Treating infected chronic wounds without antibiotics

Harling, C[1], Secker, T[2], Hands, C[2] Voegeli, D[3], Keevil, W[2], Leighton, T G[1]

[1] Faculty of Engineering & the Physical Sciences, University of Southampton
[2] Faculty of Environmental & Life Sciences, University of Southampton
[3] Faculty of Health & Wellbeing, University of Winchester

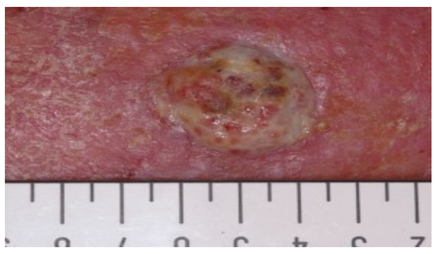
Introduction

Chronic Wounds

➢ Are wounds that fail to heal in the normal manner

- 2.2 m patients each year in the UK
- >£5 billion pa cost for the NHS[1]
- Pain, reduced mobility, social isolation for the patient

infection is largest remediable cause of chronicity



Chronic infected venous leg ulcer
www.dermnetnz.org

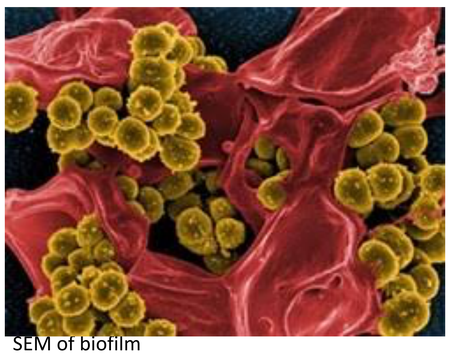
Biofilms

Chronic wound infections are normally in the form of a biofilm[3] which offer infecting organisms

- Increased adhesion to wound bed
- Protection against host defences
- Relative resistance to antibiotics

thereby increasing the difficulty of treatment

Why not just remove the biofilm?



SEM of biofilm
www.slectscience.net

Acoustically Activated Fluid Stream (AAFS)

A bubble in a fluid exposed to a sound field may exhibit a number of behaviors. With increasing sound amplitude, initial radial oscillations are replaced by Faraday waves on the bubble's surface.[2] These 'bubbles with waves' can remove contaminants from surfaces. Acoustic radiation forces propel the bubbles into crevices thereby making this technology suitable for cleaning irregular surfaces such as wounds. Creating this environment in a fluid stream allows the cleaning action to be directed at a wound.

Micrograph of trapped bubble showing Faraday waves (courtesy T G Leighton)

Results

1. Washing

Images from epifluorescent microscopy

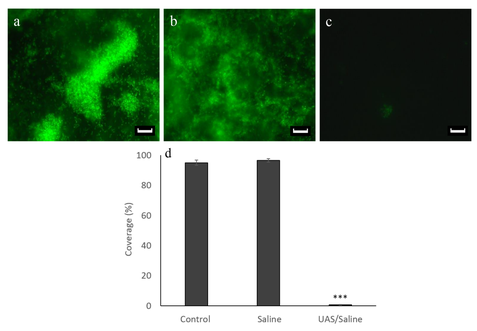
a. Sample with biofilm

b. Sample with biofilm washed with saline

c. Sample with biofilm washed with AAFS saline

Scale bar is 10 µm

Graph shows percentage coverage of GFP tagged bacteria



2. Healing

H&E stained sections through wound bed

a. Sample with no biofilm & no wash

b. Sample with no biofilm & saline wash

c. Sample with no biofilm & AAFS saline wash

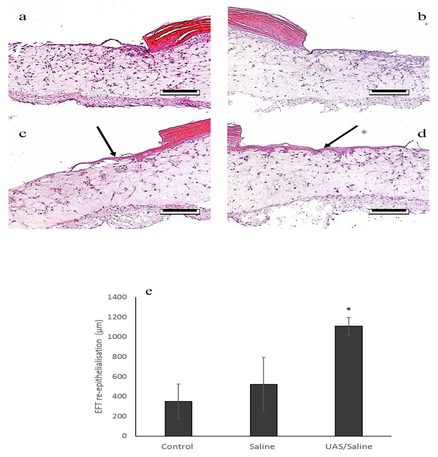
d. Sample with no biofilm & AAFS saline wash

Scale bar is 500 µm

Arrows in c. & d. indicate the tongue of re-epithelialisation

Graph shows length of tongue of re-epithelialisation growing across the wound bed

No damage to the tissue is seen



3 Keratinocyte activation

Sections through the wound bed stained for cytokeratin 14

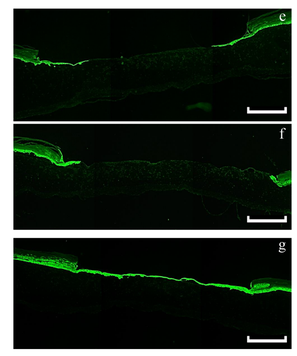
e. Sample with no biofilm & no wash

f. Sample with no biofilm & saline wash

g. Sample with no biofilm & AAFS saline wash

Scale bar is 500 µm

Activated keratinocytes spreading across the healing wound bed



Methods

- Pre wounded Epiderm Full Thickness (EFT®; Mattek Corp., USA) reconstituted human epithelium samples were used
- *Pseudomonas aeruginosa* PA01 tagged with green fluorescent protein were used to form a 24 hour biofilm
- Samples were treated according to the table below
- Samples were imaged using epifluorescent microscopy with image analysis for percentage coverage with ImageJ
- Sections from the samples were stained with Hematoxylin & Eosin or anti-cytokeratin 14

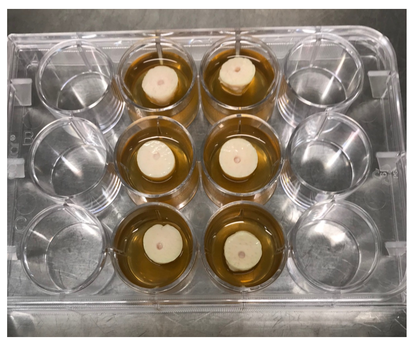
EFT® Sample	Biofilm present?	Saline Wash?	AAFS Wash?
Control 1	-	-	-
Control 2	+	-	-
Control 3	-	+	-
Control 4	-	-	+
Saline wash	+	+	-
AAFS saline wash	+	-	+

Conclusions

- AAFS washing of pre-wounded EFT® samples infected with an early biofilm removes 99.99% the infection ($p<0.001$)
- AAFS washing of pre-wounded EFT® samples without biofilm causes and increase in the re-epithelialisation of the wound
- The re-epithelialisation of the wound involves the normal mechanism of keratinocyte activation involving the production of cytokeratin 14
- Washing of pre-wounded EFT® samples does not cause damage or disruption to the underlying structures

Future work

- These experiments are being replicated using more mature biofilms and also multi species biofilms on a pig skin explant model.
- Subsequently, similar experiments will be conducted on human surgical salvage skin explants to generate performance and safety data for 'Fist in Human' trial
- When performance and safety data available, a small clinical trial will be conducted next year



Pig skin explants on media

References

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2 Leighton, T G. 2014 Proc Inst Acoust. **36(3)** 58-86
3 Malone, M 2017 J Wound Care. **26(1)** 20-25

Christopher Harling MRes FRCP
c.c.harling@soton.ac.uk
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UNIVERSITY OF Southampton