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A PLASMA POLYMER SURFACE FOR THE CO-CULTURE OF HUMAN DERMAL FIBROBLASTS AND HUMAN EPIDERMAL KERATINOCYTES FOR WOUND HEALING.

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INTRODUCTION For wound cover, reconstructive surgery and for promoting healing in chronic non-healing wounds a number of approaches have been employed to expand skin cells in the laboratory and then transfer them to the patients wound bed. Several studies have employed animal derived products such as Collagen I for the culture of human epidermal keratinocytes and transfer of an integrated sheet to wound bed models. Against this background, this laboratory has recently developed a chemically defined surface for the culture and transfer of keratinocytes [1,2,3]. These are synthetic surfaces capable of influencing and controlling cell physiology either directly or through an adsorbed protein layer. These thin polymeric films, typically a few nm in thickness, are produced from continuous wave radio frequency induced plasmas of volatile organic compounds.

The objective of this study was to further develop the wound healing potential of keratinocytes cultured on this plasma polymer surface by including a growth arrested layer of fibroblasts to exploit the well documented interdependency of keratinocytes and fibroblasts. Accordingly we aim to determine a culture surface appropriate for coculture of fibroblasts and keratinocytes. Lethal gamma-irradiation allows fibroblasts to remain viable but unable to divide. This fibroblast feeder layer in turn secretes mitogens to promote keratinocyte colony formation. Potentially the inclusion of a fibroblast feeder layer will enhance the culture or "performance" of cultured keratinocytes in promoting wound healing.

MATERIALS AND METHODS: Surfaces containing 0, 1.5, 3.5 and 10% acid were prepared at a base pressure of $3x10^{-3}$ mbar. Surface chemistry was controlled by the incorporation of an octa-1,7-diene diluent into the monomer feed and by parameters such as power input, flow rate and deposition time. Plasma polymerised films were characterised using X-ray Photon Spectroscopy. Human dermal fibroblasts and epidermal keratinocytes were cultured with foetal calf serum on plasma polymer coated 24 well plates. Cell proliferation was assessed, with respect to positive and negative controls using MTT and DNA assays [1,2,3].

RESULTS Of the surfaces examined both keratinocytes and fibroblasts showed optimal attachment and proliferation over a 7 day period to a 100% acrylic acid surface (advancing contact angle 46°). This surface contained approximately 9.2% carboxylate groups. Fabricated at 10W it was stable to dissolution as assessed by advancing and receding contact angle measurements. The performance of cells on this surface was similar to their growth on Collagen I, a well-established substratum for the proliferation of keratinocytes. The 100% acrylic acid surface also supported the attachment of irradiated fibroblasts which did not increase in number. Keratinocytes formed good colonies and were able to expand more rapidly in the presence of a fibroblast feeder layer both on collagen I and on the 100% acrylic acid surface.

Figure 1*: DNA assay illustrating the effect of an irradiated dermal fibroblast feeder layer on the proliferation of keratinocytes cultured on a 100% acrylic surface and on collagen I after 7 days in culture.*

DISCUSSION Human keratinocytes and fibroblasts have been successfully cultured on plasma co-polymer surfaces containing carboxylate groups. Optimum proliferation of both cell types was observed on a pure acrylic acid surface, fabricated at 10W. The hydrocarbon dilutent, octa-1,7-diene, allowed control of the resulting functional group concentration by promoting cross-linking of the other monomer. Further work will now examine the potential of the keratinocytes to transfer to *in vitro* wound bed models and evaluate the contribution of the fibroblast to this culture system.

CONCLUSION The data reported shows that by using a plasma polymer surface as a synthetic substrate, it is possible to effectively co-culture human epidermal keratinocytes with a non-proliferative fibroblast feeder layer to achieve an increased rate of keratinocyte proliferation. This coculture system offers an attractive approach to the delivery of keratinocyte for clinical use.

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