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GaAs/AlGaAs heterostructure based photonic biosensor for rapid detection of *Escherichia coli* in phosphate buffered saline solution

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ABSTRACT

We have investigated photonic biosensing of bacteria based on photoluminescence (PL) of GaAs/AlGaAs heterostructures. The method takes advantage of the GaAs PL sensitivity to the perturbation of the semiconductor near-surface electric field induced by the charge of bacteria immobilized in its vicinity. Maintaining the balance between device sensitivity and stability in the biosensing (aqueous) environment is one of the key parameters allowing successful biosensing. To immobilize bacteria, we have employed a network of biotinylated antibodies interfaced with biotinylated polyethylene glycol thiols through the link provided by neutravidin. Post-processing of thiolated samples in ammonium sulfide was applied to increase the stability of the biosensing architectures while allowing biosensing at an attractive level of detection. *Escherichia coli* was detected in phosphate buffered saline solutions at 10^3 CFU/mL; however, it appears that even greater sensitivity levels are feasible with this technique.

1. Introduction

The need to detect pathogenic bacteria rapidly and with high sensitivity is well documented in the literature [1–3]. While traditional bacteria detection techniques involving culture-based methods [4] are sensitive and relatively inexpensive [2], they are time-consuming and labour intensive [5–7]. The polymerase chain reaction (PCR) [8] offers considerable benefits over culture based methods including rapidity and equivalent sensitivity; however, the complexity of this technique and the requirement of highly qualified personnel for both sample preparation and interpretation make it unattractive for many users [9]. Automated PCR systems are expensive and target a limited number of microorganisms. Piezoelectric biosensors [10] hold good potential for detection of food-born and water-born microorganisms, but they are affected by relatively low sensitivity [7]. Electrochemical impedance spectroscopy (EIS) represents a sensitive approach to detect pathogenic microorganisms with a low limit of detection and a large linear range [11–13]. However, the EIS analysis, in addition to time consuming sample preparation procedure, has suffered due to the difficulties in accurate interpretation of experimental data and assigning a correct modeling [14]. Thus, the fabrication of an EIS device capable of delivering attractive results has remained an elusive task (see, *e.g.*, [15] and references therein). In that context, the surface plasmon resonance (SPR) technique has demonstrated detection of pathogenic microorganisms, such as *O157:H7* [16], but the cost and the generally large size of SPR instruments limit their application to the laboratory environment [6]. Table 1 illustrates typical detection limits and time to detection of *Legionella pneumophila*, *Escherichia coli* and *Salmonellae* bacteria achieved with common detection techniques. It is worth mentioning that the requirement of a low limit of detection could result in a significantly increased time to detection. For instance, it takes around 3 h for a dual wavelength fluorometry technique to detect 10 CFU/mL of *Enterococcus faecalis*, whereas detection of these bacteria at less than 1 CFU/mL requires more than 5½ h [17].

Photoluminescence (PL) emitting semiconductors offer an attractive alternative in developing biosensing devices due to the sensitivity of the PL effect to the presence of electrically charged molecules, such as viruses or bacteria trapped in the vicinity of the

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Table 1
Typical detection limits and time to detection of selected bacteria achieved with some common detection techniques.

Detection technique	Bacteria	Time of analysis	Detection range (CFU mL^{-1})	Detection limit (CFU mL^{-1})	Ref.
Colony count	*L. pneumophila*	5–14 days	2.5–994	1	[18]
Enzyme-linked immunosorbent assay (ELISA)	*E. coli*	Next day	10^3–10^4	1.2×10^3	[19]
PCR-ELISA	*E. coli*	5 h	10^0–10^4	10^2	[20]
PCR-electrophoresis	*E. coli*	2 h	10^1–10^4	10^3	[20]
Real-time PCR	*E. coli*	5 h 20 min	5–5×10^4	5	[21]
Piezoelectric	*E. coli*	30–50 min	10^3–10^8	10^3	[22]
Quartz Crystal Microbalance (QCM)	*Salmonellae*	60 min	10^5–5×10^8	10^4	[23]
QCM	*E. coli*	170 min	10^3–10^8	10^3	[24]
EIS	*E. coli*	Not quoted	3×10^1–3×10^4	2	[12]
SPR	*E. coli*	10–30 min	10^4–10^8	10^4	[16]
SPR	*L. pneumophila*	2 h 20 min	10^2–10^9	10^2	[25]

semiconductor surface. Examples of devices built on such a principle include colloidal QDs of CdSe, ZnS, CdS, ZnSe, CdTe and PbSe [26–29] and bulk GaAs [30–32]. Protection (passivation) of cleaned and etched surfaces of semiconductors from oxidization plays an important role in functioning of PL-based biosensors [33], and investigation of electronic or photonic properties of semiconductors surrounded by aqueous environments [34–36]. The common approach in passivation of GaAs involves sulfurization from ammonium sulfide [37,38] or coating with vacuum evaporated Ga_2O_3 [39] and Si_3N_4 films [40,41]. Deposition of self-assembled monolayers (SAMs) of n-alkanethiols [$HS(CH_2)_nR$] on the surface of GaAs is attractive because the SAM head group (HS) provides means of sulfurization while its terminal group (R) could be implicated in building biosensing architectures [42–45]. While full chemical passivation of GaAs has been successfully achieved and demonstrated, *e.g.*, in the operation of molecular controlled semiconductor resistor (MOCSER) devices [36], working with a not entirely passivated GaAs surface allows to investigate the role of the photocorrosion effect for biosensing. This is possible since electrically charged molecules, if immobilized in the vicinity of the semiconductor surface, could modify both band bending of a semiconductor and the concentration of photo-holes generated at the semiconductor surface. A detailed discussion of this approach has been published elsewhere [46].

Previously, we used SAM functionalized GaAs/AlGaAs microstructures and demonstrated PL-based detection of *E. coli* in phosphate buffered saline (PBS) solution at 10^4 CFU/mL [41]. In the present study, we have investigated detection of *E. coli* using SAM functionalized GaAs/AlGaAs microstructures with their surface also coated with sulfur precipitated from an ammonium sulfide (AS) solution. This additional processing step yields microstructures with the increased photonic stability [47] expected to play an important role while addressing detection of bacteria at reduced concentrations.

2. Experimental methods

2.1. Materials

A nominally undoped epitaxial GaAs/$Al_xGa_{1-x}As$ ($x = 0.35$) heterostructure used in this study (J0149) comprised a 500 nm thick layer of GaAs that emitted a strong PL signal at 869 nm excited with a homogenized beam of a 532 nm laser. Grown above that layer were 3 and 8 nm thick GaAs layers separated by 100 and 10 nm thick $Al_xGa_{1-x}As$ layers. Fig. 1 shows schematically a cross-section of the device and a typical PL emission plot observed at 869 nm during photocorrosion of the GaAs cap.

Biotinylated polyethylene glycol (PEG) thiols and hexadecane thiols (HDT) were obtained from Prochimia Surfaces (Gdansk, Poland) and Sigma–Aldrich (Ontario, Canada), respectively. Polyclonal biotinylated antibodies against *E. coli* were purchased from ViroStat, Inc. (Portland, ME) and a PBS solution (10×, pH 7.4) was purchased from Sigma (Oakville, Canada). Neutravidin was bought from Molecular Probes (Invitrogen, Burlington, Canada). The solvents used to remove impurities from the surface of the samples are OptiClear (National Diagnostics (Mississauga, Canada)), acetone (ACP, Montréal, Canada) and isopropanol (2-propanol) (Fisher Scientific, Ottawa, Canada). Ammonium hydroxide 28% (NH_4OH) was purchased from Anachemia (Richmond, Canada) and ammonium sulfide (48%) from Sigma–Aldrich (Ontario, Canada). The streptavidin-coated microbeads used in a modeling study of bacteria immobilization were purchased from Bangs Laboratories, Inc. (Indiana, United States). *E. coli* K12 and *Bacillus subtilis* bacteria were obtained from the Department of Biology of the Université de Sherbrooke (Quebec, Canada). The *E. coli* and *B. subtilis* bacteria were grown in Luria Bertani (LB) and minimal broths, respectively,. Before use, the bacteria were stored in 50% glycerol at −26 °C.

2.2. Preparation of the GaAs (001) surface and biofunctionalization

Semiconductor samples of 2 mm × 2 mm were cleaned using OptiClear, acetone and isopropanol in an ultrasonic bath (5 min for each). After the cleaning step, the samples were dried using a flow of nitrogen and then etched in a concentrated solution

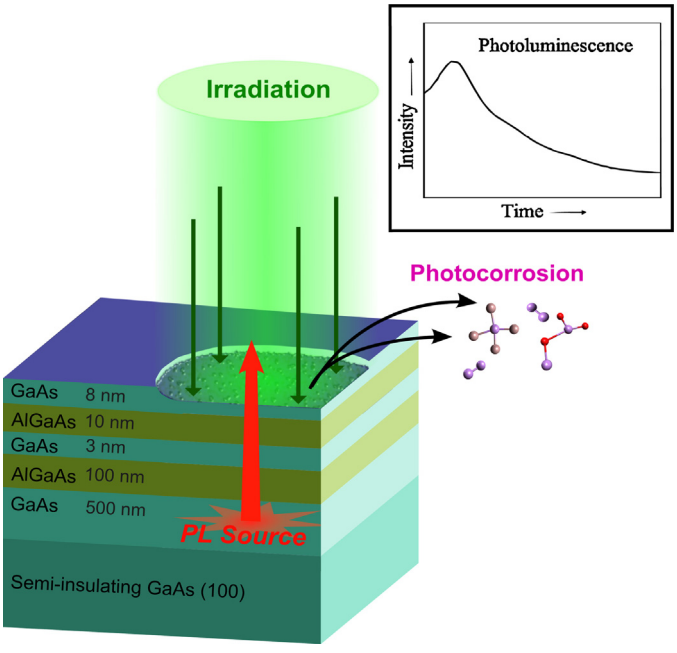

Fig. 1. Schematic of the GaAs/$Al_{0.35}Ga_{0.65}As$ heterostructure employed for biosensing. The inset shows PL emission at 869 nm observed during photoocorrosion of the GaAs cap.

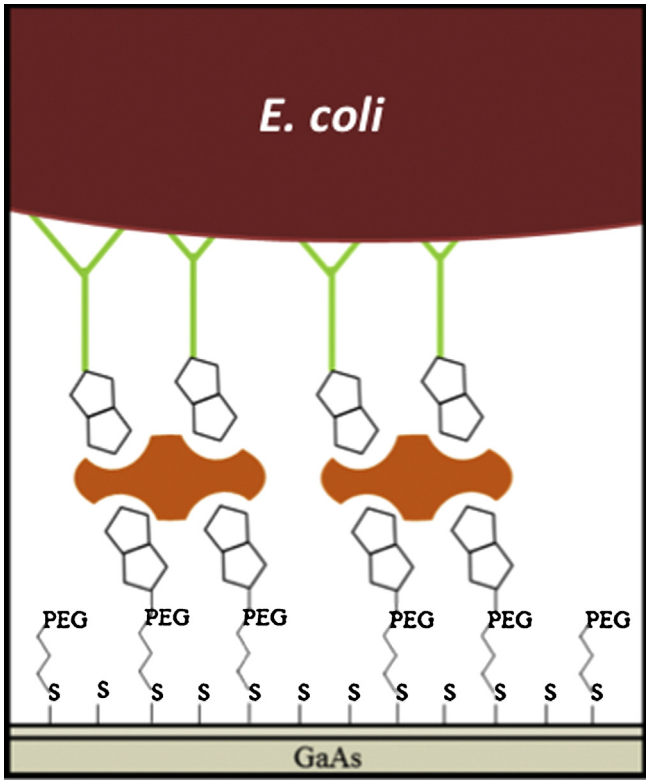

Fig. 2. Schematic illustration of the biosensor structure functionalized with PEG SAM and biotin conjugated antibodies exposed to *E. coli* K12.

of ammonium hydroxide (28%) for 2 min at room temperature to remove native oxides. Following rinsing with deoxygenized ethanol, the samples were incubated for 20 h at room temperature in a mixture of biotinylated PEG thiol and HDT (1:15) diluted in the deoxygenized ethanol at a final concentration of 2 mM. PEG-based thiols were used because they are known to decrease non-specific binding [48]. After the thiolation step, the samples were rinsed with deoxygenized ethanol to remove surplus thiol molecules physisorbed on the surface. Thereafter, the samples with biotin terminated SAMs were exposed to 0.1% AS for 15 min and then rinsed with deionized (DI) water. The preparation process continued by incubation of the samples for 2 h in PBS (1×) solution containing 0.2 mg/mL of neutravidin. This step was followed by the exposure of the neutravidin-coated samples to biotinylated antibodies against *E. coli* diluted in PBS (1×) at 0.1 mg/mL, corresponding to a concentration of 0.625 μM. The samples were stored for 1 h in antibody (Ab) solution at room temperature. After the Ab coating step, the samples were exposed to different concentrations of *E. coli* bacteria suspended in PBS (1×) and their PL emission was recorded by *in situ* measurements. For the negative control tests, the biofunctionalized samples with *E. coli* Ab were exposed to 10^5 CFU/mL of *B. subtilis* bacteria. The structure of the biosensor used for bacteria detection is schematically shown in Fig. 2. The validation of a similar PEG-thiol SAM structure for immobilization of influenza A virus was investigated by us previously using Fourier transform IR spectroscopy (FTIR) [49].

2.3. Immobilization of streptavidin coated microbeads

To investigate the 2-dimensional immobilization efficiency of bacteria on the surface of functionalized biochips, we employed a model consisting of 3 μm diameter microbeads coated with streptavidin and a network of biotinylated PEG thiol SAMs. The SAM formation was achieved by following the standard thiolation procedure. GaAs (0 0 1) samples of 2 mm × 2 mm, after etching in ammonium hydroxide (28%) and rinsing with deoxygenized ethanol, were incubated for 20 h at room temperature in a mixture of biotinylated PEG thiols and OH-terminated PEG thiols (1:5) diluted in the deoxygenized ethanol to a final concentration of 2 mM. After this step, the samples were rinsed again with deoxygenized ethanol and exposed for 30 min to beads suspended in PBS (1×) at 10^5 beads/mL. At the final step, the samples were rinsed with PBS (1×) and then with DI water. The microbead-coated samples were analyzed with a ZEISS optical microscope with the total magnification of 500× working in DIC mode. The same procedure was applied for the bacteria exposed samples. High-resolution imaging was carried out with an atomic force microscope (AFM, Digital Instruments Dimension 3000). For the AFM images, etched single-crystal silicon tips of around 5–20 nm radius with a rectangular, single beam cantilever (TESP from Digital Instruments) were applied. The cantilever spring constant and the resonance frequency were 20–100 N/m and 200–400 kHz, respectively. For this measurement, the bacteria exposed samples were rinsed with DI-water and the bacteria were fixed with glutaraldehyde (2.5%). The AFM measurement operated in a tapping mode and the measurement has been carried out in an air environment. The AFM images were analyzed with WSxM 3.0 software and the brightness and contrast were slightly enhanced using Adobe Photoshop CS4 software.

2.4. PL based GaAs/AlGaAs biosensor

Due to the discontinuity of the bulk properties of GaAs and $Al_xGa_{1-x}As$, the presence of significant density surface states is expected on the surface of these materials. This, and the exposure to air, water or other medium will result in a significant band bending of both GaAs and $Al_xGa_{1-x}As$. The associated near-surface electric field plays an important role in the behavior of carriers (electrons and holes) excited in this region. For n-type semiconductors, photo-excited holes (h^+) will be driven towards the interface and photo-excited electrons (e^-) towards the bulk. The net result is a decreased chance of radiative e^-–h^+ recombinations and, consequently, a reduced intensity PL signal [50]. In contrast, the process of surface passivation of III–V semiconductors manifests by an enhanced PL emission due to the reduced concentration of surface states and decreased surface recombination velocity (SRV) of electrons and holes [44,45,51–54]. An illustration of such a process is enhanced PL observed during thiolation of GaAs samples [51,52,55–58]. Due to the interaction with the depletion layer of GaAs, the electric field of an interfacial dipole layer formed by SAM decreases the number of non-radiative recombination events and, consequently, results in an increased PL signal [54]. The increase of PL emission has also been observed depending on the length of a molecular chain, its wetting characteristics and time of immersion of GaAs in a thiol solution [59]. However, it is known that alkanethiol SAMs provide only partial passivation of the GaAs (0 0 1) surface [43,44], resulting in relatively unstable SAM-GaAs interfaces in a water environment. This instability is related to the formation of photo-holes at the surface GaAs irradiated with photons of energy exceeding its bandgap energy. The photo-holes induce photocorrosion of GaAs via the formation of surface oxides that dissolve into solution due to their thermodynamic instability in an acidic environment. The process, described by the following reaction [60]:

$$GaAs + 2H_2O + 6h^+ \rightarrow Ga^{3+} + HAsO_2 + 3H^+ \quad (1)$$

indicates that photocorrosion of an n-type semiconductor strongly dependents on the presence of positively charged minority carriers (h^+) on its surface. To address the problem of a limited surface passivation provided in such a case by SAM, we have investigated post-processing of alkanethiol SAM coated GaAs (0 0 1) with AS

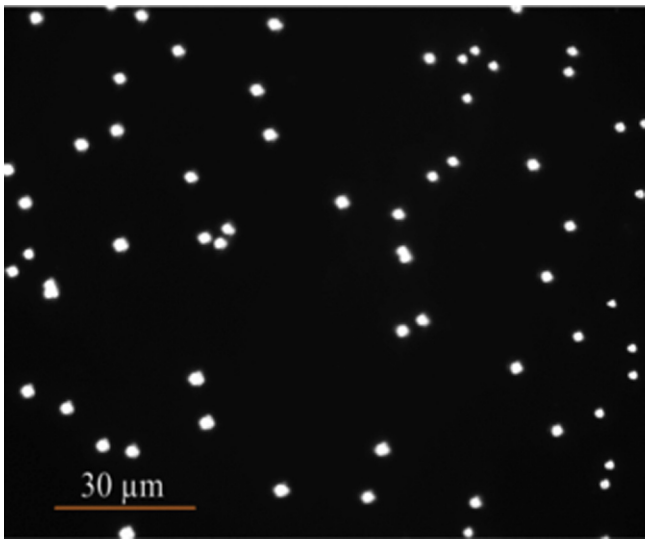

Fig. 3. Microscopic image of a functionalized surface of GaAs (001) following the exposure to a PBS solution of streptavidin coated microspheres at 10^5/mL.

and, based on X-ray photoelectron spectroscopy and FTIR measurements, we have demonstrated that this treatment increases the number of sulfur atoms reacting with Ga and As without measurable modification of the quality of SAM [45].

The process of photocorrosion was investigated with a custom designed (Photon etc., Montreal) hyperspectral imaging PL mapper (HI-PLM) designed for collecting *in situ* PL spectra of laser irradiated samples [61]. Normally, GaAs and AlGaAs layers are etched at a rate determined by the parameters of an excitation source (laser) and electrolytes of different etching power, such as PBS, water or ammonium hydroxide. As etching removes surface defects and GaAs and, consequently, reveals the AlGaAs layer, one can expect to observe measurable changes of the PL signal originating from the 500 nm thick GaAs layer of a structure depicted in Fig. 1. Furthermore, as positively charged objects, such as bacteria decorated with positive ions extracted from the solution, approach the semiconductor surface, the band bending at the surface is reduced, resulting in reduced transport of photo-excited holes to the surface. This process is expected to slow down photocorrosion with the efficiency increasing with the concentration of bacteria trapped in the vicinity of the semiconductor surface.

3. Experimental results and discussions

3.1. Immobilization of functionalized microspheres and E. coli bacteria on GaAs (001)

Fig. 3 shows a uniformly covered surface of GaAs with microbeads following the exposure to a microbead solution at 10^5/mL. The mechanism of attachment of microbeads is based on the streptavidin-biotin interaction characterized by the dissociation constant, K_d, of 4×10^{-14} M [62]. A significantly greater dissociation constant, $K_d = 10^{-9}$ M [63] characterizing antigen–antibody complexes, suggests that for a comparable concentration of binding sites, the surface concentration of the investigated microbeads should be greater than that of bacteria.

In spite of these differences, we systematically observed greater surface coverage with *E. coli* for comparable volume concentrations of microbeads and bacteria. Fig. 4 shows examples of optical images obtained for *E. coli* Ab functionalized samples exposed to PBS, 10^5 CFU/mL of *B. subtilis* and 10^5 CFU/mL of *E. coli*. The inset in Fig. 4c shows an AFM picture of one of the *E. coli* bacterium immobilized on the Ab functionalized surface of GaAs. The long and short axis of this glutaraldehyde fixed bacterium, respectively 2 and 1 μm, are consistent with the typical dimension of a *E. coli* bacterium reported in literature [64,65]. For the negative control test, the maximum number of *B. subtilis* immobilized on the biochip was at 0.13 bacteria/100 μm^2. This compares to 1.1 bacteria/100 μm^2 of *E. coli*, and to 0.5 microbeads/100 μm^2 observed for the same volume concentrations. The average number of bacteria immobilized on the 100 μm^2 of GaAs was 0.9 and 0.09 for 10^5 CFU/mL of *E. coli* and 10^5 CFU/mL *B. subtilis* respectively. The relatively greater surface coverage achieved with *E. coli* in comparison to that with microbeads is surprising given the relatively large dissociation constant characterizing the antibody-bacteria interaction. Our measurements show that the zeta potential of streptavidin coated microbeads in their buffer is around −16 mV, whereas, the zeta potential of *E. coli* bacteria in PBS (1×) at pH 7.4 is around −19 mV. Thus, the almost identical zeta potentials of the beads and bacteria cannot account for the observed differences in the surface coverage. Plausible reasons of the reduced binding efficiency of the investigated microbeads could be low concentration of neutravidin sites on surface of microbeads as well as the increased curvature and rigidity of the microbead surface when compared to *E. coli* that reduces the number of neutravidin molecules interacting with the surface. Furthermore, steric hindrance occurring between larger-than-bacteria microbeads and the streptavidin biding sites could also lead to a reduced binding efficiency.

3.2. Photonic detection of E. coli in PBS

After functionalization of the GaAs/AlGaAs biochips with *E. coli* Ab, as described in Section 2.2, the samples were exposed either to PBS or to different concentrations of bacteria diluted in PBS, while their PL emission was recorded *in situ* over the period of up to 2 h. Following the initial 30 min exposure to bacteria suspensions, the samples were rinsed with PBS to reduce the contribution to the PL signal from the bacteria nonreacted with Ab. The 30-min exposure time was assumed sufficient for allowing a reasonable amount of bacteria in the microfluidic chamber to interact with the antibody sites of the biochip surface [66]. This step could be refined further,

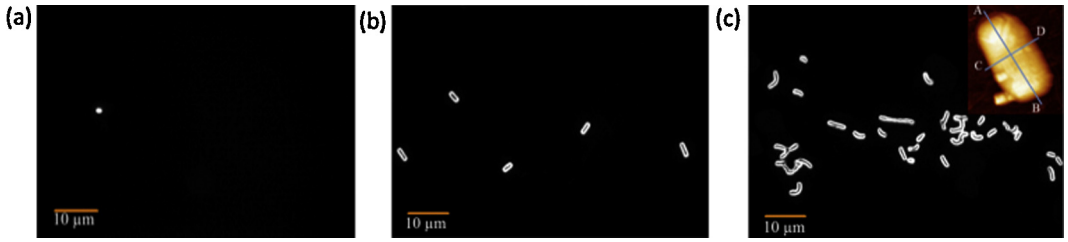

Fig. 4. Optical images of the GaAs samples biofunctionalized with *E. coli* Ab and exposed for 30 min to (a) PBS (1×) solution, (b) 10^5 CFU/mL of *B. subtilis* (0.1 bacteria/100 μm^2), and (c) 10^5 CFU/mL of *E. coli* (1.1 bacteria/100 μm^2). Inset shows an AFM image of an individual *E. coli* (AB = 2 μm, CD = 1 μm).

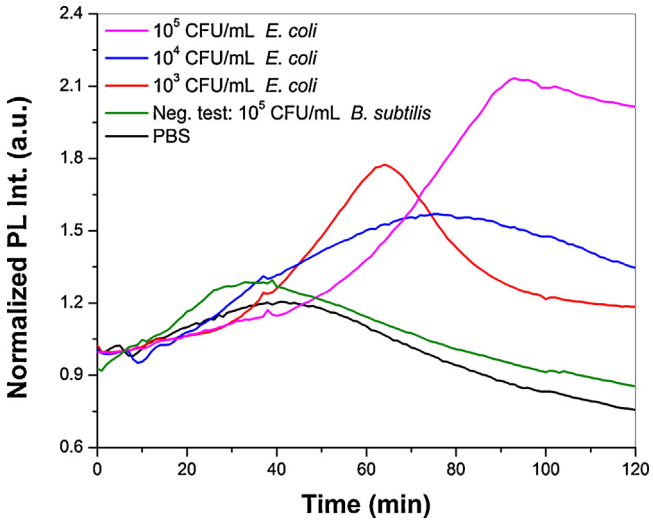

Fig. 5. Normalized PL intensity measured *in situ* for the samples exposed to different concentrations of bacteria. The samples were exposed to bacteria at $t = 0$ and then were rinsed with PBS after 30 min of bacteria exposure.

e.g., by increasing the exposure time or recirculating the stream of bacterial solution over the biochip surface.

Fig. 5 shows a series of time-dependent PL intensity plots for samples exposed to *E. coli* at 10^3, 10^4 and 10^5 CFU/mL, and to *B. subtilis* at 10^5 CFU/mL. For comparison, we also show a PL plot collected from an Ab biofunctionalized chip exposed to PBS. A characteristic feature of each plot is the presence of a maximum revealed at some time from the onset of the experiment. In agreement with data from the literature [67] and our XPS experiments [46], the increasing intensity of the PL signal is related to the formation Ga_2O_3 that passivates the surface of GaAs. However, during this process, GaAs is slowly dissolved and the PL signal begins decreasing as the thickness of the GaAs layer falls below the critical level where the etch rate of GaAs exceeds the rate of Ga_2O_3 formation. The photocorrosion rate of the GaAs/AlGaAs heterostructure correlates with the concentration of *E. coli* in solution: the greater the bacteria concentration the slower the photocorrosion rate and the formation of the characteristic maximum is delayed. A plausible mechanism behind this behavior is related to a strong interaction between negatively charged bacteria that become decorated with positive ions available in the PBS solution and, upon immobilization in the vicinity of an Ab functionalized semiconductor surface, attract electrons from the bulk of the semiconductor. This will result in a reduced concentration of photo-excited surface holes and lead to a reduced rate of photocorrosion [60], in proportion to the average concentration of the positive charge of the object immobilized in the vicinity of the semiconductor. Additionally, immobilized bacteria could reduce mass transfer of the products of photocorrosion from the semiconductor surface, changing the chemical environment and reducing photo-oxidation rates. Fig. 5 shows an example of normalized PL intensity plots measured *in situ* for a series of samples exposed to different concentrations of bacteria. Note that the *E. coli* antibody functionalized biochip exposed to *B. subtilis* generated PL response comparable to that from the biochip exposed just to PBS. This result confirms a highly specific character of the biosensing architecture employed in these experiments.

Similar sets of experiments were repeated 3 times enabling us to determine temporal position of the PL maxima and calibrate response of the biochips, as well as to evaluate an experimental error of the measurements. The results, summarized in Table 2, indicate an attractive reproducibility of the measurements. It can be seen that the PL maximum position related to the negative test has been determined with ±5% and the error of the maximum position

Table 2
PL peak position for *E. coli* Ab functionalized samples exposed to PBS and different concentrations of *E. coli*. A negative test was carried out for *E. coli* Ab functionalized samples exposed to 10^5 CFU/mL of *B. subtilis*.

Medium	Concentration	PL peak position (min)
PBS	1×	32.3 ± 25%
E. coli	10^3 CFU/mL	64.5 ± 1.1%
E. coli	10^4 CFU/mL	77.0 ± 1.8%
E. coli	10^5 CFU/mL	90.5 ± 3.9%
B. subtilis	10^5 CFU/mL	41.3 ± 5%

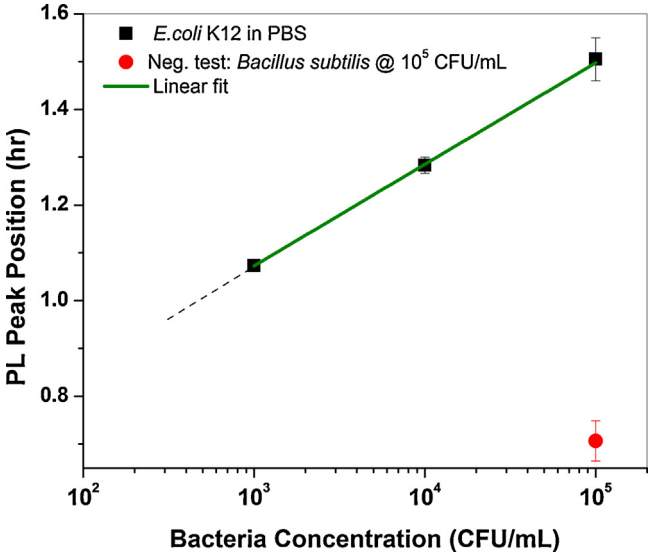

Fig. 6. PL peak position *vs.* different concentrations of bacteria. The PL peak position for the sample exposed to PBS is 32.3 ± 25%.

for 10^5 CFU/mL of *E. coli* does not exceed ±3.9%. A relatively large error of ±25% related to the position of the maximum observed for the biochips exposed to the PBS solution seems to be related to the more aggressive photocorrosion rate induced in that case and a stronger dependence of the result on the diffusion of a fresh etching medium towards the semiconductor surface.

Fig. 6 shows a semi-logarithmic plot of the position of PL maxima as a function of the investigated concentrations of bacteria. Both specificity and detection at 10^3 CFU/mL are well illustrated. It appears that a direct detection of bacteria in less concentrated solutions should be feasible pending the development of a protocol for defining the biochip response to the PBS solution with better accuracy. This development, in addition to the introduction of advanced methods for bacteria concentration, *e.g.*, based on filtration, electrophoresis or chemotaxis, has the potential to lead to an effective detection of bacteria at ~1 CFU/mL.

4. Conclusion

We have investigated detection of *E. coli* K12 suspended in PBS solution using an innovative method involving PL monitoring of the photocorrosion effect in GaAs/$Al_xGa_{1-x}As$ nano-heterostructures. The rate of photocorrosion depends on the concentration of the electric charge immobilized in the vicinity of the surface of PL emitting semiconductor biochips, and it slows down with increasing concentrations of bacteria in PBS solutions surrounding the antibody functionalized biochips. Post-processing of alkanethiol SAM functionalized biochips with ammonium sulphide, which increases their photonic stability in a biological environment, enabled us to demonstrate a direct detection of *E. coli* at 10^3 CFU/mL. This represents one order improved limit of detection in comparison to

that reported by us previously for the same bacteria [41], but it appears that the detection of an even lower concentration of bacteria should be feasible pending the development of a protocol for defining the biochip response to the PBS solution with better accuracy. This development, in addition to the introduction of advanced methods for bacteria concentration, *e.g.*, based on filtration, electrophoresis or chemotaxis, have the potential to lead to an effective detection of bacteria at ~1 CFU/mL.

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