**Effect of Cell Seeding Density and Concentration of Growth Factor on the Reconstruction of Dermal Papilla-like Tissues Employing Umbilical Cord Mesenchymal Stem Cells**

Bo-Young Yoo,1 Youn-Ho Shin,2 Hee-Hoon Yoon,1 Kye-Yong Song2, Jung-Keug Park1†

Corresponding Author: jkpark@dongguk.edu

1Dongguk University Research Center of Biotechnology, Seoul, Korea 2Dept. of Pathology, Chung-Ang University, Seoul, Korea

**Introduction**
Alopecia is not life threatening, but patients who undergo alopecia often experience severe mental stress. In addition, the number of individuals afflicted by alopecia has been increasing steadily. The most effective treatment of alopecia developed to date is auto hair transplantation. To overcome the limitations associated with current therapies for the treatment of alopecia, many researchers have attempted to revive hair follicles by in vitro culture of hair follicle cells and subsequent implantation in the treatment area. The purpose of the present study was to optimize the reconstruction of DPLTs. As in the case of MSCs, when compared to differentiated cells, DPLTs require an additional step to induce differentiation into dermal papilla cells.

**Materials and Methods**
For explant outgrowth, six pieces (each 2 mm x 2 mm) were attached onto 100 mm tissue culture dishes by contacting the connective tissue onto the dish surface. Human mesenchymal stem cells originating from the umbilical cord were subjected to a monolayer culture in DMEM supplemented with 10% FBS until the cells occupied approximately 80% of the culture dish. Next, the culture medium was replaced with dermal papilla-forming medium (DPFM) that contained 10 ng/ml hydrocortisone. Additionally, various growth factors were added to the culture medium in the different experimental groups. Specifically, the control group consisted of cells cultured in DPFM without any supplemental growth factor. The treatment group consisted of cells cultured in DPFM supplemented with HGF (rhHGF, R&D system, U.S.A.), recombinant human epidermal growth factor (EGF, Sigma, U.S.A.) or nerve growth factor (NGF, R&D system, U.S.A.). The medium was replaced with fresh medium at about 3-day intervals for 3 weeks, after which the culture was treated with Accutase at concentrations ranging from 20 to 40 μl/cm² to detach the cells from the culture dish [1].

**Results**
Adding group of EGF or HGF, mesenchymal stem cell marker expression was diminished. And collagen 4 and laminin expression increased during preconditioning independently of the added growth factor. However, the morphogenic specific marker, versican, was expressed strongly in cells that were treated with EGF or HGF.

**Discussion and Conclusions**
As in the case of MSCs, the dermal papilla like tissues required a differentiation step for dermal papilla cells to form prior to use in the cells. This generally requires the use of hepatocyte growth factor (HGF), which is relatively expensive. To reduce the cost of cell therapy using MSCs, we tested the effects of cell inoculation density and growth factor during differentiation. We found that EGF could be used to replace HGF during differentiation, which would reduce the cost of hair cell therapy.

**References**

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