Supporting Information

ZIF nano-dagger coated gauze for antibiotic-free wound dressing

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EXPERIMENTAL SECTION

Synthesis Methods

ZIF-L coated gauze and Ag gauze

ZIF-L coated gauze was prepared as follows. Cotton gauze (Guardian, Singapore) was coated with ZIF-L at room temperature in an aqueous system with a molar ratio of 2-methylimidazole (2-MeIm) to zinc ions of 7. The gauze was fixed on glass slide and placed vertically in a reaction container with 200 mL of aqueous solution of 2-MeIm (0.35 mol/L). Twenty milliliters of $Zn(NO_3)_2$ aqueous solution (0.5 mol/L) were introduced dropwise. After stirring for 3 h, the coated gauze was removed, washed with ethanol thoroughly, and then dried in an oven at 60 °C.

The Ag-coated gauze (UrgoTul® Silver gauze) was purchased commercially.

Micro-organisms and growth conditions

E. coli (ATCC 8739) and *S. aureus* (ATCC 6538) were used as model wound pathogens to challenge the bactericidal functions of ZIF-L coated gauze. The bacteria were stored frozen at -80 °C, and were grown overnight at 37 °C in Mueller Hinton broth (MHB) prior to experiments. Subsamples of these cultures were grown for 3 h, and diluted to give an optical density value at 600 nm (OD600) of 0.07 (measured with a microplate reader; Tecan, Spark 10M), which corresponded to ~ 3×10^8 colony forming units of bacteria per mL (CFU/mL) (McFarland's Standard 1; confirmed by plate counts).

Antimicrobial efficacy of ZIF-L coated gauze

The bactericidal activity of ZIF-L coated gauze was tested against *E. coli* and *S. aureus* using modified JIS L 1902:2002 method. Briefly, ZIF-L coated gauze samples (~ 0.2 g) were cut, weighed and kept in sterile glass vials. Exponentially growing bacteria with OD600 of 0.07 were further diluted 100 times (~ 10^6 CFU/mL) and used as test inoculum. Five hundred

microliters were inoculated onto each sample. The samples were incubated at 37 °C. After 24 h, 20 ml of saline was added to each vial, and the mixture was shaken to wash off the bacteria from the gauze. The solution was serially diluted by 10 folds. A hundred microliters of each diluted solution were streaked across an agar plate (Luria-Bertani broth with 1.5% agar) in duplicate and incubated at 37 °C. Colony forming units were counted after overnight incubation, and the survival bacteria on each sample were calculated and expressed as CFU/mL accordingly. All the experiments were run in triplicates.

Scanning Electron microscopy (SEM)

Surface morphology of ZIF-L coated gauze was investigated using field emission scanning electron microscopy (FESEM, JEOL JSM-7400F, Japan) with samples sputter-coated with a 2- to 3-nm layer of platinum to provide a conductive surface.

The morphological changes of bacteria on ZIF-L coated cotton gauze were also examined by FESEM. Briefly, bacteria suspended in Dulbecco's phosphate buffered saline (DPBS) (10^6 CFU/mL, 200 μL) were seeded onto ZIF-L coated gauze and uncoated gauze. After incubation at 37 °C for 3 h, the samples were washed with PBS twice, followed by fixation with 2.5% glutaraldehyde in PBS for 2 h at room temperature. The fixed bacteria were dehydrated with a series of graded ethanol solutions (25%, 50%, 75%, 95% and 100%) for 15 min each. After drying for 2 days, the samples were coated with platinum for imaging.

Powder X-ray diffraction

Powder X-ray diffraction (PXRD) experiments were conducted on a Brucker D8 advance.

Hemolysis assay

The hemolytic activities of ZIF-L particles and ZIF-L coated gauze were measured. Fresh mouse red blood cells (RBCs) were diluted with sterile DPBS to give a stock suspension of RBCs (4 vol% blood cells).

For ZIF-L particles, 100-μL aliquots of RBC suspension were mixed with 100 μL of nano-dagger suspensions (ranging from 4 mg mL^{-1} to 8 μg mL^{-1} in serial two-fold dilutions in DPBS). After 1 h of incubation at 37 °C, the mixtures were centrifuged at 2000 rpm for 5 min. Aliquots (100 μL) of the supernatant were transferred to a 96-well plate. OD readings were recorded at 576 nm with microplate reader to assess hemoglobin release. A control solution that contained only DPBS was used as a reference for 0% hemolysis. Absorbance of red blood cells lysed with 0.5% Triton-X was taken as 100% hemolysis. Hemolysis by ZIF-L particles was calculated using the following formula.

$$\% \text{ Hemolysis} = \frac{OD576\ (samples) - OD576\ (PBS)}{OD576\ (Triton - X) - OD576\ (PBS)} X\ 100$$

For the testing of gauze samples, the diluted mouse blood (2 vol% blood cells, 4.0 mL) was added into a sterile tube containing uncoated cotton gauze, ZIF-L coated gauze, or UrgoTul® Silver gauze (1 cm × 1 cm). RBCs (4 vol% blood cells, 2.0 mL) treated with 0.1% Triton-X (2.0 mL) were used as a positive control, and RBCs (4 vol% blood cells, 2.0 mL) mixed with 2.0 mL of DPBS were used as a negative control. The tubes were incubated at 37 °C. During incubation, the samples were occasionally re-suspended by inversion (maximum once per hour). At different time intervals, aliquots of 300 μL were taken out from each tube and

centrifuged at 2000 rpm for 5 min. Next, 100 μL of supernatant were transferred to a 96-well plate. Hemolysis was measured and calculated by the same method as described above for ZIF-L particles. Data were expressed as mean and standard deviation (S.D.) of quadruplicates, and the tests were independently repeated twice.

Stability test

To evaluate the stability of ZIF-L coating on cotton gauze, the ZIF-L coated cotton gauze was incubated in freshly diluted mouse blood. The method is similar to hemolysis assay except that the incubation time is 7 days at room temperature. After experiment, the ZIF-L coated gauze was washed 2 times with sterile water and dried at room temperature. Then its morphology was investigated using FESEM after sputter-coating with platinum.

In vitro cytotoxicity

The effects of ZIF-L particles or ZIF-L coated gauze on cell viability were examined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

Primary human dermal fibroblasts (HDF) (ATCC PCS-201-012) were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies) and 100 IU/mL penicillin-streptomycin (Life Technologies), and maintained at 37 °C under a humidified atmosphere with 5% CO_2. Cells were allowed to expand to 80–90% confluence before passaging with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) (Life Technologies). Before treatment, cells were seeded onto 96-well plates at ~ 10^4 cells per well. After 2 days, to test the cytotoxicity of ZIF-L particles, media were replaced with 100 μL of fresh DMEM containing ZIF-L particles (with final concentrations of 2, 4, 8, 16, 31, 62.5, 125, 250, 500, 1000 and 2000 μg/mL). For cytotoxicity test of coated gauzes, the media were replaced with 100 μL of fresh DMEM containing one piece of ZIF-L coated gauze (0.8 cm × 0.8 cm) or Urgptul® Silver gauze (0.8 cm × 0.8 cm).

Cells were further cultured at 37 °C for 1 h and treated with 100 μL of CellTiter-Glo® 2.0 Reagent. After incubating at room temperature for 10 min to stabilize luminescent signal, the luminescence of each well was measured with a microplate reader (Tecan Safire). Cell viability was expressed as the ratio of the number of viable treated cells to that without treatment. Experiments were conducted in triplicates, and consistent results were obtained.

Mouse model

Male C57BL/6Jinv mice, 6–8 weeks old, were obtained from InVivos Pte Ltd, Singapore. The mice were randomly grouped. In each group, a total of five mice were used. All the animals were maintained on a 12-h light/dark cycle with access to food and water *ad libitum*. All animal procedures were approved by Institutional Animal Care and Use Committee, Biological Resource Centre, Singapore (protocol number: 171289).

The animal studies were modified from literature. Before surgery, mice were anesthetized via intraperitoneal injection of sodium pentobarbital solution (350 mg/kg). The skin on the back and neck of each mouse was shaved with an electric razor, followed by a depilatory agent. A 6-mm punch biopsy tool was used to remove the epidermis and dermis, and create

two symmetrical full-thickness skin defects on the back of each mouse. Each defect was inoculated with 20 μl of *S. aureus* (10^6 CFU/ml). After 5 min, 1 cm × 1 cm of ZIF-L coated gauze, uncoated gauze, or Ag-coated gauze was placed to cover the wounds. Next, the wound was covered up with Tegaderm (3M, Singapore), and the mice were further wrapped with a bandage (Coban, 3M, Singapore).

Bacterial counts and histology in wound tissue

After 11 days observation, mice were euthanized with CO_2. The wounds were excised aseptically and then homogenized in 1 mL of DPBS. Viable bacteria in DPBS solution were counted by making 10-fold serial dilutions and culturing the dilutions on LB agar for 24 h at 37 °C, after which CFUs were counted manually. Data were expressed as mean and S.D. of triplicates.

For histological analysis, thin cross-sections of wounds at maximum diameter were prepared on glass slides. Staining with hematoxylin and eosin (H&E) was performed. Standard immunohistochemistry techniques were used, and images of the stained tissues were captured with an Olympus IX-83 inverted microscope (Tokyo, Japan).

Statistical analysis

Data were expressed as means ± S.D. of the mean; S.D. was indicated by error bar. Student's *t*-test was used to determine significance among groups. A difference with $P < 0.05$ was considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

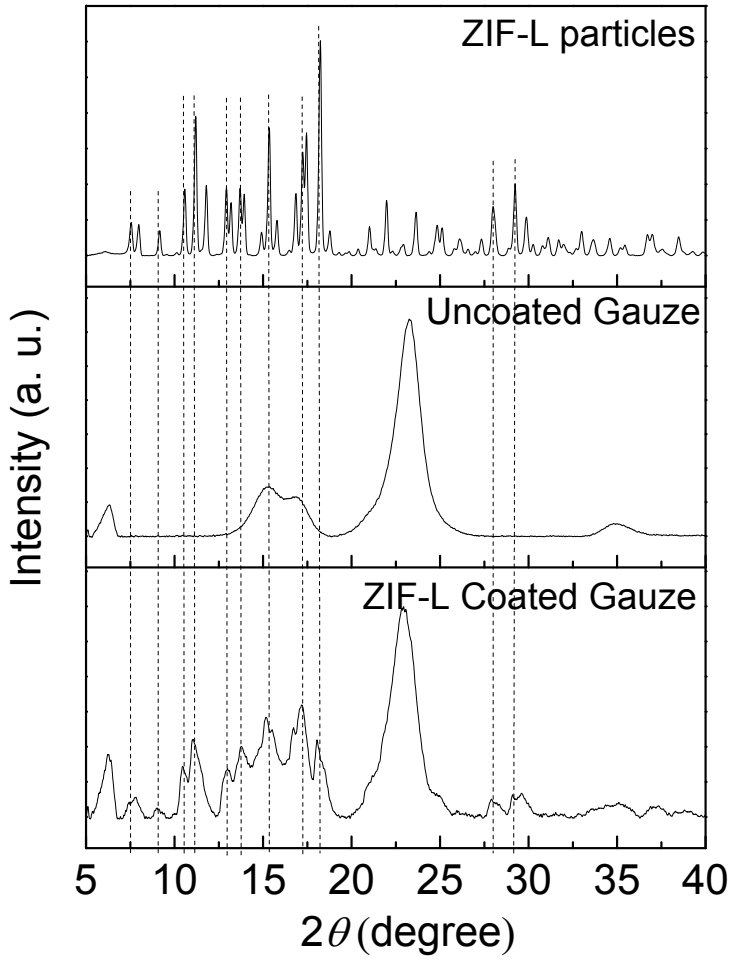

Figure S1. XRD patterns of ZIF-L particles, uncoated cotton gauze and ZIF-L coated cotton gauze.

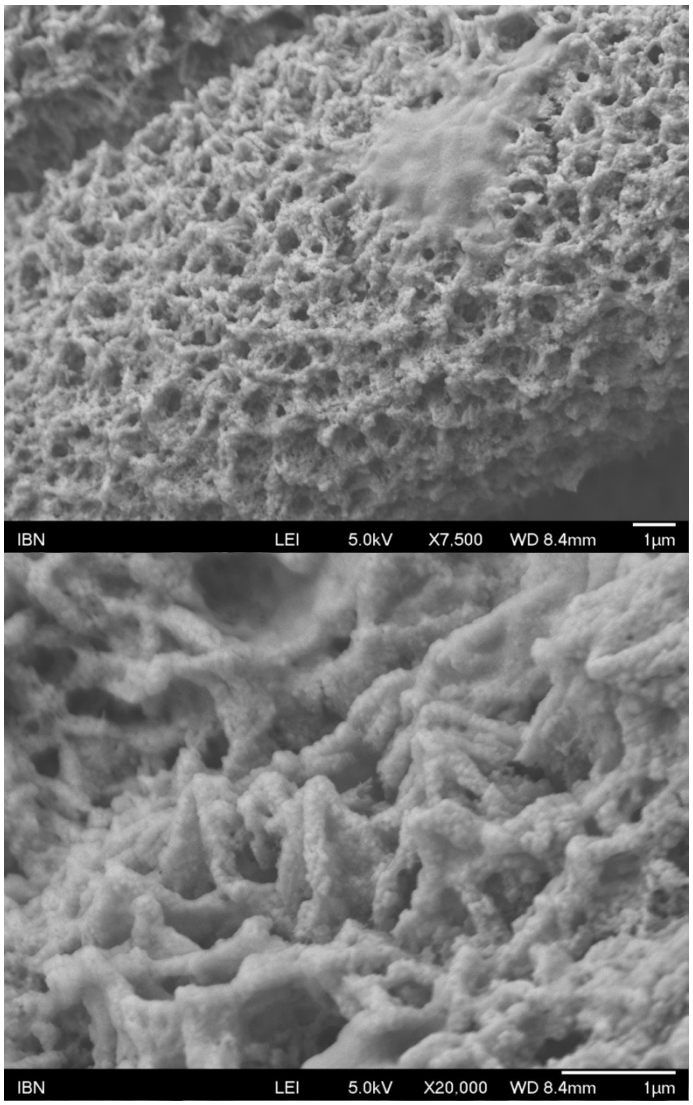

Figure S2. SEM image of ZIF-L coated cotton gauze after Incubation in diluted mouse blood solution for 7 days.

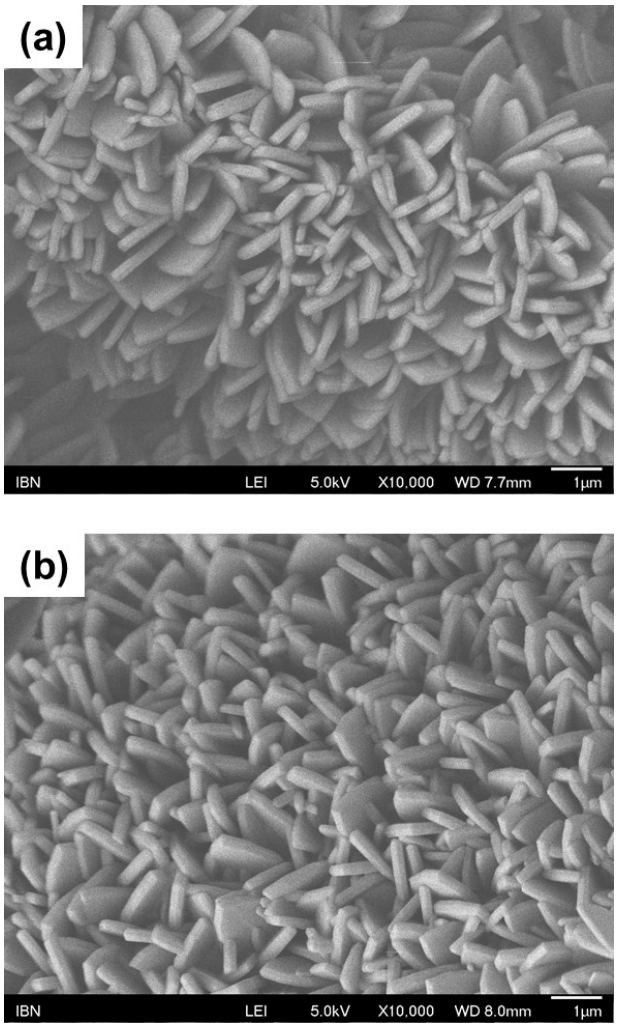

Figure S3. SEM images of ZIF-L coating on cotton gauzes obtained from different suppliers: (a) cotton gauze obtained from First Aid Supplies Pte Ltd, Singapore; (b) cotton gauze obtained from Guardian Health & Beauty, Singapore.