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

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This work demonstrates the development of a new class of SERS substrates that allows for the simultaneous: (i) filtration of bacteria from any solution (blood, urine, water, or milk), (ii) immobilization of bacteria on the SERS platform, and (iii) enhancing the Raman signal of bacteria. The proposed platform is based on an electrospun polymer mat covered with a 90 nm layer of gold.

Surface-Enhanced Raman Spectroscopy (SERS) was discovered 40 years ago and nowadays is widely used as an analytical method.[1] SERS offers an exceptional enhancement factor (EF) of the intensity of vibrational signals. For this reason, it is used as a tool in chemistry and biochemistry,[2] forensic science,[3] environmental research,[4] homeland security,[5] and other areas. SERS is also undergoing rapid development as an ultra-sensitive analytical method in the biological field.[6] There is a great interest in detecting bacteria, especially in environmental samples.[7] The SERS method allows the acquisition of spectra from single bacterial cells on a timescale of seconds, without the need for sample amplification by bacterial culture and by polymerase chain reaction (PCR). One of the biggest problems with this promising method is the lack of cheap, stable platforms with, preferably, a high enhancement factor. It also lacks a simple technique for placing bacteria cells onto a SERS platform directly from solutions like water or blood. The highest signal enhancement factors are found for structures with sharp edges, intersections, and periodic dips, as well as for spanning the range of sizes from nanometers to single micrometers. Such structures guarantee a large number of "hot spots", *i.e.*, places where local electromagnetic fields can be dramatically enhanced.[8] A large variety of sizes and structures can be provided by polymer nano- and microfibers made using different methods,[9] and one of the most popular is electrospinning (ES).[10]

The electrospinning method (ES) has been well known for many years, but was rediscovered in the 1990s.[11] In this technique a polymer solution is pushed through a steel needle, while a high voltage (usually 10–20 kV) is applied between the needle and a metal receiver. The electrospinning process has many advantages: it is simple, low-cost, and fast. Additionally, one can tune the properties of the fibers with such parameters as applied voltage, distance between the electrodes, viscosity, surface tension, conductivity of the polymer, feeding rate, and others.[12] For this reason, we have observed a rise in interest in using electrospinning as a method for the preparation of SERS substrates.[13]

So far, only a few approaches have been made to create a fiber-based SERS platform. Usually, SERS platforms are flexible, and they consist of free-standing polymer fibers with embedded (or decorated) metal nanoparticles of gold and/or silver.[14] The role of the nanoparticles is to create "hot spots" on the surface of the fiber and to enhance the signal from the analyte. For instance, Zhang *et al.*[15] coupled ES and soft photolithography. They used SU-8 (epoxy-based negative photoresist) to electrospin fibers, and thereafter, photolithography to prepare a pattern. The resulting structure was covered with a thin layer of silver and was used as a SERS platform. Lee *et al.*[16] presented a way to assemble the gold nanorods into electrospun polymer fibers that exhibited potentially interesting SERS properties. However, no research has yet been reported on the use of electrospun SERS substrates to detect and identify biological samples.

In this communication, we present: (i) a new, simple method of preparing a SERS platform made of electrospun fibers, (ii) an extremely efficient method for the filtration and immobilization of bacteria from fluids, directly on these SERS platforms.

We obtained the fibers from a commercial company (MECC Co., Ltd., Japan) and used them successfully as a SERS platform. Three different polymers were used: poly(vinylene fluoride) (PVDF), poly(L-lactide acid) (PLLA), and nylon. PLLA fibers were used as nonwoven (PLLA-a) and woven (PLLA-b) mats. To prepare the SERS platform, we cut polymer mats (area of

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0.25 cm^2 or 1 cm^2) and covered them with 90 nm of gold *via* PVD sputter coater (Leica, EM MED020). Afterwards, they were placed into a Petri dish to avoid contamination from the air. The details of the gold deposition and a discussion about the influence of the polymer fibers on the morphology of the gold layer are described in the ESI.†

We demonstrate that our platform can be used for both biological and non-biological samples. We believe that this method can be of interest for researchers working in the field of SERS and willing to use nanofibers in their experiments, but who have no access to electrospinning facilities. Moreover, scientists working on electrospinning may use it to readily prepare SERS substrates. The proposed method is cheap, fast (the platform can be prepared within *ca.* 30 minutes), and clean (we do not use any organic or inorganic substances to modify the fibers). We also developed a special technique for placing bacteria onto the platform. The technique is simple and allows researchers to immobilize and concentrate bacteria cells within a small area of the platform.

Periodic structures of the nanosized fibers combined with a thin layer of gold worked as efficient SERS platforms. We tested them with solution of *p*-mercaptobenzoic acid (*p*-MBA) (see ESI† for detailed information). In order to verify the possibility of using our platform for biological samples, we tested it with *Escherichia coli* and *Staphylococcus aureus*. The EFs obtained for *p*-MBA are on the level of 1.2×10^4–3.6×10^6, which makes this method a promising way of preparing SERS platforms for laboratory practice, especially for the detection and identification of bacteria. The platforms we developed are characterized by the following features:

(i) They enhance the Raman signal by up to 10^6 times.

(ii) They can filter out bacteria from the liquid in which the bacteria are located and where the concentration of the bacteria is small, *e.g.*, blood, urine, water, wastewater, or food products such as milk and juices.

(iii) They can immobilize bacteria on their surface, so that the bacteria cannot move and escape from the area of the laser beam.

The most important aspect is that the SERS platform enhances the weak bands of bacteria and at the same time allows filtering the bacteria from any solution (blood, urine, water, or milk). Moreover, such structures immobilize bacteria, so that during the measurement they do not move across the platform. A SEM image of *E. coli* immobilized from urine on a PLLA-b mat platform is shown in the ESI (Fig. S1†). On the basis of our previous experiments,[17] we conclude that such movement is a very serious problem in experiments aimed at detecting and identifying unknown bacteria.

The morphologies of the SERS substrates were monitored by scanning electron microscopy (SEM); a few representative images are presented in Fig. 1. Additional pictures are placed in the ESI (Fig. S2†).

In order to test the deposition process, we carried out EDX (Energy-dispersive X-ray spectroscopy) analyses (see ESI, Fig. S3–S6†). We registered a strong gold signal but no signal from the polymer (PLLA, PVDF, nor nylon), concluding that the layer of gold on the fibers is uniform and that all SERS signals come from molecules/pathogens localized on the surface of gold.

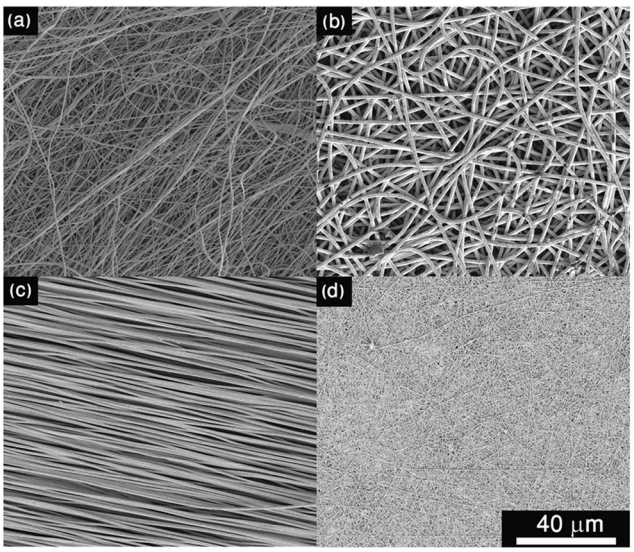

Fig. 1 SEM images of the polymer mats covered with 90 nm of gold, working as SERS platforms. We tested four polymer mats: (a) PVDF, (b) PLLA-a, (c) PLLA-b, and (d) nylon. The PLLA-a is a nonwoven mat, whereas PLLA-b is a woven mat. We present additional SEM pictures in the ESI.†

The analytical application of fiber-based platforms was verified using two bacteria species (*Escherichia coli* and *Staphylococcus aureus)* suspended in 0.9% NaCl solution. Additionally, *E. coli* was detected in tap water, urine, and in apple juice (see ESI, Fig. S7†). In order to place the bacteria on the platform we used the setup presented in Fig. 2.

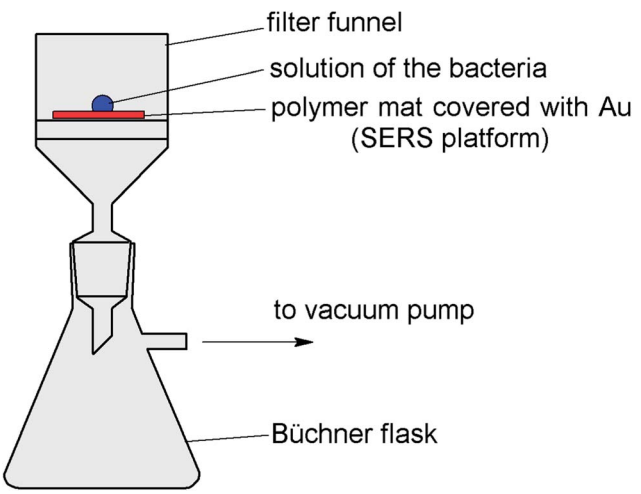

Fig. 2 The scheme of embedding bacteria on the SERS platform directly from the solution. The vacuum pump generates a partial vacuum which creates a flow of liquid from the suspended droplet with bacterial cells. The spaces between the polymer fibers are small enough to keep the bacterial cells on the surface of the polymer mat, while the fluids pass through the polymer mat and filter funnel to the Büchner flask.

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The setup consists of a Büchner flask, a filter funnel, and a vacuum pump (Büchi V-750 with V-850 vacuum controller). The procedure of depositing bacteria was as follows. We used the bacteria suspended in solution (see ESI† for details on the preparation of bacteria solution). The platform was placed on a Büchner flask and then on the platform we placed a single droplet of solution. After switching on the pump, the vacuum caused the movement of the fluid through the polymer mat, while the bacteria remained on the surface. This could be repeated several times, with the concentration of bacteria increasing after each step. This procedure was successfully used to trap bacteria from water and blood.

Fig. 3a shows the average SERS spectra of *S. aureus* recorded from four analyzed polymer platforms (PVDF, PLLA-a, PLLA-b, and nylon). For all types of platforms, the typical Raman signatures of bacteria were observed. We chose one platform (PLLA-b) to perform a detailed analysis to check the possibility of identification of the bacteria from the fluid.

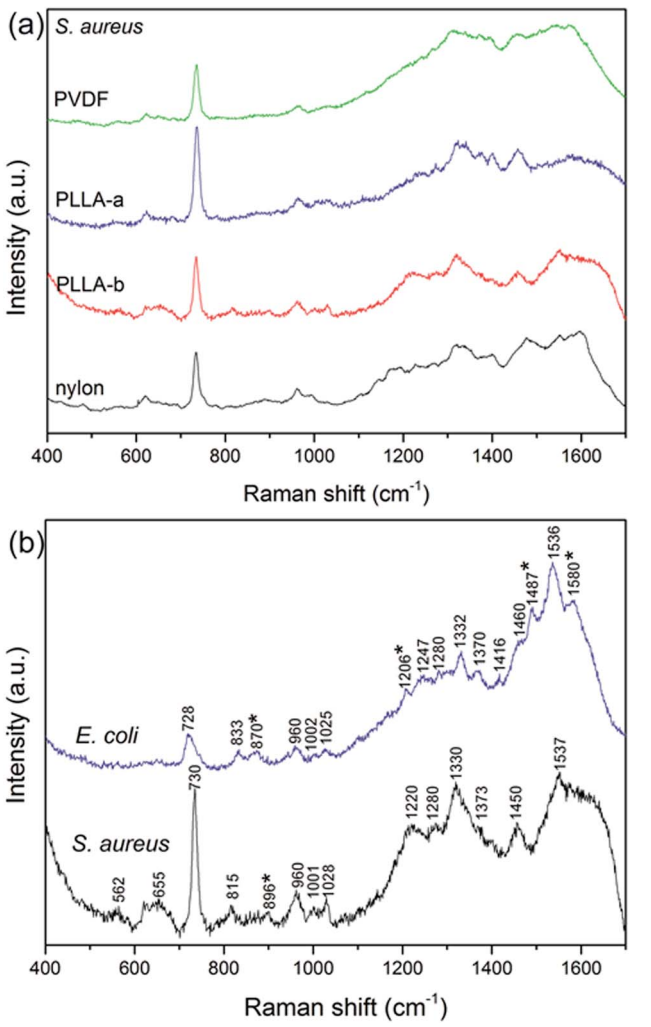

Fig. 3 (a) SERS spectra of *S. aureus* recorded from four Au-coated polymer mats: PVDF, PLLA-a, PLLA-b and nylon. (b) Raman spectra of *E. coli* and *S. aureus* collected from Au-coated PLLA-b (woven) polymer mat. The bands marked with an asterisk (*) were not assigned (they were not found in the literature). All the used polymer mats were covered with 90 nm of gold *via* PVD method.

Fig. 3b presents the spectra of *E. coli* and *S. aureus* with a characteristic band vibration at about 730 cm^{-1}. Typical Raman bands of proteins, phospholipids, and polysaccharides can be observed in these bacteria spectra. The band located at about 720–730 cm^{-1} is observed in the case of many bacterial species. It is assigned to the C–N stretching mode of the adenine part of the lipid layer in the cell wall or to the purine ring breathing mode.[18,19] Jarvis *et al*., on the other hand, attributed the same peak to the glycosidic ring mode from the cell wall peptidoglycan building blocks, *N*-acetyl-D-glicosoamine (NAG) and *N*-acetylmuramic acid (NAM).[20] We can also note spectral features which come from tyrosine, phenylalanine, C–C oscillations, and from phosphate binding in the DNA, respectively.[20] Close examination of the SERS spectra of *E. coli* and *S. aureus* also indicates other common peaks that are assigned to amide III (1280 cm^{-1}), CH_2 vibrations (1450 cm^{-1}), and amide II (1537 cm^{-1}).[18] Table S1 (see ESI†) presents band assignments for both *E. coli* and *S. aureus* bacteria.[18,19] It is important to note that *E. coli* and *S. aureus* exhibit differences in their SERS spectra, thereby enabling species identification. For example, the bands at 870, 1206, 1487, and 1580 cm^{-1} appear only in the SERS spectrum of *E. coli,* whereas they are not observed in *S. aureus*. Additionally, the bands at 562 and 655 cm^{-1} are observed only in the case of the *S. aureus* spectrum.

As exemplified by Fig. 3b, we were able to detect and identify both *E. coli* and *S. aureus*, filtrated and immobilized on a PLLA-b polymer mat.

The reproducibility of the Raman signal plays a crucial role in the practical application of SERS spectroscopy. Fig. S8 in the ESI† shows the example of SERS spectra of *E. coli* recorded from different spots within the same sample. To get statistically valid results, the strong band at 730 cm^{-1} and a weak peak at 960 cm^{-1} were chosen to calculate the relative standard deviation (RSD). The RSD of the intensity of these Raman vibrations in the 20 SERS spectra collected on the same platform are 15% and 17%, respectively. The reproducibility of the SERS signals recorded from different platforms prepared using the same method was also studied, and the achieved RSD was 22%.

In summary, we have demonstrated a fast and simple solution to crucial problems in the SERS measurement of bacteria, namely:

(i) How to deposit bacteria directly from the solution onto the SERS platform, and.

(ii) How to immobilize bacteria onto the surface of the platform for the time of measurement.

We propose a novel method of using an electrospun polymer mat covered with a gold layer *via* Physical Vapor Deposition (PVD) method. In our procedure, the electrospun polymer mat has two functions: it acts as a filter and a SERS platform at the same time. We propose a method of depositing bacteria on the platform with the use of vacuum pump, and we demonstrate its usefulness for the identification and detection of *E. coli* and *S. aureus*. In our approach, we remove the step of transferring the bacteria from the filter onto the SERS platform, as it is impractical and can cause contamination of the sample.

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Additionally, the structure of the mat immobilizes bacteria in one place, which increases the concentration of bacteria in one spot and prevents the bacteria from movement during measurement. Since we use commercially available polymer mats, the method can be applied worldwide in all laboratories, even in those that do not have an electrospinning setup.

We obtained reproducible SERS spectra, both across a single platform and between different platforms, with enhancement factors for *p*-MBA of the order of 10^6. The platforms proved to be very stable in time, and reveal good enhancement of the bacteria bands, which further confirms their applicability in fundamental biological studies and medical diagnostics. The platform based on an electrospun polymer mat, together with our novel procedure for the deposition of bacteria, opens up a new possibility for the fast detection of bacteria in medicine, biology, forensics, and environmental sciences.

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