Supporting Information

Rational design of flexible microneedles coupled with CaO_2@PDA-loaded nanofiber films for skin wound healing on diabetic rats

Zhiyong Zeng [a], Guohua Jiang [a,b,*], Yanfang Sun [c,*], Uladzislau E. Aharodnikau [d], Khaydar E. Yunusov [e], Xiaofei Gao [a], Tianqi Liu [a], Sergey O. Solomevich [d]

[a] School of Materials Science and Engineering, Zhejiang Sci-Tech University, Hangzhou, 310018, China

[b] International Scientific and Technological Cooperation Base of Intelligent Biomaterials and Functional Fibers, Hangzhou, 310018, China

[c] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, Zhejiang, 310018, China

[d] Research Institute for Physical Chemical Problems of the Belarusian State University, Minsk, 220030, Belarus

[e] Institute of Polymer Chemistry and Physics, Uzbekistan Academy of Sciences, Tashkent, 100128, Uzbekistany

Experimental Section

Materials: Calcium chloride ($CaCl_2$, analytical reagent), poly(vinylpyrrolidone) (PVP, Mn≈5800), potassium permanganate ($KMnO_4$), ammonia solution (NH_4OH, 25~28%), ethanol and hydrogen peroxide (H_2O_2, 30%) and dopamine hydrochloride were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). 2, 2-Dipenyl-1-picrylhydrazyl (DPPH), gelatin (medicinal grade, type B), polycaprolactone (PCL, Mn=80000), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methyl propiophenone (Irgacure 2959), normal saline (NS), metformin hydrochloride (Met), dimethyl sulfoxide (DMSO), streptozotocin (STZ) and 1,1,1,3,3,3-hexafluoro- 2-propanol (HFIP) were provided by Sigma-Aldrich Shanghai, China). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), MTT cell proliferation and cytotoxicity Assay Kit, trypsin-EDTA Solution (with phenol red), calcein/PI cell viability/cytotoxicity assay kit and phosphate buffer solution (PBS) were obtained from Bytotime Biotechnology Co., Ltd. (Shanghai, China). All the chemical reagents were used without further purification except ultrapure water, which was produced in a water purification system. The GelMA was prepared according to the previous reports.

Photothermal effect of CaO_2@PDA in vitro: 1 mL of CaO_2@PDA NPs in water (0.1, 0.2, 0.3, and 0.4 mg/mL) was added into centrifuge tubes respectively, and then irradiated with NIR laser (808 nm, 1.6 W/cm^2) for 8 min. The temperature was monitored at each determined time point, and thermal images were collected by thermal imager (325, Fotric, America). In order to investigate the photostability of CaO_2@PDA, 1 mL of CaO_2@PDA (0.1 mg/mL) was irradiated with 808 nm laser for 10 min and then cooled down to the room temperature repeated for five cycles.

In vitro antioxidant ability: The antioxidant capacity of CaO_2@PDA NPs and PCL/Gel-NPs was evaluated by measuring their capacity to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the previously reports [2]. Firstly, the DPPH concentration was fixed at 100 μM DPPH in ethanol, and then 35, 70, and 90 μg

of CaO_2@PDA NPs was dispersed in 1 mL of the above ethanol solution, respectively. The mixture solution was incubated at room temperature in a dark for 5, 10, 20 and 40 min. Then, the wavelength of DPPH was measured at 517 nm by UV-vis spectrophotometer. The degradation of DPPH was determined as the following equation：

$$\text{Scavenging ratio}=\left[1-\frac{A_s}{A_c}\right]\times 100\% \quad (1)$$

where A_s and A_c refers to the absorbance of the sample (DPPH+CaO_2@PDA NPs) and the control group (DPPH+ethanol) at 517 nm respectively.

Intracellular ROS scavenging was evaluated by incubating CaO_2@PDA NPs with L929 cells. L929 cells (6×10^5 cells in each sample) were co-cultured with different concentration of CaO_2@PDA NPs (50 and 100 μg/mL) for 12 h, and then incubated with 100 mM H_2O_2 for 30 min (5%, 37 ℃). Then, 1 mL of DCFH-DA (10×10^{-6} M) was added in each group and incubated for 30 min. After that, each group was washed three times with PBS. The fluorescence intensity of each group was measured by confocal laser scanning microscope (CLSM). The cells in groups incubated with normal cell culture medium were defined as the control. The cells treated with only H_2O_2 were undergo the similar treatment described above.

In vitro cytotoxicity test: In order to evaluate the cytotoxicity of the CaO_2@PDA NPs, MTT assays were performed by co-culture of CaO_2@PDA NPs with L929 cells and 3T3 cells. Briefly, the L929 and 3T3 cells were seeded respectively in a 96-well plate at an initial density of 6×10^4 in DMEM that contained 10% FBS for 12 h. Then, the cells were treated with various concentration of CaO_2@PDA NPs (25, 50, 100, 200, and 400 μg/mL) for 24 h at 37 ℃. Next, the cultural medium was removed and washed with PBS, and then 100 μL of DMEM was added into each well. After that, 20 μL of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h. After removing the cultural medium, 150 μL of dimethyl sulfoxide (DMSO) was added into each well.

Subsequently, the absorbance at 490 nm was tested by employing microplate reader (Thermos labsystems, LM-MK3, America). The cytotoxicity of the CaO_2@PDA NPs was determined by the following equation:

$$\text{Cell viability}=\frac{OD_e}{OD_c}\times 100\% \quad (2)$$

where OD_e and OD_c were the absorbance of experiment group and control group.

The cytocompatibility of the PCL/Gel-NPs was evaluated through a leaching pattern test according to the previous report [4]. The sterilized PCL/Gel-NPs nanofibers (2.8 cm^2) were incubated in DMEM without FBS for 30 min to obtain the leaching solution. MTT assays were selected to evaluate the cytotoxicity of PCL/Gel-NPs nanofibers. 1 mL of L929 cells (6×10^4) in DMEM were co-cultured with the leaching solution (1 mL). The rest of MTT assay procedures were similar to the above operation means. To observe the cell proliferation and viability after co-culturing 12 h, 24 h, and 48 h, calcein/PI cell viability/cytotoxicity assay kit were used to label the live or dead L929 cell and observed by CLSM. All the tests were conducted three times in each group.

In vitro antibacterial property: In order to evaluate the photothermal antibacterial activity of PCL/Gel-NPs nanofibers, Gram-negative bacteria (*E. coil*) and Gram-positive bacteria (*S. aureus*) were treated with PCL/Gel-NPs nanofibers in five groups: PBS, PCL/Gel-NPs + NIR (0 min), PCL/Gel-NPs + NIR (5 min), PCL/Gel-NPs + NIR (10 min), and PCL/Gel-NPs + NIR (15 min). Briefly, *E. coil* and *S. aureus* were diluted 100 times with PBS, and then PCL/Gel-NPs nanofibers with round section (diameter: 1 cm) were immersed in PBS (5 mL) that containing 300 μL of bacteria and exposed to 808 nm laser for 0, 5, 10, and 15 min. The PBS group was used as a control. Then, the all groups were incubated at 37 ℃ for 20 h. After incubation, 100 μL of co-culturing bacteria solution was spread uniformly onto LB agar plates. Subsequently, the LB agar plates were incubated for 24 h at 37 ℃. Finally, the number of colonies on the plate were recorded and calculated. For observing the morphology of *S. aureus*, the incubated

S. aureus were fixed with glutaraldehyde (2.5%) for 12 h at 4 ℃. Next, *S. aureus* were dehydrated with a sequential treatment of 30, 50, 70, 90, and 100% ethanol for 3 min and characterized by SEM.

The bacterial viability was determined as following equation:

$$\text{Bacterial viability}=\frac{CFU_t}{CFU_o}\times 100\% \quad (3)$$

where CFU_t and CFU_o were the colonies exposed NIR for 0, 5, 10, and 15 min and colonies of control group.

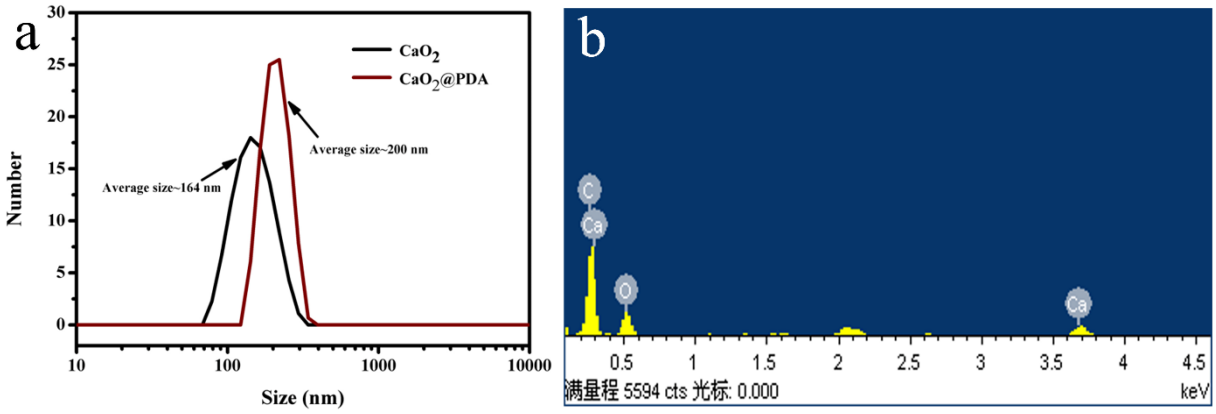

Figure S1. Size distribution of CaO_2 and CaO_2@PDA nanoparticles (a) and energy-dispersive X-ray spectroscopy (EDS) pattern of CaO_2 and CaO_2@PDA nanoparticles.

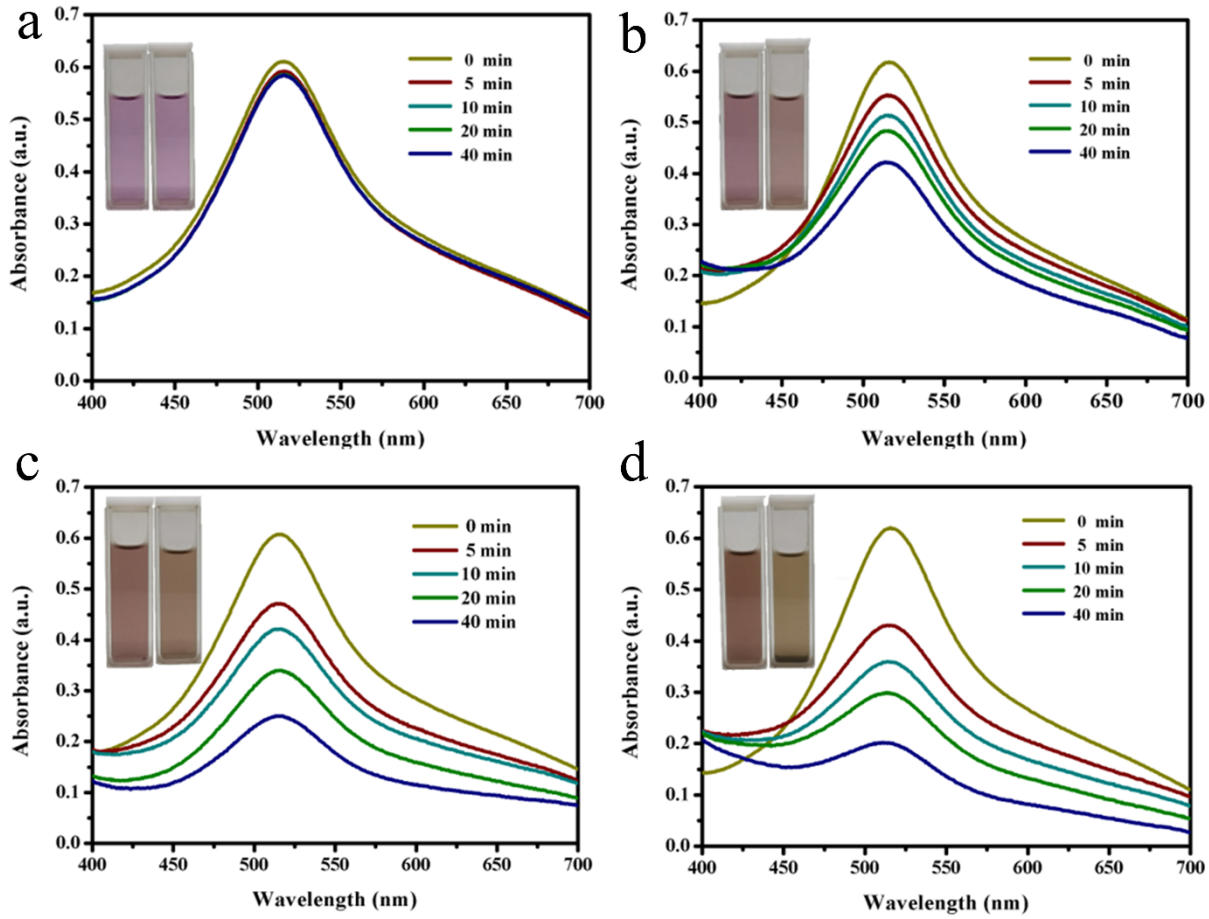

Figure S2. UV-vis spectra of antioxidant activity of CaO_2@PDA nanoparticles evaluated by DPPH assay.

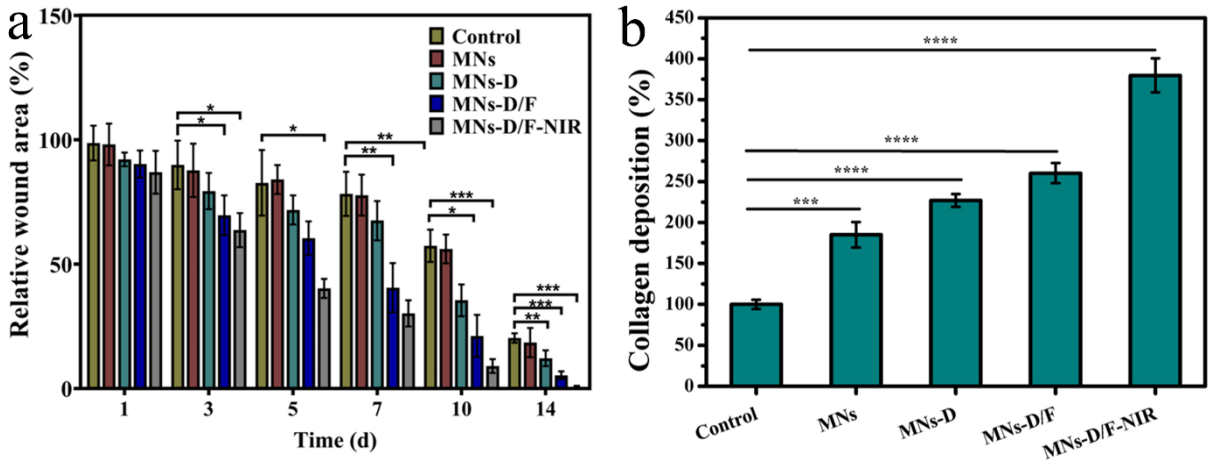

Figure S3. Relative wound area (a) and deposited collagen in the wound area (b) after treated by flexible microneedle dressings against control group.

References

[1] L Han, P Li, P Tang, X Wang, T Zhou, K Wang, F Ren, T Guo, X Lu. Nanoscale. 2019, 11, 15846.

[2] Y Liang, Z Li, Y Huang, R Yu, B Guo. ACS Nano. 2021, 15, 7078.