APPLICATION NOTES

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Using Labskin to investigate the wound healing process

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OBJECTIVE

To develop methods for the reproducible wounding of Labskin and demonstrate the use of Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI) to directly measure mass spectra from the tissue during the wound healing process.

METHODS

- Each Labskin sample was wounded with a scalpel blade using controlled depth penetration & assessed every 24h for 5 days.
- Samples were either formalin fixed paraffin embedded (FFPE) for histology or embedded in 20% gelatin and flash frozen ready for mass spectrometry imaging.
- FFPE tissue samples for histology were sectioned (5 µm) and stained with haematoxylin and eosin (H&E).
- Fresh frozen samples were sectioned (10 µm), sprayed with MALDI matrix and analysed for lipids.

RESULTS

The wounded model mimics the initial wound healing response through the migration of keratinocytes into the wound site immediately post wounding.

Figure 1 - Photographic image of wounding Labskin with scalpel blade

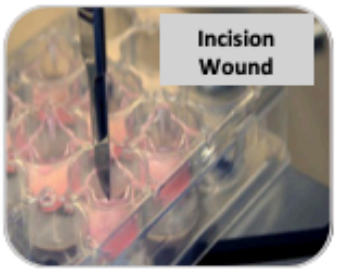
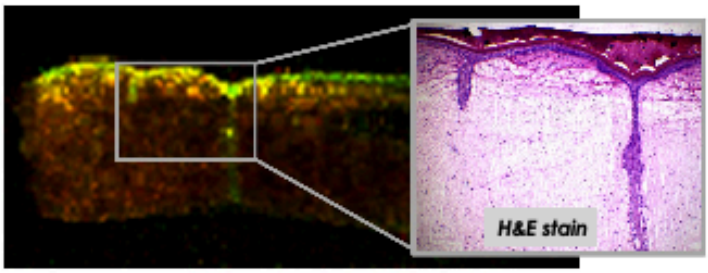


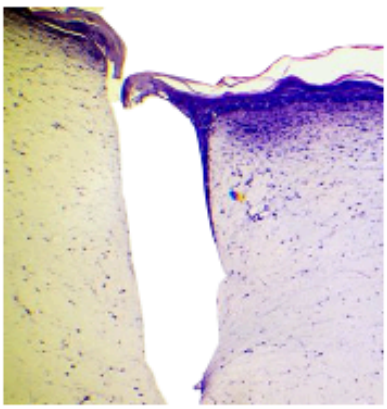
Figure 2 - MSI image and corresponding H&E image of Labskin 3 days post wounding. MSI image of two distinct ion species in the epidermis (green = m/z 721.4) and dermis (red = m/z 725.4)



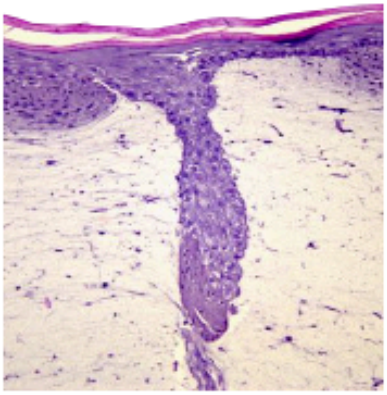
SUMMARY

With care, Labskin can be wounded in a reproducible manner. The wound healing process can be studied using a variety of techniques including MALDI-MSI. Therefore, Labskin can be used to assess the activity of ingredients and formulations in the wound healing process and benchmark against products of recognised activity.

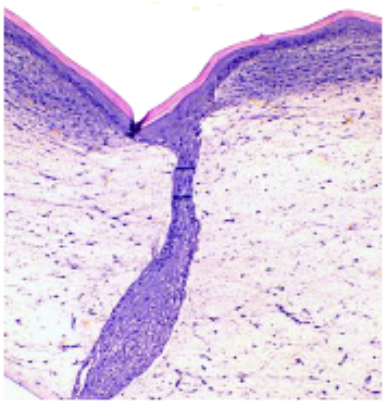
Day 0



Day 3 post-wounding



Day 5 post-wounding



Labskin can be used within the same experimental design to evaluate several endpoints including cytokine responses (i.e. IL-1a, IL-6, IL-8, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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Labskin to assess performance of cosmetic ingredients and formulations making anti-ageing claims

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OBJECTIVE

Repeated application of Retin-A and a consumer emollient to LabSkin full thickness living skin equivalent to determine the effect on procollagen I production in dermal fibroblasts by immunohistochemistry (IHC).

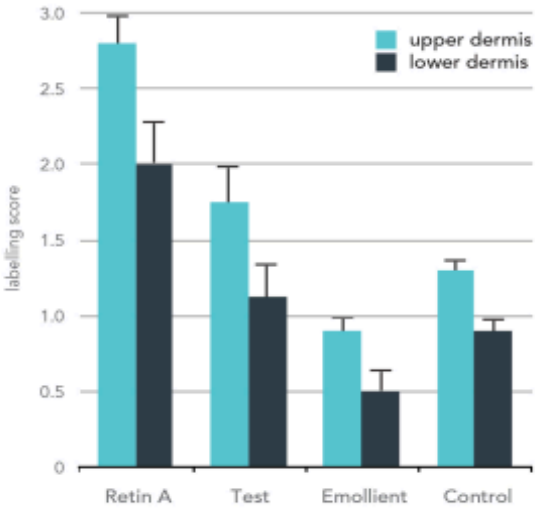
METHOD

- Products were applied to the surface of LabSkin every day for 2 weeks.
- At each application, 20 µL of product was pipetted onto the surface and spread using a sterile glass rod.
- After treatment, 3 x 8 mm punch biopsies were removed from each LabSkin unit.
- Samples were formalin fixed and paraffin embedded for histology.
- Sections of 5 µm were prepared and immuno-histochemistry was carried out to assess the expression of Procollagen-1 in dermal fibroblasts.

RESULTS

Procollagen I immunolabelling associated with fibroblasts within the dermis of LabSkin was assessed by image scoring.

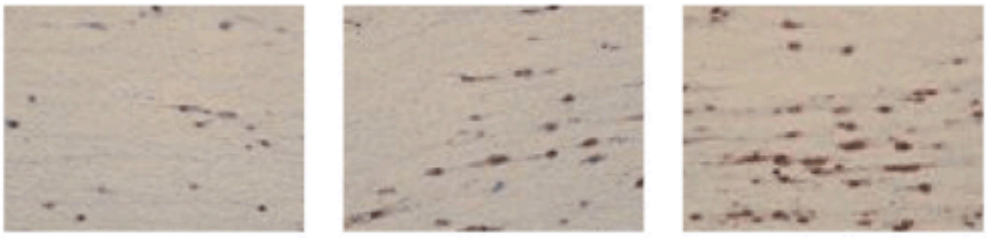
Figure 1 - Affect of products on procollagen production



SUMMARY

Assessment of Procollagen production by immuno-histochemistry (IHC) in dermal fibroblasts in LabSkin full thickness living skin equivalent can be used to support anti-ageing claims for cosmetics ingredients and formulations benchmarked against products of recognised activity i.e. Retin-A

DERMAL FIBROBLAST LABELLING SCORING



grade 1 grade 2 grade 3

Labskin can be used within the same experimental design to simultaneously evaluate multiple factors including cytokine responses (i.e. IL-1a, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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Using Labskin to investigate dermatophyte colonisation

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OBJECTIVE

To develop methods for the reproducible invasion of Labskin with *Trichophyton rubrum* for use in evaluating anti-dermatophyte treatments.

METHOD

- Labskin were incubated for 5 days in the presence of the fungus, at 37°C in 5% CO_2.
- 3 Labskin inserts were pre-treated with product before application of 2.5 x 10^4 cm^{-2} conidia of *Trichophyton rubrum*.
- 3 Labskin inserts were inoculated then treated once with product at day 4.
- 3 Labskin inserts were inoculated then treated with product twice, once at day 2 and once at day 4.
- At day 5, 5 mm biopsy punches were removed from the Labskin to be used for colony counting.
- Samples were formalin fixed and paraffin embedded for histology.
- Haematoxylin and Periodic Acid Schiff (PAS) stains were used for visualising fungal cells.

RESULTS

Fungal colonisation of the epidermis was observed in all inoculated samples. Differences in product efficacy were detected, when comparing treatment schedules.

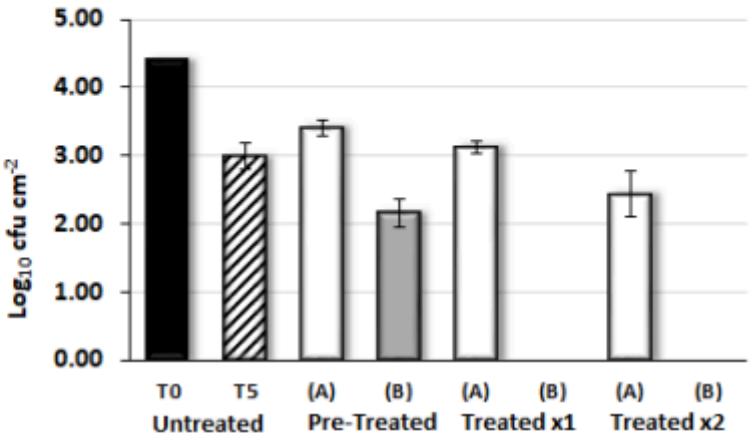
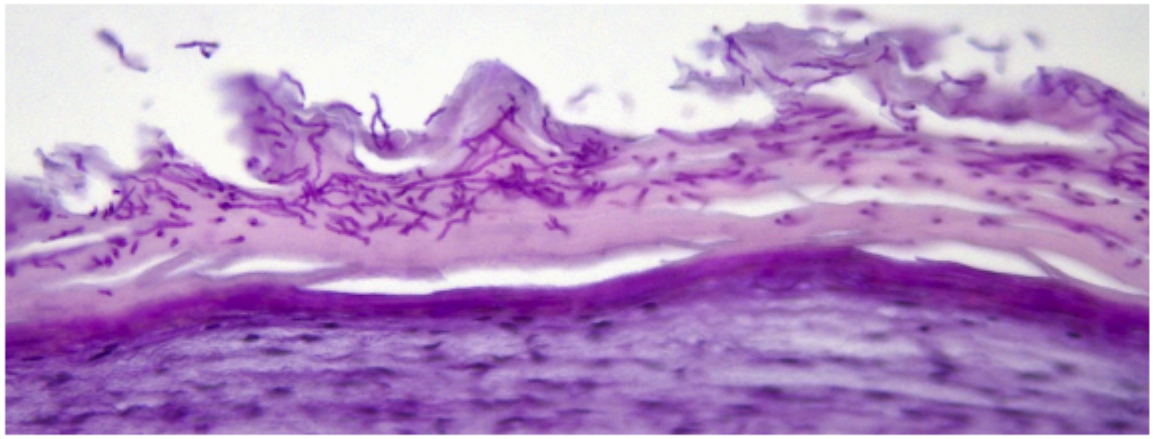


Figure 1 - Activity of topical antifungal treatments A & B against *Trichophyton rubrum* on Labskin

SUMMARY

Labskin was effectively colonised by the dermatophyte *Trichophyton rubrum*. Four days post-inoculation, fungal hyphae interspersed layers of cornified tissue but the tissue beneath remained intact making the model ideal for study of anti-dermatophyte treatments.



Labskin can be used within the same experimental design to simultaneously evaluate multiple factors including cytokine responses (i.e. IL-1a, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

Figure 2 - *Trichophyton rubrum* colonising Labskin 4 days after inoculation. Stained with Periodic Acid Schiff & Heamotoxylin.

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IL-18 Sensitisation Assay in Labskin Tissues

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OBJECTIVE

To determine Labskin's response as an *in vitro* skin model using an IL-18 / MTT based *in vitro* sensitisation assay comparing sensitising and non-sensitising materials.

METHODS

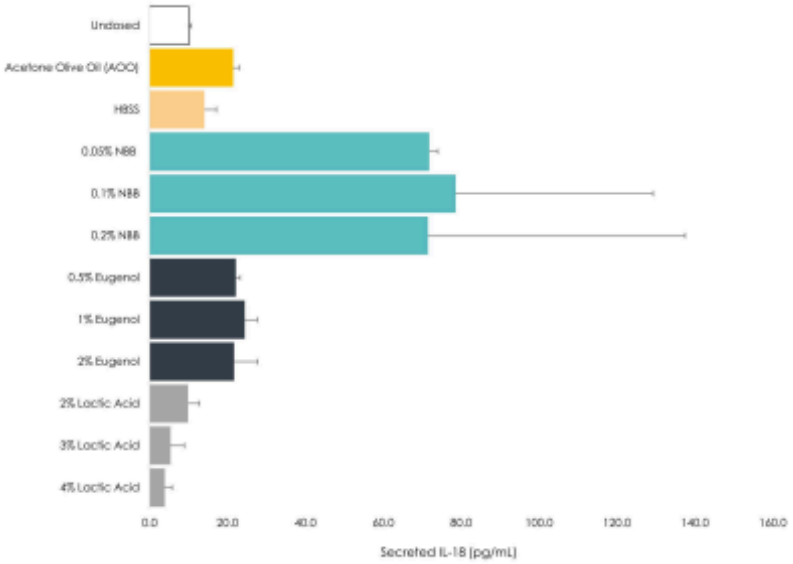
- Labskin Maintenance Medium (4.5 mL) was added to each well. The experiment was conducted using the 12 well plate configuration.
- Tissues were dosed (52 µL) with known sensitising materials (4-Nitrobenzyl bromide [NBB] and Eugenol) alongside a known non-sensitising material (Lactic Acid).
- Tissues were incubated with 1mL of MTT (1 mg/mL) in a 12 well plate for 3.5 hrs.
- Isopropanol (2 mL) was placed in each 12 well plate insert and then an additional 2 mL Isopropanol was added to the top of the tissues. Extraction occurred overnight.
- The following day 200 µL aliquots were removed and read by spectrophotometer (OD 540-690) for viability. The media was analysed by ELISA (MBL) for IL-18 secretion into the culture media.

RESULTS

The non-sensitiser Lactic Acid did not have any increase in IL-18 compared to vehicle control (AOO [Acetone: Olive OIL]), which is expected.

However, the sensitising material Eugenol did not have IL-18 increases over the vehicle control, which should have had a response. Fold increases in IL-18 for NBB was detected.

Figure 1 - IL-18 Secretion detected in culture media



SUMMARY

Overall, this experiment demonstrates that Labskin full thickness *in vitro* skin model is capable of responding to sensitising/allergic materials.

Labskin can be used within the same experimental design to evaluate several endpoints including cytokine responses (i.e. IL-1a, IL-6, IL-8, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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Assessing the safety of hygienic tampons through effects on the growth of the normal vaginal microflora and the production of TSST-1 by Staphylococcus aureus

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OBJECTIVE

An in vitro safety assessment of tampon material evaluated by the effect on the growth of the normal vaginal commensal Lactobacillus acidophilus and the growth and TSST-1 production by pathogenic Staphylococcus aureus.

METHODS

- Materials were incubated in liquid cultures of Lactobacillus acidophilus and Staphylococcus aureus for 24 hours.
- Growth was assessed at regular intervals by culture optical density and viable cell counts. The effect of growth on culture pH was also monitored.
- Samples were taken at regular intervals and processed for the assessment of Toxic Shock Syndrome Toxin 1 (TSST-1).
- TSST-1 was quantified using a validated in-house ELISA.

RESULTS

Figure 1 - Growth of Staphylococcus aureus in the presence of test tampon material

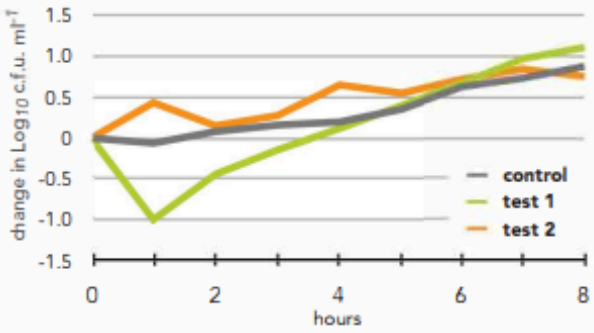


Figure 2 - TSST-1 in culture supernatants of Staphylococcus aureus in the presence of test tampon material

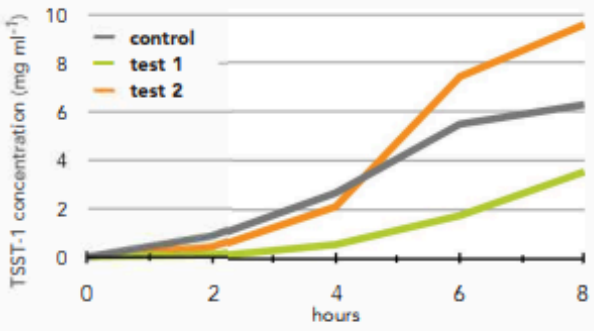


Figure 3 - Growth of Lactobacillus acidophilus in the presence of test tampon material

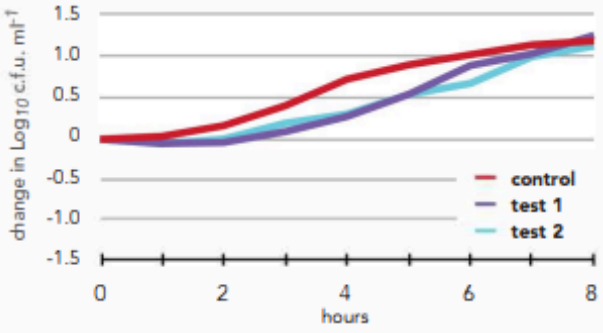
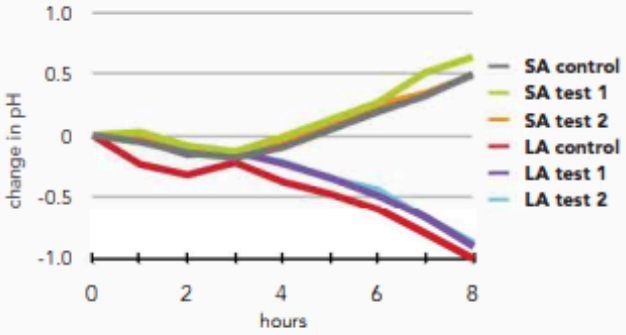


Figure 4 - Change in growth medium pH for S. aureus (SA) and L. acidophilus (LA) in the presence of test tampon material



SUMMARY

Assessment of the growth and bioactivity of vaginal commensal and pathogenic microorganisms can be used to support safety claims for hygienic tampons benchmarked against existing products.

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Characterisation of in vitro Permeation of Ibuprofen

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OBJECTIVE

Characterisation of *in vitro* permeation of Ibuprofen (IBU) using Labskin to evaluate uses as an alternative to human or animal experimentation.

METHOD

- Labskin was mounted in static glass Franz diffusion cells and equilibrated at 32°C ± 1°C. PBS was used as the receptor phase. Figure 1.
- Excess surface water was removed and TEWL measurements taken using an AquaFlux AF200 (Biox Systems).
- Infinite dose: 1mL of saturated IBU solution in propylene glycol (PG) was tested. Receptor fluid was replaced after each sample.
- Finite dose: 3.6µL of IBU solution (1.5% w/v in 5% PG : 95% isopropyl alcohol) was added to the donor compartment. 200 µL samples were collected and replaced by fresh PBS.
- All experiments were conducted for 24h and samples assayed by UV-HPLC.

RESULTS

- TEWL for Labskin remained constant over the time-course of the experiments. (Figure 2).
- Labskin demonstrates less variability (Figure 3) than typically observed for human or porcine skin and other human skin equivalent tissue culture models (Netzlaff, F., et al., European Journal of Pharmaceutics and Biopharmaceutics, 2005. 60: p.167-178)
- The flux values observed for the infinite dose study (Figure 4) were approximately 1.5 times higher for Labskin compared with human epidermis (Watkinson, R.M., et al., Skin Pharmacology and Physiology, 2009. 22: p. 225-230)
- The total amount of IBU permeated across Labkin after application of 3.6 µL of the formulation was 46.2 µg cm^{-2} compared to 17 µg cm^{-2} across human epidermis (Figure 5)

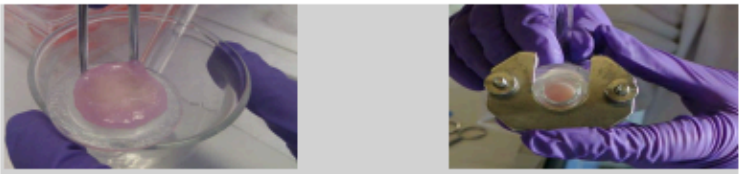


Figure 1 - Mounting in the receptor compartment of a Franz diffusion cell & Franz diffusion cell final assembly.

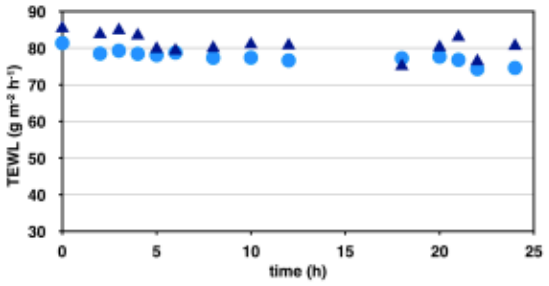
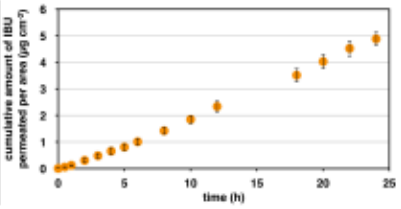


Figure 2 - TEWL values measured on Labskin, no formulation control for 24h infinite (●) and finite (▲) dose study.

Figure 3 - Cumulative amount of ibuprofen permeated from a saturated solution of IBU in PG for 24h across Labskin at 32°C



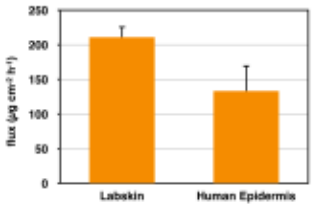
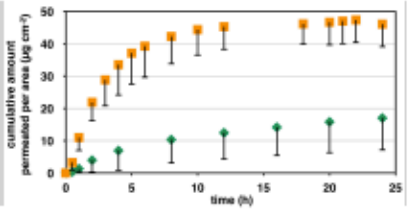


Figure 4 - Steady-state fluxes of ibuprofen through different membranes from saturated solutions in PG (mean ± SD)

Figure 5 - Cumulative amount IBU permeated from 95:5:1.47 (IPA:PG:IBU) solution over 24h across Labskin (■)and human epidermis (◆)at 32°C (n=5, Mean ± SD)



SUMMARY

Although IBU permeated through Labskin more quickly than human skin in vitro, permeability is comparable to porcine ear tissue - currently the closest animal model to human skin.

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Labskin to assess drug penetration with different drug formulations

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OBJECTIVE

To assess whether the addition of a penetration enhancer to a product formulation containing terbinafine will increase terbinafine penetration into Labskin using Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI).

METHODS

- Labskin was treated with terbinafine hydrochloride (5 mg/mL) in actone/olive oil (80:20 v/v) with or without a penetration enhancer for 24 hours
- Samples flash frozen ready for mass spectrometry imaging
- Frozen samples were sectioned (10 µm), coated with MALDI matrix (a-cyano-4-hydroxycinnamic acid) and analysed for *m/z* ratios associated with terbinafine (*m/z* 292.2 and *m/z* 141)

RESULTS

There was increased Terbinafine in the Labskin with the addition of the penetration enhancer in the production formulation.

Figure 1 - Sections of Labskin treated with Terbinafine without (Tissue 1) or with (Tissue 2) a penetration enhancer

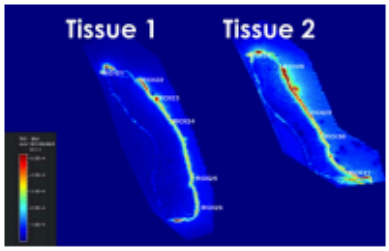
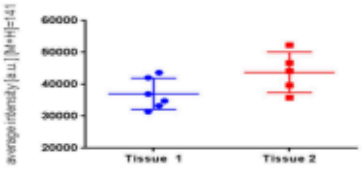


Figure 2 - Graph showing intensity data for terbinafine in Labskin without (3.41± 0.61mg/g of tissue) or with (4.2 ± 0.813 mg/g of tissue) the penetration enhancer



SUMMARY

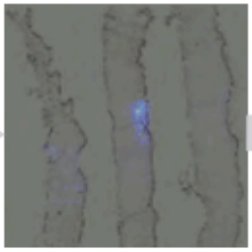
The combination of MALDI-MSI with Labskin can be used to assess and quantify the penetration of ingredients and formulations against products of recognised activity over time.

ACKNOWLEDGEMENTS - Worked conducted by C. Russo, Sheffield Hallam University, UK

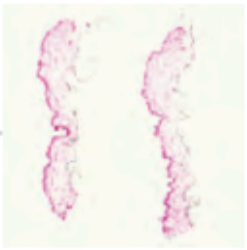
Schematic Representation of the Mass Spectrometry Imaging Workflow

Labskin was incubated with product for the assessed time period

Samples were flash frozen, cryosectioned, mounted onto slides and coated with MALDI matrix



Collated mass spectra was analysed to create mass spectrometry images



Sample can be re-imaged by conventional techniques after MS analysis

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Labskin to assess performance of ingredients and formulations making anti-dandruff claims

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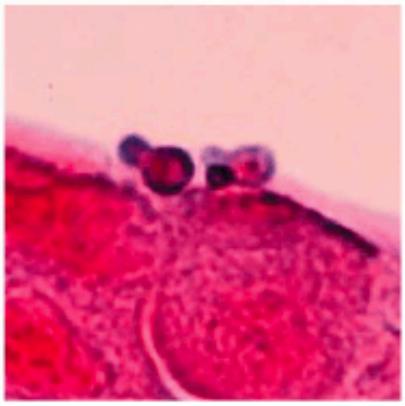
OBJECTIVE

Application of ingredients for the treatment of dandruff to Labskin full thickness living skin equivalent to determine the immediate and residual effects on the viability of Malassezia globosa and Malassezia restricta.

METHODS

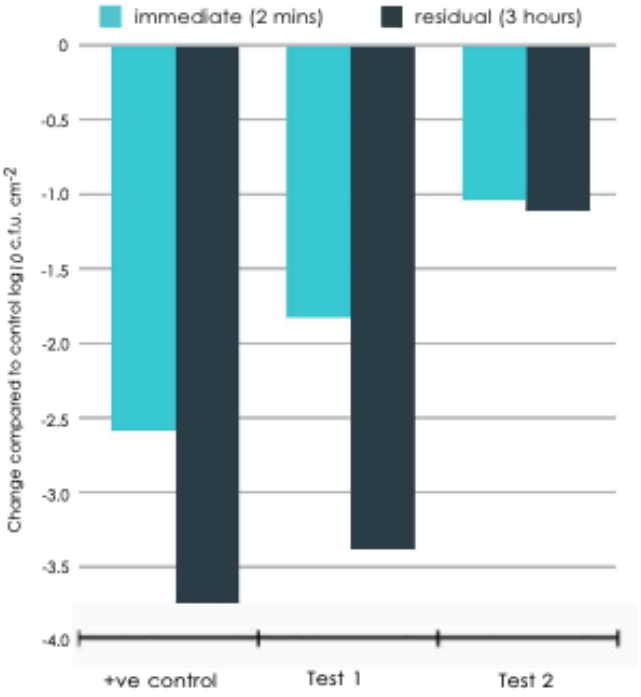
- To assess immediate anti-fungal activity, products were applied to the surface of Labskin pre-colonised for 24h with Malassezia globosa and Malassezia restricta in GS-24. After 2 minutes exposure, the skin surface was washed using a modified scrub wash with neutraliser and viable Malassezia enumerated on RM-SMA agar medium.
- To assess residual anti-fungal activity, products were applied to the surface of Labskin for 2 minutes and then removed by washing. The skin surface was then colonised with Malassezia globosa and Malassezia restricta in GS-24 and incubated. After 3h, the skin surface was washed using a modified scrub wash and viable Malassezia enumerated.

Figure 1 - Budding Malassezia on LabSkin



RESULTS

Malassezia were recovered from the surface of LabSkin using our modified scrub wash technique and viable cells enumerated on our specially formulated RM--SMA growth medium.



SUMMARY

Compared to classic *in vitro* antimicrobial testing protocols (MIC, MBC etc.) where materials are presented in solution, Labskin provides a living, skin-equivalent testing surface which supports the growth of Malassezia in a phenotypically-relevant manner.
The Labskin model can be used to evaluate ingredients and formulations benchmarked against products of recognised clinical activity, and its enhanced predictivity can help to de-risk the move from *in vitro* screening to clinical assessment.

Labskin can be used within the same experimental design to evaluate several endpoints including cytokine responses (i.e. IL-1a, IL-6, IL-8, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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Analysing Labskin using different 'Omic' technologies

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Objective:

To highlight the diversity in the different 'Omic' approaches including genomic, proteomic, lipidomic and metabolomic analysis, which can be applied to Labskin.

Each approach provides a comprehensive and potentially global assessment of multiple molecules simultaneously in a high throughput manner.

Method:

Genomic Analysis: Microarray analysis was used to assess genomic changes in Labskin inoculated with *S. epidermidis* and *S. aureus* (Figure 1).

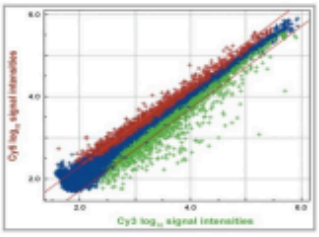
Proteomic and metabolomic analysis: Mass Spectrometry (MS) and MS imaging (MSI) analysis was used to characterise and quantify xenobiotic metabolising enzymes in Labskin (Figure 2).

Lipidomic analysis: MSI analysis with Labskin was performed to identify biomarkers associated with the wound healing process (Figure 3).

Results:

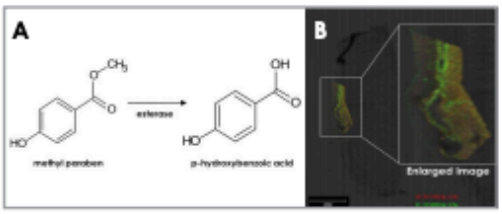
Genomic analysis

Figure 1 - Labskin gene expression response to *S. epidermidis* & *S. aureus* using a whole human genome oligo microarray (red = up regulation, blue = unchanged and green = drown regulation in gene expression).



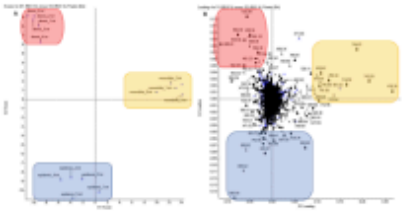
Proteomic and metabolomic analysis

Figure 2 - (A) Diagram showing metabolism reaction of SB-MSI probe methyl paraben with esterase to form p-hydroxybenzoic acid. (B) MSI image of Labskin treated with methyl paraben highlighting esterase activity (red = methyl paraben and green = p-hydroxybenzoic acid).



Lipidomic analysis

Figure 3 - Principal component analysis-discriminant analysis of a (A) scores plot and (B) loadings plot from MSI data representing all three regions of interest (red (dermis), yellow (wound site), blue (epidermis)) in wounded Labskin.



Summary:

Labskin combined with different 'Omic' technologies generates a vast amount of information in a high throughput manner. These techniques allow each stage of the central dogma of molecular biology to be assessed to obtain a greater understanding of changes to the skin environment.

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Using Labskin to characterise xenobiotic metabolising enzymes

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SKIN SCIENCE

Objective:

To compare xenobiotic metabolism distribution and levels between Labskin and *ex vivo* human skin to determine whether Labskin could be used as a prediction tool for xenobiotic metabolism in human skin through proteomic and substrate based mass spectrometry imaging (SB-MSI) analysis.

Method:

- *Ex vivo* skin was obtained from the Human Tissue Bank (university of Bradford).
- Proteomics: Samples were homogenised, treated with detergent and debris removed. The crude fraction was centrifuged and the cytosolic fraction collected and digested ready for analysis using label-free quantification and peptide identification.
- SB-MSI: Samples were treated with SB-MSI probes (e.g. methyl paraben) on the surface for 48 hours. Samples were snapped frozen, sectioned (12 µm), coated with MALDI matrix ready for analysis.

Results:

Proteomic analysis found consistent expression of several xenobiotic metabolising enzymes (XME) expressed between Labskin and *ex vivo* skin. The XME profile between Labskin and *ex vivo* skin were agreeable (Table 1).

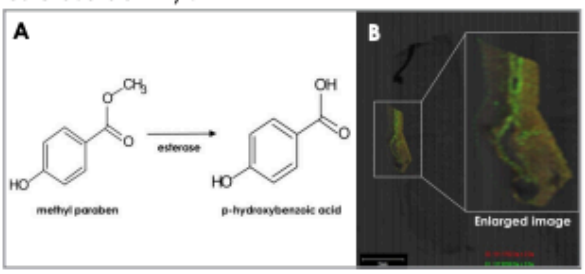
SB-MSI found esterase activity for the metabolism of methyl paraben into p-hydroxybenzoic acid (Figure 1).

Results cont.:

Table 1 - Expression of soluble phase I & II XMEs in Labskin and *ex vivo* human skin. The variation in colour highlights the similarities in XME expression between Labskin and *ex vivo* skin (fmol/µg, ND = not detected).

Phase	Protein Name	Labskin 05	Labskin 06	Human Skin 07	Human Skin 52	Human Skin 56	Human Skin 57	Human Skin 58	Human Skin 60
I	Aldo-keto reductase 1A1	ND	2.1	1.3	ND	ND	3.8	5	ND
	Aldehyde dehydrogenase 1A1	ND	2.4	9.2	12.9	ND	19.8	6.3	18.9
	Carboxylesterase 1	ND	0.3	15.3	17.3	ND	6.3	2.6	19
	Esterase D	2.6	4.5	14.7	14.1	7.7	19.5	14.4	22.2
II	Glutathione S-transferase P1-1	56.3	22.7	50.8	54.5	65.3	150.2	137.7	129.1

Figure 2 - (A) Diagram showing metabolism reaction of SB-MSI probe methyl paraben with esterase to form p-hydroxybenzoic acid. (B) MSI image of Labskin treated with methyl paraben highlighting esterase activity .



(red = methyl paraben, green = p-hydroxybenzoic acid)

Summary:

Label-free proteomic analysis highlighted similar distribution of XMEs in Labskin and *ex vivo* human skin. The location of esterase activity was identified using a XME probe (methyl paraben) in Labskin.

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Immunohistochemistry analysis of Labskin

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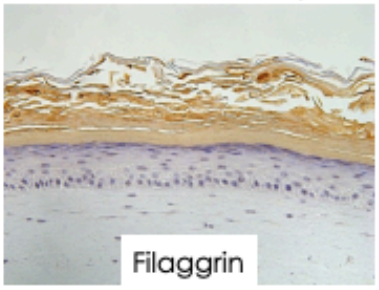
Summary:

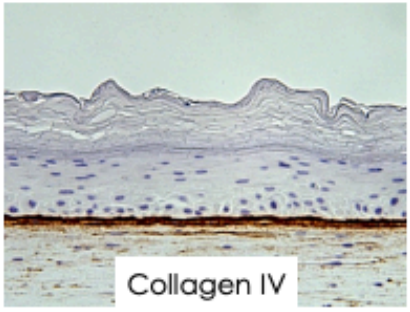
Labskin shows the same immunohistochemistry markers as human skin and is compatible with conventional paraffin wax embedding and cryo sectioning preparation techniques and a wide range of staining and immunohistochemistry protocols. (All examples were prepared by paraffin wax embedding).

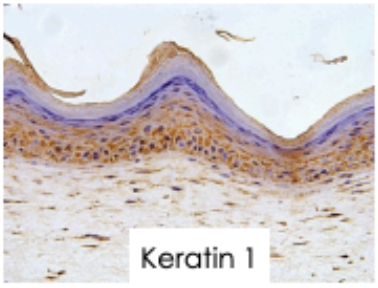
Labskin Structure Analysis

Barrier function analysis

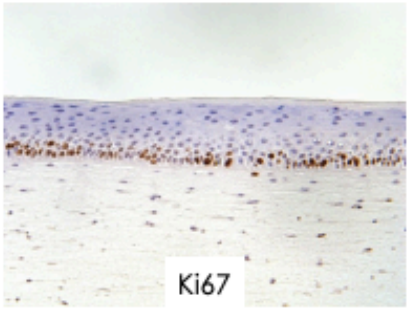


Basement membrane analysis

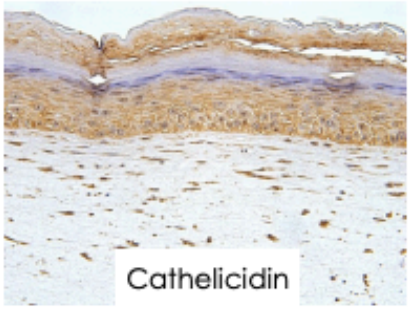


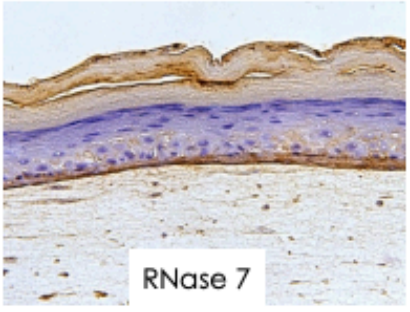


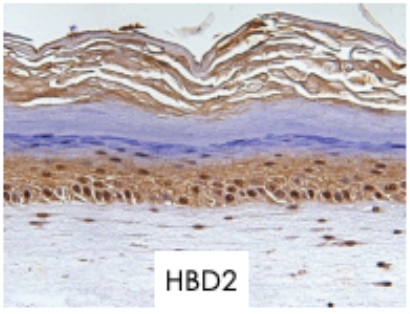
Cell proliferation analysis



Labskin Microbial Response Analysis







Labskin can be used within the same experimental design to simultaneously evaluate several endpoints including cytokine responses (i.e. IL-1a, IL-6, IL-8, PGE2, TNF a, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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Labskin1.1 Stratum Corneum Thickness

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Objective:

To determine the rate of formation of the stratum corneum (SC) in Labskin[1.1] up to 30 days air-liquid-interface (ALI).

Method:

Labskin was fixed in duplicate on days 5, 7, 9, 12, 14, 16, 19, 21, 23, 26, 28 and 30 ALI. The sections were H&E stained and stratum corneum thickness was measured in the middle and each end of the section using Image J software.

Results:

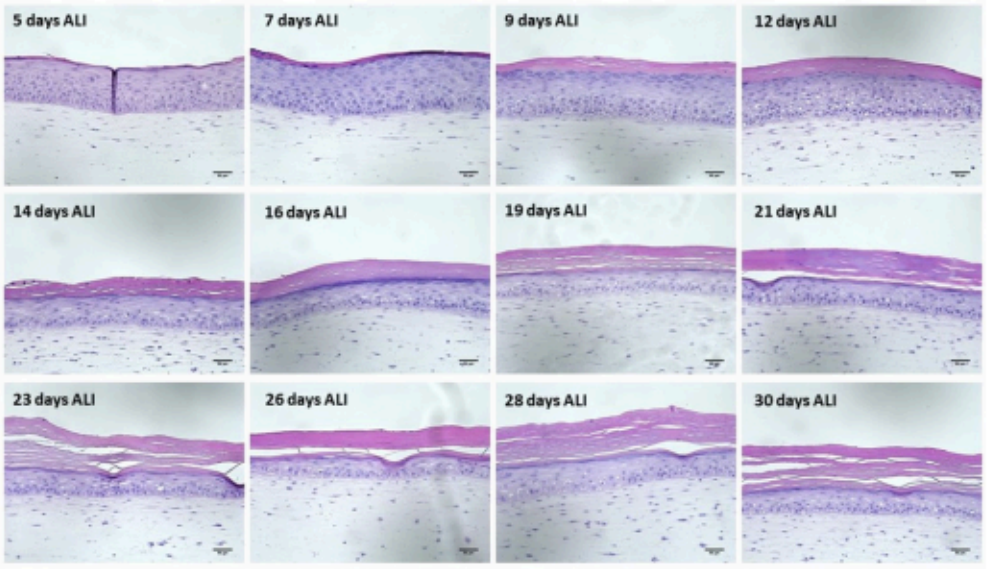


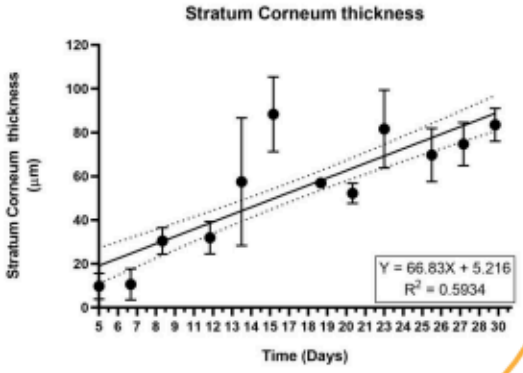
Figure 1 (left)

H&E images of the Labskin sections show that the stratum corneum gets thicker with time.

The Labskin epidermis reduces over time as the Stratum Corneum gets thicker.

Figure 2 (right)

The stratum corneum gets thicker as the Labskin ages. Variability in measurement is due to separation of the SC.



Summary:

The Labskin has a thick epidermis from day 5 to day 12 ALI. As the days proceed the epidermis becomes thinner and the SC gets thicker.

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Assessing different wound dressings on wounded and infected Labskin

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Objective:

To determine whether an iodine or a silver impregnated wound dressing will cease the penetration of *S. aureus* into the dermal layer in infected and wounded Labskin.

Additionally, to assess the wound healing properties of the iodine and silver impregnated wound dressing.

Method:

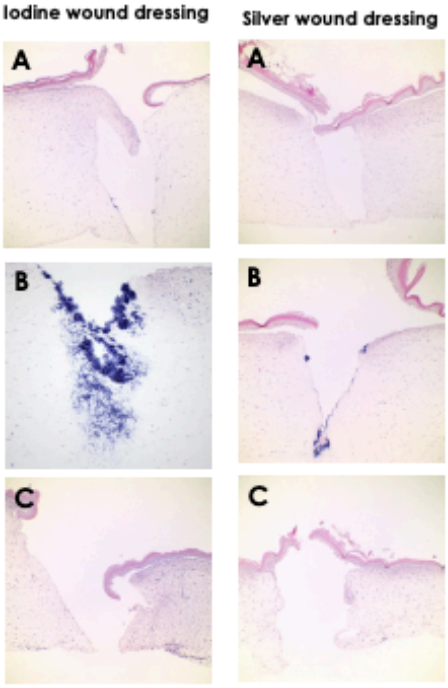
- Each Labskin sample was wounded with a scalpel blade, some samples were then immediately infected with *S. aureus*.
- All samples were incubated for 24h.
- After 24h, some samples had wound dressings applied directly on top of the wound site and then incubated for another 24 h ready for analysis.
- Samples were assessed by histology where they were sectioned (5 µm) and stained with haematoxylin and eosin (H&E).

Results:

- Both the iodine and silver impregnated wound dressing reduced the *S. aureus* penetration into the dermis after 24h incubation compared to the samples without the wound dressing.
- The iodine wound dressing prevented keratinocyte migration into the wound site whereas, the silver impregnated wound dressing did not affect keratinocyte migration.

Results cont.:

Figure 1 - H&E images of Labskin inoculated with *S. aureus* for 24 h and then a wound dressing applied for 24 h. (A) H&E images of Labskin inoculated with *S. aureus* and treated with wound dressing, (B) H&E images of Labskin inoculated with *S. aureus* and (C) H&E images of Labskin cultured with just the wound dressing.



Summary:

Both types of wound dressing did reduce the *S. aureus* infection into the dermal layer thus, reducing the cytotoxic effect of the bacteria.

The iodine wound dressing hindered keratinocyte migration compared to the silver impregnated wound dressing.

Therefore, the silver wound dressing may be more beneficial to aiding the wound healing process in an infected wound environment.

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Assessing different wound dressings on biofilm-infected wounded Labskin

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Objective:

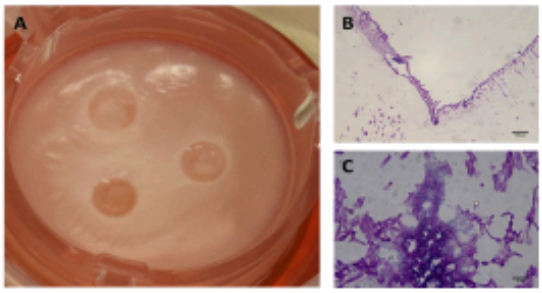
To determine whether a manuka honey, iodine or a silver impregnated wound dressing will decrease the growth of *S. aureus and C. albicans* when forming a polymicrobial biofilm on wounded Labskin.

Method:

- Each Labskin sample was wounded with a biopsy punch and immediately infected with *a mix of* S. aureus *and* C. albicans.
- All samples were incubated for 48h to allow biofilm formation
- After 48h, some samples had wound dressings applied directly on top of the wound site and then incubated for another 72 h.
- Samples were assessed by microbial viable counting

Results:

Figure 1 - Biofilm formation on wounded Labskin and PAS staining. (A) Polymicrobial biofilm growing in wounded Labskin[4,5] (B). PAS staining showing a biofilm infecting a wound (10x). (C) High magnification of biofilm showing yeast and bacteria growing together (100x)



Results continued:

Figure 2 - Application of wound dressing after 48 hours of biofilm formation

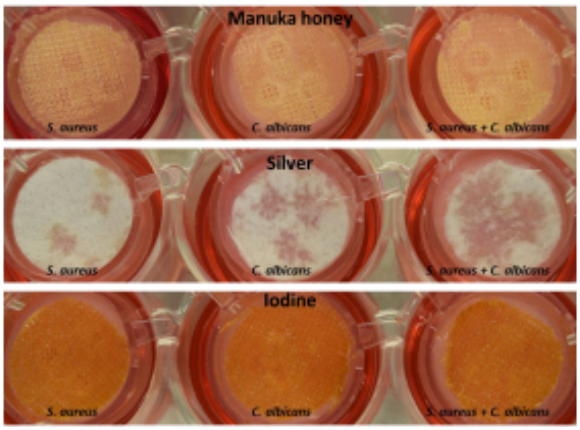
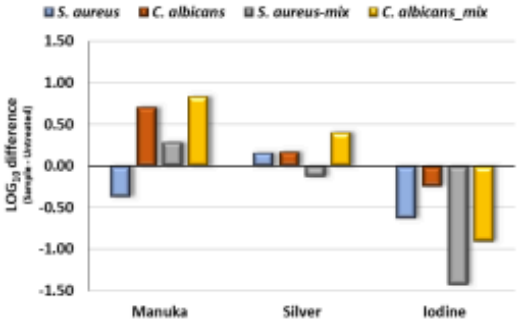


Figure 3 - Viable counts after 72 hours of treatment Log_{10} difference compared to Untreated control.



Summary:

Mono and polymicrobial biofilms were successfully developed in the Labskin wounded model. The wounds were infected with bacteria, fungi or a mix of both microbes.

Only the dressing containing iodine has a clear antimicrobial effect.

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Release of pro-inflammatory cytokines IL-1α and IL-23 by Labskin after colonization with several microbes

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Objective:

To quantify the release of Interleukins IL-1α and IL-23 by Labskin. Comparisons will be made between uncolonised Labskin and Labskin colonised with *S. aureus* (after 6 and 24h post-inoculation), *S. epidermidis*, *C. albicans*, *P. acnes*, 3x mix Normal microflora and wounds infected with Interkingdom Biofilms.

Method:

- Unwounded Labskin were colonised with *S. aureus*, *S. epidermidis*, *P. acnes*, *C. albicans* or a mix of normal skin microflora and incubated for 24 hours. Labskin medium was taken after 24 hours and the amount of cytokines assessed by ELISA.
- Wounded Labskin were infected with 3 mixes containing different numbers of *S. aureus* and *C. albicans*. Labskin medium was taken after 24 and 48 hours for cytokine assessment.

Results:

- A small amount of **IL-1α** was detected after 24 hours but only in those Labskin colonised by *C. albicans* or *P. acnes* A small amount of **IL-23** was detected after 24 hours when Labskin was colonized with individual microbes. However, when Labskin was colonized by the mix of three microbes that amount was increased from less than 10 pg/mL up to 40 pg/mL.
- All wounded Labskin produced high levels of **IL-1α** increasing with the number of microbes added. A small amount of **IL-23** was detected in uninfected wounds. All infected wounds produced high levels of **IL-23** increasing with the number of microbes added.

Results cont.:

Figure 1 - Comparison between **IL-1α** and **IL-23** production by Labskin[1,1] colonised with several microorganisms.

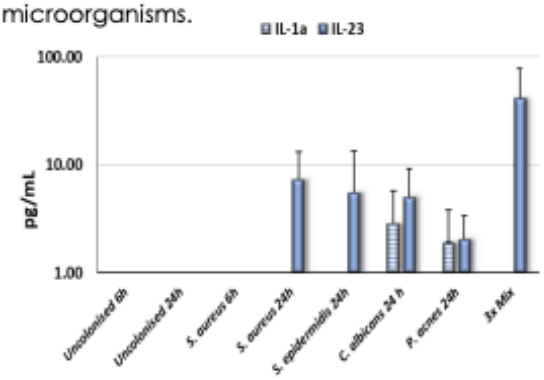
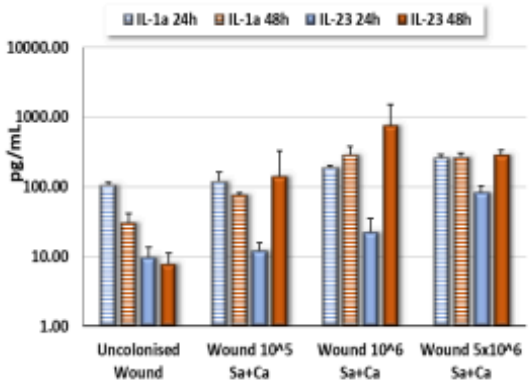


Figure 2—Comparison between **IL-1α** and **IL-23** production by wounded Labskin4.5 infected with a mix of *S. aureus and C. albicans*.



Summary:

In conclusion, **IL-23** production has shown to be regulated by the number and species of micro-organisms in contact with Labskin, whereas **IL-1α** is more likely regulated by a different type of insults.

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