Detection of bacteria using a photoluminescence-based quantum semiconductor device

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***Abstract*-The detection of *Escherichia coli* is demonstrated *in situ* using photoluminescence emission from antibody biofunctionalized GaAs/AlGaAs epitaxial microstructures. Current approach allows detecting 10^4 CFU/ml of bacteria in less than 120 minutes.**

I. Introduction

Traditional methods of bacteria detection require the use of sophisticated analytical laboratories, often in centralized facilities, which requires considerable capital and a highly skilled workforce. There are many cases where this approach is inadequate, mainly due to the long time-to-result period. For example, some standard methods, such as the ISO 11731-2:2004 and ISO 6222:1999 for the detection of *Legionella pneumophila* require up to 10 days to yield results, as they rely on the ability of micro-organisms to multiply to visible colonies [1]. Thus, there is a need for new, easy to use technologies capable of rapid, selective and sensitive detection of various pathogens. We have proposed that strong photoluminescence (PL) of III-V epitaxial microstructures, e.g., quantum dots (QD) could be used to study biochemical reactions on surfaces of III-V semiconductors [2].

II. Biosensing Architectures

The functioning of a III-V semiconductor microstructure as a biosensing device depends on the ability to maintain its stable response over an extended period of time. Indeed, the exposure of bio-functionalized, but unprotected III-V surfaces to oxygen and air atmosphere could degrade their electrical and optical properties [3]. Two different architectures were used to investigate biosensing response of GaAs/$Al_{0.33}Ga_{0.67}As$ epitaxial microstructures. Firstly, the sensor surface (GaAs) was functionalized with biotinylated polyclonal antibodies using polyethylene-glycol (PEG) hexadecanethiol (HDT) and neutravidin. The use of thiols helps to address the surface stability issue as it has been demonstrated that inorganic sulphur compounds allow passivation of GaAs surfaces. A PEG thiol-based architecture has recently been reported by us for the successful immobilization of influenza A virus [4]. For the second architecture, the sensor surface was coated with silicon nitride (Si_3N_4) prior to functionalization using glutaraldehyde. The GaAs surface covered with Si_3N_4 is protected from environmental exposure, while maintaining the functionalization ability.

III. Detection Method

The detection method is based on optical monitoring of the perturbation of the near-surface electric field induced by specifically immobilized bacteria. It is known that the near surface electric field associated with the electron (e) and hole (h) band bending leads to the spatial separation of charge carriers and, consequently, to a reduced photoluminescence emission [5]. It is expected that bacteria that carry negative electric charge, following their immobilization on the surface will modify band bending of the semiconductor band structure. Both Gram-negative bacteria, such as *E. coli*, and Gram-positive bacteria exhibit negative surface charge formed as a result of the dissociation of related chemical groups present on the bacterial surface. Thus, for an n-type semiconductor, the immobilization of bacteria is expected to reduce both band bending and the near-surface electric field. This should lead to an increased rate of e-h recombination and, consequently, an enhanced PL emission [6].

IV. Experimental Results

An atomic force microscopy (AFM) image of a selected area of the antibody-functionalized surface of (001) GaAs exposed to 10^6 CFU/ml of *E. coli* shown in Figure 1. A PEG-thiol-biotin-neutravidin architecture was applied in this case. Two characteristic features are present in the image: the small ones (≈50nm) correspond to the precipitation of salts on the sample, most likely coming from the exposure of the sample to PBS for an extended period of time, and the large feature corresponds to an immobilized bacteria, measuring approximately 1.5 μm x1.3 μm x 350 nm. The average concentration of *E. coli* immobilized from the 10^6 CFU/ml solutions was found in the range of 0.55 - 0.7 bacteria/100 μm^2, depending on the biofunctionalization architecture [6].

Figure 2 shows the time-dependent photoluminescence signal measured from biofunctionalized samples exposed to a PBS solution, 10^6 CFU/ml of *L. lactis* (control experiment) and *E. coli* at 10^4 and10^6 CFU/ml. It can be seen that, following the PBS washing step, applied at 30 min from the onset of the experiment, the PL signal from the samples exposed to 10^4 and 10^6 CFU/ml of *E. coli* solutions decreased by approximately 35

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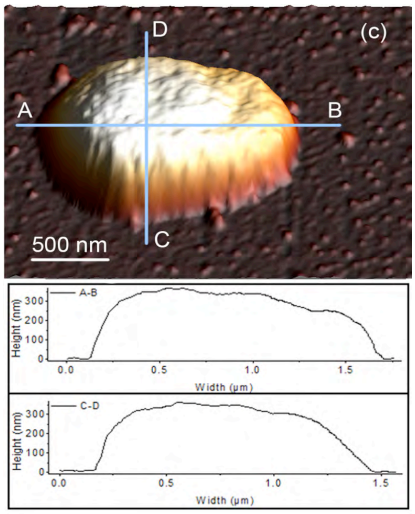

Figure 1. AFM image and cross-sectional scan of an *E. coli* bacterium present on the biofunctionalized surface of GaAs.

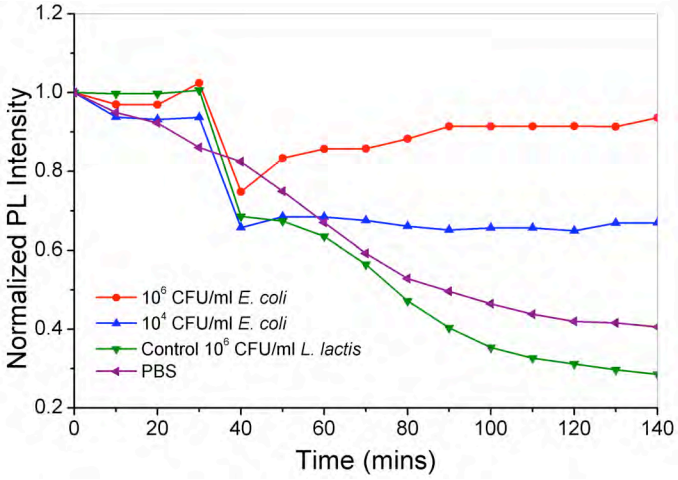

Figure 2. Time-dependent PL intensity signal from an antibody functionalized GaAs/AlGaAs epitaxial microstructure exposed to 10^4 and 10^6 CFU/ml of *E. coli* in a PBS solution. The reference and control experiments were carried out for pure PBS solution and 10^6 CFU/ml of *L. lactis* in PBS.

and 15 %, respectively. The control experiment showed also a 35% decrease of the PL signal following the washing step. However, in contrast to the 10^4 CFU/ml *E. coli* data, this signal (*L. lactis)* continued to decay in the same manner as the PBS signal, confirming a negligible response of the biosensing surface to a non-specific interaction. The net increase of the PL signal, observed at 80-120 min from the onset of the experiment, appears to saturate and it is proportional to the concentration of bacterial solutions used to expose the functionalized samples. The results indicate that the investigated method allows detection of *E. coli* at 10^4 CFU/ml within less than 120 min.

CONCLUSIONS

We have investigated a photonic method of detecting *E. coli* using PL emission from GaAs/$Al_{0.33}Ga_{0.67}As$ epitaxial microstructures. The negative electric charge of the bacteria immobilized on the surface of antibody-functionalized microstructure contributed to the net increase of PL emission observed *in situ* from liquid bacterial solutions. The samples exposed to different concentrations of bacteria allowed monitoring the dynamics of the bacteria immobilization observed over a period of several hours. The results indicate that the investigated method allows detection of *E. coli* at 10^4 CFU/ml within less than 120 min. However, optimization of the method should lead to increased sensitivity as well as potentially shorter time to detection. We observed qualitatively similar results for samples that were washed and dried following their exposure to PBS, *L. lactis* and *E. coli* solutions [7].

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