Comparison of articular cartilage images assessed by high-frequency ultrasound microscope and scanning acoustic microscope

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Abstract

Purpose The purpose of this study was to compare images of a newly developed high-frequency ultrasound imaging system (HFUIS) and scanning acoustic microscope (SAM) and to calculate their Pearson product moment correlations with a view to applying HFUIS for clinical use.

Methods Cylindrical cartilage–bone complexes from adult male Sprague-Dawley rats were obtained. The specimens were immersed in normal saline and scanned by HFUIS. Intensity by HFUIS was normalised by reflection from a steel plate at the same distance. After the scanning, specimens were fixed with paraformaldehyde, decalcified and embedded in paraffin. Thinly sliced tissues were prepared for SAM evaluation. After the scanning, three layers of articular cartilage (superficial, middle and deep) were independently evaluated and their relationships calculated.

Results The superficial and deep layers indicated high relative intensity, whereas the middle layer showed nonho-

mogeneous relative intensity by HFUIS. A high relative intensity by HFUIS and high sound speed area by SAM had strong correlations (Pearson product moment correlation, superficial layer 0.704, middle layer 0.731).

Conclusions HFUIS produced high-resolution images of the articular cartilage and its intensity was strongly correlated with sound speed by SAM.

Introduction

Articular cartilage is a viscoelastic connective tissue with a smooth surface, which serves as a cushion between joint bones. During mechanical loading, interstitial water flows out of the tissue to the joint space together with the synovial fluid, which guarantees almost frictionless motion between tangentially contacting joint surface [1]. Osteoarthritis (OA) is a common joint disorder and estimated to cause symptoms in 20–40% of the elderly population [2, 3]. Its symptoms are pain, joint stiffness and muscle weakness [4]. Intra-articular injection of hyaluronic acid and ingestion of glucosamine are suggested for treating early knee OA [5]. If conservative treatment fails, patients require surgical intervention such as total arthroplasty to relieve pain [6]. However, surgical implants are not permanent and need revision.

OA is characterised by collagen network disruption, proteoglycan depletion, water content increase and deterioration of mechanical properties of the articular cartilage [7]. Injury to the collagen network is thought to represent the point of no return in OA progression [8]. Because superficial cartilage degradation is the first sign of OA, its early detection would be of diagnostic importance [9]. Once the superficial layer of the articular

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cartilage is damaged, its mechanical properties deteriorate and degenerative changes begin to accumulate. If the early change is properly diagnosed, disease progression may be slowed by medication or lifestyle changes [10]. Therefore, early detection of the pathological changes in the articular cartilage is essential in order to treat the disease successfully [1].

Modalities for diagnosing knee OA include noninvasive techniques such as plain X-rays, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US); and invasive techniques such as arthrography and arthroscopy. MRI is a powerful tool for evaluating the articular cartilage, although location and economic limitation for routine use remain [6] and its resolution ability is not high. Arthroscopy is the only method by which to evaluate surface morphology and softness of the degenerated articular cartilage. However, it reveals only macroscopic changes and cannot be used repeatedly because of its invasiveness. US is quick and inexpensive, and its resolution higher than MRI. US plays an important role in assessing musculoskeletal soft tissues [11], articular cartilage and subchondral bone [9]. Though US is a skill-dependent method, there is a significant correlation between in vivo US and histological evaluation of the OA cartilage [12].

A scanning acoustic microscope (SAM) characterises biological tissues by estimating the elastic parameters based on sound speed with a central frequency of 80 MHz [13]. SAM allows visualisation of thinly sliced tissues [14, 15] or single-layered cultured cells [16]. In our previous study, articular cartilage degeneration induced by joint immobilisation demonstrated a decrease in sound speed [17].

Because preparation of sliced tissues is needed for SAM, it is not suitable for chronological evaluations. An intravascular US (IVUS) catheter is clinically approved for evaluating atherosclerosis and has the potential to evaluate articular cartilage [18] and subchondral bone, which are both involved in OA progression [19]. Surface roughness and articular cartilage integrity were quantitatively evaluated with IVUS and qualitative information on repaired tissues was assessed [18].

High-frequency US, signal-processing software and imaging technique advances in newer-generation acoustic microscopy allow imaging without thinly sliced tissues. The group lead by Foster et al. commercialised a high-frequency (40–60 MHz) B-mode imaging device as an ultrasound biomicroscope for animal experiments [20] and skin imaging [21]. HFUIS with a central frequency of 120 MHz provides noncontact high-resolution US impedance and 3D US imaging [22]. Articular cartilage softening is the initial sign of degeneration [9]. Intraoperative evaluation of the articular cartilage and lesion assessment are important in orthopaedic operations. The purpose of this study was to assess normal articular cartilage by HFUIS and SAM and calculate their correlations with the intention of applying HFUIS to IVUS systems.

Materials and methods

HFUIS

A schematic illustration of HFUIS is shown in Fig. 1. The system has been reported previously [22] and is

Fig. 1 High-frequency ultrasound imaging system

Fig. 2 High-frequency ultrasound imaging system (HFUIS) image of the articular cartilage clearly visualising three layers: superficial, middle and deep. The superficial and deep layers expressed high relative intensities

described briefly as follows. An electrical impulse is generated by a high-speed switching semiconductor. The electric pulse is used to excite a polyvinylidene fluoride (PVDF) transducer with a central frequency of 120 MHz and a focal length of 3.2 mm. HFUIS has three measurement modes: (1) conventional C-mode acoustic microscopic imaging of excised, thinly sliced tissue; (2) US impedance imaging of the tissue surface; (3) 3D US imaging visualising the inside of tissue. A 3D high-frequency US microscope creates conventional C- and B-mode images and 3D images reconstructed from B-mode images. Intensity was normalised by the reflection from a steel plate at the same distance.

Fig. 3 Comparison of the articular cartilage images: **a** high-frequency ultrasound imaging system (HFUIS), **b**: haematoxylin and eosin stain, **c** scanning electron microscopy (SAM), **d** colour scale bar for SAM. Superficial layer indicates high relative intensity by HFUIS and corresponds well to that of high sound-speed area by SAM

SAM

The system used in this study has been reported elsewhere [14–17] and thus is briefly described as follows. Reflections from the tissue surface and from the interface between tissue and glass are received by the same transducer. The central frequency is 80 MHz. Frequency domain analysis of the reflection enables the separation of these two components and calculation of tissue thickness and intensity by Fourier transformation the waveform [13]. Normal light microscopic images corresponding to the stored acoustic images are captured (DMLB 100 HC light microscope; LEICA, Wetzlar, Germany). Relative intensity by HFUIS and sound speed of the articular cartilage by SAM are calculated with gray-scale images using commercially available image analysis software (PhotoShop CS2, Adobe Systems Inc., San Jose, CA, USA). SAM images with a gradation colour scale are also produced for clear sound-speed visualisation. Optical and acoustic images are compared to ensure morphological congruence in the analysis. The articular cartilage is divided into three layers (superficial, middle and deep) and analysed independently. We divided the articular cartilage—from the surface of to the margin of the high-intensity areas (subchondral bone) in HFUIS and high sound-speed areas (subchondral bone) in SAM—into five areas. We defined one fifth from the surface as superficial layers and the rest as middle layers.

Tissue preparation

The protocol for the experiments was approved by the Animal Research Committee of the Tohoku University School of Medicine. We used six adult male Sprague-Dawley rats (16 weeks). The knee capsule was cut with a surgical knife and the joint opened after administration of an overdose of sodium pentobarbital. After resection of ligaments and meniscus, a

Fig. 4 Comparison of three layers of the articular cartilage: **a** high-frequency ultrasound imaging system (HFUIS), **b** scanning electron microscopy (SAM). Intensity of the superficial and deep layers was significantly higher than that of the middle layer by HFUIS. There was no statistical difference among the three layers by SAM

cartilage–bone complex (3.8 mm diameter) was obtained from the medial midcondylar regions of the tibia with a cylindrical bar (Trephine Bur, cat. No.13006, Technika Inc., Tokyo, Japan). Specimens were immediately immersed in saline at room temperature before scanning with HFUIS. After scanning, specimens were immersed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, overnight at 4°C. The fixed specimens were then decalcified in 10% ethylenediaminetetraacetate (EDTA) in 0.01 M phosphate buffer, pH 7.4, for one week at 4°C. After dehydration through a graded series of ethanol solutions, specimens were embedded in paraffin. The embedded tissue was cut into 5- μ m sagittal sections from the medial to the lateral side of the joint, as in previous reports [15]. After deparaffinisation, the sections were assessed by SAM. Sections stained with haematoxylin and eosin (H&E) sections were used to ensure morphological congruence in the analysis.

Statistics

The three individual layers were analysed using one-factor analysis of variance (ANOVA) with Bonferroni/Dunn post hoc multiple comparisons. Differences among layers were analysed with a paired t test and expressed as mean $\pm$ standard deviation (SD). Correlation coefficients were calculated by Pearson product moment correlation. $P < 0.05$ was accepted as statistically significant.

Results

HFUIS clearly visualised three layers of articular cartilage (Fig. 2). The superficial and deep layers indicated high relative intensity. The middle layer gradually became low relative intensity from the articular cartilage surface. The

Fig. 5 Pearson product moment correlations of the three layers of the articular cartilage: **a** superficial layer, **b**: middle layer, **c** deep layer. Though the deep layer had low correlation, the superficial and middle

layers had strong correlations between high-frequency ultrasound imaging system scanning electron microscopy

high signal-intensity areas by HFUIS corresponded well with high sound-speed areas by SAM; however, the middle layer did not (Fig. 3). Among the three layers, signal intensity of the middle layer by HFUIS was significantly lower than that of the other two layers (Fig. 4a). There was no statistically significant difference among the three layers of sound speed by SAM (Fig. 4b). In the superficial and middle layers, there were strong positive correlations between the relative intensity and sound speed (Pearson product moment correlation, superficial layer 0.704; middle layer 0.731) (Figs. 5a, b). There was a low positive correlation between them in the deep layer (Pearson product moment correlation 0.258) (Fig. 5c).

Discussion

The US feature of normal articular cartilage is a homogeneous anechoic band with a sharp superficial cartilage profile and subchondral bone profile [6]. The lack of echoes is due to a uniform transmission of sound waves with high water content [23] and densely packed, regularly organised, collagen [24]. The major features of OA cartilage by US were loss of margin sharpness, loss of cartilage band clarity, and thickness reduction [24]. On US, higher spatial resolution is reached by increasing the frequency of the imaging system [25]. Because the central frequency is 120 MHz and spatial resolution is 15 μm [22], HFUIS clearly distinguished three layers of articular cartilage with high resolution compared with conventional high-frequency B-mode imaging modalities. Furthermore, it could clearly visualise the non-homogeneous middle layer of the articular cartilage. HFUIS has a potential to evaluate articular cartilage histology. SAM clearly visualised the mechanical property, based on sound speed, of the articular cartilage. In our previous report, articular cartilage degeneration induced by joint immobilisation demonstrated decrease in sound speed [17]. As SAM requires sliced tissues, it is not adaptable for in vivo real-time and sequential evaluations. However, it is a powerful tool for evaluating tissue elasticity and distribution in the targeted tissue in situ.

This study reports, for the first time, the Pearson product moment correlation of the articular cartilage between relative intensity by HFUIS and sound speed by SAM. The superficial and middle layer showed strong correlations; however, the deep layer showed a weak correlation. Because the deep layer profile might include the calcified zone and the subchondral bone, it was difficult to specify subchondral bone only by US. Further, decalcification might influence the mechanical property of the subchondral bone for SAM evaluation. For these reasons, the deep layer seemed to indicate weak correlations.

In clinical situations, applying HFUIS in a weight-bearing portion of the knee may be difficult because of the patella. Overlying soft tissues, such as synovitis with synovial fluid, may also influence echogenicity of articular cartilage [23, 24]. The earlier US features of OA are loss of cartilage-band clarity and margin sharpness [6]. As for OA articular cartilage histology, there was significant correlation between in vivo US histological grading [12]. Because of the softening, the early change in articular cartilage is only detectable by arthroscopic examinations. Therefore, HFUIS has a potential to detect early changes in articular cartilage besides its colour and morphology. Because the subchondral bone is also affected by OA progression, further study is needed to specify articular cartilage echogenicity and distinguish the subchondral bone by US [18].

Conclusions

HFUIS produced high-resolution images of the articular cartilage, and its intensity was strongly correlated with sound speed by SAM.

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