Cell Interaction Distance Modulates Chondrocyte Responses on Co-Cultured Scaffolds

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Introduction: Rotator cuff tears often result from avulsion of the tendon from bone at the insertion site [1], which consists of a fibrocartilaginous transition that subdivides into non-mineralized and mineralized regions. Regeneration of this multi-region interface is essential for functional load transfer from tendon to bone and integrative rotator cuff repair. To this end, biphasic scaffolds mimicking the native interface have been found to support chondrocyte-mediated deposition of contiguous non-calcified and calcified fibrocatilage-like matrices [2]. Given the compositional difference between scaffold phases, it is not known how cells seeded on one phase of the stratified scaffold can influence the response of cell cultured on the adjacent phase, and what are the ramifications of these interactions in multi-tissue formation and homeostasis. **It is hypothesized** that cell interaction distance between phases will play a role in directing cell response on the co-cultured phases of biphasic scaffolds. To test this hypothesis, the **objective of this study** is to compare cell response on single-phased and co-cultured biphasic scaffolds, and evaluating chondrocyte growth and biosynthesis as a function of distance between scaffold phases.

Materials and Methods: ***Scaffold fabrication***: Single phased aligned polylactide-*co*-glycolide (PLGA 85:15, Lakeshore Biomaterials, AL) nanofibers with HA (15% w/w, PLGA-HA) or without HA (PLGA) were fabricated by electrospinning[3]. The biphasic scaffold was produced by direct electrospinning of PLGA-HA on top of PLGA. ***Study Design:*** Groups include single culture of chondrocytes on PLGA or PLGA-HA nanofibers, co-cultured on the segregated PLGA and PLGA-HA single phases separated by 1 mm thick spacer (co-culture 1) and co-cultured on separate phases of biphasic PLGA/PLGA-HA scaffold(co-culture 2) . Thus the distance between cells seeded on different phases (PLGA vs. PLGA-HA) is: single phase (infinite) >co-culture 1 (1mm) >co-culture 2 (0mm). Bovine chondrocytes obtained by enzymatic digestion were seeded on the scaffolds ($6x10^4$ cell/cm^2, starting cell number same for all groups at day 0), and cultured for 1, 14 and 28 days. Cell viability (n=2, Live/Dead), cell number (n=5, Picogreen), ALP activity (n=5), collagen and glycosaminoglycan (GAG, n=5) production and gene expression (col I, II, III and aggrecan, n=3) were determined over time. ***Statistical analysis:*** One-way (gene expression) and two-way ANOVA (cell proliferation and matrix production) and the Tukey-Kramer *post-hoc* test (*p<0.05).

Results: Cells attached and proliferated on all scaffolds over time, with no significant difference observed between groups. In contrast, ALP activity peaked in the PLGA-HA single-cultured group at day 28, while remaining at basal levels for all other groups over time (Figure 1). By day 28, both collagen and GAG were significantly lower in the co-culture 1 group, with collagen deposition being the highest in the PLGA-HA single cultured group (Figure 2). At day 28, aggrecan expression is upregulated in the co-culture 2 group when compared to the PLGA only group (Figure 4).

Discussion: In this study, the separation distance between scaffold phases decreased from infinity (single-phased PLGA or PLGA-HA) to a well-defined distance (1 mm in co-culture 1), and to none (co-culture 2). The results described above suggest that by increasing the distance between scaffold phases, or in other words, the interaction distance between cells cultured on PLGA or PLGA-HA, cell mineralization potential, matrix deposition and gene expression profile of chondrocytes can be modulated. It was found that chondrocytes cultured on PLGA suppressed the ALP activity of chondrocytes cultured on PLGA-HA, as seen in both co-cultured groups. Moreover, compared to single-cultured controls, both GAG and collagen syntheses were suppressed in co-culture 1 group, while this effect was absent in the co-culture 2 group. It is possible that the paracrine suppression of biosynthesis experienced in segregated co-culture is rescued by enhanced cell-cell contact on the biphasic scaffold used in the co-culture 2 group. These findings yield new insights into the mechanism of cellular interaction on complex scaffolds and future studies will explore the effects of these interactions for interface regeneration and integrative rotator cuff repair.

References: 1. Iannotti, J.Am.Acad.Orthop.Surg., 1994; 2. Moffat et al. 57th ORS Annual Meeting, 2011; 3. Moffat et al., Tissue Eng. 2009.
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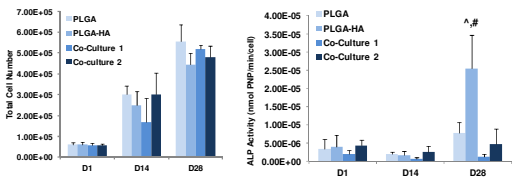

Figure 1. Cell proliferation(left) and ALP activity of chondrocytes on different groups over the period of culturing time *(^: significant different over time,# significant different over other groups at the same time point)*. Results show that the presence of PLGA phase suppressed ALP peak at day 28

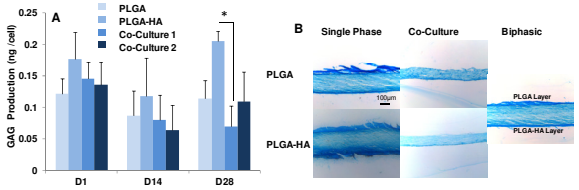

Figure 2. GAG production (A) and alcian blue stain (B) . Results show that GAG production is suppressed in the co-culture 1 group compared to the PLGA-HA group. Scale bar=100 μm

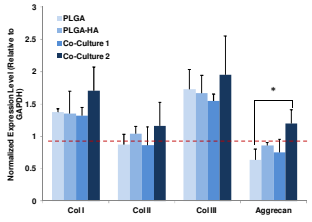

Figure 3.
Gene expression at day 28. Results show that aggrecan is upregulated in the co-culture 2 group compared to PLGA group