

Decellularized Meniscus Scaffolds and Cartilage Progenitor Cells for Total Meniscal Repair

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DISCLOSURES: PMG and RG are inventors on a non-provisional patent application for MEND technology.

INTRODUCTION: The meniscus is divided into two major regions: the red zone and the white zone. The inner, white zone has low healing capacity due to its poor vascularity and high mechanical loading, resulting in poor long-term prognoses for patients with radial meniscal tears. The current standard of care, arthroscopic partial meniscectomy, removes the damaged portion of the meniscus, however, this creates increased contact forces between the tibial and femoral cartilage. Alternative approaches to meniscal repair such as cadaveric allografts, are limited by donor availability, imperfect mechanical matching, and limited repopulation with autologous cells causing a nearly 30% revision rate³. In our previous work, we developed a scaffold for hyaline cartilage repair by decellularizing red zone meniscus and selectively digesting elastin bundles creating a wealth of channels for cell-seeding³. In this effort, we used cartilage progenitor cells (CPCs), a superior cell source due to their rapid proliferation rate and robust and stable differentiation⁴. We validated this approach that combines decellularized meniscus (MEND) scaffolds with CPCs to repair hyaline cartilage in an airway rabbit model. Following the success of this work both *in vitro* and *in vivo*, we hypothesized that MEND seeded with CPCs can produce engineered constructs with similar mechanical, biochemical, morphological, and phenotypic properties to native white zone meniscus thus highlighting the potential of this graft material for repairing the most challenging part of the meniscus.

METHODS: Cell Source Comparison for Cartilage Tissue Engineering: Cartilage tissue was collected from the ears of Yucatan miniature pigs, and treated sequentially with pronase (70U/mL, 30 minutes, 37°C) and collagenase II (300 U/mL, 4 h, 37°C) to extract cells. CPCs were separated from chondrocytes (CCs) via selective fibronectin-adhesion as we previously described⁵. CPCs were cultured to passage 4 in DMEM with 10% FBS, 2% Penicillin-Streptomycin-Fungizone, 5 ng/ml FGF-2, 5 ng/ml ascorbic acid, and 1 ng/ml TGF-β1 (CPC expansion medium). Pellets of CPCs or CCs were formed (3x10⁶ cell/pellet) and differentiated for three weeks in chondrogenic medium (DMEM with 10 µg/ml insulin, 5.5 µg/mL transferrin, 5 ng/mL selenium, 50 µg/mL L-ascorbic acid, 2 mM L-glutamine, 10⁻⁷ M dexamethasone, 10 ng/mL TGF-β3), prior to comparison via histology, immunofluorescence, and RT-qPCR. Alamar blue assay was used to assess metabolic activity of cells at P4 (4000 cells/well in a 48-well plate) at days 1, 4, and 7. Decellularization: Porcine menisci cross sections were devitalized by four freeze/thaw cycles (-20°C to 25°C), followed by treatment in 0.1% pepsin in 0.5 M acetic acid under shaking (150 rpm) at 37°C for 24 hours. Thereafter, samples were washed in PBS treated with 0.3 U/mL elastase in 0.2 M Tris-base (pH 8.6) for 24 hours for microchannel creation via selective elastin removal. After washing in PBS, cylindrical scaffolds of 6mm in diameter were created with a biopsy punch from the posterior-end (red-zone) of decellularized cross-sections that present maximum porosity. Recellularization and Differentiation: Sterile decellularized meniscus (MEND) scaffolds were inserted in a transwell and seeded with 3x10⁶ CPC/scaffold. Then a 1%-20% serum gradient was established across the transwell inserts holding MEND to drive cell invasion for MEND recellularization, with a daily renewal of media. After six days of invasion, seeded MEND underwent differentiation in chondrogenic medium for six weeks with medium renewed twice/week. MEND constructs were analyzed by histology, compression testing, biochemical assays, and RT-qPCR.

Integration: After removing part of the white zone from a live, fresh, meniscal segment to model partial meniscectomy, a 6mm incision was performed from the remaining white zone into the red zone meniscus using a scalpel. Then, acellular or seeded MEND was sutured into this incision with part of the construct protruding to substitute the excised part of the white zone, and cultured in chondrogenic medium for one week, with integration and phenotype assessed via histology.

RESULTS: CPCs exhibited significantly higher proliferation rates compared to CCs (Fig.1A) and immunofluorescence of pellets showed less signal for hypertrophic markers, collagen I and X, in CPCs than CCs whilst collagen II signal was markedly higher for CPCs than CCs (Fig. 1B-G). CPCs were therefore deemed the preferred cell source for cartilage engineering. Following decellularization and enzyme treatment, well-defined microchannels were present with an average diameter of 6.87µm (Fig.2A-C), and DNA was below 50ng/mg dry tissue (Fig.2D). After three weeks of chondrogenic differentiation of CPCs in MEND, the matrix was remodelled creating lacunae which resemble white zone native meniscus (Fig. 3A&B), and the mechanical properties of the CPC-laden MEND constructs matched those of red zone meniscus (Fig. 3C). By six weeks of differentiation collagen and glycosaminoglycan (GAG) secretion matched that of white zone meniscus and exceeded levels in red zone meniscus (Fig. 3D&E).

DISCUSSION: The robust chondrogenic properties and high proliferation rates of CPCs represent immense promise as a new cell source for meniscal tissue engineering. Meniscus decellularization via sequential enzymatic digestion without the use of surfactants is a first-of-its-kind methodology that led to the production of abundant microchannels capable of supporting uniform cell re-seeding without compromising meniscal construct integrity and properties. Following differentiation, CPC-MEND constructs mimicked native meniscus in terms of mechanics, biochemistry, structural and cellular phenotype. *In vitro* integration of native tissue with acellular MEND scaffolds is providing preliminary insight into the regenerative response of these engineered meniscal scaffolds. Future investigations must explore the immunogenicity of MEND *in vivo* and performance under physiological loading.

SIGNIFICANCE: Due to its low vascularity, white zone meniscus has lower healing capacity compared to the red zone which is richer in blood-vessel. CPC-laden MEND scaffolds that create engineered cartilaginous grafts with similar properties to both white and red zones of the native meniscus, therefore, provide a potential versatile graft option for meniscal repair.

REFERENCES: [1] Bilgen *et al.*, *Advanced Healthcare Materials*, 2014[2] Logerstedt *et al.*, *J Orthop Sports Phys Ther*. 2010, [3] Buma *et al.*, *Biomaterials*. 2004, [4] Gehret, *et al.*, *Biorxiv*, 2023 [5] Ali Akbari Ghavimi *et al.*, *Biorxiv*, 2023.

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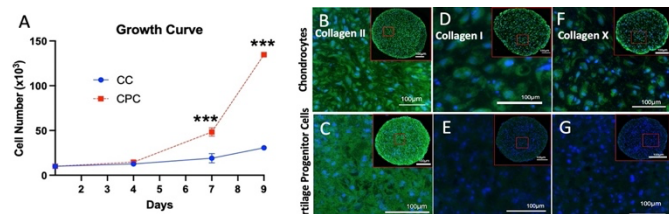


Figure 1: (A) CPCs proliferate faster than CCs. (B) CC collagen II IF presents less deposition than in (C) CPC IF. CCs pellets show more hypertrophy as demonstrated in (D) collagen I and (F) collagen X deposition as compared to (E) collagen I and (G) X in CPCs.

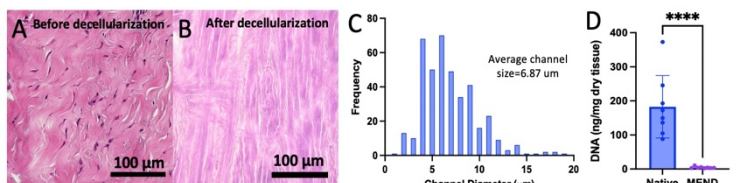


Figure 2: H&E (A) native and (B) decellularized meniscus. MEND (C) channels size distribution and (D) DNA content/weight.

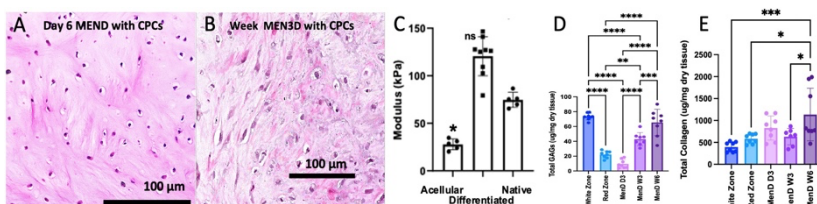


Figure 3: CPC-MEND after (A) 6 days of invasion and (B) 3 weeks of differentiation. (C) Bulk modulus from compression testing, (D) GAG content/weight and (E) collagen weight.

For all figures: data represented as \pm s.d.,
***p<0.0001, **p<0.001, *p<0.01,
**p<0.01, *p<0.05