Repair of calvarial defects with customized tissue-engineered bone grafts. I. Evaluation of osteogenesis in a three-dimensional culture system.

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Abstract:

Bone generation by autogenous cell transplantation in combination with a biodegradable scaffold is one of the most promising techniques being developed in craniofacial surgery. The objective of this combined in vitro and in vivo study was to evaluate the morphology and osteogenic differentiation of bone marrow derived mesenchymal progenitor cells and calvarial osteoblasts in a two-dimensional (2-D) and three-dimensional (3-D) culture environment (Part I of this study) and their potential in combination with a biodegradable scaffold to reconstruct critical-size calvarial defects in an autologous animal model [Part II of this study; see Schantz, J.T., et al. Tissue Eng. 2003; 9(Suppl. 1):S-127-S-139; this issue]. New Zealand White rabbits were used to isolate osteoblasts from calvarial bone chips and bone marrow stromal cells from iliac crest bone marrow aspirates. Multilineage differentiation potential was evaluated in a 2-D culture setting. After amplification, the cells were seeded within a fibrin matrix into a 3-D polycaprolactone (PCL) scaffold system. The constructs were cultured for up to 3 weeks in vitro and assayed for cell attachment and proliferation using phase-contrast light, confocal laser, and scanning electron microscopy and the MTS cell metabolic assay. Osteogenic differentiation was analyzed by determining the expression of alkaline phosphatase (ALP) and osteocalcin. The bone marrow-derived progenitor cells demonstrated the potential to be induced to the osteogenic, adipogenic, and chondrogenic pathways. In a 3-D environment, cell-seeded PCL scaffolds evaluated by confocal laser microscopy revealed continuous cell proliferation and homogeneous cell distribution within the PCL scaffolds. On

osteogenic induction mesenchymal progenitor cells (12 U/L) produce significantly, higher ($p < 0.05$) ALP activity than do osteoblasts (2 U/L); however, no significant differences were found in osteocalcin expression. In conclusion, this study showed that the combination of a mechanically stable synthetic framework (PCL scaffolds) and a biomimetic hydrogel (fibrin glue) provides a potential matrix for bone tissue-engineering applications. Comparison of osteogenic differentiation between the two mesenchymal cell sources revealed a similar pattern.