Introduction: The complex mechanical environment of cartilage during loading creates a range of tissue stresses, strains and pressures, as well as fluid and ion flows. As such, it is difficult to determine the role of each individual stimulus in the biosynthetic response observed in chondrocytes. One stimulus believed to play a role in chondrocyte metabolism is fluid flow which has been shown to alter biosynthesis in monolayer studies in a dose-dependent manner.[1,2]. However, experiments have shown metabolic differences between chondrocytes cultured in a monolayer versus those in 3D scaffolds.[3]. Intracellular calcium concentration (Ca²⁺) is known to be a mediator of mechanical signaling pathways in chondrocytes,[4] and flow-induced Ca²⁺ signaling has been demonstrated in monolayer studies[2] but at fluid velocities 1000 times higher than shown to affect chondrocyte metabolism during cyclic compression of explants[5]. The objective of this study was to measure the flow-induced Ca²⁺ response of chondrocytes cultured within alginate scaffolds as a physiological model for better understanding mechanotransduction in a 3D environment.

Materials and Methods: Chondrocytes and 2% weight/volume LVG alginate suspension were molded directly into custom-made devices (Fig 1) using an injection molding technique[6] at a seeding density of 10x10⁶ chondrocytes/mL and incubated 12-20 hours in F-12 supplemented with 100U/ml penicillin, and 100μg/ml streptomycin. Cells were labeled with the calcium indicator Fluo-4 AM to visualize intracellular calcium. Gels were rinsed in situ with Tyrodes buffer plus 200μM sulfipyrazone and 5mM glucose before securing coverslips to the devices, slightly compressing the gel. The devices were incubated for 40 minutes to allow cells to equilibrate before attachment to a syringe pump. Flow velocities were determined using particle tracking methods[7] with 39 nm polystyrene beads with and without the presence of alginate gels. Cells were imaged with a Zeiss LSM 510 confocal microscope, whereby images containing 30-60 cells in the field of view were taken every 5-10 sec throughout the imaging period. Cells were imaged 5-6 min without flow and 5-6 min with flow. The mean intensity was plotted against time to reveal the temporal variation (dI/dt) in intracellular Ca²⁺. Ca²⁺ transients were defined for each cell individually by fitting all positive dI/dt to a Gaussian distribution and defining a Ca²⁺ response as any dI/dt falling outside 3 standard deviations, a modification of published methods.[2,8]. The fraction of cells that demonstrated a Ca²⁺ transient before and after the application of flow was determined. Data was normalized by subtracting the fraction responding without flow from the fraction responding with flow. One way analysis of variance was used to compare the level of Ca²⁺ signaling between gels exposed to different levels of fluid flow with Tukey post-hoc analysis (α = 0.05). All values are reported as averages ± standard deviations.

Results: The fluid velocities within alginate gels determined using particle tracking were found to be 2.5±0.66 μm/s, 7.2±1.8 μm/s, and 12.6±2.6 μm/s (Fig 2). These velocities were 20% of the velocities seen in the absence of an alginate gel at the same flow rates. After the 40 min equilibration period the fraction of cells responding without flow was 0.07±0.06 (Fig 3A). The calcium signaling response of the cells in the gels increased with the application of fluid flow and showed a dose-dependent response to fluid velocity (Fig 3B). A significant increase in Ca²⁺ signaling occurred with a fluid velocity change from 0 to 2.5 μm/s (p=0.01), 2.5 to 7.2 μm/s (p=0.02), and 7.2 to 12.6 μm/s (p=0.001).

Discussion: This study indicates that the application of fluid flow to chondrocytes seeded in a 3D alginate scaffold results in intracellular Ca²⁺ signaling that may be acting through a mechanotransduction pathway. The signaling response is seen at much lower fluid velocities than seen in monolayer studies [2]. One possibility for the observed reduction in velocity required for signaling may be due to changes that occur as chondrocyte morphology shifts as it spreads on the surface in monolayer[3]. As such, this may represent an important phenotypic mechanotransduction behavior that is regulated by cell shape or surface attachment.

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