

***In vitro* release kinetics of bupivacaine and trans-2-decenoic acid from electrospun chitosan membranes with direct acylation of 2-decenoic acid**

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Statement of Purpose: Musculoskeletal infections remain the main cause of hospitalization after combat and non-combat-related injuries in the U.S. military due to antimicrobial-tolerant biofilm formation. Therefore, there is a need to provide infection prevention and pain relief immediately after injury and until hospitalization. We have developed novel bio-polymer membranes made of electrospun chitosan membranes acylated with 2-decenoic acid (ECX), an inhibitor of *S. aureus* biofilm. After stabilization with covalent attachment of trans-2-decenoic acid (T2DA), we hypothesize that T2DA will be released slowly and released more rapidly in acidic environments mimicking wounds.

Methods: Preliminary release studies were conducted on hexanoyl chloride-modified chitosan (HC), ECX, and commercially available gauze. The experimental groups were HC loaded with Bupivacaine, HC loaded with T2DA, gauze loaded with Bupivacaine, gauze loaded with T2DA, ECX loaded with Bupivacaine, and ECX (n=3). Loading concentrations were 0.15 mg for Bupivacaine and 0.5 mg for T2DA. Experimental groups were placed in a sterile 90% phosphate-buffered saline (PBS) and 10% bovine serum. The release kinetics of both therapeutics were evaluated with eluates collected every 3 hours up to 12 then every 12 hours for a total of 72 hours. High-performance liquid chromatography (HPLC) was used to assess the release of therapeutics from the membranes. The release of therapeutics was also assessed in a 5-pH acetate-buffered saline (ABS).

Results:

Bupivacaine-loaded groups (figures A, B) showed a substantial burst release at the first time point. The release in the acidic media was also larger than that of the neutral-pH media. This could be due to the higher solubility of Bupivacaine in acids. Burst release of T2DA occurred in gauze and to a lesser extent in HC as they eluted 64 µg and 20 µg of T2DA by the 3-hour timepoint, respectively (figures C, D). ECX consistently eluted approximately 8 µg of T2DA at every time point without the initial burst. Furthermore, the percentage of T2DA eluted by ECX at the 72-hour timepoint is 40% of the loaded amount and is less than the other groups, making it potentially suitable for releasing therapeutics at a sustained rate for longer periods than 72 hours. Similar to Bupivacaine, T2DA released in ABS by the end of the 72 hours is more than that of PBS,

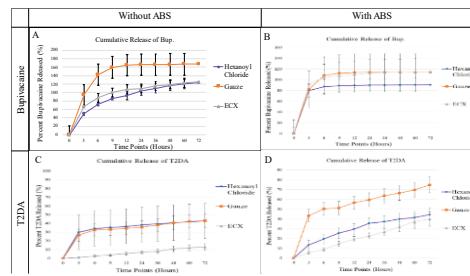


Figure 1. Graphs of the release kinetics of Bupivacaine and T2DA with and without ABS. (Figure A, B) cumulative release of Bupivacaine over 72 (Figure C, D) cumulative release of T2DA over 72 hours (n=3). Data is represented as mean \pm standard deviation.

implying that therapeutics would be released at a higher rate when the wound is infected and has a lower pH. Our results indicate that covalently attaching T2DA to chitosan addresses the issue of “burst release” allowing for zero-order drug release kinetics. Repeating the elution study in a 5-pH media revealed that therapeutics elute more quickly in acidic environments such as infected wounds.

Conclusions: Covalently bound T2DA eluted at a consistent rate from ECX and did not display the initial burst release of T2DA displayed by the HC and gauze at the first timepoint indicating that ECX addresses the challenge of burst release and the inability to load therapeutics within the acylated nanofibers. The increased burst of bupivacaine from the 3 groups implies that some of the molecules were on the surface of the membrane and not contained within the acylated chitosan nanofibers. ECX consistently released Bupivacaine until the last timepoint. The cumulative release of Bupivacaine shows that over 100% of the loaded therapeutic was released. This error may be due to errors in standard solutions, standard curve fitting, or poor detection of Bupivacaine by the HPLC.

References: Wells, C. M., et al. (2021). "Synthesis and Characterization of 2-Decenoic Acid Modified Chitosan for Infection Prevention and Tissue Engineering." *Mar Drugs* 19(10).

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