DYNAMIC DEFORMATIONAL LOADING SIGNIFICANTLY ENHANCES THE TRANSPORT OF DEXTRAN MOLECULES INTO AGAROSE HYDROGELS

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INTRODUCTION: In an attempt to engineer a functional tissue replacement for articular cartilage repair, dynamic deformational loading, mimicking the in vivo loading environment, has been shown to enhance the extracellular matrix elaboration and increase the mechanical properties of chondrocyte seaded agarose hydrogels [1-3], relative to free swelling controls. One mechanism by which the increase in mechanical properties occurs is hypothesized to be due to enhanced transport of nutrients and/or growth factors under dynamic loading [4].

The transport of solutes in biological tissues is the fundamental process supplying cells with nutrients and removing waste products. Though transport occurs through both passive diffusion and convective flow, we hypothesize that dynamic loading significantly enhances the convection of solute molecules, such as dextran, into the agarose gels [5]. The goal of the current study is to determine the effect of unconfined compression dynamic loading on the transport of neutral dextran molecules in an acellular agarose model system. Since the use of frictionless impermeable platens in unconfined compression limits the transport of solutes to the lateral surface of the agarose disk (Figure 1a), the effect of increased surface area on solute transport, through the use of porous versus impermeable loading platens, is also investigated.

METHODS: Acellular type-VII agarose gel was cast at 2% w/v, and cylindrical disks (0.5mm, 2.3mm thick) were prepared. Constructs were loaded in presence of 0.5 mg/ml of Texas Red-conjugated dextran (3kDa or 70kDa; Molecular Probes, Eugene, OR) in PBS. Loading Protocol: Agarose constructs were loaded up to 6 hours in a custom unconfined compression loading device under either static (SL) or dynamic (DL) deformation at 37 °C. A 10% static tare strain was applied to both SL and DL groups, and an additional 10% dynamic sinusoidal strain was applied at 1Hz to the DL group. Both SL and DL groups were loaded either (a) 2 frictionless impermeable platens or (b) 1 porous steel porous wire mesh platen and 1 impermeable platen (Figure 1a,b). Loading protocols were chosen to ensure that no lift-off occurred with either platen type. Solute Content Measurement: Upon completion of loading, aliquots of the bathing solution were collected. Each disk was resuspended in 200µl of PBS, pulverized, and stored at 4°C for 48 hours to allow the solute to diffuse out of the gel. The gel/solute mixture was centrifuged and the supernatant containing the fluorescently labeled solute was extracted. The dextran concentration was measured using a fluorescent plate reader (SpectraFluor Plus, Tacon, Research Triangle Park, NC) and a solute specific standard curve. The solute concentrations inside the gel were normalized to the bathing solution concentration and are expressed as concentration ratios. Statistical analysis (three-way ANOVA) was performed to test the effect of time, loading configuration, and platen type on the solute concentration. Tukey HSD post hoc test was applied with p<0.05 considered statistically significant.

RESULTS: The concentration ratio of 3kDa and 70kDa dextran increased nonlinearly with increasing time under all loading configurations, reaching equilibrium by 3 and 6 hours, respectively (Figure 2). At all time points, the concentration ratio of the 3kDa dextran was significantly greater than the 70kDa dextran (e.g. 0.75±0.03 vs. 0.28±0.04 under SL-Impermeable for 3 hours). For both platen types, DL resulted in significantly greater dextran concentration than SL (70kDa: Impereable: 0.64±0.04 vs. 0.28±0.04, p<0.0001; Porous: 0.51±0.04 vs. 0.37±0.03, p=0.0002).

SL-porous resulted in a significantly greater concentration than SL- impermeable for both 3 and 70kDa dextran (p<0.05). However under DL, the 70kDa dextran concentration ratio was significantly greater with impermeable platens than with a porous platen (0.64±0.04 vs. 0.51±0.04; p<0.001). No significant difference of 3kDa dextran was measured for DL-porous vs. DL-impermeable at all time points (3 hours: 0.97±0.02 vs. 0.96±0.02, p>0.5).

DISCUSSION: Transport, the fundamental process by which biological tissues are supplied with nutrients and dispose of waste products, may occur through passive diffusion and convective flow. In this study, the uptake of dextran molecules under static loading is the outcome of the passive diffusion behavior of dextran in agarose. The concentration ratio at equilibrium (the partition coefficient) of 3 kDa molecules was significantly higher than that of 70 kDa dextran, which is consistent with the inversely proportional relationship between partition coefficient and molecular weight. Dynamic deformational loading (DL) resulted in a significantly higher steady state solute content relative to statically loaded (SL) controls. Based on a theoretical analysis using mixture theory to model the transport of neutral solutes in a neutrally charged gel [5], this experimental outcome is believed to be a result of convective flow of the dextran molecules into the gel matrix, and is consistent with previous findings examining the role of loading on solute transport in cartilage explants [4,6,7]. DL-impermeable resulted in an 18% and 56% increase of 3kDa and 70kDa dextran respectively, over SL-impermeable controls by 3 hours. The greater degree of enhancement of dynamic loading for larger molecular weight molecules (i.e. 70kDa) is consistent with previous studies [4,8] as well as theoretical predictions [5]. A previous tissue engineering study has shown that the stimulatory effect of dynamic loading is synergistically enhanced in the presence of IGF-1 (MW=8kDa) and TGF-β1 (MW=25kDa) [3]. The results of the current study suggest that dynamic physiological loading enhances transport of these growth factors and other cellular nutrients, which may result in significant enhancement in the mechanical properties and matrix elaboration. The use of one porous platen accelerates the rate at which solutes are transported into the gel by providing more surface area for flow to occur — as demonstrated by the radially limited distribution of 70kDa dextran with impermeable platens (Figure 1e) and by the 25% increase in 70kDa dextran under SL-porous vs. SL-impermeable (Figure 2b). However, DL-porous either resulted in a lower (70kDa) or equal (3kDa) dextran partition coefficient than DL-impermeable. This finding indicates that the interaction between the two mechanisms of enhanced solute transport (i.e. increased convection via dynamic loading and increased diffusion via porous platen) does not result in an additive enhancement of total solute content under dynamic loading with a porous platen. One possible explanation for this finding is that solutes convected into the disk through the radial edge via dynamic loading may flow out of the disk at the porous filter interface where fluid and solute exchange may be occurring.

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