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Tendon and ligament as novel cell sources for engineering the knee meniscus

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SUMMARY

Objective: The application of cell-based therapies in regenerative medicine is hindered by the difficulty of acquiring adequate numbers of competent cells. For the knee meniscus in particular, this may be solved by harvesting tissue from neighboring tendons and ligaments. In this study, we have investigated the potential of cells from tendon and ligament, as compared to meniscus cells, to engineer scaffold-free self-assembling fibrocartilage.

Method: Self-assembling meniscus-shaped constructs engineered from a co-culture of articular chondrocytes and either meniscus, tendon, or ligament cells were cultured for 4 weeks with TGF-β1 in serum-free media. After culture, constructs were assessed for their mechanical properties, histological staining, gross appearance, and biochemical composition including cross-link content. Correlations were performed to evaluate relationships between biochemical content and mechanical properties.

Results: In terms of mechanical properties as well as biochemical content, constructs engineered using tenocytes and ligament fibrocytes were found to be equivalent or superior to constructs engineered using meniscus cells. Furthermore, cross-link content was found to be correlated with engineered tissue tensile properties.

Conclusion: Tenocytes and ligament fibrocytes represent viable cell sources for engineering meniscus fibrocartilage using the self-assembling process. Due to greater cross-link content, fibrocartilage engineered with tenocytes and ligament fibrocytes may maintain greater tensile properties than fibrocartilage engineered with meniscus cells.

Introduction

Meniscus pathology plays an important role in the development of knee osteoarthritis. Increasing evidence suggests a prominent role for the meniscus in load transmission as well as lubrication and nutrition of articular cartilage[1]. Accordingly, a meniscus tear results in an unequal load distribution within the knee joint and abnormal loading of underlying articular cartilage. Further, meniscus injuries are commonly accompanied with catabolic events that initiate inflammatory processes leading to articular cartilage degeneration[2,3]. Unfortunately, the common treatment of meniscectomy increases contact forces on articular cartilage by up to 350%, and often leads to osteoarthritis[1,4]. Thus, total meniscectomy has been abandoned, and partial meniscectomy is also losing favor compared to meniscus repair and replacement[5–7]. Treatment strategies that restore the functional role of the meniscus within the knee are the most promising approaches toward effective management of a meniscus tear and knee osteoarthritis in the future.

Among the options that ensure restoration of meniscus function, tissue engineering stands out as having high potential despite numerous technical challenges. Engineering a meniscus *de novo* has gained increased interest over the last decade[1,8]. Promising results have been reported with both cellular and acellular approaches, as well as with synthetic and natural scaffolds[9,10]. However, tissue engineering of the meniscus faces many hurdles, such as acquiring large numbers of allogeneic or autologous cells, matching scaffold degradation and tissue formation to one another, the poor mechanical properties of many engineered tissues, and generating mechanical anisotropy that mimics the organization of native tissues[1,11].

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To address these challenges, a scaffold-free tissue engineering approach that uses a co-culture of meniscus cells together with articular chondrocytes has been proposed[12,13]. Termed the self-assembling process[12,14], this technique has demonstrated success in creating scaffold-free constructs that can replicate the geometry and biochemical composition of native tissue[15,16]. In particular, prior work has shown that a 1:1 ratio of articular chondrocytes and meniscus cells produces appropriately shaped meniscus constructs with greater matrix content than constructs engineered with meniscus cells alone[13,15]. Self-assembling meniscus constructs with mechanical properties approaching native tissue values have been reported[17]. Additionally, two different cell types have been used in co-culture toward creating tissue heterogeneity[18]. Yet, in terms of tensile properties, self-assembling tissues have not yet achieved the values that the native meniscus exhibits, which has been reported as having a Young's modulus of 50–150 MPa[1]. Additionally, this technique requires a large number of cells, likely from an autologous source or an allogeneic donor. Additional efforts toward improving the tensile properties and the clinical translatability of self-assembling engineered menisci could represent a key advance in meniscus tissue engineering.

Within musculoskeletal tissues, articular cartilage represents the end of a continuum, demonstrating a hyaline cartilaginous profile rich in glycosaminoglycan (GAG) molecules, while tendon and ligament occupy the other end of this continuum with a more fibrous profile rich in collagen[19]. The meniscus stands at the middle of this continuum, as it combines both hyaline and fibrous characteristics, and thus may be referred to as a fibrocartilaginous tissue. Due to the high collagen content and large number of pyridinoline cross-links (40–60 nmol/g of tissue for tendon and ligament, as compared to 15–30 nmol/g for hyaline cartilage[19]), the tensile properties of tendon and ligament are superior in comparison to other musculoskeletal tissues. The native cells residing in tendon and ligament, known as tenocytes and ligament fibrocytes, respectively, are responsible for maintaining tissue composition, and may potentially be explored in a co-culture model to create tissues with more fibrous biochemical profiles and greater tensile properties. Furthermore, increasing the number of cell types that menisci can be engineered from improves the feasibility of acquiring large numbers of cells and increases clinical translatability.

The objectives of this study were threefold: (1) to evaluate the feasibility of using tenocytes and ligament fibrocytes as compared to the gold standard of meniscus cells in a co-culture model for meniscus tissue engineering, (2) to study whether the incorporation of alternative cell sources in co-culture would result in improvements to the biochemical and biomechanical content of engineered menisci, and (3) to measure pyridinoline cross-links and correlate their presence with the mechanical properties of engineered tissues created from various co-cultures. Meniscus cells, tenocytes, and ligament fibrocytes were harvested from bovine tissues and co-cultured with articular chondrocytes for 4 weeks in self-assembling meniscus-shaped tissues. After culture, constructs were assessed with a range of assays to determine engineered tissue quality. We hypothesized that tenocytes and ligament fibrocytes would be applicable toward meniscus tissue engineering, that tenocytes and ligament fibrocytes would improve the functional properties of engineered tissue, and that pyridinoline content in the various co-cultures would be correlated with tensile properties.

Materials and methods

Media formulation

The serum-free chondrogenic media formulation used throughout the study consisted of Dulbecco's Modified Eagle Medium (DMEM) with 25 mM glucose/GlutaMAX™ (Life Technologies, Carlsbad, CA), 1% v/v penicillin/streptomycin/fungizone (Lonza, Basel, Switzerland), 1% v/v insulin/transferrin/selenium (BD Biosciences, San Jose, CA), 1% non-essential amino acids (Life Technologies, Carlsbad, CA), 100 μg/mL sodium pyruvate (Thermo Fischer Scientific, Waltham, MA), 50 μg/mL ascorbate-2-phosphate (Sigma, St. Louis, MO), 40 μg/mL L-proline (Sigma), 100 nM dexamethasone (Sigma), and 10 ng/mL TGF-β1 (Peprotech, Oak Park, CA).

Isolation of cells

Meniscus, tendon, and ligament tissues were harvested from bovine calf knee joints (Research 87). The inner four-fifths of the meniscus from both hind legs were taken to obtain meniscus cells. The midsections of the long digital extensor and semitendinosus tendons were harvested to obtain tenocytes, while the midsections of the posterior cruciate, patellar, and anterior cruciate ligament (ACL) were harvested to obtain ligament fibrocytes. Extreme care was taken to remove sheath, adipose, and synovial tissue from all specimens. Tissues were minced to pieces roughly 2–4 mm^3 in size, washed with phosphate-buffered saline (PBS), and digested with 0.25% w/v pronase at 7 units/mg (Sigma) for 1 h followed by 0.2% w/v collagenase type 2 at 250 units/mg (Worthington Biochemical, Lakewood, NJ) for 18 h at 37°C/5% CO_2. Digestion solutions contained DMEM, 3% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), and 2% penicillin/streptomycin/fungizone. Digest solutions were strained through a 70 μm filter and washed three times. Cells were then counted and cryopreserved in chondrogenic media with 20% fetal bovine serum and 10% dimethyl sulfoxide (Sigma). Cell viability observed by trypan blue was >95%.

Self-assembly of constructs

After thawing, cells were seeded in non-adherent, ring-shaped agarose molds based on the rabbit meniscus. Each mold was saturated with chondrogenic media for 48 h before seeding. Wells were 9.5 mm long and 7 mm wide, and seeded with 5×10^6 articular chondrocytes combined with either 5×10^6 meniscus cells, tenocytes, or ligament fibrocytes within a volume of 180 μL. For comparison, a group of constructs seeded with 10×10^6 articular chondrocytes was cultured in parallel. Constructs were left undisturbed for 4 h before feeding with chondrogenic media, and then fed each day thereafter. At 7 days of culture, constructs were removed from their agarose wells and kept in free-floating culture. Each ring-shaped construct could be cut into two halves to represent the two compartments of the native meniscus. Following 4 weeks of culture, constructs were portioned for histological staining, biochemical assays, and biomechanical testing.

Histology and immunohistochemistry

Tissue samples were cryoembedded in HistoPrep (Thermo Fisher Scientific) and sectioned to 12 μm. After formalin fixation, sections were stained with Safranin-O/Fast Green or Picrosirius Red. To stain collagens type I and II, Vectastain ABC kits (Vector Labs, Burlingame, CA) were used. Sections were incubated with mouse anti-collagen type I antibody diluted 1:1000 (Accurate, Westbury, NY) or rabbit anti-collagen II antibody diluted 1:300 (Cedarlane Labs, Burlington, NC). Bovine articular cartilage, meniscus, and tendon served as staining controls.