Acoustic Activity of PSMA-targeted Nanobubbles Internalized into Prostate Cancer Cells

Reshani Perera, Pinunta Nittayacharn, Eric Abenojar, Al de Leon, Gopal Ramamurthy, Xinning Wang, James P. Basilion, and Agata A. Exner
Case Center for Imaging Research, Department of Radiology, Case Western Reserve University, Cleveland, OH, USA

Background, Motivation and Objective: Active targeting of nanobubbles (NBs) to the prostate specific membrane antigen (PSMA) enhances rapid accumulation and retention in PSMA-expressing tumors. We have shown in past work that active targeting enhances tumor extravasation of *intact* NBs with extended retention, which results in prolonged signal enhancement in the tumors over 25 min that can be visualized with clinical nonlinear ultrasound [Perera. et.al, IEEE IUS, 2018]. One hypothesis for the prolonged tumor enhancement is that the PSMA-targeted NBs are internalized into their target cancer cells and the internalization delays C_3F_8 dissolution. Thus, the purpose of this study was to study the effect of receptor-mediated endocytosis of PSMA-NBs on their acoustic activity and intracellular persistence.

Statement of Contributions/Methods: Targeted C_3F_8 NBs were prepared by incorporating PSMA-1-DSPE-PEG into a cocktail of DBPC, DPPE, DPPA lipids and propylene glycol and glycerol in PBS, followed by gas exchange and mechanical agitation. PSMA positive PC3pip prostate cancer cells were plated at the density of $1x10^6$ and 24h later cells were exposed to PSMA-NB or untargeted NB for 1h. Then cells were washed with PBS and incubated at 37°C. At each time point, cells were trypsinized, suspended in media and imaged in a phantom (Fig. 1a) using contrast harmonic imaging (Toshiba AplioXG, 12 MHz, 0.1 MI, 0.2 fps). Rhodamine labeled bubbles were used for confocal imaging. Cells were stained with endosome marker LysoTracker Red and DAPI and fixed before imaging.

Results/Discussion/Conclusion: PSMA-NB and NB had diameters of 277±11nm and 285±2.6nm, respectively and both had concentrations of $4x10^{11}$ NB/ml. The US signal was significantly higher for cells exposed to PSMA-NB at all-time points compared to untargeted NB. Even after 24-48h, PSMA-NB treated cells showed a 4-6 fold higher signal than NB (Fig.1b-c). Confocal images show NB internalization into the cells (Figure 1d; red). PSMA-NB showed higher internalization than untargeted NB and considerable colocalization with endosomes. Our results demonstrated the active targeting of NB to PSMA selectively enhances cellular internalization and these intact bubbles exhibit persistent acoustic activity.

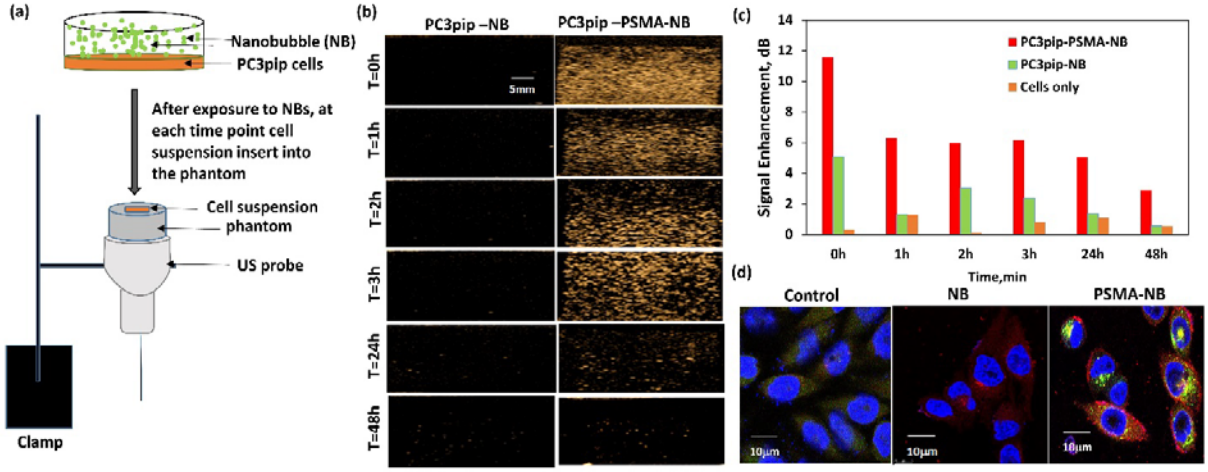

Figure 1. (a) Schematic diagram of the experimental setup (b) Representative US images of PSMA-NB, NB treated PSMA positive PC3pip cells at different times post treatment. (c) Signal enhancement of PSMA-NB, NB treated cells at different times post treatment. (d) Representative confocal images of PSMA-NB and NB distribution in PC3pip cells (blue-nuclei, red-NB, and green-endosome).