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CHARACTERIZATION OF DEPTH-DEPENDENT MECHANICAL PROPERTIES IN BIO-TITANIUM HYBRID OSTEOCHONDRAL TISSUE ENGINEERED CONSTRUCTS

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INTRODUCTION

With cartilage autografts and allografts in short supply, tissue engineered osteochondral (OC) grafts offer an alternative [1]. These constructs are comprised of a chondrocyte-seeded hydrogel region and a porous, bone-like base. Our laboratory has shown growth of more robust osteochondral constructs on clinically-relevant metal substrates (eg. tantalum) as opposed to devitalized bone, and these constructs have been evaluated *in vivo* [1,2]. Due to the presence of the base, it is expected that transport of nutrients and chemical factors in OC constructs will differ from transport in chondral-only constructs (Fig. 1, *bottom-left*). Depth-dependent mechanical properties of chondral-only constructs have been measured, yielding a "U-shaped" strain profile, in which the construct is stiffest on the edges and softest in the center. However, depth-dependent properties have not been measured in tissue engineered OC grafts [3].

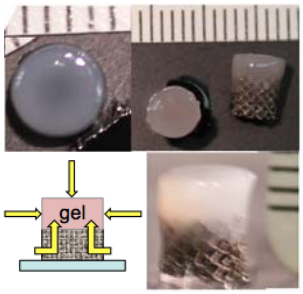

Figure 1. *Top*: Chondral Control (*left*) and OC constructs (right) at day 21; *Bottom*: Schematic of transport into OC construct (*left*), OC construct at day 42 (*right*). Scale bars: 1 mm.

METHODS

Equal volumes of 4% w/v agarose (Type IX, Sigma) and 120 million cells/mL suspension (passage 2 canine chondrocytes [1]) were mixed and cast atop cylindrical titanium disks (⌀4x4mm), which were 3D printed followed by sintering, cleaning, and passivation (Stryker Orthopaedics, Mahwah, NJ). This material is utilized clinically for orthopaedic implants to facilitate bone integration, and is here used as a bony substrate for fabrication of engineered OC grafts This yielded constructs of 60 million cells/mL in 2% w/v agarose. The chondral region of these constructs was ~2 mm thick (Fig. 1). A chondral-only slab was cast at the same concentration between two glass panes and constructs were punched out to serve as the control (dimensions: ⌀4 mm x ~2mm). Constructs were cultured in chondrogenic media and supplemented with TGF-β3 continuously [1]. Throughout a 42-day period, constructs were mechanically tested in unconfined compression (E_Y - Young's Modulus; G* - Dynamic Modulus), according to [1].

After bulk mechanical testing, constructs (n=3 per group) were cut in half, stained with a LIVE/DEAD kit (Invitrogen) for contrast, and compressive strains were applied using a custom device [4]. Images were acquired using a confocal microscope at strain increments of 2.5% strain (2-4 strain steps acquired for each sample; Fluoview FV1000, Olympus). Images were analyzed using texture correlation techniques (Vic2D, Correlated Solutions) to determine local strains. Data was cropped minimally to remove edge effects. For each image, strains were separated into 5 equal regions through the thickness,, averaged, and normalized to the bin value of minimum strain (ie. the most compressive strain) for each group (Fig 3. *right*) in order to facilitate presentation. The normalized data was then averaged for each group. Note that since it is difficult to know the orientation of the OC constructs in the custom device, the strain data was aligned based on the maximum (ie. least compressive) strain value as this would not affect the symmetric strain profile seen previously for

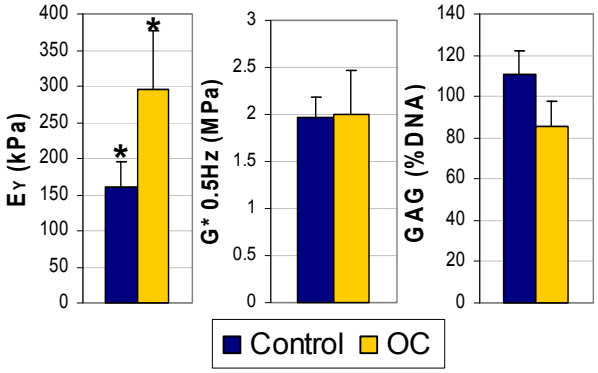

Figure 2. *Left*, Young's Modulus (Day 0: 7.21 kPa); *Middle*, Dynamic Modulus (0.5 Hz) (Day 0: 4.79 x 10^{-2} MPa); *Right*, GAG Content normalized by DNA (Day 0: 1.51%) at day 28. * $p \leq 0.05$.

chondral-only constructs. For all data, outliers outside two standard deviations from the mean were removed. A one-way ANOVA (α=0.05) with Tukey's HSD post-hoc test (Statistica) was used to determine significance.

Following mechanical testing, OC constructs were removed from their bases using a razor blade. Constructs were digested with proteinase K and biochemically assayed for glycosaminoglycan (GAG, DMMB dye-binding assay) and DNA (Picogreen dsDNA assay) content.

The surface area blocked by the titanium base was calculated using photos processed and thresholded using Photoshop (Adobe) and quantified using ImageJ (NIH).

RESULTS

Whereas gel-alone constructs had one axial surface occluded by the culture dish surface during culture, the titanium base blocked ~42% of the lower gel surface area of OC grafts (Fig. 1). By day 28, the Young's modulus of the OC grafts reached native values and was 2X greater than the control ($p \leq 0.05$; Fig 2, *left*). The dynamic modulus and GAG content was not significantly different between groups (Fig 2 middle & bottom).

Depth-dependent properties of the control group demonstrated a "U-shaped" strain profile, with the greatest compressive strains measured in the center, and the lowest strains at the construct's extremities (Fig 3, *left*). A linear increase in compressive strain was observed in OC constructs (Fig. 3). Note that statistical differences were not reported between groups as each group was normalized by its respective minimum value to facilitate presentation.

DISCUSSION

Osteochondral constructs grown on porous titanium bases yielded tissue construct properties similar to the chondral (gel-alone) control, both mechanically and biochemically, and significantly surpassed the control's Young's modulus at day 28 of culture. The corresponding dynamic modulus of the OCs was similar to control, reflecting the OC's decreased capacity to pressurize under dynamic loading due to fluid exudation through the porous base in addition to the radial surfaces, as described in [5].

The differences in transport boundary conditions between the control and OC constructs resulted in distinct depth-dependent tissue properties. The expected "U-shape" strain profile was observed for the

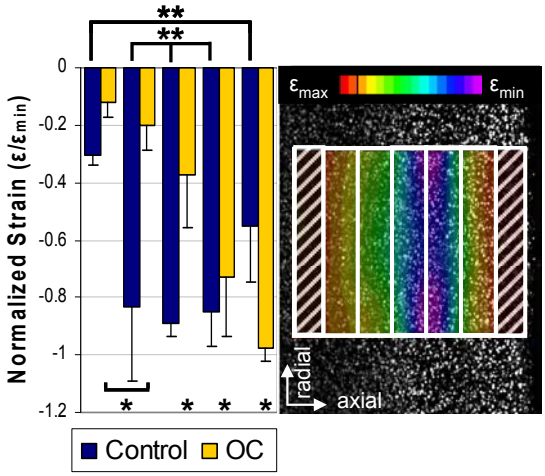

Figure 3. *Left*, Depth-dependent strains. Each bin represents 15% of the construct's thickness. Strains normalized and aligned by the minimum strain. Asterisks indicate significance (** - Control, * - OC). *Right*, Representative Strain Map (control) with horizontal (through the thickness) binning as described. Hatched bins represent cropped data. *,** $p \leq 0.05$.

control constructs; whereas the OC group's profile exhibited a gradient of strain across the depth of the construct. Comparing the external bins shows a ~10 fold increase in local strain.

Differences in mechanical properties between OC constructs and controls are expected yet their nature is as of yet not fully characterized. The presence of the underlying base both blocks some surface area depending on the base's porosity, an estimated 42% here, which may block nutrient transport , though it may elevate the construct off the culture dish bottom, enhancing transport. Such nutrient transport differences may account for differences in mechanical properties both on a bulk and a depth-dependent scale.

This study begins to characterize the depth-dependent mechanical properties of OC constructs. Future studies will investigate these properties on a larger scale, mapping the local strains with effort to quantify the characteristics of the osteochondral interface and the surface. Additionally, we will also extend our studies to investigate the depth-dependent properties of OC grafts subjected to applied deformational loading in culture. Loading is anticipated to further promote tissue development, providing a physical stimulus as well as enhanced transport of solutes [6].

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