Efficacy of Betaisodona®, Repithel® and other preparations with antiseptic properties typically in the German speaking nations against biofilms formed by *Pseudomonas aeruginosa*

Authors: Hans Hoekstra[1], Samantha Westgate[2], Stefan Mueller[3].

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[1]Burns Research Institute, Amsterdam, The Netherlands. [2]Perfectus Biomed Limited, Cheshire, UK. [3]Mundipharma Research GmbH & Co.KG, Limburg, Germany.

Introduction:

Microorganisms forming biofilms are among the leading agents of persistent infections of chronic wounds. Debridement and topical antisepsis were identified as effective measures against biofilms (Leaper, 2012).

Aims:

Evaluate the efficacy of antiseptics against pre-formed *Pseudomonas aeruginosa* biofilms that had been established using an *in vitro* biofilm model.

Test agents:

Agent code	Agent format	Active agent	Concentration tested
PHX	Hydrogel	0.1% PHMB PVP-Iodine	10% and 100%
OCT	Hydrogel	Octenidine	10% and 100%
BET	Ointment	10% PVP-Iodine	3.3%, 10%, 33% and 100%
REP	Hydrogel	3% PVP-Iodine	10% and 100%
CHG	Liquid	0.1% Chlorhexidine	10% and 100%
NAG	Dressing	Silver nanocrystals	100%
PBS	Liquid	n/a	100%

Table 1. Test agents and concentrations used throughout the study. PBS – Phosphate buffered saline control.

Methods:

- Repeatable *P. aeruginosa* biofilms were established on polystyrene coupons for 72 hours using a CDC reactor.
- Coupons were removed from the reactor and treated for 4 hours and for 24 hours with the antiseptic treatments.
- Liquid and gel products were tested at label claim and after 1:10 dilution, except Agent BET, which was additionally, diluted 1:3 and 1:30, so that povidone iodine concentrations would equal those present in Agent REP. Dressing NAG was tested at the commercial concentration only.
- Following treatment coupons were rinsed 3 times in PBS in order to remove planktonic isolates. Remaining biofilm material was recovered by sonication and quantified using colony counts.
- All samples were tested in triplicate.

Results:

Four hour treatment

- No viable biofilm material was recovered following 4 hours of treatment with agents PHX, OCT, BET, REP, and CHG, at both 100% and 10% concentrations (Figure 1).
- Four hours of treatment with Dressing NAG did not result in a significant reduction in recoverable viable biofilm material compared to control samples (Figure 1).

Figure 1. Quantity of viable *Pseudomonas aeruginosa* recovered from coupons that had been treated for 4 hours with the test agents, at the commercial concentration and at 10% of the commercial concentration.

Twenty four hour treatment

- No viable biofilm material was recovered following 24 hours of treatment with agents PHX, OCT, BET, REP, and CHG, at both 100% and 10% concentrations (Figure 2).
- A 2 Log reduction compared to the positive control was reported after 24 hours of treatment with dressing NAG (Figure 2).

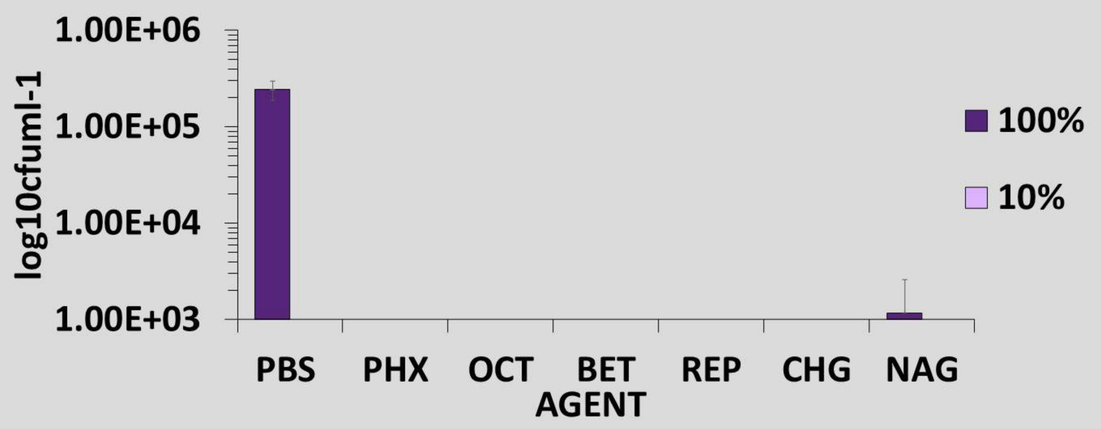

Figure 2. *Pseudomonas aeruginosa* recovered from coupons after 24 hour treatment with the different test agents at the commercially available formulation (100%) and at 10% of this formulation.

- When Agent BET was diluted 1 :3 and 1;30 the povidone iodine concentrations were equivalent to those found in agent REP. Even after the extensive dilutions no viable bacterial were recovered following treatment with Agent BET.

Discussion:

- In a clinical setting, dilution by wound fluids or absorption into dressing materials can occur. The agents that significantly reduced the amount of recoverable biofilm encased bacteria after dilution would make appropriate candidates for the treatment of biofilm infected chronic wounds.
- In an *in vivo* chronic wound situation there are a number of additional variables including; the presence of mixed species populations, proteases including MMP's and the nutritional status of the patient, to name just a few. These additional factors would require further testing in order to determine their influence on the chronic wound situation.
- BET and REP demonstrated an ability to significantly reduce the level of recoverable viable *P. aeruginosa* biofilm material.
- The species of *P. aeruginosa* investigated in this study typically produces biofilms that are extremely difficult to treat. Consequently this data may be indicative of an ability to treat *in vivo* biofilms within problematic wounds such as diabetic ulcers, pressure ulcers and burn wounds.
- Limitations of the model are pathogens apart from *P. aeruginosa*, especially resistant organisms and spore forming bacteria.

• Conclusions:

Due to the difficult to treat nature of the biofilm forming strain of *P. aeruginosa* used in this study, this data suggests that the agents that were active when applied at 10% of their commercial concentration may be appropriate for the treatment of chronic wounds, that are prevented from healing due to the presence of a *P. aeruginosa* biofilm. Povidone iodine containing products (as liposomal and conventional formulation) were effective against *P.aeruginosa* at concentrations markedly below label claim, which is often encountered in relevant clinical situations.