Manufacturing Cultured Epithelial Autografts for Treatment of Major Burns

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Cultured Epithelial Autografts (CEA), first developed by Rheinwald and Green,[1] have been used in some specialised burns units to treat patients with massive burns with an attempt by researchers and clinicians to optimise and overcome limitations involved. The preparation of CEA on a fibrin matrix has been regarded the most important development thus far[2] and has been shown to have several advantages including less primary contraction and higher take rates [3] .We are conducting a small prospective clinical trial as a proof of concept study, to demonstrate that CEA manufactured on a fibrin matrix, under the principles of Good Manufacturing Practice (GMP) and Therapeutic Goods Administration (TGA) guidelines, may be successfully produced from our laboratory.

Initial skin biopsies from the patients are digested with dispase and trypsin. Isolated keratinocytes are then co-cultured with 3T3-J2 (mouse) feeder fibroblasts as a first passage and co-cultured again on sheets of fibrin matrix (Tisseel® Baxter), as a second passage. CEA sheets of 10 x 10cm are then either applied on their own or in combination with widely meshed autologous split skin grafts (SSG) on vascularised allografts / Integra® dermal substitute.

We have achieved a system that reliably produces 7-12 CEA sheets in less than 19 days. Data collected from four patients show a cell recovery range of 0.22 -6.42 x 10^6 cells per cm^2 of biopsy digested. The colony forming efficiencies (CFE) of cultures range from 0.2-3.1% and 17-36% for passages 1 and 2 respectively. Microbial testing results have shown 3 episodes of contamination at the biopsy collection / early culture stage which agreed with contaminated swabs taken from patients' wound beds. Improvements to preparing biopsy site on the patients have been adopted to avoid product rejection. Future directions include further investigations to increase yield of cells and replacing the few animal sourced materials and cells used in this process, with human sourced materials and cells.

References:

1. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975; 6: 331-344.
2. Green H. The birth of therapy with cultured cells. Roots 2008; 30: 897-903.
3. Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y. Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. Transplantation 2000; 70(11): 1588-98.

Nominated Stream for Poster Presentations

[ ] Research