Bovine Lactoferrin, A Novel Antimicrobial Adjunct For Use As An Antimicrobial Agent In Battlefield-relevant Open Fractures.

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INTRODUCTION: Up to 50% of type 3b compound fractures are complicated by biofilm infections [1]. *Staphylococcus aureus (S. aureus),* the most common cause of infection, resides within a protective biofilm. Lactoferrin (Lf), a glycoprotein with antimicrobial and immunomodulatory properties, is found naturally in human and animal milk [2]. Bovine Lf (bLf) is a potent stimulator of bone growth adding to its appeal as a treatment for compound fractures [3]. The goal of this study was to test the ability of bLf as an adjuvant to antibiotics in the context of compound fracture infections. Specifically, we aimed to: 1) *Demonstrate the in vitro anti-biofilm properties of bLf.* 2) *Translate the in vitro antibiofilm activity of bLf to an in vivo periprosthetic, biofilm infected tibia rodent model.*

METHODS: *In vitro: S. aureus* Xen-36 biofilm was generated in a standard bioreactor on metal coupons and tested against cefazolin (CEF) and flucloxacillin (FLU) with or without bLf. Viable counts of *S. aureus* were recovered and enumerated. *In vivo:* Surgery was performed on adult male rats (n = 40), where the craniomedial tibia was exposed, and a 1.5 mm defect created and inoculated with Ten µL (≈10^7 CFU) of *S. aureus* biofilm. A 2 mm length stainless steel pin was placed within the medullary cavity of the tibia, and the wound treated with STIMULAN® beads loaded according to 4 treatment groups: 1) Control (empty), 2) bLf 3) FLU, 4) bLf and FLU. All rats received subcutaneous FLU 200 mg/kg immediately prior to surgery. At day 7 post infection, rats were re-anaesthetised for bioluminescent and x-ray imaging, euthanized, and tibial explant tissue/metal pins collected for bacterial colony enumeration.

RESULTS SECTION: *In vitro:* bLf augments the anti-biofilm activity of antibiotics such as CEF and FLU *in vitro (*Figure 1A), and in combination with FLU significantly eradicates *S. aureus* biofilm infection *in vivo* when compared against controls (Figure 1B). *In vivo* imaging confirms correct placement of metalware and inoculum (Figure 1C).

DISCUSSION: This study has shown the promising *in vitro* and *in vivo* effects of bLf, one of the main limitations observed within this study was the prolonged setting time of STIMULAN® beads when loaded with bLf, taking at least 12 hours to fully set.

SIGNIFICANCE/CLINICAL RELEVANCE: bLf holds promise as an anti-infective in battlefield relevant open fractures, supported by the anti-biofilm properties of bLF highlighted within this research. Further work is required to translate these finding into clinical practice in military conflicts.

REFERENCES: 1) Gustilo R, et al., *J Trauma.* **24**:742-746, 1984. 2) Gould G, et al., *J Food Prot* **59**:82-86, 1996. 3) Cornish J, et al., *Endocrinology* **145**:4366-4374, 2004.

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IMAGES AND TABLES:

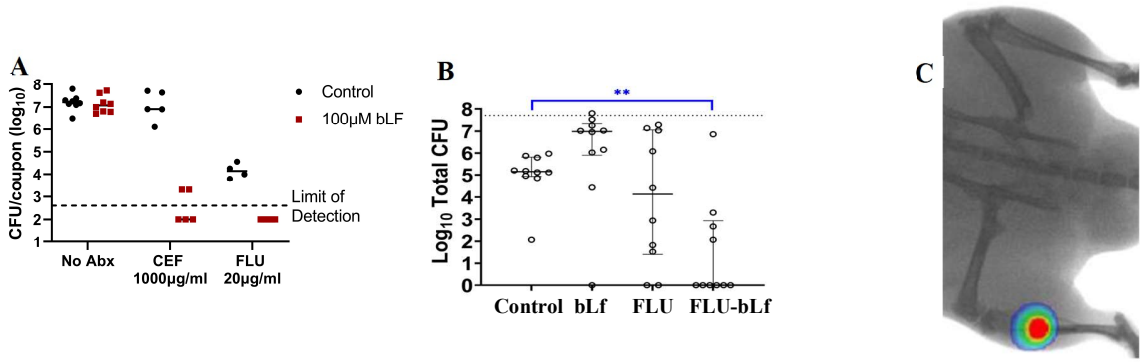

Figure 1: A) Viable counts (CFU) recovered from biofilm coupons treated with CEF or FLU in the presence (red) and absence (black) of bLf. One-way ANOVA with Šídák correction showed that the inclusion of bLF significantly improved the antibiofilm activity of CEF ($p<0.001$) and FLU ($p<0.0001$) **B)** One-way ANOVA with Tukey's test demonstrated that FLU-Lf beads significantly eradicated *S. aureus* compared with controls (empty bead) ($p<0.001$). **C)** Bioluminescence and x-ray imaging of a rat, periprosthetic, biofilm infected tibia.