Antimicrobial Peptide Delivery from Degradable Polymer Thin Films

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Abstract— The overuse of antibiotics has led to a rise in antibiotic resistance of common hospital dwelling bacteria such as *Staphylococcus aureus*. Novel broad-spectrum antimicrobials that operate without further contributing to a rise in antibiotic resistance are needed to tackle this problem. The effective delivery of antimicrobial peptides, is of interest due to their wide range of activity against gram positive and gram negative bacteria, as well as low onset of bacterial resistance. Our work focuses on using polyelectrolyte multilayer films for the delivery of these novel therapeutics targeting *S. aureus* infections. Such constructs could easily be implemented in localized delivery systems, applied directly to a wound site. We have examined hydrolytically degradable layer-by-layer constructed films for the delivery of an antimicrobial peptide, ponericin G1. This peptide exhibits a low *S. aureus* minimum inhibitory concentration, as well as low blood cell lysis. Poly(β-amino esters), containing hydrolysable ester bonds, have been incorporated into multilayer films in order to controllably deliver functional doses of ponericin G1 over the desired release time scales. Current results show this technique to be highly effective in delivering functional ponericin over many days. The exact release profiles show a strong dependence on the polyanions used during film construction.

I. Introduction

Large doses of broad spectrum antibiotics are often required for an effective treatment of bacterial infection. The overuse of such antibiotics has been accompanied with a rapid increase in drug resistant bacteria, such as *Methicillin Resistant Staphylococcus aureus* (MRSA). Treatment possibilities slowly become exhausted as bacteria gain further resistance to more potent drugs. Localized delivery rather than systemic delivery of novel drugs that do not cause bacterial resistance to arise is very desirable. Examples of such a class of therapeutics are antimicrobial peptides. These highly potent, broad spectrum peptides are part of the eukaryote innate immune system and display a wide range of activity against gram positive, gram negative, and drug resistant bacteria [1].

Layer-by-layer assembled thin films can be used to create a system capable of sustained delivery of therapeutic doses of antimicrobial peptides that can be administered locally. In this technique, the sequential adsorption of complementary charged polymer groups onto a charged substrate, leads to the electrostatically driven buildup of a polymer thin film [2]. Charged drugs (such as an antimicrobial peptide) containing layers can also be assembled in these architectures. Film architectures and construction conditions can lead to highly tunable incorporation of a large variety of drugs in these films. In this work, we have attempted to use layer-by-layer assembled thin films to demonstrate sustained delivery of functional antimicrobial peptide, ponericin G1, found in the venom of predatory ants. This peptide has a low minimum inhibitory concentration against *S. aureus* of 16-32 μM [3]. Hydrolytically degradable poly(β-amino esters) were incorporated in the film to attempt a surface-erosion based release of the drug rather than primarily diffusion based release, demonstrating control over drug release profiles [4,5].

II. Experimental Methods

Film deposition

All films were deposited on silicon, which was cleaned with methanol and DI-H_2O and plasma etched. Film construction followed the following tetralayer architecture: (poly(β-amino ester)/polyanion/ponericin G1/polanion)$_n$, where n represents the number of tetralayers deposited. Glycosaminoglycans and biocompatible polymers were utilized as polyanions.

Release characterization

Films were released in phosphate buffered saline at 37ºC. Micro-BCA assay, as well as fluorescence measurements, was used to examine the presence of peptide in the release samples.

Bactericidal activity

Film release samples were used in a macrodilution assay against *Staphylococcus aureus 25923* [6]. All tested solutions were passed through a 0.2 μm sterile filter.

III. Results and Discussion

In all of the films constructed it was found that the growth mechanism of the films largely correlates with the amount of ponericin incorporated as well as the ponericin release profile, which persisted over many days. Thick films with exponential growth profiles incorporate a larger amount of therapeutic compared to films with linear growth profiles. As has previously been noted, it is expected that a large portion of the drug resides in the top layers of these exponentially growing films. Here, we would expect to see a burst release of drug. When exposed to aqueous solutions, the large amount of drug rapidly diffuses down its concentration gradient out of the film

and into the solution. This burst can be helpful in the case of bacterial infection, serving to eradicate the problem early on.

It should also be noted that all film released solutions that were tested for bactericidal activity, maintain their activity against *Staphylococcus aureus*. The film deposition and release strategies do not inactivate the peptide, a demonstration of the benefits of the layer-by-layer deposition process versus other film assembly techniques.

IV. CONCLUSIONS

In this work, we have demonstrated the effective incorporation and release of a broad spectrum antimicrobial peptide, ponericin G1, from hydrolytically degradable polyelectrolyte thin films. The activity of the released therapeutic against *S. aureus* is maintained. The uses of various film architectures in the layer-by-layer deposition technique demonstrate different film growth and release mechanisms.

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REFERENCES

[1] C. Loose, K. Jensen, I. Rigoutsos, and G. Stephanopoulos, "A linguistic model for the rational design of antimicrobial peptides," *Nature*, vol. 443, pp. 867-869, 2006.

[2] G. Decher, "Fuzzy nanoassemblies: toward layered polymeric multicomposites," *Science,* vol. 277, pp. 1232-1237, 1997.

[3] J. Orivel, et al., "Ponericins, new antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*," *The Journal of Biological Chemistry,* vol. 276, pp. 17823-17829, May 2001.

[4] J. Zhang, N. J. Fredin, J.F. Janz, B. Sun, and D.M. Lynn, "Structure/property relationships in erodible multilayered films: influence of polycation structure on erosion profiles and the release on anionic polyelectrolytes," *Langmuir,* vol. 22, pp. 239-245, 2006.

[5] D.M. Lynn and R. Langer, "Degradable poly(β-amino esters): synthesis, characterization, and self-assembly with plasmid DNA," *Journal of the American Chemical Society,* vol. 122, pp. 10761-10768, 2000.

[6] A.L. Barry, et al., "Methods for determining bactericidal activity of antimicrobial agents: approved guideline," *National Committee for Clinical Laboratory Standards*, vol. 19, pp. M26-A, 1999.