Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Bio-sensing surface with high biocompatibility for enhancing the Raman scattering signals as enabled by Mo-Ag film

Dongzhen Chen[a,*], Yang Li[a], Lijun Wang[b], Yingjie Wang[b], Pan Ning[b], Powan Shum[c], Xinhai He[a], Tao Fu[b,*]

a Xi'an Key Laboratory of Textile Composites, Key Laboratory of Functional Textile Sensing Fiber and Irregular Shape Weaving Technology, School of Materials Science and Engineering, Xi'an Polytechnic University, Xi'an 710048, China

b Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China

c Asahi Group Co. Ltd, Kwun Tong, Hong Kong, China

Table S1. Full width at half maximium, peak position, and grain size of Mo-Ag films

Sample name	Full width at half maximium (deg.)	Peak position, 2theta (deg.)	Grain size (nm)
Mo film	0.337	40.58	24.9
Mo-Ag film (I)	0.453	40.504	18.5
Mo-Ag film (II)	0.512	40.206	16.3
Mo-Ag film (III)	0.513	40.171	16.3
Mo-Ag film (IV)	0.604	40.071	13.8
Mo-Ag film (V)	0.845	39.569	9.9

Table S2. Atomic radius and crystal lattice constant of the elements.

	Ag	Cr	Mo	W	Ti	Nb
Radius (Å)	1.34	1.18	1.30	1.30	1.32	1.34
Constant (Å)	/	2.884	3.147	3.165	/	/

Table S3. Cumulative ion concentrations of Mo-Ag film (II) sample soaked for different time measured by ICP-MS.

Time (h)	[Ag] (ng/mL)	[Mo] (ng/mL)
12	8.04	5.32
24	17.59	10.09
72	21.30	19.16

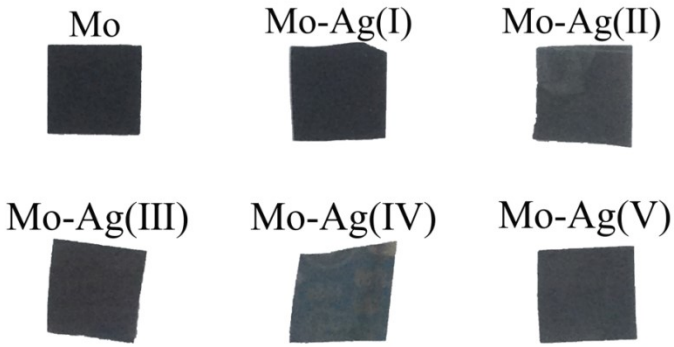

Fig. S1. The photographs of Mo film and Mo-Ag films, the films on a surface of glass sheet with size about 1.2×1.2 cm^2

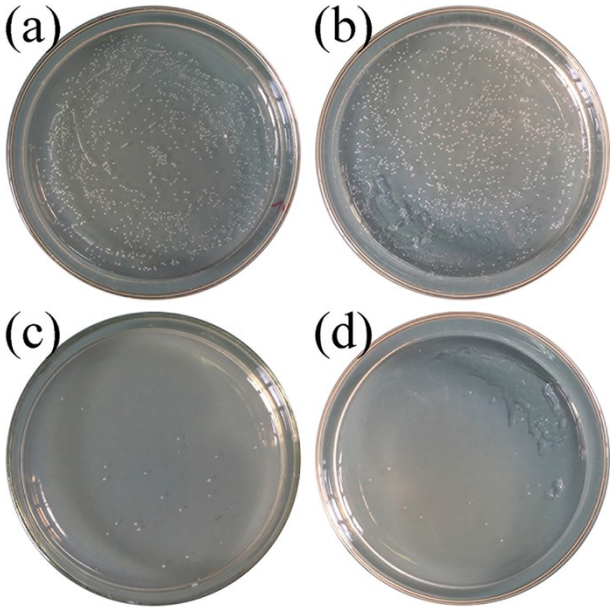

Fig. S2 Representative antibacterial test result of slide glass and the Mo-Ag film samples against *E. coli*: (a-d) glass, Mo film, Mo-Ag (I) film and Mo-Ag (II) film

Antibacterial property of the samples was assessed by the agar plate counting method against *Escherichia coli* (*E. coli*) as the typical pathogenic and environmental bacteria. The samples were sterilized with UV light for 30 min per side. Then, 10 μL of *E. coli* suspension (concentration of 10^5 colony forming units/mL) was dropped onto film surface of the samples, and the surface was covered soon by a piece of sterile filter membrane. After placed in wet atmosphere in dark at room temperature for 3 h, the samples were rinsed with 3 mL sterile water to collect the survival bacteria. After stirring by a vortex mixer for 1 min, 100 μL of the suspension was dropped on the preformed Luria-Bertani agar plate, and spread evenly using a sterile spreader. The inoculums were cultured at 37°C for 24 h for observation. The samples were tested at least twice independently.

The preparation of Cr–Ag film[1]

The Cr-Ag films were deposited on glass slides by a magnetron sputtering system. The substrates were degreased, ultrasonically cleaned and blown dry in flowing nitrogen gas in subsequence. In the sputtering system, two Cr elemental targets and one silver target (purity > 99.9%) were fixed for the deposition of Cr-Ag films. The substrates were mounted on the substrate holder that was rotated at a speed of 10 rpm. The vacuum chamber was evacuated and then pure Ar working gas (99.999% purity) was introduced. After plasma ion etching, the films were deposited with the Cr targets current of 4.0 A, the Ag target current of 0-2.0 A, the bias of −60 V and the deposition time of 15-30 min.

Calculation of SERS Enhancement Factor (EF)

In order to further indicate the SERS intensity of the substrate, we calculate the enhanced factor of Mo-Ag film for MG test, the enhancement factor (EF) is calculated using the following formula:

$$EF = (I_{SERS} \times N_{SERS}) / (I_{RS} \times N_{RS})$$

In the formula, I_{SERS} represents the SERS signal intensity of the characteristic peak at 1178 cm^{-1} obtained from the detection of MG on the substrate, the concentration of the MG solution used is 1×10^{-7} mol/L, and the characteristic peak intensity is about 693.6; I_{RS} represents the Raman intensities at 1178 cm^{-1} collected from MG with the concentration of 0.01 mol/L, and the characteristic peak intensity is about 265.9; N_{SERS} is the number of MG molecules that are dropped onto the Mo-Ag film and irradiated by the laser ; N_{RS} is the number of MG molecules that are dropped onto the silicon wafer and irradiated by the laser.

The calculation process of N_{SERS} and N_{RS} is the same. The specific formula is as follows:

$$N = (V \times C \times NA) / S_2$$

$$N_{SERS} = N \times S_1$$

In this formula, N is the number of MG molecules per unit area; S_1 represents the area of the laser spot, about 0.785 μm^2; V is the volume of the MG solution dropped; C represents the concentration of the MG solution; NA is avogadro's constant (about 6.02×10^{23}); S_2 is corresponding area that adsorbed MG molecule at Mo-Ag film.

Drop 10 μL of MG solution (0.01 mol/L) on the silicon wafer, forming a deposition area of about 1.486×10^7 μm^2, and the corresponding surface area of the Mo-Ag film is 1.6×10^9 μm^2. The Raman signal collection is carried out under the same experimental conditions, and the diameter of the laser spot is about 1 μm. The calculation results of N_{SERS} and N_{RS} are 2.95×10^2 and 3.18×10^9, respectively. Finally, the calculated EF of Mo-Ag film to MG molecule is about 2.8×10^7.

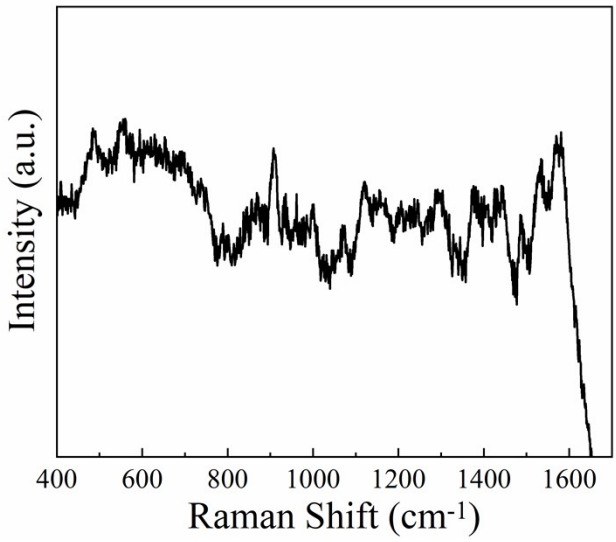

Fig. S3 SERS spectrum of *S. aureus*

Table S4. The detailed Raman band assignment of MG.

Raman band assignments	Observed /cm^{-1}	Reported /cm^{-1} [2]
Out-of-plane vibrations of Phenyl-C-Phenyl	412	417
Ring skeletal vibrations of radical orientation	913	917
Ring C-H in-plane vibrations	1178	1171
N-Phenyl stretching	1388	1398
Ring C-C stretching	1589	1595
Ring C-C stretching	1615	1617

Table S5. The detailed Raman band assignment of thiram.

Raman band assignments	Observed /cm^{-1}	Reported /cm^{-1} [2]
S-S stretching	560	556
CH3N stretching	933	928
C-N stretching,CH3 rocking	1149	1150
C-N stretching,CH3 deformation	1383	1387
C-N stretching,CH3 deformation	1513	1508

Table S6. The detailed Raman band assignment of *E.coli*.

Raman band assignments	Observed /cm^{-1}	Reported /cm^{-1} [3-5]
S-S bond stretching	524	530
Adenine from flavin	687	690
Nucleic acids (cytidine, uracil)	800 889	772-900
G (external ν(C-N))	1176	1180
Phe,AmideIII;δ(=CH) in lipids	1207	1206
N−H and C−H bend	1293	1295-1336
υ(COO-) and δ(C-H) proteins	1393	1396
δ(CH2)	1432	1434
Nucleic acids	1618	1627

Table S7. The detailed Raman band assignment of *S.aureus*

Raman band assignments	Observed /cm^{-1}	Reported /cm^{-1} [6-8]
Tyrosine	653	640
C=C deformation	936	955
Phenylalanine (in protein); β-carotene (pigmentation)	1000	1004
C-C-stretch; phospholipid bilayer; β-carotene (pigmentation)	1162	1158
$\delta(CH_2)$ amide III (bacteria)	1242	1240
Amide III (in Protein), DNA/RNA, lipids	1294	1290
ν(NH2) adenine, polyadenine, DNA (bacteria)	1331	1330
ν(COO-)and δ(C-H) Proteins(bacteria)	1376	1388
β-carotene (pigmentation)	1533	1520

References

1. L. J. Wang, Y. J. Wang, P. W. Shum, Y. F. Hou and T. Fu, *Coatings*, 2021, **11**, 1153.

2. J. Huang, D. Ma, F. Chen, D. Chen, M. Bai, K. Xu and Y. Zhao, *ACS Appl. Mater. Interfaces,* 2017, **9**, 7436-7446.

3. T. Fang, W. Shang, C. Liu, J. Xu, D. Zhao, Y. Liu and A. Ye, *Anal. Chem.*, 2019, **91**, 9932-9939.

4. D. Chen, P. Ning, Y. Zhang, J. Jing, M. Zhang, L. Zhang, J. Huang, X. He, T. Fu, Z. Song, G. He, D. Qian and X. Zhu, *ACS Appl. Mater. Interfaces*, 2020, **12**, 20138-20144.

5. M. Kögler, J. Itkonen, T. Viitala and M. G. Casteleijn, *Sci. Rep.*, 2020, **10**, 2472.

6. H. Wang, Y. F. Zhou, X. X. Jiang, B. Sun, Y. Zhu, H. Wang, Y. Y. Su, Y. He, *Angew. Chem., Int. Ed.* 2015, **54**, 5132-5136.

7. C. C. Lin, Y. M. Yang, P. H. Liao, D. W. Chen, H. P. Lin, H. C. Chang, *Biosens. Bioelectron*. 2014, **53**, 519-527.

8. D. Chen, L. Zhang, P. Ning, H. Yuan, Y. Zhang, M. Zhang, T. Fu and X. He, *Nano Res.*, 2021, **14**, 4885–4893.