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A Cationic Gadolinium Contrast Agent for Magnetic Resonance Imaging of Cartilage

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Abstract

A new cationic gadolinium contrast agent is reported for delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC). The agent partitions into the glycosaminoglycan rich matrix of articular cartilage, based on Donnan Equilibrium Theory, and its use enables imaging of the human cadaveric metacarpal phalangeal joint.

Magnetic resonance imaging (MRI) is a widely used medical imaging technique for visualization and diagnosis of soft, hydrated tissues. Often metal-based contrast agents,^{1–4} either as small molecules,^{5, 6} polymers,⁷ or nanoparticles,^{8–10} are employed to: 1) enhance signal intensity by locally altering the longitudinal and transverse relaxation rates of water;¹¹ 2) target specific sites via attachment of antibodies, receptor-specific ligands, etc;¹² and/or 3) assess the biochemical state of the tissue by responding to pH, pO₂, or enzymatic activity.^{13, 14} The identification of early pathological changes (biochemical, biomechanical, or structural) in tissue through imaging prior to morphological changes represents a significant hurdle, and, thus, opportunities exist for improved care in many diseases including osteoarthritis (OA). OA is the most prevalent form of arthritis in the world with 10% of the population over 60 years old suffering from this disease.¹⁵ Early diagnosis may provide opportunities for interventions that can significantly slow the progression of the disease, or reverse it altogether.¹⁶ Thus, imaging modalities that can ascertain cartilage structure and properties are of keen interest, and delayed gadolinium enhanced MR imaging

of cartilage (dGEMRIC) is one such technique that has progressed to evaluation in clinical trials.¹⁷ dGEMRIC relies on diffusion of a negatively-charged gadolinium contrast agent, gadopentetic acid (Magnevist®), into cartilage tissue and the corresponding tissue distribution being inversely related to the negatively-charged glycosaminoglycan (GAG) content.^{18, 19} This information is advantageous because the loss of GAGs, which maintain the hydrostatic pressure and resilience of cartilage, is a broadly accepted early indicator of OA.²⁰

Despite advances, progress in validating this novel OA imaging method for the usual clinical end points of pain and function has been challenging. One potential improvement to this technique is to substitute the negatively charged contrast agent with a positively charged one that is electrostatically attracted to the GAGs. Herein, we describe the dGEMRIC technique using a new positively charged contrast agent (i.e. dGEMRIC+). Specifically, we report: the synthesis and characterization of the cationic gadolinium-based MRI contrast agent Gd(DTPA)Lys₂ (**1**, Scheme 1), the Donnan Equilibrium mathematical relationships for partitioning in cartilage, dGEMRIC+ imaging of and diffusion kinetics of **1** into *ex vivo* bovine cartilage, and dGEMRIC+ imaging of a human cadaveric metacarpal phalangeal (MCP) joint.

The design of the cationic contrast agent, **1**, was based on the gadolinium chelate of diethylenetriamine-pentaacetate (Gd(DTPA)²⁻, gadopentetic acid, Magnevist®), which possesses a formal overall charge of -2, and is the FDA approved contrast agent used with the dGEMRIC technique. We modified the gadopentetic acid by linking two lysine amino acids to the diethylenetriaminepentaacetate ligand, to render a structure that bears an overall positive charge at physiological pH. Recent developments show that positively charged contrast agents penetrate deeper into cartilage and are more sensitive to GAG concentration gradients than negatively charged or neutral contrast agents.^{21–26} The synthesis of the cationic contrast agent **1** was accomplished in three steps (Scheme 1). First 2-{*bis*[2-(2,6-dioxomorpholino)ethyl]amino}acetic acid was reacted with *tert*-butyl 6-(2-aminoethylamino)-6-oxohexane-1,5-diylidicarbamate (**2**) without the use of a base²⁷ to afford a Boc-protected chelating agent **3**. Subsequent heating of **3** and GdCl₃ in a pyridine/methanol mixture, followed by purification by membrane dialysis, gave the Boc-protected Gd-chelate **4**. After the removal of Boc-groups in the presence of HCl, the imaging agent **1** was isolated as a white powder and characterized (see Scheme 1, SI Figure 2a–b and Supplemental Information). Cytotoxicity of the contrast agent (**1**) was evaluated against NIH 3T3 fibroblasts using an MTS assay in line with the FDA guidelines for developing medical imaging products. Fibroblasts were exposed to **1** and gadopentetic acid at concentrations of 0.1 mM and 1.0 mM, as well as 0.2 mM and 2.0 mM lysine for 24 hours. Similar to untreated control wells, none of the treatment groups exhibited toxic effects (> 95% viability; SI Figure 1).

As a first step towards imaging, we performed a contrast agent uptake study in cartilage and used Donnan Equilibrium Theory to determine a working contrast agent concentration for **1** based on the partitioning of gadopentetic acid. Donnan Equilibrium theory,

$\left(\frac{[i]_t}{[i]_b}\right)^{\frac{1}{z_i}} = \text{const.}$, describes how a charged species (i) with overall charge (z) distributes across an interface from the bath (b) to the tissue (t).¹⁹ This theory can be applied to describe the relationship between contrast agents with different charges distributing into

cartilage: $\left(\frac{[Gd^{-2}]_t}{[Gd^{-2}]_b}\right)^{\frac{1}{-2}} = \left(\frac{[Gd^{+4}]_t}{[Gd^{+4}]_b}\right)^{\frac{1}{+4}}$. Our objective was to achieve an equal concentration of **1** ($[Gd^{+4}]_b$) and gadopentetic acid ($[Gd^{-2}]_b$) in the cartilage tissue from the contrast agent bath such that, $[Gd^{+4}]_t = [Gd^{-2}]_t = [Gd]_b$ yielding the relationship:

$Gd_b^{+4} = \frac{[Gd]_t^3}{[Gd^{-2}]_b^2}$. Clinically, gadopentetic acid is administered at 0.2 mmol/kg resulting in a concentration of approximately 1.0 mM in the blood and synovial fluid. Therefore, we performed a pilot contrast agent uptake into cartilage study with 1.0 mM gadopentetic acid and determined that approximately 0.47 mM of the contrast agent partitioned into a bovine

cartilage plug. Therefore, $Gd_b^{+4} = \frac{[Gd]_t^3}{[Gd^{-2}]_b^2} = \frac{0.47 \text{ mM}^3}{1.0 \text{ mM}^2} = 0.10 \text{ mM}$. This corresponding bath concentration for **1** is approximately 1/10th that of gadopentetic acid or approximately 0.10 mM. When the same cartilage plug was immersed in a 0.10 mM bath of **1**, 0.44 mM of the contrast agent partitioned into the tissue (a 6% difference from 0.47 mM with 1.0 mM gadopentetic acid). Based on the premise that positively charged contrast agents are attracted to cartilage rather than repelled like negatively charged contrast agents, we hypothesized that at equal bath concentrations of **1** and gadopentetic acid, **1** will afford a greater T1 signal change in the tissue. Furthermore, using a bath of **1** at 1/10th the concentration of gadopentetic acid will afford sufficient T1 signal in tissue and resulting change in T1 signal from baseline for imaging as 1.0 mM gadopentetic acid.

To test these hypotheses, *ex vivo* contrast enhanced MR imaging of bovine cartilage plugs was performed using saturation-recovery T1 MRI sequences at room temperature and at 8.5 T. Three equivalent sets ($n = 3$ per group) of bovine osteochondral plugs were cored from the knee of a healthy 1–2 year old cow. The cartilage plug sets were immersed into baths of 0.10 mM gadopentetic acid, 1.0 mM gadopentetic acid, or 0.10 mM of **1** and imaged repeatedly for 12 hours. The diffusion time-course is shown in Figure 1 and the results are summarized in Table 1. Owing to the large bath volume (50 mL) compared to cartilage volume ($V_{\text{cartilage}} < 2 \text{ mL}$, i.e. $< 4\%$ of total), the concentration of the contrast agents in the baths remained approximately constant over the imaging period. After 12 hours, the T1 relaxation time in the tissue (T1 at 12 hr) was significantly lower for 0.10 mM **1** than 0.10 mM gadopentetic acid because the attractive electrostatic potential to GAGs resulted in greater accumulation of **1** in the tissue. The tissue to bath uptake percentage of **1** ($375 \pm 38\%$) was significantly greater ($p < 0.001$) than gadopentetic acid ($56 \pm 21\%$; SI Figure 3). After 12 hours, the changes in T1 from time zero were similar even though **1** (0.10 mM) is at 1/10th the concentration of gadopentetic acid (1.0 mM) (Table 1).

The diffusion of 0.10 mM **1** and 1.0 mM gadopentetic acid into the cartilage was fit to the exponential decay equation $f(t) = \alpha e^{-t/\tau} + \beta$ (SI Figure 4). For **1**, $\tau = 2.42$ hours and for

gadopentetic acid, $\tau = 1.14$ hours. About 95% of the equilibrium state ($3 \times \tau$ value) was reached in 7.25 hours for **1** and 3.45 hours for gadopentetic acid. The diffusion-out of the contrast agents from the tissue into large saline baths showed that both **1** and gadopentetic acid are cleared from the cartilage within 12 hours (SI Figure 5).

Depth-wise, articular cartilage possesses three distinct zones: superficial, middle and deep. The tissue fixed negative charge density increases from the superficial to middle to deep zones due to the respective increase in GAG concentration.²⁸ Cross-sectional views of articular cartilage imaged with the dGEMRIC technique and gadopentetic acid showed a pattern where the T1 values increased from the superficial to middle to deep zones corresponding to a decrease in concentration of gadopentetic acid from superficial to middle to deep zones (lower T1 values corresponded to higher contrast agent concentrations; Figure 2 left). Cross-sectional views of tissue imaged with dGEMRIC+ using **1**, revealed the opposite pattern, where the T1 values decreased with depth corresponding to increasing concentrations of the cationic contrast agent from the superficial to middle to deep zones (Figure 2 middle). Contrast agent **1** distributed in a similar pattern as the expected GAG distribution, unlike gadopentetic acid. The imaging results obtained with **1** also mirrored those observed with traditional histological Safranin-O staining (Figure 2 right).

To evaluate the potential of dGEMRIC+ to image human cartilage, we used **1** and saturation-recovery T1 MRI sequences to image the metacarpal phalangeal joint (MCPJ) isolated from a 71-year-old male cadaver. OA of the finger joints, especially the MCPJ, is commonly related to occupational activities²⁹ and is representative of a discreet synovial joint prone to develop OA. The MCPJ was *ex vivo* imaged at baseline using an 8.45 T MRI, then re-imaged after immersion in 50 mL of 0.1 mM **1** for 24 hours. The average tissue T1 decreased from 1123 ms at baseline to 705 ms with contrast ($T_1 = 418$ ms) confirming uptake of **1** into the tissue. Sagittal plane T1 weighted MR section images through the MCPJ clearly demonstrate the hyaline cartilage corresponding to the articulating surfaces of the metacarpal and proximal phalanx (Figure 3A). When compared at a short recover time (900 ms), the cartilage is clearly brighter after immersion in **1** (Figure 3C) than in the baseline image (Figure 3B). This contrast enhancement is due to greater signal recovery from uptake of **1** into the cartilage tissue.

Conclusions

A new cationic MRI contrast agent, **1**, is described for imaging articular cartilage. Contrast agent **1** partitions into *ex vivo* bovine cartilage plugs and human finger cartilage joints, and is cleared from the cartilage within 12 hours post immersion. Moreover, **1** affords sufficient T1 signal in cartilage for imaging at 1/10th the effective dosage of gadopentetic acid, which may help alleviate toxicity concerns from the use of gadolinium contrast agents at high concentrations. Contrast agent **1** accumulates in the glycosaminoglycan rich matrix of articular cartilage and the results highlight the importance of designing chemical probes that reflect tissue composition for functional imaging. Further development of contrast agent **1** and other contrast agents (MRI- or CT³⁰-based) that enhance anatomic imaging and provide pertinent information on the underlying biochemical composition will improve diagnosis of disease and subsequent treatment for patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

Synthesis of Gd(DTPA)Lys₂ (**1**), a cationic small-molecule gadolinium contrast agent and the chemical structure of gadopentetic acid (Magnevist®). Overall yield 31%.

Figure 1.

Diffusion of contrast agents over 12 hours into bovine osteochondral plugs. 0.10 mM **1** cartilage (■) and bath (*); 0.10 mM gadopentetic acid cartilage (■) and bath (*); 1.0 mM gadopentetic acid cartilage (■) and bath (*).

Figure 2.

Cross sectional views of bovine osteochondral plugs oriented with the articular surface on top and the bone below. The T1 MRI pattern of gadopentetic acid (left) is the inverse of that of Gd(DTPA)Lys_2 (**1**) (middle). This is consistent with the known distribution pattern of GAG seen with Safranin-O histology (right).

Figure 3.

(A) T1-weighted sagittal view of the MCP joint of the hand. The cartilage (arrows) lines the ends of the metacarpal and proximal phalanx where the bones articulate. Saturation-recovery of the boxed region after 900 ms recovery is shown at baseline (B) and after diffusion of **1** (C). The greater signal intensity is due to the contrast agent in cartilage shortening the T1 signal.

Table 1

Summary of Contrast Agent Uptake into Bovine Osteochondral Plugs

		T1 at 0 hr	T1 at 12 hr	Tissue T1	Bath T1 average^a
0.10 mM	bath	963 ms	1009 ms	-	981 ± 31 ms
Gadopentetic Acid	tissue	959 ± 37 ms	898 ± 15 ms	61 ± 25 ms	-
1.0 mM	bath	200 ms	189 ms	-	202 ± 13 ms
Gadopentetic Acid	tissue	1049 ± 130 ms	293 ± 94 ms [*]	756 ± 142 ms	-
0.10 mM 1	bath	1127 ms	1175 ms	-	1137 ± 23 ms
	tissue	1187 ± 34 ms	422 ± 13 ms	765 ± 45 ms	-

^a Average includes all timepoints,^{*} p < 0.0001