AgNPs/nGOx/Apra Nanocomposites for Synergistic Antimicrobial Therapy and Scarless Skin Recovery

Ming Zhao[a,b,1], Mingqing Zhou[a,b,1], Peng Gao[d,1], Xueyun Zheng[e], Wen Yu[a,b], Zhenyuan Wang[a,b], Jingchao Li[c*], Jiaheng Zhang[a,b*]

[a] Research Center of Printed Flexible Electronics, School of Materials Science and Engineering, Harbin Institute of Technology, Shenzhen 518055, PR China

[b] Sauvage Laboratory for Smart Materials, Harbin Institute of Technology, Shenzhen 518055, P.R. China

[b] National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou 215006, PR China

[c] Shanghai Engineering Research Center of Nano-Biomaterials and Regenerative Medicine, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, PR China

[d] National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou 215006, PR China

[e] Guangdong Provincial Key Laboratory of Fermentation and Enzyme Engineering, South China University of Technology, Guangzhou 510006, PR China

* Corresponding authors.

E-mail address: jcli@dhu.edu.cn (Jingchao Li); zhangjiaheng@hit.edu.cn (Jiaheng Zhang)

[1] These authors contributed equally to this work.

Materials and methods

Zone of inhibition test for antimicrobial activity. 5 μL of synthesized AgNPs dispersion (0.4 mg/mL、0.3 mg/mL、0.2 mg/mL、0.1 mg/mL 0.05 mg/mL) was dropped onto small filter paper disks (Φ6 mm), followed by complete oven-drying at 37 °C. 100 μL of *E. coli* BL21-EGFP suspension (10^6 cfu/mL) was poured and spread evenly over the surface of LB agar petri plates. Then five AgNPs-loaded filter paper disks were evenly spaced. The agar plate was incubated at 37 °C overnight to allow bacterial growth. Average diameters of zone of inhibition were measured and recorded. Note that the inhibition zone assay for nGOx and apramycin (Apra) followed the same procedure except that their working concentrations were: 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL, 0.001 mg/mL, 0.0001 mg/mL (nGOx) and 0.5 mg/mL, 0.05 mg/mL, 0.005 mg/mL, 0.0005 mg/mL, 0.00005 mg/mL (apramycin) respectively.

Synthesis of ROS-labile linker (TSPBA). ROS-labile crosslinker, N^1-(4-boronobenzyl)-N^3-(4-boronophenyl)-N^1,N^1,N^3,N^3-tetramethylpropane-1,3-diaminium (TSPBA) was synthesized according to the following procedure: 4-(bromomethyl) phenylboronic acid (1 g, 4.6 mmol) and , *N,N,N',N'*-tetramethyl-1,3-propanediamine (0.2 g, 1.5 mmol), were dispersed in dimethylformamide (DMF) (50 mL) at 60 °C under magnetic stirring. After 24 h, the resulting mixture was poured over tetraphydrofuran (THF) (100 mL) to precipitate the reaction product, followed by washing the precipitates with THF several times before oven-drying at 60 °C overnight to obtain the final product (0.5 g, yield 60 %).

Characterization of *in-vitro* drug release profiles. To determine the release profiles of the nanocomposite from dissolving microneedle arrays, the nanocomposite-loaded MN patch containing the optimized combination of nGOx, AgNP and Apra was fabricated. The MN patch was placed in 1 mM H_2O_2 solution (5 mL) at 37 °C to trigger degradation. At 10-minute intervals, 200 µL of aliquots were removed from the incubation mixture for release analyses. Contents of the nGOx, AgNPs and Apra were determined by the BCA assay, the UV/Vis absorbance at 425 nm, and a polarimeter respectively. The time-dependent drug release curves were plotted.

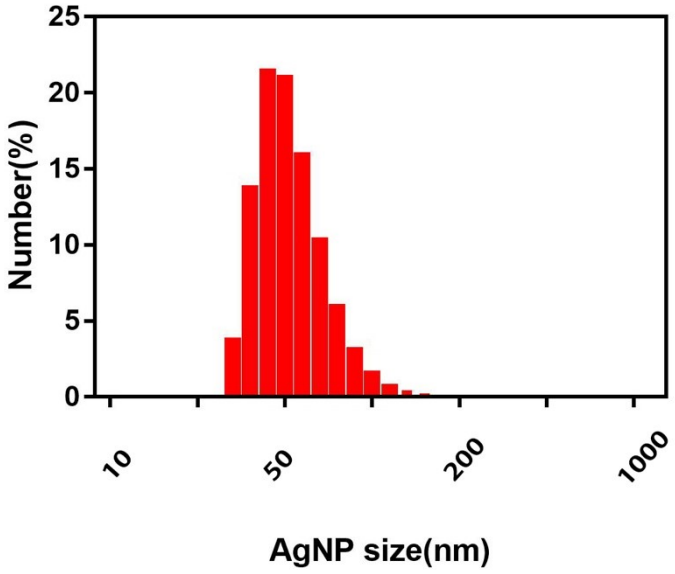

Figure S1 Size distributions of the silver nanoparticles measured by DLS.

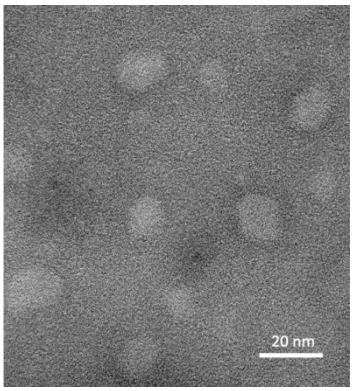

Figure S2 TEM characterization of nGOx

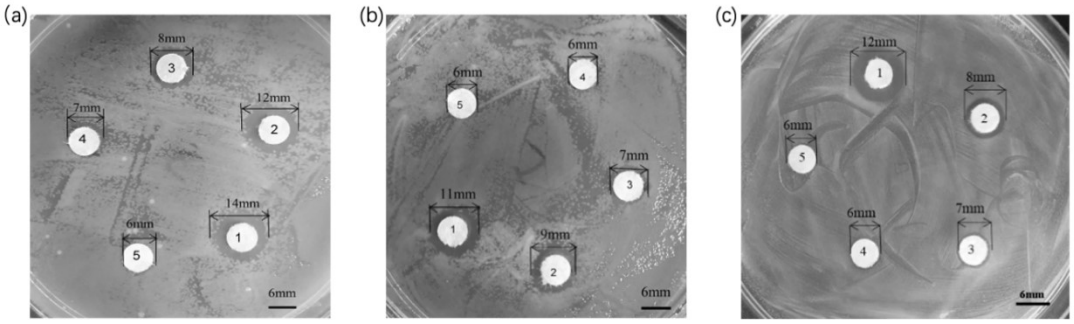

Figure S3 Zone of inhibition test for antibacterial activity. (a) Zone of inhibition test for antibacterial activity of AgNPs at 0.4 mg/ml (1), 0.3 mg/ml (2), 0.2 mg/ml (3), 0.1 mg/ml (4), and 0.05 mg/ml (5). (b) Zone of inhibition test for antibacterial activity of nGOx at 1 mg/ml (1), 0.1 mg/ml (2), 0.01 mg/ml (3), 0.001 mg/ml (4), and 0.0001 mg/ml (5). (c) Zone of inhibition test for antibacterial activity of Apra at 0.5 mg/ml (1), 0.05 mg/ml (2), 0.005 mg/ml (3), 0.0005 mg/ml (4), and 0.00005 mg/ml (5).

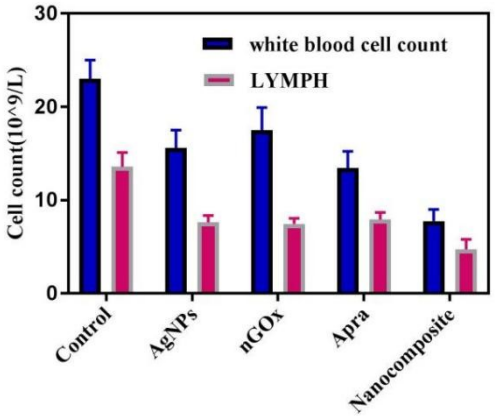

Figure s4 Analysis of white blood cell and lymphocytes numbers

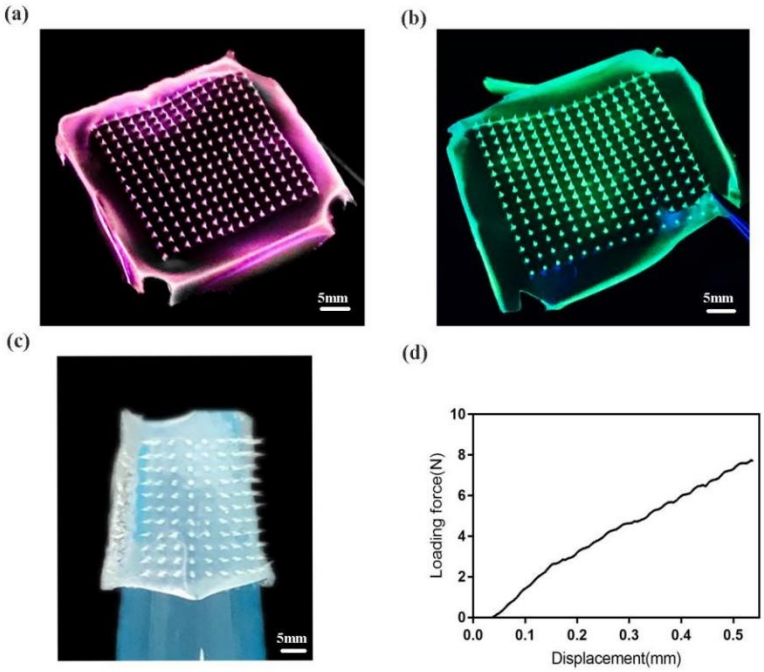

Figure S5 Fabrication and characterization of the microneedle array patch. (a) A fluorescence microscopy image demonstrating rhodamine-labeled AgNPs evenly distributed in the microneedle matrix. (b) A fluorescence microscopy image showing FITC-labeled nGOx evenly distributed in the microneedle matrix. The overlapping of the two fluorescence signals indicates the close association between AgNPs and nGOx. (c) A photograph of the microneedle patch. Scale bar: 5 mm. (d) Mechanical strength measurement of the microneedle array patch.

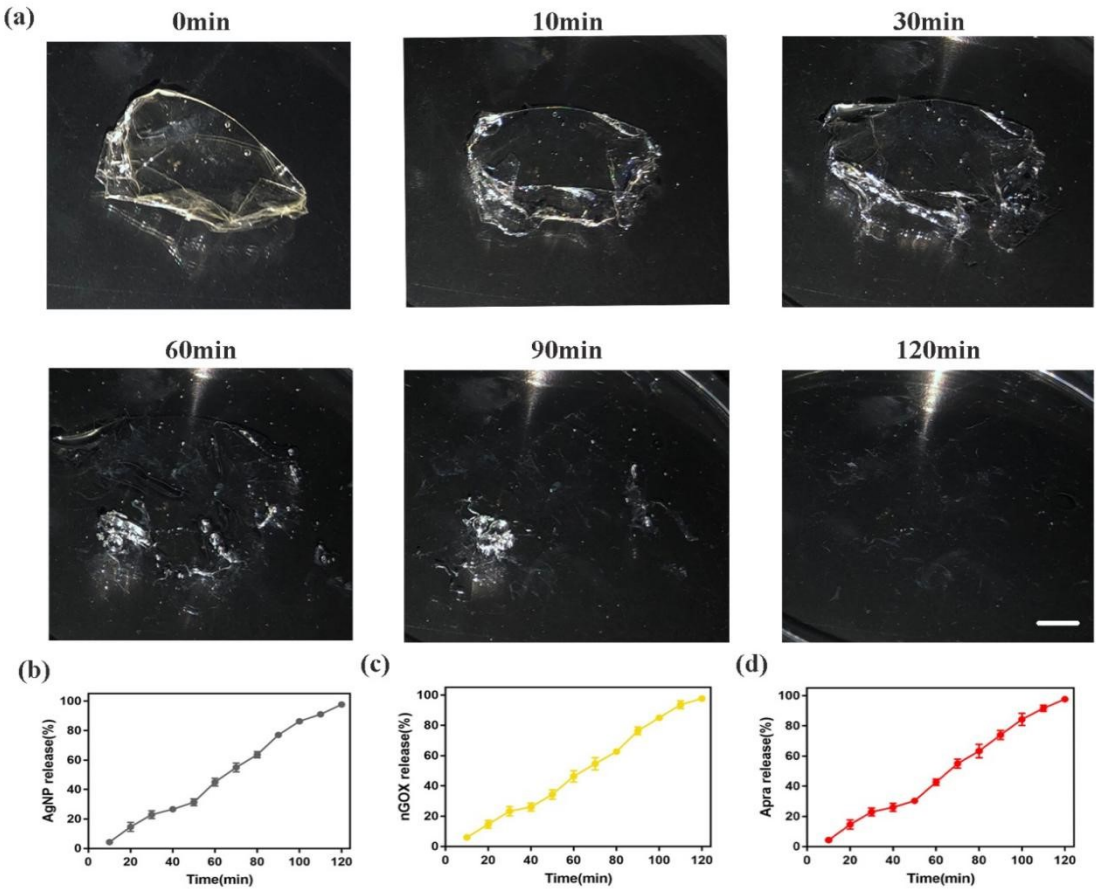

Figure S6 Degradability of the microneedle patch in response to H_2O_2. (a) Degradation of the microneedle patch in 1× PBS solution containing 10 mM H_2O_2 at 37 °C. AgNPs (b), nGOx (c) and Apra (d) release profiles from the microneedle batch when immerged in 10 mM H_2O_2, 1× PBS solution at 37 °C.

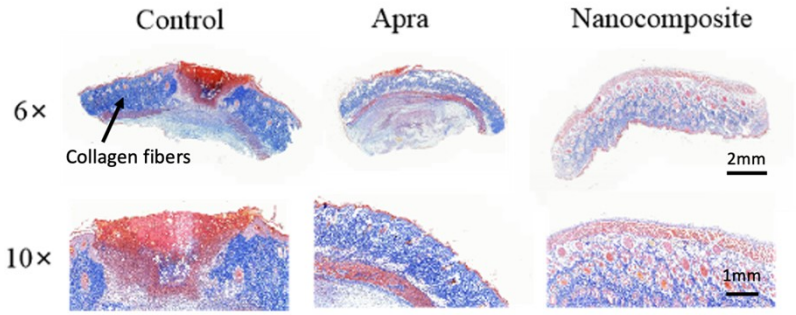

Figure S7 Masson's Trichrome staining of infected skins with/without nanocomposite treatments. Collagen fibers were stained in blue.

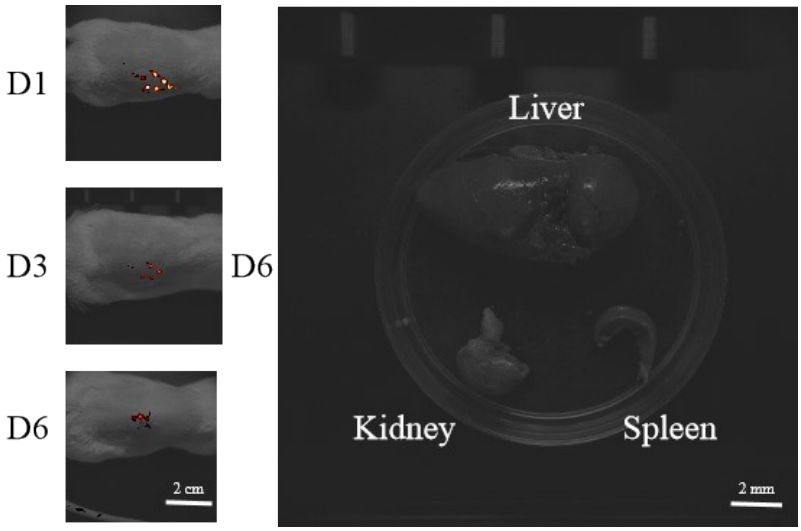

Figure S8 Biodistribution characterization of the nanocompsoites