BONE MARROW STROMAL CELL DIFFERENTIATION ON POROUS POLYMER SCAFFOLDS

* Martin, I., **Shastri, V., Padera, R.F., **Langer, R., Vunjak-Novakovic, G, +Freed, L.E. * Division of Health Sciences and Technology, Massachusetts Institute of Technology, E25-342, 77 Massachusetts Avenue, Cambridge, MA, 02139. Phone: (617) 253-3413, Fax: (617) 258-8827, E-mail: imartin@mit.edu. +Phone (617) 253 3858. E-mail: lfreed@mit.edu

Introduction: Bone marrow stroma contains a heterogeneous population of progenitor cells for bone, cartilage, adipose, and fibrous tissues. Bone marrow stromal cells (BMSC) can be easily harvested and quickly expanded in vitro, which makes them good candidates for the development of autologous tissue equivalents. In this work, we studied the in vitro differentiation of mammalian BMSC on two types of three-dimensional (3D), highly porous polymer scaffolds, with the goal of engineering cartilaginous and bone-like tissues of predefined size and shape.

Methods: Bovine BMSC were obtained from the femorae of 2-3 week old calves and expanded in monolayers for approximately 3 weeks (2 passages) in medium supplemented with 1 ng/ml fibroblast growth factor-2 and 10 nM dexamethasone (dex). Expanded cells were seeded on two types of polymer scaffolds (5 mm diameter, 2 mm thick polymer discs): (a) fibrous, nonwoven meshes made of poly(glycolic acid) (PGA, Fig. 1a) [1] or (b) sponges made of an 80:20 blend of poly(lactic-co-glycolic acid) (128,000 MW) and poly(ethylene glycol) (10,000 MW) (PLGA/PEG, Fig. 1b) [4]. In both groups, seeding was done in well mixed flasks using 2.5 million cells per scaffold. The resulting cell-polymer constructs were cultured for up to 4 weeks in mixed Petri dishes. Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (FBS) and 50 µg/ml ascorbate was either used as control medium or supplemented with different combinations of 10 or 100 nM dex, 5 µg/ml insulin, 10 ng/ml transforming growth factor beta (TGFb1) and 7 mM betaglycerophosphate (bGP). Samples were assessed histologically (safranin-O/von Kossa), immunocytochemically (alkaline phosphatase, collagen types I and II) and biochemically (DNA, glycosaminoglycans (GAG)). Results are presented as average ± standard deviation. Statistical significance ($p < 0.02$) was assessed by ANOVA in conjunction with Tukey's studentized range test.

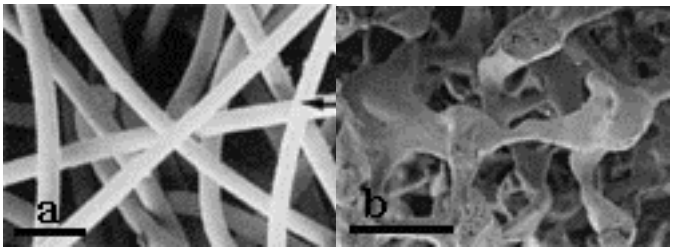

Fig. 1. Scanning electron micrographs of (a) PGA and (b) PLGA/PEG scaffolds. Bars = (a) 20 µm and (b) 300 µm.

Results: BMSC constructs based on PGA scaffolds first contracted and then collapsed during in vitro cultivation (Fig. 2a), in contrast to those based on PLGA/PEG sponges, which maintained their original dimensions (Fig. 2b). After 4 weeks of culture in control medium, the number of cells on the PGA scaffolds was only 2.64 ± 0.15 million, indicating that cell proliferation was negligible, while it increased to 4.55 ± 0.29 million on the PLGA/PEG sponges.

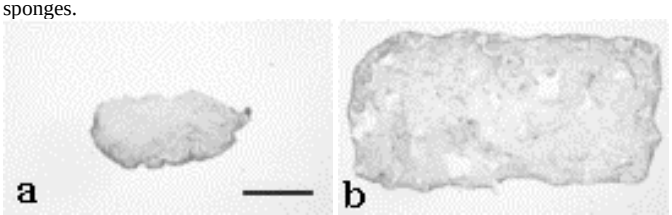

Fig. 2. Histological cross-sections of (a) BMSC-PGA and (b) BMSC-PLGA/PEG constructs cultured for 4 weeks in control medium, which were initially comparable in size. Bar = 1 mm.

BMSC-PLGA/PEG constructs cultured for 4 weeks in control medium resembled loose fibrous tissue, consisting mostly of collagen type I and with negligible GAG content (0.30 ± 0.07 % of wet weight, ww). Supplementation of control medium with dex (100 nM) and insulin did not increase the GAG content (0.25 ± 0.01 %ww). However, further supplementation with TFGb1 induced chondrogenic differentiation of BMSC, as assessed biochemically by a significant increase in GAG (1.31 ± 0.34 %ww) and histologically by positive staining for GAG (Fig. 3a) and collagen type II.

Supplementation of control medium with dex (10 nM) and bGP induced osteogenic differentiation of BMSC, as assessed by expression of alkaline phosphatase and collagen type I, and by the presence of abundant mineralized extracellular matrix (ECM) (Fig. 3b). Foci of mineralization were found throughout the construct cross-section, although their density appeared higher within 0.5 mm from the external surface.

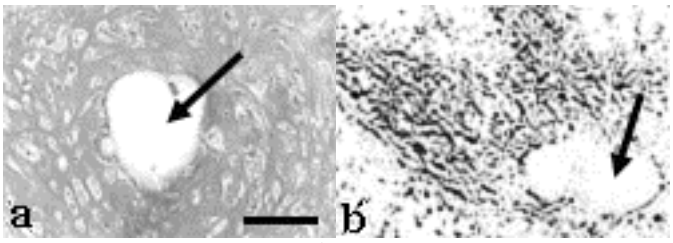

Fig. 3. Histological cross-sections of BMSC-PLGA/PEG constructs, cultured in medium containing (a) chondrogenic or (b) osteogenic supplements. Both sections were stained with Safranin-O/von Kossa. In (b), dark spots indicate calcified ECM deposits. Arrows indicate undegraded polymer. Bar = 50 µm.

Discussion: Our results demonstrate that differentiation of mammalian BMSC into 3D cartilaginous or bone-like tissues requires both a structurally stable porous scaffold and specific medium supplements. In particular, the structure of the PLGA/PEG sponges, which consisted of a single, continuous polymer phase, appeared to prevent BMSC from becoming too densely packed, while allowing cell-to-cell contacts, important in the initiation of mesenchymal cell differentiation. The nonwoven PGA mesh, which was previously used to engineer cartilaginous constructs starting from freshly harvested chondrocytes [1] and cartilaginous and bone-like tissue starting from chick embryo BMSC [3], were not found to support mammalian BMSC growth and differentiation. This implies that the choice of a suitable biomaterial in tissue engineering may depend not only on the specific tissue to be regenerated, but also on the cell source.

Culture medium compositions that induce chondrogenic differentiation of mammalian BMSC in 3D cultures have been previously reported for serum-free systems [2]. Here we show that even in the presence of serum, TGFb plays an essential role. This significantly distinguishes mammalian from avian BMSC, which can differentiate in vitro into chondrocytes in medium supplemented only with 10% FBS and ascorbate [3]. To the best of our knowledge, this study represents the first successful use of mammalian BMSC to engineer in vitro large three-dimensional cartilaginous tissues that retain their initial size and shape.

Osteogenic differentiation of BMSC at the periphery of 3D scaffolds has been previously reported by several research groups. Here we show that PLGA/PEG polymer sponges, in conjunction with appropriate biochemical factors, may support extracellular matrix mineralization not only at the construct periphery, but also in the inner tissue phase. In vivo studies are currently underway to explore the potential of the engineered cartilaginous and bone-like tissues to repair skeletal defects.

Acknowledgments: Funding of this work was provided by N.A.S.A. (Grant NAG9-836) and by N.S.F. (Grant 9525913). We thank A.J. MacKay and J. Milotis for their technical help.

References: [1] LE Freed, G Vunjak-Novakovic, RJ Biron, DB Eagles, DC Lesnoy, SK Barlow, R Langer. Biodegradable polymer scaffolds for tissue engineering. Bio/Technology 12:689-693, 1994. [2] B Johnstone, TM Hering, AI Caplan, VM Goldberg, JU Yoo. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238:265-272, 1988. [3] I Martin, RF Padera, G Vunjak-Novakovic, LE Freed. In vitro differentiation of chick embryo bone marrow stromal cells into cartilaginous and bone-like tissues. J Orthop Res 16:181-189, 1988. [4] V Shastri, I Martin, R Langer. A versatile approach to produce 3-D polymeric cellular solids. 4th US-Japan Symposium on Drug Delivery Systems, 1:36, 1997.

** Dept of Chemical Engineering, Massachusetts Institute of Technology

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.