Biofilms in Preclinical Testing – A Robust and Innovative *in Vitro* and *in Vivo* 3D Printed Model System

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Biofilms are known to stall the wound healing process. Researchers, and wound clinicians have studied biofilms for decades. Research has been performed utilizing *in vitro* and *in vivo* models of wound healing and biofilms. However, creating a reliable and reproduceable model to study these robust, persistent, and hard to kill biofilms proves to be challenging.

Coenye and Nelis (2010) outlined numerous *in vitro (*microtiter plate-based model, flow displacement biofilm model, and the cell-culture based model) and *in vivo* (Caenorhabditis elegans model and a multitude of vertebrate animal infection models) model systems for the testing of biofilms.[1] Coenye and Nelis go on to review *in vitro* wound biofilm models such as the Lubbock chronic wound biofilm model and Graftskin models which mimic what is seen in chronic wounds, and *in vivo* wound biofilm models in which porcine and rodent, partial and full thickness, wounds are innoculated with bacteria to form biofilms. More recently, Yang et al., (2013, 2016) out of Greg Schultz's laboratory developed a porcine ex vivo model to study mature biofilms.[2,3]

With so many model systems to choose from, deciding which is best for testing can be difficult. However, many biofilm models are either immature biofilms or do not provide reproducible results. Thus, there is a critical need for a reliable and reproduceable model system to study biofilms *in vitro* and in chronic, hard to heal wounds.

In our lab, we have developed such a method, a 3D printed polymicrobial biofilm model.[4] Our 3D printed biofilms have 10^7 CFU (*S. aureus* and *P. aeruginosa)* per 5 mm disk, are resistant to antibiotic and antimicrobial treatments, have less than a 2% standard deviation in colony forming units (CFU) among replicates, and can be employed for both *in vitro* and *in vivo* testing. We have demonstrated results that are reproducible *in vitro* utilizing fluorescent biomarkers of poly-*N*-acetylglucosamine (PNAG) to measure the integrity of the biofilm, as well as the fluorescent live/dead ratio of the bacteria within the biofilm.

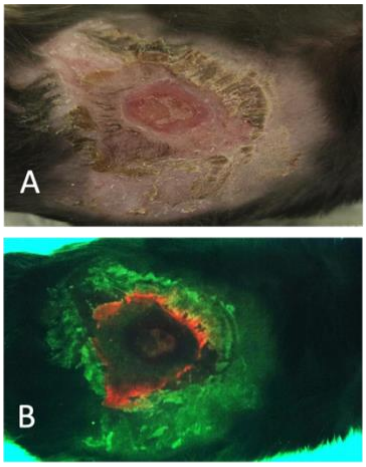

Figure 1. 3D Printed Biofilm Model
A: DB Mouse Digital Wound Image,
B: Moleculight® i:x Image of Biofilm

Additionally, our 3D printed polymicrobial biofilms have proven to be reliable and reproducible *in vivo* in a diabetic mouse delayed healing model. The 3D printed polymicrobial biofilms were implanted and absorbed into freshly created 10 MM wounds and within a week's time the biofilm spread across the entire wound bed. In the diabetic mouse model, we found that the biofilms were persistent, and regrew, even after sharp debridement. A Moleculight® i:x Device, which can detect infection up to 8 MM below the skins surface, was used to fluorescently image the wounds, and the imaging suggested that the biofilms created a deep tissue infection in this delayed diabetic mouse wound healing model which is similar to the clinical observations of ring of bacterial biofilms ("**Ring of Fire**") of the callous tissue in the periwound bed in diabetic foot ulcers reported by Drs. Armstrong, Edmonds, and Serena (2023).[5]

With such promising, reliable, and reproducible data, both *in vitro* and *in vivo,* our 3D printed biofilm model proves to be a new standardized method for studying polymicrobial wound infections with persistent biofilms. It is our goal that this model will assist clinical scientists to develop targeted therapies to disrupt the biofilms to provide better healing outcomes for patients.

References

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