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Robust carbon dots based antibacterial CDs-PVA film as wound dressing for antibiosis and wound healing

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Materials

ε-Polylysine was purchased from Meilun Biotechnology Co., Ltd. Agar, peptone, and yeast powder were purchased from Seans Biochemical Technology Co., Ltd. Sodium chloride, sodium hydroxide, citric acid, and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. Live/Dead Cell Double Staining Kit and SYTO 9/PI kit were purchased from Jiangsu Kaiji Biotechnology Co., Ltd. MTT was purchased from Shanghai Yuanye Biotechnology Co., Ltd. Cell culture medium (DMEM) was purchased from Gibco.

Characterization

Fourier transform infrared (FTIR) spectroscopy was obtained by a Bruker Vertex 70 IR spectrophotometer. UV-Vis absorption spectra were performed on a UV-2450PC spectrophotometer (Shimadzu, Japan). The fluorescence spectra were measured by LS-55 fluorescence spectrometer (Perkin-Elmer, USA). Transmission electron microscopy (TEM) images were acquired by JEM-1011 electron microscope (JEOL Co., Japan). The zeta potential was measured by Zeta-sizer Nano ZS (Malvern Instruments Ltd., UK). The bacterial confocal images were obtained using Zeiss confocal laser microscope (ZEISS LSM 700).

Preparation of CDs

Citric acid (40 mg) was dissolved in water (5 mL) and added to a round-bottomed flask. The solution was heated to 180 ^{o}C and maintained for 10 min. Then, the aqueous

solution (5 mL) of ε-polylysine (200 mg) was added, and the reaction continued for 20 min. The crude product was cooled to room temperature and dissolved in water. The solution was centrifuged at 10000 r/min for 10 min to remove the precipitate. The supernatant was purified in a dialysis bag (MWCO: 3500 Da) for 24 h, and freeze-dried to obtain CDs.

Fabrication of CDs-PVA composite films

Different mass of CDs (3, 5 and 8 mg) was mixed with PVA (100 mg) aqueous solution (5 mL). The mixture was cast on the glass slides to prepare PVA_3, PVA_5 and PVA_8 composite films.

Hemolysis Test

The hemolysis rate of the sample was measured by a microplate reader at the OD value of 540 nm, and the hemolysis ratio was calculated. The calculation formula is:

$$\text{Hemolysis rate (\%)} = [(ODx - ODo) / (ODy - ODo)] \times 100\%$$

Where ODx is the OD value of the sample; ODo is the OD value of untreated red blood cells as the negative control; ODy is the OD value of the red blood cells treated with 0.1% Triton X-100 as the positive control.

Cytotoxicity assay

Cytotoxicity of PVA, PVA_3, PVA_5 and PVA_8 films was determined by MTT assay. NIH 3T3 cells were grown to log phase, cells were trypsinized and counted. The

cell solution was diluted to 1.0 × 10^4/mL, and the diluted cell solution was inoculated into a 96-well plate, adding 200 μL to each well, and incubated in a 37 °C incubator for 24 h. PVA, PVA_3, PVA_5 and PVA_8 films were then cut into circles with d = 8 mm, immersed in the culture medium, and then placed in a cell culture incubator for 24, 48 and 72 h. Three replicate wells were set for each film and time point, and two groups of experiments were performed in parallel. One group was taken to remove film and culture medium, and then AM staining solution was added. After incubation at room temperature for 30 min, imaging was performed under a confocal microscope to observe the staining of living cells. In the other group, after removing the film and the culture medium, 20 μL of MTT reagent was added to each well for 4 h under the dark condition, the cell culture medium was aspirated and 150 μL of DMSO solution was added to each well, shaken for 5 min. The absorbance at 490 nm was detected by a microplate reader, and the OD value of the obtained duplicate wells was averaged, which was taken as the OD value of the target sample and the cell viability was calculated.

Antibacterial experiments

In the agar diffusion method: Firstly, The 10^5 CFU/mL *E. coli* or *S. aureus* bacterial solution was prepared. The diluted bacterial solution (200 μL) was taken out and spread evenly on the plates. Secondly, PVA, PVA_3, PVA_5 and PVA_8 were cut to 8 mm in diameter and placed in the center of plates. Then, the plates were placed in a 37 °C incubator for 24 h and taken out for analysis.

In the liquid culture method: the films (PVA, PVA_3, PVA_5 and PVA_8) with diameter of 8 mm were soaked in PBS (1 mL) for 24 h and then removed. *E. coli* and *S. aureus* were incubated with the leaching solution or PBS (control groups) for 4 h. The mixtures (200 μL) were taken out to coat on the plates. The plates were placed in a 37 ^{o}C incubator for 24 h and taken out for analysis.

Bacterial live and dead test

200 μL of 10^5 CFU/mL bacterial solutionwas added dropwise to a 96-well plate. Then SYTO 9/PI staining solution was added, and after incubation at room temperature for 30 min, images were imaged under a confocal microscope to observe the staining of live and dead bacteria.

In vivo evaluation of wound healing

All of the animal experiments were performed according to the legal requirements of the Animal Welfare and Ethics Committee of Changchun Institute of AppliedChemistry, Chinese Academy of Sciences and all of the recommendations of the Guide for the Care and Use of Laboratory Animals were followed. Twenty-four mice were randomly divided into eight groups. The excisions with diameter of 8 mm were made on the back of mice. The wound areas were inoculated with *E. coli* or *S. aureus* for 2 h, then covered with PVA, PVA_3 and PVA_5 for 2 d, respectively. Photographs were taken on the day 1, 3, 5, 7, and 14, respectively, and the wound healing rate was calculated. The same experimental operation was performed for the *S.*

aureus group and the *E. coli* group. All the treated mice were sacrificed after the 14th day, and the wound surface skin was taken for tissue section staining experiment to observe the wound healing.

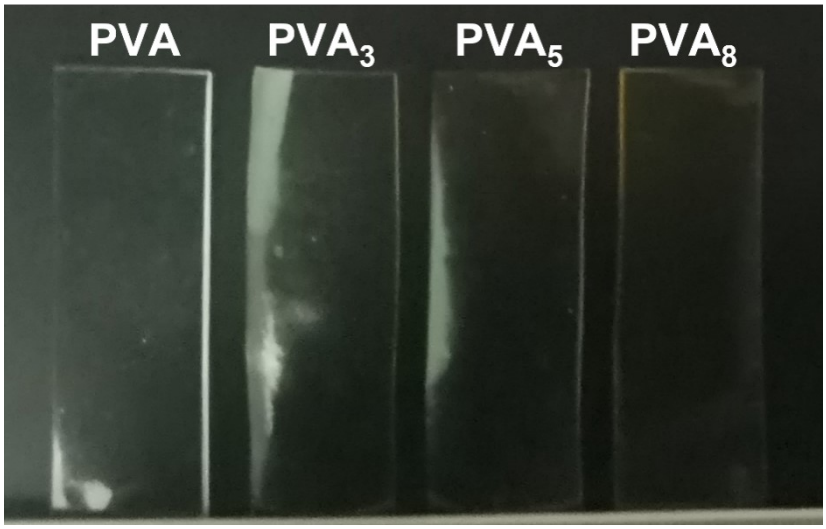

Figure S1. The photo of PVA, PVA_3, PVA_5 and PVA_8 under natural light.

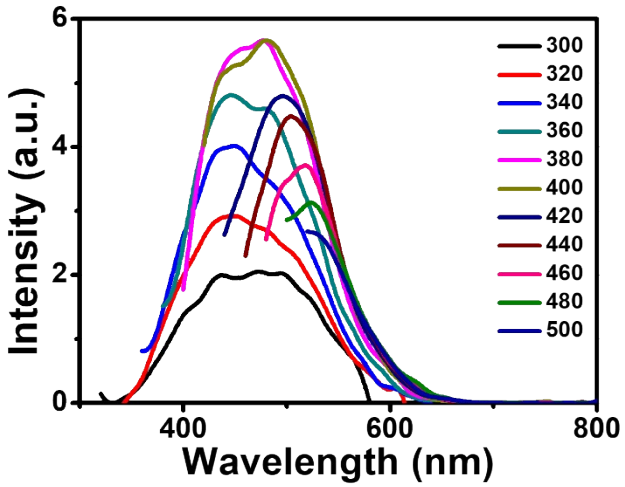

Figure S2. Fluorescence spectrum of PVA_3.

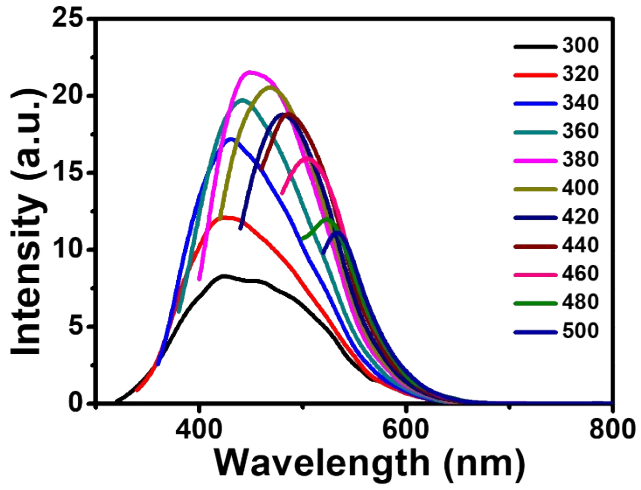

Figure S3. Fluorescence spectrum of PVA_8.

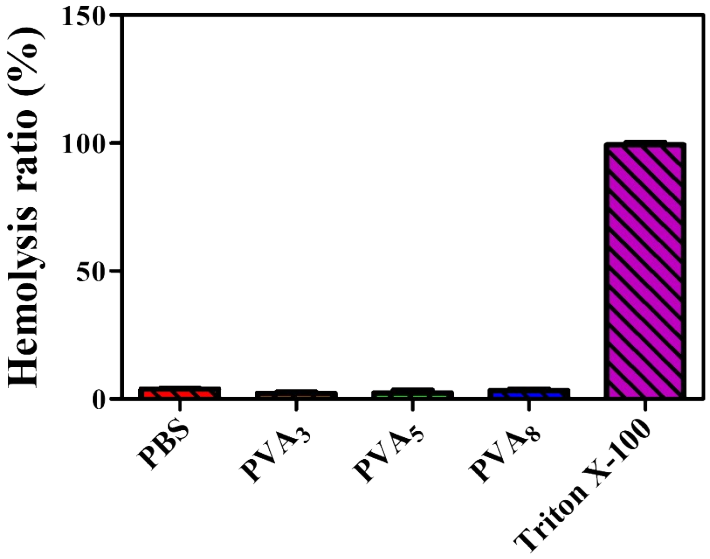

Figure S4. Quantitative analysis of hemolysis test.

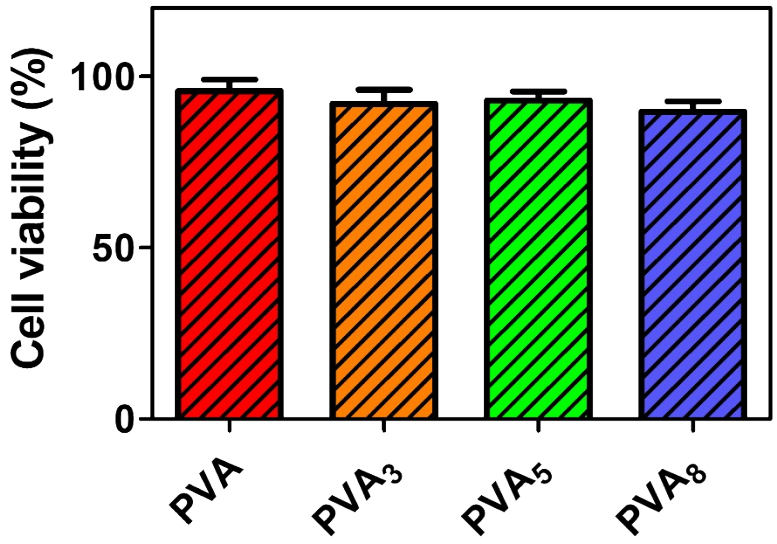

Figure S5. MTT assay of NIH 3T3 cells treated with PVA, PVA_3, PVA_5 and PVA_8 for 4 h.

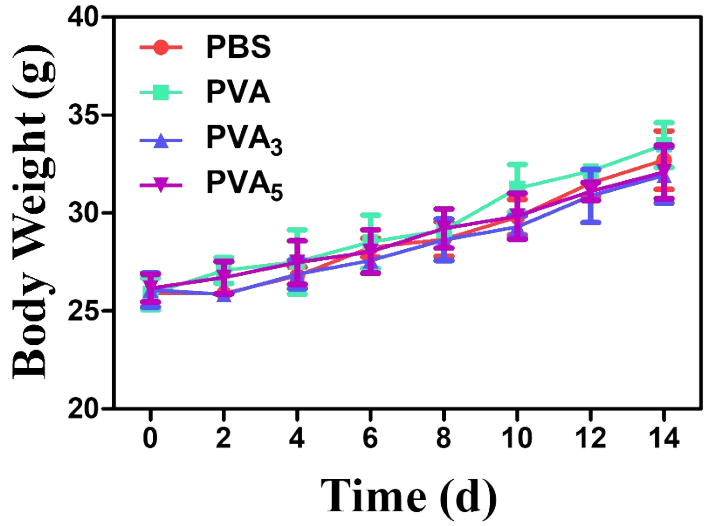

Figure S6. Body weight of mice in the PBS, PVA, PVA_3, and PVA_5 groups.