A hydroxyapatite-targeted gadolinium contrast agent for MRI of breast cancer microcalcifications

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Introduction: Clinical diagnostic mammography by x-ray and computed tomography identifies breast cancer primarily by detection of microcalcification [1], but cannot delineate between hydroxyapatite (HA) and calcium oxalate (CO), the respective forms of microcalcification in malignant and benign tumors. Solid calcified structures have very short transverse relaxation times (T_2), making them difficult to image by conventional MRI. The advent of ultra-short echo time (UTE) MRI has enabled detection of short T_2 calcified structures, but still does not imply chemical composition. We previously developed an HA-targeted gadolinium contrast agent [2], Gd-DOTA-Ser-PAM (GDSP), in which pamidronate (PAM), the HA-targeting ligand, is conjugated to a gadolinium chelate (Gd-DOTA) by a serine (Ser) linker. Here, the first reported *in-vivo* UTE MRI of the HA-targeted contrast agent following systemic delivery demonstrates in two animal models: the relative specificity of the contrast agent for HA over CO, and the application towards clinical detection of HA microcalcifications in malignant breast cancer.

Methods: *Experiments* - First, the *in-vivo* specificity of the contrast agent was evaluated by MRI before and after systemic uptake in mice (*N=4*) with HA and CO crystal slurry implants. Second, the application to detection of breast cancer microcalfications was examined by uptake imaging in female rats (*N=2*) with breast cancer tumors expressing HA microcalcifications. All of the animal experiments were IACUC approved. Implantation, inoculation, imaging, and contrast injections were all performed with the animals anaesthetized with 2.5% isoflurane/O_2 balance. *Slurry implant model*- 50 mg of HA and CO, each suspended in 150 µL saline, were implanted bilaterally by subcutaneous injection (16G) over the right and left flanks of nude mice. The implants were injected one day prior to imaging to control for natural migration and packing of the crystals. *Tumor microcalcification model*- Breast cancer tumors with hydroxyapatite calcifications were grown following the model by Liu et al. [3]. Fischer 344 female rats were inoculated with R3230 cancer cells and administered systemically bone morphogenetic protein-2 (BMP-2). CT was performed to monitor the tumors for calcifications, which presented after two weeks, before attempting MR contrast imaging. *Pre- and post-contrast MR imaging*- MRI was performed on a 1.5 T whole-body scanner (GE Healthcare) with a custom-built birdcage transmit/receive coil. Pre-contrast MRI was acquired before systemic injection (by tail vein) of the targeted contrast agent GDSP (0.13 mmol/kg). After an uptake period of 1-4 hrs, post-contrast MRI was acquired. In both of the pre-contrast and post-contrast MRI sessions, axial UTE images were acquired at short and long TE (slice thickness = 3 mm, FOV = 9 cm, matrix = 256×199, NEX = 2, α = 90°, TE = 100/20000 µs, TR = 200 ms). Fast spin-echo imaging (FSE) was used to localize the slurries/tumor, and fast gradient echo imaging (FGRE) was collected as a conventional standard. *CT imaging*- The location and morphology of the CO/HA crystal implants and tumor calcifications were validated using continuous helical micro-CT (NanoSPECT/CT, Bioscan, DC) with an 8 W X-ray and CMOS-CCD detector (55kVp, 145mA, 48 mm pitch, 1 sec. exposure, 240 angles). The CT images were reconstructed (matrix = 170x170, 0.1 mm isotropic voxels) and registered to the MR slices using an MR/CT visible fiducial marker (InvivoScope, Bioscan). *Image analysis*- The pre- and post-contrast images were compared slice-by-slice to localize areas with enhanced signal intensity (SI) in the crystal implants and tumor calcifications (ImageJ, MATLAB). The relative change in mean ROI signal intensity, from before and after contrast injection, in regions of amplified signal was used to estimate the binding specificity of the contrast agent to HA over CO implants, and towards HA-calcifications in the breast cancer tumor model.

Results: UTE subtraction images (short-long echoes) demonstrating targeted contrast in the crystal implants are shown in Figure 1. The pre-contrast image (Fig1a) shows the baseline signal intensities for HA (solid arrow) and CO (dashed arrow). In the post-contrast image (4 hr uptake), the rim of the HA slurry implant, where contrast agent has bound to the crystals, is highlighted (Fig1b). The CO crystal also intensifies, but to a lesser degree. The mean SI of an ROI over the crystal rims show a 30% enhancement in HA, and a 7% enhancement in CO: hence, the relative specificity for HA over CO is 4.3-fold (Fig1d). The CT image (Fig1c) shows the otherwise indistinguishable crystal implants. Figure 2 demonstrates targeted uptake to tumor calcifications. The pre-contrast UTE image shows a signal void in the calcified region of the tumor (Fig2a). In the post-contrast image (1 hr uptake), the signal intensity of the calcified region is amplified by the bound contrast agent (Fig2b): the targeted dose produced a 210% SI enhancement (Fig2d).

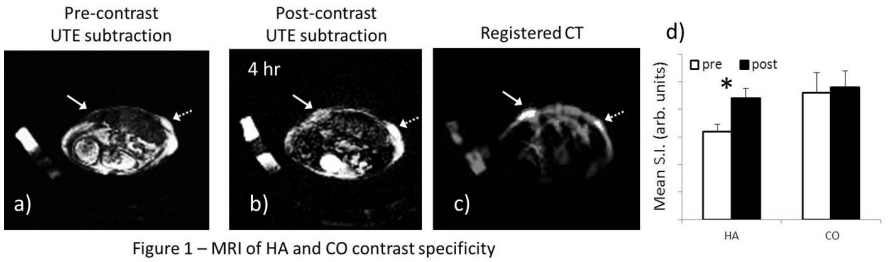

Figure 1 – MRI of HA and CO contrast specificity

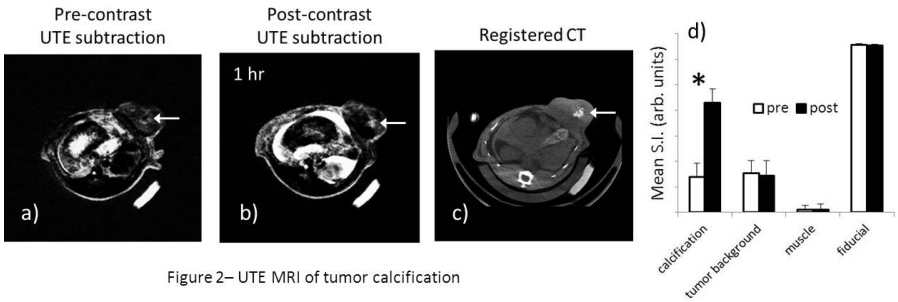

Figure 2– UTE MRI of tumor calcification

Discussion: The HA-targeted contrast agent was detected by UTE MRI following systemic delivery *in-vivo*. Contrast delivery to subcutaneous HA and CO crystal implants in mice showed that binding occurred around the border of the implants and that there was a 4.3-fold preferred specificity towards HA over CO. Pre- and post-contrast MRI of breast cancer calcifications in rats detected delivery of the contrast agent throughout the entire calcified region with SI enhancements greater than 200%. We suspect that differences in vascularization and crystal density between the slurry implant model and the biologically-induced calcifications account for why the contrast agent is delivered to only the implant rims, but to the entire body of the calcifications. The relative specificity for HA over CO may improve with less competitive binding at lower doses [4]. Future experiments will investigate the dose response characteristics of the targeted contrast agent. The optimal uptake time at which to image, the minimum detectable dose, and the smallest detectable calcifications will each be sought. The ability to distinguish HA microcalcifications by UTE using a targeted contrast agent may help position MRI for screening malignant breast cancer.

References: [1] M. Rosselli Del Turco et al. *Radiologia Medica*. 1986; 72: 7-12. [2] J. Marmurek. *19th ISMRM Meeting*. 2011; Abstract# 1665.
[3] F. Liu et al. *Molecular Imaging*. 2008; 7(4): 175-86 [4] K.R. Bhushan. *JACS*. 2008; 130(52): 17648-9.