Intracellular delivery and ultrasonic activation of folate receptor-targeted phase-change contrast agents in breast cancer cells *in vitro*

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Abstract:
Breast cancer is a diverse and complex disease that remains one of the leading causes of death among women. Novel, outside-of-the-box imaging and treatment methods are needed to supplement currently available technologies. In this study, we present evidence for the intracellular delivery and ultrasound-stimulated activation of folate receptor (FR)-targeted phase-change contrast agents (PCCAs) in MDA-MB-231 and MCF-7 breast cancer cells *in vitro*. PCCAs are lipid-coated, perfluorocarbon-filled particles formulated as nanoscale liquid droplets capable of vaporization into gaseous microbubbles for imaging or therapy. Cells were incubated with 1:1 decafluorobutane (DFB) / octafluoropropane (OFP) PCCAs for 1 hour, imaged via confocal microscopy, exposed to ultrasound (9 MHz, MI = 1.0 or 1.5), and imaged again after insonation. FR-targeted PCCAs were observed intracellularly in both cell lines, but uptake was significantly greater ($p < 0.001$) in MDA-MB-231 cells (93.0% internalization at MI = 1.0, 79.5% at MI = 1.5) than MCF-7 cells (42.4% internalization at MI = 1.0, 35.7% at MI = 1.5). Folate incorporation increased the frequency of intracellular PCCA detection 45-fold for MDA-MB-231 cells and 7-fold for MCF-7 cells, relative to untargeted PCCAs. Intracellularly activated PCCAs ranged from 500 nm to 6 microns (IQR = 800 nm – 1.5 microns) with a mean diameter of 1.15 ± 0.59 (SD) microns. The work presented herein demonstrates the feasibility of PCCA intracellular delivery and activation using breast cancer cells, illuminating a new platform toward intracellular imaging or therapeutic delivery with ultrasound.

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