Isolation and Cultivation of Adult Human Keratinocyte Stem Cells for Regeneration of Epidermal Sheets

*J Movaffagh [1], A Tabatabaee [2], MH Amoozegar [3], SA SajadiTabassi [4], N Amiri [5]

[1]Targeted Drug Delivery Research Center, Stem Cell Center , Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Dept. of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[3]Dept. of Surgery, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[4]Pharmacological Research Center of Medicinal Plants, Faculty of Medicine , Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
[5]Targeted Drug Delivery Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Keratinocyte stem cell is one of the adult stem cells that inhabits the skin and contributes to skin function and renewal. Adult stem cells are best defined by their capacity to self-renew, and to maintain tissue function for a long period of time. These findings indicate the importance of these cells for clinical applications including regenerative medicine, tissue engineering and gene therapy. In full-thickness damage or injury including burns, the cultured epidermal autografts (CEAs) may be placed directly onto muscle or fascia.
Methods: A small split thickness skin biopsy (1×2 cm) was obtained aseptically to isolate stem cells. The biopsy was cut into thin pieces and treated with trypsin at 4° C overnight (cold trypsin method) to obtain a single-cell suspension. The cells were seeded at a density of 3×104 cells/cm2 onto a preformed mitomycine-C treated 3T3 cell as feeder layer in DMEM medium supplemented with 10% fetal bovine serum (FBS) and other special supplements. Clonogenic keratinocytes divided and colonies quickly expanded and pushed away the 3T3 feeder layer cells, which then detached from the culture vessel and eliminated with medium changes. Primary cultures were usually subcultured when the cells were in exponential growth phase .Colonies of keratinocytes were expanded and after 7-10 days fused and formed a coherent stratified epithelium. Confluent cultured epithelia were detached enzymatically as coherent sheets from the surface of the culture flasks and transferred onto petrolatum- impregnated gauze.Histological studies of cultured epithelium were also carried out.
Results: In our experience from 1 cm2 of skin sample, 2,5- 4×106 cells were obtained. It resulted in keratinocytes suspensions which consisted at least 90% single cells. Cultured keratinocytes proliferated and after 8-10 days became confluent. The area of cultured epithelium detached from T-25 and T-75 culture flasks was approximately 12-15 cm2 and 35-40 cm2 respectively. Histological studies showed that 10-day old cultured epithelium had 3-4 cell layers consisting of small basal cells and big scquamous cells with large nucleus. Also in the basal layer few melanocytes with melanin pigments in the cells cytoplasm were found.The 20-day old cultured epithelium had 8-10 layers consisting of small and round basal cells, scquamous cells and 2-3 layers of keratinized cells.
Conclusion: Culture of keratinocyte stem cells could result in multilayer epithelium that creates a good cosmetic appearance upon transplantation. This could re-generate an epidermis that is resistant to trauma and infections. It can be considered as an appropriate substitution in skin loss conditions.

Keywords: Epidermal Sheets, Skin Adult Stem cells, Keratinocyte.

Oral Presentation

***Corresponding Author:** J Movaffagh, Targeted Drug Delivery Research Center, Stem Cell Center , Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.