Evaluating the Efficacy of a Multicomponent Wound Dressing for Simultaneous Antibacterial Activity and Moisture Management in an *In-Vitro* Wound Model

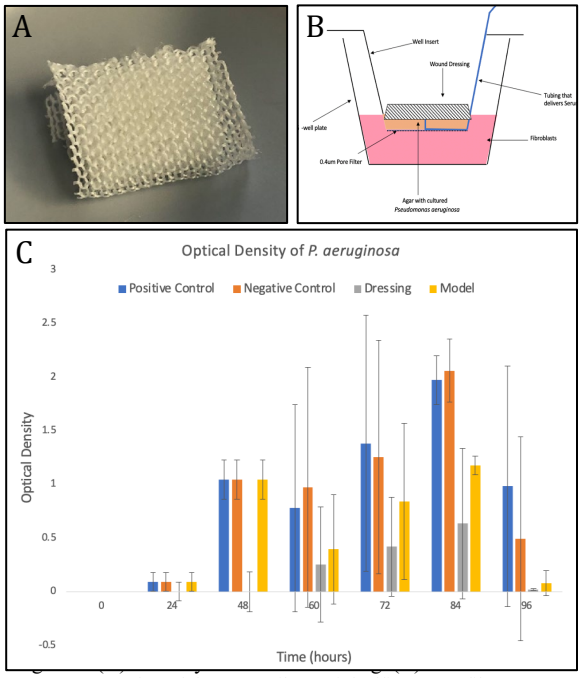
Elizabeth Gianino, Jordon Gilmore
Department of Bioengineering, Clemson University

Statement of Purpose: Basic research in understanding the complex mechanisms underlying changes in inflammation and wound healing associated with diabetes and peripheral neuropathy currently relies heavily on *in vivo* models[1]. However, this method proposes some ethical concerns related to a lack of verification and validation testing before animal trials which lead to the potential for animal subject waste and high expense. For wound healing and diabetic models, in particular, there is a strong need for implementing *in vitro* wound models for controlling and understanding key variables in chronic inflammation, while reducing ethical concerns surrounding premature animal trials.

The objective of this aim is to design and develop a basic standardized *in vitro* wound model for testing of a multi-component biomaterial wound dressing that examines the cross talk between healthy fibroblasts and *Pseudomonas aeruginosa.* This multi-level analysis includes the evaluation of changes in oxidative stress, antibacterial efficacy, moisture absorption and wicking, and metabolic activity for healthy fibroblast proliferation.

Methods: The model consisted of a 3T3 fibroblast cell culture to act as a baseline skin substitute. Infection was introduced to the fibroblasts using a substrate of *P. aeruginosa* on a fine layer of agar placed in a transwell insert. The pores of the insert were 0.4 μm to initiate a crosstalk between both cell lines without direct contact. Vascular function was modeled using 0.03 inner diameter tubing to deliver porcine serum as a surrogate for constant wound exudate production. The multi-phasic wound dressing was composed of an antibacterial Poly-ethylene glycol (PEG):Poly-l-lactide (PLLA) contact (woven) layer, a polyurethane (PU) absorptive layer, and two PLLA moisture wicking textile layers. Dressing efficacy was determined after 48 hours in co-culture with respect to antibacterial activity by measuring inhibition of *P. aeruginosa*, moisture absorption, oxidative stress via Amplex™ Red assay, and fibroblast viability via AlamarBlue™ and MTT™ assays.

Results: An image of the multicomponent, medical textile and absorptive wound dressing and an illustration of the *in-vitro* wound model can be seen un Figures 1A and 1B, respectively. The dressing appeared to maintain bacterial inhibitition by more than 40% of both controls throughout 48 hours of administration (Figure 1C). The combination of the PU foam and two moisture wicking layers maintained absorption for 44 hours before implementing evaporative properties. Finally, there is an overall upward profile of cell viability for both assays for the first 36 hours of administration.



in-vitro wound model. (C) Antibacterial efficacy profile.

Conclusions: This study explored the efficacy of our multiphasic wound dressing by looking at several variables simultaneously necessary for optimal chronic wound healing without the need for premature animal and *ex-vivo* modeling. There remains some fine-tuning of both the model, such as exploring the cross-talk between biofilm formation and fibroblast growth for both cultures. Outcomes of this work suggest the potential in the design and development of our multiphasic wound dressing and its ability to stop and prevent infection, absorb excess moisture, and promote the start of cell proliferation for repair and remodeling.

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References: [1] Gardiner NJ., et al. *International Review of Neurobiology*. 2016, 127:53-87