Bactericidal effect of Hypochlorous Acid in Biofilm

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INTRODUCTION

Biofilm occurs when certain bacteria are environmentally challenged. The bacteria produce an adhesive matrix, become embedded within it, and are thereby protected from external hazards. Unless biofilm is disrupted it is very difficult to kill the encased bacteria with biocides or antibiotics. We recently demonstrated that direct contact low frequency ultrasound (DCLFU) has the ability to disrupt biofilm and convert the encased bacteria into a planktonic state, thereby making them susceptible to biocides, such as stabilized hypochlorous acid (HClO)(Vashe®, SteadMed Medical, Fort Worth, Texas, USA)1. Enzymes such as Pulmoxzyme® (human deoxyribonuclease, rhDNase)(Genentech, South San Francisco, California, USA) and Dispersin B® (BioVectra, Charlottetown, PEI, Canada) disrupt biofilm by attacking the matrix structure at key molecular junctions2,3. As part of the DCLFU study, biofilm was grown on metallic discs then treated with DCLFU with either saline or HClO as the irrigating solution. The residual biofilm on the metallic discs was then stained with crystal violet. During the control phase, in which the irrigating fluid delivered through the DCLFU hand piece without power rinsed the discs, the discs for saline and HClO irrigation both stained positively for residual biofilm with crystal violet1. Due to the extremely effective bacterial killing by HClO in the post DCLFU planktonic phase, we decided to more thoroughly evaluate the residual biofilm during the control portion of the trials. The current study was, therefore, performed to determine the viability of bacteria encased in biofilm after exposure to external saline and to external HClO without disrupting the biofilm matrix.

METHODS

Staphylococcus epidermidis (RP62A) biofilm was grown on titanium alloy metallic discs which were placed in plastic wells. Four discs each were then individually irrigated through the DCLFU hand piece without power with saline or HClO for 10 seconds. Immediately after irrigation, the discs were removed from the wells, rinsed with phosphate buffered saline (PBS), and physically scraped to remove residual biofilm. The scraped material was then agitated in PBS, serially diluted and plated on Trypticase Soy Agar enhanced with yeast extract. The plates were incubated for 24 hours.

RESULTS

Figure 1. Titanium alloy discs covered in S. epidermidis biofilm, rinsed with stabilized HClO for 10 seconds, then stained with Crystal Violet.

Figure 2. After a saline rinse and crystal violet staining, the stained biofilm was scraped off, sonicated, plated, and incubated for 24 hours. The saline treated discs grew out a mean of 10^6 CFUs/ml. When irrigation was performed with HClO the matrix scraped off, sonicated, plated, and incubated there was no growth from any disc.

CONCLUSIONS

Stabilized HClO is a strong bactericidal agent and can effectively kill S.epidermidis in biofilm after a 10 second rinse, without disrupting the biofilm matrix.

REFERENCES


DISCUSSION

Biofilm formation is elicited when bacteria in the planktonic state are stimulated by a sensed environmental threat. The bacteria produce an adhesive polymeric matrix which encases the bacteria and adheres to a surface. The basic structure of biofilm is a Poly-N-acetylglucosamine (PNAG) and extracellular DNA (eDNA). The structure of biofilm inhibits phagocytosis and provides enhanced antibiotic resistance. There is an emerging understanding that biofilms are pervasive in chronic wounds4. Management of biofilm has become a popular area of interest in the wound healing community, as the destruction or removal of biofilm appears to enhance healing5.

HClO is a naturally occurring inorganic chemical that is produced by white cells in the process of killing invading bacteria. It is a powerful oxidizing agent which kills bacteria, fungi, viruses and spores. HClO is a weak acid which occurs only in an aqueous state when chlorine is dissolved in water. It is unstable as it exists in an equilibrium: Cl2 + H2O → HClO + HCl. Stabilized HClO is produced by acidifying NaClO (sodium hypochlorite or Dakin’s solution) with HCL to a pH range of 3.5-4.0. This reaction, when properly adjusting concentrations and quantities, yields HClO in 0.9% saline6.

The successful use of HClO in the treatment of biofilm has been documented in the laboratory and clinical setting1,6-9. In our initial experiments dealing with the ability of direct contact low frequency ultrasound’s effect on biofilm1, we found that HClO was so powerful as an antimicrobial that we repeated the controls in which the irrigation for the device was allowed to run over the established biofilm and the effluent was cultured. In that experiment all of the bacteria liberated from the biofilm, which was destroyed by ultrasound, were killed when HClO was used as an irrigant. In the repeat study the biofilm itself was examined for bacterial viability. The biofilm matrix was preserved after HClO irrigated the colony. However, all of the encased bacteria within the biofilm were killed by a brief irrigation episode. Consequently, the PNAG/eDNA matrix did not protect the bacteria within a biofilm against biocidal contact with HClO.

Stabilized HClO is a strong bactericidal agent and can effectively kill S.epidermidis in biofilm after a 10 second rinse, without disrupting the biofilm matrix.