Supplementary information

Development of a Janus nanofiber patch with antibacterial and anti-

oxidative properties for urethral regeneration

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Materials

Poly-L-lactic acid (PLLA) was purchased from Haishan Technology Co., Ltd. (Wuhan, China). Bulk MAX (Ti₃AlC₂) was obtained from XFNANO Material Tech. Co., Ltd. (Nanjing, China). Ce(NO₃)₃·6H₂O, LiCl, HCl, hydrofluoric acid, 25% NH₃·H₂O, tryptone, agar, and yeast extract were acquired from Sinopharm Chemical Reagent CO., Ltd. (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3,3',5,5'-Tetramethylbenzidine (TMB), 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), and 2,2,6,6-Tetramethyl-4-piperidone monohydrate (TEMP) were purchased from Aladdin Bioengineering Co. Ltd. (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) with high glucose, fetal bovine serum (FBS), 0.25% trypsin, and phosphate-buffered saline (PBS) were acquired from Pricella Biotechnology Co., Ltd. (Wuhan, China). The Live/dead bacterial staining kit and the calcein-AM/PI double stain kit were sourced from Yeasen Biotechnology Co., Ltd. (Shanghai, China). Reactive oxygen species (ROS) detection kit was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Silkworm cocoons were purchased from Jiaxing Silk Co., Ltd. (Jiangsu, China). Antimicrobial peptide CRRI-3, with the amino acid sequence REERWF-NH2, was synthesized and identified by Sangon Biotech Co., Ltd. (Shanghai, China). Other chemicals and reagents were used as received.

Materials characterizations

The morphology of Ti₃C₂ nanosheets, CeO₂ nanoparticles, MCe heterojunction, and composite nanofibers were observed using scanning electron microscopy (SEM). The diameter of nanofibers was statistically calculated using Image J sofeware. MCe heterojunction was dispersed into ethanol, dropped onto copper grids for morphological observation of transmission electron microscopy (TEM) at a voltage of 80 kV. The element composition of MCe heterojunction was analyzed using energy dispersive spectrometer (EDS). The surface chemical state and crystal structure of MCe heterojunction were detected using X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD), respectively.

Mechanical property testing

The nanofibers were cut into strips measuring 5 cm in length and 1 cm in width.

Tensile tests were conducted using a universal testing machine (UTM). The thickness of the nanofibrous membrane was measured using electronic calipers. The nanofiber strips were fixed in a clamp with an initial distance of 2 cm between the clamps. The clamp stretching speed was set at 2 mm/min. The stress-strain curves were recorded. At least three samples were used for statistical analysis.

Hydrophilicity test

The hydrophilicity of the nanofibers was evaluated using a contact angle measurement device (CAMD). Samples were fixed on a sample plate, and 10 μ L of normal saline was dropped onto the upper surface of the nanofibers using a micro syringe. Photos were taken immediately, and the water contact angle (WCA) was measured using the goniometer method.

Electron paramagnetic resonance (EPR) analysis

For O^{2-} detection, methanol (100 µL), xanthine oxidase and xanthine were mixed and reacted for 5 min, followed with the addition of MCe and CeO₂ dispersions. After another 5 min, DMPO (250 mM) was added as a trapping agent. The solution was immediately transferred to a sample tube to record the EPR spectrum of O^{2-} .

For \cdot OH detection, double distilled water (ddH₂O, 50 µL), H₂O₂ (10 mM, 50 µL), and FeCl₂ (1 mM, 20 µL) were mixed and reacted for 5 min, followed by the addition of MCe and CeO₂ dispersions. After another 5 min, DMPO (250 mM) was added as a trapping agent. The solution was immediately transferred to a sample tube to record the EPR spectrum of \cdot OH.

For ${}^{1}O_{2}$ detection, ddH₂O (50 µL), H₂O₂ (10 mM, 50 µL), and sodium hypochlorite (NaClO) (10 mM, 20 µL) were mixed and reacted for 5 min, followed by the addition of MCe and CeO₂ dispersions. After another 5 min, TEMP (250 mM) was added as a trapping agent. The solution was immediately transferred to a sample tube to record the EPR spectrum of ${}^{1}O_{2}$.

Biocompatibility evaluations

Biocompatibility in vitro

L929 cells and HUVEC cells are cultured using DMEM high-glucose medium, and seeded onto a 96-well plate at a density of 1500 cells per well. The nanofibers were

added onto the medium and co-incubated at 37 °C for 5 days. At regular time intervals, the viability of cells was detected by CCK-8 assay. The cells are seeded onto the upper surface of PC nanofibers and SMC nanofibers for 5 days. At regular time intervals, the samples were collected and fixed with 4% paraformaldehyde for 30 min. The morphology of cells adhered onto the nanofibers was observed by SEM.

Biocompatibility in vivo

This study was conducted with the approval of the Animal Care and Welfare Committee of Zhongnan Hospital, Wuhan University (WP20230604). Twelve healthy SD rats, weighing 180-200 g, were purchased from Slike Jingda Lab Animal Co., Ltd. (Hunan, China). All SD rats were fed in a specific pathogen-free environment for 7 days to reduce stress. A subcutaneous implantation model was established. The samples were cut into pieces with a diameter of 1 cm. SD rats were anesthetized with 3% pentobarbital and disinfected with 0.5% iodine tincture. The skin on their back was incised. The subcutaneous connective tissue was freed to place the samples underneath. The skin was sutured, and the rats were conventionally fed. After 15 days of fed, fresh blood was collected from the rats using coagulation tubes, and plasma was obtained by centrifugation for immediate detection of a series of biochemical indicators. Additionally, the materials, heart, liver, spleen, lung, kidney and brain of the rats were collected for H&E staining assay.

| Antigens | Species antibodies raised in | Dilution (IF) | Supplier |
|----------|------------------------------|------------------|---|
| AE1/AE3, | Mouse,monoclonal | 1:100 | Santa Cruz Biotechnology, USA, cat. # SC-81714 |
| TGF-β | Mouse, monoclonal | 1:100 | Novus Biologicals, USA, cat. # NBP2-46108 |
| Ki67 | Mouse, monoclonal | 1:3000 | Novus Biologicals, USA, cat. # NBP3-05537 |
| CD206 | Mouse, monoclonal | 1:400 | Proteintech Group, China, cat. # 60143-1-I |
| TGF-β | Rabbit, monoclonal | 1:200 | Bioss Biotechnology, China, cat. # bs-0086R |
| CD86 | Rabbit, monoclonal | 1:200 | Cell Signaling Technology, Inc. USA. cat. # 19589T |

Table S1. List of primary antibodies

| Table S2. List of secondary fluorescent antibodies | | | | |
|--|------|----------|--|--|
| Secondary detection system used | Host | Dilution | Supplier | |
| Anti-Rabbit-IgG (H+L)- 488 | Goat | 1:500 | Proteintech Group, China, Cat. # SA00013-2 | |
| Anti-Mouse-IgG (H+L)- 488 | Goat | 1:200 | Proteintech Group, China, Cat. # SA00013-1 | |



Fig. S1. XPS spectrum of MCe heterojunction, including C 1s (A) and Ti 2p (B).



Fig. S2. (A) Quantitative results of DPPH assay (n = 3); (B) Quantitative results of TMB assay (n = 3). Values are shown as the Mean \pm SD. For the significant comparison, *n.s.* indicates no significance, ****P* < 0.001.



Fig. S3. Live/dead cell staining images of HUVEC cells. Scale bar: 200 μ m.



Fig. S4. (A-B) The migration ability of L929 cells were evaluated by scratching assay (n = 3). Values are shown as the Mean \pm SD. Compared to P.C. group, ***P < 0.001.



Fig. S5. Blood biochemical tests, including (**A**) albumin (ALB), (**B**) creatinine, (**C**) Na⁺, (**D**) K⁺ and (**E**) Ca²⁺ (n = 3). Values are shown as the Mean \pm SD. Compared to P.C. group, *n.s.* indicating no significant.



Fig. S6. IF staining images of AE1/AE3. Scale bar: 500 μ m.



Fig. S7. IF staining images of TGF-β. Scale bar: 500 μm.



Fig. S8. IF staining images of Ki-67. Scale bar: 500 $\mu m.$



Fig. S9. IF staining images of CD206. Scale bar: 500 $\mu m.$



Fig. S10. (A) IF staining images of CD86. Scale bar: 500 μ m; (B) Quantitative results of CD86 (n = 3); (C) Relative content of IL-6 at Week 5 (n = 3); (D) Re lative content of TNF- α at Week 5 (n = 3). Values are shown as the Mean \pm SD. For the significant comparison, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.