Ultrasound indentation of normal and spontaneously degenerated bovine articular cartilage

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Summary

Objective: We have previously developed a handheld ultrasound indentation instrument for the diagnosis of cartilage degeneration. The instrument has been demonstrated to be capable of quantifying mechanical and acoustic properties of enzymatically degraded and normal bovine articular cartilage in vitro and in situ. The aim of this study was to investigate the sensitivity of the instrument to distinguish between normal and spontaneously degenerated (e.g., in osteoarthritis) articular cartilage in vitro.

Design: Thirty articular cartilage samples were prepared from the bovine lateral patellae: 19 patellae with different degenerative stages and 11 patellae with visually normal appearance. Cartilage thickness, stiffness (dynamic modulus) and ultrasound reflection from the cartilage surface were measured with the handheld instrument. Subsequently, biomechanical, histological and biochemical reference measurements were conducted.

Results: Reproducibility of the measurements with the ultrasound indentation instrument was good. Standardized coefficient of variation was ≤6.1% for thickness, dynamic modulus and reflection coefficient. Linear correlation between the dynamic modulus, measured with the ultrasound indentation instrument, and the reference dynamic modulus was high (r=0.993, n=30, P<0.05). Ultrasound reflection coefficient, as determined from the cartilage surface, showed high linear correlations (typically r²>0.64, n=30, P<0.05) with the cartilage composition and histological or mechanical properties. The instrument was superior compared to visual evaluation in detecting tissue degeneration.

Conclusion: This study indicates that the ultrasound indentation technique and instrument may significantly improve the early diagnosis of cartilage degeneration. The results revealed that visual evaluation is insensitive for estimating the structural and mechanical properties of articular cartilage at the initial stages of degeneration.

Key words: Ultrasound, Indentation, Articular cartilage, Diagnosis, Osteoarthritis.
obtained by direct mechanical testing. Dashefsky introduced a pressure transducer for measuring the stiffness of cartilage during arthroscopy. With this technique, Dashefsky found that softening of cartilage was also related to visually intact cartilage surface in patients with chondromalacia patellae. These results propose that more quantitative methods should be used in osteoarthritis diagnostics or monitoring of the healing process e.g., after cartilage repair surgery.

During the past few years, different quantitative techniques have been introduced for the diagnosis of cartilage quality. However, most of these techniques are still in preclinical stage. Clinically, it is important that diagnosis is sensitive and that different stages of cartilage degeneration could be quantitatively identified. However, there are only few studies, to our knowledge, that have investigated the capability of an arthroscopic diagnostic technique to distinguish between progressive stages of cartilage degeneration.

In our recent study, a handheld ultrasound indentation instrument for the diagnosis of cartilage degeneration was developed. The instrument distinguishes sensitively the normal and enzymatically degraded cartilage from each other in vitro, and enables an objective registration of the site-dependant variation of cartilage properties in the bovine knee joint in situ. In spontaneously degenerated cartilage (e.g., in osteoarthritis), however, tissue changes are not as specific as after enzymatic degradation and the instrument should be capable of detecting these natural degenerative alterations as well. In the present study, the capability of ultrasound indentation instrument to distinguish different degenerative stages of bovine articular cartilage was investigated in vitro. Cartilage stiffness and acoustic properties, determined with the handheld instrument, were compared with the mechanical properties, assessed with the reference device, as well as related to histological structure, water content and uronic acid content of the tissue. The aim of this study was to further validate the ultrasound indentation instrument and establish a next step towards its use in arthroscopic surgery.

**Materials and methods**

**ARTICULAR CARTILAGE SAMPLES**

Intact bovine knees ($n$ = 75) were obtained from the local abattoir (Atria Oyj, Kuopio, Finland). Knee joints were opened within $5 \text{ h}$ of post mortem and the lateral facets of patellar cartilage surfaces were visually classified to four different degenerative grades: grade 0 = intact cartilage surface ($n$ = 11), grade 1 = slightly discoloured but otherwise smooth ($n$ = 5), grade 2 = superficial defect in cartilage ($n$ = 6) and grade 3 = deep defect in cartilage ($n$ = 8). We found 30 patellae to be relevant in this study. Eleven randomly selected visually intact samples were considered to be sufficient number for the intact group. Since majority of samples appeared to be visually intact, only a limited number of samples could be included in other groups. Grading was conducted by two of the authors. Subsequently, a cylindrical osteochondral sample (diameter = 19 mm, $n$ = 30) was taken from the classified site of the patella. Before measurements, samples were immersed in phosphate-buffered saline (PBS) containing protease inhibitors [5 mM ethylenediaminetetraacetic acid (EDTA) (Riedel-de-Haen, Seelze, Germany) and 5 mM benzamidine HCl (Sigma Chemical Co., St. Louis, MO, USA)] and stored in a freezer ($-20\,^\circ\text{C}$) for 2 weeks.

Before ultrasound indentation measurements, samples were thawed and glued on a bottom of a plastic container filled with PBS containing protease inhibitors. After the measurements, samples were split into two blocks (Fig. 1). The first block of the osteochondral sample was prepared for biomechanical and biochemical reference measurements. For biomechanical reference measurements, a small cylindrical (diameter = 3.7 mm) full-thickness cartilage sample, taken from the ultrasound indentation site (Fig. 1), was detached from the subchondral bone using a biopsy punch and a razor blade. The remaining adjacent cartilage was detached with a razor blade, immersed in PBS containing protease inhibitors, stored in a freezer ($-20\,^\circ\text{C}$) for 2 months and thawed prior to the biochemical analyses. The second block of the osteochondral sample was processed for the histological evaluation (Fig. 1).

**MEASUREMENTS WITH THE ULTRASOUND INDENTATION INSTRUMENT**

The design of the ultrasound indentation instrument has been described in a previous study. Briefly, the instrument consists of an unfocused miniature contact ultrasound transducer (10.5 MHz, broadband (bandwidth: 5.5–15.5 MHz, −6 dB), diameter 3 mm, Panametrics XMS-310, Panametics Inc., Waltham, MA, USA) mounted on the tip of a commercial arthroscopic indentation instrument (Artscan 200, Artscan Oy, Helsinki, Finland). The instrument enables simultaneous measurements of cartilage thickness, deformation and applied stress during indentation using an ultrasound transducer and a strain gauge (Appendix A).

Dynamic modulus of the samples was quantified with the ultrasound indentation instrument by inducing manually two series of instantaneous compressions on the sample [215 kPa prestress (duration 3 s), followed by a compressive strain of 4%, Fig. 2(a)]. The final dynamic modulus was obtained as a mean of these two measurements. Thickness and deformation of the samples were determined with the time of flight principle using a predefined speed of sound. In the present study, the predefined speed of sound was set to be 1627 m/s, i.e., the mean ultrasound speed in the bovine cartilage, as determined in an earlier study.

The dynamic modulus of the samples was calculated along Hayes et al., as presented more in detail in Appendix A.

After indentation measurements, ultrasound reflection from the cartilage surface was determined. In order to keep the transducer at a constant distance from the articular surface, a 3.5 mm long plastic sleeve was attached over the ultrasound transducer [Fig. 2(b)]. For each sample, the maximum peak-to-peak echo amplitude was measured twice and the average of amplitudes was calculated. The reflection coefficient was determined by calculating the ratio of the maximum amplitude recorded from the PBS–cartilage interface and the maximum amplitude recorded from the PBS–air interface (a perfect reflector) at the distance of articular surface. For further details, see Appendix A.

**BIOMECHANICAL REFERENCE MEASUREMENTS**

Biomechanical reference measurements were performed with a custom made high-resolution material testing instrument (resolution 5 mN and 0.1 µm for the force and position, respectively). Samples were tested using a stress-relaxation protocol (10% prestrain, 10% strain, 2 mm/s ramp speed, relaxation time 2400 s) in unconfined compression geometry. Dynamic modulus and the Young's
modulus at equilibrium were determined as a stress per strain ratio, instantaneously after 10% step and after 2400 s relaxation time, respectively.

HISTOLOGICAL AND BIOCHEMICAL ANALYSES

Mankin et al. presented in 1971 a histological–histochemical grading system for cartilage degeneration. Using the ‘Mankin score’, it is possible to identify different stages of degeneration by evaluating cartilage structure, cell alterations, safranin-O staining (i.e., glycosaminoglycan content) and tidemark integrity (Table I). The histological degenerative grade of the samples was obtained with this method. Before evaluation, the samples were randomized and blind-coded. Evaluation was conducted by three investigators and the final Mankin score was obtained as a mean value.

Prior to determination of the water content, cartilage samples were immersed in phosphate-buffered saline containing protease inhibitors. After this, wet weight of the samples was measured
using a high-resolution balance (Mettler AE240, Mettler-Toledo AG, Switzerland). Subsequently, samples were freeze-dried and the dry weight of the samples was determined. Water content of the cartilage tissue was calculated from this information.

Freeze-dried cartilage samples were moistened and then cut into small pieces. Guanidinium chloride (4 M, Fluka, Buchs, Switzerland) in 50 mM sodium acetate (pH 5.8) containing 100 mM epsilon-amino-n-caproic acid (Sigma), 5 mM benzamidine HCl (Sigma) and 10 mM disodium EDTA (Riedel-de-Haen, Sellze, Germany) was used to extract tissue proteoglycans at 4°C for 30 h. After collection of the extract, the residual tissue was washed with PBS, and digested for 24 h at 60°C with 0.05% proteinase K (Roche, Mannheim, Germany) in 10 mM EDTA and 100 mM sodium phosphate buffer (pH 7.4). Uronic acid content of the extract and residual tissue was analysed separately from the ethanol-precipitated samples36. The amounts of uronic acid in the extract and tissue residue were summed to obtain the total uronic acid content of tissue, which was then normalized with the wet weights of the specimens.

STATISTICAL ANALYSES

Kruskall–Wallis post hoc-test was used to reveal whether ultrasound indentation measurements could discern between the visually different degenerative grades. Spearman’s correlation test was used for the comparison of the Mankin score with other parameters. Pearson’s correlation test was used when comparing other parameters. Reproducibility of the measurements with the instrument was calculated as a standardized coefficient of variation (sCV) for the duplicated measurements37. SPSS statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Mean values (±SD) of all measured parameters obtained with the ultrasound indentation instrument (dynamic modulus \(E_{Dynt}\), ultrasound reflection coefficient \(R\), thickness \(h\)), from the reference mechanical measurements (dynamic modulus \(E_{DynRef}\), Young’s modulus at equilibrium \(E_{Ref}\)) and from the histological and biochemical analyses (Mankin score, water content \(H_2O\), uronic acid content \(Uronic\)) are presented in Table II. Along the progressive degeneration of cartilage, as judged by the visual or histological evaluation, acoustic and mechanical properties of the tissue were impaired with concurrent increase of tissue water content and decrease of uronic acid content (Table II). Reproducibility (sCV) of the measurements with the ultrasound indentation instrument was 2.7, 0.7 and 6.1% for the cartilage dynamic modulus, ultrasound reflection coefficient and thickness, respectively.

Table I

<table>
<thead>
<tr>
<th>Grade</th>
<th>III Safranin-O staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Normal</td>
<td>0</td>
</tr>
<tr>
<td>b. Slight reduction</td>
<td>1</td>
</tr>
<tr>
<td>c. Moderate reduction</td>
<td>2</td>
</tr>
<tr>
<td>d. Severe reduction</td>
<td>3</td>
</tr>
<tr>
<td>e. No dye noted</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>IV Tidemark integrity</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Intact</td>
<td>0</td>
</tr>
<tr>
<td>b. Crossed by blood vessels</td>
<td>1</td>
</tr>
<tr>
<td>c. Cloning</td>
<td>2</td>
</tr>
<tr>
<td>d. Hypocellularity</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>V Complete disorganization</th>
</tr>
</thead>
<tbody>
<tr>
<td>e. Clefts to calcified zone</td>
<td>5</td>
</tr>
<tr>
<td>f. Clefts to transitional zone</td>
<td>3</td>
</tr>
<tr>
<td>g. Clefts to radial zone</td>
<td>4</td>
</tr>
<tr>
<td>h. Clefts to calcified zone</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>6 Complete disorganization</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Complete disorganization</td>
<td>6</td>
</tr>
</tbody>
</table>

Table II

Articular cartilage properties (mean±SD) obtained from the ultrasound indentation measurements [dynamic modulus \(E_{Dynt}\), ultrasound reflection coefficient \(R\), thickness \(h\)], from the reference mechanical measurements [dynamic modulus \(E_{DynRef}\), Young’s modulus at equilibrium \(E_{Ref}\)] and from the histological and biochemical analyses [Mankin score, water content \(H_2O\), uronic acid content \(Uronic\)].

<table>
<thead>
<tr>
<th>Intact</th>
<th>Slightly discoloured</th>
<th>Superficial defect</th>
<th>Deep defect</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{Dynt}) (MPa)</td>
<td>9.2±5.8</td>
<td>2.4±0.3</td>
<td>2.1±1.0</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>(R) (%)</td>
<td>3.6±1.2</td>
<td>2.2±0.7</td>
<td>1.0±0.4</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>(h) (mm)</td>
<td>1.7±0.3</td>
<td>2.2±0.2</td>
<td>2.4±0.5</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>(E_{DynRef}) (MPa)</td>
<td>7.5±5.6</td>
<td>1.5±0.6</td>
<td>1.2±0.6</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>(E_{Ref}) (MPa)</td>
<td>0.28±0.12</td>
<td>0.23±0.11</td>
<td>0.27±0.17</td>
<td>0.06±0.04</td>
</tr>
<tr>
<td>Mankin score</td>
<td>0.7±1.1</td>
<td>1.0±1.0</td>
<td>3.8±1.8</td>
<td>6.8±2.5</td>
</tr>
<tr>
<td>(H_2O) (%)</td>
<td>80.3±2.0</td>
<td>82.0±1.3</td>
<td>83.6±3.0</td>
<td>83.5±2.0</td>
</tr>
<tr>
<td>Uronic (µg/mg)</td>
<td>8.9±3.4</td>
<td>6.7±1.0</td>
<td>5.4±1.3</td>
<td>4.3±1.6</td>
</tr>
</tbody>
</table>

The samples were visually classified to different degenerative grades.
Correlation coefficients between different parameters obtained with the ultrasound indentation instrument, reference material testing device or histological and biochemical analyses are presented in Table III. Linear correlation between the dynamic modulus, measured with the ultrasound indentation instrument, and the reference dynamic modulus was high \( r = 0.993, \) \( n = 30, P < 0.05 \), Fig. 3(a). Furthermore, the linear correlation within degenerated (soft) samples was also strong \( r = 0.802, n = 24, P < 0.05 \), Fig. 3(b). Mankin score, water content, uronic acid content and cartilage stiffness were significantly interrelated with each other. Cartilage dynamic and equilibrium modulus were positively correlated \( r = 0.717, P < 0.05 \) with the tissue uronic acid content and negatively correlated \( r = -0.586, P < 0.05 \) with the tissue water content (Table III).

Articular surface ultrasound reflection coefficient showed a significant linear correlation \( n = 30, P < 0.05 \) with the uronic acid concentration \( r = -0.847, \) Fig. 4(a)]. Mankin score \( r_{\text{Spearman}} = -0.847, \) reference dynamic modulus \( r = -0.863, \) Fig. 4(c) and degenerated samples: \( r = 0.576, n = 24, P < 0.05 \), Fig. 4(d)] and water content \( r = -0.663, \) Fig. 4(d)].

Visually different degenerative grades of articular cartilage were distinguished effectively with both mechanical and ultrasound reflection measurements, as conducted with the novel instrument (Fig. 5). However, large variation of the mechanical and acoustic properties was observed within visually intact (grade 0) cartilage samples (Fig. 5).

Based on the microscopical grading, mean value of Mankin score for the visually intact samples was 0.7 (range = 0–3) and for the degenerated samples 4.3 (range = 0–10). Statistically significant difference \( P < 0.05 \) in the dynamic modulus was detected between the intact samples and the samples with deep defects. For ultrasound reflection coefficient, a statistically significant difference \( P < 0.05 \) was obtained between the intact samples and the samples with superficial or deep defects and, furthermore, between the discoloured samples and the samples with deep defects.

Discussion

In 1995, we introduced an arthroscopic indentation instrument for the quantitative in vivo measurement of human articular cartilage stiffness. Later, the instrument was applied for the characterization of spatial variation of mechanical properties of normal human knee cartilage, the diagnostics of tissue softening in chondromalacia patellae as well as for monitoring cartilage repair after autologous chondrocyte transplantation. Recently, the instrument was shown to predict changes in proteoglycan content of equine cartilage as well as to diagnose the stage of cartilage degeneration in situ in progressive osteoarthritis.

The finite and variable thickness of articular cartilage affects the indentation response and may induce
uncertainty on the results, especially when measuring thin cartilage. To avoid the uncertainties related to unknown tissue thickness, combined indentation and ultrasound measurements have been applied. Ultrasound indentation enables a simultaneous measurement of tissue thickness, indentation deformation and stress and, thereby, quantification of tissue intrinsic mechanical properties. In addition, based on the encouraging studies on the sound backscattering or attenuation in the cartilage and bone, ultrasound measurements with the novel instrument may be used to characterize the mechanical and structural properties of cartilage and subchondral bone. In the present study, the ability of the handheld ultrasound indentation instrument to distinguish healthy and degenerated bovine patellar cartilage from each other was investigated in vitro.

Mechano-acoustic properties of cartilage, i.e., dynamic modulus, ultrasound reflection coefficient and thickness were measured in a reproducible way with the instrument. The measured parameters were significantly related to tissue water and uronic acid contents, suggesting capability of the technique to provide information on the cartilage composition. Furthermore, distinct differences between the visually different degenerative stages could be observed with the measurements of dynamic modulus and ultrasound reflection (Fig. 5). The extensive variation of acoustic and mechanical properties within visually intact cartilage samples (Fig. 5) revealed the insensitivity of visual evaluation of cartilage quality. This finding was confirmed by the histological grading (Mankin score) and the biomechanical reference measurements. Some of the visually intact samples showed signs of early degeneration, such as depletion of proteoglycans in superficial tissue, as verified by histological and functional reference measurements. In addition, the ultrasound indentation instrument

**Fig. 4.** Significant linear correlations were established between the ultrasound reflection coefficient, as measured with the ultrasound indentation instrument, and the uronic acid concentration (a), Mankin score (b), reference dynamic modulus (c) or water content (d). Spearman’s correlation was used for the comparison of ultrasound reflection coefficient and Mankin score, and Pearson’s correlation was used when comparing ultrasound reflection coefficient with other parameters.

**Fig. 5.** Mean values (±SD) of dynamic modulus and ultrasound reflection coefficient, as measured with the novel instrument. The instrument distinguished sensitively visually different degenerative grades. However, the large SD with intact samples suggests the insensitivity of visual grading of cartilage quality.
distinguished sensitively between the normal and histologically degenerated cartilage.

Collagen is mainly responsible for the dynamic compressive, tensile and shear stiffness, while proteoglycans and water control the equilibrium compressive stiffness and the viscoelastic creep and stress-relaxation behaviour of articular cartilage. Dynamic indentation and ultrasound reflection from the articular surface are sensitive methods for revealing the changes in the superficial collagen network. To fully characterize the functional integrity of the tissue, it would be important to quantify the time-dependent mechanical properties of cartilage along with the dynamic stiffness and articular surface reflection coefficient (i.e., quality of superficial collagens). Previously, manual 20 s creep measurements were successfully conducted with the ultrasound indentation instrument. However, to fully interpret the results of creep measurement and to avoid possible uncertainties related to the measurement protocol and variable tissue thickness, valid theoretical approach should be applied. Our next aim is to use finite element techniques to clarify effects of tissue structure, composition and thickness on the cartilage creep during ultrasound indentation.

Several studies have suggested that ultrasound techniques can be used to characterize the roughness or fibrillation of articular surface. In the present study, ultrasound reflection from the articular surface was a significant measure of degenerative stage, water content and mechanical properties of articular cartilage (Fig. 4). In contrast to our earlier studies, the ultrasound reflection coefficient of the cartilage surface correlated linearly with the proteoglycan (uronic acid) content of the uncalcified tissue. This correlation may be secondary and follow from the fact that cartilage degeneration is a complex process with parallel alterations in proteoglycans, collagen network and tissue water content. Nevertheless, ultrasound reflection from the articular surface seems to provide a single, easy-to-use parameter for diagnosing the cartilage integrity.

High frequency ultrasound may provide additional information on the quality of subchondral bone, as suggested in an earlier study. Measurement of the ultrasound speed and attenuation in the trabecular bone has been used in clinical diagnostics of osteoporosis. A recent study suggested that ultrasound backscattering is strongly related to the density and microarchitecture of human calcaneal bone in vitro. Possibly, ultrasound backscattering may be used to evaluate the structure and properties of the subchondral bone. However, when evaluating the subchondral bone, the ultrasound attenuation in the overlaying cartilage should be taken into account.

As a conclusion, the results of the present study suggest that the ultrasound indentation instrument may significantly improve diagnostics of early osteoarthritis. However, further theoretical and experimental study is needed to fully validate the instrument. In addition, some technical modifications are needed before the instrument can be applied clinically in vivo. These modifications include an arthroscopically controllable system for keeping the transducer at a known distance from the articular surface during ultrasound reflection measurements.

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A.1. Ultrasound indentation measurements

Based on the theory of elasticity (Hayes et al. 1972), Young’s modulus of the indented cartilage can be calculated according to formula:

\[ E = \frac{F(1-\nu^2)}{2\omega \kappa h} \] (1)

where F is the force, \( \nu \) is the Poisson’s ratio of cartilage, \( a \) is the contact radius of the indenter, \( \omega \) is the deformation, \( \kappa(a/h) \) is a theoretical correction factor due to finite cartilage thickness and \( h \) is the cartilage thickness. Although cartilage is a biphasic tissue consisting of a solid matrix and an interstitial fluid phase, cartilage behaves as an incompressible (i.e., \( \nu = 0.5 \)) single phase elastic material under instantaneous compression.

In ultrasound indentation technique, cartilage is compressed manually with the ultrasound transducer (dia. = 3.0 mm) and the ultrasound signal is collected simultaneously [Fig. 2(a)]. In our instrument, force (F) induced onto the tissue is measured with the strain gauge inside the instrument. Thickness (h) and deformation (\( \omega \)) of the cartilage are detected in real-time from the ultrasound signal using the predefined value for speed of sound (in this study 1627 m/s) and the information on the ultrasound flight time from the bone–cartilage interface. Subsequently, the dynamic modulus (i.e., instantaneous Young’s modulus) of the cartilage is calculated according to formula (1).

It is not possible to measure the speed of sound with the present technique, thus it has to be assumed for the determination of thickness and deformation. In our previous study, we measured the cartilage speed of sound at different anatomical sites: medial tibial plateau, femoral medial condyle, patella, lateral side of the patellofemoral groove and ankle. At these sites, the use of a constant speed of sound (1627 m/s) value induced maximum errors of 7.8% on cartilage thickness and of 6.2% on cartilage dynamic modulus, as determined with the ultrasound indentation technique. We concluded that these maximum errors are acceptable in clinical measurements.

For the determination of ultrasound flight time (i.e., time between the cartilage surface and the subchondral bone), Hilbert envelope was calculated for the reflected ultrasound signal and the envelope was Hamming-windowed. These operations were conducted in order to enhance the tracking of the reflected signal. Subsequently, ultrasound flight time was determined from the point of the maximum value of the Hamming-windowed Hilbert-envelope.

A.2. Ultrasound reflection measurements

For the determination of the ultrasound reflection coefficient from cartilage surface, an external sleeve was attached over the ultrasound transducer [Fig. 2(b)]. The sleeve held the transducer at a constant distance (3.5 mm)
from the cartilage surface. The transducer, attached sleeve and the cartilage sample were immersed in PBS and the sleeve was gently pressed against the cartilage surface during measurements. Perpendicularity between the transducer and the cartilage surface was ensured by gently aligning the transducer to obtain the maximum echo amplitude. Theoretically, the transducer is perpendicular to the articular surface when the maximum amplitude is obtained for the reflected ultrasound signal.

As a reference, the maximum amplitude of the reflected ultrasound signal from the PBS–air interface was collected at the distance (3.5 mm) equal to that applied in cartilage measurements. The PBS–air interface is a perfect reflector for the reflected ultrasound signal. Since more than 99% of the ultrasound is reflected from the interface, the following formula:

\[ R = \frac{A_1}{A_0} \]

where \( A_1 \) is the maximum peak-to-peak amplitude obtained in the cartilage measurement (reflection from the PBS–cartilage interface) and \( A_0 \) is the maximum peak-to-peak amplitude obtained from the PBS–air interface.

References


