

Ultrastructural evidence for multi-lineage differentiation of human dental pulp stem cells

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INTRODUCTION: Human dental pulp is the mucoid connective tissue that occupies the central space of the tooth and is completely surrounded by dentin. Several studies already suggested the presence of a stem cell population in this tissue. In order to evaluate their potential usefulness in future clinical applications, it is essential to further explore the lineage specific tissues formed by these stem cells. Therefore in this study, we performed an ultrastructural investigation of multi-lineage differentiated human dental pulp stem cells (HDPSCs).

METHODS: Human dental pulp was mechanically and enzymatically digested. Cells were subcultured and characterized immunocytochemically using the mesenchymal stem cell markers STRO-1, CD29, CD44 and CD146. Then cells were cultured in different conditioned media triggering adipogenic, osteogenic and chondrogenic differentiation. Differentiation was evaluated at the immunocytochemical level using anti-FABP, anti-osteocalcin and anti-aggrecan antibodies respectively. Furthermore, differentiation was investigated using specialized histological stainings (Oil Red-O, Alizarin Red and Alcian Bleu staining respectively) and electron microscopy.

RESULTS: Adipogenic differentiation was performed with a very low efficiency (data not shown). In contrast, osteogenic and chondrogenic differentiation resulted in the production of lineage specific extracellular matrix. Osteogenic differentiation resulted in the presence of many multi-lamellated organelles which were one of the distinctive characteristics of the cytoplasm (Fig. 1a). Furthermore, the extracellular matrix consisted of an irregular collagen matrix, intermingled with calcified nodules showing some hydroxy-apatite needles (Fig. 1b). Also fully calcified structures were observed, always in close relationship with the collagen matrix (Fig. 1c)

The cytoplasm of chondrogenic induced stem cells was characterized with the accumulation of large matrix vesicles, which tended to fuse with one another (Fig. 2a and b). This resulted in rupture of the cell membrane, thereby releasing these large vesicles in the ECM. Interaction with collagen fibres resulted in the presence of calcified cartilage fragments (Fig. 2c).

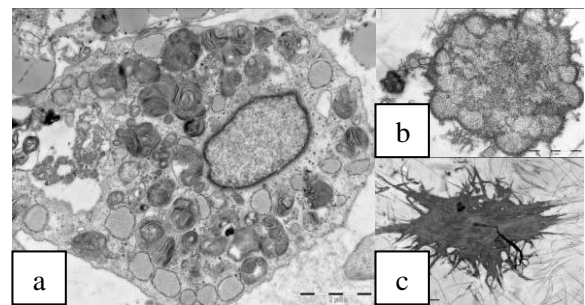


Fig. 1: Illustration of osteogenic differentiation potential of HDPSCs

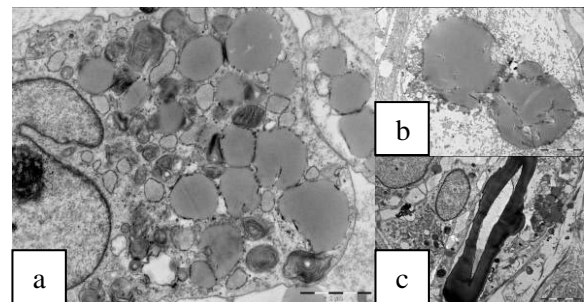


Fig. 2: Illustration of chondrogenic differentiation potential of HDPSCs

DISCUSSION & CONCLUSIONS: This research confirms the multi-lineage differentiation potential of HDPSCs. Furthermore, ultrastructural analysis of differentiated cells gives new insights into the lineage specific production of extracellular matrix.

REFERENCES: ¹ Gronthos, S., M. Mankani, et al. (2000). "Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo." *Proc Natl Acad Sci U S A* **97**(25): 13625-30.