a hydrogel [8–11]. When including proteins in the chitosan solution, the utilization of a co-deposition process results in a composite chitosan and protein hydrogel [12–14]. The co-deposited chitosan/protein hydrogel has broad applications in tissue engineering, biomedicine and biosensing [15–18].

In our previous work, we have electrodeposited a multilayered hybrid hydrogel by the co-deposition of chitosan and layered double hydroxides (LDHs) [19]. LDHs are lamellar structured anionic clays with positively charged brucite-like layers and counter ions in the interlayer [20–22]. A model protein, insulin, was loaded to the hybrid hydrogel by previous adsorption on LDHs. The co-deposition was performed in the presence of 0.25% (w/v) NaCl in order to form a hydrogel. However, the chitosan/LDHs hydrogel suffered from weak mechanical strength and quick disintegration, which affects the long-term use of the hybrid hydrogel. Recently, it was reported that the presence of salt in the chitosan solution has a remarkable effect on the structure and features of the deposited chitosan film [23]. In the absence of salt, the chitosan chains tend to have extended conformations due to electrostatic repulsions between the charged chains and form a compact membrane. Presumably, the drug release behavior from the chitosan membrane would differ from that of the hydrogel deposited in the presence of NaCl.

In this study, we fabricated a free-standing multilayered chitosan/LDHs hydro-membrane by co-deposition in the absence of salt. Under this condition, the thickness of the chitosan/LDHs hydro-membrane is much thinner than that of the hydrogel deposited in the presence of salt, while its mechanical property is improved substantially. Importantly, the hydro-membrane has a higher insulin loading capacity and applying a negative poten-

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tial triggered the release of insulin. In addition, the chitosan/LDHs hydro-membrane shows no cytotoxicity to L929 cells. Our study suggests that the chitosan/LDHs hydro-membrane co-deposited in the absence of salt has great potential in controlled drug release.

2. Experimental

2.1. Materials

Chitosan provided as a coarse powder was purchased from Sigma (deacetylation degree: 85%, molecular weight: 200 kDa). Bovine insulin was purchased from Sigma with a molecular weight of 5733.49 Da. Titanium plates with a thickness of 100 μm were purchased from Baoji Titanium Company, Shanxi. Magnesium chloride and aluminum chloride were purchased from Shanghai Reagent Co., (China). All reagents were of analytical grade and were used without further purification.

2.2. Electrodeposition of Chitosan/LDHs hydro-membrane

The layered double hydroxides, $Mg_2Al(OH)_6Cl{\cdot}xH_2O$, was prepared by a co-precipitation and hydrothermal method according to our previous work [19]. The freeze-dried LDHs were dispersed evenly in chitosan solution (1%, pH = 5) based on different mass ratios of chitosan to LDHs. To electrodeposit a chitosan/LDHs hydro-membrane, no salt was added to the chitosan/LDHs suspension. A titanium plate with a dimension of 4 cm × 2 cm × 100 μm used as the cathode and a platinum wire used as the anode were partially immersed into the chitosan/LDHs suspension. The distance between the two electrodes was maintained at 1 cm. A constant current ($-0.185\ mA/cm^2$) was applied to the two electrodes for 1 h to get a hydro-membrane with a thickness of 0.2 mm. The hydro-membrane on the titanium plate was rinsed briefly with distilled water. For comparison, the hybrid hydrogel was deposited at similar conditions in the chitosan/LDHs suspension with the addition of 0.25% (w/v) NaCl.

To load insulin on chitosan/LDHs, insulin was firstly loaded to LDHs (INS-LDHs) by an adsorption method and INS-LDHs was added to the chitosan solution for co-deposition. To measure the amount of insulin loaded in the chitosan/LDHs hydrogel or hydro-membrane, the samples were dissolved in pH 1.2 HCl solution followed by centrifugation. The concentration of insulin in the HCl solution was measured by its absorbance at 280 nm.

2.3. Release of insulin from chitosan/INS-LDHs hydrogel or hydro-membrane

The spontaneous release of insulin from chitosan/INS-LDHs hydrogel or hydro-membrane was performed in 0.1 mol/L phosphate buffer at pH 7. At predetermined time intervals, 200 μL of the release medium was withdrawn and 200 μL of fresh buffer was added to get a constant volume. The insulin concentration in the supernatant was analyzed by UV–vis. Each assay was carried out in triplicate.

For the electrochemically controlled insulin release, a titanium plate with a deposited chitosan/INS-LDHs hydro-membrane was partially immersed in 0.1 mol/L phosphate buffer, and a platinum wire was used as the counter electrode. The electrochemically controlled insulin release was activated by applying a voltage of −1 V, −3 V and −5 V with 0 V as a control. At predetermined time intervals, 200 μL of the release medium was withdrawn and 200 μL of fresh buffer was added back. The cumulative release of insulin was measured by UV–vis. In addition, the electrical potential was applied as "on-off" mode and the time sequence of the voltage was "on" for 30 min and "off" for 30 min. At the "on" step, the voltage was set as −5 V or −1 V. Each assay was carried out in triplicate.

2.4. Cytotoxicity assays and cell morphology

The cytotoxicity of the hydro-membrane was assessed using the MTT Cell Proliferation Assay. The hydro-membrane was sterilized by exposure to UV radiation for 8 h and extracted in DMEM at 37 °C for 24 h. The mouse fibroblast (L929, provided by department of medicine, Wuhan University) cells were seeded in a 96-well plate at a density of 5×10^3 cells/ml and incubated for 24 h until a monolayer was formed. The medium was replaced by complete DMEM (150 μL) and hydro-membrane extract (50 μL). The control group was added with the same volume of compete DMEM. At the indicated time points (1 d, 2 d, 3 d), 20 μL of MTT (0.1 mmol/L) was added into each well. Then, the wells were incubated for 4 h and washed gently by phosphate buffered saline (PBS) three times. The MTT formazan purple crystals were dissolved by adding DMSO (150 μL) into each well and incubating for 10–15 min. Finally, the absorbance was read by a microplate reader at 490 nm. The experiment was repeated three times for each sample and the results were presented as the mean ± standard deviations.

To observe the proliferation of L929 cells on the hydro-membrane, L929 cells were cultured on the hydro-membrane ($1 \times 1\ cm^2$) at 37 °C for 24 h. Then the hydro-membrane was washed by PBS (pH 7.4) twice to remove the unattached cells. Subsequently, 3% glutaraldehyde was added into wells to fix attached cells on hydro-membrane and the hydro-membrane was kept at 4 °C for 24 h. After being washed gently by PBS twice, the hydro-membrane was wrapped with tinfoil and flash-frozen in liquid nitrogen, then freeze-dried overnight. The morphology of cells adhered to the surface of hydro-membrane was examined with FE-SEM (Zeiss, Germany). The cell attached on 24-well plates directly was used as a control.

2.5. Characterization of chitosan/INS-LDHs hydro-membrane

The morphology of the chitosan/INS-LDHs hydro-membrane was observed by Field Emission Scanning Electron Microscopy (FE-SEM, Zeiss, Germany). The insulin absorbance at 280 nm and the optical transmittance of the hydro-membrane were measured by UV spectrophotometry (UV-1780, Shimadzu). The tensile strength and elongation at break of the hydro-membrane were measured on a universal testing machine (CMT6350, Shenzhen SANS Test Machine Co., Ltd., Shenzhen, China) with a tensile rate of 2 mm/min according to ISO527-3:1995.

3. Results and discussion

Chitosan can be electrodeposited on the cathode due to the increased pH gradient locally generated near the electrode [24]. The presence of salt in chitosan solution largely affects the chain conformation of chitosan, thus leading to chitosan deposits with different features. Fig. 1 compares the optical images of the electrodeposits from chitosan/LDHs solutions containing either 0 or 0.25% NaCl by using the same current density ($-0.185\ mA/cm^2$) and deposition time (1 h). As shown in the top images (Fig. 1A), a transparent and thin hydro-membrane (thickness 0.2 mm) was deposited on the titanium plate in the absence of NaCl. In contrast, an opaque and thick hydrogel (thickness of 2 mm) was observed on the titanium plate (Fig. 1B) when depositing in the presence of 0.25% NaCl. The discrepancy was resulted from the effect of NaCl on the deposition process of chitosan. Salt is known to screen electrostatic repulsions, alter the chitosan solution's properties [25,26] and promote aggregation [27], thus the absence of NaCl can form a hydro-membrane with more inter-polymer associations [23].