



Monocyte Cell Membrane-derived Nanoghosts for Targeted Cancer Therapy

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ELECTRONIC SUPPLEMENTARY INFORMATION

Experimental Section

Materials:

RPMI 1640, fetal bovine serum (FBS) and PBS were purchased from Life Technologies (Carlsband, CA, USA). Doxorubicin.HCl was purchased from LC Laboratories (Woburn, MA, USA) and PLGA from Sigma-Aldrich (St. Louis, MO, USA; # 430471). MCF-7 cells were obtained from ATCC (Manassas, VA, USA). Primary antibodies used were anti-sodium potassium ATPase (Abcam, Cambridge, UK; ab76020), anti-Integrin $\alpha 4$ (Cell Signaling Technologies, Beverly, MA, USA; D2E1, #8440) and anti-GAPDH (Cell Signaling Technologies, #2118). Secondary antibodies (anti-rabbit IgG, HRP-Linked, #7074) were purchased from Cell Signaling Technologies.

Synthesis of PLGA NPs:

Initially, 1 mg of Dox.HCl was neutralized using 3 molar excess of triethyl amine. 10 mg of PLGA and 1 mg of neutralized Dox were weighed and dissolved in acetonitrile and added dropwise to 3 mL of DI water while stirring. The solution was kept under continuous stirring for 2 h for NP self-assembly. The PLGA NPs were centrifuged at 12,000 rpm for 10 min and washed twice with DI water. The PLGA NPs were resuspended in DI water and stored at 4 °C.

To synthesize FITC-loaded PLGA nanoparticles, 0.2 mg of FITC and 10 mg of PLGA were dissolved in acetone (1 ml) and added dropwise to 3 ml of DI water. The reaction mixture was stirred continuously for 2 h, followed by purification of FITC-loaded PLGA nanoparticles using 10 k Da MWCO filters. The concentrated nanoparticles were resuspended in DI water and used for drug-uptake experiments.

Cell culture

U937 monocytes and MCF-7 cells were grown in RPMI-1640 medium with 10% FBS and 1% penicillin-streptomycin. Mouse skeletal myocytes were grown in DMEM medium supplemented with 10% fetal bovine serum. The cells were incubated at 37°C in the presence of 5% CO₂.

Cell membrane extraction:

U937 monocyte cell membranes were extracted using a previously reported method.¹ Briefly, U937 cells were harvested and washed 3 times with PBS, resuspended in hypotonic lysis buffer with phosphatase inhibitor and kept on ice for 5 min. The cells were then homogenized using a Dounce homogenizer (Sigma-Aldrich). The homogenate was centrifuged at 3000 x g for 5 min and the supernatant was further centrifuged at 10,000 x g for 20 min. The pellet was discarded and the supernatant was further centrifuged at 100,000 x g for 35 min. The supernatant was discarded, and the pellet was washed with PBS and stored at 4 °C for further use.

Synthesis of nanoghosts:

The U937 monocyte cell membrane extract was first extruded through a polycarbonate membrane of 400 nm pore size. Next, the membranes were mixed with Dox-loaded or FITC-loaded PLGA NPs and extruded through a polycarbonate membrane of 200 nm pore size, to produce uniformly sized nanoghosts below 200 nm in diameter. The nanoghosts were further centrifuged at 300 x g for 4 min to remove any precipitates formed during the extrusion process.

Nanoparticle characterization:

The particle size, polydispersity index (PDI) and zeta potential of freshly prepared nanoghosts and PLGA NPs (1 mg/mL) were determined by dynamic light scattering (scattering angle: 90°) using a DTS Zetasizer 3000 HAS (Malvern Instruments, Worcestershire, UK). Dox loading was quantified by measuring the absorbance of doxorubicin at 480 nm against a standard curve using a Nanodrop 2200 reader (Thermo Scientific, Wilmington, DE, USA). All measurements were done in triplicates.

Nanoghost membrane protein characterization:

The protein concentrations of cell membranes isolated from U937 or nanoghosts were first standardized using the Bradford

assay. 15 µg of protein from each sample was loaded and electrophoretically separated by SDS-PAGE (Mini-PROTEAN® TGX™ Precast Gels, Bio-Rad), followed by primary and secondary detection of three markers GAPDH, NaK ATPase and CD49d, signal development using ECL reagent (Amersham ECL Prime Western Blotting Detection Reagent, GE Healthcare, Pittsburgh, PA, USA) and imaging (GBox Western Blot Imaging System, Syngene, Frederick, MD, USA). Western blot studies were repeated twice.

In vitro drug release:

Dox release from nanoghosts and PLGA NPs was determined over 72 h in phosphate-buffered saline (PBS, pH 7.4) at 37 °C. Briefly, 100 µL aliquots of nanoghosts and PLGA NPs containing equal amounts of Dox were placed in mini dialysis vials (Thermo Fisher, Wilmington, DE, USA) that have a molecular weight cut-off of 3,500 Da. The vials were immersed in 4 L of PBS under constant stirring at 100 rpm. Samples were retrieved at pre-determined time points and the amount of Dox remaining was measured by absorbance at 480 nm using a NanoDrop 2200 reader.

NP serum stability:

Dox-loaded nanoghosts and PLGA NPs (1 mg/mL) were dissolved in PBS containing 10% FBS. NP diameters were then monitored at various time points (0, 1, 3, 5, 7, 24, 48, 72, 96 and 120 h) by dynamic light scattering.

Cellular uptake studies:

MCF-7 cells were seeded onto 12-well plates with a seeding density of 100,000 cells/well and incubated for 24 h at 37 °C in the presence of 5% CO₂. Dox-loaded nanoghosts and PLGA NPs were incubated with the cells for 2, 4 and 6 h. Subsequently, the cells were trypsinized and washed twice with PBS. The median fluorescence intensity of Dox-positive cells and percentage of Dox-positive cells were analysed using flow cytometry.

Cytotoxicity studies:

MCF-7 cells were seeded into 96-well plates at a seeding density of 3,000 cells/well and incubated for 24 h at 37 °C in the presence of 5% CO₂. Dox-loaded nanoghosts and PLGA NPs were incubated at various concentrations with the cells for 6 h. Subsequently, the cells were washed and replaced with fresh media for another 72 h. Cell viability was determined using an MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) according to the manufacturer's instructions.

References

- 1 R. H. Fang, C. M. J. Hu, B. T. Luk, W. W. Gao, J. A. Copp, Y. Y. Tai, D. E. O'Connor and L. F. Zhang, *Nano Lett.* 2014, **14**, 2181-2188.