WOUND BIOFILMS: DEVELOPMENT OF A NOVEL BASAL PERFUSION WOUND BIOFILM MODEL

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ABSTRACT
INTRODUCTION: Biofilms have been implicated in wound infection, the aim of this study was to develop a representative wound biofilm model to maintain a stable, diverse sessile population of potential wound pathogens.

METHOD: A novel basal perfusion wound biofilm model has been developed to reproduce the basolateral nutrient delivery and oxygen diffusion of wound biofilms. In this model, filters are partially immersed in continuously delivered nutrient medium at a flow rate comparable to an exudative wound. Biofilms are then established using aerobic and anaerobic bacteria previously reported in wound infections (Bacteroides fragilis, MRSA, Pseudomonas aeruginosa and Streptococcus pyogenes). In the current investigation, filters were sequentially inoculated to ensure maintenance of all species. Biofilms were sampled daily for up to 8 d (n=3) and viable counts were undertaken to identify establishment of populations. Biofilms were additionally visualised using environmental scanning electron microscopy (ESEM) and fluorescent microscopy.

RESULTS: ESEM of modelled wound biofilms revealed microcolonies and putative biofilm exopolysaccharide, indicative of biofilm formation throughout the filters. The presence of exopolysaccharide was confirmed using differential fluorescent staining and visualisation by fluorescent light microscopy. Each bacterium was recovered from the biofilm model and maintained dynamic equilibrium c. 24 h after inoculation, with cell densities of 26 Log10 CFU/cm2 that were maintained for 8 d.

CONCLUSION: The present study indicates that the wound biofilm model is capable of stably maintaining a diverse microbial biofilm population, including a range of aerobic and anaerobic wound pathogens. This model represents a platform with which the efficacy of dressing and treatment regimes can be tested topically and/or systemically against bacterial wound consortia in the biofilm model of growth.

REFERENCES

OBJECTIVES
To develop a multi-species, clinically relevant chronic wound biofilm model

BIOFILMS: FREQUENTLY ASKED QUESTIONS
What is a biofilm?
• Biofilms are surface attached microorganisms encased within a layer of exopolysaccharide (EPS; polysaccharides, proteins, nucleic acids and lipids)3,4.
• Biofilms are ubiquitous2 and microorganisms can attach to most surfaces3 eg. human tissue such as a wound bed, medical implanted devices or to other microorganisms.
• The NIH estimate that ~ 80% of all human infections are biofilm-related4, with the majority of all chronic infections attributed to biofilms5. Biofilms are difficult to treat because they can be 100-1000 less susceptible to antimicrobials than their free-floating or "planktonic" counterparts4.

Are all biofilms equal?
• No. Biofilm formation is a continuous process. Therefore, it can be difficult to compare results generated from different in vitro biofilm models: the most challenging models tend to grow a biofilm over several days, generating a mature biofilm with reduced antimicrobial tolerance.

What about wounds?
• Biofilms have been identified in chronic wounds by scanning electron microscopy7 and light and confocal microscopy.
• A number of in vitro biofilm models are in the literature/commercially available, however, none adequately reproduce the unique characteristics of a wound biofilm.

DESIGN OF WOUND BIOFILM MODEL
• In a wound, bacteria grow upon and invade the wound bed and surrounding tissue and thus derive their nutrients from the wound bed. Such infected wounds may be topically treated with antimicrobial products. In addition, wound biofilms often contain many microbial species. When developing the wound model it was important to replicate this environment to understand how these biofilm communities develop and to be able to topically apply antimicrobials.
• Therefore, key attributes of the "basal perfusion wound biofilm model" (Fig. 1) included the ability to:
  • Mimic the wound environment by continuous feeding the biofilm from the substratum
  • Support a defined, multi-species biofilm, including aerobic and anaerobic bacteria
  • Apply dressings or topical antimicrobial agents to the biofilm in situ
  • Grow biofilms under saturated conditions to simulate an infected exudative wound
  • Grow biofilms for a prolonged period of time to simulate chronic biofilms.

VALIDATION OF WOUND BIOFILM MODEL
• Bacteria selected for incorporation in the basal perfusion wound biofilm model were based upon their occurrence in wounds and their pathogenic impact upon healing outcomes.
• Growth of the bacteria was maintained aerobically in the wound biofilm model.
• Stable maintenance of individual microbial species was confirmed by removal of biofilms on filters every 24 h and culture (Fig. 2).
• Differentiation of bacteria and EPS was conducted by staining samples with a selective dye solution and viewing by epifluorescent microscopy (Fig. 3).
• Environmental scanning electron microscopy of samples was also revealed microcolonies and putative biofilm EPS throughout (Fig. 4).

SUMMARY
• Biofilms are surface attached microorganisms encased within a layer of EPS and can be 100-1000 less susceptible to antimicrobials than free-floating bacteria.
• Biofilms have previously been reported in wounds.
• We have developed an in vitro "basal perfusion" wound biofilm model that enables the maintenance of a multi-species biofilm consisting of a range of potential wound pathogens.
• The basal perfusion wound biofilm model mimics a wound in that the biofilm bacteria are continuously fed from the wound bed and is able to stably maintain a multi-species biofilm over a prolonged period of time.
• The basal perfusion wound biofilm model will enable the testing of wound care products topically to the in vitro wound bed under clinically relevant conditions.