Straightforward Immunosensing Platform Based on Graphene Oxide-Decorated Nanopaper: A Highly Sensitive and Fast Biosensing Approach

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Immunosensors are nowadays a crucial tool for diagnostics and drug development. However, they often involve time-consuming procedures and need at least two antibodies in charge of the capture and detection processes, respectively. This study reports a nanocomposite based on graphene oxide-coated nanopaper (GONAP) facilitating an advantageous immunosensing platform using a single antibody and without the need for washing steps. The hydrophilic, porous, and photoluminescence-quenching character of GONAP allows for the adsorption and quenching of photoluminescent quantum dots nanocrystals complexed with antibodies (Ab-QDs), enabling a ready-to-use immunosensing platform. The photoluminescence is recovered upon immunocomplex (antibody-antigen) formation which embraces a series of interactions (hydrogen bonding, electrostatic, hydrophobic, and Van der Waals interactions) that trigger desorption of the antigen-Ab-QD complex from GONAP surface. However, the antigen is then attached onto the GONAP surface by electrostatic interactions leading to a spacer (greater than \(\approx 20\) nm) between Ab-QDs and GONAP and thus hindering nonradiative energy transfer. It is demonstrated that this simple—yet highly sensitive—platform represents a virtually universal immunosensing approach by using small-sized and big-sized targets as model analytes, those are, human-IgG protein and *Escherichia coli* bacteria. In addition, the assay is proved effective in real matrices analysis, including human serum, poultry meat, and river water. GONAP opens the way to conceptually new paper-based devices for immunosensing, which are amenable to point of care applications and automated diagnostics.

1. Introduction

Immunosensors capitalize on the selectivity and sensitivity of antibody–antigen interactions so as to capture and detect analytes in biological or environmental samples.[1] Being highly specific techniques, immunosensors are the most extensively used detection approaches for the analytical determination of clinically relevant biomarkers.[2,3] They are also important drug screening platforms and prominent proteomic tools.[4,5] Consequently, they are a cornerstone in diagnostics and biological research. In fact, there are different configurations (direct, indirect, sandwich, competitive) and various technologies exploiting immunoassays such as microarray, lateral flow, and enzyme-linked immunosorbent assay.[1] Nevertheless, they often require time-consuming labors (e.g., multiple washing steps) and/or at least two antibodies in charge of the capture and detection of the analyte, respectively. In addition, most of them are not particularly easy-to-use or amenable to portability.[6] Given this paucity,
the technological and scientific community is actively working on the development of cost-efficient and simple approaches facilitating innovative immunosensing approaches.\(^7\text{-}15\)

We have previously reported bacterial cellulose nanopaper as an advantageous biosensing platform, since it offers a myriad of outstanding properties,\(^16\text{-}17\) including environmental sustainability, biodegradability, excellent chemical-modification capabilities (so as to be functionalized), optical transparency, and several other physicochemical properties (low density, hydrophilicity, high porosity, high flexibility, high surface area, and high crystallinity).\(^18\text{-}19\) Moreover, we have been studying the interaction between photoexcited quantum dots and graphene oxide (GO), offering innovative approaches in biosensing based on nonradiative energy transfer, which is highly efficient due to the high surface area and excellent photoluminescence-quenching nature exhibited by GO,\(^20\) even when compared with other carbon forms.\(^21\) Herein, we engineered a hydrophilic, porous, and photoluminescence-quenching nanohybrid material made of graphene oxide-coated nanopaper (GONAP). Although the optical properties of GO have been exploited in several immunosensing systems, they often require both, a capture antibody and a detection antibody.\(^22\text{-}23\) GONAP represents an advantageous immunosensing platform that uses a single antibody and requires no-washing steps. This nanocomposite facilitates adsorption and quenching of photoluminescent quantum dots nanocrystals conjugated with antibodies (Ab-QDs), allowing a ready-to-use immunosensing platform. As the immunocomplex creation involves hydrogen bonding, electrostatic, hydrophobic, and Van der Waals interactions, the complex antigen-Ab-QDs undergo a desorption form GONAP surface upon immunocomplex formation and the photoluminescence is then recovered given that the antigen is anchored onto the GONAP surface working as spacer between GONAP and Ab-QDs and hindering highly efficient nonradiative energy transfer. The immunosensing platform can be turned “On” by either big-sized analytes (pathogens) or small-sized analytes (proteins).

2. GONAP Biosensing Platform

Bacterial cellulose nanopaper (BC, a film of nanocellulose) synthesized by *Acetobacter xylinum* was employed in the proposed immunosensing platform. BC has been previously characterized in terms of average fiber diameter (\(~45 \pm 10\) nm), fiber length (\(>10\) μm), crystallinity (\(~82\%\) crystallite size (\(>6.3\) nm), average tensile strength (\(>345\) MPa), Young’s modulus (\(>17.3\) GPa), and strain-at-break (\(>7\%\)\).\(^16\) A water-based dispersion of single layer GO sheets with average lateral dimension range of \(~500\) nm and C/O ratio about one unit (supplier’s data) was exploited to build the GONAP nanocomposite. As BC exhibits hydroxyl groups onto the surface and GO also has hydroxyl groups onto the basal plane, they can be easily coupled via hydrogen bonding (see the Experimental Section). Streptavidin-decorated CdSe@ZnS QDs with an average size \(\sim 14 \pm 2\) nm and a maximum emission wavelength at \(\sim 665\) nm were employed as photoluminescent agents in the proposed immunosensing platform. Scanning electron microscopy (SEM) micrographs of bare BC, GONAP, and Ab-QDs-GONAP are shown in Figure 2. respectively.

Various concentrations of GO decorating BC were evaluated and compared with bare BC in order to select the most efficient photoluminescence-quenching concentration judiciously. Herein, 150 μg mL\(^{-1}\) of GO in milliQ water was selected as the optimum concentration and the most appropriate for the immunosensing platform (see Figures S1 and S2 in the Supporting Information), which achieved the maximum quenching efficiency (around 50%) when compared with bare BC. Additionally, the concentration of QDs and anti-*E. coli* antibody

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**Figure 1.** Operational concept of the immunosensing approach (schematic representation, not to scale). The hydrophilic, porous, and photoluminescence-quenching character of GONAP allows for the adsorption and quenching of Ab-QDs, whereas photoluminescence recovery is triggered by the immunocomplex formation phenomenon, which involves a series of forces and interactions detaching the antigen-Ab-QD complex. Nevertheless, the antigen is then attached onto GONAP surface working as spacer between GONAP and Ab-QDs and hindering highly efficient nonradiative energy transfer. The immunosensing platform can be turned “On” by either big-sized analytes (pathogens) or small-sized analytes (proteins).
and the incubation time for the immunoreaction were carefully selected based on the most sensitive response, taking the analysis of the blank sample as reference. It was found that the optimum concentrations of QDs and Ab are 100 × 10⁻⁹ M and 0.9 mg mL⁻¹, respectively. Consequently, [GO] = 150 µg mL⁻¹, [QDs] = 100 × 10⁻⁹ M, and [anti-E. coli Ab] = 0.9 mg mL⁻¹ were employed for sensitive detection of foodborne pathogen (E. coli O157:H7). Moreover, the optimum incubation time was 30 min for capturing the target bacteria by the specific Ab. The same aforementioned optimization procedures were carried out for protein detection, whereas the optimum concentrations were ≈100 µg mL⁻¹, 100 × 10⁻⁹ M, and 0.2 mg mL⁻¹ for GO, QDs, and antihuman IgG Ab, respectively, for protein detection, while an optimum incubation period of 2 h was found the most appropriate for protein detection (see Figure S5 in the Supporting Information). It is well known that the suitable incubation time for any immunoreaction strongly depends on the analyte size, thus there is a significant difference between E. coli and protein detection in this parameter (E. coli size = 0.5 × 1.5 μm, human IgG size = 12 nm). Although the size of these analytes is completely different, the biosensing mechanism is driven by the same aforementioned principle that eventually leads to a spacer between photoexcited QDs and GONAP (greater than ≈20 nm), hindering a highly efficient nonradiative energy transfer phenomenon. Thus, the proposed biosensing system is able to detect both small-sized and big-sized analytes as demonstrated below.

2.1. GONAP for Pathogen Detection

The performance of the proposed immunosensing platform as a pathogen detection device was evaluated by using tenfold serial concentrations of E. coli O157:H7 (10⁻⁶ Colony Forming Unit “CFU” mL⁻¹) in a standard buffer. Blank sample (buffer containing zero bacteria) was studied to distinguish the presence and the absence of the target analyte. The photoluminescence intensity ratios (F₁/F₀) of the test and the blank spots were estimated in dimensionless units by dividing the final photoluminescence intensity (F₁) into the initial photoluminescence intensity (F₀) of the same GONAP spot, which determine the presence or absence of the target pathogen. Furthermore, the F₁/F₀ ratio allows for the measurement of tiny amounts of the analyte circumventing analytical problems due to the original intensity of F₀, which can be considered the background signal. Given the operational concept of the immunosensing platform, the photoluminescence of the test spots is expected to increase upon addition of the pathogen, whereas that of the blank spots is expected to be relatively constant or decrease slightly due to the removal of the excess of some Ab-QD complexes after the contact with the liquid sample. Figure 3a shows how the F₁/F₀ ratio of the analyzed blank sample was around 0.8, while serial dilutions of E. coli obtained a F₁/F₀ ratio greater than this value. The proposed immunosensing platform showed a highly sensitive response to the presence of the target bacteria with a wide detection range, from 10 to 10⁵ CFU mL⁻¹, where the F₁/F₀ ratio increased gradually with E. coli concentration at the range from 10 to 10⁶ CFU mL⁻¹ with a full saturation of the system at 10⁶ CFU mL⁻¹. As experimental evidences, Figure 3b shows images of the photoluminescent performance of GONAP immunosensing platform targeting E. coli and Figure 3c displays a SEM micrograph of the platform after adding the target bacteria. Moreover, from that logarithmic response, the estimated detection limit of E. coli in standard buffer was about 55 CFU mL⁻¹, which has been calculated by the mean value F₁/F₀ ratio of the blank plus three times its standard deviation, see Figure S10A in the Supporting Information (threshold line).

In order to investigate the effect of washing steps on GONAP immunosensing platform, a series of assays was carried out via GONAP immunosensing platform and washing steps were performed using 100 µL of PBST followed by 100 µL milli-Q water. After discarding the washing solution, a fluctuating response in the F₁/F₀ ratio with serial E. coli concentrations has been observed. This could be attributed to the weak attaching forces between GONAP and the complex antigen-Ab-QDs. This fluctuating response is shown in Figure S4 in the Supporting Information. Importantly, we discovered that the overall optimal performance of the proposed immunosensing platform does not require washing steps.

2.1.1. GONAP Specificity in Pathogen Detection

We also explored the specificity of GONAP immunosensing platform targeting E. coli in the presence of another nontarget bacterial strain form the same “Enterobacteriaceae family.” Salmonella typhimurium was selected as a nonspecific pathogen for conducting this experiment. Different concentrations of the target and nontarget bacteria were simultaneously analyzed in standard

Figure 2. Scanning-electron micrographs of GONAP platform. a) Bare bacterial cellulose nanopaper. b) GONAP. c) Ab-QDs on GONAP.
buffer in order to assess the specificity of the developed assay as follows: (a) blank standard buffer, (b) low concentration of *E. coli* (10² CFU mL⁻¹), (c) high concentration of *E. coli* (10⁵ CFU mL⁻¹), (d) high concentration of *S. typhimurium* (10⁵ CFU mL⁻¹), (e) a mixture of high concentration of *E. coli* (10⁵ CFU mL⁻¹) and low concentration of *S. typhimurium* (10² CFU mL⁻¹), and (f) a mixture of high concentration of *S. typhimurium* (10⁵ CFU mL⁻¹) and low concentration of *E. coli* (10² CFU mL⁻¹). It was found, as illustrated in Figure 3d, that the $F_1/F_0$ ratio of the nontarget pathogen (*S. typhimurium*) even at high concentration was below the threshold of the limit of detection limit (LOD) and very similar to blank one. Additionally, the presence of the nontarget pathogen in the same media with the target one (*E. coli*) does not affect the response of the immunoassay; since the response produced by a mixture of [*E. coli* (10⁵ CFU mL⁻¹) + *S. typhimurium* (10⁵ CFU mL⁻¹)] was very similar to that of *E. coli* (10⁵ CFU mL⁻¹) alone, likewise, the response of a mixture [*E. coli* (10⁵ CFU mL⁻¹) + *S. typhimurium* (10² CFU mL⁻¹)] was very close to that of *E. coli* (10⁵ CFU mL⁻¹) alone. These results indicate the high specificity and selectivity of the developed immunoassay even in the presence of other competing nonspecific bacteria.

2.1.2. GONAP for Pathogen Detection in Real Samples

Although the application of any developed assay in buffer solution is very important for optimization, the analysis in real samples with minimal sample preparation is crucial in emergent biosensing platforms.[7] Therefore, serial concentrations of *E. coli* O157:H7 were inoculated in poultry meat and river water to be assayed by the proposed GONAP-based pathogen detection platform. Blank solutions of both poultry meat extract and river water were used as a negative control in this experiment. As shown in Figure S5 in the Supporting Information, the $F_1/F_0$ ratios of the blank solutions of poultry meat extract and river water were around 0.7 and 0.8, respectively. While the presence of the target bacteria increases the $F_1/F_0$ ratio to higher values. The obtained results illustrated in Figure S5A,B in the Supporting Information show that the proposed pathogen detection platform has a highly sensitive response to the presence of *E. coli* in complex matrices of poultry meat and river water at wide detection ranges accounted for 50–1.5 $\times$ 10⁵ CFU g⁻¹ and 50–10⁵ CFU mL⁻¹, respectively. Whereas, the $F_1/F_0$ ratios raised gradually with increasing bacterial concentrations with logarithmic responses at the ranges 50–1.5 $\times$ 10⁴ CFU g⁻¹ and 50–10⁴ CFU mL⁻¹ in poultry meat and river water, respectively (Figure S5 in the Supporting Information). From these logarithmic responses,
it was estimated that the limits of detection of E. coli in poultry meat and river water are 65 and 70 CFU g or mL⁻¹, respectively. These relatively low limits of detection in real samples without broth enrichment indicate the capability of E. coli detection in real food and water samples at levels <1 CFU g⁻¹ and 1 CFU mL⁻¹, respectively, after ~2 h of broth incubation. Although there was a slight influence by the matrix of real samples due to the effect of the microenvironment changes (the local viscosity, pH, ionic strength, polarity, and hydrogen-bonding capability of the matrix) on the photoluminescence of QDs, it does not affect the feasibility of the assay in real samples and confirms the possibility of using this novel immunoassay for pathogen detection in other complex real samples. As detailed in the Supporting Information, recovery tests were performed in order to investigate the accuracy and the performance of the developed immunoassay in complex matrices and standard buffer. These results confirming an acceptable accuracy level of the proposed system are shown in Table 1.

2.2. GONAP for Protein Detection

In addition, we explored the overall performance of GONAP immunosensing platform for the detection of a human protein. Human IgG has been employed as a model protein. First, a polyclonal antihuman IgG antibody (pAb) was used for the immunoassay. Several concentrations of human IgG ranging from 3.125 to 50 ng mL⁻¹ in standard buffer were investigated (under optimized condition, Figure S3 in the Supporting Information). As shown in Figure S9 in the Supporting Information, the \( F_L/F_0 \) ratio of the blank buffer was around 0.9 units, while the presence of the target analyte (IgG) obtained greater values due to the aforementioned operational principle of the proposed immunosensing system. A detection range from 3.125 to 25 ng mL⁻¹ was obtained (Figure S9A in the Supporting Information). A scanned photo of GONAP before and after various amounts of IgG (from top and downward: 3.125, 6.25, 12.5, and 25 ng mL⁻¹) presented that the photoluminescent intensity is correlational with the amount of protein, see Figure S9B in the Supporting Information. From that logarithmic relation, a limit of detection accounted for 1.91 ng mL⁻¹ was obtained (Figure S11A in the Supporting Information).

To investigate whether this innovative immunoassay can only be accomplished by integrating polyclonal antibodies or not, a monoclonal antibody targeting human IgG (mAb) was also employed. Interestingly, it was found that GONAP immunosensing platform is also able to operate using monoclonal antibodies. In fact, mAb provided greater fluorescence intensities than those obtained using polyclonal antibodies when both were compared using human IgG concentration of 3.125 ng mL⁻¹ (Figure S11D in the Supporting Information). For human IgG detection using mAb, the detection range was 195 pg mL⁻¹–3.125 ng mL⁻¹ (Figure 4a), and provided a lower limit of detection than that of pAb, accounted for 1.60 ng mL⁻¹, as calculated from the logarithmic response in Figure S11B in the Supporting Information. Importantly, pAb are expected to perform a sandwich-like immunocomplex due to its ability to bind multiple sites of the antigen, whereas mAb cannot perform a sandwich-like configuration due to its ability to bind a single site of the antigen. Hence, these results suggest both, that the complex antigen-Ab-QD is likely to be anchored by the antigen side and that GONAP is also able to anchor sandwich-like immunocomplexes (Figure S6 in the Supporting Information), enforcing the virtually universal operational principle of GONAP immunosensing platform. Moreover, scanning electron microscopy revealed that mAb promotes a higher population density of complexes antigen-Ab-QD upon analyte addition (Figure 4d) when compared to that promoted by using pAb (Figure 4c). This observation clarifies the high sensitivity obtained by using mAb.

The selectivity study of the proposed GONAP-based immunoassay for protein detection was also investigated in the presence of nonspecific immunoglobulin type and using pAb (it should be remarked that polyclonal antibodies are often less specific than monoclonal antibodies). These experiments are described in the Supporting Information, whose results indicate the high specificity of the developed immunoassay using pAb even in the presence of other competing nonspecific type of immunoglobulin (Figure S7 in the Supporting Information). Likewise, we successfully explored the efficiency of the protein sensing platform in complex matrices by screening different concentrations of human IgG (HIgG) in human serum (Figure S8 in the Supporting Information).

2.2.1. GONAP for Protein Detection in Real Samples

Moreover, in order to compare the performance of the developed immunoassay in real samples and standard buffer, spike and recovery tests were done using human immunoglobulin-depleted serum as a real matrix. Three concentrations of human IgG within the respective detection range were spiked in human immunoglobulin depleted serum samples, and then the recovery percentages from human serum were estimated and compared with those of standard buffer. It was found that the recoveries of human IgG from human serum ranged from 93 to 98%, as listed in Table 2. These recovery percentages

Table 1. Spike and recovery assay results.

| Real samples       | Spiked bacteria [CFU mL⁻¹ or CFU g⁻¹] | \( F_L/F_0 \) in standard buffer | \( F_L/F_0 \) in real matrices | Recovery [%] |
|--------------------|---------------------------------------|----------------------------------|---------------------------------|--------------|
| Poultry meat       | 10⁴                                   | 1.145                            | 1.118                           | 97.60        |
|                    | 10³                                   | 1.205                            | 1.184                           | 98.30        |
|                    | 10²                                   | 1.396                            | 1.305                           | 93.47        |
| River water        | 10⁴                                   | 1.101                            | 1.061                           | 96.35        |
|                    | 10³                                   | 1.231                            | 1.201                           | 97.60        |
|                    | 10²                                   | 1.359                            | 1.323                           | 97.30        |

\(a\)Performed in standard buffer; \(b\)Performed in real matrices. The experiment was done by spiking 10⁵, 10⁴, and 10³ CFU mL⁻¹ of E. coli in standard buffer, poultry meat, and river water (n = 3 for each sample), and the recovery percentages of bacteria from real samples were estimated by comparing with standard buffer.
indicate that the complex matrix of human serum does not affect the reliability of the proposed immunoassay and confirm the possibility of its application in real sample analysis.

3. Conclusion

Taking advantage of the hydrophilic, porous, and photoluminescence-quenching character of GONAP, we developed an advantageous and highly transformative immunosensing platform requiring no-washing steps and exploiting a single antibody. The immunosensing mechanism is triggered by an immunoreaction leading to both desorption of previously anchored Ab-QDs and attachment of the complex antigen-Ab-QD. This configures a spacer (> ∼ 20 nm) between GONAP and the Ab-QDs, disrupting highly efficient nonradiative energy transfer. Fast (30 min), highly sensitive, and selective detection and quantification of a pathogen (E. coli) have been recorded at limits of detection accounted for ≈ 55, 65, and 70 CFU mL$^{-1}$ or g$^{-1}$ in standard buffer, poultry meat, and river water, respectively, without previous broth enrichment. This result indicates the ability to detect <1 CFU mL$^{-1}$ or g$^{-1}$ of E. coli after ≈2 h of sample-broth enrichment. Moreover, the proposed device showed a quick (120 min) and sensitive detection of human protein at a detection limit of for 1.60 ng mL$^{-1}$.

In addition, this innovative immunosensing platform is able to show an acceptable level of accuracy (recovery values between 93 and 98%). Although the specificity and sensitivity (in terms of percentage of false positive/negatives, respectively) of this approach has not been determined in the present stage of this research, the successful application of this immunoassay in real matrices analysis opens up innovative capabilities in food, environmental, and biological samples analysis. Additionally, this paper-based platform is easy-to-use, cost-effective, and suitable for portability, point of care applications, automated devices, and multianalyte detection as well.

4. Experimental Section

All commercial reagents were of analytical grade and handled according to the material safety data sheets suggested by the suppliers. BC nanopaper was purchased from Nanonovin Polymer Co. (Mazandaran, Iran). GO was purchased from Angstron Materials (Dayton, OH, U.S.A.). Poly-L-lysine coated glass slides (Cat.No. 22247-1) were purchased from...
Polysciences Europe GmbH (Hirschberg an der Bergstrasse, Germany). Anti-E. coli antibody (biotin) (pAb, ab68451), sheep antihuman IgG H&L (biotin), and mouse monoclonal H2 antihuman IgG Fc (biotin) (mAb, ab97966) were obtained from Abcam (Cambridge, U.K.), and streptavidin–quantum dot 655 was from Life Technologies (Carlsbad, CA, USA). Phosphate-buffered saline (PBS) tablet (P4417), bovine serum albumin (BSA), and Tween-20 were purchased from Sigma-Aldrich (Madrid, Spain). E. coli O157:H7 (CECT 4783, E. coli) and Salmonella enterica subsp. enterica serovar typhimurium LT2 (CECT 7227, S. typhimurium) strains were obtained from the Colección Española de Cultivos Tipo (CECT, Valencia, Spain). IgG and IgA from human serum were purchased from Sigma-Aldrich (Madrid, Spain). Human immunoglobulin IgG IgA/IgM/IgE depleted serum was purchased from Selecta 2000210 oven (JP Selecta s.a., Barcelona, Spain) was used for drying graphene oxide nanocomposites. SEM was performed through a Magellan 400L SEM High Resolution SEM (FEI, Hillsboro, OR, USA). Phosphate buffered saline (PBS) (10 × 10^{-3} M, pH 7.4) at 50 °C for 30 min under vigorous stirring. After that, GONAP was separated from the GO suspension and washed five times with PBS (10 × 10^{-4} M, pH 7.4). Three parallel experiments analyzing the same sample were carried out to ensure repeatability. Then the mixture was incubated at room temperature for 30 min. Afterward, bacterial suspensions were discarded and the test and control spots were left to dry at room temperature before reading the final intensity of the photoluminescence (F1) using the microarray scanner. ImageJ 1.50i (Wayne Rasband, National Institutes of Health, Maryland, USA) was used to analyze both initial and final photoluminescence intensities to calculate the intensity ratio (F1/F0). LOD of the developed immunoassay was estimated by calculating the average F1/F0 of blank samples plus three times the standard deviation. The specificity of the assay was evaluated using S. typhimurium as nonspecific bacteria (separately and in the presence of E. coli O157:H7).

Validating GONAP Immunosensing Platform for E. coli Detection in Real Matrices: The performance of the developed immunoassay platform in bacteria detection was evaluated in complex matrices using poultry meat and river water as model samples. The same aforementioned procedure carried out for E. coli inoculated in standard buffer was applied for previously prepared tenfold serial concentrations of E. coli O157:H7 inocula in poultry meat and river water; however, the concentration of QDs was increased to 120 × 10^{-3} M in case of river water. LOD in real samples was estimated by the same above-mentioned method in standard buffer.

To assure whether the performance is affected by the difference between the buffer used to prepare the standard curve and the real sample matrix or not, spike and recovery experiment was conducted to assess the precision of the developed immunoassay platform in complex sample types. This experiment was conducted by spiking 10^2, 10^3, and 10^4 CFU mL^{-1} or g of E. coli O157:H7 in standard buffer, poultry meat, and river water (three replicates for each bacterial concentration in each sample), and the recovery percentages of the bacteria from real samples (poultry meat and river water) were calculated as compared with the standard buffer.

Using GONAP Immunosensing Platform for Protein Detection in Standard Buffer: The aforementioned procedure of E. coli detection was adapted in case of protein (human IgG) detection. Briefly, human IgG was prepared at different concentrations in the standard buffer (3.125–100 ng mL^{-1}) and stored in the fridge at 4 °C until use. 100 µL of the analyte suspensions were added on the previously prepared spots of GONAP immunosensing platform for human IgG. After incubation for 2 h at room temperature, the analyte suspension was discarded and the spots were left to dry at room temperature before reading the final photoluminescence intensity using a microarray scanner. Afterward, the analysis of the images and calculation of the LOD of IgG were done by the same above-mentioned methods used in E. coli detection. The
specificity of the assay was evaluated using human IgG as a nontarget protein (separately and in a mixture with human IgG).

Validating GONAP Immunosensing Platform for Protein Detection in Human Serum: The performance of the developed immunoassaying platform for protein detection was evaluated in complex real matrices using human immunoglobulin-depleted serum as a real matrix. The same aforementioned procedure carried out with human IgG inoculated in standard buffer was conducted for various concentrations of human IgG (7–700 ng mL⁻¹) in human immunoglobulin-depleted serum. The LOD of human IgG in human serum was estimated by the same abovementioned method in E. coli detection. Similar to E. coli detection procedures, a spike and recovery test was conducted for IgG by spiking 6.25, 12.5, and 25 ng mL⁻¹ of IgG in both standard buffer and human immunoglobulin-depleted serum (at least three replicates for each protein concentration) and the recovery percentages of protein from human serum were calculated as compared with standard buffer.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biophotonics, diagnostics, immunoassays, nanocomposites, optical biosensors, paper-based devices

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