Selective Depletion of *S. aureus* Restores the Skin Microbiome and Accelerates Tissue Repair

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Introduction

The Challenge: Wound Infection

The microbiota is essential for skin health, but dysbiosis, as seen with aging and diabetes, can impair skin function and increase infection risk (**1**). Wound infections are a major contributor to poor healing, often preceding over 90% of wound-related amputations (**2**). These wound infections are recalcitrant due to the presence of antimicrobial resistant (AMR) pathogens. Moreover, when we treat with antibiotics, we can deplete the resident microbiota and promote the growth of AMR pathogens (**3**; **Figure 1**). Thus, there is urgent need to develop new, pathogen-selective antimicrobials to replace traditional antibiotics.

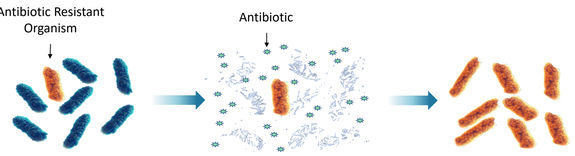
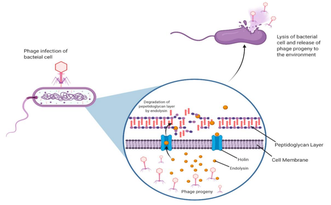


Figure 1. *Antibiotics deplete the resident microbiota, promoting AMR pathogen growth.*

The challenge is to develop antimicrobials that kill pathogens without harming the commensal microbiota

Could Endolysins Be the Key to Tackling Antimicrobial Resistance?

Bacteriophage-derived peptidoglycan-degrading enzymes (endolysins) are emerging as an innovative antimicrobial therapy. The coevolution of bacteriophages with bacteria means that endolysin resistance is rare, while endolysins are often targeted against certain bacterial hosts, thus preventing selective pressure on the resident microbiota (**4**).



Bacteriophage mode-of-action

Aim

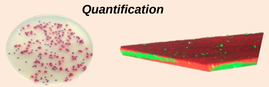
To assess the efficacy and selectivity of a novel endolysin engineered against the common skin/wound pathogen, *Staphylococcus aureus.*

Methods

i) Screening endolysin efficacy in *Staphylococcus* infection models

Human Ex Vivo Skin

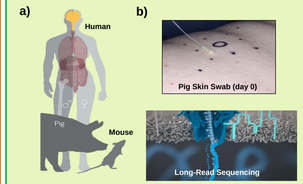
Quantification



Enumeration ***Live:Dead Staining***

ii) Microbiome profiling

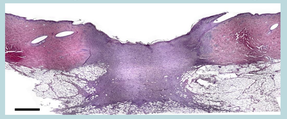
DNA isolation from swabs & long-read sequencing



a) Pig-mouse-human skin comparison
b) Endolysin effects on "real-world" microbiome

iii) Tissue level effects of endolysin

Histological characterisation of healing

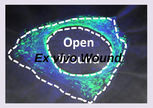


a) H&E staining
b) Macrophage profiling (Iba1 & Arg1)

iv) Translational relevance

Effects of Staphylococcus on human skin repair

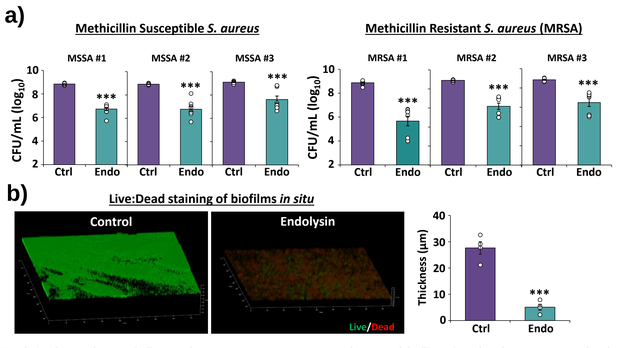
Scan me for protocol



Histology and RNA-Seq

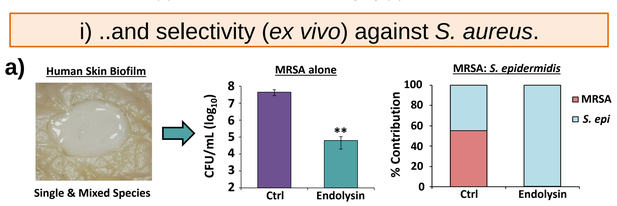
Results

i) Endolysin shows efficacy (*in vitro*)…



Endolysin substantially reduces *S. aureus* membrane biofilm load, demonstrated via bacterial enumeration (**a**) and CLSM Live:Dead imaging (**b**). *** = $P < 0.001$.

i) ..and selectivity (*ex vivo*) against *S. aureus*.

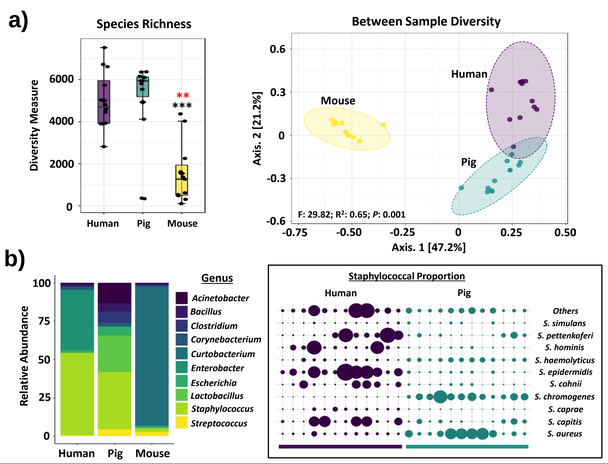


Endolysin selectively inhibits *S. aureus* in human *ex vivo* skin wounds. **a)** Quantification of bacteria in single and mixed species biofilms. **b & c)** Visual assessment of biofilm load in human skin. ** = $P < 0.01$, *** = $P < 0.001$. Bar = 200µm.

We need to develop "real world" microbiome models that are truly translational

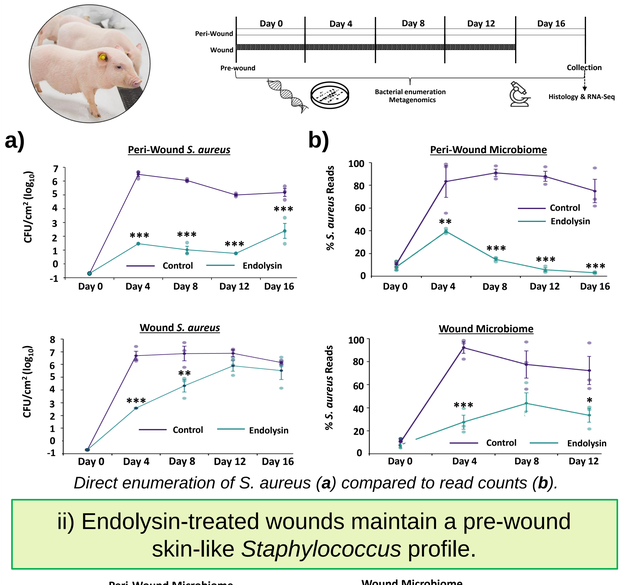
ii) Pigs provide a human-relevant model to assess staphylococcal modulation.

Long-read metagenomic profiling (Nanopore) demonstrates high similarity in skin bacterial diversity between humans and pigs (**a**) with more similar contribution of *Staphylococcus* at the genus and species level (**b**). ** = $P < 0.01$, *** = $P < 0.001$. Red ** versus pig.



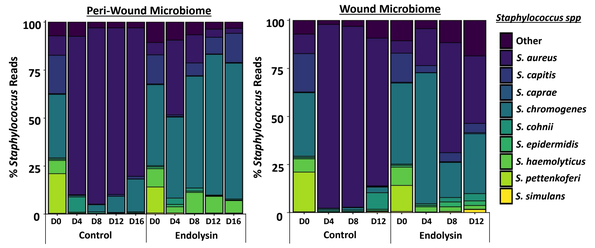
Next step: First interventional study in pigs to assess microbiome modulation with selective antimicrobials

ii) Endolysin selectively depletes endogenous *S. aureus*



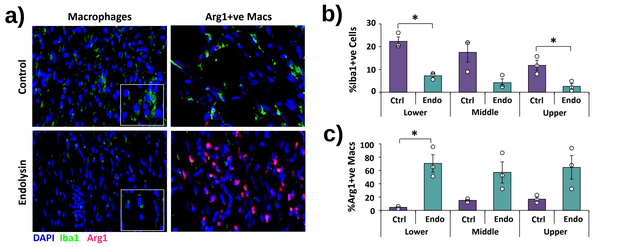
*Direct enumeration of S. aureus (**a**) compared to read counts (**b**).*

ii) Endolysin-treated wounds maintain a pre-wound skin-like *Staphylococcus* profile.



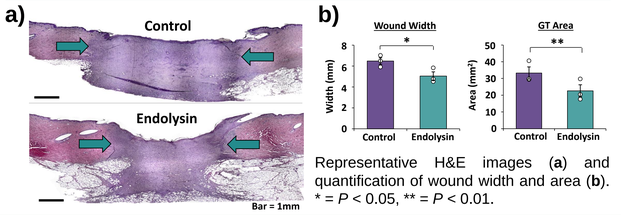
Temporal profiling of *Staphylococcus* proportions in peri-wound skin and wounds following endolysin treatment. D0 = prior to wounding.

iii) Staphylococcal modulation dampens inflammation...



Endolysin treated wounds show lower numbers of total macrophages (Iba1+ve) and a higher proportion of those macrophages are anti-inflammatory (Arg1+ve). Representative images in **a**. Quantification in **b** & **c**. Bar = 25µm. * = $P < 0.05$.

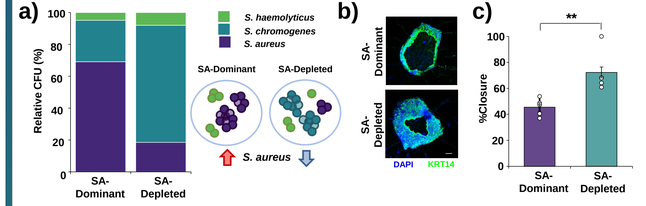
iii) …and significantly accelerates wound repair.



Representative H&E images (**a**) and quantification of wound width and area (**b**). * = $P < 0.05$, ** = $P < 0.01$.

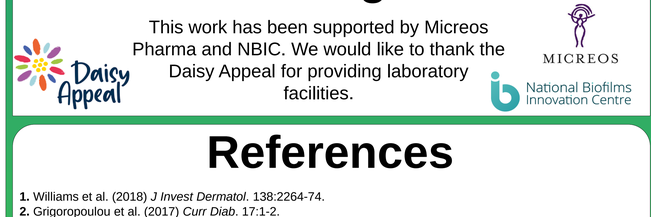
What is the role of the S. aureus dominated microbiome in human wound repair?

iv) An *S. aureus*-dominated microbiome delays human wound repair…



Porcine staphylococci grown in mixed communities mimicking *S. aureus* (SA) dominant (vehicle-modelled) and SA-depleted (XZ.700-modelled) microbiomes. Relative proportion shown in **a**. Corresponding wound closure assessed in **b** and **c**. Bar = 200µm. $P < 0.01$.

iv) …and alters host response.



S. aureus (SA) dominant mixed communities upregulate key pro-inflammatory pathways.

Summary

Our novel results provide the first demonstration that selective modulation of endogenous *S. aureus* promotes healing.

In addition, we reveal the suitability of pigs as a human-relevant model of the skin microbiome.

These findings provide the basis for developing translationally relevant microbiome models to assess targeted antimicrobial therapies.

To read more

ORIGINAL ARTICLE

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