Evaluation of the Accuracy of Articular Cartilage Thickness Measurement by Conventional and Real-time Spatial Compound Ultrasonography

INTRODUCTION

Articular cartilage thickness has previously been quantified by B-mode ultrasonography [1, 2]. However, cartilage surface and cartilage-bone borders have been decided manually in those studies. In addition, no studies have adopted real-time spatial compound ultrasonography for measuring cartilage thickness. The purpose of this study was to develop a method to objectively quantify articular cartilage thickness in vitro using both conventional and real-time spatial compound B-mode ultrasonography and to evaluate the accuracy of measurement.

MATERIALS AND METHODS

Cartilage samples

Knee joints were obtained for a 6-month- and a 3-year-old pig from a slaughterhouse (Tokyo Shibaura Zouki, Tokyo, Japan), as we assumed that thickness could differ between pigs at different ages. Femoral condyle articular cartilage was used in this study, since cartilage size and shape are relatively similar to those of human knee articular cartilage. After slaughter, whole bodies of pigs were kept at 3 °C in a refrigerated room. On the third day, the hind limbs were detached and sent to our laboratory under the same temperature. In our laboratory, limbs with intact knee joints were packed in plastic bags, degassed manually, sealed hermetically and stored at -20 °C. On the day of the experiment, soft tissues including joint capsules and ligaments were removed after the limbs were thawed in normal saline solution (Osuka Pharmaceutical, Tokyo, Japan) at room temperature. Osteochondral blocks with the surface size of 20 × 20 mm from the medial femoral condyle were acquired by cutting the bone with a band saw (SWD-250; Fujiijwara Sangyo, Miki, Japan), then fixed on a custom-made acrylic sample holder (30 × 30 × 13 mm; Murai & Co., Tokyo, Japan) with resin (GC-Ostron; GC Corporation, Tokyo, Japan). During preparation, samples were continuously cooled and moistened using normal saline solution.

Acoustic measurement

A B-mode 10.0-MHz linear ultrasound probe (UST-5411; Aloka, Tokyo, Japan) connected to ultrasound device (Prosound ALPHA 10; Aloka) was attached to a holding arm, which was fixed to a stage with an x,y micrometer for horizontal adjustment to enable identification of the location of cartilage measurement. In the water tank, osteochondral blocks and the transducer surface were placed in 20 °C water. The distance between transducer surfaces and the sample was kept as the transducer focus distance. Edges of the sample were identified by ultrasound signals, and the center of the sample was then identified from those points. B-mode images of the center line of the sample holder were acquired (Fig. 1A). Image settings were for both conventional imaging and real-time spatial compound imaging superimposed with three frames each from a different viewing angle of -20, 0, and 20 degrees to the right angle. System settings were optimized for imaging the cartilage surface. Brightness line data of 32 points at 0.5-mm intervals in each image were obtained from both the 6-month- and 3-year-old pigs (Fig. 1B). The cartilage surface and cartilage-bone border of the specimen were defined as the peaks of each reflected signal. Cartilage thickness (Tc-US) was measured as the distance between peaks, which was adjusted by the ultrasound speed for each age from our past study [3].

Optical thickness measurement

The specimen fixed to the custom-made sample holder was mounted on a diamond saw device (Minitom; Struers, Westlake, OH), which offers an accuracy of 10 μm for adjustment of the cutting plane. A center-cut plane of the acrylic sample holder was created, corresponding to the B-mode ultrasound image plane. Subsequently, each sample was mounted on a glass slide, covered with a cover glass after dripping normal saline onto the sample surface to keep the cartilage moistened and inhibit deformation during measurement due to drying. Cartilage thickness was measured using optical measuring microscope (MM-400; Nikon, Tokyo, Japan). Using this optical measuring microscope, points of line data acquisition on the cartilage surface and direction of the US beam were able to be identified from the position and orientation of the acrylic sample holder surrounding the cartilage sample. Thickness of the cartilage (Tc) along the beam direction was measured at each point.

Mean and standard deviation (SD) of Tc for each sample were calculated. Linear regression analysis was performed and Pearson’s coefficient of correlation was used to compare Tc-US to Tc. A correlation was considered significant for values of p<0.05.

RESULTS

In all B-mode line data, peaks of reflected ultrasound signals from the cartilage surface and cartilage-bone border were clear enough to be identified. Mean Tc and Tc-US (conventional, spatial compound) for both samples are shown in Table 1. Tc-US was significantly correlated with Tc in both the 3-year- and 6-month-old pigs (p<0.0001 each) (Fig. 2). Pearson’s coefficient of correlation tended to be slightly higher with spatial compound in each sample.

Table 1. Mean Tc and Tc-US (mm). Values are provided as mean ±SD.

<table>
<thead>
<tr>
<th>Age</th>
<th>Samples</th>
<th>Mean Tc-US</th>
<th>Mean Tc</th>
</tr>
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<tbody>
<tr>
<td>6-month-old pig</td>
<td></td>
<td>2.40 ± 0.39</td>
<td>2.46 ± 0.42</td>
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<tr>
<td>3-year-old pig</td>
<td></td>
<td>1.49 ± 0.10</td>
<td>1.45 ± 0.18</td>
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Figure 1: A) B-mode ultrasound image of the sample is shown. B) Line data acquired from the dotted line in the ultrasound image (A). Peak of the reflected ultrasound signal were defined as the surface and border of the tissue.

Figure 2. Scatter plot of each ultrasound image setting and sample. Linear regression analysis shows good agreement between Tc and Tc-US in all plots.

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REFERENCES: