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Bioengineering of cultured epidermis from adult epidermal stem cells using Mebio gel sutable as autologous graft material

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Closure of burn wound is the primary requirement in order to reduce morbidity and mortality that are otherwise very high due to non-availability of permanent wound covering materials. Sheets of cultured epidermis grown from autologous epidermal keratinocyte stem cells are accepted world over as one of the best wound covering materials. In a largely populated country like ours where burn casualties occur more frequently due to inadequate safety practices, there is a need for indigenous research inputs to develop such methodologies. The technique to culturing epidermal sheets in vitro involves the basic Reheinwald-Green method with our own beneficial inputs. The technique employs attenuated 3T3 cells as feeders for propagating keratinocyte stem cells that are isolated from the epidermis of an initial skin biopsy of about 5 cm2 from the patient. The cultures are then maintained in Dulbecco's modified Eagle's medium strengthened with Ham's F12 formula, bovine fetal serum and various specific growth-promoting agents and factors in culture flasks under standard culture conditions. The primary cultures thus established would be serially passaged to achieve the required expansion. Our major inputs are into the establishment of (1) an efficient differential trypsinization protocol to isolate large number epidermal keratinocytes from the skin biopsy, (2) a highly specific, unique and foolproof attenuation protocol for 3T3 cells and (3) a specialized and significant decontamination protocol. The fully formed epidermal sheet as verified by immuno-histochemical and light & electron microscopic studies, is lifted on to paraffin gauze by incubating in a neutral protease. The graft is then ready to be transported to the operating theatre for autologous application. We have a capability of growing cultured epidermal sheets sufficient enough to cover 40 per cent burn wound in 28 days. The preliminary small area clinical applications undertaken so far revealed quicker healing proving the importance and usefulness of the method.

With this new approach a large number of moderate to severely burned patients could be saved in several burn centers across our country with reduced hospitalization period.

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However, the cell based therapeutic option in burn-wound healing by the application of *in vitro - cultivated* sheets of epidermis from autologous epidermal keratinocyte stem cells uses no matrix. This technque is sufficient for burn wounds of 2nd & 3rd mixed degree. The burn wounds predominantly of 3rd?and 4th?mixed degree can not be healed by the thin cultured epidermis, thus requiring a cellular or cellular scaffold that more or less mimic for graft take in deeper burns.

With this aim, we are presently attempting to create such a scaffold using Mebiol gel, which could support the cultured epidermis for better transfer to the wound bed. Additionally, the usefulness of Mebiol gel in growing epidermal sheets without the necessity of FBS and/or animal origin feeder cells but using human feeder cells will also be tested.

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