Published in final edited form as:

Phys Med. 2015 December ; 31(8): 1075–1079. doi:10.1016/j.ejmp.2015.07.141.

Using MRI to detect and differentiate calcium oxalate and calcium hydroxyapatite crystals in air-bubble-free phantom

Devkumar Mustafi*, Xiaobing Fan, Bo Peng, Sean Foxley, Jeremy Palgen, and Gillian M. Newstead

Department of Radiology, The University of Chicago, Chicago, Illinois, USA

Abstract

Calcium oxalate (CaOX) crystals and calcium hydroxyapatite (CaHA) crystals were commonly associated with breast benign and malignant lesions, respectively. In this research, CaOX ($n = 6$) and CaHA ($n = 6$) crystals in air-bubble-free agarose phantom were studied and characterized by using MRI at 9.4 Tesla scanner. Calcium micro-crystals sizes ranged from 200 – 500 microns were made with either 99% pure CaOX or CaHA powder and embedded in agar to mimic the dimensions and calcium content of breast microcalcifications *in vivo*. MRI data were acquired with high spatial resolution T_2 -weighted (T_2W) images and gradient echo images with five different echo times (TEs). The crystals areas were determined by setting the threshold relative to agarose signal. The ratio of crystals areas were calculated by the measurements from gradient echo images divided by T_2W images. Then the ratios as a function of TE were fitted with the radical function. The results showed that the blooming artifacts due to magnetic susceptibility between agar and CaHA crystals were more than twice as large as the susceptibility in CaOX crystals ($p < 0.05$). In addition, larger bright rings were observed on gradient echo images around CaHA crystals compared to CaOX crystals. Our results suggest that MRI may provide useful information regarding breast microcalcifications by evaluating the apparent area of crystals ratios obtained between gradient echo and T_2W images.

Keywords

Air-bubble-free agarose phantom; breast cancer; ductal carcinoma *in situ*; magnetic resonance imaging; microcalcifications

Introduction

Detection and accurate characterization of microcalcifications by X-ray mammography (XRM) increases sensitivity to early breast cancers. The majority of tumors in the breast contain calcium deposits that form in the ducts and lobules [1], and these microcalcifications

*Address Correspondences to: Devkumar Mustafi, Ph. D., Associate Professor in Research, Department of Radiology, The University of Chicago, 920 East 58th Street (CLSC-109), Chicago, IL 60637, USA, Phone: 773-834-8008, Fax: 773-834-3193, dmustafi@uchicago.edu.

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are useful radiological indicators of breast carcinoma [2–4], especially ductal carcinoma *in situ* (DCIS) [5, 6]. While XRM is the primary modality and the gold standard for detection of microcalcifications associated with breast lesions [7–9], it is not the only modality used in breast cancer staging. Magnetic resonance imaging (MRI) has excellent soft tissue contrast that has significantly improved sensitivity over mammography for breast cancer staging [6, 10, 11]. However, conventional clinical MRI typically does not detect breast calcifications, unless they are very large. The efficacy of MRI in detecting calcified lesions was found to be poor in calcified intracranial lesions [12, 13].

Detection of microcalcifications with MRI has been previously investigated [2, 14, 15]. However, relatively little is known concerning the sensitivity of MRI to small microcalcifications associated with breast cancer or early neoplastic changes. Previous studies reported on MRI of large calcium crystals in an agarose or in a gelatin phantom [14, 15] and calcified intracranial lesions [12, 13]. The sizes of Ca-crystals used in phantom studies were too large (1.5–7.0 mm in diameter or 100–400 mg/mL) to be considered relevant to clinical mammography [14, 15]. It is important to detect and differentiate between calcium oxalate (CaOX) and calcium hydroxyapatite (CaHA) crystals, which are the two prevalent types of calcium deposits found in breast tissue, generally associated with benign and malignant lesions [3], respectively.

In this study, we used MRI methods *in vitro* for the identification and characterization of small CaOX and CaHA crystals (0.125 mm^3) for probing microcalcifications in breast cancer. High-resolution MR images at a 9.4 Tesla scanner were acquired of these crystals imbedded in air-bubble-free agar phantoms. Calcium crystals are detectable in MR images because they cause a signal void. True sizes of crystals were determined by spin echo images; their amplifications and the variations in magnetic susceptibility between agar and Ca-crystals were precisely determined by gradient echo sequences. The ‘blooming artifacts’ due to susceptibility gradients on MR images around the micro-crystals on gradient echo images were investigated, characterized and differentiated between CaOX and CaHA micro-crystals.

Materials and methods

Calcium Micro-crystals

Calcium micro-crystals (referred to as ‘crystals’) were made with either 99% pure calcium oxalate (CaOX) or calcium hydroxyapatite (CaHA) powder (Aldrich, St. Louis, MO, USA). An aliquot of 100 mg of fine powder was pressed into a thin disc (~0.5 mm in thickness and ~10 mm in diameter) under a vacuum using a commercially available hydraulic press with 21,000 lbs of pressure for about 90 sec by Perkin-Elmer KBr (model # 186-0025, Norwalk, CT, USA). These discs were then carefully cut into small crystals and their sizes measured under a microscope (model# 49266, Meiji Techno Co, Tokyo, Japan) with an electronic digital caliper (MG Tool Company, New York, NY, USA). All crystals used in study were ~500 microns in depth and their dimensions ranged from ~200 to ~500 microns.

Phantoms

For each phantom, a 0.5% Ultra-pure™ agarose (Invitrogen, Carlsbad, CA, USA) solution was made upon stirring and heating agar in double distilled water at approximately 75 °C for several minutes. Then, hot agar solution was transferred into a 45 mL cylindrical glass tube (I-Chem, Fisher Scientific, Pittsburgh, PA, USA) and placed in a 70 – 75 °C water bath through a funnel and glass wool to prevent dust and small particles from entering the phantom. A cap with a rubber septum was used to seal off the tube. A long hypodermic needle (22 G × 4'; Popper and Sons, Inc., New Hyde Park, NY, USA) connected to a helium source, along with a small syringe needle (22 G × 1') to allow the venting of gases from the tube, was inserted through the septum. The agar solution was helium purged for twenty minutes while kept in the hot water bath. The purpose of the helium flush was to replace all of the air in the phantom with a gas that could be more easily removed by applying a vacuum. After purging, the helium needle was removed and the syringe outlet needle was re-positioned through the center of the rubber septum. The syringe needle was then connected to a vacuum source, and the agar solution was allowed to cool down slowly to a solid state while under vacuum. It was important to prevent the agar from solidifying too rapidly so that there was ample time to remove excess helium gas; the remaining air pockets from the viscous agarose solution were slowly removed using vacuum for a longer period of time while the phantom still in a fluid state. To accomplish this, the phantom was kept in the water bath over two hours as it was allowed to cool down to room temperature from ~70 °C.

After several trials, it was established that the agarose solution was of the right viscosity at 35 °C for crystal implantation. At this temperature, the agar is fluid enough so that the introduction of the crystal would not produce air pockets in the solution, but viscous enough to prevent the crystal from sinking to the bottom of the phantom. Once the water bath, in which the phantom was submerged, cooled down to 35 °C, a crystal was introduced through another needle (22 G × 4'). Alternatively, when the crystal size was < 0.5 mm in length, the crystal was simply dropped onto the surface of the solidifying agar. A 5 µL micro-pipette with the ends sealed off was used as a rod to gently push the crystal into the center of the phantom, which was then immediately placed back under vacuum. The phantom remained under vacuum overnight and was imaged the following day.

The typical CaOX or CaHA crystal embedded in 30 mL agarose phantom was approximately 0.10 mg in weight. After crystals were embedded in agar, we inspected the crystal orientations in agar phantoms by a microscope. For the purpose of reliable and accurate comparison, only crystals of the similar size and nearly identical orientations, with respect to the main magnetic field, were studied. A total of six crystals of each type were used in this study. In each vial only one (either CaOX or CaHA) crystal was placed. In one trial we placed both crystals in the same vial containing air-bubble-free phantom as illustrated in Figure 1. Figure 1a illustrates a diagram for the production of crystals embedded in a bubble-free phantom. Figure 1b shows a picture of one CaOX crystal and one CaHA crystal embedded in agar.

Magnetic resonance imaging

MR imaging studies were performed using a 9.4 Tesla Bruker Biospec 33 cm horizontal bore scanner (Billerica, MA, USA) equipped with 20 cm bore high-performance self-shielded gradients. The phantom with crystals was wrapped with self-adhering athletic tape to secure inside a 30 mm quad coil (Rapid MR International, Columbus, OH, USA). Multi-slice fast spin echo T₂-weighted (T2W) images using a RARE (Rapid Acquisition with Relaxation Enhancement) pulse sequence (TR/TE_{effect} = 5000/55 ms, field of view (FOV) = 25.6 mm, array size = 256², slice thickness = 0.5 mm, RARE factor = 8, number of excitations (NEX) = 2) were acquired first to localize crystals. Then multi-echo gradient echo (GE) images (TR = 500 ms, TE = 5.4 – 40 ms, FOV = 25.6 mm, array size = 256², slice thickness = 0.5 mm, flip angle = 30°, NEX = 2) were acquired using a FLASH (Fast Low Angle Shot) pulse sequence for selected slices from T2W images. In the remaining text, we refer to the GE images at TE 5.4 ms and 40 ms as GE_{TE5} and GE_{TE40}, respectively.

Data analysis

Programs written in IDL software (Exelis VIS, Inc., Boulder, CO, USA) were used to display and analyze images. Since there was no measurable MRI signal of the crystal, a 'hole' in the image represented the crystal. A crystal area was determined by setting the threshold relative to the agarose MRI signal. Regions-of-interest (ROIs) were manually traced over a slightly larger area that contained crystals. Then a threshold of signal intensity less than 0.5 times the average signal intensity in the agarose was used in the calculations of crystal areas. The area ('hole') measured from T2W image was considered as the real crystal size. Then the ratios of crystal area obtained from GE images divided by T2W images were calculated for five different TEs. In order to facilitate comparisons between CaOX and CaHA crystals, it's natural to use a simple function to characterize the ratios of crystal areas as a function of TEs. Unfortunately, the plots of ratios as a function of TEs were clearly not linear. Therefore, we naturally selected a simple non-linear radical function which accurately described the plots. We fitted ratios of crystal areas as a function of TEs with the following radical function:

$$\text{area ratio} = A \sqrt{TE - TE_i} + B, \quad (1)$$

where coefficient $A (1/\sqrt{\text{ms}})$ is related to ratio of area stretch/shrink, coefficient B (unit less) is initial ratio of area, and TE_i (= 5.4 ms) is the initial TE used in the GE acquisition in this study. Due to susceptibility of the crystal, a ring appeared around some crystals [16]. The area of the ring was also measured from GE_{TE40} images from those pixels that had intensity higher than the threshold value within traced ROIs, slightly larger than the appearing crystal area. A threshold of signal intensity greater than 1.25 times of the average agarose signal intensity was used in the ring area calculations. Then a ratio of this ring area to corresponding crystal area determined by GE_{TE40} images was calculated and compared between two types of crystals.

For all statistical analyses, non-parametric Mann–Whitney *U* test was used to compare average measurement parameters between CaOX and CaHA due to a small sample size. A

p-value of < 0.05 was considered a statistically significant difference between the two groups.

Results

For each crystal, we measured the apparent crystal area (as a hole) from both T2W and GE images using the methods described above. Figure 2 shows MR images of crystals of CaOX (blue arrow) and CaHA (red arrow) for: (a) T2W image and (b – f) GE images for TE = 5.4, 10, 20, 30, and 40 ms, respectively. The blooming artifacts due to apparent crystal can be seen clearly on GE images and the area of artifacts grows as function of TE values. The average ($\pm$ standard deviation) crystal area measured from T2W, GE_{TE5} and GE_{TE40} images are given in Table 1. Although there was no statistical difference for average crystal area measured by T2W images ($p > 0.05$) between two types, when measured from GE_{TE5} images the average crystal area for CaHA was more than twice as large ($p < 0.05$) as CaOX crystal area.

In addition to blooming artifacts, as shown in Fig. 2, we also observed a ring structure around the crystals which had relatively higher signal intensity than agarose. Figure 3 shows two examples of GE images with such rings for CaOX (top row) CaHA (bottom row) at five different TE values: (a) 5.4 ms, (b) 10 ms, (c) 20 ms, (d) 30 ms, and (e) 40 ms. The ring around the CaHA crystal is much more prominent than that seen surrounding the CaOX crystal, possibly due to stronger susceptibility gradients. The detailed analysis on GE_{TE40} images showed that we could clearly see rings for 2 out of 6 and 4 out of 6 for CaOX and CaHA crystals, respectively. The average ratio of area-of-ring to area-of-crystal obtained from GE_{TE40} images is given in Table 1. The ratio for CaHA was larger than CaOX, but was statistically insignificant ($p > 0.05$).

To characterize CaOX and CaHA crystals, we calculated the ratio of crystal areas measured from GE images divided by those measured from the T2W image. Then the ratios as function of TE values were fitted with the radical function. Figure 4 shows an overall plot of ratio for these two types of crystals. The average ($\pm$ standard deviations) for fitted parameters (A and B) and goodness-of-fit parameter (R^2) are given in Table 1. Although the average parameter 'A' was the same for both crystals, the average parameter 'B' of the CaHA crystals was two times larger ($p < 0.05$) than that of the CaOX crystals.

Discussion

The characteristics of CaOX and CaHA crystals in air-bubble-free phantoms were investigated by MRI. The dark region surrounding the crystal observed on T2W images suggests exclusion of mobile water from the agar by the imbedded calcium crystals. The much larger apparent crystal size observed on GE images can be attributed to magnetic field gradients at the calcium-agar interface. The ratio of apparent area of the crystals measured between GE images and T2W images is increased as function of TEs. CaHA crystal has stronger blooming effects due to the greater susceptibility in comparison with the CaOX crystal. Susceptibility effects are emphasized by the bright ring around the crystals, which is more prominent for CaHA crystals. Based on these results, it appears that the magnetic field

gradients produced by CaHA crystals are much larger than those produced by CaOX crystals, resulting in larger spatial distortions.

The reason for the significantly larger magnetic field gradients observed around CaHA crystals is not clear. The small difference in calcium content of these two complexes measured from T2W images does not explain the large difference in susceptibility effects. A more likely explanation is that water may penetrate the more hydrophilic CaHA crystals [17, 18] to a greater degree than in CaOX crystals – as suggested by the poorly defined margins of the CaOX crystals – and this could decrease magnetic susceptibility gradients. Another possibility is that the more polar and hydrophilic compounds [18] in CaHA may have different effects on the local structure of water, ions, and macromolecules than in CaOX. More studies are required to determine the reason for the significantly larger magnetic field gradients surrounding CaHA crystals, and accurately measure the susceptibility differences that produce these gradients [16].

There have been a few published reports describing the MR imaging of calcifications in agarose or gelatin phantoms [14, 15]. The previously reported sensitivity to calcifications in phantoms was very modest and the sizes of Ca-crystals were too large to be considered clinically relevant to breast imaging [14, 15]. In this study, we tested calcium crystals closer to *in vivo* dimensions in the breast [19, 20], with at least two orders of magnitude less calcium content than those previously studied [14]. Our preliminary results should inspire more studies on using MRI to detect and differentiate these two types of crystals. Hopefully with modern clinical MR technology, it could be possible to use MRI to detect microcalcifications associated with breast lesions in the future.

Although the detection of microcalcs by MRI would increase sensitivity and specificity for breast cancer detection in clinics, the feasibility of this method in clinical settings is extremely challenging. However, we believe the present study provides a rationale for future clinical investigations. In addition to the clinical application, its role in small animal imaging research is also important. For example, in *in situ* mammary cancer in a mouse model of breast cancer, an aggressive form of *in situ* cancer, comedo-type necrosis, is often found in transgenic mouse models of breast cancer. The comedo-type necrosis is characteristic of microcalcifications in the lumen surrounded by cancerous epithelia [21]. Therefore, for both clinical and research settings, further development of MRI methods to characterize the MRI appearance of these microcrystals is needed.

The present results were obtained at a high magnetic field, which facilitated analysis of the appearance of benign and malignant Ca-crystals on MR images. Our preliminary studies suggest that the present results can be reproduced at clinically relevant field strengths of 1.5 and 3.0 Tesla and lower spatial resolution [22]. The magnetic field gradients produced by microcalcifications are smaller at lower field because the heterogeneity of breast tissue (e.g. interfaces between fat and parenchyma) produces magnetic field gradients that can reduce sensitivity to microcalcifications. On the other hand, the homogeneous B_0 fields and narrow water resonances obtained with clinical scanners, combined with sophisticated detectors with multiple elements, increase sensitivity to modest susceptibility gradients. Naturally occurring micro-calcifications are often about half the size of the synthetic crystals we were

able to manufacture, and thus produce gradients over a smaller volume. However, with voxel sizes that are typically achieved with 3 Tesla scanners and 16 channel detectors, gradients from individual microcalcifications on the order of 200 microns in diameter, or clusters of smaller microcalcifications, may be detectable using high spectral and spatial resolution MRI [22].

Compared to our straight forward approach, susceptibility weighted imaging (SWI) has often been used for imaging of calcification for better sensitivity than GE imaging [2]. However, SWI is a technique that produces post-processed images, weighting the magnetic susceptibility contrast of the phase image on the magnitude image. The TE and mask selection may change the SWI information, affecting the diagnostic reliability. In the future, we plan to use SWI to study the micro-calcium crystals and compare with images from GE acquisitions.

Conclusion

Micro-calcium crystals imaged in this study were designed to mimic the dimensions and calcium content of breast microcalcifications *in vivo*. There was a significant difference in blooming artifacts due to the susceptibility between CaHA and CaOX crystals. The CaOX and CaHA crystals are most commonly associated with benign and malignant breast lesions, respectively. If similar results can be obtained in human breast, this would increase synergy between MRI and X-ray mammography and improve analysis of breast microcalcification clusters. Since the present results were obtained under ideal conditions, more work is needed to translate these results to breast cancer diagnostics at lower field strengths, which are commonly used in clinical settings.

Acknowledgments

This work was supported by grants from the National Institutes of Health – NCI: P30-CA014599, RO1-CA133490 and RO1-CA167785. We would like to thank Ms. Erica Markiewicz for carefully reading the manuscript. JP gratefully acknowledges the American Cancer Society, Illinois Division for a summer fellowship.

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Highlights

- Investigated calcium oxalate and hydroxyapatite crystals in air bubble free phantom.
- These two crystals are commonly associated with breast benign and malignant lesions.
- Calcium hydroxyapatite crystal has stronger susceptibility causing blooming artifacts.
- Susceptibility effects are emphasized by the bright ring around crystals.
- MRI may provide useful information regarding breast microcalcifications.

Figure 1.

(a) The diagram illustrates the experimental setup for making phantoms of calcium crystals embedded in bubble-free agarose. The equipment contains: (i) water bath, (ii) rubber septum cap, (iii) liquid agar, (iv) helium gas needle (v) gas outlet needle, and (vi) needle under vacuum. (b) A photograph of phantom shows one CaOX crystal (left) and one CaHA crystal (right) embedded in agar in an air-sealed tube.

Figure 2. MRI of a phantom with a CaOX crystal (blue arrow) and a CaHA crystal (red arrow) for: (a) T2W image, and (b – f) GE images for TE = 5.4 ms, 10 ms, 20 ms, 30 ms, and 40 ms, respectively. Each image display FOV is 25.6×25.6 mm.

Figure 3. A close look of blooming artifacts obtained from GE images for (a) CaOX crystal (top row) and (b) CaHA (bottom row) for TE = 5.4, 10, 20, 30 and 40 msec (from left to right, respectively). Each display image FOV is 5×5 mm.

Figure 4.

The plot of average ($\pm$ standard deviation) ratios of crystal areas measured from GE images divided by measured from the T2W images as a function of TE values for six CaOX crystals (blue line) and six CaHA crystals (red line). The solid red and blue lines were fitted to the average of curves from CaOX and CaHA crystals, respectively, by using Eq. (1).

Table 1

The average ($\pm$ standard deviations) measured parameters from MRI for CaOX and CaHA crystals, and parameters fitted to Eq. (1). The bold p-values with stars indicate the statistical significant between CaOX and CaHA.

Type of measurements	CaOX (n=6)	CaHA (n=6)	p-values
Crystal area (mm ²) measured by T2W images	0.22 $\pm$ 0.07	0.28 $\pm$ 0.06	0.078
Crystal area (mm ²) measured by GE _{TE5} images	0.25 $\pm$ 0.06	0.60 $\pm$ 0.16	0.005*
Crystal area (mm ²) measured by GE _{TE40} images	1.04 $\pm$ 0.29	1.68 $\pm$ 0.55	0.020*
Ratio of area-of-ring/area-of-crystal measured by GE _{TE40} images	0.32 $\pm$ 0.33	0.40 $\pm$ 0.26	0.810
A ($1/\sqrt{\text{ms}}$)	0.65 $\pm$ 0.18	0.66 $\pm$ 0.11	0.936
B	0.98 $\pm$ 0.29	1.98 $\pm$ 0.42	0.005*
Goodness-of-fit (R ²)	0.96 $\pm$ 0.04	0.98 $\pm$ 0.02	0.810