# *In Vitro* MRI Identification and Characterization of Small Calcium Crystals: Implications for Breast Cancer

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**Abstract**: We tested several MRI methods *in vitro* for the identification and characterization of small calcium crystals for probing microcalcifications in breast cancer. High-resolution MR images were acquired of small crystals imbedded in air bubble-free agar phantoms. 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.

**Introduction**: Microcalcifications (microcals) associated with breast lesions are an important radiological indicator of breast carcinoma. The presence of calcium oxalate (CaOX) microcals is associated with benign breast lesions. In contrast, the microcals associated with malignant breast lesions are generally calcium hydroxyapatite (CaHA). Some studies have demonstrated that MR can detect microcals through their susceptibility effects. However these studies did not compare CaOX and CaHA under controlled conditions. The dual aim of the present study was to develop an MRI method for identifying small Ca-crystals (~0.5 mm in each dimension) imbedded in air bubble-free agar phantoms and to characterize the MRI appearance of these microcrystals.

**Methods**: For *in vitro* MRI studies at 9.4 T, we developed a reproducible method for making small Ca-crystals, as thin discs, imbedded in agar. Special precautions were taken to remove air bubbles from the agar by flushing helium gas followed by vacuum suction in sealed glass tubes, illustrated in the figure below. The dimension of a typical crystal imbedded in agar was ~0.135 $mm^3$. High resolution MR images of CaOX and CaHA crystals were compared using spin echo (SE), gradient echo (GE) and echo planar spectroscopic imaging (EPSI) sequences with slice thickness of ≤0.5 mm and in-plane resolution of ~0.1 mm. P-values between the two groups of the apparent crystal sizes were determined.

**Results**: SE images accurately depicted the true size of the Ca-crystals and were consistent with precise caliper measurements. The crystal sizes on MRI, as measured by their cross-sectional area, increased ~6-fold for calcium oxalate and ~11-fold for calcium hydroxyapatite in gradient echo images when the echo time increased from 5 msec to 40 msec, as illustrated in the figure below ($p < 0.0003$; $n = 7$). This is presumably due to the difference in magnetic susceptibility between the calcium crystals and agar. EPSI is particularly sensitive to such variations. $\mathbf{B}_0$ maps, spectral asymmetry and water peak height image analyses produced from EPSI datasets were used to isolate features specific to these susceptibility discontinuities, and, thereby, localize Ca-crystals (data not shown here).

**Discussion**: The elimination of air bubbles from agar media made it possible to accurately evaluate the appearance of microcalcs on MRI, and especially local field distortions caused by bubbles. Although GE images significantly amplified crystal sizes due to blooming artifacts, images were distorted. Calcifications were amplified and easily seen in water peak height images derived from high resolution EPSI data. Differences in apparent size of CaOX vs. CAHA crystals on MRI may be due to small differences in magnetic susceptibility. The crystals may also have differing interactions with bulk water that affect MR image contrast. The results suggest that microcals can be detected by MRI and that CaOX and CAHA may be distinguishable. Further studies are in progress to identify microcals in mouse mammary glands.

**Conclusion**: Detection of microcalcs by MRI would increase sensitivity and specificity for breast cancer detection.

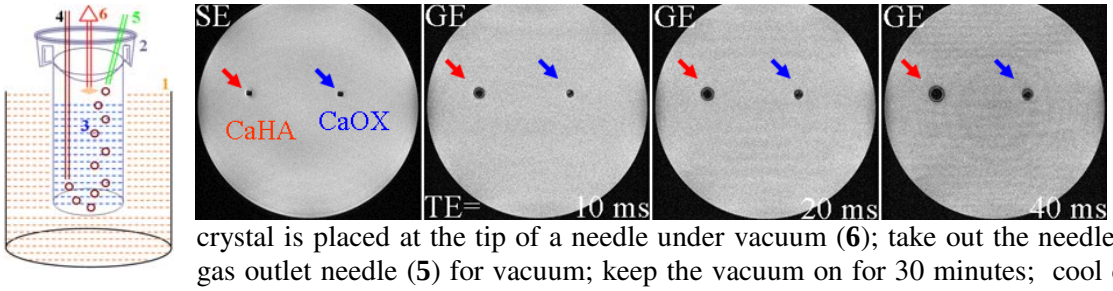


**Figure**: (**Left**) Purge the liquid agar (**3**) in a special glass tube with rubber septum (**2**) with helium gas (**4**) at > 70 °C in a water bath (**1**) for 15-20 minutes while a small Ca-crystal is placed at the tip of a needle under vacuum (**6**); take out the needle (**4**) for helium gas; use the gas outlet needle (**5**) for vacuum; keep the vacuum on for 30 minutes; cool down the bath slowly while vacuum is on; put the Ca-crystal containing needle down and place the Ca-crystal inside the agar in the middle of the tube; cut off the vacuum line and slowly remove the needle (**6**); once the agar medium is fairly uniform, transfer the phantom tube to a vacuum desiccator. (**Right**) We illustrate an axial view of MR images containing two small Ca-crystals in agar phantom. A spin echo MR image with TR/TE of 5000/55 msec and gradient echo MR images with TR of 1000 msec and TEs (as indicated in the figure) are shown. CaHA and CaOX crystals in MR images are indicated by red arrows and blue arrows, respectively, and both crystals are labeled in the spin echo image.