# A serum-free and feeder-free protocol for expanding human keratinocytes on biodegradable microcarriers for the treatment of severe burn injuries

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**INTRODUCTION:** Autologous keratinocytes are used in the treatment of severe burns to augment wound healing. Cells are commonly expanded in serum-containing medium in the presence of lethally irradiated mouse fibroblast feeder cells. Application to the wound bed in single cell suspension is damaging to the cells and often results in significant cell loss. We previously demonstrated improved wound healing outcomes in the porcine model of wound repair with cells delivered using biodegradable gelatin microcarriers instead of as cell spray[1]. We present here an improved method of culturing human keratinocytes on biodegradable microcarriers under serum-free and feeder-free conditions.

**METHODS:** Keratinocytes were isolated from discarded human skin and cultured in a serum-free, feeder-free CnT-07 medium (CellnTech) until sub-confluent (5-6 days). Gelatin Cultispher-G microcarriers (Sigma-Aldrich) were seeded with 5 x $10^6$ cells and cultured for a further 4 days in a stirring glass bioreactor. Cell phenotype was assessed by microscopy, proliferation assay (CCK-8, Sigma-Aldrich) and qRT-PCR (Bio-Rad).

**RESULTS:** Using a serum- and feeder-free culture medium, we obtained gelatin microcarriers with sub-confluent keratinocyte cultures after 4 days.

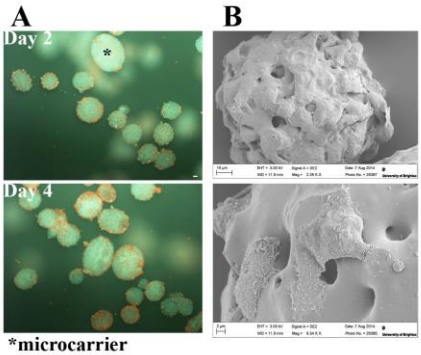


*Fig. 1: Microscopy reveals sub-confluent keratinocyte cultures. (A) Acridine orange staining indicates proliferating cells (RNA – orange, DNA – green, scale bar = 100 μm). (B) Scanning electron microscopy indicates distribution and cell shape of keratinocytes on microcarriers, scale bars as indicated.*

Proliferation was rapid. Cell phenotype on microcarriers (MC) was assessed by qRT-PCR and compared to cells prior to culturing (P0) and cells on tissue culture plastic at passages 1 (P1 TCP) and 2 (P2 TCP). Significant differences were detected between cells prior to and after culturing, but not between cells grown on tissue culture plastic and those grown on microcarriers.

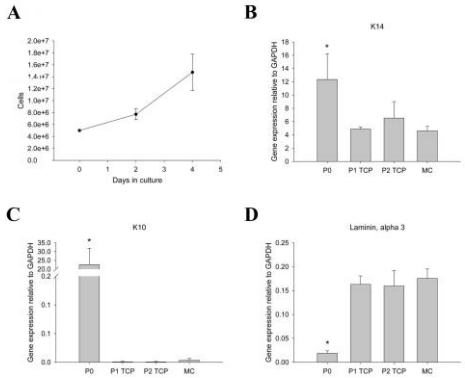


*Fig. 2: Cell growth and phenotypic analysis. (A) Proliferation assay indicates rapid growth on microcarriers. (B) qRT-PCR of cytokeratin 14 (K14), cytokeratin 10 (K10) and Laminin, alpha 3 indicates no significant differences between cells grown on microcarriers and on tissue culture plastic, n=3, p<0.05.*

**DISCUSSION & CONCLUSIONS:** Severe burns are often treated with autologous keratinocytes within 10 – 20 days of hospital admission. Using the culture protocol reported here, highly proliferative keratinocytes are available for transplantation within 10 days. The use of microcarriers for transplantation ensures the cells are not damaged by enzymatic digestion during removal from tissue culture flasks. Using a serum-free and feeder-free medium system would further limit the risk to patients associated with use of xenobiotic substances.

**REFERENCES:** [1] M. Eldardiri, Y. Martin, J. Roxburgh, et al (2012) *Tissue Eng Part A* **18 (5-6)**:587-97.

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