INTRODUCTION

Arthritis is a chronic disease which causes disability around the world. There are many types arthritis and osteoarthritis is the most common and is associated with the development of degeneration in the network of collagen fibres and proteoglycan (Broom 1982). However, relatively there has been little study of the role of the collagen fibres on the biomechanics of articular cartilage. The aim of this study is to examine the relationship between the collagen network and mechanical properties of articular cartilage.

Articular cartilage is an inhomogeneous biological tissue and it composed of chondrocytes and an extracellular matrix made up of collagen fibres, proteoglycans and water. Traditionally, articular cartilage is classified into three zones, the superficial zone, transitional zone and radial zone. The superficial zone is characterized with discoid chondrocytes and the collagen fibres are parallel to the surface of the cartilage (Stockwell & Meachim 1979; Ghadially 1983). The transitional zone features more spherical chondrocytes than those in the superficial zone (Stockwell & Meachim 1979; Ghadially 1983). The orientation of collagen fibres in this zone remains uncertain but many traditional microscopic studies suggested that the collagen fibres curve from their radial orientation in the radial zone to a tangential orientation (Stockwell & Meachim 1979; Broom 1982; Ghadially 1983). It is widely accepted that the collagen fibres anchor to the subchondral bone and grow upwards to the radial zone (Stockwell & Meachim 1979; Broom 1982; Ghadially 1983). The chondrocytes in the radial zone cluster into columns perpendicular to the surface of the articular cartilage (Stockwell & Meachim 1979; Broom 1982; Ghadially 1983).

During normal activities, articular cartilage is subjected to a wide range of tension, compression and shear. The internal structure and composition of articular cartilage permit the cartilage to fulfill its functions without causing pain. The proteoglycans are negatively charged macromolecules that attract water molecules and create a swelling potential called osmotic pressure in articular cartilage (Maroudas & Muir et al. 1969). The osmotic pressure generated by the proteoglycans is reinforced by the three-dimensional collagenous network so that the articular cartilage is provided with resilience and a load carrying ability (Maroudas & Muir et al. 1969). It is said the proteoglycans are entrapped with less than 20% of the volume which they would occupy in their free state (Muir 1979) in the collagenous network. Therefore, the tensile strength of collagen fibres and concentration of proteoglycan play crucial role in determination of the mechanical response and functions of articular cartilage. Degenerated collagen fibres have inadequate strength to constrain the swelling potential.

Thus, the cartilage becomes softer and thicker than normal and cannot support loads.

METHODS

A fibre optic laser scanning confocal microscope (FLSCM, Optiscan Pty Ltd, Melbourne) utilizes a single mode optic fibre to function as a pinhole in traditional laser scanning confocal microscopy (LSCM) for transmitting the exciting and emission lights between the object and detector. Using a portable scanning head weight of about 2 kg, which is robust in alignment, the FLSCM offers not only an equivalent image capability but also flexibility for many potential applications (Delaney, Harris et al. 1994) such as confocal arthroscopy. As shown in Figure 1, the FLSCM was modified with specially designed compression test apparatus, comprised of a linearly variable differential transformer (LVDT, RS Component Ltd), proportional pressure regulator (Festo Ltd) and a load cell (Entrance Inc), to study the biomechanical function of articular cartilage in relation to its collagen fibres.

Cylindrical articular cartilage specimens, approximately 3mm in diameter and 3mm thick, were obtained from human femoral heads and the condyles of bovine knee respectively. The human cartilage samples were taken from regions of arthritic femoral head which showed no visual arthritic sign. Bovine specimens were obtained from a local butcher within 24 hours slaughter. The human specimens were preserved in formalin for 24 hours while the bovine specimens were preserved in 9g/L saline solution and stored at ~20°C (repeated thaw was avoided). Prior to the experiment, the specimens were immersed into 0.2% Phosphormolybdic Acid to reduce the interference from the brightness of the chondrocytes during imaging of the collagen fibres using the FLSCM. The specimens they were then stained by 1g/L Picric Sirius Red Acid. After this, the specimens were washed several times by 9g/L saline solution and put into a special specimen holder and was mounted in the compression apparatus fixed on the platform of the FLSCM. Using computer program F900e (Optiscan Pty Ltd, Melbourne), three dimensional image stacks of collagen fibres were acquired before and after compression of the articular cartilage at 5MPa for cow specimen and 3.8MPa for human specimen. The displacement of the articular cartilage was recorded by a dynamic signal analysis (HP Ltd) via the LVDT. The compression applied to the specimens was supplied by a compression actuator (Festo Ltd) underneath the specimen holder via the digital proportional pressure regulator and monitored by the load cell situated right under the specimen holder. Using Voxblast (Vaytek Inc), the image stacks obtained were reconstructed into 3D images as shown from Figures 2 to 5.
RESULTS AND DISCUSSION

3D images reconstructed from image stacks of bovine cartilage show the collagen fibres in the superficial zone have a three-dimensional orientation, shown in Figure. 2. and Figure. 3. They are obliquely oriented to the surface of the cartilage. However, the fibres appear parallel to the surface of articular cartilage when observed from the direction above the surface of the articular cartilage where most traditional 2D microscopic observations (such as light microscopy and electron microscopy) have been taken place.

The cartilage specimens which were visually observed to have no arthritic signs show microscopic disruptions in the collagen network (Figure. 4.). As shown in Figure. 5., those cartilage specimens demonstrated the increased defects in the collagenous network upon application of 3.8 MPa compression on the cartilage. This would be one reason that arthritis causes pain, during normal use of synovial joints. Therefore, the findings support the view that disruptions of the collagen network are progressing in the early degenerated cartilage such as softened cartilage. The use of physiotherapy, which utilizes load-reduced exercises such as swimming, can relieve pain of arthritis. Also, as shown in Figure. 6., the human cartilage demonstrated a significantly lower mechanical stiffness than healthy bovine cartilage. However, whether the difference in the mechanical stiffness between bovine and human cartilage is due to state of healthy condition, different species, body location and preparation techniques requires further study.

Figure 1: A diagram of the modified FLSCM. The force applied to the cartilage specimen is supplied from a digital proportional pressure regulator via a pressure actuator and monitored by the load cell. The displacement impacted to the cartilage specimen is acquired by a dynamic signal analyzer via a LVDT.

Figure 2: 3D image of collagen fibres of bovine articular cartilage shows the collagen fibres has 3D orientation and obliquely oriented to the surface of the cartilage.

Figure 3: Collagen fibres of the bovine cartilage in Figure. 2. show appear parallel to the surface of articular cartilage when observation was taken from the direction above the surface of articular cartilage.

Figure.4: There are microscopic disruptions in the collagen network in the human cartilage from arthritic femoral head. The cartilage specimen did not have visual arthritic signs.
Figure 5: There are increases of the defects in the collagen network of the cartilage specimen in Figure 4, with application of 3.8MPa compression.

Figure 6: Cartilage specimens taken from arthritic femoral head show significant lower stiffness than normal bovine cartilage specimens in creep test.

SUMMARY

The collagen fibres play an important role in determination of the biomechanical properties of articular cartilage. A method has been developed to study directly the relationship between the mechanical properties and the three-dimensional structure of collagen fibres of articular cartilage. The structure, the mechanical properties and the interrelationship between the structure and biomechanics of articular cartilage have been studied.

REFERENCES