SKELETAL TISSUE REGENERATION WITH STEM CELLS

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Cells with chondrogenic potential have been isolated from many postnatal tissues including bone marrow, synovial tissue, fat and periosteum. Methods for their isoloation, expansion and differentiation have been developed in recent years, with the growing interest in using these cells in tissue engineering applications for skeletal tissue repair and regeneration. In vitro systems for their differentiation have been developed that work well for the examination of the process of chondrogenesis. However, there are many challenges to their use in creating cartilaginous tissues on the scale and of the quality required for in vivo implantation in skeletal pathologies. These challenges include controlling the differentiation to create chondrocytes that do not go on to hypertrophy, as would be found in permanent cartilage. The elaboration of the correct extracellular matrix, both in content and organization is also difficult to control. Furthermore, the size of implants required to fix human skeletal tissue defects means that scaffolds to hold cells and shape the tissue regenerate are needed.

In recent years, we have developed tissue culture systems that allow for expansion of large numbers of cells with reparative potential. We have also experimented with scaffolds of various types, examining cell behavior and matrix production. Many scaffolds have been explored for their potential usefulness in tissue engineering of cartilage. We have reported on the use of hyaluronan/collagen composite scaffolds and their usefulness in conjunction with adult human bone marrow-derived stem cells in vitro, and with these cells of several animal species, both in vitro and in vivo. These biocompatible and biodegradable scaffolds are an example of the sponge and mesh-like scaffolds that have potential clinical use. There are also scaffolds that are solidified from an initial aqueous solution that allows cell mixing before creation of the scaffold. Derivatives of poly(ethylene oxide) (PEO) are widely utilized in these hydrogels due to their hydrophilicity, biocompatibility, and intrinsic resistance to cellular adhesion and protein adsorption. Although there are reports of chondrogenesis of mesenchymal progenitor cells within this type of scaffold, the production of clinically useful load-bearing cartilage with sufficient matrix production remains an issue. We have developed a photopolymerizable semi-interpenetrating network composed of PEO and PEODA in which the extensive chondrogenic differentiation of bone marrow-derived mesenchymal progenitor cells can be achieved in a hydrogel of clinically significant size (Figure 1). Many factors can influence cell viability, differentiation and matrix elaboration in these gels. We have explored the relationship of crosslink size, density, gelation time, interpenetrating network size and also cell-related factors to the extent of chondrogenic differentiation that occurs in this type of gel. In the optimized gels, chondrogenic differentiation occurred in all areas of the cell-seeded constructs with extensive elaboration of a cartilaginous matrix throughout the construct. This photopolymerizing system may have applications in the repair of cartilaginous tissues.

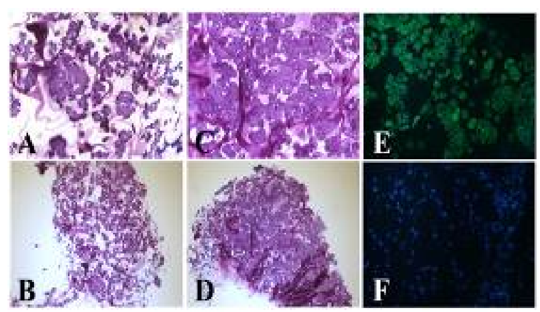

Figure 1 *Sections of cultured cell-seeded constructs stained with. toluidine blue (A-D), after 3 (A,B) and 6 (C,D) weeks in culture. Immunohistochemistry for type II collagen (E,F), under permissive (E) or non-permissive conditions (F).*