MR/OPTICAL IMAGE-GUIDED TWO-COMPONENT N/AO-DELIVERY SYSTEMS TARGETING HER2/NEU OVEREXPRESSING CANCER CELLS

Sudath Hapuarachchige1, Wenlian Zhu1, Yoshinori Kato2, and Dmitri Artemov1

1Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, 2The Johns Hopkins University School of Medicine, 1Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MARYLAND, United States

Introduction
The overexpression of Her2/neu, epidermal growth factor receptor (EGFR), which regulates the cell proliferation and differentiation, leads to the poor prognosis in human breast cancer [1]. The affinity of cancer therapeutics can be increased by the targeted drug delivery followed by controlled internalization in the cancer cells. The two-component delivery system overcomes the barriers of extravasation from vessels and diffusion to the target site. The first component is a high-affinity targeting molecule such as functionalized monoclonal antibody (mAb). The secondary component cross-links the primary components forming a network only on the targeted receptor overexpressing cancer cells which leads to the macro-scale internalization and efficient delivery of high dose of therapeutics to the cytoplasm. This strategy exhibits the low effect on non-specific binding sites and on normal cells expressing basal levels of the receptor. The new two-component delivery system consists of a target specific biomolecule/antibody and a carrier molecule/platform. As a first targeting component, we selected the antibody, trastuzumab (Herceptin®) which specifically binds extracellular domain of the Her2/neu receptors. It also provides secondary binding motif for cargo molecule/platform. The cargo-carrier is a multi-task molecule/platform which transports of therapeutic, carries imaging agents for optical/MRI imaging, and can chemoselectively bind to the target-specific biomolecule. It acts as a biocompatible nanoparticle carrier systems similar to nanocapsules and nanoparticles [2].

Methods
The target specific trastuzumab was functionalized with terminal azido PEGylated linkers and labeled with fluorochrome (Rhodamine for \textit{in vitro} and NIR CF-680 for \textit{in vivo} studies). The degree of functionalization and labeling was determined and optimized by MALDI-TOF and absorbance/fluorescence methods. The cargo carrier, BSA was substituted with DTPA and Gd, functionalized with strained promoted alkyne, dibenzocyclooctyne (DIBO) and labeled with fluorophores (Alexa-488 for \textit{in vitro} and NIR CF-750 for \textit{in vivo} studies). The degree of valency, DTPA-Gd substitution and fluorescent marker labeling was determined and optimized by MALDI-TOF, quantitative MRI, and absorbance/fluorescence methods. The two-component \textit{nano}-delivery system was studied in \textit{vitro} using Her2/neu overexpressing, human breast cancer BT-474 cells. The cells were first treated with modified Herceptin, Her(PEG$_2$-Az)$_2$(Rhod)$_2$ (10 µg/mL) and incubated at 37°C for 30 min for immunolabelling on Her2/neu receptors. The secondary component, BSA(DIBO)$_2$(Alexa-488)$_2$ was added (2 µM) and imaging using a confocal fluorescent microscopy. The carrier molecule was also substituted with the DTPA ligand followed by Gd to obtain BSA(DTPA-Gd)$_2$(DIBO)$_2$(Alexa-488)$_2$.

Results
In the \textit{in vitro} cell labeling fluorescent experiment, the colocalization of two spectral fluorescent images exhibits the presence of two components in same physical location after 1,2-dipolar cycloaddition forming a stable and bio-neutral 1,2,3-triazole linker. The MRI cell pellet study of the delivery system exhibits the significant drop in $T_1$ compared to the control. For the BT-474 human breast cancer xenograft, SCID/nude mice were implanted with the pellets containing 0.72 mg of 17α-estradiol (sustained released 90 days) using a trocar and inoculated with BT-474 cells (5x10$^6$ cells, mixed with equal volume of Matrigel) in left thoracic mammary pad. The xenografted mouse was injected with modified herceptin via tail vein followed after 4 h by the secondary component. Quantitative $T_1$ maps were reconstructed from 3D $T_1$-weighted RARE sequence acquired pre-injection and at 1,3,6, and 12 h post-injection of modified BSA with TR = 250 ms, 500 ms, 1s, 2s, and 4s using a 9.4T Bruker MRI spectrometer. \textit{In vivo} optical images of mice were obtained using a KODAK \textit{in vivo} multispectral imaging system.

Discussion
Confocal fluorescent images of BT-474 cells in \textit{in vitro} study exhibit the strong emission signal of two fluorophors ($\Delta \lambda_{\text{em}}$/ $\Delta \lambda_{\text{em}}$=64/56 nm) and good spatial colocalization of two components after cell labeling with the two-component delivery system. Efficient cell surface labeling demonstrates the high affinity binding of the receptor by functionalized trastuzumab. The cargo-carrier can also chemoselectively bind with the modified mAb under physiological conditions. Based on encouraging MRI imaging results of cell pellets, we applied two-component delivery system to \textit{in vivo} imaging and delivery study using Her2/neu overexpressing BT-474 xenografts in mouse models. The change of fluorescent intensity in the specifically labeled tumor with respect to the control is shown in Figure 2 (a). \textit{In vivo} NIR fluorescent imaging are shown in Figure 2 (b) control and (c) treated mice. A mouse without PEG$_2$-Az on Herceptin was used as the control. The change of the mean tumor $T_1$ times in control and treated mice are shown in Figure 2 (d). MRI images of mice highlighting the tumor-sites are shown in Figure 2 (e) control and (f) treated mice. Initial increase of fluorescent intensity and drop of $T_1$ in both control and treated mice can be interpreted by EPR effect, but clearance was significantly faster in controls. MRI imaging exhibits specific $T_1$ reduction for the two component delivery system, suggesting possible translational role of non-invasive image-guided strategy for cancer therapy.

References