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Towards Cell Therapeutics for Bullous Keratopathy Using Corneal Endothelial Precursor Cells; Successful *In Vitro* Expansion After Transportation in Indian Conditions and Characterization

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Background:

Bullous Keratopathy affects thousands of people in India every year, for which many experimental studies have been reported on cell therapy using corneal endothelial precursors. Successful culture of the endothelial cells and precursors taken from cadaver eyes, processed immediately after harvesting it from corneal buttons have been reported from developed nations under stringent processing conditions. But corneal endothelium removed from the button, transported at varying temperature conditions for long and then successfully cultured have not yet been reported anywhere. In Indian conditions we have done this work keeping in mind, a feasible model of a centrally equipped laboratory which can serve remote places within its reach making such cell therapeutics using in vitro cultured endothelial precursors available in distant places also.

Materials & Methods:

Endothelial layer from the corneal button were removed by an experienced ophthalmologist in Trichy and was transported to the NCRM laboratory, Chennai. The transportation was done at varying temperature conditions ranging from 22 Deg C (*Train coach*) to 30 Deg C (*Outside temperature*) taking an average of 10 hrs till processing, using two methods of preservation. Group I; Specimens were suspended in DMEM + BFGF+EGF + B27+ Collagenase type1 (Basal culture medium). Group II: Specimens embedded in TGP above which the same basal culture medium was added. Upon arrival in the laboratory, specimens were trypsinized and grown as a sphere suspension culture for 2 wks. Gr. I in the basal culture medium and Gr.II in TGP topped with basal culture medium. Viable cells in both groups counted immediately upon processing and thereafter at different intervals during the process of culture expansion and subjected to characterization by RT-PCR.

Results:

Corneal endothelial precursors cells could be grown successfully as sphere forming assay. Immediately after processing following the transportation, viability of cells in Gr.II were significantly higher. The no. of single cells forming spheres was more in Gr. II, whereas in Gr. I aggregation of developing spheres were common. Spheres from both the groups proven positive for neuronal marker B-3Tubulin and negative for Cytokeratins K3 and K12, thereby proving that they are nothing but corneal endothelial precursor cells.

Conclusion:

We could evolve a method by which endothelial cells removed as a layer from corneal button could be transported in Indian conditions in a safe and efficient method yielding viable cells and further successful *in vitro* expansion of corneal endothelial precursor cells. After further confirmatory steps, if the cells could be used for cell therapeutics in patients suffering from Bullous Keratopathy, this would be a major breakthrough and help thousands of patients recover their vision in the country.