0241

A Doped Hydrogel Overcomes Inflammatory Environment: an Osteoblasts/Macrophages Co-Culture

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Objectives The objective of this *in vitro* investigation was to evaluate the synergistic effect of osteoblasts and M1 macrophages differentiation, by direct crosstalk, in osteogenesis and osteoclastogenesis when cultured in a NP12 peptide loaded 3D collagen scaffold.
Methods A 3D collagen injectable tissue scaffold was created and NP12 peptide was loaded (50 nM). The cell-specific role and cell differentiation were investigated through osteoblast and proinflammatory macrophage-specific gene markers, phosphatase alkaline production and by histological tissue analyses. At least three independent studies were analyzed per experimental group. Data were analyzed for normalization and statistical significance was analyzed using Student's *t* tests ($P<0.05$).
Results When macrophages were exposed to NP12, the M1 macrophages tended to express a higher pro-inflammatory phenotype after 48h. Osteoblasts cultured in NP12 doped scaffolds after 24h had a diminished pro-inflammatory expression, but after 48h RANKL and TNFa and were about 50% higher and alkaline phosphatase production was lower (10-times reduced) than in those osteoblasts not exposed to NP12. Further, when osteoblasts and macrophages were cultured together, an alkaline phosphatase elevated production (about 50% higher) and a decreased expression of most of the inflammatory markers (IL1B and TNF were 10-times reduced) was encountered in the presence of NP12 at 24h or 48h, if compared to cells that were not exposed to NP12. Osteoblasts curbed the osteoclastogenic differentiation of macrophages, reducing their pro-inflammatory lineages and the release of signaling factors. The osteoblast within the 3D coculture demonstrated increased ALP activity but express RANKL significantly different than the osteoblasts cultured with macrophages in a 3D collagen matrix with NP12.
Conclusions NP12 in contact co-culturing has an anabolic effect on bone tissue in a bacteria-derived inflammatory environment. Osteoblasts and M1 macrophages in co-culture at the NP12-loaded scaffold reduced M1 pro-inflammatory phenotype and induced osteogenesis. Grant PID2020-114694RB-I00 funded by MCIN/AEI 10.13039/501100011033.