Comparing knee articular chondrocytes and costal chondrocytes as a cell source for generating articular self-assembled neocartilage

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INTRODUCTION: Cartilage lesions, which lead to degenerative changes within the joint such as osteoarthritis (OA), do not heal naturally and the current treatment options are limited. While biologic cartilage repair products have been heavily investigated, their clinical translation is dependent on the identification of an appropriate cell source. Costal chondrocytes have been utilized to generate self-assembled neocartilage implants that regenerate the temporomandibular joint disc in the Yucatan minipig model [1]. While there has been success in repairing the fibrocartilaginous temporomandibular joint disc, there has not been a direct comparison between the ability of costal chondrocytes (CCs) and knee articular chondrocytes (ACs) to produce functional articular neocartilage. Therefore, the objective of this study was to assess functional output of self-assembled neocartilage constructs derived from CCs and ACs and compare the functional properties to those of adult knee cartilage. This study specifically focuses on the preclinical evaluation phase of the translational vector, in which an animal model is required. The Yucatan minipig was selected because the FDA specifies that preclinical evaluation of products intended to repair knee cartilage must be evaluated in a large animal model [2]. The results from this study will inform which cell sources are appropriate to be used in preclinical evaluation of products intended to repair articular cartilage of the knee and other locations with articular cartilage degeneration (e.g., facet joint, hip).

METHODS: Knee articular cartilage and rib cartilage were obtained from juvenile Yucatan minipigs (5-8 months in age) as analogous to the intended donor source (i.e., healthy young tissue). As a native tissue comparator, knee tissue was also isolated from skeletally mature minipigs (1.5-2 years in age). Cells were expanded using conservative chondrogenic expansion methods to passage 3 before being subjected to a rejuvenation step in which they were placed in aggregate culture with a cocktail of growth factors to restore the chondrogenic phenotype to passaged chondrocytes. Rejuvenated cells were then utilized to generate self-assembled neocartilage by seeding the cell into non-adherent wells. The constructs were cultured for one month before evaluation of functional properties. Histologic evaluation of constructs and native tissue was carried out through hematoxylin and eosin, safranin-O, and picrosirius red staining. Total collagen content was assessed using a chloramine-T hydroxyproline assay, and glycosaminoglycan (GAG) content was assessed via a Blyscan Glycosaminoglycan Assay kit. Creep indentation and uniaxial tensile tests were performed to assess the mechanical properties. All statistical analyses were conducted using Prism 9 with AC and CC values compared through a t-test with an n=7-10 per group.

RESULTS: Constructs derived from both ACs and CCs were morphologically appropriate (i.e., round and flat in morphology) (**Figure 1A**). However, AC constructs were significantly smaller in diameter than CC constructs (5.2±0.1 mm vs. 5.6±0.1 mm, respectively, $p<0.0001$) and thinner than CC constructs (0.44±0.01 mm vs. 0.50±0.04 mm, respectively, $p=0.002$). Histologically, ACs and CCs produced constructs with similar tissue organization and staining intensities (**Figure 1B**). Collagen content for AC and CC constructs was similar (12±3% vs. 12±2% collagen/dry weight, respectively), and GAG content displayed the same trend (33±3% vs. 39±2% GAG/dry weight, respectively). In terms of mechanical properties (**Figure 1C**), the Young's modulus (10.2±2.2 MPa) and ultimate tensile strength (3.7±1.0 MPa) of constructs produced by ACs were significantly higher than the Young's modulus (6.5±2.0 MPa) and ultimate tensile strength (2.3±0.7 MPa) of constructs produced by CCs ($p=0.001$ Young's modulus, $p=0.001$ UTS). However, the compressive aggregate modulus was comparable between the two groups (497±97 kPa AC constructs vs. 447±107 kPa CC constructs). Native adult knee medial condyle tissue had 36±13% collagen/dry weight, 13±5% GAG/dry weight, a Young's modulus of 13.3±7.0 MPa, and an aggregate modulus of 430±121 kPa.

DISCUSSION: Here the functional properties of self-assembled neocartilage derived from AC and CC cell sources were evaluated and compared to native adult knee medial condyle tissue values. The AC and CC cell sources yielded constructs that were comparable in morphology and tissue organization. The tensile Young's modulus values of the AC and CC constructs reached 77% and 48% of native tissue values, respectively, while compressive aggregate modulus values reached 115% and 104% of native tissue values. Overall, the morphology and functionality of constructs derived from both tissue sources were appropriate. Obtaining healthy donor tissue from the rib is logistically advantageous to the knee. Therefore, the translation of cartilage repair products that utilize CCs may facilitate the acquisition of viable donor tissue thereby facilitating translation of new products.

CLINICAL RELEVANCE: The results from this study demonstrate that chondrocytes from juvenile knee articular cartilage and rib cartilage produce neocartilage with appropriate morphology and functional properties that approach those of native tissue. Therefore, both cell sources may be viable options for the generation and translation of articular cartilage implants.

REFERENCES: [1] Vapniarsky, N., *et al.* Tissue engineering toward temporomandibular joint disc regeneration. *Sci. Transl. Med.* **10**, eaaq1802 (2018). [2] U.S. FDA. Guidance for Industry: Preparation of IDEs and INDs for Products Intented to Repair or Replace Knee Cartilage. *www.FDA.gov* (2011).

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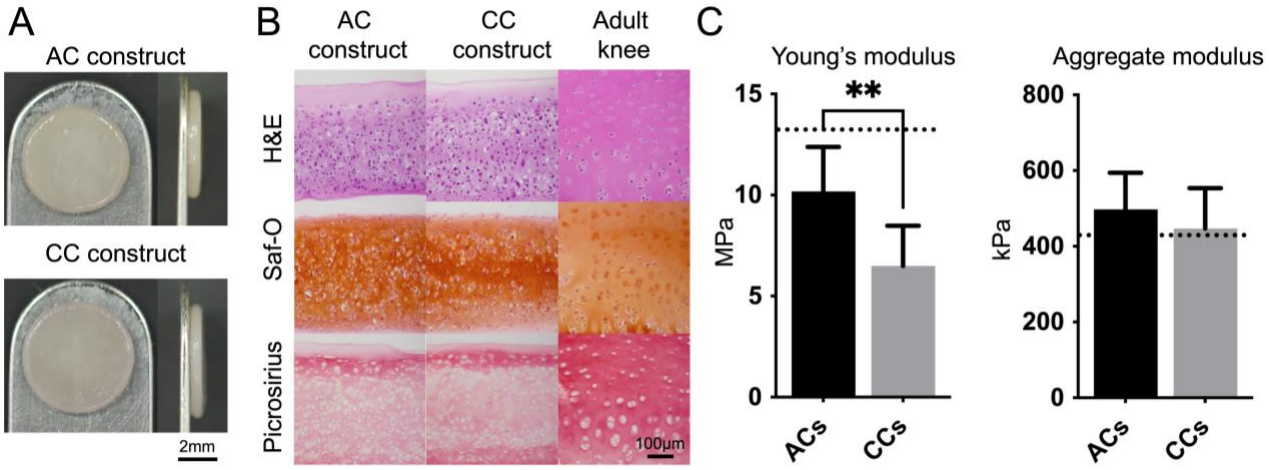

Figure 1: A. Morphology of AC and CC constructs. B. Histology of AC and CC constructs in comparison to native adult medial condyle tissue. C. Mechanical properties of AC and CC constructs in comparison to native adult medial condyle tissue (represented by dashed line) (**$p<0.01$, n=7-10 per sample).