COVER SHEET

NEW DIRECTIONS FOR THE CHARACTERISATION OF CARTILAGE HEALTH IN VIVO

Cameron P. Brown, Adekunle Oloyede, Hayley R. Moody, Ross W. Crawford
Queensland University of Technology
2 George St, Brisbane Q 4001
Australia
Email: cp.brown@qut.edu.au

ABSTRACT
This paper outlines the motivation and preliminary investigations into the possibility of a new method of characterising cartilage health in vivo. Current in vivo indentation techniques, which rely on stiffness measurements alone, are unable to adequately distinguish between healthy and degraded tissue. By considering the tangential strain in the articular surface during an indentation test, it is hypothesised that a simple and more sensitive indicator of cartilage integrity may be possible. The present study investigates the effects of low levels of degradation on the tangential surface strain under the indentor. Preliminary results suggest that this technique is highly sensitive, and can discriminate between degeneration and the natural variations across the joint. From these investigations we propose that the relationship between indentation and the surrounding strain field might be able to distinguish between healthy soft tissue and degraded tissue in vivo, important information not available from the current techniques.

This study forms the basis of a series of further experimental and modelling investigations to develop these ideas with the goal of creating a more effective diagnostic method for the surgeon.

KEY WORDS
Cartilage, arthroscopic indentation, osteoarthritis, surface tangential strain

1. Introduction

Currently, the surgeon has no accurate way of judging, short of his or her visual impression, the integrity of the articular cartilage encountered at surgery. This visual impression is important, but it is now widely accepted that visually normal cartilage is not necessarily healthy. The validation of this impression with the physical properties of the tissue, with respect to the characteristics and severity of osteoarthritis, will be an invaluable addition to the diagnostic and decision making process.

With the development of localised treatment technologies such as tissue replacement, it has become critical to develop precise and objective measures which are capable of detecting articular cartilage degeneration at an early stage. The ability to optimise and leave the maximum amount of native host cartilage in a joint would provide a significant benefit in the issues of most relevance to the patient relating to the reduction in recovery time, rehabilitation and costs.

Due to the high degree of structure-function coupling in articular cartilage, any change in the integrity of the constituents of the matrix, or in their interactions with each other, must manifest as a change in biomechanical performance. Recent publications [1, 2] have suggested that the afore-mentioned alteration in mechanical properties may be notable before any gross morphological change is apparent. This would possibly make the mechanical test a useful indicator of early stage degeneration.

Previous indentation testing of both normal and osteoarthritic cartilage is extensive yet broad in its approach. A general understanding of the bulk properties of cartilage and the subsequent changes due to osteoarthritis have been reported. These include changes such as softening [1, 3, 4], different stress relaxation behaviour [4, 5], and increased permeability [1]. In-depth studies of the outward progression of the disease and its relation to osteoarthritic severity, however, have not been attempted. Studies of the mechanical state of the cartilage surrounding different grades of osteoarthritis will help to define the progression of osteoarthritis and appropriate treatment options. This is especially important for localized treatments with respect to ensuring the complete removal of diseased cartilage, increasing the success of treatment and decreasing the risk of re-occurrence.

Some of the effects of degenerative changes on the indentation properties of cartilage surrounding an osteoarthritic site have been investigated by Kempson et al. [6]. They found that cartilage surrounding a focal defect, although visibly normal, became softer. This early softening of cartilage may be due to a decreased proteoglycan content [7, 8] and/or a change in the integrity of the collagen network [9]. Although the

studies of Kempson et al. showed that a non-visual progression of osteoarthritis exists around a focal defect, their supporting data was not related to a known level of severity nor to any other factors necessary for a full insight into the biomechanical properties of cartilage. It therefore has little immediate relevance to treatment.

With these findings, there has been increased interest in developing arthroscopic systems that can quantify and qualify the mechanical properties of articular cartilage in situ [10-14]. Such systems tend to concentrate on the perceived stiffness alone. Buschmann et. al. [15] have used the swelling potential under indentation to detect matrix degradation.

It has been shown that although the biophysical properties of isolated general matrix may change significantly with degeneration, a measurement of in situ stiffness by indentation may be an unsatisfactory indicator of the tissue's health, provided the articular surface is intact. In their study on the physical indicators of cartilage health, Broom and Flaschmann [16] found that on-bone compliance tests alone were insensitive to degenerative changes in the structural integrity of the underlying matrix that could be readily detected by microscopic and swelling analyses.

It should also be noted that, irrespective of health, the basic indentation properties of a tissue will vary considerably over the surface of a joint, between joints and between people. We therefore stress the importance of distinguishing between these natural variations and variations due to disease or degradation. This important distinction will need to be made for any in vivo test to be successful. The present study investigates the ability of a tangential surface strain measurement, when coupled with a stiffness measurement, to detect early arthritic changes in a joint and thus aid surgical decision making.

2. Method

It is widely accepted that in the earliest stages of osteoarthritis, there is a disruption of the collagen structure in the superficial layer leading to a loss of proteoglycans [17, 18]. Broom et al [19] studied the structural changes in the matrix surrounding focal arthritic regions and found significant changes in the collagen integrity of macroscopically normal, intact tissue, particularly in the superficial zones. This suggests, that from a diagnostic viewpoint, an analysis of the superficial layer may provide an additional and more detailed insight into disease progression. The ease of accessibility of the articular surface, coupled with its compromised integrity early in disease progression, make it a prime target for diagnosis.

2.1. Sample Preparation

Macroscopically normal and intact bovine joints were taken from prime oxen within 24 hours of slaughter and wrapped in a 0.15M saline soaked cloth and stored at -20°C. Prior to testing, the joints were thawed in saline, sectioned into 20 mm squares and labelled according to their position on the patella groove. To ensure a flat testing surface, the cartilage samples were taken off the bone and immediately glued to Palapress® (Heraeus Kulzer GmbH & Co. Kg) dental resin disks. A fine grid was imprinted using a waterproof marker and the samples were blot dried and returned to saline to recover for 2 hours at 4°C.

To investigate the effects of different modes of degeneration, normal samples were compared with artificially degraded samples. Macroscopically normal samples were mechanically tested immediately after sample preparation. After testing under compressive loading, samples were placed in 0.15M saline containing 30 U ml-1 collagenase (Sigma C0773 protease free, Sydney, Australia) for 4 hours at 37°C (n=4). These were then blotted dry and placed in 0.15M saline for 2 hours at 4°C before retesting. The samples were then subjected to a further 4 hour collagenase treatment and retested.

2.2. Experimental Set-up

Figure 1 presents the arrangement for mechanical testing. A custom-built indenter was used to facilitate the photographing of the indentation imprint and stretching of the area around the indenter. This indenter comprises a 6 mm diameter glass disk glued onto a 20 mm thick glass plate of 100 mm diameter. The plate was set in a stainless steel frame with an angled mirror to allow direct viewing through the indenter.

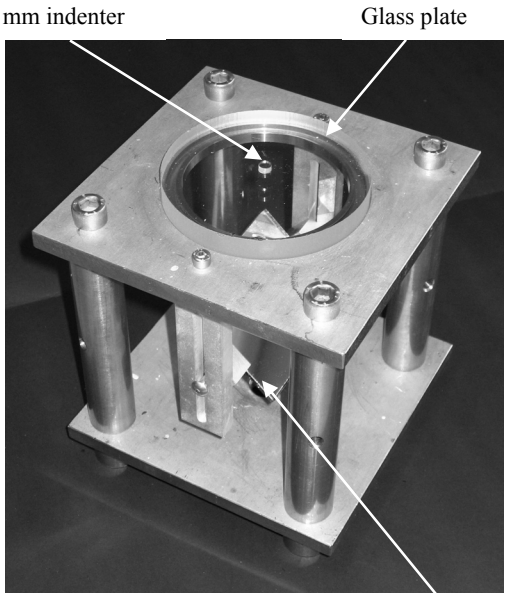

Figure 1: Experimental apparatus

2.3. Mechanical Testing

Samples were subjected to a static indentation load of 85N, corresponding to approximately 0.33MPa, on a Hounsfield Testing Machine (Hounsfield Testing Equipment, Salsford, England). This figure was chosen to represent a pressure that can be expected to be applied by a surgeon at arthroscopy. Axial force and displacement vs. time data were taken using a custom-built data logger with DeLogger software (Data Taker). Lateral "stretch" at the surface both under and surrounding the indentation was captured optically at different stages of the indentation using a Minolta Dimage 7i digital camera, and analysed using ImageJ software (National Institutes of Health, USA).

For tangential strain analysis, the x-y coordinates of the centroid of each grid point were tracked throughout the indentation. The magnitude of the displacement of each point was compared to the radius and averaged to give a strain value.

A superimposed plot of the tangential deformation, or "stretch", in the articular surface under indentation is illustrated in Figure 2. Under a plane-ended indentor, the articular surface undergoes deformation in a radial direction, increasing in magnitude with increased distance from the centre of indentation. In the region adjacent to the indentor, the visible articular surface strain continued in an outward direction for up to 2 mm. The inside end of each ray in Figure 2 shows the initial position of a point, while the outer end shows its position after 20s at 0.33 MPa.

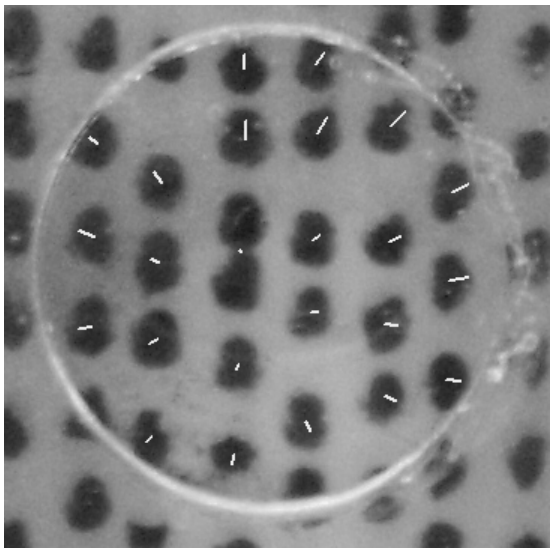

Figure 2: The radial strain pattern under indentation

3. Results

The axial strain at 0.33 MPa at different positions on the same normal patellar groove is shown in Figure 3. Figure 4 shows the variation in the tangential strain for the same indentation. These figures are indicative of the natural variations across a normal joint as measured by each technique.

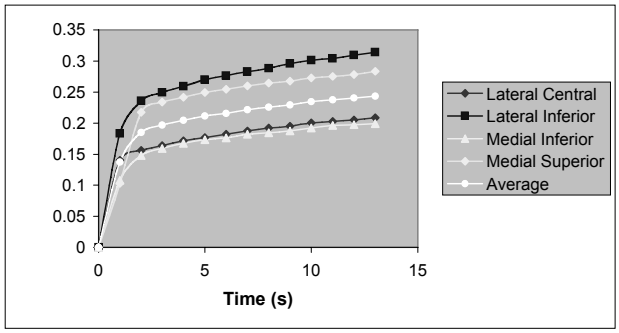

Figure 3: The axial strain varies considerably across different areas of the joint surface in normal samples

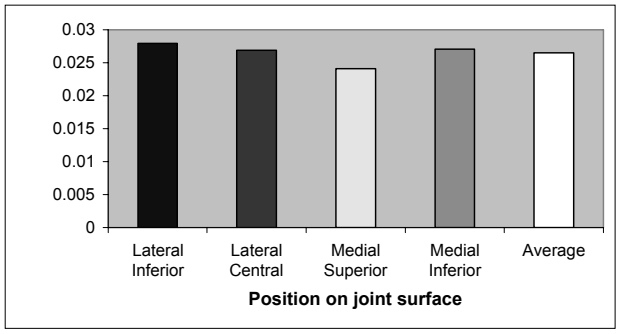

Figure 4: The tangential strain for the same indentation is less sensitive to the normal variation

Representative data from a specimen subjected to collagenase treatments are shown in Figures 5 and 6. These show the axial and tangential strain for normal samples before, and after 4 hrs and 8 hrs degeneration in collagenase. Common degeneration protocols use 24 and 48 hrs to represent mild and significant degeneration respectively. This test is therefore indicative of the sensitivity of the different techniques. Results from a visually normal and intact region adjacent to an osteoarthritic defect are included for comparison.

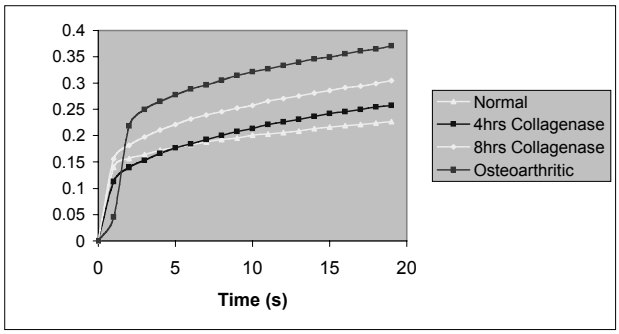

Figure 5: Axial strain increases with increased degeneration

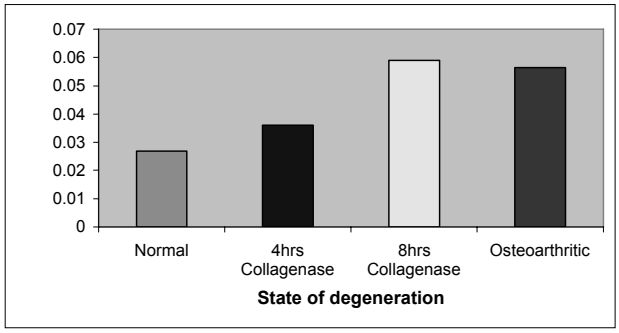

Figure 6: Tangential strain in the same indentation increases significantly with increased degeneration

4. Discussion

The parallel alignment of the collagen network in the superficial layer produces a strain-limiting behaviour [20, 21], which plays a significant role in maintaining cartilage thickness under compression. This strain limiting effect is governed by the integrity of the collagen fibres and their interactions. In this study we have investigated the ability of a tangential strain, or "stretch", measurement to detect the changes associated with arthritis in its earliest stages.

We can observe from Figures 3 and 4, the significant but natural variation (23% Standard Deviation) that would be expected in axial strain across a normal joint. The variation in tangential strain under indentation, however, is considerably smaller (6% Standard Deviation). The tangential strain technique, as shown in Figure 6, however, appears sensitive to very small changes in the integrity of the collagen network induced by collagenase treatment.

Although a measurement of compliance is also sensitive to the degenerative changes induced in this experimental program, it is limited by its inability to distinguish small degenerative changes from the natural variations across the joint and between joints. The average strain at 13s for normal samples was 0.24 with a standard deviation of 0.06. The strains presented in Figure 5 for normal, 4 hrs and 8 hrs collagenase treatment are 0.21, 0.23 and 0.28, respectively. Each of these values fall within the standard deviation of the normal average, suggesting that such results alone are unreliable in detecting small changes.

Further testing considers the relationship between the basic indentation and tangential strain data as a Poisson's ratio-type characteristic. Unlike a classically defined Poisson's ratio, the ratio of the in-plane strain to the axial strain will vary significantly with time, depth, strain rate and the biomechanical integrity of both the surface and the underlying general matrix. We hypothesise that distinct relationships between axial and tangential in-plane strains exist for healthy and degenerate tissues. This hypothesis is currently being tested on human osteoarthritic joints and correlated with histological gradings.

5. Conclusion

We conclude that the development of tangential (surface) strain in articular cartilage is sensitive to anatomical changes, including small changes in the collagen network, and can be employed for distinguishing structural and biomechanical alterations due to disease and biomechanical/biochemical degradation. By considering the relationship between the basic indentation data and the concomitant tangential strain, a more sensitive indication of the condition of the entire cartilage matrix is possible. Such a technique would allow a more meaningful analysis of health to be made at arthroscopy. This is especially important in determining the extent of the disease for tissue replacement and tissue removal at surgery.

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