The cell migration effects of decellularized porcine placental extracellular matrix in viable wounded human skin *ex vivo*

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Introduction

- Decellularized extracellular matrix (ECM) medical devices can facilitate healing of hard-to-heal wounds[1]
- Porcine placental ECM is an ECM scaffold containing collagen, fibronectin, laminin, elastin, hyaluronic acid, and glycosaminoglycans, while being largely free of cells, cell debris, and DNA[2]
- The composition and structure of ECM products is reported to increase the M2:M1 macrophage response therefore preventing the wound from remaining in the inflammatory phase [3,4]
- We evaluated a decellularized porcine placental ECM product* on a wounded *ex vivo* human skin model for its cell migration response

STUDY OBJECTIVE

To examine the effects of novel porcine placental decellularized ECM product* on the healing of wounded human skin in a viable *ex vivo* model using a range of microscopic tissue and cell staining techniques

Methods

Test device

- Porcine placental ECM*

Protocol

- ECM product-treated and -untreated wounded skin models were incubated for up to 6 days at 37°C
- Skin samples were fixed and embedded before performing cross sectioning
- Histological slides were stained with Cytokeratin 17 primary antibody (1:200). Primary antibody was removed and fluorescently labelled with the secondary antibody, Alexa Fluor 647 (1:500) (λex = 650, λem = 671)
  - Cytokeratin 17 was selected as a marker for wound healing as it is a protein marker associated with cellular migration
- Slides were additionally counterstained with DAPI stain (1:1000) (λex = 350, λem = 465) to visualize cell nuclei
- Slides were observed under a LSM800 Zeiss Confocal Laser Scanning Microscope
- Histological characterization was performed to determine the structural and cellular components of the skin model. Hematoxylin and Eosin (H&E) and Masson trichrome staining were used to identify cells as well as the epidermal and dermal components of the skin
  - Hematoxylin stained the nuclei blue, whilst Eosin stained extracellular components pink.
  - Masson trichrome staining was used to stain the collagen blue, the nuclei dark brown, muscle tissue red, and the cytoplasm pink

Results

- H&E staining of ECM-treated wounded skin showed cellular migration to the wound area (**Figure 1**)
- Masson trichrome staining of ECM-treated wounded skin showed cellular migration (**Figure 2**)
- Low amounts of Cytokeratin 17 were observed in the untreated control (**Figure 3**)
- Higher upregulation of Cytokeratin 17 marker were observed in the ECM-treated wounded skin, indicating more cell migration (**Figure 4**)

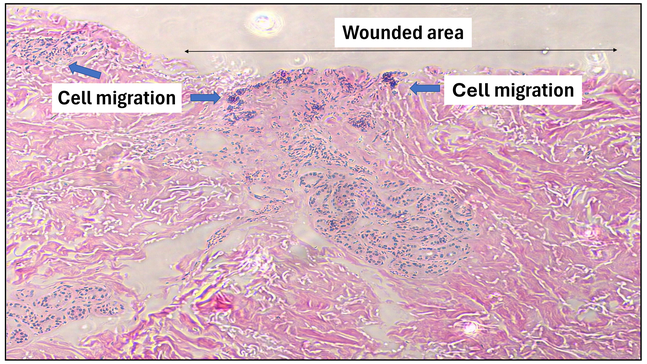

Figure 1. H&E staining of ECM-treated wounded skin

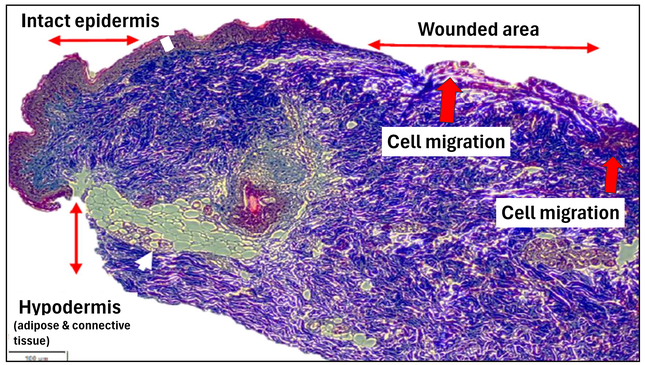

Figure 2. Masson trichrome staining of ECM-treated wounded skin

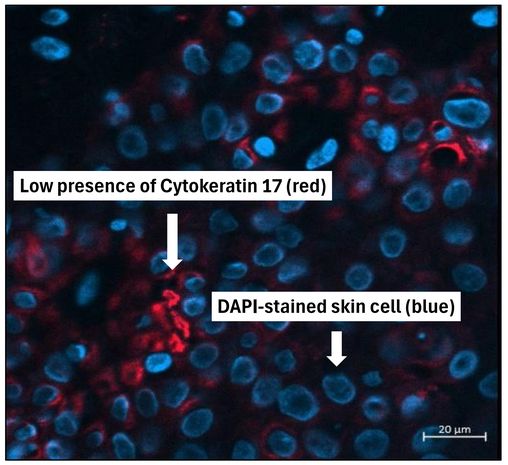

Figure 3. Cytokeratin 17 in untreated control wounded skin

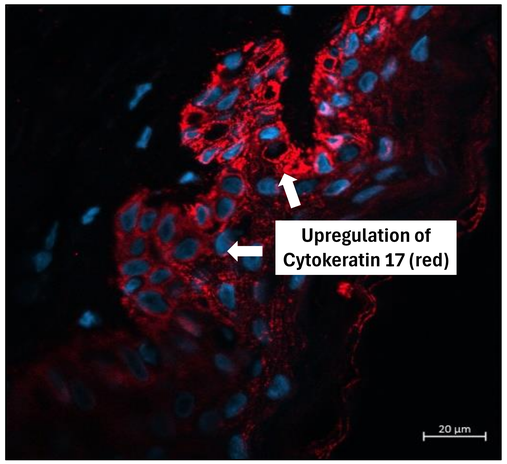

Figure 4. Cytokeratin 17 ECM-treated wounded skin

Discussion

- Immunological analysis shows an increase in the presence of cellular migration marker, Cytokeratin 17, suggesting that cell migration was encouraged by the ECM product
- H&E staining, Masson trichrome staining, and Cytokeratin 17 immunolabelling results suggested an increase in cellular migration when wounded *ex vivo* human skin was treated with a porcine placental decellularized ECM product
- Further studies in similarly complex models using a range of microscopic and immunological techniques, and examining other cellular responses, such as Ki67 associated with cell proliferation, may help to confirm these findings

CONCLUSION

The porcine placental decellularized ECM product was shown to encourage cell migration in an *ex vivo* wounded skin model using a range of microscopic staining techniques

References & Footnotes

1. Cramer M, Badylak, SF. Extracellular Matrix-Based Biomaterials and their influence upon cell behavior. Ann Biomed Eng 2020; 48: 2132-2153.

2. FDA 510(k) summary K193552: www.accessdata.fda.gov/cdrh_docs/pdf21/K211902.pdf (accessed March 2024).

3. Sicari et al. The promotion of a constructive macrophage phenotype by solubilized extracellular matrix. Biomat 2014; 35: 8605-8612.

4. Keane et al. Consequences of ineffective decellularization of biologic scaffolds on the host response. Biomat 2012; 33: 1771-1781.

*InnovaMatrix® AC (Convatec Inc)

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