Osteoarthritis and Cartilage (2009) **17,** 26–32

doi:10.1016/j.joca.2008.05.012

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

pQCT study on diffusion and equilibrium distribution of iodinated anionic contrast agent in human articular cartilage – associations to matrix composition and integrity

T. S. Silvast B.S.†¶*, J. S. Jurvelin Ph.D.†‡, M. J. Lammi Ph.D.§‖ and J. Töyräs Ph.D.†¶

† *Department of Physics, University of Kuopio, Kuopio, Finland*

‡ *Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland*

§ *Department of Biomedicine, Anatomy, University of Kuopio, Kuopio, Finland*

‖ *Department of Biosciences, Applied Biotechnology, University of Kuopio, Kuopio, Finland*

¶ *Department of Clinical Neurophysiology, Kuopio University Hospital and University of Kuopio, Kuopio, Finland*

Summary

Objective: X-ray imaging of articular cartilage using anionic contrast agents has been introduced for quantification of tissue glycosaminoglycan (GAG) concentration. In this *in vitro* study we investigated diffusion and equilibrium distribution of an anionic contrast agent in human articular cartilage and related the results to tissue composition and integrity.

Methods: Osteochondral cylinders ($d = 4.0$ mm, $n = 24$) were prepared from femoral medial condyles (FMCs, cartilage thickness 2.13 ± 0.54 mm, mean ± standard deviation [SD]), and tibial medial plateaus ([TMPs]1.99 ± 0.38 mm) of human cadaver knees. Samples were immersed for 24 h at room temperature in 21 mM concentration of anionic contrast agent Hexabrix™. The X-ray absorption maps and profiles were measured before immersion, and after every 2 h of immersion using clinical peripheral quantitative computed tomography (pQCT).

Results: An increase in X-ray attenuation along cartilage depth, indicating a characteristic density profile increasing from superficial to deep tissue, could be seen in pQCT images acquired without contrast agent. The complete diffusion of the contrast agent into cartilage took more than 12 h. However, the uronic acid concentration correlated with the contrast agent concentration in femoral cartilage ($r = -0.76$, $n = 12$, $P = 0.004$) as early as after 2 h of immersion, and the linear correlation was virtually unchanged during the remaining 22 h. Similarly, the histological tissue integrity (Mankin score) correlated positively with the contrast agent concentration in tibial cartilage ($r = +0.75$, $P = 0.005$) after 2 h of immersion. The X-ray absorption profiles before immersion, i.e., without the contrast agent, and after 24 h of immersion were significantly correlated ($r = -0.76 \pm 0.34$, mean ± SD).

Conclusions: Although the complete contrast agent diffusion into human articular cartilage *in vitro* took more than 12 h, significant apparent correlations were revealed between the spatial proteoglycan (PG) and contrast agent distributions already after 2 h of immersion. At the stage of incomplete penetration, however, the spatial contrast agent concentration distribution cannot directly reflect the true PG distribution as the Donnan equilibrium has not been reached. However, in degenerated cartilage the diffusion rate increases. Obviously, this can lead to the reported correlation between the bulk PG content and the bulk contrast agent concentration already at the early stages of diffusion.

Key words: X-ray tomography, Articular cartilage, Proteoglycan, Contrast agent, Diffusion, Osteoarthrosis.

Introduction

Mechanical properties of articular cartilage depend strongly on the integrity of collagen network and proteoglycan (PG) concentration[1,2]. In intact cartilage the water concentration decreases and glycosaminoglycan (GAG) (and PG) concentration increases with depth[3–6]. In intact cartilage, the fixed charge density (FCD) increases with depth, or remains constant in the deep zone[3–6]. Collagen concentration is lowest in the middle zone. In terms of collagen and PGs the solid fraction increases with depth. In osteoarthrotic cartilage water concentration increases particularly in the middle zone, PG concentration decreases and collagen network becomes disrupted[1,3]. These degenerative changes in composition and structure make cartilage softer and prone to mechanical damage[1,7]. In contrast to osteoarthrosis, FCD increases and water concentration decreases in articular cartilage along ageing[7]. The methods capable for non-invasive quantitative diagnostics of the determinants of mechanical properties, e.g., tissue PG concentration, would have significant clinical potential.

The delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) serves as non-invasive clinical means to estimate cartilage PG concentration[8]. However, dGEMRIC may overestimate the GAG concentration in deep cartilage[9]. The recent studies have demonstrated modest capability of dGEMRIC to predict cartilage mechanical properties at clinical field strengths[8–16]. The contrast enhanced cartilage tomography (CECT), an X-ray analogue to dGEMRIC, has been proposed for quantification of GAG concentration of cartilage[17,18]. Both iodine and gadolinium based anionic contrast agents have been applied in previous CECT studies[17–19].

*Address correspondence and reprint requests to: Dr Tuomo S. Silvast, Department of Physics, University of Kuopio, PO Box 1627, FI-70211 Kuopio, Finland. Tel: 358-1716-2339; Fax: 358-1716-2585; E-mail: tuomo.silvast@uku.fi, http://www.luotain.uku.fi

Received 19 June 2007; revision accepted 19 May 2008.

After chemically induced depletion of PGs an increased penetration of negatively charged or neutral contrast agent molecules, and a decreased penetration of positively charged contrast agent molecules into cartilage have been reported[17,19,20]. These findings are in line with the assumption that the equilibrium distribution of negatively charged contrast agent is inversely proportional, and positively charged contrast agent directly proportional to the negatively charged PGs present in cartilage. However, in our recent study, depth-dependent PG depletion was not accurately reproduced by spatial contrast agent profiles, although the PG depleted cartilage showed increased accumulation of the contrast agent (ioxaglate)[19]. This finding suggests that other factors (i.e., spatial water and collagen content), in addition to the spatial PG concentration, may affect the contrast agent accumulation.

Depending on the sample size and experimental geometry various different immersion times have been applied for contrast agent: *in vivo* 0.5–7 h for gadopentetate[10,21–31], and *in vitro* 1–24 h for gadopentetate and 1–27 h for ioxaglate[9–20,32,33]. A previous study on bovine tissue reported a continuous decrease of the T_1 relaxation time in accordance with the contrast agent accumulation into cartilage, measured after every 30 min, and a maximum decrease was achieved after 11 h of immersion[34]. In osteoarthrosis, the contrast agent penetration into the cartilage is reported to speed up[27]. *In vivo* intravenous administration, and fluid circulations induced by joint exercise, may speed up the penetration of contrast agent into articular cartilage[10,25,27,31,35].

In the present study, the potential of CECT was characterised with human articular cartilage *in vitro*. In particular, for the first time, we investigated the diffusion behaviour and equilibrium distribution of the anionic iodine based contrast agent using clinical peripheral quantitative computed tomography (pQCT). We also related the CECT results to tissue composition (PG and water concentration) and histological integrity (Mankin score).

Materials and methods

The permission to collect human samples was granted by the Finnish National Authority for Medicolegal Affairs (TEO 1781/32/200/01). Osteochondral cylinders (diameter 16.0 mm) were drilled from femoral medial condyles (FMCs, $n = 12$, cartilage thickness 2.13 ± 0.54 mm, mean $\pm$ standard deviation [SD]) and tibial medial plateaus (TMPs, $n = 12$, cartilage thickness 1.99 ± 0.38 mm) of human cadaver knees (age 26–78 years, mean age 55 years, $n = 12$) and stored in a freezer at $-20^{\circ}C$[13]. The drilling was not found to affect cartilage integrity at the tissue under investigation. Prior to experiment the samples were thawed and smaller osteochondral cylinders (diameter 4.0 mm) were punched (Fig. 1). The cartilage layer in samples exhibited different degenerative appearance, as revealed by semiquantitative histological Mankin scoring[36]. The water concentration and uronic acid concentration of the samples, prepared from the tissue adjacent to the samples of the present study, were obtained from our earlier study[37]. The spatial GAG concentration profiles were determined using optical density measurement of Safranin-O stained sections[38].

Each sample was immersed for 24 h at room temperature in 100 ml of phosphate buffered saline (PBS) containing 21 mM iodinated anionic contrast agent (Hexabrix™, Mallinckrodt, St. Louis, MO, USA), and inhibitors of proteolytic enzymes: 5 mM of ethylenediamine tetraacetic acid disodium salt (EDTA, VWR International, Fontenay, France) and 5 mM of benzamidine hydrochloride hydrate (Sigma–Aldrich Inc., St. Louis, MO, USA). Hexabrix™ is an ionic dimer containing ioxaglate meglumine and ioxaglate sodium. Both salts disintegrate into anionic ioxaglate^{-1}. In this experiment, the diffusion was allowed from every direction. However, the diffusion through subchondral bone was found insignificant. The immersion solution was continuously slowly agitated and sheltered from daylight, except when the samples were transferred to pQCT imaging. Before measurements the samples were quickly rinsed in PBS, blotted and enclosed in a polythene tube with

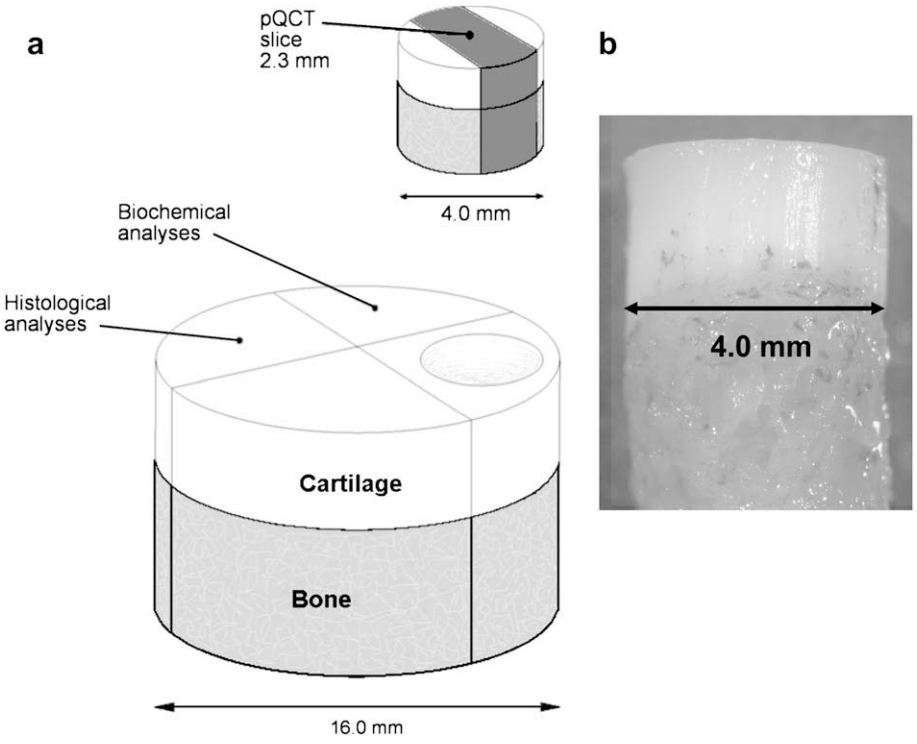

Fig. 1. Sample preparation. Osteochondral cylinders ($d = 16$ mm) were drilled from human FMCs ($n = 12$, cartilage thickness 2.13 ± 0.54 mm), and TMPs ($n = 12$, cartilage thickness 1.99 ± 0.38 mm). Smaller osteochondral cylinders were punched for the contrast agent experiment and the pQCT measurements. Biochemical and histological analyses were conducted for the cartilage extracted from the remaining part of the 16 mm cylinder.