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Supporting Information

Polyphenol-Based Nanoparticles Enhancing Doxycycline Efficacy for Acne Therapy

Lian Wang,†[a,b] Junmei Song,†[c] Hao Wang,[b] Tianyou Wang,[d,e] JiaQi Li,[a,b] NingRui Cao,[a] Yiwen Li [c], Zhipeng Gu, *[c] and Xian Jiang*[a,b]

[a]Department of Dermatology, West China Hospital, Sichuan University, Chengdu 610041, China

[b]Laboratory of Dermatology, Clinical Institute of Inflammation and Immunology, Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu 610041, China

[c]College of Polymer Science and Engineering, State Key Laboratory of Advanced Polymer Materials, Sichuan University, Chengdu 610065, China

[d]Chongqing University of Chinese Medicine, Chongqing 402760, PR China

[e]Chongqing Key Laboratory of Innovative Traditional Chinese Medicine and Health Intervention, Chongqing Academy of Chinese Materia Medica, Chongqing 400065, China

† These authors contributed equally.

*Corresponding authors: Zhipeng Gu, E-mail: guzhipeng2019@scu.edu.cn; Xian Jiang, E-mail: jiangxian@scu.edu.cn.

Antioxidant Performance of DE NPs

The in vitro antioxidant experiment included DPPH and $ABTS^{\cdot+}$ scavenging tests.

DPPH Assay. Fresh DPPH ethanol solution (1 mM) and DE NPs solutions (1 mg/mL) were prepared for the measurements. Then, 300 μL DPPH solution and 100 μL DE NPs solutions were added to 2600 μL ethanol, and the absorbance at 517 nm was recorded at different time points.

ABTS Assay. 54.04 mg ABTS was dissolved in deionized water, followed by the addition of 9.93 mg potassium peroxodisulfate. Then, the solution was gently stirred at room temperature in the dark overnight. DE NPs solutions were also prepared for subsequent tests. Subsequently, 100 μL ABTS solution and 100 μL DE NPs solutions were added to 2800 μL water, and the absorbance at different time points at 734 nm was recorded.

In vitro Drug Release

3 mL DE NPs solutions (5 mg/mL) were transferred into the dialysis apparatus (molecule weight cut-off, 3.5 kDa). Then, it was placed in a bottle containing 80 mL of phosphate-buffered saline (PBS) at pH 7.4 and 5.5, and then left to oscillate at 37 °C at 100 rpm. At specified time intervals, 500 μL of PBS solution was removed and the absorbance at 346 nm was measured by UV-visible spectrophotometry to obtain a cumulative Doxy release curve. At the same time, an equal amount of buffer was added to maintain the total volume of the solution constant.

Cytotoxicity, Anti-inflammatory and Antibacterial and Antioxidant capacity of DE NPs on *C. acnes*-induced Acne Model In vitro

To further elucidate NPs's impact on acne cellular models, a series of in vitro experiments were carried out. Firstly, *C. acnes*-induced acne model in vitro was established. After different treatments, the cell counting kit-8 (CCK8) assay and acridine orange (AO) and propidium iodide (PI) dual staining were conducted to examine the cytotoxicity. Moreover, RT-qPCR, ELISA analysis and immunofluorescence staining were used to explore anti-inflammatory ability. The minimum inhibitory concentration (MIC) and time-kill curve (TKC) analysis were also applied to test the antibacterial ability. Finally, both the flow cytometer and fluorescence microscope were used to evaluate antioxidant capacity.

C. acnes Culture. The *C. acnes* (ATCC 6919, Institute of Microbiology, Guangdong Academy of Sciences) with frozen stocks were thawed and revived in a 37 °C anaerobic incubator. The bacteria were then cultured on Columbia blood agar plates until single colonies appeared. Single colonies were selected and further cultured anaerobically at 37 °C for two generations. Afterwards, a small volume of bacterial suspension was inoculated into fresh brain heart infusion broth (BHI) liquid medium and incubated anaerobically at 37 °C for 40 hours until reaching the logarithmic growth phase, during which bacterial metabolism is very actived and cells are highly viable.

HaCaT Cell Culture. The human immortalized keratinocyte cell line (HaCaT), obtained from the Cell Bank of the Chinese Academy of Sciences, was cultured in MEM (Gibco, USA) supplemented with 10% FBS (ExCell, China) at 37℃ in a humidified incubator with a 5% carbon dioxide atmosphere. The culture medium was replaced every two days, and cells were subcultured upon reaching confluence.

C. *acnes Induced HaCaT Cells Models and DE NPs Treatment.* HaCaT cells at 40-60% confluence were cultured in FBS-free MEM and stimulated with *C. acnes* for 24 h later to establish

an acne-like cell model (namely model group) in vitro. Moreover, after another 24 h, EGCG, Doxy, and DE-1-3 were added in *C. acnes*-induced HaCaT cells for 24 h.

*The RT-qPCR and ELISA analysis foe evaluating the anti-inflammatory effect of DE NPs On C. acnes induced HaCaT models***.** Total RNA was extracted from HaCaT cells using mRNA Extraction Kit (AG, China). According to the instructions provided in the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa), the reverse transcription procedure was performed and mRNA was then reverse transcribed into cDNA. Next, quantitative PCR was carried out with TB Green® Premix Ex TaqTM Ⅱ (TaKaRa) by real time PCR instrument (Bio-Rad). The gene expression levels were quantified relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the ΔCT method. Each gene's fold change was then normalized against the control group. The primers were synthesized by Beijing Tsingke Biotech Co., Ltd and detailed primer sequences for the target genes are listed in Table S1. For ELISA test, the culture medium collected from HaCaT cells treated with *C. acnes* and different agents was subjected to centrifugation, and the supernatants were then used for ELISA analysis. The human IL-1β, IL-6, IL-18, TNF-α ELISA kits (Invitrogen, America) were utilized following the manufacturer's protocols.

The Impact of DE NPs on C. acnes Viability Over Time. Firstly, the preserved *C. acnes* was retrieved from frozen storage and cultured under anaerobic conditions until the second generation,which were collected with an OD600 of 0.8, washed twice with PBS, and then were resuspended in fresh Brain-Heart Infusion Medium (BHI) liquid culture medium（Hopebio，China） to an OD600 of 0.2. Next, drugs solution (EGCG, Doxy, DE-1, DE-2, and DE-3) were added to 1 mL of bacterial suspension (OD600=0.2) to achieve a final concentration of 200 μg/mL of Doxy in the bacterial suspension. Then, 100 μL of bacterial suspension was inoculated into wells B3-G11 of a 96-well plate, and 200 μL of bacterial suspension containing EGCG, Doxy, DE-1, DE-2, and DE-3 was inoculated into wells B2-F2. A total of 100 μL of the suspension was transferred from wells B2-F2 to the third column, and serial dilution of the drug concentration was performed down to the eleventh column. Fresh BHI liquid medium (100 μL) was added to row H as a negative control, while row G was used as the bacterial control. Each experimental group was set with three replicates. The remaining wells were sealed by adding 200 μL of PBS. The plate was incubated in an anaerobic incubator for 48 hours. The culture medium was carefully removed from the 96-well plate, and was then placed in a 65 °C oven for overnight drying to allow bacterial adhesion to the

bottom. Methanol (50 μL) was added to each well for bacterial fixation, followed by the addition of 1% crystal violet solution for staining for 5 minutes. The crystal violet solution was discarded, and the wells were rinsed twice with PBS. Finally, 200 μL of anhydrous ethanol was added to dissolve the crystal violet.

Time-kill curve (TKC) analysis was applied to test the impact of DE NPs on *C. acnes* viability over time. The *C. acnes* suspension was treated with EGCG, Doxy, DE-1, DE-2, and DE-3, respectively. At specified time intervals (0, 8, 20, 28, 40, 45, 50, 65, 70 and 86 hours), colony counts (CFU/mL) were assessed using the agar plate count technique. After sampling, a tenfold serial dilution up to 1×10^6 CFU/mL was performed. Next, the dilution with 10 μL was dropped onto a CNA blood agar plate. After 48 hours, colony counting was conducted.

In Vivo Study of DE NPs for Acne Therapy

To explore the effect of DE NPs on acne *in vivo*, the acne-like mice models were established. Furthermore, the hematoxylin and eosin (H&E) staining and immunohistochemical analysis were used to detect the efficacy.

Mice Feeding. Mice were housed in a pathogen-free animal facility. BALB/C mice were purchased from Beijing Huafukang Biotechnology Co., Ltd. The mice were kept in a room with controlled environmental conditions, maintaining a temperature of 22 ± 2.0 °C and relative humidity around 50% ± 5%. They experienced a 12-hour light and 12-hour dark cycle and received standard rodent diet along with tap water. All procedures involving animals were carried out under blinded conditions to ensure unbiased results.

The Pathological Changes and Inflammation Score. Skin specimens were initially fixed using formalin and subsequently embedded in paraffin wax. Thin tissue slices, each measuring approximately 4 μm in thickness, were prepared and subjected to H&E staining. The inflammation score was defined as: grade 0 indicating no observable alterations; grade 1 representing infiltration of a small number of cells; grade 2 corresponding to a moderate degree of cell infiltration; and grade 3 reflecting widespread infiltration. Moreover, the main organs were also collected for H&E staining to evaluate morphological differences.

Determination of Inflammatory Biomarkers. Immunohistochemical analysis was used to detect the inflammatory biomarkers in the skin. Mice skin specimens were first fixed in formalin, followed by paraffin embedding. Sections of 5 μm thickness were then prepared for subsequent use. The

immunohistochemistry procedure was performed following established protocols.[31] The skin sections were incubated with primary antibodies targeting IL-1β (diluted 1:100; Cell Signaling Technology, catalog number 12242), IL-18 (diluted 1:500; Proteintech, catalog number 60070-1-Ig), and TNF-α (diluted 1:100; Zenbio, catalog number 346654). For negative controls, the primary antibodies were omitted to ensure specificity. The mean percentage of stained cells was assigned scores as follows: 0 for zero or almost clear, 1 for less than 25%, 2 for 25 to 50%, 3 for 51 to 75%, and 4 for greater than 75%.

Characterization

Phenom Pro microscope was used for obtaining scanning Electron Microscope (SEM) images. Aqueous solutions of materials (1 mg/mL) were settled onto the substrate of mica, and then rotated to remove the excess water. The prepared samples were fully dried before testing. PerkinElmer Lambda 650 UV/Vis spectrophotometer was used for UV-vis spectra with slit of 2 nm. PHI Quantera SXM spectrometer using Al Ka radiation and spectra was used for X-ray photoelectron spectroscopy (XPS) at the pass energy of 160 eV for survey spectra and 20 eV high-resolution spectra of C 1s, O 1s, N 1s regions with the 300 ms dwell time.

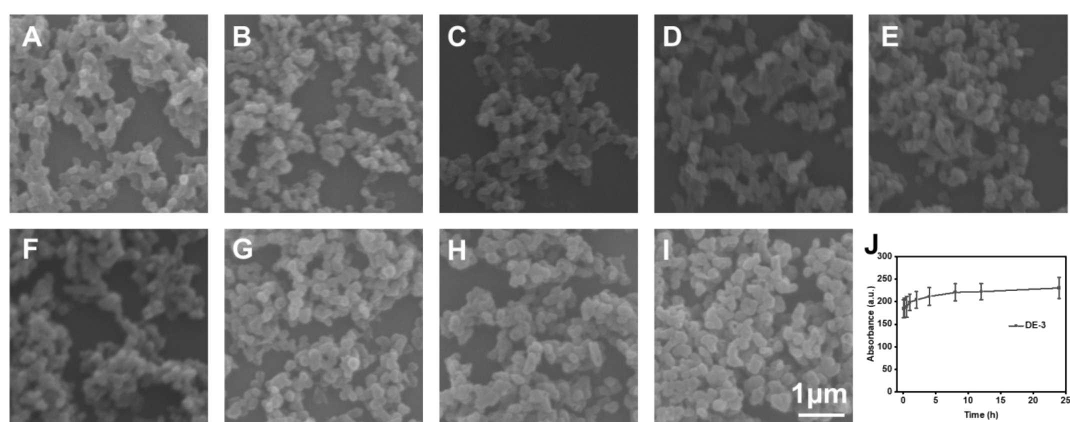

Fig. S1 Scanning electron microscopy (SEM) revealed variation of the particle sizes with reaction time.

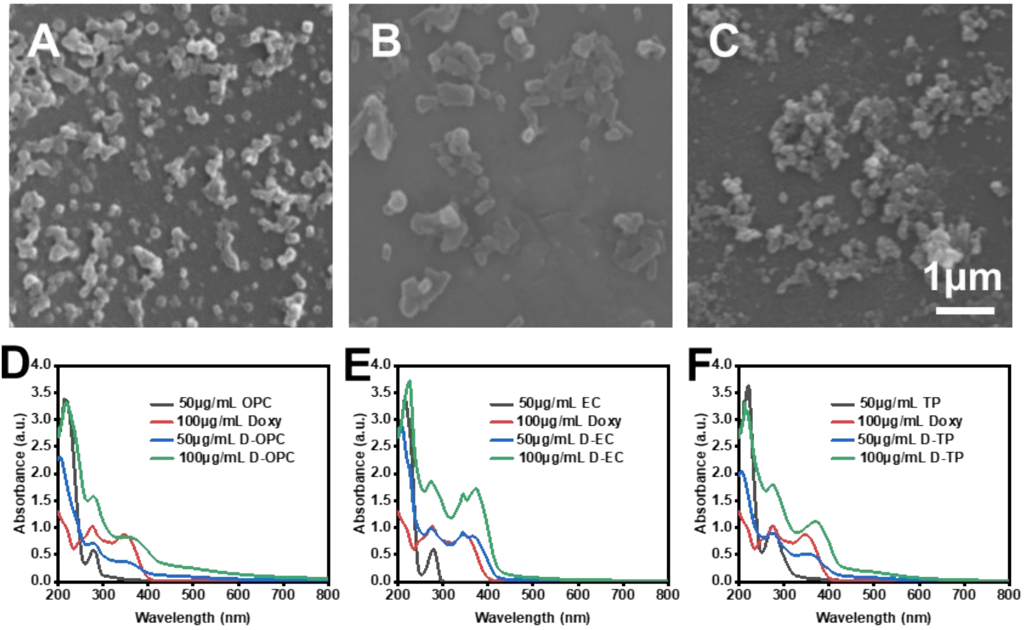

Fig. S2 The preparation method is applicable to other polyphenolic substances, such as oligomeric proantho cyanidins (OPC), epicatechin (EC), and tea polyphenols (TP).

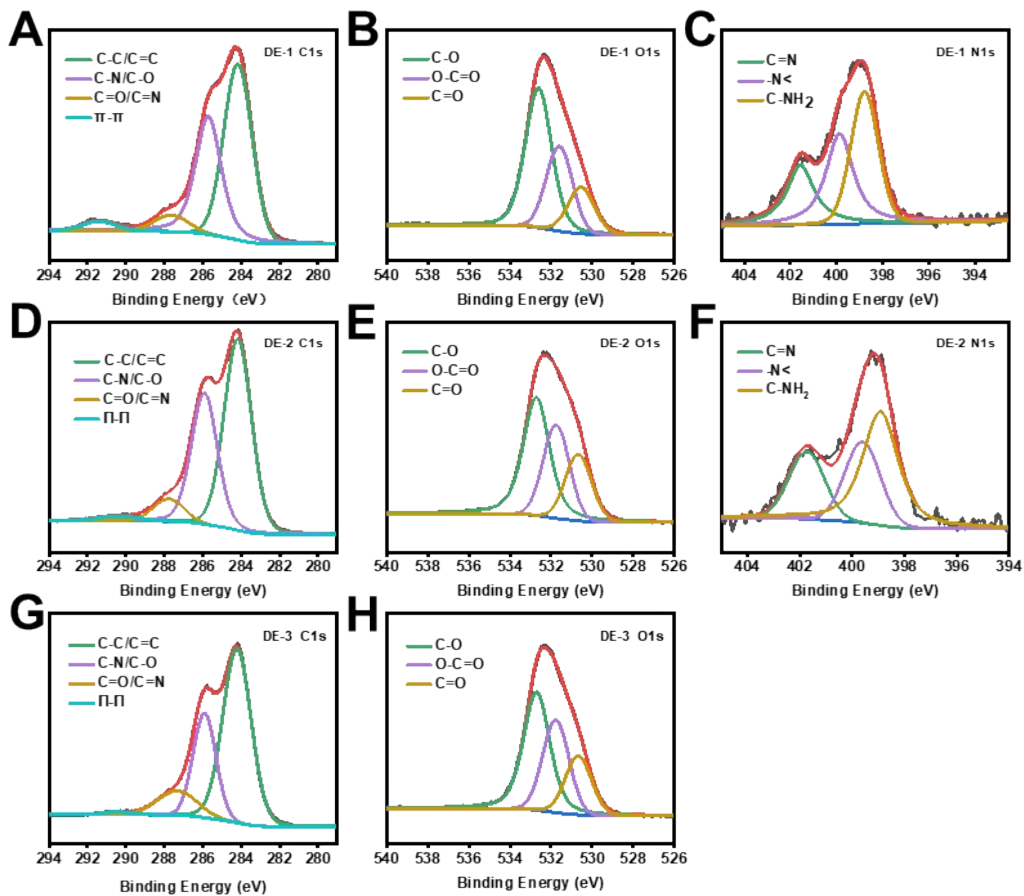

Fig. S3 The C1s high-resolution spectrum concludes C-C/C=C (284.2eV), C-N/C-O (285.9eV), and

C=O/C=N (287.3 eV) and the O1s high-resolution spectrum concludes C=O (530.7 eV), O-C=O (531.8 eV), and C-O (532.7 eV).

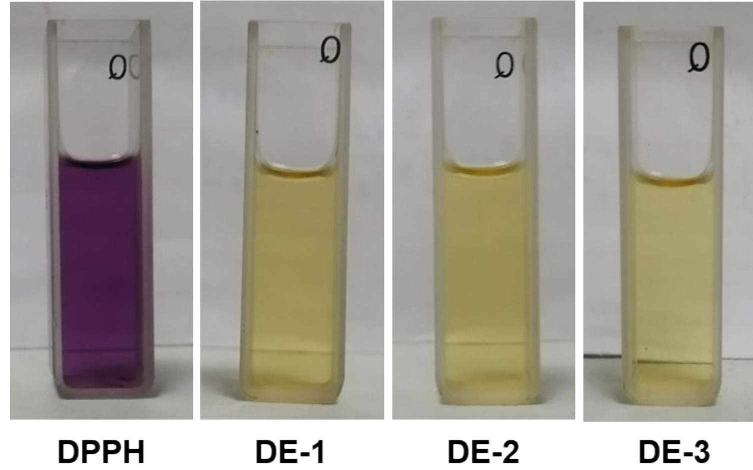

Fig. S4 The appearance and color of DPPH and DE_S.

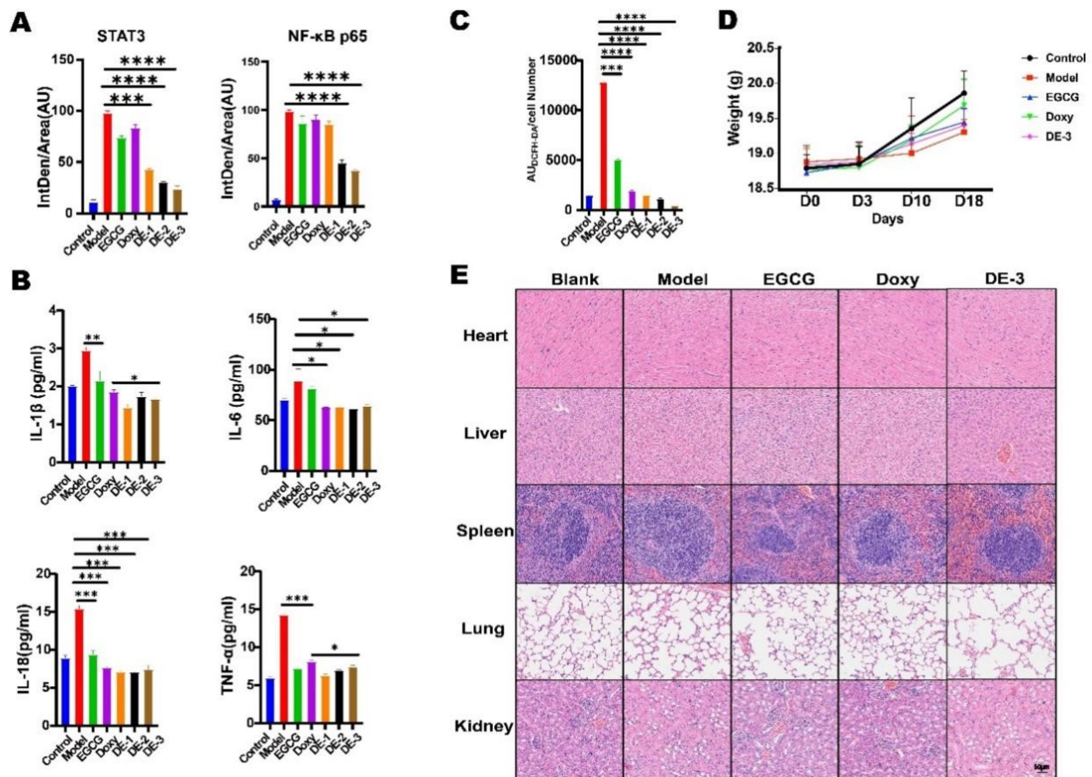

Fig. S5 A The quantitative analysis from the immunofluorescence staining of NF-κB p65 and STAT3 in HaCaT cells. B The quantitative analysis of ELISA tests including IL-1β, IL-6, IL-18, and TNF-α in HaCaT cells. C The quantitative analysis of ROS levels from the fluorescence microscopy assay in HaCaT cells. D The body weight trends in mice. E The representative H&E

staining images of main organs in mice.

Table S1 The sequence of primers

Name	Sequence(5’-3’)	
Human IL-1β	F	AGTACCTGAGCTCGCCAGT
	R	AAGCCCTTGCTGTAGTGGTG
Human IL-6	F	ACTCACCTCTTCAGAACGAATTG
	R	CCATCTTTGGAAGGTTCAGGTTG
Human TNF-α	F	TGCACTTTGGAGTGATCGGC
	R	CTCAGCTTGAGGGTTTGCTAC
Human IL-18	F	CTGCTGCAGTCTACACAGCTTC
	R	TAGAGGCCGATTTCCTTGGTC
Human GAPDH	F	CTCTGCTCCTCCTGTTCGAC
	R	ATGGTGTCTGAGCGATGTGG