Pure Hypochlorous Acid (pHA) Wound Solution Dissolves Materials within Artificial Eschar, penetrating it to Inactivate Bacterial Biofilms

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INTRODUCTION

A wound bed is often covered with eschar, necrotic tissue and slough which all harbour microbial communities known as biofilms and inflammatory chemokines which unless removed can hinder the healing process. Hypochlorous acid (HOCl) is a relatively small uncharged molecule which can freely diffuse across the bacterial cell membrane and react readily with a wide range of biological molecules resulting in bacterial cell death. However, it is relatively non cytotoxic to mammalian cells for reasons associated with evolutionary biology. For many years, due to its inherent antimicrobial properties, HOCl based cleanser has been used as a preservative in wound irrigation solutions for wound bed preparation.

METHODS

An in vitro artificial wound eschar (AWE) and slough model was applied to study the penetration and antibiofilm efficacy of a HOCl based irrigating solution, referred to as a pure Hypochlorous Acid (pHA) solution*. AWE was prepared by homogenising collagen, fibrinogen and elastin in a phosphate buffer solution and then clotting with thrombin. The rate of penetration of pHA solution through AWE was measured electrochemically by monitoring the change of open circuit potential of a platinum sensor coated with an AWE layer upon exposure to pHA. The HOCl concentration penetrating to the electrode surface beneath was established using a calibration curve determined for the sensor in solution without the AWE layer. In conjunction, AWE layers were formed on top of 24 hour biofilms using Pseudomonas aeruginosa and Staphylococcus aureus. (Table 1). The antibacterial efficacy of pHA through AWE was evaluated after 24 hours exposure. Eschar breakdown was studied upon exposure to the cleanser fluid with collagenase and PBS as controls.

RESULTS

- pHA penetration through the AWE reduced the underlying biofilm, with the extent of penetration being inversely proportional to the AWE thickness. The penetrated irrigation solution achieved >6 log reduction on the 24 hour biofilm models with complete inactivation being achieved for S. aureus biofilms in ~1mm AWE. (Fig 1)
- Confocal microscopic images of the AWE-Biofilm model upon exposure to Vashe for 24 hours revealed an abundance of non-viable bacterial cells and disruption to the biofilm in comparison with the untreated biofilm models for both P. aeruginosa (Figure 2) and S. aureus (Figure 3).
- Traces of viable cells were present in the biofilm models covered by the thicker layers of AWE, more prominent for P. aeruginosa, than S. aureus (Figs 2 and 3).
- The HOCl/pHA based cleanser dissolved elastin from the eschar with no significant difference on the elastin dissolution level compared to collagenase (Figure 4).
- This response in log reduction shown above was emulated within the electrochemical measurements of Vashe penetrating through the various thickness layers of AWE. The data shows a rapid initial response in concentration followed by an inflection and a steady increase in concentration of time; the level of response is reduced as the thickness of the AWE layers increase. (Figure 5)

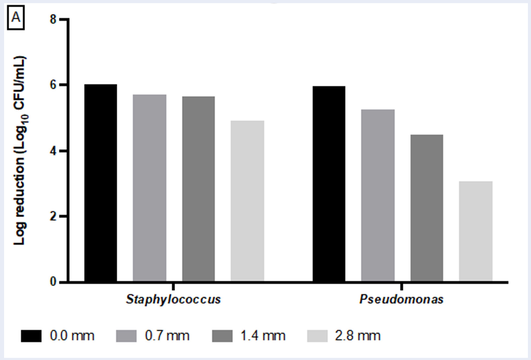


Figure 1. Log reduction (Log10 CFU/mL) of AWE-Biofilm model with various AWE thicknesses using pure Hypochlorous Acid (pHA) Cleanser Solution

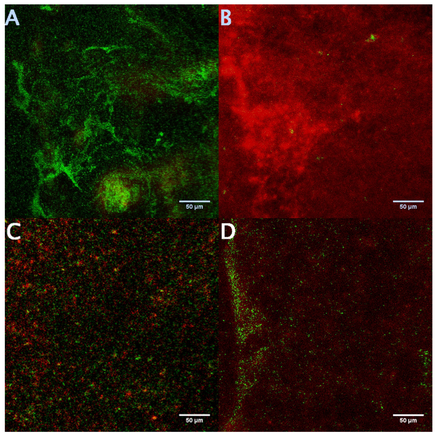


Figure 2. Microscopy images of the AWE-Biofilms, using P. aeruginosa, before (A) and after treatment with pHA for 0.7 mm (B), 1.4 mm (C) and 2.8 mm (D) thickness AWE. An abundance of viable (green) cells are present in the untreated control in comparison to the other which exhibit an increased volume of non-viable (red) cells; the greatest concentration being observed at 0.7 mm (n=3).

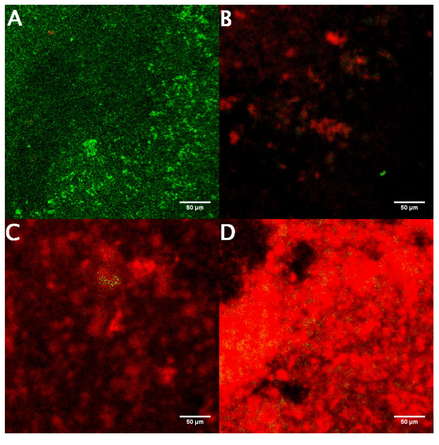
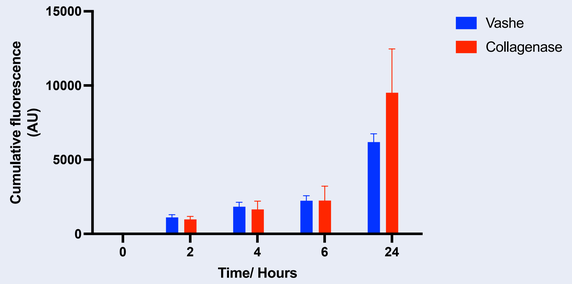


Figure 3. Microscopy images of the AWE-Biofilms, using S. aureus, before (A) and after treatment with pHA for 0.7 mm (B), 1.4 mm (C) and 2.8 mm (D) thickness AWE. An abundance of viable (green) cells are present in the untreated control in comparison to the other which exhibit an increased volume of non-viable (red) cells (n=3).

		AWE layer thickness			
Pseudomonas aeruginosa		**0.0 mm**	**0.7 mm**	**1.4 mm**	**2.8 mm**
	Mean Log CFU/ml	9.29	9.04	8.87	8.79
	Std. Deviation	0.10	0.04	0.09	0.31
		AWE layer thickness			
Staphylococcus aureus		**0.0 mm**	**0.7 mm**	**1.4 mm**	**2.8 mm**
	Mean Log CFU/ml	8.01	7.76	7.74	7.09
	Std. Deviation	0.04	0.11	0.16	0.04

Table 1. Descriptive statistics (Log10 CFU/mL) of the control AWE-Biofilm models at each of the varying thicknesses of AWE (n=3).

Figure 4. The cumulative fluorescence data obtained from the fAWE model upon exposure to pHA and collagenase for elastin-rhodamine (n=3).



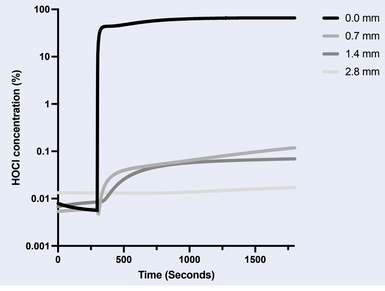


Figure 5. Concentration of HOCl (%) upon exposure to pHA as measured at the surface of the sensor beneath the various thicknesses of AWE (n=1).

DISCUSSION

The in vitro AWE model demonstrated that the rate and amount of HOCl in wound solution penetrated through the AWE layer was dependent on the thickness of AWE with strong antibiofilm potential following penetration through the AWE. It was also found that the HOCL solution had an effect in breaking down the AWE components, particularly elastin. This has potential effect in desloughing wounds.

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