Supporting Information

Copper Nanocluster Doped Luminescent Hydroxyapatite Nanoparticles for Antibacterial and Antibiofilm Applications

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Figure S1. (A) Photostability of copper nanoclusters and doped hydroxyapatite nanoparticles. The organic fluorophore rhodamine was used as standard. As compared to copper nanoclusters or rhodamine (organic dye), doped hydroxyapatite nanoparticles were found to be more stable. (B) Quantum yield measurement of doped hydroxyapatite nanoparticles using quinine sulfate as the reference.
Figure S2. TEM images showing (A) undoped hydroxyapatite nanoparticles (scale bar at 100 nm), (B) copper nanoclusters (scale bar at 10 nm), SAED images of (C) doped hydroxyapatite nanoparticles, (D) undoped hydroxyapatite nanoparticles, (E) copper nanoclusters, (F) HRTEM image of doped hydroxyapatite nanoparticles (scale bar at 5 nm).
Figure S3. TEM-EDX spectra showing elemental composition of (A) doped hydroxyapatite nanoparticles with higher copper concentration and (B) undoped hydroxyapatite nanoparticles. (C) FESEM-EDX spectrum of doped hydroxyapatite nanoparticles.

Figure S4. FESEM images of doped hydroxyapatite nanoparticles (A) low magnification, (B) high magnification (scale bar at 100 nm).
Figure S5. Zeta potential measurements of (A) doped hydroxyapatite nanoparticles, (B) undoped hydroxyapatite nanoparticles, (C) copper nanoclusters and (D) kanamycin loaded doped hydroxyapatite nanoparticles.
Figure S6. Concentration of copper calculated based on the standard curve obtained from atomic absorption spectroscopy.
A

Intensit

m/z

470 475 480 485 490 495 500

B

Kanamycin

Intensit

m/z

470 475 480 485 490 495 500

C

Intensit

m/z

470 475 480 485 490 495 500

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**Figure S7.** LCMS spectra of (A) free Kanamycin (structure of kanamycin is shown), (B) kanamycin loaded doped hydroxyapatite nanoparticles (kanamycin structure is shown corresponding to the m/z value = 483.27), (C) doped hydroxyapatite nanoparticles.

**Figure S8.** Confocal images showing the control bacterial cells (*Pseudomonas aeruginosa*) without the uptake of copper nanoclusters doped hydroxyapatite nanoparticles. (A) fluorescence image, (B) bright field image and (C) merged image. The images are having a scale bar of 5 µm.
Figure S9. Bacteria (*Pseudomonas aeruginosa*) grown on nutrient agar. (A) control, bacteria treated with kanamycin loaded doped hydroxyapatite nanoparticles at their (B) minimum inhibition concentration (MIC-12 µg/mL), (C) minimum bactericidal concentration (MBC-35 µg/mL); bacteria treated with free kanamycin at its (D) minimum inhibition concentration (MIC-3 µg/mL) and (E) minimum bactericidal concentration (MBC-6 µg/mL).

Figure S10. FESEM images of (A) control bacteria (*Pseudomonas aeruginosa*), (B) bacteria treated by kanamycin loaded doped hydroxyapatite nanoparticles at their MIC. The images are taken at a scale bar of 1 µm.
Figure S11. Epifluorescence images of Nile red stained *Pseudomonas aeruginosa* biofilm treated with free kanamycin at its MBIC<sub>50</sub>. (A) Fluorescence image (B) bright field image. The biofilm treated with free drug showed low fluorescence intensity due to reduced lipid content. The images are having a scale bar of 100 µm.

Figure S12. Epifluorescence images of AO/PI stained *Pseudomonas aeruginosa* biofilm treated with free kanamycin at its MBIC<sub>50</sub>. (A) AO stained, (B) PI stained, (C) Merged image of biofilm. The red region in the merged image marks the area with dead bacterial cells whereas green marks the area with live cells. The images are having a scale bar of 100 µm.
| Bacterial strains | Doped HAP + Drug (MIC) | Drug (MIC) |
|-------------------|------------------------|------------|
| **Gram negative** |                        |            |
| *Escherichia coli* (GFP) | 20 µg/mL | 4 µg/mL |
| *Pseudomonas aeruginosa* MTCC 2488 | 12 µg/mL | 3 µg/mL |
| *Escherichia coli* (DH5α) | 400 µg/mL | 290 µg/mL |
| **Gram positive** |                        |            |
| *Staphylococcus aureus* MTCC 96 | >900 µg/mL | 400 µg/mL |
| *Bacillus subtilis* MTCC 1305 | >900 µg/mL | 400 µg/mL |

**Table S1.** Data representing the minimum inhibition concentration (MIC) of kanamycin loaded doped hydroxyapatite nanoparticles and free kanamycin for both Gram positive and Gram negative bacteria. MIC for kanamycin loaded doped hydroxyapatite nanoparticles could be determined only for Gram negative bacteria whereas no MIC was obtained in case of Gram positive bacteria.