Electronic Supplementary Information

Ultrasound-triggered perfluorocarbon-derived "nanobombs" for targeted therapies of rheumatoid arthritis

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Materials and animals

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids Inc. (AL, USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG2000) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)-2000]-folate acid (DSPE-PEG2000-FA) were purchased from Shanghai Advanced Vehicle Technology Co. Ltd (Shanghai, China). Dexamethasone (Dex) was purchased from Meilun Biotechnology Co. Ltd (Dalian, China). Perfluoropentane (PFP) was obtained from J&K Chemical Ltd (Shanghai, China). All other chemicals in analytical reagent grade were obtained from Aladdin Biochemical Technology Ltd (Shanghai, China) and used as received unless otherwise stated. Murine monocytic cell line macrophages (RAW264.7) were supplied by Core Facility of West China Hospital, Sichuan University (Chengdu, China). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), fluorescein diacetate (FDA), propidium iodide (PI), 4',6-diamidino-2-phenylindole (DAPI), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO), and rhodamine phalloidin were purchased from Solarbio Ltd (Beijing, China). SD rats were obtained from Dossy Experimental Animal Co., Ltd. (Chengdu, China). The Sichuan University Animal Ethics Committee has approved all the experimental protocols.

Temperatures and pH effected drug release tests

The same amount of PFP-Dex@NDs-PEG-FA was put into a dialysis membrane bag in Mw 3500 and immersed in different pH of PBS at different temperatures in a shaking water bath. The cumulative drug concentration in the release medium was determined by measuring the absorbance with an UV-Vis spectrophotometer (L5S, INESA, China) at 240 nm. All measurements were performed three times.

Live/dead cell assay of HUVECs

HUVECs were seeded in to a 96-well plate at a density of approximately 1.25×10^3 cells/well and incubated with PBS, Dex, PFP-Dex@NDs-PEG-FA, and PFP-

Dex@NDs-PEG-FA (166.7 μg/mL of Dex). For the live/dead assay, the cells were stained with fluorescein diacetate (FDA) and propidium iodide (PI) with fluorescence microscopy observation. Briefly, the cells were incubated with 100 μL of 10 μg/mL FDA in PBS (pH 7.4) at 37 °C for 5 min and then with 100 μL of 20 μg/mL PI in PBS (pH 7.4) at 37 °C for 5 min. Upon removal of the FDA/PI solution and subsequently rinsed with PBS, the FDA/PI stained cells were imaged with a fluorescence microscope (AX10 imager A2/AX10 cam HRC, Zeiss, Germany).

Table S1. Qualitative scoring system used to assess severity 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 involvement of multiple joints

Table S2. The grading scheme was used to assess the severity of histology

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

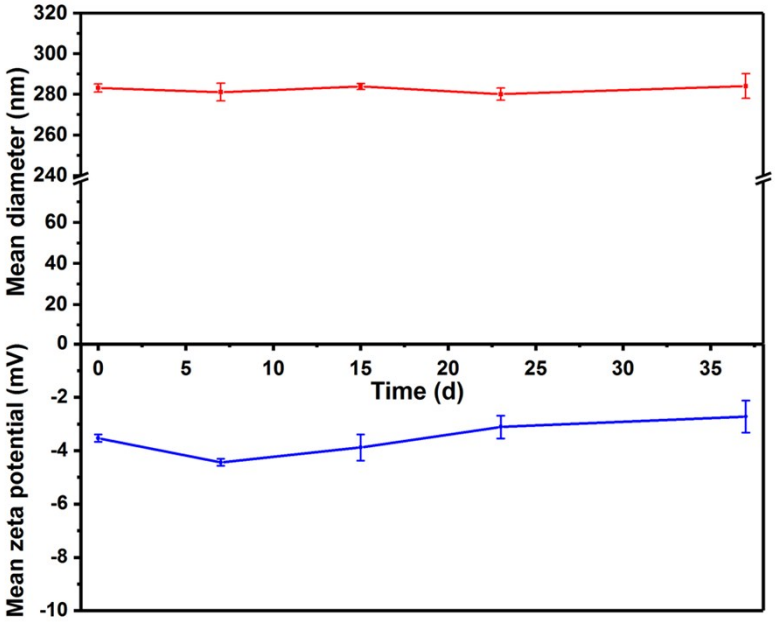

Fig. S1. The stability of PFP-Dex@NDs-PEG stored at 4 °C under different periods of time (n = 3).

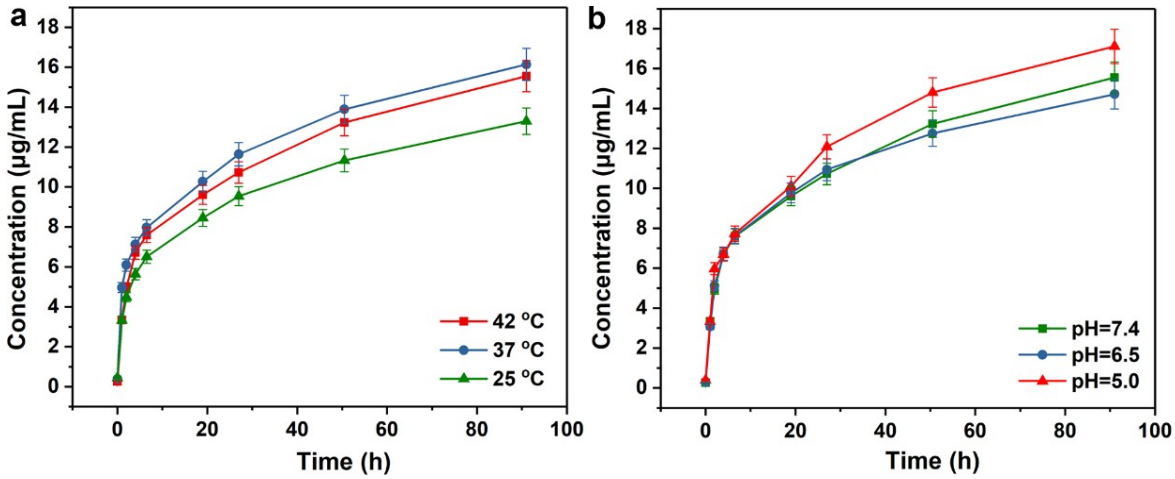

Fig. S2. Dex release curves from PFP-Dex@NDs-PEG-FA under (A) different temperatures and (B) different pH (n = 3).

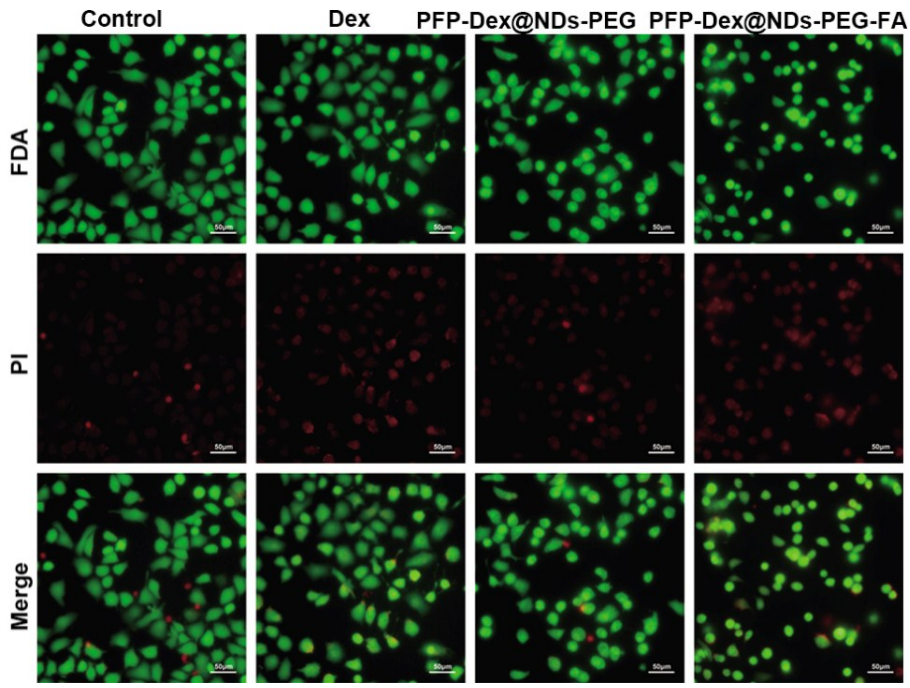

Fig. S3. Fluorescence microscope images of HUVECs viabilities treated with PBS, free Dex, PFP-Dex@NDs-PEG and PFP-Dex@NDs-PEG-FA after incubation for 24 h. The images from top to bottom presented live cell (green), dead cell (red) and merge of two images. Scale bars: 50 μm.

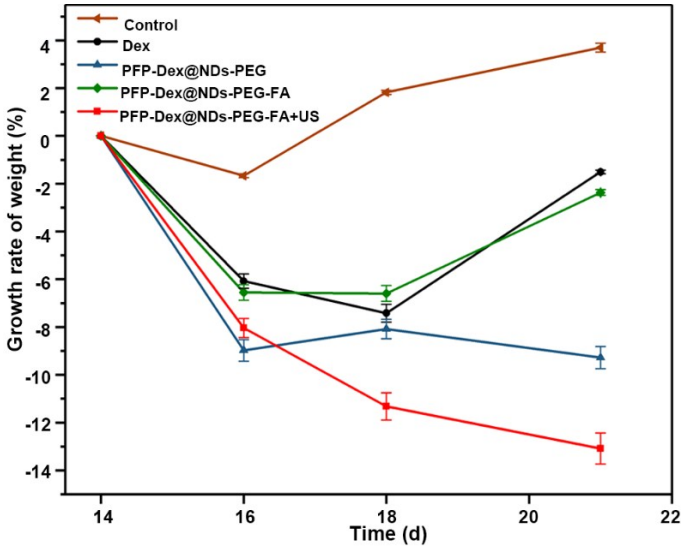

Fig. S4. The weight growth rate of control, free Dex, PFP-Dex@NDs-PEG, PFP-Dex@NDs-PEG-FA, and PFP-Dex@NDs-PEG-FA + US groups assessed from the first day of treatment (n = 6).