

# PHMB Gelling Fiber shows Powerful Antibiofilm Action with Low Cytotoxic Effects

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## Introduction

One of the fundamental challenges in developing effective wound care products is to offer enhanced antimicrobial efficacy whilst remaining kind to the healing tissue. Biofilms are one of the most challenging forms of infection, where the bacteria are contained within a protective extracellular matrix, termed extracellular polymeric substance (EPS). In this form bacteria are up to 1000 times more resistant to antimicrobial agents than planktonic bacteria<sup>1</sup>, presenting a substantial clinical challenge. Biofilms are present in an estimated 90% of chronic wounds<sup>2</sup>, resulting in a significant clinical problem. Many dressings use silver ions as an effective antimicrobial agent; however silver is known to be highly cytotoxic producing an unfavourable environment for the healing tissues.

Presented below are data on a new high gelling fiber dressing containing polyhexamethylene biguanide (PHMB) which has demonstrated highly effective antibiofilm action whilst maintaining a low cytotoxic profile.

## Methods

### Biofilm

Biofilms were established on polycarbonate coupons using a CDC biofilm reactor<sup>®</sup>. Following biofilm formation, the coupons were rinsed in sterile saline solution to remove planktonic bacteria then dressings were applied to the coupons. The time 0 control was enumerated via sonication to detach the biofilm and quantify the baseline CFU (colony forming units). The test coupons were enumerated after 48 and 168 hours. Log reductions were calculated compared to time 0.

### Cell Viability and Scratch assay

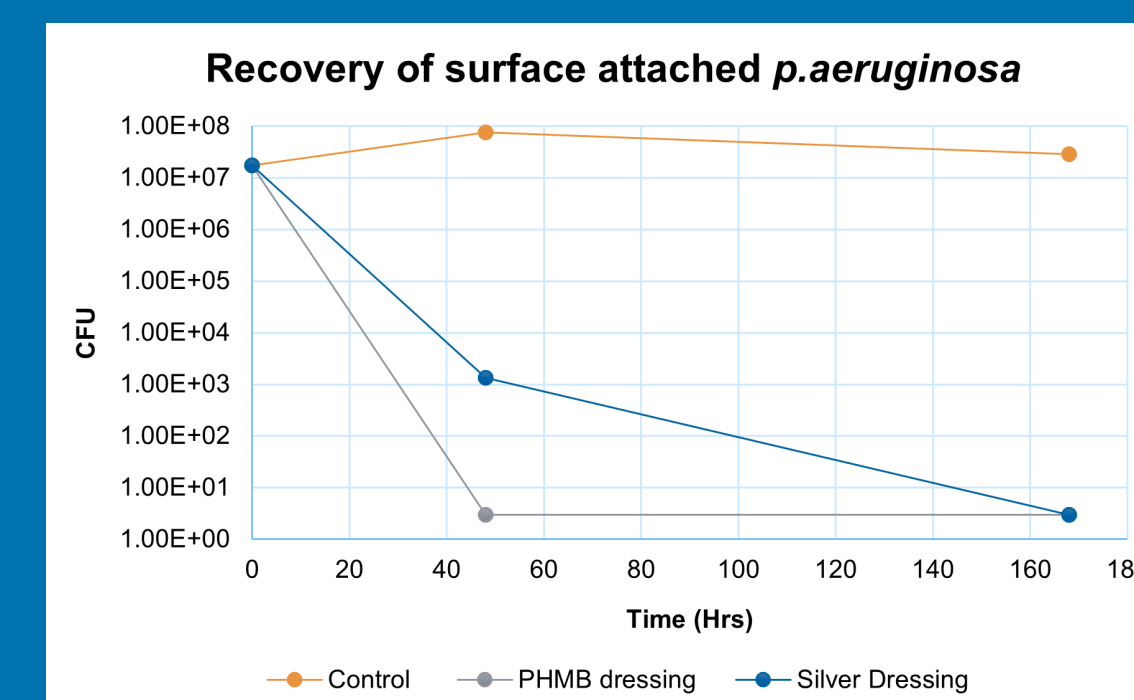
Dressings were placed into culture media for 24 hours at 37°C on a shaking platform at an extraction ratio of 0.1mg/ml. Extracts were added to L929 fibroblast cells for 24 hours then the viability was measured using a resazurin based fluorescence assay.

Scratches were made in confluent cell layers then the baseline was stopped by addition of crystal violet stain. Dressing extracts were added to the scratched cells then incubated for 24 hours. Scratched cells were then stained by addition of crystal violet stain then imaged on the inverted microscope.

## Results

### Biofilm

The high gelling fiber PHMB dressing totally eradicated an established *Pseudomonas aeruginosa* biofilm after just 48 hours and this eradication was maintained over 7 days (168hr). The silver based dressing only achieved a log reduction of 4.1 after 48 hours and by 7 days (168hrs) had reached total eradication.



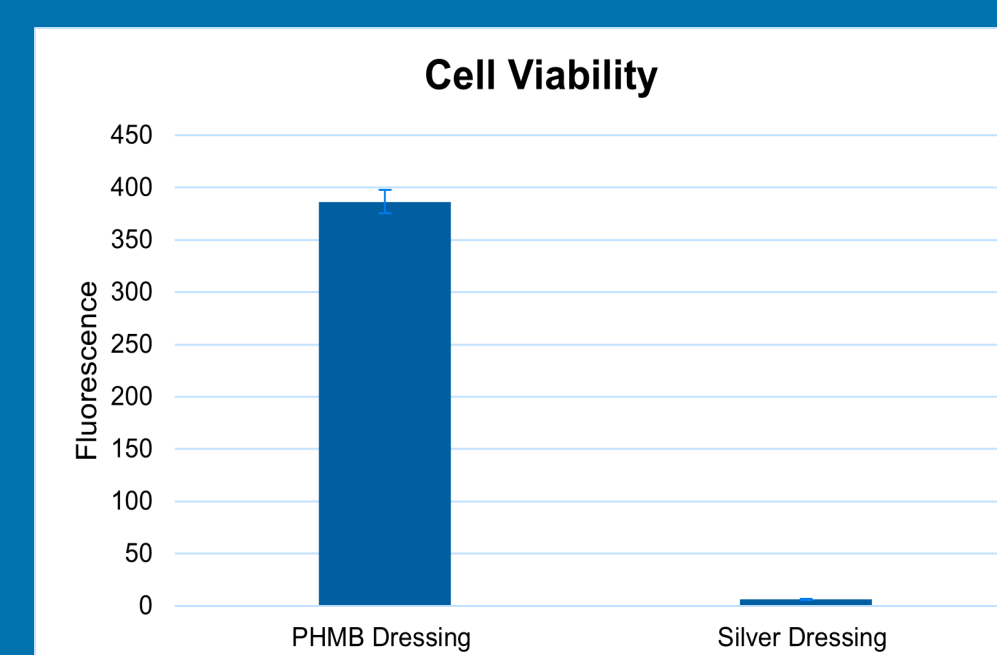
**Figure 1** – The recovery of surface attached *Pseudomonas aeruginosa* from polycarbonate coupons at time 0 and following incubation with dressings for 48 and 168 hrs.

	Log reduction of <i>Pseudomonas aeruginosa</i>	
Time	48hrs	168hrs
PHMB Dressing	>7.2	>7.2
Silver Dressing	4.1	>7.2

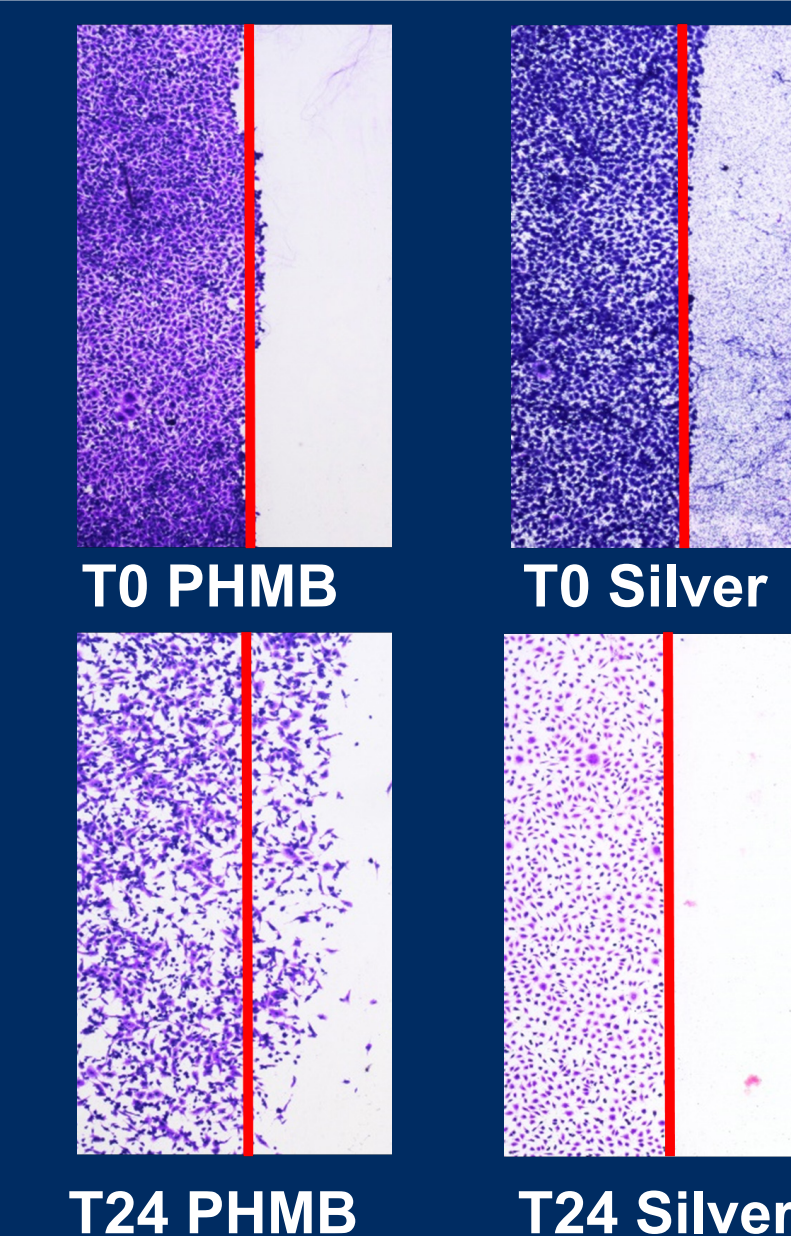
**Figure 2** – Log reduction data of surface attached *Pseudomonas aeruginosa* calculated from time 0 control.

### Cell Viability and Scratch Assay

The viability of fibroblast cells remained high showing only mild cytotoxicity following treatment with the extract of the PHMB gelling fiber dressing. However extracts from a silver fiber dressing were severely cytotoxic and lead to a total loss of cell viability (figure 3).



**Figure 3** – Cell viability was measured after 24 hours of incubation with dressing extracts. The silver dressing was found to be severely cytotoxic while cells incubated with PHMB extracts maintained viability.



Scratches were made in confluent fibroblast layers then treated with dressing extracts for 24hrs. Following this the scratches were stained and imaged. Cells treated with PHMB extract have infiltrated the scratched area and begun to heal the scratch after just 24 hours, however cells treated with silver extract were unable to heal the scratch at all (figure 4).

**Figure 4** - Scratches were made in confluent fibroblast cell layers (scratch edges marked by red line) then treated with dressing extracts for 24 hours. Images were taken at 4x magnification.

## Discussion

Biofilm represents a significant clinical challenge, where an estimated 90% of chronic wounds have biofilm present<sup>2</sup>. *Pseudomonas aeruginosa* is an opportunistic pathogen capable of developing both biofilm and antibiotic resistance and is responsible for many nosocomial infections. The antimicrobial PHMB gelling fiber dressing is capable of eradicating a biofilm within 48 hours and sustaining eradication for the whole 7-day wear time, compared to a silver based dressing where *Pseudomonas aeruginosa* was still present after 48 hours. There is no evidence of PHMB resistance developing in microorganisms. In fact, PHMB provides a sustained antimicrobial effect<sup>3,4</sup>. In addition to its antimicrobial effect the PHMB gelling fiber dressing confers a low cytotoxic profile. It has been demonstrated that cells treated with PHMB gelling fiber dressing extracts remain viable and able to infiltrate a scratch wound to allow it to heal. However silver dressing extract is found to be highly cytotoxic and cells are unable to heal a scratch wound at all, as demonstrated by the data presented.

These two characteristics of high antimicrobial efficacy and low cytotoxicity help in the management of infected wounds and those at risk of infection, helping cells to remain healthy to support progression of wound healing.

### References:

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2. C Attinger and R Wolcott (2012): Clinically Addressing Biofilm in Chronic Wounds. J Advances in wound care V1 (3)
3. Butcher,M. (2012) PHMB: an effective antimicrobial in wound bioburden management. British Journal of Nursing 21 (12) S16-21.
4. Chindera, K et al. The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. Sci.Rep 6, 23121; doi: 10.1038/srep23121 (2016).

Data on file –LD077-22, LD134-22, LD021-23,  
PHMB Dressing - PHMB gelling fiber dressing, AMS (is not cleared in USA, is not CE marked)  
Silver dressing - AQUACEL<sup>®</sup> Advantage, ConvaTec  
AQUACEL<sup>®</sup> is a registered trademark of ConvaTec Inc.



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