Tantalum Oxide Nanoparticles Reveal Proteoglycan Content of Human Articular Cartilage and Enable Detection of Superficial Lesions in Computed Tomography

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INTRODUCTION: Customization of nanoparticles (NPs), including modifying their size, shape, and charge, enables utilizing them in specific application to include carriers for drugs or contrast agent for imaging soft tissues. Notably, the negatively charged nature of the cartilage extracellular matrix, due to tissue health reflecting proteoglycans, serves as a compelling factor, as it naturally attracts positively charged contrast agents. Tantalum oxide nanoparticles (Ta_2O_5-NPs) present an example, adept at efficiently attenuating X-rays. Their versatile attributes, including the potential for size and surface chemistry modifications, render them ideal candidates for customization of contrast-enhanced computed tomography (CECT) imaging of cartilage. In this study, we tailor two different Ta_2O_5-NPs by varying their surface charge. Cationic Ta_2O_5-NPs are coated with positively charged trimethylammonium to bind to negatively charged proteoglycans in cartilage. To improve their biocompatibility, a nonionic poly(ethylene) glycol (PEG) coating is also incorporated. In contrast, the electrically neutral Ta_2O_5-NPs are synthesized exclusively using the PEG coating. Our objective is to investigate whether these physicochemical properties affect the nanoparticle diffusion, and investigate how the uptake is sensitive to proteoglycan content. Additionally, if their entry into the cartilage matrix is limited, we can leverage this information to assess the suitability of these particles for precise segmentation purposes. This investigation holds the promise of yielding more effective contrast agents, ultimately enhancing diagnostic imaging capabilities.

METHODS: Ta_2O_5-NPs were synthesized following a previously described protocol[1] with a modification to the ratio between PEG and trimethylammonium ligands to 1:2**.** The hydrodynamic diameter and Zeta potential of the NPs were characterized using dynamic and electrophoretic light scattering. Osteochondral plugs (diameter = 4 mm, $n = 32$) were obtained from the knee joints of human cadavers ($N = 7$), and halved for CECT experiments and histology. Histological sections were stained with Safranin-O, and imaged using digital densitometry to determine proteoglycan content. CECT sample edges were sealed using cyanoacrylate to allow NP diffusion only through articulating surface. Separate sample groups were immersed into either cationic ($n = 23$) or neutral Ta_2O_5-NP bath ($n = 9$) for 72 h. NP concentration in each bath was 30 mg/ml. Temperature, pH, and osmolality were fixed to 37 °C, 7.4, and 400 mOsm, respectively. The plugs were imaged using a Nikon XT H 225 µCT scanner (150 kVp, 25 W, 0.5 mm Cu filter) in air before immersion and after 1, 3, 6, 10, 24, 48, and 72 hours of immersion. Contrast agent partition was calculated by dividing the contrast agent induced X-ray attenuation in each sample with the X-ray attenuation of the contrast agent bath. After the diffusion experiments, we induced superficial lesions on the samples immersed in the neutral Ta_2O_5-NP bath using a scalpel. We then imaged these samples again in the electrically neutral Ta_2O_5-NP ($n = 5$) bath and in phosphate-buffered saline (PBS) mimicking synovial fluid ($n = 4$).

RESULTS: The hydrodynamic diameters of cationic and electrically neutral NPs were 2.77 ± 0.77 nm and 3.08 ± 1.24 nm, respectively. The Zeta potential was 25.63 ± 4.71 mV for cationic NPs and -0.98 ± 1.10 mV for electrically neutral NPs. Cationic Ta_2O_5-NP contrast agent diffused into human cartilage, while electrically neutral Ta_2O_5-NP contrast agent did not (Fig. 1). However, electrically neutral Ta_2O_5-NPs facilitated the detection of cartilage surface and superficial lesions (Fig. 2). Cationic contrast agent partition correlated positively (Pearson $r = 0.54$, $p = 0.008$) with proteoglycan content (Fig. 3).

DISCUSSION: Cationic Ta_2O_5-NP contrast agent target proteoglycans reflecting their quantity, which is important as proteoglycan loss occurs in early osteoarthritis[2]. The size of the two NPs is almost identical, nevertheless, the electrically neutral NPs did not diffuse into the cadaveric knee joint articular cartilage. This result suggests the intake of NPs is primarily driven by their charge. However, we have previously demonstrated the collagen content and the organization of collagen fibrils to influence the diffusion of NPs[3]. Consequently, we anticipate the potential to optimize NPs to provide insights into fibril network fibrillation, which serves as another indicator of early osteoarthritis. Acute cartilage damage results typically from accidental impacts, and potentially leads to post-traumatic osteoarthritis[4]. Early detection of such injuries is critical, as their development can only be prevented if detected early[5,6], and the electrically neutral Ta_2O_5-NPs offer a promising approach to injury detection.

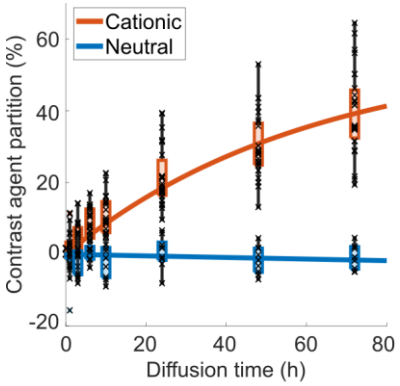

Figure 1: Cationic Ta_2O_5-NP contrast agent (red) diffused into human cartilage, but electrically neutral Ta_2O_5-NP contrast agent (blue) did not.

SIGNIFICANCE/CLINICAL RELEVANCE: Here we introduce potential applications for two tailored Ta_2O_5-NPs designs: 1) diagnosing cartilage internal conditions using proteoglycan binding cationic Ta_2O_5-NPs; and, 2) revealing cartilage morphology, including superficial lesions, employing electrically neutral Ta_2O_5-NPs.

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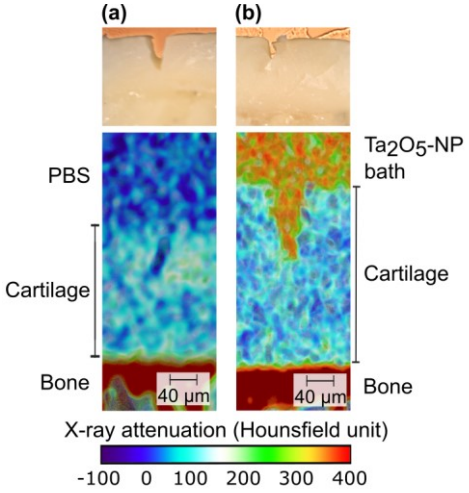

Figure 2: In upper row photos of the samples and µCT images below. **(a)** The surface and superficial lesion are invisible in µCT when imaged in phosphate-buffered saline (PBS). **(b)** In electrically neutral Ta_2O_5-NP contrast agent bath, the surface and superficial lesion become visible.

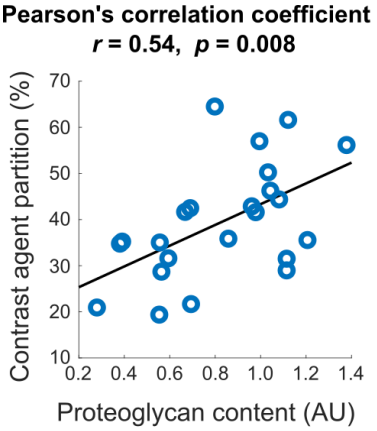

Figure 3: Cationic Ta_2O_5-NP contrast agent correlated positively with proteoglycan content (AU) at 72h. (y = 22.55 x + 20.84)