

Real-Time Pathogen Determination by Optical Biosensing Based on Graphene Oxide [†]

Mariana D. Avila-Huerta ¹, Edwin J. Ortiz-Riaño ¹, Diana L. Mancera-Zapata ¹ and Eden Morales-Narváez ^{1,*}

Biophotonic Nanosensors Laboratory, Centro de Investigaciones en Óptica, A. C., Loma del Bosque 115, Lomas del Campestre, León, Guanajuato 37150, Mexico; mavila@cio.mx (M.D.A.-E.); johan@cio.mx (E.J.O.-R.); dianamancera@cio.mx (D.L.M.-Z.)

* Correspondence: eden@cio.mx

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Abstract: Pathogenic bacterial contamination in food is a public health concern. It represents a health and safety consumer risk that could cause several diseases and even death. Currently, the food industry uses culture-based assays to determine the presence of pathogens as a gold standard method. Although this method is highly accurate, it is often time-consuming and expensive [1,2]. In this regard, the development of biosensing platforms results as an alternative for the reduction of time and cost of pathogenic bacteria detection in food [2]. In this work, we report the development of a single-step bacterial detection platform based on graphene oxide. Non-radiative energy transfer between graphene oxide coated microplates (GOMs) and photoluminescence bioprobes (PLBs) is presented in absence of the target analyte, but in presence of analyte, PLBs exhibit strong photoluminescence due to the distance between GOMs and PLBs. These PLBs are based on quantum dot (Qds)-antibody (Ab) complexes, thereby resulting as a biorecognition and interrogation element. *Escherichia coli* was used as model analyte. In optimal conditions, the bacterial detection platform reached a limit of detection around 2 CFU mL⁻¹ in 30 min, enabling a fast and sensitive alternative for bacterial detection. The biosensing platform was also used to test food industry samples achieving a qualitative response, that allows determining the presence of *E. coli* during the first 30 min of the assay. This biosensing strategy potentially offers a low-cost and quick option for the food industry to assure the quality of the product and consumer safety [3].

Keywords: biosensing; pathogenic detection; immunosensing; photoluminescence

1. Introduction

Pathogenic bacterial contamination in food is a public health concern. It represents a health and safety consumer risk that could cause several diseases and even death. Detection of bacteria is crucial not only in food quality assessment, but for clinical diagnostics and environmental monitoring [1,2,4–7]. The most commonly used methods for pathogenic detection are polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA) and culture-based assays on differential culture media. Although these methods provide very accurate results, they require specialized technical skills, high-cost, cumbersome procedures time consuming labor (in case of culture-based assay, up to 72 h) [1,2,5,8,9]. To offer a solution against the disadvantages of these methods, some biosensing systems based on nanomaterials had been developed. We proposed a single-step bacterial detection platform based on graphene oxide coated microplates (GOMs). Non-radiative energy transfer between GOMs and photoluminescence bioprobes (PLBs) is presented in absence of the target analyte, but in presence of analyte, PLBs exhibit strong photoluminescence due to the distance between GOMs and PLBs.

These PLBs are a quantum dot-antibody complex, thereby resulting as a biorecognition and interrogation element (see Figure 1). We explained this behavior between GOMs and PLBs because of the distance between the complex (PLB-bacteria) and GOMs, and because the low affinity between the complex and the GO. Also, it was analyzed industrial food samples, such as cauliflower, from a frozen vegetable processor company.

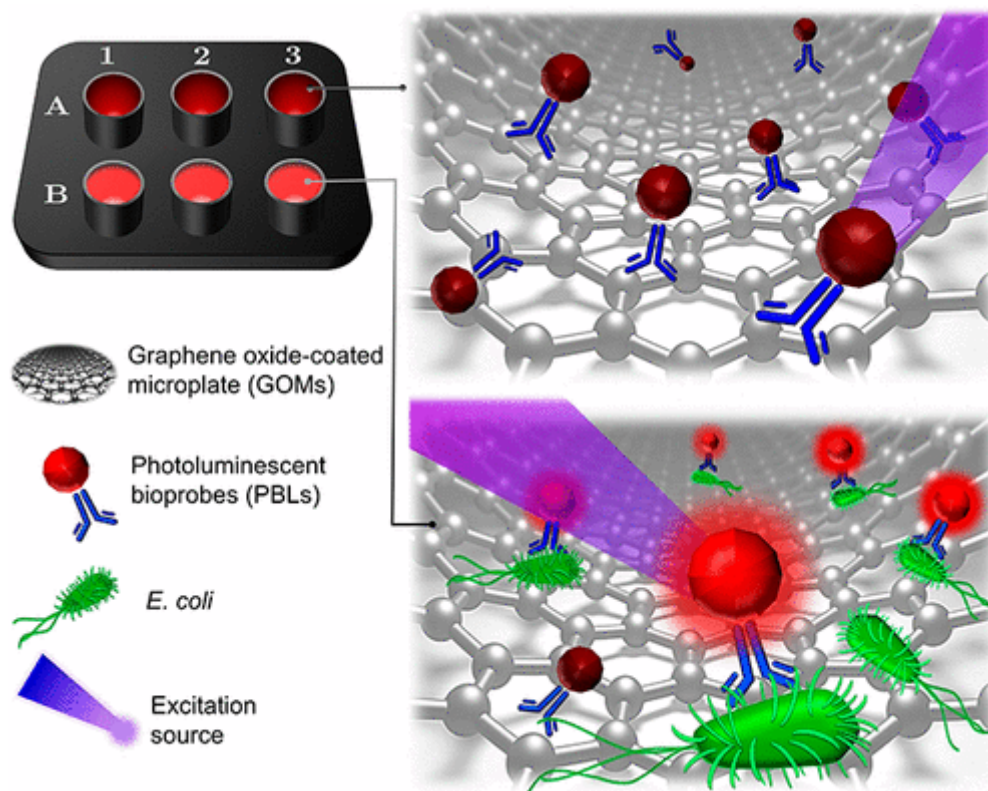


Figure 1. Operational principle of the bacterial detection platform. GOMs deactivate the photoluminescence of the PLBs that do not have interaction Antibody-analyte (top of figure). And the PLBs that interact with the target bacteria maintain their photoluminescence. Adapted with permission [3]. Copyright 2020, ACS.

2. Materials and Methods

Sterilized culture-treated 96 well microplates were purchased from Corning, monolayer GO aqueous suspension was purchased from Angstrom Materials, PBS tablets and Tween 20 from Sigma-Aldrich, bovine serum albumin (BSA) from Sigma-Aldrich, biotinylate polyclonal Anti-E. coli was purchased from Abcam, Qdot™ 655 Streptavidin Conjugate were purchased from Invitrogen. GO dilutions and buffers were prepared with fresh ultrapure water, Milli-Q system from Millipore. Immunobuffer was prepared using PBS supplemented with 0.5% Tween 20 (v/v) and 1% of BSA (w/v). Standard samples of bacteria were diluted in PBS. The photoluminescence experiments were performed using a Cytation 5 multimode reader (BioTek).

GOMs preparation. Microwell plates are easily decorated with GO via hydrophilic interactions, since GO has hydrophilic, so does the microplates. 100 μL of the suspension, with an appropriated concentration, are incubated overnight in the microplate, then, to remove those GO submicrometric sheets that were not attached onto the microplates, washed at least 3 times with ultrapure water.

PLBs preparation. This conjugation was performed by mixing 100 μL of streptavidin-QDs with 100 μL of biotinylated anti-E. coli. These reagents were diluted in immunobuffer, and the reaction was performed during 45 min of mixing at 650 rpm.

Single-step test. Once the microwell plate coated with GO and Ab-QD is prepared the immunoassay requires a single step, deposit 100 μL of sample and 100 μL of Ab-QD in a microwell. The resulting photoluminescence was then recorded throughout 120 min (excitation wavelength: 365

nm, emission wavelength 660 ± 20 nm). Typically, at least three parallel experiments in the same conditions were performed to evaluate precision. The limit of detection was calculated as the average of the control sample added to three times the standard deviation.

Preparation of industrial sample. Industrial samples of cauliflower were kindly provided by La Proxima Estación S.P.R. de R.L. Prior to the analysis the samples were diluted in PBS. Also, these samples were analyzed using 3 M Petrifilm E. coli/Coliform Count (EC) Plates, before being analyzed with the proposed technology.

3. Results

Each biomaterial (antibody) and nanomaterial (GO and QDs) were optimized in terms of concentration to reach an adequate performance of the biosensing system. Then the platform was characterized. Once the bacterial detection platform was optimized and characterized, we investigated the specificity of the platform. And finally, we test the analytical behavior with some industrial food samples.

3.1. Optimización of the Reagents and Characterization

We decorated GOMs with different concentrations of GO (from 1100 to 1600 $\mu\text{g mL}^{-1}$) to find the concentration maximizing modulation of the photoluminescent quenching ratio I_f/I_0 ; where I_f is the photoluminescence intensity of PLBs or bare QDs at time x (generally from 5 to 120 min), and I_0 is the photoluminescence intensity of PLBs at time 0. This range of concentrations was previously reported by our research group [10]. We found that the best behavior was with $[\text{GO}] = 1200 \mu\text{g mL}^{-1}$.

To optimize the analytical behavior of the studied bacterial detection platform in terms sensitivity, we explored the performance of the biosensing system using different concentrations of the PLBs. Particularly, PLBs with a final concentration of QDs-antibody at 0.1125 nM and 0.9 $\mu\text{g mL}^{-1}$, respectively, were observed to offer the best analytical performance in terms of both sensitivity and coefficient of variation for the analysis of samples of *E. coli* with concentrations from 5 to 10^6 CFU mL^{-1} . This behavior can be observed in 2A–C.

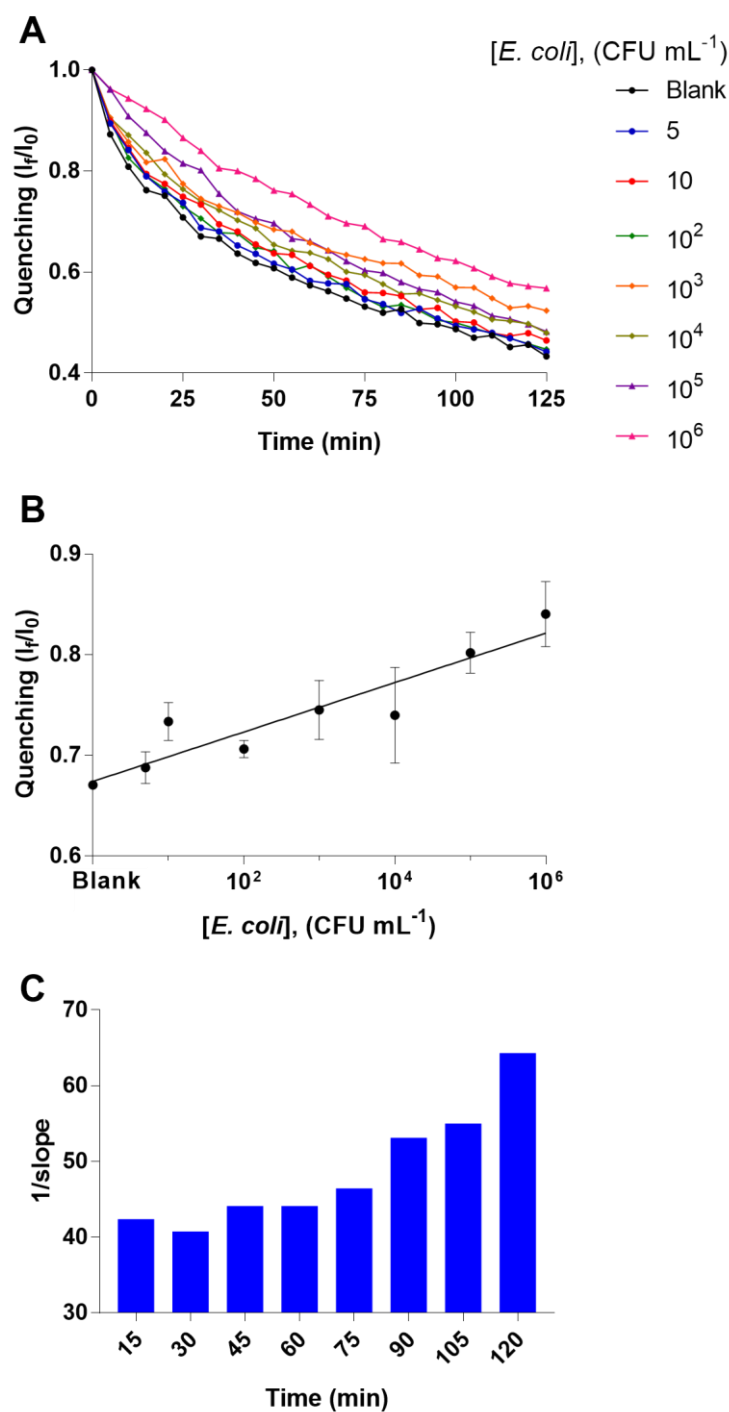


Figure 2. Performance of real-time bacterial detection in optimal conditions. [QD] = 0.1125 nM, [Ab] = 0.90 $\mu\text{g mL}^{-1}$ and GOMs prepared with GO at 1200 $\mu\text{g mL}^{-1}$. (A) Real-time quenching profile of different *E. coli* concentrations.; (B) The calibration curve resulting at 30 min of the assay. The error bars represent the standard deviation of three parallel experiments. (C) Evolution of the sensitivity of the assay in terms of 1/slope values. Adapted with permission [3]. Copyright 2020, ACS.

3.2. Study of Specificity

Additionally, we investigated the specificity of the proposed bacterial detection platform by using *Salmonella typhimurium*. We analyzed different concentrations of the interference bacteria (from 5 to 10^6 CFU mL⁻¹). We also analyzed other samples containing a relatively low concentration of *E. coli* (10^3 CFU mL⁻¹) and a relatively high concentration of *Salmonella* (10^5 CFU mL⁻¹), other with

a relatively high concentrations of both strains (10^5 CFU mL⁻¹). The results of these tests can be observed in Figure 3A–D.

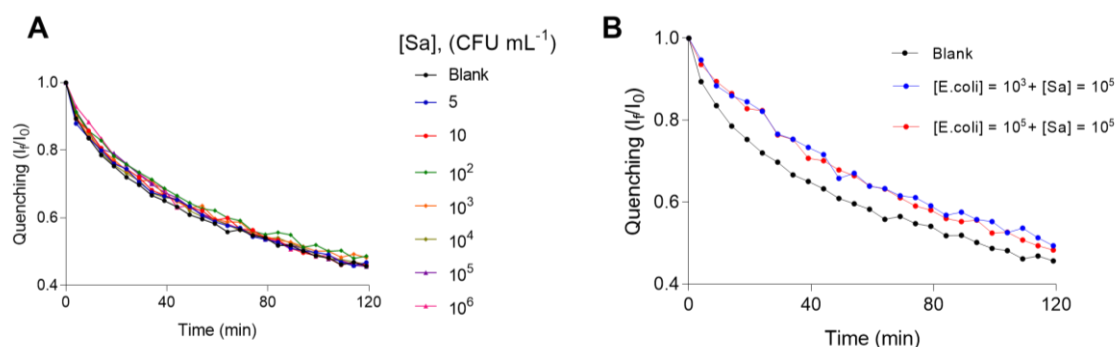


Figure 3. Experiments related to selectivity of the proposed bacterial detection platform operating in optimal conditions; **(A)** Real-time performance of the biosensing system with different concentrations of *Salmonella* as a potential interference; **(B)** Real-time performance of the analysis of samples containing *E. coli* and *Salmonella* at different concentrations. Blank samples were also included as a reference. Adapted with permission [3]. Copyright 2020, ACS.

3.2. Industrial Food Samples

In order to test the feasibility of the proposed technology in the real world, we analyzed industrial samples of cauliflower, these samples were previously determined as positives or negatives via a culture-based method. Particularly, we explored the performance of the real-time quenching profile of four positive samples and four negative samples, respectively, see Figure 4A–C.

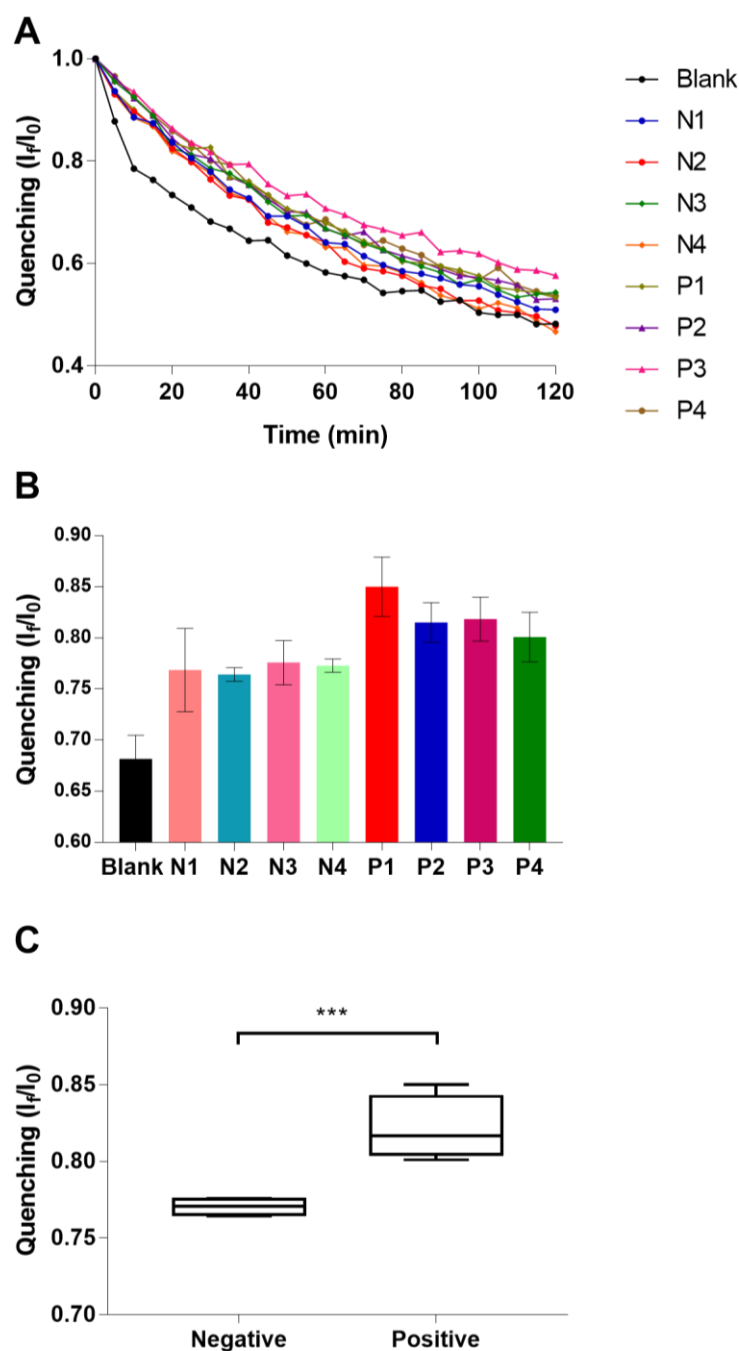


Figure 4. Analysis of industrial samples of cauliflower via the proposed bacterial platform. (A) Real-time quenching profile of the analyzed samples (four negatives, four positives); (B) Analytical performance of the proposed technology with real samples at 30 min; (C) Distribution of the respective If/I0 values at 30 min of the assay. The box plots show the median, 25th, and 75th percentiles and the extreme values of the respective distribution. Adapted with permission [3]. Copyright 2020, ACS.

4. Discussion

According to the behavior of the platform in terms of quenching profile (I_f/I_0), we determined that microplate coated with GO $1200 \mu\text{g mL}^{-1}$ shows the best performance for the real-time interrogation of the bacterial detection. Then, different concentrations of PLBs were studied in GOMs prepared with GO $1200 \mu\text{g mL}^{-1}$ and we include some blank sample analysis as control. PLBs with a final concentration of QDs-antibody at 0.1125 nM and $0.9 \mu\text{g mL}^{-1}$, respectively, were observed to offer the best analytical performance in terms of both sensitivity and coefficient of variation.

Although there are some concentrations (10 and 10^2 CFU mL⁻¹) that do not behave as we expected, the precision limits are acceptable. In optimal conditions, the bacterial detection platform reach a limit of detection of 2 CFU mL⁻¹ at 30 min, reducing the time analysis regarding culture-based methods and improving sensitivity regarding ELISA (review Figure 2C). For specificity assay, the quenching profile of samples of Salmonella are very similar to blank sample, which confirm the selective character of this novel bacterial detection platform. In the industrial food sample analysis, we could observe that despite the inner matrix effect of the sample, the proposed technology is able to discriminate the negative samples from the positives. Then, analyzed statistically the obtained I_f/I_0 values the two groups of samples. These two groups of samples result statistically different, reaching the lower P value at 30 min of the assay ($P = 0.004$), it is shown in Figure 4C.

5. Conclusions

We developed a novel optical platform based on GOMs to pathogenic bacterial determination, this technology operates with a single antibody, avoid wash stages and cumbersome procedures, it is not time consuming and highly sensitive (around 2 CFU mL⁻¹). For industrial samples, the bacterial detection platform is capable to classify between negative and positive samples in around 30 min. This biosensing strategy potentially offers a cheaper and faster option for the food industry to assure the quality of the product and consumer safety.

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