Bacterial Eradication in Existing Microbial Biofilms by Zuragen™ (Methylene Blue, Citrate, Antiseptic Solution)

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Introduction

•In biofilm, communities of sessile bacteria are embedded in a hydrated matrix of extracellular polymeric slime comprised of polysaccharides, proteins and nucleic acids. In the biofilm microenvironment of high osmolarity, low nutrient levels, and high cell density, bacteria have different growth rates and gene transcription than planktonic bacteria.

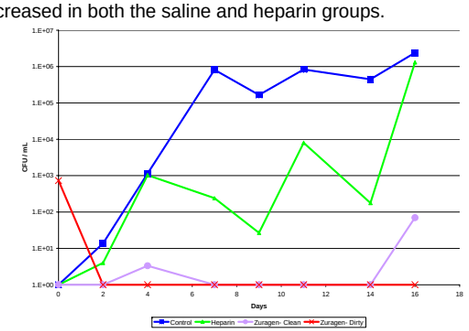
•Biofilms are inherently resistant to both antimicrobial agents and host defenses. Up to 60% of bacterial infections treated by physicians are related to biofilm formation.

•To prevent Catheter Related Blood Stream Infections (CRBSI) in tunneled hemodialysis catheters the ideal catheter lock would: provide broad spectrum efficacy against both planktonic and biofilm bacteria, avoid inducing bacterial resistance, and be safe when injected intravenously.

Previous Findings

•Zuragen™ [0.05% methylene blue (MB), 7% sodium citrate pH 6.2 and other antiseptic compounds] was tested against gram positive and gram negative bacteria and fungi. The components in this formulation demonstrated a synergistic antimicrobial efficacy against all bacteria. The final product was able to effectively kill most tested planktonic microorganisms within minutes of incubation. The concentrations of more resistant strains were diminished to zero in one hour or less. All organisms had an MIC of 25% or less of the original concentration of Zuragen™.

•In pre-clinical studies, a hospital isolate of *S. aureus* was used to evaluate the anti-biofilm properties of Zuragen™. Plastic coupons were alternately exposed to microorganisms in human serum for 3 hours and then Zuragen™ solution for 2 days over a 14 day period. Zuragen™ eradicated CFU in biofilm from 10^3 CFU/mL to zero even after repeated exposure to *S. aureus* in plasma, while bacterial counts increased in both the saline and heparin groups.



Zuragen™ effectiveness against biofilm: *S. Aureus* colonization and elimination

Recent Studies

•Additional studies were undertaken to further evaluate biofilm elimination using a wide range of bacterial strains and fungi. The information regarding Zuragen's™ efficacy against biofilm was obtained by applying a variety of microscopic methods.

Materials & Methods

•Resistance development studies were carried out with both gram negative and positive bacteria. Microorganisms were challenged against Zuragen™ in the concentration range from below to above the previously established MIC. Each day surviving organisms were exposed to fresh Zuragen™ solutions containing nutrients. Viability of microbes was checked by plating on TSA plates.

•In present studies, biofilm of tested microbes was developed on circular polycarbonate or glass coupons (d = 0.5 in). Sterile coupons were incubated with bacteria in LB broth containing 0.2% glucose (37° C @ 160 RPM). Biofilm developed in 1 to 7 days. If incubation was longer than 1 day, coupons were submerged into fresh medium at 24-hour intervals.

•At the end of the incubation, coupons were gently rinsed in sterile saline to remove planktonic bacteria. Coupons were divided into two groups: control which served as a baseline for biofilm grown, and treated discs which were additionally exposed to Zuragen™ for 1 hour. Biofilm was removed by vortexing and sonicating. Serial dilutions were plated for bacterial counts.

•To evaluate if human plasma proteins may enhance biofilm resistance to Zuragen™, sterile coupons were immersed in 10 mL of human plasma and incubated overnight (37° C @ 160 RPM). They were then rinsed with saline, dried, and treated as described above.

•Optical evaluations follow standard method protocols i.e. fixation with glutaraldehyde and critical drying for Scanning Electron Microscopy (SEM), and staining with LIVE/DEAD® BacLight™ bacterial viability dye for Fluorescence Microscopy (FM).

Results

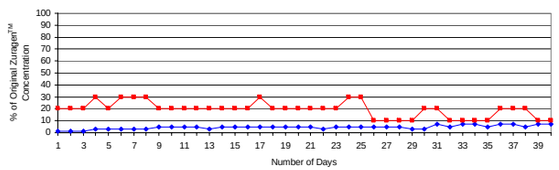


Figure 1- Lack of resistance of *S.aureus* (blue) and *E. coli* (red) to Zuragen™ expressed as Zuragen™ concentration versus time.

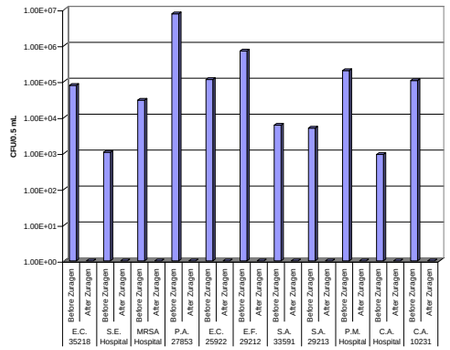


Figure 2- Bacterial quantitation from biofilm grown on polycarbonate coupons before and after Zuragen™ treatment.

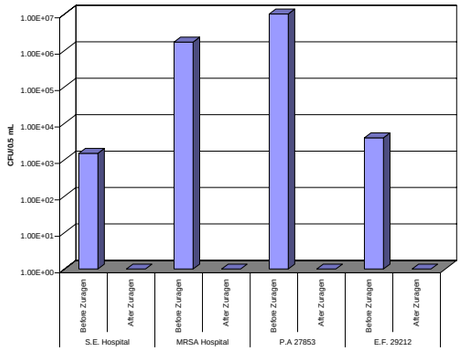


Figure 3- Bacterial quantitation from biofilm grown on polycarbonate coupons, pre-treated with human plasma, before and after Zuragen™ treatment.

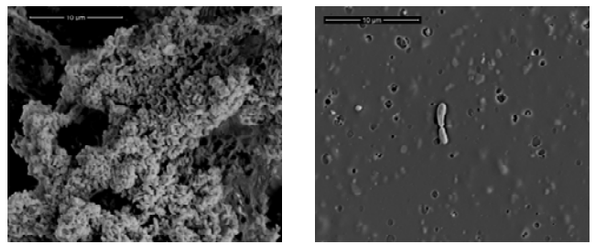


Figure 4- Scanning Electron Microscopy (SEM) of *P. aeruginosa* biofilm on polycarbonate coupons before (left) and after (right) Zuragen™ treatment.

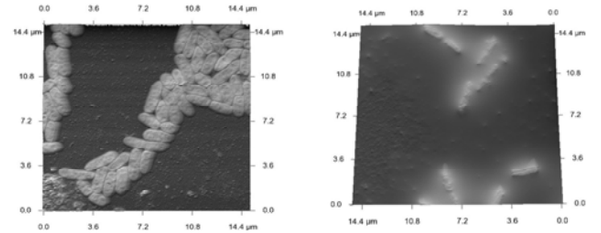


Figure 5- Atomic Force Microscopy (AFM) of *P. aeruginosa* biofilm developed on glass disc before (left) and after treatment with Zuragen™ (right). The small percentage of remaining cells show a high level of structural disruption.

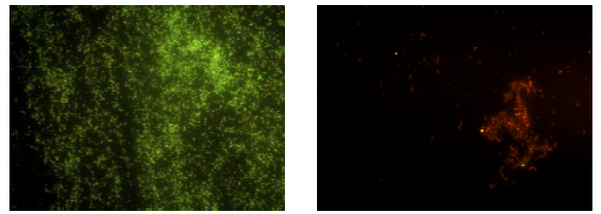


Figure 6- Fluoresence Microscopy (FM) of *S. aureus* biofilm with LIVE/DEAD® BacLight™ bacterial viability dye staining. Dense biofilm with mainly live cells (green- shown on left) was drastically reduced after 1 hour Zuragen™ treatment. Remaining cells are stained red (right) indicating the anti-biofilm efficacy of Zuragen™.

Discussion

•Studies showed that treated bacteria did not develop resistance above the earlier determined MIC over a long period of time (data in **Figure 1** represents the first 40 days of this experiment).

•Zuragen™ is bactericidal against biofilm colonization. One hour treatment of biofilm growth on polycarbonate or glass coupons with Zuragen™ diminished viable cells practically to zero. This happened also with most resistant strains from hospital isolates (**Figure 2**). Pre-treating the coupons for 24 hours in human plasma did not reduce the potency of Zuragen™ **(Figure 3).**

•On the basis of optical results (SEM, AFM, and FM), Zuragen™ eradicates microorganisms from polycarbonate or glass discs by killing bacteria and removes them from the surface to a high degree (**Figure 4, 5,** and **6**). After treatment with Zuragen™, few bacterial cells survived. Those which remained on the coupon surface were dead or degraded, as confirmed by FM and AFM.

•A clinical trial (AZEPTIC) evaluating the effect of Zuragen™ versus heparin on CRBSI incidence in CVC dialysis is nearing completion*.

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