The Influence of Dynamic Loading on Bio-Titanium Hybrid Osteochondral Tissue Engineered Constructs

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INTRODUCTION: Tissue engineered osteochondral (OC) grafts consisting of a chondrocyte-seeded hydrogel region and an underlying porous, bone-like base provide potential substitutes for allografts and autografts [1]. Previous *in vitro* studies in our laboratory have shown that osteochondral constructs grow better on clinically-relevant metal substrates (e.g., tantalum) rather than devitalized bone [2]. Dynamic loading (DL) has been shown to yield engineered cartilage with better functional properties, allowing for greater transport of larger molecules, such as 70 kDa dextran which is a size similar to that of the growth factor TGF-β3, into constructs [3-5]. Modeling has suggested that DL will also benefit the engineering of OC constructs [6]. This study investigates the influence of DL on transport properties in OC constructs with highly porous titanium bases and evaluates cartilage tissue development on such bases.

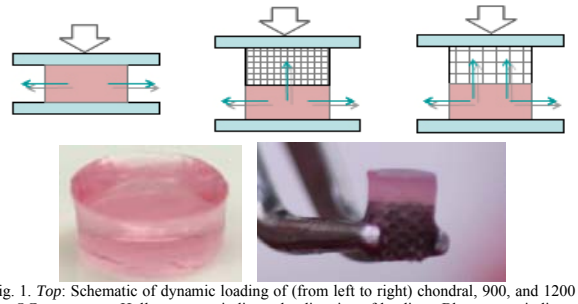

Fig. 1. *Top*: Schematic of dynamic loading of (from left to right) chondral, 900, and 1200 µm OC constructs. Hollow arrows indicate the direction of loading. Blue arrows indicate the path of loading-induced fluid transport. *Bottom*: Photo of ø4 mm cellular chondral and OC construct on a titanium base with 900 µm pore size at day 0.

METHODS: Study 1: Cylindrical titanium disks (ø10 x 4 mm, 900 or 1200 µm pores) which were 3D printed followed by sintering followed by cleaning and passivation (Stryker Orthopaedics, Mahwah, NJ) were lyophilized then weighed. Acellular OC constructs were cast using 2% w/v agarose (Type VII, Sigma) atop these disks. Acellular chondral constructs were cast between glass panes and punched to ø10 x 2.34 mm. Constructs were placed in baths of 500 µg/mL 70 kDa fluorescein-conjugated dextran solution in phosphate buffered saline (PBS) for 1 hour with half of each group undergoing dynamic loading (DL), leaving the other half in a free swelling (FS) condition. DL was performed using a custom built mechanical loader at 10% strain (~ 200 µm) at 1 Hz (Fig. 1). Constructs were then removed, rinsed with PBS, and blotted. They were then placed in 2 mL of PBS for 24 hours. PBS solution was aspirated and read on a Biotek Synergy 4 Microplate Reader for fluorescence. Constructs were lyophilized and weighed. From this value the respective base's weight was subtracted to acquire the amount of agarose, and each construct's dextran concentration was calculated. Study 2: Equal volumes of 4% w/v agarose and 60 million cells/mL suspension (P4 juvinile bovine chondrocytes) were mixed and cast atop titanium discs of 900 µm pore size (ø4 x 4 mm) for a final concentration of 2% w/v agarose and 30 million cells/mL in the ~2.3 mm thick chondral region. Chondral control constructs were cast at the same concentrations. Constructs were cultured in chondrogenic media and supplemented with TGF-β3 for the first 14 days. Throughout a 42-day period, constructs were mechanically tested in unconfined compression according to (E_Y, equilibrium modulus) [1]. Constructs were biochemically analyzed by digesting (OC constructs were removed from their bases using a razor blade) in proteinase K then assaying for glycosaminoglycan (GAG, DMMB dye-binding assay), DNA (Picogreen dsDNA assay), and collagen (orthohydroxyproline assay) content. A two-way ANOVA (α=0.05) with LSD post-hoc test was used to determine significance.

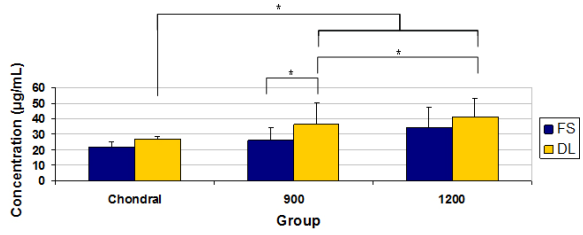

Fig 2. Concentration of dextran within constructs of chondral, 900, and 1200 µm OC constructs following 1 hr of soaking under FS and DL conditions. Asterisks indicate statistical significance among the groups.

RESULTS: Study 1: The FS groups exhibited a trend of increasing dextran concentration with increasing substrate porosity. DL OC constructs showed significantly higher dextran concentration than the chondral group (Fig. 2). DL showed significant concentration differences with increasing pore size. DL also yielded higher dextran concentrations than FS in each group, significantly so for the OCs with the 900 µm pore size. Study 2: OC constructs grew to a similar extent as the controls with no statistically significant difference in equilibrium modulus (Fig 3. *top*), GAG (*bottom*), or collagen (not shown) content.

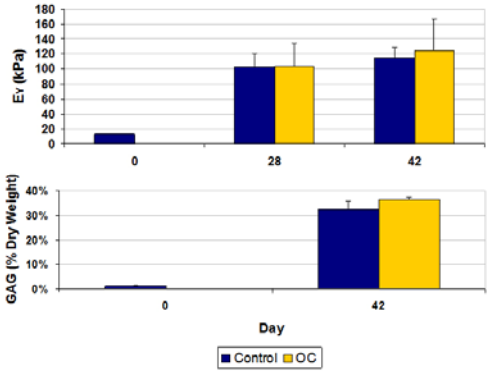

Fig. 3. Equilibrium modulus (*top*) and glycosaminoglycan content normalized by dry weight (*bottom*) showing no statistically significant differences, n= 4 constructs/group for day 0, 5 for day 14, and 6-9 for day 42.

DISCUSSION: Previous studies have shown DL to yield more robust tissue engineered cartilage than that grown in a FS environment [1], due in part to enhanced solute transport with loading-induced convection [7]. While this effect has been predicted for OC constructs [6], this study begins to validate it experimentally. Study 1 shows that FS constructs with porous bases results in a trend of increased solute transport. Additionally, DL yields significantly more transport into OC than into chondral-only constructs with increasing pore size. These observations are expected as there is more unrestricted chondral region surface area through which molecules can transport (Fig. 1). Study 2 confirms the ability of chondrocytes to grow on the porous titanium bases used for the acellular studies. This study promotes the use of DL as a method for increasing transport in engineered cartilage OC constructs, which may facilitate fabrication of large anatomically-shaped constructs suitable for replacing entire articular surfaces [8]. Future studies will assess the growth of cell-seeded osteochondral constructs under DL cultivation conditions.

SIGNIFICANCE: Dynamic loading has been predicted to benefit the tissue engineering of osteochondral grafts. This abstract explores its effect on transport in constructs with highly porous titanium bases, while confirming the base's biocompatibility.

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