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Engineered vesiscles from gingival stem cells: a new approach in 3D printed bone tissue regeneration

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In the bone regeneration field, properties of 3D scaffold could be improved using cellular and their released products. Even if previously documented, poly-(lactide) (PLA) scaffolds were not thoroughly evaluated for their design-related characteristics. The aim of the study was to investigate the properties of 3D printed PLA scaffolds for bone regeneration obtained through 3D printing, evaluating the differences in terms of structural properties, in vitro and in vivo cellular responses induced by different scaffold structures. Biofabrication is to generate a construct with biological function. In particular in our reserach we describe the fully process, including printing scaffold step, in vitro culture phase and subsequently in vivo transplantation.

Five porous scaffold designs (A-B-C-D-E) were fabricated from a poly-(lactide) (PLA) filament. Scaffold structural parameters, such as porosity and pore size, were measured using scanning electron microscopy, and micro-computed tomography. Nano-topographic surface features were investigated by means of atomic force microscopy.

Over a 112-day period, scaffolds were hydrolytically degraded and changes in weight, pH and mechanical properties were measured during degradation.

Osteogenic differentiation of hPDLSCs on different scaffold designs after 21 days of culture was measured by means of RT-PCR and Western Blot.

In vivo study was performed using C57BL/6 mice and was designed in 5 different groups:

- Group1: Scaffold loaded with hPDLSCs
- Group2: Scaffold loaded with conditioned medium (CM) derived from hPDLSCs
- Group3: Scaffold loaded with exosomes (Exo) purified from CM
- Group 4: Scaffold loaded with engineered exosomes (e-Exo), exosome treated with PEI (poly ether imide)
- Group5: Scaffold, used as control.

Histological analysis were performed after 60 days of in vivo transplantation and morphological evaluations revealed a high bone tissue formation and osteogenic cells commitment in group 3 and 4 when compared to other groups.

From these results, the cell-laden PCAMSC scaffold offers a significant advantage in the TM regeneration in a rat subacute TM perforation model. It may offer attractive opportunities in the conservative clinical treatment.

This study demonstrated that scaffold of group 3 and 4 significantly improved bone tissue regeneration in animal model and successfully showed new bone deposition in situ compared to the control scaffold (group 5) and group 1 and 2. Based on the results, we believe that the bioprinted scaffold may become a novel treatment for tissue regeneration approach therapy.

Keywords

Tissue engineering, mesenchymal stem cells, bone tissue regeneration, exosomes, conditioned medium