Stage I

Summary

In this study, the optimal conditions for obtaining and processing dental pulp stem cell cultures (DPSC) for regenerative medicine, as well as the influence of conservation conditions on their specific properties, were analyzed. Fifteen DPSC cultures were obtained and divided into 3 groups, age-related and analyzed over 9 passages, both before and after cryopreservation. Optimal parameters were established for harvesting and transporting the samples, as well as for isolation and expansion of DPSC cultures from the 3 groups using an enzymatic method, which showed an efficiency of 75%.

Morphological analysis has demonstrated similar characteristics and presence of nodular formations in all analyzed DPSC cultures, regardless of age. In the case of cryopreserved DPSC, the specific mesenchymal stem cell morphology was maintained (fusiform, elongated, fibroblast-type cells). Cytometry data have shown that isolated DPSC have a mesenchymal stem cell phenotype and they express specific markers CD29, CD44, CD73, CD90 and CD105 in a high percentage (> 94%), regardless of age or cryopreservation.

Cell viability of cryopreserved DPSC was investigated by both Trypan blue method and the Live/Dead method and indicated a high percentage of cell recovery (~ 80-90%), demonstrating the effectiveness of 10% glycerine as cryopreservation agent, similar to that of 5% DMSO. Comparative analysis of cell proliferation was performed by determining cell number, growth curve, population doubling, and doubling time over 9 passages. The results indicated a decrease of proliferative capacity with $2x10^6$ cells and an 18-hour delay in population doubling time in samples of group III (adult) after cryopreservation, compared to groups I and II (young).

The potential of osteogenic differentiation of DPSC from the 3 groups was analyzed by determining the specific markers (osteopontin, osteocalcin, calcium, alkaline phosphatase, collagen type I-Col) after 21 days of cultivation in the osteoinduction culture medium. The morphological analysis indicated DPSC transition to the osteoblastic phenotype and the presence of characteristic cuboidal cells. In addition, calcium deposition was highlighted by Alizarin Red S staining. The expression of both markers, osteopontin and osteocalcin, observed by immunofluorescence, demonstrated the total differentiation of DPSC in osteoblasts. Quantitative data regarding the metabolic activity of osteogenic differentiated DPSC have demonstrated that the donor's age and the freezing process did not influence the process, but only the amount of the secreted marker (calcium, alkaline phosphatase, Col type I). Significant higher values ($p < 0.05$) were recorded for groups I and II (16-29 years) before freezing, compared to group III (32-43 years) after freezing, with differences between 20-35%.

Biomimetic equivalents were prepared by combining three polymeric components of the extracellular matrix, of protein and polysaccharidic nature: Col type I, chondroitin 4-sulfate (CS) and fibronectin (FN), in a weight ratio of 10:1:0.001 (variant A) and 10:1:0.005 (variant B), followed by lyophilization. Cross-linking of 3D porous sponges was chemically performed, using a solution of 30 mM carbodiimide.

The physicochemical and biochemical characterization of sponges indicated porosity values higher than 70% and an effective cross-linking that allows control of their biodegradation in inflammatory conditions. The structural analysis showed a uniform distribution of glycosaminoglycans on Col surface, in both sponge variants, and CS presence was confirmed by

immunohistochemistry. Electron microscopy allowed visualization of Col fibers that presented an
orderly disposition in the non-crosslinked variant A and indicated a heterogeneous aspect with thicker fibers in case of variant B crosslinked sponges. CS was also observed as electron-dense deposits in regions with aggregated Col or with a point distribution in areas with lax Col.

Cytotoxicity tests demonstrated that experimental variants A and B, cross-linked and non-cross-linked were biocompatible with the cell types present in the oral cavity (osteoblasts, fibroblasts). Variant A with lower FN content presented physico-chemical, structural and biochemical features of interest for an equivalent matrix for repair of periodontal tissue, as a scaffold for cell infiltration, adhesion and proliferation.