Development and assessment of non-infected and infected skin models using MALDI-MSI

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Introduction:

Infections are known to have a detrimental effect on wound healing leading to chronic wound formation.[1] Chronic wounds are becoming a burden for western societies due to increased incidences of obesity, diabetes and ageing populations.[2] This health crisis has a vast effect on wound care management resources and budgets worldwide.[3] Effective diagnosis and wound management has been shown to reduce costs and chronic wound formation.[4] Therefore, is it vital to understand the molecular mechanisms involved in the wound healing process. Labskin is a human living skin equivalent model (LSE), which when used in combination with Mass Spectrometry Imaging (MSI) is able to assess biological changes within non-infected and infected wounds to identify biomarkers associated with wound healing. Here we report the development of a novel incision method for wounding LSE: infection of these wounds and their study by MALDI-MSI.

Method:

Labskin, (Innovenn UK Ltd, UK) was used in all experiments. Samples were wounded with a scalpel blade where infected samples were immediately infected with *Staphylococcus aureus*. Samples were assessed every 24 hours for up to 4 days post injury and or infection.

Samples were sectioned at wound site and MSI samples were coated in MALDI matrix (α-cyano-4-hydroxycinnamic acid or N-(1-naphthyl) ethylenediamine dihydrochloride) and analysed for lipids (mass range: 400-1200 m/z) in $[M-H]^+$ or $[M-H]^-$ mode on either a Applied Biosystems QSTAR-TOF or a Bruker Autoflex III MALDI-TOF. Whereas, histology samples were stained using haematoxylin and eosin (H&E).

Results:

Non-infected wound models

Keratinocytes migrated into the wound bed and closed the wound site by day 4 post injury simulating the initial *in vivo* wound healing response. MALDI-MSI was able to distinguish between lipids found only in the epidermal (721.4 m/z) or dermal (725.4 m/z) layers (see **Figure 1**). The technique was also able to detect lipids specific to the wound site (727.6 m/z – see **Figure 2**). Additionally, principal component analysis-discriminant analysis (PCA-DA) shown in **Figure 3** highlights a range of individual lipids from the three distinct grouped areas (epidermis, dermis and wound site).

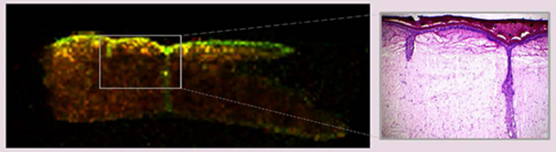

Figure 1: MSI of non infected incision model 4 days post wounding, green = 721.4 m/z and red = 725.4 m/z. H&E image of same sample, 4x magnification.

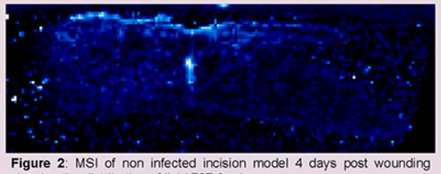

Figure 2: MSI of non infected incision model 4 days post wounding showing the distribution of lipid 727.6 m/z.

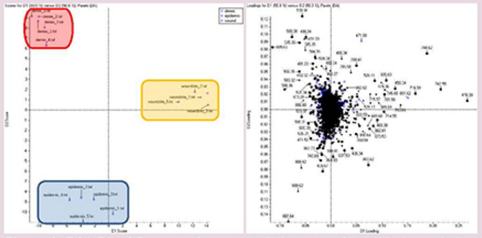

Figure 3: PCA-DA of 5 spectra from each distinct part of Labskin (wound site, epidermis and dermis) identifying lipids from each group.

Infected wound models

Staphylococcus aureus permeated the dermal layer immediately post wounding and produced a cytotoxic effect in the surrounding area killing fibroblasts and keratinocytes (see **Figure 4**). The cytotoxic effect gets greater the longer the *Staphylococcus aureus* is present in the sample killing all the cells within Labskin after 72 hours. MSI was able to identify limited lipids by day 4 as the fibroblasts and keratinocytes had died in the Labskin (see **Figure 5**).

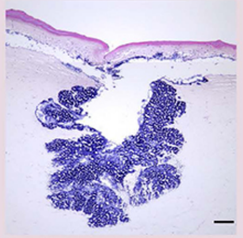

Figure 4: H&E image of incision wound bed, 4 days post wounding and infecting with Staph. aureus. 10x magnification.

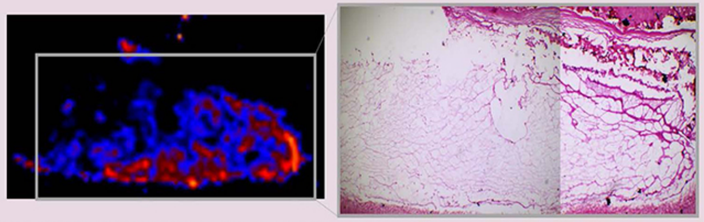

Figure 5: MSI of infected incision model 4 days post wounding at 728.4 m/z. H&E image of same sample, 4x magnification.

Conclusion:

The non-infected model showed full wound closure by day 4 thus, mimicking the initial *in vivo* wound response. As well as identifying specific lipids directly in the healed wound site. These *in vitro* wounded models provide an excellent platform to assess the wound healing process in depth, whilst MSI has shown to be a novel diagnostic tool for identifying specific wound healing markers.

References:

1. Landén et al., *Cellular and Molecular Life Sciences*, **2016**, 73, p3861
2. Sen et al., *Wound Repair Regen.*, **2009**, 17, p7632.
3. Guest et al., *BMJ*, **2015**, 5, pe009283
4. Morgan. *J. of Community Nursing*, **2015**, 29, p17