
Abstract

Osteoarthritis (OA) is an age-related disease of the joint in which the tissues degenerate and the function of the joint is disrupted. Key load-bearing tissues that break down as part of knee osteoarthritis include the articular cartilage and the meniscus. These tissues are complex cartilaginous tissues, consisting of around 70% water confined in a network of collagens and proteoglycans. During OA, this network is disrupted by processes that are not yet fully understood resulting in inferior mechanical properties of the tissues and eventually pain and loss of mobility for the patient.

Traditionally the pathologies of cartilaginous tissues are studied via magnetic resonance imaging (MRI)-based techniques as well as histological slicing and staining, which carry several limitations. MRI is a powerful technique that is usable both in vivo and on ex vivo samples but is limited in resolution and often produces complex signals that can be difficult to interpret. Histology provides excellent characterization of cell features and matrix composition but is limited to 2D and requires ex vivo samples, fixation and slicing, which alters the state of the tissue and consequently limits sequential experiments.

Several novel approaches seeking to address these limitations exist, the work in this thesis being focused on examining whether new insights into tissue properties and the degeneration process can be gained by three main techniques: synchrotron phase-contrast tomography, neutron tomography, and quasi-elastic neutron scattering (QENS) for the examination of *ex vivo* samples. More specifically, the aim was to study in 3D distribution of cells, protein matrix density, and water diffusivity within tissues as well as potential changes induced by OA-related degeneration.

Phase-contrast in X-rays circumvents many of the limitations with applying X-rays to the study of soft tissue by producing contrast not only from absorption but also from transitions between materials. The addition of the powerful beams produced

by synchrotron sources enables rapid, high-volume measurements while preserving the tissue in a close to native state.

Neutrons can be used for tomography in a similar manner to X-rays, but interact strongly with certain light elements, particularly hydrogen. Additionally, they are sensitive to isotopes, which allows heavy water to be used as a contrast agent. Furthermore, the low energies of neutrons allow small changes in energy to be detected, which enables the study of water or other molecular dynamics via QENS.

The first study employs synchrotron phase-contrast tomography to examine human articular cartilage samples. Samples from both donors and total knee replacement patients were imaged and chondrocytes segmented. Results show several degeneration features in similar or better quality than histology as well as depth-dependent associations between cell properties and degeneration level.

The second and third studies employ neutron tomography to study protein density and water diffusion in cartilage and meniscus. Human articular cartilage samples were imaged in an exploratory study, showing the best contrast in heavy water-treated samples as well as revealing clear differences in depth-dependent protein matrix density depending on disease state. Then in the third study, bovine cartilage and meniscus samples were imaged during the exchange of water and heavy water, showing long-range macroscopic diffusion in the tissue.

The fourth study employed QENS to directly probe water dynamics in bovine articular cartilage. Samples from different depths were measured, producing a measure of fast water dynamics and slower molecular movements at the nanosecond scale. Results show that the restriction of water diffusion within the cartilage is more complex than a simple reduction of diffusion speeds at short enough length scales.

In summary, the work presented in this thesis shows the potential of synchrotron and neutron techniques for the study of *ex vivo* cartilaginous tissues. Initial experiments have provided a baseline for what these techniques reveal, showing the potential for future experiments, such as altered water dynamics with loading or degeneration, or novel ways of categorizing cartilage degeneration based on phase-contrast volume imaging.