CHONDROCYTE AND OSTEOBLAST INTERACTION ON A DEGRADABLE POLYMER CERAMIC SCAFFOLD

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INTRODUCTION

Osteoarthritis is the predominant form of arthritis, with 21 million Americans suffering from this degenerative condition[1]. A recent area of research in the treatment of osteoarthritis is the formation of osteochondral grafts[2,3] and the design of functional interfaces facilitating bone and cartilage integration. Recreation of the osteochondral interface is believed to be critical in initial graft integration and in ensuring long term functionality. In addition to formulating *in vitro* co-culture models of osteoblasts and chondrocytes[4], we are also developing implantable osteochondral grafts by co-culturing osteoblasts and chondrocytes on degradable polymer-ceramic scaffolds. The objective of this study is to examine the interaction of these two cell types on a composite scaffold of polylactide-co-glycolide and bioactive glass (PLAGA-BG). The PLAGA-BG scaffold system (Fig. 1a) is biodegradable and osteointegrative, and has been shown to support the growth and phenotypic expression of osteoblasts *in vitro*[5].

Since the interaction between osteoblasts and chondrocytes as well as the mechanisms of these interactions are not well understood, this study will focus on examining the viability, attachment morphology, and growth of osteoblasts and chondrocytes during co-culture on the 3-D scaffold system (Figs. 1b, 1c). We hypothesize that the presence of osteoblasts or the extracellular matrix produced by these cells on the composite scaffold will have an effect on the subsequent attachment and growth of chondrocytes.

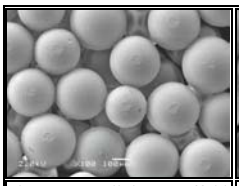

Fig. 1a Acellular Scaffold, x100

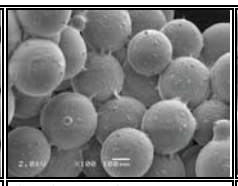

Fig. 1b Co-Culture, x100, Day 1

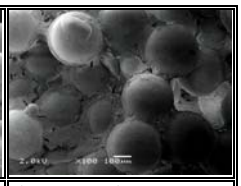

Fig. 1c Co-Culture, x100, Day 7

MATERIALS AND METHODS

Synthesis of Polymer-Ceramic Composite - PLAGA-BG composites were fabricated in both disc and microsphere forms. Composite discs were formed via a standard solvent-casting technique, while the microspheres were synthesized by modifying a method used by Laurencin *et al*[6]. Briefly, PLAGA 50:50 granules were dissolved in methylene chloride, after which BG granules were added to achieve a 25% mixture. The mixture was then poured into a 1% polyvinyl alcohol solution. The suspension was stirred and the spheres allowed to harden. The 3-D scaffold construct (7.5x18.5 mm) was fabricated by sintering the microspheres at 70°C for 20 hours.

Cells and Cell Culture - Bovine articular chondrocytes were isolated from the carpometacarpal joints of calves (2-6 months) following enzymatic digestions[7]. Primary bovine osteoblast cultures were established from cortical bone fragments taken from the same calves. All cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acid (NEAA) and 1% antibiotics (Mediatech), and maintained in a 5% CO2 incubator at 37°C.

Cell Viability and Osteoblast-Chondrocyte Co-Culture - Cell viability was assessed on PLAGA-BG thin films, where the substrates were seeded with $5.0x10^5$ chondrocytes/disc, and the discs were cultured in supplemented DMEM for 4 days. For co-culture, the scaffolds were first pre-seeded with $2.5x10^5$ osteoblasts and cultured in supplemented DMEM for 2 days. Next, chondrocytes ($4.0x10^5$) were seeded onto the osteoblasts pre-cultured scaffolds. Scaffolds seeded with only osteoblasts or chondrocytes at the same densities served as controls. Both short-term and long-term co-culture experiments were conducted. Scaffolds were analyzed at 0.5, 3 and 8 hours, as well as at 1, 3, and 7 days.

Characterization of Co-Cultured Composite Substrates Scanning electron microscopy (SEM) was used to document cell attachment and growth during co-culture and on control scaffolds. At designated time points, the scaffolds were fixed in Karnovsky's fixative, and serially dehydrated in ethanol. Samples were pre-coated

and analyzed using JEOL-5600LV and LEO 1455 SEM systems. Cell viability was assessed using the Live/Dead assay (Molecular Probes), and imaged using confocal microscopy (Olympus).

RESULTS AND DISCUSSION

The objective of this study is to examine the interaction of osteoblasts and chondrocytes on a 3-D composite scaffold. Extensive SEM analysis revealed that significant interactions occurred between osteoblasts and chondrocytes during co-culture.

Although the PLAGA-BG scaffold has been shown to supported the growth of osteoblasts[5], it was uncertain whether it could also support the growth of chondrocytes. To promote osteointegration, Ca-P nodules were designed to form on the scaffold under physiological conditions. It was a potential concern that these nodules may affect the growth of chondrocytes. Positive live stain demonstrated that the scaffold supported chondrocyte viability and growth in culture (Figure 2).

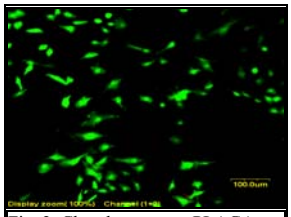

Fig. 2 Chondrocytes on PLAGA-BG disc, x20, 2 days, live (bright).

Cellular Attachments - Differences in cellular attachment were observed between the chondrocyte control scaffolds and the co-cultured scaffolds. On the co-cultured scaffolds, focal adhesions were evident between the spherical chondrocytes and the surface, indicated by the arrow in Figure 3b. No comparable focal adhesions were observed on the chondrocyte controls at the same time point. It is likely that during co-culture, chondrocytes are actively attaching to the extracellular matrix (ECM) pre-formed by the osteoblasts. The chondrocytes may also only form the focal adhesions in the presence of osteoblasts.

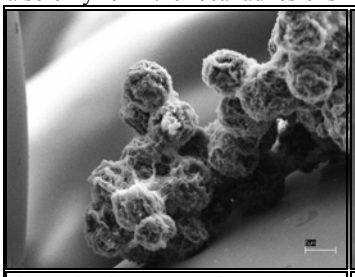

Fig. 3a Chondrocyte Control, x2000, 30 Min.

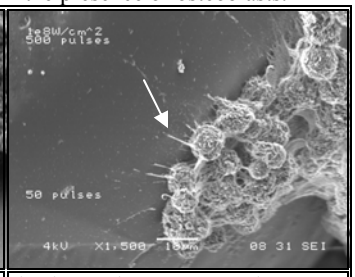

Fig. 3b Co-culture, x1500, 30 Min.

Cellular Morphology - It was observed that chondrocyte morphology changed between 8 hours and 1 day of culturing. At the 8 hour time point (Fig 3a), the chondrocytes assumed a spherical morphology as opposed to the 1 day time point, where the chondrocytes appear to have flattened along the surface of the microspheres. This can be seen in Figure 4b, where the nodules on the surface of the microspheres correspond to the flattened chondrocytes. These nodules are most likely chondrocytes instead of calcium phosphate nodules, since calcium phosphates nodules are approximately 1-5 μm in diameter and these nodules are 5-10 μm [8].

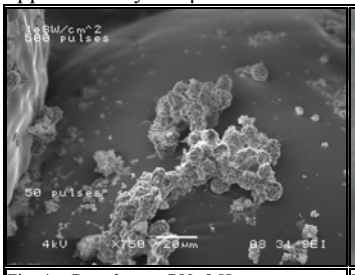

Fig. 4a Co-culture, x750, 8 Hours

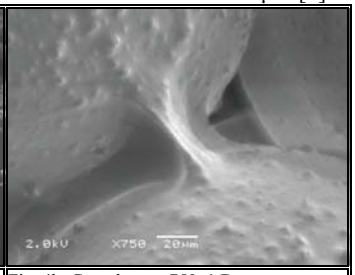

Fig. 4b Co-culture, x750, 1 Day

Chondrocyte-Osteoblast Interaction - Differences in cell growth morphology are evident between the chondrocyte control and the co-cultured scaffolds after 1 day of culture. In Figure 5b, semi-spherical chondrocytes can be seen on the surface of the microspheres. On the chondrocyte control (Fig. 5a) however, the chondrocytes have lost their phenotypic morphology and have conformed to the microsphere surface. One explanation for the observed differences in morphology, is that direct interactions with osteoblasts or their ECM delayed the spreading of the chondrocytes, and may in turn help to maintain the chondrogenic phenotype.

These interactions may have also enhanced the proliferative capabilities of both chondrocytes and osteoblasts. It was observed that after 7 days of culture, more extensive growth was observed on the co-cultured scaffolds (Fig. 1c, 6b) as compared to the chondrocyte (Fig. 6a) or osteoblast controls.

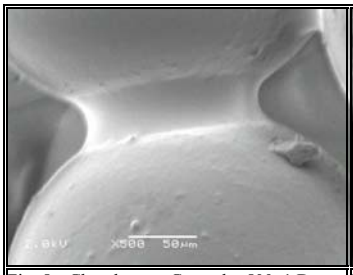

Fig. 5a Chondrocyte Control, x500, 1 Day

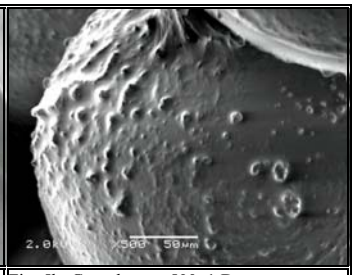

Fig. 5b Co-culture, x500, 1 Day

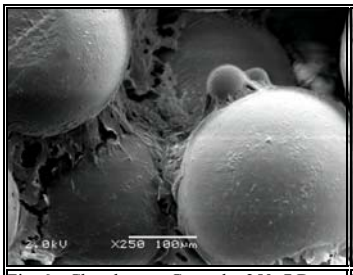

Fig. 6a Chondrocyte Control, x250, 7 Days

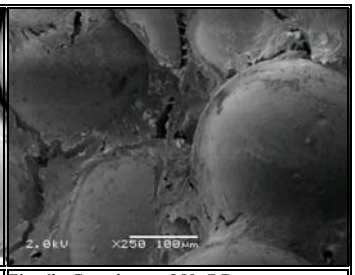

Fig. 6b Co-culture, x250, 7 Days

CONCLUSIONS

The results of this investigation demonstrate the significant adaptation of chondrocyte cellular behavior when co-cultured with osteoblasts on PLAGA-BG scaffolds. It is possible that direct interactions with osteoblasts or their ECM delayed the spreading of the chondrocytes, and may in turn help to maintain the chondrogenic phenotype. These results provide some of the first reported examinations of osteoblast and chondrocyte interactions *in vitro*. Phenomenological level observations gained from this study have facilitated the formation of new hypotheses aimed at achieving fundamental understanding of the interactions between osteoblasts and chondrocytes. Future studies will focus on determination of the precise surface chemistry and modifications effected by the osteoblasts, which resulted in the observed chondrocyte attachment and growth patterns. This novel information will be utilized to further engineer osteochondral grafts for the treatment of osteoarthritis.

REFERENCES

1)CDC statistics, 2001. 2) Gao et al, Tissue Eng., 7, 363-71, 2001. 3) Schaefer et al, Biomat., 21, 2599-2606, 2000. 4) Jiang et al, ORS Annual Meeting 2003 (accepted). 5) Lu, H.H. et al, J. Biomed Mater. Res., In Press. 6) Laurencin et al, J. Biomat. Sci. (Poly. Ed.), p.661, 1996. 7) Mauck et al, J.Biomech., 122, 252-60, 2000. 8) Hench et al, J.Amer.Cerm.Soc., 74(7), p.1487, 1991.