Electrically biased GaAs/AlGaAs heterostructures for enhanced detection of bacteria

Mohammad R. Aziziyan, Walid M. Hassen, Jan J. Dubowski*

Interdisciplinary Institute for Technological Innovation (3IT), CNRS UMI-3463, Université de Sherbrooke, 3000 boul. de l'Université, Sherbrooke, Québec J1K 0A5, Canada
Department of Electrical and Computer Engineering, Faculty of Engineering, Université de Sherbrooke, 2500 boul. de l'Université, Sherbrooke, Québec J1K 2R1, Canada

ABSTRACT

We have examined the influence of electrical bias on immobilization of bacteria on the surface of GaAs/AlGaAs heterostructures, functionalized with an alkanethiol based architecture. A mixture of biotinylated polyethylene glycol (PEG) thiol and hexadecanethiol was applied to attach neutravidin and antibodies targeting specific immobilization of *Legionella pneumophila*. An electrochemical setup was designed to bias biofunctionalized samples with the potential measured versus silver/silver chloride reference electrode in a three electrode configuration system. The immobilization efficiency has been examined with fluorescence microscopy after tagging captured bacteria with fluorescein labeled antibodies. We demonstrate more than 2 times enhanced capture of *Legionella pneumophila*, suggesting the potential of electrically biased biochips to deliver enhanced sensitivity in detecting these bacteria.

Keywords: Biosensing, Bacteria Detection, Band Engineering, Debye Length, GaAs/AlGaAs Heterostructure, Surface assembly monolayer (SAM), *Legionella pneumophila*

1. INTRODUCTION

Detection of bacteria with biosensors has been investigated as an attractive alternative to traditional methods of bacteria counting due to its potential for a cost effective, rapid and specific analyte recognition [1]. Rapid detection becomes critical especially when dealing with outbreaks of diseases characterized by high mortality rates induced by bacteria, such as *Legionella pneumophila* (*L. pneumophila*). This pathogen can be dispersed via aerosol of hot-water systems and cooling tower water [2]. Numerous biosensing systems have been investigated for rapid detection of bacteria based on a variety of transducer effects that determine their sensitivity [3]. Recently, a photoluminescence (PL) monitored photocorrosion of GaAs/AlGaAs biochips has emerged as an attractive approach for rapid detection of bacteria [4-6]. The PL effect can provide remarkable information due to the sensitivity to surface and interfaces located phenomena. Generally, optical excitation and photonic data collection does not impose complicated prearrangements, such as creation of high quality ohmic contact; hence, PL based study becomes convenient and useful specifically for studying with high resistivity materials [7]. It has been suggested that PL of epitaxial quantum dots provides a novel platform for optical biosensing capable of rapidly detecting pathogens captured on the surfaces of III–V semiconductors [8]. Moreover, PL of GaAs/AlGaAs nano heterostructures has been used for detection of *Escherichia coli* and *L. pneumophila* suspended in phosphate buffered saline solutions [4-6]. Thus, photonics of nanoscale materials such as semiconductor quantum heterostructures and quantum dots has become an attractive tool for biosensing.

* jan.j.dubowski@usherbrooke.ca; Phone: 819-821-8000 x62528; FAX: 819-821-7937; Web: http://www.dubowski.ca/

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A primary step of bacteria detection with biosensor systems is an efficient immobilization of the bacteria on the transducer part. Most of bacteria are negatively charged under normal physiological conditions and at pH around 5 to 7 [9]. Furthermore, formation of a semiconductor/electrolyte interface is associated with redistribution of ions and, at equilibrium, semiconductor energy bands bend upward in an n-type semiconductor creating a negatively charged surface [6]. Thus, before immobilization with an antibody (Ab) through intermolecular interactions, bacteria experience an electrostatic interaction that plays an important role in their adhesion to the biosensor surface, or in the interaction with an Ab functionalized surface. A reasonable model of bacteria interaction with the surface could be provided using colloidal particles described by the DLVO (Derjaguin, Landau, Vervey, Overbeek) theory [10] that, in its simplest form, considers only van der Waals attraction and double layer repulsion forces to give a picture of how colloidal particles and surface interact electrostatically [11]. By theoretical plot of these two forces, Yuehuei et al. [12] explained how a double layer repulsion limits the particles that undergo Brownian thermal fluctuation from reaching the surface. The repulsion between a charged surface and bacteria could be decreased by increasing the ionic strength of the solution [13], however, this could limit the performance of biosensing systems based on the electrostatic interaction due to reduction of the Debye length [14].

Band bending of semiconductors and its consequences on electronic state of surfaces and interfaces has been extensively discussed in literature, indicating that tuning of this parameter could lead to formation of an electrostatic interaction facilitating bacteria immobilization [15, 16]. In view of this, one can create conditions where less negative surface of electrically biased GaAs could attract a greater number of bacteria. However, selection of a bias potential for a GaAs/AlGaAs biochip plays an important role since at high anodic potentials, and transport of electrons to the GaAs valence band results in decomposition of this material, while at high cathodic potentials hydrogen evolution could occur [17].

In this paper we report on the role of electrical bias of GaAs/AlGaAs biochips, biofunctionalized against *L. pneumophila,* in achieving an enhanced immobilization of this pathogen.

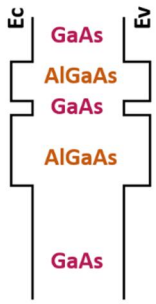
2. MATERIALS, PREPARATION AND CHARACTERIZATION DETAILS

2.1 Materials

Samples used in this experiment were GaAs/AlGaAs nano-heterostructures (Wafer J0152) consisting of undoped epitaxial layers of GaAs and $Al_{0.35}Ga_{0.65}As$ grown on a semi-insulating GaAs (001) substrate. Figure 1 presents details of this multilayer structure along with its energy band diagram. We note that similar nano-heterostructures were used for detection of bacteria in phosphate buffered saline (PBS) solutions [5, 6]. The chemical reagents were obtained as follow: biotinylated polyethylene glycol (Bio-PEG) from Prochimia Surfaces (Gdansk, Poland), hexadecanethiol (HDT) from Sigma-Aldrich (Ontario, Canada), neutravidin from Molecular Probes (Invitrogen, Burlington, Canada), polyclonal biotinylated and fluorescein isothiocyanate (FITC) conjugated antibodies against *L. pneumophila* from ViroStat, Inc. (Portland, Maine), PBS 10X, pH 7.4 from Sigma (Oakville, Canada), OptiClear from National Diagnostics (Mississauga, Canada), acetone from ACP (Montréal, Canada), isopropyl alcohol (IPA) from Fisher Scientific (Ottawa, Canada), ammonium hydroxide 28% (NH_4OH) from Anachemia (Richmond, Canada). Deionised (DI) water, 18.2 MΩ, was produced with a Millipore purification system built by Culligan (Quebec, Canada). The *L. pneumophila* ssp1 samples were provided by Magnus Chemicals Ltd. (Boucherville, Canada). For the reported here experiments, we worked with UV light killed bacteria. Throughout the text, we use term “bacteria/mL” for UV-treated bacteria.

Description	Material	X	T [nm]
GaAs (Cap layer)	GaAs		8
Barrier	$Al_xGa_{1-x}As$	0.35	10
Well	GaAs		3
Barrier	$Al_xGa_{1-x}As$	0.35	100
GaAs (Emitter)	GaAs		500

(a)



(b)

Figure 1. (a) Details of J0152 GaAs/AlGaAs nano-heterostructure, and (b) corresponding energy band diagram.

2.2 Functionalization process of biochips

We used 2 mm × 2 mm chips diced from a GaAs/AlGaAs wafer. The backside of the chips was coated with gold before the biofunctionalization step. Details on creating Au contact at the backside of chips can be found elsewhere [6]. Gold coated chips were all degreased sequentially in ultrasonic bath of OptiClear, acetone and IPA for 5 min each, then dried with flow of nitrogen and immersed in ammonium hydroxide (28%) for 2-min, in order to remove their native oxide layer. Next, they were immersed in deoxygenized anhydrous ethanol and immediately transferred to solution of thiol (0.15 mM Bio-PEG and 1.85 mM HDT diluted in deoxygenized anhydrous ethanol) and kept there around 20 hours. Then, biochips were rinsed with anhydrous ethanol and 1XPBS then post processed with neutravidin (0.2 mg/mL in 1XPBS for 1h) and then, after rinsing with 1XPBS, incubated for 1 h in a solution of Ab against *L. pneumophila* (0.1 mg/mL in 1XPBS). At this stage, the biochips were rinsed with 1XPBS and transferred to microfluidic setup for bacteria immobilization tests. At the end of bacteria incubation process, biochips were exposed for 1 h to FITC conjugated antibodies (50µg/ml in 1XPBS) to survey presence of bacteria via fluorescence microscopy. As illustrated in Figure 2, *L. pneumophila* will be sandwiched between two antibodies.

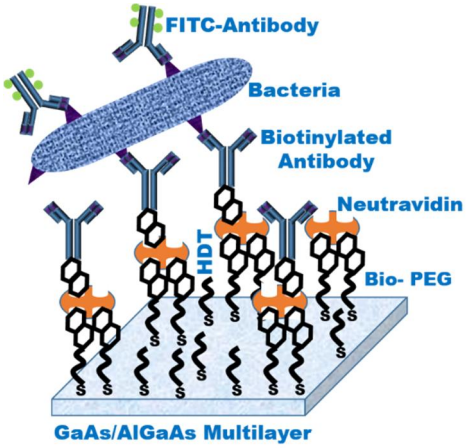


Figure 2. Bioarchitecture of Bio-PEG/HDT/Neutravidin/Antibody for immobilization of *L. pneumophila*. After trapping the bacteria, they are stained with FITC conjugated antibodies for fluorescence microscopic identification.

The influence of electric bias on GaAs biochips was studied for biochips biased at E= -0.2, 0 and 0.2 volts and exposed for 30 min to a solution containing *L. pneumophila* with concentration at 10^5 bacteria/mL. For the applied bias voltages, the

current-voltage characteristics of GaAs/AlGaAs heterostructures suggests that anodic dissolution or hydrogen evolution reactions of this material play a negligible role [18].

2.3 Immobilization experiments with biased biochips

After the Ab coated biochips were rinsed with 1XPBS, they were placed inside a flow cell, which allowed for exposure to different environments facilitated with a peristaltic pump. Figure 3 displays a schematic view of the flow cell setup. After biasing a biochip, we injected *L. pneumophila* suspended in 1XPBS at,10^5 bacteria/mL and incubated for 30 min under a continuous flow of a bacterial solution at 0.04 mL/min. Next, the biochip was removed from the flow cell and rinsed with DI water to wash salts and physisorbed bacteria from surface of GaAs. Bacteria immobilized on the surface of biofunctionalized GaAs were stained with FITC conjugated antibodies, and characterized by a fluorescence inverted microscope (Olympus, IX71). The light source emits between 450 and 490 nm (blue light) and images were observed at 515 nm using a DP71 digital camera [19]. For characterization of electrically biased biochips, we used an electrochemical analyser (CH Instruments, CHI604C) and in all tests, the working electrode was GaAs with a Au contact, counter electrode was also Au, while the reference electrode was Ag/AgCl.

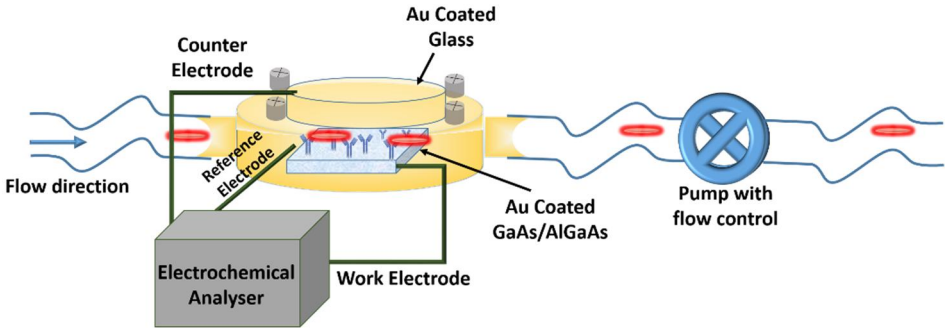


Figure 3. Schematic view of employed microfluidic setup connected to pump and electrochemical analyser.

3. EXPERIMENTAL RESULTS AND DISCUSSIONS

Figure 4 shows fluorescence microscopy image of a GaAs surface after FITC tagging and systematic analysis of bacteria immobilization. The florescence image is obtained by 3 seconds exposure time. FITC dyes are clearly observed on the surface of GaAs, representing the *L. pneumophila*. We systematically assessed the number of FITC dyes and compared obtained numbers to verify influence of each bias point on the trapping process. As shown by Figure 4(b), for sample biased at 0.2 V we could increase the surface coverage more than 2 times relative to sample biased at 0 V. Conversely, we degraded bacteria immobilization efficiency for sample biased at -0.2 V. The anodic potential actually increased the band bending of the GaAs/AlGaAs heterostructure at its interface with the electrolyte; hence, it depletes more charge carriers from space charge and creates a more positive depletion region. While the variation of the Helmholtz layer, responsible for double layer repulsion, is small compared to variations of the space charge region due to applied bias [17], presumably, anodic bias provides more attraction force for deflection of negatively charged bacteria towards the GaAs surface. We also carried out a control test by exposing a biofunctionalized biochip directly to FITC conjugated antibodies (no bacteria were immobilized initially). As shown by the inset of Figure 4(a), there was no noticeable unspecific binding of FITC conjugated antibodies to the surface of that biochip.

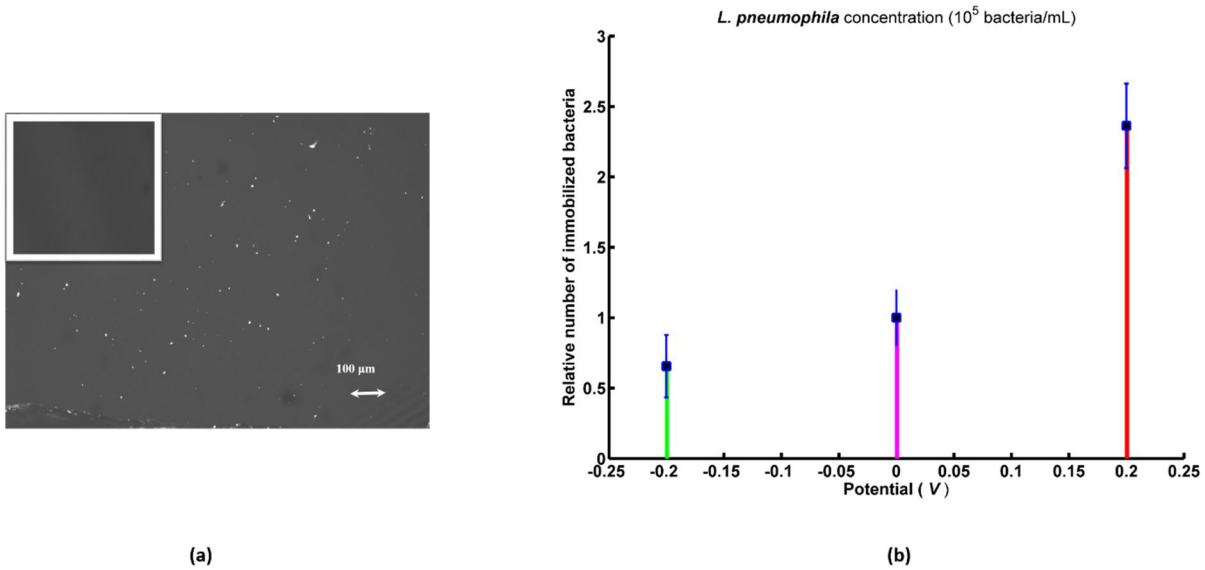


Figure 4. (a) Fluorescence microscopic image showing the presence of *L. pneumophila* on the surface of Ab functionalized biochip exposed to 10^5 bacteria/mL in comparison to a control sample exposed to FITC conjugated Ab only, as shown by the inset; (b) relative number of bacteria captured at different electrical bias conditions normalized to bacteria counted for an unbiased (E = 0 V) sample.

4. Conclusion

We studied the impact of an electrical bias on immobilization of *L. pneumophila* on the surface of biofunctionalized GaAs/AlGaAs nano-heterostructures. We used bio-PEG/HDT/Neutravidin/Antibody architectures to specifically capture *L. pneumophila*. Electrical bias was realized in an electrochemical cell with a 3-electrode configuration employing Ag/AgCl as a reference electrode. The presence of immobilized bacteria was studied by employing FITC conjugated antibodies and a fluoresce microscopy technique. We demonstrate that electrical control of band bending could be applied to moderate bacteria-semiconductor interaction. Under increased band bending conditions achieved with E = 0.2 V, the repulsion semiconductor-bacteria could be reduced, leading to a 2X increased surface concentration of the negatively charged *L. pneumophila*. Given that the limit of detection with GaAs/AlGaAs nano-heterostructure biochips is determined by the critical number of bacteria immobilized on the biochip surface, our investigations suggest that an enhanced sensitivity of detection could be achieved by electrical biasing of the biochip.

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REFERENCES

[1] L. D. Mello, and L. T. Kubota, "Review of the use of biosensors as analytical tools in the food and drink industries," *Food Chemistry,* vol. 77, no. 2, pp. 237-256, 2002.

[2] D. F. Yaradou, S. Hallier-Soulier, S. Moreau, F. Poty, Y. Hillion, M. Reyrolle, J. Andre, G. Festoc, K. Delabre, F. Vandenesch, J. Etienne, and S. Jarraud, "Integrated real-time PCR for detection and monitoring of Legionella pneumophila in water systems," *Applied Environmental Microbiology,* vol. 73, no. 5, pp. 1452-1456, March, 2007.

[3] B. Byrne, E. Stack, N. Gilmartin, and R. O'Kennedy, "Antibody-based sensors: principles, problems and potential for detection of pathogens and associated toxins," *Sensors (Basel),* vol. 9, no. 6, pp. 4407-45, 2009.

[4] V. Duplan, E. Frost, and J. J. Dubowski, "A photoluminescence-based quantum semiconductor biosensor for rapid in situ detection of Escherichia coli," *Sensors and Actuators B: Chemical,* vol. 160, no. 1, pp. 46-51, 2011.

[5] E. Nazemi, S. Aithal, W. M. Hassen, E. H. Frost, and J. J. Dubowski, "GaAs/AlGaAs heterostructure based photonic biosensor for rapid detection of Escherichia coli in phosphate buffered saline solution," *Sensors and Actuators B: Chemical,* vol. 207, Part A, pp. 556-562, 2015.

[6] M. R. Aziziyan, W. M. Hassen, D. Morris, E. H. Frost, and J. J. Dubowski, "Photonic biosensor based on photocorrosion of GaAs/AlGaAs quantum heterostructures for detection of Legionella pneumophila," *Biointerphases,* vol. 11, no. 1, pp. 019301, 2016.

[7] T. H. Gfroerer, "Photoluminescence in Analysis of Surfaces and Interfaces," *Encyclopedia of Analytical Chemistry*, R. A. Meyers, ed., pp. 9209–9231, Chichester, UK: John Wiley & Sons Ltd., 2006.

[8] J. J. Dubowski, "Novel Quantum Dot based Approach for Biosensing," in Lasers and Electro-Optics Society, 2006. LEOS 2006. 19th Annual Meeting of the IEEE, 2006, pp. 302-303.

[9] A. T. Poortinga, R. Bos, W. Norde, and H. J. Busscher, "Electric double layer interactions in bacterial adhesion to surfaces," *Surface Science Reports,* vol. 47, no. 1, pp. 1-32, 2002.

[10] K. C. Marshall, "Mechanisms of Bacterial Adhesion at Solid-Water Interfaces," *Bacterial Adhesion: Mechanisms and Physiological Significance*, D. C. Savage and M. Fletcher, eds., pp. 133-161, Boston, MA: Springer US, 1985.

[11] M. Katsikogianni, and Y. F. Missirlis, "Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions," *European cells & materials,* vol. 8, pp. 37-57, 2004.

[12] Y. H. An, R. B. Dickinson, and R. J. Doyle, "Mechanisms of Bacterial Adhesion and Pathogenesis of Implant and Tissue Infections," *Handbook of Bacterial Adhesion: Principles, Methods, and Applications*, Y. H. An and R. J. Friedman, eds., pp. 1-27, Totowa, NJ: Humana Press, 2000.

[13] D. C. Mays, "Using the Quirk-Schofield Diagram to Explain Environmental Colloid Dispersion Phenomena," *JOURNAL OF NATURAL RESOURCES & LIFE SCIENCES EDUCATION* vol. 36, pp. 45-52, 2007.

[14] E. Stern, R. Wagner, F. J. Sigworth, R. Breaker, T. M. Fahmy, and M. A. Reed, "Importance of the Debye Screening Length on Nanowire Field Effect Transistor Sensors," *Nano Letters,* vol. 7, no. 11, pp. 3405-3409, 2007.

[15] K. Rajeshwar, "Fundamentals of Semiconductor Electrochemistry and Photoelectrochemistry," *Encyclopedia of Electrochemistry: Semiconductor Electrodes and Photoelectrochemistry* A. J. Bard, M. Stratmann and S. Licht, eds.: Wiley-VCH Verlag GmbH, 2002.

[16] Z. Zhang, and J. T. Yates, Jr., "Band bending in semiconductors: chemical and physical consequences at surfaces and interfaces," *Chem Rev,* vol. 112, no. 10, pp. 5520-51, Oct 10, 2012.

[17] M. V. Lebedev, T. Masuda, and K. Uosaki, "Charge transport at the interface of n-GaAs (100) with an aqueous HCl solution: Electrochemical impedance spectroscopy study," *Semiconductors,* vol. 46, no. 4, pp. 471-477, 2012.

[18] T. Fink, and R. M. Osgood, Jr., "Photoelectrochemical Etching of GaAs / AlGaAS Multilayer Structures " *Journal of the Electrochemical Society,* vol. 140, no. 9, pp. 2572-2581, 1993.

[19] L. Neng, H. Xiaohuan, and J. D. Jan, "Selective area in situ conversion of Si (001) hydrophobic to hydrophilic surface by excimer laser irradiation in hydrogen peroxide," *Journal of Physics D: Applied Physics,* vol. 47, no. 38, pp. 385106, 2014.