Supplementary information

Lotus Root Inspired Implant System with Fever Responsive Characteristic and 3D Printing Defined Nano-antibiotic Release Patterns

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implants.

Experimental Section

1.1 Materials: Polylactic acid (PLA, D=1.75mm) for 3D printing was provided by Print-Rite Unicom Image Products CO,.LTD. of ZhuHai. Silver nitrate ($AgNO_3$) was provided by Alfa Aesar Co (Tianjin, China). Phosphate buffer saline (PBS), starch and fluorescence isothiocyanate were purchased from Aladdin Co (Shanghai, China). Ethanol, Glucose and Silver sulfadiazine (Valencia Reagent Limited; 98%) were provided by West Long Chemical Co. (Shanghai, China). LB broth, Agar powder and medical sterilization alcohol (75%) were provided by Jiangxi Department of Health Enterprise Co. (Jiangxi, China).

1.2 Characterization: Silver nanoparticles (Ag NPs) was observed by X-ray diffraction (D3 ADVANCE type, German Bruker-AXS Company). Transmission electron microscope (JEM-2010 type; Japan Electronics Co.) was employed to measure the size of Ag NPs. The intensity of FITC and the cytocompatability of materials were measured by enzyme-linked immunosorbent assay (SpectraMax M5 type, Tianjin Science and Technology Development Co.) and H&E staining were observed by fluorescence microscope. SLA 3D printer (Pegasus Touch, America) was used to print the modular experimental apparatus. The mechanical strength of designed implant was measured by computer controlled servo pressure high-temperature testing machine (GAW2000, SANS).

1.3 Preparation of 3D Printing Implants: All of the implants were designed by 3D software CATIA. Two 3D printers (SLA Pegasus Touch, America and MakerBot Replicator Z18) was used to print the models of the implant scaffolds.

1.4 FITC Loading and In Vitro Release Study: Fluorescein isothiocyanate (FITC) (0.5 mg/mL) was injected into the internal cavity of 3D printing implants, then the FITC loaded implants were placed at petri dishes with 43 mL PBS (pH 7.4). To simulate the environment of blood circulation, FITC release was carried out in constant temperature vibrator with a vibration rate of 50 times/min for 50 minutes at 37°C. After incubation for a specific time of interval, 100 μL sample was taken out to 96 well plate for measuring the fluorescence intensity *via* ELIASA microplate reader, the time points were 2 min, 6min, 10min, 15min, 20min, 25min, 30min, 40min, and 50min. The experiment was repeated three times.

1.5 Synthesis of Silver nanoparticles: Firstly, 1 g soluble starch was dissolved into 95 mL deionized water and heated with stirring to form starch solution. Secondly, 5 mL $AgNO_3$ (1mol/L) solution was added with the starch solution and stirred in the dark to form starch solution containing $AgNO_3$. Thirdly, After 10 mL glucose solution (4 mol/L) was quickly added to the above solution with stirring to form a homogeneous mixture solution. Lastly, the reaction was placed in an ultrasonic power (560W, temperature 40°C) for 20 min, the mixture was placed under a dark environment to self-reaction for another 6 h.

1.6 Anti-microbial Assay in vitro: To demonstrate antimicrobial property of released Ag NPs, five of the most common clinical strains—*Escherichia coli*, *Shigella*, drug resistant *S. aureus*, standard *S. aureus*, *Pseudomonas aeruginosa* were chose to assess the practical bactericidal effect of the released silver nanoparticles. Firstly, 5 ml Luria-Bertani broth, 100 μL bacterium suspension were mixed in 10 ml tubes, and then 200 μL silver nanoparticles sealed by thermosensitive material tetradecyl alcohol was added into the tube. Instead of silver nanoparticles, 200 μL PBS and 200 μL water was added into the tube as control experiment respectively. Next, the three samples were heated in water bath pot at 45 °C and then the bacterial solution was

cultured in orbital shaker for 6 h. Last, 100 μL of the co-culture media was taken out for different degree of dilution and 50 μL of the diluent was used to coat on the plates which were placed in the constant temperature incubator (37 °C) for 24 hours later. The antibiotic potency of silver nanoparticles was compared by plate colony-counting method.

1.7 Anti-microbial Assay in vivo: All experiments were performed in cpmpliance with the relevant laws and approved by the Institutional Animal Care and Use Committee at Institute of Translational Medicine, Nanchang University. To verity that hyperthermia could be induced by infection, KM mice and rabbit were injected with drug resistant *S. aureus* subcutaneously. Temperature was measured every day via infrared thermal imager. In order to demonstrate the antimicrobial property of Ag NPs loaded implant in vivo, 200 μL silver nanoparticles loaded implant sealed by thermosensitive material tetradecyl alcohol was implanted subcutaneously at the dorsum of rabbit, and then injected with 1mL drug resistant *S. aureus* was injected at the site of wound. The rabbit was feed at 25°C for 1 week. At last, the skin tissue was sliced and treat with H&E staining.

1.8 Cytocompatability Assay in vitro: 3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM,GIBCO) with 10% fetal bovine serum (FBS) in a humidified incubator at 37°C under the conditions of 5% CO_2, and the culture medium was replaced once every day. Cells were seeded in 96 well plates after digested by trypsin and grown overnight. PLA wafers were added to the 96 well plates and incubated with cells for 24 h, 48 h and 72 h respectively. Then, 10 μL CCK8 reagent were added in cell culture dish, followed by an additional 2h incubation at 37°C. Afterward, Enzyme standard instrument was used to measure the absorbance at 450nm.

1.9 Statistical Analysis: All data were expressed as means±standard deviations (SD). The statistical was performed using Student's t test and one-way analysis of variance (ANOVA) at confidence levels of 95 and 99 % (originPro version 7.5)

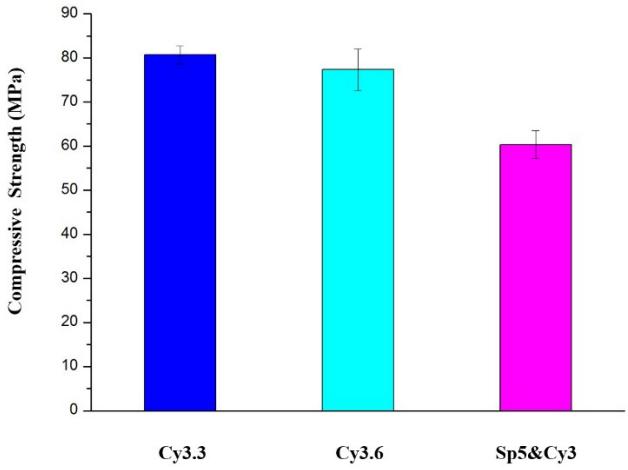


Figure S1： The mechanical strength of designed implant (Solid cylinder, Cy3.6 and Sp5&Cy3).

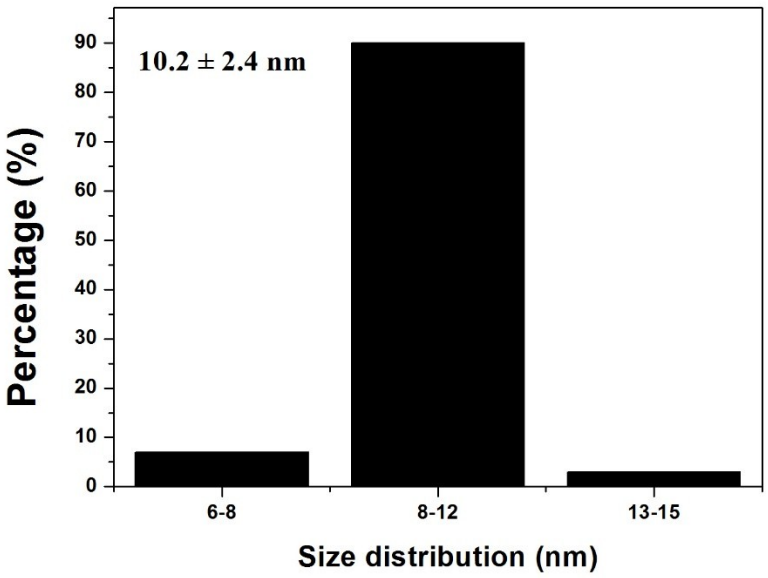


Figure S2： The size distribution of Ag NPs (n=300).

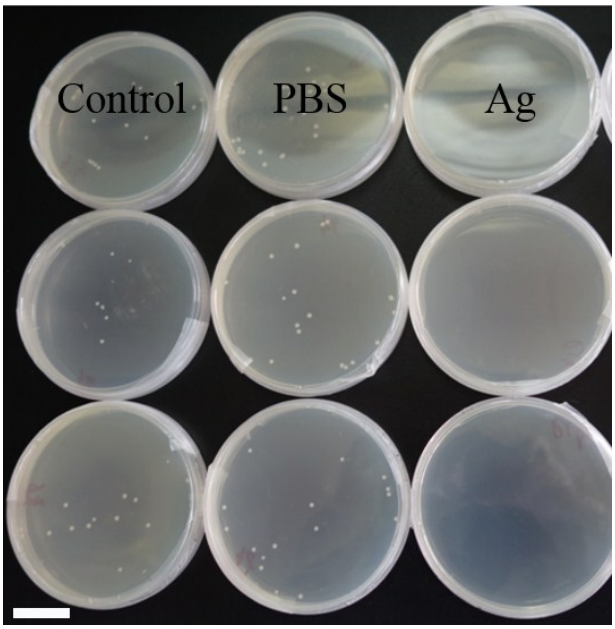


Figure S3： The bacterial colony of three groups after the drug resistant *S. aureus* solution were cultured on the petri dish for another 24 h. (Scale bar: 20 mm)

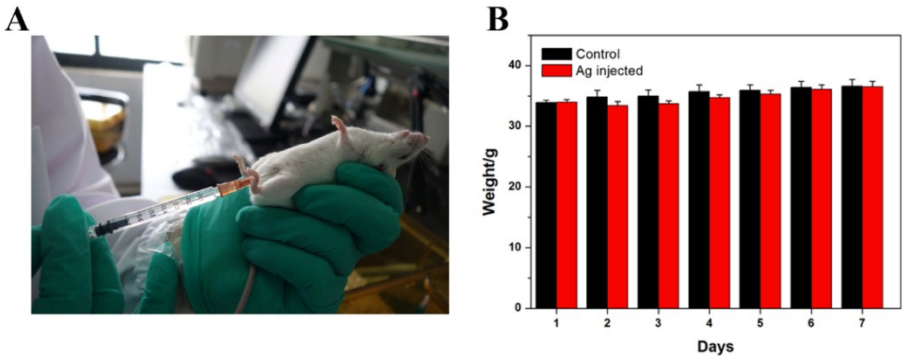


Figure S4：(A) Photo of Ag NPs injection to mice intraperitoneally; **(B)** The weight change of mice treated with Ag NPs injection and normal mice.

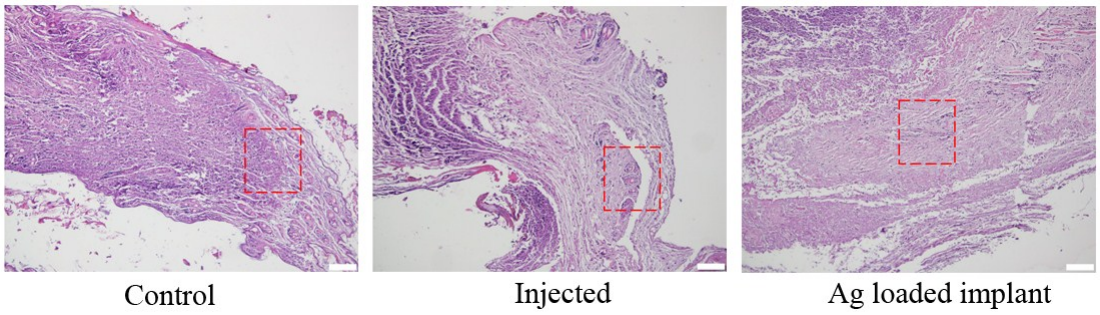


Figure S5： H&E stained skin of rabbit with different treatment in wide range (Enlarged H&E stained photos, marked with red block, were shown in Figure 5c). (Scale bar: 200 μm)

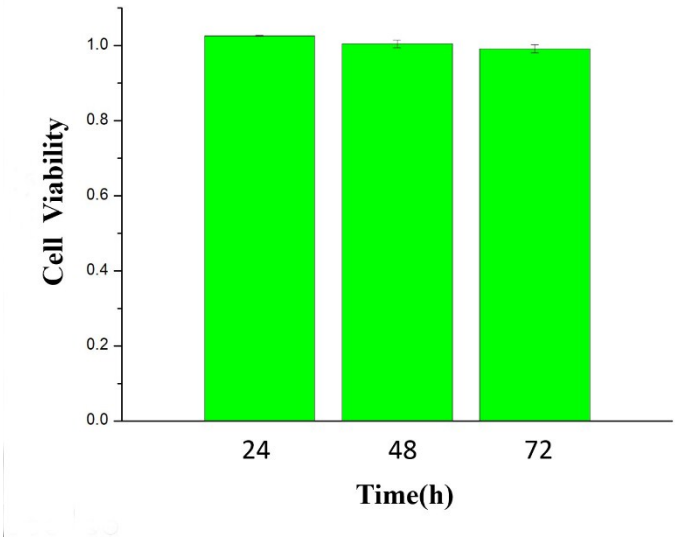
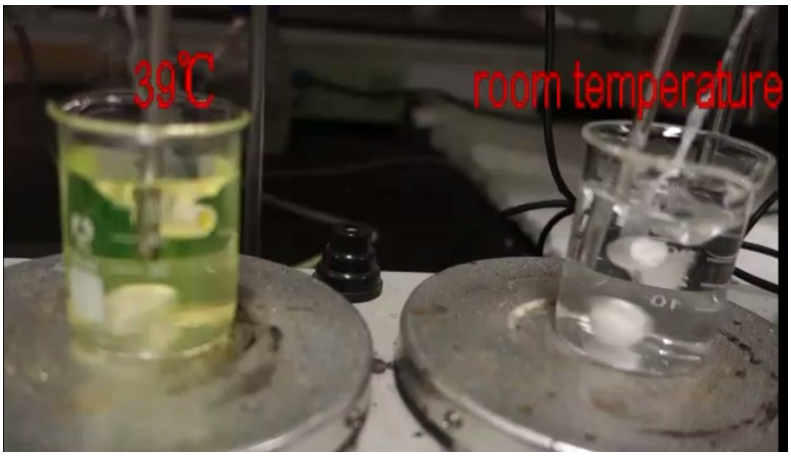


Figure S6: Cell viability of 3T3 cells adhered to PLA wafers for different times (n=6).



Video S1： Screenshot of Video demonstrating FITC releasing with the change of temperature.

Table 1: The FITC loaded amount and the released efficiency of three designed implants.

	Cy3.3	Cy3.6	Sp5&Cy3
Loaded FITC (0.5 mg/mL)	0.45 mL	0.8 mL	0.85 mL
Released efficiency	97.6%	98.3%	98.5%