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The micromechanics of the superficial zone of articular cartilage

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SUMMARY

Objective: To investigate the relationships between the unique mechanical and structural properties of the superficial zone of articular cartilage on the microscopic scale.
Design: Fresh unstained equine metacarpophalangeal cartilage samples were mounted on tensile and compressive loading rigs on the stage of a multiphoton microscope. Sequential image stacks were acquired under incremental loads together with simultaneous measurements of the applied stress and strain. Second harmonic generation was used to visualise the collagen fibre network, while two photon fluorescence was used to visualise elastin fibres and cells. The changes visualised by each modality were tracked between successive loads.
Results: The deformation of the cartilage matrix was heterogeneous on the microscopic length scale. This was evident from local strain maps, which showed shearing between different regions of collagen under tensile strain, corrugations in the articular surface at higher tensile strains and a non-uniform distribution of compressive strain in the axial direction. Chondrocytes elongated and rotated under tensile strain and were compressed in the axial direction under compressive load. The magnitude of deformation varied between cells, indicating differences in either load transmission through the matrix or the mechanical properties of individual cells. Under tensile loading the reorganisation of the elastin network differed from a homogeneous elastic response, indicating that it forms a functional structure.
Conclusions: This study highlights the complexity of superficial zone mechanics and demonstrates that the response of the collagen matrix, elastin fibres and chondrocytes are all heterogeneous on the microscopic scale.

Introduction

Articular cartilage forms a low friction shock absorbing layer at the ends of bones in synovial joints. It contains few cells and consists mainly of a highly hydrated proteoglycan gel constrained within a network of type II collagen[1]. Cartilage structure changes with depth from the articular surface and is classified into three zones (superficial, transitional and deep) based on the organisation and degree of alignment of the collagen fibres (Fig. 1)[1,2].

The superficial zone has several distinct structural features and we are interested in the role these play in its unique mechanical properties. In this zone, the collagen fibres are aligned parallel to the articular surface compared to the perpendicular fibres in the deep zone (Fig. 1). The proteoglycan content and fixed charge density are lower than in the deep tissue[3,4]. In addition it contains an extensive network of elastin fibres which are roughly aligned with the collagen fibres in the plane parallel to the surface[5–9]. Their role is completely unexplored and is therefore a target of this investigation. The superficial zone chondrocytes are morphologically different, being disc shaped in the plane parallel to the articular surface unlike the more spherical chondrocytes in the deeper zones. Their pericellular matrix[10] also differs, with both elastin[7] and lipids[11] being present only in the superficial zone pericellular matrix. They also have metabolic differences with a lower rate of proteoglycan and protein synthesis than in the deeper zones[12] and a different metabolic response to tensile loading[13].

The bulk mechanical properties of this zone of cartilage are reasonably well defined. It has a lower compressive modulus than the deeper tissue[4] resulting in more matrix deformation and larger changes in chondron volume[14] under compressive load. Its high water content[15] and permeability result in interstitial fluid being exuded into the joint cavity under minimal compressive loading[16] which is important to joint tribology[17].

Although tensile loads are not generally considered to be physiological, Neu *et al.*[18] demonstrated that compressive loading

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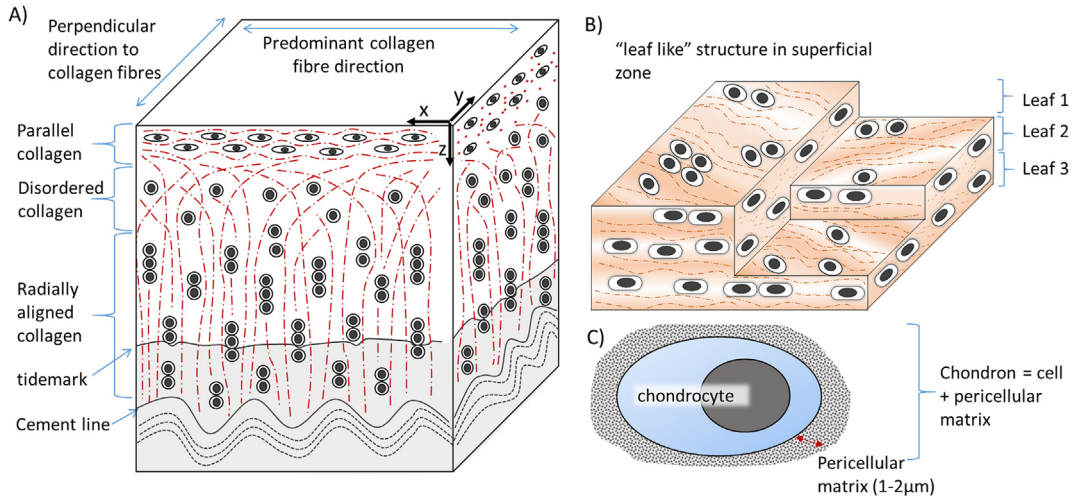

Fig. 1. Schematic diagrams to show the change in cartilage structure with depth (A), the leaf like structure at the surface (B) and a chondron (C).

gives rise to tensile forces parallel to the surface in the superficial zone cartilage. Previous tensile testing studies demonstrated that the tensile modulus is largest in the direction parallel to the predominant collagen fibre orientation[19–21], and the tensile modulus decreases with depth, with the superficial zone being up to 5 times stiffer than the deep zone[20–22]. The tensile properties vary across joints in line with loading patterns *in vivo*[23] and the tensile modulus decreases with fibrillation, osteoarthritic degeneration[23] and age[24,25]. Collagen fibres are able to support very high tensile loads and under compressive loading the applied pressure, coupled with osmotic swelling generated by the proteoglycans is arrested by tension in the collagen fibres, preventing lateral over-expansion. Under transient loading conditions the proteoglycans also play a role in determining the rate of redistribution of interstitial fluid[24].

In this study we combine microscopic observations with mechanical loading to look at the microstructural response of cartilage to both compressive and tensile loads. This was made possible through the use of two multiphoton microscopy techniques; two-photon fluorescence (TPF) and second harmonic generation (SHG). TPF differs from single photon fluorescence as the fluorophores are excited by the simultaneous absorption of 2 infrared photons instead of a single visible photon[26]. In cartilage, endogenous fluorescence provides contrast for the cells and elastin fibres[5,7]. In SHG 2 infrared photons are simultaneously absorbed and a single visible photon is emitted at exactly half the original wavelength and this only occurs in highly ordered crystalline materials which lack inversion symmetry[27]. In cartilage, SHG reveals the collagen matrix[5,28,29]. Like confocal microscopy they allow 3D imaging at submicron resolution. They avoid the need for sectioning, fixing or staining of the sample making them ideal for imaging in mechanical studies as the material properties should remain unaltered[21,30–33]. This work builds upon a previous study using TPF to investigate the response of articular cartilage to tensile load, which revealed unexpected heterogeneity in the microscopic strain fields[21].

We first investigate the elastic moduli and Poisson's ratios of the superficial zone under tensile and compressive loads, taking advantage of multiphoton microscopy to measure their micron-scale variations in fresh tissue. We then investigate the microstructural bases of these properties, paying particular attention to the behaviour of the elastin fibres and cells. Finally we discuss the effect of early degenerative changes.

Methods

Tissue preparation

Thoracic legs from 16 horses aged 3–14 years were obtained from a local abattoir (Potters, Taunton). Horses older than 14, skeletally immature or lame were excluded from the study. The metacarpophalangeal joints were opened and cartilage plugs were removed from the proximal phalanx within 3 h of euthanasia. ten samples were used for tensile testing and 6 for compressive loading. As cartilage is stiffer when strained parallel to the predominant collagen fibre direction, cartilage strips were taken both parallel and perpendicular to the predominant collagen fibre orientation as defined by split line measurements (see Supplementary information) in order to fully characterise its tensile properties in this plane.

Multiphoton microscopy

SHG and TPF were excited using the 810 nm output of a Ti:Sapphire laser (Mira 900 Coherent) with 100fs pulses and a 76 MHz repetition rate.

For the tensile loading experiments, we imaged with a modified, non-inverted confocal laser scanning microscope (FluoView 300 and BX51 Olympus UK) with a 1NA 60x long working distance dipping lens (LUMPLFLN 60XW Olympus UK). For the compressive loading experiments, we imaged with a modified inverted confocal laser scanning microscope (FluoView 300 and IX71 Olympus UK) with a 1.2NA 60x objective (UPlanSApo Olympus UK).

For both experiments, SHG and TPF were collected simultaneously in the epi-direction. The signal was separated from the laser fundamental by a long pass dichroic filter (670dcxr Chroma technologies) and a colour glass filter (CG-BG-39 CVI laser) and the SHG and TPF were directed onto two separate photomultiplier tubes (PMTs) (R3896 Hamamatsu Japan) by a long pass dichroic filter (Semrock Di02-R405). Additional filters were placed in front of the PMTS (Semrock FF01-405/10 and Semrock FF01-520/70 for SHG and TPF respectively).

To avoid photodamage the average laser power at the sample was maintained below 20 mW.

Mechanical loading

For tensile loading [Fig. 2(A)], strains were applied using micrometers accurate to 10 μm (MT1/M Thorlabs) and the