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SIP-Based Thermal Detection Platform for the Direct Detection of Bacteria Obtained from a Contaminated Surface

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Surface detection of bacteria has been proven difficult and time-consuming. Different recovery techniques yield varying numbers of bacteria. Subsequently, bacterial culturing, used for identification of these bacteria, will take several hours. In this article, the potential of a newly developed thermal biomimetic sensor for the detection of bacteria on surfaces is described. Previously this thermal biomimetic sensor has proven to be able to detect and quantify different bacteria in various liquid media such as buffer and spiked urine samples. In this article, laboratory surfaces are contaminated with increasing concentrations of *Escherichia coli*. Bacteria are recovered from the surfaces using commercially available swab rinse kits (SRK). A calibration curve is created by coating chips with surface-imprinted polymers (SIPs), serving as synthetic bacteria receptors, and exposing them to increasing concentrations of *E. coli*. Next, concentrations of *E. coli* in the SRK buffer are measured and quantified. The results show that it is possible to detect *E. coli* recovered from surfaces. Although quantification has been proven difficult as the dynamic range of the sensor is relatively narrow and the bacterial load obtained by using SRK is low, the sensor is able to give an indication about the concentration present on the surface. The results in this article illustrate that the thermal biomimetic sensor is a fast, low-cost, and label-free device useful in surface detection of *E. coli*, and seems a promising tool for future on-site bacterial detection.

1. Introduction

Bacteria are all around and frequently grow in biofilms on different surfaces.[1] Bacterial contamination of surfaces plays an important role in transmission of these bacteria. Especially in controlled environments such as the health care environment, prevention of bacterial contamination is extremely important in the prevention of infection.[2]

Over the years, surface detection of bacteria has been proven difficult and time consuming. Biofilms are difficult to overcome, the yield of bacteria by using different recovery techniques is varying and culturing of bacteria, used for the identification of bacteria, takes several hours.[3] In recent years, sensitive bacterial identification assays have been developed based on molecular techniques such as polymerase chain reaction (PCR). Although, these techniques allow for fast and accurate bacterial screening, they are often confined to a lab environment, involve sample preparation and require analysis by skilled professionals using expensive readout equipment.[4]

Biosensors could offer a fast and low-cost alternative and exploit the affinity natural receptors have for bacteria or bacteria-specific proteins. In addition, they do not require labeling or sample pre-treatment. Bacterial capturing by biological receptors is typically translated into a measurable signal by electrochemical,[5] optical,[6] or microgravimetric transducers.[7] Although these assays are usually very sensitive and selective, the number of commercial biosensor applications remains limited due to the poor chemical and physical stability of the biological receptor layer. Additionally, receptor synthesis is complex and costly and biomolecule immobilization usually involves coupling through carefully optimized linkers.[8]

Many of the issues associated with the use of biological recognition elements in a sensor platform can be overcome by using synthetic receptors that are more robust than their natural counterparts but have a similar affinity for their target. Surface-imprinted polymers (SIPs) are an interesting class of synthetic receptors that have been used over the past 20 years for e.g., bacteria detection.[9,10] The integration of SIPs into biomimetic

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sensing platforms has been achieved by coupling these imprinted thin layers to sensitive readout techniques based on microgravimetry,[11,12] optics,[13,14] or electrochemistry.[15,16]

The authors of this article introduced a novel thermal readout technique in 2012, that has been used in combination with several natural and synthetic receptors for the detection of point mutations in DNA,[17] small organic molecules,[18] proteins,[19] and human cells.[20] This so-called heat-transfer method (HTM) is based on the attenuation of the heat-transfer pathway in response to biochemical changes occurring at the solid–liquid interface. The technique has proven to be very interesting in terms of biosensing due to its simple and low-cost nature. The technology has evoked a lot of interest, as it is in fact a temperature control unit, classically incorporated into commercial sensing devices, and a readout platform in one. This makes the device low-cost and easy to miniaturize which emphasizes its potential for point-of-care sensing. In 2016, the concept was extended toward the detection of bacteria,[21] an application that has a lot of commercial potential as it could be used in diverse fields ranging from the food industry to medical diagnostics. It was demonstrated that it is possible to use the technique to detect bacteria in spiked urine samples at concentrations relevant for the diagnosis of urinary tract infections in a fast and reproducible manner.[22]

Until now the sensor has been tested in controlled liquid media, spiking both buffer solutions and sterilized urine samples with different bacterial species. Here we assess the potential of the thermal biomimetic sensor in the detection of bacteria on surfaces. To this extent laboratory benches were contaminated with increasing concentrations of *Escherichia coli* (*E. coli*). Recovery of the bacteria was done using a commercially available swab rinse kit (SRK). To faithfully determine the *E. coli* concentration in the swabbed samples, SRK medium solutions were spiked with increasing concentrations of *E. coli* and the resulting sensor response was registered in order to construct a dose–response curve. Results were benchmarked using the gold standard of bacterial culturing. The results from these experiments indicate that it is possible to detect *E. coli* obtained directly from a contaminated surface using the thermal biomimetic sensor. In addition, the sensor is able to give an indication of the concentration of bacteria that was present on the surface.

2. Experimental Section

2.1. Bacterial Culture

E. coli (ATCC® 8739™) strains were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Growth medium was prepared in the form of Luria broth (LB solution) by dissolving 10 g of tryptone, 5 g of yeast, and 10 g of sodium chloride in 1 L of deionized water. LB broth was inoculated with a single colony of *E. coli* and incubated overnight at 37 °C while shaking at 125 rpm. Bacteria were grown till an optical density of 1 was obtained at 600 nm (8×10^8 cells mL^{-1}). Bacteria were harvested by centrifugation and pellets were washed and resuspended in either PBS solution or SRK medium to achieve desired concentrations.

2.2. Surface Imprinting Procedure

Polyurethane synthesis was initiated by dissolving 122 mg of 4,4′-diisocyanatodiphenylmethane, 222 mg of bisphenol A, and 25 mg of phloroglucinol in 500 µL of anhydrous tetrahydrofuran (THF). All reagents were used as received from Sigma–Aldrich N.V (Diegem, Belgium) and had a purity of minimally 99.9%. This mixture was stirred at 65 °C for 200 min under an inert nitrogen atmosphere until the polymer solution reached its gelling point. Then, the solution was diluted in a 1:5 ratio in THF and spin coated for 60 s at 2000 rpm onto 1 cm^2 aluminum substrates. In parallel, polydimethylsiloxane (PDMS) stamps were covered with cells to stamp the cells into the spin-coated polyurethane layer. PDMS stamps were made using the Sylgard 184 silicone elastomer kit (Malvom N.V., Schelle, Belgium). Then, the *E. coli* suspension in PBS (400 µL) was applied onto the PDMS stamp. After 20 min of sedimentation time, the excess fluid was removed by spin coating at 3000 rpm for 60 s to create a dense monolayer of cells on the stamp surface. The cell-covered stamp was gently pressed onto the polyurethane layer and cured for 18 h at 65 °C under nitrogen atmosphere. After curing, the stamp was removed from the surface and bound bacteria were removed by rinsing the layer with 70% ethanol and PBS, leaving behind selective binding cavities on the polyurethane surface.[22]

2.3. Measurement Methodology

Measurements were performed using a thermal readout unit that was previously introduced by the authors (**Figure 1**) [23].

SIP-coated aluminum chips ($10 \times 10 \times 1$ mm^3, Brico N.V., Leuven, Belgium) were attached to a copper heat provider that is heated by a power resistor (Farnell, Grâce-Hollogne, Belgium). The temperature of the copper (T_1) was measured by a thermocouple (type K, diameter 0.5 mm, TC Direct, Nederweert, the Netherlands). and registered by a TC-08 thermocouple data logger (Picotech, UK) and transferred to a Labview-based program (National Instruments, Zaventem, Belgium). A software-based proportional-integral-derivative (PID) controller uses this information to adjust the voltage over the power resistor in order to keep T_1 at a constant temperature of 37 °C.

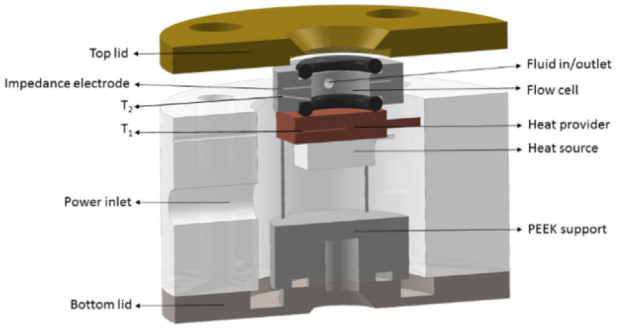

Figure 1. Schematic representation of the thermal readout methodology. Samples are heated by a power resistor through a copper heat provider. The temperature of the heat provider (T_1) and the liquid inside the flow cell (T_2) are registered to monitor heat transport through the chip. Reproduced with permission.[23]

The sample is exposed to bacteria solutions by means of a polyether ether ketone (PEEK) liquid flow cell, with an inner volume of 160 µL, facing the functionalized chip surface. The flow cell is shielded by two O-rings defining a contact area of 36 mm^2 between the chip and the liquid. The temperature of the bacteria solution (T_2) was monitored by a second thermocouple at a distance of 1 mm from the chip surface.

2.4. Construction of a Calibration Curve

A dose-response experiment was performed to verify if the SIP-based sensor is able to detect bacteria quantitatively in SRK medium. Prior to exposing the chips to bacteria, the system was left to stabilize for an hour. Next, the cell was sequentially exposed to concentrations of 5×10^3, 1×10^4, 2×10^4, 5×10^4, 1×10^5, 5×10^5, and 2×10^6 CFU/mL of *E. coli* dilutions suspended in SRK medium at a flow rate of 0.25 mL min^{-1}. Between each addition, the system was flushed with SDS and SRK solution to regenerate the sensor surface.

2.5. Surface Contamination and Bacterial Recovery

E. coli suspensions in PBS of different concentrations were used to contaminate 10 × 10 cm squares on laboratory benches. Before contaminating, all surfaces were sterilized with 70% ethanol to ensure no additional bacteria would be present. Prior to the experiment, all ethanol was evaporated to avoid bacterial dying upon contact. Bacterial solutions were prepared as described in Section 2.1 to give a concentration of 10^8–10^9 CFU/mL. Serial dilutions of this stock solution were made in PBS to acquire concentrations of 10^8, 10^7, 10^6, and 10^5 CFU/mL. Next, 0.5 mL was transferred into the designated 10 × 10 squares by pipetting onto the surface. Bacteria were evenly distributed using L-shaped cell distributers (Dimensions: L 146 × W38 × Ø 4 mm) with even pressure. Bacterial recovery was performed using the SRK rinse kit (obtained from COPAN, Italy) once the surfaces had dried after 20 min. Each surface was swabbed twice, while rotating the swab head perpendicular as recommended by the manufacturer. Recovered bacteria were dissolved in 10 mL of SRK solution by placing the swab into the test tube and vortexing for 1 min.

2.6. Thermal Detection of Bacteria in Swab Solutions

E. coli concentrations of collected swab solutions were analyzed using the thermal detection platform. To this extent, the system was stabilized in pure SRK medium for an hour. Afterwards, swab solutions obtained from surfaces fouled with different concentrations of bacteria, were injected into the flow cell. Between adjacent exposure runs, the sensor surface was regenerated by flushing with SDS and SRK medium. For each fouling concentration, three samples were analyzed. The responding effect size for every fouling concentration was compared to the calibration curve to determine the recovered amount of bacteria. These results were validated using the gold standard technique; culturing the swabbed solutions and counting the bacterial cultures on an agar plate.

2.7. Validation by Cell Culturing

Swabbed bacterial solutions were plated on LB agar plates. For each collected solution, serial dilutions were made with a 1:10 dilution factor. Solutions collected from surfaces with initial contaminations of 10^8, 10^7, 10^6, and 10^5 CFU/mL were diluted 5, 4, 3, and 2 times, respectively. A total of 100 µL of each dilution was used to plate and colonies were grown overnight in an incubator at 37 °C. For the lowest dilutions, 50 µL of the solutions were also plated. After the colonies grew, their numbers were determined. Since for the higher concentrations, the plates were fully covered and the number of single colonies could not be determined these plates were excluded from the calculations. The concentration in CFU/mL was calculated using Eq. (1):

$$\text{CFU/mL} = \frac{\text{colonies}}{\text{volume solution}} \times \frac{1}{\text{dilution factor}} \quad (1)$$

3. Results and Discussion

3.1. Calibration Curve

Previous research has indicated that it is possible to determine the concentration of bacteria in both PBS buffer and spiked urine samples.[21,22] However, since the commercial swab rinse kit dilutes the swabbed bacteria in the accompanying SRK solution, a calibration curve was made in this medium (**Figure 2**).

The data in Figure 2 show that, at a constant T_1 value, a concentration-dependent drop in T_2 can be observed when exposing the SIP to increasing amounts of *E. coli* in SRK solution. T_2 drops from 35.19 ± 0.09 °C at baseline to 34.35 ± 0.12 °C at the highest concentration. The effect size at each concentration, defined as the absolute drop in T_2, is used to construct a dose–response curve. The detection limit appears to be in the low 10^4 CFU/mL range with a dynamic range that spans about an order of magnitude before the signal saturates. These results are comparable to results previously obtained in spiked urine samples[22] and indicate that the composition of the SRK medium does not interfere with the potential for bacterial concentration determination of the proposed sensor platform.

3.2. Detection of Bacteria Obtained from a Surface by SRK

To assess the potential of the proposed platform for application in bacterial "on the bench" screening, laboratory benches were contaminated as described in Sections 2.5 and 2.6. The swab solutions (diluted in 10 mL SRK medium) were collected and sequentially injected into the measuring chamber (0.25 mL min^{-1} for 12 min). To faithfully determine the actual concentration of bacteria in the swab solution, the results were projected onto the calibration curve obtained in the previous section (**Figure 3**).

The results in Figure 3 indicate that an insignificant drop of 0.18 ± 0.19 °C in T_2 is registered at fouling concentrations in the 10^5 bacteria per mL range, which makes sense as only 0.5 mL

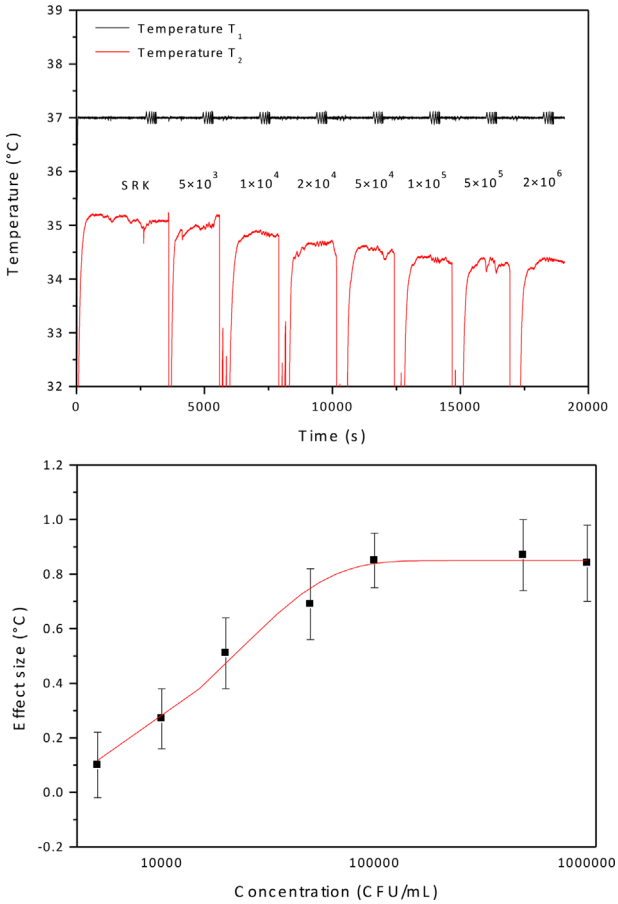

Figure 2. Construction of a dose–response curve. The sensor was calibrated by exposing the SIP layer to an increasing concentration of *E. coli* cells in SRK buffer. A concentration-dependent drop in the temperature of the cells can be observed (top). These data were used to construct a dose-response curve (bottom).

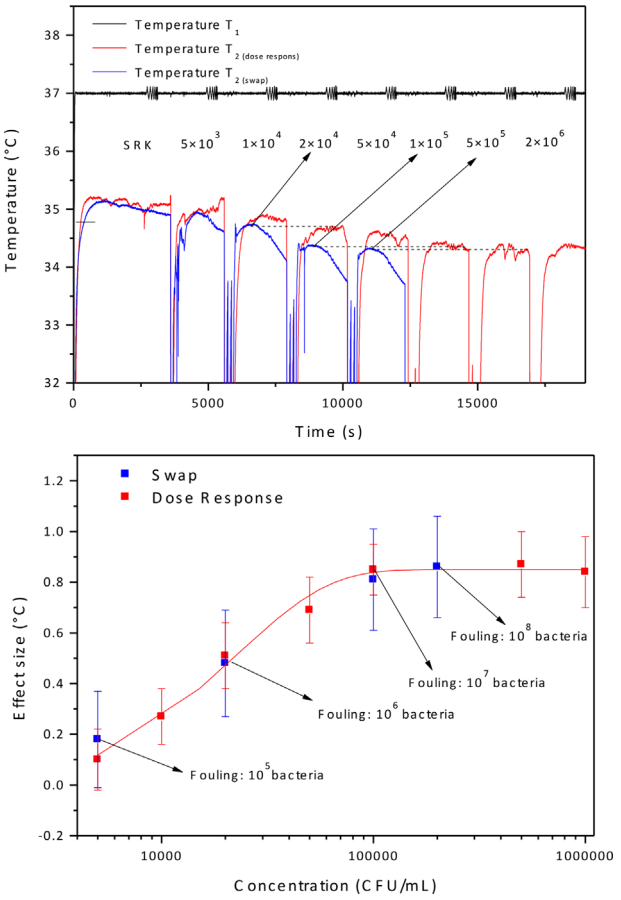

Figure 3. Swab samples of contaminated surfaces were injected into the flow cell and their time-dependent temperature profile (top) and effect size (bottom) were compared to the calibration curve (red curve) to determine the concentration in the swab solution (blue curve).

was used for fouling and the swab was diluted in 10 mL buffer. Likewise, the saturated effect size of $0.86 \pm 0.18\,^{\circ}C$ observed at higher fouling concentrations makes sense. Nevertheless, a gradual trend can be observed that confirms bacterial presence at higher concentrations and provides an indication about the native concentration as evidenced by the intermediate value at a fouling concentration of 10^6 bacteria. However, the absolute value of the registered concentration seems to be an order of magnitude lower than can be expected when considering the fouling concentration and dilution factor. To explain this properly, the results were benchmarked with a sensitive gold standard technique; the culture method.

3.3. Validation of Results with Culture-Based Gold Standard

The results obtained in the previous section were benchmarked using bacterial culturing. Bacterial solutions obtained with SRK were transferred to a culture plate and incubated overnight. The resulting colonies were counted and compared to both the native fouling concentration and the results obtained with the thermal SIP-based sensor (**Table 1**). This analysis demonstrates that the SRK procedure is able to recover between 16 and 65% of *E. coli*.

This can partly be explained by the fact that determining the concentration with an optical density measurement only gives an indication about the concentration range rather than an exact concentration. Although the combination of HTM and SRK seems to lead to a moderate underestimation of the number of bacteria present on a surface, these results confirm that it is possible to provide the end-user with an indication about the presence of bacteria of interest and give a fast indication about their concentration.

4. Conclusions

The results of the study presented in this article demonstrate that the proposed thermal SIP-based platform is able to detect *E. coli* obtained from a contaminated surface using a commercial swab rinse kit. In addition, the experimental data show that the obtained concentration in the linear part of the sensor are in the same range as the results obtained with the same sample using the gold standard culture-based assay.

Table 1. Validation of results obtained from an experiment were lab benches were fouled with increasing concentrations of bacteria. These benches were swabbed using the commercial swab rinse kit and diluted in 10 mL of SRK medium. These swabbed solutions were analyzed with both the thermal biosensor and the gold standard culture method, indicating that the gold standard is still more sensitive and accurate.

Fouling conc.	Culturing recov.	HTM recov.
2.8×10^5	1.8×10^4	NA
2.8×10^6	9.3×10^4	2.4×10^4
2.8×10^7	1.7×10^6	1.0×10^5
2.8×10^8	4.5×10^7	5.0×10^5

This illustrates the potential of the device as a low-cost and fast alternative to modern-day screening tools. However, due to the limited sensitivity and binding capacity of the SIP layer and the resulting narrow dynamic range, practical applicability of the sensor in commercial applications for bacterial screening will still require some chemical and biomedical engineering of both the device and receptor layer. By creating SIPs with a higher surface coverage, both sensitivity and binding capacity will be improved, leading to a broader dynamic range of the resulting sensor. Additional progress can be made by combining the thermal readout unit with modern day device engineering and microfluidics. In this way, sample volumes will become smaller and by adding a pre-filtration step the concentration of bacteria in the sample can be increased.

The sensor seems a promising tool in the detection of onsite bacterial detection. Especially in a controlled health care environment, the device could play a role in portable bacterial detection assays that enable healthcare workers to screen various surfaces within their facility identifying the presence of bacteria and subsequently taking hygienic measures, thereby preventing bacterial transmission and possibly infection.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

bacterial culture, bacterial detection, heat-transfer methods, swab rinse kits, thermal interface resistance

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