

Ultrastructural and biochemical analysis of *in vitro* odontoblast differentiation

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The purpose of this study was to develop an *in vitro* model of odontoblast differentiation and dentin formation which allows to deeply and better study the process of odontoblast differentiation and protein matrix deposition and mineralization.

Human dental pulp cells were obtained from extracted molar teeth of donors under informed consent. Cells were induced to odontoblast differentiation for 7, 14, 21 and 28 days by adding in the cell medium dexamethasone, β -glicerophosphate, ascorbic acid and TGF- β 1.

Alizarin red staining, electron microscopy, western blotting were carried out to demonstrate the odontoblast differentiation process, extracellular matrix deposition and mineralization.

Alizarin red staining results showed a gradual calcium deposition during the treatment, reaching the highest signal at 28 days of stimulation. Electron microscopy demonstrated the deposition of fibrillar structures in the extracellular matrix, connected with extracellular matrix proteins. Western blotting analysis showed the expression of collagen type I and dentin matrix protein 1, markers for the odontoblast phenotype.

The *in vitro* odontoblast differentiation model described if an highly repetitive model and it represents a potential and promising tool to explore the extracellular matrix deposition and mineralization process in physiological and pathological conditions.

Keywords: odontoblast like cells, differentiation, extracellular matrix protein, DMP1, Collagen type I