Electronic Supporting Information

Electrospun polymer mat as a SERS platform for immobilization and detection of bacteria from fluids

Tomasz Szymborski, Evelin Witkowska, Witold Adamkiewicz, Jacek Waluk, and Agnieszka Kamińska**

Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy: measurements were carried out on dried samples using a Renishaw inVia Raman system equipped with a 785 nm HeNe laser. For bacteria detection 785 nm laser diode was used. The light from the laser was passed through a line filter and focused on a sample mounted on an X–Y–Z translation stage with a 50× microscope objective, NA = 0.25. The Raman-scattered light was collected by the same objective through a holographic notch filter to block Rayleigh scattering. A 1800 grooves/mm grating was used to provide a spectral resolution of 5 cm^{-1}. The beam diameter was approximately 5 μm.

Bacteria culture and SERS sample preparation: bacterial species used in the experiment were obtained from the Department of Bacterial Genetics, University of Warsaw, Poland. To multiply microbial organisms, we cultivated them in liquid LB (Lysogeny broth) growth medium followed by incubation in a shaker (150 rpm) at 30 °C for 24 h. After that the bacteria were centrifuged for 10 min at 4000 rpm, dispersed, and the LB growth medium was discarded. Then the bacteria were redispersed in saline solution (sterile 0.9% NaCl solution) and centrifuged for 10 min at 4000 rpm (not to destroy the cell membrane). The centrifugation process in the saline solution was repeated 4 times to obtain solution of clean bacterial cells. Purified bacteria were finally dispersed in following fluids: saline solution, tap water, urine and apple juice. In each case we obtained a concentration of *E. coli* at the level of 10^2 CFU/mL. The density of bacterial cells was determined by counting the amount of colonies which have grown on the Petri dish from a known amount of medium. Count was taken after one day of cultivation at 37 °C. Before carrying out Raman measurements 1 ml of an aqueous bacterial solution was placed over the SERS substrate. Measurements were taken after 5 minutes.

Procedure of gold sputtering: to sputter a layer of gold we used PVD equipment from Leica, model EM MED020. The gold target was obtained from Mennica Metale Szlachetne, Warsaw, Poland. The size of the gold target was 54 mm in diameter, thickness 0.5 mm, and gold purity was 3N. The vacuum during the gold sputtering was on the level of 10^{-2} mbar. The current of sputtering was 25 mA. No adhesive layer (chromium or

titanium) was sputtered on the polymer mat before sputtering gold. After the deposition process the samples were placed into a sterile Petri dish.

Three different thicknesses of gold (30, 90, and 200 nm) were tested to find optimal conditions for SERS enhancement. The 30 nm gold layer was not thick enough both to obtain SERS signal of the analyte (*p*-MBA and bacteria species) and to 'screen' the polymer. Therefore, we recorded the Raman spectra of the polymer (PLLA, PVDF, or nylon). In the case of the polymer mat covered with 90 nm of gold we observed the highest SERS enhancement without any signals from polymer. We achieved the same level of enhancement for 200 nm gold layer. For further experiments we chose 90 nm layer of gold due to fact that this particular thickness gives high enhancement factor and the fact that 90 nm is more cost-effective. Also, the process of sputtering of 90 nm of gold takes 20 minutes instead of 45 minutes for 200 nm layer.

Morphology of the gold layer: the morphology of the gold layer deposited on the polymer mat can affect the SERS enhancement factor. We have used three polymers: PVDF, PLLA (woven and non-woven) and nylon, which have different physical and chemical properties. In our experiments we did not use any adhesive layer, i.e. chromium or titanium between polymer mat and gold layer. It means that the bare polymer mat may determine the morphology of deposited gold. In each case we did not obtain uniform and flat layer of gold, but we did obtain the layer of gold semi-spheres ranging from 30 to 100 nm in diameter. For example, for PLLA-a polymer mat (with the thickest fibers) the average diameter of deposited gold nanostructures was 45 nm, whereas in the case of nylon polymer mat (with the thinnest fibers) it increased up to 100 nm. The close examination of the SEM images (see Fig. 1 in the article and Fig. S2 in ESI) reveals that the diameter of gold semi-spheres depends on the polymer type, diameter of the polymer fibers and their arrangement (woven, non-woven and number of fibers per area unit). The calculated EF clearly revealed that all these parameters determine the SERS efficiency of obtained platform.

Figure S1. SEM image of *E. coli* immobilized on PLLA-b polymer mat covered with 90 nm layer of gold. The bacteria were suspended in urine and placed on the mat with method and setup presented in main article.

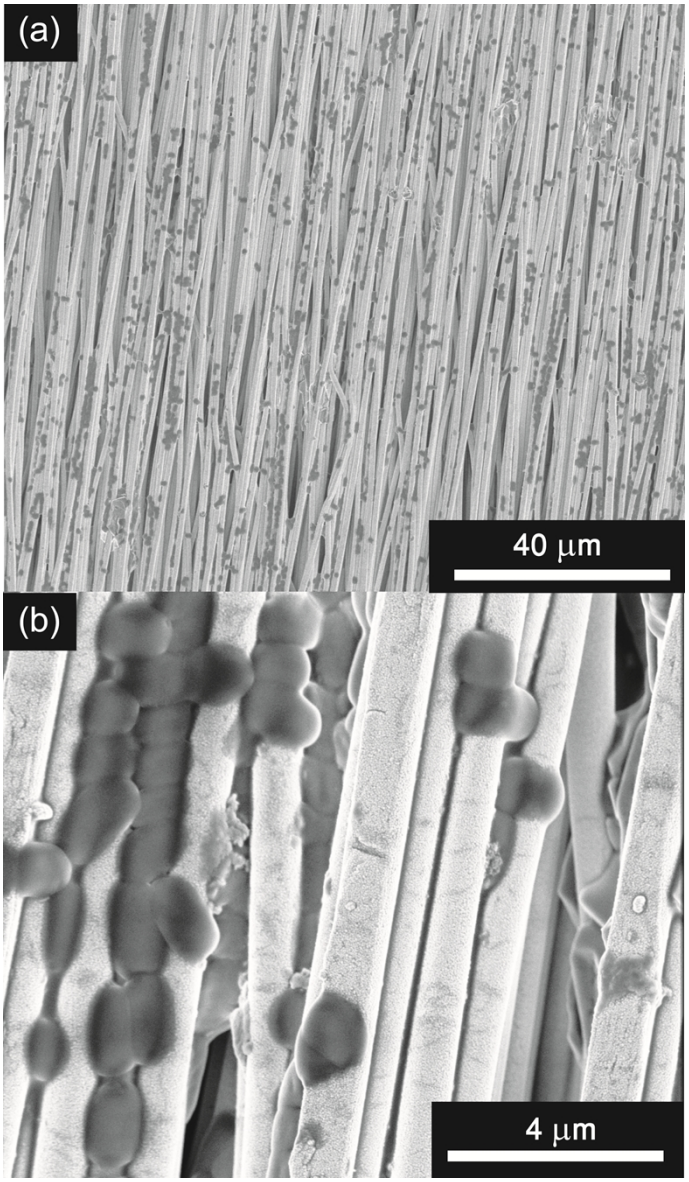


Figure S2. Polymer fibers made by electrospinning covered with 90 nm of gold via PVD process. Type of polymer (a) PVDF, (b) PLLA-a, (c) PLLA-b, (d) nylon.

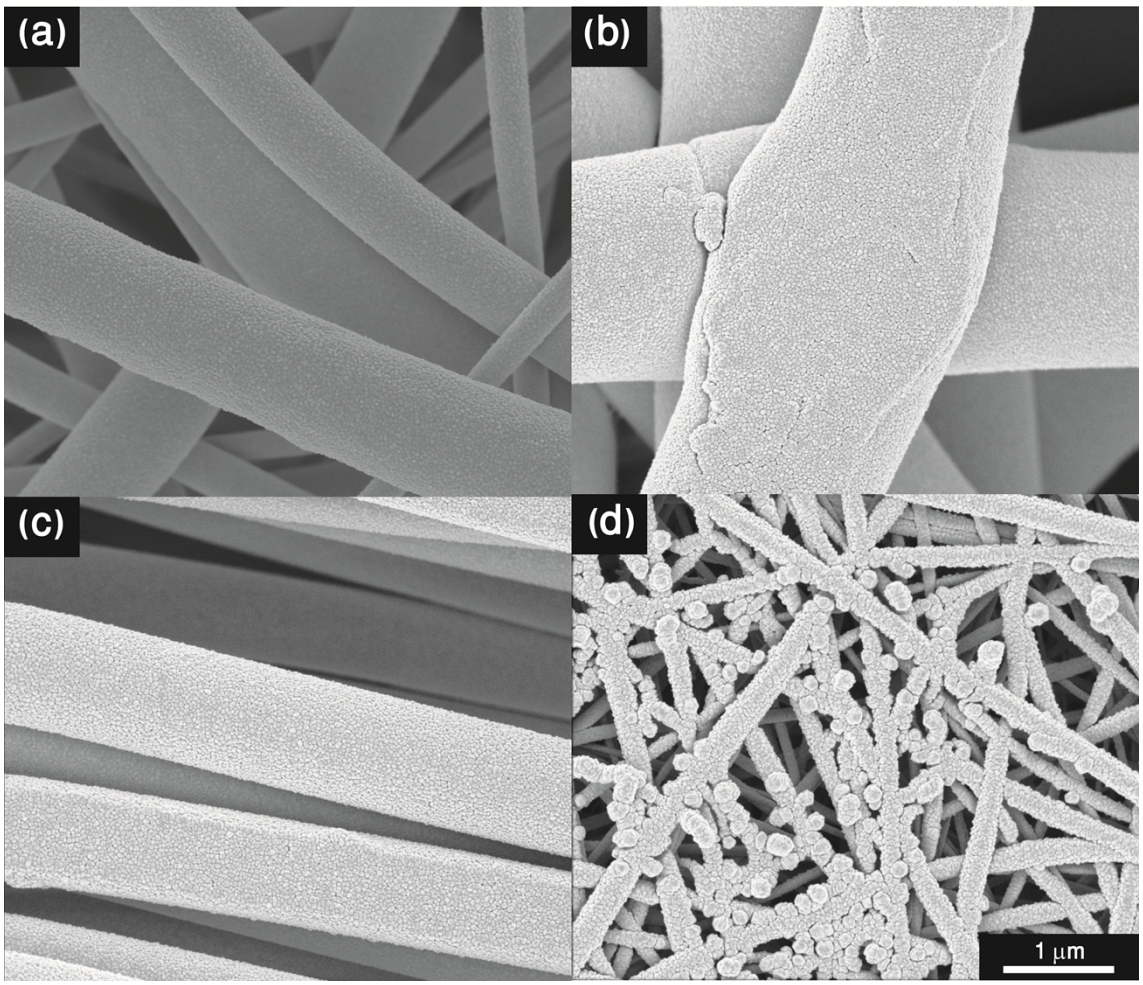
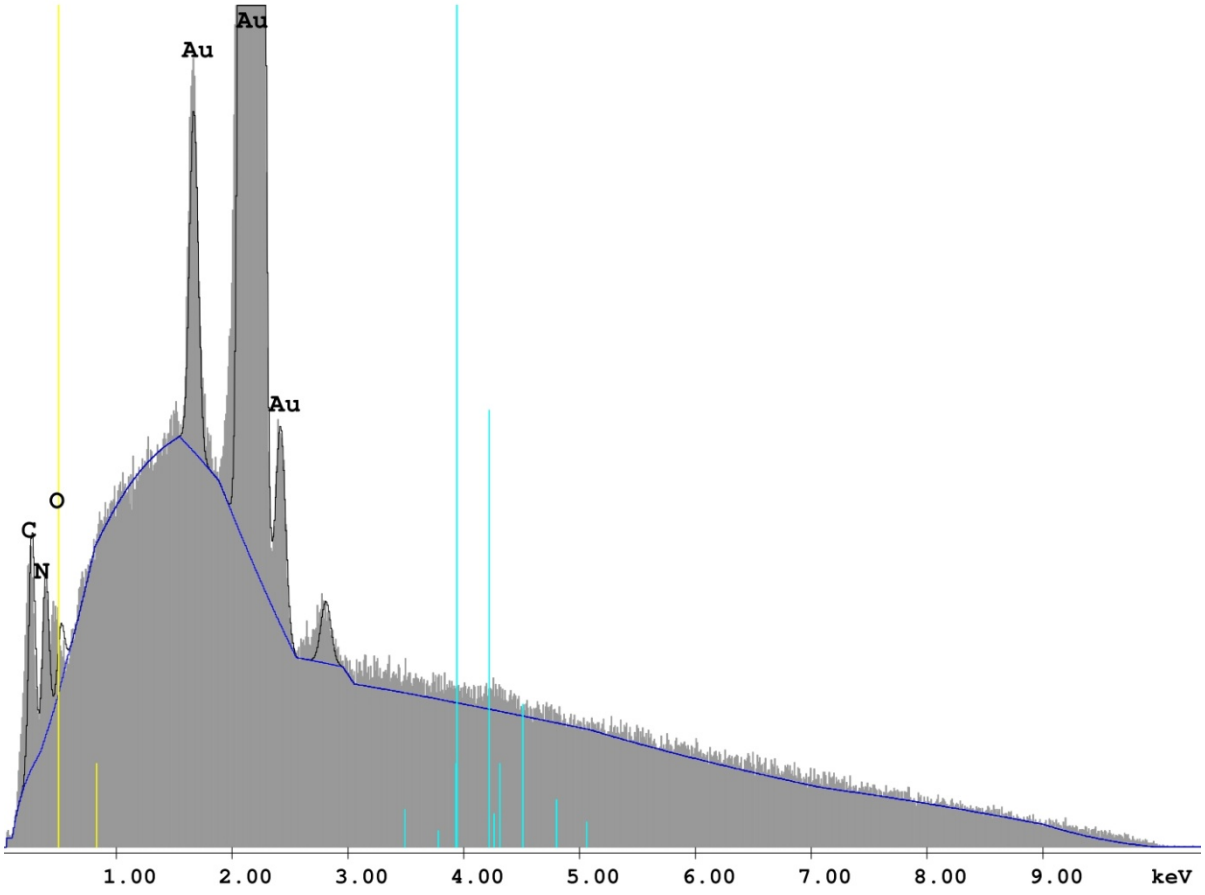


Figure S3. EDX results for nylon polymer mat covered with 90 nm of gold.

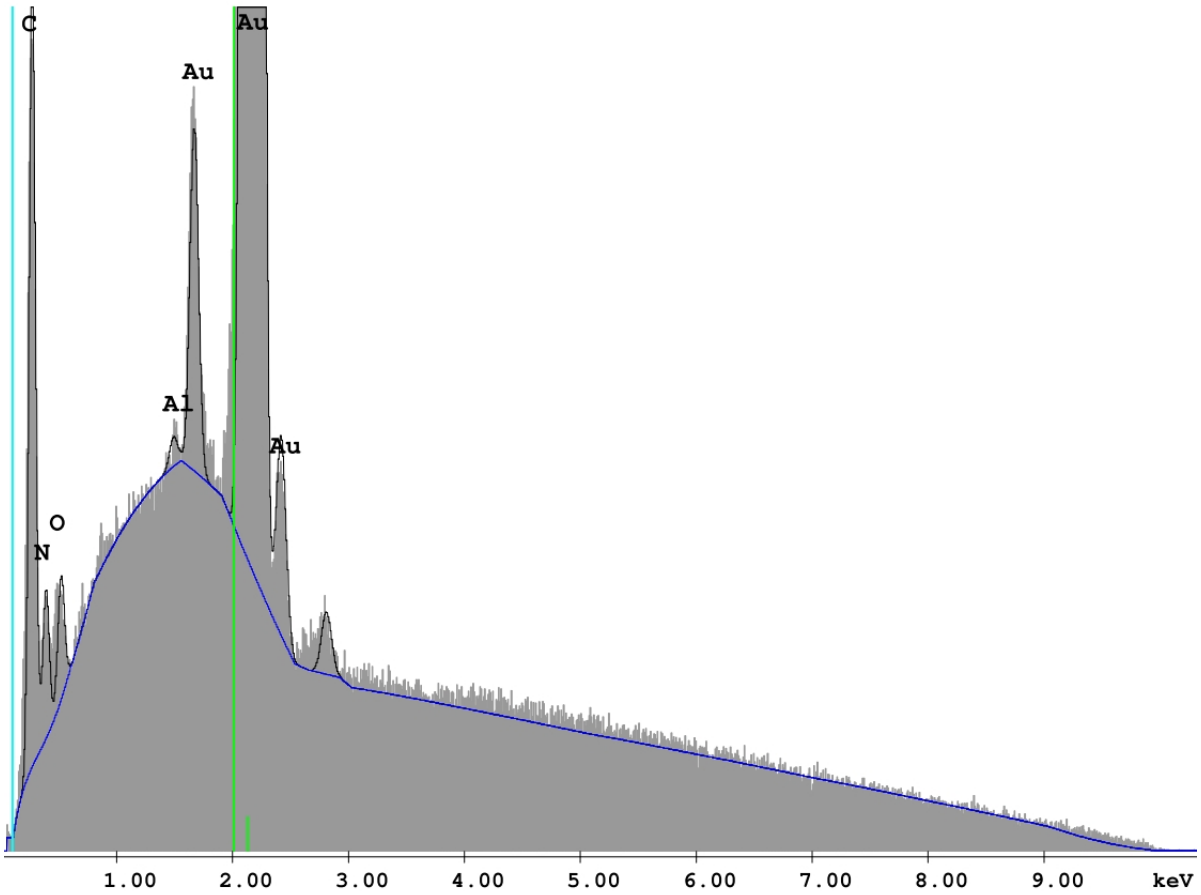


EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	1.49	16.72	0.0057	1.5731	0.2440	1.0000
N K	1.49	14.37	0.0059	1.5511	0.2536	1.0000
O K	0.33	2.80	0.0015	1.5300	0.2946	1.0000
AuM	96.68	66.11	0.9447	0.9708	1.0066	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	46.63	21.13	2.02	2.21
N K	32.64	29.86	2.94	1.09
O K	12.19	43.76	8.19	0.28
AuM	1861.10	99.71	0.24	18.67

Figure S4. EDX results for PLLA-a polymer mat covered with 90 nm of gold.

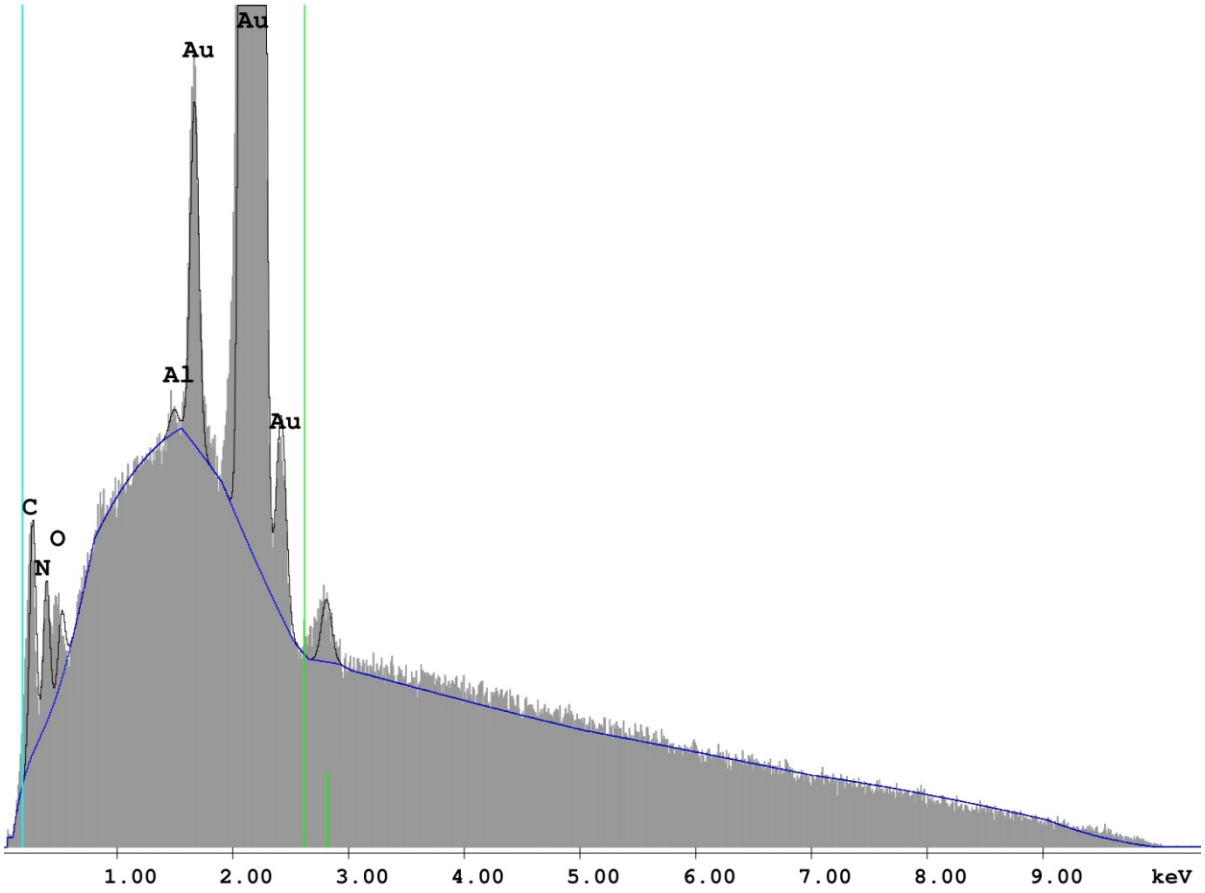


EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	4.58	38.29	0.0179	1.5282	0.2558	1.0000
N K	1.34	9.59	0.0051	1.5070	0.2536	1.0000
O K	0.65	4.11	0.0029	1.4869	0.3011	1.0000
AlK	0.11	0.40	0.0012	1.4268	0.7546	1.0000
AuM	93.32	47.61	0.8918	0.9434	1.0129	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	128.90	18.62	1.00	6.92
N K	25.08	25.01	3.46	1.00
O K	21.06	33.66	4.46	0.63
AlK	6.33	106.95	23.44	0.06
AuM	1551.76	81.06	0.27	19.14

Figure S5. EDX results for PLLA-b polymer mat covered with 90 nm of gold.

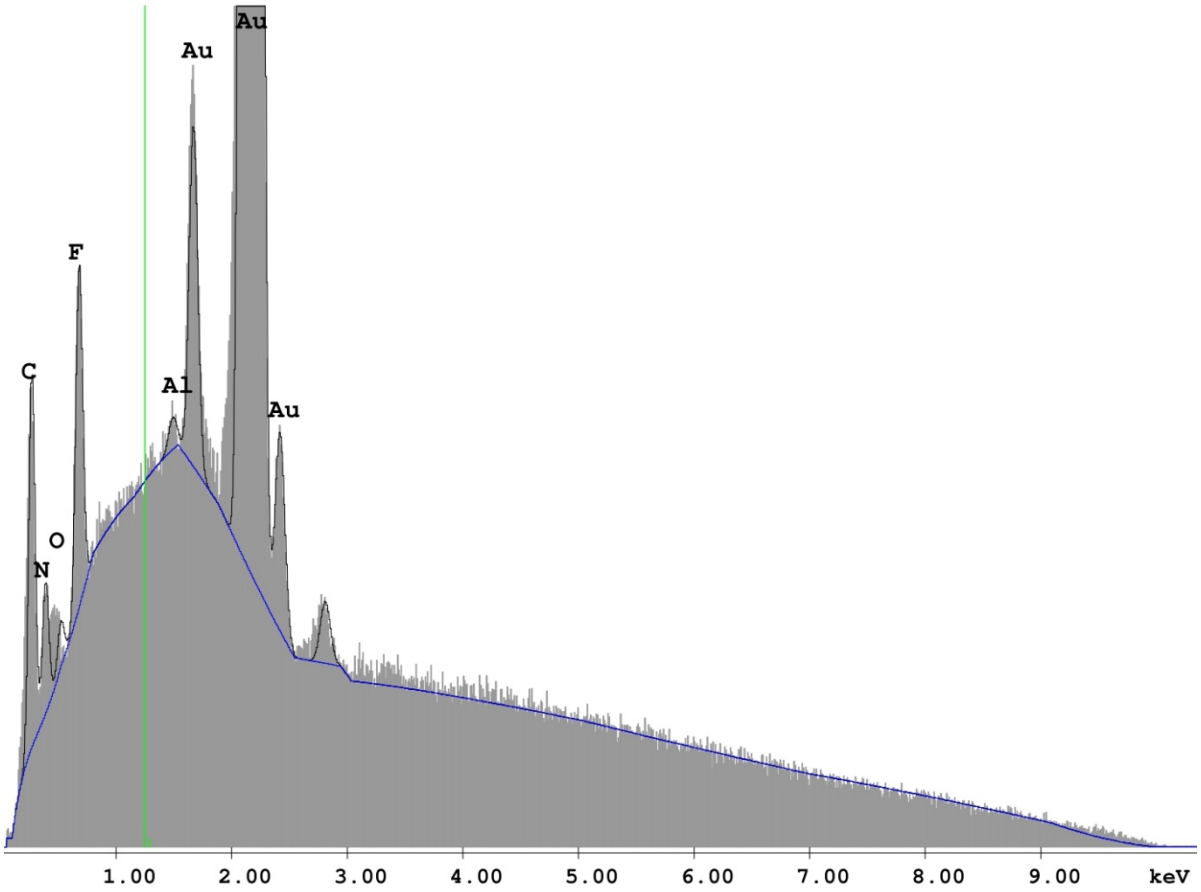


EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	1.48	16.76	0.0056	1.5743	0.2424	1.0000
N K	1.31	12.76	0.0051	1.5522	0.2521	1.0000
O K	0.38	3.21	0.0017	1.5312	0.2935	1.0000
AlK	0.09	0.44	0.0009	1.4723	0.7422	1.0000
AuM	96.74	66.83	0.9457	0.9715	1.0062	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	47.15	25.37	2.10	1.86
N K	29.26	34.08	3.37	0.86
O K	14.16	45.88	7.27	0.31
AlK	6.04	141.56	28.15	0.04
AuM	1911.01	103.78	0.24	18.41

Figure S6. EDX results for PVDF polymer mat covered with 90 nm of gold.



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	2.28	21.97	0.0088	1.5484	0.2482	1.0000
N K	1.18	9.78	0.0046	1.5269	0.2559	1.0000
O K	0.24	1.75	0.0011	1.5063	0.3003	1.0000
F K	1.71	10.43	0.0087	1.4077	0.3621	1.0000
AlK	0.12	0.51	0.0013	1.4465	0.7467	1.0000
AuM	94.47	55.56	0.9109	0.9557	1.0090	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	66.88	24.05	1.60	2.78
N K	24.05	32.42	3.92	0.74
O K	8.34	43.53	11.71	0.19
F K	64.84	58.22	2.08	1.11
AlK	7.42	120.68	21.26	0.06
AuM	1682.94	85.61	0.26	19.66

Figure S7. Raman spectra of *E. coli* bacteria on PLLA-a polymer mat. *E. coli* was suspended in apple juice, tap water and urine and placed on the polymer mat using experimental setup described in the article.

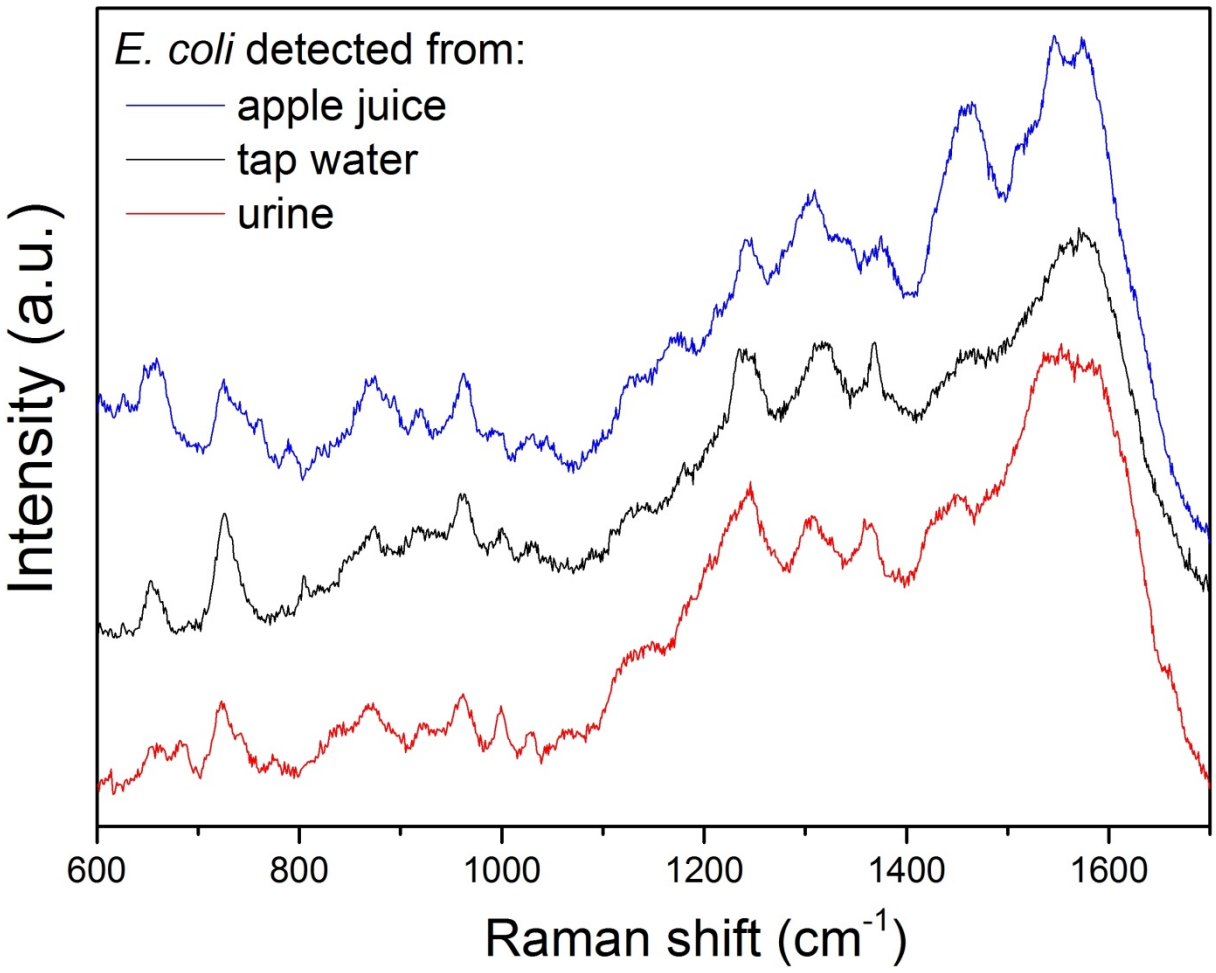
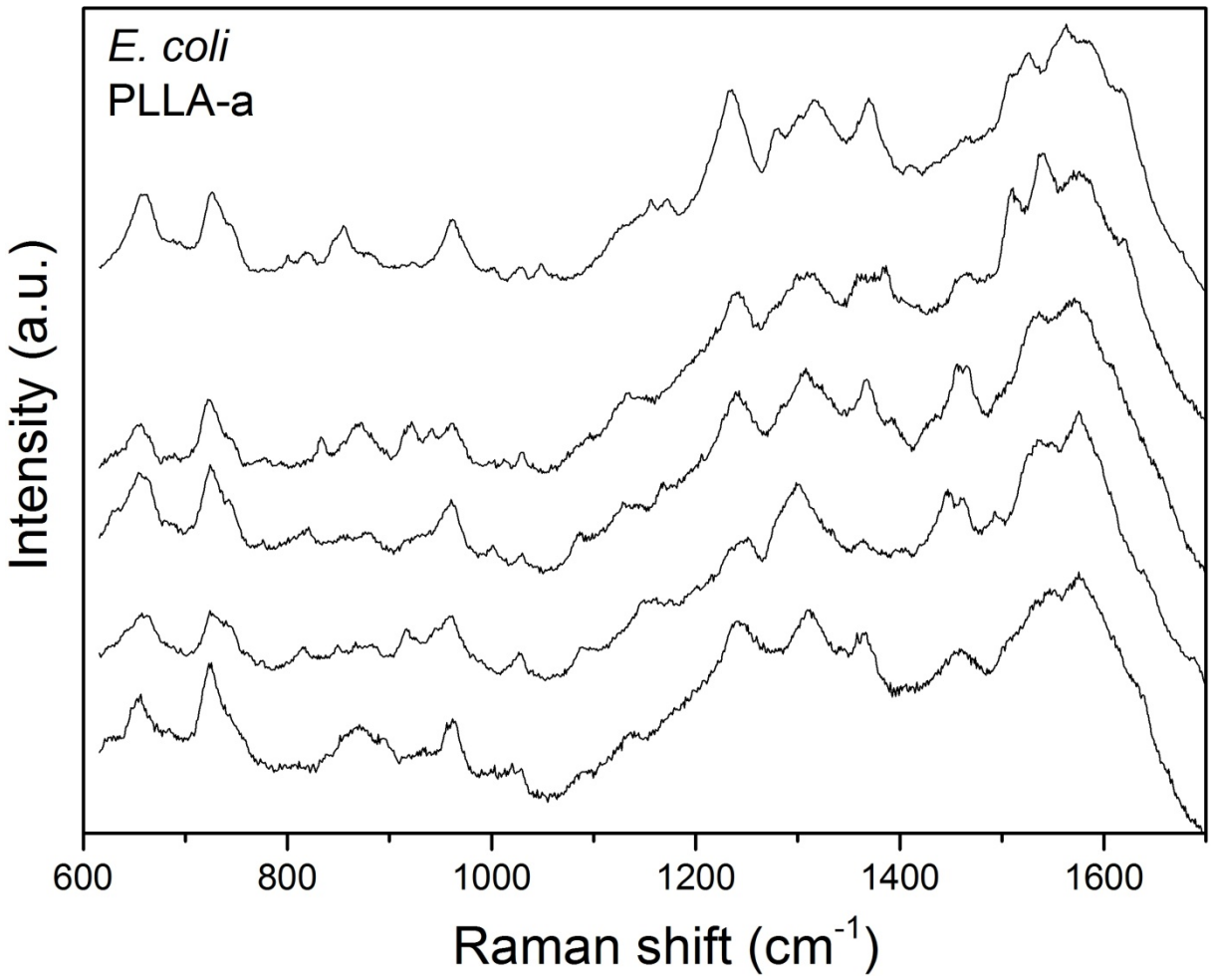


Figure S8. Raman spectra of *E. coli* on a SERS platform made of PLLA-a polymer fiber covered with 90 nm of gold. The spectra were taken in different spots within the same platform.



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In order to verify the properties of the obtained platforms we calculated the enhancement factors and *p*-MBA was chosen as standard analyte. The obtained spectra, collected in **Figure S9**, are typical for *p*-MBA, and can be treated as a fingerprint of this molecule. All the observed vibrations come from *p*-MBA moieties.[1] **Table S1** summarizes band assignments for the normal Raman spectrum of *p*-MBA and its SERS spectrum.

The normal Raman and SERS spectra of *p*-MBA were recorded by excitation with a 785 nm laser. To estimate the enhancement ability of the obtained structures, the surface enhancement factor (EF) was calculated according to the following formula:

$$EF = \frac{I_{SERS} N_{NR}}{I_{NR} N_{SERS}} \quad (1)$$

where N_{SERS} and N_{NR} refer to the number of molecules adsorbed on the SERS probe within the laser spot area and the number of molecules probed by regular Raman spectroscopy, respectively. I_{SERS} and I_{NR} correspond to the SERS intensity of *p*-MBA on the modified surface and to the normal Raman scattering intensity of *p*-MBA in the bulk. I_{NR} and I_{SERS} were measured at 1077 cm^{-1}.

The SERS samples were prepared by dipping the substrate in 9.0 mL of 1.0×10^{-6} M solution of *p*-MBA. The number of molecules contained in the solution was 5.4×10^{15} ($6{,}02 \times 10^{23}$ molecules/mol $\times$ 9.0×10^{-3} L $\times$ 1.0×10^{-6} mol/L = 5.4×10^{15} molecules). The surface area irradiated by the laser beam (5 μm in diameter) was 19.6 μm^2. Therefore, about 4.2×10^9 molecules were present in the laser beam spot. The normal Raman spectrum was observed for

a cell filled with a pure *p*-MBA acid liquid (154.19 g/mol; density of 1.06 g/cm^3). The effective illuminated volume for our setup is 2×10^3 μm^3. This value was confirmed by registering Raman spectra of silicon while varying the distance from the focal plane. Under these conditions, $N_{NR} = 8.1 \times 10^{12}$ molecules were irradiated by the laser. From these data of the relative intensity and the number of molecules sampled from the regular Raman and SERS measurements, the enhancement factor was calculated for each sample. In **Figure S9** we present *p*-MBA spectra acquired from all four polymer platforms. In the same Figure we present the value of the calculated EF for each of them.

The EF for PLLA and nylon polymer mats has the value of 10^6. The PVDF polymer mat shows EF in the range of 10^4. For this reason we have chosen PLLA polymer mats for further investigations, i.e. for immobilization and detection of bacteria.

Figure S9. Raman spectra of *p*-MBA obtained on four different polymer mats covered with 90 nm of gold: (a) PVDF, (b) PLLA-a, (c) PLLA-b and (d) nylon. We present the enhancement factors (EF) for all of them. The EFs for PLLA and nylon are of the order of 10^6, which is suitable for most applications in chemistry, biology or forensic science.

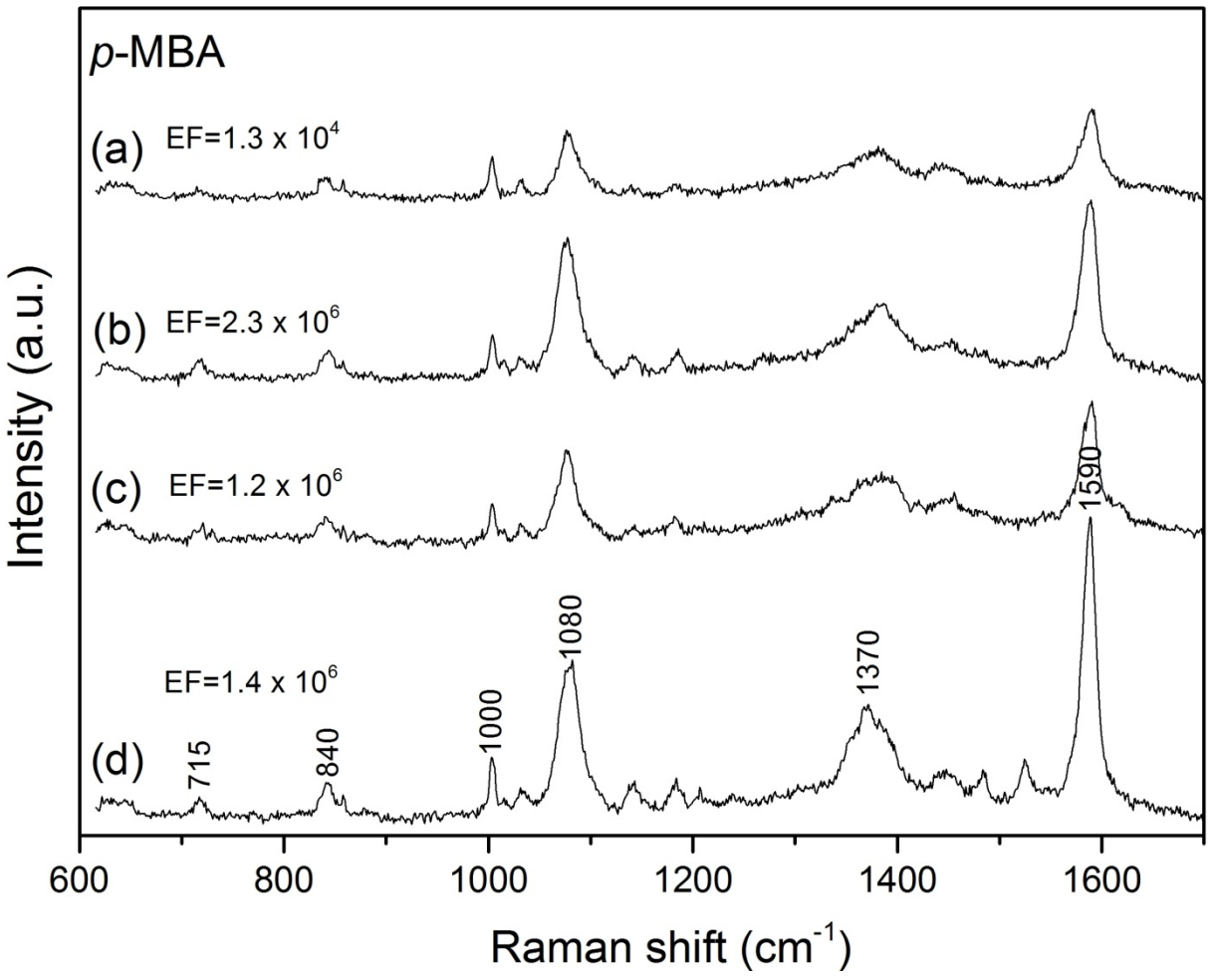

Table S1. Band assignments for *E. coli* and *S. aureus* bacteria. The spectra was acquainted for PLLA-a polymer mat covered with 90 nm of gold (data based on [18]).

	Assignment	Range	*E. coli*	*S. aureus*
1.	C-O-C ring deformation	540-575	-	+
2.	guanine, tyrosine	655-675	-	+
3.	Adenine	725-740	+	+
4.	C-O-P-O-C (RNA backbone)	805-815	-	+
5.	tyrosine	825-860	+	-
6.	C=C deformation	930-990	+	+
7.	phenylalanine	1000-1010	+	+
8.	carbohydrates, mainly C-C (skeletal)	1025-1060	+	+
9.	Amide III (random)	1220-1295	+	+
10.	adenine, guanine, CH deformation	1330-1345	+	+
11.	(COO–)	~1370-1380	+	+
12.	(COO–) amino acids	~1410-1420	+	-
13.	CH_2 deformation	1440-1475	-	+
14.	amide II	1510-1560	+	+

Table S2. Band assignments for *p*-MBA (data based on [1]).

Wavenumber [cm^{-1}]	**Band assigment**
~715	γ(CCC) aromatic ring vibrations
~840	$\delta(COO^-)$
~1000	substitued benzene ring vibrations
~1080	ν_{12} aromatic ring vibrations
~1370	$\nu_s(COO^-)$
~1590	ν_{8a} aromatic ring vibrations

[1] A. Michota and J. Bukowska, *J. Raman Spectrosc.* 2003, **34**, 21.