1.1 Materials

Paclitaxel (PTX, purity 99%), Dalian Meilun Biotechnology Co., Ltd. PAMAM (Generation 5, terminal amino groups, dissolved in methanol, with the concentration of 0.1 mg/mL), Weihai Chengyuan Molecular New Materials Co., Ltd., Shandong, China. Hyaluronic acid (HA, molecular weight 10 kDa), Shandong Freda Biotechnology Co., Ltd., China. Piperine (PIP, purity 98%), 3,3'-dithiodipropionic acid (DTDP), 4-dimethylaminopyridine (DMAP), and N-hydroxysuccinimide (NHS) were purchased from Energy Chemical (Anhui, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) was purchased from Aladdin Chemical (China). Coumarin 6 (C6) was purchased from Shanghai Biochempartner Co., Ltd. (China); DiD (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine,4-Chlorobenzenesulfonate Salt) was purchased from Beyotime Biotechnology (Shanghai, China); MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) was purchased from Biosharp (China); Annexin-FITC/PI apoptosis detection kit was purchased from APExBIO Technology LLC. All other chemical reagents were of analytical grade.

The multifunctional microplate reader was Varioskan LUX, Thermo Scientific, USA. The real-time imaging system was IVIS Lumina III, PerkinElmer, USA. The flow cytometry used a FACSCelesta instrument (Agilent, USA). The blood samples were tested by a biochemical analyzer (c311, Roche, Switzerland).

1.2 Cell culture and Animals

The murine ovarian epithelial cancer cell line ID8 in the experiment was obtained from the Key Laboratory of Gynecological Oncology, West China Second University Hospital, Sichuan University. It was cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S), and maintained in a humidified incubator with 5% CO_2 at 37 °C. The cell line was passaged every 4–6 days. All assays were performed in the respective cell media.

The 4–5-week-old female C57BL/6 mice (SPF grade) used in the experiment, weighing 16–18 g, purchased from SPF (Beijing) Biotechnology Co., Ltd. All animal procedures were carried out in accordance with the "Regulations on the Housing and Use of Laboratory Animals" of Sichuan University.

1.3 In Vitro Cytotoxicity Study

ID8 cells were seeded into 96-well plates at a density of 5×10^3 cells per well and incubated in a humidified incubator at 37°C with 5% CO_2. After the cells adhered, ID8 cells were treated with different drug formulations containing different concentrations of PTX and PIP. Negative control groups (cells and culture medium without drugs) and positive control groups (culture medium only, without cells) were included. The effects of drug-free blank carriers on the viability of ID8 cells were also evaluated. After 48 h of drug incubation, 5 mg/mL MTT solution was added to each well and incubated for 4 h. The supernatant was then carefully removed, and 200 μL of DMSO was added to each well. The plate was shaken in the dark for 10 minutes to fully dissolve the MTT formazan crystals.

The formula for calculating the Q value is as follows:

$$Q = E_{(AB)} / [E_A + (1 - E_A) \times E_B]$$

Where $E_{(AB)}$ is the inhibition rate of the combination treatment, E_A or E_B are the inhibition rates of a single drug. A Q value between 0.85 and 1.15 indicates an additive effect, whereas a Q value less than 0.85 or greater than 1.15 indicates antagonistic or synergistic interactions, respectively.

1.4 Morphological Assessment of Cell Apoptosis

ID8 cells were seeded into 24-well plates at a density of 1×10^6 cells per well and incubated overnight. Subsequently, treatments of PTX, PS, HPS, and HPSI were added, each with a PTX concentration of 5 μM. Wells without drug treatment served as the blank control group. After 48 h of incubation, the original culture medium in the wells was removed, and the cells were washed three times with PBS. Then, 200 μL of 4% paraformaldehyde solution was added to each well, and the cells were fixed at room temperature for 10 minutes. The 4% paraformaldehyde solution in the wells was removed, and the cells were washed three times with PBS. Then, 200 μL of DAPI solution was added to each well and incubated at room temperature in the dark for 10 minutes. After incubation, the cells were washed three times with PBS to remove excess dye. Finally, 200 μL of PBS was added to each well to prevent cell shrinkage and maintain normal cell morphology. Nuclear changes were observed using an inverted fluorescence microscope.

1.5 In Vitro Annexin V-FITC /PI Apoptosis Detection

ID8 cells were seeded at a density of 1×10^6 cells per well in 12-well plates and incubated overnight. Subsequently, the cells were treated with PTX, PAMAM-SS-PAMAM, HPS, and HPSI, each containing PTX at a concentration of 5 μM. Wells without drug served as the blank control group. After 24 h of incubation, the original culture medium from each well was collected into centrifuge tubes. The cells

were then trypsinized, collected into centrifuge tubes, and centrifuged at 300 × g for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in PBS, followed by another centrifugation step to discard the supernatant. The cell pellet in each centrifuge tube was then resuspended in 500 µL of 1× Annexin V Binding Buffer (working solution) from the kit. Following the manufacturer's instructions, the samples were prepared and analyzed by flow cytometry.