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Biocompatibility of decellularised human amniotic membrane

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INTRODUCTION: There is a clinical need for an immunologically compatible surgical patch with a wide range of uses including, use as a wound dressing and as a substrate for cell delivery. A novel detergent based protocol was modified to remove all cellular components from human amniotic membrane (HAM) in order to render it non immunogenic. The study aim was to investigate the biocompatibility of acellular human amniotic membrane *in vitro* and *in vivo*.

METHODS: HAM was decellularised according to the method developed by Wilshaw *et al.*, (2006). *Sodium dodecyl sulphate quantification:* Radiolabelled C^{14} SDS was used to spike the wash buffer during decellularisation. The amount of radiolabelled SDS in each wash buffer and in the decellularised tissue was determined using a micro-plate scintillation counter and a standard curve of known C^{14} SDS concentrations.
In vivo biocompatibility study: Samples of fresh, decellularised and fresh glutaraldehyde-fixed HAM were implanted subcutaneously into groups of three mf-1 mice (two samples per mouse). The mice were sacrificed after three months. Explants were fixed, paraffin embedded and serially sectioned. Sections were stained using haematoxylin and eosin, Von Kossa's and labelled using monoclonal antibodies against CD 3, CD 4, CD 34 and F4/80.
Cell seeding: Primary human keratinocytes were seeded onto the basement membrane side of decellularised HAM. Following 24 hours the tissue was processed for SEM analysis. Primary human fibroblasts were also allowed to proliferate on the stromal side for up to four weeks, the rate of proliferation was monitored using the commercially available ATPLite-M® kit (PerkinElmer). The cell viability of the seeded cells was determined by means of the commercially available live/dead kit (Molecular Probes) and viewed using a confocal microscope and a conventional fluorescein longpass filter. Replicate samples (n=3) were fixed, paraffin embedded and serially sectioned. Sections were stained using haematoxylin and eosin.

RESULTS: The SDS assays indicated there to be 60 $pg.g^{-1}$ SDS present in decellularised HAM and 3.19×10^{-4} % (w/v) was found to be present in the final wash buffer. Two of the decellularised samples, three of the fresh samples, whereas all six of the glutaraldehyde fixed samples were recovered from the mice after three months. The histology demonstrated a thick fibrous capsule and poor tissue integration of fresh HAM along with evidence of calcification. Decellularised HAM demonstrated good integration and a thin fibrous capsule. Immunohistochemical labeling of explanted decellularised HAM showed a low T-cell infiltrate with higher numbers of macrophages and endothelial cells, suggesting that the tissue might be being remodelled. Primary human keratinocytes demonstrated good attachment to decellularised HAM following 24 hours (Figure 1). Proliferation studies indicated that fibroblast numbers increased from 1.9×10^3 cells to 3.5×10^4 cells over a period of 14 days (Figure 2). Live/dead staining showed that decellularised HAM was able to maintain the viability of seeded human fibroblasts and keratinocytes over a period of 21 days.

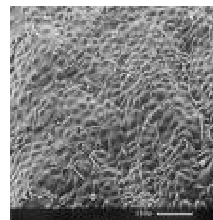

Figure 1 *SEM micrograph of primary human keratinocytes seeded onto basement membrane of decellularised human amniotic membrane after 24 hours.*

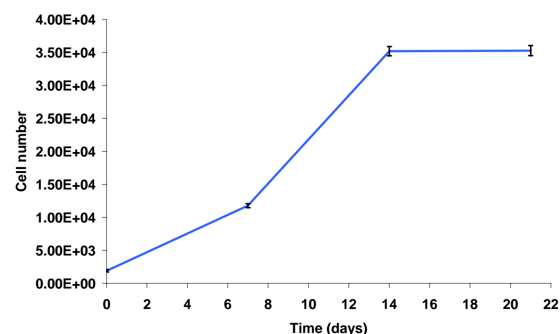

Figure 2 *Proliferation rate of primary human fibroblasts cultured on the stroma of decellularised HAM.*

DISCUSSION & CONCLUSIONS: The acellular matrix appeared to be biocompatible *in vivo*. The acellular matrix has shown potential for the attachment and proliferation of primary human fibroblasts and keratinocytes, as well as maintaining the viability of seeded cells.

REFERENCES: [1]S. Wilshaw, J. N. Kearney, J, Fisher & E. Ingham. (2006) *Tissue Engineering*.