

Stage III

Summary

This study continued the activity of evaluation of cell differentiation capacity on experimental variants of 3D cellular systems consisting of dental pulp stem cells (DPSC) spheroids, prepared in different sizes and osteogenic differentiated. It were performed, for the first time, morphological and ultrastructural analyses using optimized techniques of optic and electronic microscopy. The acquired images showed a unique structure of cellular aggregates with high viability even after 21 days of cultivation, proliferative capacity at the periphery and differentiated cells with an abundant specific matrix inside. Microscopy observations were confirmed by sensible quantitative analyses results for specific osteogenic markers, such as procollagen type I, calcium and alkaline phosphatase, which underlined the influence of DPSC spheroids size and incubation period. These data allowed the selection of functional variants of 3D DPSC spheroids, having their own secreted matrix, useful for *in vitro* experimental tests.

Conditioned culture media were harvested from DPSC cultivated as monolayers and 3D spheroids, after the first week of cultivation, due to their rich content in secreted bioactive factors (secretome) and were tested in osteoblasts cultures. Both types of conditioned media exerted stimulative effect on osteoblasts metabolism and cell proliferation. These results confirmed the paracrine effect of DPSC and the utility of their presence at the wounded tissue situs, to speed up the healing process.

A lab technology for the achievement of 3D cellular systems was optimized using a scaffold of biopolymeric hydrogel based on a composite mixture of collagen, chondroitin sulfate and fibronectin and DPSC. The performed tests highlighted their viability, cell adhesion and a higher cell proliferation rate, compared to that of DPSC control cultures without polymeric substrate.

In addition, investigations on the biological activity of the obtained 3D cellular systems and their utility in treatment protocols for periodontal disease were carried out in experimental models *in vitro*. They allowed the optimization and obtaining of 3D functional system variants of scaffold matrices for spheroids of DPSC with antioxidant, anti-inflammatory and regenerative properties for increasing the cell survival after transplantation.

Dissemination activities were fulfilled by publication of 2 original articles in journals with visibility and one chapter book in an international volume, and also by communication of 2 papers at international conferences, which were published in international indexed journals. In addition, a patent application was registered at OSIM, based on original results achieved during project development. The web page of the project was updated.