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Construction and deconstruction of PLL/DNA multilayered films for DNA delivery: Effect of ionic strength

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Abstract

Through the layer-by-layer (LbL) self-assembly technique, DNA was incorporated into the multilayered films with poly-L-lysine (PLL). The effect of ionic strength on the construction and deconstruction of the PLL/DNA films was investigated. It was found that the salt concentration of the deposition solution had a significant effect on the construction of the films, which might attribute to the effect of salt ions on the conformation of polyelectrolytes and interaction between PLL and DNA molecules. A salt-induced deconstruction of the PLL/DNA films was observed. The extent of the deconstruction increased with the salt concentration in the incubation solution. The mechanism of the deconstruction was discussed. Taking the advantages of the LbL technique, the erasable PLL/DNA films could deposit onto a variety of surfaces, such as vascular stent, intervention catheter and tissue engineering scaffold, to serve as a novel DNA delivery system.

Keywords: DNA delivery; Effect of ionic strength; Layer-by-layer (LbL); Self-assembly; Nanofilms

1. Introduction

During the past several decades, gene therapy has become the research focus worldwide and has been advanced considerably. The main objective in gene therapy is constructing an efficient gene delivery system and transferring the DNA to the targeted tissues and cells [1]. Controlled delivery of DNA from coating [2,3], hydrogel [4,5], tissue engineering scaffolds [6] and nanoparticles [7,8] have been developed. However, although a great deal of effort is being expended, the development of efficient and safe gene delivery systems remains the main challenge for gene therapy.

Recently, the polyelectrolyte multilayered (PEM) ultrathin films, which is prepared by layer-by-layer (LbL) self-assembly technique [9], has been extensively researched. Through controlling the parameters of construction, such as the ionic strength and pH value, a variety of properties of films including films thickness, roughness, molecular structure, surface wettability and surface charge can be precisely engineered [10]. The new development of LbL PEM films is in the field of biotechnical applications includes biomaterials, biomedicine and bioengineering. For example, construction of cytophilic and cytophobic films [11], procoagulation and anticoagulation films [12] and enzymes-loading films [13] have been reported. Many kinds of biomacromolecules includes proteins [14], polysaccharides [15] and DNA [16–20] can be employed to construct PEM films by LbL technique.

DNA is a very important genetic material. However, it is also an interesting anionic polyelectrolyte. Since, Lvov et al. [16] fabricated the assembly of thin films by LbL deposition of alternate layers of DNA and poly(allylamine), several multilayered films that incorporated DNA have been successfully assembled includes DNA/poly(dimethyldiallylammonium chloride) (PDDA) [17], DNA/poly(ethylenimine) (PEI) [18], DNA/polyamine [19] and DNA/dye [20]. Recently, with the hypothesis that DNA incorporated into ultrathin films on the medical implants might efficiently reach the targeted cells [21], we have fabricated DNA films through LbL deposition of DNA and poly-L-lysine (PLL) [22,23]. Because of the biodegradability of PLL, the PLL/DNA ultrathin films can be degraded under physiological conditions by enzymes; meanwhile, the DNA was released. Such system could provide precise control over DNA release and it is very importance for gene therapy. Thus, this kind of DNA films could be served as a DNA delivery system.

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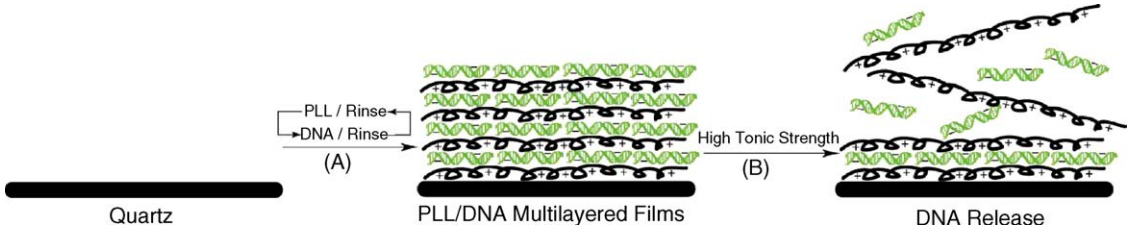

Fig. 1. The schematic construction and deconstruction of PLL/DNA multilayered films via layer-by-layer as DNA delivery system. (A) Construction of the films via layer-by-layer deposition of PLL and DNA, and (B) salt-induced deconstruction of the films.

Taking the advantages of LbL technique, the PLL/DNA ultrathin films can be deposited onto a variety of kind and shape of surface such as vascular stents, intervention catheters and tissue engineering scaffolds. Furthermore, the films thickness can be controlled with nanoscale. Nanoscale LbL films with ability to release of DNA might advance these medical implants in the field of gene therapy.

In this paper, we further research the effect of ionic strength on the construction and deconstruction of PLL/DNA multilayered films (Fig. 1). The research may present an insight of PLL/DNA films. Through controlling the ionic strength in solution, it is found that the amount of DNA embedded into the films can be effectively engineered. The topographic feature of films' surface is changed correspondingly. It is interesting that the films is erasable upon changing in the ionic strength in incubation solution, which might serve as another method (besides enzymatic degradation) to controlled release of DNA. Consequently, this system may be applied in special tissues or organs (such as kidney) with high ionic strength. These properties could lead PLL/DNA ultrathin films to a versatile DNA delivery system.

2. Experimental

2.1. Materials

PLL (MW 80,000) was purchased from Sigma. Deoxyribonucleic acid (DNA, fish sperm, sodium salt) and *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid] (HEPEs, free acid, high purity grade) were purchased from AMRESCO. Ethidium bromide (EtBr) was purchased from BBI. Water used was distilled three times, pH 6.06 (PB10, Sartorius, Germany). All reagents were used as received.

2.2. Construction of PLL/DNA multilayered films in varying ionic strength solution

Alternating PLL/DNA multilayered films were LbL assembled onto quartz substrates (10 mm × 20 mm). The first layer of PLL was adsorbed onto the quartz surface by immersing the quartz substrate in 0.5 mg/mL PLL/HEPEs buffer (20 mM HEPEs, pH 7.4) at varying NaCl contents for 10 min. The PLL-covered substrates were then dipped in water three times, followed by drying under a stream of N_2. DNA was then attached to the PLL layer by immersing the quartz substrate in 0.5 mg/mL DNA/HEPEs buffer (20 mM HEPEs, pH 7.4) at the same NaCl content as PLL solution for 10 min. The process was alternately repeated until a desired number of bilayers were deposited. Before the measurements, all samples were dried in the vacuum desiccator under room temperature for at least 12 h.

2.3. Characterization of construction of PLL/DNA multilayered films

The build-up process of PLL/DNA films was measured on a UV–vis spectrophotometer (CARY 100 BIO, USA). The surface topographic feature of films with different bilayer number was monitored on an atomic force microscope (AFM, SPA400, Seiko, Japan) by tapping mode in air with 2 μm × 2 μm scanning range. The root mean square (RMS) roughness values were obtained from the AFM software simultaneously. Every kind of sample was carried out at three or four different places on the surface and typical images and roughness values were presented. All measurements were carried out under room temperature.

2.4. Deconstruction of PLL/DNA multilayered films in solution at varying ionic strength

A salt induced deconstruction study was carried out through putting the sample into the incubation solution at varying NaCl concentration. Unless stated, $(PLL/DNA)_{10}$ films constructed at 0.5 M NaCl were selected as the deconstruction samples. Multilayered films were exposed to a system containing 0.5, 0.75, 1, 1.25 and 1.5 M NaCl at 37 °C for 12 h. The samples were then removed from the incubation solution, washed and dried in the vacuum desiccator under room temperature for at least 12 h.

2.5. Characterization of deconstruction of PLL/DNA multilayered films

A UV–vis absorption at 260 nm of multilayered films on quartz substrates was measured on a UV–vis spectrophotometer (CARY 100 BIO, USA). The relative absorbance was presented, with 0 representing the absorbance of $(PLL/DNA)_{10}$ multilayered films, while 1 designating the absorbance of bare quartz substrates. The incubation solution that contained released DNA was added (100:1, vol.%) EtBr solution (50 μg/ml) and then measured on a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan) with an excitation wavelength of 530 nm, a 5 nm slit, and an emission wavelength of 590 nm with a 5 nm slit. All measurements were carried out under room temperature.