Effects of Low Oxygen Tension during Expansion on Chondrogenic Potential of Osteoarthritis Chondrocytes

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Statement of Purpose: Primary chondrocytes are a frequently studied source of cells for cartilage tissue engineering approaches, both biomaterial-scaffold-based and scaffold free. Before seeding cells into a scaffold, chondrocytes are often monolayer expanded *in vitro*. During monolayer expansion, chondrocytes tend to dedifferentiate and lose their characteristic gene and protein expression profiles [1]. This change in phenotype can lead to poor biochemical and biomechanical properties of subsequent engineered cartilage. Low O_2 tension *in vitro* culture is one approach to minimize dedifferentiation and optimize chondrogenic potential. The rationale for this approach is that low O_2 tension is present during chondrogenesis and in adult articular cartilage. In addition, oxygen concentration gradients in chondrocyte-seeded scaffolds contribute to cell and tissue heterogeneity [2, 3]. Therefore, low O_2 tension may be a requirement of *in vitro* culture to maintain chondrocyte phenotype. Previous studies have shown that low O_2 *expansion* (1 % to 5 %) [4, 5] of chondrocytes is sufficient to enhance the biochemical quality of the extracellular matrix (ECM) produced post-expansion in both bovine and rabbit models. However, it is not known if low O_2 expansion improves chondrogenesis of human chondrocytes and particularly osteoarthritic (OA) chondrocytes, which are of high clinical relevance. As a step toward determining whether incorporation of low O_2 expansion with scaffold-based cartilage tissue engineering approaches would be beneficial, the objective of this study was to test the hypothesis that low O_2 expansion promotes chondrogenesis of human OA chondrocytes with increased chondrogenic characteristic gene expression and extracellular matrix production.

Methods: Human articular cartilage tissue was harvested from femoral condyles of 4 OA patients (n = 4, age = 51-62) under SUNY Upstate Medical University and Syracuse University institutional review board protocols. Chondrocytes from cartilage tissue slices from visibly unaffected areas of the joint were collected and expanded under 5 % and 21 % of O_2. By the end of passage 2 (P2), monolayer cells under each condition were pelleted for micromass culture for 3 weeks under 21 % O_2 as previously described [5]. To allow quantitative comparison of markers of chondrocyte phenotype, total RNA was extracted from monolayer chondrocytes at the end of P2 culture and from pellets at the end of 3 weeks culture. Realtime-qPCR reactions were then performed. Quantification of GAG and DNA content were performed following modified DMMB method [6] and picoGreen (QIAGEN) manufacture's instruction respectively. To examine cartilage matrix deposition and protein distribution, histological staining of Safranin-O and Toluidine Blue were performed together with immunohistochemical staining of ColI and ColII as previously described [5]. Realtime-qPCR and GAG/DNA results were analyzed by paired t-test.

Results: Realtime-qPCR results of monolayer cells by the end of P2 culture showed higher ACAN and ColII expression in chondrocytes expanded under 5% O_2 than under 21 % O_2 (Figure 1), suggesting that low O_2 tension can promote chondrogenic characteristic gene expression in OA chondrocytes. In contrast, there was no significant difference in GAG content (normalized to DNA) between the 5 % and 21 % O_2 groups (Figure 2).

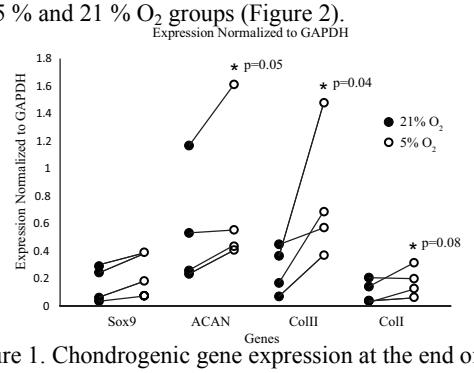

Figure 1. Chondrogenic gene expression at the end of monolayer expansion normalized to GAPDH. Human OA chondrocytes expanded under 5 % O_2 tension showed significantly higher ACAN and ColII expression than expanded under 21 % O_2.

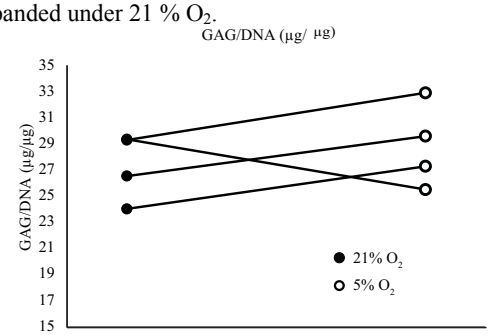

Figure 2. GAG/DNA quantification showed no significant difference between the 5 % and 21 % O_2 groups.

Conclusions: These results suggest that low O_2 condition (5 %) may promote chondrogenic characteristic gene expression in monolayer cells. However, improved ECM production observed in previous animal models may not be achieved when applied to tissue engineering using human OA chondrocytes. Examination of the mechanical properties of the engineered tissue is needed to confirm the effects of low O_2 expansion on human OA chondrocytes and to determine the potential for low O_2 expansion in scaffold-based approaches.

References: 1. Benya PD, Cell. 1982;30: 215-224. 2. Lewis MC, Biotech. and Bioeng. 2005;91:607-615. 3. Malda J, Biotech. and Bioeng. 2004: 86: 9-18. 4. Egli RJ, J Ortho Res. 2008; 26:977-985. 5. Henderson JH, Tissue Engi. 2010;16: 1585-1593. 6. Barbosa I, Glycobiology. 2003;13: 647-653.