

Technical Note

Versatile Electrochemical Sensing Platform for Bacteria

Sabine Kuss, Rosa Alexandrina de Sousa Couto, Rhiannon M.
Evans, Hayley Lavender, Christoph Tang, and Richard G Compton

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.9b00326 • Publication Date (Web): 27 Feb 2019

Downloaded from <http://pubs.acs.org> on March 11, 2019

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.



ACS Publications

is published by the American Chemical Society, 1155 Sixteenth Street N.W.,
Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society.
However, no copyright claim is made to original U.S. Government works, or works
produced by employees of any Commonwealth realm Crown government in the course
of their duties.

Versatile Electrochemical Sensing Platform for Bacteria

Sabine Kuss^{†,‡}, Rosa A.S. Couto[§], Rhiannon M. Evans[#], Hayley Lavender[‡], Christoph C. Tang[‡], Richard G. Compton^{*,†}

[†]Department of Chemistry, Oxford University, South Parks Road, Oxford, OX1 3QZ, United Kingdom

[‡]Laboratory for Bioanalytics and Clinical Chemistry, University of Manitoba, Department of Chemistry, Winnipeg, MB, R3T 2N2, Canada

[§]REQUIMTE, LAQV, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

[#]University of Oxford, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, United Kingdom

[‡]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Electrochemical detection, Chronoamperometry, Cytochrome c Oxidase, Sexually Transmitted Diseases, N,N,N',N'-tetramethyl-para-phenylene-diamine, *E. coli*

ABSTRACT: Bacterial infections present one of the leading causes for mortality worldwide, resulting in an urgent need for sensitive, selective, cost efficient and easy to handle technologies to rapidly detect contaminations and infections with pathogens. The presented research reports a fully functional chemical detection principle, addressing all of the above mentioned requirements for a successful biosensing device. At the examples of *Escherichia coli* and *Neisseria gonorrhoeae*, we present an electrochemical biosensor, based on the bacterial expression of cytochrome c oxidase, for the selective detection of clinically relevant concentrations within seconds after pathogen immobilization. The generality of the biochemical reaction, as well as the easy substitution of target specific antibodies make this concept applicable to a large number of different pathogenic bacteria. The successful transfer of this semi-direct detection principle onto inexpensive screen printed electrodes for portable devices represents a potential major advance in the field of biosensor development.

INTRODUCTION

Bacterial infections represent one of the leading causes of mortality worldwide,¹ whereby most infections occur through contaminated water, food and bodily fluids. According to the World Health Organization, outbreaks and epidemics related to bacterial infections, such as *Escherichia coli* (*E. coli*), meningococcal diseases or sexually transmitted infections (STIs) are frequently reported on all continents.²⁻⁴ Although numerous reports for the electrochemical detection of bacteria have been published,⁵⁻⁸ no point-of-care device has entered the global market yet, due to the challenge of combining a rapid detection mechanism with sensitivity, selectivity, cost-efficiency, and a facile handling procedure.

Over the past two decades, it has become evident that electrochemical sensing methods can not only overcome the limitation of both culture and biochemical methods in terms of assay time, sensitivity and specificity, but furthermore offer more convenience of handling, as this technology can be implemented into a handheld device where technical training becomes minimal. One of the key advantages of electrochemical sensing for bacteria is its sensitivity, and detection limits of one to only a few culture forming units (CFU) for different bacteria have been achieved in the literature.⁹⁻¹³

In general an electrochemical current on-signal,¹⁴⁻¹⁶ based on the direct measurement of bacterial metabolites, compares favorably to an off-signal,¹⁷ which can be obtained by impedance spectroscopy and is often prone to false positive results. The aspect of selectivity is commonly addressed through the use of nanomaterials,¹⁸⁻²¹ wherein the potential risk of nanomaterial release into the environment should not be neglected.²² The development of paper/plastic based substrates for electrochemical detection of pathogens presents an inexpensive and easy-to-handle alternative to standard electrodes.^{10-11, 23}

Herein, we report a fully functional electrochemical detection assay for the recognition of pathogenic bacteria, which addresses all of the above mentioned criteria. Using the examples of *E. coli* and *Neisseria gonorrhoeae* (*N. gonorrhoeae*) bacteria, we demonstrate the versatility of the proposed recognition strategy across cell lines and it is suggested that the outlined principle can be adapted to any organism expressing cytochrome c oxidase. Due to its significant impact on the food industry in correlation with food contamination and food related illnesses²⁴, *E. coli* represents an ideal target for the proof-of-concept of our electrochemical sensing principle. Furthermore, we address the health issue of STIs, which

accounts for about 340 million new infections annually worldwide⁴ and the number of infected individuals is increasing every year, particularly in North America²⁵ and Europe.³ People of all ages can be affected, and since STIs are highly contagious even at early stages, and their reoccurrence rate is high if not thoroughly cured,²⁶ an early, reliable and accurate method of diagnosis is crucial. One of the current analysis techniques for urine or blood samples is the long and old established method of cell culture, which is easy to handle, but holds a delay of three to four days until a conclusive diagnosis can be reached.²⁷ Modern methods, such as polymerase chain reaction (PCR) or ligase chain reaction, reduce the analysis time, but involve difficult and expensive equipment and material.²⁸ Due to these limitations in STI detection and diagnostics, an accessible and cost-effective technology to detect STIs is an urgent need in public health monitoring. We have recently shown that the use of N,N,N',N'-tetramethyl-para-phenylene-diamine (TMPD) during chronoamperometry measurements on pathogenic bacteria led to a quantification of cytochrome c oxidase activity in *E. coli* and *Bacillus subtilis*, which could be seen for the first time in aerobically grown *E. coli* bacteria.¹⁶ This sensing principle is based on the expression of cytochrome c oxidase in target bacteria, oxidizing TMPD, which is then regenerated at an electrode. An enhanced electrochemical current thereby indicates the presence of bacteria. However, bacteria could not be detected in solution and had to be dropcasted onto the electrode surface in order to achieve measurable electrochemical signals. The present manuscript overcomes these limitations, by combining immunohistochemistry and electrochemical sensing to provide the concept for a potential electrochemical sensing device. Herein we develop the previous non-selective chemical principle¹⁶ into a fully applicable selective detection method for medically pertinent organisms at clinically relevant concentrations. We show the selective immobilization of target bacteria to gold macroelectrodes by employing thiol chemistry and antibody binding, allowing the subsequent sensitive quantification of cytochrome c oxidase activity within seconds. This allows the specific detection of organisms in solution, which is a crucial aspect for a biosensing device. The presented detection of bacteria based on an electrochemical on-signal thereby reduces the risk of false positive results, compared to off-signals which are observed due to blockage of the electroactive surface of the electrode by bacteria or any kind of impurities. Furthermore, the direct interaction of the redox species with bacteria greatly reduces the chance of interferences and reducing the costs of the experimental equipment, as expensive instrumentation, such as for impedance spectroscopy is not required. The presented analytical detection method was transferred onto screen printed electrodes (SPEs) by evaluating several commercially available sensing platforms, allowing the possibility of point-of-care applications. In addition, a limit of detection for *E. coli* is presented and the applicability to the detection of *N. gonorrhoeae* is illustrated.

EXPERIMENTAL SECTION

Chemical reagents. All chemicals were purchased from Sigma-Aldrich, if not indicated otherwise. Phosphate buffered saline (PBS, 0.17 M) solution was prepared by adding 8 g sodium chloride ($\geq 99\%$), 0.2 g potassium chloride ($\geq 99\%$), 1.44 g sodium phosphate dibasic ($\geq 99\%$), 0.24 g potassium phosphate dibasic ($\geq 99\%$) and was completed to 1 L using nanopure water with a resistivity not less than 18.2 M Ω cm at 25°C (Millipore water purification system). N,N,N',N'-

tetramethyl-paraphenylene-diamine tetrafluoroborate (TMPD-BF₄) was prepared following the method of Yamauchi et al.²⁹ Crystals were characterized by their brownish purple appearance and a melting point of 125 to 127°C.

Immobilization of Bacteria. The number of bacteria in solution was determined by optical density at a wavelength of 600 nm (*E. coli*³⁰ OD₆₀₀ of 1.0 = 8.0 x 10⁸ cells mL⁻¹; *A. faecalis*³¹ OD₆₀₀ of 0.14 = 3.0 x 10⁷ cells mL⁻¹). The capture of bacteria at gold electrodes, following the procedure by Maalouf et al.,³² was carried out by the formation of self-assembled monolayers (SAMs) on the gold substrates. HSCH₂[CH₂]₉CH₂NHCOBiotin (ProChimia Surfaces, Poland) was used as biotinylated thiol and octanethiol functioned as a spacer. In a 1:1 mix of ethanol and chloroform, 0.2 mM of biotin-thiol and 0.8 mM of spacer was mixed and exposed to gold electrodes for about 24 hr at room temperature. Electrodes were washed thoroughly with PBS by carefully immersing the electrodes multiple times in solution. To block any unspecific binding sites electrodes were exposed for 30 min to 1 μ M bovine serum albumin (BSA) in nanopore H₂O and again washed with PBS. Incubation in 10 μ M neutravidin in nanopure H₂O for 45 min (ThermoFisher, USA) resulted in the replacement of BSA at the biotinylated thiols due to the high affinity of avidin for biotin, with a disassociation constant of 10-15 M.³³ A 1:200 dilution of a commercially available biotin-tagged polyclonal anti-*E. coli* antibody (GeneTex, USA, GTX40765) was prepared in PBS and exposed to the electrodes for 45 min to achieve its binding to neutravidin. Incubation with a cell suspension while gently tapping the vial occasionally for 45 min at 37°C resulted in the immobilization of target bacteria on the surface of the electrodes.

Electrochemical Measurements. Gold macroelectrodes (Basi, USA, 3 mm diameter) were employed as working electrodes and an in-house fabricated gold microelectrode with a diameter of 6.9 μ m was used to determine the concentration of TMPD-BF₄ in solution by cyclic voltammetry.¹⁶ Working electrodes were polished prior to experiments using a water-alumina mix (1.0, 0.3 and 0.05 μ m, 30 seconds for each grade) on microcloth polishing pads (Buehler, USA).³⁴ After polishing, macroelectrodes were sonicated for approximately 2 min to remove alumina powder from the electroactive surface. A platinum mesh and a saturated calomel electrode were used as counter and reference electrode, respectively. All bioelectrochemical experiments were carried out in a thermostated (37°C) electrochemical cell inside a Faraday cage, using a modular potentiostat (PGSTAT302N, Autolab, UK).

Chronoamperometry was carried out respecting a delay time of 45 s after the electrode was brought in contact with the TMPD-BF₄ solution to maximize the electrochemical response of the target bacteria.¹⁶ Hence, following an open circuit potential for 45 s, an oxidative potential of 250 mV was applied for 5 s, followed by a potential step to -150 mV for 5 s. All potentials are indicated vs. a saturated calomel reference electrode (SCE).

RESULTS AND DISCUSSION

Immobilization of target bacteria on gold substrates and electrodes. To enable the electrochemical detection of specific bacteria, gold substrates, macroelectrodes as well as screen printed electrodes (SPEs) were modified by thiol-chemistry, following the method of Maalouf et al.³² As shown in Figure 1A, a biotin-thiol and spacer-thiol self-assembled monolayer (SAM) was formed on a gold surface, employing HSCH₂[CH₂]₉CH₂NHCOBiotin and octanethiol as biotinylated

thiol and spacer, respectively. Following the blockage of unspecific binding sites by bovine serum albumin (BSA), the incubation with neutravidin resulted in the replacement of BSA at the biotinylated thiols. A commercially available polyclonal anti-*E. coli* antibody was added, binding to neutravidin, and

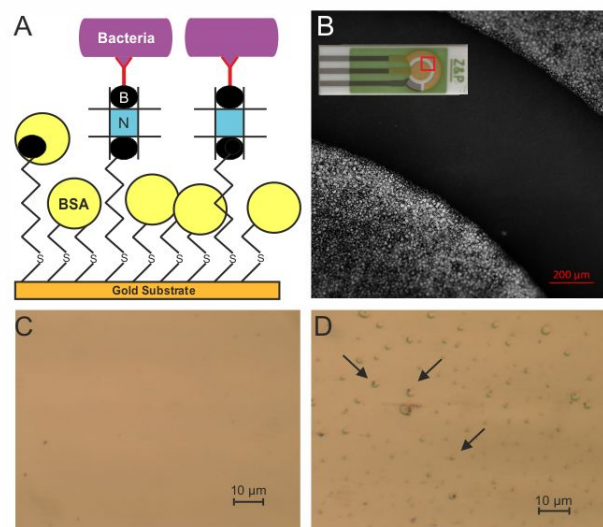


Figure 1. Surface modification of gold substrates and electrodes. A) Schematic representation of surface modifications. Self-assembled monolayers of biotinylated thiols, capped with BSA, allows binding of neutravidin (N), with high affinity to a biotinylated antibody (red), which captures target bacteria. B) Fluorescent imaging of different substrates on an SPE containing immobilized biotinylated fluorescent beads instead of an antibody, illustrating the precision of the presented electrode modification method. C) Optical micrographs of a bare gold substrate compared to D) a fully functionalized gold surface with immobilized *E. coli* bacteria. Arrows pointing to three immobilized bacteria as examples.

immobilizing target bacteria on the substrate during incubation with a cell suspension. Optical micrographs shown in Figure 1D demonstrate the successful immobilization of *E. coli* bacteria on a fully functionalized gold substrate compared to a control surface, lacking the SAM (Fig. 1C). The accuracy of the presented electrode modification method and its applicability to screen printed electrodes (SEPs) was demonstrated by replacing the antibody by biotinylated fluorescent beads (Fig. 1B). Fluorescence microscopy reveals the successful binding of beads at the gold surface of a fully functionalized SPE. The rapid and sensitive detection of pathogens by electrochemistry based on the binding of specific target bacteria on gold electrodes is described in the following.

Electrochemical recognition of bacteria and control measurements. Our previous studies reported the recognition of bacterial cytochrome c oxidase using the compound N,N,N',N'-tetramethyl-paraphenylene-diamine (TMPD) by electrochemistry.¹⁶ The oxidation of TMPD consists of two electron transfer reactions (Supplementary Fig. 1). At the first oxidation step, the radical cation TMPD^{•+} is formed, which has a characteristic blue color³⁵, as seen in the oxidase test in cell culture applications. This step is highly reversible, both chemically and electrochemically, whereas the product of the second electron transfer is unstable and decomposes quickly. Due to the rapid oxidation of TMPD by atmospheric oxygen, the radical cation salt N,N,N',N'-tetramethyl-paraphenylene-

diamine tetrafluoroborate (TMPD-BF₄) was synthesized according to the procedure of Yamauchi et al.,²⁹ and exposed to bacteria, dropcasted onto gold macroelectrodes. Due to the expression of cytochrome c oxidase, the immobilized bacteria are capable of oxidizing TMPD to its radical cation TMPD^{•+}, which is then regenerated to TMPD at the macroelectrode, resulting in a statistically significant increase in electrochemical current in the presence of the bacteria.¹⁶ The electrochemical detection of specific pathogens was not addressed in our previous publication and is presented in the following.

As a proof-of-concept *E. coli* bacteria were immobilized onto a gold macroelectrode by exposing a functionalized macroelectrode to a bacteria suspension at a concentration of 10⁹ cells per mL for 45 min at 37°C. It should be noted that the fractions of living and dead bacteria in solution was not determined in order to mimic the conditions of a real sample, such as urine. Hence, we purposely use the term “cells per mL” instead of “culture forming units (CFU)”. In this study, six bare gold macroelectrodes function as control sensors. As shown in Figure 2A, in the presence of *E. coli* bacteria, a significant increase in electrochemical current was recorded. This increase is attributed to the oxidation of TMPD by the bacteria, as no significant change in current is observed at a functionalized electrode in the absence of *E. coli* (Supplementary Fig. 2), excluding potential influences on the electrochemical current signal by the SAM. Furthermore, all individual chemical compounds of the SAM were tested for their electroactivity and found not to result in a faradaic current, as shown in Supplementary Figure 3.

To assess the specificity of the antibody for the *E. coli* detection, *Alcaligenes faecalis* (*A. faecalis*) bacteria were employed as control organisms. *A. faecalis* are Gram-negative rod-shaped bacteria, which can be found in soil, water and the general environment of humans.³⁶ These bacteria express cytochrome c oxidase, which can be electrochemically detected when organisms are dropcasted onto a macroelectrode, as shown in Figure 2B. Exposing a macroelectrode containing *A. faecalis* to a 2 mM TMPD-BF₄ solution while applying a potential at the electrode exceeding the formal potential for the reduction of TMPD^{•+}, an increase of electrochemical current is observed. A turnover number of 6.4 × 10⁸ L mol⁻¹ s⁻¹ was determined, representing a 70% increase of cytochrome c oxidase activity compared to aerobic *E. coli* as reported previously.¹⁶ Please see supporting information for detailed calculations. However, when incubating a modified electrode containing an anti-*E. coli* antibody to *A. faecalis*, no significant current increase was observed, demonstrating the specificity of the method for the detection of

E. coli, as no *A. faecalis* can bind to the surface of the macroelectrode (Fig. 2C). These control studies demonstrate that the electrode modifications do not compromise the electrochemical concept behind the recognition of specific target bacteria at macroelectrodes and the specific detection of *E. coli* can be achieved within seconds after bacteria immobilization.

Specific detection of pathogenic bacteria at SPEs. To transfer the presented concept to substrates suitable for the operation within a portable handheld device, the applicability of the proposed methodology was tested on commercial disposable SPEs, purchased from Zimmer&Peacock. Prior to the formation of SAMs, redox chemistry of TMPD was carried out. It should be noted that all SPEs were used as received, without the need for polishing or pre-treatment prior to the

electrochemical studies. Sensors provided by Zimmer&Peacock revealed a stable electrochemical behavior during voltammetry. Next to a clean and stable electrochemistry, SPEs by Zimmer&Peacock were easy to handle and withstood organic solvents easily. Other SPEs were

evaluated, but did not compare favorably with SPEs from Zimmer&Peacock, which were selected for further studies.

To determine a detection limit for the recognition of *E. coli* at SPEs, functionalized SPEs were exposed to cell suspensions ranging from 5×10^3 to 1×10^7 cells per mL (Fig. 3A) and incubated for 45 min at 37°C . Following incubation, chronoamperometric measurements revealed a detection limit of 10^5 cells per mL (Supplementary Fig. 4). The electrochemical measurements at all concentrations were performed in triplicates and are shown as average with an error bar representing three times the standard deviation. Three functionalized SPEs in the absence of bacteria functioned as controls, whereby their average is represented as dotted line in figure 3A.

The detection limit is recorded as bacteria concentration, which results in an electrochemical current exceeding three times the standard deviation for each condition. Interestingly, recent reports suggest urinary tract infections diagnosed with a CFU value of less than 10^5 per mL resulted in the inappropriate use of antibiotics³⁷, as the human immune system usually copes well with the exposure to pathogens below this concentration. For the development of biosensors, it is recommended to focus less on reaching detection limits below 10^5 CFU, but rather focus on selective, rapid, easy to handle, portable and compact devices.⁸ The sensibility of the presented methodology therefore sits at the immediate threshold for clinically significant bacterial concentrations and directly addresses current sensing needs. To illustrate the convenience of the proposed method the reusability of single sensors for the recording of both baseline and bacterial signal was assessed. A freshly prepared functionalized SPE was exposed to TMPD- BF_4 during chronoamperometry. The same sensor was reused after one hour of incubation in PBS (Supplementary Fig. 5A). No degradation in the electrochemical current was observed, demonstrating the stability of the electroactive surface area. Furthermore, two individual functionalized SPEs were employed to record the electrochemical signal before the incubation with *E. coli* and the same sensors were reused to detect immobilized *E. coli* bacteria after a 45 min exposure to a cell suspension of 10^6 cells per mL at 37°C . As shown in Supplementary Figure 5B for two individual SPEs, the presence of *E. coli* bacteria resulted in a significant current increase. These results demonstrate that a single SPE is sufficient to record both the initial baseline current as well as the electrochemical current signal as a result of bacteria immobilization at the surface of the SPE.

Our findings using the example of the pathogen *E. coli* illustrate the usefulness of the proposed procedure for the detection of contaminations in the food industry and clinical applications in connection with *E. coli*. To widen the impact of the technology within the medical sector, the *E. coli* antibody was replaced by a polyclonal anti-*N. gonorrhoeae* antibody. Figure 3B shows chronoamperometry in 2.0 mM TMPD- BF_4 , carried out at a bare SPE, an SPE containing the SAM layer including the anti-*N. gonorrhoeae* antibody, as well as a fully functionalized SPE after exposure to 10^7 cells per mL *N. gonorrhoeae* in solution for 45 min at 37°C . A significantly enhanced electrochemical current was recorded in the presence of *N. gonorrhoeae*. A turnover number of $1.2 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ was determined, which corresponds to about 200% of the value obtained for *A. faecalis*. The straightforward substitution of antibodies and successful recognition of specific target bacteria demonstrates the

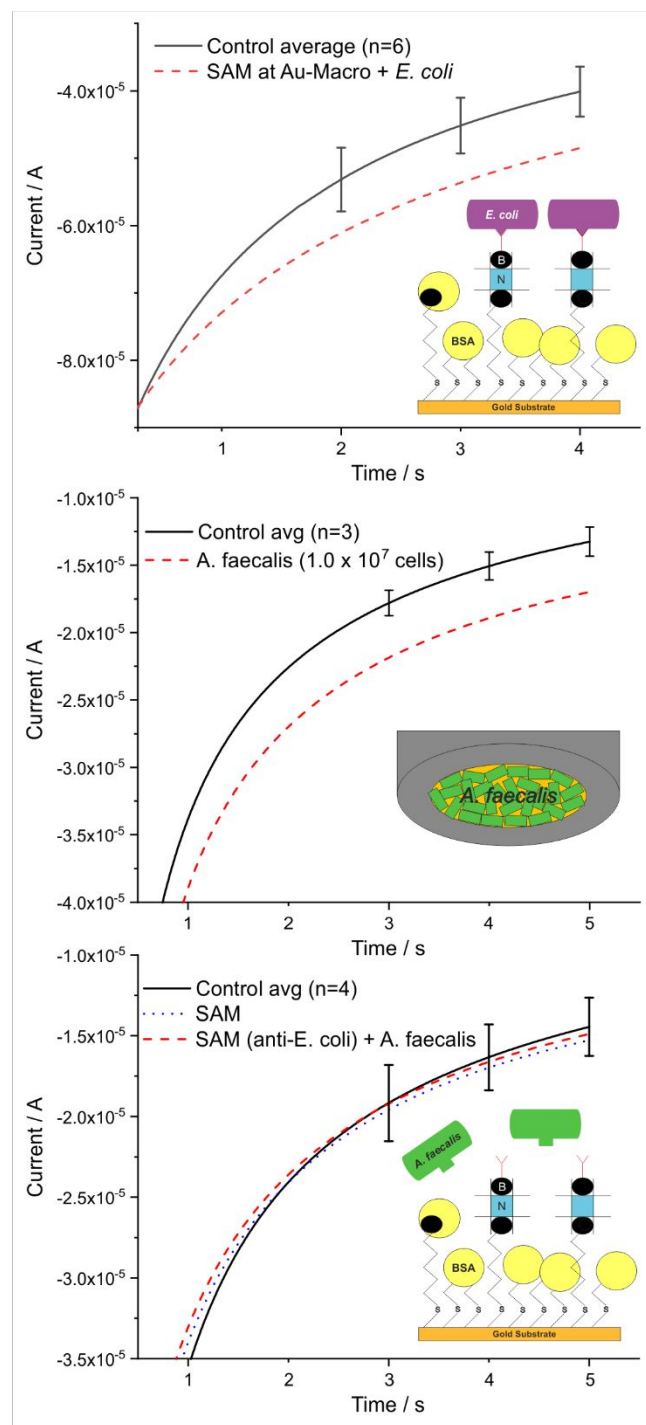


Figure 2. *E. coli* recognition and control studies. A) An electrochemical current increase is observed following the binding of *E. coli* to a fully functionalized macroelectrode (red, dashed line), compared to a bare electrode (black, full line). B) Dropcasted *A. faecalis* bacteria are detected at a macroelectrode shown as increase in electrochemical current (red, dashed line). C) The exposure of *A. faecalis* to a functionalized electrode containing an anti-*E. coli* antibody does not result in an enhanced electrochemical current signal (red, dashed line) compared to control electrodes (black, full line = bare electrode, blue, dotted line = modified electrode in the absence of bacteria). All error bars are representing three times the standard deviation.

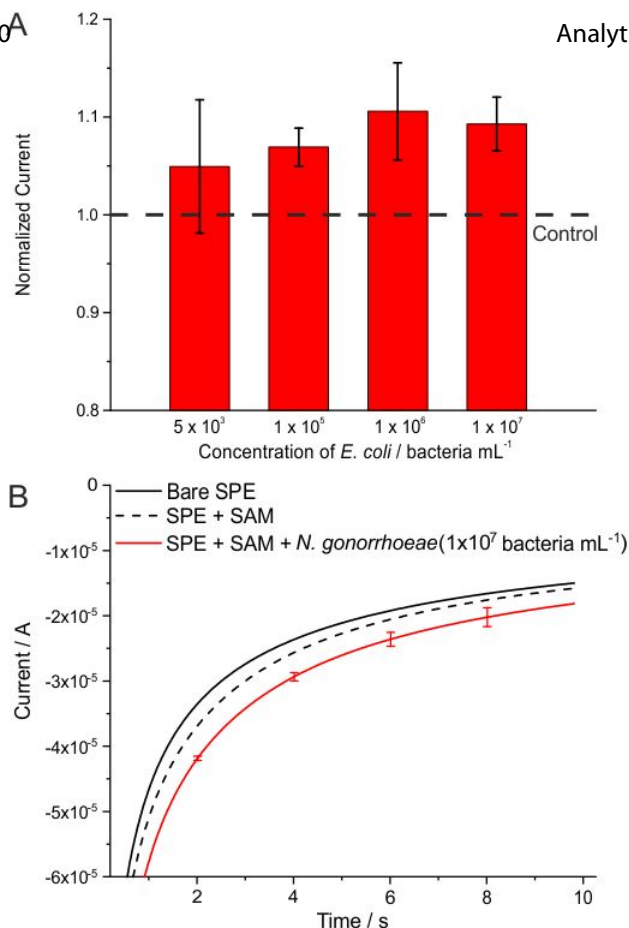


Figure 3. Detection of bacteria at SPEs. A) Detection of various *E. coli* concentrations, revealing a detection limit of 1×10^5 bacteria mL⁻¹. B) Detection of *N. gonorrhoeae* at functionalized SPEs. Immobilized bacteria result in a significantly enhanced electrochemical current (red), in contrast to a blank electrode (black, full line) and a functionalized sensor, in the absence of bacteria (black, dotted line). Error bars are representing three times the standard deviation.

versatility of the proposed method. Given the generality of the biochemical reaction, by exchanging the target specific antibody, such biosensors could be envisioned for a large number of different pathogenic bacteria that exhibit cytochrome c oxidase, including species related to food contaminations, sexually transmitted infections, and other diseases. Furthermore, the use of disposable SPEs in combination with an antibody reduces the current costs of diagnosis to under \$5.00 per test, as, SPEs are produced at high volumes for less than \$1.00 and a volume of 500 μ l of antibody is sufficient to produce about 500 to 600 functionalized SPE sensors.

CONCLUSION

Herein we report an electrochemical method for the sensitive, rapid, selective, cost-efficient and easy-to-perform detection of specific bacteria. Using the examples of *E. coli* and *N. gonorrhoeae*, the detection of pathogens was achieved at clinically relevant concentrations. The immobilization of target bacteria on SPEs by thiol-chemistry and antibody binding can be applied to a large diversity of bacteria that exhibit cytochrome c oxidase, enabling the specific detection of pathogens within seconds by electrochemistry. As pathogens are successfully detected at inexpensive SPEs, which are commonly used for portable potentiostatic devices, results can in principle be obtained with the push of a button. Furthermore, the proposed use of polyclonal antibodies is ideal to provide a first alert for a contamination or infection with a suspected bacteria type, which can then initiate the analysis of samples by more rigorous techniques. This approach will find broad application, such as in the food industry, as well as veterinary and human medical health services.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Correspondence should be addressed to R.G.C. (richard.compton@chem.ox.ac.uk).

Author Contributions

The manuscript was written through contributions of all authors. S.K. designed the research, conducted experiments, analyzed data and wrote the manuscript. R.A.S.C. conducted experiments and prepared figures. R.M.E. contributed *E. coli* and *A. faecalis* cultures and H.L. provided *N. gonorrhoeae* bacteria. C.C.T. provided funding, materials and facilities for *N. gonorrhoeae* cultures. R.G.C. directed the research, is the corresponding author and wrote the manuscript. All authors have given approval to the final version of the manuscript.

Funding Sources

European Commission under the Marie Curie Programme (grant number 702009)
Portuguese Fundação para a Ciência e a Tecnologia (PD/BD/127797/2016)
UK Biological and Biotechnology Sciences Research Council (Grant BB/N006321/1 awarded to Prof. Fraser A. Armstrong)
Wellcome Trust Senior Investigator award to C.M.T. (Grant 102908/Z/13/Z)

ACKNOWLEDGMENT

S.K. thanks the support from the European Commission under the Marie Curie Programme (grant number 702009). The contents reflect only the authors' views and not the views of the European Commission. RASC acknowledges the Portuguese Fundação para a Ciência e a Tecnologia for her PhD fellowship (PD/BD/127797/2016) of the PhD Programme in Medicines and Pharmaceutical Innovation (i3DU). RME is supported by the UK Biological and Biotechnology Sciences Research Council (Grant BB/N006321/1 awarded to Prof. Fraser A. Armstrong). H.L. acknowledges the Wellcome Trust Senior Investigator award to C.M.T. (Grant 102908/Z/13/Z). All authors thank Dr

Fraser A. Armstrong for providing facilities and resources for the culture of *E. coli* and *A. faecalis* bacteria.

REFERENCES

- (1) Department of Health and Social Care. Annual Report of the Chief Medical Officer: Infections and the rise of antimicrobial resistance; 2011. Accessed 10.12.2018.
- (2) World Health Organization (WHO), Emergencies preparedness, response (<https://www.who.int/csr/don/archive/disease/en/>). Accessed 07.12.2018.
- (3) World Health Organization/Europe. Report: Centralized Information System for Infectious Diseases (CISID). 2014. Accessed 21.10.2016
- (4) Satterwhite, C. L.; Torrone, E.; Meites, E.; Dunne, E. F.; Mahajan, R.; Cheryl Bañez Ocfemia, M.; Su, J.; Xu, F.; Weinstock, H. *Sexually Transmitted Diseases* **2013**, 40, 187-193.
- (5) Monzó, J.; Insua, I.; Fernandez-Trillo, F.; Rodriguez, P. *Analyst* **2015**, 140, 7116-7128.
- (6) Kokkinos, C.; Economou, A.; Prodromidis, M. I. *Trends in Analytical Chemistry* **2016**, 79, 88-105.
- (7) Justino, C. I. L.; Duarte, A. C.; Rocha-Santos, T. A. P. *Trends in Analytical Chemistry* **2016**, 85, 36-60.
- (8) Kuss, S.; Amin, H. M. A.; Compton, R. G. *Chemistry – An Asian Journal* **2018**, 13, 2758-2769.
- (9) Bekir, K.; Barhoumi, H.; Braiek, M.; Chrouda, A.; Zine, N.; Abid, N.; Maaref, A.; Bakhrouf, A.; Ouada, H. B.; Jaffrezic-Renault, N.; Mansour, H. B. *Environmental Science and Pollution Research* **2015**, 22, 15796-15803
- (10) Bhardwaj, J.; Devarakonda, S.; Kumar, S.; Jang, J. *Sensors and Actuators, B: Chemical* **2017**, 253, 115-123.
- (11) Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S. *Analytical Chemistry* **2017**, 89, 3613-3621
- (12) Primiceri, E.; Chiriaco, M. S.; De Feo, F.; Santovito, E.; Fusco, V.; Maruccio, G. *Analytical Methods* **2016**, 8, 3055-3060.
- (13) Thiha, A.; Ibrahim, F.; Muniandy, S.; Dinshaw, I. J.; Teh, S. J.; Thong, K. L.; Leo, B. F.; Madou, M. *Biosensors and Bioelectronics* **2018**, 107, 145-152.
- (14) Thet, N. T.; Jenkins, A. T. A. *Electrochemistry Communications* **2015**, 59, 104-108.
- (15) Elliott, J.; Simoska, O.; Karasik, S.; Shear, J. B.; Stevenson, K. J. *Analytical Chemistry* **2017**, 89, 6285-6289.
- (16) Kuss, S.; Tanner, E. E. L.; Ordovas-Montanes, M.; Compton, R. G. *Chemical Science* **2017**, 8, 7682-7688.
- (17) Lee, J. Y.; Kim, B. K.; Kang, M.; Park, J. H. *Scientific Reports* **2016**, 6:30022, 1-6.
- (18) Yamada, K.; Choi, W.; Lee, I.; Cho, B. K.; Jun, S. *Biosensors and Bioelectronics* **2016**, 77, 137-143.
- (19) Yang, Z.; Wang, Y.; Zhang, D. *Biosensors and Bioelectronics* **2017**, 98, 248-253.
- (20) Sepunaru, L.; Tschulik, K.; Batchelor-McAuley, C.; Gavish, R.; Compton, R. G. *Biomaterials Science* **2015**, 3, 816-820.
- (21) Ye, W.; Guo, J.; Bao, X.; Chen, T.; Weng, W.; Chen, S.; Yang, M. *Materials* **2017**, 10, 603-614.
- (22) Nowack, B.; Bucheli, T. D. *Environmental Pollution* **2007**, 150, 5-22.
- (23) Liu, H.; Zhou, X.; Liu, W.; Yang, X.; Xing, D. *Analytical Chemistry* **2016**, 88, 10191-10197.
- (24) McClure, P. *World Journal of Microbiology and Biotechnology* **2000**, 16, 749-755.
- (25) Martin, I.; Jayaraman, G.; Wong, T.; Liu, G.; Gilmour, M. *Sexually Transmitted Diseases* **2011**, 38, 892-898.
- (26) Low, N.; Broutet, N.; Adu-Sarkodie, Y.; Barton, P.; Hossain, M.; Hawkes, S. *Lancet* **2006**, 368, 2001-2016.
- (27) Singh, R.; Dhand, C.; Sumana, G.; Verma, R.; Sood, S.; Gupta, R. K.; Malhotra, B. D. *Journal of Molecular Recognition* **2010**, 23, 472-479.
- (28) Lee, S. R.; Chung, J. M.; Kim, Y. G. *Journal of Microbiology* **2007**, 45, 453-459.
- (29) Yamauchi, J.; Fujita, H. *Bulletin of the Chemical Society of Japan* **1990**, 63, 2928-2932.
- (30) Agilent, T., *E.coli Cell Culture Concentration from OD600*. **2017**. (<http://www.genomics.agilent.com/biocalculators/calcODBacteria.jsp>). Accessed 01.06.2018.
- (31) Yamamoto, K.; Oishi, K.; Fujimatsu, I.; Komatsu, K. I. *Applied and Environmental Microbiology* **1991**, 57, 3028-3032.
- (32) Maalouf, R.; Fournier-Wirth, C.; Coste, J.; Chebib, H.; Saïkali, Y.; Vittori, O.; Errachid, A.; Cloarec, J.-P.; Martelet, C.; Jaffrezic-Renault, N. *Analytical Chemistry* **2007**, 79, 4879-4886.
- (33) Green, N. M. *Biochemical Journal* **1963**, 89, 580-585.
- (34) Cardwell, T. J.; Mocak, J.; Santos, J. H.; Bond, A. M. *Analyst* **1996**, 121, 357-362.
- (35) Michaelis, L.; Schubert, M. P.; Granick, S. *Journal of the American Chemical Society* **1939**, 61, 1981-1992.
- (36) Bizet, J.; Bizet, C. *Journal of Infection* **1997**, 35, 167-169.
- (37) Kwon, J. H.; Fausone, M. K.; Du, H.; Robicsek, A.; Peterson, L. R. *American Journal of Clinical Pathology* **2012**, 137, 778-784.

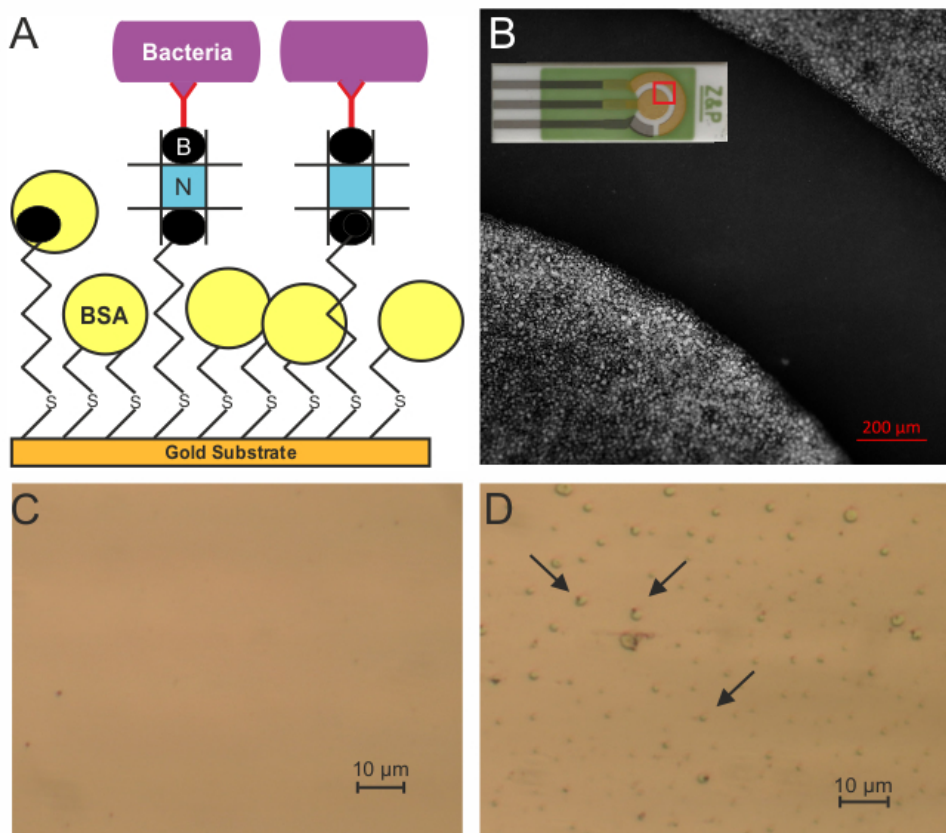


Figure 1. Surface modification of gold substrates and electrodes. A) Schematic representation of surface modifications. Self-assembled monolayers of biotinylated thiols, capped with BSA, allows binding of neutravidin (N), with high affinity to a biotinylated antibody (red), which captures target bacteria. B) Fluorescent imaging of different substrates on an SPE containing immobilized biotinylated fluorescent beads instead of an antibody, illustrating the precision of the presented electrode modification method. C) Optical micrographs of a bare gold substrate compared to D) a fully functionalized gold surface with immobilized *E. coli* bacteria. Arrows pointing to three immobilized bacteria as examples.

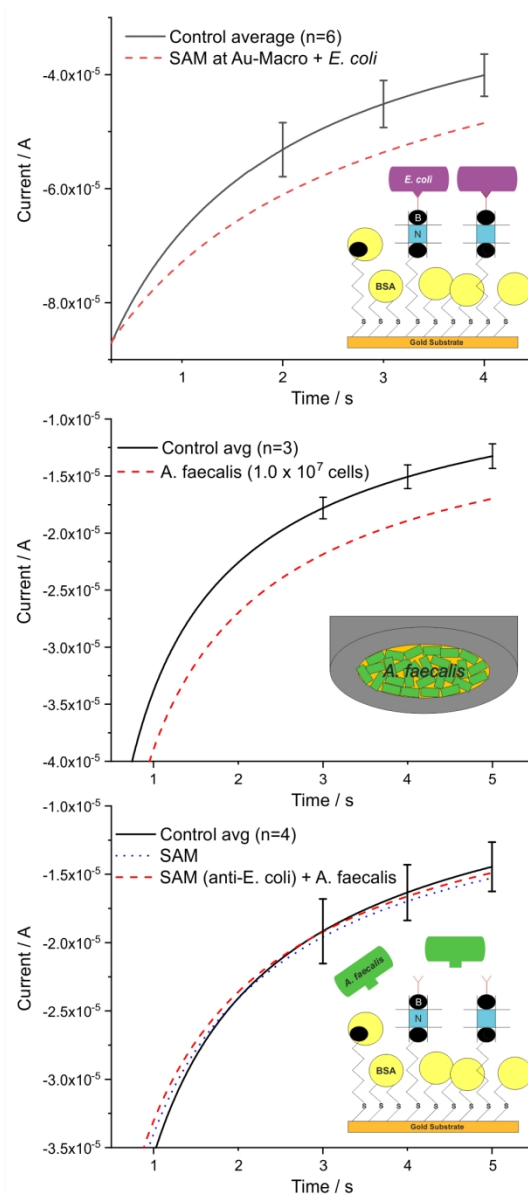


Figure 2. *E. coli* recognition and control studies. A) An electrochemical current increase is observed following the binding of *E. coli* to a fully functionalized macroelectrode (red, dashed line), compared to a bare electrode (black, full line). B) Dropcasted *A. faecalis* bacteria are detected at a macroelectrode shown as increase in electrochemical current (red, dashed line). C) The exposure of *A. faecalis* to a functionalized electrode containing an anti-*E. coli* antibody does not result in an enhanced electrochemical current signal (red, dashed line) compared to control electrodes (black, full line = bare electrode, blue, dotted line = modified electrode in the absence of bacteria). All error bars are representing three times the standard deviation.

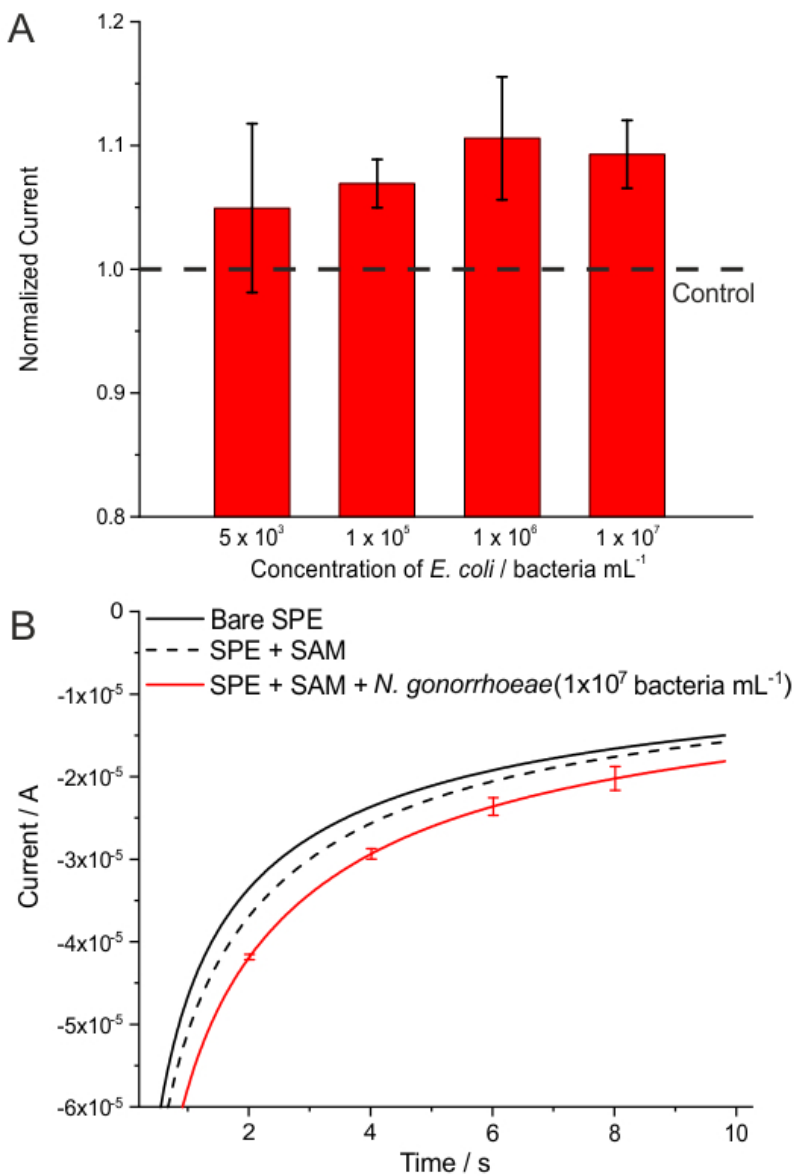


Figure 3. Detection of bacteria at SPEs. A) Detection of various *E. coli* concentrations, revealing a detection limit of 1 x 10⁵ bacteria mL⁻¹. B) Detection of *N. gonorrhoeae* at functionalized SPEs. Immobilized bacteria result in a significantly enhanced electrochemical current (red), in contrast to a blank electrode (black, full line) and a functionalized sensor, in the absence of bacteria (black, dotted line). Error bars are representing three times the standard deviation.

