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Preparation and characterization of macrophage membrane camouflaged cubosomes as a stabilized and immune evasive biomimetic nano-DDS

Supplementary Information

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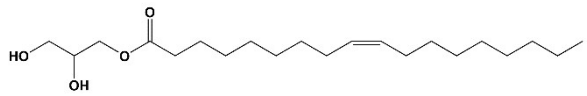
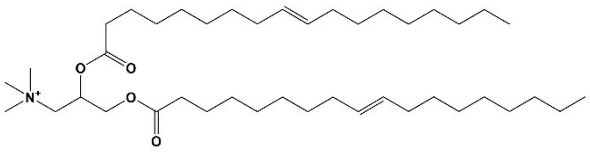
Content

1. **Chemical structures of CB ingredients**
2. **Summary of previous studies about CBs surface modification**
3. **Cell membrane protein/phospholipid Quantification**
4. **MTT assay result**
5. **Colon26 internalization efficacy investigation using CLSM**
6. **Mouse plasma preparation**
7. **Biodistribution study results**
8. **DOX encapsulation efficacy investigation**
9. **Additional SAXS investigation results**

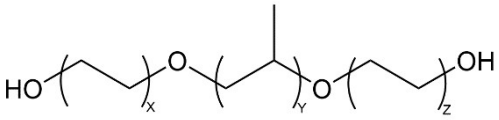
1. Chemical structures of CB ingredients



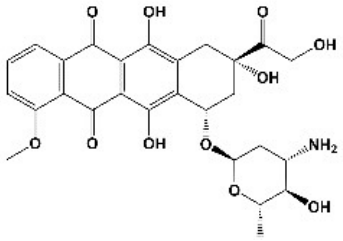
Monoolein



DOTAP



Pluronic F127



Doxorubicin

Figure S-1. Chemical structures of CB ingredients used in this study.

2. Summary of previous studies about CBs surface modification

Table. S-1 Previous studies about CBs surface modification

Surface Modifier	Modification Material	Function	Ref
Polymer	PEG	Stabilize CBs; Extend the circulation timespan *in vivo*.	1, 2, 3, 4, 5
	Poly-ε-lysine	Stabilize CBs in serum; Sustain drug release.	6
	Chitosan	Sustain drug release; Enhance bioavailability.	7
		Enhancing the immune response for vaccines.	8
	Hyaluronic acid	CD44 targeting ability	9
	Biotin-based block copolymer	Active targeting	10
Protein/Peptides	Antimicrobial peptides	Antibacterial	11
	Affimer	Cancer cell targeting	12
	Lactoferrin	Cancer cell targeting	13
	Cell-Penetrating Peptides	Skin penetration	14
	Odorranalectin	Improve brain drug delivery	15
Other	Folate	Tumor targeting	16, 17

3. Cell membrane protein/phospholipid quantification

Extracted cell membranes were further analyzed and quantified by the membrane-associated proteins and membrane-associated phospholipids, respectively using a Pierce® BCA Protein Assay Kit and, LabAssay™ Phospholipid Kit (Fuji Film). Generally, cell membrane vesicles extracted from 1×10^8 J774.1 contain ~1.53 mg cell membrane-related protein and ~0.21 mg phospholipid. The protein to phospholipid ratio was ~7.29.

4. MTT assay result

MTT assays were performed using HEK293 cells. Cells were seeded in a 96-well plate at a density of 1×10^4 cells/well and cultured for 24 hours. After removing the medium, fresh medium containing various concentrations of CBs (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, calculated based on MO weight) was added to the wells. After 24 and 48 hours of incubation, MTT solution was added to each well at a final concentration of 0.5 mg/mL, followed by 4 hours of incubation. The 96-well plates were then centrifuged at 1000 g for 5 minutes, and the medium was carefully removed before adding DMSO (100 μL per well) to dissolve the formazan crystals. The optical density (OD) of the resulting solution was measured at 570 nm using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer, Bio Rad, USA).

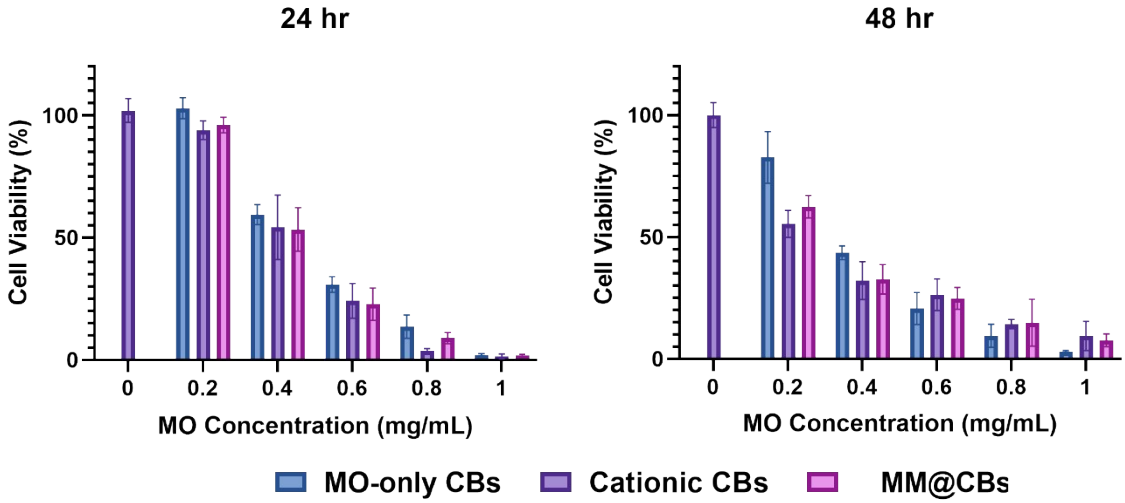


Figure S-2. MTT Assay Results. HEK293 cells were treated for 24/48 hours with MO-only CBs, cationic CBs, and MM@CBs at different concentrations (calculated according to the MO concentration). Error bars represent ± *s.d.* n=3.

5. Colon26 internalization efficacy investigation using CLSM

The Colon26 internalization efficacy investigation was carried out as described in experimental section.

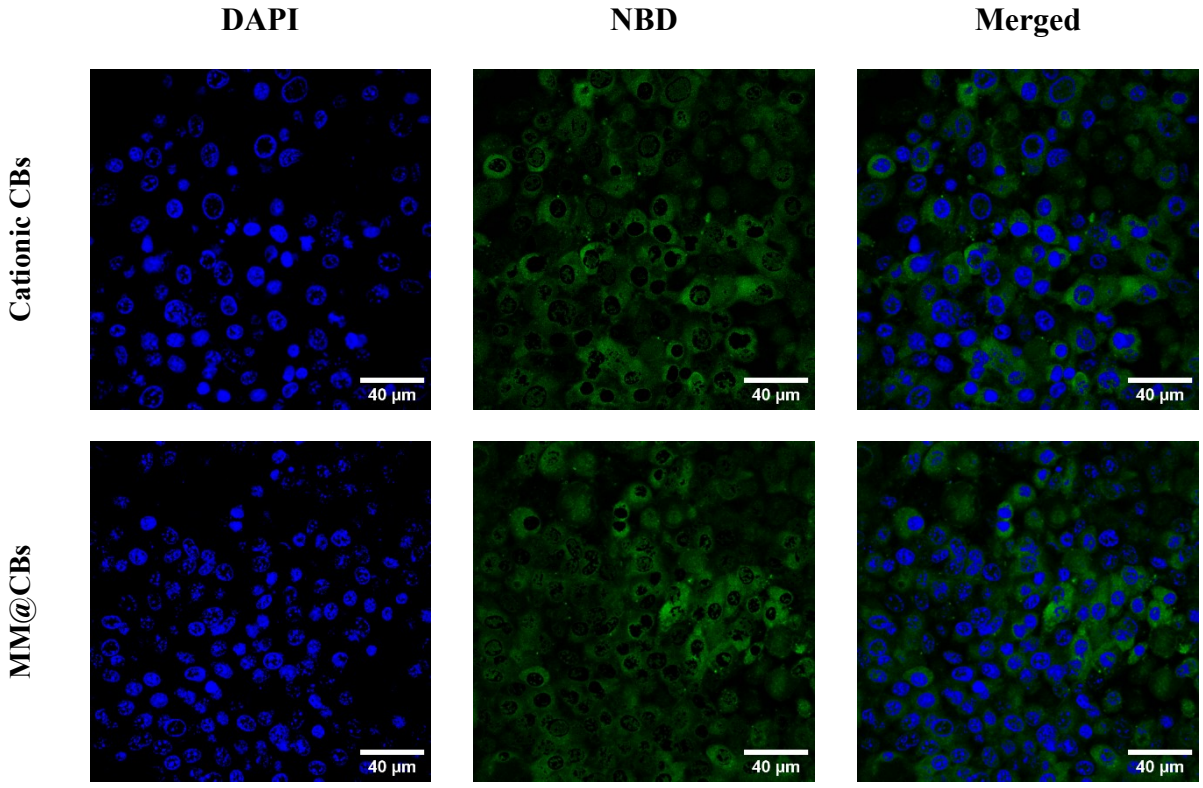


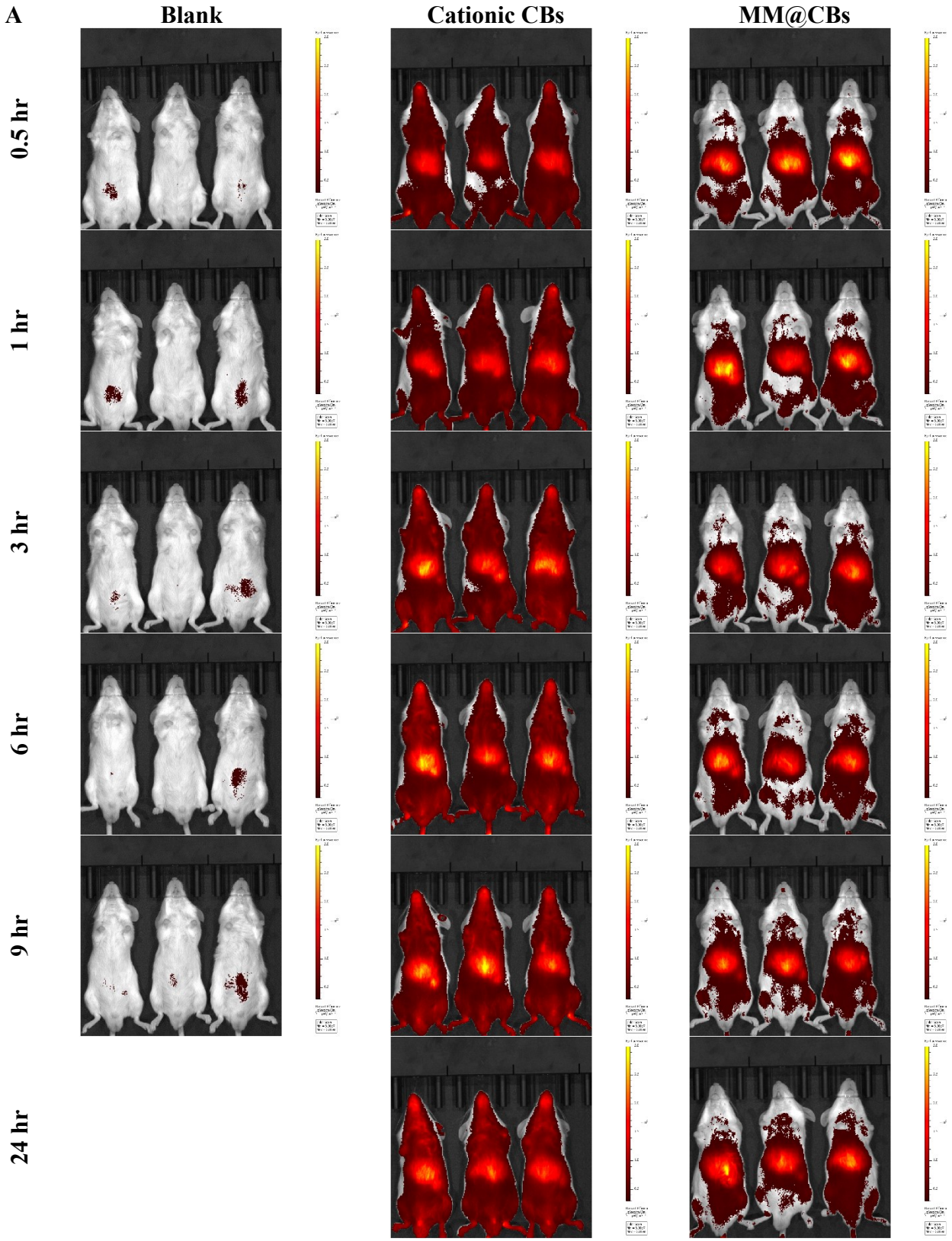
Figure S-3. Colon26 internalization efficacy investigation. Confocal laser scanning microscopy (CLSM) images of Colon-26 cells after 4 hours treatment with NBD-PE doped cationic CBs and MM@CBs (0.15 mg/mL MO). The three columns are corresponding to the DAPI channel, NBD channel and merged pictures respectively. Scale bar = 40 µm.

6. Mouse plasma preparation

Blood samples were collected from BALB/c mice via the inferior vena cava under anesthesia. Whole blood was centrifuged at 1800 g and 4℃ for 15 min. The supernatant was then ultracentrifuged at 50,000 g and 4℃ for 30 min. The resulting plasma was collected for further experiments.

For DLS and ζ-potential analysis, 50 μL of plasma was diluted in 1 mL of ultrapure water. The hydrodynamic diameter and ζ-potential were measured in triplicate at 25℃. The mean hydrodynamic diameter of the mouse plasma was 35.42 ± 0.63 nm, with a polydispersity index (PDI) of 0.53 ± 0.01. The ζ-potential was –19.50 ± 2.92 mV.

59 **7. Biodistribution study results**



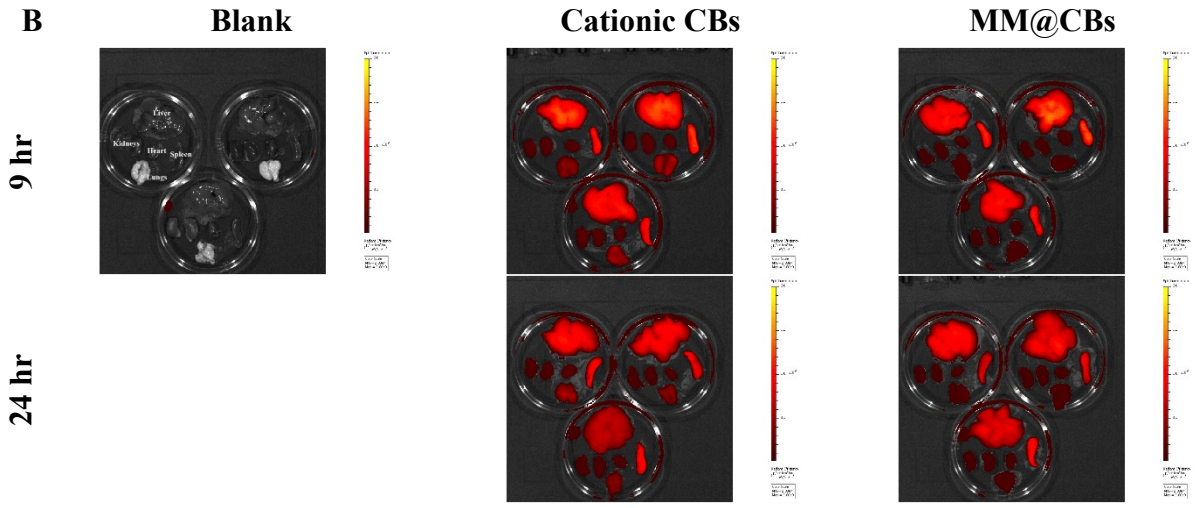


Figure S-4. Biodistribution investigation results. (A) *In vivo* imags of BALB/c mice after *i.v* injection of PBS (blank control group), cationic CBs and MM@CBs (100 µL, 2 mg/mL MO) at 0.5, 1, 3, 6, 9 and 24 hr post-injection. **(B)** *Ex vivo* images of collected mice organs at 9 and 24 hr post-injection. Color scale ranges from 3×10^7 to 3×10^8 ($\frac{p/sec/cm^2/sr}{\mu W/cm^2}$), *n*=3.

60

8. DOX encapsulation efficacy investigation

DOX concentration was analyzed using a fluorescence spectrometer (FP-8500, JASCO, Japan), excitation wavelength was set at 485 nm and the fluorescence intensity was detected at 556.5 nm. The DOX concentration-fluorescence intensity was calibrated (0-10 μM range).

The eluted free DOX solutions were diluted 6 times before fluorescence spectrometer measurement. The free DOX concentrations were calculated according to the calibration curve. The DOX encapsulation efficacy results are shown below.

Table.S-2 DOX encapsulation efficacy result

CB Formulation	Cationic CB + DOX	MM@CB +DOX
Total DOX con. (μM)	280.46	233.72
Eluted Free DOX con. (μM)	22.65 ± 0.44	32.04 ± 0.19
Encapsulation Efficacy (%)	91.93 ± 0.16	86.29 ± 0.08

9. Additional SAXS investigation results

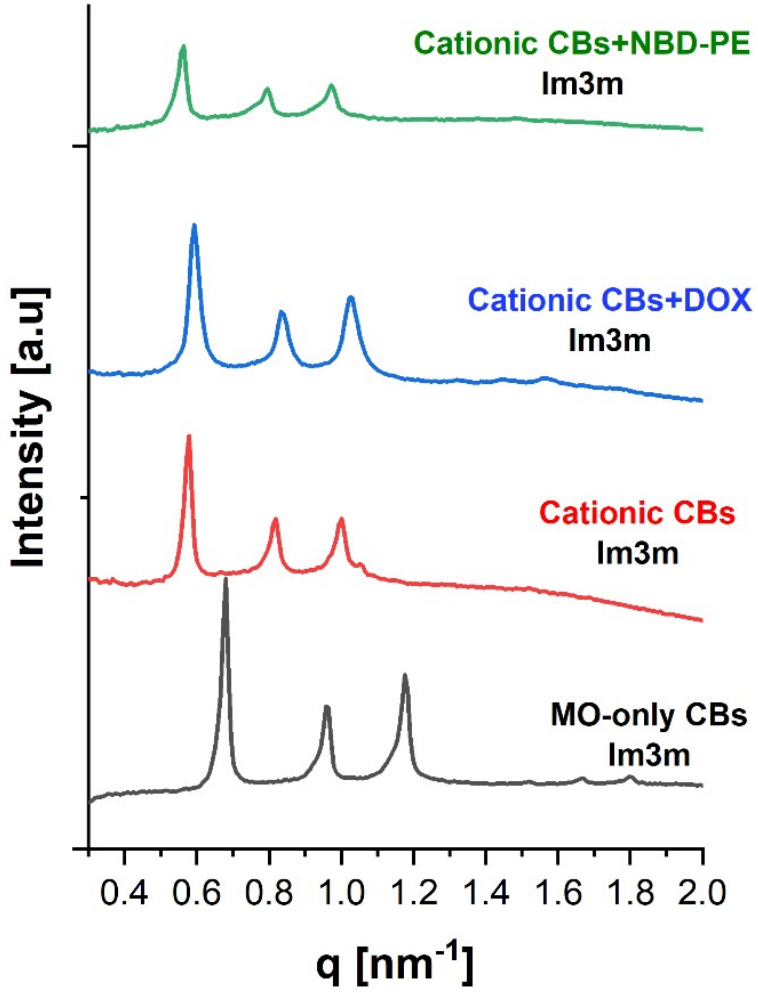


Figure S-5. Additional SAXS patterns. From bottom to top: MO-only CBs, cationic CBs, cationic CBs doped with 0.5 wt% DOX, and cationic CBs doped with 0.5 wt% NBD-PE. All the samples contain 20 mg/mL MO. Measurements were carried out at 37°C.

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