Multilayer films addressing bleeding and infection

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Statement of Purpose: Among military casualties, exsanguinating hemorrhage (i.e. bleeding out) is the leading cause of death (Kelly 2007). We have recently developed a hemostatic dressing with layer-by-layer (LbL) assembly for administration of thrombin (Shukla 2012a). In addition to hemorrhage, the leading cause of morbidity of those soldiers surviving beyond the first few hours is infection (Zapor 2005). Here we report the development of a film that incorporates both a hemostat (thrombin) and an antibiotic (vancomycin) with uniquely controlled release profiles such that thrombin is released rapidly while vancomycin is released in a sustained manner.

Methods: Thrombin (Biopharm Laboratories) and tannic acid (Sigma Aldrich) were deposited from 1 mg/mL and 2 mg/mL solutions, respectively, in 10 mM sodium phosphate buffer, pH 7.4. Films were assembled utilizing the LbL technique where a single bilayer of $(\text{thrombin/tannic acid})_n$ consisted of immersion for 15 minutes in thrombin solution, 10 s, 20 s, and 30 s washes in H_2O, then 15 min in tannic acid solution, and then washes. Bilayers of $(\text{PLL/PMLA-Vanco})_n$ were constructed similarly from 1 mg/mL solutions of PLL and PMLA-Vanco. Conjugates of vancomycin to poly(β-L-malic acid) (PMLA-Vanco) were synthesized by previously described methods (Greenwald 2003 and Ding 2011). Degrees of conjugation were assessed by quantification of the fluorescence (λ_{ex} = 280nm; λ_{em} = 355 nm). Therapeutic release was performed in physiologically relevant conditions of PBS, pH 7.4 at 37°C. Vancomycin concentration was determined via HPLC (Shukla 2012b) and thrombin concentration was determined by the time to coagulation of a fibrinogen solution (Sigma Aldrich) by a ST4 coagulation analyzer. Antibacterial efficacy against *Staphyloccocus aureus* (ATCC 25923) was determined by a turbidity assay as previously described (Shukla 2012b).

Results: To combat hemorrhage and infection, we aimed to create specifically tuned release profiles that address each issue. We created a composite film by directly assembling a $(\text{thrombin/tannic acid})_{25}$ film atop a $(\text{PLL/PMLA-Vanco})_{80}$ film.

Hemostatic Film. Growth behavior of the (thrombin/tannic acid) film was characteristic of a linearly growing film, where films increase rapidly as a function of bilayers (Figure 1). Total coagulation activity of thrombin loaded in these films ranged between 8 and 14 U/cm^2, depending on the number of bilayers.

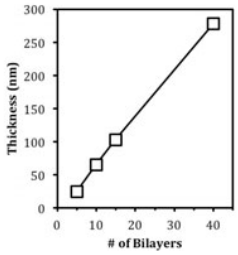
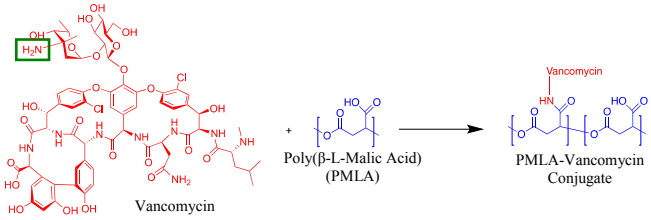


Figure 1. Growth profile of $(\text{thrombin/tannic acid})_n$ films

Antibiotic Film. Because of the pH sensitivity of thrombin, we developed a new LbL architecture capable of assembly at physiological pH by conjugating vancomycin to PMLA through an amide bond between the former's disaccharide amine (Scheme I, green box) and the latter's pendant carboxylic acid. We found that MIC values between the PMLA-vanco conjugate (1.2 μg/mL) and PMLA-vanco conjugate released from $(\text{PLL/PMLA-vanco})_{80}$ films (1.8 μg/mL) were comparable to the free drug (1.1 μg/mL).



Scheme I. Vancomycin conjugation to PMLA

Composite hemostatic and antibiotic film. In combining the hemostatic and antibiotic films, we found that thrombin was rapidly released while vancomycin release was controlled to multiple days.

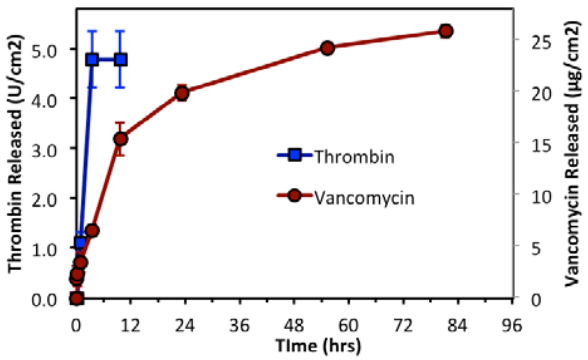


Figure 2. Release profiles of thrombin and vancomycin from composite films.

Conclusions: Wounds sustained in the battlefield can be sources of great mortality and morbidity. We aimed to address the uncontrollable hemorrhage by employing a rapid thrombin release film, while fighting infection with a sustained release vancomycin film. To overcome the challenge of incorporating two uniquely different types of molecules (a 72kDa enzyme and 1.5kDa small molecule), we chemically attached the latter, vancomycin, to a hydrolytically degradable polymer backbone. This allows us to assemble a robust LbL film with two distinct release profiles that uniquely address their unique requirements.

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