A Multifunctional Electrospun Wrap to Treat Infection and Enhance Bone Regeneration

Sarah Jones[1], Taneidra Buie[1], Annika Balakrishnan[1], Evan He[1], Joseph Wenke[2], Elizabeth Cosgriff-Hernandez[1]
1. Department of Biomedical Engineering, The University of Texas at Austin 2. Department of Orthopedic Surgery and Rehabilitation, The University of Texas Medical Branch

Statement of Purpose: In the Masquelet technique, a bone cement spacer is used to preserve the defect site during decontamination and soft tissue remodeling. The subsequent membrane formation is critical for bone healing, as it serves as a barrier to slow rapid graft resorption associated and provides regenerative cues to promote a better wound healing environment. Current hurdles in this technique are persistent infection that requires revision surgeries and/or prevents bone consolidation and receding membrane vascularity. We aim to develop a wrap for the spacer that can sustain infection control and enhance membrane vascularity to improve the environment guiding bone formation. To this end, a multifunctional wrap is electrospun with two distinct fibers. The first fiber population will be a reinforcing polyester fiber that releases antibiotics and improves membrane handling properties. The second fiber population is electrospun from decellularized tissue matrix that has been shown to increase vascularization, stem cell recruitment, and regenerative macrophage polarization by releasing vesicular growth factors and natural protein degradation products. By co-electrospinning the decellularized tissue matrix with the loaded polyester fibers, we will generate a durable, antimicrobial wrap that can concurrently enhance vascularity and regenerative properties in the induced membrane.

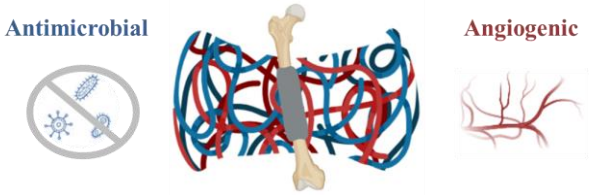

Figure 1: Schematic of dual-fiber bone wrap.

Methods: Poly(lactic-co-glycolic acid) (PLGA) was dissolved in hexafluoroisopropanol (HFIP) with the antibiotic gentamicin sulfate and electrospun to form a wrap at a 0.7 mL/h flow rate and +12kV charge. Release kinetics of gentamicin *in vitro* were determined by soaking the wraps in DI water, collecting release over 6 weeks, and quantifying with a ninhydrin assay. The *in vivo* antimicrobial activity was assessed in a contaminated traumatic fracture model in Sprague Dawley rats. The wrap was placed around the defect for 2 weeks, then bacterial load was quantified by plating supernatants of tissue and counting resultant colony forming units (CFUs). For the bioactive fibers, porcine small intestine submucosal layer (SIS) was isolated via freeze/thaw and mechanical delamination and decellularized via established methods.[1] The resulting SIS was dried, homogenized, and sieved to isolate particles from 90 – 425 µm, then dissolved in HFIP and homogenized or dissolved in HFIP and acetic acid. SIS fibers were electrospun with a 0.7mL/h flow rate and 14kV charge. The angiogenic capacity was evaluated via a tube formation assay. An SIS sheet was soaked in basal media (0.5% FBS in EBM-2) for 7 days. Human umbilical vein endothelial cells (HUVECs) were cultured (1.2E5 cells/cm^2) on Matrigel in SIS-conditioned media, stained with Calcein AM, and imaged with fluorescence. Resulting tubes were counted with respect to area.

Results: A gentamicin-loaded PLGA wrap was electrospun with a 2.02 ± 0.44 µm fiber diameter. The wrap released gentamicin *in vitro* above the minimum bactericidal concentration (MBC = 1.5 µM) for 6 weeks. The wrap also reduced bacterial load around a contaminated bone defect below the threshold known to inhibit bone healing (10^3 CFU/g) after 2 weeks, **Figure 2a,b**.[2] Preliminary studies have been conducted to fabricate an electrospun wrap composed of SIS and evaluate its angiogenic capacity. As an initial indication of the angiogenic capacity, SIS-conditioned media resulted in a significant increase in endothelial cell tube formation compared to a negative control and was similar to a positive VEGF control, **Figure 2c,d**. Current studies are identifying electrospinning parameters to spin the decellularized tissue matrix using a combination of tissue homogenization, tissue particle size restriction, and solution homogenization and acidity.

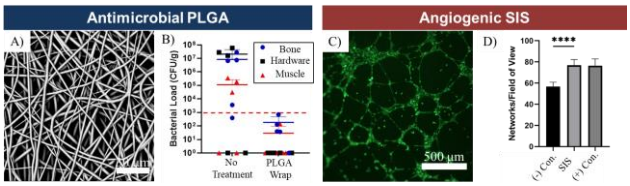

Figure 2: A) PLGA wrap B) Bacterial load with and without PLGA wrap C-D) HUVEC tube formation in SIS-conditioned media

Conclusions: These findings demonstrate the potential of developing a wrap that can impart long-term infection control and enhance membrane vascularization to ultimately improve bone healing in critical-sized bone defects. A PLGA fibrous wrap has been shown to support long-term antibiotic release, and preliminary SIS studies have shown *in vitro* angiogenic capacity with initial efficacy in electrospinning into a fibrous wrap. Future studies are focused on co-electrospinning both the PLGA and SIS fibers into one cohesive wrap with antimicrobial and angiogenic properties with the goal of improving clinical outcomes of the Masquelet technique.

References: (1) Ji, Y., et al. J Biomed Mater Res A 2019, 107(3), 689-97; (2) Buie, T., et al. J Biomed Mater Res A 2021, 109(11), 2255-68;