GENERATION OF AN IMPLANTABLE CARTILAGINOUS GRAFT USING MESENCHYMAL STEM CELLS DERIVED FROM HUMAN BONE MARROW.

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Introduction: Bone marrow derived mesenchymal stem cells (MSCs) have been shown to differentiate into the cell lineage of myoblast, adipocyte, chondroblast and osteoblast phenotype, when cultured under a permissive microenvironment. It has been previously shown that expanded human articular chondrocytes (HACs), when seeded on a Millipore filter coated with collagen type II and cultured in a serum free medium containing TGF-β2, consistently result in the formation of articular cartilage graft (ACG) [1, 2]. In the present study, we demonstrate that human mesenchymal stem cells, isolated and expanded from adult bone marrow, have the potential to differentiate into a cell lineage of chondroblast phenotype and eventually form a hyaline-like cartilaginous graft for use in the repair of chondral and osteochondral defects in vivo.

Materials and Methods: Human bone marrow samples were obtained from Biowhittaker from donors ages 19 to 34 years of age. The samples were aliquoted and diluted using a dilution buffer (2% FBS, 0.6% Sodium Citrate) slowly overlaid onto Histopaque in equal volumes. The samples were centrifuged at 1000x g for 30 minutes. The mononuclear cells at the interface were recovered, washed twice with MACS buffer (0.5% BSA, 2mM EDTA pH 7.2), and centrifuged for 10 minutes at 1000 x g. Cells were seeded at 50,000-60,000 cells/cm² in DMEM L-Glucose/10% FBS, bFGF 10 ng/ml and gentamycin. Non-adherent cells were removed 6-72 hrs after initial plating. At the end of 2nd, 3rd and 4th passages, the plated cells were harvested and seeded (2x10⁶ cells/cm²) onto Millipore CM filters coated with collagen type II in either DMEM/20% FBS or DMEM/HSA/ITSx. At the first medium change, ascorbic acid (100 µg/ml) and TGF-β2 (5 ng/ml) were added to the cultures. ACG’s were incubated for 1-4 weeks in a humidified environment, fixed in 10% formalin, embedded in paraffin and sectioned. Grafts were evaluated for the presence of hyaline-like cartilage using Toluidine Blue, Safranin O, H&E staining, and types I and II collagen immunoreactivity. Sulfated glycosamino-glycan content was quantified using the 1,9-dimethylmetheleneblue assay. Gene expression was evaluated using RT-PCR and quantitative real-time PCR methods.

Results and Summary: Human bone marrow derived MSCs, when cultured in monolayer, initially assume a fibroblast-like appearance and then grow into tight colonies. Upon expansion and subsequent seeding onto the collagen coated filter, the MSCs proliferate and form a homogenous multi-layered tissue. At two weeks of culture, the tissue graft showed the appearance of chondrocytes with the formation of extracellular matrix at the top layer of the graft (Fig. 2, A-C). By 4 weeks, all the cells in the graft appear to have fully differentiated from a fibroblastic morphology into a chondrogenic pathway, with round-shaped hyaline-like chondrocytes embedded in an extensive extracellular matrix (Fig. 2, D-F) as indicated by Toluidine blue and immunoreactivity for type II Collagen. Preliminary molecular analysis indicates expression of aggrecan and collagen type II in MSC grafts is comparable to that in HAC grafts (Fig 1). We have also quantified the expression of collagens type I, II, and X, aggrecan, versican, osteocalcin and osteopontin, for molecular evidence of differentiation. This data suggests that our in vitro culture conditions can reproducibly trigger the differentiation of human bone marrow derived MSCs into a chondrogenic pathway.