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Tuning cell adhesive properties via layer-by-layer assembly of chitosan and alginate

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ABSTRACT

Understanding the mechanisms controlling cell-multilayer film interactions is crucial to the successful engineering of these coatings for biotechnological and biomedical applications. Herein, we present a strategy to tune the cell adhesive properties of multilayers based on marine polysaccharides with and without cross-linking and/or coating with extracellular matrix proteins. Chemical cross-linking of multilayers improved mechanical properties of the coatings but also elicited changes in surface chemistry that alter the adhesion of human umbilical vein endothelial cells. We evaluated a strategy to decouple the mechanical and chemical properties of these films, enabling the transition from cell-adhesive to cell-resistant multilayers. Addition of chitosan/alginate multilayers on top of cross-linked films decreased endothelial cell adhesion, spreading, and proliferation to similar levels as uncross-linked films. Our findings highlight the key role of surface chemistry in cell-multilayer film interactions, and these engineered nanocoatings represent a tunable model of cell adhesive and non-adhesive multilayered films.

Statement of Significance

Multilayered films based on marine-derived polysaccharides were obtained by layer-by-layer (LbL). Biological tests with human umbilical vein endothelial cells (HUVECs) showed the potential of these films to tailor cell adhesion, spreading and proliferation. These multilayered films promise to be versatile and tunable model of cell adhesive and non-adhesive films.

1. Introduction

Layer-by-layer (LbL) assembly can produce hierarchical films with fine and precise control over the film properties [1–7]. Since its discovery, LbL assembly has been shown to be a simple, versatile and elegant bottom-up approach for template-assisted assembly of materials [4,8–10]. This method is based on the sequential adsorption of multivalent molecules on virtually any type of substrate via electrostatic, non-electrostatic, or a combination of thereof [2,11–13]. The overall properties of the film can be controlled simply by adjusting processing parameters: nature of polyelectrolytes, functional groups, molecular weight, charge density, concentration of the adsorption species, adsorption and rising time, pH, ionic strength, and humidity [14–19].

By using LbL assembly, an unprecedented variety of different components (i.e. hundreds of different materials) can be used. The nature and intrinsic properties of the building blocks dictate the bulk properties of the film [2,4,20]. Natural origin polymer-based multilayers have received particular attention due to their ability to impart unique properties to the polyelectrolyte multilayers (PEMs), such as cytocompatibility, biodegradability, presence of cell recognition sites, and natural similarity to the biological tissues [21–26]. Herein, two polysaccharides were used as polyelectrolytes: chitosan (CHI) and alginate (ALG). Multilayers based on these polysaccharides are stable over a pH range of 3–9, but exhibit high hydration levels and low mechanical strength, which impair cell adhesion [27–29]. Post-assembly modifications can be used to tune these properties, namely adsorption or immobilization of

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extracellular matrix (ECM) proteins such as laminin (LM) and fibronectin (FN) as well as chemical cross-linking of the films.

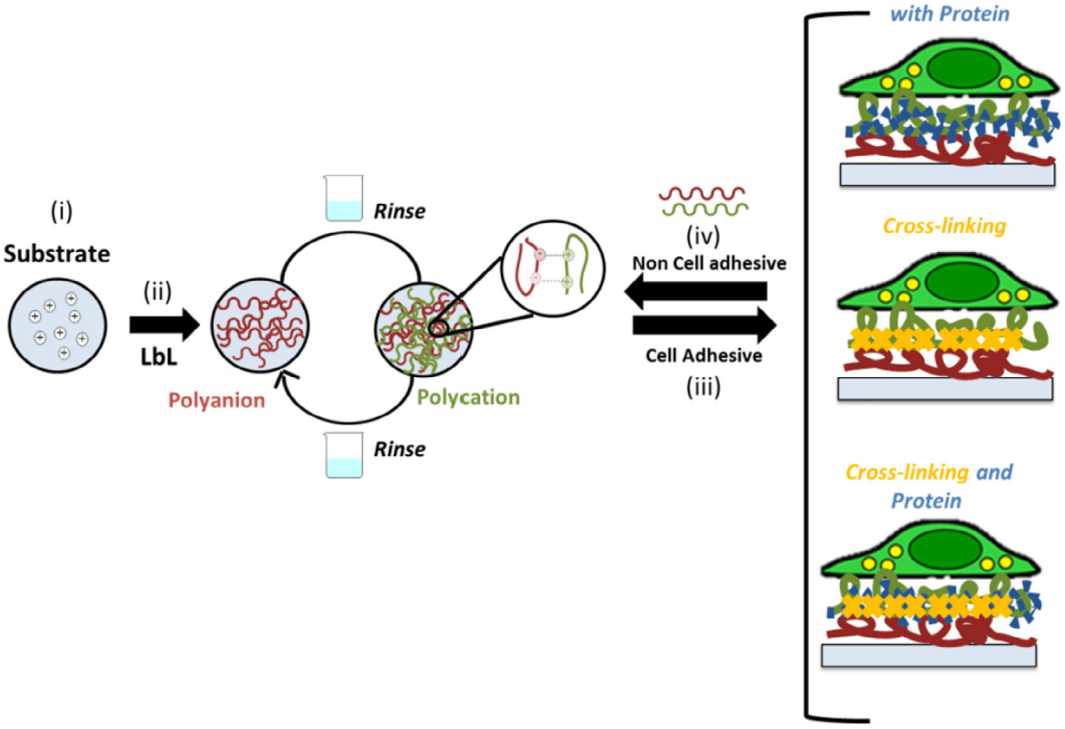
Multilayers with poor cell adhesion ability are ideal candidates for an adsorption or chemical attachment of an ECM layer. The kinetics of protein adsorption is governed by electrostatic interactions and other secondary interactions such as hydrophobic interactions and hydrogen bonding [30]. The advantages of covalent immobilization over the adsorption strategy are that the biomolecules immobilized are not easily removed by physical force (e.g., rinsing) and are robust enough to withstand the possible harsh conditions of *in vivo* exposure [31]. Using this approach, it is also possible to avoid the diffusion of biomolecules along the multilayers, allowing a spatial control over its distribution [30].

Modulation of mechanical properties is a common approach used to tailor the bulk properties of multilayered films. Different approaches can be used to tune mechanical properties including the incorporation of nanocolloids [32], assembly pH [33], or by using covalent cross-linkers such as glutaraldehyde, [34,35] 1-eth yl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) in combination with sulfo- N-hydroxysulfo-succinimide (EDC/s-NHS) [36–38] and genipin [29,39]. Genipin is a naturally derived chemical from the gardenia fruit that has been extensively investigated due to its ability to cross-link amine-containing polymers [40,41] in diverse systems, including multilayered films [39,42]. Using genipin as cross-linker, a wide range of films of increased stiffness can be obtained by simply varying the reaction time or the concentration of the cross-linker [43,44]. Genipin cross-linker, however, is reported to have effects on the wettability and roughness of the film. Thus, upon cross-linking of multilayers with genipin, it is challenging to decouple the mechanical and chemical properties of the films. In a previous study, Discher and co-workers achieved the decoupling of both parameters using polyacrylamide gels grafted with increased densities of collagen [45]. In the present work, we performed a systematic study of the cell adhesive properties of CHI/ALG multilayers upon cross-linking and ECM protein adsorption or immobilization (Scheme 1). Although the production and characterization of such multilayered films have been reported, to the best of our knowledge, this is the first time that such films have been used to decouple the effects of mechanical and chemical cues on cell adhesion.

2. Materials and methods

2.1. Production of multilayered films

The multilayers were processed using CHI of medium molecular weight (M_w 150–300 kDa, 90% degree of deacetylation, Heppe Medical Chitosan, Germany) and low viscosity ALG (538 kDa, ~250 cP, ref.71238, Sigma Aldrich, USA). PEMs were constructed by alternately immersing the tissue culture-grade polystyrene substrate (96 well plate, TCPS, Corning, USA) into a polyethylenimine solution (PEI, 0.5 mg mL^{-1}, 0.15 M sodium acetate buffer, pH 5.5) for 10 min, with an intermediate rinsing step of 10 min. TCPS coated with PEI presents a positive charge, providing support for the multilayer assembly. The substrates were then immersed in an ALG solution (1 mg mL^{-1}, 0.1 M sodium acetate buffer/0.15 M NaCl, pH 5.5) for 6 min. After rinsing with 0.1 M sodium acetate buffer/0.15 M NaCl (4 min), the same procedure was followed for CHI deposition (1 mg mL^{-1}, 0.1 M sodium acetate buffer/0.15 M NaCl 0.15 M, pH 5.5). Multilayers were generated by repeating the ALG/CHI deposition for five cycles. After buildup, the multilayers were cross-linked with genipin (Wako chemical, USA). Briefly, genipin solutions (0.125–5.0 mg mL^{-1}) were prepared by dissolving the adequate amount of lyophilized genipin into dimethyl sulfoxide (DMSO, Sigma Aldrich USA)/sodium acetate buffer (0.15 M NaCl, pH 5.5) mixture (1:4, v/v). The cross-linked films were then



Scheme 1. Schematic representation of layer-by-layer (LbL) adsorption of polyelectrolytes based on electrostatic interactions. (i) The substrate (polystyrene well plates) was first modified with polyethylenimine (PEI) and extensive washed. (ii) The positive charged substrate was coated with polysaccharides that shared marine origin (ALG and CHI) using LbL assembly. Between the polyelectrolyte depositions a washing step was performed. (iii) The adhesive properties of ALG/CHI multilayers can be tuned by the addition of ECM proteins, chemical cross-linking or a combination of thereof. (iv) Upon the addition of ALG/CHI on the top of the cell adhesive ALG/CHI multilayers the films became non-adhesive.