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Effect of crosslinking in cartilage-like collagen microstructures

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ABSTRACT

The mechanical performance of biological tissues is underpinned by a complex and finely balanced structure. Central to this is collagen, the most abundant protein in our bodies, which plays a dominant role in the functioning of tissues, and also in disease. Based on the collagen meshwork of articular cartilage, we have developed a bottom-up spring-node model of collagen and examined the effect of fibril connectivity, implemented by crosslinking, on mechanical behaviour. Although changing individual crosslink stiffness within an order of magnitude had no significant effect on modelling predictions, the density of crosslinks in a meshwork had a substantial impact on its behaviour. Highly crosslinked meshworks maintained a 'normal' configuration under loading, with stronger resistance to deformation and improved recovery relative to sparsely crosslinked meshwork. Stress on individual fibrils, however, was higher in highly crosslinked meshworks. Meshworks with low numbers of crosslinks reconfigured to disease-like states upon deformation and recovery. The importance of collagen interconnectivity may provide insight into the role of ultrastructure and its mechanics in the initiation, and early stages, of diseases such as osteoarthritis.

1. Introduction

Articular cartilage performs an impressive mechanical function, which is underpinned by a hierarchical structural configuration of type II collagen, proteoglycans, and interstitial fluid (Huber et al., 2000). As one of the main determinants of function in the tissue, changes to the collagen meshwork are central to disease processes (Hwang et al., 1992; Hunziker, 1999; Brown et al., 2012; Stolz et al., 2009). Collagen-collagen interactions dominate the cohesive strength of the matrix (Broom and Silyn-Roberts, 1990) and therefore the resistance to mechanical damage progression.

The breakdown from the intermeshed, pseudo-random collagen configuration at the micrometer scale to form aligned fibre bundles has been identified as a mechanically irreversible step in the damage process (Brown et al., 2012) and has long been associated with abnormal cartilage softening (Broom, 1982). A recent study has further found localised regions of collagen meshwork disruption and reconfiguration at early stages of disease before the appearance of histological changes (Brown et al., 2014). An improved understanding of the mechanics of the collagen meshwork, and the implications of its properties and connectivity, is therefore of interest for osteoarthritis pathogenesis, diagnostics and the design of regenerative medicine strategies.

Computational modelling provides an ideal platform from which to explore mechano-structural changes. In recent years, increasingly sophisticated constitutive models have been developed to represent tissue-level cartilage mechanics (Wilson et al., 2006; Pierce et al., 2013; Ateshian et al., 2009; Wilson et al., 2005; Deneweth et al., 2013; Wilson et al., 2007). With this sophistication has come an improved representation of structure. Wilson et al., for example, integrated the relation between permeability and tissue composition with a viscoelastic constitutive relation within a fibril-reinforced model for predicting the equilibrium and transient response of articular cartilage during compression, indentation and swelling tests (Wilson et al., 2006). Ateshian et al. applied continuous fibre angular distributions to model the solid matrix of cartilage and successfully predicted experimental observations of the tissue's equilibrium response to mechanical and osmotic loading (Ateshian et al., 2009).

However, such models do not incorporate details from individual fibres but instead consider the impact of average fibril orientations on the scale of continuum. In particular, while there have been advances in the theoretical mechanics of upscaling (e.g. Quintard and Whitaker, 1994; Cushman et al., 2002; Davit et al., 2013), whereby material properties at the sub-continuum scale are systematically incorporated into the constitutive relation of continuum models with controlled accuracy, such approaches are generally not tractable for collagen

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networks and cartilage (Buehler, 2006; Klika et al., 2016). Hence, it is currently not feasible to assess the impact of fibril-scale changes and individual crosslinks in a continuum model. Thus one must instead adopt a bottom-up approach and represent collagen fibrils individually; furthermore elastic dominated constitutive relationships are indicated for fibrils (Buehler, 2006) in contrast to entropic dominated models at lower scales. An exemplar of such a model has already been explored by Lee et al. (2014), who concentrate on elucidating the stress strain relationship of material made from networks of collagen fibrils. In contrast, here, our objective is to explore how crosslink properties and densities within a collagen meshwork representing cartilage impact on mechanical performance and structure, in particular fibril alignment as it is a signature of cartilage pathology (Broom, 1982; Chen and Broom, 1998; Brown et al., 2012).

2. Methods

2.1. Collagen structure simulation

A 2-D model of collagen structure was implemented in C/C++. For simplicity collagen fibrils were not represented as Euler-Bernoulli filaments; instead each fibril was modelled as a series of 1-D springs of length approximately 1 μm connected at nodes, via torque free pin joints. Assuming a fibril diameter of 100 nm and a linear stress-strain relation, the material property of the fibrils were calculated by fitting experimental data from a single fibril tensile test (van der Rijt et al., 2006) to give a force-strain relation of 4 μN/unit strain (equivalent to a Young's modulus ≈500 MPa). Crosslinks were implemented with a linear force-strain relation on the assumption of small deformation, with parameters chosen a posteriori due to a lack of available data. It should be noted that crosslinking in this model refers to inter-fibril connectivity, and does not probe, for example, enzymatic or AGE-related crosslinking (Buehler, 2006; Chen et al., 2002). Validation of the fibril implementation was performed using a single fibril of length 20 μm (20 segments) under load in one dimension. Validation of crosslink behaviour was similarly performed by linking two fibrils in series. Each spring had the linear force-strain relation

$$F = k\left(\frac{L' - L_0}{L_0}\right), \tag{1}$$

where L' was the displaced length of the spring, L_0 was the resting length of the spring, F was force and k is the spring constant, a material property. The error in the numerical solution, relative to the analytical solution, was considered by calculating the spring length on reaching equilibrium (ie. all spring movement was below a very small tolerance). The accuracy of numerical solution was better than 99.9% (see Table 1).

Thirty collagen meshwork configurations were constructed to simulate the pseudo-random collagen microstructure observed in electron micrographs (Teshima et al., 1995; Stolz et al., 2009; Broom et al., 2001), with anisotropy and connectivity forming the Benninghoff arcades (Benninghoff, 1925) at larger scales. A representative structure is given in Fig. 1A. Each node, shown in yellow, is connected to two neighbours on the same fibre by linear springs, shown in green. All structures were based on 30 parallel sets of 24 springs, giving a total length 24 μm, initially aligned with the y-axis and separated by 1 μm in the x direction. Nodes were then randomly displaced in x direction within ±1 μm. Crosslinks (shown in red in Fig. 1) were incorporated based on proximity of nodes from adjacent fibrils. Proximity thresholds of 0.3 μm, 0.5 μm and 0.8 μm were applied to each structure, with a linear stress-strain relation for the crosslink varied between 2 and 12 μN/unit strain. Once a crosslink formed in the model, it was not allowed to break.

Table 1
Numerical spring length error under one dimensional tensile test load for an isolated spring segment (single spring) and a single, 20-segment spring (single fibril).

	single spring	single fibril		
Spring resting length	1 μm	0.5 μm	1 μm	2 μm
Error(%)	−0.006	−0.022	−0.003	−0.003

Uniaxial tensile loading, in the direction of predominant fibril alignment, was applied as an exemplar to simplify the complex stress environment of the tissue while capturing the tensile response of the collagen meshwork to macroscale applied loads (Wilson et al., 2004; Thambyah and Broom, 2006). In each simulation, the end nodes of each fibril were fixed in the x-direction and subjected to a constant tensile force of 0.5 μN in the y-direction. Node positions were time-stepped according to a linear overdamping law. When the structure reached equilibrium, the load was released and the structure allowed to recover. Node positions were recorded at original, equilibrium and recovered positions and passed to MATLAB (2015a, The MathWorks Inc., Natick, USA) for analysis.

Strains and fibril organisations were calculated for each simulation. Fibril strains in each structure were calculated based on the change in distance between adjacent nodes. Bulk strains were calculated based on the mean distance between the fibril end nodes. Organisation was classified using an anisotropy parameter r_{mean} based on polarised optical parameters used for cartilage measurements (Houle et al., 2015; Couture et al., 2015; Campagnola and Loew, 2003), providing a means for comparison with experiments. In particular r_{mean} is defined by

$$r_{mean} = \frac{1}{N}\sum_{i=1}^{N}\frac{\delta Y_i - \delta X_i}{\delta Y_i + 2\delta X_i}, \tag{2}$$

where N is total number of springs, $\delta X_i = |\delta x_{i+1} - \delta x_i|$, $\delta Y_i = |\delta y_{i+1} - \delta y_i|$ and δx_i and δy_i are the x and y positions of the ith node. Note for instance that when $r_{mean} = 1$, the fibrils are aligned in the direction of loading.

3. Results

3.1. Effect of crosslink density

Representative microstructures before and after stretching (along the y direction) and after relaxation are shown in Fig. 1. Qualitatively, different numbers of crosslinks resulted in substantially different configurations after loading. Highly-crosslinked microstructures maintained a 'normal' configuration with loading (Fig. 2A). Microstructures with lower crosslink densities were more aligned with the direction of loading, and formed fibre bundles (Fig. 2B) similar to those observed in electron microscopy of osteoarthritic cartilage (Fig. 2C (Chen and Broom, 1998)).

For a given structure and crosslinking threshold, the crosslink density was inversely proportional to the anisotropy measure of the pre-loaded meshwork structure (Fig. 3), with $r^2 > 0.9$ (Pearson's correlation coefficient).

Quantitative differences due to crosslink density were also observed. Due to the random modification of node positions for each microstructure, threshold distances for crosslinking produced a range of crosslink densities in the structures. The reconfiguration of fibrils under loading, quantified by the above anisotropy parameter r_{mean} (Eq. (2)) is presented in Fig. 4. At low crosslink densities, fibrils aligned with the direction of load, with minimal recovery. At higher crosslink densities, the microstructures resisted realignment (Fig. 4A) and recovered their isotropy to a greater extent (Fig. 4C). The maximum recovery of isotropy (% recovered r_{mean} relative to r_{mean} at equilibrium), however, was only 35%.

For a fixed crosslink proximity threshold, bulk strain data clustered into groups. Within these groups (i.e. black, green or blue dots in