Journal of the Mechanical Behavior of Biomedical Materials 75 (2017) 390–398

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

How a decreased fibrillar interconnectivity influences stiffness and swelling properties during early cartilage degeneration

Mieke Nickien[a,b,*], Ashvin Thambyah[a], Neil D. Broom[a]

[a] Department of Chemical and Materials Engineering, The University of Auckland, New Zealand
[b] Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands

ARTICLE INFO

Keywords:
Cartilage
Degeneration
Collagen network de-structuring
Stiffness
Swelling

ABSTRACT

Objective: The functional coupling between the fibrillar network and the high-swelling proteoglycans largely determines the mechanical properties of the articular cartilage matrix. The objective of this new study was to show specifically how changes in fibrillar interconnectivity arising from early cartilage degeneration influence transverse stiffness and swelling properties at the tissue level.

Design: Radial zone transverse layers of cartilage matrix were obtained from intact and mildly degenerate bovine patellae. Each layer was then subdivided to assess tensile stiffness, free-swelling response, glycosaminoglycan (GAG) content, and micro- and ultra-structural features.

Results: The tensile modulus was significantly lower and the degree of swelling significantly higher for the degenerate matrix compared to the intact. Scanning electron microscopy revealed a homogeneous response to transverse strain in the intact cartilage, whereas large non-fibrillar spaces between fibril aggregates were visible in the degenerate matrix. Although there were no significant differences in GAG content it did correlate significantly with stiffness and swelling in the intact samples but not in the degenerate.

Conclusions: The lower degree of fibril network interconnectivity in the degenerate matrix led to both a decreased transverse stiffness and reduced resistance to osmotic swelling. This network 'de-structuring' also resulted in a reduced functional interaction between the fibrillar network and the proteoglycans. The study provides new insights into the role of the fibrillar network and how changes in the network arising from the degenerative cascade will influence tissue level behaviour.

1. Introduction

Articular cartilage is a high weight-bearing biological material that consists of several distinct phases contributing to its mechanical function (Urban et al., 1979; Eisenberg and Grodzinsky, 1985; Lai et al., 1991; Buschmann and Grodzinsky, 1995; Lu et al., 2007; Korhonen and Herzog, 2008). First is the solid phase consisting mainly of the collagen fibrillar network, together with the chondrocytes and proteoglycans (PGs). Second is the fluid phase largely made up of water (up to 80% of tissue weight), and third is the ionic phase arising mostly from the presence of negatively charged PG aggregates. The collagen fibrils provide the constraining network within which the entrapped hydrophilic PGs can draw in water from the interstitial space and thus create within the matrix an intrinsic swelling pressure. This hydrostatic pressure is further enhanced by an osmotic swelling pressure arising from the excess concentration of mobile counter ions electrostatically drawn to the fixed negative charges of the PG chains. The matrix swelling pressure is balanced by the tensile resistance of the fibrillar network as well as the frictional drag on fluid flow when the matrix is deformed. Intuitively therefore, from a purely structural standpoint, one could suggest that the permeability of the matrix, and hence the microstructure of the solid phase and especially the fibrillar network, is of fundamental importance in governing the weight-bearing properties of cartilage.

It is therefore not surprising that cartilage mechanics studies attempting to incorporate the 'triphasic' nature of cartilage (Lai et al., 1991; Lu et al., 2007), while partially successful, have several underlying structure-related limitations (Taylor and Miller, 2006; Julkunen et al., 2013). First, the solid phase model does not consider the structural heterogeneity of the fibrillar network and associated complexity in its permeability. Second, the model also assumes that the solid phase is linear elastic and intrinsically non-dissipative. Third, the zonally-differentiated fibrillar architecture, and associated permeability variation, is ignored.

In view of the above limitations various hyperelastic models have been developed in order to account for (i) the non-linearity in the solid

* Corresponding author at: Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands.

http://dx.doi.org/10.1016/j.jmbbm.2017.07.042
Received 30 March 2017; Received in revised form 10 July 2017; Accepted 26 July 2017
Available online 03 August 2017

phase cartilage stress-strain response (Lai et al., 1991; Holmes and Mow, 1990; Guo et al., 2015), (ii) the strain dependent permeability that more realistically represents the changing porosity and hence fluid flow with increasing compression of the matrix (Ateshian et al., 1997; Chahine et al., 2004), and (iii) the zonally differentiated ultrastructure via fibril-reinforced biphasic models (Julkunen et al., 2013; Wilson et al., 2005). These advances in modelling have greatly improved not only the simulation of actual cartilage mechanics, but also advanced our understanding of the mechano-structural complexity of articular cartilage and the development of strategies to optimise the relationship between model complexity, cost and relevant function (Julkunen et al., 2013).

In addition to the zonally oriented fibrils in a single primary direction, some cartilage models also incorporate a randomly oriented network of secondary fibrils in order to better mimic the creep response (Wilson et al., 2004; Julkunen et al., 2008). One important complexity that has, however, yet to be addressed in modelling work is that regarding the ultrastructural reality of the fibrillar matrix. Electron microscopy has revealed that the collagen matrix is configured by a dense interwoven fibrillar network which includes lateral or transverse interconnections between the radial fibrils so as to maintain a cohesive structure (Broom and Silyn-Roberts, 1989; Broom et al., 2001; Chen and Broom, 1998; Chen and Broom, 1999). Besides there being some evidence of actual physical entwinement between fibrils (Broom and Silyn-Roberts, 1989), it is thought that the bulk of the transverse connectivity is achieved by some form of biochemically-mediated interfibril linkage (Broom et al., 2001; Chen and Broom, 1998; Chen and Broom, 1999). There have been studies that show such chemical interactions can be achieved by non-enzymatic glycation products and linking proteins such as small leucine-rich proteins (SLRPs), cartilage oligomeric protein (COMP), collagen IX and certain matrillins (Nickien et al., 2013). In the degenerative process such as that associated with osteoarthritis, the loss of transverse interconnectivity in the fibrillar network, also referred to as 'de-structuring', occurs very early and even before macro level mechanical properties can be detected (Nickien et al., 2013; Thambyah et al., 2011). Thus, to a large extent the mechanical significance of this de-structuring, remains unknown.

Previous publications have shown a decreased transverse matrix stiffness (Akizuki et al., 1986; Roberts et al., 1986; Kempson et al., 1973; Kempson, 1982; Temple et al., 2007; Temple-Wong et al., 2009; Bader et al., 1981) or an increased matrix swelling tendency (Chen and Broom, 1999; Maroudas, 1976; Venn and Maroudas, 1977; Maroudas and Venn, 1977; Bank et al., 2000; Broom and Flachsmann, 2003) in degenerate cartilage but have not investigated the effect of fibrillar interconnectivity on its combined stretching and swelling properties. Experimental work on degenerate tissue with established fibrillar de-structuring has revealed that the manner in which loads are transmitted laterally away from directly loaded regions of the matrix is severely limited following a loss of fibrillar interconnectivity (Thambyah et al., 2011; Thambyah and Broom, 2007). However, these studies still offer little insight into the effectiveness of the fibrillar network in constraining the swelling pressure as a function of its level of de-structuring and loss in connectivity. Cartilage computer models may include mechanisms of damage of the collagen network or ground substance due to overloading or degeneration, with related softening, but de-structuring is not yet included in these approaches (Mononen et al., 2011; Hosseini et al., 2014; Mononen et al., 2016). A more rigorous understanding of this coupled solid-fluid response and importantly from a microstructural standpoint, should contribute to the development of improved models of cartilage mechanics and also incorporate more effectively the influence of early degenerative changes.

The motivation for this study therefore was to establish the extent of fibrillar connectivity as a defining factor in cartilage mechanical behaviour. The study hypothesis was that the decreased fibrillar interconnectivity in the degenerate cartilage matrix will lead to increased swelling and a decreased transverse stiffness.

2. Methods

2.1. Tissues

The bovine patella has been reported as a suitable model for human osteoarthritis, and was therefore utilized in this study (Hargrave-Thomas et al., 2013). Fresh bovine patellae were obtained from prime male animals slaughtered at ~2–3 years of age and from mature female animals slaughtered at ~5–9 years, and then stored at −28 °C for a period of no more than 6 months. Prior to any experimentation each patella was thawed in cold running water and then stained with Indian ink to reveal any surface fibrillation (Meachim, 1972). Only patellae exhibiting localised staining profiles of G0 (from male animals showing no uptake of India ink) and G2 (from female animals showing softening and swelling of cartilage with slight India ink uptake) were used in this study (Outerbridge, 1961).

2.2. Sample preparation and macroscopic analysis

A single cartilage-on-bone block with en face dimensions of ~15 × 15 mm and ~ 20 mm of subchondral bone was obtained from the distal-lateral quadrant of each patella. This site was selected both because of its relatively planar articular surface and, most importantly, because it is the region on the articular surface of the patella exhibiting the earliest signs of fibrillation (Broom and Flachsmann, 2003; Thambyah and Broom, 2007; Hargrave-Thomas et al., 2013). The four sides of the sample were then ground with 40 grit carborundum paper to ensure that the exposed cartilage/bone cross-sections were initially flush and the sample was equilibrated in 0.15 M saline at 4 °C for one hour. Two of the flush-ground sides of the blocks were then photographed and measurements of cartilage on-bone lateral bulging and radial thickness were obtained. These macro-level measurements provided an additional, albeit crude, means of confirming the relative normality or degeneration of the AC based on its propensity for both lateral and radial swelling.

For the normal samples with their lesser thickness of AC the uppermost 400 μm layer containing the tangential and transition zones was removed using a sledging microtome and discarded. The next 400 μm layer of AC was then similarly removed and this furnished a test sample that consisted almost entirely of the radial zone matrix adjacent to but not including any calcified cartilage (see Fig. 1). The absence of any matrix texture other than radial was confirmed with differential interference contrast (DIC) microscopy. For the much thicker degenerate samples a layer of up to 800 μm could be removed and still furnish another 400 μm thick layer of radial zone cartilage immediately adjacent to the calcified zone. The above procedure produced transverse samples for experimentation that were consistently taken from the deeper general matrix and not affected by the penetration of radial fissures associated with the fibrillated tissues.

The isolated layers were then subdivided into four parts: the first (~5 × 15 mm^2) for mechanical assessment, the second (~3 × 3 mm^2) for swelling studies, the third (~5 × 5 mm^2) for biochemical assays, and the fourth for micro- and ultra-structural analysis (see Fig. 1).

2.3. Tissue stiffness measurements

A 15% isostrain modulus perpendicular to the radial direction was obtained from each of the ~5 × 15 mm^2 transverse samples as follows: - Based on the 400 μm layer thickness and measured width the sample's cross-sectional area was determined. The sample was then inserted in custom-built minigrips fitted to an Instron® testing machine (model 5543) equipped with a 50 N load cell. To ensure there was no pre-strain in the sample prior to testing the sample was hung axially aligned from the upper grip and then introduced into the lower grip while aiming for a pre-load gauge length of ~12 mm. The sample was then carefully extended without any detectable increase in load only until any obvious