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Site-specific cell-tissue interactions in rabbit knee joint articular cartilage

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ABSTRACT

Relationships between cartilage structure and superficial *in situ* chondrocyte deformations were investigated from 6 different knee joint locations ($n=10$ knees). Depth dependent cartilage structure and composition were quantified with microscopic/microspectroscopic methods. Medial tibial cartilages had the lowest superficial collagen content, highest collagen orientation angle, and highest proteoglycan content in the pericellular matrix relative to that in the extracellular matrix, coupled with the largest chondrocyte deformations. In contrast, femoral groove and lateral tibial cartilages had the highest superficial collagen contents, lowest collagen orientation angles, and low normalized proteoglycan contents in the pericellular matrix, coupled with the smallest chondrocyte deformations. To study cell-tissue interactions further, observations ($n=57$) from all locations were pooled and a multivariable linear regression was performed. Cell width deformations ($R^2=0.57$) correlated with collagen orientation angle (standardized regression coefficient $\beta=0.398$) and collagen content ($\beta=-0.402$). Cell height deformations ($R^2=0.52$) also correlated with collagen orientation ($\beta=-0.248$) and collagen content ($\beta=0.455$). Cell volume change upon cartilage compression ($R^2=0.41$) correlated with collagen content ($\beta=0.435$) and proteoglycan content ($\beta=0.279$). In conclusion, higher collagen and proteoglycan contents combined with lower collagen orientation angle in the extracellular matrix were related to reductions in superficial chondrocyte deformations. Also, a steep gradient of proteoglycan content from the extracellular to the pericellular matrix was associated with increased cell deformation, particularly in the medial tibial plateau cartilage.

1. Introduction

Articular cartilage consists mainly of water and a solid matrix of a densely woven collagen fibrillar network (mainly type II collagen), which confines negatively charged proteoglycan (PG) aggregates and cartilage cells, chondrocytes (Cohen et al., 1998). Chondrocytes are responsible for the synthesis and maintenance of molecules in the extracellular matrix (ECM), and they are important regulators of joint health (Bader et al., 2011). The distribution, morphology and synthetic activity of chondrocytes vary within the depth of articular cartilage (Clark et al., 2006; Youn et al., 2006) and between knee joint locations (Quinn et al., 2005); as does the structure and function of articular cartilage (Fox et al., 2009; Mäkelä et al., 2014). However, properties of the pericellular matrix (PCM) appear more uniform within cartilage zones (Guilak et al., 2005). Subsequently, the PCM can act as a protective layer to reduce cell strains or as a transducer that amplifies cell strains, depending on the mismatch between PCM and surrounding matrix properties (Choi et al., 2007). PCM modulus is lower than that of the surrounding ECM, varying approximately from 10 to 200 kilopascals (Alexopoulos et al., 2003; Nguyen et al., 2010). As in the ECM, the stiffness of PCM is greater in the collagen split-line direction (McLeod et al., 2013; Wilusz et al., 2013). Overall, PCM is important in modulating the stress-strain and fluid-flow environments around chondrocytes (Alexopoulos et al., 2005; Guo et al., 2014).

Cartilage mechanical and structural properties as well as dimensions, such as stiffness and thickness, vary between locations of the knee joint (Jurvelin et al., 2000; Mäkelä et al., 2014) and regionally within locations (Jurvelin et al., 1986). For example, in rabbits, the patellar cartilage is thicker and softer than the opposing femoral groove cartilage (Fick et al., 2015; Räsänen and Messner, 1996; Turunen et al., 2013a). This difference could be related to adaptations of cartilage to improve patellofemoral joint congruency under various loading conditions (Froimson et al., 1997). Structure and function of cartilage are also altered in a site-specific and depthwise manner after disruption of the knee joint mechanics, for example, due to meniscectomy or anterior cruciate

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ligament transection (Appleyard et al., 2003; Mäkelä et al., 2014). However, information on variations in depthwise cartilage composition between anatomical regions could further improve our understanding of cartilage adaptation to its mechanical environment.

The cellular responses of chondrocytes are ultimately determined by chemical and mechanical signals in their microenvironment (Ramage et al., 2009). Chemical signals include hormones, growth factors, cytokines and other various molecules found in the ECM (Goldring et al., 2006; Zhang, 2014). Mechanical signals include interstitial fluid pressure, fluid velocity and tissue strain, which are ultimately dependent on the properties of cell surroundings, such as the collagen and PG content (Alexopoulos et al., 2005; Julkunen et al., 2009; Nguyen et al., 2009). Chondrocytes sense and react biologically to mechanical forces within articular cartilage at least in part through stretching of the cell membrane and through regulation of the associated mechanosensitive ion channels (Mobasheri et al., 2002) or through direct force transmission via the cell cytoskeleton (Dowling et al., 2013). As the underlying mechanical signals vary spatially, the microenvironments within cartilage and consequently the responses of chondrocytes vary spatially. Here, we focus on the way collagen, proteoglycan and cell strain vary spatially within articular cartilage from various rabbit knee joint sites. More specifically we are investigating how composition of the ECM and PCM relates to previously measured deformations of superficial chondrocytes in six different rabbit knee joint locations (Fick et al., 2015).

These chondrocyte deformations (changes in volume, height and width) were highly site-specific and differences between opposing anatomical regions were evident (see Fig. 1 for details). Cartilage composition was also previously analyzed for each location, but only at a single depth (10% of tissue thickness). Chondrocyte width and height deformations were strongly correlated with collagen orientation angles (Fick et al., 2015), possibly reflecting site-specific adaptations in cell-tissue interactions for optimal cartilage function. It has been suggested that lower ECM PG content or lower ECM collagen fibril modulus lead to higher cell volume loss in mechanically loaded cartilage (Tanska et al., 2013). Moreover, decrease of superficial collagen content combined with disruption of the collagen network orientation has been observed to enhance cell volume recovery after hypotonic loading (Turunen et al., 2013b). These studies imply that cartilage PG and collagen contents affect cell deformations, but surprisingly, no correlation with cell deformations was found previously (Fick et al., 2015). This could be due to an artifact of pooling all samples by location prior to regression, or the choice of the depth (superficial 10%) used in the analysis of tissue composition. Previous studies have also shown that the mechano-chemical properties of the PCM contribute significantly to chondrocyte deformations (Guilak et al., 2006). Especially, high pericellular fixed charge density (FCD) has been related to decreased cell aspect ratio (height/width) and increased von Mises stresses on chondrocytes (Korhonen et al., 2008), as well as increased cell volume loss during compression (Tanska et al., 2013). These observations imply that high pericellular FCD may increase compression of superficial chondrocytes. As such, it would be important to experimentally validate if this is indeed the case.

We hypothesize that cartilage from sites with lower superficial cell deformations and local tissue strains (such as groove and lateral plateau) have higher PG or collagen contents, or lower collagen orientation angles in the superficial layer of cartilage—and subsequently stiffer local ECM—compared to the corresponding contact knee joint locations. In addition, we hypothesize that chondrocytes from sites that have high pericellular PG content relative to the surrounding ECM, and subsequently high PCM/ECM stiffness ratios, undergo high cell deformations (such as medial tibial and patellar chondrocytes). To investigate our hypotheses, collagen content, collagen fibril orientation angle and proteoglycan content were quantified in a depthwise manner throughout the articular cartilage. In addition, pericellular proteoglycan content was determined. These structural parameters were then compared to the corresponding local cell responses. Finally, cell-tissue interactions were studied using linear multivariable correlation analyses.

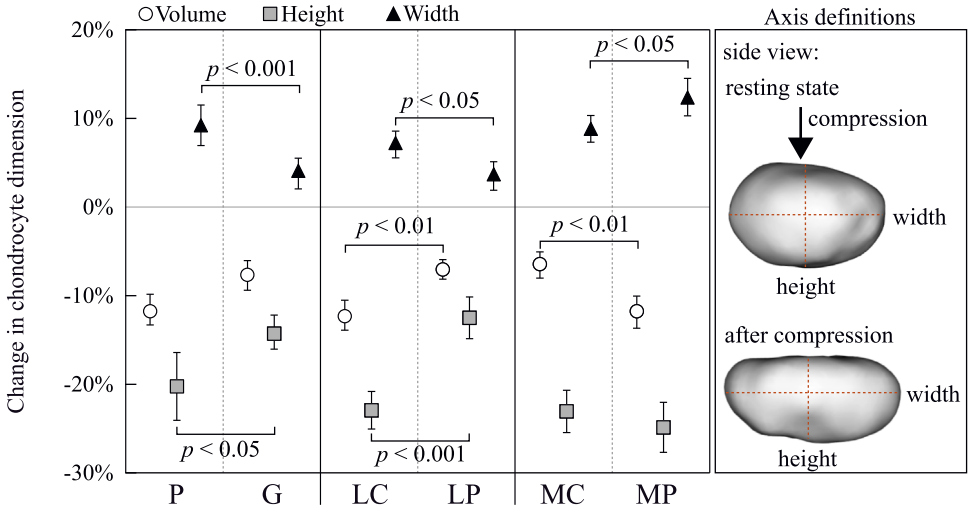

Fig. 1. Previously measured site-specific chondrocyte deformations due to indentation loading and the assigned direction of the cell axes (Fick et al., 2015). These measurements were conducted using a custom confocal laser scanning microscopy system that allows simultaneous indentation and imaging of samples by using a flat-ended cylindrical light-transmittable indenter (diameter 2 mm). Image stacks (512 × 512 pixels, 0.41 × 0.41 μm resolution) were obtained in 0.5 μm increments and the same chondrocytes were analyzed before and after indentation, while keeping the sample compressed. Compression was performed using a nominal pressure of 2 MPa, applied at a rate of 10 μm/s and held for 20 min. The 95% confidence interval range of the resulting mean tissue strain was 12.4–16.4%. Volume, height and width changes of superficial zone chondrocytes due to indentation were compared between groups using an ANOVA statistical test followed by a Tukey post hoc test; differences between anatomical contact sites are shown. Values are given as mean ± 95% confidence intervals. Notations: patella (P), femoral groove (G), lateral/medial femoral condyle (LC/MC) and lateral/medial tibial plateau (LP/MP).