

Supplementary Information

A superlattice composite of Zn-Fe layered double hydroxide and graphene oxide for antitumor application

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1. Experimental

1.1. *Sample preparation*

Commercial pure titanium slices with the dimensions of 10 mm \times 10 mm \times 1 mm were polished with abrasive papers series and followed by ultrasonically cleaning with acetone, ethanol, and ultrapure water in succession. Corresponding samples were marked as Ti. Then, cathode electrophoresis deposition was performed using an electrolyte solution containing 0.5 mL of graphene oxide (GO) aqueous solution (6mg/mL, provided by Hangzhou Gaoxin Technology Co., Ltd), 25 mg of ferric chloride hexahydrate, 54.9 mg of zinc nitrate hexahydrate and 250 mL of ethanol. Fe^{3+} and Zn^{2+} ions were absorbed on GO making GO positively charged and deposited on Ti surface by cathode electrophoresis with a constant potential of 40 V for 1 min. The samples were labeled as ZnFe@GO. Subsequently, ZnFe@GO samples were put into a Teflon-lined stainless-steel autoclave containing 50 mL of 1M NaOH and 25.2 mg NaHCO_3 and reacted at 120 °C for 12 h. Corresponding samples were marked as ZnFe-LDH@GO.

1.2. *Surface characterization*

Surface morphologies of Ti, ZnFe@GO, and ZnFe-LDH@GO were observed by field emission scanning electron microscope (FESEM, Magellan 400, FEI, USA). An atomic force microscope (AFM, Bruker Multimode 8 system) was used to observe the sample surface topography. X-ray diffraction (XRD, D8 advance, Bruker, Germany) patterns of Ti, ZnFe@GO, and ZnFe-LDH@GO were detected with a $\text{Cu K}\alpha$ radiation ($\lambda = 1.541 \text{ \AA}$). Raman spectra were acquired from 500–3000 cm^{-1} using a Raman microscope system (LabRAM, Horiba Jobin Yvon, France).

1.3. *Surface wettability*

Water contact angles were detected from Ti, ZnFe@GO, and ZnFe-LDH@GO to investigate the surface wettability by contact angle measurement (Automatic Contact Angle Meter Model SL200B, Solon, China). In detail, 2 μL of ultrapure water was dropped on the sample surface and the photograph was taken using a camera inside the system. Then, the water contact angle value was obtained by analyzing the photographs.

1.4. *Cell compatibility*

1.4.1. *Cell culture*

The cholangiocarcinoma cell line RBE (purchased from the Cell Bank of Chinese Academy of Science) was used to investigate the cell compatibility of various samples. The RBE cells were cultured with RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% of the antimicrobials penicillin and streptomycin (Antibiotic-Antimycotic, Gibco, USA) in a humidified atmosphere with 5% CO_2 at 37 °C. Cell culture media were refreshed every three days. For cell seeding, the RBE cells were cultivated with trypsin/EDTA (0.05% trypsin, 0.02% EDTA, Gibco, Invitrogen) solution to detach the cells and cultured with various samples.

1.4.2. *Cell adhesion and spreading*

To investigate the initial cell adhesion and spreading of RBE cells on Ti, ZnFe@GO, and ZnFe-LDH@GO, RBE cells with a cell density of 5.0×10^4 cells/mL were seeded on various samples and cultured for 1, 4, and 24 h. At each time point, RBE cells on the samples were rinsed with PBS, fixed with 4% paraformaldehyde (PFA, Sigma, USA), permeabilized with 0.1% Triton-100 (Amresco, USA), and stained

with FITC-Phalloidin (Sigma, USA). Finally, fluorescence images of RBE cells adhered on the samples were taken using a confocal laser scanning microscope (CLSM, Leica SP8, Germany).

1.4.3. *Cell proliferation and morphology*

An alamarblue assay (AbD Serotec Ltd., UK) was utilized to evaluate the cell proliferation rate of RBE cells cultured on various samples. To be specific, 1 mL of RBE cells with a cell density of 5.0×10^4 cells/mL were seeded on Ti, ZnFe@GO, and ZnFe-LDH@GO and cultured for 1, 3, and 5 days. At each time point, the samples with cells were transferred into a new 24-well plate and cultivated with 500 μ L of culture media containing 10% alamarblue for another 2 h. Then, 100 μ L of culture medium mentioned above was transferred into a 96-well plate and the fluorescence intensity was detected with an excitation wavelength of 560 nm and emission wavelength of 590 nm using a microplate reader (BioTek, USA). The cell proliferation rate is positively related to the fluorescence intensity. For cell morphology observation, RBE cells cultured on Ti, ZnFe@GO, and ZnFe-LDH@GO for 1, 3, and 5 days were fixed with 2.5% glutaraldehyde overnight and dehydrated with 30%, 50%, 75%, 90%, 95%, and 100% ethanol solutions. Finally, cell morphologies were observed using a scanning electron microscope (SEM, S-3400, HITACHI, Japan).

1.4.4. *Live/dead cell staining*

A live/dead cell staining kit (Biovision, USA) was utilized to evaluate the cell viability of RBE cells cultured on various samples. To be specific, 1 mL of RBE cells with a cell density of 5.0×10^4 cells/mL were seeded on Ti, ZnFe@GO, and ZnFe-

LDH@GO and cultured for 5 days. Then, RBE cells on the samples were rinsed with PBS and a live/dead cell staining kit with 5 μM of propidium iodide and 2 μM of calcium-AM were added and cultured for another 15 min. Finally, fluorescence images of RBE cells were observed using a confocal laser scanning microscope (CLSM, Leica SP8, Germany).

1.5. *In vivo antitumor activity*

Animal experiments were approved by the Animal Care and Experiment Committee of Shanghai Rat&Mouse Biotech. Co., Ltd. To establish the tumor model, 200 μL of mouse breast cancer 4T1 cells with a cell density of 1.0×10^7 cells/mL were injected into the back of BALB/c mice. Two weeks later, the tumor-bearing mice were anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection and followed by implantation of the samples contacting the tumor directly. Then, the tumor dimensions and body weight were measured every two days. The tumor volume (V) was calculated according to the equation: $V = 1/2 \times \text{tumor length} \times \text{tumor width}^2$ and the relative tumor volume was obtained by normalizing the tumor volume to its initial volume. The relative weight of mice was obtained by normalizing the weight of mice to its initial weight. At last, the mice were sacrificed at 16 days after implantation surgery. Then, the tumor tissues and organs including the heart, liver, spleen, lung, and kidney were collected, fixed, dehydrated, and stained with hematoxylin-eosin (H&E).

1.6 *Statistical analysis*

Data were expressed as the mean \pm standard deviation. The results were analyzed by GraphPad Prism 5 software. The statistically significant differences were analyzed

using a two-way ANOVA. A value of $p < 0.05$ indicated a statistically significant difference.