

# Biofilm Formation is Durably Prevented on Novel Polymethylmethacrylate Designed to Increase Antibiotic Elution

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**INTRODUCTION:** Prefabricated polymethylmethacrylate (PMMA) articulating spacers containing antibiotics and a proprietary pore structure to increase antibiotic elution have recently become commercially available for two-stage revision of infected arthroplasties. Clinical studies of those spacers reported promising early term follow-up results [1,2]. Antibiotic elution from these spacers is characterized by a rapid-burst phase for 1-2 days and an extended slow-release phase for >28 days. This *in vitro* study determined whether biofilm formation is prevented by antibiotic release during the rapid-burst phase and/or the slow-release phase.

**METHODS:** *S. aureus*-Xen36 was incubated for 1 or 3 days (37°C) with PMMA discs with the proprietary pore structure either without antibiotics or containing high dose gentamycin and vancomycin, or with orthopaedically-relevant, positive-control discs (UHMWPE or cobalt-chrome). Planktonic bacterial growth and biofilm formation were measured by CFU counting and resazurin reduction assays. Experiments were repeated >4 times. Individual symbols in the figures denote the mean for each group from an independent experiment. Horizontal lines represent the geometric means from the independent experiments. Statistical significance was determined by one-way ANOVA with Tukey multiple comparison tests (PRISM version 9.4.1); \*: p<0.01, \*\*: p<0.001, \*\*\*: p<0.0001.

**RESULTS:** Inclusion of a PMMA disc with antibiotics during a 24-hour incubation in *S. aureus* cultures blocked planktonic growth and biofilm formation both on the disc and on the polystyrene culture surface (Fig 1 & not shown). 3-day experiments were performed to assess biofilm formation more stringently. No detectable planktonic bacterial growth or biofilm formation occurred in cultures with PMMA containing high-dose antibiotics, whereas biofilms readily formed on PMMA without antibiotics, cobalt-chrome, and UHMWPE (Fig 2 & not shown). Biofilm maturity was confirmed by a 100-fold decrease in sensitivity to vancomycin (not shown). To determine whether the antibiotic slow-release phase is sufficient to block biofilm formation, PMMA discs with antibiotics were pre-eluted for 14 days with multiple saline changes prior to bacterial inoculation. No detectable biofilms formed on PMMA discs after antibiotic elution (Fig 2).

**DISCUSSION:** Biofilm formation was blocked during both the rapid-burst phase and the extended slow-release phase of antibiotic elution. In contrast, biofilms can readily form on conventional PMMA loaded with antibiotics, on the proprietary PMMA without antibiotics, and on Co-Cr and UHMWPE. Limitations of this study include that only a single strain of bacteria was tested. However, the combination of gentamycin and vancomycin is expected to provide broad-spectrum activity against both Gram-negative and Gram-positive bacteria, including methicillin-resistant *S. aureus*. Additionally, only a single time-point of antibiotic pre-elution prior to bacterial inoculation was investigated. We therefore did not determine the length of time that antibiotic release from PMMA with the proprietary pore structure remains sufficient to prevent biofilm formation. However, the slow-release phase from the proprietary PMMA is maintained for >28 days (data available on the company website, osteoremedies.com) whereas the slow-release phase from conventional PMMA typically lasts for <14 days. Future studies to directly compare biofilm formation on the proprietary and conventional PMMAs after multiple time periods of antibiotic pre-elution time are warranted.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Antibiotic release during both the rapid-burst phase and the slow-release phase prevented biofilm formation on PMMA with the proprietary pore structure. Two recent studies demonstrated ~15% reinfection rate after two-stage revisions using commercially prefabricated all-PMMA articulating spacers with the proprietary pore structure designed to increase antibiotic elution [1,2]. Clinical studies with longer follow-up will be needed to determine whether the results of the current *in vitro* study translate into improved infection eradication rates.

**REFERENCES:** [1] DeBoer 2020 J Arthroplasty 35(3S):S53-S56 [2] Buller et al 2021 J Arthroplasty 36:3979-3985

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