Layer-by-Layer Antimicrobial Hydrogel Thin Films

Svetlana Pavlukhina[1], Altida Patimetha[2], Matthew Libera[2], and Svetlana A. Sukhishvili[1]
[1]Dept. of Chemistry, Chemical Biology and Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030
[2]Dept. of Chemical Engineering and Materials Science, Stevens Institute of Technology, Hoboken, NJ 07030

Purpose: Weak polyelectrolyte layer-by-layer (LbL) single-component thin films are capable of binding functional therapeutic molecules, such as dyes or drugs, within the body of the film and releasing them at a later stage in response to pH variations as an external trigger [1]. While one class of drugs consists of traditional low-molecular-weight substances [2], another important class of therapeutic compounds includes proteins, such as growth factors and/or antibacterial polypeptides [3]. Embedding protein therapeutic compounds within polymer films coated onto a solid surface, such as that of a bone-regeneration construct or an orthopedic implant, will provide benefits of controlling tissue growth and infection. Motivated by the need to construct drug-releasing antibacterial coatings, in this study we use single-component poly(methacrylic acid) (PMAA) surface-bound LbL hydrogel films, which are crosslinked with ethylenediamine (EDA), as matrices for the pH-controlled loading and release of positively charged functional molecules and antibacterial agents , such as lysozyme (Lys), gentamicin (Gent), poly-L-lysine (PLL) and a polypeptide JFLO.

Materials and Methods: Poly(N-vinyl pyrrolidone) (PVPON) with M_w 2,500 was purchased from Polysciences, Inc.; PMAA with M_w 150, 000 was received from Scientific Polymer Products, Inc.; N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) was purchased from Fluka. Hydrochloric acid, sodium hydroxide, dibasic and monobasic sodium phosphate, poly(ethyleneimine) (PEI; M_w 70, 000), EDA, PLL, Lys, and Gent (10 mg/mL solution in deionized water) were purchased from Sigma-Aldrich. The polypeptide JFLO was synthesized by Gen Script (Piscataway, NJ). All chemicals were used without further purification. To enhance the multilayer adhesion to the Si substrate, two bilayers of PEI/PMAA were first deposited as a precursor film. Hydrogen-bonded PVPON/PMAA multilayers were then deposited using the LbL technique. These were converted to hydrogel by selective chemical crosslinking of the PMAA [2]. The cross-linking procedure included activation of PMAA carboxylic groups with EDC, and subsequent treatment with EDA solution. To remove PVPON and the activation agents, the crosslinked multilayers were exposed to 0.01 M phosphate buffer (PhB) at pH=7.5 for 2 hours. Guest molecules were then incorporated into the hydrogel films by soaking the dried films in various solutions. $(PMAA)_{10}$ films crosslinked with EDA were exposed to: PLL (0.1mg/mL, 0.01M PhB at pH 7.5, 0.6), Lys (0.1 mg/mL, 0.01M PhB at pH 7.5, 0.6 mL), polypeptide JFLO (200 μM, 0.01 M PhB at pH 7.5, 0.6 mL), or Gent solution (1 mg/mL, 0.01M PhB at pH 7.5, 0.6 mL). The thickness of dry and swollen films was measured by a home-built phase-modulated ellipsometer. *In situ* deposition and cross-linking of PVPON/PMAA films were also followed by ATR-FTIR using a Bruker Equinox-55 Fourier transform infrared spectrometer equipped with a narrow-band mercury cadmium telluride detector. The adhesion and growth inhibiting properties of the films were tested with the introduction of *S. epidermidis* (*S. epi)* bacteria (strain NJ9709, from a patient at University Hospital, Newark, NJ) in tryptic soy broth (TSB) at an initial concentration of $5x10^6$ colonies/mL.

Results: Large amounts of Lys, Gent, PLL, or polypeptide JFLO (6:1; 1.5:1; 1.5:1; 2:1 are the mass-to-mass ratios to dry hydrogel, correspondingly) were loaded within the PMAA matrix at pH 7.5 through an electrostatic charge-compensation mechanism. Subsequent release of loaded molecules was triggered by protonation of PMAA groups in the hydrogel at lower pH values. The ionic-strength and pH-induced release profiles varied with the nature of the loaded compound. Figure 1 demonstrates the effect of pH on retention of JLFO within the hydrogels. Importantly, after culture times of 3 and 6 hours, the hydrogels loaded with polypeptide JFLO showed significantly less *S. epi* adhesion than its counterparts, and growth was completely inhibited.

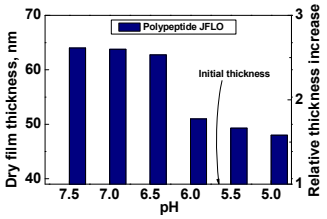

Figure 1. Effect of pH on retention of JLFO within $(PMAA)_{10}$ hydrogels. Concentration of NaCl was 0.2 M.

Conclusions: PMAA hydrogel thin films are capable of loading large amounts of the polypeptide JLFO. The JLFO-loaded hydrogels did not leach the antibacterial agent at physiological pH and were highly resistant to attachment of *S. epi* bacteria. Release of JLFO could be then triggered by lowering the solution pH.

References:

1. Peppas, N.; Leobandung, W.J. Biomat. Sci. Polymer Edn, 2004, 15, 125.
2.Kharlampieva, E.; Erel-Unal, I.; Sukhishvili, S.A. Langmuir, 2007, 23, 175.
3.Macdonald, M.; Rodriguez, N.M.; Smith, R.; Hammond, P.T. J. Controlled Release , 2008, 131, 228.