Tantalum Oxide Nanoparticles for use in Contrast Enhanced Computed-Tomographic Imaging of Articular Cartilage

Taylor B. Lawson[1,2], Amit Patwa[1], Brian D. Snyder[1,2,3], Mark W. Grinstaff[1]
[1]Boston University, Boston, MA; [2]Beth Israel Deaconess Medical Center, Boston, MA; [3]Boston Children's Hospital, Boston, MA

Disclosures: Taylor B. Lawson (N), Amit Patwa (N), Brian D. Snyder (N), Mark W. Grinstaff (N)

Introduction: Articular cartilage is a biphasic material comprised of a 3D poroelastic collagen network and hydrated matrix phase of negatively charged sulfated and carboxylated glycosaminoglycans (GAGs) that confer articular cartilage its unique elastohydrodynamic tissue properties. A decline in GAG content is an early sign of osteoarthritis (OA) associated with degradation in the tissues mechanical performance. Minimally invasive techniques to image cartilage and assess GAG content are of significant interest, as diagnosing OA at an early stage will facilitate interventions that slow or halt disease progression[1]. We are evaluating a new nanoparticle (NP) based contrast agent for computed tomography (CT) assessment of functional performance including GAG quantification. NPs have shown promise as contrast agents; they can be tailored to a particular purpose by manipulating their composition, size, surface structure and charge density[2]. In this study, we examined the effect of NP diameter, and we hypothesize that the smaller diameter NPs will permeate the tissue to a greater extent, resulting in improved coefficients of determination between X-ray attenuation and GAG content. To develop NP based contrast agents to facilitate CT imaging of articular cartilage, the NP must be optimized to measure the composition and structure of articular cartilage that define the tissue's functional performance. In particular, it is necessary to define the range of NP sizes that will permeate the tissue's porous structure and partition throughout the anisotropic zonal morphology of articular cartilage in proportion to the matrix GAG concentration. Tantalum is an ideal NP material for CT imaging as it posseses a higher *k*-edge (67.4 keV) than conventional iodinated contrast agents, resulting in more X-rays attenuated at clinical CT scanning voltages, lessening low energy filtration. Tantalum oxide was incorporated into the core-shell of nanoparticles, with positively charged tetra-ammonium ligands to coordinate to the anionic GAGs and a short polyethylene glycol shell layer to improve biocompatibility. The effect of NP size on the ability to assess GAG content via CECT, in cadaveric human metacarpal phalangeal joints, was assessed by comparing NPs of an average size of 3.4 vs. 7.8 nm. We hypothesize that the smaller diameter NPs will permeate the tissue to a greater extent, resulting in improved coefficients of determination between X-ray attenuation and GAG content.

Methods: The tantalum oxide (Ta_2O_5) core was formed by hydrolysis of tantalum (V) ethoxide, $Ta(OEt)_5$. The size of the NPs was controlled by the amount of deuterated water and isobutyric acid added to n-propanol. Two nanoparticle solutions were prepared with average size of 3.4 nm and 7.8 nm. Cationic silane ligands were coupled to the NP surface in n-propanol under reflux conditions (Figure 1). The NPs were purified by dialysis (3,500 MWCO, Snakeskin, Thermo Sci., Billerica, MA) over 72 h, and filtration through 0.22 M PES membrane (Corning Inc, Corning, NY). Volatiles were removed by lyophilization resulting in Ta_2O_5-NPs as a white powder. NPs were characterized by 1H NMR and FTIR. The hydrodynamic size of the nanoparticles was measured by dynamic light scattering (DLS) at 25 °C. The zeta potential of the nanoparticles was measured using Brookhaven Instruments ZetaPALS Zeta Potential Analyzer. Disarticulated second and third metacarpophalangeal joint (MCPJ) were dissected from 6 cadaveric (Medcure, Portland, OR) hands (9 donors, mean age: 73 years). After removing soft tissues, transaxial uCT images (36-um^3, 70 kVp, 113-uAmp, 300-ms integration; Scanco Medical AG, Bruttisellen, Switzerland) were obtained of the joint articular surfaces in air and after 24 hours of immersion in NP-saline solutions iso-osmotic to synovial fluid (400mOsm, pH 7.4). After converting to DICOM format, the average x-ray attenuation of the articular cartilage was determined. Following 24-hr saline washout, the cartilage was removed and GAG content assessed using 1,9-dimethylmethylene blue (DMMB) colorimetric assay.

Results: Both cationic nanoparticle sizes, resulted in a positive linear correlation between X-ray attenuation and GAG concentration (Figure 2). Contrast enhanced CT attenuation using smaller NPs accounted for nearly 90% of the variation in cartilage GAG concentration ($R^2 = 0.89$), compared to 54% using larger diameter NPs ($R^2 = 0.54$) (Figure 2). The color map generated from the small diameter NP enhanced CT scans reflect dissemination of the particles throughout the different cartilage layers with uniform attenuation throughout the three cartilage layers, in both samples with high and low GAG content (Figure 3). Conversely, the color map generated from large diameter NPs resulted in higher attenuation at the surface, which decreased towards the subchondral bone (Figure 3).

Discussion: Cationic NPs of average size 7.4 nm resulted in lower X-ray attenuation values, compared to NPs of average size 3.8 nm or less. This suggests that smaller NPs are better able to penetrate into the middle and deep zones of articular cartilage, where the GAG content is relatively higher, and the porosity is lower. Our lab previously reported on the contrast agent CA4+ in comparison to the Ioxaglate, where it was shown that CA4+ enhanced CT attenuation, reflects GAG distribution within human MCPJs, and accounts for 86% of the variation of GAG content[3]. Whereas, Ioxaglate, an anionic, clinically used CECT agent, did not partition within the tissue with expected inverse distribution of GAG content. The correlation between CECT attenuation and GAG content was also weak and not significant ($R2 = 0.12$)[3]. The results demonstrate that NP size, relative to tissue porosity, is a critical contrast agent design criterion.

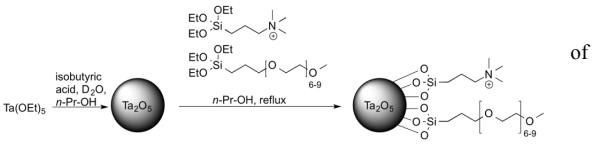

Figure 1. Tantalum oxide nanoparticle synthesis

Significance: Tantalum oxide nanoparticle based contrast agent detect glycosaminoglycan content within articular cartilage and the resulting CECT attenuations are sensitive to the biochemical composition of articular cartilage. Imaging techniques sensitive to early detection of OA, when chondroprotective or chondroregenerative technologies will be most effective in slowing or halting OA progression, are of significant interest.

References: [1] Sophia Fox AJ, Bedi A, Rodeo SA. The Basic Science of Articular Cartilage : *Sports Health*. 2009;1(6):461-468. [2]Freedman JD, Lusic H, Snyder BD, Grinstaff MW. Sythesis of Tantalum Oxide Nanoparticles for Imaging Articular Cartilage Using X-Ray Computed Tomography. *Angew Chemie - Int Ed*. 2014;53(32):8406-8410. [3] Lakin BA, et al. Contrast-Enhanced CT Facilitates Rapid, Non-Destructive Assessment of Cartilage and Bone Properties of the Human Metacarpal. *Osteoarthr Cartil*. 2015;23(12):2158-2166.

Acknowledgements: NIH (R01GM098361), Harvard Catalyst Program, NSF Graduate Research Fellowship (BGC).

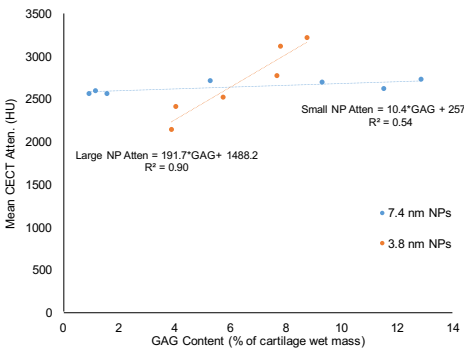

Figure 2. Correlations for NPs of a large (7.4 nm) and smaller (3.8 nm) average size vs GAG content (% of cartilage wet mass).

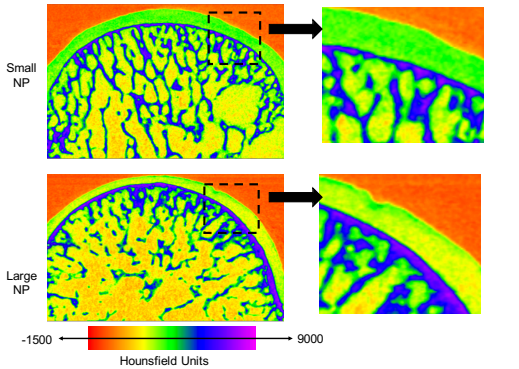

Figure 3. Representative color maps from a central, sagittal, CECT slice for a metacarpal sample with **a)** small NPs and **b)** large NPs. Color scale bar indicates corresponding CECT attenuation in Hounsfield Units (HU) for all CECT color maps