# Resorbable, bilayer polymer membranes for multi-modal drug release

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**INTRODUCTION:** The use of resorbable membranes to facilitate bone tissue regeneration has been well-established.[1] The design objective of such membranes in the past has been primarily to serve as a physical barrier to a) prevent infiltration by non-osseous tissue, b) contain component-leakage from the defect site, and c) direct osteoregeneration as scaffolding for periosteum tissue.[1-2] While capable in maintaining the aforementioned objectives, the potential of these membranes for enhanced bone healing due to targeted drug delivery has been underutilized. In particular, infection at potential bone graft site and necrotized tissue remains a concern in conjunction with a desire to increase regeneration rate through the use of osteogenic growth factors. Therefore, a need exists for the synthesis of a multi-modal drug release platform that maintains the essential properties of a physical barrier for optimized bone tissue regeneration. Models for different multi-layered polyelectrolyte coatings on poly(lactic-co-glycolic acid) films for front-loaded vancomycin release and film-embedded polylysine release were examined in this study.

**METHODS:** Fluorescein isothiocyanate (FITC)-labeled Poly-L-lysine (MW 7493, Sigma) was embedded in single-layer films by solvent casting technique. Briefly, poly(lactic-co-glycolic acid) [PLGA, LA:GA at 75:25, Lactel Absorbable Polymers] was dissolved in a mixture of acetone and DMSO. FITC-labeled Poly-L-lysine peptide was added to the mixture in a ratio of 1:5000 peptide to PLGA and sonicated. Subsequently, the formulation was applied to glass wells and dried at 37°C for 5 hours, then at 50°C under -25 mmHg vacuum for 12 hours. The films were then cut to size after cooling by a 14 mm diameter metal punch. Blank films were similarly prepared, with water replacing peptide solution during the loading step. Peptide-loaded films were examined for release profile over a 54 day period. Films were incubated in 400 ul PBS at 37°C under static conditions. Buffer was collected and replaced at time 0 hrs, 3 hrs, 6 hrs, 12 hrs, 24 hrs, and then every 24 hrs until the end of study. The amount of peptide released was analyzed by fluorescence spectrometry.

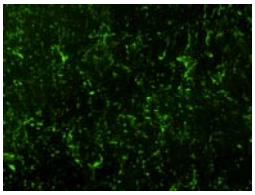

**Figure 1.** FITC-labeled Poly-L-lysine peptide (green) embedded in PLGA film observed fluorescent microscopy. Polylysine demonstrates primarily homogenous distribution.

Vancomycin (Vanc)-loaded films were prepared by layer-by-layer (LBL) coating technique. Blank PLGA films were initially coated with the polyelectrolyte primer polyethyleneimine. Two surface-coating methodologies were subsequently examined by varying the pH of polyanion polyacrylic acid (PAA) and the polycation polyallylamine hydrochloride (PAH) to vary the amount of incorporated vancomycin. Layered constructs consisted of four layers of PAA/PAH and five layers of PAA/Vanc/PAA/PAH. Blanks were synthesized by substituting PAH for Vanc in the methodology. The release profiles were examined in PBS at 37°C at every 24 hours for 6 days. Vancomycin amount released was analyzed by absorbance spectrometry.

**RESULTS:** When examining polylysine release (Fig 2a), an initial burst release was observed in the initial hours, with subsequent continuous base-line release till successive secondary and tertiary burst releases. Cumulatively, the continuous release resulted in a linear release profile, while the latter showed as an exponential rise (Fig 2b). Maximal cumulative release, thus, was observed at around 44-48 days, at a time when the films demonstrated the most fragmentation. This is highly suggestive of release being tied to bulk degradation of the polymer.

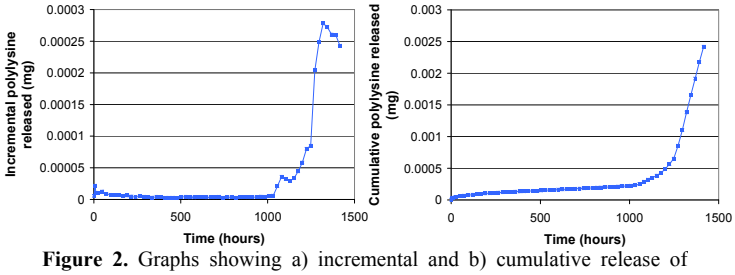


**Figure 2.** Graphs showing a) incremental and b) cumulative release of polylysine from PLGA films over 59 days.

Vancomycin release was compared against drug coated with PAA at pH 7.1/PAH at 4.5 (Vanc 1) and PAA at 3.5/PAH at 7.5 (Vanc 2). The profile suggested front-loading of vancomycin, with an initial burst release, followed by gradual reduction in release after the first day to baseline levels by day 3, where it remained thereafter (Fig 3). Absorbance-based release results from blanks indicate possible interference from polyelectrolyte film by delamination. Vanc 2 demonstrated significantly greater release (15 ug/ml) than either blanks and an observed better vancomycin release than Vanc 1.

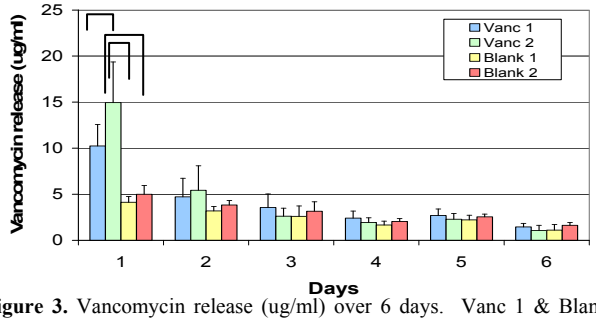


**Figure 3.** Vancomycin release (ug/ml) over 6 days. Vanc 1 & Blank 1 groups were coated with PAA at pH 7.1/PAH at 4.5 and Vanc 2 and Blank 2 groups with PAA at 3.5/PAH at 7.5. Statistical differences are grouped

**CONCLUSIONS:** Polylysine released from PLGA films occurred in a burst releases with maximal release occurring at the latter stages of the experiment, suggestive of degradation-release mechanism. Vancomycin release followed a front-loaded scheme with maximal release at 24 hours. Such a release profile is desirable with recent publications suggesting the effectiveness of similar treatment.[3] Future work must investigate the synergestic release of polylysine and vancomycinn from true bilayer-films.

**REFERENCES:**

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