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

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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 molecules/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 nanospheres [2].

Methods

The target specific trastuzumab was functionalized with terminal azido PEGylated linkers and labeled with fluorochrome (Rhodamine for *in vitro* and NIR CF-680 for *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 *in vitro* and NIR CF-750 for *in vivo* studies). The degree of valency, DTPA-Gd substitution and fluorescent marker labeling was determined and optimized by MALDI-TOF, quantitative MRI, and aborbance/fluorescence methods. The two-component *nano*-delivery system was studied *in vitro* using Her2/*neu* overexpressing, human breast cancer BT-474 cells. The cells were first treated with modified Herceptin, Her$(PEG_4\text{-}Az)_{22}(Rhod)_2$ (10 μg/mL) and incubated at 37° for 30 min for immunolabelling on Her2/*neu* receptors. The secondary component, $BSA(DIBO)_{10}(Alexa\text{-}488)_2$ was added (2 μM) and imaged using a confocal fluorescent microscopy. The carrier molecule was also substituted with the DTPA ligand followed by Gd to obtain $BSA(DTPA\text{-}Gd)_{12}(DIBO)_{10}(Alexa\text{-}488)_2$.

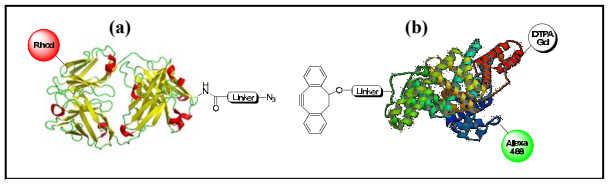

Figure 1. Two delivery components (a) Her$(PEG_4\text{-}Az)_8$ $(Rhod/CF\ 680)_2$ (b) $BSA(DTPA\text{-}Gd)_{12}(DIBO)_{10}(Alexa\ 488/CF750)_2$

Results

In the *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-dipolarcycloaddition 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 (5×10^6 cells, mixed with equal volume of Matrigel) in left thoracic mammary pad. The xenographed 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. *In vivo* optical images of mice were obtained using a KODAK *in vivo* multispectral imaging system.

Discussion

Confocal fluorescent images of BT-474 cells in *in vitro* study exhibit the strong emission signal of two fluorophors ($\Delta\lambda_{emi}$/ $\Delta\lambda_{emi}$=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 *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). *In vivo* NIR fluorescent imaging are shown in Figure 2 (b) control and (c) treated mice. A mouse without PEG_4-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.

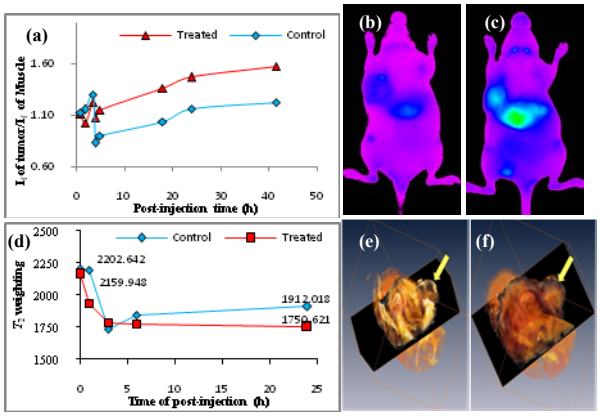

Figure 2. *In vivo* optical imaging (a), (b), (c) and MRI imaging (d), (e), (f) of two-component *nano*-delivery

References

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2. Hawkins MJ, Soon-Shing P, Desai N. Protein nanoparticles as a drug carrier in clinical medicine. Adv Drug Delivery Rev 2008;60:876-885.