In Vivo Evaluation of Heterotypic Cellular Interactions on a Tri-Phasic Scaffold for Soft Tissue-to-Bone Integration

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Statement of Purpose: The anterior cruciate ligament (ACL) is the most commonly injured knee ligament. Soft tissue-based reconstruction grafts are limited by donor site morbidity and the lack of a functional interface with bone tissue[1]. The natural ACL-bone interface consists of three regions: ligament, fibrocartilage interface, and bone[2-4]. The degree of graft integration is a critical factor governing its clinical success, and interface regeneration will improve long term graft outcome. Our interface tissue engineering effort has focused on biomimetic scaffold design and the optimization of interaction between relevant cell types through co-culture.

This study describes the *in vivo* evaluation of heterotypic cell-cell interactions on a triphasic scaffold with controlled heterogeneity mimicking that of the native ligament-bone interface. This scaffold (Fig 1) consists of three distinct yet continuous phases: Phase A for soft tissue, Phase C for bone, and Phase B for interface development. Each phase of the tri-phasic scaffold was designed with specific composition and geometry suitable for the tissue type to be regenerated. Fibroblasts, chondrocytes, and osteoblasts were seeded on Phases A, B, and C, respectively. The effects of *in vivo* culture on tissue ingrowth, phase-specific matrix production and scaffold mechanical properties were determined.

Methods: *Scaffold Fabrication*- Phases A, B, and C consist of poly(lactide-*co*-glycolide) (PLGA, 10:90) knitted mesh, PLGA (85:15) microspheres, and PLGA(85:15)/ Bioactive Glass (45S5, BG) composite microspheres, respectively. The microspheres were formed via an emulsion method[5], and continuous triphasic scaffolds were formed by sintering above the T_g[6].

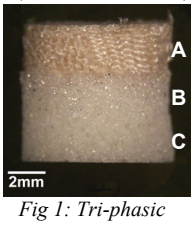

Fig 1: Tri-phasic scaffold

Cell-Cell Interactions on Scaffolds- Bovine osteoblasts (OB) and ACL fibroblasts (FB) were obtained by explant culture, and chondrocytes (CH) were digested from articular cartilage. FB ($5x10^5$ cells/scaffold) and OB ($2.5x10^5$ cells/scaffold) were seeded on Phases A and C, while CH ($5x10^5$ cells/scaffold) were loaded into Phase B in 0.5% agarose. FB+CH+OB (tri-culture), FB+OB (co-culture), and acellular groups were investigated.

In Vivo Implantation and Evaluation- Following 3 days of *in vitro* culture, scaffolds were implanted into subcutaneous pouches in the dorsa of athymic rats (NIH-rnu). At 2, 4, and 8 weeks, the animals were sacrificed and tissue infiltration and phase-specific matrix production were assessed by histological and immunohistochemical analysis. Scaffold mechanical properties were determined under uniaxial compression, cell proliferation was measured by fluorometric DNA quantitation, and phase-specific mineralization was evaluated by both microCT and von Kossa staining.

Results: *Tissue Ingrowth and Matrix Distribution*- An extensive collagen-rich matrix was prevalent in the three phases of the seeded scaffolds and was considerably more abundant than in the acellular group at weeks 4 and 8 (Fig 2). Total cell number increased in all groups tested up to week 4, with the highest cell number measured in the tri-culture group. In addition, phase-specific matrix distribution was observed over time. Specifically, a fibrocartilage-like matrix rich in types I and II collagen as well as glycosaminoglycans was found between Phases A and B in the tri-culture group at weeks 4 and 8. In addition, a mineral rich matrix was detected by microCT only in Phase C for all experimental groups.

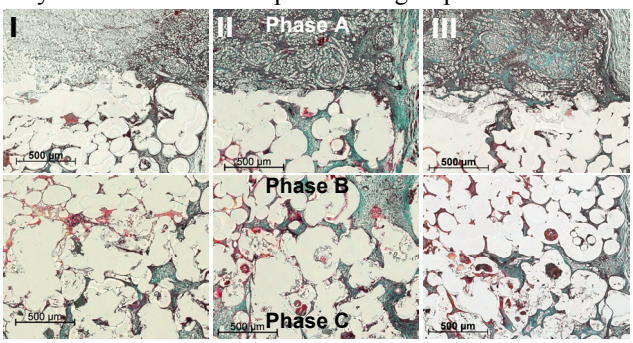

Fig 2: Tissue ingrowth into (I) acellular, (II) co-culture, and (III) tri-culture scaffolds after four weeks in vivo. (Modified Goldner's Masson Trichrome stain, 5x, bar = 500 µm).

Mechanical Properties- With polymer degradation, an initial 40% decrease in compressive modulus was found. This was followed by an increase in elastic modulus for the co-cultured and tri-cultured groups from week 4 to 8, attributed to the extensive tissue infiltration and matrix production seen in the seeded groups (Fig 3).

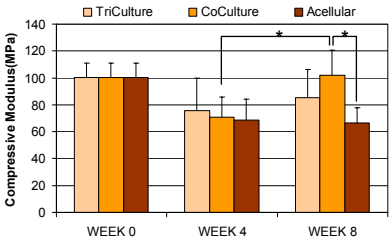

Fig 3: Compressive modulus of explanted triphasic scaffolds.

Discussion: The biomimetic, triphasic scaffolds supported the growth and matrix production of FB, CH, and OB *in vivo*. By 8 weeks, tissue ingrowth was extensive throughout the seeded scaffolds, compensating for hydrolytic polymer degradation. The highest increase in modulus was observed in the tri-culture group, suggesting that the interaction of these cell types is important for structural integrity and functionality. The phase-dependent distribution of mineralized matrix and fibrocartilage-like matrix is indicative of the potential for the development of interface-like tissue as well as multi-tissue formation on the tri-phasic scaffold.

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ACKNOWLEDGMENTS: NIH-NIAMS and Wallace H. Coulter Foundation.