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● *Original Contribution*

SPECIES-INDEPENDENT MODELING OF HIGH-FREQUENCY ULTRASOUND BACKSCATTER IN HYALINE CARTILAGE

Nils Männicke,* Martin Schöne,* Jukka Liukkonen,† Dominik Fachet,‡ Satu Inkinen,† Markus K. Malo,† Michael L. Oelze,§ Juha Töyräs,†¶ Jukka S. Jurvelin,† and Kay Raum*

*Berlin-Brandenburg Center for Regenerative Therapies and Berlin-Brandenburg School for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany; †Department of Applied Physics, University of Eastern Finland, Kuopio, Finland; ‡Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, Berlin, Germany; §Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA; and ¶Diagnostic Imaging Center, Kuopio University Hospital, Kuopio, Finland

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Abstract—Apparent integrated backscatter (*AIB*) is a common ultrasound parameter used to assess cartilage matrix degeneration. However, the specific contributions of chondrocytes, proteoglycan and collagen to *AIB* remain unknown. To reveal these relationships, this work examined biopsies and cross sections of human, ovine and bovine cartilage with 40-MHz ultrasound biomicroscopy. Site-matched estimates of collagen concentration, proteoglycan concentration, collagen orientation and cell number density were employed in quasi-least-squares linear regression analyses to model *AIB*. A positive correlation ($R^2 = 0.51$, $p < 10^{-4}$) between *AIB* and a combination model of cell number density and collagen concentration was obtained for collagen orientations approximately perpendicular (>70°) to the sound beam direction. These findings indicate causal relationships between *AIB* and cartilage structural parameters and could aid in more sophisticated future interpretations of ultrasound backscatter. (E-mail: kay.raum@charite.de) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Apparent integrated backscatter, Cartilage, Ultrasound, Osteoarthritis, Backscatter, Ultrasound spectroscopy, Quantitative ultrasound, Backscatter coefficient.**

INTRODUCTION

Hyaline cartilage is a connective tissue covering the joint surfaces and mediates both friction of adjacent joints and shock absorption. In addition to water, the principal constituents of the cartilage matrix are collagen, proteoglycans and chondrocytes, which are organized heterogeneously with respect to quantity, size and orientation in an arcade-shaped depth-dependent structure (Männicke et al. 2014a). Osteoarthritis (OA) is a joint disease with high socioeconomic impact resulting in a decrease in quality-adjusted life years (Pinto et al. 2012). OA is characterized by progressive degenerative changes in cartilage structure and matrix, chondrocytes and subchondral bone (Buckwalter and Mankin 1998). The first signs of OA are fibrillation or disruption of the superficial cartilage layers, leading to tissue softening and increased surface roughness. Degeneration of the cartilage matrix is characterized by a loss of proteoglycans (aggrecans), followed by a disruption of the collagen network in later stages. To date, none of the established non-invasive imaging modalities are able to assess these degenerative tissue alterations concurrently. The diagnosis of early OA, in particular, is highly important, as it could aid in the development of treatment strategies that aim to arrest or revert the disease progression at an early stage (Chu et al. 2014).

Ultrasound biomicroscopy refers to high-frequency ultrasonic imaging of biological tissues and cells (typically with frequencies >20 MHz), which is capable of visualizing cartilage tissue. The fine spatial resolution on the order of 50–100 μm (Gelse et al. 2010) gives access to a variety of quantitative parameters via temporal separation of the received signals. The most common ultrasound parameters are surface reflection amplitude and surface roughness as surrogates for alterations of cartilage matrix stiffness and surface roughness, respectively. These parameters have been found to significantly vary in

Address correspondence to: Kay Raum, Berlin-Brandenburg School for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: kay.raum@charite.de

the course of OA (Kaleva et al. 2008; Nieminen et al. 2009; Saarakkala et al. 2011; Wang et al. 2010) and have been associated with collagen depletion (Nieminen et al. 2002; Töyräs et al. 1999; Wang et al. 2010), surface fibrillation (Saarakkala et al. 2004; Schöne et al. 2013) and biomechanical competence (Gelse et al. 2010; Töyräs et al. 1999) of the tissue. In addition to changes detectable at the cartilage surface, the reflection intensity at the subchondral bone boundary was reported to increase in osteoarthritic samples (Jaffre et al. 2003; Saarakkala et al. 2006). This increase was related to increased bone density and trabecular thickness in the subchondral bone tissue *in vitro* (Liukkonen et al. 2013). Moreover, a degeneration-related decrease in acoustic attenuation of the cartilage matrix was hypothesized to significantly increase the intensity of the subchondral bone reflection (Saarakkala et al. 2011).

Relating the analysis of backscattered ultrasound signals originating from the cartilage matrix to osteoarthritic changes was proposed 20 years ago (Cherin et al. 1998; Kim et al. 1995). The conventional parameter chosen to assess these changes is the apparent integrated backscatter (*AIB*), which quantifies the average backscattered energy within the frequency bandwidth of the transducer. Findings suggest that alterations in collagen packing density and collagen orientation may influence *AIB*. In particular, (i) a decrease in *AIB* with aging in a Wistar rat animal model (Cherin et al. 2001) and (ii) an increase in *AIB* for repair cartilage compared with intact cartilage tissue (Gelse et al. 2010; Laasanen et al. 2006; Viren et al. 2010) were reported. In other work, a decrease in *AIB* was observed after acute impact injury (Viren et al. 2012), whereas a weak but statistically significant increase was reported after enzymatic depletion of collagen and proteoglycans using collagenase and trypsin, respectively (Wang et al. 2010). In contrast, Pellaumail et al. (2002) reported no statistically significant differences in *AIB* after trypsin treatment. However, a direct comparison of these results is difficult, as the frequency ranges used for the calculation of *AIB* varied between studies.

In a recent study, we found that quantitative ultrasound parameters based on envelope statistics and the frequency-dependent spectral slope are more sensitive to degeneration stages of human cartilage than conventionally applied integrated backscatter amplitude parameters (Männicke et al. 2014a). Moreover, in a classification approach using ultrasound based parameter pairs as predictors for cartilage degeneration, we found that parameters of the envelope statistics are feature candidates throughout all degeneration stages, whereas integrated backscatter amplitude and spectral slope appeared to be particularly good predictors for advanced and early stages of degeneration, respectively (Männicke et al. 2014b).

Nevertheless, the diagnostic value of quantitative ultrasound parameters in assessment of cartilage degeneration is limited as long as structural sources giving rise to the backscattered signal remain unknown. Collagen concentration and chondrocyte number density were reported to increase backscatter amplitude in agarose gels (Inkinen et al. 2014) and collagen hydrogels (Mercado et al. 2014; Mercado et al. 2015), strengthening the hypothesis that both the extracellular collagen matrix and chondrocytes contribute to ultrasound backscatter (Männicke et al. 2014a). However, the specific contributions of collagen and cells to backscattered signals have not been clarified for the naturally occurring concentration, composition and orientation of collagen and cells in hyaline cartilage. Therefore, the aims of this study were to characterize hyaline cartilage of different species and to apply species-independent regression models to elucidate causal relationships between ultrasonic backscatter signals measured in the frequency range between 30 and 50 MHz with underlying chemical and structural parameters.

METHODS

Samples

Cartilage specimens were obtained from patellas of human ($N = 16$), bovine ($N = 8$) and ovine ($N = 10$) origin. Human samples were acquired from both left and right lower limbs of human cadavers at the Institute of Anatomy in Lübeck, Germany ($N = 12$) and the Central Finland Central Hospital in Jyväskylä, Finland ($N = 4$). The donor ages were in the range 24–92 years (mean ± standard deviation: 70 ± 20 years). All bovine and four ovine samples were acquired from local abbatoirs (Väisäsen Kotiliha Oy, Iisalmi, Finland, and HKScan Oyj, Outokumpu, Finland, respectively) at estimated animal ages of 1–3 years. Moreover, six ovine specimens were obtained from elderly sheep (4–8 years). At the given animal age ranges, full maturation of human, bovine and ovine cartilage samples was expected. Approval for the experiments was granted from the ethics commissions and approved by local institutional review boards.

Prior to experiments, patellas were stored at −20°C for at least 48 h and up to 10 years (human samples). Using a hollow-core drill (Biltema, Helsingborg, Sweden, diameter = 10 mm), osteochondral cylinders were drilled from the central part of the lateral patella facet. The biopsies comprised the full thickness of cartilage and at least 2 mm of subchondral bone. Immediately after explantation, scalpel cuts at the proximal, medial and lateral sites were set to indicate the anatomical