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Polymer mat prepared via Forcespinning™ as a SERS platform for immobilization and detection of bacteria from blood plasma

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ABSTRACT

One of potential applications of nano- and microscale polymer fibers is SERS-active platforms for the detection of biological compounds and microorganisms. This paper demonstrates the polymer mat obtained with Forcespinning™ technique used to detect the bacteria from blood plasma. Forcespinning™ is a new method of manufacturing of polymer fibers which can be applied to variety of polymer materials, e.g. polyethylene, nylon, PA6 and others. The method is based on the centrifugal force to draw fiber from molten polymer, which allows tuning the diameter of the fiber from tens of nanometers up to micrometers. Wide range of diameters makes the forcespun polymer mat an excellent material to filter bacteria from fluids (e.g. blood plasma, water). Covering the mat with Au:Ag alloy turns it into a SERS platform able to immobilize, detect, and identify bacteria. We provide proof-of-concept, showing detection of *S. aureus*, *P. aeruginosa*, and *S. Typhimurium* from blood plasma.

1. Introduction

The surface-enhanced Raman scattering (SERS) was discovered 40 years ago and has now evolved into a mature analytical technique. This technique relies on amplification of the Raman signal, which is relatively weak, possibly via the formation of the charge-transfer complex between the analyte and the SERS substrate, but mainly through the electromagnetic interaction of light with metal which 'produces' surface plasmons, coherent delocalized electron oscillations across the surface [1]. Surface-enhanced Raman spectroscopy is a very sensitive method. The enhancement of Raman signal depends on many factors, including the type of metal and examined chemical compound, the frequency of incident light, effective Raman cross-section, and the structure of the SERS substrate. The SERS signal is highly dependent on the interaction between adsorbed molecules and the surface of nanostructures of gold, silver, or copper, as surface plasmons from small uniform particles have a resonance frequency at which they absorb or scatter light most efficiently. In general, gold and silver are most often used as SERS substrates because they are air-stable materials, while Cu is more reactive. However, all three metals have LSPRs (Localized Surface Plasmon Resonance) that cover most of the visible and near infrared wavelength range and therefore, they are suitable for use with the visible and NIR laser systems commonly utilized during Raman measurement.

The SERS technique has numerous applications. It may be used, e.g., to detect and identify chemical compounds (toxins, drugs) and biological substances (including DNA, viruses, bacteria, cancer cells), for examination of single molecules, or in art for pigment identification [2]. SERS is fast, sensitive, and specific. In addition, there is no need to use expensive reagents and labels or to employ qualified personnel to carry out the experiment. As a result, the measurement is not associated with additional costs. Moreover, there is a possibility to obtain a lot of spectra in a very short time through mapping function. Therefore, over the last ten years, the interest in SERS of bacteria has increased rapidly [3–7].

One of the obvious reasons of the rise of the popularity of SERS method is its exceptional sensibility. The second cause is the increasing number of infections in hospitals in recent years [8]. Therefore fast, simple and repeatable detection method of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella Typhimurium* is an important issue. The use of SERS in the detection and identification of bacteria gives the opportunity to detect a single bacteria in an analytical sample, such as clinical cerebrospinal fluid (CSF) or blood plasma.

Current methods of detection of bacteria are based on solid state SERS platforms [9–11] or involve colloids [12] or nanoparticles [13]. The main disadvantage of using solid state platforms is the need of previous filtration of bacteria from the matrix (e.g. blood, urine, water, saliva, etc.) and then moving the bacteria to the platform. This makes the procedure long, complicated and prone to contamination. The solution is a combination of filter and SERS platform. Due to small dimensions of the bacteria (ca. 1–5 μm in diameter) the dimensions of filter pores must be in nanoscale, with openings smaller than 1–5 μm (depending on the type of bacteria). One of the possible materials that

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can be used as a SERS-active platform are polymer mats which consist of polymer fibers with diameters below 1 μm.

Nanofibers are an important class of materials that found application in filtration [14], tissue engineering [15], protective clothing [16], battery separators [17,18] and energy storage [19], just to name a few. Nanofibers are usually made as non-woven or woven mats. In a non-woven mat the orientation of the fibers is random, whereas the woven one is aligned in one direction. For detection of bacteria both mats can be used, as shown in a publication of Szymborski et al. [20] The crucial parameter of the used mat are free spaces between fibers, which are smaller than the dimension of the bacteria. It allows to filter bacteria from fluids and to immobilize them on the mat surface to ensure repeatable and fast measurement of the SERS spectra.

Currently there exist several methods to produce woven and non-woven nano- and microfibers [21], such as electrospinning (ES), dry-spinning [22], wet-spinning [23] and dry-jet wet spinning. Among these, electrospinning [24] is the most common method to produce nanofibers [25]. Electrospinning process involves a syringe with a steel needle and a polymer solution inside, and a metal target. The solution is delivered at a constant rate through the syringe needle, whereas a high voltage DC (ca. 10 kV) is applied between the needle and the target. At a critical voltage electrostatic forces overcome the surface tension forces, causing a fluid jet to emerge from the needle toward a grounded collector. Polymer jet travels toward, and finally deposits on the metal collector. After leaving the needle the solvent evaporates, therefore solid polymer fibers are deposited on the collector. The ES method is well known and non-complicated, both in laboratory and on the industrial scale. However, it has also disadvantages, including:

i) the use of high voltage, ca. 10 kV,

ii) low fiber yield,

iii) it is limited to solvents with a certain range of the dielectric constant.

Therefore, other methods of production of nanosized fibers have been developed [21]. One of them is Forcespinning™ (FS).

The concept of Forcespinning™ technology was developed by Lozano et al. [26]. This method uses centrifugal force instead of the electric field to create the fibers. In FS, solid polymer or polymer solution is heated above the melting temperature and fed into a spinneret with multiple orifices. The spinneret rotates at high speeds (up to 20,000 rpm); therefore, it is creating a centrifugal force which drives the melted polymer via orifices. A jet of molten polymer is ejected from the orifice to the collector, when the centrifugal force (and hydrostatic pressure) is higher than capillary forces that tend to restrict the flow of the polymer in the orifice. The molten polymer solidifies into a nanosized polymer fibers (a non-woven mat) at the collector. The advantage of this method is that conductivity or dielectric constant of the material are not the relevant parameters for the solution properties. Therefore, the scope of possible materials which can be used to create nanofibers and non-woven mat is broader than in the ES method. To date, materials like cellulose [27], polyamide (PA) [28], polyethylene terephthalate (PET) [29], polyacrylonitrile (PAN) [30], polylactid acid (PLA), polycaprolactone (PCA) [31], polyethylene oxide (PEO) [32], polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) [33] have been successfully used to prepare nanofibers using the FS method. The wide range of variables (speed of rotation, temperature of the spinneret and the air inside the device, type of the polymer and its concentration, distance of the collector) make this method excellent for preparation of non-woven polymer mats with controllable parameters. Such polymer mats are, after sputtering with gold or silver, ideal materials for active SERS platforms, where one can filter, immobilize and detect bacteria.

Introducing the FS method to SERS substrates production allows the reduction of detection time, which is already very short compared to other detection methods (e.g., PCR). In the future it will allow fast detection of bacteria in large volumes of fluids without the need for prior bacteria culturing, purification, or DNA amplification.

In this paper we demonstrate the use of non-woven polymer mats manufactured via Forcespinning™ method as SERS platforms. To provide enhancement of the Raman signal we sputtered 90 nm layer of Au:Ag alloy. By applying the Ag:Au alloy we have combined the characteristic features of both metals: high chemical stability of Au with very high Raman scattering enhancement for Ag. The difference in the enhancement factor (EF) can reach 4 orders of magnitude for SERS measurements performed on platforms with pure Ag as compared to pure Au top layers [34].

Such a SERS-platform was used as a filter to separate microorganisms from blood plasma and immobilize them on the surface of the mat during measurements. Using our developed platforms we successfully detected and identified *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella Typhimurium*.

2. Materials and methods

Bacteria culture and SERS sample preparation: S. aureus, P. aeruginosa and S. Typhimurium used in the experiment were obtained from the Department of Bacterial Genetics, University of Warsaw, Poland. The whole procedure of the preparation of bacterial samples is presented in Fig. 1. To multiply microbial organisms, we cultured them in LB (Lysogeny broth) growth medium at 37 °C for 24 h in an incubator shaker (150 rpm). 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 5 min at 4000 rpm (in order not to destroy the cell membrane). The centrifugation process in the saline solution was repeated 3 times to obtain solution of clean bacterial cells. Purified bacteria were finally dispersed in saline solution and in EDTA human blood plasma to obtain a concentration of 10^3 CFU/mL of each solution. 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 24 h of cultivation at 37 °C on LB agar medium. The mixtures were next placed in the syringes and then by pressing the syringe plungers were placed over the SERS substrate. Measurements were taken after ~5 min.

Preparation of the SERS platform: we used commercially available nylon mats from FibeRio^R Technology Corporation (USA), prepared via Forcespinning™ method. The mat was covered with 90 nm of Au:Ag (50:50) alloy via physical vapour deposition (PVD) sputter coater (Leica, EM MED020) with a current of 20 mA for 20 min, which was an equivalent of 90 nm of the thickness. The same thickness of the layer was selected previously [20]. No adhesive layer (chromium or titanium) was sputtered on the polymer mat before sputtering Au:Ag alloy. The Au:Ag target was obtained from Mennica Metale Szlachetne, Warsaw, Poland. The size of the gold target was 54 mm in diameter, thickness of 0.5 mm; gold purity was 3N. After sputtering, the round shaped mats of ~10 mm in diameter were cut off and placed in a sterile Petri dish.

SEM measurements: of Forcespinning™ mat covered with Au:Ag alloy were done under high vacuum using the FEI Nova NanoSEM 450 with an accelerating voltage of 3 kV (Forcespinning™ mat before use as a SERS platform) or 2 kV for measurements of FS mat with *S. aureus* bacteria on the surface.

Filtering and immobilization of bacteria on the platform: the bacteria suspended in saline solution and blood plasma were placed in the syringes. At the end of every syringe we placed filter holders with Au:Ag FS nylon SERS platform. After pressing the plunger of the syringe, the pressure caused movement of the fluid through the nylon mat, whereas the bacteria remained on the surface. This could be done with any volume of a fluid, up to ~20 mL (a standard syringe volume).

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. The light from the laser passed through a line filter and was focused on a sample

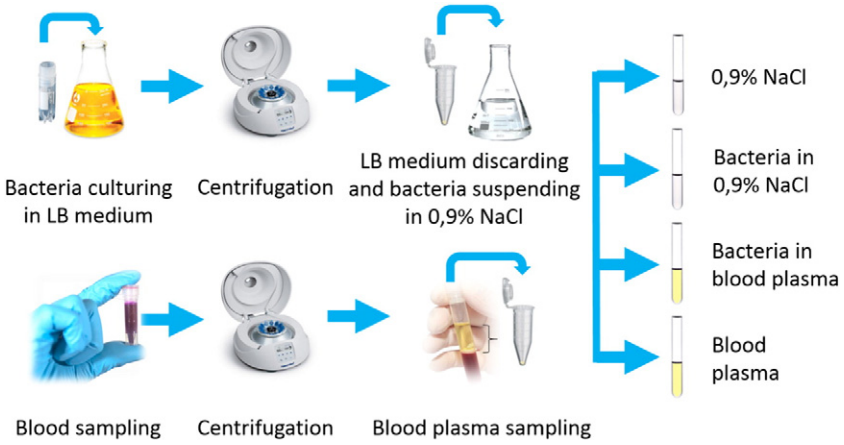

Fig. 1. The scheme presenting preparation of bacterial samples for SERS measurements.

mounted on an X–Y–Z translation stage with a 50× microscope objective, NA = 0.25. The microscope was equipped with 1200 grooves per mm grating, cutoff optical filters, and a 1024 × 256 pixel Peltier-cooled RenCam CCD detector, which allowed registering the Stokes part of the Raman spectra with 5–6 cm^{-1} spectral resolution and 2 cm^{-1} wavenumber accuracy. The beam diameter was approximately 5 μm and the power at the sample was 5 mW. The experiments were performed at ambient conditions using a back-scattering geometry.

The recording of the spectra was started immediately after placing, via syringe, the analyzed fluid onto a SERS-active surface. During the period of about 30 min, SERS spectra were repeatedly recorded, with the time of 60 s required for completing a single SERS spectrum. The obtained spectra were processed with the Wire3 software provided by Renishaw (Fig. 2).

3. Results

3.1. Morphology of Forcespinning™ mat via SEM measurements

The morphology of the Au:Ag FS nylon SERS substrates was analyzed by Scanning Electron Microscopy (SEM) (Fig. 3a–d).

The forcespun polymer mats consists of fibers with diameter of ca. 20 μm, and the latter consists of nanofibers of different cross-sectional diameters with the average diameter of 261 nm (see Fig. 3g for the histogram of the diameters of the nanofibers). The polymer mat works as a support for the nanofibers on the top, which makes our SERS platform durable and easy to work with, i.e., the platform does not deform under the influence of the fluid. The SERS enhancement factor is connected both with the fiber diameter and with the morphology of the Au:Ag nanostructures covering nylon fibers [20] (Fig. 3c). Because bacteria are bigger than the spaces between the nanofibers, they cannot pass through the mat and are retained in the mat cavities (Fig. 3e, f). This results in Au:Ag nanostructures surrounding bacterial cells and thus, in high Raman signal enhancement. Moreover, such immobilization of bacteria makes the measurement easy to perform, because bacteria cannot change their position during registration of the spectra.

3.2. SERS investigation of S. Typhimurium, P. aeruginosa and S. aureus

First we employed new Au:Ag FS nylon SERS platforms to demonstrate their ability to immobilize and enhance the Raman signal of three different bacteria species: *S. aureus*, *P. aeruginosa* and *S. Typhimurium*. The SERS spectra of these bacteria are shown in Fig. 4.

Bands at ca. 730, 782, 1034, 1100, 1330 and 1452 cm^{-1} can be observed in each spectrum. However for *P. aeruginosa* the peaks at 726 and 1330 cm^{-1}, assigned to the adenine part of flavin adenine dinucleotide (FAD) and to amide III (protein), respectively, are very weak in comparison with two other bacteria species. The band at ca. 782 cm^{-1} originates from cytosine or uracil, that at 1034 cm^{-1} most probably from C—C stretching in phospholipids, the band at 1104 cm^{-1} from C—O—C stretching, and the band at 1450 cm^{-1} from CH_2 deformation. Except from these bands each bacterial species shows its characteristic peaks. The SERS spectrum of *S. Typhimurium* (Fig. 4a) reveals several characteristic bands at ca. 650, 958, 1273 cm^{-1}assigned to guanine, C═C deformation and amide III (random), respectively. There is also a band at 1376 cm^{-1}. In case of *P. aeruginosa* (Fig. 4b) which has the most distinctive SERS spectrum in comparison with the other bacteria, except from the strong band at 675 cm^{-1}, assigned to guanine or tyrosine, we can also observe small peaks at 850, 885, 1205 and 1306 cm^{-1}. In case of *S. aureus* (Fig. 4c) showing very strong band at 730 cm^{-1}, we can also observe peaks at 956, 1244, and 1403 cm^{-1}. The last one can be linked to the COO^- symmetric stretching.

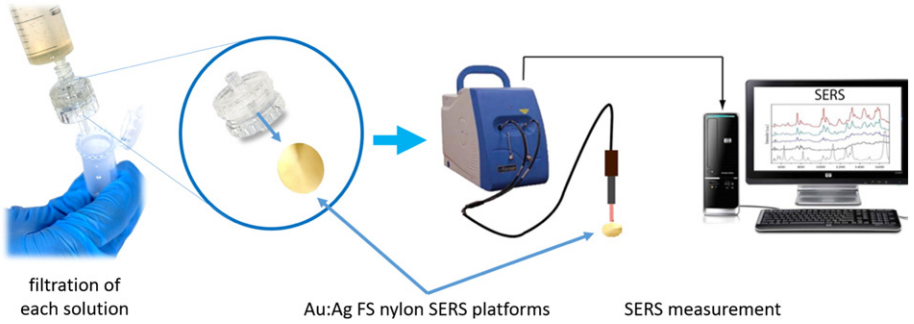

Fig. 2. The scheme presenting fast and simple preparation of a sample for SERS measurements. The syringe creates the flow of the liquid. The bacterial cells stay on the surface of the nylon mat, whereas the fluid passes through the polymer mat and filter funnel to the flask. The polymer is taken out and serves as a platform for SERS measurements.

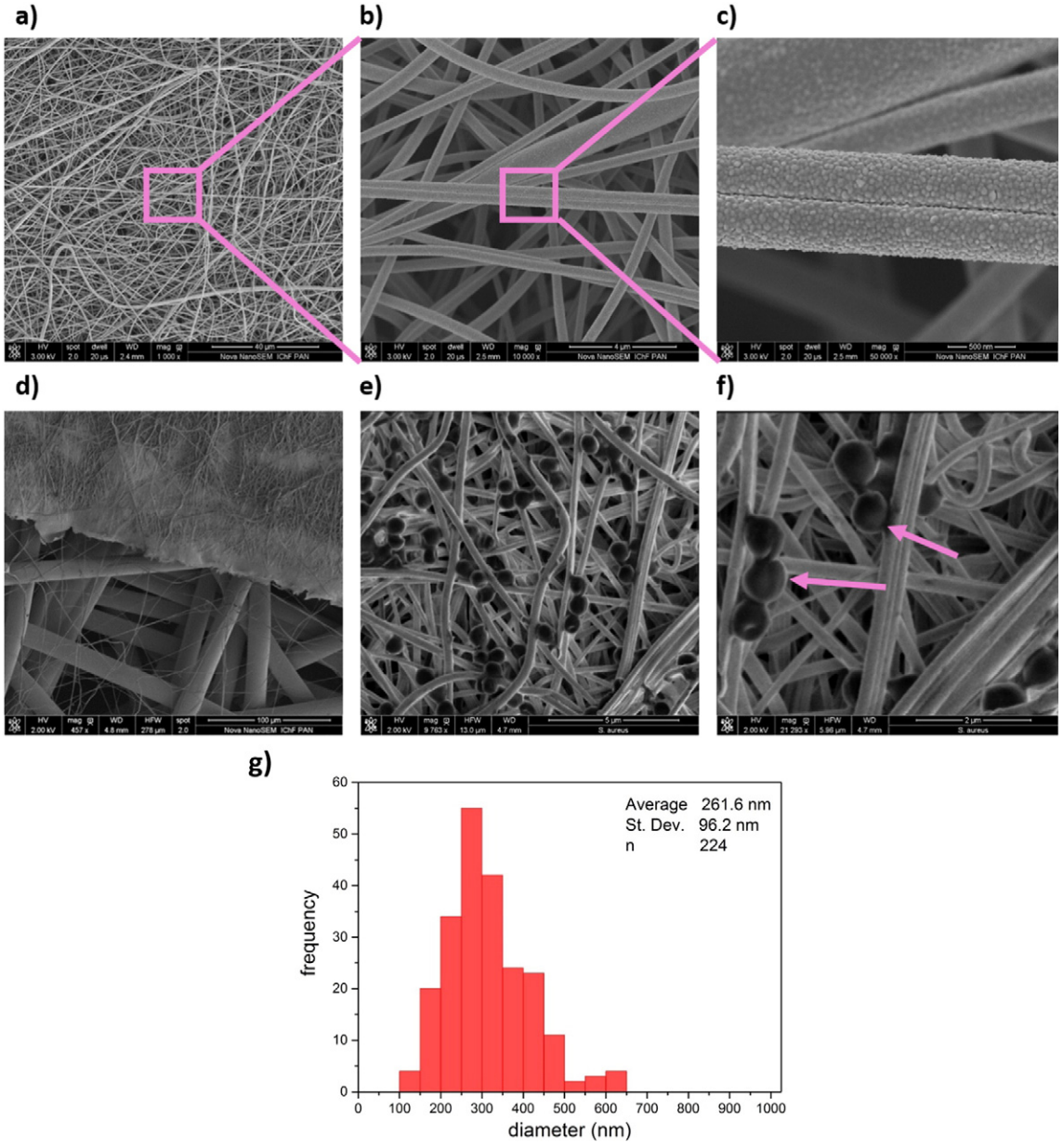

Fig. 3. SEM images of the nylon mats prepared via Forcespinning™ method, covered with 90 nm of Ag:Au alloy, working as SERS platforms: (a, b, c), morphology of the forcespun polymer mat at different magnifications, visible single polymer fibers covered with small nanostructures of Au:Ag alloy, (d) Au:Ag FS nylon SERS platforms consists of two layers: bottom which comprises fiber having diameter of micrometers, and top, which comprises nanofibers with a mean diameter of 261 nm, (e, f) *S. aureus* attached to and immobilized on Au:Ag FS nylon SERS platforms at different magnifications, and (g) histogram of diameters of nylon nanofibers on top of the forcespun polymer mat.

As can be noticed, each spectrum of the examined bacteria has its individual spectral fingerprint. The majority of the observed bands and their assignment are included in Table S1.

Additionally, the reproducibility of the SERS signals of *S. aureus*, *P. aeruginosa* and *S. Typhimurium* were calculated and presented in Supplementary materials. Fig. S2 shows the example of SERS spectra of *S. Typhimurium* recorded from different spots within the same sample. To obtain statistically valid results, the strong signal at 730 cm^{-1} and a weak peak at 958 cm^{-1} were chosen to calculate the relative standard deviation (RSD). The RSD of the intensity of these vibrations in the 30 SERS spectra recorded on the same platform are 8% and 12%, respectively. The reproducibility of the SERS signals of *S. Typhimurium* recorded from different platforms prepared using the same method was also studied, and the achieved RSD was 14%.

3.3. Detection of *S. aureus*, *P. aeruginosa* and *S. Typhimurium* from fluids

In this study, the possibility of the detection and identification of studied bacteria: *S. aureus*, *P. aeruginosa* and *S. Typhimurium* from saline solution and human blood plasma samples using developed Au:Ag FS nylon SERS substrates has been also demonstrated.

Fig. 5 depicts the recorded SERS spectra of *S. aureus* bacterial cells filtered from EDTA human blood plasma and from saline solution.

It can be easily noticed that the saline solution does not give characteristic SERS spectrum (Fig. 5a). In turn, EDTA human blood plasma (Fig. 5b) reveals few weak bands mainly at ca. 647, 723 1000, 1122, 1353, 1451 and 1550 cm^{-1}. Based on literature [35], the sharp bands due to aromatic amino acids at 1000 cm^{-1} can be assigned to phenylalanine, the band at 1122 cm^{-1} to tyrosine, and at 1450 cm^{-1} to CH_2

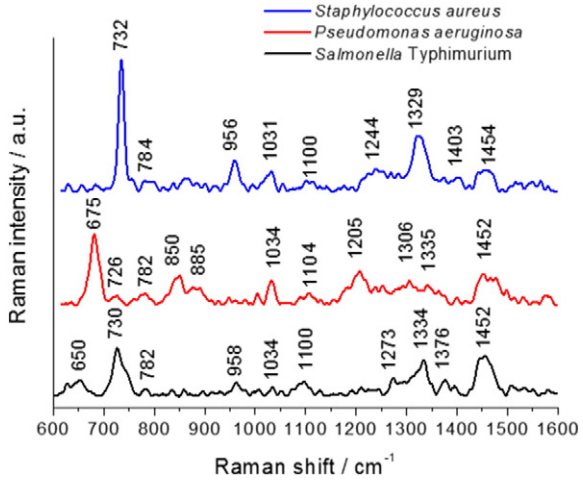

Fig. 4. Average SERS spectra of *S. aureus*, *P. aeruginosa* and *S. Typhimurium* filtered from saline solution and recorded on Au:Ag FS nylon SERS platforms. All the used polymer mats were covered with 90 nm of gold via PVD method. For all spectra, excitation wavelength was at 785 nm, laser power was 5 mW, and acquisition time was 60 s. Each SERS spectrum of examined bacteria: *S. aureus*, *P. aeruginosa* and *S. Typhimurium* was averaged from 30 measurements in different places across the SERS surface using mapping mode.

deformation. The peak at 1550 cm^{-1} can be linked to amide II. The majority of these peaks, namely 1000, 1122, 1353, 1451 and 1550 cm^{-1}, give their contribution to SERS spectrum of *S. aureus* filtered from blood plasma (Fig. 5d). However, the spectrum of bacteria is so strong compared to EDTA human blood plasma spectrum that bacteria can be easily detected and identified in the surrounding of blood plasma without the need of any previous purification. Even in the case of filtering bacteria from liquids giving strong bands in the SERS spectrum there is no problem with flushing Au:Ag FS nylon SERS platforms containing attached bacterial cells by using a clean syringe with a portion of pure saline solution. Similarly, as exemplified by Fig. S3, we were able to detect and identify also *P. aeruginosa* and *S. Typhimurium*, immobilized on Au:Ag FS nylon SERS platforms from EDTA human blood plasma. The spectra depicted in Fig. S3 exhibit the spectral features (marked with an asterisk - *) characteristic for these particular bacterial species (see Fig. 4).

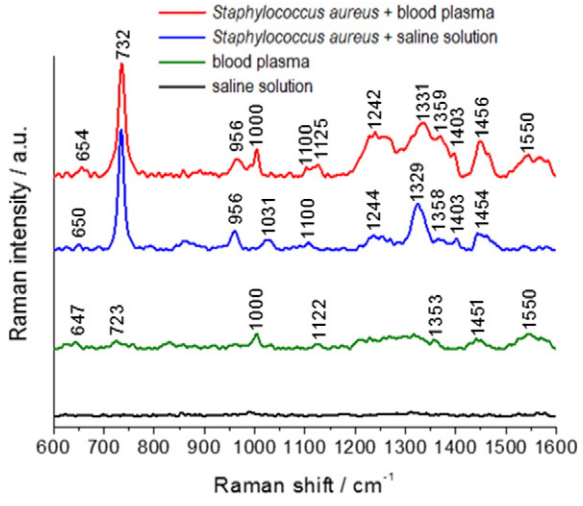

Fig. 5. SERS spectra of a) saline solution (0.9% NaCl) (control 1), b) blood plasma (control 2), c) *S. aureus* mixed with saline solution, and d) *S. aureus* mixed with blood plasma, recorded on Au:Ag FS nylon SERS platforms. All the used polymer mats were covered with 90 nm of gold-silver alloy via PVD method. For all spectra, excitation wavelength was at 785 nm, laser power was 5 mW, and acquisition time was 60 s. Each SERS spectrum was averaged from 30 measurements in different places across the SERS surface using mapping mode.

4. Conclusions

We have developed and tested a SERS-active platform based on a polymer nanofiber mat made via Forcespinning™ method. The advantages of using forcespun mats as SERS platforms are as follows:

i) The mat consists of two layers: bottom, working as a support and top, working as SERS-active layer. This layered structure is mechanically stable and durable during measurements.

ii) Contrary to other methods like electrospinning, Forcespinng™ can be applied to a wide range of polymers and other materials (i.e., ceramics), creating new opportunities for SERS platforms.

iii) The top nanosized layer of fibers can be easily tuned, thus allowing to efficiently filter bacteria from any fluid (blood, urine, water).

We have combined our SERS platform with previously developed method of filtration and immobilization of bacteria on polymer mats using underpressure [20]. We provide a proof-of-concept, showing detection of *S. aureus*, *P. aeruginosa* and *S. Typhimurium* from blood plasma. Simplicity, versatility and low cost of our SERS-active polymer mat make it a promising and attractive tool for scientists involved in detection and identification of bacteria species, especially from medical samples.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.msec.2016.10.027.

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