CHARACTERIZATION OF CHONDROGENESIS IN A PERFUSION BIOREACTOR: EFFECTS OF MEDIA PH AND FLUID FLOW ON MATRIX ASSEMBLY

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INTRODUCTION Demand for autologous cartilage for reconstructive surgery and structural repair necessitated by injury and disease such as osteoarthritis is very high. Due to the low yield of cartilage from primary patient harvests, primary explant cartilage must be augmented by tissue engineering techniques to meet this demand [1]. The use of polylactide acid (PGA) and poly-l-lactic acid (PLLA) as scaffold materials to localize cell and promote focal matrix accumulation has shown much promise [2]. Based on previous studies that demonstrated chondrocyte metabolism is dependent upon interstitial pH, concerns have been raised on the use of biodegradable polymers that produce acidic fragments [3]. The growth of chondrocytes on PGA matrices has been augmented by the use of flow bioreactors, which increase transport of nutrients to and waste away from cells [1]. A flow rate of 1 µm/s directed through the culturing tissue has been engineered to optimize the maintenance of this ideal pH, 6.9 to 7.2, which has been suggested to stimulate cartilage matrix biosynthesis [4]. The objectives of this study were to 1) characterize ECM assembly by chondrocytes in PLLA/PGA scaffolds in static culture and in a perfusion bioreactor and 2) to assess the relative roles of cell metabolism and polymer degradation in regulating environmental pH in both static and bioreactor culture.

METHODS AND MATERIALS Circular disc scaffolds of non-woven PGA mesh of 15 µm diameter were cut to a 12.7 mm diameter and a 100 µm thickness and were coated with PLLA by solvent evaporation [5]. Chondrocytes were isolated and purified from freshly slaughtered bovine calf thickness and were coated with PLLA by solvent evaporation [5]. The bioreactor media gradually decreased in pH from 7.4 to 6.96 over a two week time period (Fig. 1). In contrast, rapid, successive decreases in pH from 7.4 to 6.58 of seeded static cultures were observed over three-day intervals. Unseeded PLLA/PGA matrices in static culture showed successive moderate decreases in pH from 7.4 to 7.11 in periods between media changes. Histological examination of safranin-O/fast green stained samples by light microscopy revealed columns of chondrocytes and ECM aligned in the direction of media flow in the bioreactor (Fig. 2a). No orientation of cell growth and ECM biosynthesis was evident in the static culture samples (Fig. 2b). Analysis of static and bioreactor culture samples showed a 118% increase (p<0.05) in DNA content in the bioreactor compared to static culture (Fig. 3). At four weeks, GAG concentrations of bioreactor culture samples were 184% higher (p<0.05) than in static culture (Fig. 4) confirmed by the increased presence of proteoglycan in the safranin-O/fast green stained bioreactor samples. By two weeks in bioreactor culture, a 155% (p<0.05) increase in hydroxyproline content was observed in bioreactor samples compared to static controls (Fig. 5), and four week analysis revealed a 130% (p<0.05) increase.

DISCUSSION This study focused on the comparison of cell proliferation and ECM biosynthesis in static and bioreactor culture systems and, in particular on the role of media pH on these processes. Comparison of media pH in seeded and unseeded static controls demonstrated a sharper decrease in the pH of the seeded constructs. These findings suggest that cell metabolism, not scaffold degradation, is the primary regulator of media pH in this system. This is of great importance given that more acidic environments are known to inhibit cartilage matrix assembly [3]. The dynamic bioreactor system was more effective at maintaining stable pH even compared to static controls of the same volume (data not shown), which likely contributed to increased proliferation and matrix synthesis. Additionally, the axial flow through the sample induced in the bioreactor produced focal areas of columnar cell orientation and matrix assembly. Given the known stimulatory effects of fluid velocities of this magnitude (~1 µm/s) [4], it is likely that fluid flow, as well as pH regulation, contribute to the higher rates of matrix assembly observed in samples cultured in the bioreactor.


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