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TITLE: Amniotic Epithelium- A Possible Stem Cell Source for Pulp Regeneration?

ABSTRACT BODY:

Objectives: The aim of regenerative endodontics is to replace damaged pulp tissue and restore its biological function. Transplantation of multipotent dental pulp stem cells (DPSC) may facilitate pulp-like tissue formation, however, their availability is limited. Therefore, we aimed to investigate the use of pluripotent human amniotic epithelial cells (HAEC), which can be obtained in large numbers from the placenta, as an alternative stem cell source.

Methods: HAEC were isolated from placentas by trypsinization of the amniotic membrane and characterized by flow cytometry (CD44/CD49f/CD105/CD326), whereas DPSC were obtained from primary cultures of human pulp after magnetic-activated cell sorting (STRO-1). Dentine matrix proteins (eDMP) were extracted and concentrated as previously described. Both cell types were cultured with eDMP to induce odontogenic differentiation, with osteogenic differentiation medium (StemPro™) to promote osteogenic lineage commitment and with 10% fetal bovine serum as a control. Morphological changes were documented after 7 days by fluorescence imaging after DAPI and phalloidin staining to visualize cell nuclei and the actin cytoskeleton. Expression of genes related to dentinogenesis and proliferation (COL1A1/IBSP/BGLAP/BMP4/TGFB1/NES/GPX3/IGFBP2/S100A4) was investigated by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) after 1, 7 and 14 days (n=4). The mineralization capacity was assessed by alizarin red staining after 21 days. Data were analysed nonparametric tests (Mann-Whitney U) at an $\alpha = 0.05$ level of significance.

Results: Isolated HAEC expressed all surface antigens (CD49f>CD105>CD44>CD326), but neither morphological changes nor an increased expression of differentiation-related genes were observed during the culture period. In contrast to DPSC, which differentiated and mineralized under osteogenic and odontogenic culture conditions, HAEC only showed calcification after induced osteogenic differentiation.

Conclusions: Overall, HAEC appear to not differentiate into an odontogenic, mineralizing phenotype, which makes them an unsuitable cell source for regenerative endodontic approaches.

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