Co-culture of osteoblasts and osteoclasts in composite scaffolds support osteogenesis in vitro

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Osteoporosis is a bone pathology caused by an imbalance in bone remodelling due to excessive osteoclast-mediated bone resorption and decreased action of osteoblasts. Bone tissue engineering (BTE) is an attractive strategy to treat long bone fractures using three dimensional (3D) constructs as bone graft substitutes mimicking the 3D porous environment of native bone [1, 2]. Fused deposition modeling (FDM) was used to process thermoplastic materials with mechanical properties close to those of the natural tissue [3]. In this study, polymeric blends of poly-L-lactic acid (PLLA), polycaprolactone (PCL) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (90/5/5 %wt), blend+2.5%wt of nano-hydroxyapatite (nano-HA) and blend+2.5%wt of strontium-substituted-nano-HA (Sr-nano-HA) were fabricated into 3D scaffolds with specific geometry and evaluated for their osteogenic and osteoclastogenic potential using appropriate cell types.

Human bone marrow mesenchymal stem cells (hBM-MSCs, $2x10^4$ cells/scaffold), were seeded onto the scaffolds and cultured for 13 days. Then, human peripheral blood mononuclear cells (hPBMCs) were added ($50x10^4$ cells/scaffold). Cell proliferation and morphology were monitored via a reduction-based cell viability assay, scanning electron microscopy (SEM) and confocal microscopy. Measurement of the alkaline phosphatase (ALP) and tartrate acid phosphatase (TRAP) activity was conducted to determine the effect of the polymeric scaffolds on the osteogenesis and osteoclastogenesis respectively. Quantitative polymerase chain reaction (qPCR) was applied to quantify changes in the gene expression of osteogenesis-related markers such as osteonectin, osteoprotegerin and osteocalcin, as well as osteoclastogenenic markers including TRAP, dendritic cell-specific transmembrane protein and nuclear factor of activated T cells 1.

The cell viability assessment displays an excellent biocompatibility for all scaffold compositions allowing cells to proliferate. SEM and confocal microscopy images reveal well-spread cells depicting a physiological morphology. After 14 days, the ALP activity is more than two-fold higher in the co-culture compared to the hBM-MSC mono-culture. The TRAP activity results showed a significantly lower TRAP activity in the co-culture than in the hPBMC mono-cultures at all tested time points and materials. Osteogenesis related markers examined with qPCR were significantly higher in the co-culture than in the mono-cultures of hBM-MSCs.

The results confirm that hydroxyapatite and strontium as components of composite scaffolds with controlled spatial architecture enhance osteogenesis and reduce osteoclastogenesis in co-culture due to the communication of the two cell types in relation to the individual monocultures, suggesting their potential as implants to treat degenerative bone pathologies such as osteoporosis.

References

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