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● *Original Contribution*

QUANTITATIVE EVALUATION OF ENZYME-INDUCED PORCINE ARTICULAR CARTILAGE DEGENERATION BASED ON OBSERVATION OF ENTIRE CARTILAGE LAYER USING ULTRASOUND

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Abstract—Enzyme-induced articular cartilage degeneration resembling osteoarthritis was evaluated using a newly defined acoustic parameter, the "averaged magnitude ratio" (AMR), which has been suggested as an indicator of articular cartilage degeneration. *In vitro* experiments were conducted on porcine cartilage samples digested with trypsin for 2 h (n = 10) and 4 h (n = 13) and healthy control samples (n = 13). AMR was determined with 15- and 25-MHz ultrasound, and the integrated reflection coefficient (IRC) and apparent integrated backscattering coefficient (AIB) were also calculated for comparison. The Young's modulus of superficial cartilage was measured using atomic force microscopy. Performance of the AMR differs between 15 and 25 MHz, possibly because of frequency-related attenuation and resolution of ultrasound. At the proper settings, AMR exhibited a competence similar to that of IRC and AIB in detecting cartilage degeneration and could also detect differences in deeper positions. Furthermore, AMR has the advantages of being easy to measure and requiring no reference material.
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Key Words: **Cartilage, Ultrasound, Backscatter, Quantitative, Degeneration, Osteoarthritis.**

INTRODUCTION

Osteoarthritis (OA) is a degenerative disease of articular cartilage that is prevalent among elderly people (Heidari 2011). OA leads to cartilage fibrillation, softening, ulceration and even loss of cartilage (Knecht et al. 2006). Different methods have been proposed to detect OA-induced degenerative changes in hyaline cartilage, including arthroscopy, magnetic resonance imaging and ultrasound (Iagnocco 2010; Kiviranta et al. 2007; Spahn et al. 2009). Quantitative ultrasound (US) methods have the potential to assess the state of cartilage degeneration non-destructively with higher sensitivity and accuracy.

Various acoustic parameters have been proposed to evaluate the properties and state of articular cartilage. These include basic features of ultrasound propagation in cartilage. Sound speed (Agemura et al. 1990; Nieminen et al. 2004; Patil et al. 2004; Töyräs et al. 1999, 2003) and attenuation coefficient (Nieminen et al. 2004; Ohashi et al. 2011; Senzig et al. 1992; Töyräs et al. 1999) have received much attention. Acoustic impedance (Leicht and Raum 2008) has also been measured.

Observations have also been made regarding the reflection and backscatter characteristics of articular cartilage, through which the integrity of cartilage surface or microstructures of cartilage matrix may be indirectly assessed. Some proposed parameters are determined in the time domain, including the reflection coefficient, R (Laasanen et al. 2002), or in the frequency domain, including the integrated reflection coefficient (IRC) (Chérin et al. 1998, 2001; Kiviranta et al. 2007), apparent integrated backscattering coefficient (AIB) (Aula et al. 2010; Chérin et al. 1998; Inkinen et al. 2015) and apparent frequency dependence of backscatter (Männicke et al. 2014). Cartilage surface roughness can also be evaluated with the ultrasound roughness index (URI) (Saarakkala et al. 2004; Wang et al. 2016).

Attempts involving wavelet transform have also been made. Maximum magnitude (MM) (Hattori et al. 2005; Kaleva et al. 2008; Nishitani et al. 2014) and echo duration (ED) (Hattori et al. 2003; Kaleva et al. 2008) can be derived from the wavelet map. Other parameters include

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US aggregate modulus (Sun et al. 2015) and cartilage thickness (Liukkonen et al. 2014; Wang et al. 2010).

The parameters proposed in the literature provide insight into cartilage properties and lesion detection, but each has its own limitations. For example, currently, the feasibility of precise *in vivo* measurement of the sound speed and ultrasound attenuation coefficient is very weak (Kiviranta et al. 2007). MM and ED also have disadvantages with respect to clinical application in real time because they require much computing capacity. *R*, IRC, URI and AIB concern only the surface or superficial layer of cartilage, neglecting deeper positions. However, as the OA-induced cartilage changes may also appear in deeper positions, it is possible that useful information is missed by using these superficial-layer parameters.

On the other hand, many suggested parameters involve a reference signal reflected from a reference interface under the same experimental condition. However, the reference reflector may not be identical in different studies and may exhibit different reflection characteristics. For example, in some previous studies, the reference signal may be collected from the phosphate-buffered saline (PBS)–air interface (Kaleva et al. 2008; Laasanen et al. 2005; Saarakkala et al. 2003, 2004), sodium chloride–silicon rubber interface (Sun et al. 2015), PBS–polymethylmethacrylate (PMMA) interface (Männicke et al. 2014; Schöne et al. 2013), PBS–stainless steel interface (Virén et al. 2009, 2010) or physiologic saline–steel interface (Wang et al. 2010). Also, the acoustic properties of the reference material usually are assumed to be known and fixed, but the real acoustic characteristics of reference material deviate more or less from what has been assumed. Even with similar reference material, these deviations may be quite different in different studies. In addition, in some studies, the reflection from a planar reflector was further normalized using a scattering phantom (Männicke et al. 2014; Schöne et al. 2013). These differences pose difficulties in summarizing different studies. For example, in the literature, for healthy bovine patella cartilage, the reported mean value of IRC measured under normal incidence with a 40-MHz transducer varies from about −17.6 dB (Wang et al. 2010) to about −23 dB (Virén et al. 2012), a difference of 30%. Perhaps partly for this reason, conflicting results exist regarding the diagnostic abilities of proposed parameters.

In consideration of the aforementioned problems, the objective of the present study was to find a quantitative parameter to detect degenerative changes of articular cartilage that (i) is not difficult to measure and calculate, (ii) requires no reference signal and (iii) takes not only the superficial layer but also the deeper positions of cartilage matrix into account. We moved the ultrasound transducer vertically to change the distance between the acoustic lens and the sample surface to observe the entire cartilage layer. A novel acoustic parameter, the averaged magnitude ratio (AMR), was suggested as a possible indicator of articular cartilage degeneration. Because enzymatic treatment may also cause disruption of the collagen network and loss of proteoglycans, enzymatic models resembling early-stage OA are commonly used in the literature (Moody et al. 2006; Saarakkala et al. 2004, 2011; Sun et al. 2015; Wang et al. 2008). These models have an advantage in that the degree of degeneration can be controlled through the choice of treatment duration and enzyme solution (Moody et al. 2006). Thus, in the present study, we also based our observation on enzyme-induced degeneration. Ultrasound and atomic force microscopy (AFM) measurements were carried out on healthy and trypsin-digested porcine cartilage *in vitro*. Differences in Young's modulus, AMR, IRC and AIB between articular cartilage with different degrees of degeneration were investigated, and the performance of acoustic parameters was explored.

METHODS

Specimen preparation

Thirteen mature porcine left hind knee joints without visible lesions were obtained from a local market within 12 h postmortem and stored at −20 °C until further preparation. The pigs were 5–7 months of age. For each joint, three cylindrical cartilage–bone samples ($\Phi = 8$ mm, $n = 13 \times 3$) were prepared from adjacent positions on the patellar surface of femur using a hollow drill and were arranged in the control group ($n = 13$), trypsin 2-h group ($n = 10$) and trypsin 4-h group ($n = 13$), respectively. The trypsin 2-h group included 10 samples because 3 other samples were damaged in the preparation process and thus excluded.

Samples of the control group and the trypsin 4-h group were immersed in PBS and 0.25% trypsin–EDTA solution, respectively, in separate plastic multiwall arrays and were kept in an incubator (DZF-6050AB, Zhongxingwy, Beijing, China) at 37 °C for 4 h. The trypsin 2-h group samples were first immersed in 0.25% trypsin–EDTA solution and kept in the incubator for 2 h. Then they were rinsed with PBS, immersed in PBS and kept in the incubator for another 2 h. Trypsin digestion of cartilage is often used as a simulation of OA, because trypsin digests proteoglycans and also has a slight effect in attacking collagen molecules (Brown et al. 2008; Saarakkala et al. 2011). After incubation, samples were embedded in PMMA.

AFM indentation

Before indentation measurement, samples were immersed in PBS at room temperature for 1 h. AFM indentation testing was performed using a commercial AFM (Dimension Icon, Bruker, USA) with pyramidal probe tip