# **Supporting Information**

# Biocompatible, Surface Functionalized Mesoporous Titania Nanoparticles

for Intracellular Imaging and Anticancer Drug Delivery

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# Experimental

#### Synthesis of MTNs

Typically, 2.2 mL of titanium(IV) ethoxide (Ti(OC<sub>2</sub>H<sub>5</sub>)<sub>4</sub>, Aldrich) was added drop by drop into 100 mL of ethanol (98%, Aldrich) while vigorous stirring at room temperature. After stirring until white precipitate appears (usually around 30 mins), the whole suspension was kept in a static condition at room temperature for at least 24 hours. Then, the precipitate was collected by centrifuge (5000 rpm for 10 min), washed by ethanol several times, and dried in a vacuum.

### Characterization of the synthesized MTNs

The structural properties of the samples were analyzed by X-ray diffraction (XRD) on a Rigaku ultima IV with Cu K $\alpha$  radiation (40kV, 40mA). The morphology and porous structure of the samples were observed with SEM (NovaTM Nano SEM) and TEM (JEOL-JEM 2100F, 200kV). The obtained powders were dispersed in ethanol and dropped onto copper grids for SEM and TEM observation. The porous properties of the products were analyzed using N<sub>2</sub> adsorption/desorption isotherms on a Micromeritics ASAP 2000 instrument. Sample were degased at 100 °C overnight before N<sub>2</sub> adsorption. Data were evaluated using the BET and BJH methods to calculate the surface area and pore size distribution, respectively. The appearance and distribution of elements in the samples were determined using SEM energy dispersive X-ray spectroscopy (EDS).

# Cell culture

BT-20 human breast cancer cells (ATCC® Number: HTB-19<sup>TM</sup>) were purchased from the National Health Research Institute (NHRI), Taiwan. They were maintained in flasks using DMEM (Dulbbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, 2% sodium bicarbonate, 1% amino acid, and 1% sodium pripate at 37 °C with 5% CO<sub>2</sub> in a 95% humidified atmosphere.

# MTT assay

BT-20 cells were seeded at a density of  $1 \times 10^5$  per well in 24-well plates in 0.5 mL DMEM for 24 h. After that, cells were incubated with different amounts of particles in serum-free medium for various durations, followed by the replacement of fresh serum-free medium containing 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/mL) and allowed to grow for another 4 h. The amount of dark blue formazan dye produced by the living cells was proportional to the number of living cells, and the absorbance at 570 nm was measured using a microplate reader (Bio-Rad, model 680). The LC<sub>50</sub> (the lethal concentration of 50% cell death) value was directly determined from the cell viability data.

#### **Prepartion of crystalline MTNs**

The as-synthesized MTNs (1 g) and  $H_2O$  (75 mL) were placed into an autoclave, and the autoclave was heated in an oven with 180 °C for 12h. After reaction overnight, the sample was washed with DI water and dried in vacuum. This hydrothermal treatment would change the crystalline phase of the as-synthesized MTNs from amorphous phase to anatase phase, which was confirmed by XRD (see Figure S5).

#### **Preparation of FMN-MTNs**

Flavin mononucleotide (FMN or Riboflavin 5P', a derivatives of Riboflavin (also known as vitamin  $B_2$ )) was selected as the probe because of its fluorescent property. In addition, because of the strong affinity between titania and phosphates, FMN could be adsorbed onto the surface of MTNs. MTNs (0.02 g) were added into FMN solution (3 mL,  $2.5 \times 10^{-4}$  M). After stirring for 12 h, the product was collected by centrifuge at 5500 rpms for 30 mins. The supernatant was then taken out for the fluorescence measurement. The decrease of the fluorescence intensity in supernatant represents the adsorption of FMN onto MTNs. The FMN-MTNs were then washed twice with DI water and dried in vacuum.

#### **Confocal fluorescence microscopy**

For confocal fluorescence microscopy measurements, BT-20 cells were seeded at a density of 1 x  $10^5$  cells per well in 4-well plates in 0.5 mL DMEM medium with Lab-Tek chambered coverglasses at the bottom of the wells. After incubation for 4 h, the DMEM medium was replaced by 1 mL of Doxo@FMN-MTNs (100 µg/mL) in the serum-free DMEM medium various times. The cell-plated coverglasses were then washed with medium and soaked in 4% formaldehyde in 1X PBS buffer (pH 7.4). The formaldehyde solution was then replaced by a solution containing DAPI (4',6-diamidino-2-phenylindole) PBS buffer (1 µg/mL) for staining nucleus. The DAPI-stained coverglasses were examined with a confocal fluorescence microscopy system

(Leica TCS SP5 II) using a 63x oil immersion objective lens. The blue fluorescent, DAPI-stained nuclei could be clearly observed by exciting the cells with a UV laser. The green fluorescent FMN@MTNs in the cytoplasm of the BT-20 cells was visualized by excitation at 488 nm. The red fluorescent Doxo in BT-20 cells was obtained by excitation at 543 nm.

# Results



*Figure S1.* Histogram obtained from SEM images showing the particle size distribution of the as-synthesized MTNs.



*Figure S2.* (b) A TEM image evidencing the disordered mesopores. (c) The nitrogen adsorption– desorption isotherm suggesting a BET surface area of  $237.3 \text{ m}^2 \text{ g}^{-1}$  and a pore size of 2.8 nm. (d) The MTT assay of BT-20 cells treated with various concentrations of MTNs showing a good biocompatibility.



*Figure S3.* (Left) a typical SEM image of P25 and (right) A MTT assay of BT-20 cells cultured with different concentrations of P25.



*Figure S4.* The MTT assay of BT-20 cells treated with various concentrations of crystalline MTNs. Compared with amorphous MTNs, crystalline MTNs here showed the  $LC_{50}$  was around 60 µg/mL, indicating an increased toxicity towards cells.



*Figure S5.* XRD patterns of P25 and MTN (before and after hydrothermal treatment) samples. The results indicated that the MTN before hydrothermal treatment exhibited amorphous phase. In contrast, MTN before hydrothermal treatment exhibited anatase crystallinity, same as P25.



*Figure S6.* (Top) The  $O_{1s}$  XPS spectrum of FMN-MTNs and (down) its corresponding deconvolution result.