Research Article

Microfluidic device for concentration and SERS-based detection of bacteria in drinking water

There is a constant need for the development of easy-to-operate systems for the rapid and unambiguous identification of bacterial pathogens in drinking water without the requirement for time-consuming culture processes. In this study, we present a disposable and low-cost lab-on-a-chip device utilizing a nanoporous membrane, which connects two stacked perpendicular microfluidic channels. Whereas one of the channels supplies the sample, the second one attracts it by potential-driven forces. Surface-enhanced Raman spectrometry (SERS) is employed as a reliable detection method for bacteria identification. To gain the effect of surface enhancement, silver nanoparticles were added to the sample. The pores of the membrane act as a filter trapping the bodies of microorganisms as well as clusters of nanoparticles creating suitable conditions for sensitive SERS detection. Therein, we focused on the construction and characterization of the device performance. To demonstrate the functionality of the microfluidic chip, we analyzed common pathogens (Escherichia coli DH5α and Pseudomonas taiwanensis VLB120) from spiked tap water using the optimized experimental parameters. The obtained results confirmed our system to be promising for the construction of a disposable optical platform for reliable and rapid pathogen detection which couples their electrokinetic concentration on the integrated nanoporous membrane with SERS detection.

Keywords:
Bacteria / Drinking water / Microfluidics / Porous membrane / Surface-enhanced Raman spectroscopy

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Raman spectrometry belongs to the most powerful methods for unambiguous identification of analytes. It monitors energy changes in inelastically scattered light, which carries unique information about rotational-vibrational states of the investigated molecule. Surface-enhanced Raman spectrometry (SERS) deals with the so-called surface enhancement effect, which significantly boosts the sensitivity potential of this method [1]. The essence of the enhancement phenomenon lies in the application of nanostructured metal (typically silver or gold) to the sample. Excited metal electrons collectively oscillate and create an electromagnetic field intensifying the Raman signal, usually by factor $10^6$–$10^8$. It should be stressed that the intensity of the field is highly distance-dependent and that for the maximal enhancement effect, the proximity of an analyte and nanostructure is crucial [2].

SERS indicates itself as a mature method for a wide spectrum of analytes ranging from low-molecular structures to very complex biological samples (e.g. cells or microorganisms) [3]. From the 1990s, the special interest in the field of SERS analysis of microorganisms is paid particularly to the analysis of bacteria [4]. Such Raman experiments usually follow two possible strategies nicely reviewed by Liu et al. [5]. The first, label-based approach typically deals with nanostructures with attached recognition elements (e.g. antibodies or aptamers) and SERS tags (i.e. strong Raman scatterers), which allow obtaining excellent LODs [6,7]. These measurements rely on a selective reaction between the labeled nanostructure and the target bacteria. The second,
label-free approach avoids any labeling and collects spectra directly from bacteria. For the surface enhancement effect, the bacteria are deposited on the SERS-active substrate [8], or nanoparticles are introduced to the sample by mixing [9], in-situ synthesis [10], or deposition on bacteria [11]. Although this strategy usually does not reach the sensitivity of the label-based approach, it is not limited by available antibodies and provides the real spectra of bacteria being a key to their detailed characterization [9,12,13].

Despite the remarkable potential of SERS for bacteria identification, an absence of robust and portable devices still challenges their application for in-field analysis. Such a device should integrate the ability for bacteria concentration particularly. Currently used set-ups mostly utilize pressure-assisted filtration [7,14,15], discharge-induced flow [16,17], and solvent evaporation [18] for this purpose. Unfortunately, all of these techniques require very sophisticated technologies or external apparatuses hardly compatible with a portable device. Recently, very promising achievements were obtained in microfluidic devices trapping bacteria by the dielectrophoretic principle. For example, Lin et al. used the nonuniform electric fields for trapping and SERS analysis of a single bacterium [6]. Cheng et al. developed a circular dielectrophoretic microfluidic device concentrating pathogen bacteria from human blood in less than 1 min [19].

The integration of nanoporous membranes in microfluidic devices has proven to be a fast and simple way for the preconcentration of peptides [20–22], DNA [23], fluorescent probe molecules [24,25], or microorganisms [18,26] within a single platform. Although a few membrane-based microfluidic devices have already demonstrated high sensitivities in the determination of biological relevant organisms [26], these approaches do not include on-chip detection strategies of target pathogens or the use of drinking water samples [18]. The contamination of sources of drinking water can result in many health problems including cramps, nausea, diarrhea, and strong headaches. Therefore, there is an urgent need for the development of a widely applicable detection system providing reproducible and selective results in a short-time scale.

As a result, our approach focused on the development of a three-dimensional microfluidic device with an incorporated nanoporous membrane as a tool for rapid and automated sample-in-answer-out analysis of microorganisms in water. The nanoporous membrane serves as concentration as well as on-chip SERS detection area due to the electrodration trapping of silver nanoparticles (AgNPs) and bacteria.

Herein, we report on a simple and disposable PDMS device for the concentration of bacteria from drinking tap water, which function combines filtration with the electrodridev flow. The device is composed of two perpendicular channels attached via a nanoporous polycarbonate track-etched membrane with a pore diameter of 200 nm. While one of the channels is held under a positive voltage and supplies the mixture of bacteria and nanoparticles, the second one is grounded. This creates a strong EOF dragging bacteria and silver clusters toward the membrane, where they are sterically trapped, thus, concentrated. The function of this device is very straightforward and claims neither bulky instrumentation nor complicated antibody/chemical immobilization. This, together with user-friendly size (2.5 × 2.5 cm), is a very promising prerequisite for the construction of a robust and portable device for rapid sample-in-answer-out analysis of bacteria from water samples. In this work, we focus on the optimization of the presented system using Saccharomyces cerevisiae. We prove its functionality on the concentration and SERS analysis of Pseudomonas taiwanensis VLB120 and Escherichia coli DH5α from spiked tap water. To the best of our knowledge, this approach represents the first reported three-dimensional low-cost microfluidic device, which utilizes a porous membrane for concentration as well as for the detection of pathogen microorganisms in drinking water.

2 Material and methods

2.1 Preparation of phosphate buffer solution

PBS tablets were received from Sigma-Aldrich (Germany) and used to prepare the buffered solutions. One tablet was dissolved in 200 mL of deionized water (18.2 MΩ/cm) obtained from a Smart2Pure purification system (Hartmann, Germany), yielding 10 mM phosphate buffer with 2.7 mM potassium chloride and 137 mM sodium chloride, pH 7.4 at 25°C.

2.2 Preparation of yeast samples

Dry yeast cells S. cerevisiae (Ruf, Germany) purchased at a local supermarket (Leipzig, Germany) were used for the initial optimization of the developed device. The yeast samples were prepared by dispersing 7 mg/mL of the dry yeast in PBS and vortexed to gain a homogeneous mixture without clusters of cells.

2.3 Preparation of bacteria samples

Escherichia coli DH5α, P. taiwanensis VLB120, and a constitutively fluorescent P. taiwanensis VLB120_eGFP were investigated in this study as potential bacterial drinking water contaminants [27]. For precultures, 5 mL of Lysogeny broth (LB) medium were inoculated with a single colony from an LB-medium agar plate using a sterile inoculation loop [28]. The precultures were grown overnight at 180 rpm (2.5 cm amplitude) in a Multiron Pro shaker (Infors, Switzerland). Pseudomonas taiwanensis VLB120 and P. taiwanensis VLB120_eGFP were cultivated at 30°C. Escherichia coli DH5α was cultivated at 37°C. A total of 250 μL of the respective precultures were used to inoculate 25 mL of M9 mineral medium supplemented with 2 g/L glucose (Sigma-Aldrich) and 0.01 mg/L thiamine (Sigma-Aldrich) [28]. Cell concentrations were determined via cell counting, with a Coulter...
counter (Beckman Coulter, USA). The measured concentration of E. coli DH5α was 247 × 10⁶ cells/mL, whereas the concentration of the P. taiwanensis VLB120 was determined to be 38 × 10⁶ cells/mL. After 12 h of incubation in baffled shake flasks under identical conditions as the precultures, the cell suspensions were harvested. M9 medium was removed by repeated centrifuging (5000 g, RT, 5 min) and washing the cells three times with tap water. The washed cells were collected in sterile plastic tubes for transfer. Prior to the experiments on the chip, both bacterial samples were diluted 1:9 v/v with tap water. For analysis, 5 μL of the washed bacteria (or yeast cells dispersion) was added to 5 μL of the silver colloidal suspension and thoroughly vortexed to yield a homogenous mixture.

### 2.4 Preparation of the silver colloidal suspension

AgNPs were synthesized by a standard Lee–Meisel protocol [29] based on the reduction of silver nitrate (Sigma Aldrich, USA) by sodium citrate dihydrate (Lachema, Czech Republic). The details about its synthesis and basic characterization are given in Supporting Information (Fig. S1). For the concentration of the colloidal suspension, a required portion of supernatant was removed after its centrifugation (10 000 rpm for 10 min) using a Galaxy 14D centrifuge (VWR, USA).

### 2.5 Scanning electron microscopy

For the characterization of the silver colloid as well as for the investigation of the cell-colloid interaction, SEM (Mira3, Tescan, Czech Republic) was used. These cells represent relatively large nonconductive areas, which can result in unwanted charging of the sample. Therefore, samples were sputtered with a layer of gold (15 nm) to create a conductive surface on the sample.

### 2.6 Fluorescence measurement and image processing

To optimize the performance for on-chip concentration, the process of the trapping of the constitutively green fluorescent protein (GFP)-synthesizing P. taiwanensis VLB120 eGFP on the membrane was observed using an inverse epifluorescence microscope (IX-71, Olympus, Germany) equipped with a high-pressure mercury-vapor lamp (HBO 103 W/2, Osram, Germany) and a filter cube for excitation and detection of GFP (U-MWIB, Olympus). A sequence of images was recorded by a 16-bit charged-coupled device camera (PCO, Germany) with an integration time of 1 s, controlled by open-source microscopy software (Micro-Manager, US National Institutes of Health, USA). Image processing was performed using ImageJ (National Institute of Health, USA). To compare the concentration efficiency depending on different voltages, a region of interest was defined by the channel cross-section with an area of 100 × 100 μm, and the mean intensity was calculated in this area for every recorded image.

### 2.7 Surface-enhanced Raman spectrometry measurements

SERS spectra were measured with a confocal Raman system (S&I Spectroscopy & Imaging GmbH, Germany). The Raman set-up was equipped with an epifluorescence microscope (Olympus) and 633 nm HeNe laser (CVI Melles Griot, USA) as an excitation light source. The laser was focused by a 40 x objective (Olympus) on the channel cross-section of the microfluidic device. The laser irradiation power was 10 mW with 5 s integration time and three spectral accumulations. The scattered light passed an Acton SP2750 monochromator (Princeton Instruments, Acton & Trenton, USA) with an entrance slit of 150 μm and a grating of 600 lines/mm and was detected via a ProEM® 1600 × 200 CCD camera (Princeton Instruments, USA) which was thermoelectrically cooled to −78°C. All measurements were controlled and recorded via VistaControl (S&I Spectroscopy & Imaging GmbH). The Raman spectra are plotted with the γ-axis of arbitrary units. For better clarity, the luminescent background was subtracted in all presented SERS records.

### 2.8 Device fabrication and operation

The three-dimensional concentration device consists of two perpendicularly arranged channels sandwiching a nanoporous polycarbonate track-etched (PCTE) membrane with a pore size of 0.2 μm (Pieper Filter, Germany). As most bacterial cells are in the range of a few micrometers, a membrane with a pore size of 0.2 μm was chosen as it allows fluidic transfer, whereas cells are retained. The microfluidic channels were fabricated in PDMS, Sylgard 184, Dow Corning, USA) according to established rapid prototyping and soft lithography processes which were described previously [30].

Briefly, a replica master was prepared by structuring a 60 μm thick layer of the negative photoresist SU-8 2050 (MicroChem, USA) on a clean 4-inch silicon wafer by common photolithography. A 10:1 w/w mixture of PDMS prepolymer and curing agent was degassed, poured over the SU-8 master, and cured at 80°C for at least 2 h to form upper and lower PDMS layers individually. The cured PDMS was peeled off, and the layers containing the 100 μm broad channels were separated using a razor blade. Fluidic access holes, which serve as reservoirs for sample and buffer solutions were punched on the upper PDMS layer using a stainless-steel tube (3 mm diameter). To assemble the two PDMS layers, both monoliths were treated with an oxygen plasma (Diener electronic, Germany, 100 W, 500 mTorr, 30 s) making the complementary PDMS surfaces reactive to one another.

Right after plasma treatment, 2 × 2 mm sized PCTE membrane was placed at the intersection of the crossed microfluidic channels before alignment. Both PDMS layers
were then arranged in the perpendicular geometry sandwiching the nanoporous membrane. The assembled device was immediately transferred to a hotplate and heated to 65°C for 15 min. Additionally, 1.5 kg weight was placed on the chip while heating to further support the bonding process between the nanoporous membrane and PDMS layers and to prevent the formation of gaps between the PDMS-membrane interface. With the thermal treatment, as well as the pressure on the chip, a leakage-free sealing was achieved.

Finally, the bottom of the PDMS device was bonded on a microscopic glass slide (Carl Roth, Germany) by oxygen plasma treatment to enhance the mechanical stability and facilitate to mount the chip on a microscope.

The operating principle and the design of the three-dimensional system are illustrated in Fig. 1 with an inset showing a photograph of the microfluidic chip with the integrated nanoporous membrane and electrical connections for electrokinetic transport on a fluorescence microscope. The device consists of a sample channel (lower position) and an extraction channel (upper position) with a hydrophilic porous membrane sandwiched in between. The sample channel supplies the bacteria sample mixed with the AgNPs, whereas the upper channel contains PBS. The term extraction channel is used to express the direction of the sample flow within the device.

After both channels were filled with the sample or buffer solution, the fluidic reservoirs were interfaced with platinum electrodes and connected to an eight-channel high voltage sequencer (HVS448-6000D LabSmith, USA) to generate and monitor the DC voltage across the device. The sample channel was held under a positive potential of 300 V, whereas the extraction channel was grounded. The SERS spectrum was collected from the bottom of the chip, which minimizes the potential risk of spectral interferences originating from the polycarbonate membrane.

Since PDMS as well as polycarbonate exhibits a negative zeta-potential, the application of a potential gradient across the three-dimensional system generated a cathodic EOF [31,32], dragging all the presented entities through the nanoporous membrane being an interface of both channels. Therein, the components larger than 200 nm, that is, bacteria and AgNPs clusters, were continuously trapped and enriched. If not stated otherwise, the process of the enrichment was stopped after 1 min. The beam of 633 nm HeNe laser was focused through the bottom side of the microfluidic chip on the PCTE membrane to collect the SERS spectra of the sample.

3 Results and discussion

3.1 Optimization of silver nanoparticles concentration

SERS has proven to be a powerful analytical tool for the rapid identification of chemicals, biomolecules, or microorganisms, due to its high sensitivity, inherent spectral fingerprint information, and compatibility to aqueous media. As our work focuses on the development of a low-cost, disposable device with the potential for rapid sample-in-answer-out analysis, a colloidal solution of AgNPs with an approximate size range of 60–70 nm was used, synthesized according to the Lee–Meisel protocol [29]. The crucial aspect of the high sensitivity of SERS analyses is the proximity of an analyte and the nanostructured noble metal surface. In contrast to cost-intense and sophisticated solid-state SERS substrates, like a
Figure 2. (A) Influence of silver colloid concentration on the SERS signal of yeast cells. For better clarity, the offset was used. The inserted window depicts the correlation of silver colloid concentration and the SERS intensity of the 909 cm$^{-1}$ band of the measured yeast spectra. The error bars represent standard deviation ($n = 3$). (B) SEM figure of the dried mixture of Saccharomyces cerevisiae with silver colloid (2 mg/mL).

nanostructured surface (e.g. sputtered membrane), where the analyzed cells would be in the contact with the enhancing metal only on their “sitting” side, the aggregates of nanoparticles can surround the cell and thus, enhance the signal more efficiently.

Thus, at the beginning of our work, we focused on the influence of the AgNPs concentration on the quality of the SERS signal. For these experiments, we employed yeast cells (S. cerevisiae) being widely accepted as a model microorganism. Yeast cells provide a similar size and biochemical composition to bacteria as their cell walls are created particularly from peptidoglycans and carbohydrates [33,34]. This composition creates equivalent systems for SERS analysis as well as similar electromigration properties. The SERS spectra of bacteria and yeast also show some similarities, as their dominant peaks can be addressed to biomolecules like adenine and guanine that were identified as the metabolites of purine degradation [35,36]; thus, the established workflow can be easily transferred to the target type of the sample.

AgNPs dispersions with the concentration of silver ranging from 0.1–4.0 mg/mL were prepared and mixed with the yeast sample suspended in PBS in the volume ratio of 1:1. The mixture was vortexed for 30 s, dropped onto a glass slide, covered with a coverslip, and characterized by SERS. Since the sample concentration on the porous membrane will be conducted at the same chemical composition, it should be stressed, that this experiment imitates the on-chip conditions.

Figure 2A summarizes the obtained SERS spectra and nicely demonstrates that the SERS bands become more prominent, as the AgNPs concentration increases. However, the systematic evaluation of the data showed (see insert in Fig. 2A) that after overcoming the level of 1 mg/mL no further improvement of the spectra was reached. On the contrary, the intensity of the SERS signal provided significantly higher RSD values ranging from 5% up to 26%. This trend nicely matches observations published elsewhere [37].

The surface enhancement can be achieved only if the bacteria-AgNPs distance is negligible. We assume that in our system the surface enhancement effect can be caused by two various aspects. One of them is related to the spontaneous adhesion of AgNPs/AgNPs clusters on the cell surface (driven by its biochemical composition) [33,38–40], whereas the other one relies on the formation of AgNPs clusters, which are located in the proximity of bacteria bodies. Thus, one can deduce that the observed higher RSD values could have two possible explanations: (1) the cell walls were fully saturated by AgNPs at a concentration of 1 mg/mL or (2) the high level of nanoparticle concentration provoked their aggregation with unrepeatable geometry of AgNPs clusters.

We used SEM microscopy to further investigate the interaction processes of yeast cells with AgNPs and, thus, reveals the potential source of the increased RSD value. Figure 2B shows the dried mixture of yeast cells with silver colloid (2 mg/mL). Despite the fact, that one can see nanoparticle clusters on several places of the yeast surfaces, the overall distribution of the nanoparticles suggests that the formation of clusters aside from the cell bodies is probably the more contributing phenomenon and that the nanoparticles do not interact specifically with the cell walls. Besides, it is difficult to control the immobilization of particles on the bacteria surface. We assume that most of the nanoparticle aggregates got
on the surface of the cells during the drying process, which is a needed step before SEM analysis. For more figures, see Supporting Information (Fig. S2). Based on these observations, we believe that only a minor fraction of nanoparticles will spontaneously adhere to the cell surface and travel along the channel as one entity. The majority of AgNPs form relatively large aggregates due to the ionic strength of the sample matrix and are trapped as clusters on the pores of the membrane.

Since the formation of clusters appears to be critical for the functionality of our device, we investigated the potential of our target matrix, that is, tap water, to initiate aggregate formation. Using molecular absorption spectrometry, demonstrated that this environment acts on a very similar basis as PBS buffer (see Supporting information Figs. S3 and S4) and that the nanoparticles aggregate in the time scale of minutes. The process of aggregation severely changes the shape and size of nanoparticles, due to the formation of AgNP clusters. As this would happen for colloids regardless of the dimensions of the nanoparticles, no further examination of the particle size effect was undertaken.

Based on these observations, we conducted SERS analysis of the mixture of 1.0 mg/mL AgNPs and two types of bacteria: E. coli DH5α and P. taiwanensis VLB120 (124 × 10⁶ and 19 × 10⁶ cells/mL, respectively) both being recognized as common bacterial water contaminants. This preliminary testing of our system provided very nicely resolved spectra of high intensity shown in Supporting information Fig. S6. The obtained spectrum of E. coli DH5α was in excellent agreement with the literature [10,41–43]. To the best of our knowledge, this is the first time, when the SERS spectrum of P. taiwanensis VLB120 is reported. These results confirmed the suitability of the chosen concentration level of AgNPs in the system.

### 3.2 Electrokinetic trapping of bacteria

As the concentration of pathogen microorganisms in drinking water is low (0–10⁷ CFU/mL) [44], an efficient enrichment step is required to obtain a detectable signal. Capturing bacteria on porous structures has been reported as an effective way to concentrate microorganisms on a surface [7,45,46]. Even though these methods showed good sensitivities, all of them were time consuming (>60 min) as sample manipulation and preconcentration was carried out off-chip. Also, these approaches did not provide any spectral fingerprint information [46] or were limited to the indirect detection of an indicator molecule [45].

In this work, we sandwiched a membrane with a pore size of 200 nm between two microfluidic PDMS channels. At suitable potential conditions, this results in mass transport between these channels and concentration of structures (larger than 200 nm) on the membrane surface at the same time.

During the experiments, ions can penetrate from the PBS filled extraction channel to the sample channel which is filled with tap water by diffusion and electromigration. As previously described, diffusion through the membrane is negligible (concerning the time scale of the whole experiment) and does not interfere with the measurement [30,32]. During the application of voltage, cations travel back to the extraction channel, whereas anions migrate towards the sample channel. The presence of these ions can influence the stability of nanoparticles. Nevertheless, the nanoparticles are already in an aggregated state (Supporting information Fig. S3) and the duration of the experiment (<2 min) does not represent a significant time scale for dramatic changes in the aggregate geometry. Therefore, the probable presence of ions from the PBS buffer is assumed to have a minor effect on the analysis.

To characterize and precisely monitor the enrichment process of bacteria within the microfluidic device, fluorescence observation of a GFP expressing P. taiwanensis VLB120_eGFP strain was carried out. This strain carried a genome integrated gene that coded for eGFP. The change of fluorescence intensity in the cross-section of the microfluidic channels was followed in the time scale of 50 s and gave insight into the cell-concentration efficiency of the membrane.

As one of the most important factors, the effect of the potential gradient was systematically evaluated by the application of different external voltages. A positive potential was applied to the liquid reservoirs of the sample channel (100–300 V), while the extraction channel was grounded. In these experiments, the extraction channel was filled with PBS, while the sample channel was filled with tap water (pH 6.8, 665 μS/cm). Before the application of voltage, 10 μL of a sample mixed with AgNPs was pipetted in each of the sample channel reservoirs. In our future work, we will focus on the extension of the analyzed sample volume, herein, we stuck to the volume of 10 μL, which enabled us to optimize the performance of the device.

The data in Fig. 3A demonstrate the correlation between the applied voltage and the fluorescence intensity in time,

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**Figure 3.** Concentration of P. taiwanensis VLB120_eGFP on the membrane of the microfluidic device. (A) Voltage-dependent concentration efficiency expressed as the intensity of fluorescence over time. (B) Photographs showing the process of electrokinetic concentration on the channel cross-section of the microfluidic device.
indicating that a higher potential gradient increases the speed of concentration. As the membrane is continuously covered by the bacteria, the fluorescence signal increases. However, the increase in the signal intensity weakened over time. This is particularly apparent for the application of 300 V, where the saturation of the fluorescence signal was reached in approximately 50 s. The electric current resulting from the application of 300 V to the sample channel reservoirs was measured to be 140 μA. Due to the migration of ions in the system this current dropped throughout the analysis down to 95 μA. This measurement confirmed that the application of 300 V, led to an efficient enrichment of bacteria on the nanoporous membrane. A further increase of the applied voltages resulted in the formation of bubbles at the electrodes and very unstable performance.

At the application of 300 V, a set of photographs of the channel cross-section was taken (Fig. 3B). As can be seen, the fluorescent bacteria become visibly accumulated at the channel intersection (0.01 mm²) within less than 15 s. While this proves effective concentration of bacteria cells, which cannot overcome the pores, it also opens the possibility of effective sample purification from entities smaller than 200 nm (e.g. unbound/unclustered AgNPs or cell fragments), which can pass to the extraction channel.

### 3.3 On-chip SERS detection of spiked samples

After the optimization of operational values, the system was applied to the on-chip SERS analysis of tap water spiked with bacteria. Membrane-based microfluidic systems utilizing electrokinetic-driven sample manipulation allow a fast and automated concentration of bacterial species in a miniaturized device. To prove the feasibility of the device for sample-in-answer-out SERS analyses, we used clinically relevant bacteria strains, namely *E. coli* DH5α and *P. taiwanensis* VLB120, as model microorganisms. Bacterial samples were washed with tap water, mixed with AgNPs (1 mg/mL), and loaded into the sample reservoirs. The SERS spectra were collected after 60 s of the enrichment process from the area of the channel cross-section. As the size of the used nanoparticles (60–70 nm) is smaller than the pore size of the membrane, most of the free nanoparticles can pass the pores. Thus, the concentration process works only for aggregated AgNPs and the bacteria.

Figure 4A presents the obtained SERS spectra, which are in perfect agreement with recorded standard samples (see Supporting information Fig. S5). The dominant molecular species contributing to the SERS spectra have been identified as several purine derivative metabolites involved in bacteria purine salvage pathways [35]. The most prominent bands of these spectra can be addressed to biomolecules like guanine (659 and 661 cm⁻¹, ring-breathing mode) and adenine (730 and 735 cm⁻¹, ring-breathing mode) that are secreted during sample preparation when the cells are transferred from a nutrient-rich environment to pure water as a result of the starvation response.

![Figure 4A](image-url)  
**Figure 4A:** SERS spectra of the spiked tap water by model bacterial strains after on-chip preconcentration. The thick lines show the mean spectrum. The red and blue area represents the standard deviation (*n* = 7). For analysis, 300 V was applied and 1.0 mg/mL of AgNPs were presented. For better clarity, the offset was used. (B) Repeatability of the SERS signal across the concentration zone after enrichment of *E. coli* DH5α bacteria.

While before the application of voltage, no SERS bands could be detected (gray line), the enrichment process dramatically increased the concentration of bacteria on the membrane and allowed reliable SERS detection. The most prominent bands are labeled directly in the respective spectrum.

As the bacteria and AgNPs migrated toward the detection zone at different velocities they are deposited onto the membrane with a random distribution. The robustness of the SERS signals across the concentration zone is a significant factor affecting the applicability of the developed device for fast sample in-answer-out analysis of tap water samples. At voltage-free conditions, the signal stability can be influenced by gravity and diffusion, which act against the achieved sample concentration. To investigate this characteristic, we randomly took seven SERS spectra from various places of the membrane to examine the repeatability of the signal across the membrane after the analysis of tap water spiked with *E. coli* DH5α. Figure 4B depicts that all the obtained SERS spectra own all the typical bands labeled in Fig. 4A without any...
significant variations. Thus, all the tested spots on the membrane could be reliably used for a clear and definite identification of *E. coli* DH5α. Since, the intensities of the first and seventh spectrum differ only in the range of RSDs commonly observed for SERS experiments, these observations nicely proved that the random distribution of AgNPs, gravity or diffusion do not significantly influence the quality of the obtained results.

Based on these observations and the SEM images, we assume that the aggregated AgNPs, which are smaller in size than the target cells, can occupy the free space between the entrapped bacteria on the membrane, thus, effectively enhancing the Raman signal. As a consequence, the distribution between the SERS active particles and the targeted cells on the membrane becomes more uniform. This makes the system very robust toward the signal intensity fluctuations and shows that this relatively random composition does not influence the performance of the device.

4 Concluding remarks

This work introduces for the first time a three-dimensional microfluidic device utilizing a nanoporous membrane that fulfills the function of enrichment as well as SERS detection area for bacterial pathogens. The membrane-based microfluidic device with SERS detection is a promising approach for fast and accurate identification of microorganisms such as bacteria. The presented device relies on the electrokinetic flow of the sample across the porous membrane, where bacteria, as well as AgNP clusters, are trapped, concentrated, and reliably analyzed by SERS. The integration of nanoporous membranes in microfluidic devices opens the possibility of fabricating flexible and low-cost analytical platforms that can perform rapid analysis of biological samples. The three-dimensional chip architecture together with a nanoporous membrane enables to maintain two disparate chemical environments for enrichment and detection of microorganisms within the same device, which significantly expands the scope for method development. This work aimed particularly at the construction of the lab-on-a-chip device for analysis of bacterial contamination in tap water and the characterization of its performance using spiked samples. Since the concentration of bacterial cells in drinking water is usually very low, our future work will focus on the extension of the analyzed sample volume. The presented device allows fast as well as simple operation, shows small dimensions, and excellent robustness and can be used as a disposable. It has thus a high potential for rapid point-of-care analysis of bacterial samples, particularly if combined with portable Raman instruments.

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