Methylene Blue as a Biofilm-Disclosing Agent for Fracture-Related Infection: An In-Vitro Evaluation Across Orthopaedic Implant Materials

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INTRODUCTION:

Fracture-related infection (FRI) remains a major challenge in orthopaedic trauma, and can be complicated by bacterial biofilms that impair eradication and affect decisions on debridement and implant retention.[1] Early intraoperative identification of biofilm could improve infection management. Methylene blue (MB) is a nontoxic dye with biofilm-staining and antimicrobial properties, which has previously been studied for use in arthroplasty in the setting of prosthetic joint infection, but has not yet been evaluated for organisms and biomaterials implicated in fracture-related infection.[2,3] This study examined whether MB can (1) visibly disclose biofilm on common orthopaedic implant materials and (2) correlate staining intensity with quantitative biofilm burden.

METHODS:

Biofilms of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Acinetobacter baumannii* were cultured for 48 hours on titanium, stainless steel, and polymethylmethacrylate (PMMA) coupons in a CDC Biofilm Reactor.[4,5] Coupons were immersed in 0.005 and 0.001% MB for 5 minutes and rinsed in saline. Digital photographs quantified mean blue-channel pixel intensity as a measure of staining. Biofilm burden was determined from sonicated coupons plated in triplicate to calculate colony-forming units (CFU). Stained versus unstained samples were compared, and correlations between staining intensity and log CFU were analyzed.

RESULTS:

MB produced visible, organism-dependent staining. *E. coli* and *S. aureus* exhibited the greatest blueness across all materials, while *A. baumannii* and *S. epidermidis* showed minimal discoloration (Figure 1). Titanium demonstrated slightly higher staining intensity than stainless steel or PMMA (Figure 1). Staining intensity correlated positively with log CFU when all organisms were analyzed together ($p=0.025$) (Figure 2). Stained coupons showed significantly reduced CFU counts for *A. baumannii*, *E. coli*, and *S. epidermidis* ($p < 0.001$) (Figure 3), indicating partial antimicrobial or detachment effects.

DISCUSSION:

Methylene blue selectively stained bacterial biofilms and correlated with biofilm burden, suggesting utility as a visual marker for biofilm detection. Differences among organisms likely reflect biofilm structure and matrix composition. Limitations include the in-vitro design, small sample size, single species strains, and reliance on photographic quantification. Further work using confocal microscopy and in-vivo models is warranted.

Figure 1

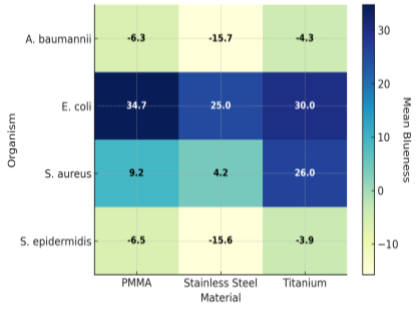


Figure 2

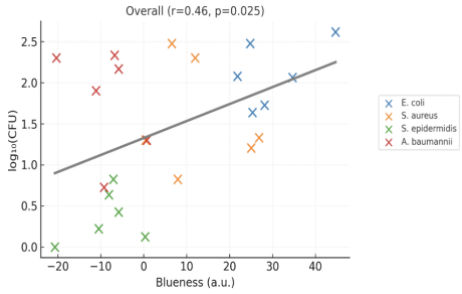
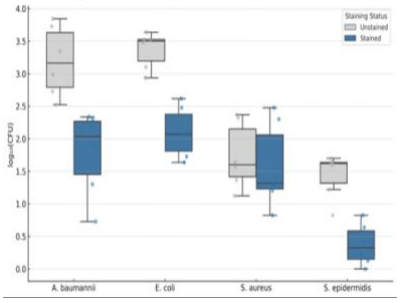


Figure 3



SIGNIFICANCE/CLINICAL RELEVANCE:

Methylene blue may offer a simple, inexpensive, and visually interpretable method for intraoperative detection of biofilm during fracture-related infection surgery, potentially guiding debridement extent and implant retention decisions to improve infection outcomes.

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