Supporting Information

Multifunctional Copper-Containing Carboxymethyl Chitosan/Alginate Scaffolds for Eradicating Clinical Bacterial Infection and Promoting Bone Formation

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1. Morphology of the scaffolds made with Cu^{2+} ions alone (CMC/Alg/Cu ions scaffold) observed by SEM.

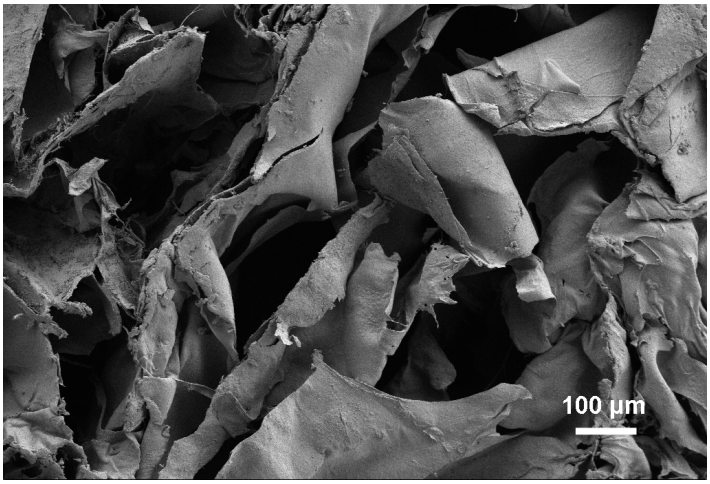

Figure S1: SEM image of the CMC/Alg/Cu ions scaffold.

2. Cytotoxicity of the CMC/Alg/Cu ions scaffolds.

Cell proliferation in the scaffolds made with Cu^{2+} ions (1 mmol/L $CuSO_4$ solution) alone was determined by standard 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay. CMC/Alg/Cu ions scaffolds weighing 10 mg were placed in the plates. A 500 μL suspension of MC3T3-E1 cells with a concentration of 2×10^5 cells/mL were seeded and incubated with the scaffolds at 37 °C in the α-Minimum Essential Medium (α-MEM, Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco). After cultured for 1 day, the media were discarded. 500 μL of the MTT solution (0.05%) was added to each well, and incubated for 4 h. 500 μL of DMSO was used to dissolve the formazan crystals, and optical densities (OD) were determined at 490 nm using a microplate reader (Multiskan GO, Thermo Scientific). Each experiment was performed in triplicate.

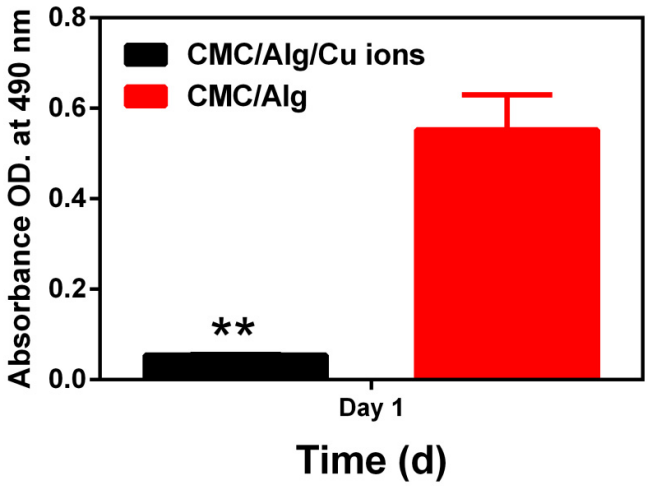

Figure S2: Cytotoxicity of the CMC/Alg/Cu ions scaffolds. Cell proliferation in the scaffolds was measured by a standard MTT assay. Significant inhibition of cell proliferation was observed in the CMC/Alg/Cu ions (1 mmol/L) scaffolds after 1 day of culture. Each value is the mean ± standard deviation of triplicate determinations; **$p < 0.01$ compared with CMC/Alg scaffolds.

3. Cytotoxicity of the Cu-containing CMC/Alg scaffolds made with different concentrations of Cu nanoparticles

Cell proliferation in the scaffolds made with different concentrations of Cu nanoparticles (10 mmol/L, 1 mmol/L, 0.1 mmol/L, and 0.01 mmol/L, respectivley) was determined by a standard MTT assay. Scaffolds weighing 10 mg were co-cultured with 500 μL cell suspension in the concentration of 2×10^5 cells/mL. After cultured for 1 and 7 days, the standard MTT assay was performed as described above. Each experiment was performed in triplicate.

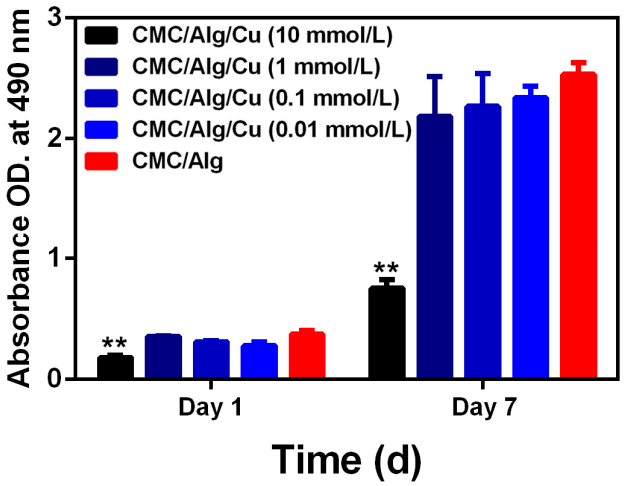

Figure S3: Cytotoxicity of the scaffolds with different concentrations of Cu nanoparticles. Cell proliferation in the scaffolds was measured by a standard MTT assay. Significant reductions were observed in the CMC/Alg/Cu (10 mmol/L) scaffolds on day 1 and 7. Each value is the mean ± standard deviation of triplicate determinations; **$p < 0.01$ compared with CMC/Alg scaffolds.

4. *In vitro* antibacterial property

Staphylococcus aureus (*S. aureus*) was used to evaluate the *in vitro* antibacterial properties of scaffolds with different concentrations of Cu nanoparticles. After cultivated in lysogeny broth (LB) medium (1% w/v tryptone, 0.3% w/v yeast extract, and 0.5% w/v $NaCl_2$) under shaking at 200 rpm at 37 °C for 12 h, the bacteria with the concentration of 2×10^6 CFU/mL were used in an antibacterial assay. 10 mg of each scaffold was introduced to 500 μL of bacteria suspension. The resultant suspension was incubated in a biochemical incubator at 37 °C for 24 h. The viable number of bacteria in the PBS was quantified by standard serial dilution. A culture suspension of 100 μL from each tube was uniformly spread on the LB agar plates, and the number of viable bacterial colonies was counted after incubation at 37 °C for 24 h.

The antibacterial rate was determined by using the formula (1).

$$\text{Antibacterial rate (\%)} = (N_{control} - N_{scaffold})/ N_{control} \times 100 \quad (1)$$

In Equation 1, $N_{control}$ is the average number of bacteria in the control groups. (CFU/sample), and $N_{scaffold}$ is the average number of bacteria in the scaffold groups (CFU/sample).

Each experiment was performed in triplicate.

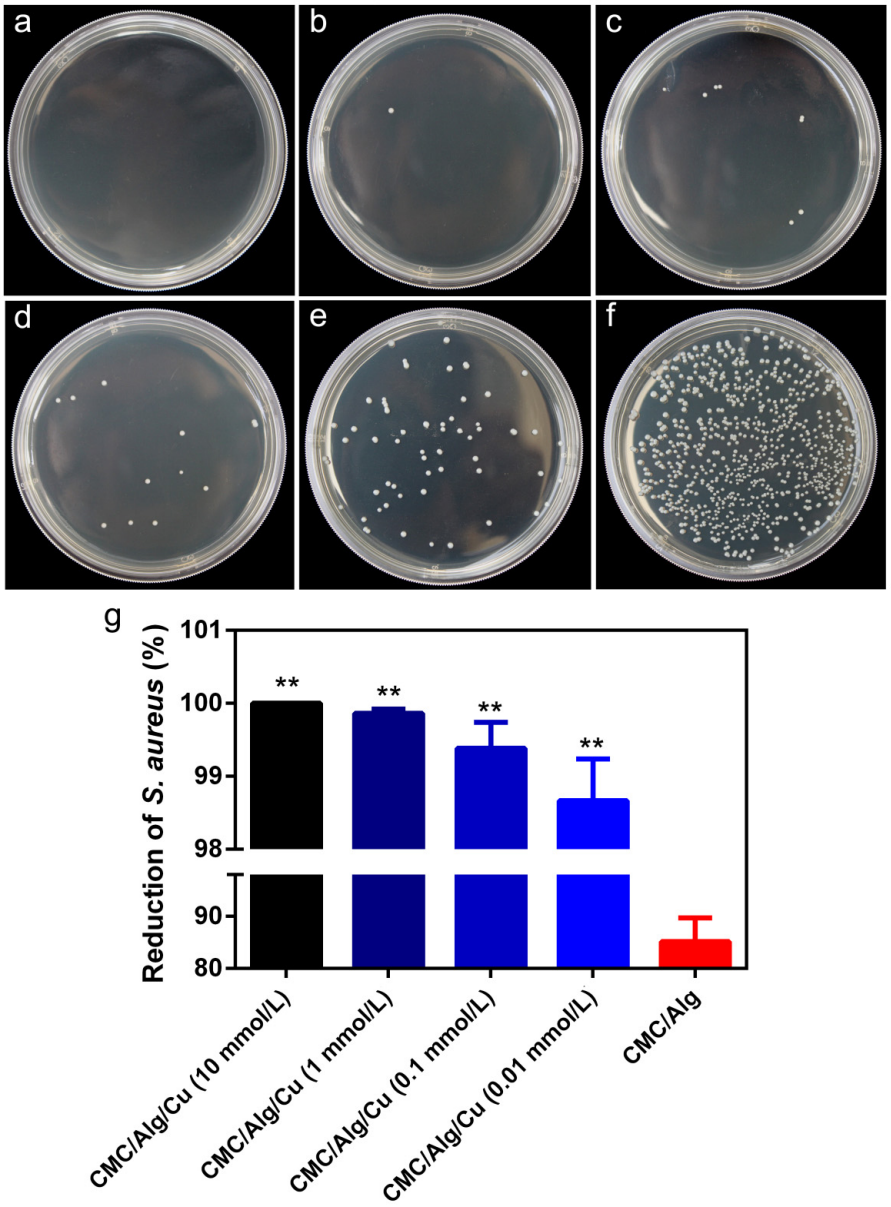

Figure S4: *In vitro* antibacterial property of the CMC/Alg/Cu scaffolds made with different concentrations of Cu nanoparticles. (a-f) Numbers of *S. aureus* colonies in the CMC/Alg/Cu with a Cu nanoparticle concentration of 10 mmol/L (a), 1 mmol/L (b), 0.1 mmol/L (c), 0.01 mmol/L (d), CMC/Alg scaffolds (e), and control (saline) group (f); (g) The antibacterial rate of these scaffolds. After the addition of the Cu nanoparticles into the CMC/Alg scaffolds, the antibacterial property was significantly enhanced. Each value is the mean ± standard deviation of triplicate determinations; $^{**}p < 0.01$ compared with CMC/Alg scaffolds.