

Electronic Supplementary Information

Ultrasound-targeted microbubble destruction augmented synergistic therapy of rheumatoid arthritis via targeted liposomes

Liyun Wang,^{a#} Bihui Zhu,^{a#} Jianbo Huang,^a Xi Xiang,^a Yuanjiao Tang,^a Lang Ma,^{a*}

Feng Yan,^a Chong Cheng,^{b,c} and Li Qiu^{a*}

^a Department of Medical Ultrasound, Laboratory of Ultrasound Imaging Drug, West China Hospital, Sichuan University, Chengdu 610041, China

^b College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, China.

^c Department of Chemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

[#] These two authors contributed equally to this article.

* Corresponding author.

E-mail: (L. Ma) malang1989@scu.edu.cn or malang1989@163.com; (L. Qiu)

qiulihx@scu.edu.cn or wsqiuli@126.com

Tel: +86-28-85464146, Fax: +86-28-85464146.

Materials and animals

The 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol were purchased from Avanti Polar Lipids Inc. (AL, USA). The 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000 (DSPE-MPEG2000) was purchased from AVT Pharmaceutical Technology Co. Ltd (Shanghai, China). The 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate (polyethylene glycol)] (DSPE-PEG2000-FA) was purchased from Nanosoft Polymers (NC, USA). The chloroform was acquired from Changlian Chemical Industry Co. Ltd (Chengdu, China). The dexamethasone sodium phosphate (DexSP) was acquired from Meilun Biotechnology Co. Ltd (Dalian, China). Sodium dihydrogen phosphate, disodium hydrogen phosphate and ethyl alcohol (99.5%) were acquired from Kelong Chemical Industry Factory (Chengdu, China). SonoVue microbubbles were obtained from Bracco Imaging S.p.A (Milano, Italy). The murine macrophage RAW264.7 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Fetal bovine serum, 1% penicillin/ streptomycin solution, and Dulbecco's modified Eagle medium (DMEM) were purchased from Gibco by Life Technologies (ThermoFisher Scientific Inc.). Female SD rats aged 6-8 weeks and weighing 180-220g were provided by Dossy Experimental Animal Co. LTD (Chengdu, China). Bovine type II collagen acetic acid solution and incomplete Freund's adjuvant for animal modeling were purchased from Chondrex Inc. (WA, USA).

FTIR spectra

Infrared spectra were obtained in the transmission mode using a Fourier transform infrared spectroscopy spectrometer (FTIR, Nicolet is50, Thermo Fisher, USA). KBr pellets of the liposomes (LPs-PEG), or Dexamethasone Sodium Phosphate (DexSP) loaded liposomes (DexSP@LPs-PEG) or DexSP loaded folate-conjugation liposomes (DexSP@LPs-PEG-FA) were prepared using a hydraulic press. FTIR analysis was performed between 4000 and 400 cm^{-1} at a resolution of 1 cm^{-1} averaging 10 scans.

XPS spectra

The chemical composition of LPs-PEG and DexSP@LPs-PEG-FA were determined from the X-ray photoelectron spectroscopy (XPS) spectra (Thermo escalab 250Xi, USA). The dried sample was then placed in the XPS chamber and subsequently excited by the X-rays with a spot area over 500 μm^2 using a monochromatic Al Ka radiation at 1486.6 eV, with the power of 150 W. The individual elemental composition of the sample was measured from the spectra using the Advantage built-in software of the instrument.

Temperatures and pH affected drug release tests

The same amount of DexSP-loaded liposomes were placed into a dialysis membrane bag (3.5 kDa, Biosharp Biotechnology Inc., Hefei, China), which was immersed in 30 mL of outer PBS with different pH (pH 5.0, pH 6.5, and pH 7.4) and temperature (22 °C, 37 °C, and 50 °C). At different time intervals, 2 mL buffer was taken out for DexSP amount measurement. The DexSP amount that passed through the dialysis membrane was quantified by measuring the absorbance at 240 nm using the UV-Vis spectrophotometer.

Table S1 Arthritis scoring system for evaluation of paw inflammation.

Score	Condition
0	Normal
1	Mild, but definite redness and swelling of the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits
2	Moderate redness and swelling of ankle or wrist
3	Severe redness and swelling of the entire paw including digits
4	Maximally inflamed limb with the involvement of multiple joints

Table S2 Histology scoring system for H&E staining of ankle joints.

Lesion	Score	Condition
Inflammatory	0	No inflammatory cell infiltrate
	1	A few inflammatory cell infiltrate

	2	A part of the joint cavity filled with inflammatory cells
	3	All the joint cavity filled with inflammatory cells
Synovitis	0	Healthy
	1	Mild thickening of the synovium
	2	Substantial thickening of the synovium
	3	Severe thickening of the synovium
Cartilage damage	0	Normal
	1	Minor destruction of the cartilage surface
	2	Clear loss of cartilage
	3	Cartilage almost absent in the whole joint
Bone destruction	0	Normal
	1	Minor signs of destruction
	2	Up to 30 % destruction
	3	More than 30 % destruction

Table S3 Modified Mankin score for Safranin O staining of ankle joints.

Lesion	Score	Condition
Structure	0	Normal
	1	Irregular surface, including fissures into the radial layer
	2	Pannus
	3	Superficial cartilage layers (≥ 6) absent
	4	Slight disorganization (cellular rows absent, some small superficial clusters)
	5	Fissures into calcified cartilage layer
	6	Disorganization (chaotic structure, clusters, osteoclast activity)
Cellular abnormalities	0	Normal
	1	Hypercellularity, including small superficial clusters
	2	Clusters
	3	Hypocellularity
Matrix staining	0	Normal/ slight reduction
	1	Staining reduced in radial layer

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|---|---|
| 2 | Reduced in interterritorial matrix |
| 3 | Only present in the pericellular matrix |
| 4 | Absent |

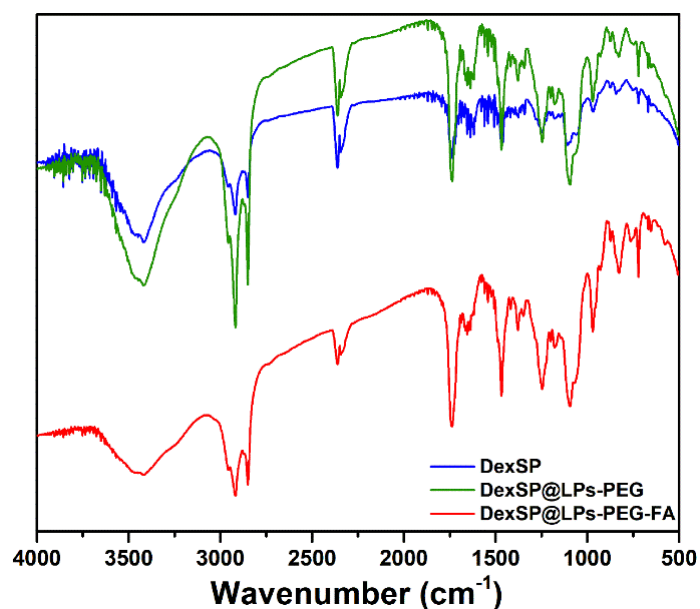


Fig. S1 FTIR spectra of LPs, DexSP@LPs-PEG, and DexSP@LPs-PEG-FA.

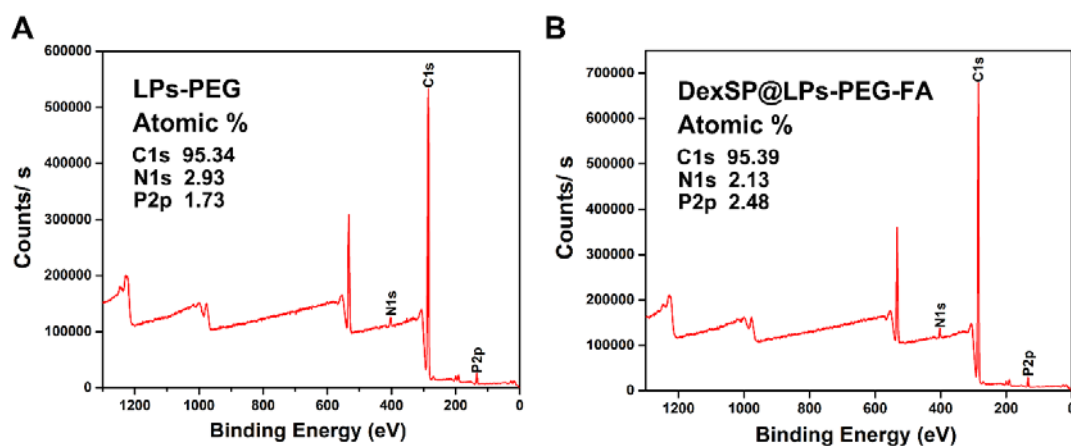


Fig. S2 XPS survey depicting the difference in chemical composition (at. %) between 2 spectra: (A) LPs-PEG and (B) DexSP@LPs-PEG-FA.

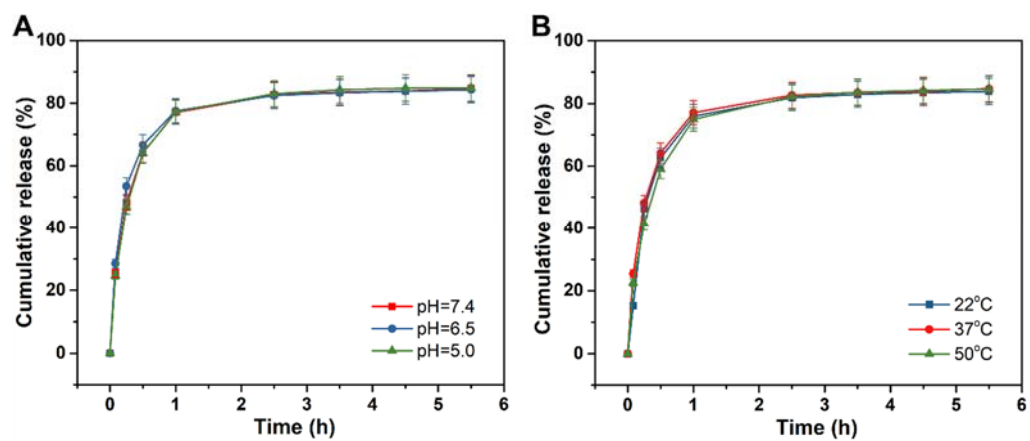


Fig. S3 Drug release of liposomes under different (A) pH and (B) temperatures.