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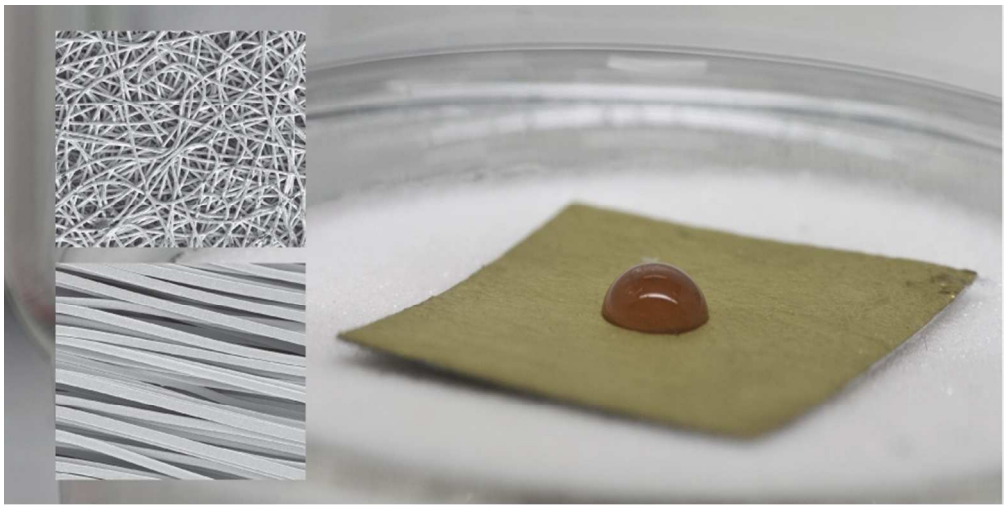

A new class of SERS substrates is presented that allows for simultaneous filtration of bacteria from any solution (blood, urine, water or milk), immobilization of bacteria on the SERS platform, and enhancing Raman signal of bacteria.
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Electrospun polymer mat as a SERS platform for 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 simultaneous: (i) filtration of bacteria from any solution (blood, urine, water or milk); (ii) immobilization of bacteria on the SERS platform, and (iii) enhancing Raman signal of bacteria. Proposed platform is based on electrospun polymer mat covered with 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 exceptional enhancement factor (EF) of the intensity of the 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 time scale of seconds, without the need for sample amplification by bacterial culture and by polymerase chain reaction (PCR). One of the biggest problems in this promising method is the lack of cheap, stable platforms with, preferably, high enhancement factor. There is also lack of simple technique of placing bacteria cell on SERS platform directly from the solution like water or blood. The highest signal enhancement factors are found for structures with sharp edges, intersections, periodical, and spanning the range of sizes from nanometers to single micrometers. Such structures guarantee a large number of 'hot spots', places where local electromagnetic field can be dramatically enhanced.[8] A large variety of size and structure can be provided by polymer nano- and micro-fibers 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, and was re-discovered in the 90s.[11] In this technique a polymer solution is pushed through a steel needle, whereas 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 electrodes, viscosity, surface tension, conductivity of polymer, feeding rate, and others.[12] For this reason we observe rise of 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 or/and 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 the way to assemble the gold nanorods into electrospun polymer fibers which exhibited potentially interesting SERS properties. However, no research was 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 SERS platform made of electrospun fibers; (ii) an extremely efficient method for 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 non-woven (PLLA-a) and woven (PLLA-b) mats. To prepare SERS platform we have cut polymer mats (area of 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 gold deposition and discussion about influence of polymer fibers on morphology of gold layer are described in 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 scientists working in the field of SERS, willing to use nanofibers in their experiments, but having 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 of placing bacteria on the platform. The technique is simple and allows 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 have 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 have tested it with *Escherichia coli* and *Staphylococcus aureus*. The EF obtained for *p*-MBA are on the level of $1.2 \times 10^4 - 3.6 \times 10^6$, which makes this method a promising way of preparing SERS platforms for laboratory practice, especially for detection and identification of bacteria. The platforms we developed are characterized by the following features:

i) they enhance the Raman signal up to 10^6 times,

ii) they can filter out bacteria from the liquid in which 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 weak bands of bacteria and at the same time allows to filter 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. SEM image of *E. coli* immobilized from urine on PLLA-b mat platform is shown in ESI (Fig. S1). On the basis of our previous experiments,[17] we conclude that such movement is a very serious problem in the 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 ESI (**Fig. S2**).

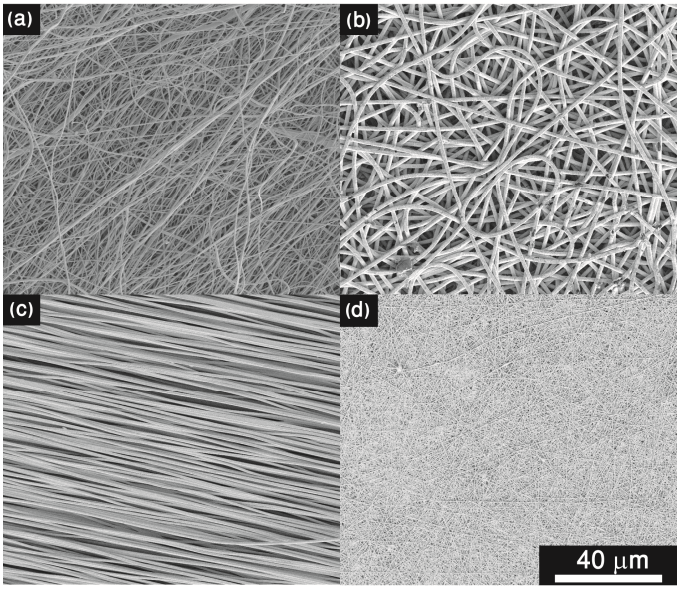

Fig. 1. SEM images of the polymer mats covered with 90 nm of gold, working as SERS platforms. We have tested four polymer mats: (a) PVDF, (b) PLLA-a, (c) PLLA-b and (d) nylon. The PLLA-a is a nonwoven mat, whereas PLLA-b is a woven mat. We present additional SEM pictures in ESI.

In order to test the deposition process we made EDX (Energy-dispersive X-ray spectroscopy) analysis (see ESI, **Fig. S3-S6**). We registered a strong gold signal and no signal from the polymer (PLLA, PVDF or nylon), concluding that the layer of gold on the fibers is uniform and all SERS signals come from molecules/pathogens localized on the surface of gold.

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 from tap water, urine and apple juice (see ESI, Fig. S7). In order to place the bacteria on the platform we used a setup presented in **Fig. 2**.

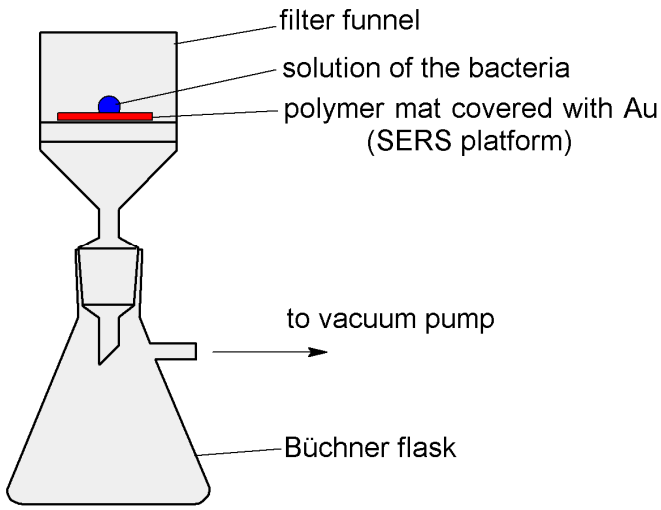

Fig. 2 The scheme of embedding bacteria on the SERS platform directly from the solution. The vacuum pump generates partial vacuum which creates flow of liquid from the suspended droplet with bacterial cells. The spaces between the polymer fibers are small enough to keep bacterial cells on the surface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

The set-up 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 considering preparation of bacteria solution). The platform was placed on a Büchner flask and then we placed on the platform a single droplet of solution. After switching on the pump the vacuum caused movement of the fluid through the polymer mat, whereas the bacteria remained on the surface. This could be repeated several times, the concentration of bacteria increasing after each step. This procedure was successfully used to trap bacteria from water and blood.

Fig. 3a shows 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 have chosen one platform (PLLA-b) to perform a detailed analysis and check the possibility of identification of bacteria from fluid.

Fig. 3b presents the spectrum of *E. coli* and *S. aureus* with a characteristic band vibration at about 730 cm^{-1}. Typical Raman bands of proteins, phospholipides, and polysacharides 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 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 DNA, respectively.[20] Close examination of the SERS

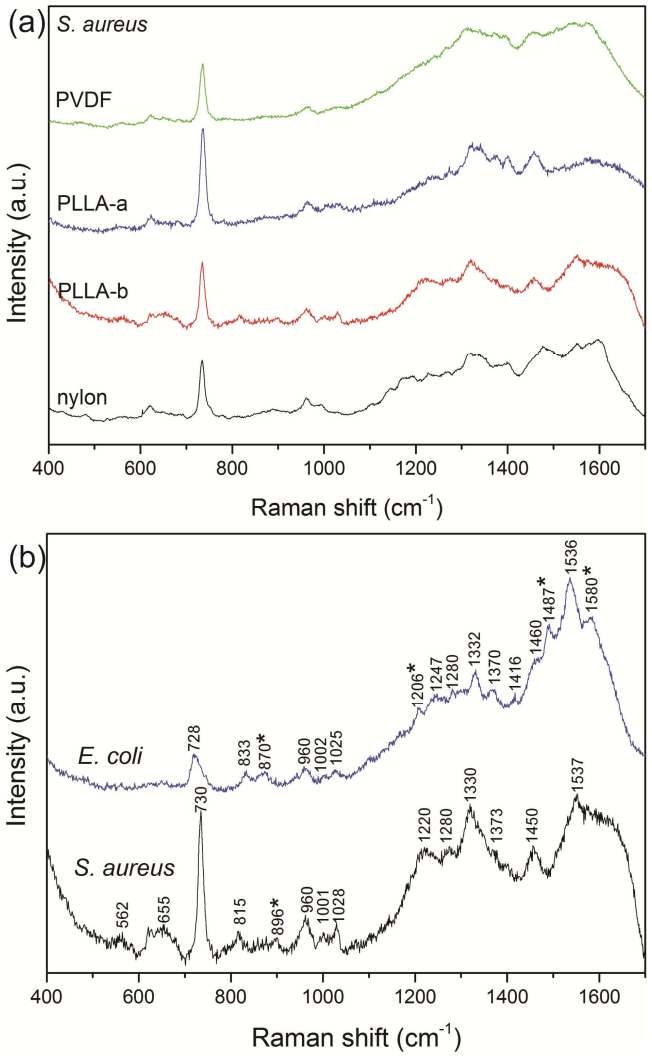

Fig. 3 (a) SERS spectra of *S. aureus* recorded from four Au-coated polymer mats: PVDF, PLLA-a, PLLA-b and nylon. (b) Raman spectra of *E. coli* and *S. aureus* collected from Au-coated PLLA-b (woven) polymer mat. The bands marked with an asterisk (*) were not assigned (they were not found in the literature).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

spectra of *E. coli* and *S. aureus* indicates also other common peaks 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 SERS spectra 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 *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 Raman signal plays a crucial role in practical application of SERS spectroscopy. Figure S8 in ESI shows 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 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 SERS signals recorded from different platforms prepared using the same method was also studied, and the achieved RSD was 22 %.

In summary, we demonstrated a fast and simple solution to crucial problems in SERS measurement of bacteria:
i) how to deposit bacteria directly from the solution on the SERS platform, and

ii) how to immobilize bacteria on the surface of the platform for the time of measurement.

We propose a novel method of using electrospun polymer mat covered with gold layer via Physical Vapor Deposition (PVD) method. In our procedure, the electrospun polymer mat has two functions: it acts as a filter and 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 identification and detection of *E. coli* and *S. aureus*. In our approach, we remove the step of transferring the bacteria from the filter to the SERS platform, as it is impractical and can cause contamination of the sample. 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 bacteria bands, which confirms its applicability in fundamental biological studies and medical diagnostics. The platform based on electrospun polymer mat, together with our novel procedure of deposition of bacteria, opens a new possibility for fast detection of bacteria in medicine, biology, forensics and environmental sciences.

Notes and references

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