Design and testing of a bioreactor for studying osteochondral fluid transport in an ex-vivo system

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INTRODUCTION: Osteoarthritis (OA) is a disease of the joint that affects more than 250 million people globally. OA is characterized by joint degeneration involving cartilage and bone [1]. The current paradigm is that chondrocytes (*i.e.*, cartilage cells) receive nutrients solely from the synovial fluid in the joint. However, recent studies [2,3] suggest that fluid transport may occur between bone and cartilage in both healthy and OA joints. Fluid transport from bone-to-cartilage could be a critical source of nutrients for chondrocytes particularly near the osteochondral interface. This presents a critical gap in knowledge and understanding of cartilage physiology: does fluid transport occur between bone and cartilage and, if so, how it is affected by cyclic compression (e.g., from loads associated with walking). We hypothesize that bone-to-cartilage fluid transport driven by cyclic compression is greater than fluid transport by diffusion only. Thus, this study aims to understand fluid transport from bone-to-cartilage and how cyclic compression affects such transport. The two objectives of this study are to: (1) design and develop a bioreactor, and (2) verify separation of the two fluid chambers through concentration studies.

METHODS: A two-chamber bioreactor was designed and manufactured (**Figure 1**). A 1.5”x1.5”x1-3/16” base with a 15/16” diameter (3/8” depth), and a threaded hole on bottom for attachment to a load cell (**Figure 1A**). The top attachment to the bioreactor that is separated by a 1/32” rubber sheet of 1.5”x1.5”x1/32” from the base has four vertical through holes for securing of two chambers, and two horizontal through holes (separated by 90°) for fluid circulation during experimental runs (**Figure 1B**). Additionally, the bottom chamber has a horizontal through hole, providing access to the fluid chamber after securing the system together. Two identical bioreactors were manufactured with polysulfone (Rigid polysulfone Sheet, McMaster-Carr). After manufacturing and upon further consideration of our experimental design small adjustments were made to the bioreactor. For the bottom chamber a ~4 mm thick by 15/16” diameter shim was added with a 6.8 mm centered hole at depth of 2.4 mm for eventual press-fit of osteochondral cores. To adjust the fluid volume top chamber to match that of the bottom chamber (~2mL) a shim was added. For testing of the bioreactor, we first pressed an impermeable plastic core (~7.8mm diameter) into a 3mm hole in a rubber sheet (1/32” thick) then placed into the bottom chamber of the bioreactor. A top chamber was then secured and attached to a peristaltic pump (**Figure 1C**). After securing the system the bottom chamber was filled with 13µM fluorescent dextran (3kDa Texas Red, Invitrogen, neutral) in PBS, the horizontal through hole was sealed (Gorilla mounting tape). For testing the pump was started, and the first aliquots (100µL in duplicate) were sampled ($t=0^-$) and replaced with 200µL of neat PBS. A second set of duplicate aliquots were sampled a few seconds later ($t=0^+$) and replaced with 200µL of PBS. Subsequently duplicate 100µL aliquots were taken every 5 minutes for 2hrs, with 200µL of neat PBS replaced each time. All aliquots were placed in a 96-well plate and the fluorescent intensity of each well was measured using a fluorescent plate reader (Synergy H1 microplate reader).

RESULTS: To verify minimal cross-over of fluorescent dextran n=3 experiments were performed with the impermeable surrogate. Preliminary results indicate a slow increase in fluorescent concentration with time, that on average peaked at ~0.02 µM around 40 minutes of diffusion.

DISCUSSION: Fluid transport within our joints plays a critical role in joint mechanics and the mechanobiological environment of chondrocytes (cartilage cells). Chondrocytes rely on fluid transport to receive biomechanical signals that drive its responses. To improve our understanding of the joint fluid transport environment we need to first understand the potential for fluid to transport from bone-to-cartilage in healthy joints. These results suggest that our two-chamber bioreactor has strong potential to allow for direct studies of the fluid transport from bone-to-cartilage within 40 minutes. Minimal increases in fluorescent intensity were found in our system, suggesting that through minor adjustments, such as a more robust rubber sheet (i.e., higher tensile strength) may further improve our baseline measurements. Furthermore, additional tests on the system such as testing the permeability of the rubber sheet without the center hole will provide further insights for improving the system. With this system we can address critical gaps in our understanding of joint fluid transport. First, by replacing the impermeable pipette with an osteochondral core we can address the potential for fluid transport from bone-to-cartilage. Further, our system is designed to attached to a custom-built loading machine, thus we can further probe osteochondral fluid transport during cyclic compression (i.e., walking). Therefore, by continuing to design, test and validate this system we are laying the groundwork for improved understanding of the bone-to-cartilage fluid transport and its impact on joint health (**Figure 2**).

SIGNIFICANCE/CLINICAL RELEVANCE: Our system shows the strong potential to be capable of resolving critical gaps in our understanding of osteochondral fluid transport. By understanding the amount of osteochondral fluid transport, we can better understand the nutrient environment present for chondrocytes.

REFERENCES: [1] Mandl, L.A(30453055), [2] Pan (19360842), [3] Pan (22197997), [3] Szarko, M (20932309)

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IMAGES AND TABLES: **Figure 1** Bioreactor A) Bottom Chamber B) Top chamber C) Assembled bioreactor in loading study; **Figure 2** Bioreactor design A) Schematic of loading study and two fluid chambers B) Diagram of boundary conditions.