Bone Tissue Engineering using P-15 Coated Scaffolds and Human Dental Pulp Stromal Cells

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Introduction
P-15 a fifteen residue synthetic peptide (GTPGPQGIAGQRGVV), is a structural analogue of the cell binding domain of Type 1 collagen [1]. P-15 adsorbed on anorganic bovine mineral (ABM-P15) scaffolds has been shown to enhance bone marrow stromal cell growth [2]. This study aimed to investigate the osteogenic potential of human dental pulp stromal cells (HDPSCs) in monolayer culture compared to human bone marrow stromal cells (HBMSCs) and on 3D ABM-P15 in vitro and in vivo.

Material and Methods
HDPSCs and HBMSCs were cultured in basal or osteogenic media for 3 weeks and osteogenic potential was investigated using alkaline phosphatase (ALP) staining and ALP specific activity (ALPSA). Gene expression of osteogenic markers (ALP, COL1, RUNX2 and OCN) was determined using RT-PCR after 1, 3 and 5 weeks in basal culture. Alternatively, HDPSCs were seeded on 3D ABM±P-15 scaffolds (CeraPedics LLC) and cultured in basal media for 6 weeks. Cell viability and growth were visualized by confocal microscopy. Diffusion chambers containing ABM±P-15/HDPSCs constructs were implanted intraperitoneally in nude mice for 8 weeks. The samples were then processed for histology.

Results
HDPSCs showed stronger ALP staining in both culture conditions compared to HBMSCs (Fig. 1). RT-PCR indicated an up regulation of all osteogenic markers in both cells at weeks 1 and 3. At week 5, there was a marked down regulation of COL1 and RUNX2 in HDPSCS compared to HBMSCs. Confocal microscopy and SEM showed ABM-P-15 promoted the HDPSCs bridge formation between the scaffold particles. Histological staining and biochemical analysis confirmed that P-15 enhanced HDPSCs ALP activity (Fig. 2) in vitro and fibrillar collagen formation in vivo compared to ABM alone.

Discussion and Conclusion
HDPSCs have higher osteogenic capacity compared to HBMSCs. ABM-P15 enhanced HDPSC ALPSA and collagen formation, suggesting that this combination of ABM-P15 plus HDPSCs could be used for bone tissue engineering.

References

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