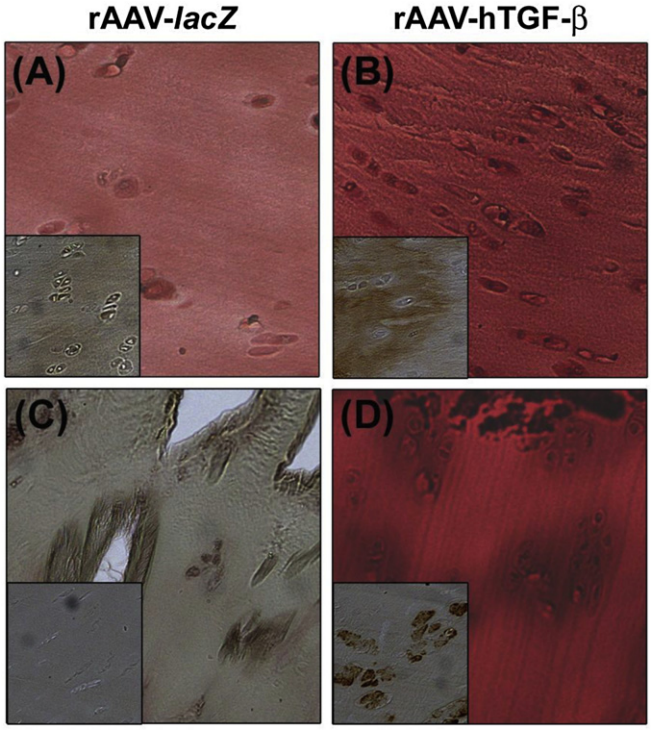

Fig. 3. Histological analyses in transduced human normal (A,B) and OA (C, D) cartilage (day 30): safranin O/H&E staining and type-II collagen immunoreactivity (insets) All at magnification x40.

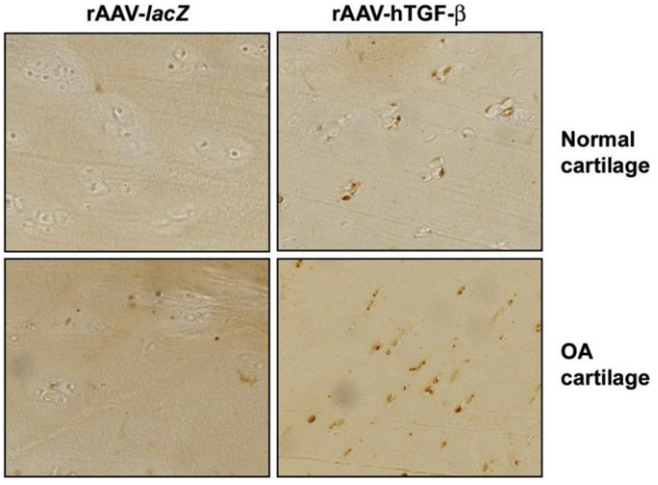

Fig. 4. Immunohistochemical detection of BrdU in transduced human normal and OA cartillage (day 30). Magnification x10.

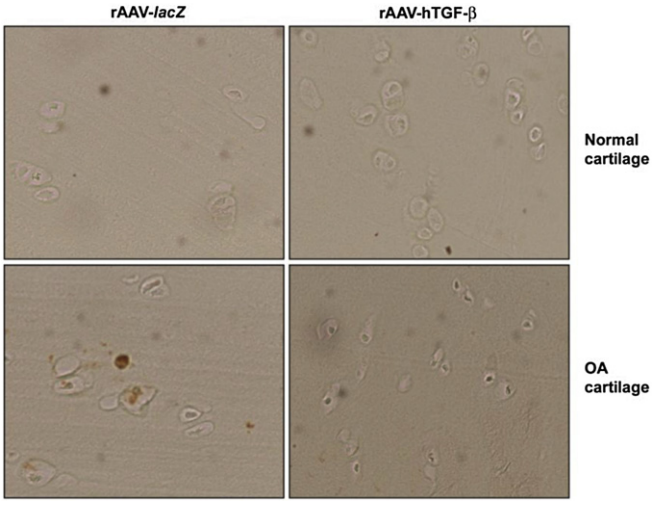

Fig. 5. Immunohistochemical detection of type-X collagen in transduced human normal and OA cartilage (day 30). Magnification x20.

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TEMPORAL EVALUATION OF SCAFFOLD-FREE CARTILAGE TISSUE ANALOGS: MECHANICAL PROPERTIES AND CELL AND MATRIX CONTENT

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Purpose: Cartilage tissue engineering aims to mimic the architecture and function of native cartilage. Although many approaches rely on the use of cells seeded-biocompatible materials, a number of alternative approaches use scaffold-free platforms. We have previously established a material-free cartilage tissue analog (CTA), and successfully demonstrated this model can maintain chondrocyte phenotype *in vitro* for extended periods. In the absence of a scaffold, cells in CTAs self-aggregate and elaborate matrix, therefore mechanical properties take longer to develop than in a scaffold-based system. Here, we evaluated the temporal development of CTA mechanical properties, and defined the relationship between construct mechanics and cell and matrix content. This study will not only help to determine patterns of growth and maturation of an engineered cartilage with the potential to repair defects, but also identify time periods at which construct properties most closely mimic juvenile or adult cartilage for the study of age-related cartilage pathology and underlying mechanisms.
Methods: Cartilage was harvested from juvenile bovine knees, minced, and digested overnight in DMEM with 1mg/mL collagenase-II. The cell suspension was filtered, washed and seeded at $1x10^6$ cells/well in our self-aggregating suspension culture model using hydrogel coated 96 well plates. CTAs were cultured in DMEM with 10%FBS 1 to 24 weeks, during which time the mechanical properties, biochemical content, histological features, and molecular aspects were evaluated. CTA and cartilage equilibrium (10% strain, stress relaxation) and dynamic (1% strain, 1Hz) compressive properties were tested in unconfined compression. GAG and DNA content was determined using the DMMB and PicoGreen assays, respectively. Histological sections were stained with Alcian blue to identify proteoglycan and nuclear fast red; cell:-matrix ratios were quantified by analyzing micrographs with ImageJ.
Results: Over the first 24 hrs a cell-rich aggregate formed, which matured via the production of cartilage ECM with time in culture. Between 4 and 12 weeks, the equilibrium modulus remained low (<50 kPa), but increased by >500% by 16-24 weeks [from 23 (+/-9) kPa to 123 (+/-88) kPa]. These values were greater than 20% of that of native adult cartilage [560 (+/-252) kPa] (Fig 1A). The dynamic modulus likewise increased over this time period, though the changes were not as dramatic (~200%) (Fig 1B).

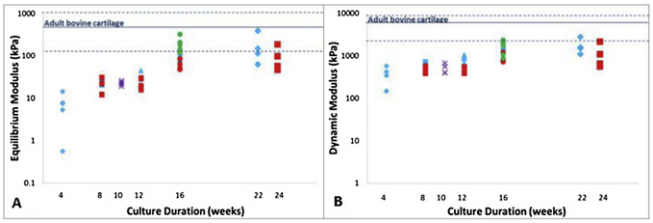

Figure 1. (A) Equilibrium and (B) Dynamic modulus of CTAs mechanically tested over 24 weeks.

CTA mechanical properties strongly correlated with GAG content across all donors, but not correlate with DNA content. The increase in mechanics over 16 weeks of culture was consistent with the histological analysis. At early times of culture, the cell:matrix ratio was quite high (0.13+/-0.03), however by 16 weeks of culture it decreased to 0.06+/0.01 matching that of native adult cartilage (0.05+/-0.01) (Fig 2A). Histological sections illustrate the increased amount of matrix per cell at 16 weeks and its similarity to native cartilage (Fig 2B).

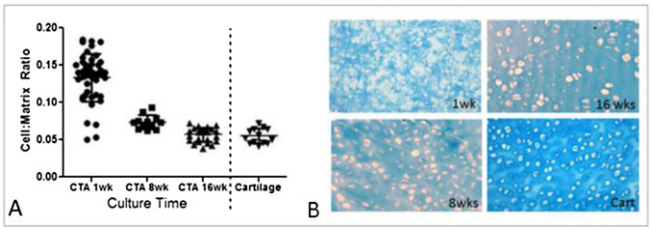

Figure 2. (A) Cell:matrix ratio and (B) representative histological sections of CTAs and native cartilage.

Conclusions: Our results show that as CTAs mature, chondrocytes produce cartilage-like ECM, with cell:matrix ratios similar to native cartilage. This transformation generates engineered cartilage constructs with near-native mechanical integrity via a scaffold-free mechanism. Taken together with our previous studies using this model, our results suggest that mechanical outcomes are more closely related to proteoglycan/GAG content, while early growth involves initial cellular establishment of the CTA. Having established the CTAs baseline mechanical properties, future studies may employ dynamic loading, which we have shown increases matrix production and may further improve mechanical properties.

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AGE-DEPENDENCE OF ZONAL CHONDROCYTE/MSC CO-CULTURES IN HA HYDROGELS FOR CARTILAGE TISSUE ENGINEERING

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Purpose: To determine whether zonal chondrocyte identity influences MSC fate decisions, and whether this influence is age-dependent.
Methods: Adult (AbMSC) or juvenile bovine MSC (JbMSC) were isolated from bone marrow, and juvenile zonal chondrocytes (JbCH) were isolated from full thickness articular cartilage (Fig 1). MSC only, zonal CH only, or a mixed population (MSC:CH = 4:1) were seeded at 60 x 10^6 cells/mL in 1% w/v photo-crosslinkable HA hydrogel. Cylindrical constructs (Ø4 x 2.25mm) were cultured in a defined medium containing 10ng/mL TGF-ß3. Cell viability and the distribution and proliferation of mixed populations of MSCs and CHs were tracked using CellTracker. Unconfined compression testing was carried out to determine mechanical properties. Sulfated glycosaminoglycan (s-GAG) and hydroxyproline contents were determined. Paraffin embedded sections (8μm) were stained with Alcian Blue for proteoglycan, and underwent immunostaining for collagen I, II, X and chondroitin sulfate. Significance was determined by two-way ANOVA with Tukey's post hoc test ($p<0.05$).
Results: JbMSCs were viable in 3D HA gels, both alone and in co-culture with CHs, and retained the cell tracker signal (Fig 2A-C) over 56 days. While AbMSCs were also viable with stably labeled in co-culture (Fig 2D-F), AbMSCs alone were less viable and aggregated together, resulting in the formation of a single mass (Fig 2G). Mechanical and biochemical properties for pure CH and co-culture groups increased with time and depended on the zonal origin of the CHs; lower properties were achieved with superficial CHs and higher properties with deep zone CHs (Fig 3). Constructs containing JbMSCs alone grew well, matching the properties of deep zone CHs (633 kPa and 6.1% WW GAG) (Fig 3A-B) while construct formed with only AbMSCs resulted in poor properties (5 kPa and 1.2%WW GAG) (Fig 3C-D). Interestingly, properties in constructs seeded with AbMSCs were markedly improved when co-cultured with zonal JbCHs (Fig 3C-D). Analysis of the efficacy of co-culture for JbMSC-JbCH showed independent contributions of each cell type, while for AbMSC-JbCH co-cultures, there was a marked synergistic effect in both EY (Fig 3E) and GAG content (Fig 3F).
Discussion: This study examined the impact of age on the chondrogenic capacity of bovine MSCs and how aging might alter response to co-culture with zonal articular CHs in a 3D platform. Zonal origin of CHs influenced construct properties both alone and in co-culture groups. The lowest properties observed were with constructs containing superficial CHs and the highest properties were with deep zone CHs, suggesting that CHs retain their zonal characteristics, even after extensive culture expansion and seeding in this HA system. While JbMSCs grew quite well on their own in HA gels, AbMSCs failed to mature. While JbCHs had no impact on the growth of co-cultures with JbMSCs, a synergistic enhancement of construct properties was observed in co-cultures of AbMSCs and JbCHs. Future work will determine the mechanism by which CHs influence MSCs in this co-culture system. Using these co-cultures, we will also determine (1) the minimal density of CHs needed to elicit the synergist growth response, (2) the potentially reduced need for TGF with co-culture, (3) the resistance of co-cultures to hypertrophic challenge in/ex vivo, and (4) co-culture mediated in vivo cartilage formation for functional and stable restoration.

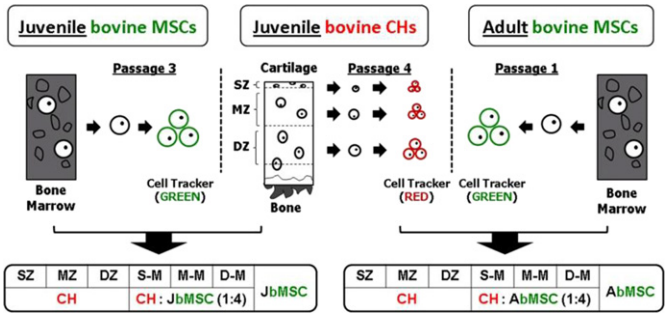

Fig 1. Co-culture of Zonal CHs and MSCs.

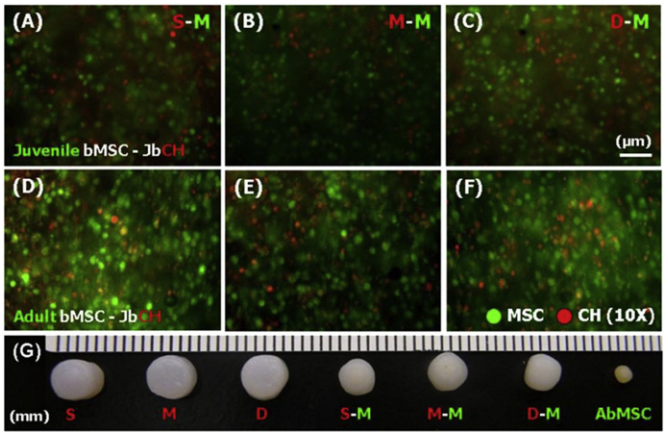

Fig 2. Visualization of CHs (Red) and MSCs (Green) (mixture ratio; CH:MSC = 1:4) and geometric changes of AbMSC- based constructs after 56 days of culture. (A-C) JbMSC-JbCH, (D-F) AbMSC-JbCH (10X; Scale bar=100 μm), (G) Co-cultures of AbMSCs and JbCHs at day 56 (Marking = 1 mm).