EVALUATION OF CELLULAR PROLIFERATION AND MATRIX ORGANIZATION IN POLYLACTIDE/ALGINATE ALAMGAM FOR CARTILAGE TISSUE ENGINEERING

Methods: This study evaluated the potential of human bone marrow-derived cells harbored within a three-dimensional matrix for cartilage tissue engineering by characterizing in vitro the chondrogenic differentiation profile, cellular proliferation kinetics, and the generation of fibrous tissue production. Bone marrow-derived cells were seeded at high density (40 x 10^6 cells/ml) in three-dimensional scaffolds consisting of either poly-L-lactic acid (PLA) alone, or an amalgam of PLA with the polysaccharide gel, alginate, and cultured in the presence of the chondrogenic factor, transforming growth factor-β1, (TGF-β1). The biodegradable PLA/alginate amalgam scaffold construct is an appropriate carrier for in vitro differentiation and subsequent transplant of the marrow-derived cells because of the ability of alginate to retain the cells in the construct and promote a round cell shape, thus providing for a high density growth format of cells encased in a removable temporary matrix. This amalgam scaffold can also direct chondrogenic differentiation when the appropriate bioactive molecules are supplied. In addition, alginate prevents chondrocytes from undergoing dedifferentiation, and when implanted in vivo induces only a minimal inflammatory reaction.1,4 PLA is a rational selection for a biodegradable polymer scaffold to bolster the alginate amalgam because polyester scaffolds of α-hydroxy acids, such as PLA, have been shown to confer mechanical stability to regenerating tissue and simultaneously support cartilage and connective tissue ingrowths.5

Results/Discussion: Cartilage formation was apparent in the TGF-β1 treated groups, on the basis of expression of chondrocyte specific gene expression assessed by reverse transcription-polymerase chain reaction (RT-PCR) (Fig. 1A), and sulfated proteoglycan rich matrix biosynthesis measured by metabolic [35S] sulfate incorporation. In addition, histological and immunohistochemical analysis revealed a cartilaginous phenotype in both the plain PLA and the PLA/alginate amalgam (Fig. 1B), cellular proliferation was simultaneously support cartilage and connective tissue ingrowths.5 shown to confer mechanical stability to regenerating tissue and because polyester scaffolds of


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Figure 1. A) RT-PCR analysis of chondrogenic gene expression in bone marrow-derived cells cultured for two weeks in various scaffold compositions with and without TGF-β1 (10ng/ml). Chondrogenic gene expression was detected only in the TGF-β1 treated constructs B) Histology and immunohistochemistry of constructs harvested after two weeks in culture treated with and without TGF-β1 (10ng/ml). Constructs included the polymer alone (PLA) or the polymer combine with alginate (PLA/Alg). Both the plain PLA and the PLA/alginate amalgam elicited robust chondrogenesis with TGF-β1 treatment. Bar = 25 µm.

Figure 2. Immunohistochemistry for BrdU, PCNA, and collagen type I of constructs harvested after two weeks in culture. Plain PLA and PLA/Alg amalgam constructs were either treated with (Treated) or without (Control) TGF-β1 (10 ng/ml). The BrdU and PCNA demonstrate that the areas of cell proliferation is restricted to the perimeter of the constructs. This same region showed collagen type I deposition in the plain PLA constructs but this fibrobastic collagen deposition was absent in the PLA/alginate amalgam constructs. Bar = 20 µm.