Supporting Information

Tracking of Multimodal Therapeutic Nanocomplexes Targeting Breast Cancer in Vivo

Rizia Bardhan[†,‖], , Wenxue Chen[⊥,ξ,‖], Marc Bartels[⊥], Carlos Perez-Torres[‡], Maria F. Botero[#], Robin Ward McAninch[#], Alejandro Contreras[#], Rachel Schiff[#], Robia G. Pautler[‡], Naomi J. Halas[†,] , Amit Joshi[⊥,*]

Materials and Methods

Multifunctional Nanocomplexes Fabrication: The nanocomplexes were fabricated similar to the procedure we previously reported (ref. 20). Au nanoshells (NS) [r_1, r_2] = [60, 75] nm were fabricated by seed mediated electroless plating of Au onto silica spheres[1]. SPIO nanoparticles were synthesized following a method reported by Kang et al.[2] and functionalized with aminated silane (3-aminopropyl triethoxysilane, Sigma) overnight and then mixed with NS. The NS coated with SPIO were encapsulated with SiO_2 by condensation of SiO_2 precursor (tetraethylorthosilicate, Sigma) in basic media and ICG molecules were doped within the SiO_2 epilayer. After SiO_2 coating the nanocomplexes were resuspended in ethanol at a concentration of ~10^9 particles/mL and ICG concentration of ~650 ± 50 nM. Streptavidin maleimide was covalently attached to the nanocomplexes via a thiolate silane linker (3-mercaptopropyl triethoxysilane, Sigma) and finally redispersed in phosphate buffer at pH 7.5. The antibody, anti-HER2 (c-erbB-2/HER-2/neu epitope specific rabbit antibody 200 µg/mL, Thermo Scientific) was mixed with a 1 mM solution of Sulfo-NHS-Biotin (Pierce) reagent and conjugated at 4 °C for 3 h. The streptavidin conjugated nanocomplexes were then mixed with the biotinylated anti-HER2

and gently stirred for 8 h at 4 °C. 50μM PEG-biotin (Nanocs, MW~5000) was finally added and nanocomplexes were redispersed in phosphate buffer at pH 7.5.

Live animal studies: The protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Baylor College of Medicine.

BT474AZ xenografts: The estrogen-dependent BT474AZ/ATCC breast carcinoma Human (in which HER2 is naturally amplified) cells were grown in RPMI 1640 medium, 1 % Penicillin-Streptomycin and 10 % fetal bovine serum (FBS). Cells were incubated at 37 °C in a 5 % CO_2 environment and were detached from culture with trypsin (0.05 %) and EDTA (0.02 %) and resuspended in media for passaging to wells. Athymic Nude-Foxn1nu female mice (4-6wk of age, 20±3g, Harlan) were implanted subcutaneously with E2 pellets (0.2 mg, 60 d release; Innovative Research of America) on the dorsal flank. The next day, mice were injected subcutaneously on the right flank near 4th mammary gland with 1×10^7 BT474AZ/ATCC cells suspended in serum-free medium mixed with Matrigel (BD Biosciences) at 1:4 ratio as described[3]. Tumors were allowed to grow to about 7-8mm in diameter before nanocomplexes injection and imaging.

MDAMB231 xenografts: The estrogen-independent human breast adenocarcinoma (Homo sapiens) cell line MDAMB231 (which expresses basal levels of HER2 receptor) were grown in DMEM medium, 1 % Penicillin-Streptomycin and 10 % FBS. Cells were incubated at 37 °C in a 5 % CO_2 environment and were detached from culture with trypsin (0.05 %) and EDTA (0.02 %) and resuspended in medium for passaging to wells. Athymic Nude-Foxn1nu female mice (4-6 wk of age, 20±3 g, Harlan) were injected s.c. on the right flank near 4th mammary gland with 3 $\times10^6$ cells/mouse, the cells suspended in serum-free medium. Tumors were allowed to grow to about 7-8 mm in diameter before nanocomplexes injection and imaging.

FOI Methodology: Tumor bearing mice were each placed on a dark platform and isoflurane was delivered in concentrations of 1-3 % in oxygen (up to 5 % for initial induction), using a precision vaporizer and ventilation. The body temperature of mice was maintained at 37 °C during anesthesia by employing a heating pad and temperature controller (FHC Bowdoin, ME, USA). The *in vivo* images were acquired using an optical imaging system in continuous wave (CW) mode with a charged-coupled device (CCD) camera (PhotonMax 512, Princeton Instruments) and a 28 mm Nikkor (Nikon) lense. Camera was custom-controlled by MatLab (The MathWorks, Inc.) software. Excitation light was generated with a 100 mW near infrared (NIR) diode at 785 nm (Thorlabs, Inc.) and diffused with the combination of a plano-convex lens and a diffuser (both Thorlabs, Inc.). The excitation light was captured by using a neutral density filter with optical density (OD) 3 (Andover Corporation). The emission light was collected using a fluorescence band pass filter at 830 ± 20 nm (Andover Corporation) and a holographic notch filter (OD 6) at 785 nm (Kaiser Optical Systems, Inc.) while rejecting excitation leakage, as suggested by Hwang et. al [4].

MRI Methodology: MR Imaging experiments were performed on a Bruker Avance Biospec, 9.4 T spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. Animals were initially anesthetized with gaseous isoflurane at 2-3 % in oxygen (up to 5 % for initial induction), and placed into a mouse holder within the magnet where they were subsequently maintained at 2 % isoflurane in oxygen. During the imaging, the animal body temperature was maintained at 37 °C and continuously monitored with a rectal probe using an animal warmed air heating system (SA Instruments, Stony Brook, NY). Imaging was performed with a multislice RARE (rapid acquisition with relaxation enhancement) sequence with a repetition time (TR) of 2805 ms, an echo time (TE) of 20 ms (TR/TE equal to 2805/20

ms) with a RARE factor of 6 leading to an effective TE of 60 ms. The imaging sequence included a 5 ms fat suppression pulse. FOV was 30×30 mm with 20 slices at 1 mm thickness. The acquisition matrix of 256×256 yielded an in-plane isotropic 117 μm resolution.

Sample Preparation for ICP-MS: The mice were sacrificed 72 h post injection, organs were collected and immediately frozen at -80 °C. The organs were then lysed in trace-metal grade aqua regia for 24 h, boiled to evaporate excess aqua regia and finally dispersed in 1 % aqua regia. Gold standards were prepared in 1 % aqua regia.

Cell Preparation for TEM: After 72 h organs were collected and a small portion of BT474AZ tumor was immediately fixed in 2.5 % glutaraldehyde and 2 mM $CaCl_2$ in 0.1 M cacodylate buffer (PH 7.39). Tissue was then cut into 1 mm cube keeping them immersed in fixative solution. Specimens were stained for 1 hour in saturated uranyl acetate + 50 % ethanol, then counter-stained for 4 mins in Reynold's lead citrate. Sections were then cut at 70-75 nm (silver sections) on an RMC MT-6000XL ultramicrotome and used for TEM. A Hitachi H-7500 TEM at accelerating voltage 80 kV was used.

Statistical Analysis: Statistical analysis was generated using unbalanced two-way ANOVA (Analysis of Variance) and differences with $p < 0.05$ were considered significant.

References

1. Brinson, B. E.; Lassiter, J. B.; Levin, C. S.; Bardhan, R.; Mirin, N.; Halas, N. J. *Langmuir* **2008,** 24, 14166-14177.

2. Kang, Y. S.; Risbud, S.; Rabolt, J. F.; Stroeve, P. *Chem. Mater.* **1996,** 8, 2209-2211.

3. Shah, C.; Miller, T. W.; Wyatt, S. K.; McKinley, E. T.; Olivares, M. G.; Sanchez, V.; Nolting, D. D.; Buck, J. R.; Zhao, P.; Ansari, M. S.; Baldwin, R. M.; Gore, J. C.; Schiff, R.; Arteaga, C. L.; Manning, H. C. *Clinical Cancer Res.* **2009,** 15, 4712.

4. Hwang, K.; Houston, J. P.; Rasmussen, J. C.; Joshi, A.; Ke, S.; Li, C.; Sevick-Muraca, E. M. *Mol. Imaging* **2005,** 4, (3), 194-204.