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Using a novel three-dimensional inter-kingdom wound biofilm model to assess antimicrobial treatments

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Background: Chronic wounds, including diabetic foot ulcers, pressure ulcers and venous leg ulcers, are becoming increasingly more common due to an aging population, and sedentary and urbanised lifestyles. It has been shown that infection is important in the recurrence and chronicity of such wounds, although this is not well characterised. Fungal infections in chronic wounds are largely ignored, despite increasing evidence that fungi are prevalent. Therefore, we aimed to develop a representative microbiological model of a chronic wound that can be used for testing of treatments used in the clinic setting, a model previously used to characterise the response to wound washes (Townsend et al., 2016). Here, we aimed to challenge the triadic interkingdom biofilm model with antimicrobials to mimic treatment regimens in the clinic.

Material/methods: The yeast, *Candida albicans*, and bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were chosen for inclusion in the model. The preparation of both the hydrogel and the cellulose matrix, as well as the level of serum contained within the hydrogel were all assessed and optimised. The biofilms are grown by first inoculating the cellulose matrix with yeast and/or bacteria before being placed onto the hydrogel for 24 h. Antimicrobials were added, both alone and in combinations, after the biofilm had matured adding directly onto the cellulose matrix for 24 h. The treatments chosen for use in this work were flucloxacillin, ciprofloxacin, and fluconazole. The efficacy of the treatments was assessed using colony forming unit (CFU) counts and live/dead qPCR.

Results: Traditional techniques showed that ciprofloxacin was the only treatment to cause a significant reduction in only the bacterial load in the model. For combination treatments, either flucloxacillin or fluconazole in combination with ciprofloxacin caused a significant decrease in bacterial CFU counts. However, it was necessary to triple treat with all three drugs to reduce both the yeast and bacterial components of the biofilm. This was confirmed by live/dead qPCR, where only the triple treatment caused a $\log_{10}$ reduction in the viable organisms present. The composition of the biofilm after this triple treatment was not dissimilar to the untreated biofilm. SEM showed structural changes in the biofilm after antibiotic treatment, with the relative abundances of each organism appearing to change post-treatment.

Conclusions: The triadic interkingdom *in vitro* biofilm model supports the use of combination treatments in the clinic to reduce bioburden within a wound. The work also strongly supports the addition of an antifungal into a treatment regimen, alongside antibiotics, to disrupt any synergistic and protective interkingdom interactions.

TOWNSEND, E. M., SHERRY, L., RAJENDRAN, R., HANSOM, D., BUTCHER, J., MACKAY, W. G., WILLIAMS, C. & RAMAGE, G. 2016. Development and characterisation of a novel three-dimensional inter-kingdom wound biofilm model. Biofouling, 32, 1259-1270.