Supplementary Material for:

Antibacterial activity of nanoporous gold against *Escherichia coli* and *Staphylococcus epidermidis*

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Energy-dispersive X-ray spectroscopy (EDXS) of nanoporous gold (NPG)

The results of EDXS of the synthesized NPG are shown in FIG. S1. The EDXS detected gold as well as other elements (oxygen, sodium, magnesium, silicon and potassium) in the glass substrate. No silver peaks were detected, which means that the residual Ag content in the NPG substrates was very small, being below the 1 at.% detection limit.

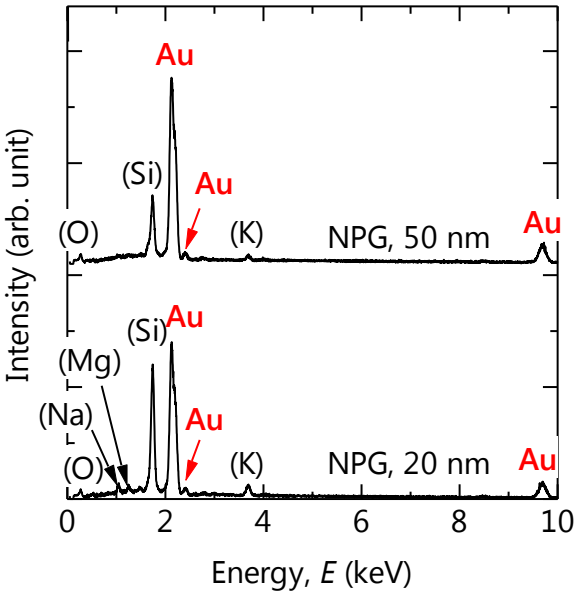

FIG. S1. EDXS results for NPG. The peaks of oxygen, sodium, magnesium, silicon and potassium come from glass substrates. (color online)

Scanning electron microscopy (SEM) image of flat gold (FG)

The SEM image of FG prepared by simple sputtering of gold on glass substrate is shown in FIG.S2. The polycrystalline and dense, but not porous, structure was observed.

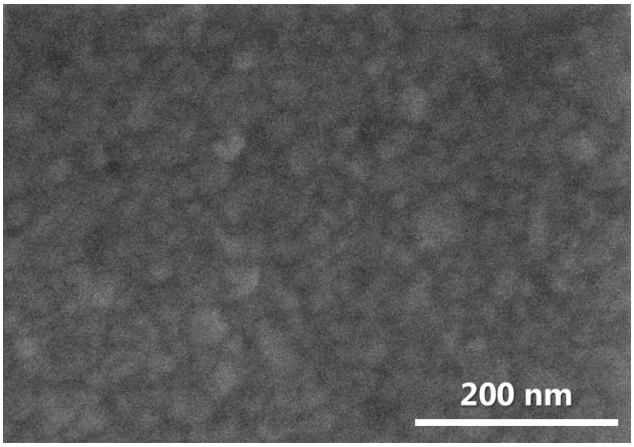

FIG. S2. SEM image for FG. Polycrystalline and dense, but not porous, structure was observed.

Time variation of viable bacterial counts (VBCs) on NPG and FG for *E. coli*

Figure S3 shows the variation in VBCs of *E. coli* on NPG and FG with incubation time, at a RH of 60%. The VBC on NPG was lower than that on FG at every incubation time. The VBCs on NPG incubated for 12–24 h were significantly lower than those on FG, at the corresponding incubation

times. The VBCs on NPG and FG were similar for incubation times of 0–12 h. Thus, antibacterial properties of NPG were evident after incubation for 12 h at a RH of 60%. This behavior is quite different from the antibacterial activity by metal ions, which typically begin decreasing the VBC immediately after commencing incubation.[1]

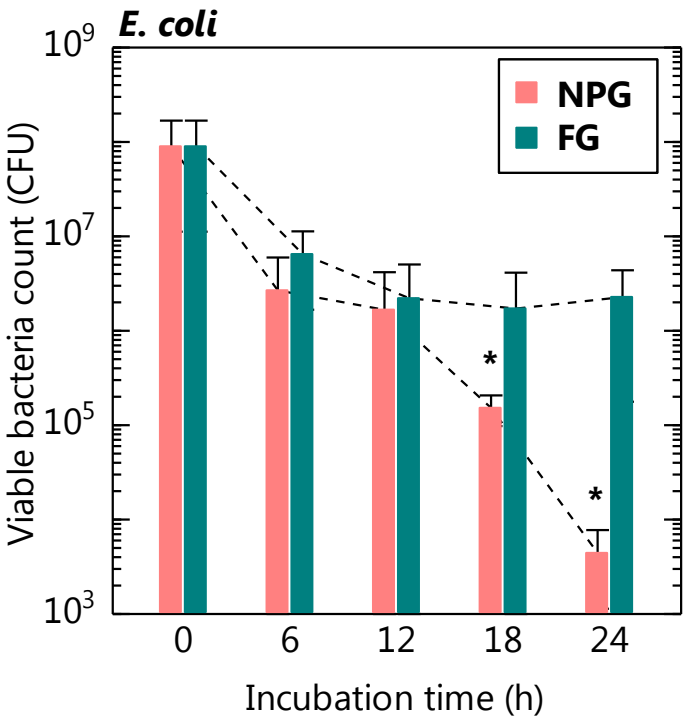

FIG. S3. Time variation of VBCs on NPG and FG for *E. coli*. * denotes significant difference between NPG and FG with $p < 0.01$. The VBC of *E. coli* on NPG significantly decreased after 12 h of incubation, while the VBC on FG did not. (color online)

Effect of metal ions on antimicrobial properties of NPG

The culturing solution was suspended on the nanoporous gold (NPG) substrate for 24 h, and the sample was then analyzed using inductively-coupled plasma atomic emission spectroscopy. The results are shown in FIG. S4. The concentrations of silver and gold in the culturing solutions were found to be < 0.05 ppm, which was the apparatus detection limit. It is well known that a concentration of at least approximately 1 ppm is necessary for realizing the antimicrobial properties of Ag ions.[1–4] Therefore, Ag ion dissolution from the NPG into the culturing solution was not responsible for the present antimicrobial properties of NPG.

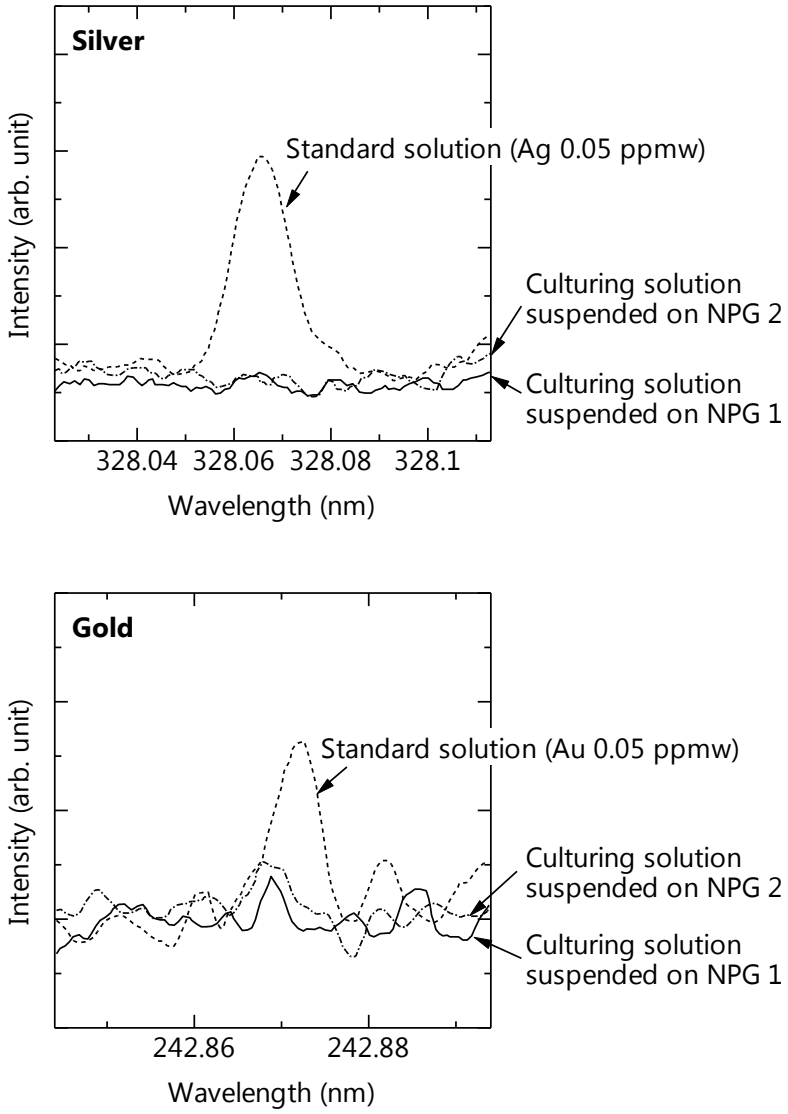

FIG. S4. The results of inductively-coupled plasma atomic emission spectroscopy analyses on culturing solution on NPG (pore size = 20 nm). No essential silver and gold dissolution was confirmed.

Effect of reactive oxygen species (ROS) on antimicrobial properties of NPG

We determined whether peroxides and reactive oxygen, which are known to be the main sources of the antimicrobial properties of copper, were responsible for the present antimicrobial properties of NPG.[5] We used the "Merckoquant peroxide test" to analyze the bacterial suspensions on the samples. First, the bacterial suspension used in the antimicrobial properties tests was incubated on NPG and FG for 12 h in humidity-controlled incubators at 298 K, under a relative humidity (RH) of 60%. Second, we immersed a test strip in the bacterial suspension for 1 s, to moisten the reaction area of the test paper. Third, we absorbed moisture on the test strip through the edge of the test strip for 15 s. Last, we compared the color of the reaction area with that of the color scale and read the corresponding values. The test strips immersed in the bacterial suspensions of both NPG and FG did not show any change in color, as shown in FIG. S5. Thus, the generation of ROS was excluded as a cause for the present antibacterial activity of NPG.

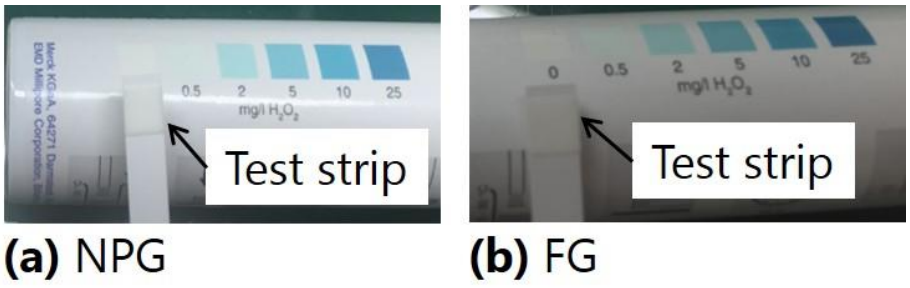

FIG. S5. The results of Merckoquant peroxide test on culturing solution on (a) NPG (pore size = 20 nm) and (b) FG. No essential ROS was confirmed. (color online)

References

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