

Background

P. aeruginosa, *S. aureus* and *C. albicans* are common wound pathogens and products are often assessed against them individually. However, in nature they do not exist in isolation. The presence of a microorganism in a culture can alter the behaviour of other microorganisms present. For example, growth of *C. albicans* with *P. aeruginosa* in a multi-species biofilm has been shown to increase the expression of *P. aeruginosa* virulence factors such as pyoverdine, rhamnolipids and pyocyanin compared to monocultures. Single species biofilm models mimic the real-world scenario considerably more than planktonic assessments, however the use of multi-species biofilm models adds additional complexity to bridge the gap between *in vitro* models and clinical testing. Multi-species biofilm infections can complicate or delay wound healing and effective treatment can aid healing.

Aim

To determine the biofilm disruption capabilities of two wound care products using a mixed species colony drip flow model.

Methodology

- *P. aeruginosa*, *S. aureus* and *C. albicans* were prepared in a bespoke growth medium.
- A Drip Flow Reactor (DFR) was prepared with hydrated, multi-layered coupons.
- The multi-species inoculum was circulated through the DFR at 5 mlhr⁻¹ at 37°C for 72 hours to establish mature biofilms (Figure 1).
- Following incubation, coupons were rinsed in Phosphate Buffered Saline (PBS) to remove planktonic organisms.
- Coupons were transferred to a bioengineered medical honey wound gel* or sandwiched between Cadexomer iodine** dressings for 24 hours at 37°C (Figure 2).
- Following treatment, coupons were rinsed in PBS, placed in neutraliser and sonicated to recover remaining attached microorganisms. The resulting suspension was enumerated.

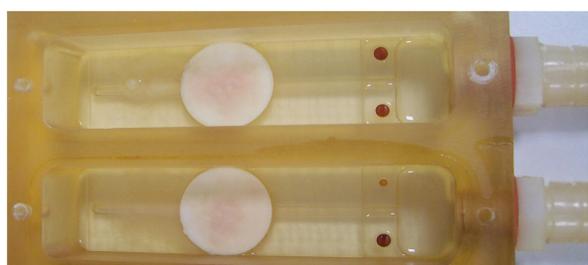


Figure 1. A photograph of pre-formed multi-species biofilms in a drip flow reactor.

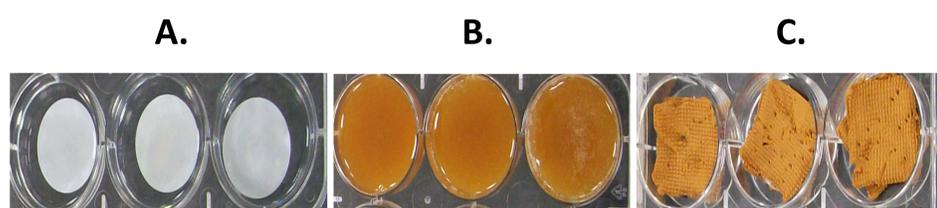


Figure 2. A photograph of biofilm surfaces during treatment. A = Negative Control, B = bioengineered medical honey wound gel* C = Cadexomer iodine**

Results

No viable *P. aeruginosa* or *S. aureus* were recovered from coupons following treatment for 24 hours with a bioengineered medical honey wound gel or a Cadexomer iodine dressing. An average of 5.82 Log₁₀cfuml⁻¹ *P. aeruginosa* and 2.13 Log₁₀cfuml⁻¹ *S. aureus* were recovered from the control following 72-hours incubation. No *C. albicans* were recovered from the control or treated coupons which was expected from this model (Figure 3).

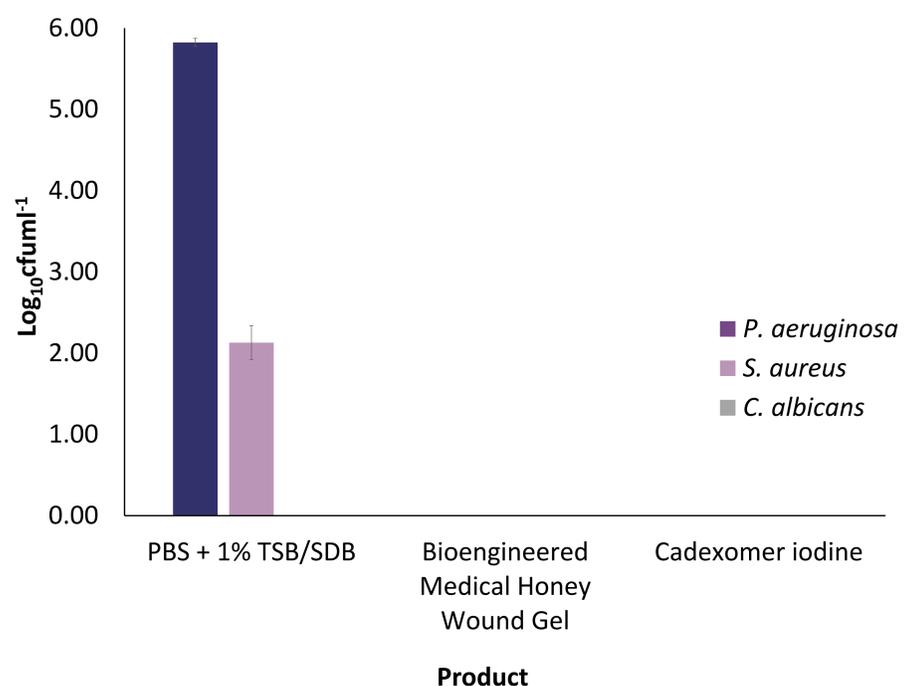


Figure 3. Quantity of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* recovered from porous surfaces containing multi-species biofilms following treatment.

Discussion and conclusions

Biofilms have been shown to be present in approximately 70% of chronic wounds where they reduce healing times. Single species biofilm models are typically used to test products. However, clinical biofilms are more likely to be composed of a number of microorganisms rather than a single species. The model used in this study utilised bacteria and fungi commonly found in chronic wounds to develop an infected wound model that is relevant to a clinical environment.

The study showed that following treatment with a bioengineered medical honey wound gel and a Cadexomer iodine dressing, no viable organisms were recovered from coupons containing pre-formed 72-hour mixed species biofilms. This suggests that both treatments were comparable as they could both treat pre-formed biofilms. Products with anti-biofilm capabilities could have useful clinical implications. Clinical studies would be required to confirm this result.

References

- Kerr J. Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from patients with cystic fibrosis. *Journal of Infection* (1994); **28**, 305–310.
- Gupta N, Haque A, Mukhopadhyay G, Narayan RP, Prasad R. Interactions between bacteria and *Candida* in the burn wound. *Burns* (2005); **31**: 375–378.
- Abigail Trejo-Hernández, Andrés Andrade-Domínguez, Magdalena Hernández and Sergio Encarnación. Interspecies competition triggers virulence and mutability in *Candida albicans*–*Pseudomonas aeruginosa* mixed biofilms. *The ISME Journal* (2014); **8**, 1974–1988.

*= Surgihoney™ RO® sterile medical honey

**= Iodoflex cadexomer dressing with iodine