

Anti-Biofilm Efficacy of a Novel Activated Zinc Irrigant and Comparison to Commercially-available Irrigants:
A Multi-organism, Multiple Timepoint Kinetic Time Kill Study

Derek Hill, DO^{1,2}, Nash Reigle, BS¹, Brandon Nutt, BA¹, Paul Attar, PhD³, Michael Scarborough¹, Korey Goldsmith¹, Ahmed Siddiqi, DO⁴
1=AZ Solutions, LLC; 2=Michigan State University; 3= BRIDGE PTS, Inc; 4=Ortho NJ
Corresponding Author: hillder@msu.edu

Introduction: Zinc salts combined with oxidizing agents release high concentrations of antimicrobial zinc ions (Zn²⁺). The oxidizing agent sodium chlorite (NaClO₂), commonly used in mouthwashes, possesses its own profound antimicrobial activity. Combining dilute zinc chloride (ZnCl₂) with dilute NaClO₂ creates a potent, synergistic, nontoxic antimicrobial solution (AZ100). We evaluated biofilm eradication of this novel solution against diverse bacterial organisms compared to leading wound irrigants.

Methods: CDC Biofilm Reactors (CBRs) create robust biofilms with strong bacterial adhesion using circulating bacterial broths. The acronym ESKAPE represents a broad range of common, virulent, nosocomial bacterial pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. Biofilms of ESKAP and *Escherichia coli* were tested against AZ100 and multiple other irrigants: 0.9% normal saline (NSS, negative control), hand-mixed 0.35% povidone-iodine (0.35% PI, positive control), and commercially-available sterile 0.5% PI, 0.05% chlorhexidine gluconate (CHG), and 0.1% polyhexamethylene biguanide/betaine (PHMB/B). Biofilms were exposed to each irrigant (n=3 per data point) for intervals of 5, 15, and 30 minutes. Colony forming units (CFUs) were counted and reported as log recoverable CFUs compared to NSS. Data were analyzed using Student’s t-test comparing each irrigant to AZ100 to 0.35%PI. P-values for clinically-relevant 5-minute exposures were reported with statistical significance set at 0.05.

Results: After more than 300 assays, AZ100 demonstrated complete eradication (>5-log reduction) of all biofilm pathogens/timepoints, except 3.2-log reduction for *E coli* at 5 minutes and 4.5-log reduction for *K pneumoniae* at 5 minutes. Commercially-available irrigants 0.05% CHG, sterile 0.5% PI, and 0.1% PHMB/B demonstrated <3-log reduction of all bacteria at 5 minutes, except 3.0-log reduction of *A baumanii* by PHMB/B and 5.6-log reduction of *E faecalis* by SLS/CA/SC, with none of these irrigants demonstrating complete eradication of all biofilms at 30 minutes. At 5 minutes AZ100 eradicated better than 23-of-24 organism/commercial irrigant assays with p<0.05, and better than 0.35% PI control against 5 of 6 organisms (p< 0.05 for four, p=0.11 for *K pneumoniae*) and equivalent against *A baumanii* (AZ100 and 0.35% PI both → complete eradication).

Discussion: AZ100 demonstrates complete biofilm eradication of a diverse array of gram-positive and gram-negative biofilm-producing bacterial pathogens with exposure at surgically-relevant timeframes (5 minutes). AZ100 was the only irrigant that exhibited >5-log reduction of 4/6 biofilm organisms at 5 minutes, and reduction better than (4/6) or essentially equal to (1/6) hand-mixed 0.35% povidone-iodine. Conversely, in the commercially-available irrigants tested, less than 3-log eradication was observed in 22/24 assays at 5-minutes, less than hand-mixed 0.35% PI in 23/24 cases (p<0.05 in 20/24), and less than AZ100 in 23/24 cases (p<0.05). Commercially-available irrigants performed statistically significantly worse than hand-mixed 0.35% povidone iodine and AZ100 in most cases. AZ100 offers a viable and potentially-superior alternative to commercially-available irrigants and 0.35% PI against a broad spectrum of virulent biofilm-producing organisms.

