

Improved technologies for translating culture of human melanocytes to the clinic

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INTRODUCTION: Obtaining pigmentary function in autologous skin grafts is a current challenge in the healthcare industry as is developing reliable robust grafting strategies for patients with vitiligo. Vitiligo is an extremely common skin condition in which melanocytes stop producing pigment and are eventually destroyed by the immune system. Whilst not life threatening, it has a very negative psychological impact on patients.

In this paper we present ongoing work aimed at developing a simple methodology for delivering cultured cells to the patient that is clinically effective with respect to pigmentation and wound healing, is low risk for the patient but also user friendly for the surgeon or dermatologist when applying cells to the patient.

METHODS: Chemically defined substrates were produced by plasma polymerisation of either allylamine, acrylic acid or a plasma co-polymerisation of two of these monomers. Four culture media were employed, two designed to support keratinocytes, Green's (10% serum) and keratinocyte defined media (KDM – serum free), and two designed to support melanocyte expansion – MCDB153 (which cannot be used clinically) and melanocyte media (M2 – serum free). Cells were seeded either as monocultures, or as a 1:1 or 2:1 ratio of keratinocytes to melanocytes. Cell attachment and melanocyte function were assessed by MTT-ESTA assays and S100 staining.

RESULTS: Melanocytes and keratinocytes both in mono- and co-cultures attached and grew well on both acid and amine plasma polymers. There was no significant preference between these surfaces but co-cultures of the two cell types on these plasma polymers fared better than individual cell cultures. Explicitly, total cell numbers (keratinocytes plus melanocytes) in Greens, KDM and M2 media were 14%, 34% and 103% respectively greater than individual cultures of either melanocytes or keratinocytes. Also, with 2:1 co-cultures of keratinocytes:melanocytes on either acid or amine surfaces the survival rate of melanocytes in the presence of keratinocytes was much higher in M2 compared to Greens media. On an acid surface, melanocyte numbers ranged from 1700 cells/cm² in Greens to 5700 cells/cm² in M2. Melanocyte numbers on an amine surface ranged

from 2500 cells/cm² in Greens to 4300 cells/cm² in M2.

Assessment of transfer of co-cultures of melanocytes and keratinocytes to an *in vitro* model of human dermis showed that melanocytes and keratinocytes can be successfully transferred from chemically defined carriers onto human dermis. This *in vitro* model of skin was then cultured in Greens media for 10 days at an air liquid interface. At this point the skin model was sacrificed for histological analysis. Figure 1 illustrates the presence of melanocytes (stained for S100 – brown endpoint – see red arrows) within the human skin model after a period of 10 days.

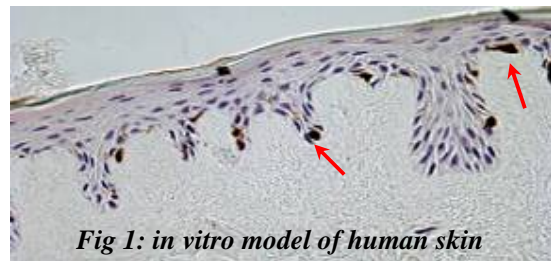


Fig 1: *in vitro* model of human skin

DISCUSSION: So far, we have demonstrated that chemically defined substrates can be used to culture both melanocytes and keratinocytes in medium (Greens) currently used in the clinic (which contains fetal calf serum sourced from New Zealand), but also in a serum free alternative – M2. We have also achieved successful transfer of melanocyte:keratinocyte co-cultures from flexible plasma polymerized silicone carriers onto an *in vitro* human wound bed model. However, in order to progress this work into a clinical environment we need to establish culture protocols within a GMP clean room and develop a cell transport media based on agar/hyaluronic acid for the transport of cells over a wide geographical area. A similar gelled media has recently been established by the group and used in a clinical environment for the treatment of chronic wounds with autologous keratinocytes.

CONCLUSION: We have developed a pre-clinical methodology for the delivery of a co-culture of autologous melanocytes and keratinocytes using a flexible and user-friendly chemically defined carrier surface. We now hope to translate this work from the laboratory into the clinic.