Stage II

Summary

In this study, the activities to characterize the cultures of dental pulp stem cells (DPSC) established in the previous phase were continued. The optimal parameters of DPSC defrosting, after applying a cryopreservation protocol for 1 year, were selected and cultivation parameters were established to differentiate stem cells to adipocytes and chondrocytes. Analysis of specific secreted markers, such as adiponectin, leptin and chondroitin sulfate, aggrecan and type II collagen, respectively, was performed by specific immunocytochemical methods and high sensitivity ELISA assays. The multidifferentiation potential of DPSC was demonstrated using *in vitro* experimental models, but it was influenced by the cryopreservation process, the cell passage and the donor age.

The activities of preparation of 3D matrices, biomimetic to the extracellular matrix (ECM), continued. Thus, experimental variants of composite hydrogels based on natural polymers, collagen type I (Col), chondroitin sulfate (CS) and fibronectin (FN), cross-linked by physico-chemical methods were obtained. It has been shown that hydrogels have fluid absorption capacity, controlled biodegradation due to cross-linking and biocompatibility towards cells from the oral cavity tissues, thus presenting biotechnological interest.

Experimental variants of 3D spheroids were obtained by self-assembling DPSC under controlled cultivation conditions. The optimal cultivation parameters were selected, namely the liquid overlay technique, the use of agarose-coated microplates, the initial cell density, the growing and cultivation period, the incubation conditions.

By means of optical microscopy and transmission and scanning electron microscopy techniques, it has been shown that obtained spheroids can have a regular shape and a controllable size. Live & Dead assay and DAPI staining demonstrated that DPSC from spheroids maintained high cell viability during cultivation.

Col-CS-FN porous matrix was used as scaffold for DPSC spheroids cultivation, in order to fabricate viable 3D systems that can be used as experimental models for laboratory testing of therapeutically useful pharmaceutical compounds or in the development of microtissues with applications in regenerative medicine. We have optimized the methods for analyzing the structure of 3D systems by optical microscopy techniques and working protocols have been established to investigate their ultrastructure by electron microscopy. The obtained results allowed the selection of parameters, such as the scaffold's surface geometry, the procedure of cell seeding on the scaffold's surface, the number of spheroids and the cultivation period.

Lab technologies have been experimented for the development of 3D matrix - DPSC spheroid systems. The cells from these systems presented the ability to secrete specific ECM proteins, such as type I collagen and fibronectin, and also the glycosaminoglycan chondroitin sulphate. This property is important in providing a micromedium similar to that *in vivo* and favors cell adhesion.