PRODUCTION OF AN *IN VITRO* MODEL OF STEM CELLS FROM DENTAL PULP TO STUDY THE EFFECTS OF MECHANICAL VIBRATIONS ON PROLIFERATION AND OSTEOGENIC DIFFERENTIATION AND TO IDENTIFY OSTEOGENIC INDUCTORS ABLE TO IMPROVE THE PHYSIOLOGICAL REPAIR OF THE TOOTH.

Mesenchymal stem cells (MSC) have the ability to proliferate and differentiate when treated with appropriate inducers. They can also be isolated from different tissues, in particular, from the dental pulp. These cells are easily accessible, plastics and can be used for a variety of clinical applications. In particular, the MSC of the pulp of deciduous teeth are characterized by a better potential for proliferation and differentiation compared to those derived from peripheral blood and bone marrow. In addition to growth factors, stem cells react to mechanical stimulation such as the low magnitude and high frequency vibration (vLMHF), which can promote osteogenic anabolic activity and therefore lends itself to a wide range of therapeutic applications, including repair or treatment of bone deterioration. The low invasiveness of the vLMHF treatment and its ability to stimulate the bone metabolism leaves us to assume their use in the orthodontic clinic, as well as to reduce pain and facilitate root resorption, also to reduce the time of orthodontic treatments performed by applying fixed or aligning devices.

GOALS

1) To date, the biological effects triggered by in vivo treatment with vLMHF are poorly understood. To better understand the cellular events involved in the clinical response, an experimental model of primary cells will be developed for the *in vitro* evaluation of possible correlations between treatments with vLMHF and osteogenic induction:

• production and validation of a simplified experimental cell model based on the use of cells extracted from the pulp of deciduous teeth and enriched in MSC cells by immuno-purification techniques. Definition of experimental conditions for purification, cultivation, enrichment in stem cells, characterization by FACS analysis, osteogenic induction and evaluation of osteoblastic differentiation;

• definition of experimental conditions for cryo-conservation of pulp tissue MSCs and evaluation of proliferative properties and inducibility following in vitro expansion after different freezing periods;

• characterization of the cellular effects of in vitro treatment cycles with vLMHF. The study includes: a) the design of a special elastic suspension support with counterweight to apply the vLMHF device to cell cultures; b) the evaluation of the effect of vLMHF on the proliferation and expression of markers of bone differentiation through the use of colorimetric assays; c) the evaluation of the effect of vLMHF on the induction of transcripts involved in osteogenic differentiation.

2) Trauma and infections can fracture or erode the protective outer layer of the tooth, dentin, exposing the dental pulp to irreversible damage. Dentists use cements or resins to clog the cavities, but these artificial materials do not integrate perfectly with the tooth and can degrade, causing re-infections and subsequent removal of the damaged part. In the dental pulp, stem cells are available to restore dentin, but only if the lesions are very small. The goal of the project is:

• identification and characterization of molecules of natural or synthetic origin capable of stimulating in vitro proliferation and osteogenic differentiation, in order to identify new strategies for reconstituting the original tooth structure, much less exposed to the risks of erosion than to artificial fillings now in use.

INSTRUMENTS AND METHODS

Basic tools for primary cell cultures (CO_2 incubator, laminar flow hood, inverted microscope, clinical centrifuge). Device and support for the application of vLMHF to *in vitro* cultures. Cellular cryo-preservation systems, *ELISA* reader, thermal cycler, electrophoresis gel instrumentation and image acquisition, RT-PCR quantitative real-time system for analysis of gene expression involved in bone formation and remodeling.

MTT assay, *FACS* analysis of stem and bone differentiation markers, cell cycle analysis with propidium iodide, colorimetric analysis of *alizarin red* and *alkaline phosphatase*.

SUBJECTS

Biochemistry, Cellular Biology and Molecular Biology.

WORKING GROUP

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