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Entrapment of uropathogenic *E. coli* cells into ultra-thin sol-gel matrices on gold thin films: A low cost alternative for impedimetric bacteria sensing

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ABSTRACT

Bacterial infections are causing worldwide morbidity and mortality. One way to limit infectious outbreaks and optimize clinical management of infections is through the development of fast and sensitive sensing of bacteria. Most sensing approaches are currently based on immunological detection principles. We report here on an impedimetric sensor to selectively and sensitive detect uropathogenic *E. coli* cells (*E. coli* UTI89) using artificial recognition sites. We show here the possibility to imprint the rod-shape structure of *E. coli* UTI 89 into ultra-thin inorganic silica coatings on gold electrodes in a reproducible manner. A linear range from to 1×10^0 –1×10^4 cfu mL^{-1} is obtained. With a detection limit for *E. coli* UTI89 below 1 cfu mL^{-1} from five blank signals (95% confidence level) and excellent selective binding capabilities, these bacterial cell imprinted electrodes brings us closer to a low cost specific bacterial recognition surfaces.

1. Introduction

Specific and sensitive detection of pathogenic microorganisms remains a scientific challenge and a problematic of high importance (Dye, 2014). Pathogen diagnosis is currently based on microorganism culturing on agar plates, being however often too long and consequently inadequate in hospital settings. Biochemical assays, molecular techniques and immunological-based methods have been proposed as alternatives (Law et al., 2014). The heterogeneous distribution and low concentration of pathogens remain challenging for these methods with detection limits of 10^4 to 10^5 colony forming units per milliliter (cfu/mL) (Wang and Salazar, 2016). Thus the need for the development of more rapid and less laborious methods becomes an urgent matter. Immobilization of pathogen bioreceptors such as antibodies onto conductive transducers proved of particular advantage for a fast, selective and quantitative read out (Amiri et al., 2018). Besides being costly, antibody based sensing devices lack the stability required to detect pathogenic species in harsh environmental conditions. There are currently no adequate solutions that allow the selective and sensitive binding to specific microorganism, that are fast in detection and screening, cheap to implement, and able to be conceptualized for a wide range of biologically relevant targets.

The main objective of this work is to develop a pathogen detection strategy that does not require expensive biological affinity agents and can be tailored at will to any microorganism with minimal change in the technological approach. We demonstrate here that imprinting *E. coli* cells into ultra-thin sol-gel film coatings allows for impedimetric pathogen detection with biologically relevant detection limits. Compared to imprinting into polymers where cross-linkers are needed (Schirhagl et al., 2010), the formation of thin imprinted organosilicate films with good diffusional penetration, important for sensing driven applications, is generally easier to achieve. Since the pioneering studies of Carturan et al. (2004) who encapsulated *S. cerevisiae* yeast cells into silica gel films and studied its catalytic activity, imprinting of whole cells into inorganic sol-gel matrices rather than polymers (Haupt, 2003) has become a widely proposed approach (Avnir et al., 2005; Eleftheriou et al., 2013; Fazal et al., 2017). These matrices have gained more interest lately for the creation of sensing devices (Borovicka et al., 2013; Chen et al., 2016). Next to porosity, nanometer-thick films are required (Kraus-Ophir et al., 2014) to allow efficient recapture of bacteria. In

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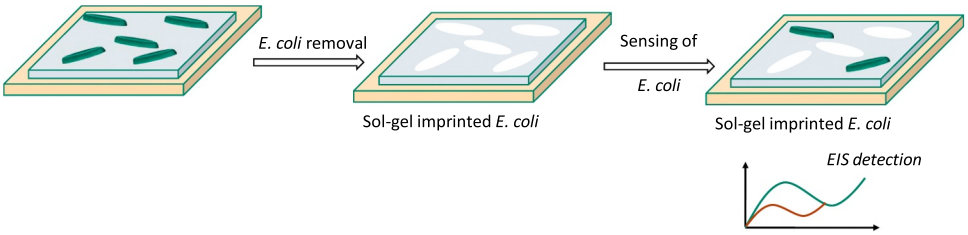

Scheme 1. Schematic steps of sol-gel imprinting bacteria on Au interface preparation, removal and sensing.

this work, we show that sol-gel imprinted E. *coli* films are also adapted for rapid and selective pathogen detection (Scheme 1).

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS), (3-mercaptopropyl)trimethoxysilane (MTMS) and (3-aminopropyl) triethoxysilane (ATES) were purchased from Sigma-Aldrich and used as received. Acetic acid, ethanol, hydrochloric acid (HCl), cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) were obtained from Merck.

2.2. Bacterial growth conditions

Escherichia coli UTI89 (wild-type strain) was used for imprinting. The cells were grown in LB/BHUI broth at 37 °C for 18–20 h with shaking at 150 rpm to reach an OD 600 nm of 0.6–1. The harvested cells were washed twice to remove the culture medium and then re-suspended by vortex for 7 min in 10 mL LC/MS Grade Water (Merck, Darmstadt, Germany) to reach 1×10^8 cfu mL^{-1}. *S. aureus* and *Pseudomonas aeruginosa* bacteria were grown in the same manner.

2.3. Imprinting of E. coli into organosilica films

The sol was prepared by mixing TEOS (1.83 mL) with MTMS (0.17 mL) in 1 mL of water followed by gently stirring for 10 min at room temperature. HCl (0.1 M, 0.25 mL) was added and the sol was stirred for 3 h, stored in ice and used soon after preparation. Bacteria imprinting was achieved by mixing fresh sol stock solution (0.5 mL) with fresh aqueous bacterial suspension (3 mL, 10^8–10^9 cfu mL^{-1}) and stirring for 2 min.

Gold coated glass slides were prepared by thermal evaporation of 5 nm of titanium and 50 nm of gold. Gold thin film electrodes were cleaned by subsequent washing in ethanol/acetone (1/1) for 15 min, distilled water and dried at 80 °C for 3 h. The cleaned electrodes were immersed vertically into the sol and removed after 30 s using a removal rate of 150 cm min^{-1} using a monitorized micromanipulator. Gelation occurred upon heating the interface at 40 °C for 8 h.

2.4. E. coli removal

The imprinted *E. coli* cells were removed from the dried films by rinsing the coated slides for 10 min in ethanol (70%) and washing with sterile double-distilled water two times. Thereafter, the surface was immersed into a solution of CTAB (2.5%) and further rinsing for 10 min with double-disstilled water.

2.5. Characterization

Scanning Electron Microscopy (SEM): SEM images were obtained using an electron microscope ULTRA 55 (Zeiss, France) equipped with a thermal field emission emitter and three different detectors (EsB detector with filter grid, high efficiency In-lens SE detector and Everhart-Thornley Secondary Electron Detector).

UV/Vis measurements: Absorption spectra were recorded using a Perkin Elmer Lambda UV–Vis 950 spectrophotometer in a 1-cm quartz cuvette. The wavelength range was 200–1100 nm.

SPR instrumentation: The surface plasmon resonance instrument used was an Autolab ESPRIT instrument (Eco Chemie, Utrecht, The Netherlands) working at 670 nm (Kooyman et al., 1991; Wink et al., 1998).

Profilometry: An optical profilometer (Zygo NewView 6000 Optical Profilometer with MetroPro software) with 1 nm height resolution was used for thickness measurements.

2.6 Electrochemical measurements

Electrochemical measurements were performed with an Autolab 20 potentiostat (Eco Chemie, Utrecht, The Netherlands). A conventional three-electrode configuration was employed using a silver wire and a platinum mesh as reference and auxiliary electrodes, respectively.

EIS experiments were carried out in an aqueous solution of a mixture of 10 mM $Fe(CN)_6^{4-}$/10 mM $Fe(CN)_6^{3-}$ in PBS (0.01 M) using the following parameters: amplitude of 10 mV at open circuit potential with a frequency range of 100 kHz-0.1 Hz. Impedance data were modeled using ZView2 software.

3. Results and discussion

3.1. Imprinting of E. coli cells into ultra-thin sol gel films on gold electrodes

A two-step sol-gel process is used for *E. coli* imprinting: a first low pH hydrolysis of a mixture of TEOS and MTMS, followed by neutral condensation-gelation step. MTMS was chosen as additive as the thiol function present in the sol-gel can promote the adhesion of the hydrophilic gel to the gold thin film electrode resulting in high quality layers. The thickness of the sol-gel film depends strongly on the sol-gel

Table 1
Influence of sol-gel precursor concentrations, dip time and immersion/removal time of gold interface on film thickness and charge transfer.

TEOS (mL)	MTMS (mL)	Dip time (Sec)	Immersion/removal rate (mm min^{-1})	d_{SPR} (nm)	$d_{prof.}$ (nm)	R_{CT} kΩ
0.92	**0.08**	**30**	**150**	**10 ± 1**	**9 ± 2**	**12.746 ± 0.345**
0.92	0.08	60	150	31 ± 2	34 ± 3	18.732 ± 0.567
0.92	0.08	30	200	11 ± 1	13 ± 2	12.45 ± 0.5678
0.92	0.08	60	200	30 ± 2	29 ± 2	17.235 ± 0.675
1.83	0.17	30	150	66 ± 1	68 ± 3	202.816 ± 0.342
1.83	0.17	60	150	96 ± 2	94 ± 4	391.548 ± 0.345

d_{SPR}: thickness form SPR measurements; $d_{prof.}$ thickness determined from profilometer measurements;

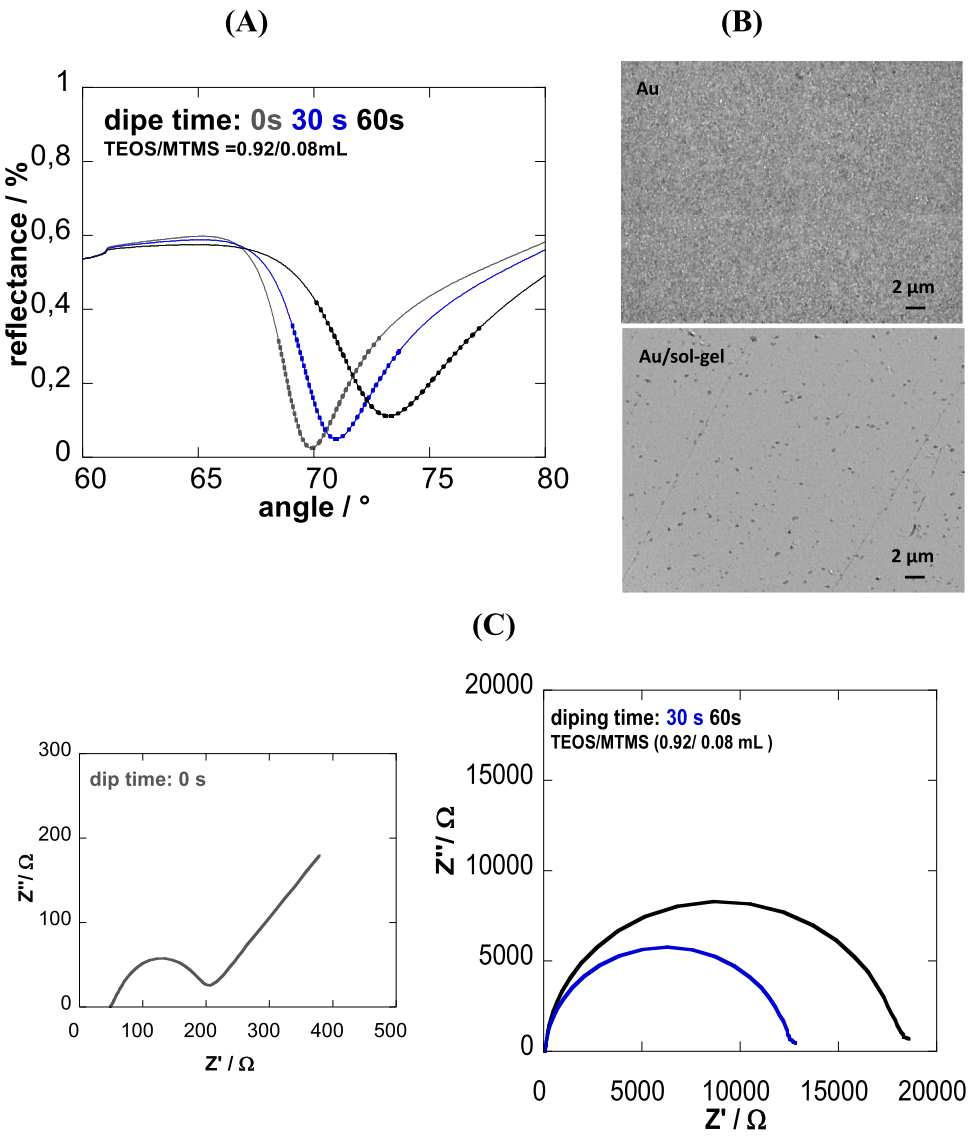

Fig. 1. (A) SPR curves of a gold electrode before (gray) and after coating with a sol-gel film upon dipping into TEOS/MTMS (0.92/0.08 mL) for 30 s (blue) and 60 s (black) using a removal rate of 150 mm cm^{-1}, full lines: fitted SPR curves (n = 1.52, λ = 670 nm, n_{Ti} = 2.36 +i3.47, d_{Ti} = 5 nm, n_{Au} = 0.197 + i3.67, d_{Au} = 50 nm, $n_{sol\text{-}gel}$ = 1.42 + i0.022), dotted lines: experimental curves; (B) SEM images of a gold surface before and after coating with TEOS/MTMS (0.92/0.08 mL) for 30 s; (C) Nyquist plots of a gold interface (before, dip time 0 s) and after coating with TEOS/MTMS (0.92/0.08 mL) for 30 s (blue) and 60 s (black) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

precursor concentrations, the length of dipping time of the gold thin electrode into the solution and removal rate of the electrode out of the sol (Table 1).

The concentration of TEOS/MTMS was most influencing the film thickness. Using a volume ratio of TEOS/MTMS of 1/0.09 mL and dipping the clean Au interface for 30 s into a fresh sol solution results in optimal sol-gel films of 10 ± 1 nm, as determined from shifts in the surface plasmon resonance angle and the reflectance intensity compared to a pure gold thin film (Fig. 1A). The silica film thickness determined by surface plasmon resonance is in good agreement with that obtained by profilometric measurements (Table 1).

The deposition of a thin sol gel film onto the gold electrode results in a very smooth surface morphology (Fig. 1B) when compared to bare gold with a visible granular surface structure. The Nyquist plots of the sol-gel coated gold electrodes upon immersion in TEOS/MTMS (0.92/0.08 mL) for 30 or 60 s in the presence of $Fe(CN)_6^{4-/3-}$ as redox probe (Fig. 1C) display a semi-circle located in the high frequency range, which corresponds to a parallel $R_{CT}//C_{dl}$ element due to the charge transfer resistance at the modified electrode/solution interface and the necessity to charge the double layer capacitance C_{dl} of this interface. While both interfaces show comparable electrolyte resistance (R_{ele} = 50 Ω) , increasing the immersion time from 30 to 60 s results in increased charge transfer resistance. Bacteria imprinting was consequently performed using condition 1 (Table 1), which insured that the film thickness is indefinitely thin compared to the morphology of *E. coli*, rod shaped bacteria of approximately 500 nm in width and 2000 nm in length. Fig. 2A depicts the SEM images of the bacteria imprinted films obtained by mixing *E. coli* UTI89 (1×10^8 cfu mL^{-1}) with a freshly prepared TEOS/MTMS (0.92/0.08 mL) sol and using a dipping time of 30 s with immersion and removal rate of 150 mm cm^{-1}. The bacteria are coated within the thin sol-gel film with preservation of the bacteria structures. Clustering of bacteria imprinting is observed in most cases with individual bacteria being attached to each other. This might be due to a partial repulsion effect between the negatively charged bacteria and the sol-gel with no evident phase separation (Shen et al., 2014). Bacterial removal was achieved by sonicating the electrodes in ethanol (70%), known to be most effective against microbial killing followed by immersion into SDS/acetic acid (5%) and further sonicated for 10 min. SDS, an anionic detergent leads to the disintegration of bacteria cell walls (Li et al., 2013); both SDS and acetic acid (Bjarnsholt et al., 2015) are known for their antimicrobial effect and to eradicate bacterial biofilms. A representative SEM image of the interface after bacteria removal is seen in Fig. 2B. Tailor-made cavities were left on the surface which can be easily accessible for bacterial binding. The removal of the *E. coli* template results in a significant decrease of the charge transfer from 22 kΩ (imprinted film) to 5.6 kΩ after removal (Fig. 2C). Without *E. coli*, the generation of imprinted cavities increased the electron transfer and decreased the diameter of the semicircle of

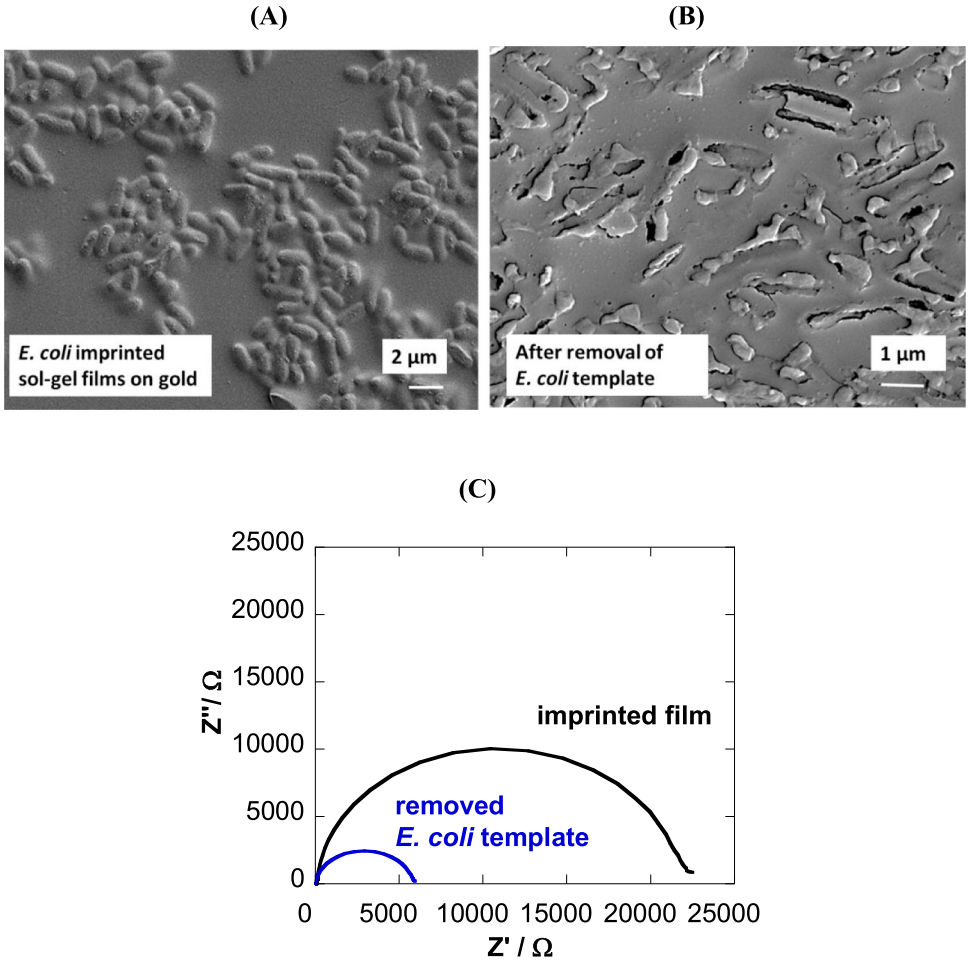

Fig. 2. (A) SEM images of a gold surface coated with *E. coli* imprinted sol-gel films formed by mixing *E. coli* UTI89 (1×10^8 cfu mL^{-1}) with a freshly prepared TEOS/MTMS (0.92/0.08 mL) sol using a dipping time for 30 s with immersion and a removal rate of 150 mm cm^{-1}; (B) SEM after removal of the *E. coli* template; (C) Nyquist plots recorded on a gold interface with imprinted *E. coli* before (black) and after (blue) *E. coli* removal (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

EIS. Subsequently, when *E. coli* was captured, impedance of the electrode had an apparent enhancement because bacteria filled the cavities (Chen et al., 2017).

3.2. Recognition performance of the E.coli templated electrode

The recognition performance of the imprinted interface was studied in the following using *E. coli* UTI89 as well as *S. aureus* as negative control. Fig. 3A shows that upon incubation with different *E. coli* concentrations, the charge transfer resistance increases. As seen from the calibration curve in Fig. 3B, where the change in the charge transfer resistance $Z^{"}$-$Z_o^{"}$ vs. log [*E. coli* UTI89] is plotted, with $Z_o^{"}$ being the initial charge transfer resistance in the absence of pathogens, a linear range from to 1×10^0–1×10^4 cfu mL^{-1} ($R^2 = 0.998$) according to $Z^{"}$ (kΩ) = 1.53 + 3.21 × log [*E.coli*] (cfu mL^{-1}) is obtained. The system displays a detection limit for *E. coli* UTI89 below 1 cfu mL^{-1} from five blank signals (95% confidence level). The performance of the developed impedimetric *E. coli* sensor is several orders of magnitude lower than other impedimetric sensors using anti-*E. coli* antibodies as sensing element (Chowdhury et al., 2012; Escamilla-Gómez et al., 2009; Maalouf et al., 2007). The sensor is also comparable to the detection limit to the BACTEC 9240 blood culture system, one of automated, continuous-monitoring systems that is widely used in clinical laboratories reaching a detection limit of 10 cfu mL^{-1} (Miller et al., 2011).

The specificity of this electrode architecture for the sensing of *E. coli* UTI89 is evidenced in Fig. 3C, where the addition of *S. aureus* to the imprinted interface results in strongly reduced signal change. The same was true for *Pseudomonas aeruginosa*, also a rod shaped Gram-positive bacterium. The good selective binding towards the rod-shaped *E. coli* over the spherical *S. aureus* bacterial cells makes this approach an effective way to produce specific bacterial recognition surfaces.

The reproducibility of the imprinting process for *E. coli* UTI89 detection expressed in terms of the relative standard deviation, was determined to be 2.7% (Fig. 3D) testing an *E. coli* concentration of 100 cfu mL^{-1}. Long-term stability of the bacteria cell imprinted electrode is insured due to the lack of bioreceptors used as recognition element. Indeed, after 1-month storage in room temperature, the loss in bacteria sensing capability was 1.1%.

3.3. Detection of E. coli UTI89 in real samples

The potential of this sensing interface for determination of *E. coli* UTI89 levels in urine spiked with *E. coli* UTI at different concentrations was finally investigated. Urinary tract infections (UTIs) represent the most common bacterial infectious diseases for humans, with *E. coli* UTI89 the most predominant pathogens responsible for 30–50% of hospital acquired and 80–90% of community-acquired UTIs (Ejrnaes et al., 2011). Fig. 4 compares the difference in the impedimetric signal for when urine was spiked with different concentrations of *E. coli* UTI89 compared to the electrochemical signal in PBS based solutions

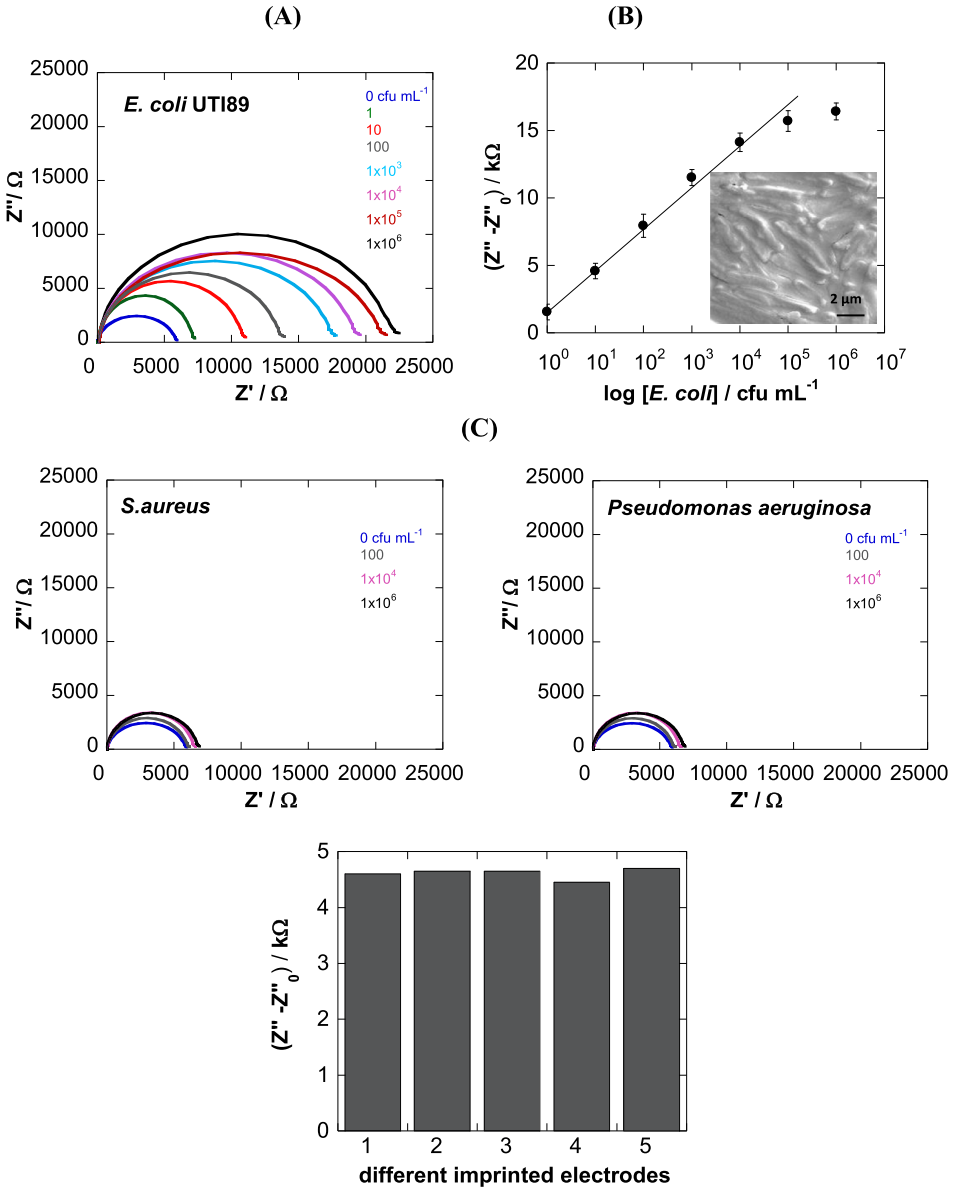

Fig. 3. (A) Nynquist plots of *E.coli* imprinted electrodes upon exposure to *E. coli* UTI89, (B) Calibration curve: inset: SEM image of the interface after rebinding; (C) Nynquist plots of *E.coli* imprinted electrodes upon exposure to *S. aureus* and *Pseudomonas aeruginosa* (D) Reproducibility of the sensor fabrication tested upon the incubation with 100 cfu mL^{-1} *E. coli* UTI89.

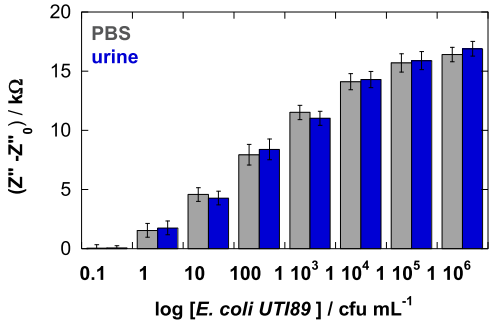

Fig. 4. (A) Comparison between the impedimetric change of *E. coli* UTI89 in PBS (gray) and when analyzed in urine (blue) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

containing of the same concentrations. The differences are within the error of the analysis technique, pointing towards the possibility to analyze real samples with the developed sensor.

4. Conclusion

In summary, surface imprinting of *E. coli* bacteria cells within TEOS and MTMS derived sol-gels has been demonstrated. The artificial recognition sites formed on the electrical interfaces allow a selective and highly sensitive detection of rod-shaped bacteria cells as exemplified here with detect uropathogenic *E. coli* cells (*E. coli* UTI89). Comparing the cross sensitivity to other bacterial cells of different shape gave a good evidence about the imprinting dependent interactions between bacterial cells and the imprinted matrix. The date presented demonstrate a truly alternative to biological recognition layers such as antibodies for bacteria detection. The obtained detection limit of

1 cfu mL^{-1} *E. coli* UTI89 in urine is highly encouraging and adapted to clinical settings, where bacterial culturing methods are still mainly used, which cannot reach such detection limits in a short time span. Furthermore, the concept can be easily adapted to other pathogen species, making the approach very versatile. While tested for *E. coli* UTI89, the sensor is not specific to only to *E. co*li strain but responses to any other *E. coli* strains.

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