Biofabrication of Spatially Defined Cartilage Grafts Using Microtissues Engineered in Different Oxygen Conditions

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INTRODUCTION:

Articular cartilage (AC) has a complex, spatially defined composition and structure which determines its biomechanical functions. Due to its avascular nature, oxygen levels in AC are relatively low (1 to 5% O_2), decreasing from the surface into the deeper regions of the tissue[1]. Cells used in the regeneration of AC have been shown to be highly sensitive to changes in oxygen tension, with a number of studies demonstrating the role of low oxygen culture conditions in promoting a chondrogenic phenotype[2,3]. Mesenchymal stem/stromal cells (MSCs) are commonly used in the engineering of AC, however such tissues have an inherent tendency to undergo endochondral ossification and form bone, potentially limiting their use in the regeneration of synovial joints[4]. Oxygen availability has been shown to be key regulator of chondrogenesis and endochondral ossification, with hypoxic conditions known to support the engineering of phenotypically stable AC using MSCs[5]. In this study, we first sought to engineer cartilage microtissues (µT) with different phenotypes (AC-like or endochondral-like) using human bone marrow derived MSCs exposed to different oxygen levels. We then sought to use such phenotypically distinct µTs as building blocks to biofabricate larger cartilaginous grafts with layered composition and organization.

METHODS:

Human MSC-derived µTs from three different donors were fabricated as previously described[6]. The µTs were kept under two different oxygen tensions: 2% O_2 and 5% O_2, and matured in presence of TGFβ3 for 2, 7 or 21 days to assess their phenotype. Statistical differences were analyzed by two-way ANOVA. In a second experiment, after 2 or 7 days of priming in altered oxygen conditions, µTs were placed in a melt electro-written (MEW) mesh (230×230 µm and approximately 60 µm height) to guide µT fusion. The MEW mesh was embedded in agarose to enable handling and coated with fibronectin to support cellular adhesion. The engineered grafts were then cultured for a total of 21 days in chondrogenic culture conditions at 5% O_2.

RESULTS:

After 21 days, chondrogenic differentiation of µT at 2% O_2 resulted in an increase in glycosaminoglycans (GAGs) deposition compared to 5% O_2 for the three donors tested. Histological analysis confirmed this result, with more intense GAG staining observed in the 2% O_2 group. In addition, negative staining for calcium deposit and type X collagen was observed at 2% O_2 while positive staining for these markers of cartilage hypertrophy was observed at 5% O_2 (Figure 1). In a second experiment, MEW meshes were successfully coated with fibronectin without altering their printed dimensions. The MEW mesh was able to collect µT and allowed them to fuse with neighboring µT, enabling the engineering of larger cartilaginous grafts of defined geometry.

DISCUSSION:

Here, we demonstrate that (i) oxygen tension can be used to engineer MSC-derived µTs with hyaline-like or endochondral phenotypes and (ii) MEW meshes can guide fusion of µT building blocks. No signs of hypertrophy were observed in µTs differentiated at 2% O_2, however longer-term *in vivo* studies are required to confirm if the resultant grafts are truly resistant to endochondral ossification. In addition, the quality of µT fusion decreases the longer they are cultured in isolation prior to fusion. We fused µTs after 7 days of culture in altered oxygen conditions to maximize the priming time whilst still enabling robust fusion. Further profiling of chondrogenic (*SOX9, type II collagen*) and hypertrophic (*RUNX2, type X collagen*) genes will be used to confirm the specificity of these different oxygen priming regimes.

SIGNIFICANCE/CLINICAL RELEVANCE:

Our study consolidates previous work by defining optimal conditions for the engineering of phenotypically distinct cartilage µT using human MSCs. In addition, the integration of a low volume MEW mesh and µTs with distinct phenotypes could allow the biofabrication of highly cellular grafts with user-defined geometry and composition while minimizing the presence of foreign material. Such approaches may lead to better graft integration to the defect site as well as superior engineered graft function.

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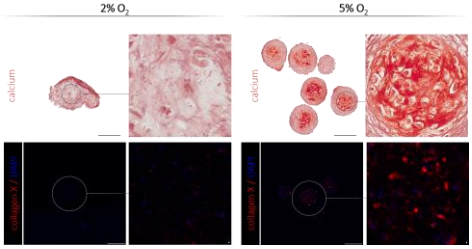

Figure 1: µT differentiated at 2% O_2 show negative staining for calcium deposit and type X collagen. Scale bar = 200 µm