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Electrochemical bacterial detection using poly (3-aminophenylboronic acid)-based imprinted polymer

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Abstract

Biosensors can deliver the rapid bacterial detection that is needed in many fields including food safety, clinical diagnostics, biosafety and biosecurity. Whole-cell imprinted polymers have the potential to be applied as recognition element in biosensors for selective bacterial detection. In this paper, we report on the use of 3-aminophenylboronic acid (3-APBA) for the electrochemical fabrication of a cell-imprinted polymer (CIP). The use of a boronic acid group bearing monomer with its ability to specifically interact with *cis*-diol allowed the formation of a polymeric network presenting both morphological and chemical recognition abilities. A particularly beneficial feature of the proposed approach is the reversibility of the *cis*-diol-boronic group complex, which facilitates easy release of the captured bacterial cells and subsequent regeneration of the CIP. *Staphylococcus epidermidis* was used as the model target bacteria for the CIP and electrochemical impedance spectroscopy (EIS) was explored for the label-free detection of the target bacteria. The modified electrodes showed a linear response over the range of 10^3 - 10^7 cfu/mL. A selectivity study also showed that the CIP could discriminate its target from non-target bacteria having similar shape. The CIPs had high affinity and specificity for bacterial detection and provided a switchable interface for easy removal of bacterial cell.

Keywords: *3-Aminophenylboronic acid, whole-cell imprinted polymers, electrochemical impedance spectroscopy, label-free detection, Staphylococcus epidermidis.*

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1. Introduction

Early stage detection and identification of pathogens are key concerns since infections have an enormous impact on social health and the global economy (Allos et al. 2004). Although conventional culture-based approaches are accurate and reliable, they are costly, requiring well-equipped laboratories and trained people, and lengthy procedures. Unfortunately, delays in results are still responsible for significant number of mortalities and morbidities, and hence there is a need for faster, cost-effective and arguably decentralised analytical methods. This need led to the development of several new approaches based on genetic analysis (Call et al. 2003; Kemp et al. 2010; Zhou 2003) and affinity assays based on antibodies or peptides (Dover et al. 2009; Skottrup et al. 2008; Yoo and Lee 2016). Unfortunately, the use of antibodies has a significant drawback due to their relatively poor stability and limited operational conditions. In order to overcome these limitations, the development and application of synthetic receptors such as aptamers (Bagheryan et al. 2016; Özalp et al. 2013; Sheikhzadeh et al. 2016) and molecularly imprinted polymers (MIPs) has been explored.

MIPs are polymers that are fabricated via chemical, photochemical or electrochemical methods in the presence of a target template material of interest (Sharma et al. 2012; Uzun and Turner 2016). This process results in the fabrication, following removal of the template, of polymeric structures containing cavities, complementary in shape and chemical properties with the template itself (Uzun and Turner 2016; Whitcombe et al. 2014). MIPs have found wide application in several areas including sensing and separation of small molecules (Schirhagl 2014), bio-macromolecules (Iskierko et al. 2016) and even larger analytes such as whole cells (Dickert and Hayden 2002; Namvar and Warriner 2007; Yilmaz et al. 2015). The simplicity of the fabrication

process together with their stability and cheapness makes MIPs attractive as artificial recognition elements for use in biosensors (Uzun and Turner 2016). Reports on cell-imprinted polymers (CIPs), MIPs obtained using whole cells as template, have been recently started to appear for various applications including in microbial fuel cells (Lee et al. 2015a), for specific cell capture, cell-sorting, separation (Schirhagl et al. 2012a) and detection (Cohen et al. 2010; Lee et al. 2015b; Lee et al. 2015c). Stamping and casting methods have been the most widely reported approaches for whole-cell imprinting (Aherne et al. 1996; Qi et al. 2013; Ren and Zare 2012; Schirhagl et al. 2012b), but more recently the use of electrochemical polymerisation (EC) has been demonstrated. Rapid bacteria detection using a QCM (quartz crystal microbalance) was reported by using electrochemically fabricated overoxidised polypyrrole CIP film on gold electrode. (Le et al. 2015a; Le et al. 2015b; Tokonami et al. 2013; Tokonami et al. 2012). Major limiting factors in CIP technology are their low affinity and the difficulties associated with the complete removal of the template (Lopez-Roldan et al. 2013; Velusamy et al. 2010).

In the work reported herein, we explored the use of a functional monomer, 3-aminophenylboronic acid (3-APBA), for the electrochemical fabrication of CIPs with improved affinity and ease of template removal. These improvements were made possible by the presence of the boronic acid group in the polymeric matrix.

Boronic acid is well known to interact, at physiological pHs or above, with 1,2- or 1,3-diols. This interaction has already been demonstrated for the detection of diol-containing molecules such as sugars, dopamine and bacteria (Badhulika and Mulchandani 2015; Badhulika et al. 2014; Deore and Freund 2003).

Electrochemically fabricated poly(3-aminophenylboronic acid) (PAPBA) based MIPs have been already reported for the detection of saccharides and proteins (Deore and Freund 2003; Rick and Chou 2006). The presence of boronic acid groups at the interface between the CIPs and solution is expected to improve, as already shown in the case of planar polymeric surfaces (Polsky et al. 2008; Xue et al. 2010), the affinity of the polymer towards bacterial cells due to presence on the outer wall of the bacteria of a variety of diol-rich biomolecules such as teichoic acid, peptidoglycan and lipopolysaccharides. Furthermore, the reversibility of the diol-boronic acid group interaction has been demonstrated to allow the selective capture and release of biomolecules and cells (Aytaç et al. 2011; Ivanov et al. 2006; Ren et al. 2009; Xue et al. 2010; Zhong et al. 2015a; Zhong et al. 2015b).

In this work, we demonstrated that the use of 3-APBA for the electrochemical fabrication of CIPs resulted in a synergistic interaction between the steric recognition of the cavities and the chemical affinity of boronic acid for diols. Furthermore, the reversibility of the boronic acid-diol interaction facilitated the release of the cell templates. Finally the use of 3-APBA-based CIP for the label-free detection of *S. epidermidis*, via electrochemical impedance spectroscopy (EIS), was demonstrated.

2. Materials and methods

2.1. Materials and Apparatus

Bacterial strains were either obtained from the Culture Collection of the University of Gothenburg, Sweden (*Escherichia coli* and *Deinococcus proteolyticus*) or donated by the

Linköping University Hospital (*Staphylococcus epidermidis* and *Streptococcus pneumoniae*). Brain heart infusion broth (Sigma-Aldrich) were used to culture bacteria. Pyrrole monomer (Sigma-Aldrich, St. Louis, USA) was distilled and stored at -20°C prior to use. Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$) were obtained from E. Merck (Germany) and 3-aminophenylboronic acid (3-APBA) monomer, *o*-phenylenediamine (*o*-PD, $\geq 98\%$), ready to use phosphate buffer saline buffer tablets (PBS, 10x, pH 7.4), sodium fluoride (NaF), concentrated sulfuric acid (H_2SO_4), Tris-HCl, sodium dihydrogen phosphate (NaH_2PO_4), concentrated hydrochloric acid (HCl), and fructose were purchased from Sigma-Aldrich (Sweden). All solutions were prepared in high purity water (18 M Ω) obtained using a Milli-Q RG system (Stockholm, Sweden).

Fabrication of CIPs was performed either on in-house fabricated Au substrates or on commercially available Au disk electrodes (GDE) (internal diameter 2 mm from CHI Instruments, USA). In-house substrates were fabricated by evaporating 2000 Å of Au and 100 Å of Ti (adhesion layer) onto a Si wafer, which was subsequently cut into 2x1 cm² electrodes. Electrochemical deposition of the polymeric films and impedimetric measurements were carried out using either an EmStat2 electrochemical workstation together with PSTrace software (Palm Sense, The Netherlands) or an IviumStat.XR electrochemical analyser coupled with dedicated software (Ivium, Eindhoven, Netherlands). An Ag/AgCl, 3 M NaCl (CHI Instruments, USA) and a Pt mesh were used as reference and counter electrodes, respectively, during the electrochemical synthesis and characterisation of the MIPs.

2.2. Bacterial cultivation

Bacterial strains were cultured in Brain heart infusion broth at 37 °C and 170 rpm overnight ($\approx$ 18 h). The cultured bacterial cells were collected by centrifugation (4000 g, 5 min) and the pellet was re-suspended in sterilised PBS. The washing process was repeated three times completely to remove impurities. The final bacterial cell concentration was adjusted by dilution guided by optical density measurement of solutions at 600 nm using a spectrophotometer (Shimadzu UV-1601 PC, Japan).

2.3. CIP and NIP fabrication

Prior to use, the GDE surface was polished on a cloth pad with wet alumina slurry (1, 0.3 and 0.05 μ m, CHI Instruments, USA) to a mirror finish, washed with deionised water and subjected to cyclic potential sweeps (0.2 – 1.5 V) in 0.5 M H₂SO₄ until obtaining a stable cyclic voltammogram. The CIP was electrochemically synthesised at a constant potential (+ 950 mV vs Ag/AgCl, 3M NaCl); the polymerisation was terminated when a predetermined amount of charge (3.2 mC) had passed thus allowing good control of the film thickness. CIPs were fabricated from 50 mM 3-APBA and 50 mM NaF (Deore and Freund 2003; Badhulika and Mulchandani 2015) in 10x PBS (pH 7.4) in the presence (cell-imprinted, CIP) and in the absence (non-imprinted polymers, NIP) of *S. epidermidis* (10^9 cfu/mL final concentration). The fabricated CIPs were then treated for 30 min with fructose (20 mM), for competitive detachment of the cells from the surface (Polsky et al. 2008), followed by washing with plenty of running deionised water to remove the bacterial cells from the polymer network. Finally, prior to storage, the electrodes were immersed in phosphate solution (pH 2.2) for 20 min to remove bound fructose and to

reactivate the boronic groups of the polymer (Springsteen and Wang 2002). The activated electrodes were stored in Tris-HCl (0.1 M, pH 8.5) until further use (Polsky et al. 2008).

2.4. Bacteria detection test

The ability of the CIPs to detect the target bacteria, *S. epidermidis*, was evaluated by incubation, in quiescent conditions, of the modified CIP electrode in 10x PBS (pH 7.4) containing defined concentrations of bacterial cells for 30 min. Electrochemical impedance spectroscopy was carried out in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) in PBS. Impedance measurements were in the frequency range of 0.1–100,000 Hz at a bias potential of 0.0 mV vs. OCP potential. The amplitude of the applied sine wave potential was 5 mV. The Nyquist plots obtained were fitted to an equivalent circuit to extract the value of the charge transfer resistance (R_{ct}).

2.5. Scanning electron and fluorescent microscopies

The Scanning Electron Microscopy (SEM) images were taken using a Leo 1550 Gemini SEM operating at 8.0 keV for polymer characterisation purpose. For evaluating selectivity of CIP, we performed fluorescent microscopy observations (ZEISS, Germany). CIPs separately prepared on Au planar electrodes (1 cm²) were used for these microscopy studies. All cell samples were visualised by staining with ethidium bromide (0.05%) for 45 min.

3. Results and discussions

3.1. Electrochemical whole-cell imprinting

CIPs based on PPy (Tokonami et al. 2013) and poly(*o*-phenylenediamine (Po-PD) polymer were successfully fabricated (Fig. S1-Supporting Information) following the previously described

methods detailed in brief in the supporting information (Figure S1). In order to allow the further use of the CIPs removal of the template is required, but this step is not simple in the case of large biomolecules and whole cells. Mild conventional approaches, such as washing and soaking in water (Cheng et al. 2001), sonication, mild acidic and alkaline treatment (Ameli and Alizadeh 2012; Gómez-Caballero et al. 2008), which have been successfully applied to the removal of small templates are not effective in the case of whole cells (Levi and Srebnik 2010). On the other hand, applying harsh conditions to remove the template damage the polymer and created cavities (Lorenzo et al. 2011). In the case of casting methods (Iskierko et al. 2016), due to the feeble interactions between the cell and the polymer, templates can be removed by physical treatments like sonication or soaking and washing with water (Ren et al. 2013; Schirhagl et al. 2012a). Unfortunately, in the case of electrochemically fabricated CIPs, the use of such moderate conditions were insufficient to detach bacterial cells from the polymer network (Fig. S2-Supporting Information). Attempts to remove bacterial cells, following an approach inspired by the work of Tokonamy *et al.* [28] (treatment of the CIPs with lysozyme (10 mg/mL) at 4°C for 2 h followed by washing in 10% Triton for 80 min, and constant potential treatment (+ 980 mV) in 0.1 M NaOH solution) was not successful. As can clearly be seen from Fig. S2 significant damages in the cavities and/or the partial retention of the bacterial cell wall were observed. In order to overcome this significant problem, CIPs using 3-APBA as functional monomer were fabricated (Gao et al. 2013; Li et al. 2013).

Figure 1 is a schematic of the rationale behind the use of 3-APBA for the fabrication of CIPs. When pre-mixed with the template cell, the phenylboronic acid groups are expected to attach to the *cis*-diols present on the bacterial surface (a). The subsequent electrochemical polymerisation captures the bacterial cells in the polymeric network (b); monomers previously present on the

bacterial surface are expected to be integrated in the polymer network adding, in this way, to create the structural and chemical affinity. By removing bacterial cells from the polymer, complementary cavities remain at the polymer surface, which not only present structural (shape and size) specificity, but also chemical specificity via the diol molecules found on the surface of the cell walls of the template (*S. epidermidis*).

Location of Figure 1

Electrochemical fabrication of PAPBA based CIPs and the removal of cells from the polymer network, by competitive displacement with fructose and washing with deionised water, were successfully achieved. Typical chronoamperometric, curves recorded during the fabrication of the CIPs and the NIPs, and a schematic description of the polymerisation process, are reported in Fig. S3. Both the deposition processes were consistent with the deposition profile reported for the chronoamperometric electrodeposition of aniline (Yano et al. 1997). In the ortho-position to an amino-group, the boronic acid group does not hamper the polyaniline growth (Nikitina et al. 2015). At the beginning of the process, both the amperograms presented a decrease in current, which was probably associated with a slow seeding processes taking place at the electrode surface. This initial step is then followed by a region characterised by fast current ramping associated with first a nucleation step followed by a bulk deposition of the film. Comparing the amperograms obtained with those reported in the literature for aniline (Yano et al. 1997), the presence of the boronic acid substitution and of the less conductive material, i.e. bacteria,

significantly slowed the seeding step. This is due to the fact that the presence of an electron attracting group significantly reduced the reactivity of the aniline making the polymerisation process slower. Fig.2a shows the SEM images of bacterial cells captured in the PAPBA polymer network after electrochemical polymerisation. The shape of the complementary cavities on the polymer surfaces after removal of the bacteria cells are clearly seen in Fig.2b. It should be noted that the potential used during the electrochemical fabrication of the polymer is crucial for the success of this process. If the potential is too low ($<+900$ mV) a poor film is deposited, while too high potential ($> +980$ mV) results in films with stronger interconnection with the template that could not be completely removed from the CIP. On the other hand, the use of $+950$ mV resulted in the fabrication of highly reproducible films from which the easy and quantitative removal of the bacterial cells was possible.

These significant differences can probably be ascribed to the fact that a higher potential gives a faster polymerisation which results in the formation of rougher polymers (G.B. Street 1983; Golabi et al. 2016; Xu et al. 2005) and more branched polymers that could result in a high surface area providing a good adhesion of the bacterial cells and subsequent irreversible entrapment of them. Consequently, there is a limited potential window that allows the fabrication of CIPs with the desired properties.

Location of Figure 2

The effect of polymer thickness was also evaluated to optimise the synthesis and efficient bacterial removal processes. Fig.3 shows three CIPs with different polymer thickness; these were obtained by stopping the electrochemical polymerisation process after different charges had been passed. Fig. 3a shows a thin CIP (≤ 20 nm) in which the complementary cavities have insufficient specificity for the target cell, *S. epidermidis*. In Fig.3b, the CIP thickness is appropriate (≤ 100 nm) for both specific capture and release of the target cells. Figure 3c shows a thick CIP (≥ 250 nm) in which *S. epidermidis* is stuck in the thick polymer layer making the release process difficult. To release the bacterial cells from the optimised CIP network, the film was incubated with fructose (20 mM solution), which is known to have a strong affinity for boronic acid groups (Ma and Yang 2005; Polsky et al. 2008; Torun et al. 2009). The treatment with fructose solution, followed by washing with deionised water resulted in the quantitative removal of the bacterial cells from the CIP (Fig. 2b). The bound fructose molecules were then removed by further treatment at low pH to reactivate the boronic groups present in the CIPs.

Location of Figure 3

3.2. Electrochemical evaluation of cell-imprinted polymer

EIS was used to characterise the fabricated polymers and the removal step from CIP. Fig. 4 shows the EIS of bare Au electrode, NIP and CIP before and after removal of the bacterial cells (modified CIP). The presence of the NIP (blue line) and of the CIP (red line) resulted in a significant increase in the high frequency semicircle, when compared to the bare GDE. This

result confirmed the successful formation of the polymer layer onto the GDE. Notably a much higher resistance was recorded in the case of the CIPs; this result is consistent with the presence of bacterial cells in the polymer network, which have a significant electrostatic repulsion effect on the redox probe used in the measurement due to their negative surface charge. A significant change was observed in the impedance of the CIP after the treatment process for the removal of the bacterial cells; this significant loss in charge transfer resistance was ascribed to (i) the loss of electrostatic repulsion and (ii) to the high porosity of the polymer film after cell removal.

After bacterial removal and regeneration of the boronic acid groups, the regenerated CIP electrodes were incubated with different concentrations (10^1 to 10^8 cfu/mL) of target bacteria, *S. epidermidis*. Fig. 5A shows the EIS response of the CIP incubated in solutions with different bacterial concentrations. The capture of bacteria cells in the CIP network partially blocks the electron transfer between the redox probe and the electrode surface with a subsequent increase of the overall charge transfer resistance of the film beside the repulsive interactions with probe molecules as mentioned before. Fig.5B shows the linear relationship of the bacterial concentration and the EIS response of the electrode. The optimum incubation time was 30 minutes, where the highest differences in the responses of NIP and CIP were detected (Figure S4, Supporting Information).

In order to minimise the variability between different CIP sensors, the relative variation of R_{ct} , $[\Delta R/R(\Omega)]$ was used as analytical response (Nasef et al. 2010). This value was calculated as follows:

$$\Delta R/R(\Omega) = (R_{cta} - R_{ct}\Omega)/R_{ct}\Omega \quad (\text{eq. 1})$$

Where R_{cta} is the value of R_{ct} after incubation with the concentration of target bacteria and $R_{ct}\Omega$ is the value recorded for the CIP prior to incubation with bacteria.

The binding isotherm of the MIP-modified electrode was fitted using a model for two types of simultaneous binding; at the sites of specific recognition inside the polymer film and on the surface of the electrode due to non-specific adsorption (Karimian et al. 2013):

$$\frac{R - R_0}{R_0} = \frac{B_{MAX}C}{K_D + C} + N_S C$$

Where: C is the bulk concentration of the target, B_{MAX} is the maximum number of binding sites in the MIP; K_D is the equilibrium dissociation constant; and N_S is the binding constant for non-specific adsorption. The value of K_D obtained by fitting is 1.28×10^{-8} M (R^2 0.94) cfu/mL.

Location of Figure 4

Location of Figure 5

3.3. Selectivity studies

The affinity of the CIPs was evaluated by comparing their EIS responses to different concentrations of *S. epidermidis* with those recorded in the case of NIP. Fig.6a shows the normalised EIS responses of the CIP and NIP for *S. epidermidis*.

As it can be seen from Fig. 6a, the response obtained for CIP was significantly higher than those for NIP at the lower target concentrations, confirming the improved affinity due to the presence

of the cavities. Nevertheless, the difference in response was progressively reduced with increasing bacteria concentration in the solution. This result is consistent with the presence of boronic groups on the both the CIP and NIP surfaces. The presence of the boronic acid groups in electrochemically fabricated PAPBA films have been already shown to increase cell adhesion onto the surface (Wannapob et al. 2010; Xue et al. 2010) and also found application in bacterial detection (Wannapob et al. 2010).

In order to confirm this, fluorescence imaging experiments were performed with CIP, NIP and polyaniline (NIP-PA) that had a similar polymer backbone, but that did not have the boronic acid groups. As shown in Fig. 6b, when the three surfaces were put in contact with 10^6 cfu/mL of *S. epidermidis* adsorption and capture of the bacteria were recorded. Although the expected high non-specific adhesion at the NIP surface was observed, the CIP still showed higher affinity for the target bacteria.

Location of Figure 6

The selectivity of CIPs was also investigated by comparing the capture of *S. epidermidis* with those recorded for non-target bacterial strains, *D. proteolyticus*, *E. coli* and *S. pneumoniae*. Fig. 7 shows the different normalised EIS responses of the CIPs for the target and non-target bacteria at a concentration of 10^4 cfu/mL. The results of this experiment clearly demonstrate the ability of CIPs to discriminate the target from similar bacteria, in terms of shape and size, such as *S.*

pneumoniae and *D. proteolyticus*. Table S1 shows the imprinting factor and selectivity factor for the imprinted polymer (Uzun et al. 2009).

Location of Figure 7

4. Conclusions

We demonstrated the use of 3-APBA as a functional monomer for the electrochemical fabrication of CIP with high affinity for bacteria. The proposed CIP, when fabricated under optimal conditions (potential and total charge), presented two significant advantages: (i) a high affinity, due to the combination of morphological and chemical recognition of the target; and (ii) easy removal of template, by the use of a competitive process, without significant damage to the polymer structure. CIPs specific for *S. epidermidis* were fabricated on GDE, via constant potential methods, and used for the label-free detection, via EIS, of the target bacteria. The CIP sensor was shown to respond linearly to *S. epidermidis* concentrations in the range 10^3 to 10^7 cfu/mL. Furthermore, the CIP-based sensor delivered, at least at low concentrations, a significantly higher response than the corresponding NIP, confirming the importance of the cavities in the recognition process. In addition, the CIP sensor was shown to be highly specific for *S. epidermidis* when compared to other non-target bacteria such as *D. proteolyticus*, *E. coli* and *S. pneumoniae*.

On the one hand, the presence of boronic acid groups resulted in significant benefit by improving the template removal and increasing the specific interaction within the imprinted cavities. However, the presence of boronic acid groups on the surface of the polymer as well as in the

imprinted cavities, tended to increase non-specific absorption, as evidenced by the results with NIPs. Further work will include strategies to deactivate this undesirable side effect. Despite this shortcoming, the introduction of a functional derivatives in addition to established cell imprinting, led to substantial improvement in the performance of the CIP and a promising strategy of for cell capture, sensing and release. We envisage this as a viable approach for inexpensive, simple and rapid detection of pathogens in food and possibly environmental samples.

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Scheme and Figure Captions

Figure 1. Schematic representation of whole cell imprinting. Boronic acid groups attach to the surface of bacteria (a). Electropolymerisation preserves the structure of the cell at the polymer network (b). Bacterial cells are removed and the complementary cavities remain (c). Cavities containing boronic acid groups increase affinity of the preserved complementary shape of target bacteria (d).

Figure 2. SEM images of cell-imprinted polymer. (a) *S. epidermidis* captured in PAPBA imprinted polymer, and (b) the remaining shape-complementary cavities after removing bacterial cells.

Figure 3. SEM images of cell-imprinted polymer. *S. epidermidis* captured in PAPBA with different thicknesses, ($A \leq 20\text{nm}$, $B \leq 100\text{ nm}$, and $C \geq 250\text{ nm}$). Polymers were fabricated at constant a potential of +950 mV. The CIP solution contained 50 mM 3-APBA in 10x PBS containing *S. epidermidis* (10^9 cfu/mL) and 50 mM NaF.

Figure 4. Electrochemical impedance spectra at bare GDE, CIP, NIP and modified CIP.

Experimental details: 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1) in PBS. Impedance measurements were in the frequency range 0.1–100,000 Hz at a bias potential of 0.0 mV vs. OCP potential. The amplitude of the applied sine wave potential was 5 mV.

Figure 5. EIS results for cell-imprinted polymer incubated with different concentrations of *S.*

epidermidis (a) and the calibration curve of $\Delta R_{ct}/R_{ct}$ versus log concentration of *S. epidermidis*.

The data are averages of at least three independent experiments and error bars indicate standard deviations (b). Experimental details: 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1) in PBS. Impedance measurements were in the frequency range 0.1–100,000 Hz at a bias potential of 0.0 mV vs. OCP potential. The amplitude of the applied sine wave potential was 5 mV.

Figure 6. Affinity of CIP for its target, *S. epidermidis*. A) Normalised EIS response of CIP and APBA-NIP to *S. epidermidis*. B) Fluorescent images of *S. epidermidis* adhered to NIP made by Polyaniline (PA-NIP), PAPBA-NIP and PAPBA-CIP. The data are averages of at least three independent experiments and error bars indicate standard deviations.

Figure 7. Specificity of the PAPBA-CIP for its target *S. epidermidis* vs *D. proteolyticus*, *E. coli* and *S. pneumoniae* in the concentration of 10⁴ cfu/mL. The data are averages of at least three independent experiments and error bars indicate standard deviations.

A 2 μ m

Mag = 11.35 K X
WD = 5.2 mm

EHT = 6.00 kV
Signal A = InLens Photo No. = 457

B 3 μ m

Mag = 4.15 K X
WD = 7.1 mm

EHT = 6.00 kV
Signal A = InLens Photo No. = 169

A

1 μm

Mag = 34.66 K X
WD = 7.0 mm

EHT = 8.30 kV
Signal A = SE2

Photo No. = 926

B

2 μm

Mag = 19.74 K X
WD = 5.4 mm

EHT = 6.00 kV
Signal A = InLens

Photo No. = 472

C

100 nm

Mag = 94.92 K X
WD = 4.8 mm

EHT = 6.30 kV
Signal A = InLens

Photo No. = 1287

A

B

A

B

A

B

C

D