A. Caserta, J. Neil, J. Volmer, L. Ring, J. Lenn, M.B. Brown

MedPharm Ltd., Chancellor Court, 50 Occam Road, Surrey Research Park, Guildford, GU2 7YN, UK / 4222 Emperor BLVD, Suite 320 RTP, NC, USA

Development of an early wound model in human *ex vivo* skin explant as a preclinical model of wound healing

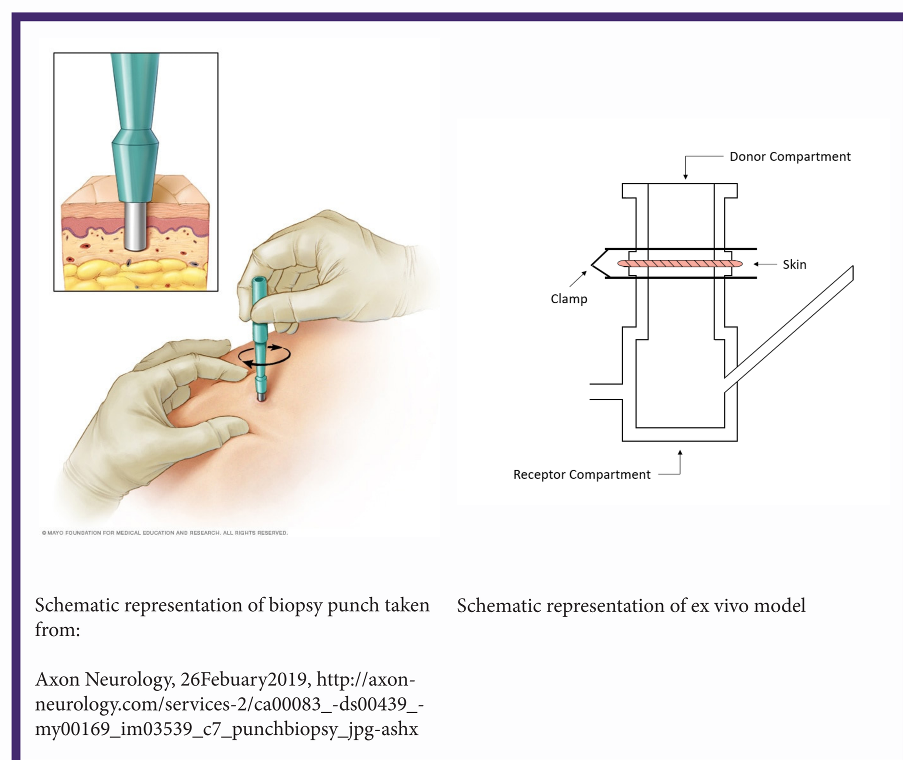
Introduction and Aim

Animal models have been widely used in the wound healing field and have provided invaluable insights into crucial pathways of wound repair processes. However, translation of drug candidates identified in animal models to human therapies has been problematic and there has been a considerable interest in developing alternative models that could be better suited to preclinical evaluation.

The aim of the present work was to develop a model based on the use of ex vivo human skin explants, which could be employed to explore wound treatments, and to obtain a better understanding of the healing cascade of keratinocyte differentiation, collagen and fibrin deposition, inflammatory inhibition and healing time.

As part of the method development work, the expression of a series of biomarkers involved in the aid of wound healing, such as targeting growth factors (PDGF, FGF and CTGF), extracellular matrix proteins (MMP9), collagen (Col1a2), cytokines (IL1α and IL6), keratinocyte (KRT16) and involucrin were investigated.

The regeneration of human skin over the course of 11 days after making excisional wounds by punch biopsy with and without topical treatment with petroleum jelly was also evaluated.



Schematic representation of biopsy punch taken from:

Axon Neurology, 26Febuary2019, http://axon-neurology.com/services-2/ca00083_-ds00439_-my00169_im03539_c7_punchbiopsy_jpg-ashx

Schematic representation of ex vivo model

Methods

Full thickness healthy skin obtained from elective abdominoplasty surgery was immediately defatted.

A 4 mm punch biopsy was used to wound the epidermis and upper dermis and skin mounted on to static cells cultured with a modified DMEM/Ham media.

In the first study, samples were without topical treatment, incubated at 37°C with replacement of media every other day, and then harvested at 2, 5, 8 and 11 days. Upon harvest, half of the tissue was stored in RNAlater for PD analysis via RT-qPCR and the other half fixed in 10% NBF for H&E histology. In the second study, topical treatment with Petroleum Jelly was included on the wounded tissue, incubated at 37°C with replacement of media every other day, then harvested at 5, 8 and 11 days. Upon harvest, the tissue was split in half, one half was stored in RNAlater for PD analysis via RT-qPCR and the other half was sent for H&E histology.

Analysis of gene expression by RT-qPCR: Each sample was normalised to a control gene (GAPDH). Fold change of each sample was normalised to its wounded timepoint.

Nuclei counts of newly forming collagen were quantified by counting 3 individual 1mm^2 regions per tissue sample.

Results

Fig 1. (A) Pictures taken over the course of 11 days with observed wound closure. (B) Gene expression of wound-associated biomarkers analyzed on day 2, 5, 8, and 11. Fold change with SEM of each sample was normalised to its unwounded timepoint. 3 donors, n=3 replicates per donor.

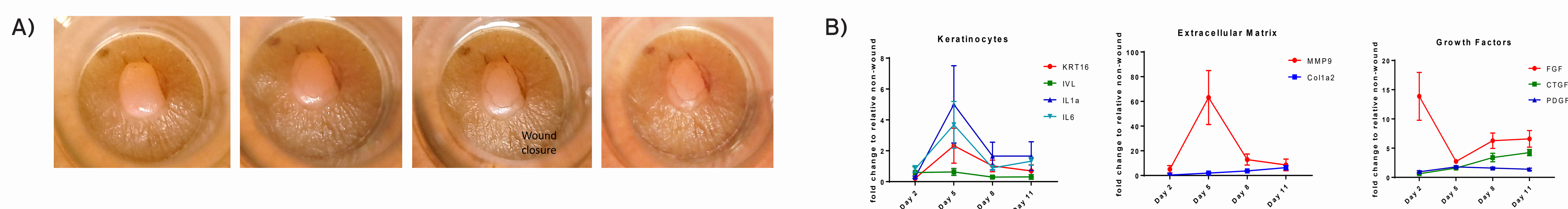
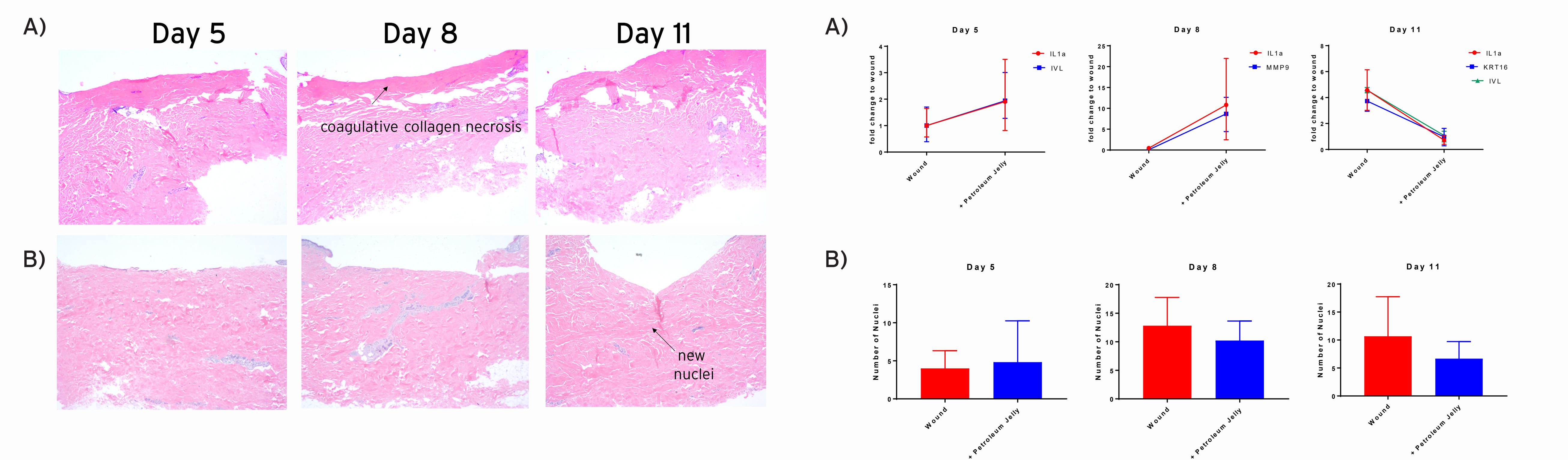


Fig 2. (A) Hematoxylin and Eosin stain at 4X magnification with no treatment (top), and Petroleum Jelly (bottom). (B) Gene expression by RT-qPCR for samples untreated and treated with Petroleum Jelly. Fold change with SD of each sample was normalised to its unwounded timepoint. 1 donor, n=3 replicates. (C) Nuclei count of newly formed collagen in wounded and treated tissue; nuclei/mm2.



Conclusion

Gene expression of KRT16, IL1a and IL6 as well as MMP9 peaked at 5 days post-wounding while Col1a2 and growth factor CTGF and FGF steadily rose over the regeneration period. Fibroblast growth factor increased expression at day 2 is expected to be an artifactual readout from the surgery process. Over the course of 11 days, there is a visual, histological and gene expression evidence of wound healing.

The keratinocyte-associated biomarkers evaluated had peak expression of cytokines and MMP9 on day 5. MMP9 induces keratinocyte migration which could be why we see it expressed early. Col1a2 started a slight incline on day 8, this has a direct effect on fibroblasts which pump out collagen during the proliferative phase. With growth factors FGF and CTGF, an upregulation occurs from day 5 and onward.

Coagulative collagen necrosis in the untreated wound was observed by H&E which was alleviated in the wounded tissue with Petroleum Jelly (PJ) treatment. In the PJ-treated tissue newly formed disbursement of nuclei indicating cell proliferation and new collagen formation signal regeneration. Evidence of angiogenesis was also observed (data not shown). In the gene expression, an induction of expression of IL1a in PJ-treated compared to wounded is shown at day 5 and day 8, as well as increased gene expression of IVL and MMP9. Several genes declined at day 11 compared to the wounded tissue suggesting a shift in wound healing stage at this time. This trend was reflected in the nuclei count which increased from day 5 to day 8 in both tissues, however no further increase was observed at day 11.

The investigation of wound accelerating therapeutics can be invaluable to evaluate keratinocyte differentiation, collagen deposition, inflammatory inhibition and healing time with this model. However, the lack of inherent tension on the skin sample in static cells and absence of infiltration of immune cells precludes this model from direct translation to in-vivo wound healing.

Booth 30
www.medpharm.com

GUILDFORD, UK
DURHAM, NC, USA

References

S.Patel, A. A. (2016). Biomarkers for wound healing and their evaluation. Journal of wound care , 10.

Stojadinovic Olivera, M. t.-C. (2013). Human Ex Vivo Wound Healing Model. Research Profiles, 10.

Sara Ud-Din, A. B. (2016). Non-animal models of wound healing in cutaneous repair: In silico, in vitro, ex vivo, and in vivo models of wound and scars in human skin. Wound Healing Society, 13.