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# Monitoring growth and antibiotic susceptibility of *Escherichia coli* with photoluminescence of GaAs/AlGaAs quantum well microstructures

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ABSTRACT

Development of quick and reliable methods to investigate antibiotic susceptibility of bacteria is vital to prevent inappropriate and untargeted use of antibiotics and control the antibiotic resistance crisis. The authors have developed an innovative, low-cost and rapid approach to evaluate antibiotic susceptibility of bacteria by employing photoluminescence (PL) emission of photocorroding GaAs/AlGaAs quantum well (QW) biochips. The biochips were functionalized with self-assembled monolayers of biotinylated polyethylene glycol thiols, neutravidin and biotinylated antibodies to immobilize bacteria. The illumination of a QW biochip with the above bandgap radiation leads to formation of surface oxides and dissolution of a limited thickness GaAs cap material ($\leq 10$ nm) that results in the appearance of a characteristic maximum in the PL plot collected over time. The position of the PL maximum depends on the photocorrosion rate which, in turn, depends on the electric charge immobilized on the surface of the GaAs/AlGaAs biochips. Bacteria captured on the surface of biochips retard the PL maximum, while growth of these bacteria further delays the PL maximum. For the bacteria affected by antibiotics a faster occurring PL maximum, compared with growing bacteria, is observed. By exposing bacteria to nutrient broth and penicillin or ciprofloxacin, the authors were able to distinguish in situ antibiotic-sensitive and resistant *Escherichia coli* bacteria within less than 3 h, considerable more rapid than with culture-based methods. The PL emission of the heterostructures was monitored with an inexpensive reader. This rapid determination of bacterial sensitivity to different antibiotics could have clinical and research applications.



## 1. Introduction

Monitoring bacterial growth and reactions to specific environments plays an important role in the fields of medicine, food production, and pharmaceutical research with great importance for small-scale, as well as industrial-scale production of natural or genetically engineered bacterial products such as enzymes or hormones (Garneau and Moineau, 2011; Kee et al., 2013; Nayak et al., 2009; Schuler and Marison, 2012; Sonnleitner et al., 1992; Versalovic et al., 2011). Antimicrobial resistance has been recognized as a global problem (Gootz, 2010) related to inappropriate or untargeted use of antibiotics (Engel, 2009; Fleming-Dutra et al., 2016; Kee et al., 2013). Part of the solution lies in effective, rapid and low-cost diagnostic tools to guide optimal use of antibiotics (WHO, 2015). Microbiological antibiotic susceptibility tests (AST) help medical personnel predict the reactions of bacteria to specific drugs and allow them to prescribe appropriate treatments (Jorgensen and Ferraro, 2009). Conventional techniques such as broth microdilution and Kirby-Bauer disk diffusion tests for AST (Poupard et al., 1994; Versalovic et al., 2011) are analytical diagnostic methods which do not provide same-day results (Chiang et al., 2009; Kee et al., 2013; Versalovic et al., 2011) nor can they be easily applied outside laboratory settings. The polymerase chain reaction (PCR) can also be applied for investigation of bacterial susceptibility to antibiotics by detecting resistance markers like the *mec*A gene of *Staphylococcus aureus* (Hombach et al., 2010). However, genetic methods require background knowledge of resistance genes (Quach et al., 2016) and they lack standardization (Cockerill, 1999). In addition, resistance of bacteria to antibiotics might be related to presence of different genes, while the PCR technique only evaluates the presence of specific genes (Cockerill, 1999). An approach based on electrochemical reduction of resazurin, a redox-active molecule, has recently been proposed

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for evaluation of antibiotic susceptibility of *Escherichia coli* claiming that the results could be delivered in one hour (Besant et al., 2015). Surface plasmon resonance (SPR) and plasmonic nanohole arrays represent other approaches to monitor antibiotic susceptibility of bacteria, with a time-to-result for *E. coli* claimed to be 2 h (Chiang et al., 2009; Kee et al., 2013). However, the cost of sensitive SPR systems (Lazcka et al., 2007) and the demanding fabrication process necessary to make uniform plasmonic nanoholes (Kee et al., 2013) are inhibitory factors restricting application of these techniques to laboratory environments. Clearly, development of a simple, rapid and sensitive method for detection of growth and antibiotic sensitivity of bacteria at low cost remains an attractive but elusive goal for clinical diagnostics, food and water control industries. Photoluminescence (PL) emitting semiconductors have the potential to be applied in the biosensing field due to the sensitivity of the PL signal to reactions taking place at the surface of these materials (Adamowicz et al., 1998; Gfroerer, 2006; Lebedev, 2001; Moumanis et al., 2006; Tomkiewicz et al., 2009). For instance, Budz et al. (2010) demonstrated operation of a GaAs-based biosensor for PL detection of adenosine 5′-triphosphate (ATP), and we used a PL-based approach to detect *E. coli* at $10^4$ CFU/mL (Duplan et al., 2011). PL spectroscopy has also been employed to characterize photocorrosion of semiconductors, such as laser-induced etching of GaAs (Joshi et al., 2009). Since the photocorrosion of III-V semiconductors is driven by the surface presence of photo-generated holes (Ruberto et al., 1991), electric and/or electrostatic interactions occurring at the semiconductor surface could increase/decrease concentration of photo-generated holes and, consequently, accelerate/delay the photocorrosion process. Based on this approach, we have developed a PL-monitored photocorrosion method for detection of *E. coli* (Nazemi et al., 2015) and *Legionella pneumophila* (Aziziyan et al., 2016). Furthermore, we have reported that functionalized surfaces of GaAs/AlGaAs biochips remain relatively stable in biological fluids, and provide satisfactory conditions for cultivating *E. coli* (Nazemi et al., 2016). In this report, we discuss the innovative concept of PL monitored photocorrosion of GaAs/AlGaAs biochips for evaluating the growth and susceptibility of bacteria to antibiotics. Using as an example *E. coli* K12 and *E. coli* HB101, we identified the sensitivity of these bacteria to penicillin and ciprofloxacin in less than 3 h. This approach has a considerable advantage over alternative biosensing techniques due to the application of commercially available GaAs/AlGaAs microstructures, suggesting that related experiments could be realized with low-cost devices.

## 2. Experimental methods

### 2.1. Materials

The biochips were fabricated from an epitaxially grown GaAs/$Al_{0.35}Ga_{0.65}As$ quantum well (QW) wafer (10-413) obtained from Azastra Inc. (Ottawa, Canada). A cross-sectional view of the wafer microstructure is shown in Fig. 1a. It comprises 30 pairs of 6 nm thick GaAs QWs and 10 nm thick $Al_{0.35}Ga_{0.65}As$ barriers grown on a 500 nm thick buffer layer of GaAs that was deposited on a semi-insulating GaAs (001) substrate. The QW architecture is capped with a 10 nm thick GaAs layer. The irradiation of such a microstructure with above bandgap radiation, such as that of a 532 nm laser, induces a PL signal dominated by the QW emission at 829 nm, as shown in Fig. 1b.

The solvents used for cleaning the QW wafers were OptiClear, acetone and isopropanol (2-propanol) which were purchased from National Diagnostics (Mississauga, Canada), ACP (Montréal, Canada) and Fisher Scientific (Ottawa, Canada), respectively. Anhydrous ethanol was obtained from Commercial Alcohols Inc. (Brampton, Canada). Ammonium hydroxide ($NH_4OH$) from Anachemia (Richmond, Canada) was employed to remove oxides from the surface of the wafers. Deionized (DI) water with an electrical resistivity of 18 MΩ cm was obtained through a domestic purification system connected to the city water supply. Ultra-high purity nitrogen 5.0 UHP (99.999%) used for deoxygenation of anhydrous ethanol, and high purity nitrogen 4.8 HP (99.998%) used for drying the chips were both purchased from Praxair Canada Inc. (Mississauga, Canada). Hexadecane thiol (HDT) and biotinylated polyethylene glycol (PEG) thiol were bought from Sigma-Aldrich (Oakville, Canada) and Prochimia Surfaces (Gdansk, Poland), respectively. A 48% aqueous solution of ammonium sulfide (AS) was obtained from Sigma-Aldrich (Oakville, Canada). Phosphate buffered saline (PBS) solution (10X, pH 7.4) and polyclonal biotinylated antibodies (Ab) against *E. coli* were purchased from Sigma (Oakville, Canada) and ViroStat, Inc (Portland, ME), respectively. Neutravidin (NA) was bought from Molecular Probes (Invitrogen, Burlington, Canada). Penicillin, ciprofloxacin and Luria Bertani (LB) medium were purchased from Sigma-Aldrich (Oakville, Canada). Live *E. coli* K12 and *E. coli* HB101 bacteria were obtained from the Department of Biology, Université de Sherbrooke (Quebec, Canada). The bacteria were grown in LB broth and kept overnight at 37 °C before starting experiments.

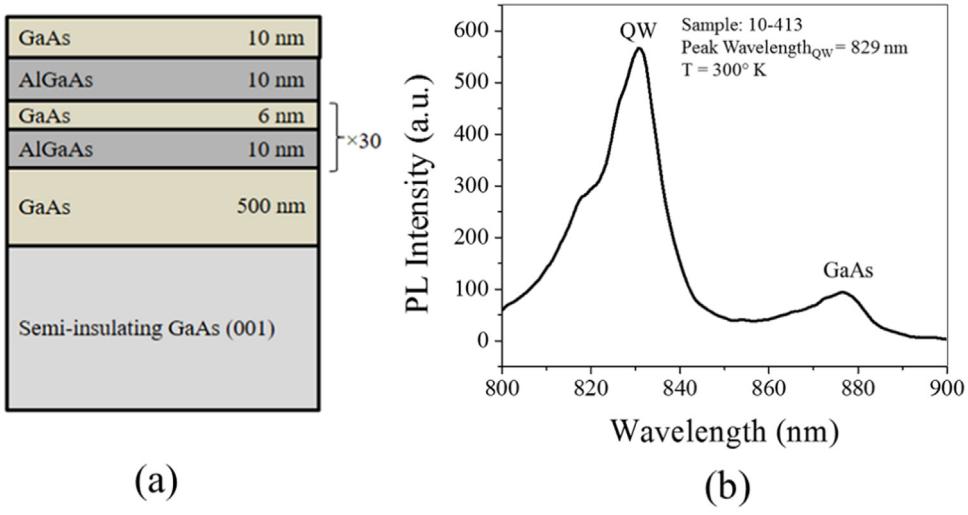



**Fig. 1.** Cross-section of the GaAs/AlGaAs QW microstructure employed for the fabrication of biochips (a), and an example of the PL emission observed at RT from the microstructure irradiated with a 532 nm laser (b).

### 2.2. Biofunctionalization of the GaAs/AlGaAs biochips

Chips of 2 mm × 2 mm were separated from the QW wafer and cleaned in an ultrasonic bath sequentially in acetone, OptiClear, acetone, and isopropanol for 5 min each. Thereafter, the chips were dried with compressed high-purity nitrogen and etched in $NH_4OH$ (28%) for 2 min at room temperature to remove native surface oxides. Following this step, the freshly etched chips were rinsed with deoxygenated anhydrous ethanol and immersed for 20 h in 0.13 mM biotinylated PEG thiol and 1.87 mM HDT in deoxygenated anhydrous ethanol. After the thiolation step, the biochips were exposed to AS 0.1% for 15 min and rinsed with deionized (DI) water. Following this step, the samples were incubated for 2 h in NA dissolved in PBS (1X) at 0.2 mg/mL. The preparation process continued by exposure of the NA-coated biochips to 0.1 mg/mL of biotinylated polyclonal *E. coli* antibodies for 1 h at room temperature. Previously, this architecture had been successfully used by us for detection of *E. coli* bacteria (Nazemi et al., 2015).

### 2.3. Methodology of monitoring bacterial growth and their reactions to antibiotics

We investigated growth and antibiotic susceptibility of two strains of *E. coli* bacteria, *E. coli* K12 (penicillin-sensitive) and *E. coli* HB101 (penicillin-resistant). Freshly cultured bacteria were employed to reduce the lag phase and to be able to monitor antibiotic susceptibility in less than 3 h. The procedure to prepare freshly cultured bacteria is described in Supporting information.

To monitor bacterial growth, Ab-coated biochips were placed in an ULTEM™ flow cell and exposed to 1 mL of freshly cultured bacteria at $2 \times 10^8$ CFU/mL that was injected into the flow cell at a flow rate of 0.1 mL/min. The biochips were then incubated for an additional 20 min to allow capture of bacteria on the surface of the biochips. It was assumed that 30 min was sufficient to achieve this goal as suggested by reports of antigen-antibody binding reactions (Hlady et al., 1990). Thereafter, LB broth was injected into the flow cell for 30 min (with flow rate of 0.1 mL/min) and the biochips were incubated in LB at room temperature for an additional 4 h without any further injection to allow the bacteria to grow. The photocorrosion process was investigated in situ using a custom designed quantum semiconductor photonic biosensor (QSPB) reader (Aziziyan et al., 2016) capable of rapidly collecting PL maps of 2 mm × 2 mm biochips excited with a light-emitting diode (LED) operating near 660 nm, i.e., delivering the above QW bandgap (829 nm) radiation. The QSPB reader employed a long pass filter with a transmission wavelength threshold at near 820 nm and a CCD camera to record the PL signals. The biochips were irradiated with 6 s pulses, 35 mW/cm$^2$ each, delivered in every 60 s period with the help of a computer-programmed shutter. The 829 nm PL emission of photocorroding biochips was recorded for up to 5 h. This procedure is neither technically complicated nor expensive. The cost of a QSPB reader is less than $3k, which in comparison to traditional optical systems for detection of bacteria, such as those based on Raman spectroscopy (Wu et al., 2016) offers an economically attractive approach. The necessary apparatus occupies a footprint of less than 30 cm × 30 cm.

As a reference experiment, one series of the Ab-coated samples were exposed to 1 mL of UV-killed bacteria (the preparation procedure of UV-killed bacteria is explained in Supporting information) at $2 \times 10^8$ cell/mL in the same way that live bacteria had been tested. The susceptibility of the bacteria to penicillin G at 50 μg/mL, or ciprofloxacin at 10 μg/mL, was investigated by adding antibiotic solutions to fresh LB, following the initial 30 min exposure of the biochips to LB. The antibiotic solutions were injected into the flow cell for 15 min at a flow rate of 0.1 mL/min and the biochips were incubated in antibiotics for an additional 225 min without any further injection. To study the effect of penicillin and ciprofloxacin on PL emission of the biochips, we compared the PL plots collected from Ab functionalized biochips exposed for 30 min to PBS (1X) and 4.5 h to LB, with the PL plots of the biochips exposed for 30 min to PBS (1X), 30 min to LB and 4 h to either penicillin or ciprofloxacin in LB solutions. These antibiotics have different mechanisms of bacteria-antibiotic interaction. Penicillin inhibits cell wall synthesis of bacteria (Yocum et al., 1980), which could result in bacterial lysis or detachment from the surface, whereas ciprofloxacin inhibits DNA synthesis and might not affect the bacterial surface proteins that are involved in capture or cause lysis (LeBel, 1988).

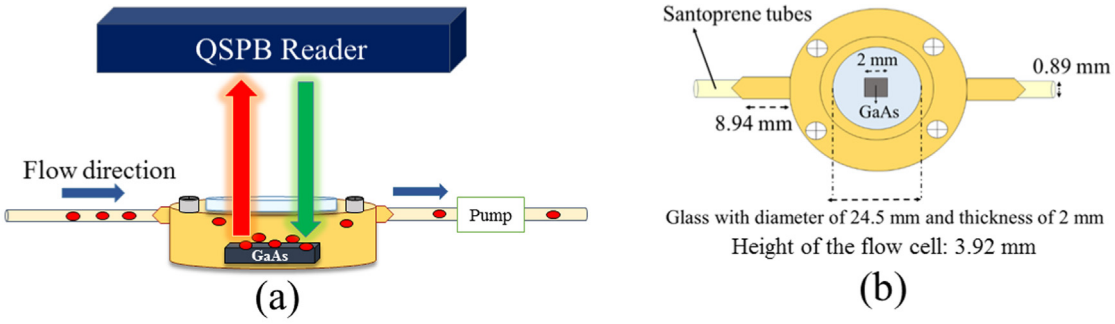
A schematic illustration of the experimental setup is presented in Fig. 2. The setup consists of the flow cell for holding the biochips, the peristaltic pump, and the QSPB reader for collecting PL of photocorroding biochips. All the experiments have been carried out at ambient temperature and repeated at least three times to provide average value and standard deviation. After finishing the PL-monitored photocorrosion experiments, the biochips were analyzed with an optical microscope (Zeiss, Axiotech) to detect changes in bacterial cell shape and biochip surface coverage (see Supporting information for details).

## 3. Results and discussion

### 3.1. Photonic monitoring of growth and reaction of E. coli K12 to antibiotics

An example of normalized time-dependent PL intensity plots from biochips exposed to penicillin-sensitive live and UV-killed *E. coli* K12 bacteria during incubation in LB without antibiotics, or with penicillin, or ciprofloxacin is shown in Fig. 3. The formation of characteristic maxima in these plots is related to the growth of surface oxides and photocorrosion of the 10 nm thick GaAs cap layer (Nazemi et al., 2015). It can be seen that the maximum of the PL plot from the biochip exposed to live *E. coli* K12 bacteria and LB medium has been delayed to near 240 min, while remaining plots exhibit maxima at less than 180 min. Furthermore, the PL maxima from the biochips exposed to bacteria and antibiotic solutions occurred earlier than those induced by UV-killed bacteria. The average position of the PL maxima for the biochips exposed to live *E. coli* K12 bacteria and LB has been determined at 231 min ± 4.9%, UV-killed *E. coli* K12 bacteria and LB at 160 min ± 3.5%, live *E. coli* K12 bacteria, LB and ciprofloxacin at 113 min ± 8.8% and live *E. coli* K12 bacteria, LB and penicillin at 107 min ± 2.6%. Moreover, the average number of bacteria immobilized on the surface of the biochips exposed to live bacteria and LB increased from 929 ± 133 bacteria/mm$^2$ to 3436 ± 244 bacteria/mm$^2$, while this number for UV-killed bacteria was 885 ± 168 bacteria/mm$^2$. In the case of exposure to penicillin and ciprofloxacin, the average number of bacteria immobilized on the surface was 169 ± 64 bacteria/mm$^2$ and 786 ± 177 bacteria/mm$^2$, respectively (see Supporting information for examples of optical images).

The position of the PL maxima for the biochips without bacteria, but exposed to PBS, LB and penicillin has been determined at 101 min ± 4.2%, to PBS, LB and ciprofloxacin at 94 min ± 9%, while exposed to PBS and LB has been determined at 83.5 min ± 5.9%. Comparable positions of the PL maxima for the biochips exposed to PBS and LB with those of the biochips exposed to PBS, LB and penicillin or ciprofloxacin solutions, suggests that the presence of the investigated antibiotics does not affect the photocorrosion of the GaAs/AlGaAs biochips. This behaviour has been verified also for penicillin and ciprofloxacin solutions at 1 mg/mL.




| | 30 min | 4. 5 h |
|---|---|---|
| Live bacteria+LB | Live bacteria | LB |
| UV-treated bacteria+LB | UV-treated bacteria | LB |
| PBS+LB | PBS | LB |

| | 30 min | 30 min | 4 h |
|---|---|---|---|
| Live bacteria+LB +Antibiotic | Live bacteria | LB | Penicillin or Ciprofloxacin solution |
| PBS+LB+ Antibiotic | PBS | LB | Penicillin or Ciprofloxacin solution |

(c)

**Fig. 2.** Schematic illustration of the experimental setup (a), top view of the flow cell (b), and time required for different steps of the experiment (c). The biofunctionalized biochips were kept in the flow cell and exposed to bacterial suspension (red ovals) and LB with or without antibiotics while their PL was recorded during the experiment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

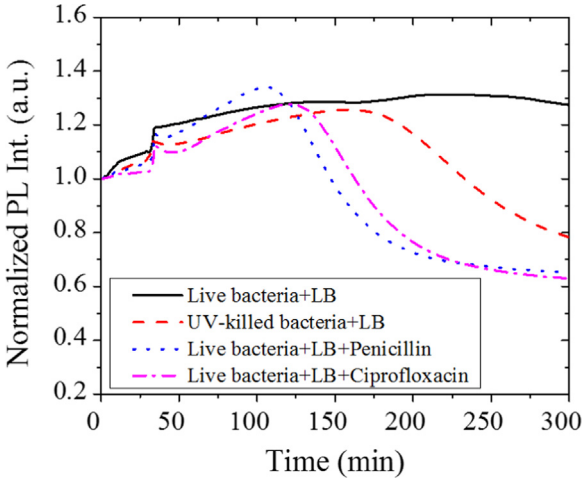



**Fig. 3.** Examples of normalized PL intensity measured in situ for biochips exposed to penicillin-sensitive live *E. coli* K12 bacteria and LB (solid line), UV-killed *E. coli* K12 bacteria and LB (dashed line), live *E. coli* K12 bacteria, LB and penicillin (dotted line), and live *E. coli* K12 bacteria, LB and ciprofloxacin (dashed-dotted line).

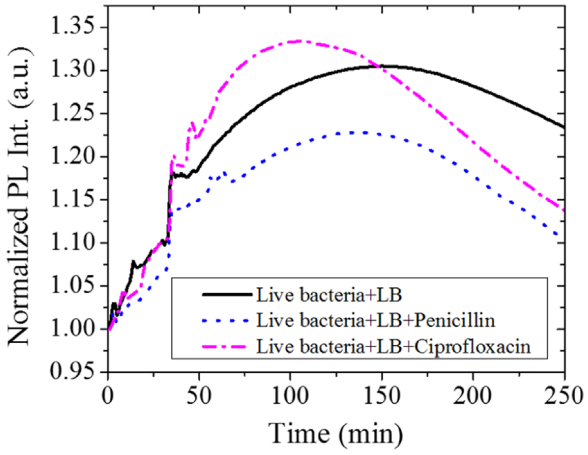



**Fig. 4.** Examples of normalized PL intensity measured in situ for biochips exposed to penicillin resistant, ciprofloxacin sensitive *E. coli* HB101 bacteria and LB (solid line), live *E. coli* HB101 bacteria, LB and penicillin (dotted line), and live *E. coli* HB101 bacteria, LB and ciprofloxacin (dashed-dotted line).

### *3.2. Photonic monitoring of growth and reaction of E. coli HB101 to antibiotics*

Fig. 4 shows an example of normalized time-dependent PL plots of the biochips exposed to penicillin-resistant live *E. coli* HB101 bacteria and LB medium with or without antibiotics (data shown only for 250 min), as described in Section. 2.3. The *E. coli* HB101 bacteria used in this study have the ability to secrete penicillinase enzyme to hydrolyze the penicillin structure which makes them penicillin-resistant (Dever and Dermody, 1991). Therefore, the presence of penicillin should not interfere with bacterial growth, as indicated by the almost identically located PL maxima produced by HB101 bacteria exposed to LB (solid line in Fig. 4) and to LB and penicillin solution (dotted line). Since *E. coli* HB101 are sensitive to ciprofloxacin, the PL maximum occurred earlier when the bacterial solution was exposed to ciprofloxacin, and is consistent with the inhibition of bacterial growth (dashed-dotted line). According to Fig. 4, the maximum of the PL plot from the biochip exposed to live *E. coli* HB101 bacteria and LB with or without penicillin has been delayed to near 160 min, while the PL plot from the biochip exposed to bacteria and ciprofloxacin exhibits maximum at less than 120 min. The position of the PL maxima for the biochips exposed to live *E. coli* HB101 bacteria and LB was at 155 min $\pm$4.6%, live *E. coli* HB101 bacteria, LB and penicillin at 147 min $\pm$3.8% and live *E. coli* HB101 bacteria, LB and ciprofloxacin at 111 min $\pm$7.6%. The exposure to LB without and with penicillin resulted in this case in the average number of bacteria immobilized on the surface of the biochips increased from $821 \pm 96$ bacteria/mm$^2$ to $2843 \pm 203$ bacteria/mm$^2$ and $2605 \pm 163$ bacteria/mm$^2$, respectively, while this number decreased to $739 \pm 190$ bacteria/mm$^2$ when the biochips were

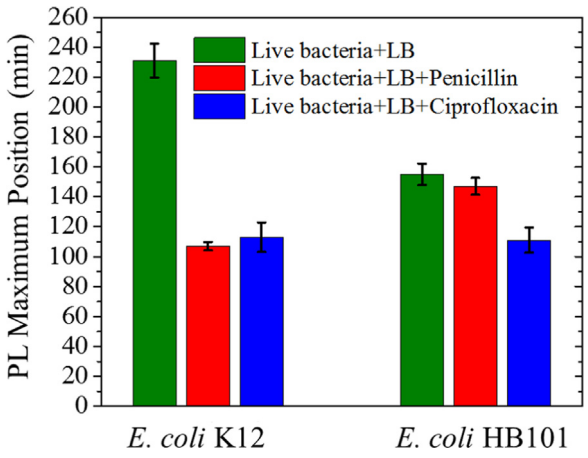



**Fig. 5.** PL maxima positions observed for Ab-coated biochips exposed to penicillin-sensitive *E. coli* K12 or penicillin-resistant *E. coli* HB101 and LB without or with antibiotics.

exposed to bacteria and ciprofloxacin (see Supporting information for examples of optical microscopy images).

In Fig. 5, we summarize the positions of the PL maxima for the biochips exposed to live *E. coli* K12 or *E. coli* HB101 and LB with or without antibiotics. The comparable position of the PL maxima for the biochips exposed to *E. coli* HB101 and LB with those exposed to *E. coli* HB101, LB and penicillin is consistent with the resistance of these bacteria to penicillin. In the case of exposure of the biochips to *E. coli* K12, LB and penicillin, we observed PL maxima sooner in comparison with the *E. coli* K12 growth control tests, which showed the sensitivity of these bacteria to penicillin. The advance in the position of the PL maxima for the biochips exposed to *E. coli* K12 or *E. coli* HB101, LB and ciprofloxacin in comparison with growth control tests demonstrates the sensitivity of both bacteria to ciprofloxacin.

### 3.3. Effect of photoelectrochemical reactions on PL of GaAs/AlGaAs biochips

The dynamics of temporal PL plots shown in Figs. 3 and 4 depends on the power of the 660 nm LED employed for excitation of the biochips, duration of the pulse, and the time allowed between the pulses for the product of photocorrosion to dissipate in the solution. Under the conditions of the current experiment, the built-in electric field in the depletion region of the semiconductor separates photo-excited electrons (e) and holes ($h^+$). The electric field driven $h^+$ arriving to the semiconductor surface will induce the corrosion of this material through the transient formation and dissolution of surface oxides dominated by $Ga_2O_3$ (Choi et al., 2002; Ruberto et al., 1991). It is the formation of $Ga_2O_3$ that reduces the density of surface states and decreases the surface recombination velocity of e - $h^+$, leading to the increased intensity of the PL signal emitted by GaAs surrounded by an aqueous solution (Passlack et al., 1995). As the surface oxides are dissolved into solution and GaAs photocorrodes, a new interface with AlGaAs is formed. This results in a quickly decreasing QW PL signal and formation of a maximum of the temporally dependent PL signal. The formation of this maximum could be accelerated or delayed depending on the electrostatic interaction between the semiconductor and molecules immobilized in its vicinity. For instance, the immobilization of electron donor molecules on the surface of an n-type GaAs (GaAs investigated in this work behaves at room-temperature as an n-type material) will decrease band bending and depletion width of this material (Zhang and Yates Jr., 2012), which would result in a decreased concentration of photo-excited $h^+$ arriving to its surface and, consequently, a delayed photocorrosion process.

The surface of most bacteria at pH greater than 4 is negatively charged, primarily due to the excessive concentration of phosphate and carboxyl groups in comparison to their amino groups (Poortinga et al., 2002). Thus, bacteria suspended in a PBS solution could become decorated with positively charged counter ions present in the solution. The close proximity interaction of these counter ions with the surface of a GaAs/AlGaAs biochip could affect (reduce) transport of photo-excited holes to the semiconductor surface, resulting in a decreased photocorrosion rate of the biochip (delayed formation of the characteristic PL maximum). This characteristic of photocorroding GaAs/AlGaAs nano-heterostructures has been previously explored by us for detection of bacteria tethered to Ab-coated biochips. The dependence of the position of PL maxima vs. different concentrations of bacteria has also been demonstrated in the form of calibration curves (Aziziyan et al., 2016; Nazemi et al., 2015). While immobilization of bacteria could be responsible for electrostatic repulsion of photo-excited $h^+$ from the surface of the investigated GaAs/AlGaAs microstructures, the growth of these bacteria would further amplify this effect and slow down the photocorrosion rate. The result would be a delayed position of the PL maximum. An alternative mechanism of the biochip interaction with bacteria could involve electric charge transfer, such as that observed between bacteria and indium tin oxide coated glass plates (Poortinga et al., 1999). It is possible that secretion of $H^+$ ions to create a chemiosmotic or proton motive force associated with bacterial metabolism (Mitchell, 2011) could reduce photocorrosion as the presence of $H^+$ ions in the vicinity of the biosensor surface could reduce the transport of photo-excited $h^+$ towards the surface through the electrostatic interaction.

The exposure of Ab-functionalized biochips to UV-killed *E. coli* K12 and LB broth produced PL maximum at t ~180 min (Fig. 3), which is delayed in comparison to that corresponding for the penicillin or ciprofloxacin-treated bacteria, but consistent with the much delayed maximum (~250 min) observed for the case of growing bacteria. The more rapidly occurring PL maxima for the biochips exposed to *E. coli* K12 and antibiotics, either penicillin or ciprofloxacin, compared with those exposed to UV-killed bacteria might be related to the drastically decreased zeta potential of these bacteria after antibiotic treatment (data not shown), consistent with the literature data concerning effect of different antimicrobial agents on reduction of zeta potential of bacteria (Alves et al., 2010; Nomura et al., 1995). In contrast, we have not observed a significant effect of UV treatment on decreasing the zeta potential of *E. coli* K12.

The exposure of Ab-functionalized biochips to penicillin-resistant live *E. coli* HB101 bacteria and LB with or without penicillin produced PL maxima coinciding with each other (Fig. 4). In contrast, the exposure to ciprofloxacin inhibited the bacterial growth, which resulted in a slightly accelerated photocorrosion (faster occurring PL maximum). Generally, the position of the PL maxima for the biochips exposed to *E. coli* HB101 and LB occurs earlier in comparison to that induced with *E. coli* K12 and LB. This result seems consistent with the weaker zeta potential of *E. coli* HB101 bacteria in 1X PBS (−18 mV) in comparison to that of *E. coli* K12 (−30 mV). Since photocorrosion of the biochips depends on the electrical charge of bacteria, the greater zeta potential of bacteria indicates stronger electrostatic interaction with the biochip and results in a much delayed PL maximum.

For bacterial concentrations lower than $2 \times 10^8$ CFU/mL, for which around 800–900 bacteria/mm$^2$ were observed immobilized on the biochip surface (see Supporting information), we were not able to monitor bacterial growth. The likely reason for this behaviour is a poisonous effect of As and Ga ions released by the

photocorroding biochip to the flow cell, which could affect the viability of bacteria (Harvey and Crundwell, 1996; Podol'skaia et al., 2002; Tanaka, 2004). We note that a non-irradiated GaAs substrate could support the growth of *E. coli* K12 at concentrations as low as $10^5$ CFU/mL (Nazemi et al., 2016). This suggests that under optimized conditions the PL-based monitoring of bacterial reaction to antibiotics should be possible for suspensions with bacteria diluted to less than $2 \times 10^8$ CFU/mL.

All the experiments reported here were carried out at ambient temperature, however, it might be possible to operate the biosensor at 37 °C. Due to the higher growth rates at 37 °C, it is expected that the results of the antibiotic sensitivity of bacteria could then be delivered in a shorter period of time. Our present results were obtained, each time, with a freshly fabricated biochip. During a 5 h long biosensing run, photocorrosion consumed the entire 10 nm thick GaAs cap and, partially at least, the 10 nm thick $Al_{0.35}Ga_{0.65}As$ layer. This left 30 pairs of GaAs/$Al_{0.35}Ga_{0.65}As$ heterostructures (see Fig. 1) that, potentially, could be used for other biosensing runs. The advancement of this concept would additionally increase the commercial value of a proposed biosensor, although such an approach exceeds the scope of the research reported in this document.

## 4. Conclusions

We have investigated an innovative method of monitoring growth and reaction of bacteria to antibiotics using PL emission of photocorroding GaAs/AlGaAs QW heterostructures. The method takes advantage of the sensitivity of the photocorrosion effect to the perturbation of the electric field induced by electrically charged bacteria immobilized in the vicinity of a biosensor surface. By monitoring the formation of PL maxima of biofunctionalized GaAs/AlGaAs biochips exposed to different bacterial solutions and antibiotics, we have demonstrated the functionality of this process for monitoring the growth and antibiotic sensitivity of *E. coli* K12 (penicillin-sensitive) and *E. coli* HB101 (penicillin-resistant) bacteria at ambient temperature in less than 3 h. The functionalization of the biochips with antibodies makes the process suitable for specific investigation of different bacteria, although it could also be applied for studying bacteria captured non-specifically, e.g., through covalent binding (Meyer et al., 2010; Nazemi et al., 2016). The reduction in time-to-result can be considered as the main advantage of this method over culture-based techniques, while a relatively simple functionalization process, the potential for automation of all the steps of the experiment and low-cost of the analysis seem to be attractive for developing clinical diagnostic applications. The method could lead to a significant progress in the pharmaceutical field and help medical personnel to rapidly identify suitable drugs for treating bacterial infections.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.112.

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