Supplementary Information

Preparation and antibacterial behaviour of nanostructured Ag@SiO_2-Penicillin with silver nanoplates

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1. Calculation of Ag and SiO_2 contents in the core-shell nanostructures

According to the TEM image, the silver plate has the length of ~ 40 nm and thickness of about 4 nm. The thickness of silica shell was ~ 7 nm.

Ag mass present in the core-shell nanoparticle:

$V_{Ag} = V_{core} = \left(\frac{\sqrt{3}}{4} * a^2 * t\right) = \left[\frac{\sqrt{3}}{4}\right] (40*10^{-7})^2 * (4*10^{-7})] = 2.7728 * 10^{-18}$ cm^3

Using d_{Ag} = 10.45 g.cm^{-3},

$m_{Ag} = 2.7728 * 10^{-18} * 10.45$ g.cm^{-3} $= 28.96 * 10^{-18}$ g

Then we calculate the SiO_2 mass present in the core-shell nanoparticle

$V_{SiO_2} = V_{cs} - V_{core} = \left[\frac{\sqrt{3}}{4}\right] * (64.28. * 10^{-7})^2 * (18*10^{-7})] - 2.7728 * 10^{-18} = 29.4322 * 10^{-18}$ cm^3

Using d_{SiO_2} = 2.1 g.cm^{-3},

$m_{SiO_2} = 29.4322*10^{-18}$ m^3 * 2.1 g.cm^{-3} = $61.80762*10^{-18}$g

So, we can calculate the mass of silver in nanostructure core-shell.

Core-shell mass $(m_{SiO_2} + m_{Ag})$	**Silver mass** m_{Ag}
$(61.80762*10^{-18}\text{g} + 28.96*10^{-18}\text{g})$	$28.96*10^{-18}$g
X	Mass of silver

The concentration of silver present in the core shell is ~ 31.9 wt%.

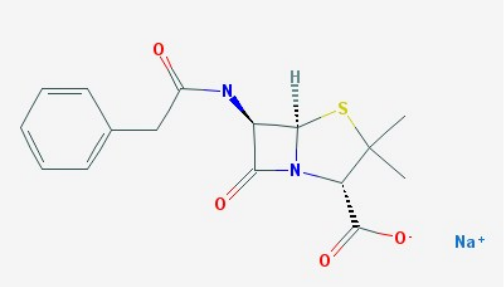


Fig. S1. Chemical structure of Penicillin G tested in this study [1].

2. Reaction mechanism of amide bond formation

The functionalization of nanoparticles occurs through the formation of an amide bond between COO^- group from Penicillin and the NH_2 present on the silica surface.

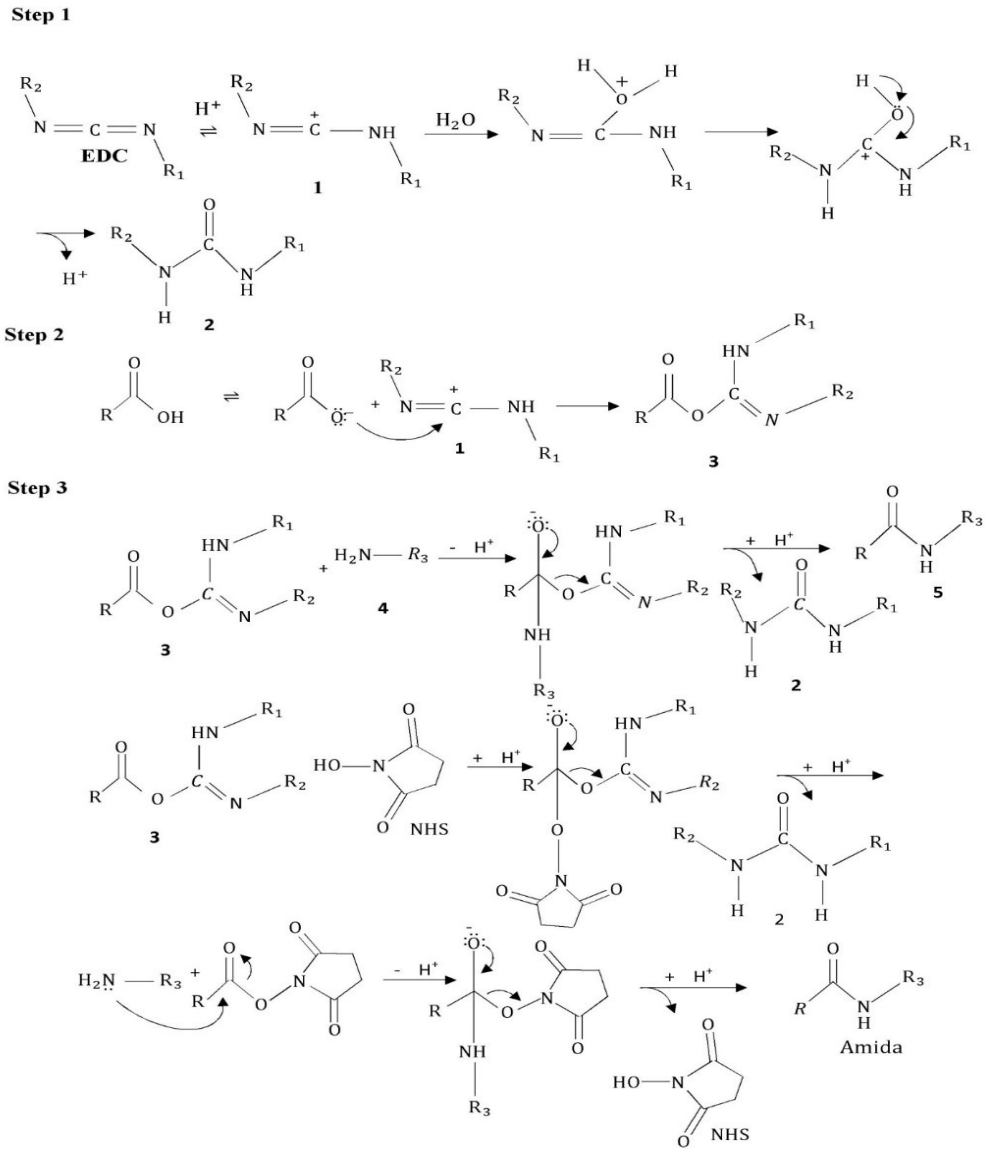
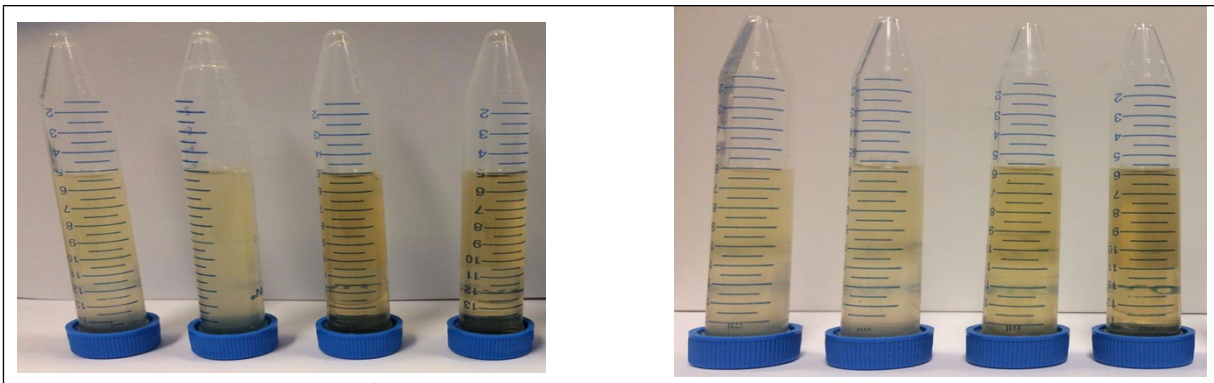


Fig. S2. Reaction mechanism of amide bond formation [2].

3. Bactericidal test

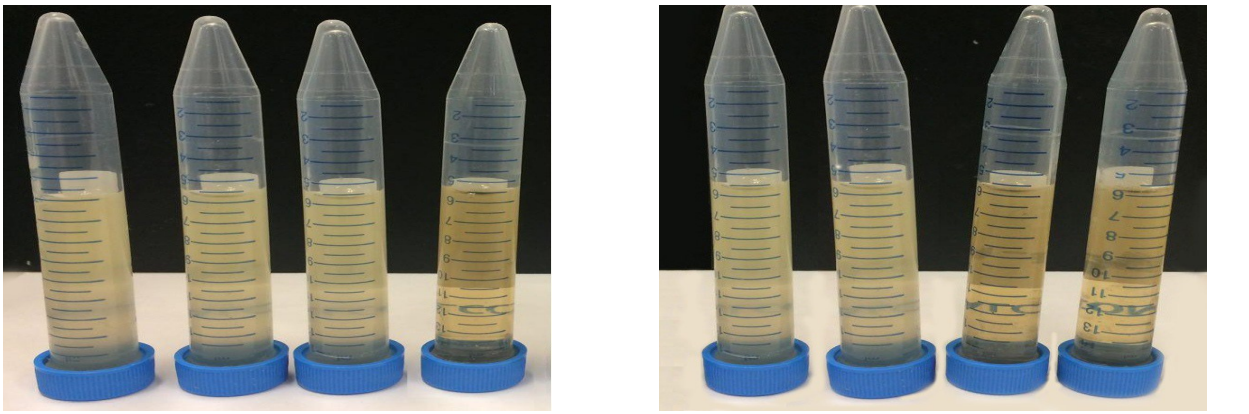
Antibacterial activity of Penicillin, $Ag@SiO_2$ and $Ag@SiO_2$-Penicillin nanoparticles were tested in vitro using broth dilution and agar well diffusion methods against MRSA and MSSA bacteria supplied from the Hong Kong local hospital

MRSA	MSSA



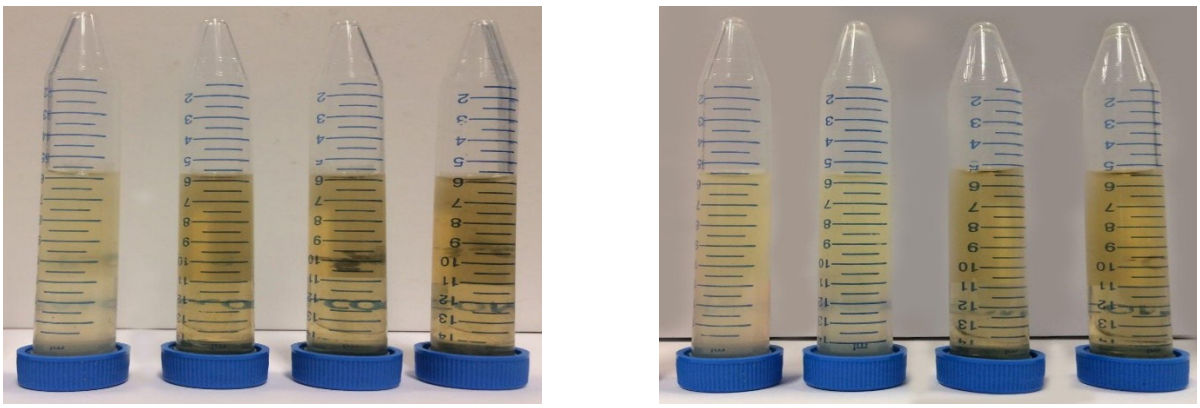
325 330 335* 340 (μg/ml) 13 15 17* 19 (μg/ml)

(Penicillin)



240 245 250 255* (μg/ml) 8 10 12* 14 (μg/ml)

($Ag@SiO_2$)



125 130* 135 140 (μg/ml) 2 4 6* 8 (μg/ml)

($Ag@SiO_2$-Penicillin)

Fig. S3. Determination of the minimum inhibitory concentration (MIC) of penicillin, $Ag@SiO_2$ and $Ag@SiO_2$-Penicillin against MRSA and MSSA bacteria. The MIC was defined as the lowest concentration of drug-inhibiting visible growth after incubation at 37 °C for 24 h.

* The minimum inhibitory concentration (MIC).

For determination of the inhabitation zone (IZ), the nutrient agar plates were seeded with a suspension of 100 µl of each bacterium after incubation for the appropriate time. Then, 30 µl of the test agents at a concentration of 310 µg/ml and 15 µg/ml were introduced into several pieces of sterilized filter paper (1 cm in diameter). Subsequently, the filter papers were dried in a bio-safety cabinet and place on the agar plates. The plates were incubated at 37 °C for 48 h. Clear zones around the papers showed the positive results and cloudiness indicates that bacterial growth has not been inhibited by the concentration of compound present in the medium. The diameter of inhibition zones was determined, and values are expressed in millimeters (mm). All experiments were made in triplicate to assess their reproducibility and the results were confirmed in three independent experiments. The results are reported as mean of zone of inhibition in millimeter. Saline was used as a negative control.

Type	**Bacteria**	
	MRSA (310 µg/ml)	**MSSA** (15 µg/ml)

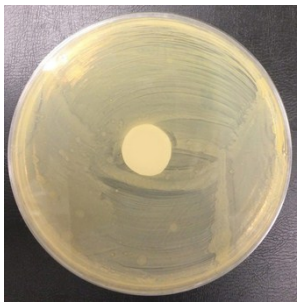
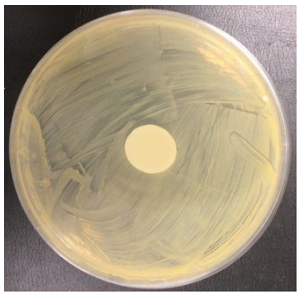
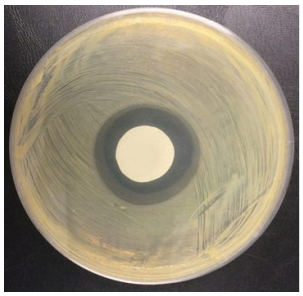
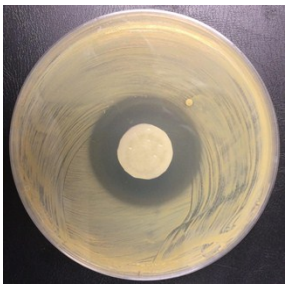
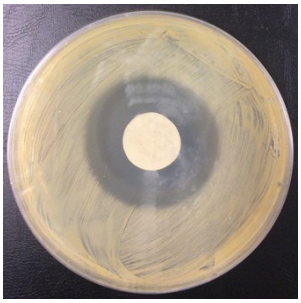
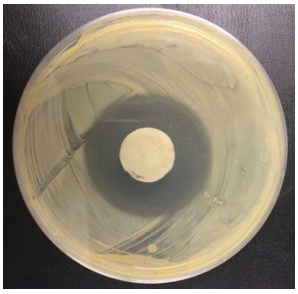
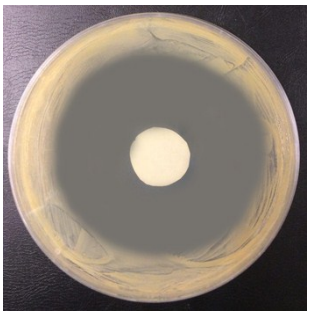
Control		
Penicillin	 7 mm	 10 mm
$Ag@SiO_2$	 15 mm	 14 mm
$Ag@SiO_2$-Penicillin	 24 mm	 21 mm

Fig. S4. Determination of the inhibitation zone (IZ) of penicillin, $Ag@SiO_2$ and $Ag@SiO_2$-Penicillin against MRSA and MSSA bacteria using disk-diffusion agar method.

TEM images showed characteristic morphological changes in the MRSA cells. The untreated bacterial cells had normal morphology with clear distinct Peptidoglycan layer of about 40 nm. In the presence of Ag-SiO_2-Penicilline, the Peptidoglycan layer and cytoplasmic membranes of the bacterial cell walls became disordered and damaged.

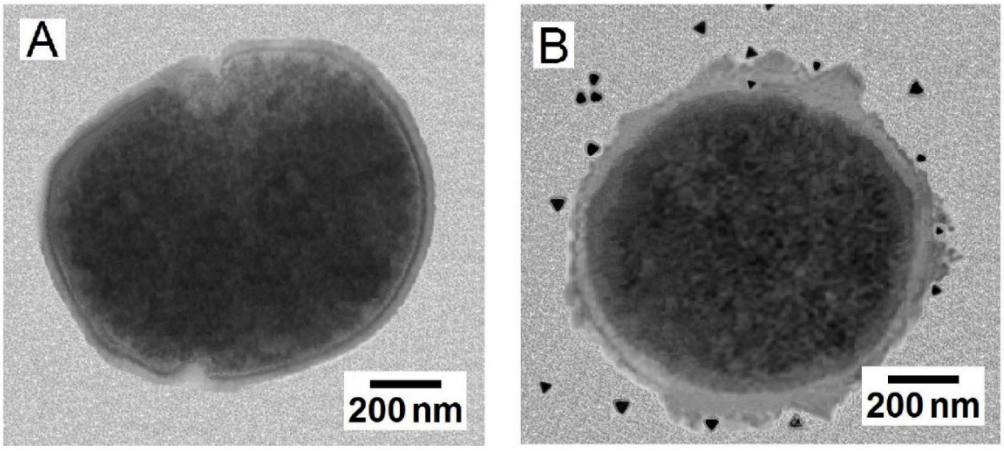


Fig. S5. TEM images of MRSA bacteria (A) untreated, (B) treated with MIC nanostructured Ag-SiO2-Penicillin

4. Cytotoxicity test

The human A431 (ATCC CRL -1555) skin cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific) supplemented with 10% (v/v) Foetal Bovine Serum (FBS), penicillin (100 U/ml) and streptomycin (100 µg/ml) and maintained in a humidified incubator (95% air, 5% CO2 at 37 °C) until 80–90% confluent. In the Following step, they were passaged using trypsin (0.05% w/v) – EDTA (0.02% w/v) solution and sub-cultured in a T25 flask. Cell culture media were changed every two days.

References:

[1]. National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD20894, USA, https://pubchem.ncbi.nlm.nih.gov/compound/penicillin_g#section=Top (Cited on September 6, 2018).

[2]. C A.G.N. Montalbetti, V. Falque, Amide bond formation and peptide coupling, Tetrahedron 61 (2005) 10827–10852.