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Matrix degradation in osteoarthritis primes the superficial region of cartilage for mechanical damage

Megan E. Cooke [a,b,*], Bernard M. Lawless [c], Simon W. Jones [b], Liam M. Grover [a]

[a] School of Chemical Engineering, University of Birmingham, Birmingham, UK
[b] MRC ARUK Centre for Musculoskeletal Ageing, QE Hospital, University of Birmingham, Birmingham, UK
[c] Department of Mechanical Engineering, University of Birmingham, Birmingham, UK

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ABSTRACT

Osteoarthritis (OA) is a degenerative disease that affects 25% of the world's population over fifty years of age. It is a chronic disease of the synovial joints, primarily the hip and knee. The main pathologies are degradation of the articular cartilage and changes to the subchondral bone, as a result of both mechanical wear and a locally elevated inflammatory state. This study compares the viscoelastic properties of cartilage that represents the biochemical changes in OA and age-matched healthy tissue. Further, the mechanical damage induced by this compressive loading cycle was characterised and the mechanism for it was investigated. The storage modulus of OA cartilage was shown to be significantly lower than that of healthy cartilage whilst having a higher capacity to hold water. Following mechanical testing, there was a significant increase in the surface roughness of OA cartilage. This change in surface structure occurred following a reduction in sulphated glycosaminoglycan content of the superficial region in OA, as seen by alcian blue staining and quantified by micro X-ray fluorescence. These findings are important in understanding how the chemical changes to cartilage matrix in OA influence its dynamic mechanical properties and structural integrity.

Statement of significance

Cartilage has a very specialised tissue structure which acts to resist compressive loading. In osteoarthritis (OA), there is both mechanically- and chemically-induced damage to cartilage, resulting in severe degradation of the tissue. In this study we have undertaken a detailed mechanical and chemical analysis of macroscopically undamaged OA and healthy cartilage tissue. We have demonstrated, for the first time in human tissue, that the mechanical degradation of the tissue is attributed to a chemical change across the structure. In macroscopically undamaged OA tissue, there is a reduction in the elastic response of cartilage tissue and an associated destabilisation of the matrix that leaves it susceptible to damage. Understanding this allows us to better understand the progression of OA to design better therapeutic interventions.

1. Introduction

In osteoarthritis (OA), a degenerative disease of synovial joints, there are both mechanical and inflammation-induced processes that result in changes to joint tissues [1–3]. Of these, the most commonly reported is degeneration of hyaline cartilage, whilst there are also pathological changes to the subchondral bone and synovium [4,5]. Cartilage is avascular and therefore has a limited capacity for self-repair, so structural damage to its surface is largely irreversible. It has three main non-water components: chondrocytes, a network of collagen type II (Col II), and proteoglycans. These proteoglycans bind water, which, in turn, makes up between 70–80% of cartilage by weight [6,7]. The distribution and alignment of these three constituents results in a graduated tissue structure which brings about distinct mechanical properties [8].

Mechanically, cartilage provides both a low-friction surface for articulation of the joint and acts to transmit compressive loading forces to the underlying subchondral bone. Similar to many other tissues in the body, it exhibits time-dependent viscous and elastic

* Corresponding author at: School of Chemical Engineering, University of Birmingham, Birmingham, UK
E-mail address: megan.cooke.research@gmail.com (M.E. Cooke).

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behaviour in both compression and tension [9]. The superficial region consists of Col II fibres oriented parallel to the articulating surface giving local tensile strength. This collagen network becomes more disordered in the middle region and in the deep zone and through the tidemark, fibres are aligned perpendicular to the articulating surface. In OA, the Col II network deteriorates, starting from the articulating surface in early OA and progressing through the tissue with increasing OA severity, as measured by Hollander et al., using Mankin grading [10]. Aggrecan, the most common proteoglycan, is found primarily in the middle and deep zones. It is the second most abundant protein in cartilage following Col II and it sits along a hyaluronic acid backbone. Individual aggrecan molecules have sulphated glycosaminoglycan (sGAG) chains (keratan and chondroitin sulphate) coming from the aggrecan core protein that form a bottle-brush structure. These sGAG chains have a negative charge resulting in a high binding affinity to water.

The binding of water to proteoglycans is partly responsible for the compressive mechanical properties of cartilage. The carboxyl and sulphate groups of chondroitin sulphate and sulphate group of keratan sulphate provide fixed charge density (FCD) to the tissue, which, in combination with repulsion between neighbouring negatively charged chains results in a high osmotic pressure [11]. In healthy cartilage this pressure is compensated by the Col II network which restricts over-swelling and allows the tissue to resist compressive forces [11]. In OA an increase in matrix metalloproteins (MMPs) and aggrecanases, particularly members of the ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family, have been identified as primary factors in the catabolism of articular cartilage matrix proteins including aggrecan and Col II [2,10]. Changes in these matrix components have been shown to affect the mechanical properties of human cartilage [12] and the dynamic mechanical properties of canine tissue with destabilisation-induced OA [13].

The viscoelastic properties of a material can be quantified by numerous methods including creep, stress relaxation and dynamic mechanical analysis (DMA). Unlike creep and stress relaxation which usually have long time constants, DMA is a dynamic testing method. By applying an oscillating force to a specimen and analysis of the out-of-phase displacement response, the frequency-dependent storage and loss moduli can be calculated. The storage modulus (E') describes a material's ability to store energy for elastic recoil while the loss modulus (E'') characterises the material's ability to dissipate energy [14]. Studies have examined articular cartilage at low strain rates [15,16] and others have used DMA to assess frequency-dependency of the viscoelastic properties of cartilage in bovine and human specimens [17]. However, the effects of OA on the viscoelastic properties of human cartilage have so far only been examined using indentation methods and these looked at femoral head cartilage, where the direction and magnitude of loading is harder to define [18].

Changes to both the mechanical and physicochemical properties of cartilage are of interest in osteoarthritis, and physical techniques for tissue analysis are becoming more commonly utilised. As such, quantitative mapping of chemical elements present in tissues is now possible using micro X-ray fluorescence. This gives good spatial resolution of elements and has been used in a number of tissues including cartilage [19–21]. Other quantitative techniques that are traditionally used in materials science but not biological analysis such as thermogravimetric analysis are also being transferred to understand the chemical structure of tissues and biomaterials. Cartilage is interesting thermogravimetrically due to its high water content, and such, the changes in hydration in different disease states and from different anatomical locations are interesting when considering the importance of water on the inherent mechanical properties of cartilage [22]. In OA, there is an observed change in the joint surface as cartilage becomes fibrillated. Interferometry is a commonly used technique in the analysis of surfaces but as yet its use in characterising biological surfaces is infrequent. These techniques can all be used to enhance knowledge and understanding of biological tissues.

Varus knee alignment as measured by the femorotibial angle has been shown to be a key factor for development and progression of OA [23]. In this position, the medial aspect of the knee joint experiences more damaging mechanical loading, as seen by a larger reduction in cartilage thickness [24]. However, the local inflammatory environment affects both aspects of the joint equally as the synovial fluid fills the joint space. In this study we have used human cartilage from the lateral aspect of the femoral condyles (Fig. 1) of OA subjects. This tissue is exposed to the same chemical environment but is macroscopically undamaged. As yet, no one has studied the links between the early mechanical and chemical changes to human OA cartilage. Therefore, the aim of this study was to determine the dynamic mechanical properties at a range of physiological frequencies and undertake a detailed chemical analysis to understand the differences observed in early, less structurally degraded, OA and healthy cartilage from the same anatomical region.

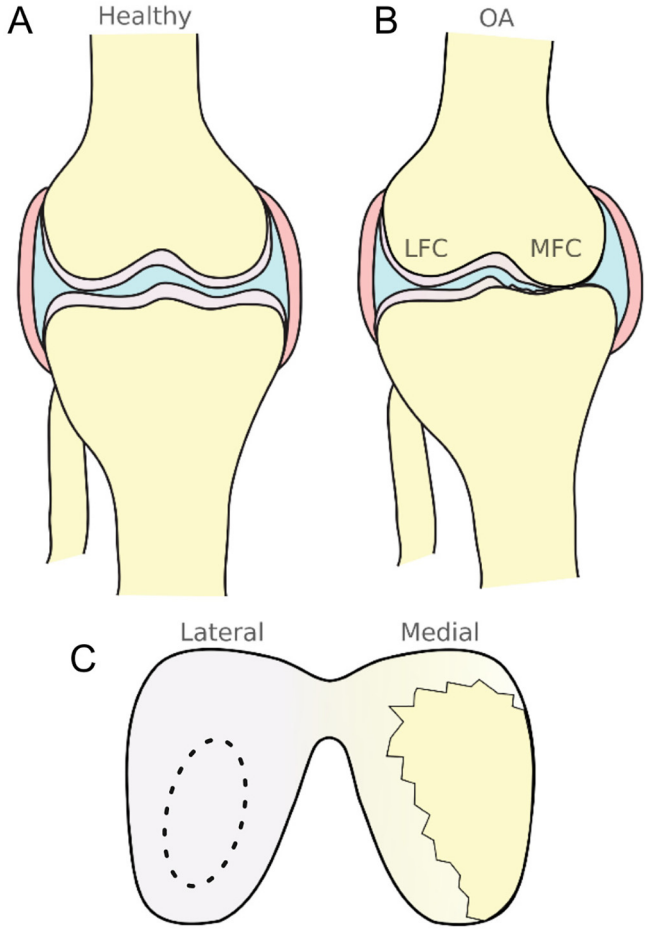

Fig. 1. Representation of healthy (A) and OA (B,C) joint structures. Varus loading in OA causes a reduced joint space in the medial aspect such that cartilage of the medial aspect including the medial femoral condyle (MFC) undergoes severe mechanical degradation, revealing the underlying subchondral bone. The lateral aspect of the joint maintains a wider joint spacing such that tissue of the lateral femoral condyle (LFC) experiences less mechanical damage. The dashed region in C indicates where cartilage explants were taken for this study.

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