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Short communication

Real-time observation of fluid flows in tissue during stress relaxation using Raman spectroscopy

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ABSTRACT

This paper outlines a technique to measure fluid levels in articular cartilage tissue during an unconfined stress relaxation test. A time series of Raman spectrum were recorded during relaxation and the changes in the specific Raman spectral bands assigned to water and protein were monitored to determine the fluid content of the tissue. After 1000 s unconfined compression the fluid content of the tissue is reduced by an average of 3.9% ± 1.7%. The reduction in fluid content during compression varies between samples but does not significantly increase with increasing strain. Further development of this technique will allow mapping of fluid distribution and flows during dynamic testing making it a powerful tool to understand the role of interstitial fluid in the functional performance of cartilage.

1. Introduction

Articular cartilage is a biphasic tissue, containing around 80% interstitial fluid. The flow and distribution of this fluid in the tissue during dynamic loading is important in resisting load, in providing the exceptionally low friction levels found at the articular surfaces and in providing nutrients and mechanical stimuli to the cartilage cells (Ateshian, 2009; Grad et al., 2011; Mow et al., 1984). Similarly fluid flows in bioreactors increase collagen production, chondrocyte viability, and the 3-D structure and tensile modulus of engineered tissue (Gemmiti and Guldberg, 2006; Vunjak-Novakovic et al., 1999; Wartella and Wayne, 2009). Understanding these flows is then crucial in our continuing quest to regrow damaged cartilage and to develop biomimetic bearings.

The prediction of fluid flows during compression has been performed in finite element (FE) models. However, the predicted flows are dependent on such variables as inclusion and orientation of collagen fibrils (Federico and Herzog, 2008), the tension-compression parameters (Soltz and Ateshian, 2000a; Wilson et al., 2005) and the specified permeability of the solid matrix (Lai et al., 1981). Some validation of FE models has been achieved through real-time measurements of fluid pressure in a compression test (Soltz and Ateshian, 2000b). However these are general measurements over the bulk volume of the cartilage and do not allow spatial variations in pressure or fluid flow to be determined.

Spatial variation in diffusion coefficients as well as the directional diffusion of water in cartilage have been measured using Magnetic Resonance imaging (MRi) under static conditions (Pierce et al., 2010; Xia et al., 1994). Whilst MRi offers the spatial resolution needed to measure fluid flow, acquisition times in the order of minutes prevent this technique from being used for real-time measurements (Binks et al., 2013).

Here we detail a new technique which allows the measurement of changes in fluid distribution in cartilage with both spatial and temporal resolution. Our technique uses confocal Raman spectroscopy measurements during an unconfined compression test. This technique has spatial resolution of less than 1 μm in the *x*-*y* plane and approximately 7 μm in the *z*-plane, and individual measurements are performed at a frequency of 10 Hz. Whilst this initial testing has been performed on osteochondral plugs the method could be extended to other tissues.

2. Methods

Osteochondral samples ($n = 8$) were obtained using an 8 mm biopsy punch from porcine femoral condyles, and frozen at −20 °C until required. Cartilage thickness after thawing was determined optically. Samples were mounted in polyethylene holders using bone cement which was cured for 1 h. Two points approximately 1 mm apart were marked on the cartilage surface with a needle dipped in Indian ink to locate the measurement area. The PE holders were press fit into a petri dish mounted on the compression stage. Throughout measurements samples were kept immersed in phosphate buffered saline.

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A custom loading device was used, driven by a linear actuator with a linear travel per step of 3 μm. Loads were recorded by a 6 axis load cell, with an axial resolution of 1/80 N (Gamma, ATI Industrial Automation, USA). The cartilage samples were compressed against a 40 mm diameter glass plate that was held fixed in space (Fig. 1).

Raman spectra were collected using an Alpha300R, confocal Raman spectrometer (WITec Gmbh, Ulm, Germany) using a laser wavelength of 532 nm, a 20x objective and a 100 μm pinhole giving an axial resolution of 7 μm. Spectra were recorded at the interface between the glass plate and the cartilage sample during compression with an integration time of 0.1 s.

2.1. Unconfined compression

For all samples two unconfined compression test lasting 20 min were performed, with 24 h recovery at 4 °C between tests. A pre-load of 0.5 N was applied to ensure contact with the cartilage surface. Contact was confirmed by acquisition of a single Raman spectra at the glass-cartilage interface. The sample was then allowed to relax for 10 min under pre-load before the stress relaxation step was started. The sample was loaded to either 10% or 20% strain at 1% strain per second. The strain was held constant for 20 min and the reaction force recorded. Simultaneously a continuous time series of 10,000 Raman spectra were acquired.

2.2. Strain recovery

For three samples a further test was conducted to determine if the proposed technique would accurately measure water content during strain recovery. As before a pre-load of 0.5 N was applied with 10 min rest before the sample was loaded to 10% compression. The position was held constant for 1000 s, the sample was then unloaded by returning the loading device to its original position at 0% strain. A time series of 15,000 Raman spectra were acquired throughout the process.

2.3. Calculation of water content

The ratio of water to protein content in the cartilage tissue was determined using the ratio of intensities of the Raman peaks at 3390 cm^{-1} due to OH stretching vibrations in water and 2935 cm^{-1} due to CH_3 stretching vibrations in proteins. This method has previously been used to determine water content in eye lenses and skin tissue (Caspers, 2003; Siebinga et al., 1991). Matlab (MATLAB and Statistics Toolbox Release 2015a, The MathWorks Inc., Natick, Massachusetts) was used to process all data. To correct for fluorescence background a 1st order baseline was fit between the spectral points of 2500 and 3800 cm^{-1}. The intensity of the water peak was calculated as the sum intensity between 3350 and 3550 and the protein peak as the sum intensity between 2910–2965 cm^{-1}. To calculate the water content the following equations as proposed in (Caspers, 2003) were used:

$$\frac{W}{P} = \frac{m_w}{m_p} \cdot R \tag{1}$$

$$\text{Water content } (\%) = \frac{m_w}{m_w + m_p} = \frac{\frac{W}{P}}{\frac{W}{P} + R} \cdot 100\% \tag{2}$$

where W is the integrated Raman signal of water, P is the integrated Raman signal of protein, m_w and m_p are the mass of water and protein respectively and R is a proportionality constant representing the water:protein signals in solutions of

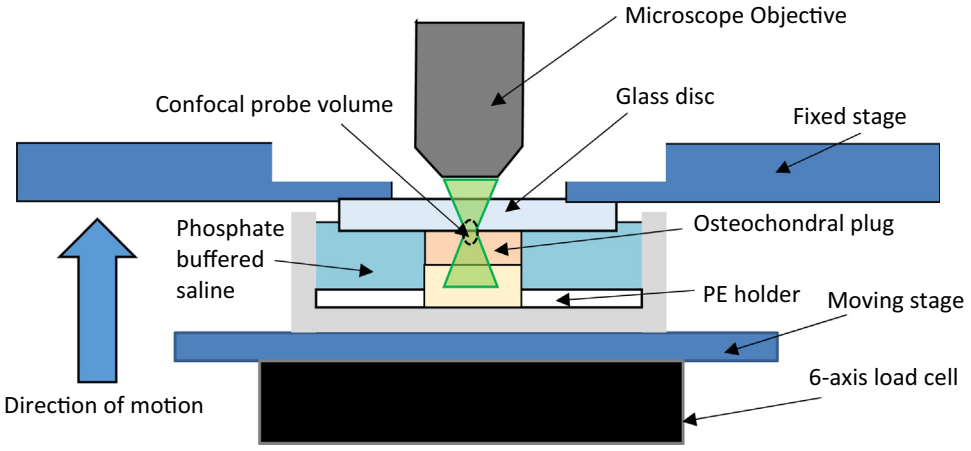

Fig. 1. Schematic of loading rig.

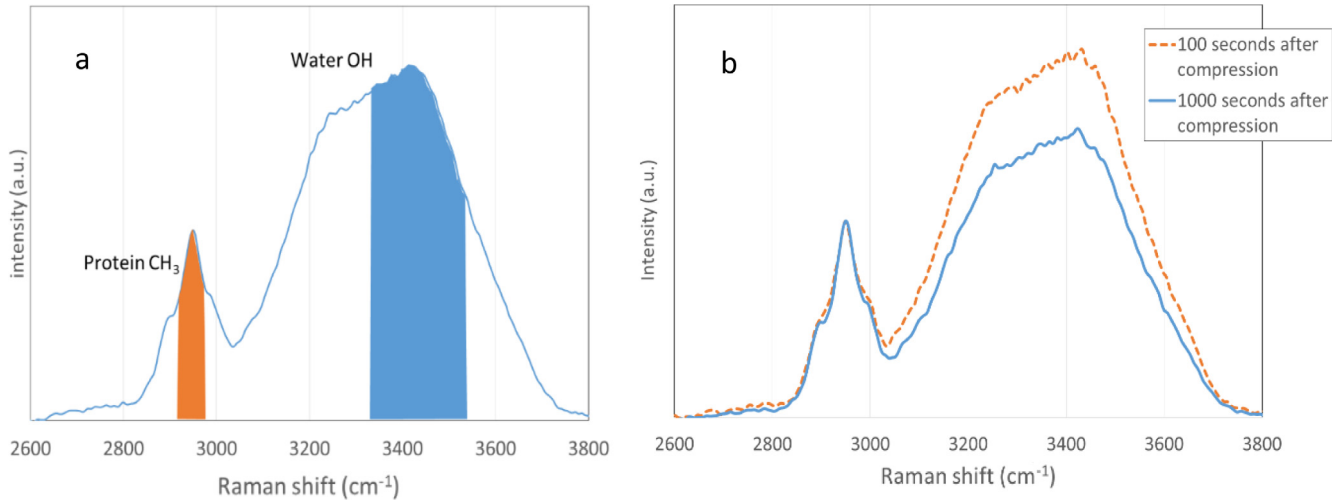

Fig. 2. Typical Raman spectra for cartilage sample recorded (a) under 0.5 N pre-load showing areas used to calculate water and protein content (b) at 100 and 1000 s after compression showing changes in peak ratio.