Supporting information

Two birds with one stone: Natural polyphenols boosted periodontitis treatment of chlorhexidine via reducing toxicity and regulating microenvironments

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Supplementary materials and methods

Characterization of Nanoparticles

Scanning Electron Microscope (SEM) images were taken using the Nova Nano SEM 450 microscope. The hydrodynamic diameter (DH) of the prepared NPs, along with the particle dispersion index (PDI), was determined using Dynamic Light Scattering (DLS), and the zeta potentials (ZP) were measured with the Malvern Nano ZS ZEN3690 instrument. UV-vis spectroscopy experiments were conducted using a PerkinElmer Lambda 650 UV/Vis spectrophotometer to explore the absorbance properties of the NP solution. 1H nuclear magnetic resonance (NMR) spectra were performed in methanol-D4 (Aldrich, 99.8 %) or DMSO-d6 (Aldrich, 99.8%) utilizing a Bruker AV III HD 400 MHz NMR spectrometer. X-ray diffraction (XRD) experiments were conducted using Rigaku Ultima IV to confirm the structural characteristics. Fourier transform infrared spectroscopy (FTIR) was performed using the Perkin-Elmer Spectrum One B system via the KBr pellet method, with a resolution of 2.0 cm^{-1}. Electrospray ionization mass spectrometry (ESI-MS) was performed on the Applied Biosystems API 2000 with cationic mode electrospray ionization. X-ray photoelectron spectroscopy (XPS) was conducted on the PHI Quantera SXM spectrometer using Al Ka radiation. To evaluate the thermal stability of NPs, thermogravimetric (TG) tests were conducted with Rigaku TG/DTA 8122 (Japan) and differential scanning calorimeter (DSC) tests were conducted with TA DSC2500 (USA). To test the response release behavior of NPs, they were dissolved in PBS, PBS + 0.1 mM H_2O_2, and PBS + 1 mM H_2O_2 and placed in a constant temperature shaker at 37°C. Centrifugation was performed at various time points (1h, 2h, 6h, 12h, 1d, 2d, 3d, 4d, 5d, 7d), and a fixed volume of supernatant was removed and replenished to keep the solution volume constant. The absorption peak at 254 nm of CHX and the reference concentration had a linear relationship, establishing a calibration curve. All concentrations were determined by measuring the absorption of the supernatant and calculating accordingly.

***In Vitro* Antibacterial Assay**

P.gingivalis is typically cultivated in BHI medium at 37°C in an anaerobic incubator (80% N_2-10% CO_2-10% H_2). To evaluate the antimicrobial capacity of NPs, the drugs (CHX, EGCG and NPs, respectively) with effective concentrations of 0.3 μg/mL, 6 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL were co-cultured with 100 μL of *P. gingivalis* bacterial solution (10^7 CFU·mL^{-1}) for 24 hours. The bacterial counts were quantified by measuring the optical density of the medium at 600 nm (OD600) using a microplate reader. After various dilutions of

the suspension, 200 μL of the suspension was spread on a blood agar plate and incubated. The colonies on the plate were counted to estimate the number of surviving bacteria. The bacterial relative viability was calculated using the formula: relative viability = (N1-N2)/(N3-N4), where N1 represents the experimental group, N2 represents the BHI with different drug concentrations, N3 represents the *P. gingivalis* bacterial solution group, and N4 represents the BHI group. The co-cultured suspension was also centrifuged, fixed, dehydrated, and lyophilized to observe the morphology of bacteria by SEM.

In the zone of inhibition (ZOI) test, 200 μL of the bacteria solution (10^8 CFU·mL^{-1}) was pipetted onto a blood agar plate and distributed evenly. Blank drug-sensitive sheets, either loaded or unloaded with drugs drugs (CHX, EGCG, and NPs) at effective concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, and 0.8 mg/mL, were placed on the plates. The plates were then incubated at 37°C in an anaerobic incubator for 48 hours. The antibacterial capacity of the NPs was evaluated by measuring the diameter of the ZOI using vernier calipers, defined as the clear region around the film on the agar surface.

DPPH and ABTS Assays

DPPH and ABTS assays were used to determine the free radical scavenging capacity of samples in ethanol and water, respectively. For DPPH assay testing, 300 μL DPPH (1 mM) was added to 2.6 mL ethanol, and then 100 μL EGCG, CHX and NPs were added (the final efffective sample concentration was 3, 6, 12.5, 25, 50 μg/mL), respectively. The UV−vis spectrophotometer (LAMBDA 650, PerkinElmer) was used to test the absorbance value of the above mixed solution at 517nm at different time (1min up to 30 min). For ABTS assay testing, ABTS radical cation solutions were obtain by dissolving 54.04 mg ABTS and 9.93 mg potassium persulfate in 15 mL deionized water, and stirred in the dark for 12 hours. After that, 100 μL ABTS radical cation solutions were added into 2.8 mL deionized water, and then 100 μL samples were added (the final efffective sample concentration was 3, 6, 12.5, 25, 50 μg/mL) , respectively. Finally, the absorbance value of the above mixed solution at 734 nm was measured at different time (1min up to 30 min) using UV−vis spectrophotometer (LAMBDA 650, PerkinElmer).

Intracellular ROS Scavenging Activity

Intracellular ROS levels were assessed by staining of the fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA). HOK cells were seeded on 6-well plates at a density of 100,000 cells per well and incubated for one day. The medium was then changed, and the

cells were incubated with DMEM containing *P.g.*-LPS (1 μg/mL) with or without drugs (EGCG and NPs). After 24 hours, the cells were rinsed with PBS and then stained with DCFH-DA. Finally, ROS levels in the cells were measured by flow cytometry, and the cells were photographed with an inverted fluorescence microscope.

Anti-inflammatory Property

The procedures for cell culture, *P.g.*-LPS, and drug treatment were performed as before. Total RNA of HOK cells was extracted using the Cell Total RNA Isolation Kit (Foregene). cDNAs were reversely transcribed using the PrimeScript RT Master Mix (Takara, Cat#RR092S) and subsequent RT-qPCR was performed using specific primers with SYBR Premix Ex TaqII (Takara, Cat#RR820A). The primer sequences used are presented in Supplementary Table S1.

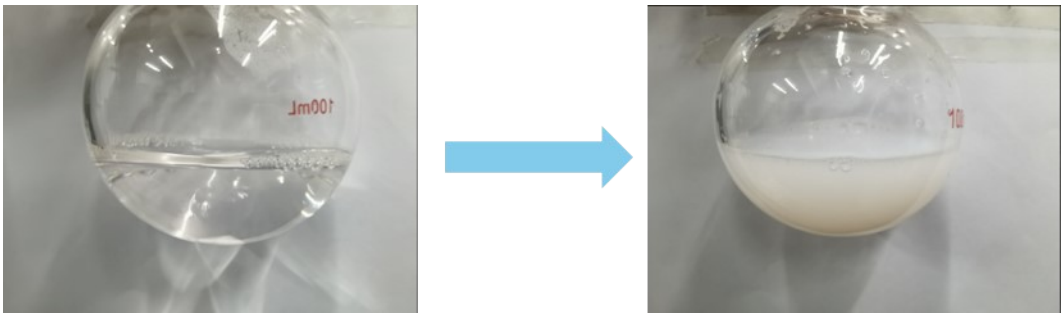


Figure S1. Images of the NPs in both their pre- and post-formation states.

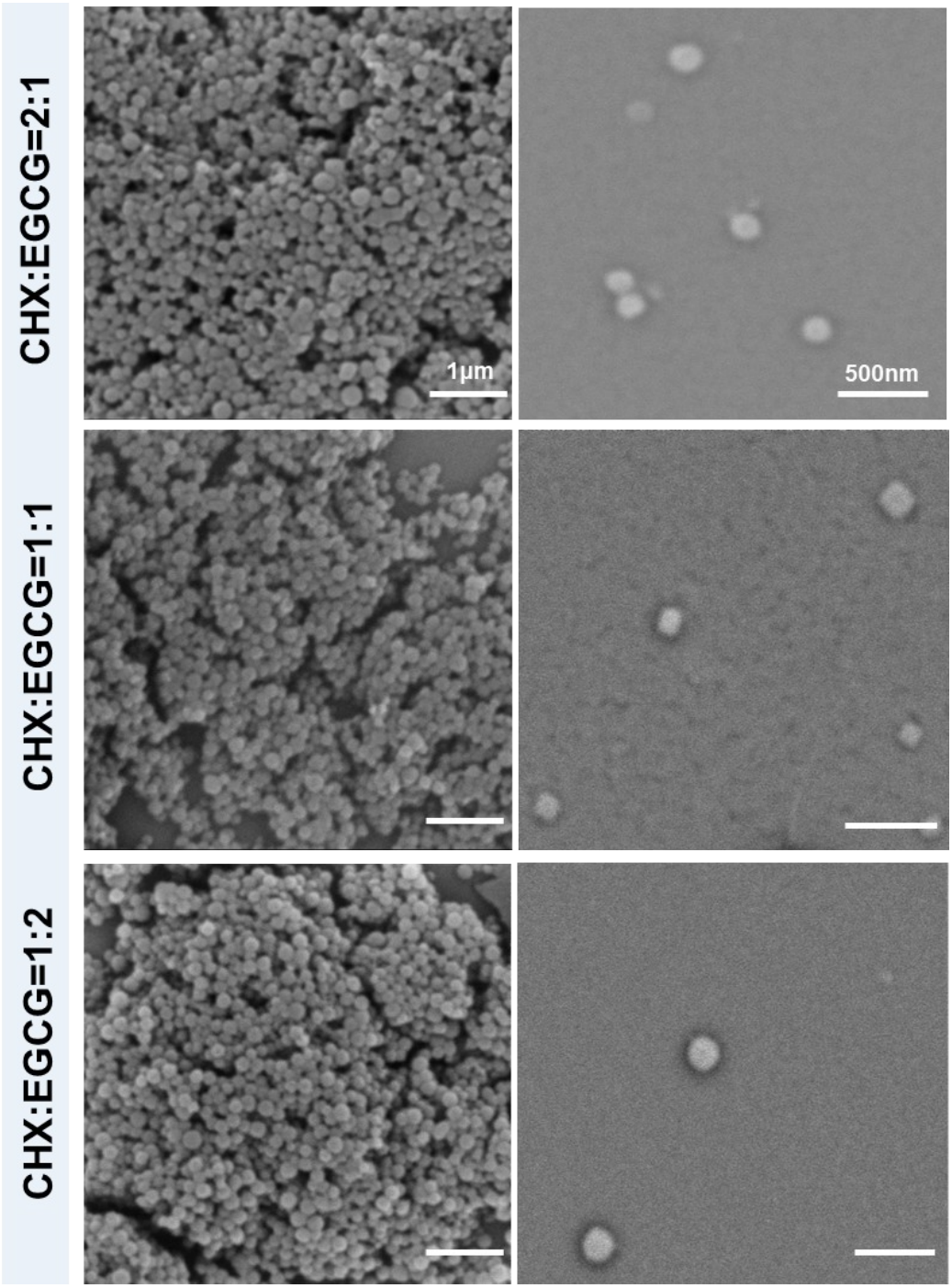


Figure S2. Represent SEM images of EGCG-CHX NPs with different CHX:EGCG ratio.

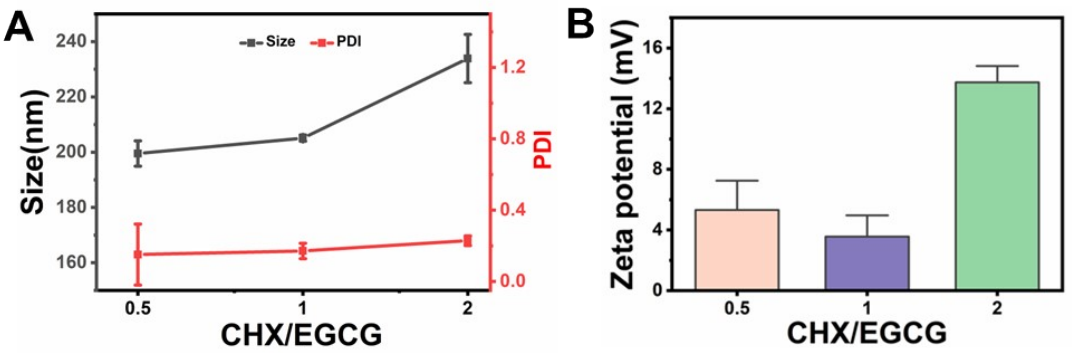


Figure S3. A) Average sizes and PDI, and B) Zeta potential of NPs with different CHX: EGCG ratio.

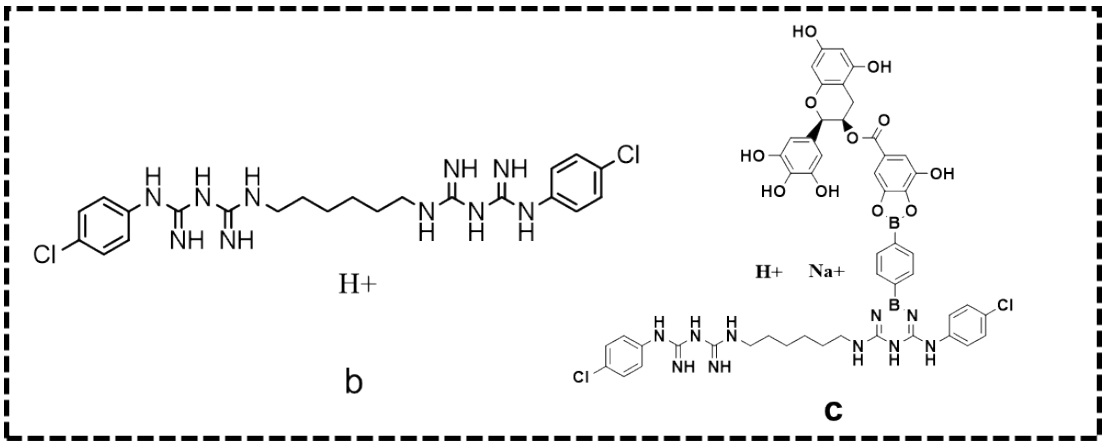


Figure S4. The other possible oligomer structures after the reaction process corresponding to the main peak from ESI-MS spectra.

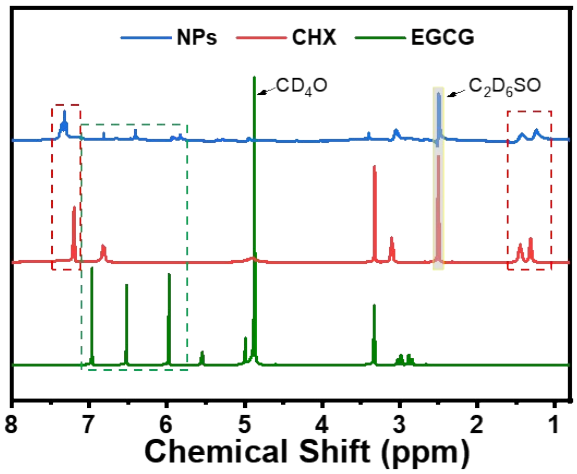


Figure S5. The 1H NMR spectra of EGCG, CHX and NPs.

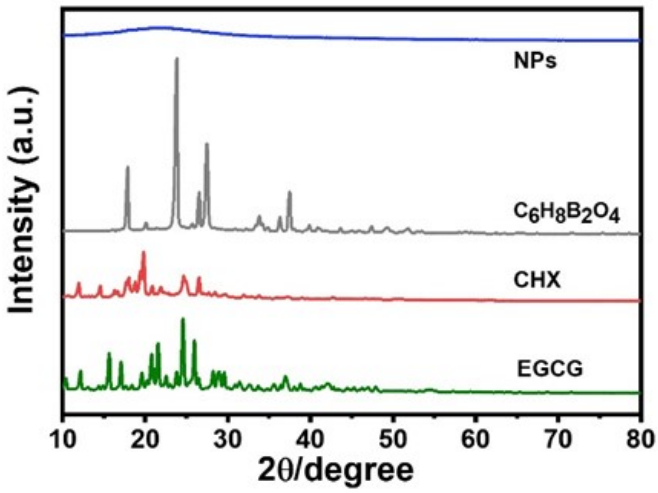


Figure S6. The XRD of EGCG, CHX, C6H8B2O4 and NPs.

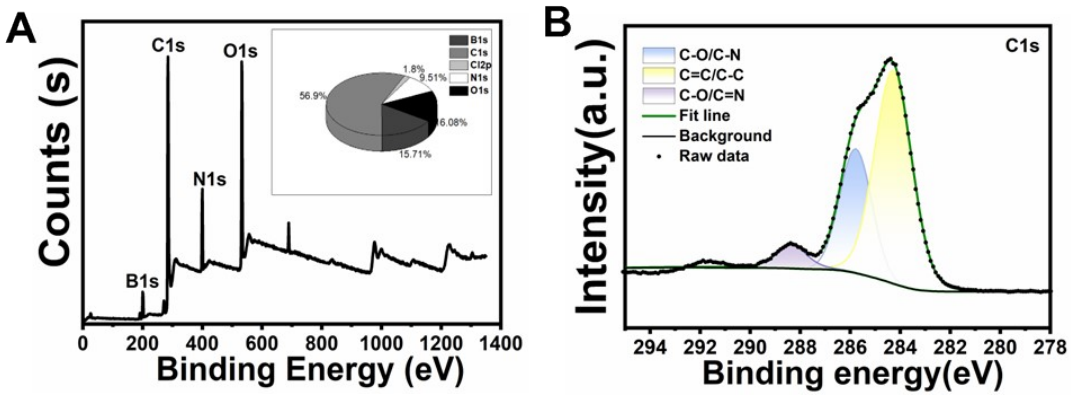


Figure S7. A) XPS survey scan and B) C 1s XPS high-resolution spectra of NPs.

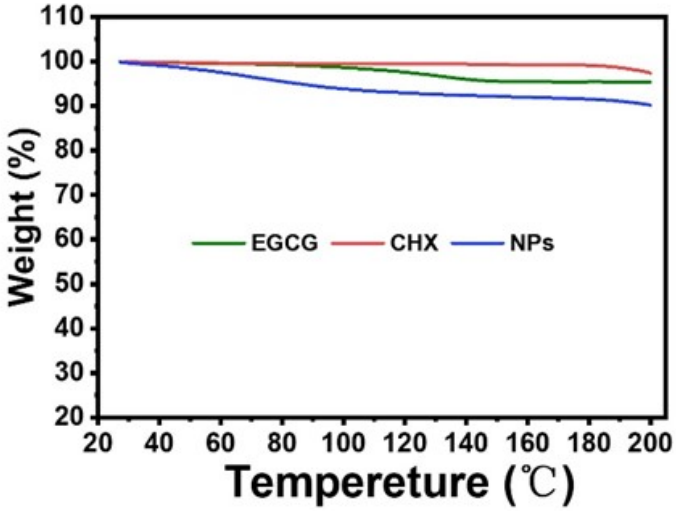


Figure S8. The TG curves of EGCG, CHX and NPs.

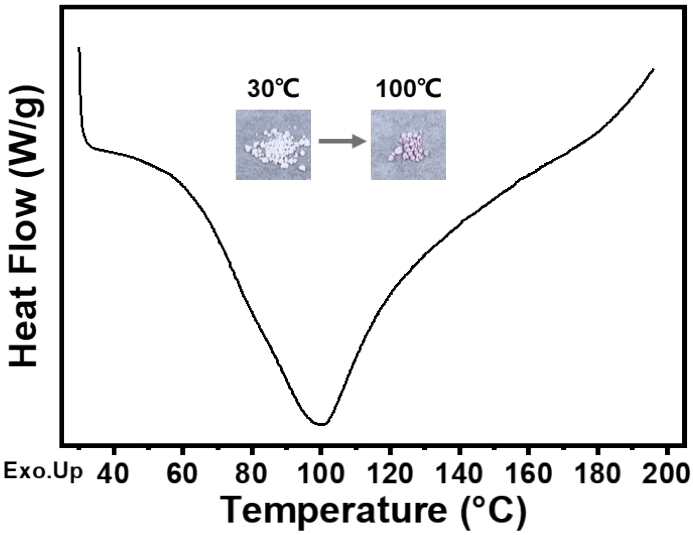


Figure S9. The DSC curve of NPs.

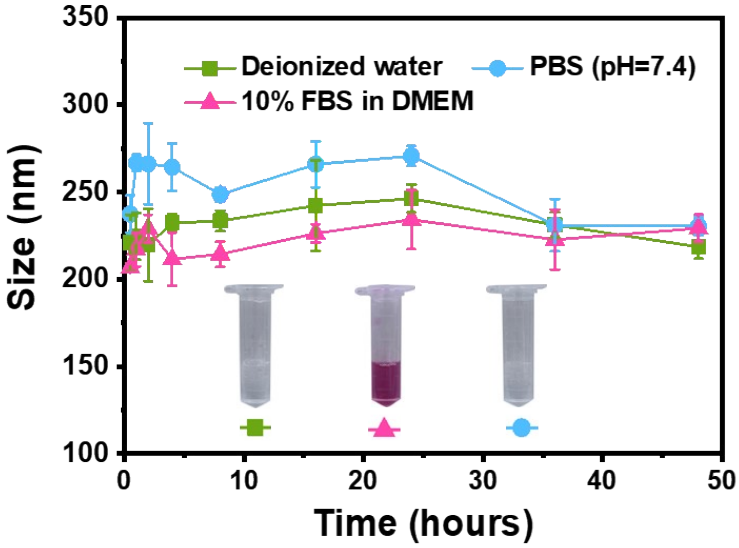


Figure S10. Particle sizes of NPs in deionized water, PBS solution (pH = 7.4) and DMEM with 10% FBS at different times.

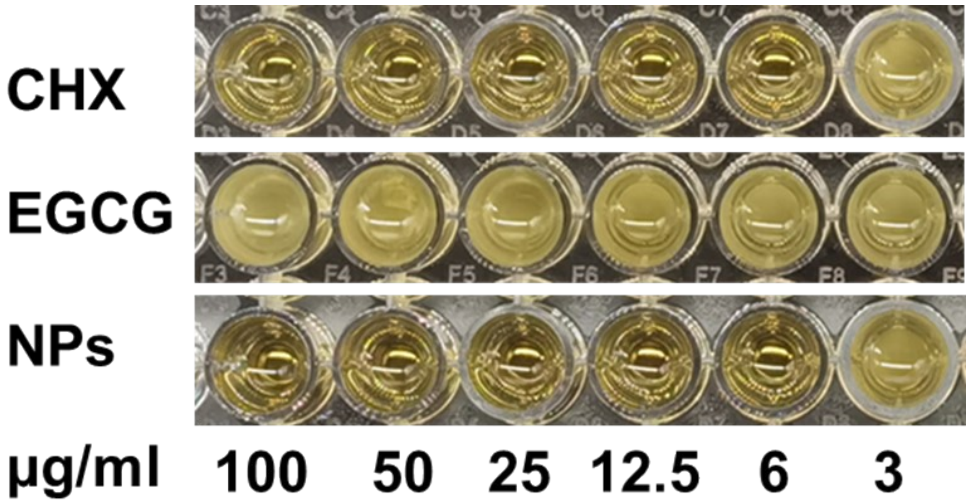


Figure S11. Images of representative microdilution plate following co-culture with *P. gingivalis.*

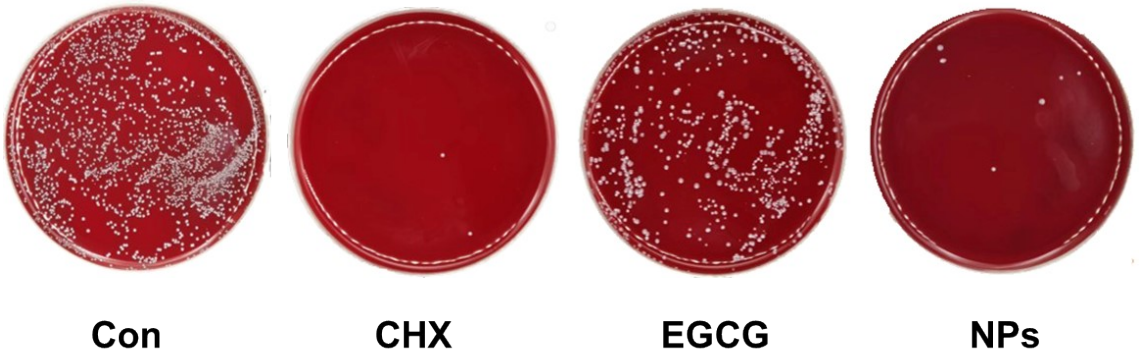


Figure S12. Images of *P. gingivalis* clones.

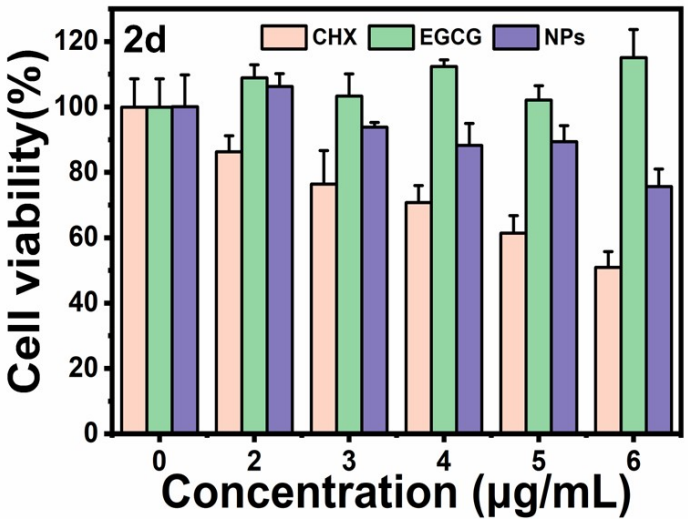


Figure S13. Cell viability of HOK cells incubated with different drugs at various concentrations (0-6 μg/mL) for 2 d**.**

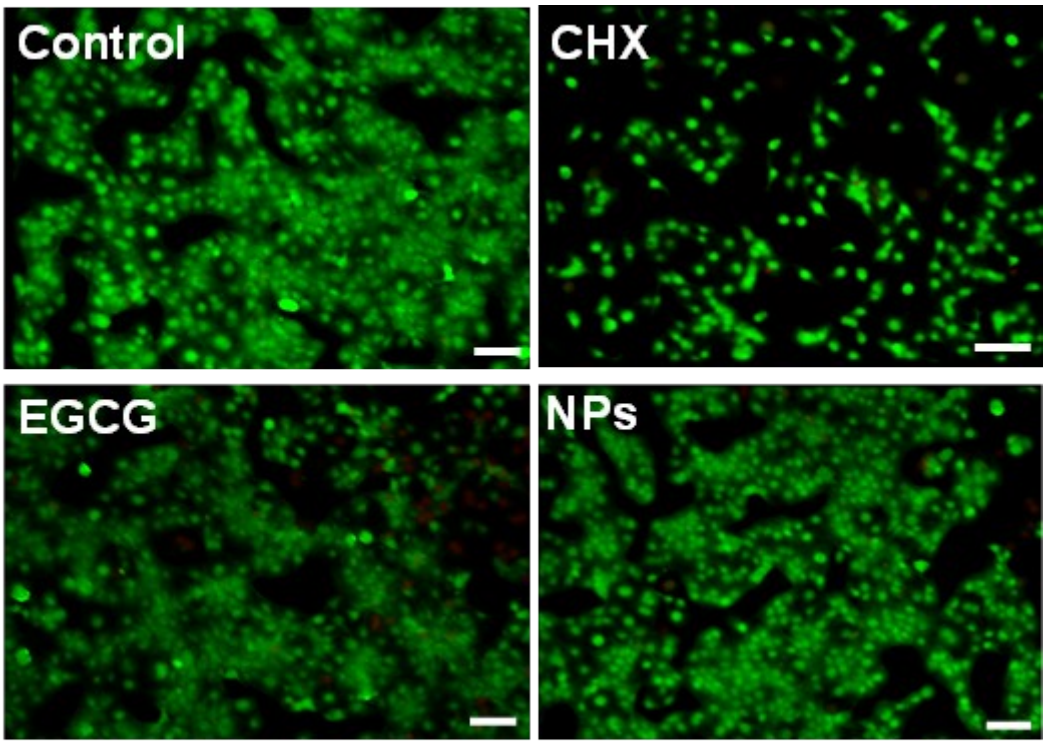


Figure S14. Images of Calcein-AM/PI staining of HOK cells incubated with different drugs at 5μg/mL for 2d. (Scar bar=200 μm)

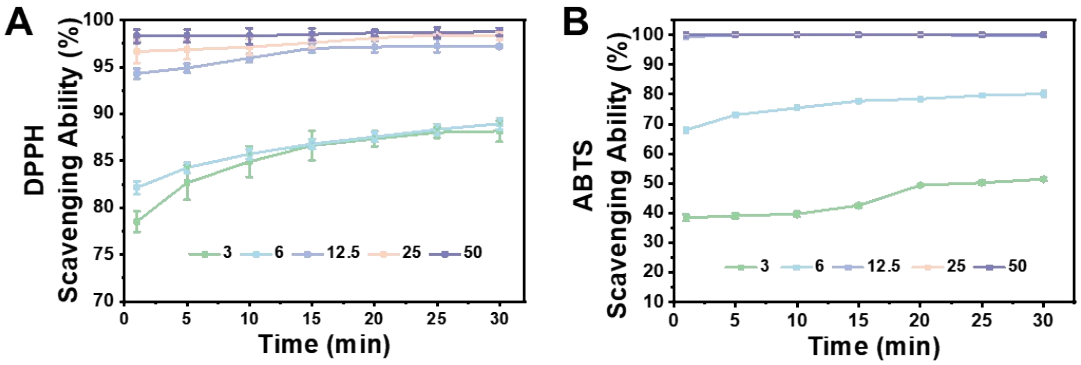


Figure S15. DPPH and ABTS scavenging activity of EGCG with different concentrations (3-50 μg/mL) at different time, respectively.

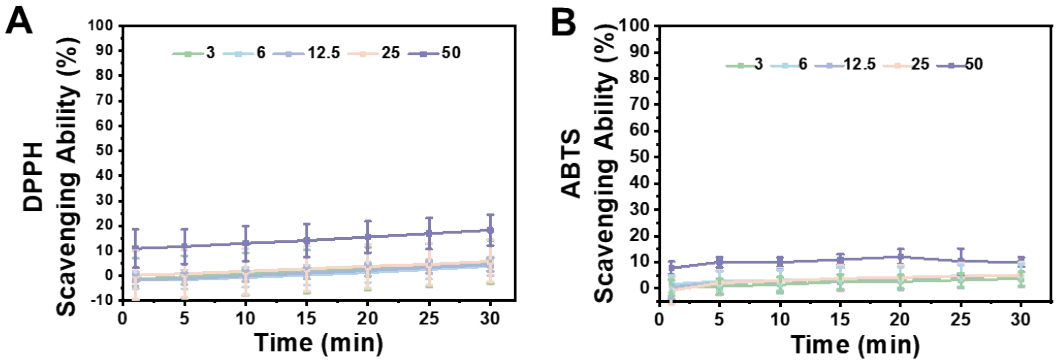


Figure S16. DPPH and ABTS scavenging activity of CHX with different concentrations (3-50 μg/mL) at different time, respectively.

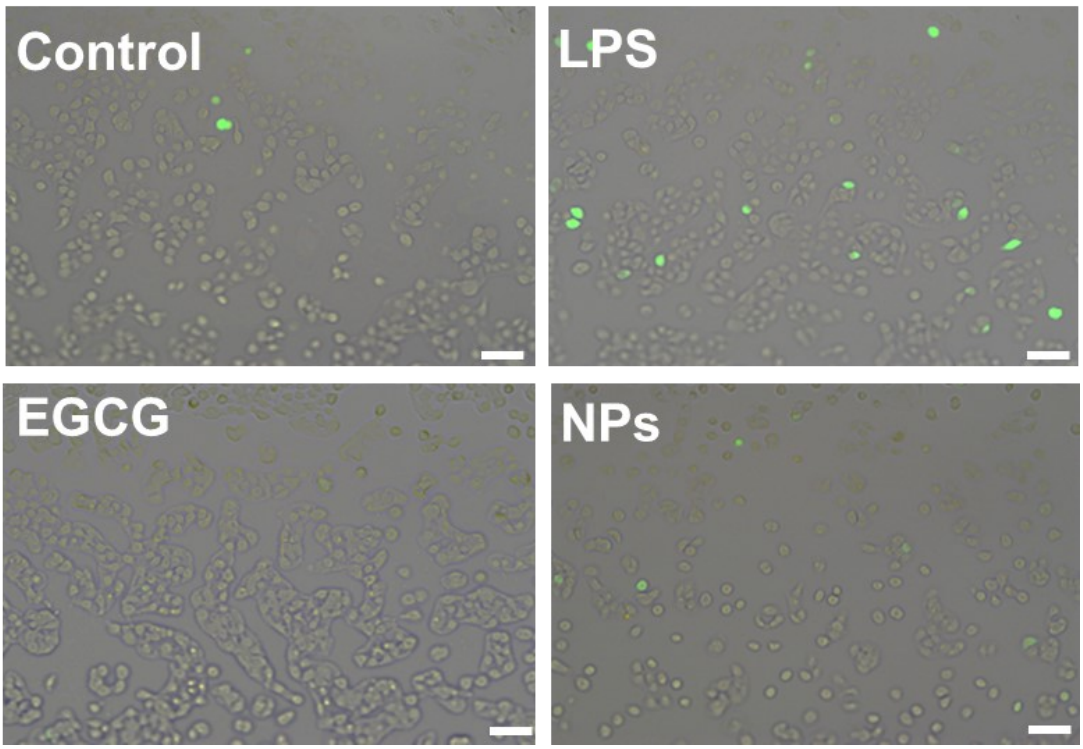


Figure S17. Images of DCFH-DA staining of HOK cells after co-incubation with *P.g.*-LPS and different drugs (5 μg/mL) for 1 d. (Scar bar=100 μm)

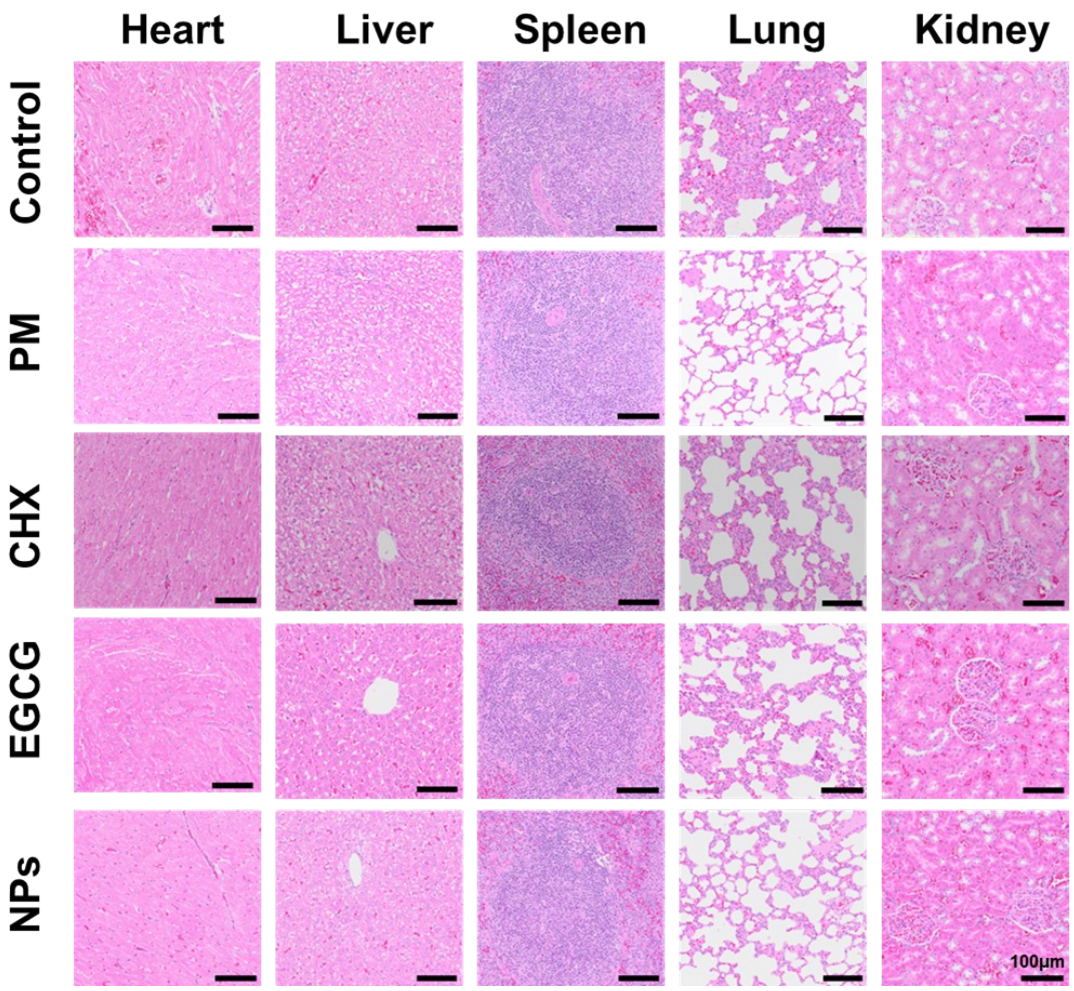


Figure S18. Images of main organs stained with hematoxylin and eosin (H&E).

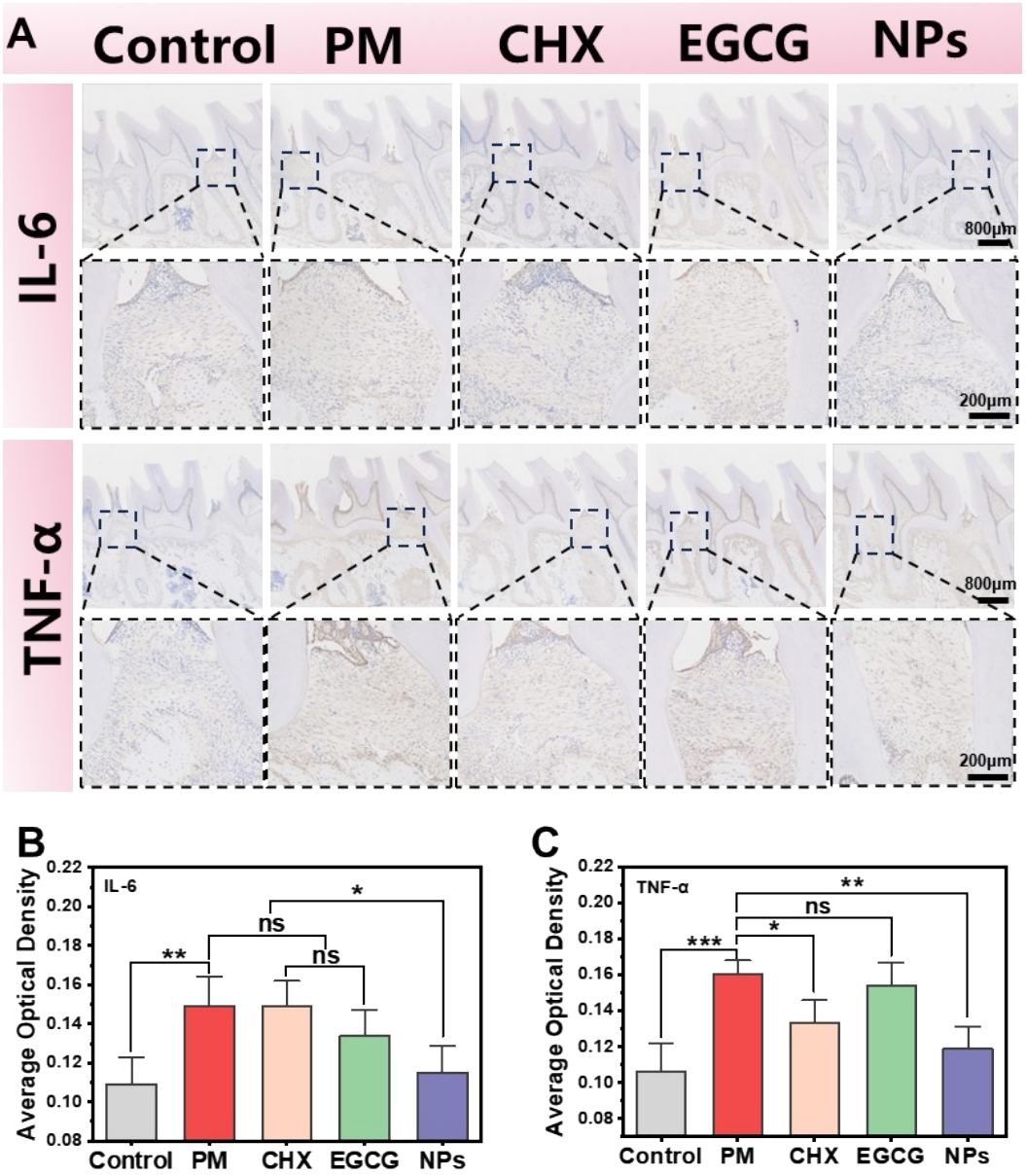
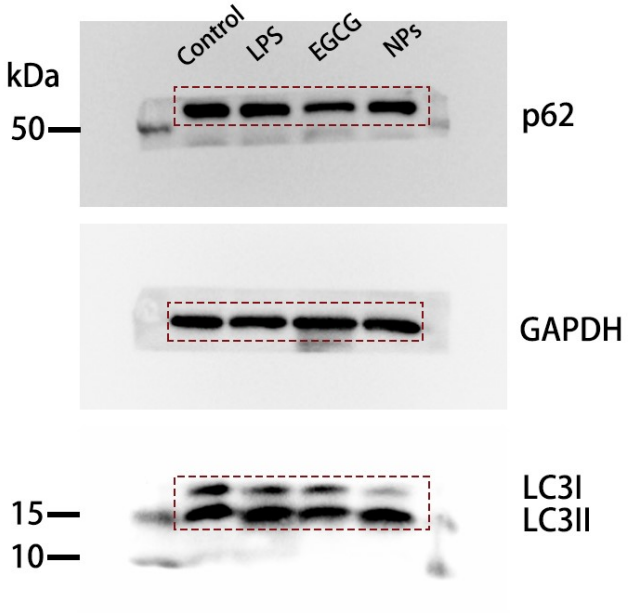


Figure S19. A) Immunohistochemical staining images for IL-6 and TNF-α of the periodontal tissue. B-C) Average optical density of IL-6 and TNF-α.

Table S1. Primers used in this study.

Gene symbol	5'- Forward primer -3'	5'- Reverse primer -3'
GAPDH	GGAAGGTGAAGGTCGGAGTC	CGTTCTCAGCCTTG ACGGT
IL-1β	TACGAATCTCCGACCACCACTACAG	TGGAGGTGGAGAGCTTTCAGTTCATATG
IL-6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
NLRP3	CGGTGACCTTGTGTGTGCTT	TCATGTCCTGAGCCATGGAAG

Extended data Figure 4F. original blots.



Extended data Figure 4F-H. plots of repeated experiments.