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● *Original Contribution*

RELATIONSHIPS BETWEEN QUANTITATIVE PULSE-ECHO ULTRASOUND PARAMETERS FROM THE SUPERFICIAL ZONE OF THE HUMAN ARTICULAR CARTILAGE AND CHANGES IN SURFACE ROUGHNESS, COLLAGEN CONTENT OR COLLAGEN ORIENTATION CAUSED BY EARLY DEGENERATION

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Abstract—We aimed to quantitatively investigate the relationship between amplitude-based pulse-echo ultrasound parameters and early degeneration of the knee articular cartilage. Twenty samples from six human femoral condyles judged as grade 0 or 1 according to International Cartilage Repair Society grading were assessed using a 15-MHz pulsed-ultrasound 3-D scanning system *ex vivo*. Surface roughness (R_q), average collagen content (A_1) and collagen orientation (A_{12}) in the superficial zone of the cartilage were measured *via* laser microscopy and Fourier transform infrared imaging spectroscopy. Multiple regression analysis with a linear mixed-effects model (LMM) revealed that a time-domain reflection coefficient at the cartilage surface (R_c) had a significant coefficient of determination with R_q and A_{12} ($R^2_{LMMm} = 0.79$); however, R_c did not correlate with A_1. Concerning the collagen characteristic in the superficial zone, R_c was found to be a sensitive indicator reflecting collagen disorganization, not collagen content, for the early degeneration samples. (E-mail: kuroki.hiroshi.6s@kyoto-u.ac.jp) © 2017 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Osteoarthritis, Cartilage, Surface roughness, Collagen orientation, Collagen content, Fourier transform infrared imaging spectroscopy, Laser microscopy, Ultrasound.**

INTRODUCTION

Osteoarthritis (OA) of the knee is one of the most common locomotor diseases (Felson et al. 1987; Hart and Spector 1993; Yoshimura 2009), and considerably decreases quality of life (QOL) (Norman-Taylor et al. 1996). Therefore, the early detection of OA and development of conservative therapies (Zhang et al. 2007) are critical in maintaining acceptable QOL. In the early-stage OA, the cartilage surface becomes rougher or exhibits fibrillation (Minns et al. 1977), and the collagen meshwork is damaged (Han et al. 2002; Poole et al. 2002). Additionally, collagen matrix disorganization occurs in the superficial zone (Panula et al. 1998; Saarakkala et al. 2010).

The ability of quantitative high-frequency ultrasound to detect cartilage degeneration in OA has been investigated. The frequency-domain reflection coefficient at the cartilage surface (integrated reflection coefficient [IRC]) has been reported to be lower in OA rat models (compared with control rats), which, it was suggested, is related to a disruption in the fibers of the collagen network (Chérin et al. 1998). In human cartilage, the IRC has been found to correlate moderately with surface roughness (Kaleva et al. 2010).

With respect to collagen content in the superficial zone, the ultrasound reflection coefficient in the time domain (R_c) has been reported to have no correlation with collagen content estimated by the amide I value from Fourier transform infrared imaging spectroscopy

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(FTIR-IS) in bovine cartilage samples (Laasanen et al. 2005); the R_c is correlated with collagen content in human early OA samples (Nishitani et al. 2014).

In arthroscopic clinical evaluations, echo intensity at the cartilage surface decreased with an increase in visually evaluated International Cartilage Repair Society (ICRS) grade (Kuroki et al. 2008). Thus, ultrasound parameters have been individually compared with aspects of degeneration, for example, ultrasound parameter versus surface roughness or ultrasound parameter versus matrix degeneration, by histologic evaluation and have been found to be moderately related to these aspects. However, there is no report that quantitatively describes the relationship between ultrasound parameters and the degeneration of the surface profile and collagen organizational characteristics. Particularly the relationship between ultrasound parameters and collagen orientation in the superficial zone has not been investigated. An arch-like structure in the collagen fibril, which means that the collagen orientation is parallel to the surface in the superficial zone, random in the middle zone and perpendicular to the surface in the deep zone, was reported to be essential from the viewpoint of functionality against load in the articular surface (Halonen et al. 2013).

The aim of the present study was to quantitatively investigate how amplitude-based ultrasound parameters, IRC and R_c, can be used to detect degeneration of the surface profile and collagen organizational characteristics in the superficial zone of mild OA-affected cartilage. As a reference measurement of degeneration, surface roughness was quantified using laser microscopy, and the collagen content and collagen orientation parallel to the cartilage surface in the superficial zone were analyzed *via* FTIR-IS. Although a higher frequency could directly measure surface roughness, a broadband ultrasound probe with a central frequency of 15 MHz was used in this *ex vivo* study to allow *in vivo* evaluation in the future, which requires tolerance for frequency-dependent attenuation through the soft tissue.

METHODS

The present study was carried out according to a protocol approved by the National Hospital Organization, Kyoto Medical Center Review Board (Approval No. 09-31). All patients provided written informed consent before participation.

Samples

Human knee osteochondral samples were obtained from six patients with OA who had undergone total knee arthroplasty. A total of 20 samples of femoral condyle from visually intact or early degenerative sites based on ICRS grading were cut into approximately 14 × 7-mm pieces (Fig. 1a) (Brittberg and Peterson 1998). Eleven samples were grade 0, and 9 were grade 1.

Ultrasound measurement

Figure 1 illustrates the ultrasound experimental system. A cartilage sample was fixed on rubber clay and placed in saline water at room temperature. A computer (NI PXIe-8133, National Instruments, Austin, TX, USA) controlled an X–Y stage through a stage controller (SHOT-202 AM, Sigma Koki, Tokyo, Japan) to move a point-focused ultrasound probe (Panametrics-NDT V313, Olympus) two-dimensionally. In this study, the x and y directions are defined to be orthogonal to the sound axis, Z. The ultrasound probe had a nominal central frequency of 15 MHz, relative bandwidth of 98% and −6-dB beam diameter at a focus of approximately 0.2 mm. The scanning pitch was 0.1 mm in both directions.

A pulser-receiver (Model 5800, Panametrics) excited the ultrasound probe and received radiofrequency (RF) analogue echo signals within a 1- to 35-MHz band. The RF analogue signals were digitized by a digitizer with a 14-bit resolution and sampling frequency of 100 MHz (NI PXI-5122, National Instruments). Ten RF signals were acquired at each scanning point and averaged to ensure an adequate signal-to-noise ratio (SNR). Typical SNRs were 44 dB for intact cartilage and 31 dB for degenerated cartilage. The averaged RF signal values were stored on the computer to analyze the ultrasound parameters IRC and R_c.

Before data acquisition, the distance between the ultrasound probe and cartilage surface was adjusted with the Z-stage, so that the cartilage surface was in focus. In addition, the inclination of the cartilage sample was visually adjusted using biaxial goniometer stages so that the ultrasound wave was transmitted almost perpendicularly to the global surface of the sample.

All ultrasound measurements were carried out at room temperature, which ranged from 20°C to 25°C.

Ultrasound reflection coefficient at cartilage surface (R_c, IRC)

The RF signal was Hilbert transformed, and its envelope was detected. The envelope was normalized (Saarakkala et al. 2004) using the amplitude of the perfect plane reflector at each depth to remove the depth dependency of the echo amplitude, as

$$r(x,y,t) = \frac{A(x,y,t)}{A_{\mathrm{ref}}(t)} \tag{1}$$

where A is the amplitude of the echo envelope, x and y indicate the probe position, t is the time of flight (TOF)