Rapid, culture-independent, optical diagnostics of centrifugally captured bacteria from urine samples

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(Received 8 May 2015; accepted 24 July 2015; published online 11 August 2015)

This work presents a polymeric centrifugal microfluidic platform for the rapid and sensitive identification of bacteria directly from urine, thus eliminating time-consuming cultivation steps. This "Lab-on-a-Disc" platform utilizes the rotationally induced centrifugal field to efficiently capture bacteria directly from suspension within a glass-polymer hybrid chip. Once trapped in an array of small V-shaped structures, the bacteria are readily available for spectroscopic characterization, such as Raman spectroscopic fingerprinting, providing valuable information on the characteristics of the captured bacteria. Utilising fluorescence microscopy, quantification of the bacterial load has been achieved for concentrations above 2×10^{-7} cells ml^{-1} within a $4 \mu\text{l}$ sample. As a pilot application, we characterize urine samples from patients with urinary tract infections. Following minimal sample preparation, Raman spectra of the bacteria are recorded following centrifugal capture in stopped-flow sedimentation mode. Utilizing advanced analysis algorithms, including extended multiplicative scattering correction, high-quality Raman spectra of different pathogens, such as *Escherichia coli* or *Enterococcus faecalis*, are obtained from the analyzed patient samples. The whole procedure, including sample preparation, requires about 1 h to obtain a valuable result, marking a significant reduction in diagnosis time when compared to the 24 h and more typically required for standard microbiological methods. As this cost-efficient centrifugal cartridge can be operated using low-complexity, widely automated instrumentation, while providing valuable bacterial identification in urine samples in a greatly reduced time-period, our opto-microfluidic Lab-on-a-Disc device demonstrates great potential for next-generation patient diagnostics at the point-of-care. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4928070>]

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections affecting humans.^{1,2} Although most infections are less severe, complications can lead to life-threatening sepsis. UTIs cause major physical discomfort to the individual and are responsible for significant healthcare

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and social costs.³ Globally, UTIs are responsible for several million visits to clinics and GPs annually, with the associated costs exceeding the billion dollar mark.³

Escherichia coli is the most common causative pathogen of a UTI. However, other Enterobacteriaceae such as *Proteus mirabilis* and *Klebsiella* spp., non-fermenters (e.g., *Pseudomonas aeruginosa*), and Gram-positive cocci (e.g., *Staphylococci* and *Enterococci*) may also play an important role.⁴ In times of increasing resistances towards antibiotics, due to their frequent use over recent decades,⁵ rapid and sensitive identification, as well as monitoring of antibiotic susceptibility of these pathogens, is urgently required for the earliest possible initiation of appropriate treatments and the reduction of the economic burden on global healthcare systems. Nevertheless, the current gold standard of bacterial identification in microbiology is still based on time-consuming cultivations, typically taking at least 24 h to identify the organisms present.^{1,6} As a result of this long diagnosis time, immediate treatment is based on calculated antibiotic therapy without any knowledge of the pathogen, thereby contributing to the development of resistant pathogen strains owing to selection pressure.⁷ A variety of novel approaches to the detection of pathogens in drastically reduced time periods have been developed recently. Some of these novel techniques have already been tested in the clinical environment such as mass-spectrometry⁸ and sequencing methods.⁹ In parallel, other techniques are still being researched in the laboratory to find the optimal pathogen identification approach which is fast, accurate, sensitive, easy to operate, and cost-effective. Capillary electrophoresis has been employed in a “three-plug-injection” method to detect bacteria in urine samples within just 10 min.¹⁰ However, this method can only be applied to detect the presence of bacteria and cannot distinguish between different bacterial species.¹⁰ In their recently published article, Kulpakko *et al.* demonstrated a detection limit as low as 1000–10 000 bacterial cells in 1 ml of urine, achieved within 25 min, utilizing a time-resolved fluorescence-based assay for *E. coli* detection. Once again this approach cannot distinguish between different bacterial species, is not applicable to dormant bacteria, and also completely destroys the cells.¹¹ Within this study, we aim to develop a novel technology which allows for rapid detection (within little more than 1 h), is inexpensive (few cents in production), is easy to operate, and has the potential to identify bacteria from typical urine samples from UTI infected patients with only minimal sample preparation.

Automated sample handling requiring only minimal amounts of the liquid can be easily achieved in microfluidic devices. Within these microfluidic chips liquids can be transported rapidly, objects of cellular size can be easily manipulated, and cellular or cell-like organisms can be analyzed under controlled environments that mimic human physiological conditions.¹² Among various different microfluidic techniques, typically based on pump-pressure driven systems, centrifugal microfluidic platforms offer specific advantages as outlined in the following.^{13,14} These “Lab-on-a-Disc” platforms are widely independent of fluidic properties such as viscosity, surface tension, pH, and conductivity, which tend to vary across body fluids. This robustness is mainly because the centrifugal field scales with the density and the square of the angular frequency; thus, the centrifugal field dominates other hydrodynamic forces related to surface tension and capillarity. These aforementioned characteristics combine to make centrifugal microfluidic platforms well suited to biological and medical applications.

Another advantage associated with the utilization of the Lab-on-a-Disc platform for the detection of potentially infectious materials is the separation of the fluid handling on chip from the driving and detection units. Instead of the complex and bulky mechanical displacement pumps associated with pump-driven system, centrifugally driven devices only require a basic spindle motor. The self-stabilizing inertia of the rotor leads to jitter-free pumping, which is more difficult to achieve in reciprocating or syringe-based devices. Furthermore, even high-performance centrifugal microfluidic platforms can be produced in a cost-efficient manner, and therefore, bear a high potential to reduce the costs of current point-of-care diagnostics. Microfluidic chips are often engineered in silicon or polymer substrates, thermoplastics, glass, quartz,^{15,16} or even paper.¹⁷ From the various polymers used in microfluidic applications, we selected polydimethylsiloxane (PDMS) as it is broadly chemically inert, thermally stable, permeable to gases, and can be easily prototyped by casting, even for the replication of submicron features.¹⁸ Future developments will also assess the use of thermoplastics as a potential substrate material, which may outperform PDMS with respect to material cost and volume production.¹⁹

In our previous work,^{13,20} we demonstrated the capture of 20- μm particles under stopped-flow conditions (i.e., particle sedimentation with the liquid on the rotor at rest) in a well-defined V-cup array, on a centrifugal microfluidic platform. The channels and chambers were arranged to facilitate the movement of the particles into the V-cups under the centrifugally induced artificial gravity field, where they remained in place due to the centrifugal force. Optical investigations such as immunochemical staining assays, as utilized in the aforementioned article, could then be carried out successfully. While fluorescence based detection methods are very commonly used in biology,²¹ and have already made their way into analysis on microfluidic chips,¹⁶ the potential of Raman spectroscopy has not been fully employed yet.²² Raman spectroscopy offers unique benefits in the field of optical diagnostics,²³ as it instantly delivers a complete molecular fingerprint of the investigated specimen without the need for time-consuming and often toxic labeling.²⁴ This technique has already demonstrated great potential with respect to the identification of bacteria directly from patient samples in previous work by Schröder *et al.*,²⁵ Kloss *et al.*,²⁶ and Rusciano *et al.*,²⁷ although the level of automation is still limited. Therefore, the combination of Raman spectroscopy with microfluidic sample management for the safe handling of real-world patient samples could lead to a major step forward in the field of biomedical diagnostics. The microfluidic system should not only carry the patient's sample but also enable capture of the bacteria locally from the dilute suspension for efficient Raman spectroscopic analysis. Recently developed approaches which utilize dielectrophoresis (DEP) for bacteria enrichment and Raman-spectroscopy based identification of the captured bacteria,^{25,28} suffer from a strong dependence of the capture efficiency from the electric conductivity of the sample. This electric conductivity can significantly vary in a bodily fluid from a patient, and therefore, extra preparation steps have to be incorporated to thoroughly adjust the conductivity to assure efficient capture of the bacteria for subsequent analysis. Centrifugally driven enrichment of bacteria within a microfluidic device offers a highly attractive alternative to the DEP approach by virtue of its independence from electric parameters, and therefore, robustness for complex bio-samples. To the best of our knowledge, only one combination of sample handling within a centrifugal microfluidic platform and Raman-based analysis has been demonstrated in the context of biomedical applications previously by Cho and Lee.²⁹ However, in the work by Cho and Lee, much larger HeLa cells were analyzed from well-defined culture, and the Raman signal was enhanced by nanoparticles using Surface Enhanced Raman Scattering (SERS).²⁹ While the SERS approach amplifies the Raman signal by several orders of magnitude, data analysis can be complicated by additional surface selection rules.³⁰ Furthermore, additional preparation of an SERS probe is required and the SERS technique is very prone to disturbances by impurities which obscure the spectra of the biological sample of interest and critically compromise reproducibility. Thus, standard Raman scattering is the more suited approach for the detection and analysis of small bacteria directly from body fluids.

In the present study, we combined particle capturing in a glass-plastic-hybrid, V-cup-based centrifugal microfluidic platform with advanced spectroscopic analysis. This enables both cost-effective device production as well as the collection of extensive chemical information from the biological sample in a highly reproducible manner, thus overcoming the issues encountered in recent work on SERS-based platforms. The efficient capture of bacteria in the micron-sized features and subsequent fluorescence and Raman analysis is demonstrated with bacteria suspensions, as well as with real-world urine samples from patients with UTI. The demonstrated analysis power for patient samples makes the presented approach of combined micro-Raman spectroscopy within a centrifugally driven Lab-on-a-Disc device a promising platform for further development towards a point-of-care diagnostic device.

METHODS

Fabrication of V-cup chips

The V-cup chips are made from polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) using standard soft lithography replica molding techniques.¹³ To begin, a mold is created by

depositing three layers of SU-8 (Microchem, USA) on a 4-in. silicon wafer. For the first layer, the negative photoresist SU-8-3025 (Microchem, USA) is deposited onto a clean silicon wafer using a spin coater (Laurel WS-400). Approximately 5 ml resist is spread onto the wafer at 500 rpm for 10 s, the spin coated is then accelerated at 300 rpm s^{-1} up to 3500 rpm and then spun for another 30 s, which results in a 20- μm thick SU-8 layer. Next, the wafer is soft-baked at 95°C for 14 min on a hot plate, and after that UV-exposed (at an energy density of 200 mJ cm^{-2}) on the mask aligner. Directly after exposure, the wafer is placed onto the hot plate at 95°C for 3 min for the post-exposure bake, cooled down to room temperature, and developed in developer (Microposit EC Solvent) for 8 min. Next, the wafer is rinsed with isopropanol (Sigma Aldrich, USA) and dried with nitrogen gas. The second and third layers are designed to increase the sample volume of the microfluidic device up to $10 \mu\text{l}$. The fabrication process follows the same steps as described above. Following the deposition of the second and third layers, the processed disc is hard baked at 150°C for 120 min. Finally, the wafer is placed in 400 μM octadecyltrichlorosilane (OTS) in heptane (both Sigma Aldrich, USA) for 120 min and then baked on a hot plate at 100°C for 20 min, which results in a hydrophobic SU-8 surface (demonstrating a contact angle of greater than 100°). PDMS is prepared by mixing a ratio of 10:1 of PDMS to crosslinking agent, which is then degassed in a desiccator for 30 min and poured onto the silicon-SU-8 mould.

After curing for 10 h at 60°C , the PDMS is carefully peeled off the mould, and fluidic inlets and outlets are punched with a 1 mm outer diameter flat-tip needle. In a final step, the surface of the PDMS structure and a $25 \times 60 \times 0.1 \text{ mm}^3$ microscope cover glass with a poly(methyl methacrylate) (PMMA, Radionics, Ireland) frame are activated in oxygen plasma (Plasma Cleaner, Harrick) at a pressure of 800 mTorr for 3 min. The plasma treated surfaces are then placed together to form bridging Si-O-Si bonds at the interface and thus form a secure bond.

Chip support system

Most centrifugal microfluidic systems utilise a polymeric substrate of a similar geometry as a conventional Compact DiscTM (CD) to contain the microfluidic features. For clinical applications, a parallel analysis of more than one sample is often desirable while still being able to exactly trace the position of the sample. Therefore, a more flexible geometric approach was deemed to be advantageous. Peytavi *et al.*³¹ reported on a plastic, disc-shaped cartridge which is able to carry a couple of individual disposable microfluidic chips, while avoiding the waste of costly materials. A similar solution was developed in this work to secure the V-cup chips safely on the rotor with the ability to load, adjust, and unload chips within seconds (Fig. 1(b)). The centrifugal holding disc was designed with the SolidWorks 2011 software and made of ABS^{plus} polymer using a 3D-polymer printer (Stratasys Dimension $\mu\text{Print Plus}$). The structure was then printed on a polycarbonate support tablet. Undercuts were filled with support material and mechanically removed after the printing process. Four chips can be analyzed simultaneously without microfluidic design restrictions, thus allowing for a high level of flexibility in analyzed samples as well as chip functionality when using the assay. A spin speed of 50 Hz is possible without any imbalances, and suggests that even higher speeds could be implemented.

Bacteria and patient urine samples

Escherichia coli ATCC[®] 25922TM and *Enterococcus faecalis* ATCC[®] 29212TM are cultivated on CASO agar (ROTH GmbH) overnight at 37°C . Bacteria are collected from one colony, centrifuged with a relative centrifugal force of 11 500 g for 5 min, and the pellets are washed twice with phosphate buffered saline (PBS, ROTH GmbH). Afterwards, bacteria are resuspended in PBS. The optical density is measured at 600 nm with an Agilent Cary 60 UV-Vis spectrometer to adjust the bacteria concentration.

For fluorescence measurements, *E. coli* XL1Blue with green fluorescent protein (GFP) expressed in a pET plasmid from Novagen is used.

FIG. 1. Workflow of the analysis of a patient's urine sample. (a) 5–10 ml of patient's urine is sourced for microbiological analysis. After a short (20 min) preparation step involving filtration, volume reduction, and medium exchange, the sample is loaded into the microfluidic chip. (b) The chip is mounted on a centrifugal chip holder, allowing the analysis of four samples at a time. (c) During centrifugation the bacteria (shown in green) experience a centrifugal force (red arrows), which results in the collection of the bacteria in the V-cups within 45 min. (d) The captured bacteria are analyzed by means of Raman spectroscopy. (e) After 1.5 min species-specific Raman spectra are available, which facilitate an assignment of the UTI pathogen in the patient's urine sample.

Anonymized urine samples (5 ml–10 ml) have been provided by the Institute of Medical Microbiology (Jena University Hospital, Germany) for testing purposes. They originate from different patients with single pathogen UTIs ($\geq 10^5$ cells ml⁻¹, *E. faecalis* and *E. coli*). To remove bigger particles such as leukocytes or epithelial cells, the urine samples are run through membrane filters of 5- μ m pore diameter (Pall Life, Acrodisc® Supor®). The filtered urine is then treated like the bacteria samples from cultivation: It is centrifuged, the pellet washed twice with PBS, and is finally resuspended in PBS. All experiments involving patient material were approved by the local ethic committee of the Jena University Hospital under the number 3701–02/13.

Capturing bacteria from suspension on the microfluidic device

Prior to use, the V-cup chips are stored in a vacuum for 30 min to remove the air from the porous PDMS. The V-cup chips are then loaded with 2 μ l PBS, immediately inserted into the chip holder, and spun at 20 Hz for approximately 20 min, during which time all of the air is removed from the array area and replaced with buffer. After these chip initialization steps, 4 μ l of bacteria suspension is loaded onto the chip. To allow the bacteria to sediment to the bottom of the chip, the chip is left at rest for 15 min. The chip is then spun at 25 Hz for 30 min to centrifuge the bacteria into the capturing structures using a DC micromotor (Faulhaber). Four different bacterial concentrations were tested in this work: 1×10^7 cells ml⁻¹, 2×10^7 cells ml⁻¹, 1×10^8 cells ml⁻¹, and 2×10^8 cells ml⁻¹.

Fluorescence measurements

To evaluate the performance of the microfluidic chips, *E. coli* are labeled with GFP. A fluorescence microscope (IX81, Olympus, Japan) collects fluorescence images. Bright field images are recorded with a 4× and 10× objective and detailed measurements are recorded with a 20× and 50× objective. Fluorescence images are carried out with 4× and 10× objectives. The shutter sampling is set to 100 ms and the gain level is set to one. The setup parameters are held constant for all measurements.

Fluorescence images are analyzed with the graphic software ImageJ.³² The mean grey value is measured from the empty measuring spot and subtracted from the mean grey value of the same spot loaded with bacteria solution.

Raman spectroscopy

A CRM 300 WITec micro-Raman setup (WITec, Ulm, Germany), equipped with a 600 lines per mm grating, is used for micro-Raman measurements. A frequency-doubled cw Nd-YAG laser beam with an excitation wavelength of 532 nm and a power of 35 mW (before passing the objective) is focused onto the sample. A 50× objective with a numerical aperture of 0.7 (Zeiss) is used to perform Raman measurements of the laboratory samples, and a 63× Nikon water immersion objective with a numerical aperture of 1.0 is used to collect the signal of the bacteria from the urine samples. The 180° backscattered light is detected by a back illuminated CCD camera (DV401 BV, Andor Technology Ltd, Belfast), with 1024 × 127 pixels, cooled down to −60 °C.

Analysis of Raman data

Data analysis is performed using the programming language “R” V3.0.0.2 (R Core Team, 2014; <http://www.r-project.org/>) using the packages “hyperSpec” (Beleites and Valter, 2014; <http://hyperspec.r-forge.r-project.org/>) and “cbmodels” (Beleites, 2014) for preprocessing functions (i.e., spectra containing cosmic spikes are removed, baseline corrections are done using fifth order polynomial fits, vector normalization is carried out using l^2 -norm). Extended multiplicative scattering correction (EMSC)³³ is applied for background correction of the patient’s samples. First, pure Raman spectra of the PDMS signal within the PBS loaded centrifugal microfluidic platform are measured and averaged to obtain one typical mean background spectrum (see Fig. 4, second line from the bottom). Second, typical Raman spectra of the two different bacterial species *E. coli* and *E. faecalis* without any additional background signal are taken from a previous study,²⁵ and averaged to obtain one mean spectrum for reference purposes. This mean reference spectrum illustrates the typical Raman signature of bacteria (see Fig. 4, third line from the bottom). Finally, these bacterial reference spectra, the pure PDMS spectrum and the bacterial spectra obtained on the Lab-on-a-disc device are processed by the EMSC algorithm to extract the true Raman signature (see Fig. 4, top line) from the superimposed bacterial Raman spectra collected within the microfluidic device (Fig. 4, bottom line). MATLAB software (The Mathworks, USA) with the PLS toolbox (Eigenvector Research, Wenatchee, WA) is used for N-FINDR calculations.

RESULTS AND DISCUSSION

Working principle

Figure 1 illustrates the workflow for the analysis of a patient’s urine sample. Usually 5–10 ml of patient’s urine arrives to routine clinical laboratories for microbiological analysis (Fig. 1(a)). If the patient suffers from a UTI, the urine sample will contain at least 10^5 cells ml^{-1} .^{4,34} A short pre-treatment step, including filtering, volume reduction, and medium replacement, is performed within 20 min. At the same time, the chip is initialized, i.e., it is removed from the vacuum and filled with buffer. The sample is injected into the prepared chip and is guided by the radial centrifugal field to the V-cup chamber, where the bacteria are captured by

the V-cups within 45 min (Fig. 1(c)). Stopped flow conditions prevent the bacteria from being flushed away by hydrodynamic flow. Raman spectra are subsequently collected and analyzed within 1.5 min (Fig. 1(d)) to yield species-specific spectral information of the bacterial pathogen within the urine sample (Fig. 1(e)).

Visualization of captured bacteria within the V-cups

Fluorescence microscopic imaging using a GFP labeled *E. coli* strain was applied to visualize how the bacteria are retained within the V-cups. Different concentrations ranging between 2×10^8 cells ml^{-1} and 1×10^7 cells ml^{-1} were loaded onto the chip. Fluorescence images from the first three rows of the loaded V-cup array are shown in Figure 2(a), and detailed bright field images of one representative capturing structure can be seen in Figure 2(b). The V-cups of subsequent rows are staggered, with the fourth row shadowing the first row. Therefore, the first three rows capture the majority of bacteria. However, the presented V-cup chip consists of more than three rows, and a small quantity of bacteria can also be found in V-cups in subsequent rows. This can be explained with complex particle sedimentation processes for small objects such as bacteria with a size below $2 \mu\text{m}$. Brownian motion³⁵ and neighboring particles and cluster of particles³⁶ can affect the trajectory of sinking particles. Furthermore, *E. coli* possess a flagellum, which makes them motile resulting in rapid and agile movements of the bacteria as soon as the centrifugal force is stopped.

At high bacterial concentrations, bright fluorescence spots indicate that the bacteria are concentrated within the cups. At the highest applied bacterial concentration, the white light image shows a completely filled cup (Fig. 2(b), top). Upon lowering the bacterial concentration, the filling level gets reduced and the fluorescent spots have a crescent-like shape resulting in a reduced overall integrated brightness of fluorescence. At the lowest applied cell concentration (of 1×10^7 cells ml^{-1}), the fluorescence can barely be detected and very few bacteria are captured within the cup.

Plotting the captured bacteria (measured as the mean grey value of the fluorescence images) as a function of the bacterial concentration yields a linear relationship (Fig. 2(c)). This relationship can be implemented as a calibration curve to quantify the bacterial load of a patient sample from the amount of captured bacteria. The calibration curve yields a limit of detection of around 2×10^7 cells ml^{-1} . However, this limit of detection does not significantly hamper the sensitivity of the presented device regarding the analysis of UTIs. Just $4 \mu\text{l}$ of the sample is needed to load the centrifugal microfluidic platform. Therefore, an easily implementable (standard procedure) and rapidly (about 20 min) performed upstream pre-concentration step (Fig. 1)

FIG. 2. Determination of the capture efficiency. (a) Fluorescence images of the first three V-cup rows filled with GFP labeled *E. coli* after centrifugation of four suspensions displaying different bacterial concentrations (from top to bottom): 2×10^8 cells ml^{-1} , 1×10^8 cells ml^{-1} , 2×10^7 cells ml^{-1} , 1×10^7 cells ml^{-1} . The bright spots indicate captured bacteria. Captured bacteria can only be detected for the three highest concentrations. The red circle marks the V-cup, which is magnified in the bright field image in (b). (c) The mean grey value of fluorescence is measured to show a quantitative relation between the captured bacteria and the bacterial concentration.

can be utilized for the analysis of samples with lower bacterial concentrations. This will be shown in detail later, for the diagnosis of real world patient's samples.

Raman spectroscopic characterization of bacteria captured in the V-cups

In contrast to typically employed PDMS and plastic microfluidic devices, the microfluidic cartridges used in this study are modified on one side to contain a glass window. A similar, multi-material hybrid was previously presented by the authors of this work.³⁷ This allows for the recording of the relatively weak Raman signal of the captured bacteria as opposed to the strong Raman bands that are characteristic of PDMS material. Figure 3 shows on the left a microscopic image of a V-cup filled with *E. faecalis*, which belong, after *E. coli*, to the most commonly encountered pathogens in urinary tract infections. A Raman scan of about $7\ \mu\text{m} \times 18\ \mu\text{m}$ is carried out to include the whole V-cup area. To obtain Raman spectra of the different components, such as bacteria and background, N-FINDR³⁸ analysis is employed. Briefly, this automated iterative algorithm carries out an unsupervised spectral decomposition of multidimensional images and yields the Raman spectra of the different components as so-called “endmembers.” The algorithm has already been successfully applied for the identification of subcellular compartments³⁹ and intracellular bacteria⁴⁰ following Raman spectroscopic imaging of cultured cells and tissue. Figure 3 depicts the N-FINDR result for one V-cup filled with bacteria showing the resulting endmember spectrum (on the right) together with their relative abundances in each pixel of the data (middle). The V-cup structure is clearly visible where PDMS is dominating the endmember spectra (first three endmembers A, B, C). The characteristic Raman bands arise from the Si–O–Si backbone with methyl side chains: Si–O–Si stretching vibration at $491\ \text{cm}^{-1}$, Si–CH₃ rocking mode at $687\ \text{cm}^{-1}$, other stretching modes at $712\ \text{cm}^{-1}$ and $793\ \text{cm}^{-1}$ referring to the bond between Si and C; symmetric and anti-symmetric deformations at $1266\ \text{cm}^{-1}$ and at $1415\ \text{cm}^{-1}$ of Si–CH₃. The methyl group itself has two CH stretching

FIG. 3. Raman spectroscopic analysis of captured *E. faecalis*. The bright field image on the left shows a V-cup filled with *E. faecalis*. The red rectangle denotes the area for which a Raman map has been recorded. N-FINDR analysis was carried out to reveal the spectral contributions and extract the pure spectra as endmembers. A–E represent the spatial distribution of five unmixed endmembers, together with their spectra on the right. The first three endmembers A, B, and C show pure Raman spectra from PDMS of the V-cup structure. Endmember D contains signal from the PDMS substrate of the bottom and a water band around $1640\ \text{cm}^{-1}$ from PBS buffer solution. Endmember E shows a typical bacterial signature; the enlarged view on the right reveals clearly distinguishable Raman bands (prominent bands are labeled). However, the background signal from PDMS is still present, with the respective bands indicated by red stars.

vibrations: the antisymmetric one at 2906 cm^{-1} and the symmetric one at 2965 cm^{-1} .⁴¹ The fourth endmember (D) also shows PDMS features, but in addition the broad O–H bending vibration of water around 1640 cm^{-1} is present and indicates regions filled with PBS buffer solution. The fifth endmember (E) (a zoomed view for better identification of the peaks of this endmember spectrum is plotted on the right side of Figure 3) contains spectral information, which can be assigned to bacteria. Typical bands can be found in the fingerprint area between 600 cm^{-1} and 1800 cm^{-1} , such as contributions from phenylalanine at 1004 cm^{-1} , the phosphate backbone of nucleic acids at 1093 cm^{-1} and 1578 cm^{-1} , CH vibrations at 1341 cm^{-1} , CH_2 vibrations at 1452 cm^{-1} , and the prominent broad amide I band around 1658 cm^{-1} .⁴²

Analysis of patient's urine samples

In order to prove the applicability of the glass-PDMS-hybrid microfluidic chip for the analysis of real world samples, urine samples delivered for routine microbiological analysis to the Institute of Medical Microbiology, within the Jena University Hospital (Germany) are used. These samples typically amount to 5 ml to 10 ml, with minimal bacteria concentrations in a significant bacteriuria of around $10^5\text{ cells ml}^{-1}$.^{4,34} Furthermore, leukocytes, which act to fight the urinary tract infection, and epithelial cells from the bladder can be found in the urine samples. Those bigger particles are removed from the urine sample with a filtration step through a filter membrane, as noted earlier. A pre-concentration of the bacteria by two to three orders of magnitude can be achieved by centrifugation and volume reduction. This pre-concentration step also involves an exchange of the medium for PBS, such that the Raman spectra of the bacteria are free of a possible fluorescence background from other components in the urine. This short sample preparation process is completed in less than 20 min (Fig. 1) and therefore can easily be carried out in parallel to the chip initialization step, ensuring high device sensitivity. Following the sample preparation step, $4\text{ }\mu\text{l}$ of the pre-processed patient sample is deposited into the V-cup chip. The bacteria are captured in the V-cups as described above and are further analyzed by means of Raman spectroscopy. A single Raman spectrum is acquired in just 5 s. Figure 4 (bottom) shows two resulting mean Raman spectra averaged from 133 spectra per sample, collected at various centers of different bacteria-filled V-cups. Besides the typical Raman bands from the PDMS background, significant spectral contributions from the bacteria can be discerned. To remove the strong PDMS background, an extended multiplicative scatter correction³³ (EMSC) is performed. Using a pure background spectrum from a V-cup filled only with PBS (Fig. 4, third line) and a generalized bacterium spectrum (Fig. 4, second line) computed from several bacteria measurements (which are collected according to our previous study²⁵), the algorithm is able to extract the true bacterial Raman spectra of the patient samples. Figure 4 (top) presents the resulting high-quality Raman mean spectra of two different pathogens, which originate from the urine of two patients with different infections. The spectra are in very good agreement with typical Raman spectra of *E. coli* and *E. faecalis* obtained in previous studies.^{25,43} The main Raman bands are labeled: 788 cm^{-1} , 1093 cm^{-1} , and 1578 cm^{-1} represent DNA contributions; 1004 cm^{-1} , 1250 cm^{-1} , and 1658 cm^{-1} correspond to contributions from proteins; 1341 cm^{-1} and 1452 cm^{-1} can be assigned to CH-vibrations.⁴² The Raman spectra of the two species can be distinguished by calculating the difference spectrum using the EMSC corrected spectra displayed in Figure 4. Figure 5 compares the post-analysis result to a difference spectrum from our previous study,²⁵ in which the bacteria were captured on a dielectrophoresis chip and Raman spectra were recorded without a background. The excellent agreement of both calculated difference spectra demonstrates the distinction of *E. coli* and *E. faecalis* from their Raman spectroscopic fingerprints recorded within the V-cups. Thus, the presented method holds high potential to identify different pathogens from patients' urine samples, thus making this approach of combining a cost-efficient centrifugal opto-microfluidic system with powerful spectroscopic analysis a promising one for rapid microbiological diagnostics.

The total duration of all of the above process steps, from the initial arrival of the patient sample into the lab, to the identification of the pathogen using Raman spectroscopy, is less than 100 min. The first 30 min of this workflow are devoted to the evacuation of the chip. If the

FIG. 4. Analysis of urine samples on the V-cup chip. Bottom: Baseline corrected and vector normalized Raman mean spectra from bacteria captured in different V-cups from two different patients suffering from UTI. Beyond the PDMS background, spectral contributions from bacteria are visible and labeled. Second line from the bottom: Raman mean spectra of the chip loaded with PBS without bacteria. Only PDMS and water signals are detected. Third line from the bottom: Generalized bacteria spectrum (left and right are identical) computed as a superposition of a typical *E. faecalis* and a typical *E. coli* mean spectrum (measured in a previous study²⁵). Top: The Raman spectrum of the empty V-cup and the generalized bacteria Raman spectrum are used in an EMSC algorithm to extract the bacterial spectrum from the recorded spectrum depicted on the bottom. The resulting spectra are in very good agreement with typical *E. coli* (left) and *E. faecalis* (right) spectra. Typical Raman peaks are labeled.

chips are stored under vacuum, this time can be saved, reducing the total analysis time to less than 70 min. Thus, the so-called “golden hour” for pathogen analysis is nearly achieved using this approach. In this work, the evacuated chips are then loaded with buffer and centrifuged,

FIG. 5. Difference spectra of EMSC corrected *E. coli* and *E. faecalis* spectra. *E. coli* and *E. faecalis* can be differentiated by their Raman signatures, shown by the calculation of the difference spectrum using the EMSC corrected spectra, illustrated in Fig. 4 (top row). The difference spectrum is compared to results obtained using a quadrupole dielectrophoresis chip from our previous study²⁵ and reveals highly coinciding features, which are labeled in the figure.

requiring 20 min in total. During that period, the patient's sample is prepared. However, future developments in this field are moving towards the integration of this pre-treatment step within the same device. Promising results which can help to increase the capture efficiency directly on the "Lab-on-a-Disc" platform have already been achieved in the area of blood cell diagnostics making use of unspecific enrichment approaches through centrifugal sedimentation,⁴⁴ as well as specific approaches based on immuno-magnetophoresis.⁴⁵ After the pre-treatment step, the bacteria are centrifuged for 45 min into the V-cup area. In order to record 100 Raman spectra of the captured bacteria, about 1.5 min is required, with the subsequent analysis completed online within seconds (once the statistical models are implemented) (Fig. 1).

Compared to established, cultivation-based microbiological methods, which require 24 h or more to identify the bacteria, an analysis time of slightly over 1 h marks a huge step forward in fast, cultivation-independent analysis. There are other approaches which have a slightly shorter analysis time, such as our previously established, hybrid dielectrophoresis-Raman approach, which can identify bacteria from urine within only 35 min.²⁵ However, the centrifugal microfluidic platform combined with Raman spectroscopic analysis presented in this study is more cost-efficient, as only low-cost, plastic-glass hybrids are required compared to their more expensive counterparts with electrode structures. Our approach also excels with respect to its robustness against the varying properties of the liquid bio-sample, as it is independent of electric conductivity. Compared to recent developments, which target the sensing of bacteria irrespective of the species level in urine, within even shorter intervals (25 min (Ref. 11) and 10 min (Ref. 10)), the Lab-on-a-Disc approach presented in this work sets itself apart by its high potential for bacterial classification and identification on a species level. Furthermore, our implementation of normal Raman spectroscopy yields high-quality Raman spectra without the need for enhancement techniques, such as SERS, which would require additional probes and lead to a higher risk of contamination as well as greater device complexity and cost.

CONCLUSION

In the present study, a cost-efficient "Lab-on-a-Disc" platform based on a glass-polymer hybrid is presented which captures suspended bacteria in an array of geometrical traps and allows for their subsequent optical analysis with a special focus on Raman spectroscopy. It has been demonstrated that the platform can detect and analyze bacteria from patients' urine samples without prior cultivation steps in under 70 min. The centrifugal microfluidic approach presented here can therefore drastically decrease the typical diagnosis time of more than 24 h in conventional, cultivation-based detection. This marks a substantial step forward compared to currently established methods in microbiological diagnosis in clinics. Characterization of the captured bacteria by label-free conventional micro-Raman spectroscopy allows rapid identification of the pathogens with their characteristic features, which is valuable for first screening analysis. Furthermore, the device outlined in this work has been easily adapted for fluorescence measurements, paving the way for the development of microfluidics-based immunochemical assays, illustrating a high potential of the presented device for numerous applications in spectroscopy-based point-of-care diagnostics.

ACKNOWLEDGMENTS

We thank Valerie Fitzgerald for providing GFP labeled *E. coli*; Tia E. Keyes for enabling first Raman test measurements in Dublin; Martin Somers, Robert Burger, and Ondřej Stráník for fruitful discussions; Claudia Beleites and Norbert Bergner for support in statistical analysis.

Financial support of the BMBF via the Integrated Research and Treatment Center "Center for Sepsis Control and Care" (FKZ 01EO1002), the EU via Photonics 4 Life (Grant Agreement No. 224014), and Science Foundation Ireland under Grant No 10/CE/B1821 is highly acknowledged.

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