Effect of Molecule Structure to the Diffusion of the Cationic Contrast Agent CA4+

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Introduction: Cationic contrast agents for contrast-enhanced computed tomography (CECT) offer a solution for the early diagnosis of osteoarthritis (OA) [1]. Of the different cationic agents, CA4+ which has four positive charges and six iodine atoms, creates high attenuation with low concentration inside articular cartilage. Thus, it provides high sensitivity for analyzing diminished GAG content, the first symptom of OA [1-3]. Reaching diffusion equilibrium however can take hours. In order to reach clinical relevance, the time period between administration and imaging needs to be minimized and less than one hour. In this work using μCT, three novel CA4+ solutions were synthesized and their diffusion in to cartilage was compared against the conventional CA4+. We hypothesize that by changing the CA4+ structure, the diffusion inside articular cartilage can be altered as the chemical nature of the cationic group (-NMe3+ or -NH3+) will influence the rate via size, hydrogen bonding, and/or electrostatic interactions.

Methods: The chemical structure of the used agents differed either in methyl degree of substitution (quaternary ammonium salts possessed fixed positive charged density irrespective of pH changes) or heterocyclic amines (possess various non-covalent interactions) as terminal functionality (Fig. 1). Used agents were conventional CA4+ (**1**), CA4-N(Me)3 (**2**), CA4-piperazine (TFA salt) (**3**), and CA4-morpholine (**4**). CA4+ powders were diluted to sodium chloride (NaCl) solution containing protease inhibitor benzamine hydrochloride (5 mM), GIBCO Antibiotic/Antimyotiv (Invitrogen, Grand Island, NY), and calcium ion chelating agent ethylenediamine tetraacetic acid (5 mM). pH was adjusted to 7.4 and osmolality to 400 mOsm. For each agent, osteochondral articular cartilage plugs (diam. 7mm, n = 5, thickness = 1.60±0.49 mm) were harvested from bovine femoral condyles, acquired from a local butcher. To allow the contrast agent to diffuse only through the articular surface, the sample sides were sealed using cyanoacrylate (Super Glue Ultragel Control, Loctite, Düsseldorf, Germany). The samples were incubated in the agents on an orbital shaker in 37 °C. Using a custom airtight holder, the plugs were imaged with μCT (μCT 40, SCANCO Medical AG, Brüttisellen, Switzerland) using 70kV tube voltage at 0, 1, 3, 6, 12, and 24 h between the incubation. After the last measurement, samples were immersed in saline and incubated similarly for 24 h, after which a final scan was performed. Using Matlab (R2017b, Mathworks, Natick, MA), native cartilage attenuation was subtracted and the contrast agent partition was calculated as the ratio of the attenuation compared to the bath. A curve of the form $A\cdot[1\text{-}e\text{^}(\text{-}t/\tau)]$ was fit to the partition data, where A is maximum partition, t is diffusion time, and the τ value is calculated as the time required to reach 63.2% of the maximum agent partition [4]. Statistical comparison for the partition parameters (A, τ) between different contrast agents was done using one-way ANOVA with Tukey's multiple comparison analysis method.

Results: With a constant concentration of 24 mg I/ml, the agent baths **1**, **2**, **3**, and **4** produced radiodensities of 1416, 1308, 1183, and 1044 HU, respectively. Partition maximum (A) (± standard deviation) was 212±28, 278±45, 175±14, and 42±16 %, for the agents **1**, **2**, **3**, and **4**, respectively (Fig 2). A of agent **2** was significantly higher (p=0.011) compared to the conventional **1** compound. Time constant τ was 2.3±0.5, 3.4±2.0, 2.3±1.1, and 2.9±0.4 h, for **1**, **2**, **3**, and **4**, respectively. Average partition after 24 h wash out was 15.6±16.2%.

Discussion: This preliminary study shows that by changing the terminal functionality (fully methylated quaternary ammonium salt, aromatic and non-aromatic heterocyclic ring), and thereby altering positive charged density or non-covalent interactions of the contrast agents, the partitions were seen to change inside cartilage. Attenuation of the agents **1**, **2**, and **3** followed the typical GAG distributions of articular cartilage (Fig. 3). Results suggest the agent **2** to be a potential candidate to substitute the conventional agent **1** CA4+. However, no difference was seen in the wash out results or in the time constant τ between the agents. Perhaps a significantly smaller molecule is needed for more effective diffusion through the small pores of the articular cartilage. Agent **4** did not diffuse inside the tissue. It is possible that the bath aggregated and precipitated during the dilution process. However, all the agent baths were clear and transparent to the eye. An agent that can attach itself effectively on the surface without penetrating it could be viable in segmentation. Variation in the bath radiodensitites suggest possible impurities in the agent solutions. Mechanical tests and biochemical analysis using the 1,9-dimethylmethylene blue (DMMB) colorimetric assay for the GAG content have been completed and will be presented. Authors acknowledge the small sample size to be a limitation of this preliminary study.

Significance: The development of sensitive, minimally invasive cartilage imaging techniques that allow assessment of cartilage thickness, morphology, equilibrium compressive modulus, and GAG content will aid in the diagnosis, treatment, and monitoring of osteoarthritis. The results herein demonstrate that minimal changes in diffusion rate occur with modification of the terminal cationic functionality and suggest that altering other molecular parts of the contrast agent (e.g., number of aromatic rings) may provide a greater difference in cartilage tissue uptake and diffusion rates.

References: [1] P. N. Bansal et al., J. Orthop. Res., 29(5):704–709, 2011. [2] P. N. Bansal, Osteoarthr. Cartil., 18(2):184–191, 2010. [3] J. A. Buckwalter et al., Instr Course Lect, 47(4):487–504, 1998. [4] R. C. Stewart et al., Radiology, 266(1):141–150, 2013.

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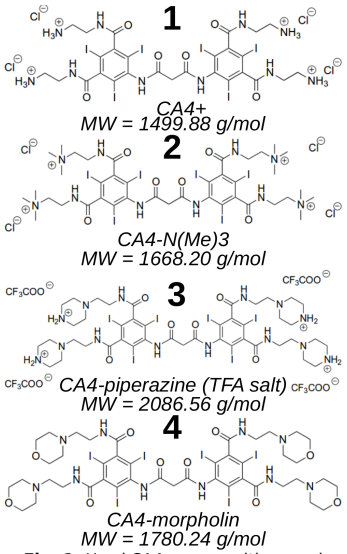

Fig. 1: Used CA4+ compositions and molecular weights.

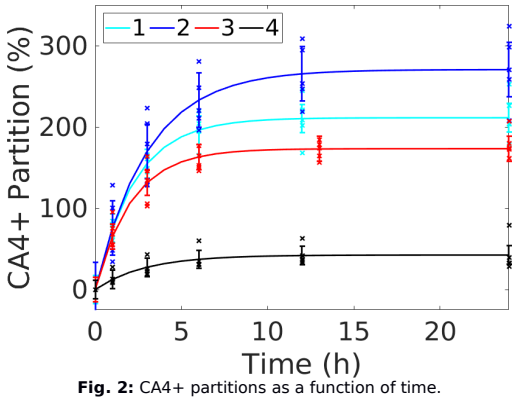

Fig. 2: CA4+ partitions as a function of time.

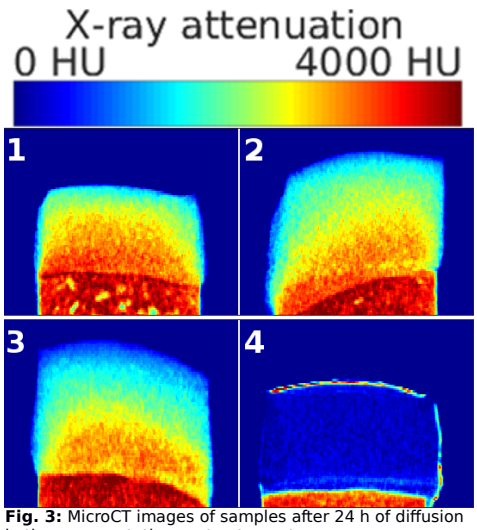

Fig. 3: MicroCT images of samples after 24 h of diffusion in the representative contrast agent.