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A Gold-polyphenol-based Nanoplatform for delivery of Lenvatinib and Photothermal Therapy induction against Hepatocellular Carcinoma Treatment

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Materials: The following materials were obtained and used without further purification: Lenvatinib

(Aladdin, China), epigallocatechin gallate (EGCG, ≥95%, Beyotime, China), chloroauric acid trihydrate ($HAuCl_4$, Macklin, China), dimethyl sulfoxide (Aladdin, China) and sodium borohydride (Aladdin, China).

Characterization: Fabrication of AE and ALE: Lenvatinib and EGCG were respectively dissolved in DMSO. Lenvatinib, EGCG, and $HAuCl_4$ were mixed at molar ratios of 2:1:1. After stirring for 10 minutes, the mixed solution was left undisturbed overnight. Subsequently, sodium borohydride (0.1 mg) was added, and the solution was stirred for another 30 minutes before being dialyzed against ultrapure water.

Photothermal activity: To assess the ALE photothermal activity, an 808 nm laser was used for irradiation and an infrared (IR) camera was applied for monitoring. First, ALE at various concentrations ranged from 0 to 100 μg/mL were exposed to an 808 nm laser at density of 0.8 W/cm^2. Next, power density was changed from 0.6 to 1.2 W/cm^2 and ALE concentration was kept at 50 μg/mL.

Cell culture: Hepatoma 22 (H22) cells were purchased from Chinese Academy of Sciences, Shanghai Institute of Cell Biology. H22 cells were cultured in RPMI-1640 (Gibco or equivalent) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. H22 cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

MTT assay: H22 cells were seeded into a 96-well plate at a density of 5×10^4 cells/well in 100 μL of RPMI-1640 complete medium and incubated for another 24 h. Then cells were divided into 4 groups (5 wells/group) including (1) control, (2) ALE, (3) AE+Laser and (4) ALE+Laser. For cells in group (2)-(4), the culture medium was replaced with fresh medium containing AE or ALE. Next, cells in group (3) and (4) were irradiated with an 808 nm laser. After incubation for an additional 24 h, 10 μL of MTT solution to each well and incubate at 37°C for 4 hours. The supernatant was removed and add 150 μL of DMSO to each well to dissolve the formazan crystals. The plate was shaken for 10 minutes to ensure complete dissolution. Then the absorbance at 570 nm was measured using a microplate reader. Cell viability was calculated using the below equation

$$Cell\ viability\ (\%) = \left(\frac{OD_{Sample} - OD_{blank}}{OD_{Control} - OD_{blank}}\right) \times 100\%$$

Western blotting assay: H22 cells were seeded into 6-well plates and divided into four experimental groups including (1) Control, (2) ALE, (3) AE + Laser and (4) ALE + Laser. After treatment, H22 cells were lysed using RIPA buffer containing protease and phosphatase inhibitors and total protein concentration was measured using a BCA assay. Equal amounts of protein were separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk in TBST for 1 hour at room temperature, followed by overnight incubation at 4℃ with primary antibodies against HSP70, HSP90 and Cleaved-Caspase 3. GAPDH was applied as a loading control. After several washing, the membrane was incubated with HRP-conjugated secondary antibodies for 1 h. Protein bands were detected using an enhanced chemiluminescence (ECL) system, and densitometric analysis was performed using ImageJ software to quantify protein expression levels relative to GAPDH.

qPCR analysis: qPCR was performed to analyze the mRNA expression levels of HSP70, HSP90, and Cleaved-Caspase 3 in H22 cells. Total RNA was extracted using TRIzol reagent, and RNA purity and concentration were measured using a NanoDrop spectrophotometer. cDNA was synthesized from 1 μg of RNA using a reverse transcription kit according to the manufacturer's instructions. qPCR was performed using SYBR Green Master Mix on a real-time PCR system, with GAPDH as the internal reference gene. Relative gene expression levels were calculated.

Tumor model establishment: Nude BALB/c mice aged 6 weeks were purchased from Vital River (China). To establish a subcutaneous H22 tumor bearing model, H22 cells were injected into the left flank of nude BALB/c mice at a concentration of 5×10^6 cells in 100 μL PBS. Tumor growth and body weight were monitored every 2 days.

Antitumor effect: Once the tumors reached 200 mm3, mice received various treatment including (1) control, (2) ALE, (3) AE+Laser and (4) ALE+Laser. At day 20, all mice were sacrificed and tumors were harvested for further analysis. The excised tumors were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for hematoxylin and eosin (HE) staining as well as Ki67 immunofluorescence.

Photoacoustic (PA) imaging: Nude BALB/c mice bearing H22 tumors were administered ALE via intravenous injection. PA imaging was conducted at different time points (0 h, 12 h, 24 h and 48 h) using a photoacoustic imaging system to evaluate the biodistribution of ALE in the tumor region.

Long-term toxicity study: Healthy nude BALB/c mice were administered ALE via tail vein injection. After the treatment period, the mice were sacrificed, and major organs including the heart, liver, kidneys, lungs, and spleen were collected for histopathological examination. The tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned for HE staining. In addition, liver and kidney function was assessed through routine biochemical tests, measuring markers such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), and blood urea nitrogen (BUN).

Statistical analysis: All data are expressed as the mean ± standard deviation (SD), with each experiment conducted independently at least three times (n = 3). Group comparisons were assessed using one-way analysis of variance (ANOVA) or Student's t-test. Statistical significance was determined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

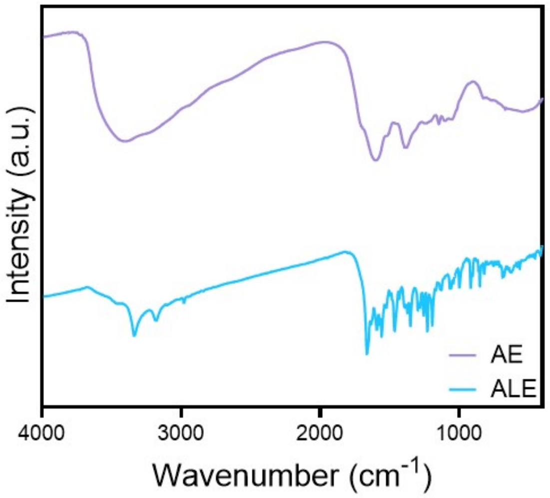

Figure S1. FTIR spectra of AE and ALE in the range of 4000–1000 cm^{-1} , showing the characteristic absorption peaks of both samples.

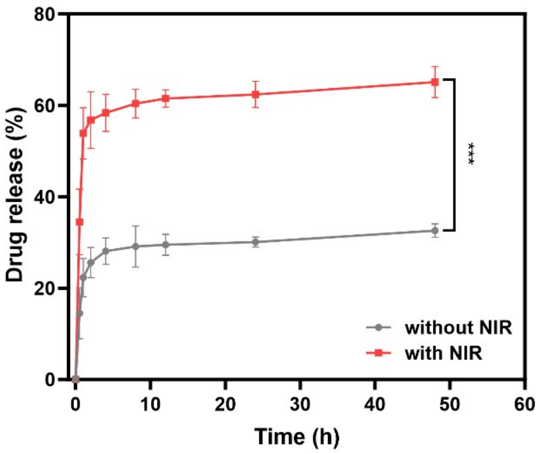

Figure S2. Lenvatinib release profile from ALE with and without laser irradiation.

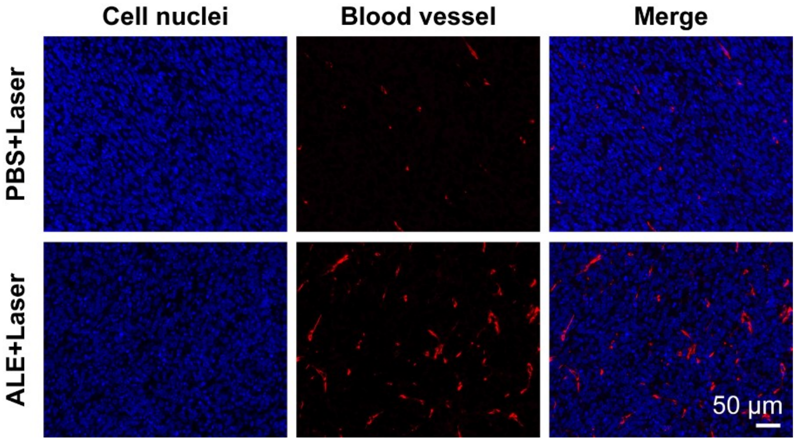

Figure S3. Representative CD31 immunofluorescence staining.

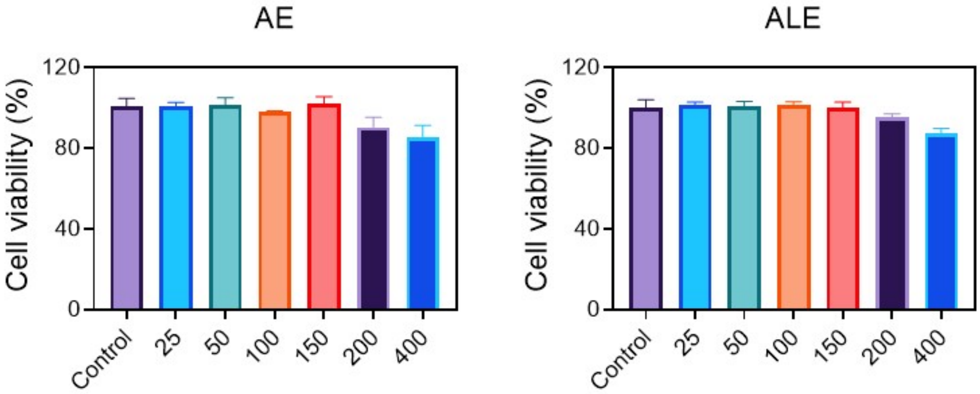

Figure S4. Cell viability of cells after treatment with various concentrations of AE and ALE (unit: μg/mL). Data are presented as mean ± SD.

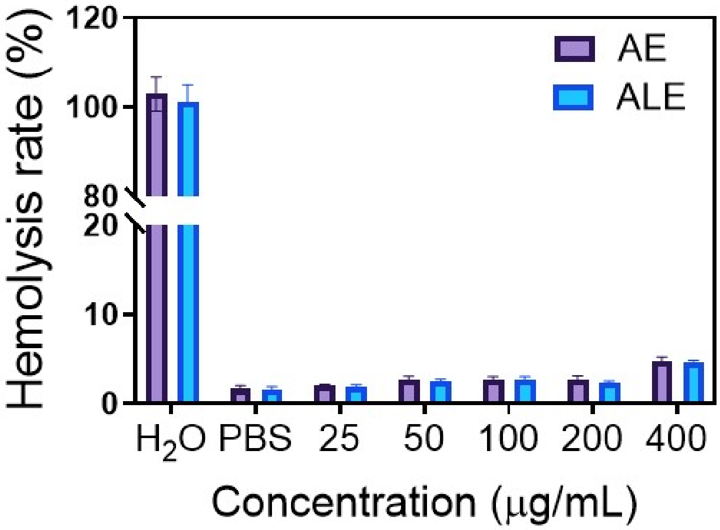

Figure S5. Hemolysis rates of AE and ALE at different concentrations (25–400 μg/mL). H_2O and PBS were used as positive and negative controls, respectively.