Evaluation of the Neurogenic and Dentinogenic Effect of Valproic Acid on Undifferentiated Mesenchymal Cells of Human Dental Pulp

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Introduction

The dental pulp is the innermost portion of the tooth and is surrounded by primary and secondary dentin. Primary and secondary dentin have odontoblastic activity and are organized into tubules which contain nerve endings. Tertiary dentin is formed as a natural defense mechanism when the pulp is exposed, through trauma or a deep carious lesion. Tertiary dentin builds up as a mass of minerals that lacks organization and cuts off the nerve communication to that outward portion of the tooth. In an effort to maintain the natural state, vitality and sensitivity, of teeth as much as possible, we are researching a method to induce mesenchymal cells (stem cells) from dental pulp to differentiate into odontoblast-like cells. In this study, we analyze the effect of Valproic Acid to induce pulp stem cells *in vitro* to produce the neurogenic and odontogenic markers similar to those found in primary and secondary odontoblasts.

Hypothesis

If Valproic Acid is applied to undifferentiated mesenchymal cells from the dental pulp, then protein markers associated with odontoblasts will start to be expressed (*in vitro*).

Materials and Methods

Isolation:
Healthy pulps of 3rd molars extracted for orthodontic or other reasons will be obtained. Extracted teeth are subjected to enzymatic digestion w/ collagenase and disphase and cultured in a growth medium; Dmem medium supplemented w/ fetal bovine serum (FBS), and an antibiotic solution. Upon obtaining a 90% cell confluence several passages will be carried out and passage 3 will be used for characterization of cells as stem cells.

Characterization of cultured Cells as Mesenchymal Stem Cells:
The Gene expression of pluripotential markers (SOX2, Nanog, OCT4, CD73, and CD90) and expression of CD31 and CD145 markers will be evaluated to rule out that they are hematopoietic. qRT-PCR will be performed and ΔΔ CT method will be used.

Evaluation of Cell Proliferation:
Concentrations of 0.1, 0.5, 1, 5, and 10 uM of Valproic Acid will be added to cell cultures and the cell proliferation will be evaluated through the method based on new DNA synthesis using the Click IT EdU Assay kit and flow cytometry at 6, 12, 24, and 48 hours. In addition, cytotoxicity will be evaluated by an MTT assay.

Evaluation of minimum therapeutic dose:
The different concentrations of Valproic Acid will be administered to each culture and the Alizarín RED test will be carried out to evaluate the mineralization in the different cultures during a period of 24-48 hours.

Evaluation of differentiation towards Odontogenic and Neural Lineage:
Three different concentrations of Valproic Acid will be added to the cultures (depending on the degree of cell proliferation and cytotoxicity) and by qRT-PCR the genetic expression that code for specific proteins of odontoblastic differentiation and neurogenic markers (Dentin Sialophosphoprotein, Collagen Type I, Nestin, S100B, SOX 10 and Protein Zero will be evaluated). The negative control will be the group of cells that grew in the culture medium without the inducing molecule.

Preliminary Results

At this point we have isolated the pulp tissue from the extracted 3rd molars. We have successfully isolated the pulp stem cells and proliferated them in a growth medium and characterized them as pluripotential. We are currently in the stages of introducing set concentrations of Valproic Acid to the cells and analyzing the expression of the protein biomarkers that are associated with odontoblastic activity.

Figure 1: Extracted lower 3rd molar to be used as a source of healthy dental pulp tissue for cellular isolation.
Figure 2: Dr. Edwin de la Cruz extracting the pulp from the 3rd molars.
Figure 3: Extracted pulp.
Figure 4: Enzymatic digestion of the pulp.
Figure 5: 10x microscopic photograph taken immediately after the tissue was placed in the growth medium.
Figure 6: 10x microscopic photograph of pulp cell colonies taken on the 4th day after the enzymatically digested pulp was placed in the growth medium.
Figure 7: 80% cell confluence on the 18th day in the growth medium, replacing the medium every 4 days.
Figure 8: 100% cell confluence demonstrated on the 24th day through a microscope at 10x.

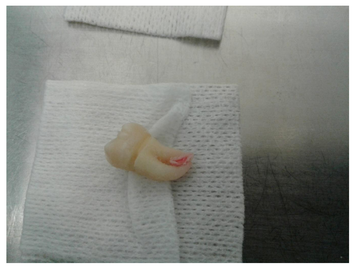

Figure 1

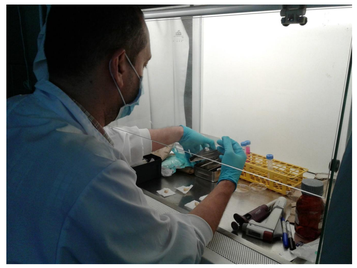

Figure 2

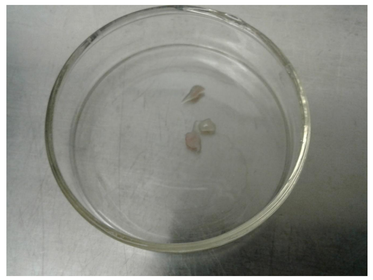

Figure 3

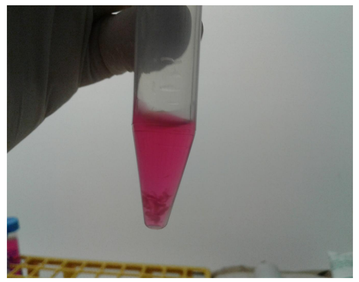

Figure 4

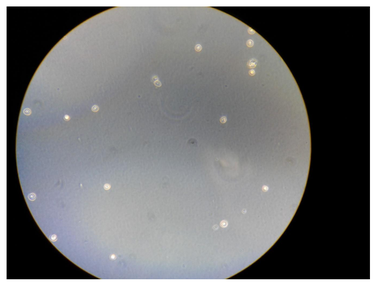

Figure 5

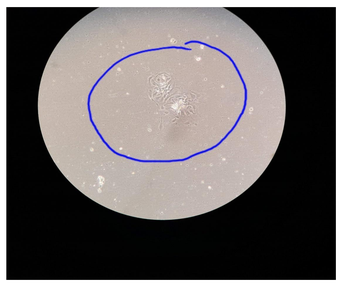

Figure 6

Clinical Implications of Research

Upon successfully replicating the protein markers found in odontoblasts, we can further the research of these odontoblastic-like cells by testing their ability to produce dentin and analyzing the structural organization formed. Furthermore, we can analyze the timeline of dentin formation and mineralization from these cells.

Although this research is in the beginning stages, this would allow potential for a newer approach to treating pulp exposures, especially partial pulpotomies (Cvek). If we are able to induce these stem cells to become odontoblasts and create dentin with proper organization, we could preserve and restore the tooth similar to its native state. This will allow the tooth to have a more natural communication with the nerve ensuring that it responds to environmental stimuli.

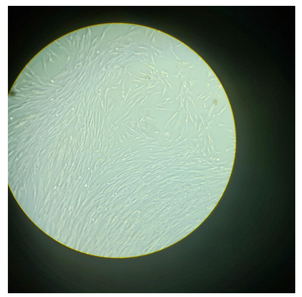

Figure 7

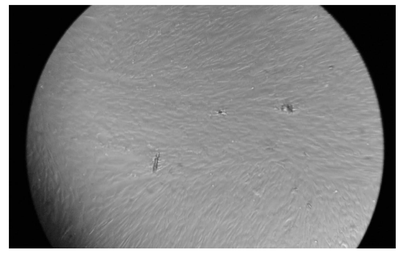

Figure 8

Acknowledgements

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