Research Study

Ex Vivo Human Bone Organ Culture Evaluation of Surgical Irrigation Solutions: Antimicrobial Efficacy and Cytocompatibility

Rebecca McMahon, PhD, Fahimeh Tabatabaei, PhD, Nina Bionda, PhD

INTRODUCTION

The rising incidence of total joint arthroplasties (TJAs) underscores a pressing healthcare challenge, particularly as the aging population increases. Currently, approximately 790,000 total knee arthroplasties and 450,000 total hip arthroplasties are performed annually in the U.S. alone, with projections indicating these numbers could soar to over 6 million knee and 2 million hip arthroplasties by 2040. While TJA is recognized for its effectiveness in alleviating joint pain and enhancing quality of life, complications such as periprosthetic joint infections (PJIs) pose significant risks. The incidence of PJIs is alarming, estimated between 1% to 2% for primary arthroplasties, and up to 20% for revisions, leading to increased morbidity and healthcare costs, with expenditures on infected revisions potentially reaching $1.62 billion annually by 2030.[1,2]

PJIs are primarily caused by pathogenic microorganisms, with gram-positive bacteria, particularly staphylococci, being the primary culprits. Implementing effective irrigation solutions during the surgical process is a proven strategy for mitigating the risk of PJIs. Intraoperative wound irrigation significantly reduces bacterial load and debris at surgical sites, effectively lowering the likelihood of infections. Using antibacterial or antiseptic solutions not only aids in wound cleansing but also minimizes contamination during the critical postoperative phase, making it a cost-effective intervention for improving surgical outcomes.[3,4]

While the primary goal of irrigation solutions is to minimize postoperative infections, it is essential to balance efficacy with cytocompatibility. Different solutions can have varying cytotoxic effects on human tissue, potentially hindering normal healing. In this study, we meticulously evaluated and compared seven different irrigation solutions **(Table 1)** using a human *ex vivo* organ culture methodology to closely replicate surgical conditions. By analyzing a range of options, including PHMB (polyhexamethylene biguanide), PVP-I (povidone-iodine), CHG (chlorhexidine gluconate), BZK (benzalkonium chloride), and acids we aimed to pinpoint the most effective solutions that provide robust antibacterial activity while ensuring tissue safety.

Pre-clinical study results may not directly correlate to human clinical outcomes.

SOLUTION	KEY INGREDIENTS
BIASURGE® Advanced Surgical Solution	Polyhexamethylene biquanide (PHMB), EDTAs, vicinal diols (VDs)
Prontosan® Wound Irrigation Solution	PHMB, betaine
Irrisept® Antimicrobial Wound Lavage	Chlorhexidine gluconate (CHG)
Surgiphor™ Antimicrobial Irrigation System	Povidone-iodine (PVP-I)
Bactisure® Wound Lavage	Benzalkonium chloride (BZK), acetic acid, ethanol
Xperience™ Advanced Surgical Irrigation	Citric acid, sodium citrate
Vashe® Wound Solution	Hypochlorous acid (HOCl)

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

METHODOLOGY

Viable human femoral heads were obtained during primary total hip arthroplasty, excluding patients with infectious diseases like HIV or hepatitis. Using a low-speed hole saw, we harvested 15mm trabecular bone cores, trimmed to 10mm, and vortexed them in saline to remove residual debris. The cores were incubated in cell culture medium (DMEM) media supplemented with 10% fetal bovine serum (FBS) to ensure proper acclimatization before experimentation.

To assess the antibacterial efficacy of the surgical irrigation solutions, 168 trabecular bone cores were collected from 41 patients (18 males and 23 females), averaging 68.5 years. Sets of four patient-matched bone cores were inoculated with *Staphylococcus aureus* (ATCC 12600) and incubated in DMEM for six hours. One core from each set was then soaked in a test irrigation solution for 10 minutes, while a second core was soaked in phosphate-buffered saline (PBS) as a control. Following the soaking, cores were placed in Dey-Engley broth to neutralize any remaining antimicrobial agents, vortexed, and centrifuged to separate the bacterial pellet from the supernatant. Serial dilutions were plated on tryptone agar for colony counting after overnight incubation, with six matched pairs processed for each irrigation solution. The third and fourth specimens from each four-core set were soaked in test irrigation solutions and saline for 10 minutes after the 6-hour incubation, rinsed with PBS, and placed in fresh media for an additional 24-hour incubation, followed by the same processing as the acute groups to evaluate surviving microbes.

To evaluate the cytocompatibility of irrigation solutions, 127 specimens from 68 patients (19 males and 20 females), with an average age of 68, were compared to patient-matched controls. After acclimation in media, cores were exposed to irrigation solutions for 10 minutes, while their matched controls were treated with saline. The exposed cores were rinsed with saline and placed in DMEM media for overnight incubation. Metabolic activity was assessed via resazurin assays, normalizing data against the controls. For long-term effects, bone cores were exposed to test irrigation fluids for 10 minutes, rinsed with PBS, and then loaded into a bioreactor system under controlled conditions, delivering media and mechanical stimuli for two weeks. After removal from the bioreactor on day 13, the specimens underwent the same assay as the acute response groups.

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RESULTS AND DISCUSSION

In this study, we used human viable *ex vivo* bone tissues for evaluating the efficacy and cytocompatibility of surgical irrigation solutions. They preserve the native 3D structure, cell-cell interactions, and extracellular matrix of bone tissue providing a more physiologically relevant environment compared to simplified *in vitro* cultures. Additionally, *ex vivo* models bridge the gap between *in vitro* experiments and *in vivo* studies, potentially improving the translation of results to clinical practice.[5]

The antibacterial efficacy of various irrigation solutions was assessed both acutely and 24 hours after exposure **(Figure 1)**. Notably, the ranking of irrigation solutions in terms of log reductions remained consistent over the 24-hour period, with more pronounced differences observed. BIASURGE (PHMB/EDTA/VDs) exhibited an impressive 3.2-log reduction in bacterial counts in the acute phase, significantly outperforming other solutions. After 24 hours, BIASURGE achieved a remarkable 6.0-log reduction, clearly surpassing the efficacy of Irrisept, Surgiphor, Xperience, and Vashe. However, it was comparable to Bactisure and Prontosan, highlighting BIASURGE's competitive edge in antimicrobial performance.

The enhanced efficacy of BIASURGE compared to Prontosan (PHMB with betaine), may be attributed to the synergistic effect of PHMB combined with EDTA and vicinal diols. EDTA acts as a chelating agent that destabilizes bacterial cell membranes and disrupts biofilms, thereby increasing the penetration and activity of PHMB. Vicinal diols further enhance membrane permeability and bactericidal efficiency, creating a multi-targeted antimicrobial mechanism that outperforms PHMB-surfactant combinations alone.[6]

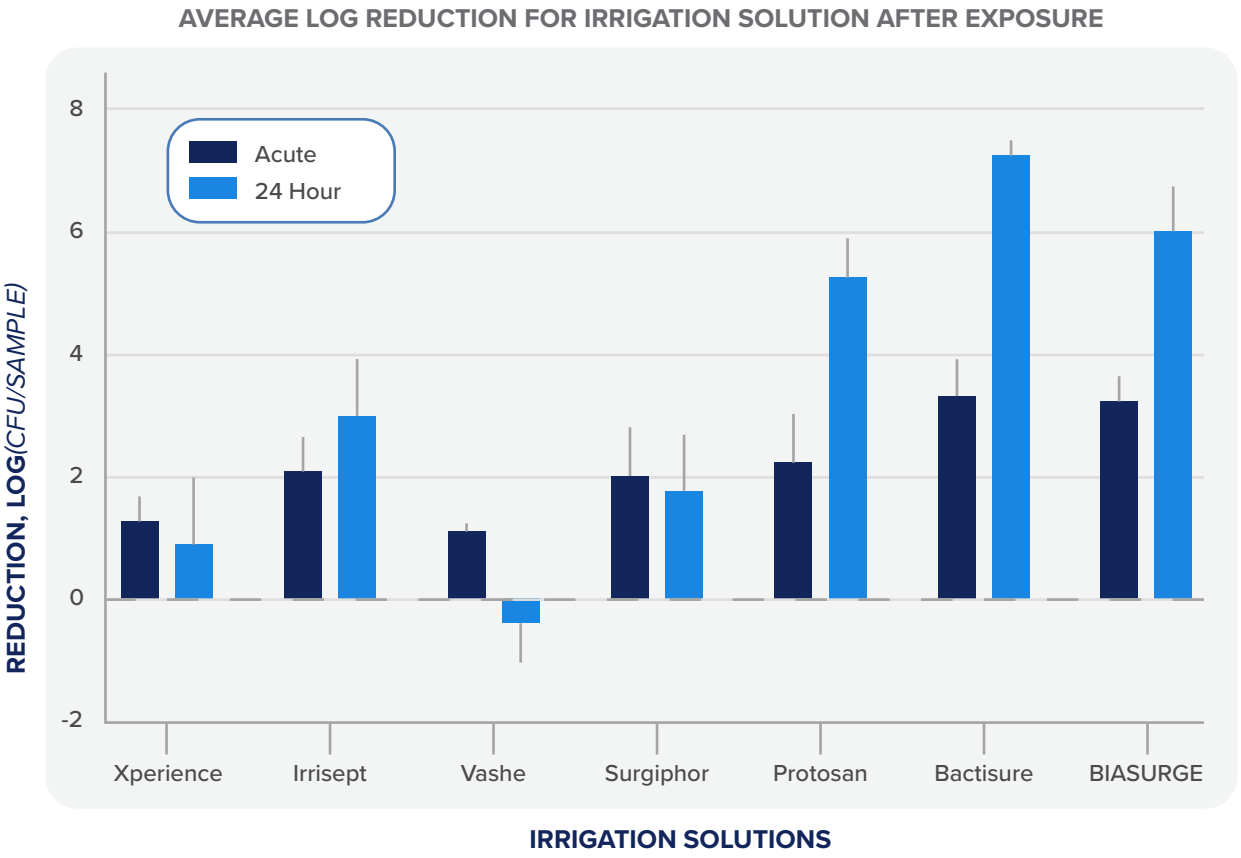

Figure 1: *Average log reduction for each irrigation solution after acute and 24-hour exposure*

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In terms of host cell response, the majority of tested solutions reduced metabolic activity following initial exposure (Figure 2). Although Xperience had the lowest antibacterial efficacy, it significantly enhanced cell viability after two weeks. Citrate supplementation, especially calcium citrate positively influences osteoblast function and proliferation; however, it can also inhibit mineral formation and growth, potentially compromising bone quality and integrity.[7] Bactisure resulted in the most significant decrease in cell viability, which persisted even after two weeks. Previous studies indicate that benzalkonium chloride in Bactisure can be cytotoxic to articular chondrocytes, and fibroblast samples treated with Bactisure became nonfunctional, regardless of exposure duration.[8,9] In contrast, the reduced metabolic activity associated with BIASURGE recovered after two weeks, showing no significant difference from the control. This suggests a favorable cytocompatibility profile for BIASURGE, highlighting its potential for safe use in surgical applications.

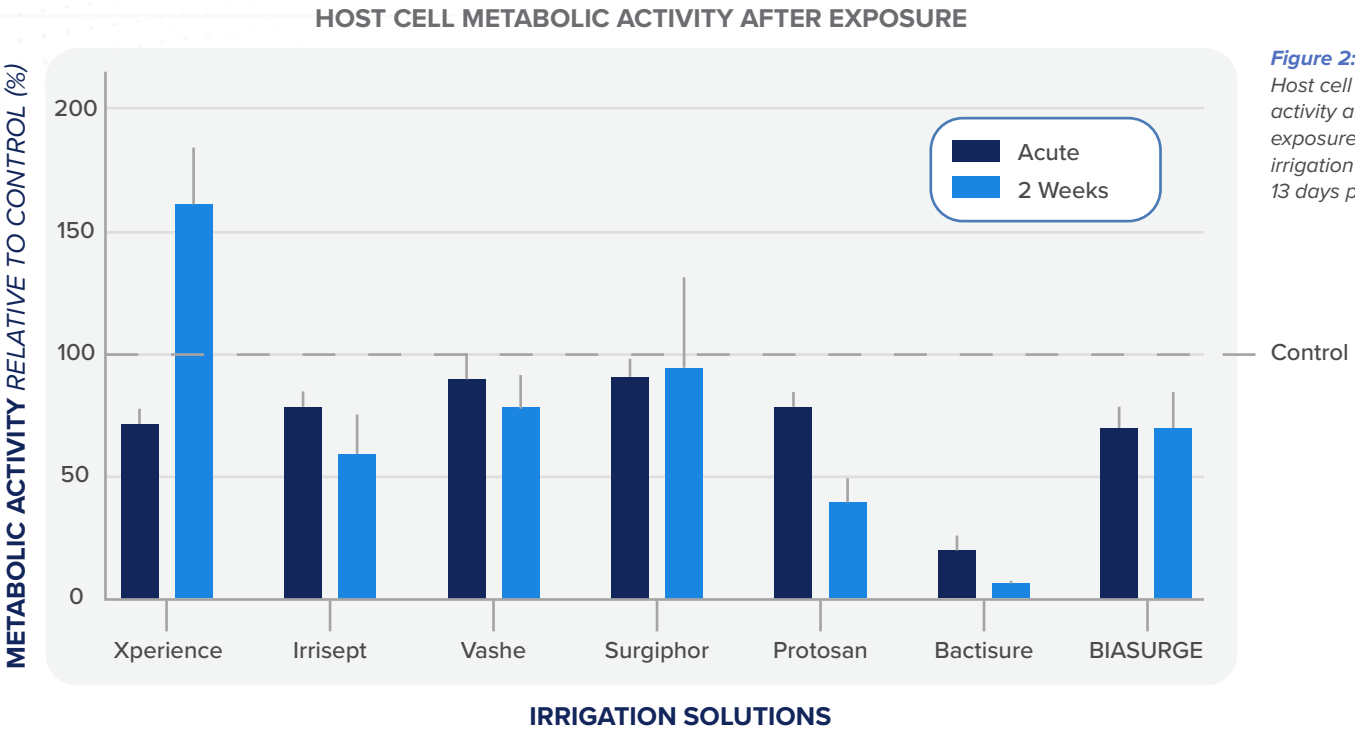

Figure 2:
Host cell metabolic activity after acute exposure to each irrigation fluid and at 13 days post-exposure

This study underscores the critical role of effective surgical irrigation solutions in enhancing patient outcomes during total hip arthroplasty. The evaluation of various formulations demonstrated that BIASURGE (PHMB/EDTA) not only excels in antibacterial efficacy—achieving a remarkable 6.0-log reduction in bacterial counts 24 hours post-exposure—but also showcases a favorable cytocompatibility profile by promoting host cell recovery.

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CONCLUSION

The ideal irrigation solution should be both efficacious and safe. As healthcare providers increasingly seek to mitigate the risk of surgical site infections, our findings highlight the importance of selecting irrigation solutions that balance both antimicrobial effectiveness and biocompatibility. BIASURGE stands out as not only highly effective in terms of antibacterial properties but also exhibiting significantly less cytotoxicity on *ex vivo* bone tissue compared to other tested solutions. This balance of efficacy and cytocompatibility positions BIASURGE as a promising choice for enhancing surgical outcomes and minimizing the risk of periprosthetic joint infections in total joint arthroplasties. Further research and clinical trials are warranted to validate these findings and potentially influence best practices in surgical irrigation protocols.

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