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The mechanical microenvironment of high concentration agarose for applying deformation to primary chondrocytes

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ABSTRACT

Cartilage and chondrocytes experience loading that causes alterations in chondrocyte biological activity. *In vivo* chondrocytes are surrounded by a pericellular matrix with a stiffness of ~25–200 kPa. Understanding the mechanical loading environment of the chondrocyte is of substantial interest for understanding chondrocyte mechanotransduction. The first objective of this study was to analyze the spatial variability of applied mechanical deformations in physiologically stiff agarose on cellular and sub-cellular length scales. Fluorescent microspheres were embedded in physiologically stiff agarose hydrogels. Microsphere positions were measured via confocal microscopy and used to calculate displacement and strain fields as a function of spatial position. The second objective was to assess the feasibility of encapsulating primary human chondrocytes in physiologically stiff agarose. The third objective was to determine if primary human chondrocytes could deform in high-stiffness agarose gels. Primary human chondrocyte viability was assessed using live–dead imaging following 24 and 72 h in tissue culture. Chondrocyte shape was measured before and after application of 10% compression. These data indicate that (1) displacement and strain precision are ~1% and 6.5% respectively, (2) high-stiffness agarose gels can maintain primary human chondrocyte viability of >95%, and (3) compression of chondrocytes in 4.5% agarose can induce shape changes indicative of cellular compression. Overall, these results demonstrate the feasibility of using high-concentration agarose for applying *in vitro* compression to chondrocytes as a model for understanding how chondrocytes respond to *in vivo* loading.

1. Introduction

Osteoarthritis (OA) is the most common joint disorder, affecting over 100 million individuals (Woolf and Pfleger, 2003). OA is most commonly associated with excessive loading of aging joints (*e.g.* caused by obesity or injury), leading to deterioration of articular cartilage and joint inflammation. Articular cartilage is located at the surfaces of joints, and serves as a low-friction material between bones. Articular cartilage is composed of articular chondrocytes (cartilage cells), a pericelluar matrix (PCM), and an extracellular matrix (ECM) (Muir, 1995). In these regions of the body (*e.g.* the knee), the articular cartilage, and thus articular chondrocytes, are subjected to almost-constant mechanical loading (*e.g.* walking, running, etc.). Repetitive action is crucial for joint health, yet excessive loading can lead to OA (Harada et al., 2005). Individuals with a history of heavy mechanical work (*e.g.* heavy lifting) are ~7-fold less likely to have OA at the age of 90 (Goekoop et al., 2011), suggesting that long-duration, but sub-injurious, mechanical loading may induce protective biological responses. Therefore, understanding the biological responses of chondrocytes to mechanical loading are extremely important to improving joint health. These data emphasize the need for development of fundamental knowledge regarding how chondrocytes and other joint cells sense and respond to mechanical loads, a process defined as mechanotransduction (Vincent, 2013). This paper characterizes the deformational environment of a stiff 3D hydrogel for use in cartilage mechanotransduction studies.

Exogenous dynamic compression can substantially alter chondrocyte metabolism in both an anabolic and catabolic manner, but the balance between matrix synthesis and matrix degradation is not yet fully understood (Buschmann et al., 1999; Fitzgerald et al., 2008). Dynamic compression can induce phosphorylation of multiple enzymes, including MAPK and SEK (Fanning et al., 2003; Bougault et al., 2008a), Akt (Niehoff et al., 2008), Erk-1 and -2 (Li et al., 2003; De Croos et al., 2007; Ryan et al., 2009), and Rho kinase (Haudenschild et al., 2008). Additionally, exogenous loading can alter Superficial Zone Protein expression (Neu et al., 2007), induce transcription of ECM genes (Bougault et al., 2008b),

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and activate RhoA (Haudenschild et al., 2008). Cyclic dynamic compression can promote Smad2 phosphorylation (Bougault et al., 2012), gene expression of MMP-13 (Nebelung et al., 2012), which is the marker for catabolic changes in the ECM, and increases in ATP release (Garcia and Knight, 2010). These studies demonstrate the sensitivity of chondrocytes to mechanical loading and indicate that a complete understanding chondrocyte mechanotransduction remains to be determined.

A variety of hydrogels have been utilized including photo cross-linked polyethylene glycol (Farnsworth et al., 2013), self-assembling peptides (Kisiday et al., 2009), alginate (Haudenschild et al., 2011), and agarose (Knight et al., 2006; Vaughan et al., 2010). Most existing studies utilize 3D microenvironments (*e.g.* agarose or alginate) for cell encapsulation with a much lower stiffness (<5 kPa) than the cartilage pericelluar matrix (25–200 kPa) (Alexopoulos et al., 2005; Darling et al., 2010). Agarose hydrogels are of particular interest because the stiffness can be taylored to match the stiffness of cartilage PCM (Normand et al., 2000) without potential complications of UV photocrosslinking (*e.g.* induction of the DNA damage response (Filatov et al., 1996)). This study characterizes the deformational environment of high-stiffness ($\sim$35 kPa) agarose gels. To our knowledge, chondrocyte mechanotransduction studies have never been performed using agarose with PCM stiffness.

Cartilage experiences a variety of *in vivo* loading. The motivation for this study is to characterize the micro-level deformation fields in a physiologically stiff, 3D culture environment, to study how chondrocytes sense and respond to mechanical loading. Using a bioreactor capable of applying sub-micron precision, displacement-controlled

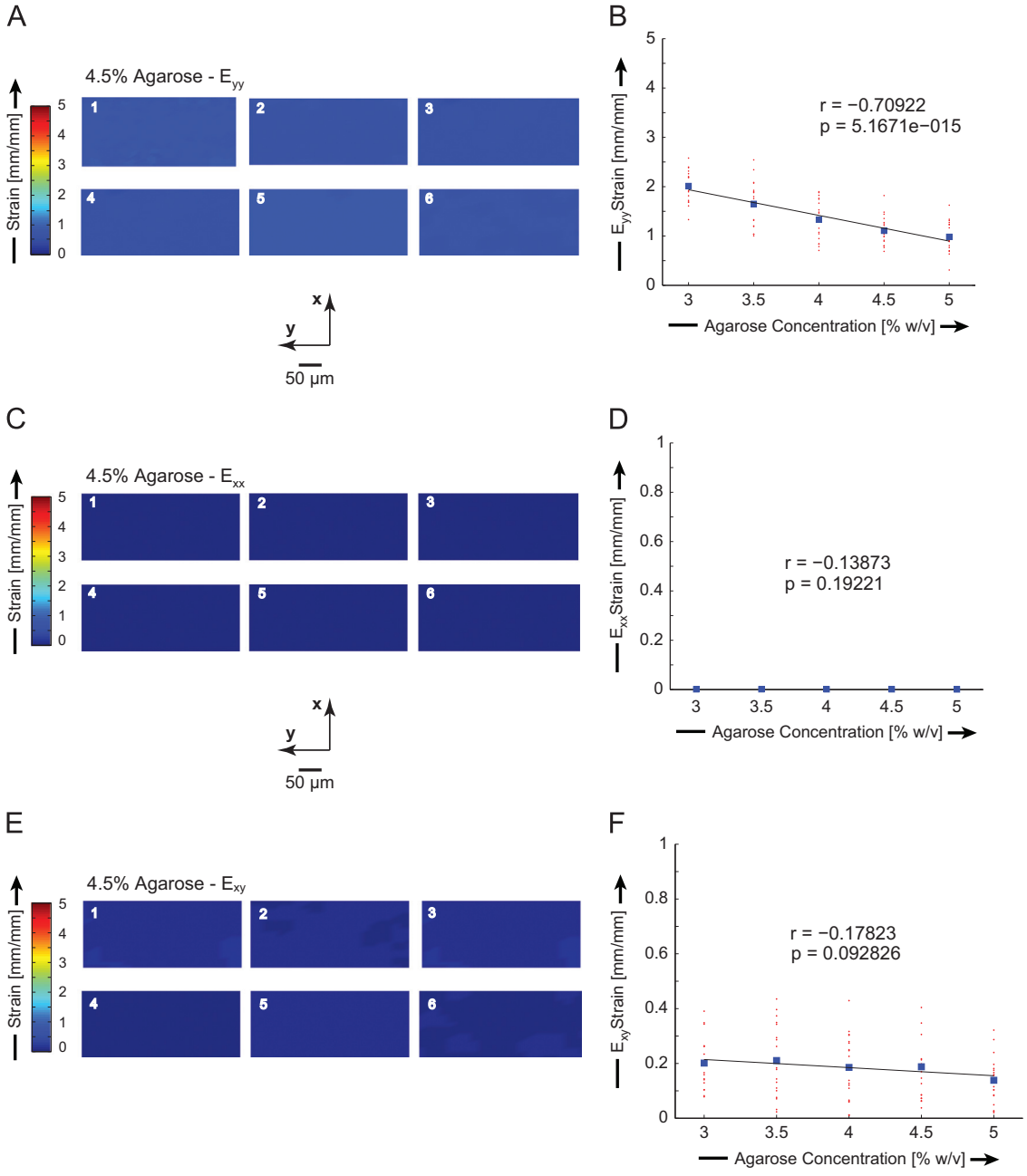

Fig. 1. Finite deformation Lagrangian strain fields within 4.5% agarose hydrogel. Strains were calculated using a finite deformation code in Matlab (Geers et al., 1996). The axial (E_{yy}), transverse (E_{xx}), and shear (E_{xy}) strain fields for 4.5% agarose are plotted. Axial strains were calculated to be 1.11 ± 0.08 [mm/mm]. Transverse strains were calculated to be 0.01 ± 0.00 [mm/mm]. Shear strains were calculated to be 0.18 ± 0.02 [mm/mm]. (A) Representative axial strain image E_{yy}. (B) Axial strain E_{yy} as a function of agarose concentration. (C) Representative transverse strain E_{xx}. (D) Transverse strain E_{xx} as a function of concentration. (E) Representative shear strain E_{xy}. (F) Shear strain E_{xy} as a function of concentration.

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