Manuka Honey/PCL Microparticles as a Bioactive Treatment for Osteomyelitis

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INTRODUCTION: Osteomyelitis, a bone infection caused by pyogenic organisms like bacteria, fungi, and mycobacteria, occurs when the bone is exposed to a substantial bacteria inoculum, ischemia, foreign objects, or trauma [1]. Adhesions, such as those from *Staphylococcus aureus*, attach to bone matrix components [2]. The primary course of treatment for osteomyelitis is antibiotics tailored to the specific bacterial culture, where bony debridement surgery is often essential. However, the pressing issue of antimicrobial resistance and tolerance exhibited by bacteria in antibiotic treatment results in treatment failure in ~20% of cases [3]. These challenges highlight the need for minimally invasive alternative treatments of osteomyelitis. In the field of tissue engineering, electrospraying is employed to create microparticles for sustained drug release. Electrosprayed microparticle delivery offers advantages including biodegradability, biocompatibility, sustained release, and improved efficacy [4]. To increase bioactivity, various additives can be explored and released from the shell of these microparticles. Honey is an antibacterial agent that has been used in medicine for ~8000 years, where Manuka honey (MH) contains the Unique Manuka Factor (UMF), which correlates to honey's antibacterial efficiency. In this study, solutions of MH/ethanol (EtOH), as well as MH/acetic acid (AA) were encapsulated within polycaprolactone (PCL) and chloroform using coaxial electrospraying to form microparticles. We *hypothesize* that microparticles fabricated using PCL and MH as the shell and core, respectively, will provide tailorable, local treatment to mitigate inflammation and infection, reducing reliance on antibiotics for osteomyelitis.

METHODS: Despite published work by our group and others utilizing MH as an antibacterial agent, no work has fabricated a delivery method using microparticles. In this study, two versions of PCL-MH microparticles were fabricated using coaxial electrospraying (Fig 1). First, 1% PCL in 10 mL chloroform (shell) and 5% MH dissolved in 95% EtOH/water (core) were combined to create core-shell microspheres. Here, the EtOH was used to improve the conductivity of the core solution. In the second group, 1% PCL in 10 mL chloroform (shell) and 5% MH was dissolved in 4% AA/water (core) to create core-shell microspheres. Here, the addition of AA was also used to improve core solution conductivity. A high voltage power supply was applied, flow rates of 0.5 mL/h for the core and 0.75 mL/h for the shell were used, and a voltage of ~9.5 kV was applied. Both particle groups were electrosprayed into a solution of 0.1% stirred polyvinyl alcohol (PVA)/water, followed by a process of cooling, centrifuging, washing, and lyophilization (Fig 1). Both types of microparticles were imaged using scanning electron microscopy (SEM; Tescan Vega3) and light microscopy to quantify average particle size. Ongoing work is focused on atomic force microscopy (AFM) to characterize the particle surface and provide additional information on size. Assessment of sheer stress on the particles by extrusion through a 15-gauge needle is also under analysis to quantify injectability. Finally, incubation in phosphate buffered saline (PBS) and a glucose assay will provide a quantitative MH release profile.

RESULTS: Recently published work from our group has illustrated that 5% MH has improved bactericidal effects against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. sanguinis* shown through bacterial clearance testing in cryogels. Prior to testing the addition of AA and EtOH, all microparticles were fabricated with only 5% MH dissolved in water for the core and 1% PCL in chloroform for the shell. Despite the usage of voltages as high as 23.5 kV, the MH solution proved to not be sufficiently conductive, evidenced by the MH leaking out of the PCL shell and a generally unstable Taylor cone. In order to improve conductivity, AA solutions and EtOH solutions were mixed with MH. To demonstrate this, 5% MH dissolved in 4% AA/water was sprayed with an average particle size of 24.0 μm and collected with a stable Taylor cone (Figs 2A and 3A). Note that 5% MH dissolved in 5% AA/water was also sprayed, but resulted in a liquid instead of particle formation and was thus could not be collected. The other solution consisting of 5% MH dissolved in 95% EtOH/water with 1% PCL in chloroform was successfully sprayed at an average particle size of 6.1 μm and collected with a stable Taylor cone (Figs 2B and 3B).

DISCUSSION: These results demonstrate that coaxial electrospraying can be used to create microparticles as effective additive (MH) delivery systems. Current studies indicate that coaxial electrospraying is a better biodegradable alternative due to reduced initial burst release, improved absorption, and higher encapsulation efficiency than emulsification. Ongoing work is focused on verifying sizing and morphology of the particles, as well as degradation. Future work will be focused on parameter and size optimization, as well as verifying similar bacterial clearance, adhesion, and biofilm formation to what our group has previously demonstrated.

SIGNIFICANCE: This novel delivery of MH-PCL microparticles has the potential to be applied to a variety of bacterial infections where antibiotic resistance is a growing concern, especially related to bone infections. These experiments represent a less-invasive, non-antibiotic centered approach that will contribute to current studies focusing on controlled and extended release drug delivery mechanisms.

REFERENCES: 1. Gimza. *Front. Immunol.*, 2021. 2. Jha, *Cureus*, 2022. 3. *Osteomyelitis (StatPearls)*, 2023. 4. Sridhar, *Biomatter*, 2013.

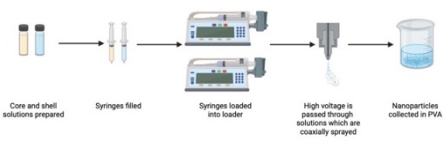

Figure 1: Coaxial electrospraying general procedure

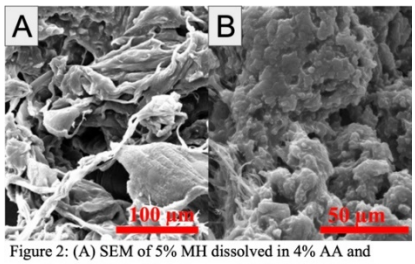

Figure 2: (A) SEM of 5% MH dissolved in 4% AA and water in 1% PCL in chloroform and (B) SEM of 5% MH dissolved in 95% EtOH and water in 1% PCL in chloroform.

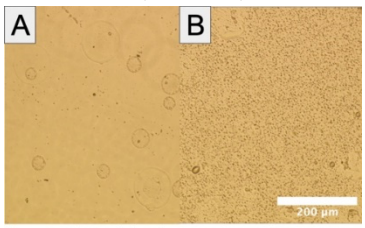

Figure 3: (A) 20x light microscope image of 5% MH dissolved in 4% AA and water in 1% PCL in chloroform and (B) 20x light microscope image of 5% MH dissolved in 95% EtOH and water in 1% PCL.