Visual Assessment of the Combined Non-Cytotoxic and Antimicrobial Effects of PHMB

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INTRODUCTION

Microbial colonisation of wounds can lead to wound infection and a delay in healing, increased pain and in the worst cases sepsis. The emergence of increasing antibiotic resistant microbial strains is cause of major concern globally. It is therefore important to consider the use of antimicrobial agents in the prevention of wound infection. One of the key considerations is to find an effective antimicrobial that has a low cytotoxic profile. Efficiently killing microorganisms while mammalian cells remain healthy and able to heal the wound.

Here we present microscopic images of common wound colonising microorganisms cultured with mammalian fibroblast cells, in the presence and absence of the antimicrobial agent PHMB (polyhexamethylene biguanide). This broad-spectrum antimicrobial agent can be used in a variety of formats, such as within wound dressings and wound irrigation solutions as well as for pre-operative washes and contact lens solutions. Here we demonstrate an effective antimicrobial action against gram-negative, gram-positive and yeast species, whilst mammalian cells remain healthy and viable.

METHOD

Microorganisms were grown on suitable agar plates then added to cell culture media at a final concentration of $1x10^3$ CFU/ml in the presence or absence of 5ppm PHMB.

Fibroblasts were cultured in 24 well plates to ~ 80% confluence, then the above described solutions were added. The co-cultures were incubated for 24 hours at 37°C 5% CO_2

The culture media was removed, and Live/Dead stain (LIVE/DEAD™ *Bac*light™ Invitrogen UK) was added to the wells and incubated. The cultures were then imaged on a Nikon Ti-S inverted microscope and visualised using ImageJ software.

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RESULTS

MRSA – (Methicillin-resistant staphylococcus aureus)

Staph aureus species are common skin commensals and represent one of the most common causes of skin wound infections (1). This gram-positive bacteria is resistant to increasing numbers of antibiotics and presents a difficult to manage infection clinically (2). Here we demonstrate that PHMB is effective at killing MRSA and leaving mammalian fibroblasts healthy and viable (Figure 1). This offers an alternative to traditional antibiotics reducing development of further resistant strains.

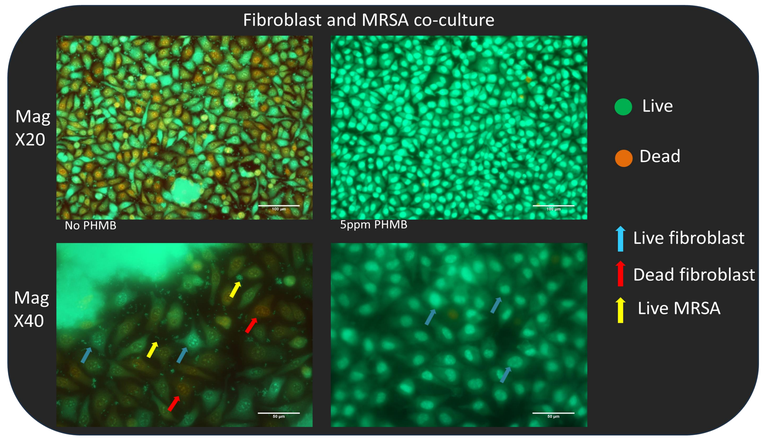

Figure 1 Mammalian fibroblasts cells co-cultured with MRSA in the presence and absence of PHMB. Fluorescence staining with live/dead stain showed that in the absence of PHMB the fibroblasts showed a loss of viability and a number of dead (orange staining) cells could be seen. The MSRA were visible as green cocci (yellow arrow). In the presence of 5ppm PHMB the MRSA was no longer viable and the vast majority of fibroblast cells were viable and healthy (green staining)

Candida albicans *Candida* species cause yeast infections in skin and cause a delay in wound healing. These fungal species are often found in chronic wounds such as diabetic foot ulcers and pressure ulcers. In addition to the yeast infection itself these species can also help bacterial species evade antibiotic treatment leading to an inability to clear an infection (3). As yeast species are not themselves killed by antibiotics, clinically they are treated separately with anti-fungal agents. Here we demonstrate that PHMB is effective at killing *Candida albicans* and leaving mammalian fibroblasts healthy and viable (Figure 2).

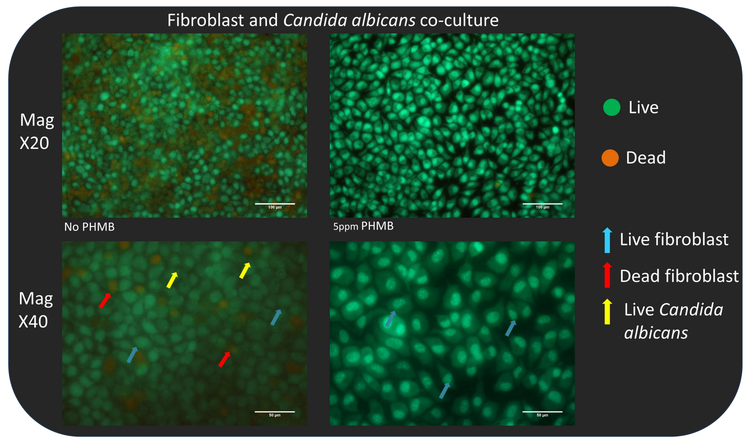

Figure 2 - Mammalian fibroblasts cells co-cultured with *Candia albicans* in the presence and absence of PHMB. Fluorescence staining with live/dead stain showed that in the absence of PHMB the fibroblasts showed a loss of viability and a number of dead (orange staining) cells could be seen. The *Candia albicans* was visible as green hazy area punctuated with cocci (yellow arrow). In the presence of 5ppm PHMB the *Candida albicans* was no longer viable and the fibroblast cells were viable and healthy (green staining).

RESULTS

Pseudomonas aeruginosa *Pseudomonas* is an opportunistic pathogen often found in deep puncture wounds as well as chronic wounds (4). This is a gram-negative bacteria with its own challenges for treatment, including the tough outer membrane associated with gram-negative species as well as a high degree of antibiotic resistance (5). Here we demonstrate that PHMB is effective at killing *Pseudomonas aeruginosa* and leaving mammalian fibroblasts healthy and viable (Figure 3).

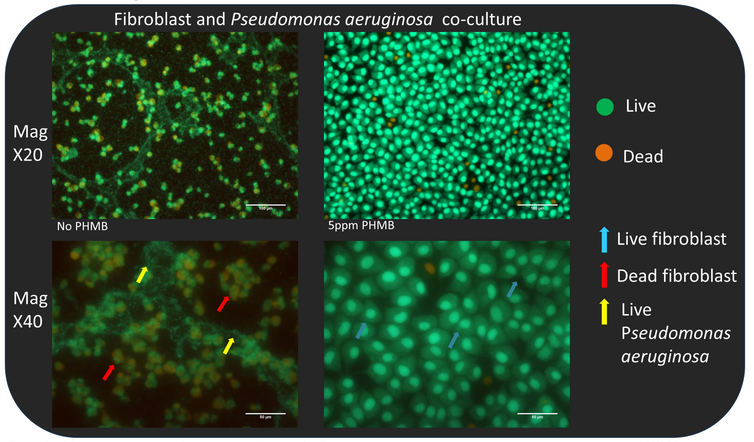

Figure 3 - Mammalian fibroblasts cells co-cultured with *Pseudomonas aeruginosa* in the presence and absence of PHMB. Fluorescence staining with live/dead stain showed that in the absence of PHMB the fibroblasts showed a loss of viability and a number of dead (orange staining) cells could be seen. The *Pseudomonas aeruginosa* is visible as green rods (yellow arrow). In the presence of 5ppm PHMB the *Pseudomonas aeruginosa* was no longer viable and the fibroblast cells were viable and healthy (green staining).

CONCLUSION

The increasing prevalence of antibiotic resistance is a major health concern, making the use of effective antimicrobial agents to help manage infection an essential consideration in wound management. We have demonstrated that PHMB is very effective at killing gram-positive bacteria, gram-negative bacteria and yeast species giving a full spectrum antimicrobial action. Additionally in the presence of PHMB mammalian fibroblasts in co-culture remained healthy and viable suggesting that in clinical use PHMB would allow patient tissues to heal a wound while managing microbial infection.

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