Supporting Information

Strontium-Doped Amorphous Calcium Phosphate Porous Microspheres Synthesized through a Microwave-Hydrothermal Method Using Fructose 1,6-Bisphosphate as an Organic Phosphorus Source: Application in Drug Delivery and Enhanced Bone Regeneration

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1. Characterization of the SrAPMs. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed using FEI Magellan 400 electron microscope (USA) and Hitachi H-800 electron microscope (Japan),

respectively. X-ray diffraction (XRD) patterns were analysed using an X-ray diffractometer (Rigaku D/max 2550 V, CuK_{α} radiation, $\lambda = 1.54178$ Å). Fourier transform infrared (FTIR) spectra were performed on a FTIR spectrometer (FTIR-7600, Lambda Scientific, Australia). The specific surface area and pore size analyzer (V-sorb 2800P, Gold APP, China) was used to measure the Brunauer-Emmett-Teller (BET) specific surface area (S_{BET}) and pore size distribution. Thermogravimetric analysis (TGA) was performed on a STA 409/PC simultaneous thermal analyser (Netzsch, Germany).

2. Bacteria Preparation and Culture of rBMSCs. The staphylococcus aureus (S. aureus, ATCC 43300) was used to evaluate the antibacterial ability of Van-HAP nanorods, Van-APMs and Van-SrAPMs. The bacterial strain was cultured on a sheep blood agar (SBA) plate at 37 ˚C for 24 h. Then, a single colony was collected and incubated in 3 mL of sterile Trypticase Soy Broth (TSB; BD Bioscences) at 37 ˚C for 24 h. The density of bacteria was determined by optical density (OD) measurements at a wavelength of 600 nm.

The rBMSCs were obtained from the tibia and femur of 4-week-old Sprague-Dawley (SD) rats. Briefly, the marrow of the midshaft tibia and femur was flushed out and suspended in complete medium (CM, Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% (v/v) penicillin/streptomycin (Gibco, USA)). The cells were cultured at 37 ˚C in a humidified atomosphere of 5% CO_2. The non-adherent cells

were removed after 48 h and the medium was changed every 2-3 days thereafter. The rBMSCs from passage 2 were used for the following experiments.

3. Antibacterial Assay. For the antibacterial assay using a modified Kirby-Bauer (KB) method,[1] 1×10^8 colony forming units (CFU)/mL bacteria suspensions of S. aureus in TSB (equivalent to approximately 0.5 M McFarland solution) were prepared. Then, 200 μL of bacterial suspension was added to the M-H agar plates and spread uniformly with a sterile cotton swab. Holes measuring 5mm in diameter and 3 mm in depth were made with a sterile stainless steel cylinder. Then, 1 or 3 mg of vancomycin-loaded or vancomycin-free samples were filled into the holes. Antibacterial activity was determined by measuring the diameter of clear circular zones on the opaque background of bacterial growth after incubation for 24 h at 37 ˚C.

For the minimum inhibitory concentration (MIC) assay, 1×10^8 CFU/mL bacterial suspensions of S. aureus in Mueller-Hinton (M-H) broth were prepared. Then, 15 μL of bacterial suspension was added to the serially diluted solutions containing different concentrations of vancomycin-loaded particles. The given bacterial concentration of the M-H broth was 5×10^5 CFU/mL. The series of samples were incubated at 37 ˚C for 24 h. Finally, the samples were visually analyzed for their turbidity and the OD values were measured at $\lambda = 600$ nm. Transparent M-H broths indicated those in which the concentration of the released vancomycin was sufficient to prevent the growth of bacteria, as opposed to the turbid M-H broths.

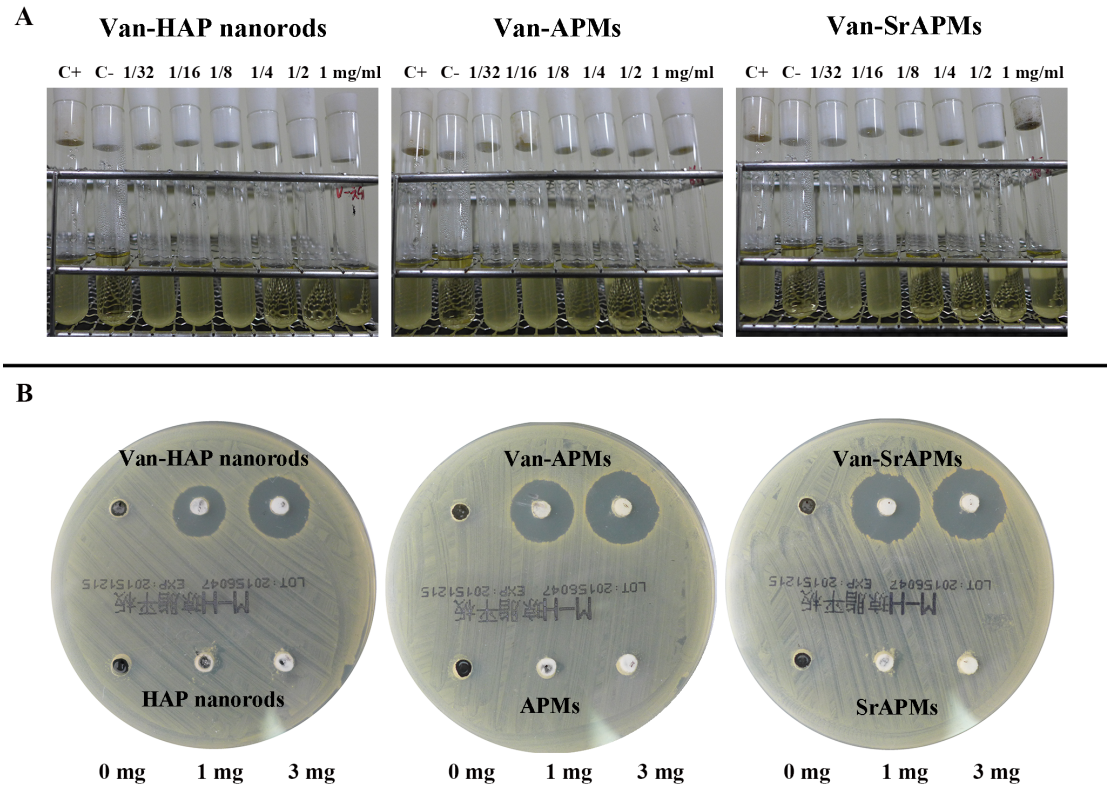

Fig S1. (A) Visual appearance of clear/disinfected and turbid/infected M-H broths inoculated with S. aureus and different amounts of Van-loaded particles, following 24 h of incubation, including the negative control (C-), incubated without bacteria, and the positive control (C+), incubated with bacteria and no Van-loaded particles. (B) Inhibition zones formed around 0, 1 and 3 mg of Van-loaded or Van-free particles following 24 h of incubation.

REFERENCES

(1) Uskokovic, V.; Batarni, S. S.; Schweicher, J.; King, A.; Desai, T. A., Effect of Calcium Phosphate Particle Shape and Size on Their Antibacterial and Osteogenic

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