Supplementary Information

Substrate-Independent Adsorption of Nanoparticles as Anti-Biofilm Coatings

Shang-Yi Yi[1,#], Hsin-Yao Lin[1,2,3,#], Ling-Chu Yang[1], Shao-Chin Tseng[4], Aileen Y. Sun[1], Chihchen Chen[2,5], and Dehui Wan[1,6,*]

[1]*Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan*
[2]*Institute of NanoEngineering and Microsystems, National Tsing Hua University,*
[3]*Department of Surgery, Division of Neurosurgery, MacKay Memorial Hospital, Taipei*
[4]*Experimental Facility Division, National Synchrotron Radiation Research Center, Hsinchu, Taiwan*
[5]*Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan*
[6]*Frontier Research Center on Fundamental and Applied Sciences of Matters, National Tsing Hua University, Hsinchu, Taiwan*

[#]These authors contributed equally to this work.

[*]To whom correspondence should be addressed. E-mail: dhwan@mx.nthu.edu.tw

S1. Adsorption of silanes on various surfaces.

First, some of the most extensively researched organosilanes are those containing amino terminal groups (–NH_2), such as 3-aminopropyltrimethoxysilane (APTMS) and 3-aminopropyltriethoxysilane (APTES), and TMS, especially for anchoring negatively charged nanoparticles (e.g., citrate or PVP-coated Ag NPs). In particular, the –NH_2 group can act as a self-catalyst for the hydrolysis of alkoxy-silanes, and catalyze siloxane bond formation, both intra- and inter-molecularly.[1] Among them, TMS is the best candidate for preparing stable amine-functionalized surfaces, by considering that the secondary amine group can catalyze siloxane bond formation, but the intramolecular catalysis of bond detachment is sterically hindered.[1] For the adsorption of TMS on various surfaces, a few possible mechanisms may be involved, including hydrogen bonding between NH_2/SiOH group and C=O of PC, electrostatic interaction between NH_3^+ group and O^- of SS, van der Waals forces between the alkyl chain and hydrophobic surface of PTFE, as well as the high affinity of amino group toward Au surface.

However, we also conducted the coating experiments on PC substrates with other silanes, such as TPS and T-2-PES. Both silane molecules contain a benzene terminal group. In **Figure S3**, the XPS peaks at 285.0, 286.5, and 532.1 eV represent the binding energies of C–C (C 1s), C–O (C 1s), and C=O–C (O 1s) units, respectively, matching the chemical composition of bare PC. After silane treatment, the appearance of additional peak for Si 2p binding energies (101.4 eV for TPS and 101.3 eV for T-2-PES, respectively) reflected the formation of a siloxane layer. Interestingly, in contrast to the amino groups of TMS, the benzene ring could not provide hydrogen bonding and electrostatic interactions with the PC surface. However, the hydrolyzed TPS and T-2-PES still possess SiOH groups, which may form hydrogen bonding with substrate surface (e.g., C=O). Thus, we assume the initial adsorption of TPS and T-2-PES onto the PC surface may be supported by van der Waals forces (e.g., π–π interaction between benzene ring and C=O) and hydrogen bonding. According the above results, not limited to TMS, some other silanes could also form a dense siloxane layer on various substrates.

S2. Size requirement for the particles on TMS-treated substrates.

Typically, NH_2-containing-silane-modified substrates are suitable for the attachment of particles with diameter between 15-150 nm.[2] For example, the AgNPs (ca. 50 nm), HGN (ca. 66 nm), and AuNPs (ca. 13 nm) have been coted on TMS-treated substrates (see **Figure S4**). Also, we successfully coated AgNPs (10 nm) and AuNPs (5 and 10 nm) on TMS-treated PC substrates (see **Figure S9**). Therefore,

the silane-based coating strategy is expected to work with a wide range of particle sizes.

S3. Evaluation on the stability of NPs arrays coated on substrates

Clinically, some medical procedures are performed in a smooth and delicate way. For example, an external ventricular drainage tube (**Figure 7a**) is inserted into cerebral ventricle through brain parenchyma gently with minimal mechanical force on the surface of catheter for preventing injury to vulnerable brain tissue. And then, the catheter is left in the brain ventricle which contains relatively static cerebrospinal fluid for a period time. In this case, we have evaluated the durability and sustained antibiofilm effect of the AgNPs coated on the substrates in a similar condition (**Figure 5b**). However, in some procedures, such as gastric tube, urinary or pig-tail catheter insertion, the implants may need to possess more resistance against the mechanical forces during implantation. Therefore, we also conducted an evaluation on the stability of NPs array coated on substrates. In **Figure S20**, the surface of the PC substrates coated with AgNPs or AuNPs was being scraped with a cotton swab 20 times to imitate the mechanical force during the catheter implantation. The color of coatings maintained the same after scraping, suggesting the great stability of nanoparticle coating.

S4. The mechanism of the anti-biofilm ability of AgNPs arrays

In general, both Ag NPs and Ag ions have been recognized to provide excellent anti-biofilm ability,[3] by damaging the biofilm structure, biofilm components and hampering bacterial metabolism.[4] For example, the physical contact between Ag NPs and bacterial cell walls can damage the membrane and then cause the leakage of cellular components.[5] Also, Ag ions can attach to the bacterial cell walls and enter into cytoplasm to destroy the intracellular structures.[6] In our case, however, we attributed the anti-biofilm ability to the sustained release of Ag ions, based on a few observations. First, the SEM and Live/Dead images (see **Figures 4e, 4f and 7c**) demonstrated that almost no bacteria (live and dead cells) could be found in those AgNPs groups, even after co-culturing with bacteria for 7 days. The results indicated that the bacteria were inhibited from attachment but not killed on the surfaces. Second, the releasing profile of Ag ions from Ag600 on PC immersed in PBS for 28 days was demonstrated in **Figure 5a**, suggesting a sustained release of Ag ions in a physiological condition. Lastly, the AgNPs were tightly immobilized on the surface, even after the immersion in PBS for 28 days (**Figure 5b**) or co-culturing with bacteria for 7 days (**Figure S19**), thus restricting their mobility and toxicity.[7] In contrast, the released Ag ions are still free to access the approaching bacteria close to the substrate

surface, consequently preventing the bacteria attachment. Therefore, we believed that the inhibition of the biofilm formation is mainly assisted by the Ag ions released from the AgNPs coating on the substrate. However, some AgNPs may deattach from the substrate and can also contribute partly to the antibacterial effect.

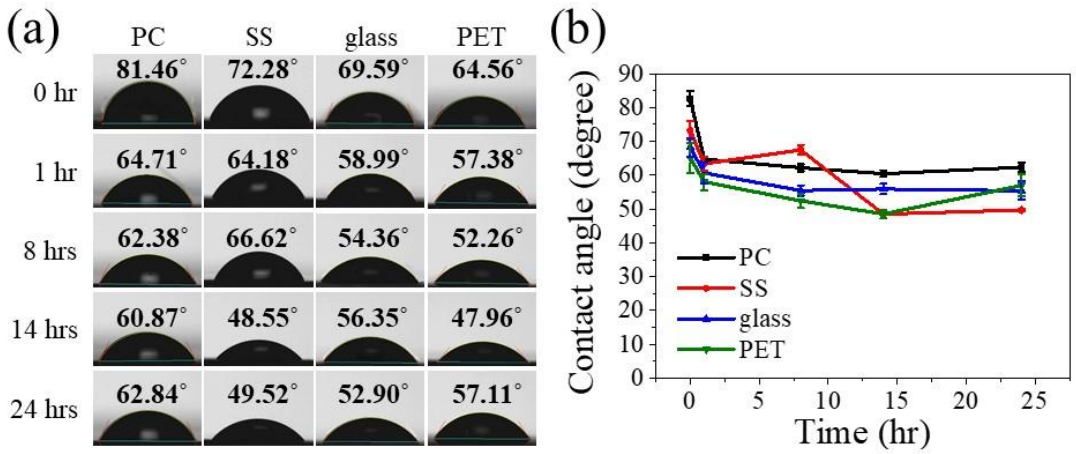

Figure S1. (a) Optical images of the water contact angles of PC, SS, glass, and PET substrates after treatment with TMS for various periods of time. (b) Relationships between the water contact angles of PC, SS, glass, and PET substrates and the TMS treatment time.

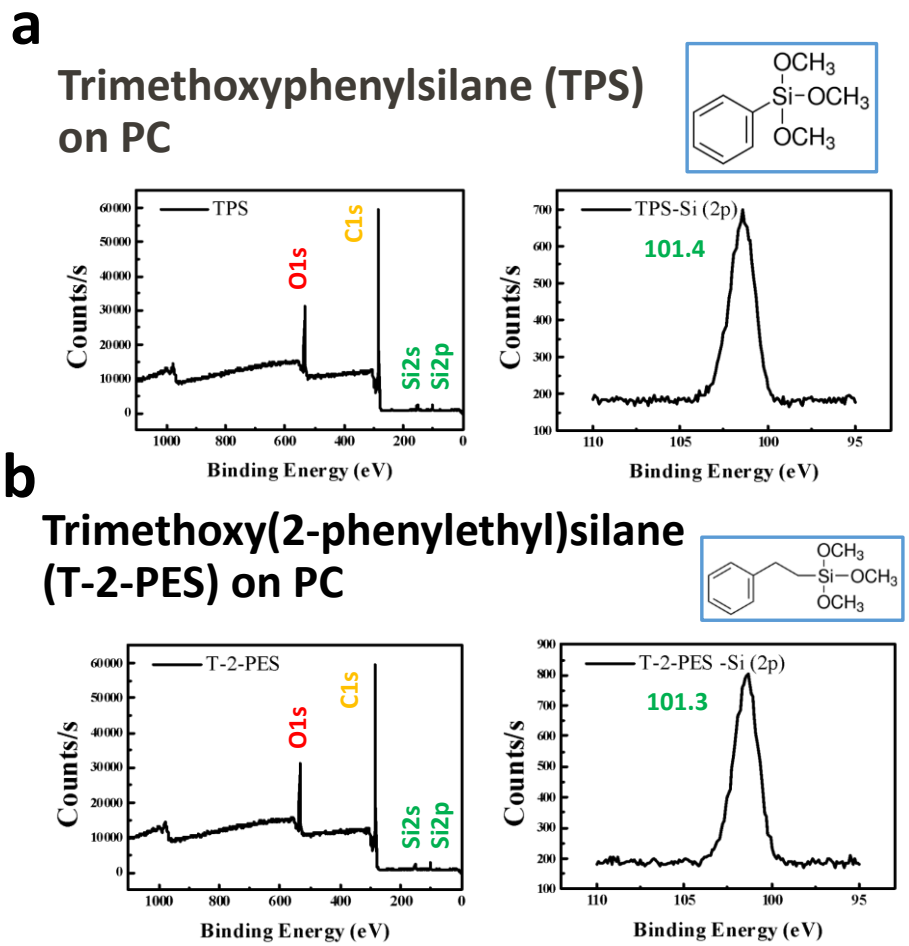

Figure S2. (a, b) XPS survey and high-resolution Si 2p spectra of a PC surface after (a) TPS and (b) T-2-PES) treatment.

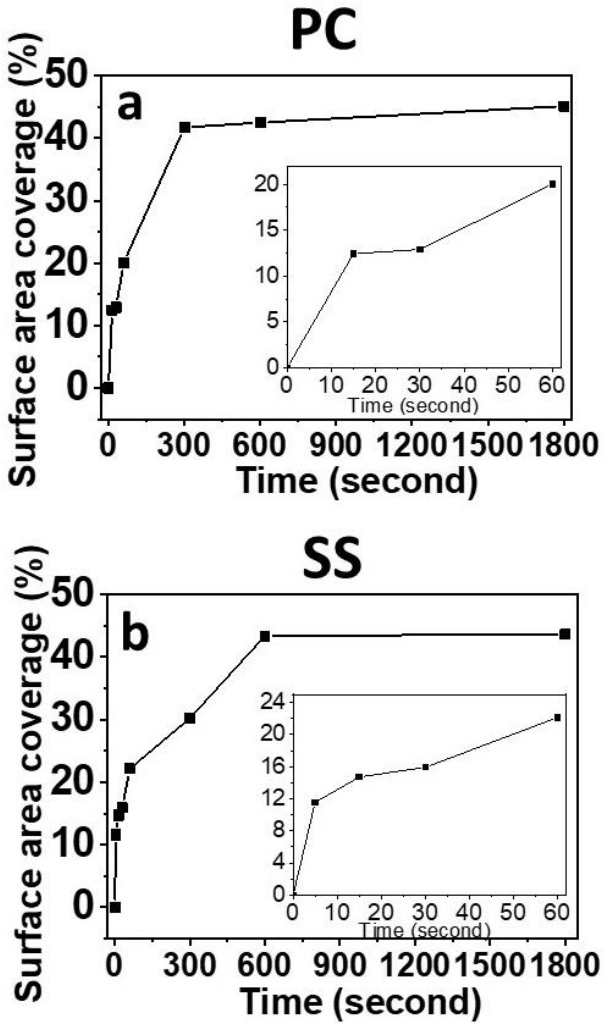

Figure S3. (a, b) Relationships between the immersion time of the AgNPs on (a) PC and (b) SS substrates and the surface area coverage; insets: corresponding profiles of surface area coverage for the immersion time in the initial 60 s.

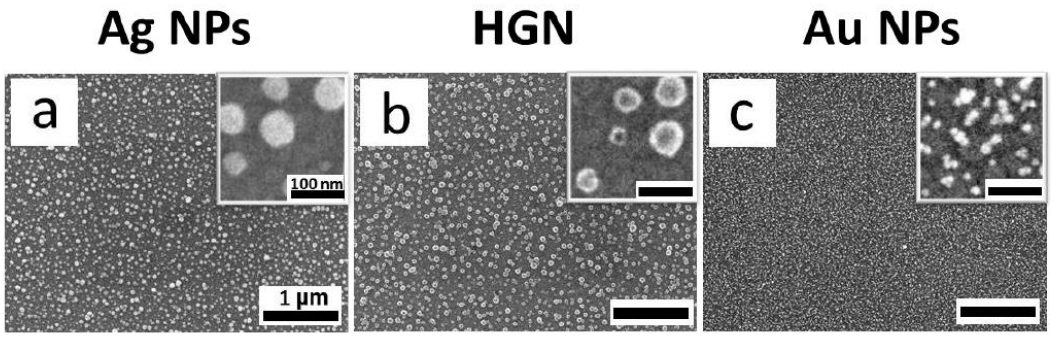

Figure S4. SEM images of AgNPs, HGNs, and AuNPs immobilized on Al substrates for 600 s.

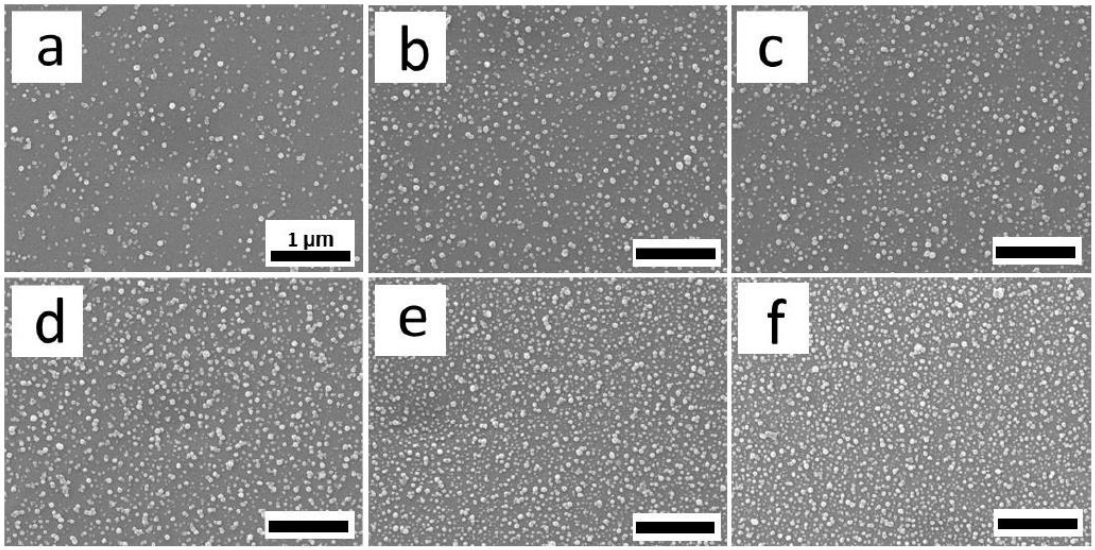

Figure S5. SEM images of AgNPs immobilized on PET substrates for coating times of (a) 15, (b) 30, (c) 60, (d) 300, (e) 600, and (f) 1800 s.

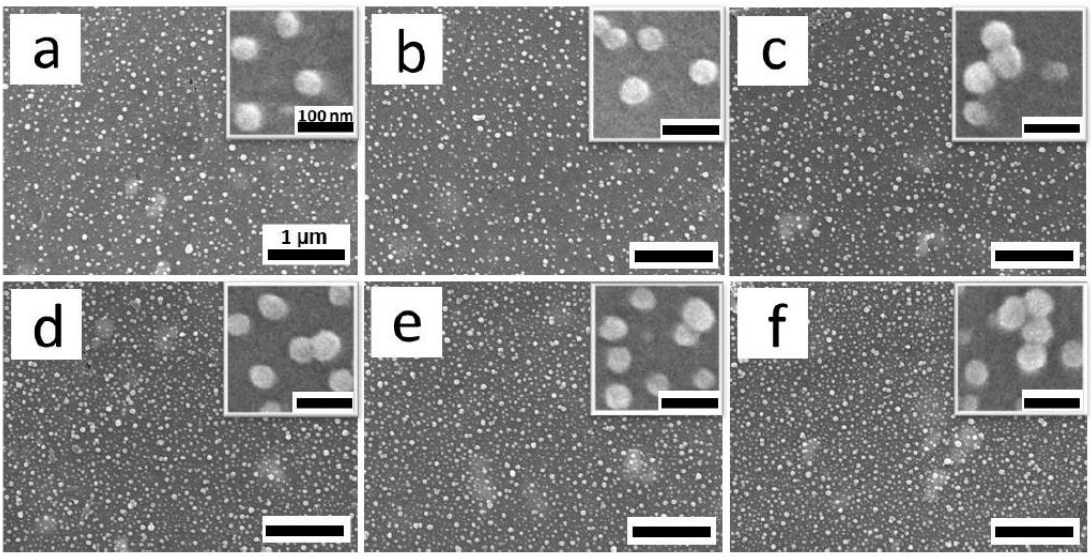

Figure S6. SEM images of AgNPs immobilized on PEEK substrates for coating times of (a) 15, (b) 30, (c) 60, (d) 300, (e) 600, and (f) 1800 s.

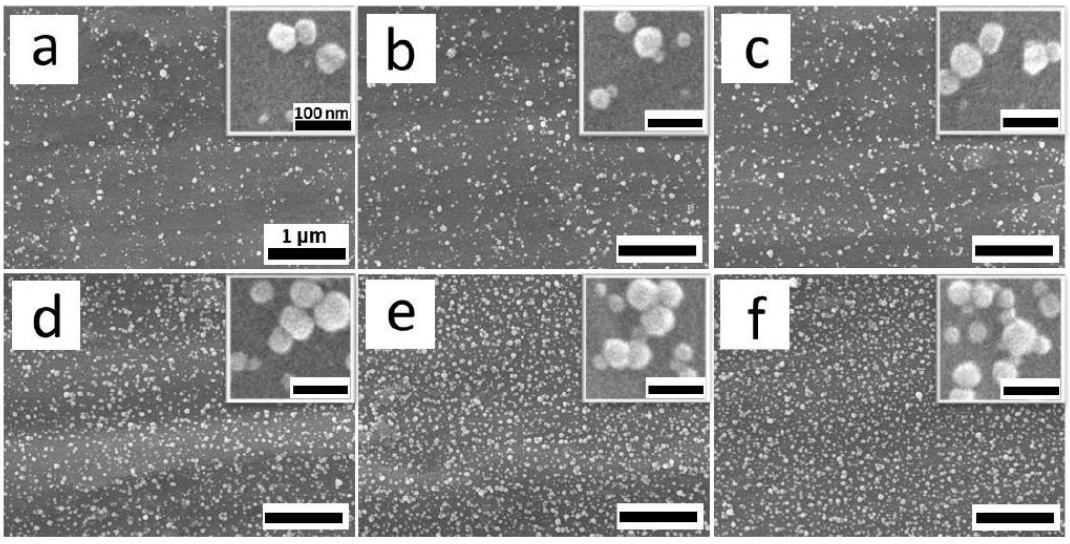

Figure S7. SEM images of AgNPs immobilized on Ti substrates with coating times of (a) 15, (b) 30, (c) 60, (d) 300, (e) 600, and (f) 1800 s.

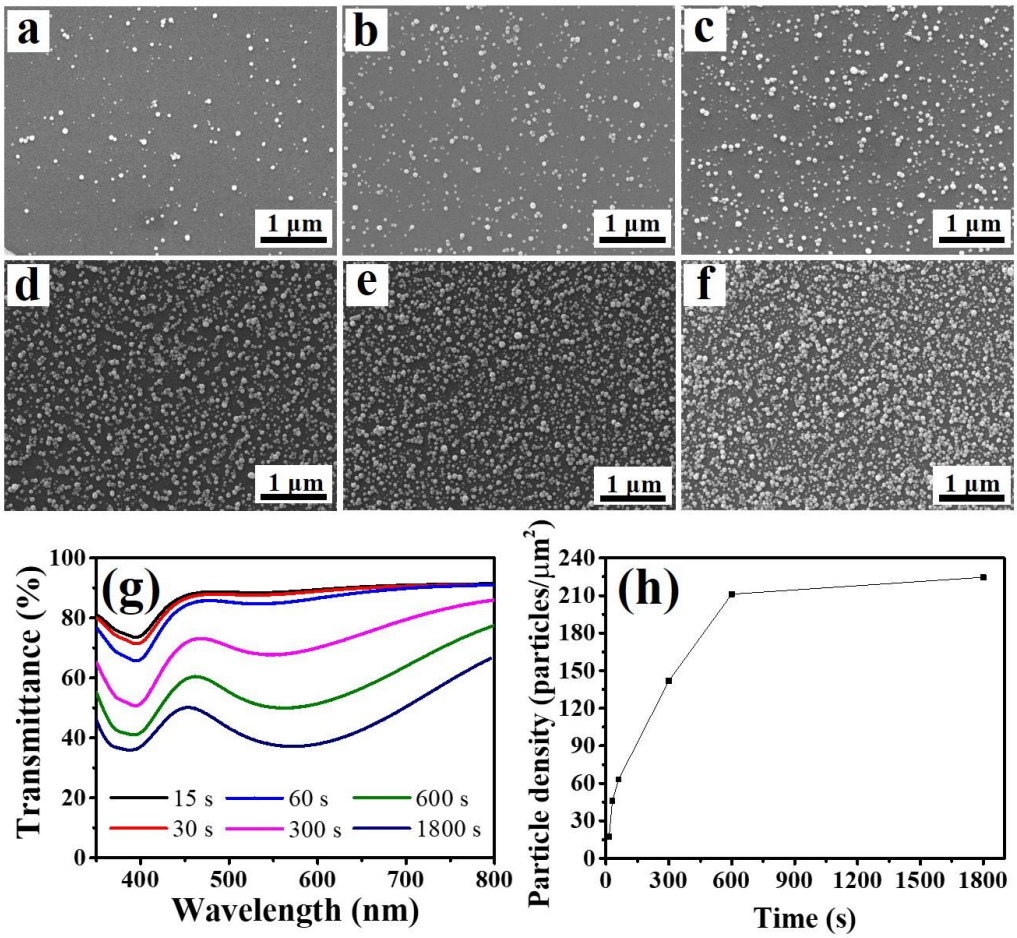

Figure S8. SEM images of AgNPs immobilized on B270 glass substrates with immersion times of (a) 15, (b) 30, (c) 60, (d) 300, (e) 600, and (f) 1800 s. (g) Transmittance spectra and (h) particle number densities of AgNPs immobilized on B270 glass substrates with various immersion times; TMS treatment time: 14 h.

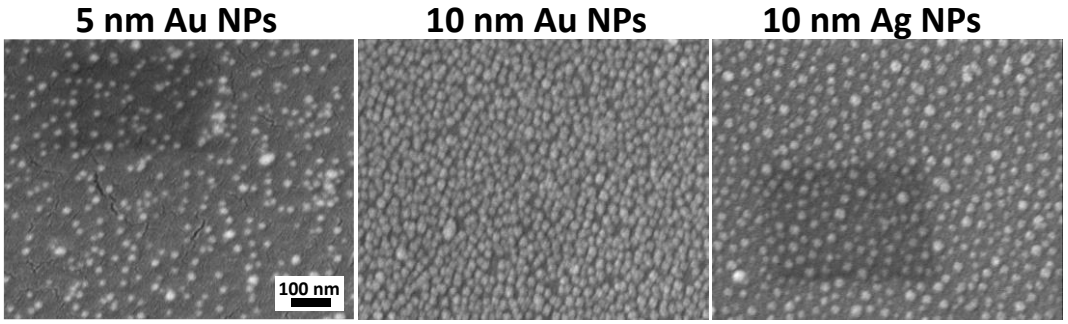

Figure S9. SEM images of 5-nm-AuNPs, 10-nm-AuNPs, and 10-nm-AgNPs immobilized on PC substrates.

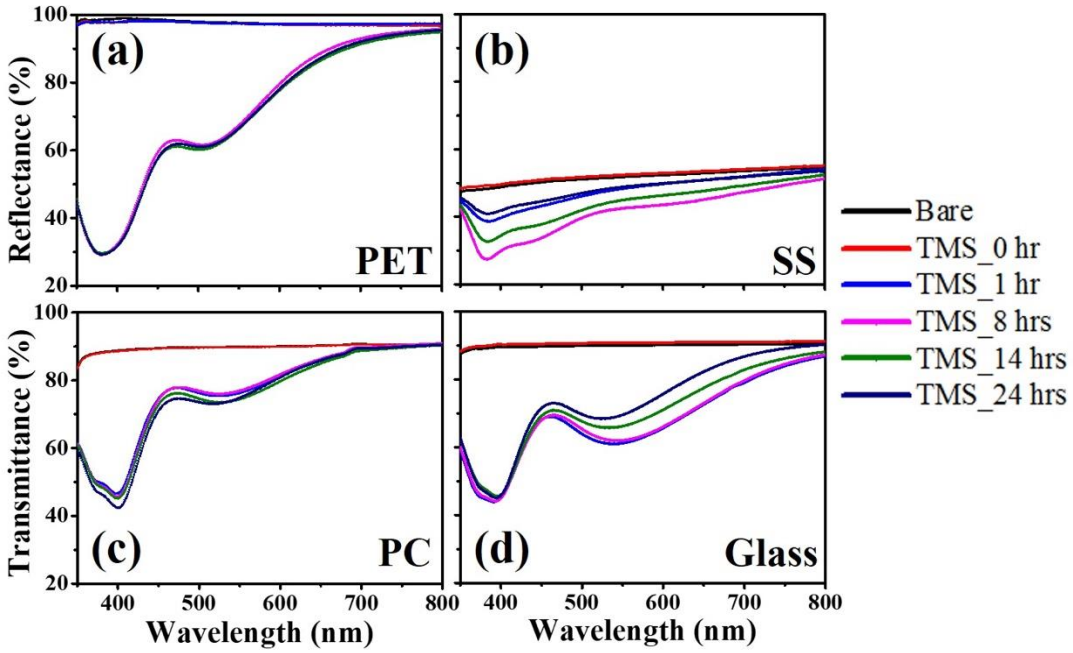

Figure S10. (a, b) Reflectance and (c, d) transmittance spectra of AgNPs immobilized on (a) PET, (b) SS, (c) PC, and (d) B270 glass substrates with various TMS treatment times. Bare substrates without AgNPs immobilization: black spectra. Substrates after various treatment times: 0 h, red spectra; 1 h, blue spectra; 8 h, pink spectra; 14 h, green spectra; 24 h, dark blue spectra. AgNP immersion time: 10 min.

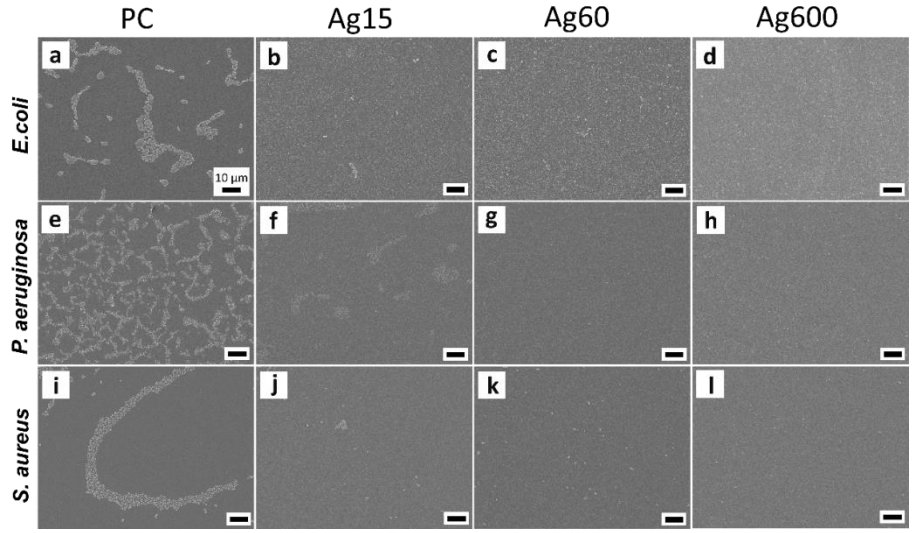

Figure S11. SEM images of biofilms formed on PC, Ag15, Ag60, and Ag600 surfaces for 4 h: (a–d) *E. coli*, (e–h) *P. aeruginosa*, and (i–l) *S. aureus*. Scale bar: 10 µm.

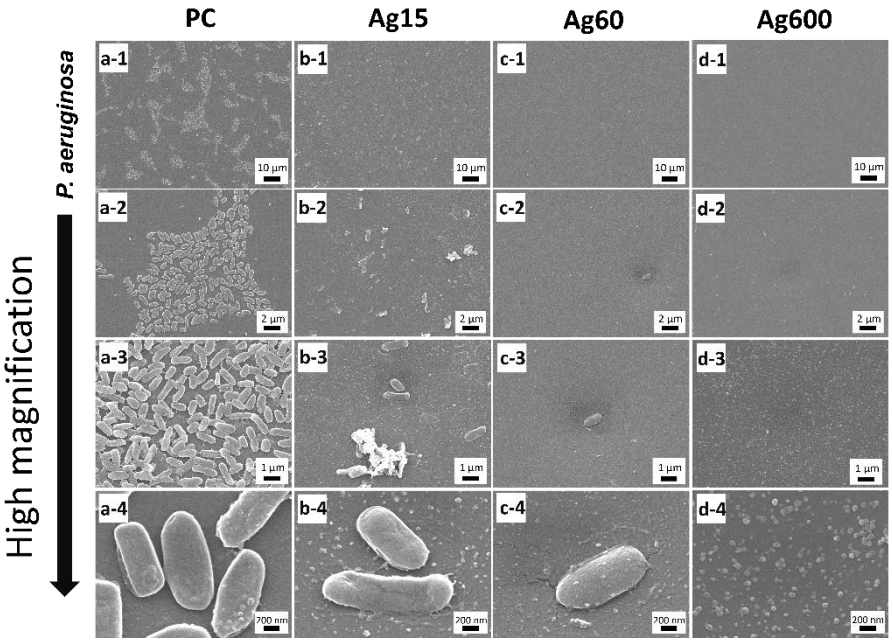

Figure S12. SEM images of *P. aeruginosa* species seeded on various surfaces for 24 h. Scale bar: 10 µm.

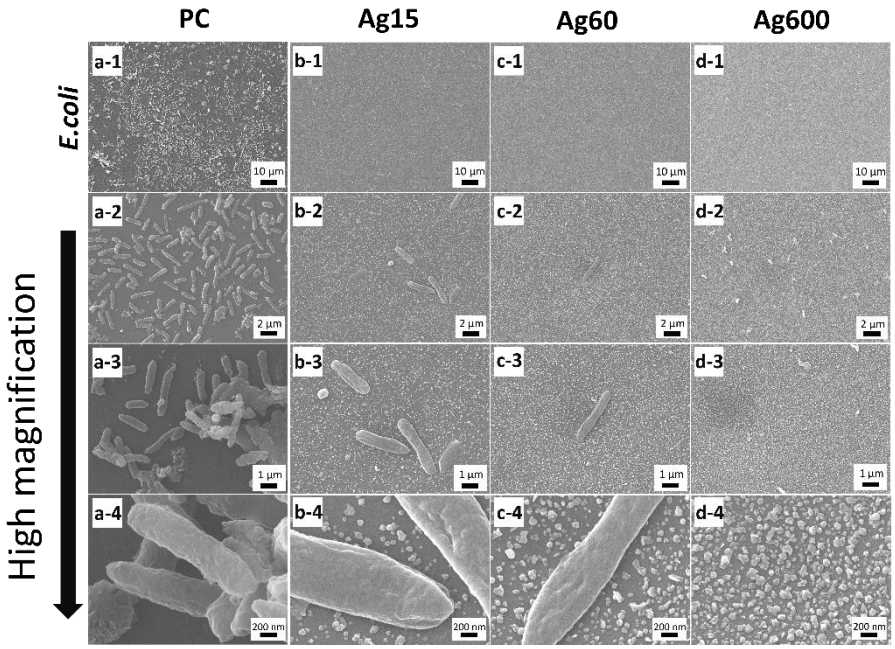

Figure S13. SEM images of *E. coli* species seeded on various surfaces for 24 h. Scale bar: 10 µm.

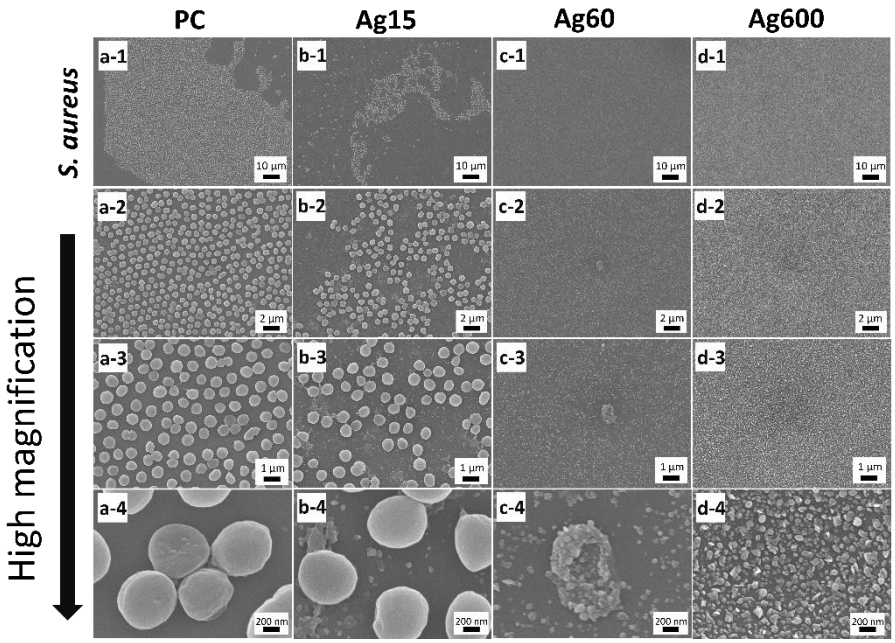

Figure S14. SEM images of *S. aureus* species seeded on various surfaces for 24 h. Scale bar: 10 µm.

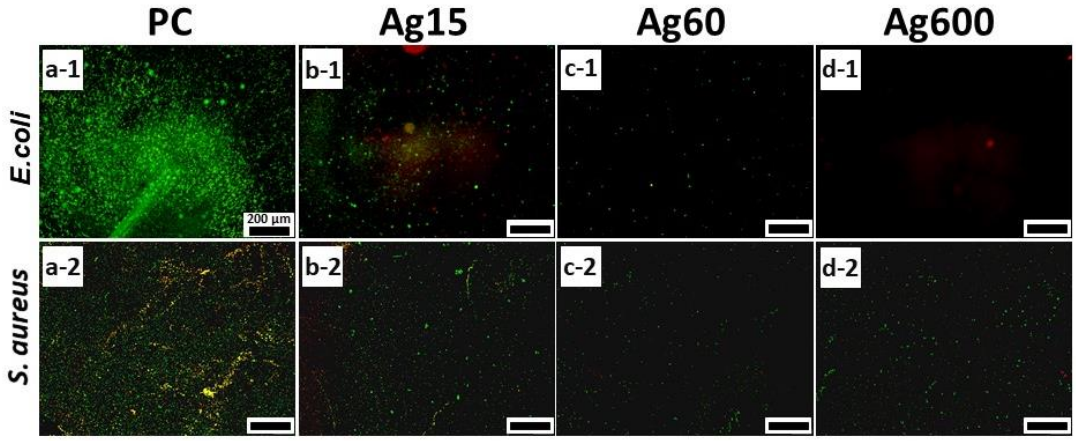

Figure S15. LIVE/DEAD fluorescence images of *E. coli* and *S. aureus* biofilms formed on various surfaces after incubation for 24 h. Scale bar: 200 µm.

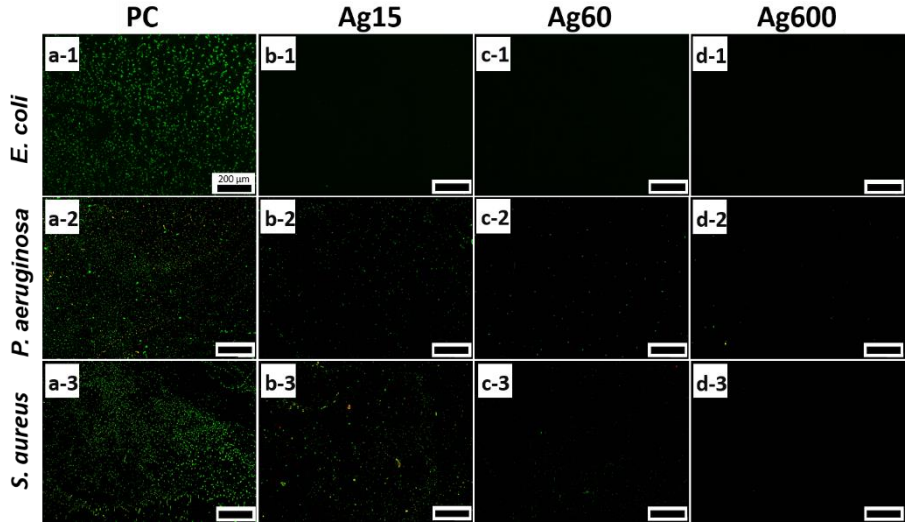

Figure S16. LIVE/DEAD fluorescence images of biofilms formed on various surfaces after incubation with *E. coli*, *P. aeruginosa*, and *S. aureus* for 4 h. Scale bar: 200 µm.

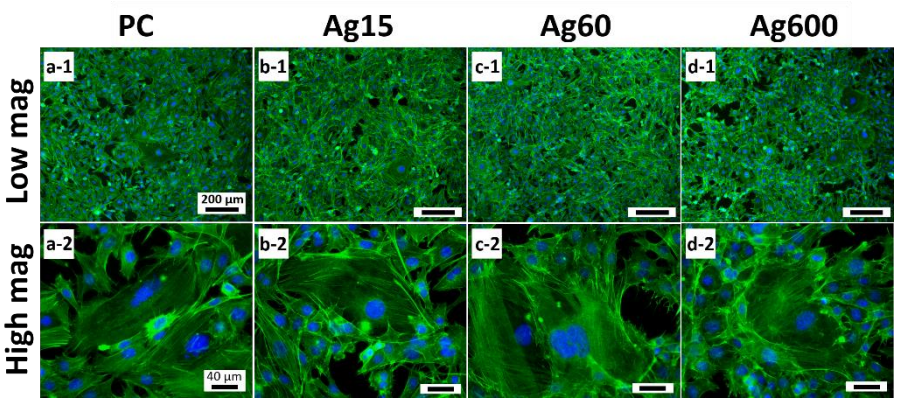

Figure S17. Cytoskeletons of NIH3T3 cells grown on various surfaces.

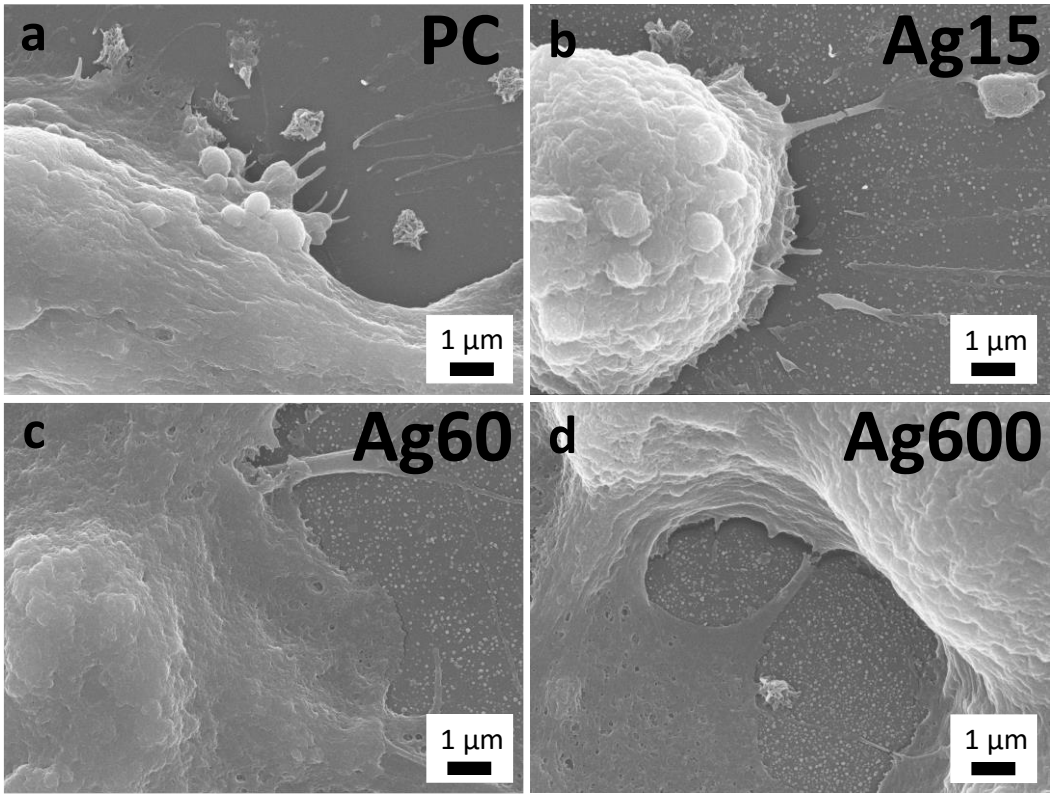

Figure S18. SEM images of cell adhesion on various surfaces.

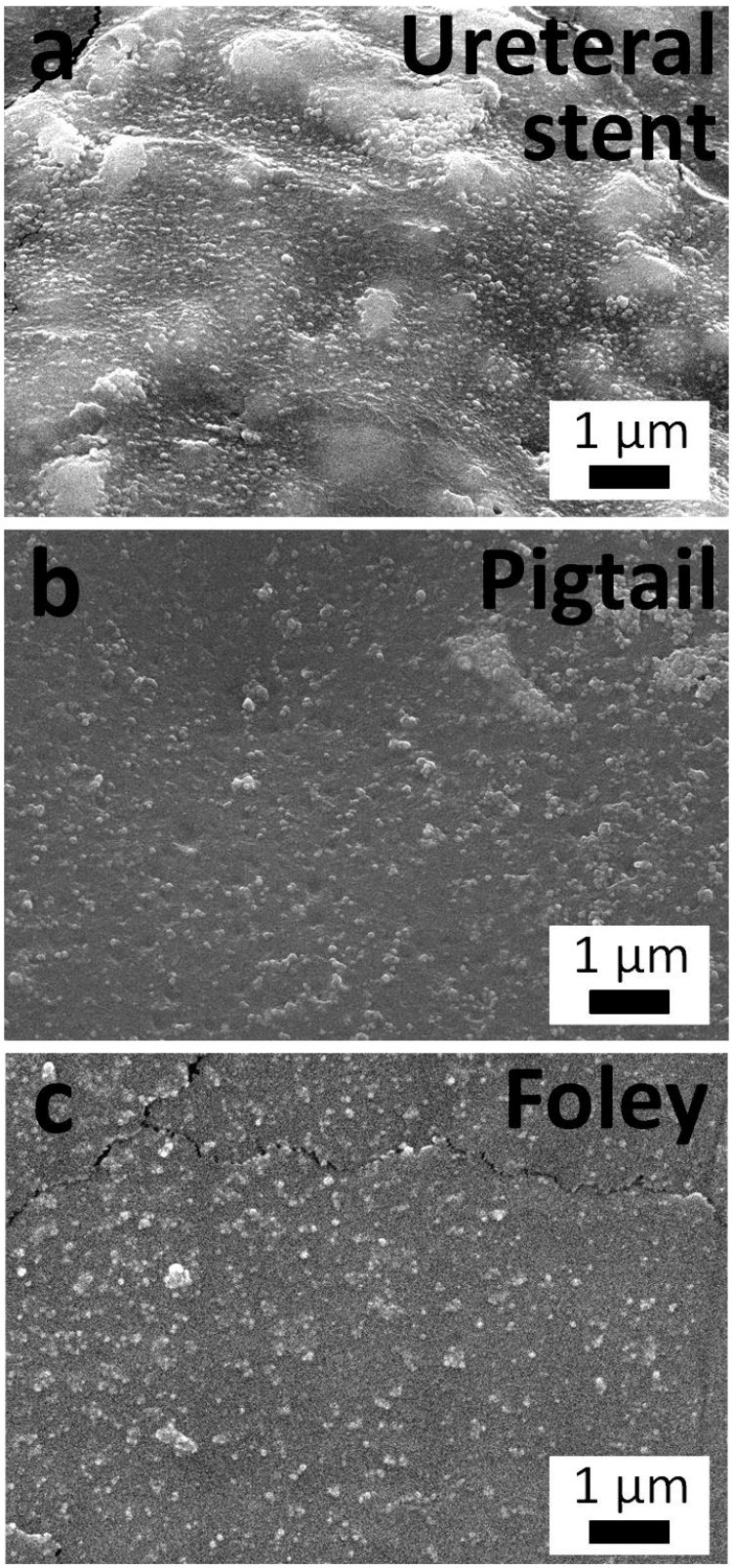

Figure S19. SEM images of three commercial medical devices, prepared with AgNPs, after co-culturing with bacteria for 7 days.

Figure S20. The images of PC substrates prepared with 10-nm (a) AgNPs and (b) AuNPs before and after being scraped with a cotton swab 20 times.

References:

1. M. Zhu, M. Z. Lerum and W. Chen, *Langmuir*, 2012, **28**, 416-423.
2. A. Githens and S. Daly, *Strain*, 2017, **53**, e12215.
3. L. Rizzello and P. P. Pompa, *Chem. Soc. Rev.*, 2014, **43**, 1501-1518.
4. A. S. Joshi, P. Singh and I. Mijakovic, *Int. J. Mol. Sci.*, 2020, **21**, 7658.
5. I. Sondi and B. Salopek-Sondi, *.J Colloid Interf. Sci.*, 2004, **275**, 177-182.
6. C. Völker, M. Oetken and J. Oehlmann, *Rev. Environ. Contam. Toxicol.*, 2013, **223**, 81-106.
7. H. Qin, H. Cao, Y. Zhao, C. Zhu, T. Cheng, Q. Wang, X. Peng, M. Cheng, J. Wang, G. Jin, Y. Jiang, X. Zhang, X. Liu and P. K. Chu, *Biomaterials*, 2014, **35**, 9114-9125.