Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supporting Information

Chitosan-derived Nanoparticles Impede Signal Transduction for T790M Lung Cancer Therapy

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1. Materials and Methods

1.1. Materials

α-Linolenic acid (ALA, 99%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI, 99.0%), 4-(Dimethylamino)pyridine (DMAP, ≥99%), Pluronic F127, 4-Carboxybenzeneboronic acid pinacol ester (PBAP, 97%), trifluoroacetic acid (TFA, 99%), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 98%) were purchased from Sigmae-Aldrich (St. Louis, MO, USA). Trypsin-EDTA and fetal bovine serum (FBS) were obtained from Gibco-BRL (Burlington, Canada). RPMI 1640 medium and penicillin-streptomycin were purchased from Bristol-Myers Squibb Trading Co. Ltd. (Shanghai, China). Hoechst 33342 was bought from the Invitrogen. Alexa Fluor® 647 conjugate of wheat germ agglutinin (WGA-647), and Pierce™ dialysis membranes were purchased from Thermo Fisher Scientific (Waltham, MA). Nuclease-free water was bought from Sangon Biotech (Shanghai, China). Giemsa stain kit was purchased from Yeasen Biotech Co., Ltd. (Shanghai, China). All chemicals were of reagent grade and used without further purification.

Dialysis membranes were purchased from Spectrum Labs (Rancho Dominguez, USA). The fluorescein-tagged negative-control miRNA (FAM-miRNA), and the antisense to miR-21 (anti-miR-21, 5′-UCA ACA UCA GUC UGA UAA GCUA-3′) were synthesized by GenePharma Co, Ltd. (Shanghai, China).

1.2. Synthesis of DCA, FP and Preparation of DCAFP

N, N-dimethyldipropylenetriamine (DMAPAPA) modified reconstructed chitosan (DC) was synthetized as previous report [Huang, G. etc., *Carbohydrate Polymers* **2020,** *227*, 115339.]. Synthesis of ALA conjugated DC (DCA) was as following step: powder of DC was dispersed in 10 mL DMSO in N_2 atmosphere, stirred for 4 h to complete dissolve, 150 μmol of ALA was added followed by EDCI and DMAP, the mixed solution was continuous stirred at 30℃ for additional 24 h (Scheme S1). The product was dialyzed in a cellulose membrane tube (molecular mass cut off 7 kDa) against flowing demineralized water for 3 days. Finally, the product DCA was freeze dried for two days under reduced pressure and stored at low temperature.

The conjugate between 4-Carboxybenzeneboronic acid pinacol ester (PBAP) and Pluronic F127 (F127) was prepared by carboxylic ester chemistry (Scheme S1). F127(2 g, 0.16 mmol) was dissolved in dry dichloromethane (DCM, 100 mL), was added dropwise in excess of PBAP (0.16g, 0.64 mmol) containing dry DCM (5 mL) at room temperature (R.T) followed by EDCI (0.13g, 0.64mmol) and DMAP (78 mg, 0.64mmol). The mixture was kept in an inert atmosphere for 24 h then the solution was concentrated using rotary evaporator and washed with an excess of diethyl ether thrice to remove the un-reacted chemicals. F127-PBAP was collected as a white powder after drying under vacuum dehydration. F127-PBAP was dissolved in dichloromethane, and added with trifluoroacetic acid (TFA). Then, the mixture was washed with saturated sodium bicarbonate, and saturated sodium chloride. The organic phase was poured into excess of cold ether, and the precipitated was dried overnight to obtain F127-PBA (FP).

Fused polymer of DCA and FP (DCAFP) was manipulated by ultrasound (65w) with the sonication using a microtip probe sonicator (XL2000, Misonix Incorporated, NY) for 5 min in ice-bath, and stay at R.T. for 30 min.

1.3. Characterization of Polymers

The ^{1}H NMR spectra of the chemicals were used to verify the chemical structures. ^{1}H NMR (400 MHz) were recorded on a Bruker DRX-400 spectrometer (Bruker, Ettlingen, Germany). The morphological examinations were performed by TEM (JEM-1230, JEOL Inc., Peabody, USA). Samples were prepared by drying a drop of the micelle (1 mg/mL) on a copper grid. The grid was dried before TEM observation. The particle size and zeta potential of the micelles were detected by DLS using a Zetasizer 3000 (Malvern Instrument, Worcestershire, UK) at 25 °C. Absorption spectra were measured using a Techcomp UV2310 UV/vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF-5301PC spectrofluorophotometer.

The CMC was determined using pyrene as a fluorescence probe. The fluorescence excitation wavelength was using a fluorescence spectrophotometer (RF-5301PC, Shimadzu, Japan). The intensity ratios of I393/I373 from the excitation spectra were plotted against the micelle concentration to determine the CMC.

The blood compatibility of DCAFP was evaluated by hemolysis tests on mouse red blood cells (RBC). 0.5 mL of the RBC stock solution were incubated with different concentrations of DCAFP. The resulting mixtures were incubated at 37 ℃ for 4 h. The absorbance of the supernatant solution of the test sample (A_{test}), positive control (A_{pos}), and negative control (A_{neg}) at 540 nm was determined by UV-Vis spectrophotometry. Each set of experiments was carried out three times and the hemolysis (%) = $[(A_{test} - A_{neg})/ (A_{pos} - A_{neg})] \times 100\%$.

1.4. Preparation and Characterization of polyplexes

The polymer/miRNA polyplexes was formed by simple electrostatic complexation followed by 30 min incubation at room temperature. For the gel retardation study, the polyplexes with various weight ratios were loaded onto a 1% agarose gel containing 1× TRIS acetate-EDTA (TAE) buffer solution and nucleic acid gel stain Gel Red reagent, and were electrophoresed at 80 V for 40 min on a Sub-Cell system (Bio-rad Laboratories, CA). The gel analyzed with a UV illuminator showed the position of the complexed miRNA relative to the naked miRNA.

1.5. Preparation of GFT-Loaded Nanomicelles

2 mL DCM mixture including 2 mg of GFT and 10 mg of DCAFP was dropwise added to 10 mL pure water with the sonication using a microtip probe sonicator (XL2000, Misonix Incorporated, NY) at 60 W power output for 5 min, and then vigorous stirred overnight to volatilize DCM. Finally, the solution was filtered through a 0.45 μm filter to remove the unloaded GFT and then lyophilized.

To determine drug encapsulation efficiency (EE) and drug loading content (DL), lyophilized DCAFP/GFT nanomicelle powders were dissolved in deionized water and centrifuged. The amount of encapsulated GFT in the supernate was detected by UV Spectrophotometer (UV-3600, Shimazu, Japan) at the wavelength of 330 nm. DL and EE were then calculated according to the following equations:

$$DL(\%) = \frac{weight\ of\ drug\ in\ micelles}{weight\ of\ drug\text{–}loaded\ micelles} \times 100\%$$

$$EE(\%) = \frac{weight\ of\ drug\ in\ micelles}{weight\ of\ feeding\ drug} \times 100\%$$

1.6. *In vitro* GFT Release

The in vitro release profiles were studied by dialyzing the GFT-loaded micelle suspension in PBS (pH 7.4 and pH 5.0) with horizontal shaking (100 rpm) at 37 ℃. The drug-loaded suspension (2.0 mL) was dialyzed against a 50.0 mL solution (MWCO = 3500 Da). At predetermined time intervals, the medium outside of the dialysis tube was collected and displaced with fresh solution. The samples were analyzed by high performance liquid chromatography (HPLC) system with a reverse-phase C18 column. The mobile phase consisted of methanol−water (78:22, v/v) at a rate of 1.0 mL/min at 25 °C. An ultraviolet detector was set to 330 nm.

In order to test the stability of storage of nanoparticle, the release profiles were studied by dialyzing the GFT-loaded micelle suspension in PBS at 4 ℃ with slight shaking (30 rpm). The medium outside of the dialysis tube was collected and displaced with fresh solution. The samples were analyzed by HPLC.

1.7. *In vitro* miRNA Release Behaviour

Free anti-miR21 and DGA was incubated at pH 5.5 or pH 7.4 DEPC H_2O in triplicate and incubated at 37 °C with gentle shaking (80 rpm). The cumulative release of miRNA was monitored and recorded at different time points. The incremental released amount of miRNA in the supernatant was measured using the Quant-iT™ RiboGreen (Invitrogen, Carlsbad, CA) assay. Cumulative release (R%) data was fitted to posttreatment time (t) in hours by the following formula:

$$R\% = Rmax \times (1 - 2^{-\frac{t}{t_{1/2}}})$$

where R_{max} is the maximum cumulative release amount, and $t_{1/2}$ is the half-life of first-order release.

1.8. Hemolysis

The blood compatibility was evaluated by hemolysis tests on mouse red blood cells (RBC) according to the method in our previous work (*Carbohydrate polymers*, 2020, **227**: 115339.). In brief, the anti-coagulated blood was collected and washed three times with PBS. The equal volume of RBC stock solution were incubated with different concentrations of DCA or DCAFP and the volume was adjusted to 1.0 mL with PBS. RBCs after incubation with water and PBS were used as the positive and negative controls, respectively. The resulting mixtures were incubated at 37 °C for 4 h and centrifuged at 1500 rpm at 4 °C for 10 min. The absorbance of the supernatant solution of the test sample (A_{test}), positive control (A_{pos}), and negative control (A_{neg}) at 540 nm was determined by UV-Vis spectrophotometry. Each set of experiments was carried out three times and the hemolysis (%) = $[(A_{test} - A_{neg})/ (A_{pos} - A_{neg})] \times 100\%$.

1.9. Cells and Animals

All cell lines used in this study were purchased from ATCC. Gefitinib-sensitive non-small-cell lung cancer (NSCLC) cell line PC9 and gefitinib-resistant NSCLC cell line H1975 was cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS). Cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2.

BALB/c female nude mice (6-8 weeks old, 18-22 g) were purchased from the Zhejiang Chinese Medical University and maintained in a pathogen-free environment under controlled humidity and temperature. The animal experiments were performed in accordance with the China Animal Protection Law (CAPN), and the protocols were approved by the Zhejiang University Animal Care and Use Committee (ZJU20190018).

1.10. ROS and Mitochondrial Membrane Potential Measurement

The H1975 cells were grown overnight in 6-well tissue culture plates. Upon reaching 70% confluence, the growth media was replaced with media containing DCA or DCAFP at concentration of 50 μg/mL. The ROS levels in cell were determined according to the manufacturer's protocol. DCFH-DA stock solution was diluted to 10μM with serum-free cell culture media. After 24 h incubation of solutions, the culture media was removed, 100 μL of DCFH-DA (10μM) was added to each well. After incubated in the dark for 30 min at room temperature, cells were suspended and the fluorescence of the oxidized DCF in cells was measured using flow cytometry.

The membrane-permeant JC-1 dye is used in apoptosis studies to monitor mitochondrial health. JC-1 dye exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~529 nm) to red (~590 nm). The mitochondrial membrane potential was detected by manufacturer's protocol of MitoProbe™ JC-1 Assay Kit (Molecular Probes, Inc. USA).

1.11. TEM of Mitochondrial Morphological

H1975 cells were seeded at a density of 3×10^6 cells in 10 cm culture plate and allowed to grow in 15 mL of growth medium at 37 °C for 24 h. Then, they were exposed to RPMI 1640 culture medium (control) or DCAFP solution at concentrations of 50 μg/mL for 24 h. The treatment medium was then removed and cells were washed three times with PBS. For the TEM analysis of cancer cells, cells from control group and from DCAFP treated groups were first fixed with 2.5% glutaraldehyde in phosphate buffer (0.1M，pH7.0) for more than 4h; washed three times in the phosphate buffer(0.1M，pH7.0) for 15min at each step; then postfixed with 1% OsO4 in phosphate buffer for 1-2 h and washed three times in the phosphate buffer(0.1M，pH7.0) for 15min at each step. The fixed cells were then dehydrated by a graded series of ethanol for about 15min at each step, then dehydrated by alcohol for 20min.In the end, transferred to absolute acetone for 20 min. The specimen were them placed in 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1h at room temperature, then transferred to 1:3 mixture of absolute acetone and the final resin mixture for 3 h and to final Spurr resin mixture for overnight. Specimen was placed in eppendorf contained Spurr resin and heated at 70℃ for more than 9 h. The specimen was sectioned in LEICA EM UC7 ultratome and sections were stained by uranyl acetate and alkaline lead citrate for 5 to 10min respectively and observed in Hitachi Model H-7650 TEM.

1.12. Cell Apoptosis

Apoptosis was measured by Annexin V-FITC/PI double staining according to the apoptosis detection kit (Boster Biological Technology co. ltd, China). Cells cultured in 6-well plates were treated with solutions. The cells were digested and washed with cold PBS buffer. Then the collected cells were re-suspended in 500 μL binding buffer and stained with 5 μL Annexin V-FITC and 5 μL PI for 15 min in the dark. The stained cells were analyzed by flow cytometry (Cytoflex, Beckman).

1.13. Cellular uptake investigation

The cellular uptake and distribution were examined by confocal microscopy. 5×10^4/well cells were seeded onto 24-well plates and were grown for 24 h. 1 μg of FAM-miRNA was complexed with DCA or DCAFP at room temperature for 20 min before use. After cell uptake for defined period, the cells were fixed with fresh 4% paraformaldehyde for 10 min and cell nuclei were counterstained with Hoechst 33342, and cell membrane was stained by WGA-647 respectively. The images were acquired on a confocal scanning laser microscope (CLSM, Nikon A1R).

Cellular uptake of DCA/miRNA and DCAFP/miRNA polyplexes was evaluated via flow cytometry using fluorescently-labeled miRNA. After incubated with polyplexes solution for 4 h at 37 ºC, the cells were collected and the fluorescence intensity of cells were analyzed using flow cytometry (Cytoflex, Beckman).

To elucidate the mechanisms underlying the cellular internalization of DCAFP/drug nanomicelles, hydrophobic dye Nile Red (NR) was encapsulated to DCAFP by the same method as DCAFP/GFT. The accumulation of NR in cells can be observed by confocal microscope. Cells were seeded on 6- well plate at a density of 2×10^5/well. After 24h, the medium was then replaced by NR or DCAFP/NR at a NR concentration of 5, 10, or 50 ng/mL and incubated for 4 h. After the medium was taken out, the cells were washed and were cultured with 1 mL fresh complete medium at defined time points. The NR accumulation in the cells were measured flow cytometry.

1.14. Cytotoxicity and Cell Proliferation

The MTT method was used to evaluate the cell viability. Prior to exposure to the solutions, the cells were seeded into a 96-well plate. After cultivation for 24 h, the cells were incubated with various concentrations of solutions at 37 °C for designated period. 5 mg/mL MTT solution was added to the 96-well plate and the mixture was incubated for 4 h. The formazan crystals were dissolved in 100 μL of DMSO per well and measured on a microplate reader (Model 680, Bio-Rad) at a wavelength of 570 nm. The untreated cells served as the 100% cell viability control and the completely dead cells served as the blank. The relative cell viability (%) related to control cells was calculated using the following formula: $V\% = ([A]_{experimental} - [A]_{blank})/([A]_{control} - [A]_{blank}) \times 100\%$, where V% is the cell viability (%), $[A]_{experimental}$ is the absorbance of the wells containing the treated cells, $[A]_{blank}$ is the absorbance of the blank, and $[A]_{control}$ is the absorbance of the wells containing the untreated cells.

Determination of live and dead cells. The cells were incubation with DC, DCA, DCAFP solutions for 24 h. After staining with Calcein-AM and PI, an Olympus IX71 biological inverted microscope (Japan) was used to observe the viable and dead cells.

1.15. Western Blot Analysis

Western blotting analysis was used in the analysis of the expression of pro caspase-3, cleaved caspase-3 in PC9 and H1975 cell lines. Membranes were blocked with Tris-buffered saline/0.1% Tween 20 (TBS/T) containing 5% bovine serum albumin (BSA) and incubated overnight at 4 °C with anti-AKT, anti-AKT (phospho S472+S474+S473) (Abcam, UK), or anti-GAPDH (Abcam, UK). The membranes were washed three times with TBS/T, incubated with goat anti-mouse horseradish peroxidase cell antibody (Boster Biological Technology co. ltd, China) for 2 h at 4 °C, washed. The membranes were then washed and immunoreactive bands were developed using an enhanced chemiluminescence reagent (GE Healthcare, Piscataway, NJ) and visualized by autoradiography kit (Kodak; Rochester, NY).

The expression of PTEN protein in PC9 and H1975 cell lines after incubated with naked anti-miR-21, DCA/anti-miR-21, or DCAFP/anti-miR-21 also analyzed by western bolt. Membranes were incubated overnight at 4 °C with anti-PTEN or anti-GAPDH.

1.16. Colony Formation and Cell Migration Assays

A total of 1500 H1975 cells per well were seeded into a 6-well plate and treated with GFT, DCAFP/GFT nanoparticles, or DGA nanoparticles (equal GFT dose of 1 μM). At the 10th day after treatment, the cells were washed with cold PBS twice and fixed with 3.7% formaldehyde. Cells were then dyed with crystal violet, and colony number in each well was counted.

Migration of H1975 cells were detected in 12-well plate. When reaching about 90% confluence, the cell layers were scratched with pipet tips and then treated with GFT, DCAFP/GFT nanoparticles, or DGA nanocomplexes at 37 °C, and wound closure was quantified at different time point.

1.17. Establishment of Animal Tumor Models

Animal experiments were performed in accordance with the regulations of China Animal Protection Network, and all protocols used in the study were approved by the Zhejiang University Animal Care and Use Committee. BALB/c female nude mice (6-8 weeks old, 18-22 g) were purchased from Zhejiang University Animal Care Center and maintained in a pathogen-free environment under controlled temperature (24 °C). NSCLC cell line H1975 cells were transplanted into the left abdominal of the mice to establish the tumor models.

To measure the effects on cancer recurrence, 18 days after 3×10^6 H1975 tumor cells were transplanted into the right flank of mice; the resulting tumors were ~400 mm^3 in volume. These tumors were then resected, leaving about 5% residual tumor tissue behind to mimic the presence of residual microtumors in the surgical bed. Briefly, animals were anesthetized in an induction chamber using isoflurane (up to 5% for induction; 3% for maintenance), and anaesthesia was maintained *via* a nose cone. The tumor area was clipped and aseptically prepped. Sterile instruments were used to remove approximately 95% of the tumor.

1.18. *In Vivo* Anti-tumor Therapy:

The animal experiments were performed in accordance with the China Animal Protection Law (CAPN), and the protocols were approved by the Zhejiang University Animal Care and Use Committee. The H1975 cells were inoculated subcutaneously in the right abdominal region of BALB/c nude mice. The tumors were allowed to grow to 100 mm^3 before the experiment began. Mice bearing H1975 tumors were randomly divided into 7 groups. Each group consisted of 5 mice treated with PBS, free GFT, anti-miR-21, DCAFP/anti-miR-21, DCAFP/GFT, and DGA namomedicine. The equivalent dose of GFT was fixed at 3 mg/kg. The treatment was performed every 3 days for 3 weeks, and the tumor growth was monitored using calipers twice a week. The tumor volume (V) was calculated using the following formula: $V\,(mm^3) = \pi/6 \times \text{length (mm)} \times \text{width (mm)}^2$.

1.19. Hematological Analysis

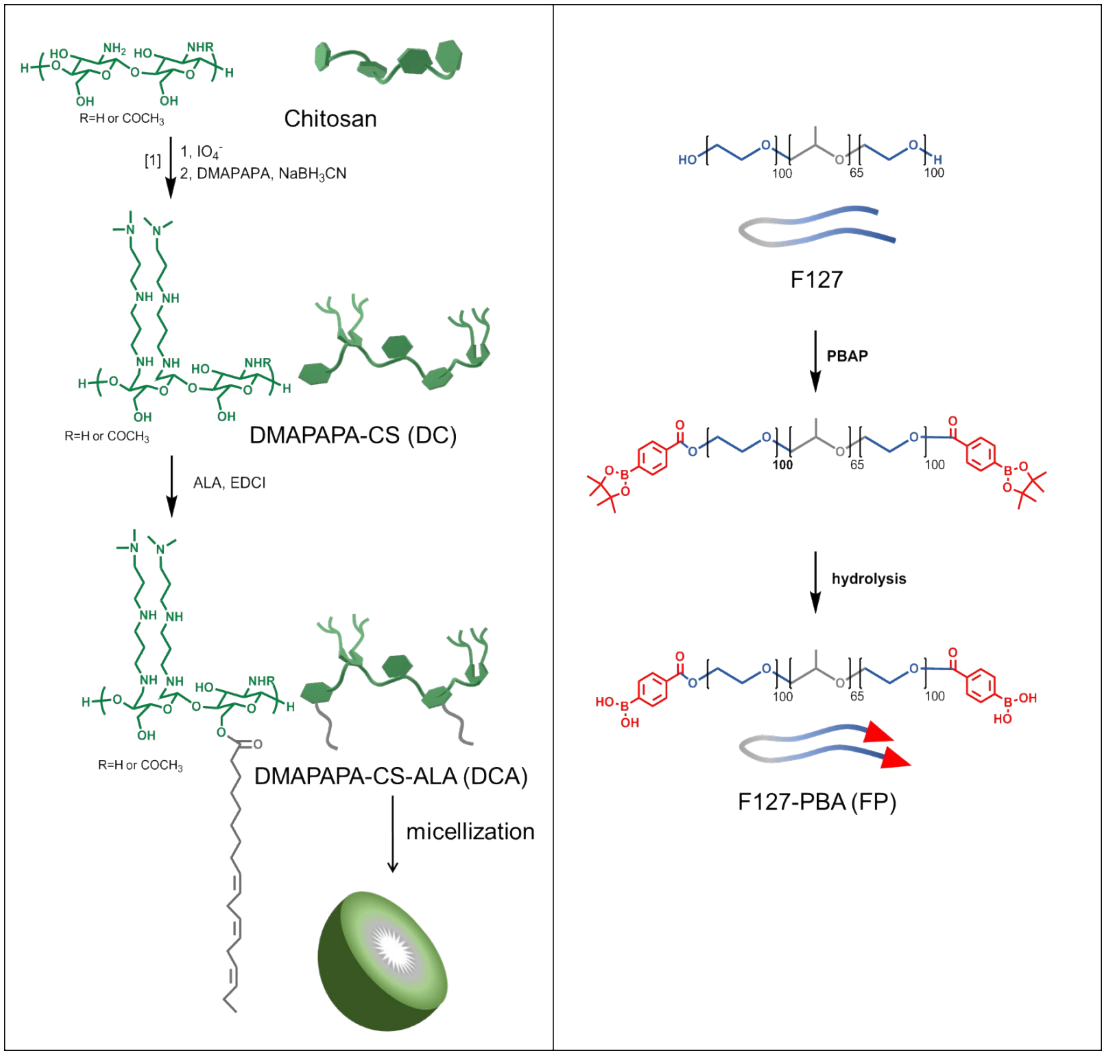
Hematological analysis was carried out to study acute side effects and toxicities caused by different formulations treatments. The female BALB/c mice (6–8 weeks old, 18-22 g) assigned into

3 groups at random (n = 3) and received the treatment of PBS, DCA (35 mg/kg), and DCAFP (35 mg/kg). The treatment schedule was the same as the *in vivo* anti-tumor experiment. The mice were sacrificed 24 h post the last injection, both whole blood and serum were collected. The hematological parameters were analyzed including aspartate aminotransferase [AST], alanine aminotransferase [ALT], and alkaline phosphatase [ALP], uric acid [UA], blood urea nitrogen [BUN].

1.20. Statistical Analyses

Experiments were repeated at least thrice. Data are the mean ± SD. Statistical calculations were made using Prism 7 (GraphPad, La Jolla, CA) with the indicated analytical methods. Data from nuclear magnetic resonance were analyzed by MestReNova (Mestrelab Research, Santiago de Compostela, Spain), and flow-cytometry data were analyzed using FlowJo 10 (FlowJo, Ashland, OR). Best-fit values and 95% CIs were calculated using indicated nonlinear regression. $p < 0.05$ was considered significant.

2. Supplementary Figures



Scheme S1. Reaction Scheme for the synthesis of DMAPAPA-CS-ALA (DCA) and F127-PBA (FP).

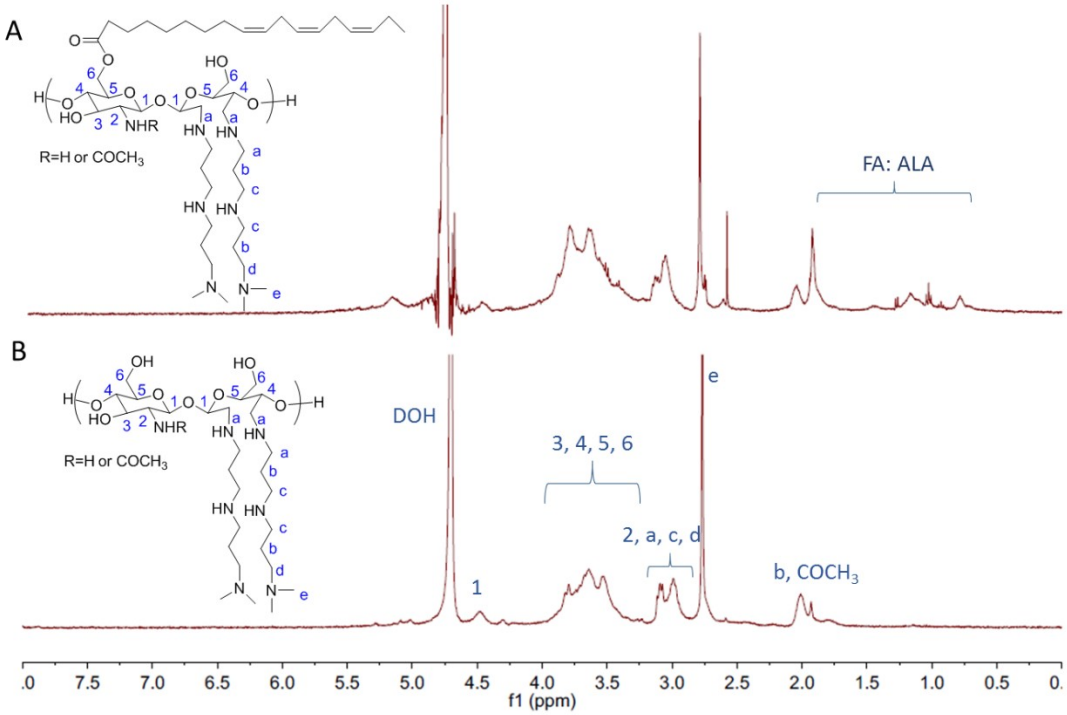


Figure S1. ^{1}H NMR spectrum of A) DCA and B) DC in D_2O.

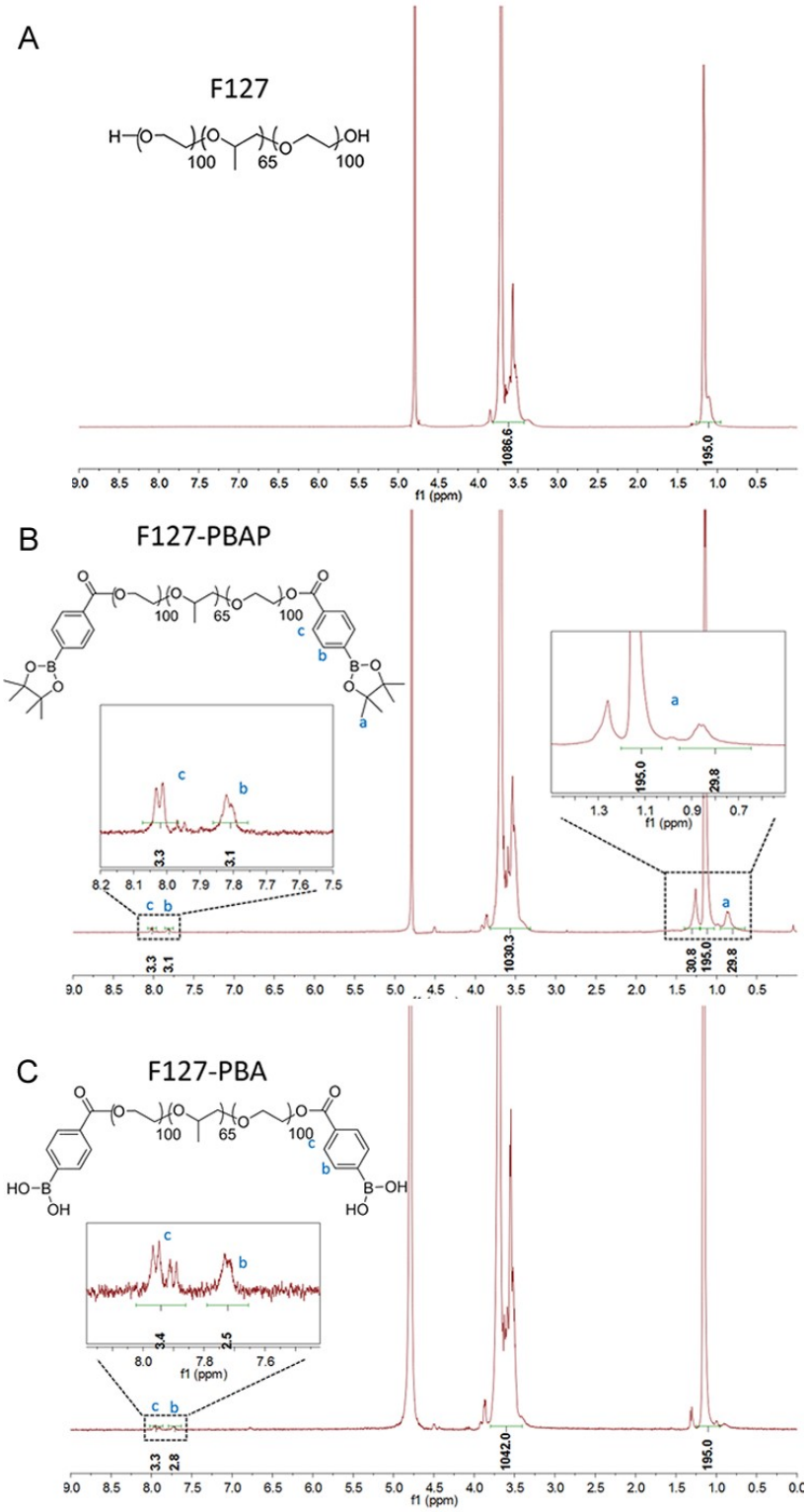


Figure S2. ^{1}H NMR spectrum of A) F127, B) phenylboronic acid pinacol ester conjugated F127 (F127-PBAP), and C) F127-PBA in D_2O.

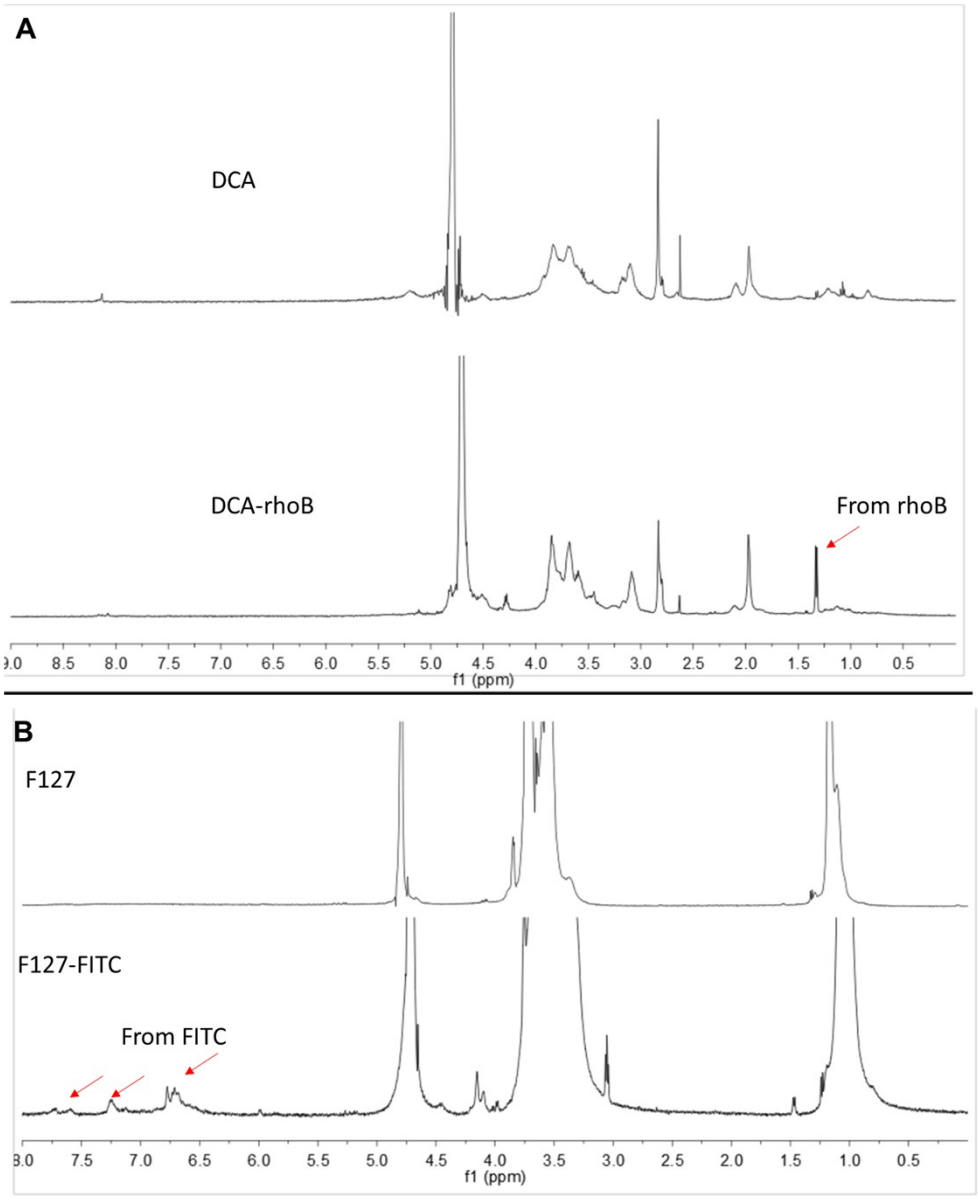


Figure S3.[1]H NMR spectrum of A) DCA-rhoB and B) F127-FITC in D_2O

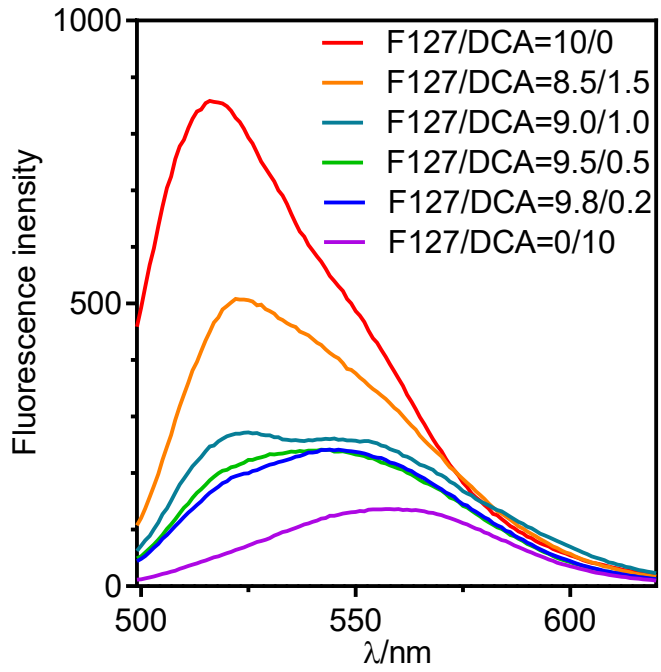


Figure S4. The fluorescence spectrum of DCAFP in different weight ratios of FP/DCA, F127 was labeled with FITC, and DCA was labeled with rhoB.

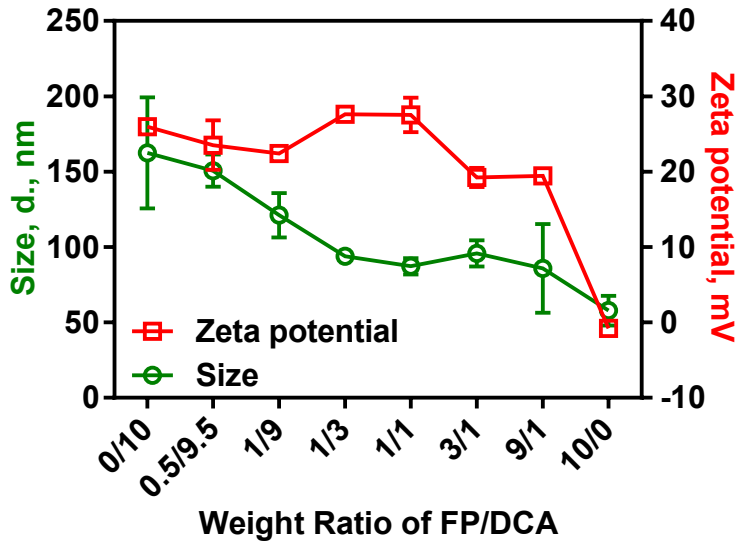


Figure S5. Size and zeta potential of DCAFP in different weight ratio of FP/DCA.

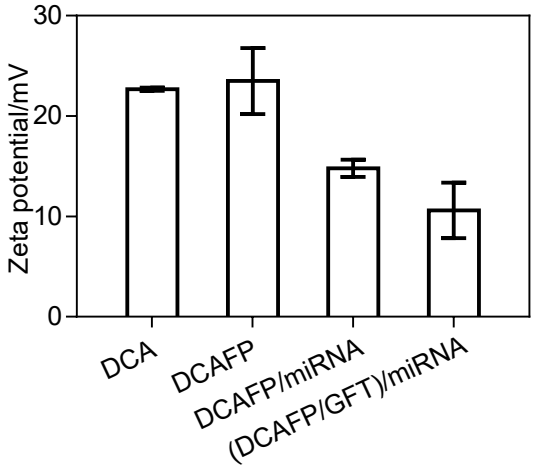


Figure S6. Zeta potential of DCA, DCAFP, DCAFP/miRNA(100/1), and (DCAFP/GFT)/miRNA (100/1).

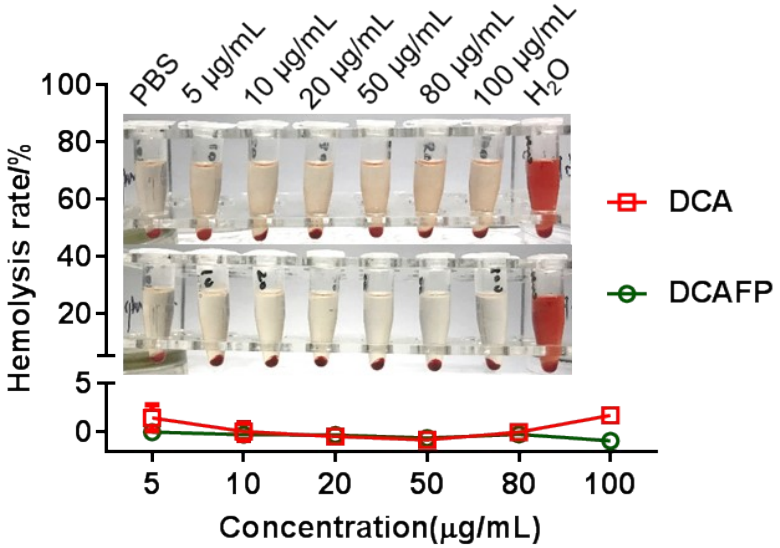


Figure S7. Blood hemolytic effects of DCA and DCAFP. The red blood cells are treated with DCA or DCAFP solutions in various concentrations from 5 to 100 μg/mL

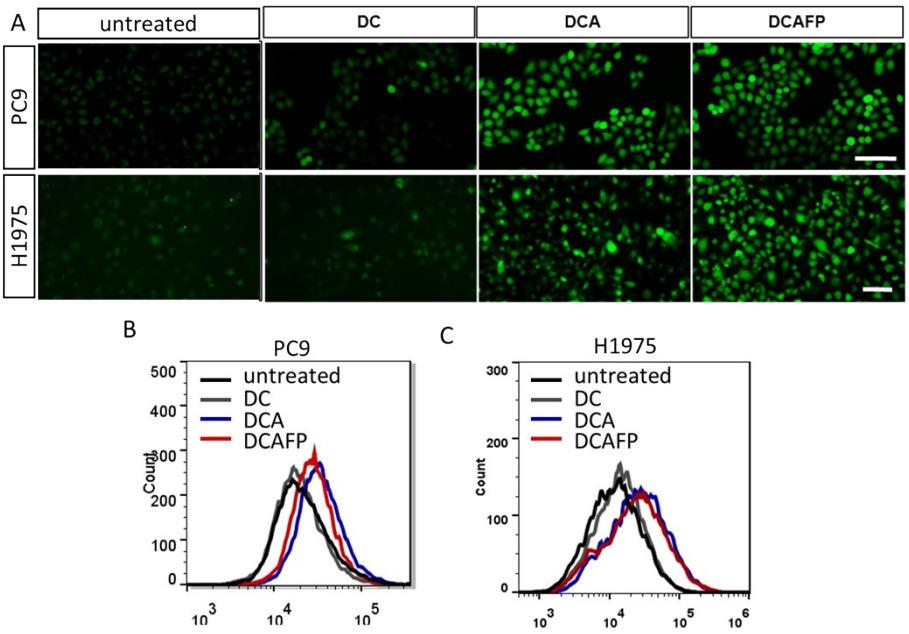


Figure S8. ROS measurement: A) microscopy images of ROS generation by DCFH-DA staining in PC9 and H1975 cells incubated with DC, DCA, or DCAFP (green for DCF; scale bar represents 100 μm). Flow cytometry analysis of ROS generation in B) PC9 and C) H1975 cells with different treatments.

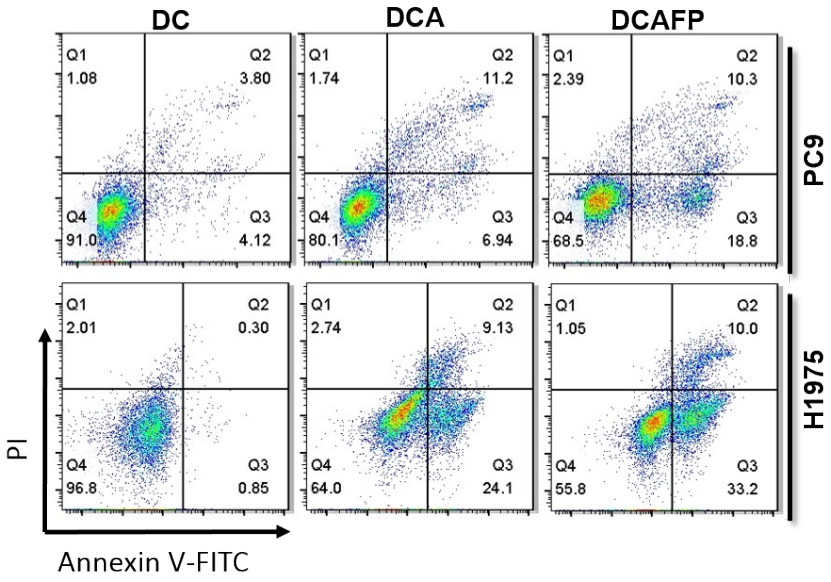


Figure S9. Cellular apoptosis assay of PC9 and H1975 cells after co-incubation with DC, DCA, or DCAFP solutions.

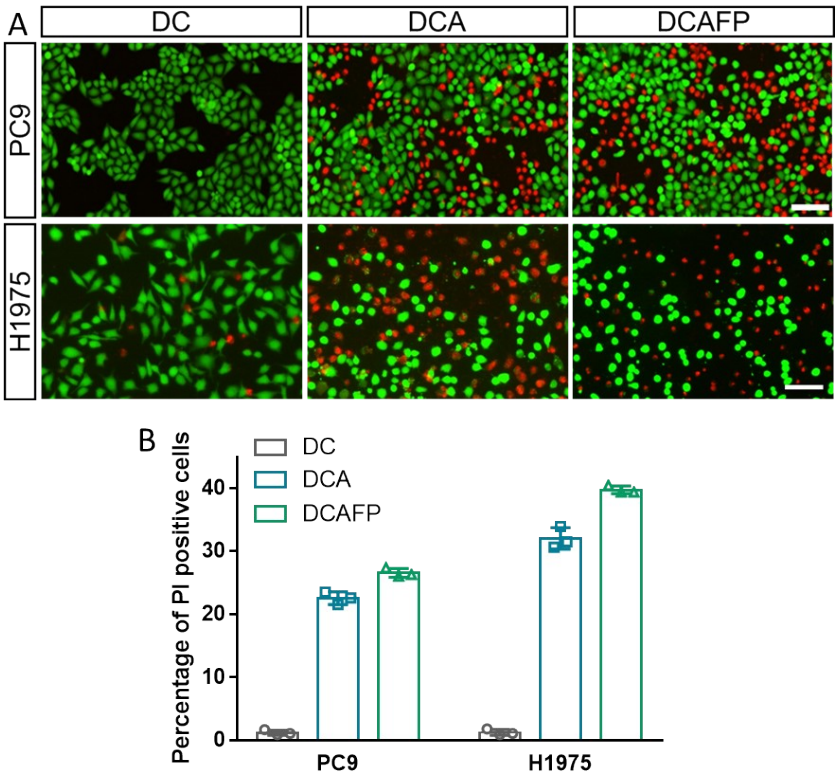


Figure S10. A) The images and B) PI-positive percentage of PC9 and H1975 cells stained by calcein AM (green)/propidium iodide (PI, red) after treating with DC, DCA, or DCAFP solutions for 24 h, Scale bar present 100μm.

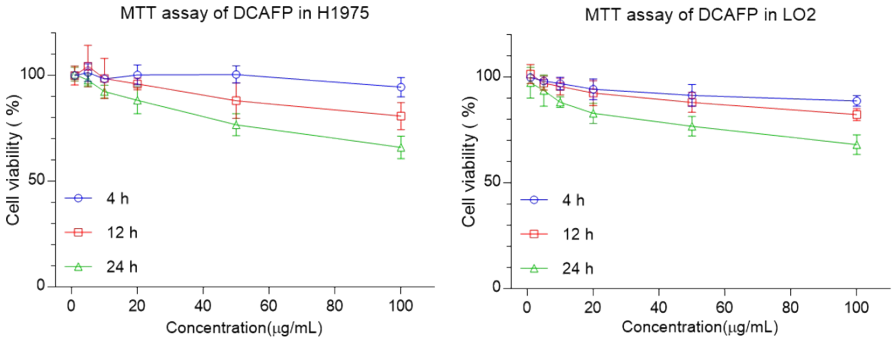


Figure S11. Cell viability of H1975 and LO2 cells co-cultured with DCAFP in different concentration for 4 h, 12 h, 24 h.

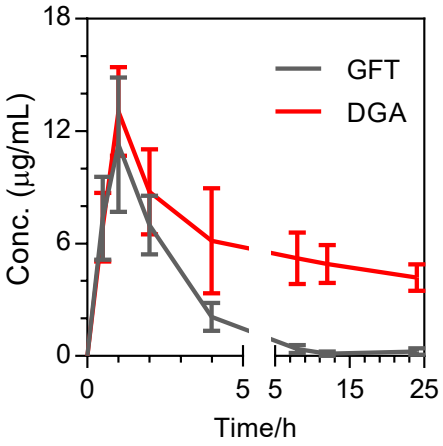


Figure S12. Plasma concentration-time curves of GFT after treatment of GFT or DGA nanomedicine at an equivalent GFT dosage of 10 mg kg^{-1} in mice (n = 3) from 0.5 h to 24 h.

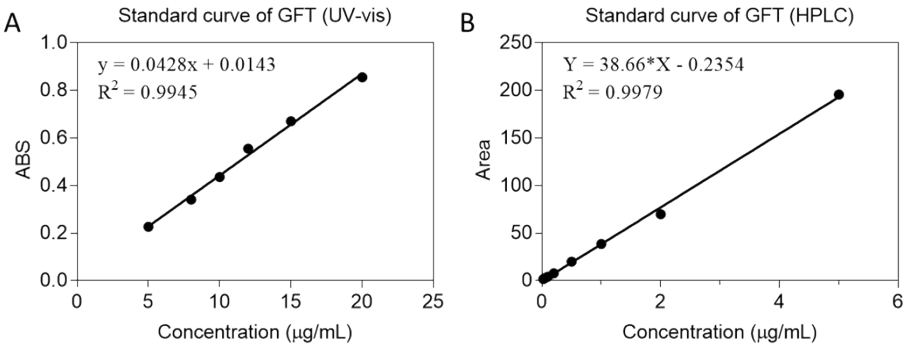


Figure S13. The standard curve of GFT by A) UV-vis and B) HPLC.

Table S1. Drug loading efficiency (DL) and encapsulation efficiency (EE) of nanoparticles.

Nanoparticles	**DL**	**EE**
DCA/GFT	3.64±1.36%	31.35±3.04%
DCAFP/GFT	7.28±0.81%	54.55±1.63%

Table S2. Size of nanoparticles determined by DLS.

NPs	**Size/nm**	**PDI**

DCA/GFT	120.9±30.51	0.219
DCAFP/GFT	129.4±9.26	0.349

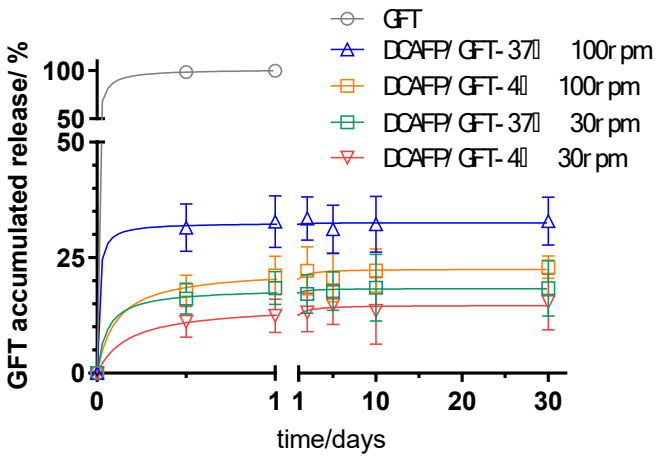


Figure S14. The GFT release from DCAFP/GFT in different condition.

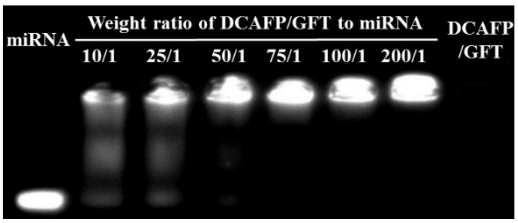


Figure S15. Agarose gel electrophoresis of the DCAFP/GFT/miRNA complexes with different weight ratios (w/w). DCAFP/GFT could completely retard migration of miRNA in the agarose gel at w/w of 75.

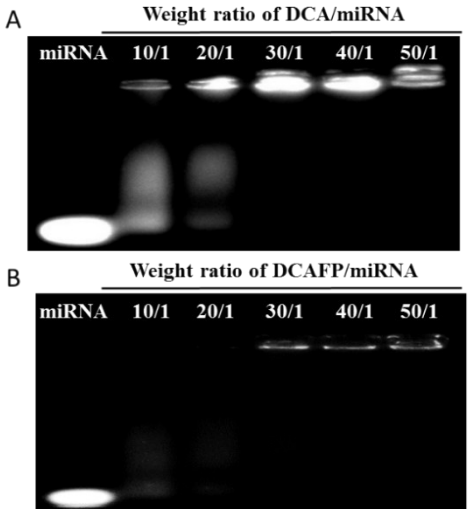


Figure S16. Agarose gel electrophoresis of the A) DCA/miRNA (NC-miRNA) and B) DCAFP/miRNA complexes with different weight ratios (w/w). Both DCA and DCAFP could completely retard migration of miRNA in the agarose gel at w/w of 30.

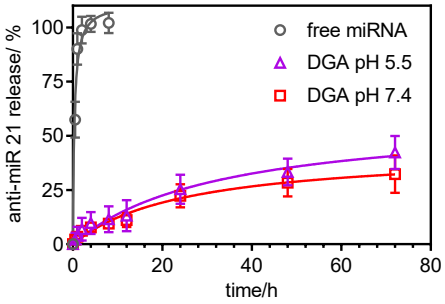


Figure S17. the in vitro anti-miR21 release behavior of DGA nanomedicine.

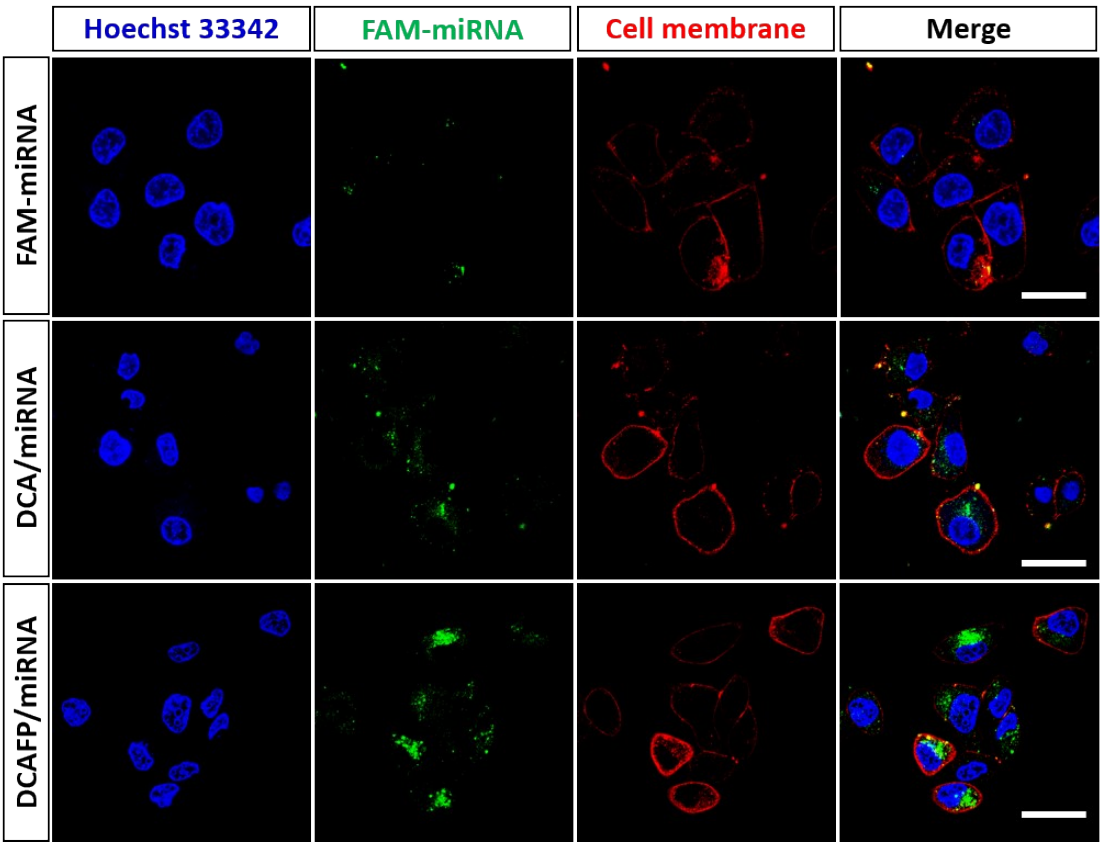


Figure S18. CLSM images of the PC9 cells after incubation with DCA/FAM-miRNA or DCAFP/FAM-miRNA (2 μg/mL FAM-miRNA) for 4 h. Hoechst 33342 (blue) and Alexa Fluor® 647 conjugate of wheat germ agglutinin (WGA-647, red) was used to stain cell nuclei and cell membrane. Scale bar present 30 μm.

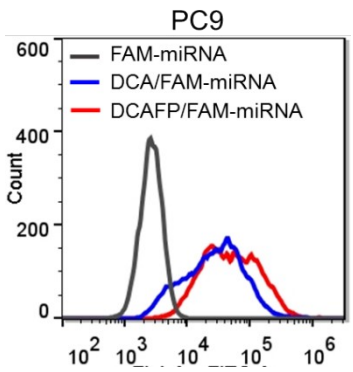


Figure S19. Cellular uptake analysis of naked FAM-miRNA, DCA/FAM-miRNA or DCAFP/FAM-miRNA (2 μg/mL FAM-miRNA) for 4 h in PC9 cells by flow cytometry

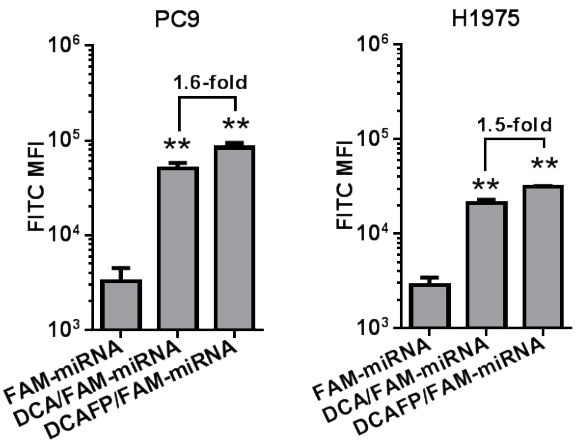


Figure S20. Flow cytometry analysis of FAM-miRNA in the PC9 and H1975 cells incubated with FAM-miRNA, DCA/FAM-miRNA or DCAFP/FAM-miRNA.

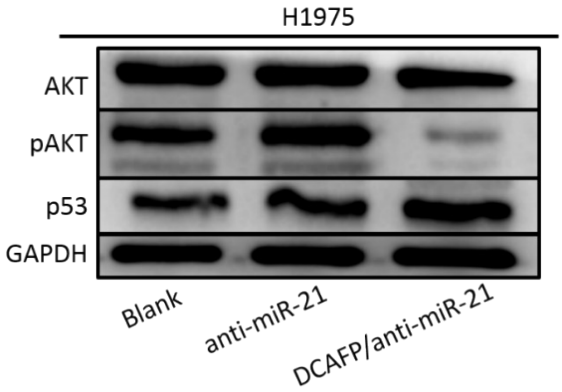


Figure S21. Analysis of AKT, pAKT, and p53 protein expression in H1975 cells from Western blot results.

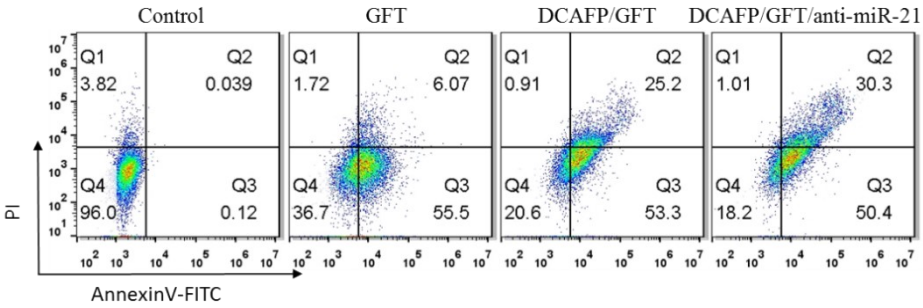


Figure S22. Cellular apoptosis assay of H1975 cells after co-incubation with GFT, DCAFP/GFT, or DGA solutions determined by Flow cytometry.

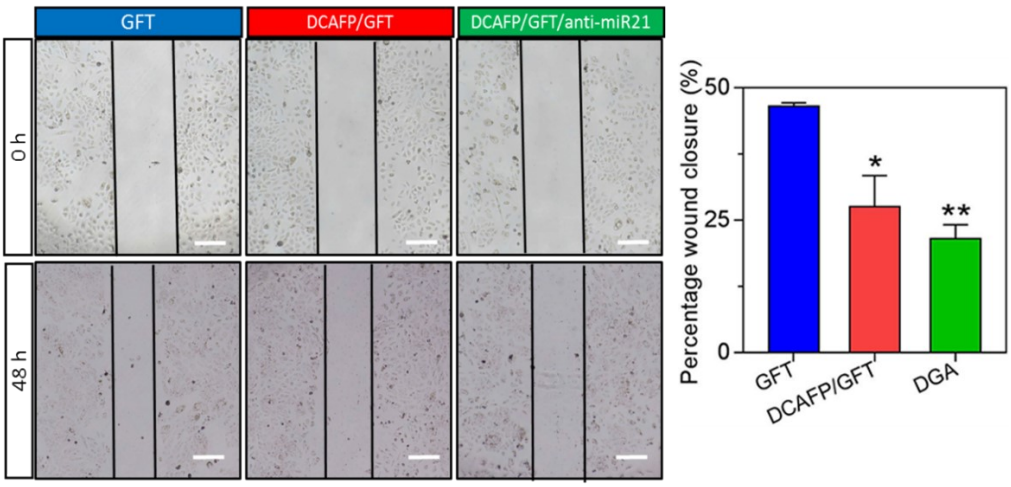


Figure S23. Wound healing assays in H1975 cells, representative images of cell migration after treatment with free GFT, DCAFP/GFT, and DGA. Scale bar present 150 μm.

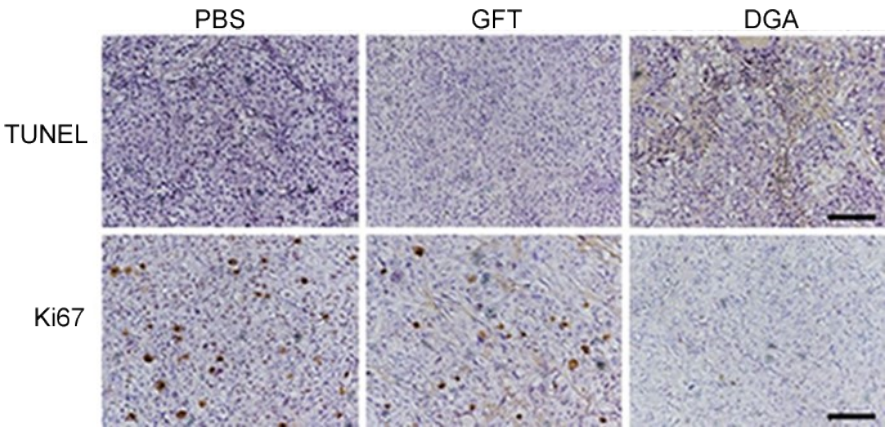


Figure S24. Ki67 and TUNEL immunohistochemical images of tumor tissues after mice were sacrificed at the last treatment after intravenous injection of various formulations.

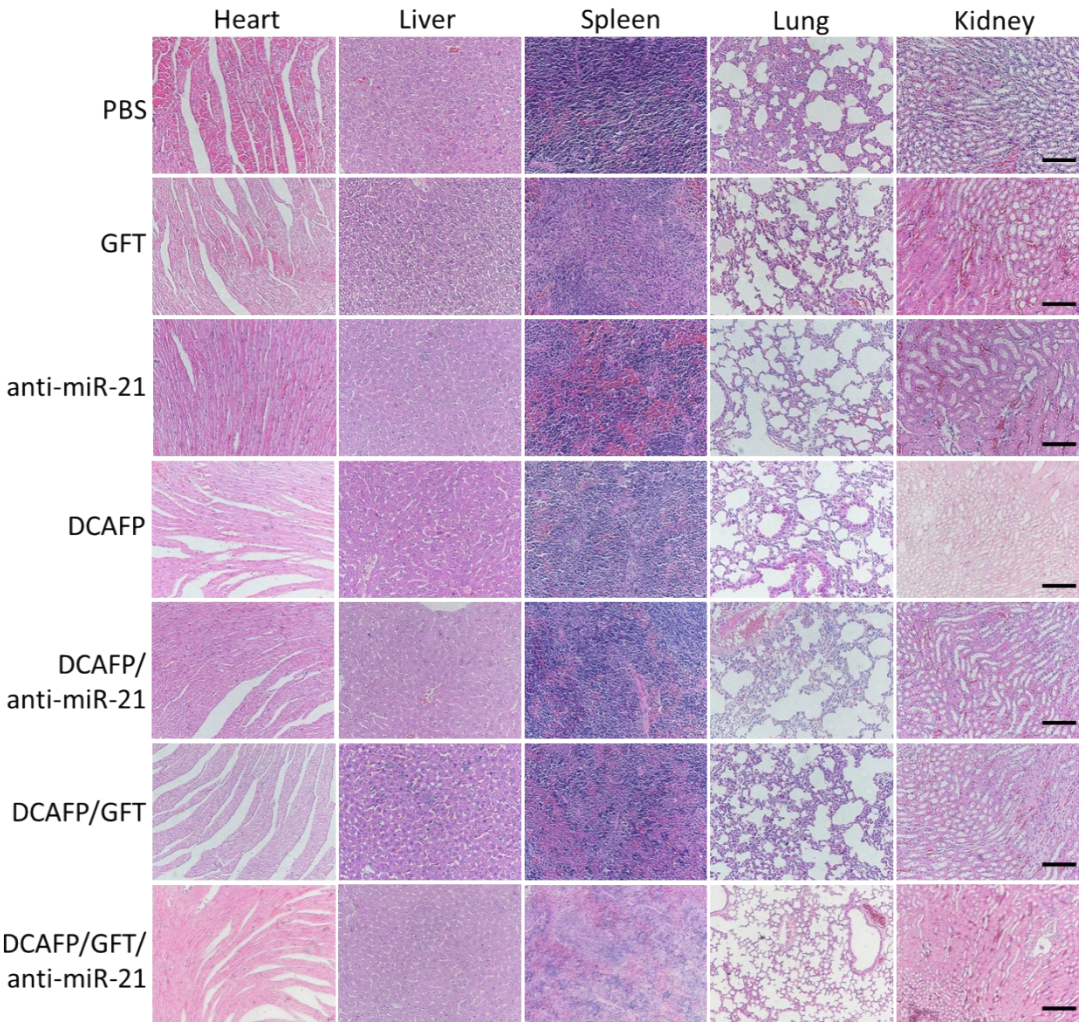


Figure S25. Histological sections of the major organs of mice with different treatments. Hematoxylin-eosin; scale bar present 100 μm.