In vitro and *ex vivo* antibiofilm testing of a controlled release antibacterial wound protectant

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Statement of Purpose: Bacteria in nature primarily reside in a biofilm phenotype [1]. Biofilms respond differently than planktonic cells to antimicrobial treatments and are linked to chronic and antibiotic-resistant infections [2, 3]—particularly in austere environments where resources to treat infected wounds can be limited. The aim of this project was to evaluate an antibacterial wound protectant for use as a drug delivery device against local biofilm-related infections following battlefield-relevant trauma. *In vitro* and *ex vivo* assays were used to screen antibiofilm efficacy of moxifloxacin-loaded formulations.

Materials and methods: Formulation development and characterization was conducted by DePuy Synthes. Final formulations consisted of solids: cholesterol (Chol, Spectrum), hydrogenated castor oil (HCO, BASF), and glyceryl mono- and di-stearates (GMSII, BASF); and oils (CRODA): soybean oil (Soy), glyceryl monocaprylocaprate (GMCC), and oleic acid (OA). Screening of orthopaedic and military relevant antibiotics was conducted at the University of Utah. Moxifloxacin was selected and loaded at varying concentrations up to 10% w/v. *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 27853 biofilms were grown on sanded borosilicate glass spheres in a 'bead' reactor (Fig. 1).

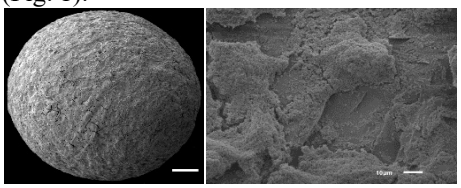

Figure 1: (Left) Scanning electron microscopy image of a roughened borosilicate glass bead. (Right) *S. aureus* biofilms on the surface of a glass bead.

For *in vitro* gel studies, ~0.2 ml of formulation was spread evenly across the base of a 6-well plate. Six mature biofilm beads were added with 2 ml of 50% BHI broth. For *ex vivo,* fresh ovine tissue was placed into the wells of a 6-well plate and ~0.2 ml of gel was added on either side. 3 biofilm beads and 2 ml PBS were added to gel coated tissue. In both *in vitro* and *ex vivo* studies, biofilm burden was quantified after 24 h of incubation using a 10-fold dilution series. Colony forming units (CFU) were counted to calculate bioburden.

Results and discussion: The 4 mm positive control glass beads consistently had 7 $\log_{10}$ CFU. Formulations (0% moxifloxacin) that contained GMCC reduced *S. aureus* but have no effect on *P. aeruginosa* bioburden (Fig. 2). *P. aeruginosa* was not inhibited by GMCC, and tended to form stronger biofilms in formulations not loaded with antibiotic (Fig. 2). *In vitro*, *S. aureus* biofilms were reduced to below detectable levels by formulations alone (Fig. 2). When loaded with 10% moxifloxacin, all formulations were effective at eradicating detectable biofilm growth on beads. The minimum effective moxifloxacin loading concentration against *S. aureus ex vivo* was 1% for GMCC containing formulations and greater than 1% for non-GMCC containing formulations (Fig. 3).

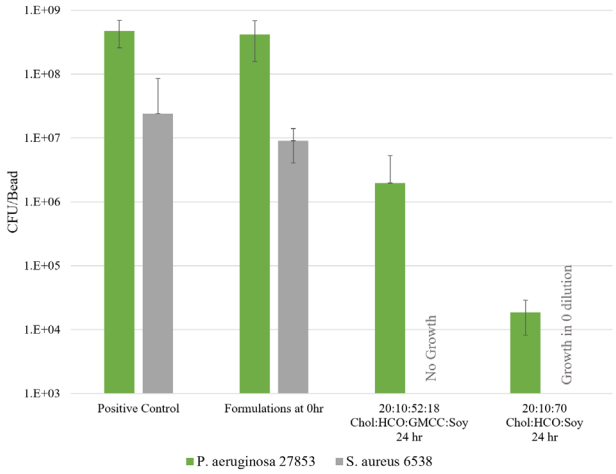

Figure 2: *In vitro* comparison of formulations alone against *P. aeruginosa* and *S. aureus* biofilms at 0 h and 24 h.

Coupled with the antibiofilm effect of GMCC in formulation, 1% moxifloxacin loaded formulation reduced

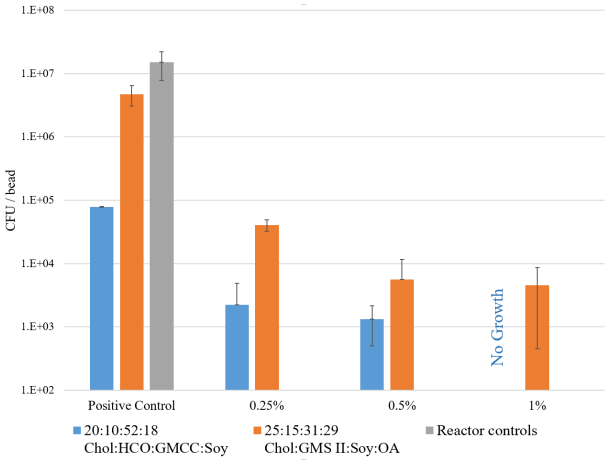

Figure 3: Comparison of two drug loaded formulations incubated overnight (24 h) in fresh tissue.

S. aureus bioburden by 7 $\log_{10}$ units *ex vivo* (Fig. 3).

Conclusion: Moxifloxacin eluted out of gels in sufficient concentrations to eradicate *S. aureus* and *P. aeruginosa* biofilms in *in vitro* and *ex vivo* assays. *P. aeruginosa* was more tolerant to treatments than *S. aureus*. *In vitro* performance of formulations appeared to be more efficacious; however, *ex vivo* results may be more indicative of how formulations may perform *in vivo*. Future work is planned to assess gels *in vivo*—particularly to explore if gels require a higher concentration (e.g., 10% w/v) of moxifloxacin to increase the potential for biofilm eradication.

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References:

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