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Dumbbell-Like Au-Fe_3O_4 Nanoparticles for Target-Specific Platin Delivery

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Pt-based platin complexes, such as cisplatin, carboplatin and oxaliplatin, as shown in Figure 1A, are well-known generations of anticancer therapeutic agents.[1] One common feature of these square planar Pt complexes is that they all contain coordination bonds of Pt-N/Pt-Cl, or Pt-N/Pt-O with two Pt-N bonds in *cis*-position. Pt-Cl or Pt-O bonds in the complex are chemically much weaker than Pt-N bonds and subject to facile hydrolysis in low Cl^- and low pH conditions, giving charged [*cis*-$Pt(NH_3)_2(H_2O)_2]^{2+}$ that are highly reactive for DNA binding through the N7 atom of either an adenine or guanine base. This binding de-stacks the double helix structure and interrupts with cell's genetics/transcription machinery and repair mechanism, leading to cell death.[2] However, these powerful platin therapeutic agents have no capability of identifying the tumor cells from the healthy ones. As a result, they tend to be taken up by any fast grown cells, tumorous and healthy ones alike, causing the well-known toxic side effects.[3]

Here we report that dumbbell-like Au-Fe_3O_4 nanoparticles (NPs) can act as a target-specific nanocarrier to deliver platin into Her2-positive breast cancer cells with high therapeutic effects. Recent research progress has revealed that antigens are often over-expressed on the surfaces of the fast growing tumor cells. These over-expressed antigens provide obvious targets for specific binding as each type of antigens can be selectively captured by a typical monoclonal antibody.[4] Therefore, linked with a monoclonal antibody, these carriers may achieve target-specific delivery through strong antibody-antigen interactions and receptor-mediated endocytosis. The dumbbell-like Au-Fe_3O_4 NPs offer an ideal platform for this delivery purpose. As shown in Figure 1B, their core structure contains magnetic Fe_3O_4 NPs and optically active Au NPs. Compared with the conventional single component iron oxide NPs used for biomedical applications,[5] the dumbbell-like Au-Fe_3O_4 NPs have the following distinct advantages: (1) the presence of Fe_3O_4 and Au surfaces facilitates the stepwise attachment of an antibody and a platin complex; (2) the structure can serve as both magnetic and optical probes for tracking platin complex in cells and in biological systems.

To produce Au-Fe_3O_4 NPs for target-specific platin delivery, we first synthesized the dumbbell-like Au-Fe_3O_4 NPs based on the published method,[6] and a series of dumbbell-like NPs are shown in Figure S1. As an example, the oleate/oleylamine coated 8 nm – 18 nm Au-Fe_3O_4 NPs (Figure S1C) were functionalized by replacing oleate/oleylamine with dopamine- and thiol-based surfactants (Figure 1B).[7] In this structure, platin was anchored on Au side by reacting Au-S-$CH_2CH_2N(CH_2CH_2COOH)_2$ with cisplatin, and the Her2-specific monoclonal antibody, Herceptin, was chosen as a targeting agent and was linked onto Fe_3O_4

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Supporting Information **Available:** Nanoparticles modification for platin delivery. These materials are available free of charge via the Internet at http://pubs.acs.org.

through PEG_{3000}-CONH-Heceptin.[7] The linkage of Au-Fe_3O_4-Heceptin was confirmed through matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (Figure S2), while the conjugation of platin-Au was characterized by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and energy dispersive spectroscopy (EDS). The elemental analyses reveal that the conjugate contains S/Pt at an atomic ratio of ~1/1 (Figure S3). This indicates that two carboxylic group's replace two Cl's in cisplatin, forming the platin complex as shown in Figure 1B. According to the weight percentage of Pt/Au (~17.8%), ~2812 platin units are bound to each Au NP. We also characterized the size dependent platin loading on Au-Fe_3O_4 NPs. Among the 3 nm-18 nm, 6 nm-18 nm, 8 nm-18 nm and 8 nm-25 nm Au-Fe_3O_4 NPs tested, larger Au NPs were capable of incorporating more platin complexes, while the size of the Fe_3O_4 had little effect on platin concentration (Table S1). This further proves that platin binds to the Au side, not to the Fe_3O_4 side, as shown in Figure 1B. The final conjugate can be dispersed in PBS. The 8 nm–18 nm Au-Fe_3O_4 NPs have a 32 nm hydrodynamic diameter as measured by dynamic light scattering (DLS) (Figure S4).

The specificity of the platin-Au-Fe_3O_4-Heceptin NPs was examined through their preferred targeting to Sk-Br3 cells that are Her2-positive breast cancer cells (Her2-negative breast cancer cells (MCF-7) were used as a control).[8] Before incubation with the platin-Au-Fe_3O_4-Heceptin NPs, Sk-Br3 and MCF-7 cells were pre-blocked with 1% BSA. The cells were then incubated with the NPs in PBS for 1 h and fixed with 4% paraformadehyde. The cells were later imaged using Leica TCS SP2 AOBS spectral confocal microscope at 594 nm – the region where the Au NPs show the strong reflection.[9] Figure 2A&B show the reflection images of Sk-Br3 cells (Figure 2A) and MCF-7 cells (Figure 2B). The brighter image (~1.5 times brighter as measured through Image J) shown in Figure 2A indicates that more platin-Au-Fe_3O_4-Heceptin NPs target to Sk-Br3 cells. We can conclude that under the same incubation concentration, Herceptin helps the preferred targeting onto Sk-Br3 cells, not MCF-7 cells. TEM image analysis on the Sk-Br3 cells reveals the presence of NPs in endosome/lysosome, which indicates that the NPs were up-taken through endocytosis process (Figure S5).

The platin release from the NP conjugate (100 μg Pt in 2 ml PBS) was analyzed in a dialysis bag (MWCO = 1,000) that was put into a 30 ml PBS reservoir at 37 °C. Cisplatin in the same Pt concentration was used as a control. The membrane of the dialysis bag keeps the bound platin and the NPs inside the bag while the released platin or free cisplatin can diffuse into the buffer reservoir from which the Pt concentration was measured by ICP-AES. The platin release data are given in Figure 2C. It can be seen that 80% of free cisplatin diffuses through the dialysis bag in 1 h while for the NP conjugate this release is reduced to only about 25% in the same incubation time. Furthermore, the Pt-releases is pH dependent (Figure 2D). At pH = 6, 70% of platin is released from the platin-Au-Fe_3O_4-Heceptin NPs after 10 h while at pH = 8, the amount of platin release is reduced to 40%. Clearly, lower pH conditions accelerate the platin release from the conjugate shown in Figure 1B. As endosome/lysosome has pH around 5, we can conclude that platin release will be accelerated once the conjugate is inside the cells through endocytosis process.

The therapeutic effect of the platin-Au-Fe_3O_4-Heceptin NPs was studied by measuring the cell viability and p53 expression in Sk-Br3 cells. The control experiments show that Au-Fe_3O_4 NPs without platin did not inhibit cell growth under all Fe concentrations we tested (Figure S6A). Once coupled with platin, however, the platin-Au-Fe_3O_4-Heceptin NPs have half maximal inhibitory concentration (IC_{50}) to Sk-Br3 cells at 1.76μg Pt /ml (Figure 3), lower than that needed for cisplatin at 3.5 μg/ml. Note that the platin-Au-Fe_3O_4 NPs without Herceptin is also toxic, but its toxicity is less than cisplatin due to their non-specificity and the slow platin hydrolysis in the conjugate. The highest toxicity to Sk-Br3 cells observed from the platin-Au-Fe_3O_4-Heceptin NPs is clearly attributed to the specific targeting and enhanced uptake of NPs

by Sk-Br3 cells. In contrast, platin-Au-Fe_3O_4-Heceptin NPs did not show obvious improvement in their toxicity to MCF-7 cells (Figure S6B).

The increase of Pt concentration within Sk-Br3 cells can also be monitored by the accumulation of p53 - a tumor suppressor protein.[10] This is easily seen in a control experiment that more p53 are present with higher concentration of cisplatin added in the cell culture medium with beta-actin as the loading control (Figure S6C).[7] We tested the p53 protein expression in Sk-Br3 cells after treatment with different NPs and cisplatin. The cells treated with platin-Au-Fe_3O_4-Heceptin NPs have the highest p53 expression (Figure S6D). This is consistent with what we observed in cell toxicity data in Figure 3, indicating that Herceptin indeed induces more uptake of platin into the Sk-Br cells, causing highly toxic effect to these cells.

In summary, we have demonstrated that the dumbbell-like Au-Fe_3O_4 NPs can serve as a multifunctional platform for target-specific platin delivery. The release of the therapeutic platin at a low pH condition render the NP conjugate more toxic to the targeted tumor cells than the free cisplatin. The methodology developed here can be generalized and the dumbbell-like Au-Fe_3O_4 NPs should have great potentials as nanocarrriers for highly sensitive diagnostic and highly efficient therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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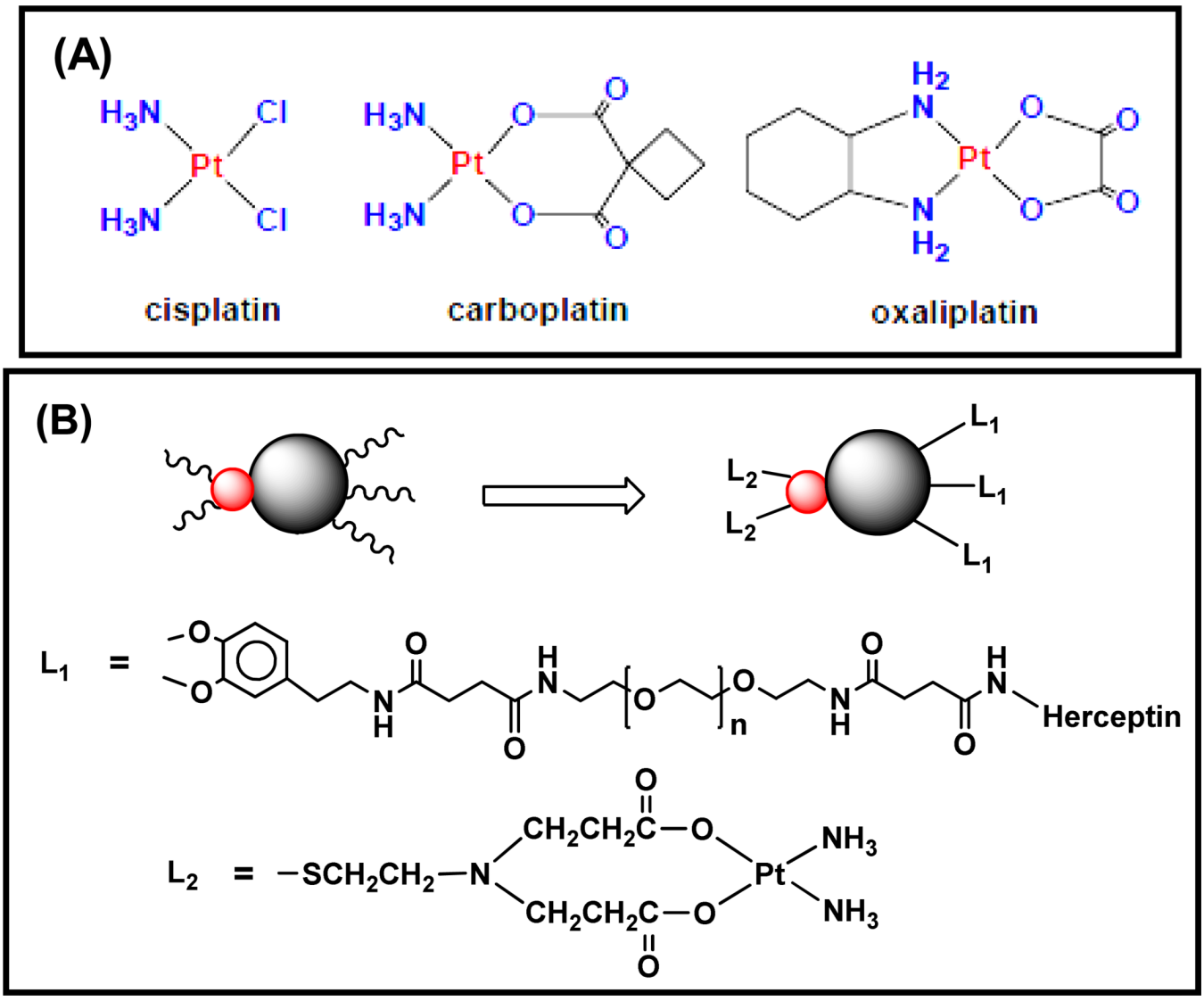

Figure 1.
(A) Structural illustration of the common therapeutic platin complexes; (B) Schematic illustration of the dumbbell-like Au-Fe_3O_4 NPs coupled with Herceptin and platin complex for target-specific platin delivery.

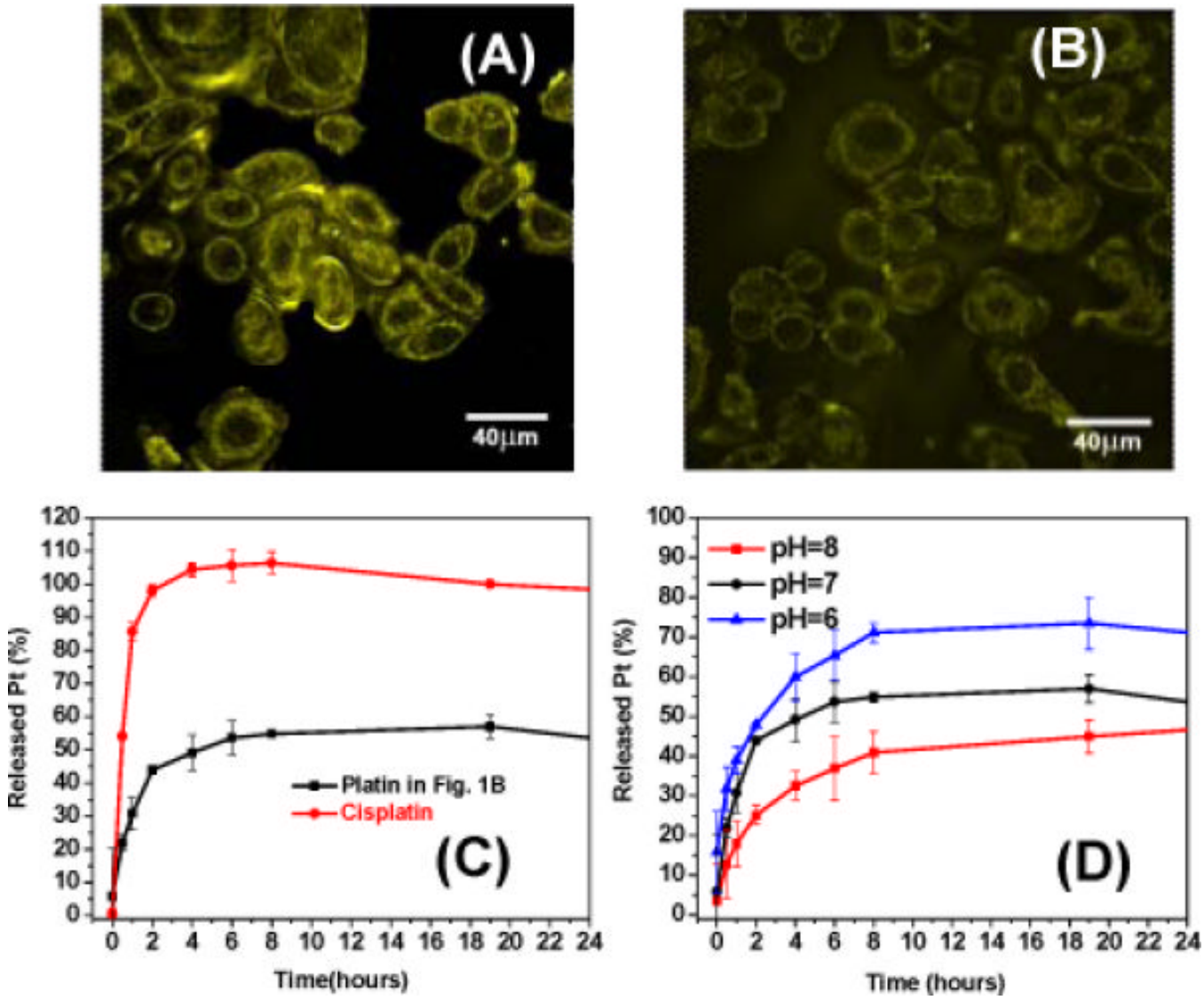

Figure 2.

Reflection images of (A) Sk-Br3 cells and (B) MCF-7 cells after incubation with the same concentration of platin-Au-Fe_3O_4-Heceptin NPs. (C) Cisplatin and platin release curves at 37° C (pH = 7); (D) pH dependent Pt-release from platin-Au-Fe_3O_4-Herceptin at 37 °C.

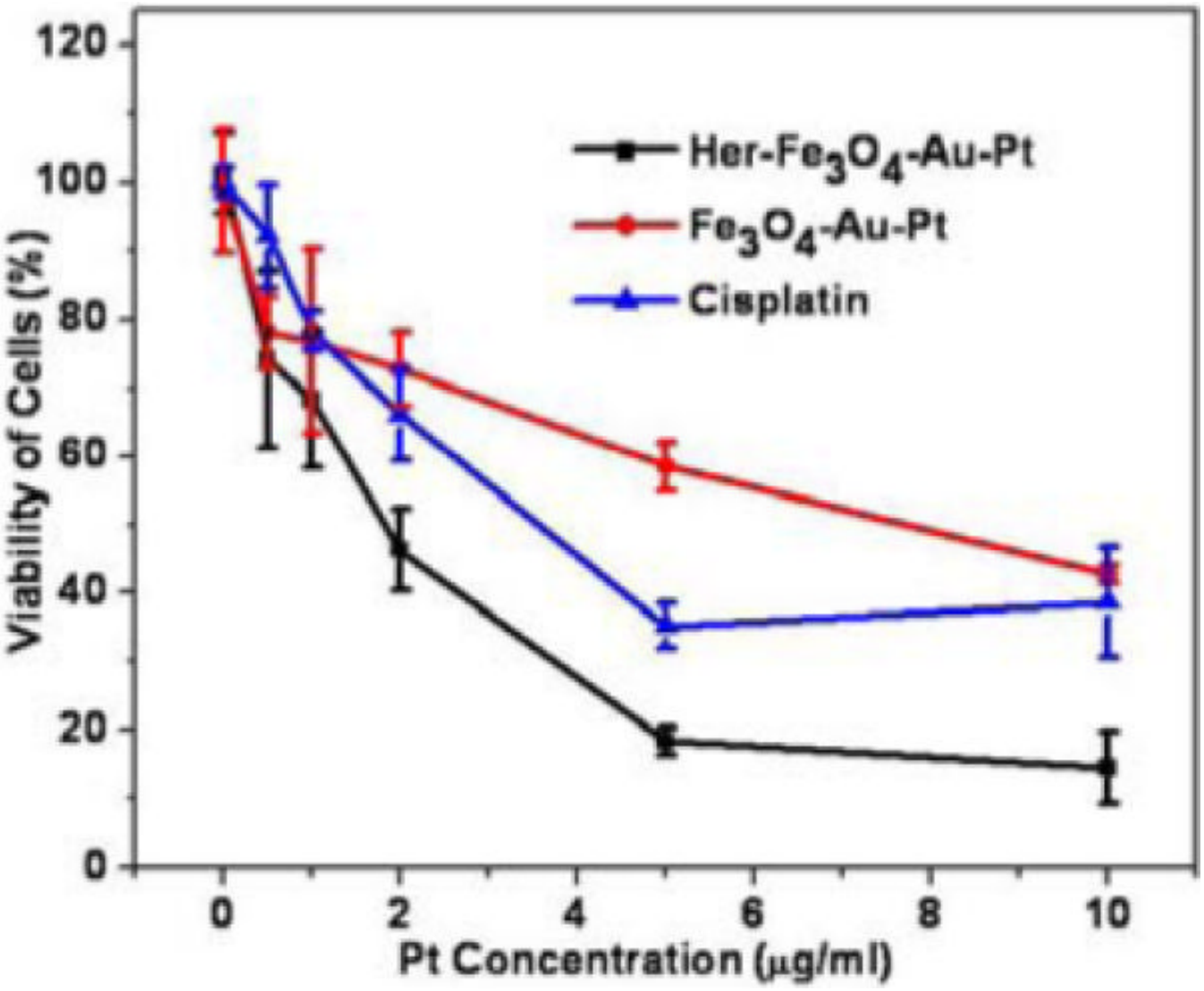

Figure 3.
Viability of Sk-Br3 cells after incubation with platin-Au-Fe_3O_4 NPs, platin-Au-Fe_3O_4-Herceptin NPs and free cisplatin.