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## Osteogenic differentiation of human dental pulp stem cells in 3D scaffolds

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The aim of this study was to characterize the *in vitro* osteogenic differentiation of dental pulp stem cells (DPSCs) in 2D and 3D cultures. DPSCs were isolated by magnetic cell sorting using antibodies against c-Kit, CD34 and STRO-1 surface antigens and then differentiated toward osteogenic lineage on 2D surface of culture flask by using an osteogenic medium. Differentiated cells express specific bone proteins such as Runx-2, Osx, OPN and OCN with a sequential expression analogous to those occurring during osteoblast differentiation and produce extracellular calcium deposits. In a second phase, DPSCs were cultured in Matrigel<sup>TM</sup>, Collagen and Fibroin 3D scaffolds in order to differentiate cells in a 3D space that mimics the physiological environment. In DPSC-Matrigel<sup>TM</sup> complexes, cells differentiate in osteoblast phenotype and form calcified nodules. DPSCs differentiated in collagen sponge actively secrete human type I collagen and after implant in immuno-suppressed rats were enveloped in a highly vascularised connectival capsule. DPSC-Collagen complex presents small areas of mineralization but appears infiltrated by inflammatory cells indicating that an immuno-response occurred. Fibroin scaffolds colonized with DPSCs after implant appear enveloped in a connectival capsule highly vascularised similarly to collagen scaffolds. In contrast the immune system invasion did not occur. DPSC are present in the whole scaffold thickness and in intimate contact with the scaffold fibers. After 20 days of implant extended areas of mineralization were stained by alizarin red. Fibroin scaffold appears more suitable for future application in regenerative medicine because it is highly histo-compatible, does not produce immune-response and offers the optimal environment for DPSCs growth and differentiation. These neo-formed DPSCs-scaffold devices may be used in regenerative surgical applications to resolve pathologies and traumas characterized by critical size bone defects.

### Key words

DPSC, Stem Cells, Osteogenic Differentiation, 3D Scaffold, Fibroin