2018

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Springer Nature

Tieteeniset aikakauslehtiartikkelit
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http://dx.doi.org/10.1007/s10439-018-2013-y

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Quantitative Dual Contrast CT Technique for Evaluation of Articular Cartilage Properties

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Submitted to Annals of Biomedical Engineering, December 2017

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ABSTRACT

Cationic CT contrast agents detect loss of cartilage proteoglycans (PGs) more sensitively than anionic or non-ionic agents. However, degeneration related loss of PGs and increase in water content have opposite effects on the diffusion of the cationic agent, lowering its sensitivity. In contrast to cationic agents diffusion of non-ionic agents is governed only by steric hindrance and water content. We hypothesize that sensitivity of iodine-based cationic agent may be enhanced by simultaneous use with non-ionic gadolinium-based agent. We introduce a quantitative dual energy CT technique (QDECT) for simultaneous quantification of two contrast agents in cartilage. We employ this technique to improve the sensitivity of cationic CA4+ by normalizing its partition in cartilage with that of non-ionic gadoteridol. The technique was evaluated with measurements of contrast agent mixtures of known composition and human osteochondral samples (n=57) after immersion (72h) in mixture of CA4+ and gadoteridol. Samples were arthroscopically graded and biomechanically tested prior to QDECT (50kV/100kV). QDECT determined contrast agent mixture compositions correlated with the true compositions ($R^2=0.99$, average error=2.27%). Normalizing CA4+ partition in cartilage with that of gadoteridol improved correlation with equilibrium modulus (from $\rho=0.701$ to $\rho=0.795$). To conclude, QDECT enables simultaneous quantification of iodine and gadolinium contrast agents improving diagnosis of cartilage integrity and biomechanical status.

KEYWORDS

Biomechanics, Cartilage, Cationic contrast agent, Contrast enhanced computed tomography, Dual energy CT
INTRODUCTION

Mechanical impact injury of articular cartilage (e.g. fall or sports related accidents) often initiates cartilage degeneration and development of post-traumatic osteoarthritis due to the limited self-repair capability of the aneural and avascular cartilage tissue. Using present diagnostic techniques clinicians cannot sensitively detect the injury at its earliest stages. Moreover, patient symptoms (pain and loss of mobility) arise, only at later stages of cartilage disease progression, thus leaving clinicians with limited treatment options at the time of diagnosis. Therefore, it is imperative to develop diagnostic methods capable of early detection of articular cartilage injuries.

The first signs of cartilage injury include disruption of superficial collagen network and loss of proteoglycans (PGs). Contrast enhanced computed tomography (CECT) enables detection of cartilage degeneration and lesions as well as estimation of tissue composition based on the diffusion of contrast agents. In CECT, anionic contrast agents are widely used to investigate cartilage tissue properties as their partition is inversely proportional to the fixed negative charge density of PGs.

Recently, cationic contrast agents were introduced for CT imaging of cartilage. Cationic contrast agent molecules, such as iodine-based CA2+ and CA4+, are attracted by the negative fixed charge (i.e. PGs) in cartilage, providing potentially a more sensitive technique for monitoring PG content in tissue compared with use of anionic and non-ionic agents. However, characteristic tissue changes related to cartilage degeneration, such as loss of PGs and increase of permeability, have opposite effects on the diffusion of cationic agents lowering their sensitivity.
Along Gibbs-Donnan theory diffusion of cationic contrast agents in cartilage is governed by PG content, permeability and water content, while with non-ionic agents, the diffusion is only controlled by permeability and water content of tissue. To improve the diagnostic potential and enhance the sensitivity of a cationic contrast agent (e.g. iodine-based CA4+), it could be used simultaneously with a non-ionic agent (e.g., gadolinium-based gadoteridol). By normalizing the partition of a cationic agent with that of non-ionic one the diagnostic potential of the cationic agent may be maximized. Further, we hypothesize that with the application of two X-ray tube voltages (50 kV and 100 kV), simultaneous quantification of iodine (I) and gadolinium (Gd) based agents is possible due to the element specific K-edges having significant effect on X-ray absorption.

The aims of the present study are to: (1) introduce a quantitative dual energy CT technique (QDECT) for simultaneous determination of the partitions of iodine and gadolinium-based contrast agents in a mixture; (2) determine the partitions of cationic and non-ionic agents in cartilage using QDECT; (3) evaluate the change in diagnostic sensitivity of the CA4+ cationic contrast agent after normalization of its partition with that of a non-ionic agent; and (4) illustrate the association of partitions of contrast agents in the cartilage with the tissue biomechanical properties.

MATERIAL AND METHODS

MicroCT scanning of Contrast Agent Solutions and Mixtures

MicroCT (Skyscan 1172, Skyscan, Kontich, Belgium) imaging was conducted using two X-ray tube voltages (50 kV and 100 kV) and isotropic voxel size of 25 × 25 × 25 µm³.
Additional filtering was used to obtain the desired X-ray spectra. With 50 kV tube voltage, 0.5 mm thick aluminum (Al) and 0.05 mm thick copper (Cu) filters provided by the microCT manufacturer were used along with a custom-made 0.1 mm thick Cu filter. When image acquisition was conducted using 100 kV tube voltage only the 0.5 mm thick Al filter was applied. Mass attenuation coefficients of contrast agents were determined at both energies by imaging series of solutions with varying iodine (0, 12, 24, 48 mg/ml) and gadolinium (0, 12, 24, 48 mg/ml) concentrations in distilled water (Fig. 1b).

Along Beer-Lambert law and Bragg’s rule of mixtures:\(^{22}\):

\[ I_E = I_{0E} e^{-\alpha_E}, \]  
\[ \alpha_E = \mu_{I_E} C_I + \mu_{Gd_E} C_{Gd}, \]  
where, \( I_{0E} \) and \( I_E \) are the intensities of the incident and transmitted X-ray beams through material with attenuation coefficient \( \alpha_E \) at energy \( E \), respectively. The concentrations of I \((C_I)\) and Gd \((C_{Gd})\) in contrast agent mixtures can be solved using the equations 3 and 4.

\[ C_I = \frac{\alpha_{100} \mu_{Gd_{50}} - \alpha_{50} \mu_{Gd_{100}}}{\mu_{I_{100}} \mu_{Gd_{50}} - \mu_{I_{50}} \mu_{Gd_{100}}}, \]  
\[ C_{Gd} = \frac{\alpha_{100} \mu_{I_{50}} - \alpha_{50} \mu_{I_{100}}}{\mu_{Gd_{100}} \mu_{I_{50}} - \mu_{Gd_{50}} \mu_{I_{100}}}, \]  
where, \( \mu_{I_{50}} \), \( \mu_{Gd_{50}} \), \( \mu_{I_{100}} \) and \( \mu_{Gd_{100}} \) are the mass attenuation coefficients of the iodine and gadolinium-based contrast agents measured using 50 and 100 kV tube voltages.

The dual energy technique was first tested with measurements of contrast agent mixtures of known iodine and gadolinium concentrations in distilled water \([I/Gd\]
concentration (mg/ml) ratios of 4.8/43.2, 9.6/38.4, 14.4/33.6, 19.2/28.8, 24.0/24.0, 28.8/19.2, 33.6/14.4, 38.4/9.6 and 43.2/4.8]. Subsequently, the true and the solved contrast agent iodine and gadolinium concentrations in the mixtures were compared (Fig. 1c).

Preparation and MicroCT imaging of Osteochondral Samples

Osteochondral samples ($n = 57$, $d = 8$ mm, Fig. 2) were drilled out from human cadaver ($n = 2$) distal femur ($n = 4$) and proximal tibia ($n = 4$) (Decision number 150/2016, Research Ethics Committee of the Northern Savo Hospital District, Kuopio University Hospital, Kuopio, Finland). For the CT experiment, the samples were cut half and their edges were sealed with cyanoacrylate (Loctite, Henkel Norden AB, Dusseldorf, Germany) to allow contrast agent diffusion only through the articular surface. The samples were immersed in an isotonic mixture of iodine-based cationic contrast agent (CA4+, $q = +4$, $M = 1354$ g/mol) and gadolinium-based non-ionic agent (gadoteridol, $q = 0$, $M = 558.69$ g/mol, ProHance™, Bracco Diagnostic Inc., Monroe Twp., NJ, USA) bath (3 ml/sample) for 72 hours at 4 °C. The bath was continuously gently stirred during the immersion of osteochondral samples. Contrast agent mixture (24 mg/ml of both iodine and gadolinium in distilled water) was supplemented with inhibitors of proteolytic enzymes [5 mM ethylenediaminetetraacetic acid (EDTA, VWR International, France) and penicillin-streptomycin-amphotericin (Antibiotic Antimycotic solution, stabilized, Sigma-Aldrich Inc., St. Louis, MO, USA)] to prevent general degradation of proteins in tissue. With the established QDECT protocol human osteochondral samples were imaged with an
isotropic voxel size of 25 × 25 × 25 µm³ using a micro-CT scanner (Skyscan 1172) before
and after immersion in the contrast agent bath.

**Determining Contrast Agent Distribution**

Micro-CT images were reconstructed using NRecon software (Bruker co., Kontich, Belgium). Further microCT image data analysis was conducted using MATLAB (R2016b, Mathworks Inc., Natick, MA, USA). Cartilage was segmented from subchondral bone manually using segmentation software (Seg3D vs 2.2, The University of Utah, Salt Lake City, UT, USA) and custom made MATLAB code to select the cartilage surface and cartilage-bone interface. Volume of interest (2000 µm × 1250 µm × cartilage thickness) was selected to obtain the depthwise X-ray attenuation profile. For each sample, native cartilage X-ray attenuation profile was subtracted from that measured after the contrast agent diffusion. The concentrations of iodine ($C_I$) and gadolinium ($C_{Gd}$) in cartilage were determined using the equations 3 and 4, respectively.

**Biomechanical testing and ICRS grading**

A custom made material testing device equipped with an actuator having resolution of 0.1 µm (PM500-1 A, Newport, Irvine, CA, USA) and a load cell with resolution of 0.005 N (Sensotec, Columbus, OH, USA) was employed for biomechanical testing of osteochondral samples. During testing the samples were immersed in phosphate buffered saline supplemented with inhibitors of proteolytic enzymes [5 mM EDTA, VWR International and 5 mM benzamidine hydrochloride hydrate (Sigma-Aldrich Inc.)]. A flat
ended metallic indenter \( d = 728 \mu m \) \( (n = 29) \) or \( d = 667 \mu m \) \( (n = 28) \) was driven in perpendicular contact with the articular surface using a pre stress of 12.5 kPa \(^{13}\). Stress relaxation protocol consisting of four compressive steps (each being 5% of cartilage thickness, 100%/s ramp rate) was implemented with a 900 s relaxation after each step.

The equilibrium modulus \( (E_{equilibrium}, \text{fit to the equilibrium points of the last three steps}) \) and instantaneous modulus \( (E_{instantaneous}, \text{the ramp phase of the third step}) \) were calculated using the Hayes model\(^{7}\). Following stress strain relaxation tests, dynamic moduli \( (E_{dynamic}) \) was determined based on Hayes model using sinusoidal loading \( (f = 1 \text{ Hz}, \text{strain amplitude} = 2 \% \text{ of cartilage thickness}) \). An experienced orthopaedic surgeon (A.J.) graded all the cartilage sample locations before sample extraction using the ICRS (International Cartilage Repair Society) grading (scale 0 to 4) \(^{3}\).

**Statistical Analysis**

Pearson’s correlation was used to analyze the relation between the true and measured iodine and gadolinium concentrations in contrast agent mixtures. Spearman’s rho \( (\rho) \) was determined to analyze the relation between contrast agent partitions in cartilage and the values of biomechanical moduli. All the statistical analysis were conducted using SPSS (vs. 23, SPSS Inc., IBM Company, Armonk, NY, USA).

**RESULTS**

Composition of the contrast agent mixture, as determined with the dual energy technique, correlated linearly with the true mixture composition \( (R^2 = 0.99, P < 0.01) \), with an average
error of 2.27 percentage points (Fig. 1c). After immersing human osteochondral samples
(n = 57) for 72 hours in the contrast agent mixture, the partitions (mean ± SD) of CA4+
and gadoteridol in cartilage were 197.6 ± 28.4% and 66.0 ± 9.2%, respectively (Table 1).
CA4+ and gadoteridol partitions determined with the dual energy technique showed
increasing and decreasing trends, respectively, along cartilage depth (Fig. 3). CA4+ and
gadoteridol partitions in the cartilage superficial zone (500 µm) correlated significantly
(0.656 ≤ ρ ≤ 0.701, P < 0.001) and (-0.566 ≥ ρ ≥ -0.583, P < 0.001), respectively, with the
cartilage biomechanical properties (Table 2). Importantly, normalizing CA4+ partition with
that of gadoteridol in the superficial cartilage improved linear correlations with the
biomechanical parameters, e.g. with \( E_{\text{equilibrium}} \) from \( ρ = 0.701 \) to \( ρ = 0.795 \) (Table 2).
Furthermore, normalizing CA4+ partition in full thickness cartilage with that of gadoteridol
improved the correlation with ICRS grading (from \( ρ = -0.385 \) to \( ρ = -0.458 \) (Table 2).

**DISCUSSION**

In this laboratory study, a QDECT methodology for simultaneous quantification of iodine
(CA4+) and gadolinium (gadoteridol) contrast agents in human articular cartilage was
developed and validated. We hypothesized that this novel technique would enable
simultaneous determination of the depthwise distribution of the two contrast agents.
Furthermore, we summarized that the diagnostic sensitivity of the cationic contrast agent
would improve by normalizing its partition with that of the non-ionic agent. Our
experimental findings confirmed both hypotheses.

Cartilage degeneration in osteoarthritis (OA) involves loss of proteoglycans (PGs),
disruption in the collagen network, and increase in cartilage water content. 5 In OA, loss
of PGs and increase in water content occur simultaneously having opposite effects on the diffusion of cationic agents. Unfortunately, this limits the diagnostic value of cationic contrast agents at clinically relevant imaging time points (<1 hour after contrast agent administration, i.e. when complete diffusion has not taken place).

In the present experiment, the equilibrium partition of the cationic agent (CA4+) was nearly three times that of the non-ionic agent (gadoteridol) and increased towards the deep cartilage. This is in line with literature reports, as cationic contrast agents have shown an uptake in direct proportion to the negative fixed charge density (i.e. PG content), which are in greater concentrations in middle and deep zones of cartilage. On the contrary, the partition of non-ionic gadoteridol decreased as a function of cartilage depth. This was also expected as cartilage water content decreases with increasing cartilage tissue depth. The permeability of cartilage to electrically neutral agents reduces with increasing cartilage tissue depth because of the increasing steric hindrance of contrast agent molecules induced by the collagen network and the highly concentrated PGs. Hence, the quantification of non-ionic agent’s partition along the tissue depth provides information of water content and permeability of the cartilage extracellular matrix.

Simultaneous determination of the partitions of both agents with the dual energy technique enabled normalization of cationic agent partition with that of the non-ionic agent. In line with our hypothesis, the normalization of the CA4+ partition in superficial cartilage improved its correlation with cartilage biomechanical properties ($E_{equilibrium}$ from $\rho = 0.701$ to $\rho = 0.795$, $E_{dynamic}$ modulus from $\rho = 0.656$ to $\rho = 0.748$) and structural integrity (ICRS grade from $\rho = -0.398$ to $\rho = -0.408$). Even though the trend towards higher correlations after normalization was observed with most reference parameters, the enhancement in
correlation was not statistically significant. This is possibly due to the relatively low sample number \( n = 57 \) in the present study. Correlation between biomechanical moduli and normalized CA4+ in full thickness cartilage did not demonstrate enhancement in CA4+ sensitivity as with the cartilage superficial layer. This could be due to superficial layer controlling cartilage indentation response.\(^9,13\)

Based on these results, the introduced QDECT technique may have significant potential for clinical diagnostics of degenerative cartilage conditions. However, the current study protocol does possess limitations. The dual energy CT imaging was conducted at diffusion equilibrium (osteochondral plugs immersed for 72 hours in contrast agent bath). In normal clinical practice, contrast-enhanced CT scanning is carried out in earlier time points, i.e. at 45 minutes after administration of the contrast agent.\(^10,19\) During the first hour, the diffusion is fast and the depthwise partitions of the contrast agents are changing rapidly.\(^8\) In such a scenario, imaging with two energies must be done as instantaneously and simultaneously as possible. This is not a problem with clinical scanners, but might jeopardize the reliability of the results when using high resolution microCT-scanners with longer imaging times. In the present study, imaging was conducted at diffusion equilibrium and therefore this is not a problem. However, this issue warrants further research and the potential of the present technique should be tested in all phases of the diffusion process and most importantly in clinically relevant time points.

To conclude, QDECT imaging enables simultaneous determination of the distribution of iodine and gadolinium-based contrast agents in cartilage. Importantly, the introduced technique improves the diagnostic sensitivity of contrast-enhanced imaging of cartilage.
ACKNOWLEDGEMENTS

Sandra Sefa, (B.Sc.) is acknowledged for assistance with the biomechanical measurements. Jaakko Sarin, M.Sc. (Tech) is acknowledged for assistance in sample extraction. Academy of Finland (Projects 269315, 307932), Kuopio University Hospital (VTR 5041746, 5041757, PY210), Instrumentarium Science Foundation (170033) and Doctoral Program in Science, Technology and Computing (SCITECO, University of Eastern Finland) are acknowledged for financial support.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

REFERENCES


Table 1. Values of biomechanical moduli (instantaneous, equilibrium and dynamic) and contrast agent partitions (mean±SD) in superficial and full thickness cartilage in osteochondral samples extracted from tibial and femoral locations in human cadaver (n = 2) knee joints (n = 4).

<table>
<thead>
<tr>
<th>Location</th>
<th>Thickness (mm)</th>
<th>Instantaneous</th>
<th>Equilibrium</th>
<th>Dynamic 1Hz</th>
<th>Superficial Cartilage (top 500 μm)</th>
<th>Full Thickness Cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CA4+</td>
<td>Gadoteridol</td>
</tr>
<tr>
<td>FLC (n = 9)</td>
<td>2.86±0.61</td>
<td>18.76±7.10</td>
<td>1.46±0.92</td>
<td>8.51±3.45</td>
<td>157.7±26.6</td>
<td>78.4±5.1</td>
</tr>
<tr>
<td>FMC (n = 9)</td>
<td>2.63±0.59</td>
<td>16.64±9.82</td>
<td>1.51±1.31</td>
<td>7.65±4.87</td>
<td>154.2±39.7</td>
<td>81.5±6.4</td>
</tr>
<tr>
<td>FG (n = 9)</td>
<td>2.61±0.41</td>
<td>13.59±9.56</td>
<td>1.12±0.92</td>
<td>6.81±4.69</td>
<td>129.1±22.2</td>
<td>77.6±6.2</td>
</tr>
<tr>
<td>TMC (n = 14)</td>
<td>2.48±0.42</td>
<td>12.06±9.30</td>
<td>0.69±0.59</td>
<td>4.85±3.63</td>
<td>125.8±25.9</td>
<td>84.1±9.8</td>
</tr>
<tr>
<td>TLC (n = 16)</td>
<td>2.65±0.84</td>
<td>10.50±10.55</td>
<td>0.65±0.62</td>
<td>4.42±4.08</td>
<td>111.4±20.6</td>
<td>82.4±10.9</td>
</tr>
<tr>
<td>Total (n = 57)</td>
<td>2.63±0.61</td>
<td>13.64±9.64</td>
<td>1.02±0.94</td>
<td>6.10±4.43</td>
<td>131.8±31.4</td>
<td>81.3±8.7</td>
</tr>
</tbody>
</table>

FLC (Femoral Lateral Condyle), FMC (Femoral Medial Condyle), FG (Femoral Groove), TLC (Tibial Lateral Condyle), TMC (Tibial Medial Condyle).
**Table 2.** Linear correlations (Spearman’s rho) between the equilibrium, instantaneous and dynamic moduli and contrast agent partition in cartilage (n = 57).

<table>
<thead>
<tr>
<th></th>
<th>Superficial Cartilage (Top 500 μm)</th>
<th>Full Thickness Cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA4+ Normalized</td>
<td>CA4+</td>
</tr>
<tr>
<td>Equilibrium Modulus (MPa)</td>
<td>0.795**</td>
<td>0.701**</td>
</tr>
<tr>
<td>Instantaneous Modulus (MPa)</td>
<td>0.693**</td>
<td>0.614**</td>
</tr>
<tr>
<td>Dynamic Modulus 1Hz (MPa)</td>
<td>0.748**</td>
<td>0.656**</td>
</tr>
<tr>
<td>ICRS Grading</td>
<td>-0.408**</td>
<td>-0.398**</td>
</tr>
</tbody>
</table>

* P < 0.05

** P < 0.01
Figure 1. (a) The simulated microCT spectra (SPEKTR vs. 3, I-star Lab, John Hopkins University, Baltimore, MD, USA) at tube voltages of 50 kV and 100 kV are presented along mass attenuation curves for Iodine (I) and Gadolinium (Gd). (b) Linear fits between contrast agents (I and Gd) concentrations and X-ray attenuation at 50 and 100 kV tube voltages are $\alpha_{I}(50\text{ kV}) = 0.0528 C_I + 0.1094$, $\alpha_{Gd}(50\text{ kV}) = 0.0432 C_{Gd} + 0.0621$, $\alpha_{I}(100\text{ kV}) = 0.0353 C_I + 0.0985$ and $\alpha_{Gd}(100\text{ kV}) = 0.0508 C_{Gd} + 0.071$. (c) Linear correlation between the true mixture compositions and the mixture compositions determined using the QDECT technique.
Figure 2. Osteochondral plugs \((d = 8 \text{ mm})\) were extracted from the marked locations of human cadaver \((n = 2)\) knee joints \((n = 4)\).
Figure 3. (a) Depthwise partitioning of (a) gadoteridol and (b) CA4+ in cartilage after 72 hours of immersion in mixture of cationic and non-ionic contrast agents. The black solid lines represent the mean partition in all the samples ($n = 57$).
Figure 4. Correlations (Spearman’s rho) between CA4+ partition (normalized and non-normalized by gadoteridol partition) in cartilage and cartilage biomechanical properties. The partition values are calculated for superficial cartilage (i.e. top 500 µm from cartilage surface). Linear fit is drawn to enable better visualization of correlation. FG (Femoral Groove), FLC (Femoral Lateral Condyle), FMC (Femoral Medial Condyle), TLC (Tibial Lateral Condyle), TMC (Tibial Medial Condyle).
(a) Attenuation (cm$^2$/g) vs. X-ray photon energy (KeV)

(b) X-ray attenuation (AU) vs. Concentration (mg/ml)

(c) (1/Gd) Measured composition vs. (I/Gd) True composition

$R^2 = 0.99$, $n = 9$, $P < 0.01$
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