Evaluation of Water Dynamics in Articular Cartilage using Isotope-Labeled Water

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INTRODUCTION: The water retention capacity of articular cartilage accounts for about 70% and is important for cartilage function and nutrition through synovial fluid. Deterioration of osteoarthritis (OA) is known to affect the water content of cartilage, initially increasing and later decreasing [1]. However, there have been no studies that clearly describe the movement of water molecules in joints. We are attempting to trace water itself by focusing on stable isotopic elements of oxygen contained in water molecules. In a previous MRI study, influx of isotope-labeled water into cartilage tissue was observed in superficial cartilage injuries (OARSI histological grade 3) [2]. However, a detailed observation is necessary because the spatial understanding of water molecules after cartilage injury is extremely limited. The purpose of this study is to apply isotope microscopy, commonly applied in meteorite and mineral analysis, to observe articular cartilage to clarify the spatial dynamics of water molecules.

METHODS: A male Japanese white rabbit at 12 weeks of age was used for the study. The femoral knee joint was excised, and a wound 0.5 mm wide and 0.5 mm deep was made laterally on the loading surface of the lateral condyle to simulate cartilage damage (Fig. 1), and immersed in 99% mol 18-O labeled water for 30 minutes. Subsequently, rapid freezing was performed using liquid nitrogen to stop the movement of water molecules. The tissue was trimmed to fit the observation pedestal, and the observation surface was smoothed using a tungsten blade. The observation was conducted using an isotope microscope that detects isotopic elements based on mass spectrometry principles. The field of view was 80 µm each, and the ratio of 18O to 16O present (18O/16O ratio) was observed at five locations; a: wound margin, b, c, d: cartilage calcification layer at a distance of 400 µm from the wound, intermediate layer, superficial layer, and e: deep cartilage layer at a distance of 800 µm from the wound. The natural 18O/16O ratio is approximately 0.002 (Fig. 2).

RESULTS SECTION: In the superficial and intermediate layers of normal cartilage, the 18O/16O ratio was approximately 30 times the natural ratio (Fig. 2c, d). In the tide mark and calcified layer, 18O/16O ratios were observed that were approximately five times higher than the natural ratio (Fig. 2b). The 18O/16O ratio around the cartilage injury was approximately 45 times higher than the natural ratio and 1.5 times higher than the superficial and intermediate layers (Fig. 2a).

DISCUSSION: The present study showed that isotope-labelled water penetrates cartilage tissue in 30 minutes. The influx of isotope-labelled water around the injured area was approximately 1.5 times higher than in normal areas. These results were consistent with previous observational studies analyzed by MRI. Surface damage of articular cartilage resulted in a reduction in the water retention capacity of cartilage tissue leading to an increased influx of water molecules.

SIGNIFICANCE/CLINICAL RELEVANCE: This is the first study in the world to successfully visualize water influx into cartilage tissue using isotope microscopy. This approach will provide a better understanding of the spatial dynamics of water metabolism in the cartilage matrix in articular cartilage diseases.

REFERENCES: [1] T. Aigner et al, Cell. Mol. Life. Sci. 59 (2002) 5-18, [2] Hosokawa et al., Cartilage 2022 Jul-Sep;13(3):19476035221111503

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IMAGES AND TABLES:

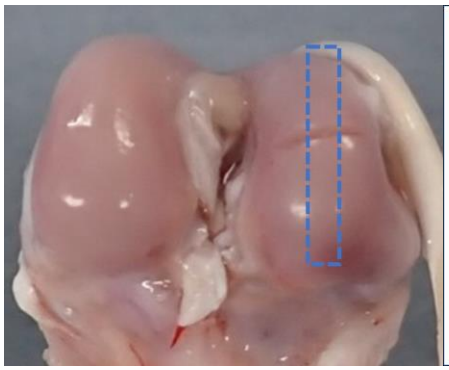

Figure 1

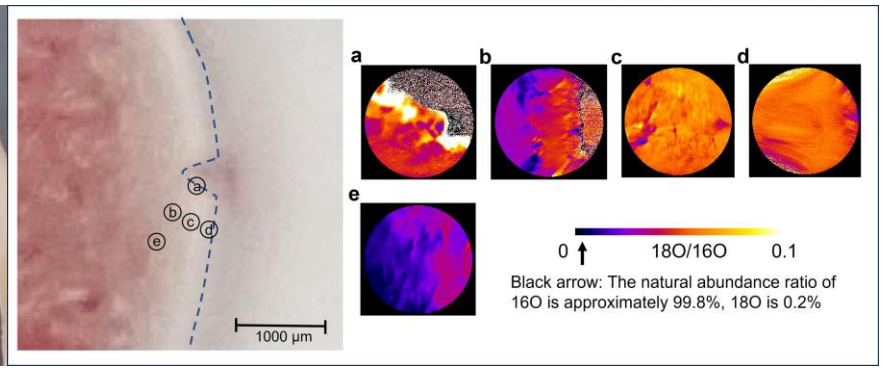

Figure 2