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ORIGINAL PAPER

Lectin-modified piezoelectric biosensors for bacteria recognition and quantification

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Abstract The use of lectins for microorganism biosensors fabrication is proposed. Lectins are immobilised onto a gold-plated quartz crystal for direct piezoelectric label-free transduction of the bacteria–lectin binding event using an electrochemical quartz crystal microbalance (EQCM). Concanavalin A (Con A) and *Escherichia coli* were used for the evaluation of the lectin immobilisation method and the biosensor performance. Adsorption on nonpolarised and polarised (−0.200 V) gold-coated quartz crystals and immobilisation through avidin–biotin binding were checked for Con A surface attachment. Lectin–bacteria binding was evaluated in all cases. With a crystal modified with Con A via avidin–biotin immobilisation we obtained a linear calibration plot between 5.0×10^6 and 2.0×10^7 cfu mL^{-1} by measuring frequency changes with *E. coli* concentration 1 h after bacteria addition. A remarkable increase in sensitivity was achieved when the analytical solution contained free biotinylated Con A, as a consequence of multiple lectin adhesion to *Escherichia coli* cell wall, which produced an accumulation of Con A–*E. coli* conjugates in the form of multilayers at the electrode surface. A detection limit of approximately 1.0×10^4 cfu mL^{-1} was achieved. Moreover nonspecific adsorptions were minimised. Using Con A and lectin from *Arachis hypogaea*, different response profiles were achieved for *Escherichia coli*, *Staphylococcus aureus* and *Mycobacterium phlei*, thus demonstrating the feasibility of bacteria discrimination. An approach involving filtering of free and lectin-bound bacteria and introduction of a filter in the measuring cell allowed a significant frequency change to be obtained for an *E. coli* concentration of 1.0×10^3 cfu mL^{-1} in order to further increase the sensitivity and discriminate between viable and nonviable cells; an approach using electrochemical measurements of bacterial catalase activity was also checked.

Keywords Lectins · Quartz crystal microbalance · Microorganisms · Biosensors

Introduction

Microorganism detection is a key issue in fields like drinking water quality assurance, food quality, biological terrorism threat control, applied medicine and fermentation technology [1]. In most cases, early detection of very low concentrations of microorganisms is essential for an effective action; therefore there is a demand for rapid, inexpensive and highly sensitive analytical tools for their detection.

Traditional methods relying on cultural techniques are time consuming (days) and expensive [2]. Methods based on bioluminescent adenosine 5′-triphosphate (ATP) measurement, electrical impedance analysis or radiometry allow microorganism detection in a much simpler way, although their sensitivity is still low, and pre-enrichment and/or culture steps are required, increasing the assay time (8–10 h) [3]. Moreover, these methods do not discriminate between species. Flow cytometry can detect about 10^2–10^3 bacterial cells per mL within a few minutes, although it cannot discriminate between organisms nor between living and dead cells [3]. Immunological and DNA techniques have provided a means of detecting and identifying microorganisms selectively, but they are complex methods, targeted at a particular pathogen or microorganism [4].

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One possible alternative principle relies on carbohydrate recognition. A high percentage of microorganisms, proteins and viruses have carbohydrates on their surface and so, in theory, there is a real possibility of using this natural phenomenon as an alternative detection and identification principle [5]. These carbohydrate structures can be recognised using lectins. Lectins are structurally diverse proteins from many sources (plants, viruses, microorganisms and animals) that selectively and reversible associate with mono- and oligosaccharide components of polysaccharide structures [6]. Lectins are specific for a particular carbohydrate structure (sometimes two or three structures), which can be found in different types of bacteria. Therefore, a particular lectin can bind to several microorganism allowing a selective but not specific recognition. Consequently, in order to identify a target pathogen, it will be necessary to study its response to several lectins. Furthermore, these proteins are readily available and inexpensive.

Nevertheless, there are very few examples to date of lectins actually being employed in biological detection systems. Ertl and Mikkelsen [6] developed lectin-based sensor arrays for qualitative discrimination of bacterial strains. They employed lectin-modified membranes that were allowed to react with bacterial cell suspensions for 100 min and were subsequently fixed at the surface of a Pt electrode in order to obtain electrochemical signals from respiratory cycle activity to identify bacterial strains. Shen et al. [7] used lectins to form bridges between a specific carbohydrate structure and bacteria containing a complementary carbohydrate pocket in order to amplify the response on a quartz crystal microbalance, but they did not use the lectin as the recognition element itself.

Therefore, the use of lectins for bacteria biosensor fabrication is proposed in this work as a rapid and simple alternative for selective microorganism detection, which could be applied routinely. In this study, lectins are immobilised onto a quartz crystal for the real-time piezoelectric transduction of the bacteria–lectin binding event using an electrochemical quartz crystal microbalance. Consequently, transduction is achieved with no need for label, and the binding event is directly monitored. Moreover, the use of an electrochemical QCM allows the simultaneous label-free monitoring of both the affinity event by measurement frequency changes and the metabolic activity of bound bacteria by amperometric measurement of hydrogen peroxide consumption by bacterial catalase on the gold-plated quartz crystal surface. In this way, it is possible to discriminate between living and dead organisms.

As a model system, the lectin concanavalin A (Con A) is employed for the rational evaluation of the lectin immobilisation method on the quartz crystal surface, by considering stability, reproducibility, elimination of nonspecific adsorption of bacteria and overall simplicity. The responses of quartz crystals modified with different lectins have been evaluated for different microorganisms such as *Escherichia coli, Staphylococcus aureus* and *Mycobacterium phlei.*

Experimental

Apparatus and electrodes

All experiments were carried out at 25±0.1 °C using a Maxtek electrochemical RQCM device. A homemade PVC measuring cell with inner dimensions of 2.0 cm×2.5 cm×4.0 cm and which had the QCM probe embedded in one of its vertical walls was used in all cases. A 1-inch gold-plated 9-MHz quartz crystal optimised to work at 25 °C was used for the frequency assays, as well as the working electrode for the electrochemical assays by means of an interface connecting the RQCM to a Voltalab 80 potentiostat. An Ag/AgCl electrode was used as the reference electrode and the auxiliary electrode consisted of platinum foil. Experiments were carried out in 15 mL of stirred aqueous buffer solutions. When measurements are compared with a blank, a dual probe system is used with the RQCM, as it allows up to three simultaneous measuring frequency channels.

Materials and solutions

Wild-type *Escherichia coli* (CCT 515)*, Staphylococcus aureus* (CCT 59), *Salmonella cholerasuis* (CTT 700) and *Mycobacterium phlei* (CCT 3009) were obtained from the Spanish Collection of Type Cultures. Concanavalin A and lectin from *Arachis hypogaea*, both wild-type and biotinylated, were supplied by Sigma, as were avidin, biotin, bovine serum albumin (BSA) and hydrogen peroxide. 1-Mercaptopropanol and mercaptopropane (Aldrich) were used to check minimisation of unspecific adsorption. Nylon membranes (Osmonics) with 0.2-μm pore size were employed. Working medium consisted of 15 mL 0.1 M acetate buffer of pH 5 unless (otherwise stated) containing 1 mM of Mn^{2+} and 1 mM Ca^{2+}, and was prepared everyday. This medium was chosen to obtain, according to the provider's instructions, optimum concanavalin A activity in its dimeric form. The presence of Ca^{2+} and Mn^{2+} salts is necessary for Con A to have the active conformation to interact with α-mannose.

Gold-plated quartz crystal pretreatment and cleaning

The crystals were immersed in a 1:1:5 solution of hydrogen peroxide (30%), ammonia (25%) and deionised water heated to a temperature of about 75 °C for 5 min then immediately rinsed with deionised water and finally dried in a gentle flow of nitrogen gas.

Lectin modification of the quartz crystal

The following approaches were used for lectin immobilisation.

Direct adsorption

QCM probe was immersed in 15 mL of stirred 0.1 M acetate buffer solution of pH 5.0 containing 1 mM Mn^{2+} and 1 mM Ca^{2+}. After frequency stabilisation, 2 mg of lectin dissolved in the buffer solution is added to the measuring cell and the frequency is allowed to stabilize again. Subsequently, the probe is rinsed with deionised water, and is then ready for bacteria binding assays.

Adsorption on a polarised surface

The same protocol as before is followed, but in this case the gold-plated quartz crystal is connected to the potentiostat, and the reference and auxiliary electrodes are immersed in the solution to allow working electrode polarisation at −0.2 V vs. Ag/AgCl. In this case, the working solution pH value is 4 to allow a positive charge of Con A, as its isoelectric point is 5.

Lectin immobilisation via avidin–biotin binding

QCM probe was immersed in 15 mL of 0.1 M stirred acetate buffer solution of pH 5.0 containing 1 mM Mn^{2+} and 1 mM Ca^{2+}. After frequency stabilisation, 1 mg of avidin dissolved in the buffer solution is added to the measuring cell and the frequency is allowed to stabilize again. Subsequently, the probe is rinsed with deionised water, and immersed in a new 15 mL acetate buffer solution. When frequency is stable, the appropriate amount of biotin-labelled lectin is added. After frequency stabilisation, the crystal is rinsed with deionised water.

Electrochemical measurement of bacterial catalase activity of the attached bacteria

Once bacteria are bound to the electrode surface via lectin interaction, the probe is rinsed with deionised water and immersed in a fresh 0.1 M acetate buffer solution. The electrode is polarised at 0.6 V vs. Ag/AgCl and when current is stabilised, hydrogen peroxide is added to the measuring cell to a final concentration of 1.0×10^{-4} M. Current change is monitored and compared with the current change of a free bacteria lectin-modified gold-coated quartz crystal in order to estimate the number of viable cells.

Bacteria cultivation

All bacteria used in this work belong to the microorganism biosafety level 2 group, except *Mycobacterium phlei* which belongs to level 1. All safety considerations concerning group 2 microorganisms were adhered to during the manipulation of these bacteria. *E. coli* and *S. aureus* cultures were grown overnight in Luria broth medium at 37 °C with aeration by shaking, which allowed the growing stationary phase to be reached. *M. phlei* cultures were grown for 48 h in Sauton broth medium at 37 °C, with aeration by shaking until the stationary phase was also reached. Then, bacterial cultures were serially diluted (tenfold steps) and 10-μL aliquots of samples were applied to LB agar or Middlebrook agar plates, respectively, and incubated for 24 h at 37 °C, for enumeration of colonies (*M. phlei* were incubated for 48 h). At the same time, the stationary-phase cultures were diluted to $10–10^8$ cfu mL^{-1} in 5.0 mL LB medium. The bacterial suspensions were added to the QCM.

Results and discussion

Lectin adsorption on the gold QCM electrode

For lectin immobilisation and biosensor performance optimisation, concanavalin A and *Escherichia coli* are used as a model system. Concanavalin A has affinity for the α-mannose and glucose residues on the *E. coli* surface [8]. The simplest biosensor design would consist of quartz crystal modification by direct lectin adsorption on the gold surface. Figure 1 shows frequency changes due to direct adsorption of concanavalin A on the gold-coated quartz electrode surface, when no polarisation was applied to the electrode, and when a potential of −0.200 V vs. Ag/AgCl reference electrode was applied.

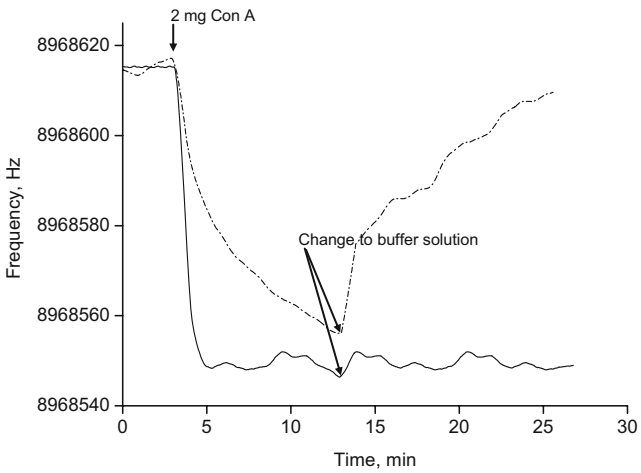

Fig. 1 Frequency changes due to concanavalin A adsorption onto a gold-coated quartz crystal immersed in a 15 mL stirred solution of 0.1 M acetate buffer of pH 4.0 containing 1 mM Ca^{2+} and 1 mM Mn^{2+}: (- - -) no polarisation of the electrode, (—) electrode polarised at a −0.200 V vs. Ag/AgCl. The protein exposure was interrupted at 13 min by exchanging the protein solution in the QCM cell for a new buffer solution

Con A was introduced into the cell in a great excess (2 mg) to allow total coverage of electrode surface. In both cases, its adsorption caused an initial rapid frequency decrease, although the Con A adsorption was much more rapid on the polarised gold surface, as was expected considering the positive charge of the protein at the working pH value. Moreover, a significant desorption of Con A was observed from the nonpolarised surface when protein exposure was eventually interrupted by exchanging the Con A solution in the QCM cell for a pure buffer solution, suggesting the polarisation of the electrode was a better option for fabrication of a stable biosensor. Three replicates for Con A deposition on polarised crystals yielded a standard relative deviation (RSD) value for frequency change of 10%. It is well known that the crystal cleaning procedure used is a key factor affecting the reproducibility of the results as well as the durability of gold-coated quartz electrodes. The method used in that work (see Experimental) proved to yield satisfactory results, and one single crystal could be reused for 25 experiments based on protein adsorption.

The Con A adsorption saturated at frequency changes of −62 and between −67 and −82 Hz for the nonpolarised and the polarised electrodes. It is noteworthy that further addition of Con A to the cells did not produce any frequency change, which confirmed surface saturation. From these values, the Sauerbrey equation can be used to estimate the coverage of Con A. Nevertheless, when using QCM in the liquid phase and applying it to nonrigid overlays several mechanisms can contribute to energy dissipation making the Saurebrey equation invalid. Rodahl et al. [9] thoroughly studied adsorption of biomolecules on the gold QCM electrode and found that even very thin (a few nanometres) biofilms dissipate a significant amount of energy owing to the QCM oscillation. However, they demonstrated that small proteins (2–6 nm in diameter) form layers that can be practically considered as rigid, and frequency changes can be generally related to mass changes. Moreover, Kim et al. [10] demonstrated by impedance characterisation that a protein layer immersed in buffer behaved as a pure mass loading within experimental error, and Janshoff et al. [11] stated that in most of protein layers, higher changes of frequency than expected are due to accumulation of solvent molecules rather than to energy dissipation. With all these considerations, we assumed the validity of Sauerbrey equation to describe our system. Therefore, changes of −62 Hz and −82 Hz corresponded to a mass change of ca. 339 ng cm^{-2} and 448 ng cm^{-2}, respectively, using the sensitive factor (C_f=0.1834 Hz ng^{-1} cm^2) for a 9-MHz crystal at 20 °C. In order to estimate the coverage of Con A, we took into account that the dimer structure, which is predominant at the pH value of the experiment, has a molecular weight of ca. 66 kDa, including 25% mass water, which is a typical average water content in proteins [12]. Accordingly, and assuming that no protein denaturalisation occurred at the pH value optimum for its activity, coverages of 5.1×10^{12} and 5.4×10^{12} Con A molecules per cm^2 were obtained, respectively. The dimensions of the dimer structure of Con A are ca. $8\times4\times4$ nm^3 [13]. This gives upper and lower limits (depending on the orientation of the molecules) of a close-packed monolayer of 414 ng cm^{-2} (6.25×10^{12} molecules per cm^2 in an upright orientation) and 166 ng cm^{-2} (3.12×10^{12} molecules in a prone orientation). It is therefore likely that the Con A dimer molecules adsorb in a predominantly upright position, covering a high percentage of the surface.

Monitoring of lectin–bacteria binding on the QCM electrode

Lectin–microorganism interaction is affected by pH and ionic conditions, temperature and the presence of metal ions. Many lectins require transition metals and/or Ca^{2+} or Mg^{2+} for activity, which is also the case of Con A. Therefore, it is important to choose the appropriate experimental conditions [14]. Pei et al. studied lectin–carbohydrate interactions on the surface of a QCM, and they observed long reaction times (ca. 2 h). Consequently, frequency changes to bacteria addition are expected to be slow.

On the other hand, a cell layer on a quartz resonator cannot be treated as an ideal rigid mass: a sole cell layer on a 5-MHz crystal would produce a theoretical frequency change of 5,600 Hz, according to the Sauerbrey equation [11]. The values generally obtained are at least one order of magnitude smaller.

It has to be remarked that the diameter of a typical cell is ca. 1 μm (2–3 μm for *E. coli*), which is significantly larger than the decay length for the shear wave in water excited by a 9-MHz AT-cut QCM (ca. 0.34 μm). Thus, only a minor fraction of a cell's mass will be reflected in the measured frequency shift [9].

Figure 2 depicts the frequency change obtained after the addition 100 μL of a 1.0×10^9 cfu mL^{-1} *E. coli* solution to the QCM cell containing 15 mL of buffer solution in which a gold crystal covered with Con A and BSA was immersed. Therefore, the final *E. coli* concentration was 6.07×10^7 cfu mL^{-1}. BSA is also added to minimize nonspecific adsorption of bacteria to the surface. In order to evaluate the blocking capacity of BSA, a control experiment was carried out in which the electrode surface was covered with BSA only. Considering that BSA acts as a rigid layer as well, the BSA coverage obtained with this probe is close to 100%. In the probe with concanavalin A, frequency changes suggest the formation of protein multilayers on the electrode surface. The addition of *E. coli* produced a frequency decrease in the control probe of 43 Hz which levelled off after 30 min. In contrast, the frequency in the probe with

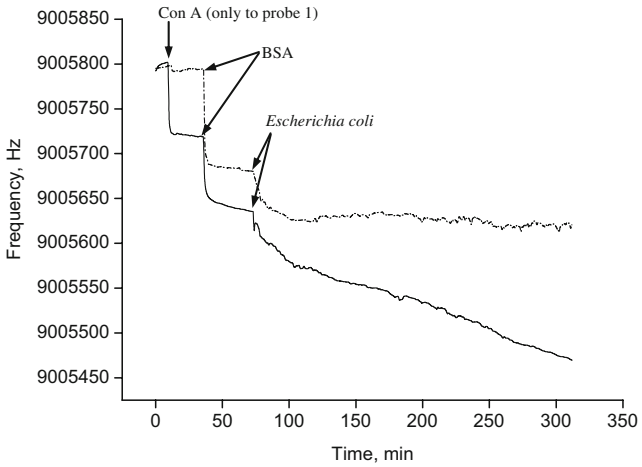

Fig. 2 Frequency changes obtained with probes 1 and 2 upon immersion in 15 mL of 0.1 M acetate buffer of pH 4.0 containing 1 mM Ca^{2+} and 1 mM Mn^{2+}; electrodes were polarised at −0.200 V vs. Ag/AgCl. Probe 1 (—): changes due to 2 mg concanavalin A 40 mg BSA and 6.7×10^7 cfu mL^{-1} of *E. coli* additions; probe 2 (- - -): changes due to 40 mg BSA and 6.7×10^7 cfu mL^{-1} of *E. coli* additions

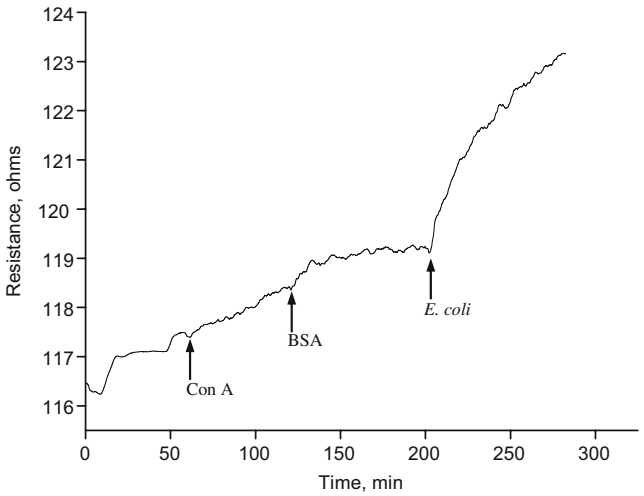

Fig. 3 Series resistance changes on the EQCM probe when Con A, BSA and *Escherichia coli* are added to the cell containing 15 mL of stirred solution of 0.1 M acetate buffer of pH 4.0, 1 mM Ca^{2+} and 1 mM Mn^{2+}. Electrode polarised at −0.200 V vs. Ag/AgCl

concanavalin A was still decreasing 4 h after bacteria addition. The ratio of responses to *E. coli* after 30 min between probes with and without concanavalin A was only 1.4, whereas after 4 h it was 3.8. Therefore, it seems feasible to separate selective from nonselective binding at longer times, as binding events are expected to be slow.

In order to evaluate the possibility of avoiding nonspecific adsorptions of bacteria, other substances have been assayed as blocking agents (short-chain thiols ending with polar and nonpolar groups) without success (data not shown). Also surfactants such a Tween-20 were tested, but no significant improvement of the results was obtained; on the contrary, their reproducibility was poorer.

It is interesting to observe the changes of motional resistance (R_1) on the electrode surface when Con A, BSA and bacteria are added to the cell (Fig. 3). Δf and ΔR measurements are routinely used as independent indicators of mass loading and viscosity at the crystal liquid interface, as it is possible to relate resistance changes to energy dissipation processes [15–17]. Small changes of R_1 suggest that a rigid film is formed on the quartz crystal electrode. From Fig. 3 it is observed that changes in resistance are very small for the protein adsorption processes, whereas big changes are obtained for bacteria layer formation (4.5 Ω in 60 min).

The addition of different *E. coli* concentrations gave rise to significantly different frequency changes, thereby suggesting the possibility of bacterial quantification (Fig. 4).

Lectin immobilisation through the avidin–biotin system

In order to achieve a more robust and sensitive biosensor, the immobilisation of biotin-labelled concanavalin A on an avidin-modified gold-coated quartz crystal electrode was explored, and its response to *E. coli* is compared with the results discussed above.

Experiments were carried out by adding avidin directly to the measuring cell containing 15 mL of acetate buffer of pH 5.0 and Ca^{2+} and Mn^{2+}; frequency changes were measured at a clean gold QCM crystal without polarisation. Following the same reasoning as with Con A, coverages close to 100% were obtained (RSD=8%) which were retained on the surface even after rinsing with pure buffer. Retention of the protein was now possible by the sulfur bonds formed between cysteine thiol groups from the protein and the gold surface, which were not possible for Con A, as its amino acid sequence has no cysteine residues [18].

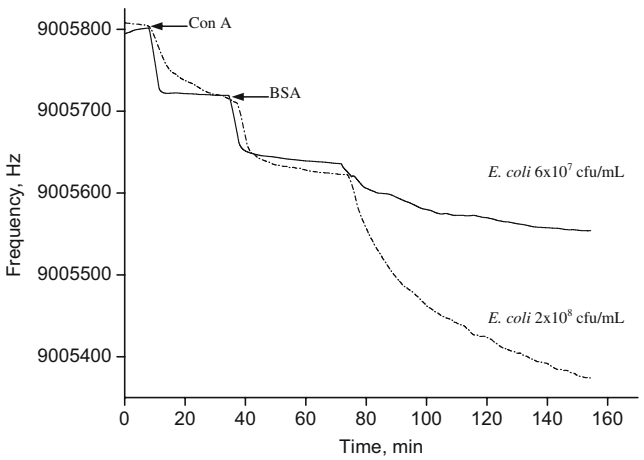

Fig. 4 Frequency changes obtained after additions of (—) 2 mg concanavalin A, 40 mg BSA and 6×10^7 cfu mL^{-1} of *E. coli*; (-·-) 2 mg concanavalin A, 40 mg BSA addition and 2.0×10^8 cfu mL^{-1} of *E. coli*. Other conditions as in Fig. 3

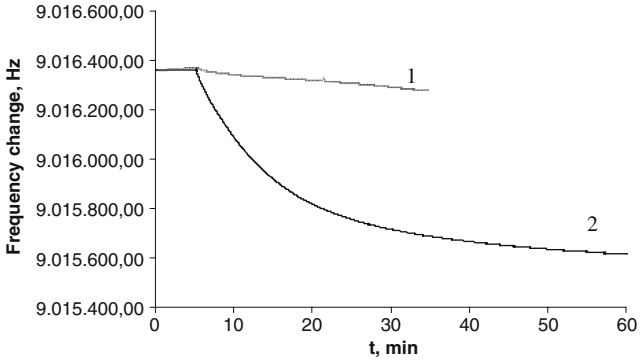

Fig. 5 Frequency changes on a gold-coated quartz crystal modified with avidin–biotin–Con A after addition of 6×10^7 cfu mL^{-1} *E. coli*: without biotin–Con A in solution (*1*) or with biotin–Con A in solution (*2*). Other conditions as in Fig. 3

The subsequent addition of biotinylated Con A to the cell yielded frequency shifts values of 77±10 Hz. These shifts are close to the total theoretical dimer–Con A coverage (ca. 76 Hz). As avidin possesses four biotin binding sites, each pair on opposite faces, under the optimal conditions each avidin molecule could accept two biotins, which for the biotin–Con A would mean two dimers. Nevertheless, due to steric hindrances, not all of the biotin recognition sites on the avidin were occupied after the addition of the biotinylated lectin. In this case, as no significant resistance changes were measured upon addition of the conjugate to the sensor surface, the avidin–biotin–Con A bilayer can still be treated as a rigid layer, and mass addition calculation using the Sauerbrey can be considered as valid.

With crystals modified with Con A via avidin–biotin immobilisation, frequency changes showed a linear response to *E. coli* concentration in the 5×10^6–2×10^7 cfu mL^{-1} range. Higher bacteria concentration produced a curvature in the calibration plot indicating system saturation. Nevertheless, depending on the bacteria concentration, frequency stabilisation after addition of microorganisms to the measuring cell can take as long as 6 h. Therefore, frequency changes with *E. coli* concentration were measured after a fixed period of time.

It should be noted that, in this configuration, avidin is located on the crystal surface and it is covered with biotinylated Con A. Therefore, although at the pH working value avidin is positively charged, the efficient surface coverage produced by the subsequent addition of biotin–Con A avoids electrostatic interaction between avidin and negatively charged bacteria.

A remarkable increase in sensitivity was achieved when bacteria were added directly to the solution containing biotinylated concanavalin A which was used for concanavalin A immobilisation, i.e. no change of solution was carried out after lectin immobilisation and the solution contained free biotin–Con A not attached to the crystal surface. In this case, after *E. coli* addition, frequency changes were eight times bigger than when no Con A is dissolved in the solution (see Fig. 5). This result is probably due to multiple lectin adhesion to *E. coli* cell wall, which produced an accumulation of concanavalin A–*E. coli* conjugates in the form of multilayers at the crystal surface.

Under these conditions, and after 1 h of reaction in the cell, a dynamic response between 2.0×10^5 and 2.0×10^8 cfu mL^{-1} could be obtained (see inset of Fig. 6), dramatically improving the detection limit to approximately 1×10^4 cfu mL^{-1} in the measuring cell. This value was estimated as three times the standard deviation of the blank signal. Figure 6 shows a typical response curve. The reproducibility of this type of response was evaluated by measuring frequency changes for eight different solutions containing 6.6×10^6 cfu mL^{-1} *E. coli*. The relative standard deviation value was 12% which

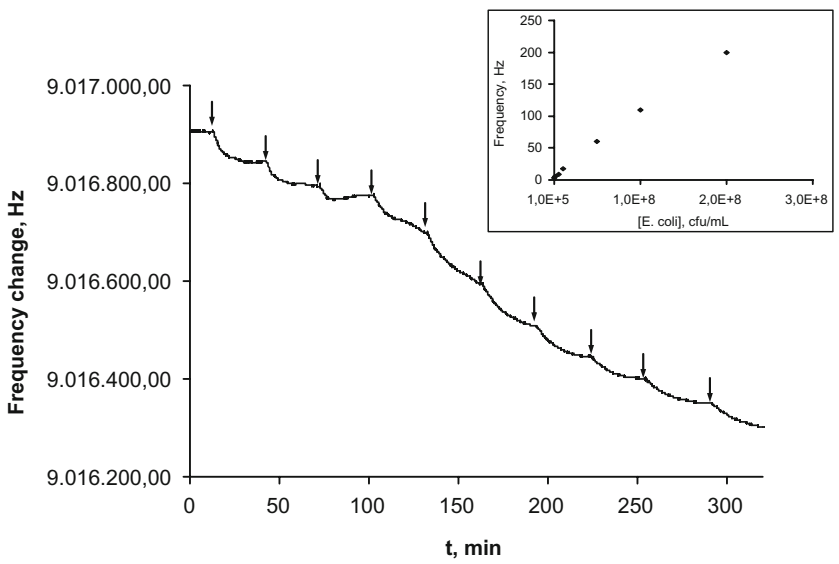

Fig. 6 Frequency changes on a gold-coated quartz crystal modified with avidin–biotin–Con A immersed in solution containing biotin–Con A for successive additions of 1.7×10^6 cfu mL^{-1} bacteria to the cell. *Inset* frequency changes versus *E. coli* concentration after 1 h of reaction in the cell

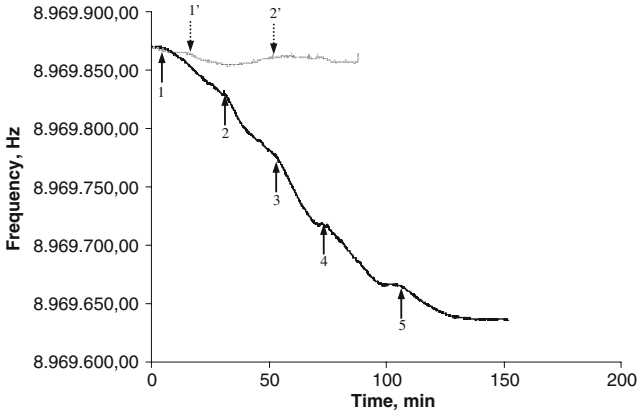

Fig. 7 Frequency changes obtained with an avidin–biotin-*Arachis hypogaea* lectin modified gold-coated quartz crystal immersed in 15 mL of 0.1 M stirred acetate solution of pH 5.0 containing 1 mM Ca^{2+} and 1 mM Mn^{2+} and free biotin-*Arachis hypogaea* lectin upon additions of *S. aureus* (*solid line*, *1* 1×10^6 cfu mL^{-1}, *2–5* 1×10^7 cfu mL^{-1} each) and *E. coli* (*dotted line*, *1′* 6×10^7 cfu mL^{-1}, *2′* 2×10^8 cfu mL^{-1}) into the measuring cell

can be considered as acceptable considering that bacteria quantification is carried out.

One of the advantages of the biosensors prepared in this way is the minimisation of nonspecific adsorptions. As an example of this, Fig. 7 shows the response of a piezoelectric resonator modified with avidin to which biotinylated *Arachis hypogaea* lectin was bound. This lectin has no affinity for *Escherichia coli*, but binds *Staphylococcus aureus* [6]. As can be observed, no significant frequency change after *E. coli* additions was obtained even for large bacteria concentrations, whereas the addition of *S. aureus* yielded well-defined frequency changes.

On the other hand, *S. aureus* has a strong affinity towards Con A, with a frequency change of 120 Hz 90 min after addition of 1×10^7 cfu mL^{-1} to the measuring cell.

Moreover, the responses to *Mycobacterium phlei* when Con A and *Arachis hypogaea* lectin were immobilised on

Table 1 Frequency responses obtained for three microorganisms using a gold-coated quartz crystal modified with either Con A or *Arachis hypogaea* lectin vis avidin–biotin bonds

Lectin	*E. coli*	*S. aureus*	*M. phlei*
Con A	+	+	−
Arachis hypogaea	−	+	+

the quartz crystal surface have also been evaluated: positive responses were observed for *Arachis hypogaea* lectin, whereas no response was obtained for Con A (Fig. 8). Therefore, using just two lectins, different response profiles can be achieved for the three microorganisms evaluated (Table 1), which demonstrates the feasibility of bacteria discrimination and quantification using a battery of lectins immobilised on the quartz crystal surface.

The results shown in Fig. 6 demonstrate the feasibility of fabrication of lectin-based piezoelectric biosensors for bacteria determination at concentrations around 10^5 cfu mL^{-1} in 1 h. Furthermore, negative controls (i.e. with no bacteria) under the optimised conditions showed no significant frequency changes and consequently no significant nonspecific adsorption. In order to increase the sensitivity of the method, a three-step approach was evaluated. Firstly, an incubation step, in which bacteria were allowed to react in solution with the biotinylated lectin for 1 h, was carried out; the solution was then filtered through a 0.2-μm-pore-size Nylon filter, and all bacteria, free and bound lectin, were retained. The filtrate containing unbound biotinylated lectin was discarded. Subsequently, the filter was introduced into the measuring cell containing 15 mL 0.1 M acetate buffer solution and in which an avidin-modified gold-coated quartz crystal was immersed. Therefore, the biotin–lectin–bacteria complex was bound on the gold-coated quartz surface via avidin–biotin bonds. Preliminary experiments with 1.0×10^3 cfu mL^{-1} *E. coli* yielded a frequency change of

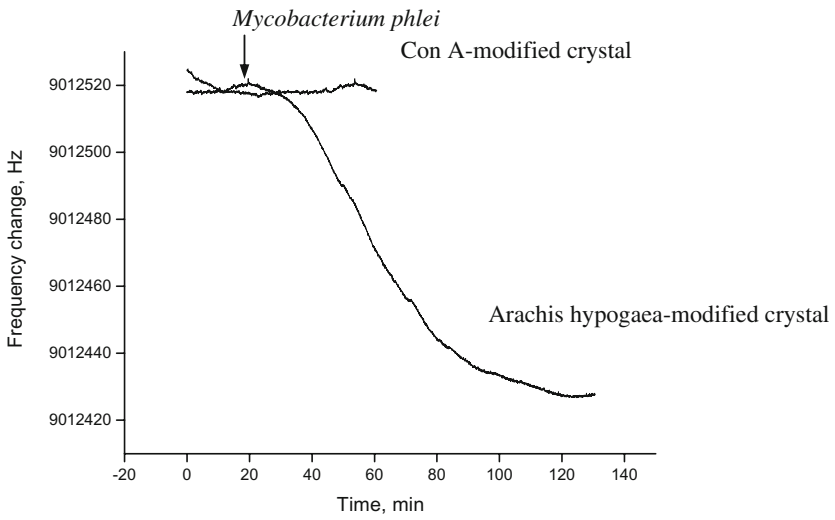

Fig. 8 Frequency changes obtained at an avidin–biotin–Con A and an avidin–biotin–*Arachis hypogaea* lectin modified gold-coated quartz crystal immersed in 15 mL of stirred 0.1 M acetate solution of pH 5.0 containing 1 mM Ca^{2+} and 1 mM Mn^{2+} and free biotin–lectin when 2.7×10^4 cfu mL^{-1} *M. phlei* is added into the measuring cell

approximately 65 Hz 60 min after the addition. Although this approach seems to offer exciting possibilities, its optimisation is still required.

Electrochemical determination of bacterial catalase activity

In order to further increase the sensitivity and discriminate between viable and nonviable cells, an approach using electrochemical measurements of bacterial catalase activity was checked. In this approach, once the bacteria were attached to the electrode surface via lectin interaction, the probe was rinsed with water and immersed in fresh 0.1 M acetate buffer solution. The electrode was then polarised to +0.6 V vs. Ag/AgCl, which is an adequate value for H_2O_2 detection, and the current was allowed to stabilize. After approximately 5 min, 10^{-4} mol L^{-1} hydrogen peroxide was added into the cell. The amperometric response was recorded and compared with that obtained for a free bacteria quartz crystal. Although the methodology has to be optimised, very promising preliminary results have already been obtained. For instance, an avidin–biotin–Con A quartz crystal in contact with an *E. coli* suspension of 1×10^5 cfu mL^{-1} for 1 h yielded a hydrogen peroxide signal 87% smaller than the crystal modified with free bacteria. These results suggest that the measurement of catalase activity on the surface-bound bacteria may allow an estimation of bacteria activity.

Conclusions

A new method is proposed for the easy and early detection, identification and quantification of microorganisms based on specific recognition through wall cell glycocalyx components of bacteria by immobilizing lectin proteins on a gold-plated quartz crystal surface. The piezoelectric transduction allows label-free real-time monitoring of the binding event. Nonspecific adsorption of nontargeted organisms has been minimised. Further development of the method would consist of building up a battery of lectin–quartz crystals as sensors to obtain a response profile for different microorganisms which would permit the identification via statistical analysis.

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