Supplementary materials

Regulating Tumor-associated Macrophage Polarization by Cyclodextrin-modified PLGA Nanoparticles Loaded with R848 for Treating Colon cancer

Haohua Yuan[1], Huan Gui[1], Shuanghui Chen[1], Lan Zhu[1], Chenglv Wang[1], Qianyu Jing[3], Hang Lv[1], Quan Wan[3], Shuyi Wang[1], Shengwen Zhou[1], Xiaodong Ren[1], Yingjie Nie[1,2*], Linzhao Li[1*]

[1] Medical College of Guizhou University, Guiyang, Guizhou Province, 550025, People's Republic of China

[2] NHC Key Laboratory of Pulmonary Immunological Diseases, Guizhou Provincial People's Hospital, Guiyang, Guizhou Province, 550002, People's Republic of China

[3] School of Preclinical Medicine of Zunyi Medical University, Zunyi, Guizhou Province, 563000, People's Republic of China

*Correspondence: Yingjie Nie, Medical College of Guizhou University, Guiyang, Guizhou Province, 550025, People's Republic of China; NHC Key Laboratory of Pulmonary Immunological Diseases, Guizhou Provincial People's Hospital, Guiyang, Guizhou Province, 550002, People's Republic of China, Tel: +86-085188274013, Email: nienyj@hotmail.com; Linzhao Li, Medical College of Guizhou University, Guiyang, Guizhou Province, 550025, China, Tel: +86-085188274016, Email: lzli@gzu.edu.cn

Material and methods

Chemicals

Ethanol (J&K Scientific Ltd., China), Trizol reagent (YEASEN, Shanghai, China), Hifair 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) (YEASEN, Shanghai, China), SYBR Green PCR master mix (YEASEN, Shanghai, China).

UV–Vis Spectroscopy

A volume of the sample solution was transferred to a colorimetric dish. The blank was corrected with acetonitrile. Subsequently, the drug absorbance of R848 was measured using an ultraviolet spectrophotometer at 254 nm.

Hemolysis test

The orbital blood of C57BL/6 mice was collected and placed in a centrifuge tube containing heparin. It was then immediately centrifuged at 3000 rpm for 15 minutes. After discarding the supernatant, an appropriate amount of normal saline was added and centrifuged again. This step was repeated until the supernatant became clear. The sediment at the bottom, which consisted of the red blood cells, was collected. An appropriate amount of physiological saline was added to adjust the volume and prepare a 2% red blood cell suspension (ensuring thorough shaking). The prepared red blood cell suspension was stored in a 4°C refrigerator for later use. CD@NPs and CD@R848@NPs nanoparticles were diluted with physiological saline to concentrations of 25, 50, 75, and 100 μg/mL respectively (total volume 0.75 ml) for the experimental group samples. The negative control group and the positive control group were diluted with physiological saline. Saline or distilled water was used as a replacement, followed by the addition of 0.75 ml of the 2% red blood cell suspension to each group. The mixtures were carefully stirred and immediately placed in a water bath with a constant temperature of 37°C for 3 hours. Subsequently, the

solutions of each group were transferred to clean 1.5 mL centrifuge tubes, centrifuged at 3000 rpm for 10 minutes, and the appearance of the supernatant in each group was observed visually.

H&E staining

The excised tissues were fixed in a 4% paraformaldehyde solution for a minimum of 24 hours. After dehydration, the tissues were embedded in paraffin and sectioned into 5 μm thick slices. The tissue paraffin sections were then deparaffinized using xylene and graded alcohol (100%, 85%, 75%) before being washed with distilled water. Subsequently, the tissue sections were stained with hematoxylin and eosin (H&E) and observed under an optical microscope (CIC, XSP-C204) to record their histological properties.

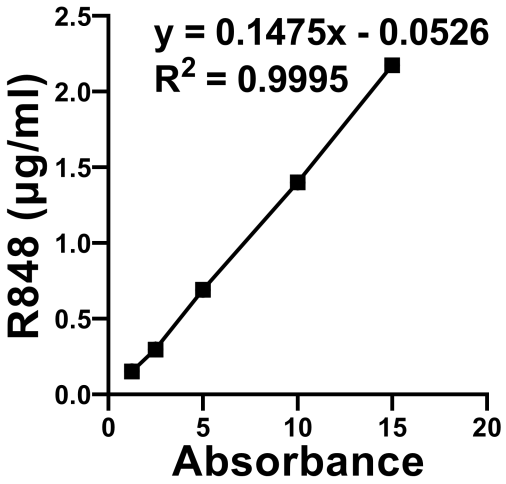
Supplementary Tables

Supplementary table 1: Primers used to assay expression levels of M1-associated genes and M2-associated genes.

Gene name	forward primer	reverse primer
β-actin	5′-ACGCATGTACGTAGCCATCC-3′	5′-CTCTCAGCTGTGGTGGTGAA-3′
arg1	5′-CAAGACAGGGCTCCTTTCAG-3′	5′-TGGCTTATGGTTACCCTCC-3′
mrc1	5′-CAGACAGGAGGACTGCGTGG-3′	5′-TGCCGTTTCCAGCCTTTCCG-3′
cd80	5′-TCAGTTGATGCAGGATACACCA-3′	5′-AAAGACGAATCAGCAGCACAA-3′
nos2	5′-CCACCCGAGCTCCTGGAAC-3′	5′-CCCTCCTGATCTTGTGTTGGA-3′
TNF-α	5′-CCCACGTCGTAGCAAACCAC-3′	5′-GCAGCCTTGTCCCTTGAAGA-3′

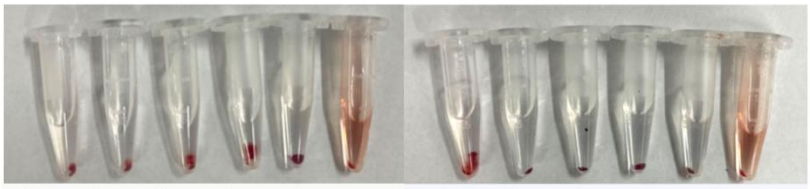
Supplementary Figures

Supplementary figure 1



Supplementary figure 1. The standard curve of R848 by UV method. The data are expressed as the mean ± SD of three independent measurements.

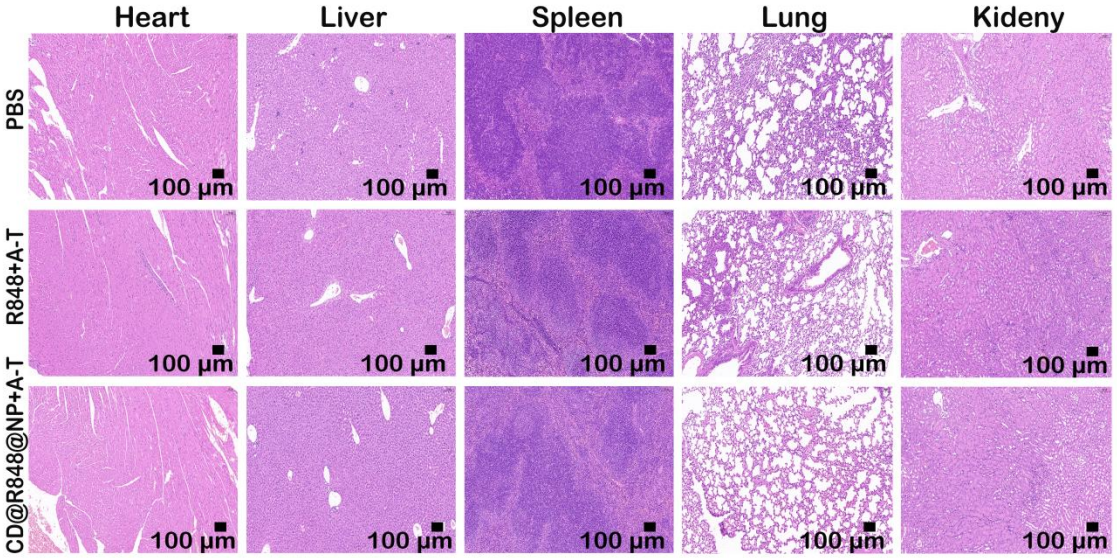
Supplementary figure 2.



PBS	+	+	+	+	+	-	+	+	+	+	+	-
ddH2O	-	-	-	-	-	+	-	-	-	-	-	+
CD@NP (μg/ml)	-	-	-	-	-	-	-	25	50	75	100	-
CD@R848@NP (μg/ml)	-	25	50	75	100	-	-	-	-	-	-	-

Supplementary figure 2. Tests on the safety assessment of CD@NP, CD@R848@NP. Determination results of CD@NP, CD@R848@NP for hemolysis rate.

Supplementary figure 3.



Supplementary figure 3. Biosafety evaluation of CD@R848@NP+A-T, R848+A-T, and PBS *in vivo*. Histopathologic analyses of H&E-stained tissue sections from heart, liver, spleen, lung and kidney of tumor-bearing mice after the indicated treatment. Scale bar: 100μm.

Notes: A-T: anti-TNFR2; R848: Resiquimod; NPs, bare PLGA nanoparticles; CD@NPs: PLGA nanocapsules modified with 2-HP-β-CD; CD@R848@NP: drug R848-loaded PLGA nanoparticles modified by 2-HP-β-CD; CD@R848@NP+A-T: CD@R848@NP+anti-TNFR2; R848+A-T: R848+anti-TNFR2.