Electronic Supplementary Material (ESI) for Analyst.

Dual-targeting SERS-encoded Graphene oxide nanocarrier for intracellular co-delivery of doxorubicin and 9-aminoacridine with enhanced combination therapy

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Quantity of HER2 antibodies

The quantitative measurements of HER2 antibodies were conducted by using immunoassay protocols with FITC-conjugated goat anti-human IgG. After the conjugation of antibodies, GO-Fe_3O_4/Au@Ag-anti-HER2 nanocomposites were obtained through magnetic separation and the quantity of antibodies in the nanocomposites was estimated indirectly by measuring the unbound antibodies in the supernatant. To achieve this goal, the supernatant was collected and the unbound HER2 antibodies concentration remaining in the supernatant was deduced from the calibration curve established by immunoassay protocols.

The immunoassay was conducted as follows. First, glass slide was treated with the piranha solution (H_2SO_4 : H_2O_2, 3 : 1, v/v) under the ultrasonic condition for 1 h and then washed with abundant deionized water. After being dried under argon gas, the glass slide was immersed in polyethyleneimine (PEI) solution (0.5%) for 2 h to modify the surface with amine groups and then rinsed with deionized water. Afterwards, the slide was immersed in glutaraldehyde (GA) solution (2.5%) for 3 h and then rinsed with deionized water, rendering the aldehyde groups modified slide. After the glass slide was dried with argon gas, HER2 antibodies with different concentrations were pipetted onto different spots on the slide. For each spot, 4 μL of HER2 antibody solution was used. The slide was then kept in a humidity chamber at 4 ℃. After 12 h

of incubation, the slide was washed three times with TBST solution and rinsing with deionized water. The slide was then immersed in BSA solution (1%, in BBS) at 4 ℃ for 12 h to block the nonspecific binding sites. After the slide was washed three times with TBST solution and rinsing with deionized water, it was dried with argon gas again. Then, 6 μL of FITC-conjugated goat anti-human IgG (0.1 mg/mL) solution were pipetted onto the HER2 antibody modified spots and incubated for 2 h at room temperature. Finally, after being rinsed to remove unbound FITC-conjugated goat anti-human IgG and dried with argon gas again, the slide was subjected to fluorescence measurements. Under excitation of 490 nm, the intensity at wavelength of 525 nm was recorded. By plotting the intensity at 525 nm as a function of the logarithm of HER2 antibody concentration, the calibration curve was established as showed in Fig. S3. The detected fluorescence intensity of FITC-conjugated goat anti-human IgG was proportional to the HER2 antibody concentration in the range of 1000 μg/mL to 0.1 μg/mL, which is linearly regressed as y = 899.3log(*c*) + 1921.3 with the correlation coefficient (R^2) of 0.997. Finally, the collected supernatant was diluted and subject to fluorescence measurement for determining the concentration. The resulting value was multiplied by the dilution factor (x10) to give the actual concentration of antibodies in the supernatant i.e. 443.9 μg/mL. Therefore, the quantity ratio of HER2 antibody to GO-Fe_3O_4/Au@Ag in the final nanocomposites was calculated to be 1.57.

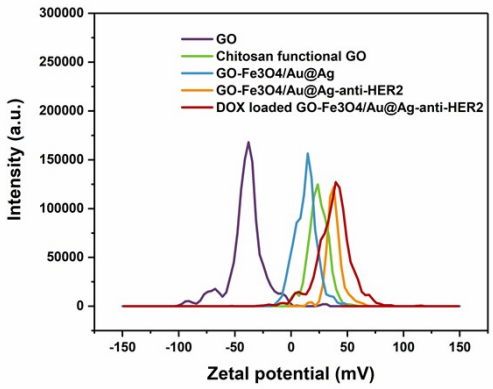

Fig. S1 Zeta potential profiles of GO, Chitosan functional GO, GO-Fe_3O_4/Au@Ag, GO-Fe_3O_4/Au@Ag-anti-HER2 and GO-Fe_3O_4/Au@Ag-anti-HER2.

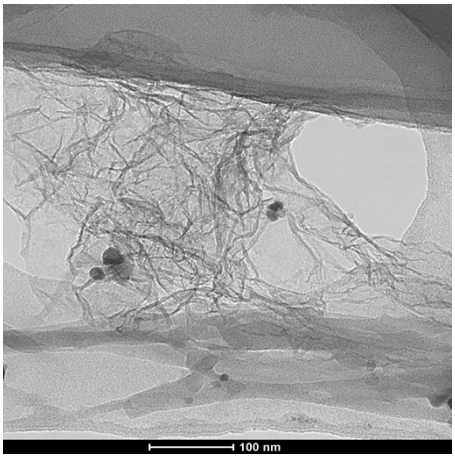

Fig. S2. TEM image of GO.

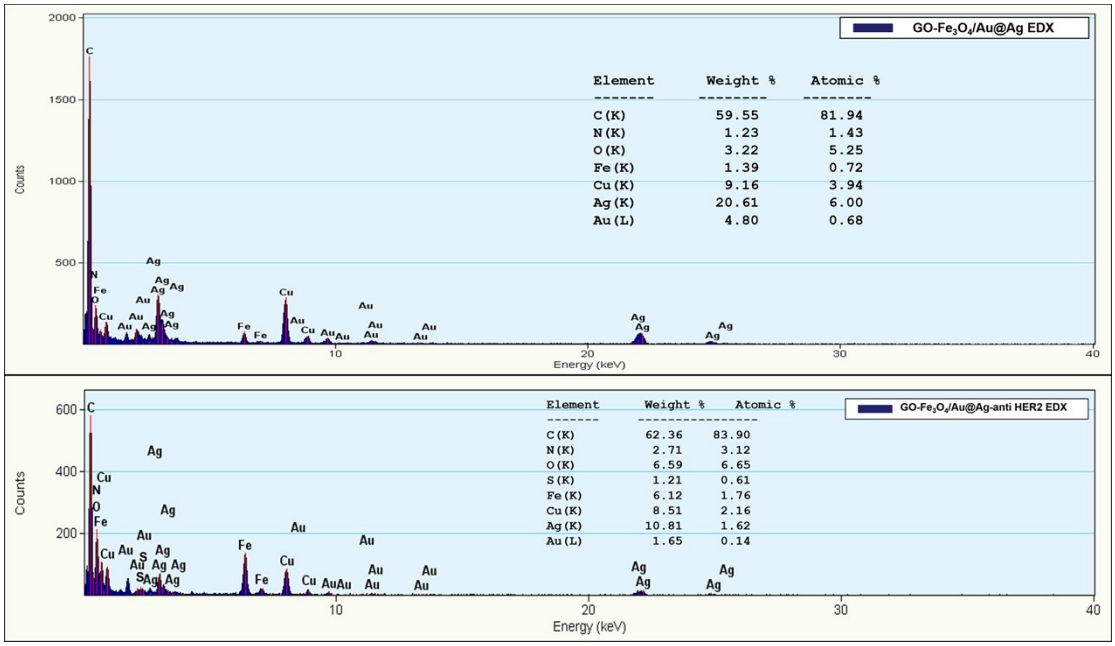

Fig. S3. EDX spectra of GO-Fe_3O_4/Au@Ag and GO-Fe_3O_4/Au@Ag-anti-HER2.

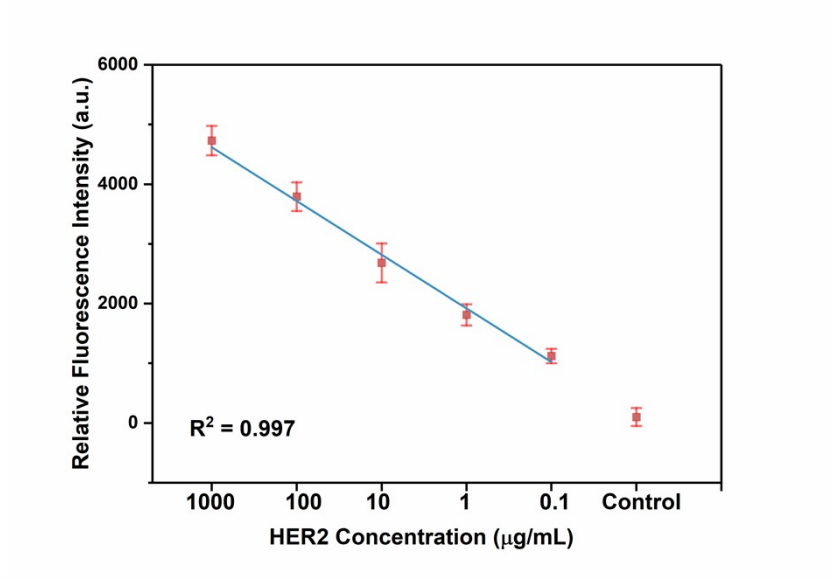

Fig. S4. plot of fluorescence intensity at 525 nm as a function of the HER2 antibody concentration. The calibration equation is y = 899.3log(*c*) + 1921.3 (y represents the fluorescence intensity at 525 nm and *c* represents the concentration of HER2 antibody). Error bars indicate the standard deviation of three measurements.

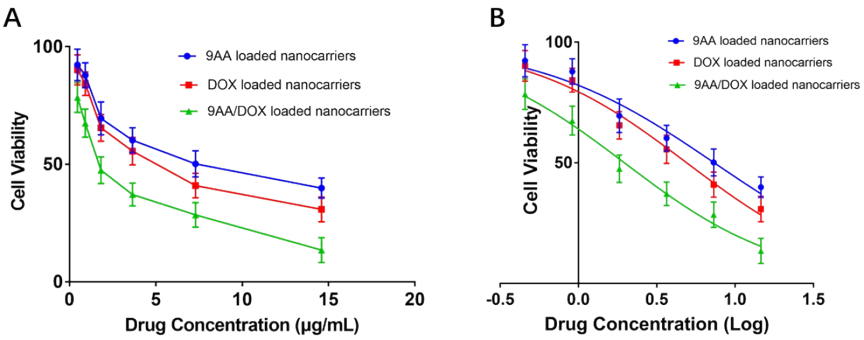

Fig. S5. Viability of SKBR3 cells incubated with 9AA loaded nanocarriers, DOX loaded nanocarriers and 9AA/DOX loaded nanocarriers for 48 h. SKBR3 cells incubated with standard culture media were used as a control. Error bars indicated the standard deviations of three measurements.