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Introduction: The early diagnosis of osteoarthritis (OS) plays a vital role for the development of effective treatments. There has been an increasing interest to use ultrasound (US) for diagnosing OA-related changes in articular cartilage (AC). It is generally understood that US is highly sensitive for degenerative changes of AC when applied invasively directly on the cartilage surface (1). On the other hand, there are also studies investigating the utility of qualitative (2) and quantitative (3) non-invasive US for the diagnosis of knee OA. In non-invasive knee US, image quality is worse compared to application directly on the cartilage surface as there is always a variable layer of soft tissue between the transducer and the AC surface. However, yet no study has been conducted to experimentally characterize which kind of degenerative changes of AC, e.g. depth and width of cartilage lesions or tissue compositional changes, can be depicted with non-invasive knee US. Therefore, the aim of this study was to visualize and characterize various kinds of degenerative AC defects (mechanical and enzymatically) in situ using bovine AC. Specifically, the effect of overlying soft tissue was investigated.

Methods: Bovine knee joints were obtained from the local slaughterhouse (Veljekset Rönkä Oy, Kemi, Finland). Eight patellae along with the surrounding soft tissue were prepared from the tibio-femoral joints. One patella was used in a pilot experiment to optimize the experimental protocol and image acquisition and one patella was used as a control. Three different kinds of damages were artificially made on four whole patellae that were in accordance with the reported changes in AC occurring in the early and late stages of OA (4). This included grinding with emery paper of average particle sizes (FEPA standard) of 428 µm, 268 µm, 141 µm, 116 µm, 93 µm, holes with varying diameter (1 mm, 2 mm, 4 mm, 6 mm) and depth (superficial and deep), and irregular mechanical damages with blunt objects. In addition to mechanical defects, a 2 cm x 2 cm blocks from two patellae was prepared and subjected to enzymatic degradation with 30 U/ml collagenase (Sigma C0773 protease free, Finland). Both patellae were imaged before immersion to get control images. First patella was imaged at intervals of 30 hours, 50 hours and 138 hours, and the second patella was imaged only after 94 hours of immersion. Commercial GE Logiq E9 device (General Electric Medical Systems, Milwaukee, WI) with 15 MHz transducer was used for US imaging of all the samples. Samples were submerged in the Phosphate Buffer Saline (PBS) with corresponding depth than in typical clinical imaging situation. US Imaging was conducted first without any soft tissue by immersing transducer in the PBS. Subsequently, imaging was repeated with the layer of soft tissue on top of the sample. Imaging parameters were: Gain= 30 dB for PBS and 47 dB for soft tissue, Frequency= 15 MHz, Focal point= at the level of cartilage. All of the imaging parameter were kept constant for both the PBS and soft tissue imaging.
Results: US imaging of the drilled holes demonstrated that both of the superficial and deep holes were very well visualized in the PBS solution (without the soft tissue, Figure 1). However, with soft tissue small holes of diameter 1 mm were not very well visualized and can be easily missed (Figure 1). The drilled holes appeared as a well-defined defect with sharp edges in PBS solution, whereas with the soft tissue they appeared as echogenic defect without any bright borders.

Mechanical irregular damages by the blunt objects showed that superficial damages can be easily seen within the PBS solution (without the soft tissue, Figure 2). However, with the soft tissue the superficial damages cannot be visualized at all. Deep damages that were extending into the middle layer of cartilage were very well visualized with the PBS solution and also with the soft tissue. Imaging of the patella with damages by emery paper revealed that the surface of cartilage appears rougher with some increment in the echogenicity (Figure 2). These appearances were clearly visible only in the PBS solution and images with the soft tissue made the damages in practice invisible (Figure 2).

Both patellar samples with enzymatic degradation demonstrated the gradual increase in the roughness of cartilage surface (Figure 3). The edges seemed to be more affected by the enzyme degradation where the complete loss of reflection from surface was also seen. In addition, there was an increase in the echogenicity of the cartilage that was more prominent in the subsequent images. However, in both of the samples the enzymatic degradation has only affected the superficial or middle layers of cartilage. The degenerative changes were clearly depicted in the PBS solution as compared to images with the soft tissue where reflections from the soft tissue structures hinders the changes due to enzymatic degradation.

Discussion: We studied different kinds of damages of AC that are reported to be representative of the actual degenerative changes in OA. We observed that ultrasound can very well visualized holes ≥2 mm that are located both deep and superficial/middle layers of AC. Mechanical irregular damages that are extending into the middle layer of the cartilage can also be visualized both in PBS and with soft tissue. However, superficial damages and surface fibrillation of AC were only observed in the PBS images. US also showed progressive changes in the cartilage as a result of the enzymatic degradation when imaged in PBS. However, the changes due to enzyme were not visualized in the soft tissue images. Consequently, the results of this experimental study supports that the soft tissue in top of the AC significantly deteriorate the diagnostic performance of non-invasive knee US for detecting mechanical and compositional degenerative changes in the AC.

Limitations of the study: 1) Acoustic properties of soft tissue as a result of multiple freezing and thawing might have changed in different imaging sessions, 2) Limited number of samples was used, and 3) Only one US device was used.

Significance: This study provides the first experimental evidence that superficial defects and fibrillation, as well as degradation of collagen content, of AC may not be visualized in non-invasive knee US imaging. Furthermore, small (~1 mm) full-thickness defects may be missed with non-invasive US. On the other hand, larger AC lesions (≥2 mm) extending middle or deep layers should be clearly seen in non-invasive knee US imaging protocols. The study has significant clinical implications as increased echogenicity of AC has traditionally believed to represent early structural or compositional changes of AC, and current results do not support that.
Figure 1: US images of deep (a,b) and superficial (c,d,e) holes with the PBS and soft tissue.

Figure 2: US images of superficial damage (a) deep damage (b) damage with emery paper of grit size 428 μm (c) and damage with emery paper of grit size 93 μm (d) with PBS and soft tissue. The white arrows show the damage and the blue arrows show the reference holes drilled to make sure that same area is imaged.

Figure 3: US transverse section of first patella before enzyme immersion (a), after 138 hours of enzymatic degradation (b), and second patella before enzyme immersion (c), after 94 hours of continuous immersion (d). The white arrows show the reference holes drilled to make sure that same area is always imaged.

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