

## MODULAR TISSUE ENGINEERED CARTILAGE SURFACES

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### INTRODUCTION

Osteoarthritis is a debilitating degenerative disease of the articular cartilage, affecting more than 21% of adults [1]. Treatments, including total joint replacement, have a limited life span in the body, while allografts and autografts have had limited success due to tissue availability [2,3]. Cartilage tissue engineering techniques have shown promise as a potential implantable biological repair strategy. Our approach employs a well-established agarose hydrogel model with cells encapsulated within the biocompatible scaffold to develop functional cartilage *in vitro* [3,4]. This technique has been successful in developing small-engineered cartilage plugs (4 mm diameter) with glycosaminoglycan (GAG) composition and compressive mechanical properties similar to native cartilage [4,5].

Cultivating large-scale engineered tissues has been difficult due to limited nutrient diffusion in larger scaffolds, resulting in inhomogeneous matrix deposition and mechanical properties [5, 6]. Modeled from the native vasculature of cartilage development, micro- and macro-channels have been added to 3D scaffolds to improve nutrient diffusion [5]. Macro-channels, cut into the existing construct, have been shown to decrease spatial variation in larger constructs and maintain the mechanical properties similar to smaller constructs. However, the channels become occluded with deposited extracellular matrix within weeks, when nutrient diffusion is critical [5].

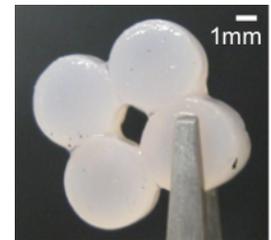
The objective of this study was to employ a novel modular tissue engineering technique to maximize nutrient diffusion during early formative stages, and, then, connect individual constructs to form larger engineered tissue surfaces (Figure 1). This technique takes advantage of the propensity of developing constructs to stick together in culture to synthesize bonds [7]. The negative space between joined constructs serve as macro-channels for increased nutrient diffusion. We hypothesize that the modular engineered tissue surfaces (METS)

will maintain the same mechanical and biochemical properties as the individual control constructs.

### METHODS

Articular cartilage was harvested from juvenile bovine knees (3 - 6wks, n = 3) and digested with Type IV collagenase serum medium (DMEM, Invitrogen Co., Carlsbad, CA, USA). Chondrocytes were expanded with serum media supplemented with a growth factor cocktail (10% FBS, 1% PSAM, 1ng/mL PDGF, 0.5 ng/mL FGF, and 0.5 ng/mL TGF) [4]. Cells were passaged twice to achieve sufficient cell numbers for tissue engineering studies and to improve matrix production in 3D cultures [8]. Once confluence was reached, passaged cells were encapsulated within agarose for a final concentration of 2% w/v agarose (Type VII, Sigma-Aldrich, St. Louis, MO, USA) at a cell density of  $30 \times 10^6$  cells/mL. Constructs were prepared using a 4 mm diameter biopsy punch, and cultured in chondrogenic media supplemented with 10 ng/mL TGF- $\beta$ 3 for the first 14 days in culture [4]. At day 7, constructs were placed into a 3D printed porous mold (9 mm x 9 mm) to cultivate constructs in groups of 4 (2 x 2; Figure 1). Individual constructs served as the control, and both groups were cultured for 28 days.

Compressive mechanical properties (i.e. equilibrium and dynamic moduli) were determined weekly for individual constructs and METS samples (n = 4/5 per group per time point). The equilibrium modulus was measured in unconfined compression at 10% strain, and the dynamic modulus was determined from sinusoidal  $\pm 1\%$  strain at 0.5 Hz. Following bulk compressive testing, each group of four was cut in half to isolate a single bond. The tensile strength of the bond was

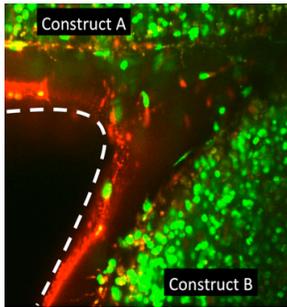


**FIGURE 1. Modular engineered tissue surface (METS).**

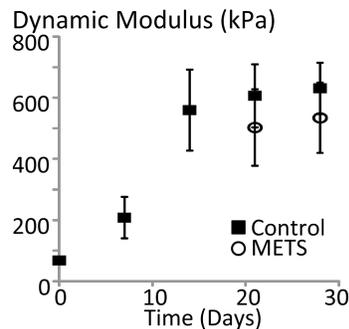
assessed using a tensile strain rate of 1 mm/min until failure. The stiffness was calculated as the slope of the force-displacement curve, and the peak force at failure was recorded. After mechanical testing, the wet weight was recorded and samples were prepared for biochemical assays.

Biochemical characterizations measured the levels of DNA, GAG and collagen in the samples. DNA was measured using a PicoGreen (Invitrogen, Co.) assay kit. GAG was measured using a DMMB assay and collagen was measured using a hydroxyproline assay [4]. Cell viability and distribution was evaluated using a LIVE/DEAD assay (Invitrogen Co.).

A one-way ANOVA was used to compare changes in mechanical and biochemical properties over time. A Student's t-test was used to compare mechanical and biochemical properties of METS and individual constructs at day 28. Significance was set at  $p \leq 0.05$ . Data are presented as mean  $\pm$  standard deviation.



**FIGURE 2. Live cells (green) in junction. Oversaturation of stain (red) used to mark contact tissue (line).**



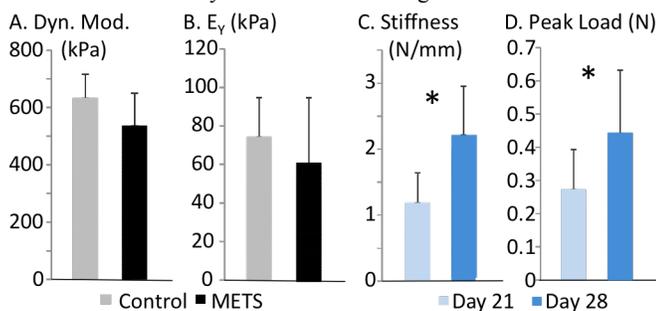
**FIGURE 3. Dynamic modulus over time ( $p > 0.5$ ).**

## RESULTS

By day 21, stable connections had formed between constructs in METS samples, allowing for bulk mechanical testing (final dimensions: 8 mm x 8 mm; Figure 1). Tissue deposition was observed optically with small fibers observed connecting constructs (data not shown). Images for cell viability demonstrated cell infiltration into the connections (Figure 2). At day 28, the bond width was  $2.2 \pm 0.3$  mm.

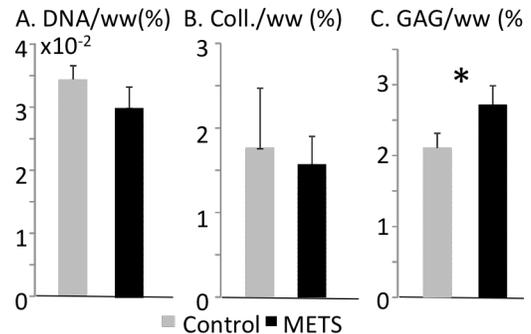
Compressive mechanical properties of METS and individual constructs at day 28 were 3-9X greater than initial properties ( $p < 0.01$ , Figure 3). At day 28, there were no significant differences in dynamic and equilibrium moduli between individual constructs and METS samples ( $p > 0.19$ ; Figure 4A & B). Tensile mechanical properties of the bonds increased over time (day 21 versus day 28,  $p < 0.05$ ; Figure 4C & D).

Biochemical assays demonstrated no significant differences in the



**FIGURE 4. (A) Dynamic and (B) equilibrium ( $E_\gamma$ ) moduli were not significantly different at day 28. (C) Stiffness and (D) peak force under tension. \* $p < 0.05$ .**

DNA and collagen contents of METS samples compared to the individual control constructs ( $p > 0.05$ ; Figure 5A & B. Interestingly, the GAG content of METS samples was 29% greater than the control ( $p < 0.01$ ; Figure 5C). Similar results were observed for GAG and collagen contents normalized by DNA content and dry weight.



**FIGURE 5. (A) DNA/ww%, (B) coll./ww%, and (C) GAG/ww% at day 28. \* $p < 0.01$ .**

## DISCUSSION

This study demonstrated feasibility of creating modular engineered cartilage using the inherent propensity of developing *de novo* cartilage to adhere to neighboring constructs. Mechanical and biochemical properties of METS samples were not significantly different from individual constructs, except for GAG content, which was greater in METS. These findings agree with previous work using macro-channels added to the scaffold during fabrication (i.e. day 0) [5, 6]. In this study, macro-channels remained free of extracellular matrix throughout the culture period (Figure 1). Moreover, the tensile stiffness of the bonds was within the range of values reported for bovine articular cartilage (0.3-2 N/mm) [9]. These results suggest that forming macro-channels later in culture is feasible and may be beneficial for maintaining long-term nutrient diffusion in the channel.

Cartilage tissue engineering has focused on optimizing properties of small tissue constructs, and researchers have been largely successful in achieving native properties for compressive modulus and GAG content [3-6]. However, these constructs are too small to be a clinically viable solution. Recent work in the literature has demonstrated methods for developing large-scale engineered cartilage tissues either through large scaffold fabrication on day 0 [5, 6] or through fractal fabrication techniques [10], where smaller engineered tissue samples are used to create larger surfaces. The findings of this study demonstrate that mature engineered cartilage plugs (4 mm diameter) can be used to develop larger surfaces with mechanical and biochemical properties of individual constructs. Further work will evaluate depth-dependent and location-dependent variations in mechanical and biochemical properties of larger METS samples. In conclusion, the METS approach presented in this study describes a method for scaling functional cartilage tissue engineering techniques towards clinically relevant dimensions.

## ACKNOWLEDGEMENTS

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## REFERENCES

- [1] Helmick, C+, *Arthr. and Rheum.*, 2008;
- [2] Hunt, H+, *Amer. J. of Sp. Med.*, 2014;
- [3] O'Connell, G+, *JKS*, 2012;
- [4] O'Connell, G +, *eCells and Mat.*, 2014;
- [5] Bian, L+, *Osteoarth. and Cart.*, 2009;
- [6] Nims, R+, *T. Eng. Part C*, 2015 – In Press.
- [7] Ng, S+, *A. Biomat.*, 2012.
- [8] Tan, AR+, *Osteoarth. and Cart.*, 2015 – In Press.
- [9] Danso, E+, *J. of Biomat.*, 2014;
- [10] Bhumiratana+, *PNAS*, 2014.