Electronic Supplementary Material (ESI) for Nanoscale.

Multi-Block Magnetic Nanorods for Controlled Drug Release Modulated by Fourier Transform Surface Plasmon Resonance

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Experimental section

Materials

A commercially available AAO template were purchased from Whatman (product no. WHA68097023, pore size ≈ 250 nm). Au (Orotemp 24 RTU) and Ag (Technic ACR silver RTU) plating solutions were purchased from Technic Inc. Doxorubicin (DOX) and tetraethyl orthosilicate (TEOS) were obtained from Sigma-Aldrich. Sodium hydroxide (NaOH) and ammonia solution were purchased from Samchun. All aqueous solutions were prepared with deionized water (Millipore) that was prepared using a milli-Q water purification system. DMEM (Dulbecco's Modified Eagle Medium) was purchased from Cellgro. Fetal bovine serum (FBS, Orgin USA) was obtained from Capricorn.

Instruments

Electrochemical deposition was performed using Autolab® equipment with a three electrochemical system. The counter electrode and reference electrode were Pt mesh and Ag/AgCl electrode, respectively. Field-emission scanning electron microscopy (FE-SEM) images were obtained using a JEOL 7100F and a JEOL 7800F. UV-vis-NIR spectra were acquired using S-3100 (Scinco) spectrophotometer and UV-3600 (Shimadzu) spectrophotometer with an optical path length of 1 mm. Fluorescent intensity of DOX was obtained with a spectrofluorometer (Jasco FP-6200). HeLa cells were cultivated in an incubator (Thermo Fisher Heracell 150i and Stage top incubator TOKAI HIT Chamber System STRG). Formazan

produced in the MTT assay was quantified using a microplate reader (Multiskan EX, Thermo Electron Corp.)

Synthetic process of Au/Ni/Au@SiO_2 (ANAS) nanorods

To form a conduction layer, an Ag layer was evaporated on the bottom side of the AAO template by a sputtering process (30 mA, 600 s). The bottom side of the AAO template was filled with Ag by electro-deposition at a potential of -0.95 V (vs. Ag/AgCl). Au block was electroplated on the Ag layer with an Orotemp 24 RTU plating solution at - 0.95 V (vs. Ag/AgCl) and homemade Nickel sulfate solution was injected and deposited on the Au surface. Au block was re-deposited on the surface of Ni block. The Ag layer was etched with nitric acid solution (35% v/v) and Au/Ni/Au NRs were removed from AAO templates using 3 M NaOH solution. After washing with distilled water three times, the Au/Ni/Au NRs were dispersed in distilled water. Silica was plated on the surface of the Au/Ni/Au NRs via hydrolysis of TEOS. Au/Ni/Au NRs (0.16 pM) were dispersed in 1 ml of 3rd D.I water. The NR solution was mixed with 0.3 ml TEOS, 0.1 ml ammonia, and 1.8 ml ethanol in a conical tube and sonicated for 1 hr. The silica-coated NRs were separated by a magnetic bar, washed three times with ethanol and 3rd D. I. water, and re-dispersed in 3rd D. I. water.

DOX loading process

ANAS NRs were dissolved with 1 ml DOX solution (1 mg/ml) and the concentration of ANAS NRs was adjusted to 0.64 pM. The mixture was shaken for 24 hr in the dark. The drug-loaded

nanocarriers were magnetically separated with a magnetic bar to remove remaining supernatant. Before the release experiment, ANAS (DOX) NRs were dispersed in 1 ml of PBS solution.

Quantitation of DOX loaded into the ANAS nanocarriers

ANAS (DOX) NRs solution (1 ml) was heated at 80ºC for 1 hr after magnetic separation of ANAS (DOX) NRs. For measurement of the amount of released DOX, the fluorescence intensity (λ_{ex} = 490, λ_{em} = 590 nm) of the supernatant was measured by photoluminescence (PL) spectroscopy.

Magnetic modulation and rotation monitoring

A magnetic stirrer (iUNO Company, dimensions: 11 cm × 12 cm × 2 cm) with 1-mT field strength was fixed in a S-3100 spectrophotometer. When the rotating magnetic field was on, the optical extinction intensity of nanocarriers was measured by kinetic measurement (at 520 nm, time interval: 25 ms).

Magnetically modulated drug release

All drug release experiments were performed in PBS solution (pH 7.2) at 25ºC. Drug-loaded nanocarriers were exposed to rotating magnetic fields. After drug release and washing, nanocarriers were removed by magnetic separation. PL intensity measurement (λ_{ex} = 490 nm, λ_{em} = 590 nm) was used to determine fluorescence intensity of released drug for various speeds of external magnetic field.

MTT Assay

MTT assay was carried out with HeLa cells to measure cytotoxicity of the magnetically modulated drug release system in an in vitro environment. HeLa cells (5,000 cells/well) were incubated in 95-well plates. After 12 hr, ANAS (DOX-treated) nanocarriers were dissolved in 0.1 ml DMEM and DMEM with DOX-treated ANAS was injected into the HeLa cell culture medium. DOX on ANAS NRs was released by application of rotating magnetic fields at 37ºC. After the magnetic-controlled release, the HeLa cells were washed with DMEM and cultured with DMEM for 12 hr. MTT solution (20 μl, 0.5 mg/ml in PBS) was added to wells. After 2 hr, the MTT solution was removed and 50 μl DMSO was added to dissolve the formazan crystals. The amount of formazan crystals was measured with a microplate reader at 520 nm.

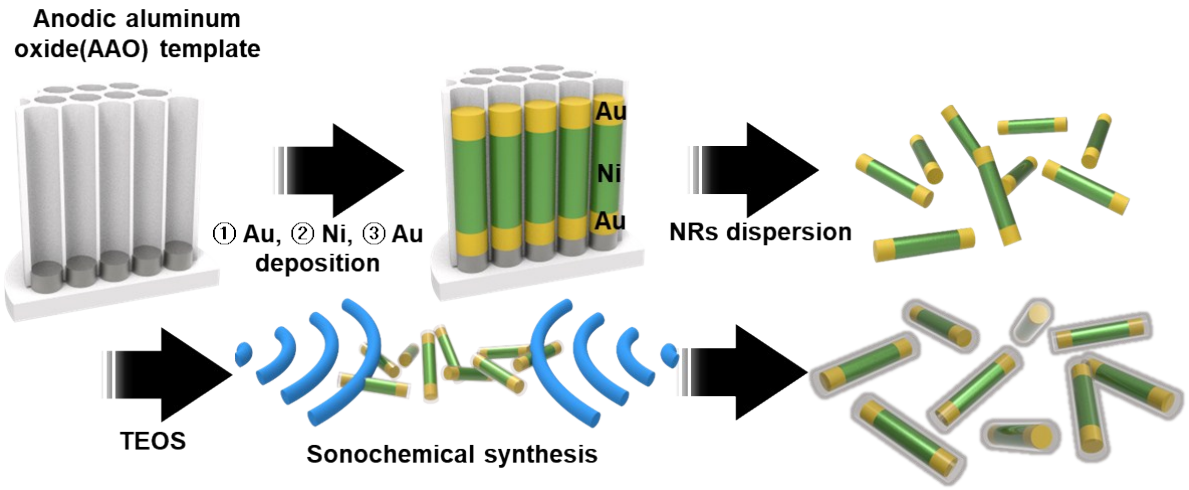

Figure S1. Schematic illustration of the synthesis of Au/Ni/Au@SiO_2 (ANAS) NRs nanocarriers. Silica layers were synthesized by sonochemical synthesis with TEOS.

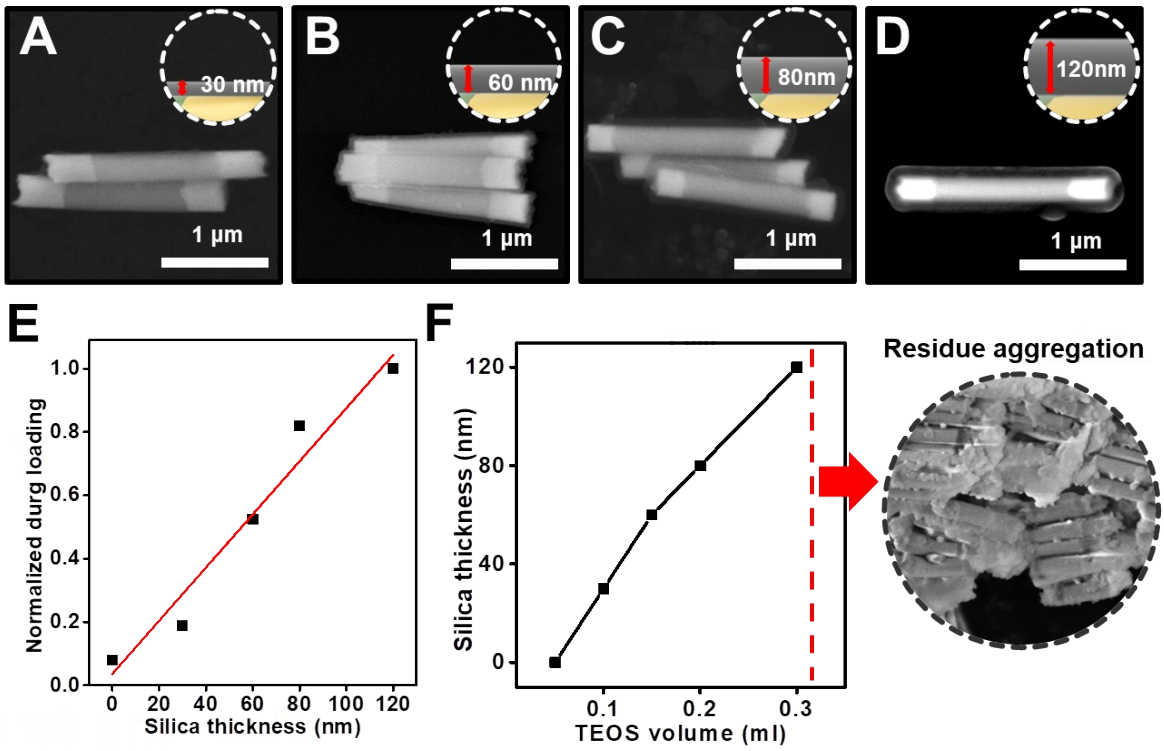

Figure S2. Silica thickness control. (A-D) FE-SEM image of ANAS carriers with different thickness of silica shell. **(E)** Loading capacity of ANAS carriers was proportional to the thickness of the silica shell. **(F)** Silica shell thickness was controlled by modulating the volume of TEOS. When the amount of TEOS was above that shown by the dashed red line, the silica formed aggregations and did not function as drug payload.

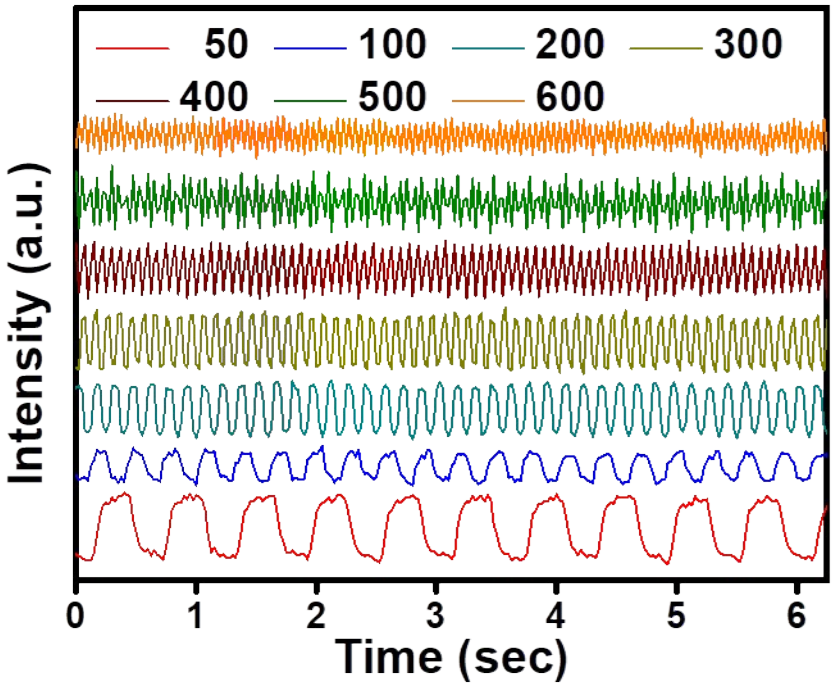

Figure S3. Raw sigmoidal functions corresponding to Figure 3C in the main text.

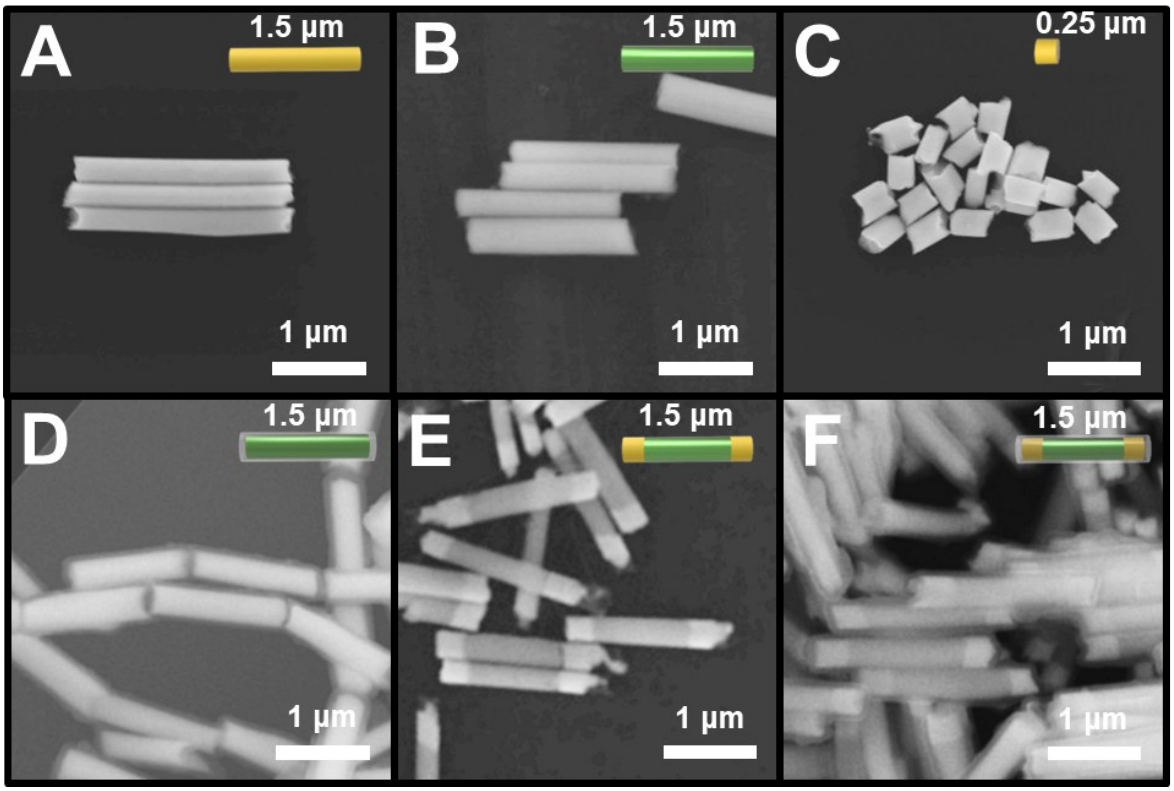

Figure S4. FE-SEM images of **(A)** pure Au NRs (1.5 µm in length), **(B)** pure Ni NRs (1.5 µm in length) **(C)** pure Au NRs (0.25 µm in length), **(D)** silica coated Ni NRs, **(E)** ANA nanorods **(F)** ANAS nanorods (silica thickness is 120 nm).

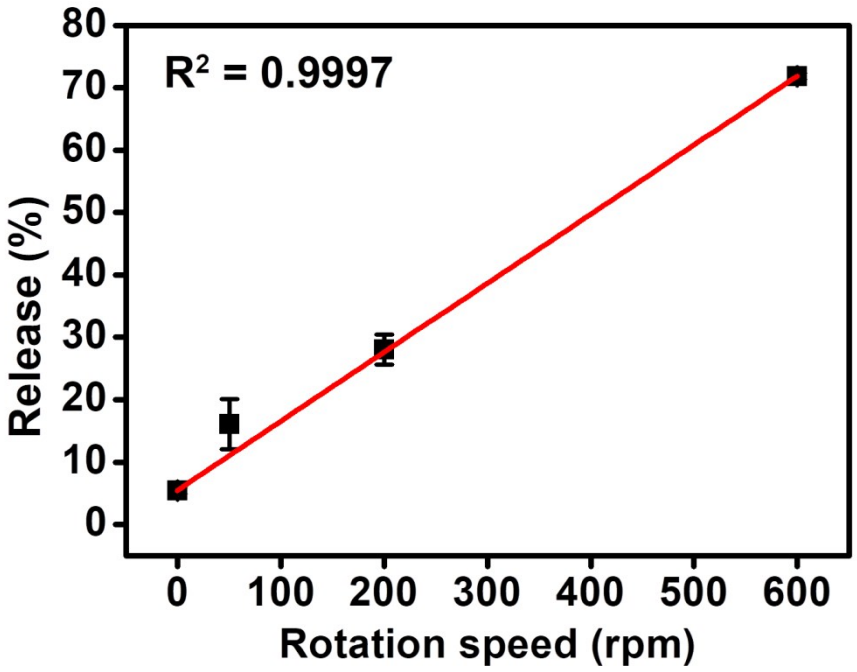

Figure S5. Release percentage of DOX from ANAS NRs as a function of external rotating magnetic field (2-hr drug release time). There is a linear relationship between the amount of released DOX and the rotating field speed, which allows us to control the release rate.

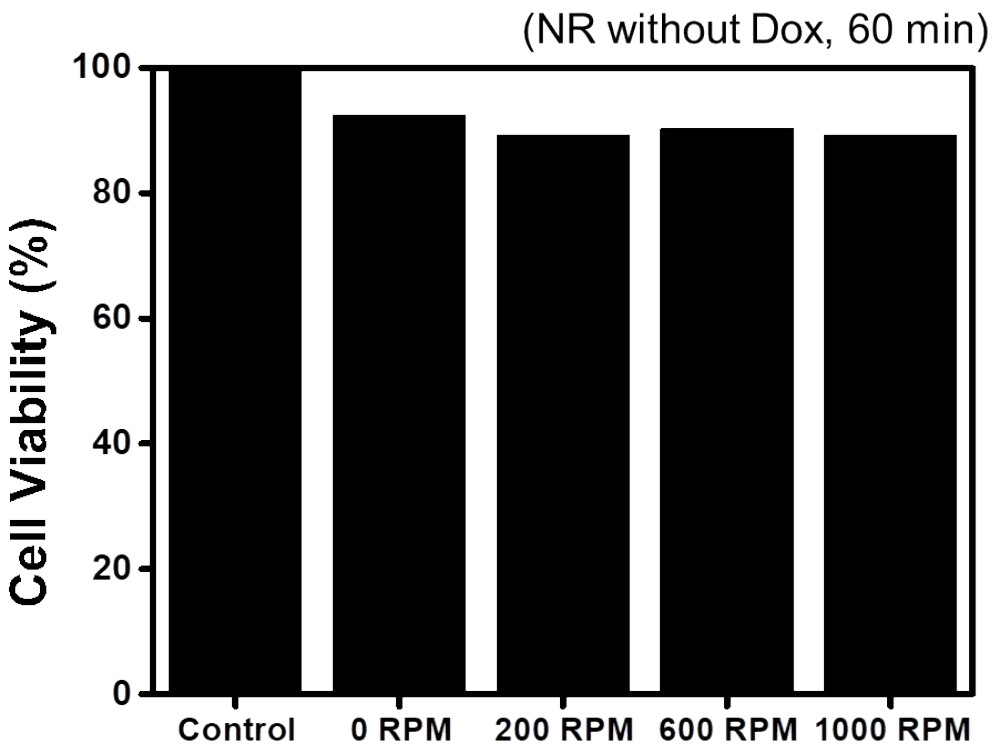

Figure S6. Biocompatibility of ANAS carriers. Control experiment to determine the cell toxicity of ANAS NRs (without DOX) and to measure the effect of mechanical rotation of NRs on cell viability.