Research Study

Preventing Biofilm Formation on Implants: The *In Vitro* Performance of Surgical Irrigation

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INTRODUCTION

Surgical site infections (SSIs) and periprosthetic joint infections (PJIs) are serious complications that can arise following arthroplasty. Infection remains the leading cause of revision surgeries, and with the increasing number of total joint arthroplasty (TJA) procedures year over year, effective infection control has never been more critical.[1,2]

One solution to reducing the risk of these complications is the use of surgical irrigation, designed to remove microbial contamination from the tissues and thus prevent colonization of implantable materials and subsequent potential for biofilm formation. Biofilms are comprised of aggregates of microorganisms and extracellular polymeric matrix, they adhere to implant surfaces, are notoriously difficult to treat with antibiotics, and significantly increase the risk of PJIs. A growing body of evidence suggests that optimizing irrigation solutions could be a game changer in reducing infection rates in joint replacement surgeries.[3,4]

This white paper presents a comparative *in vitro* analysis of the biofilm prevention effectiveness of nine commercially available irrigation solutions on four commonly used implantable materials. These solutions utilize a range of antimicrobial technologies, including acids, surfactants, polyhexamethylene biguanide (PHMB), povidone-iodine (PVP-I), chlorhexidine gluconate (CHG), benzalkonium chloride (BZK), and a triple antibiotic solution (TAS). Each solution was evaluated using a two-minute pre-treatment contact time on four different implantable materials. Two common bacteria, Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa,* known to cause surgical site infections, were used in simulated synovial fluid to mimic the conditions typically encountered in joint replacement surgeries.

METHODOLOGY

Round 0.5 inch diameter coupons of four different implantable materials (BioSurface Technologies Corporation, Bozeman, MT) without surface modifications were used in this testing: stainless steel (SS), cobalt-chromium alloy (CoCr), titanium alloy (Ti-6AL-4V), and ultra-high molecular weight (UHMW) polyethylene. Two pathogenic bacteria were used as model organisms, Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC USA300 and Gram-negative *Pseudomonas aeruginosa* ATCC 27312. The simulated synovial fluid inoculation medium was purchased from Biochemazone (item# BZ183 without preservatives). **Table 1** shows the irrigation solutions evaluated in the study.

To evaluate the effectiveness of commercial surgical irrigation solutions in preventing attachment of bacteria, coupons (n=4 per group), attached to the bottom of 24-well plates using

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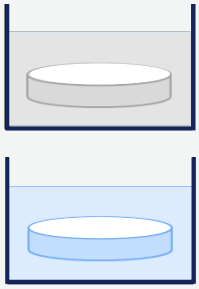
a medical grade silicone adhesive, were first exposed to surgical solutions for two minutes. After aspiration, 1x10^6 CFU of bacteria in synovial fluid was added to each coupon. The inoculated coupons were incubated at 37°C for 24 hours. Following 24 hours of contact time with the inoculum, the coupons were detached from the plate, and bacteria adhered to the material were recovered through a series of sonication debridement steps. The bacteria were enumerated using standard serial dilution and agar plating method. A schematic of this method is shown in **Figure 1.**



Pretreatment with surgical solution

2 min

Inoculation in simulated synovial fluid

24 hr

Recovery and enumeration of bacteria

Figure 1: *Schematic of the biofilm prevention model used in this study.*

SOLUTION	KEY INGREDIENTS	NOTES
Saline	0.9% sodium chloride Solution	Commonly used in wound care to wash and cleanse tissues without antimicrobial activity
Surgiphor™ Antimicrobial Irrigation System	0.5% povidone-iodine (PVP-I)	Potential cytotoxic effects of iodine on human cells; deep brown color and strong smell
BIASURGE® Advanced Surgical Solution	0.1% polyhexamethyl-ene biguanide (PHMB), chelators (EDTAs), vicinal diols	Synergistic composition with enhanced antimicrobial properties of a broad-spectrum, safe and effective antimicrobial compound
Prontosan® Wound Irrigation Solution	0.1% polyhexamethylene biguanide (PHMB)	Safe and effective antimicrobial compound
Irrisept® Antimicrobial Wound Lavage	0.05% chlorhexidine gluconate (CHG)	Significant and irreversible damage to cell; cause tissue irritation; can cause allergic reactions
Bactisure® Wound Lavage	0.13% benzalkonium chloride (BZK), 10% ethanol, 0.6% acetic acid	High cell toxicity even after brief exposure (1 minute) in a study on 3D cultures of human fibroblasts
Vashe® Wound Solution	0.033% hypochlorous acid (HOCl)	Significant erosion and wear on cobalt chrome and titanium surgical implants; bleach smell, limited efficacy in established biofilms
Xperience™ Advanced Surgical Irrigation	32.5 g/L citric acid, 31.3 g/L sodium citrate	May cause irritation or damage to sensitive tissues, reduced efficacy in established infections
Triple Antibiotic Solution (TAS)	gentamycin, bacitracin, cefazolin	Risk of antibiotic resistance development, and it may be less effective against biofilm in short contact times

Table 1: *Products tested in this study and their ingredients*

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RESULTS

The results of the assays are shown in **Figures 2 and 3.** BIASURGE Advanced Surgical Solution (BIASURGE) demonstrated superior and consistent reductions in biofilm formation for both *Staphylococcus aureus* and *Pseudomonas aeruginosa* across all tested implantable materials. This remarkable performance, with over a 6-log reduction in microbial load, especially against challenging pathogens like *P. aeruginosa,* underscores its role as a key component in infection prevention protocols designed to reduce costly revision surgeries. The simulated synovial fluid used in the study closely mimics clinical conditions encountered during joint replacement surgeries.[5] The fact that BIASURGE maintained its efficacy under these conditions further validates its use in real-world surgical applications.

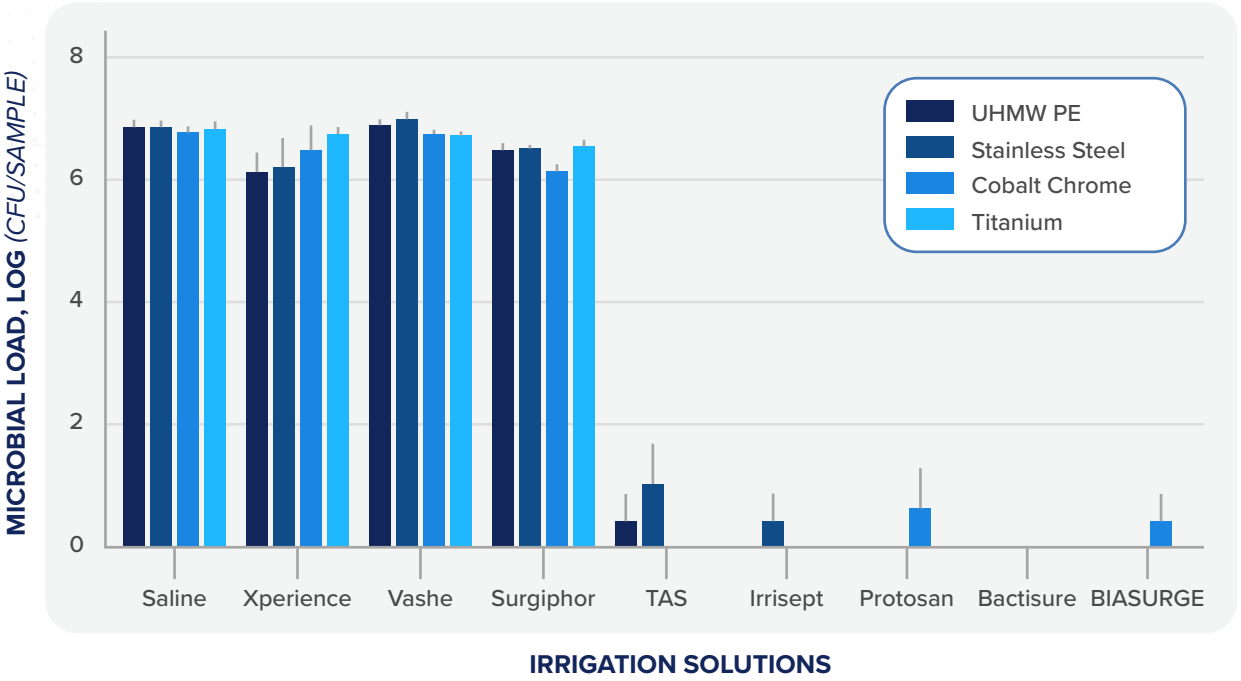

Figure 2: *Biofilm prevention effectiveness of various commercial surgical irrigation solutions on coupons after a twenty-four hour challenge with MRSA in simulated synovial fluid.*

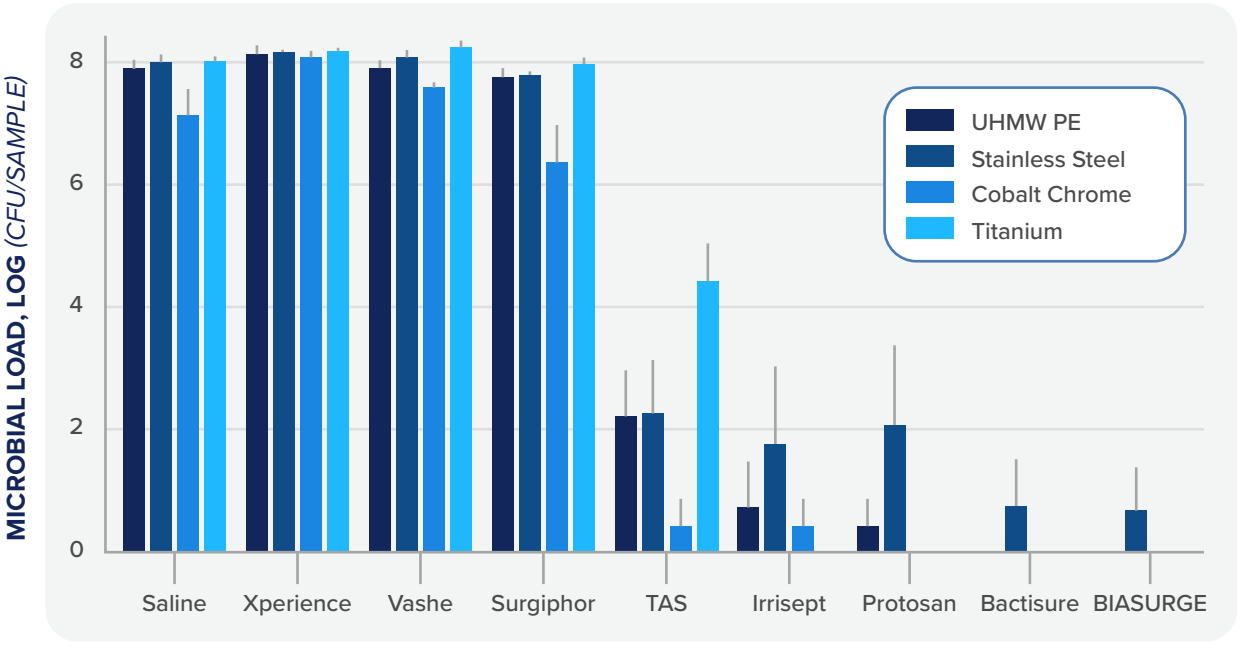

Figure 3: *Biofilm prevention effectiveness of various commercial surgical irrigation solutions on coupons after a twenty-four hour challenge with Pseudomonas aeruginosa in simulated synovial fluid.*

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DISCUSSION

Compared to the other PHMB-based solution (Prontosan), BIASURGE showed higher level of performance, which can be attributed to the synergistic antimicrobial formulation based on a proprietary blend of PHMB, EDTAs which serve as metal chelators, and vicinal diols which interact with lipids.[6]

The BZK/ethanol/acetic acid solution demonstrated favorable performance; however, there are ongoing concerns regarding its toxicity.[7] Additionally, while there is extensive corrosion testing data on metal-based materials for BIASURGE, comparable research on Bactisure is lacking and the recommended rinsing after application does not eliminate questions about its effects on metal implants during brief exposures.[8]

The material-dependent efficacy of Prontosan, Irrisept, and TAS underscores the complex interactions between irrigation solutions and specific implant materials. This complexity should be a key consideration when selecting a solution for clinical use.[9]

In contrast, the citric acid, HOCl, and PVP-I solutions were largely ineffective in preventing the formation of *S. aureus* and *P. aeruginosa* biofilms on any of the tested materials. While some studies have indicated that PVP-I solution is ineffective at eradicating *S. aureus* biofilm, our findings expand on this by demonstrating that it also fails to prevent the formation of biofilms.[10] This dual ineffectiveness highlights the limitations of these solutions in clinical settings where biofilm-related infections are a significant concern, underscoring the need for more robust solutions like BIASURGE.

CONCLUSION

The results of this study provide valuable insights into the effectiveness of various surgical irrigation solutions in preventing biofilm formation by *S. aureus* and *P. aeruginosa* on different implantable materials. One of the most significant findings is the superior performance of the PHMB-based technology in BIASURGE, which resulted in over 6-log reduction in microbial load across all implantable materials and pathogens tested compared to untreated control materials.

The study also highlights the importance of selecting an irrigation solution that is tailored to the specific implant materials used in surgeries. Solutions like BIASURGE, which consistently performed well across all materials, may be preferable for broad-spectrum protection. Conversely, other solutions, while effective in certain contexts, may require more strategic use depending on the specific implant material.

As antimicrobial resistance continues to rise and hospitals seek more cost-effective ways to reduce infections and improve patient outcomes, BIASURGE presents itself as a high-impact solution that can help mitigate these challenges while enhancing patient care. Further *in vivo* testing and clinical trials will help validate its long-term efficacy, thus improving patient outcomes in total joint arthroplasty and similar procedures.

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