

Treating Chronic Wounds With Hypochlorous Acid Disrupts Biofilm

Mon, 12/15/14 - 13:21

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Issue Number:

Volume 8 Issue 9 - November/December 2014

Author(s):

Martin C. Robson, MD

Wound healing is such a complex process that many potential factors can delay actual healing, including the presence of bacteria. Increasing evidence shows that some bacteria within chronic wounds live within biofilm communities in which bacteria are protected from host defenses and develop resistance to systemic antibiotic treatment.¹ Bacteria in biofilm behave differently from planktonic bacteria of the same organism in terms of their response to antibiotic treatment and human immunity.^{2,3} The biofilm is formed when a group of microorganisms stick to each other and become embedded within a self-produced matrix of extracellular polymeric substance composed of extracellular DNA, polysaccharides, and proteins.³ Among the most common biofilm-forming bacteria are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.² In order to disrupt biofilm within a wound, an agent must kill the bacteria and decrease the polysaccharides and proteins in the extracellular matrix of the biofilm.

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Although the exact role of biofilm in chronic wound healing remains unclear, treatments that kill microorganisms within biofilms and disrupt the extracellular matrix of the biofilm may aid in the healing of chronic wounds.⁴ Hypochlorous acid is a naturally occurring small molecule generated by white blood cells in the human body. *In vitro* testing has shown hypochlorous acid to rapidly kill important wound pathogens, including antibiotic-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. The time for inactivating these organisms is extremely rapid (< 30 seconds).⁴

An important attribute of the human body's immune system is its ability to instigate a rapid attack against invading pathogens by releasing highly potent oxidized molecules such as hypochlorous acid. After engulfing the invading pathogens, neutrophils release an oxidative burst of hypochlorous acid that very quickly destroys the engulfed bacteria, virus, or fungi. The hypochlorous acid, produced by neutrophils, kills microorganisms by binding to critical cell membrane components and affecting cell permeability. This leads to the rupture of the cell membrane and subsequent disintegration of cells.⁴ The purpose of this study was to test the efficacy of hypochlorous acid (Vashe Wound Cleanser, SteadMed Medical LLC, Fort Worth, Texas) as an agent to disrupt *Staphylococcus aureus* biofilm in a recognized biofilm model.⁵

Study Methods Used

1) *Staphylococcus aureus* biofilms were produced by circulating nutrient broth [casamino acid (0.1 g/l); yeast extract (0.1 g/l); MgSO₄·2H₂O (0.2 g/l); FeSO₄·7H₂O (0.0005 g/l); Na₂HPO₄ (1.25g/l); KH₂PO₄ (0.5 g/l); lactose (0.025 g/l)] through Tygon® tubing for 12 hours. 2) 5-10 ml of *Staphylococcus aureus* culture (108 colony-forming unit [CFU]/ml) was circulated through the tubing. 3) Biofilms were treated with hypochlorous acid for 1, 3, 5, 7, and 10 minutes. 4) After each treatment, 2 cm² pieces of tube were cut and neutralized, and bacterial numbers, residual protein, and carbohydrate content measured.⁴

Figure 1. Effect of Hypochlorous Acid on Biofilm *S. aureus* Bacterial Numbers.

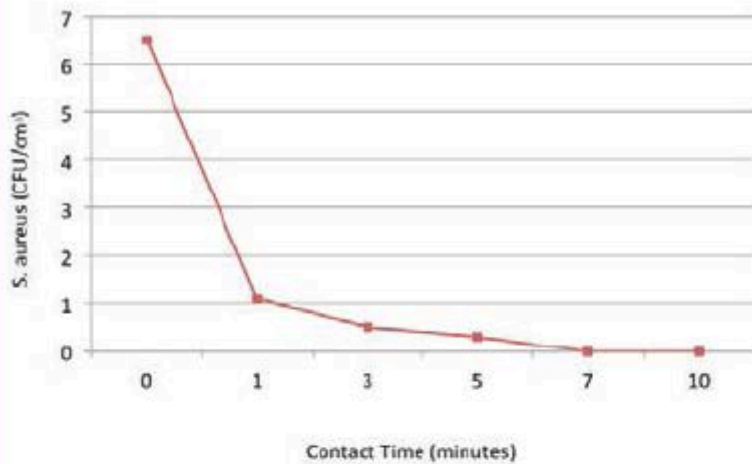
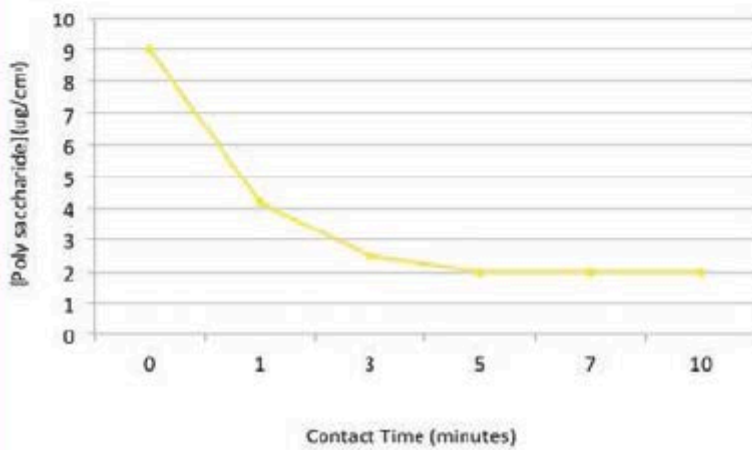


Figure 2. Effect of Hypochlorous Acid on Polysaccharide Levels Within *S. aureus* Biofilm Matrix.



Study Results Found

1) *Staphylococcus aureus* bacterial numbers were reduced by $> \log 5$ CFU/cm³ following a 1-minute exposure to hypochlorous acid. 2) A reduction of $> \log 6$ CFU/cm³ was observed after 3, 5, 7, and 10 minutes exposure to hypochlorous acid (**Figure 1**). This log reduction represented complete removal of the *Staphylococcus aureus* biofilm. Furthermore, approximately 70% of biofilm polysaccharide and $> 90\%$ of biofilm protein was removed after 5, 7, and 10 minutes of contact time (**Figures 2 and 3**).⁴

Discussion & Conclusion

Hypochlorous acid was effective at reducing *Staphylococcus aureus* bacterial numbers and at disrupting the polysaccharide and protein matrix within the biofilm model. Similar data have been reported in a *Pseudomonas aeruginosa* biofilm model using hypochlorous acid.⁶ This study indicates that hypochlorous acid may assist in the

management of “hard-to-heal” chronic wounds by decreasing the bacterial numbers and by penetrating and disrupting the polysaccharide/protein matrix of wound pathogen biofilms.

Martin C. Robson, MD, is emeritus professor of surgery at the University of South Florida, Tampa.

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