Assessment of rat articular cartilage maturation using 50-MHz quantitative ultrasonography

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Summary

Objective: The objective was to assess the relationship between maturation-related structural changes of articular cartilage and variations of acoustic parameters estimated using high frequency ultrasonography.

Design: Patellae taken from 48 immature Wistar male rats and divided into six age groups (from five to 11 weeks old) were explored in vitro using 50-MHz scanning acoustic microscopy, then assessed by histology for the analysis of the cartilage cell distribution and fibrillar collagen organization. The variation of cartilage proteoglycan and collagen content with age was evaluated. Thickness measurements performed on both B-scan images and histologic sections were compared. Ultrasonic radiofrequency signals reflected by the cartilage surface and backscattered from its internal matrix were processed to estimate the integrated reflection coefficient (IRC) and apparent integrated backscatter (AIB).

Results: One-way ANOVA indicated that acoustic parameters and thickness change significantly (P<0.05) as the animal matures because of age-related changes in cartilage composition and morphology. A moderate correlation was found between IRC and the animal age. The parameter decreased slightly but significantly over time. However, a good correlation was observed between the rat age and the AIB, which decreased significantly over time. The parameter variation was mostly related to the changes in collagen fiber orientation, and/or to a change in cell size, density and organization.

Conclusions: Current results indicate that acoustic properties of cartilage are affected by maturation-related cartilage changes. This suggests that high frequency ultrasonography may serve as a useful means for the investigation of cartilage matrix structural changes occurring under various clinical circumstances, like those observed during osteoarthritis, and for the evaluation of the efficacy of specific therapeutics.

Key words: Cartilage, Maturation, High frequency Ultrasound, Tissue characterization.

Introduction

During progression of osteoarthritis (OA), the common degenerative modifications observed in cartilage are a fibration of its superficial layer, changes in organization and activity of the chondrocytes, changes in water and proteoglycan content, and a disruption of cross-links between the collagen fibers1–3. All these phenomena take part in a remodeling of the cartilage and can induce variations of the tissue thickness.

The diagnosis of OA is delayed because its initial phase activity is clinically silent. Furthermore, current clinical non-invasive imaging modalities, such as radiography (X-ray), computed tomography (CT) and conventional echography (5–10 MHz) allow the detection of only severe cartilage lesions that characterize the late stages of OA. Difficulties in this field are partly related to the constraints of image resolution. An enhanced spatial resolution is required to detect early lesions and follow their evolution in time. Using magnetic resonance imaging (MRI), these resolution challenges can be addressed in part by the use of higher performance gradients that are becoming available from the equipment and permit thinner sections and smaller field of view. Clinical studies have demonstrated that MRI allows a direct evaluation of the patellar cartilage from its surface to its deepest zones and the detection of severe focal chondral lesions with a spatial resolution of 470×625 μm² and slice thickness varying between 2 and 3 mm4–6. Higher MRI sensitivity can be obtained by the use of advanced imaging sequences that can provide specific information about the molecular structure in general. However, the accuracy of these techniques for detecting early structural abnormalities of the cartilage still remains controversial7.

In ultrasonography, higher spatial resolution is reached by increasing the frequency of the imaging system. In a previous study8, our group reported that high-frequency (50 MHz) scanning acoustic microscope (SAM) could be used to investigate cartilage structural changes at various
stages of a chemically induced OA in rat knees. The 30-μm axial resolution of the SAM allowed the in-vitro visualization of the early and progressive lesions involving both the surface and internal matrix of the cartilage. Furthermore, we have shown that quantitative ultrasound parameters such as the integrated reflection coefficient (IRC, a quantitative index of the acoustic energy level reflected from the surface of the cartilage) and apparent integrated backscatter (AIB, a quantitative index of the level of acoustic energy backscattered from the cartilage internal structure) could reflect the overall structural and biochemical composition of cartilage matrix and their variations caused by the disease. However, for the successful clinical application of high frequency ultrasonography to the assessment of the cartilage integrity, it is necessary to establish the relationship between the changes of this complex tissue and variations of acoustical parameters.

The goal of the present work was to determine more precisely the components of the cartilage, which are involved in the variations of acoustical parameters. For this purpose, acoustic parameters were measured during the maturation of the cartilage, which is a reproducible and well-described model of cartilage changes, and correlated to qualitative analysis of histologic images and quantitative evaluation of biochemical constituents of the tissue. To the best of our knowledge, there is no report on the relationship between the variations of ultrasound parameters and age-related progressive changes of the cartilage occurring in the early maturation period.

Animals and method

ANIMALS

One set (N=48) of immature male Wistar rats, approximately 5 weeks old, was studied. These animals were divided into six groups, which were sacrificed when the animals were 5, 6, 7, 8, 10 or 11 weeks old (approximately 200 g, 250 g, 300 g, 350 g, 400 g and 450 g in weight, respectively). The rats were anesthetized and killed by cervical dislocation. Patellae were dissected within minutes of the sacrifice. They were mounted on a special holder and fixed in formaldehyde solution for tissue preparation and histomorphological analysis. Only right patellae were used for ultrasound (US) data acquisition. After the scanning, patellae were fixed in formaldehyde solution for 48 hours. They were mounted on a special holder and positioned at the focal zone in order that the cartilage surface oriented perpendicularly to the probe axis. The patellae were scanned with a displacement step of 40 μm along both the sagittal (long axis) and transverse directions to acquire three-dimensional information about the cartilage structure.

Estimation of acoustical parameters

The integrated reflection coefficient (IRC) and apparent integrated backscatter (AIB) were measured using a standard substitution method, which has already been extensively discussed in a previous paper. For the sake of clarity, the main steps of this measurement technique, which eliminates the apparatus transfer functions, are briefly described below.

Integrated reflection coefficient. The 32 first samples of the signal reflected by the cartilage surface were selected using an 80-ns-duration Hamming window. The power spectrum | S_0(f, z_0) |^2 of the windowed signal was calculated and normalized to the power spectrum | S_1(f, z_0) |^2 of the signal reflected by a steel plate. The steel plate was positioned at the same distance z_0 from the transducer as the cartilage surface. The ratio of both spectra was averaged spatially over a region of interest (ROI) that was selected in the central part of the patella, where the cartilage surface was perpendicular to the direction of sound propagation. Then, the mean ratio was converted into dB and integrated over the −6 dB frequency bandwidth.
\[ R_{\text{eq}}(f) = 10 \log_{10} \frac{|S_c(f, z_f)|^2}{|S(f, z_f)|^2} \]  

(1)

where \( R_{\text{eq}}(f) \) is the intensity reflection coefficient at the cartilage surface in dB, and (...) indicates the spatial average calculated over 45 A-lines corresponding to a ROI of 120 \( \mu \text{m} \) in the transverse direction by 600 \( \mu \text{m} \) in the sagittal direction. The integrated reflection coefficient is defined by the following integral equation:

\[ IRC = \frac{1}{\Delta f} \int_{\Delta f} R_{\text{eq}}(f) \, df \]  

(2)

where \( \Delta f \) corresponds to the frequency range of 30–75 MHz.

**Apparent integrated backscatter.** The signal backscattered from a volume \( dV \) of 100 \( \mu \text{m} \) thickness, which was located 32 \( \mu \text{m} \) beneath the cartilage surface, was selected using a 120-ns-duration Hamming window. Its power spectrum \( |S_c(f, z_f)|^2 \) was calculated and normalized to the power spectrum \( |S(f, z_f)|^2 \) of the signal reflected by a steel plate. The steel plate was positioned at the same distance \( z_f \) from the transducer as the center of the volume \( dV \). The frequency dependence, in dB, of the apparent energy backscattered by the cartilage is equal to:

\[ \mu_{\text{eq}}(f) = 10 \log_{10} \frac{|S_c(f, z_f)|^2}{|S(f, z_f)|^2} \]  

(3)

where (...) indicates the spatial average over 200 echo graphic A-scans corresponding to a ROI of 320 \( \mu \text{m} \) in the transversal direction by 1 mm in the sagittal direction, which was selected in the central region of each patella. The energy backscattered by the cartilage is given by:

\[ AIB = \frac{1}{\Delta f} \int_{\Delta f} \mu_{\text{eq}}(f) \, df \]  

(4)

where \( \Delta f \) is the frequency range from 35 to 70 MHz corresponding to the ~6 dB frequency bandwidth of the backscattered signal from the cartilage internal matrix.

**Estimation of cartilage thickness**

Ultrasonic measurements of the mean cartilage thickness were performed on two adjacent sagittal B-scan images of the central region of the patella and then averaged. On these images, two hyperechoic bands marked the cartilage boundaries. The first results from the US reflection at the cartilage surface and the second marks the hyaline cartilage–calcified cartilage–bone interface. A region, 1 mm long approximately, corresponding to 25 A-scans (dL), was adjusted manually to the cartilage boundaries in the central part of each B-scan image (Fig. 2), where the cartilage thickness is the greatest. The area \( A \) of this region was calculated in pixels and the average thickness of the cartilage (in pixels) was estimated by dividing the area \( A \) by its length dL. The average thickness in pixels was converted to micrometers using a scaling factor of 1.25 ns/pixel which accounts for the double path length, and a speed of sound of 1600 m/s.

**Histological analysis**

**Tissue preparation**

After US data acquisition, the patellae were fixed in 10% neutral buffered formalin solution for 5 days, decalcified (RDO solution, Eurobio) for 24 h and conventionally wax embedded. Sixty sagittal contiguous sections (5–10 \( \mu \text{m} \) thick) were performed in the central part of the patellae corresponding to the US acquisitions. Then the serial sections were stained with hematoxilin-eosin-safran (HES) and Toluidine blue to estimate the cellularity and the proteoglycan (PG) content, and with Picrosirius red to estimate the collagen content. The collagen fiber orientation was determined by polarized light microscopy.

Because of technical problems in preservation of the 7-week-old animal cartilages, histologic processing could not be performed at this maturation stage.

**Histologic measurement of cartilage thickness**

The mean thickness of identical site-matched regions was estimated on corresponding images of histologic sections stained with HES. These sections were selected in such a way that the cartilage thickness was the greatest, indicating that they are located in the central part of the patella in the transverse direction.
To estimate a mean ‘histologic’ thickness (HT) over the same volume of cartilage approximately, measurements were averaged over four non-adjacent histologic sections (in sagittal plane) separated by an interval of 15–30 μm. A 1-mm-long region was adjusted manually to the cartilage boundaries in the central part of each histologic sections. The difficulty encountered in histologic measurement of cartilage thickness consisted mainly in determining the deep boundary corresponding to that of the region previously selected on B-scan images. The deep boundary was set on histologic sections just beneath the deepest hypertrophic cells.

BIOCHEMICAL ANALYSIS

The contents of sulfated glycosaminoglycan and collagen were evaluated as a function of the cartilage maturation using patellae taken from additional groups of animals (N=6 per group) of the same ages and weights as those explored by ultrasound. The values of these parameters were expressed in μg per mg of dry cartilage.

For the quantification of sulfated glycosaminoglycans, the cartilage was digested in sodium phosphate buffer containing EDTA, dithiothreitol and papain. The solution was completed to 1 ml by addition of Tris/HCl. The assay was calibrated using reagent blanks and standards containing up chondroitin sulfate in the same solvent as that of the samples. The metachromatic reaction of dimethylmethylene blue was measured at 525 nm.

For the evaluation of hydroxyproline (collagen), samples of cartilage were dried and hydrolyzed in HCl. NaOH solution was added for neutralization. Hydroxyproline oxidation was initiated by adding chloramine T. Finally, aldehyde-perchlorique acid reagent was added and mixed. The absorbency was determined with a spectrophotometer at 550 nm.

STATISTICS

Distribution properties of all the data were examined. All measurements were normally distributed. However, it was necessary to reject a single high outlier from each of IRC and histologic thickness measurements. An analysis of variance (one-way ANOVA) was performed using NCSS software (version 2000, NCSS, Utah, U.S.A.) to test for significant differences between group means. For each variable, statistical significance tests and multiple comparison procedures were performed with a level of significance α=0.05.

Results

QUALITATIVE ANALYSIS

Structure of cartilage

Only sections stained with Toluidine blue and Picrosirius red (in polarizing microscopy) are shown in Fig. 3 and Fig. 4 to describe the change in cartilage structure as a function of maturation.

Immature patellar cartilage (5–10 week old). Structural organization. On histological sections stained with Toluidine blue (Fig. 3) the articular cartilage was thick with a typical endochondral bone formation at the lower border. The cartilage thickness decreased rapidly between five and 10 weeks of age. On sections stained with picrosirius red and viewed with polarizing microscopy (Fig. 4) the articular cartilage was organized in three zones: the superficial zone (approximately 5% of the cartilage thickness, varying between 20 μm at 5 weeks old and 12 μm at 8 weeks old) with orange collagen fibers oriented parallel to the cartilage surface. On corresponding sections stained with Toluidine blue, this layer contained ellipsoidal cells which were approximately 5–10 μm in width by 10 μm in length and oriented parallel to the cartilage surface. The transitional or intermediate zone (approximately 35% of the cartilage thickness, varying between 130 μm at 5 weeks and 80 μm at
at 8 weeks) where the collagen fibers display a green color and an alveolar meshwork organization with their long axis oriented parallel to the articular surface. In this zone, the cells were spherical, 10–15 μm in diameter and randomly distributed. The hypertrophic zone, which contained the largest cells (30 μm in diameter), corresponded approximately to 60% of the cartilage thickness (varying between 230 μm at 5 weeks and 140 μm at 8 weeks) contained thin green collagen fibers having a perpendicular organization and surrounding the hypertrophic cells which occupied three or four rows. The cellularity in the different zones tended to decrease progressively as a function of cartilage maturation. The cells changed in size and shape, and assembled in clusters. The cartilage–bone interface was not well delineated since it constituted a mixture of cells, marrow and bone.

Matrix of the cartilage. The matrix (PGs) of youngest cartilage was markedly stained by Toluidine blue. No spatial variation between the different zones of the tissue was observed (Fig. 3). Nevertheless, the matrix organization and, more precisely, the cells–matrix ratio was different between the transitional and the hypertrophic zones. The hypertrophic zone presented a high cells–matrix ratio, whereas the transitional zone was composed of small cells surrounded by an abundant matrix. An important decrease of Toluidine blue staining was observed between 5 and 10 weeks. The Picosirius red (collagen content) mainly stained the cartilage in the superficial and transitional layers.

Mature patellar cartilage (from 10-week-old). Structural organization. On histological sections, the articular cartilage was organized in four zones. The superficial zone had a thickness and structure that remained unchanged. The transitional zone was thinner than that of immature cartilage since it occupied approximately 18% of the cartilage thickness (approximately 45 μm). This layer remained dark with no birefringence because the collagen fibers were arching in several directions. The radial zone occupied approximately 68% of the cartilage depth and was 175 μm thick for 11-week-old rats. In this layer almost all the cells (approximately 30 μm in width by 50 μm in length) were grouped in clusters of 3–4 units that were organized in columns oriented perpendicular to the articular surface. On polarizing light microscopy, these cells were surrounded by thick and intense orange collagen fibers which were perpendicular to the cartilage surface. In 11-week-old rats, the hypertrophic layer was formed by just one row of cells surrounded by thick orange collagen fibers. Finally, the calcified zone, which allows distinction between immature and mature cartilage, was seen only in 11-week-old animals. This layer occupied 10% of the cartilage thickness. The cartilage–bone interface consisted of a large proportion of bone compared to that of marrow. Thus, this interface was sharper and easily identified.

Matrix of the cartilage. The cartilage matrix (PGs) was weakly stained by Toluidine blue. No spatial variation of PGs between the different zones of the tissue was observed. Compared to immature cartilage, the intensity of Picrosirius red staining of mature cartilage increased in the different zones and mainly in the radial zone. Thick bundles, perpendicular to the articular surface and surrounding the clusters, were observed in the deepest region of the radial zone. On polarizing microscopy, these birefringent bundles were green in their upper part and became orange near the calcified cartilage.

Quantitative analysis

Analysis of the integrated reflection and backscatter coefficients

The estimated reproducibility of IRC and AIB measurements was reported earlier and found to be 3.5% and 1%,
Individual AIB values ranged from $-36.6\text{ dB}$ to $-53.6\text{ dB}$. One way ANOVA indicated that age has a significant effect on cartilage internal structure ($P<0.05$). The group average AIB values, estimated over each group, showed a decline with age from $-40.9\text{ dB}$ for 5-week-old animals to $-51.8\text{ dB}$ for 10-week-old animals. All-pairwise multiple comparison between means showed that the difference in AIB was significant between two groups of animals sacrificed at least 2 weeks apart. However, because of the slight increase of AIB observed between 10 and 11 weeks, the AIB measured at 8 weeks of age was not significantly different from the AIB of 11-week-old rats. A least-square linear fit to AIB data over time showed a significant linear decrease of $-2.2\text{ dB}$ per week ($R=-0.89; P<10^{-4}$).

**Cartilage thickness**

The reproducibility of thickness measurement in B-scan images assessed in a previous work was 1.3%\(^9\). For both ultrasonic and histologic measurements, the mean cartilage thickness and the standard deviation were calculated over the eight animals of each group and plotted as a function of rat aging in Fig. 8. The mean ultrasonic (US) thickness decreased from $353.9\pm25.4\text{ µm}$ for the 5-week-old rats down to $227.2\pm51.5\text{ µm}$ for the 7-week-old animals, and was approximately constant ($232\pm40\text{ µm}$) for the older groups. One-way ANOVA indicated that age has a significant effect on cartilage thickness ($P<0.05$). All-pairwise multiple comparison between means showed that the cartilage thickness of the 5- and 6-week-old groups was significantly different from that of all the other groups. No significant difference was found between 7, 8, 10 and 11-week-old specimens. A least-square linear fit to US cartilage thickness data of the three first groups over time showed a significant linear decrease of $-63.3\text{ µm}$ per week ($R=-0.82; P<10^{-4}$).

Statistical analysis of thickness data obtained from histology showed the same trends as those observed on ultrasonic data. A good agreement was noted between US and histologic thickness with a highly significant positive
age has a significant effect on cartilage collagen content ($P<0.05$). All-pairwise multiple comparison between means showed that all the groups were significantly different except the collagen content at 6 weeks, which was not significantly different from that obtained at 5 and 7 weeks old.

In mature cartilage, the biochemical analysis of PGs showed a significant decrease between 10- and 11-week-old cartilage. The collagen content slightly but significantly increased to 75.01±0.70 µg per mg of cartilage at 11 weeks of age.

### Discussion

The current study investigated the relationship between maturation-related structural changes in rat patellar cartilage and high frequency ultrasound measurements. The US method, which combined high resolution imaging and local estimation of acoustic parameters, was confronted to histological and biochemical findings.

Maturation is characterized by cartilage thinning and structural modifications of the tissue mainly involving the PG and collagen content and the collagen fibrillary network orientation and density. Correspondences were observed between histologic sections and ultrasonic images, concerning the cartilage surface, the laminar appearance of matrix, and the cartilage–bone interface. The bilaminar backscatter pattern of the matrix that was observed on B-scans of immature cartilage apparently corresponded to the laminar organization of the cartilage in histologic sections. However, the thickness of the layers that were observed in US images did not exactly correspond to the thickness of the layers observed in images of histologic sections. This is due to the fact that, first, in ultrasound exploration the superficial layer of the cartilage could not be separated from the cartilage surface since its thickness is below the axial resolution of the SAM (30 µm), and second, the acoustic properties, in particular, speed of sound, may vary within the different layers.

Changes in echo intensity and thickness of the cartilage–bone interface observed in B-scan images may result from the effective remodeling of this interface during maturation, which could be observed on histologic sections. For
5-week-old animals, this interface was irregular due to the interpenetration, sometimes over a depth larger than 50 \, \mu m, of cartilage hypertrophic cells, marrow and bone trabeculae. This irregularity gave rise to thick echogenic band on the corresponding B-scan images. For 11-week-old animals, the cells were well separated from the bone and the cartilage–bone interface contained less marrow. Thus, the boundary between cartilage and subchondral bone was more smooth and regular and gave rise to a short specular and more intense echo.

The integrated reflection coefficient is primarily determined by the composition and micro-architecture of the superficial layer, and the surface roughness. In the current study, IRC measurements demonstrated a moderate correlation between the IRC values and time. A visual analysis of histologic sections obtained at different stages of maturation did not show any alteration of either the cartilage surface or the superficial layer. Since, to our knowledge, there is no reported study on the local histologic evaluation of the cartilage superficial layer as a function of the early stages of the tissue maturation, the slight decrease of IRC with maturation cannot be thoroughly explained at this time. It should, however, be noted that IRC values are subject to measurement errors. The most probable sources of error are the effect of the internal structure backscattered on IRC values (the resolution of our US system does not allow the detection of the superficial layer) and/or a strong influence of the US beam incidence angle with respect to the cartilage surface on IRC measurement (a \( 2^\circ \)-variation of incident angle induced by a 50 \, \mu m lateral displacement of the transducer resulted in an IRC variation of \(-1.7 \, \text{dB}, \) i.e. approximately 7.5%)..

The most important finding of the current study was the highly significant age-related decrease of AIB. This parameter, which is device- and operator-independent, provides quantitative information on the characteristics (concentration, size, orientation, e.g. anisotropy and acoustic impedance mismatch) of subresolution scatterers of the explored tissue. AIB values were greater by up to 11 \, dB, i.e. 27\% in immature cartilage, than that of AIB in mature cartilage. Substantial modifications of cartilage composition and structure were observed in the region of interest on histologic images during the maturation. Progressively, in the volume of US measurements, the cellularity slightly decreases, the size and the shape of cells change, and the collagen fibers change in orientation.

Regarding the modification of the matrix biochemical constituents within the whole depth of the specimens, our analysis indicated that age has a significant effect on proteoglycan and collagen content. During maturation the PG’s content progressively decreased by up to 49\%, while the collagen content slightly increased by up to 34\%.

The AIB decrease should not be associated significantly with the decrease in PG content. Indeed, a recent preliminary study reported by our group, dealing with correlation of 50 MHz ultrasound backscatter with matrix constituents of rat patellar cartilage, has shown that there was no difference between ultrasound backscatter images of PG depleted cartilage (selective degradation of PGs using hyaluronidase enzyme) and backscatter images of their control contralateral nondegraded cartilage.

With regard to cell contribution, it can be noted that in our study ultrasound backscatter does not provide resolution to the cellular level. However, the cell size (10–30 \, \mu m) is consistent with expected domains of scatterers to which ultrasound backscatter is sensitive. It would appear likely, therefore, that the apparent decrease in cell density and changes in cell diameter and organization within the region of measurement have some effect on apparent integrated backscatter during the maturation process. Previous studies performed in ocular melanoma at lower frequencies (10 MHz) demonstrated a correlation between ultrasound backscatter parameters and cell characteristics. AIB may also be affected by the increase in collagen content of the whole matrix, which may be attributed to the high fiber density observed in the mature state of the cartilage. Because of their small size, collagen fibers cannot be directly detected; however, the change in collagen fiber orientation could explain the local change in apparent integrated backscatter. Indeed, AIB value was greater when ultrasound propagated across collagen fibers than that of AIB of ultrasound propagating along the fibers. This finding is in agreement with results described by other groups in highly oriented biological tissues such as myocardium and skeletal muscles.

Finally, our findings demonstrated a good agreement between US and histologic measurements of the total cartilage thickness. The potential sources of error that may account for the difference in US and histologic cartilage thickness measurements include variations of cartilage thickness due to histologic preparation, difference in orientation of US scan plane and histologic section, and inaccuracy of speed of sound. The slope and intercept of a linear fit between US and histologic thickness data were significantly different from 1 and 0, respectively, indicating that the error between US and histologic measurements was a function of the cartilage thickness. Since cartilage thickness was found to be age-dependent, one could argue that the difference between US and histologic thickness measurement is also dependent on age. Speed of sound is known to vary as a function of the cartilage composition and micro-architecture. Thus, age-related changes in cartilage composition, water content and/or micro-structure may influence speed of sound and may potentially explain the age-related difference between US and histologic thickness measurements observed in this study.

In summary, this study reports the first results on the relationship between quantitative ultrasound and maturation-related changes in rat patellar cartilage. US technique allows local characterization of structural changes in cartilage. The decrease of the apparent integrated backscatter appears more likely to be related to changes in the cartilage anisotropy resulting from the change in collagen fiber orientation within the region of measurement. Histologic and biochemical findings indicate that chondrocytes and collagen content may contribute to the changes in ultrasound backscatter. Local measurements of other acoustic properties, such as speed of sound and the frequency variation of attenuation in immature and mature cartilage, is under way in our laboratory in order to clarify the contribution of each cartilage constituent, in particular the collagen content, to the changes in backscattered ultrasound.

This study demonstrates that quantitative measurements of ultrasound backscatter may provide a useful and sensitive index to age-related or disease-related modifications of cartilage composition or microstructure. High frequency ultrasound has the potential to be used as a non-invasive and quantitative technique for the research and evaluation of pharmacological treatments using animal models. In the future, attention should also be focused on the miniaturization of the echographic probe potentially to extend this technique to the clinical field. Such understanding provided
by this study could improve the utility of ultrasonography for
the assessment of joint cartilage and related diseases.

References