The Electrical Property of SiO$_2$/Graphene/PBA-NHS/anti O and K E. coli Antibodies as Sensing Layer for Escherichia coli Bacteria Sensor

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Abstract. This paper explains the effects of BSA/PBA-NHS/Graphene/SiO$_2$ used as a sensing layer for E. coli bacteria sensor based on the electrical properties. In this research, graphene is used as a sensing layer due to its superior properties especially its biocompatibility. The biocompatibility of graphene was examined by topological properties by AFM. Moreover, it was also confirmed by Raman spectroscopy. The topological structure and Raman spectroscopy showed good results which is indicate the graphene layer suitable for biosensing applications. Additionally, it was also confirmed that the antibody was successfully immobilized on the modified SiO$_2$/graphene/PBANHS. PBANHS is used as a linker due to its cost-effective and time-saving. The electrical properties of the sensing layer before and after the immobilized antibody shows a significant difference. When the number of E. coli bacteria increases, the current increase also became higher. The change in resistance gradually increased from 0.04 to 2.62 $\Omega$ when the number of bacteria increases from 55 to 1200 CFU/ml.

1. Introduction
Recently, research on graphene such as for applications nanoelectronics [1], sensors [2], composite materials [3], biosensing [4], and energy harvesting technology [5] still increasing. The graphene-based sensor is one of the issues that attract the attention of researchers to develop into many applications such as gas sensors, electrochemical sensors, biosensors, and many more. Graphene-based biosensor like bacteria sensor was still the most interest area to explore more. This is due to the statistic that there are still many fatalities both sick and died caused by bacteria diseases or infection. In 2007, the reports from the Joint Monitoring Programme (JMP), every year there were about 289,000 children <5 years die from diarrheal diseases (caused by E. coli bacteria). Its equal to 800 children per day, or 1 child every 2 minutes. The unsafe water supply, inadequate sanitation, and poor
hygiene are contributing 88% of these deaths [6]. Over the last decades, a significant amount of research has been undertaken to establish reliable and effective techniques for detecting water pollutants [7].

The sensing layer plays an important role in the biosensor itself. This is due to it will directly in contact with the bio component and gives a signal for the sensor. There were many sensing materials used in the research such as polyvinyl alcohol (PVA) hydrogel nanofibers [8], metal/oxide [9], and carbon [4]. PVA has outstanding biocompatibility; however, it still needs a complex setup. Moreover, metal/ metal oxide offers high endurance toward extreme environments. However, not all metals are suitable for bacterial detection. Moreover, some metals could kill bacteria instead of detecting, such as Ag (silver), brass, and copper (Cu) [10]. The most commonly used carbon as a sensing layer in graphene due to its superior properties like high specific face area and high mobility, and low electrical noise.

Some linkers have been developed in the biosensor formation depend on the purpose. 1-Pyrenebutyric acid N-hydroxysuccinimide ester (PBANHS) is one of the developed linkers as an activator reagent for the carboxylic acid which consists of hydrophobic headgroup π-π stacking system. Succinimidy ester group is possessing a binding to nucleophilic substitution strongly by amine groups on the antibody. However, the use of PBANHS can save both sensing layer preparation time reaction and cost without using EDC (1-ethyl-3-(3-dimethyl aminopropyl carboxiimide hydrochloride) coupled to (NHS) N-hydroxysuccinimide reaction to activate carboxylic acid group [11].

Furthermore, the antibody was also explored by Oh et.al on a gold sensing layer through 11- mercaptooundecanoic acid and protein G linker. The E. coli antibody was immobilized over the optical system based on the surface plasmon resonance, a reflection of light from the thin metal film that will shift the reflectance index when any binding occurred on the sensor. The sensor can sense E. coli with a limit of detection of 10^5 cell/ml [12]. The antibody of E. coli can also be functionalized as a bioreceptor in bacteria detection on graphene film using 1-pyrenebutanoic acid succinimidy ester (PASE) linker which provide amine linkage for antibody immobilization by π-π interaction with graphene film [13].

Theoretically, the material measured has a low in resistivity if the material is a conductive material, where the charge freely to move. On the other hand, if the material is non-conductive, the resistivity will be measured high. Bacteria, a living things organism, supposedly will act as a good conductor and has a low resistivity. In this paper we describe the functionalized of BSA/PBA-NHS/Graphene/SiO2 as a sensing layer for E. coli bacteria detection based on electrical properties. Moreover, the biocompatibility of the sensing layer was also briefly discussed through Raman spectroscopy and topological properties.

2. Experimental Method

The experiment was begun by preparing the sensing layer of graphene and a sample of E. coli bacteria. The graphene used is a multilayer (p-type) commercial SiO2/graphene P3106 from Graphenea, USA with a size of 0.5 × 0.5 cm². Bacteria E. coli ATCC 25922 (American Type Culture Collection, USA) was used in this research. The suspension and culture of bacteria were conducted by Chromocult Coliform Agar (CCA) 2.65 gr (w/v) (Merck, USA) and nutrient broth (Sigma Aldrich, USA) respectively. The number of E. coli bacteria was stated in the colony-forming unit (CFU).

The graphene sensing layer development was firstly initiated by mix 5% Pyrene Butyric Acid (PBA) with 5% N-hydroxysuccinimide ester (NHS) as a cross-linker on graphene. After that, 5.15 ml of 97% PBA was added into 94.85 ml of dimethylformamide (DMF) 99.8% solvent and 5.10 ml of 98% NHS was added into 94.89 ml dimethylformamide (DMF) 99.8% solvent which then both PBA and NHS solution were blended. PBA (257354) 97% and NHS (130672) 98%, and DMF was purchased from Sigma Aldrich, USA. Then, 0.5 × 0.5 cm² SiO2/graphene film was incubated in PBANHS solution for 2 h at room temperature. It was then washed once with DMF, twice with ethanol 96%, and once more with isopropanol 99%, then blow-dried using nitrogen gas. After the
cross-linking process, the modified SiO$_2$/graphene/PBANHS was incubated in a mixture solution of antibody and Phosphate Buffer Saline (PBS) (pH 7.4: 1.0 M) (Sigma Aldrich, USA) solvent. The antibody was anti–O and K *E. coli* polyclonal antibodies ab31499 (liquid phase) (Abcam, USA). Anti–O and K *E. coli* polyclonal antibodies were diluted in PBS (1:2000 v/v). 1 μl of antibody was added into 2000 μl PBS solvent. The modified SiO$_2$/graphene/PBANHS was put into the antibody dilution overnight at 4°C. Then, the layer was rinsed with DI water and PBS. Next step, 80 mg of Bovine Serum Albumin (BSA) was mixed into 2 mL of DI water 96% (A2153) (Sigma Aldrich, USA) in lyophilized powder to block nonspecific agents.

The characterizations that were carried out in this research are topologically imaged by AFM, Raman spectroscopy, and the electrical properties. Topological characterization is important to indicate the topographic information of the sample. Based on local properties such as friction, height, and magnetism that the AFM can provide, the presence and quality of the anti-*E. coli* antibodies on the graphene layer can also be confirmed. The topological image of the sensing layer was observed using Park System XE-100 (Park system Ltd, Korea non-contact mode since the bacteria was the presence on the sample, it acts as the precaution to avoid the contamination to the cantilever tip. The surface roughness analysis before and after immobilized was done by 3D topographic image and by using height parameters such as average roughness ($R_a$), root mean square roughness ($R_q$) and ten-point average roughness ($R_z$). The height parameter was chosen instead of the case of roughness indicator because it is the most significant parameter among the local properties.

Raman spectroscopy analysis was performed on the sensing layer to confirm the functionalization process of the linker molecules and antibodies that lying on the top of single graphene. To obtain more accurate data from Raman spectroscopy, calibration was performed before the usage. The standard sample used for calibration, was a graphene/SiO$_2$/Si thin film because graphene on silicon has a unique Raman peak located at three locations which are D, G, and 2D peak and also strong Raman peak in around 520 cm$^{-1}$ area that corresponds to single crystal silicon. The Raman spectrometer used in this experiment was the Horiba XploRA PLUS.

The electrical characterization of the functionalized sensing layer with anti-*E. coli* antibodies on the graphene layer were carried out using a two-point probe (Newport Corp., USA). The current and voltage electrodes on the sensing layer were measured and act as a reference. Using the two-probe method, the fixed connector platform was set up to measure the electrode of the sensing material before and after immobilization. Besides, a two-point probe also was utilized to test the resistance of the sensing layer when the sensing layer was treated with different concentrations of *E. coli* bacteria. The water sample with 1.0 ml was dropped on the sensing chamber. The resistance of the source and drain electrode was measured by scanning the potential difference between -3 V to 3 V. The I-V curve that appeared on the screen monitor using Oriel instruments software was recorded to know the resistance value which is the inverse of the slope in the I-V curve.

3. Results and Discussion

3.1 Topological structure of sensing layer

The topological structure of the graphene/SiO$_2$/Si layer and after linked with the anti-*E. coli* antibodies with the BSA was observed qualitatively 3D image using atomic force microscope (AFM). The average roughness ($R_a$), maximum peak-to-valley height ($R_z$), and root-mean-square roughness ($R_q$) of bare graphene and with immobilization of the anti-*E. coli* antibodies and the BSA were listed in Table 1 as the quantitative results. It indicated that the coverage of denatured BSA film and anti-*E. coli* antibodies on graphene/SiO$_2$/Si surface is in nanoscale in size.

The height profile result corresponding by horizontal line scan based on the image in Figure 1 is illustrated in Figure 2. Bare graphene in Figure 1 (a) has a thin and flat appearance with a height of approximately 2.5 nm which may correspond to the multilayer state of graphene. The height of the surface was increased due to the conjugated with the anti-*E. coli* antibodies by around 6.0 nm which was in line with the previous research [14][15]. Based on experimentally result reported by Wu *et al.*
the thickness of PBA-NHS linker was around 0.5 nm while the thickness of anti-E. coli antibodies were around 4.2 nm [14].

Also, the following roughness parameters are presented in Table 1. The topography images gave rough estimation for both graphene and after immobilized with anti-E. coli antibodies and BSA. The results of Ra, Rz, and Rq demonstrated the increase in the surface roughness of the sensing layer after sensing immobilization. However, the quantitative data roughness in Table 1 shows that the value of Ra and Rq was not changed significantly compared to the value of Rz. It can be concluded that for the sensing layer that had high porosity, the value of Rz showed more significant change when compared to Ra and Rq value. AFM result confirmed that anti-E. coli antibodies were successfully attached to graphene thin film based on the distributions of the particles.

![Image](image_url)

**Figure 1.** Topology of (a) graphene/SiO2/Si, and (b) anti-E. coli antibodies with BSA/PBA-NHS/graphene/SiO2/Si

| Treated of sensing layer | Ra (nm) | Rq (nm) | Rz (nm) |
|-------------------------|--------|--------|--------|
| Graphene/SiO2/Si        | 0.669  | 0.986  | 2.327  |
| Anti-E. coli antibodies  | 1.955  | 2.876  | 11.169 | with BSA/PBA-NHS/graphene/SiO2/Si |
3.2. Raman spectra of sensing layer

Figure 4 shows the Raman spectrum of the graphene/SiO$_2$/Si layer and after immobilized with the anti-
$E.\text{coli}$ antibodies and the BSA with the scanning image in Figure 3. The peaks of D, G, and 2D peaks
located at 1348.46, 1580.48, and 2718.20 cm$^{-1}$ respectively show the common Raman peak of
graphene. The intensity ratio of G peak was slightly higher than 2D peak indicated that the graphene
sample was multilayer graphene [16]. Graphene quality is indicated by the low-intensity ratio of D and
G peaks (ID/IG) of graphene which is lower than 0.1 a.u in Raman spectrum [17]. The intensity ratio
of D and G peaks (ID/IG) of this graphene used was 0.08 a.u indicated that the high quality of
graphene was applied. Smaller ID/IG peak intensity ratio can be assigned to lower defects or disorders
in the graphitized structure [18].

When the anti-$E.\text{coli}$ antibodies were linked with the graphene layer by PBA-NHS linker, the
change of peak intensity can be observed. From the result, it can be observed that the D, G, and 2D
peaks were increased and slight right shifted. This attribute to the aromatic pyrene group presented on
PBA-NHS resonance stacked with $\pi-\pi$ interaction of graphene as reported by Wu et al. [19].

The Raman markers of the side chain groups of antibodies as a protein derived from the localized
vibrational mode of specific groups. Raman marker bands of aromatics in protein were well
recognized in visible Raman spectra [20][21][22]. The Raman spectra of the anti-$E.\text{coli}$ antibodies
and the BSA in both compositions were shown in Table 2 with its band assignment. It can be seen that
the conformation of amide I and amide III region with the backbone skeletal stretch acted as a
fingerprint that represented anti-$E.\text{coli}$ antibodies and BSA as a protein were presented on the sensing
layer [23][24][25].
Figure 3. Raman image of graphene before and after undergoing immobilization

![Raman Image Before and After Immobilization](image)

Figure 4. Raman shift of graphene and after added anti-\(E.\) coli antibodies and BSA as the protein

![Raman Shift Graph](image)

Table 2. Raman spectra of anti-\(E.\) coli antibodies and BSA as the protein

| Raman peak (cm\(^{-1}\)) | Tentative assignment                                      |
|--------------------------|----------------------------------------------------------|
| 1062                     | Backbone skeletal stretch. Assigned to \(C_\alpha-C_\beta\), \(C_\alpha-N\) stretches |
| 1101                     |                                                          |
| 1241                     |                                                          |
| 1277                     |                                                          |
| 1309                     |                                                          |
| 1309                     | Amide III region. Assigned to NH bending and \(C_\alpha-N\) stretching mode |
| 1397                     |                                                          |
| 1426                     |                                                          |
| 1514                     |                                                          |
| 1547                     |                                                          |
| 1641                     | Amide I region. Assigned to C=O stretching of carbonyl group |

3.3 Electrical properties
To monitor the magnitude of each subsequent functionalization the I-V measurement was performed before and after anti-\textit{E. coli} antibodies. The resistances were calculated from the I-V slope and transferred in Table 3 up to ten samples to know the value of the differences of all initial immunosensor samples. The driving current at the given voltage shows the decreasing value after the immobilization process. Hence, it’s worth noting that the resistivity of the sample increased after the immobilization process. This observation was attributed to the additional substance that contains charge either via the \(\pi-\pi\) stacking interaction between linker and pyrene or via anti-\textit{E. coli} antibodies and BSA stacking. The decrease of current after the anti-\textit{E. coli} antibody immobilization agreed with a previous study reported by Huang \textit{et al.}\[26].

From the results can be observed that the initial resistivity from each immobilized sample was different from each other. The device for measuring the electrical change was utilized with the calibration function using the Wheatstone bridge that read the initial value by null value so that the resistivity measurement is valid.

Table 3: Resistivity of sensing layer by I-V characterizations before and after the immobilization process

| Number of samples | Resistivity of bare graphene (\(\Omega\)) | Resistivity of graphene with anti-\textit{E. coli} antibodies (\(\Omega\)) | Resistivity change of the samples (\(\Delta\Omega\)) |
|-------------------|------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| 1                 | 7.428                                    | 8.150                                           | 0.722                                         |
| 2                 | 6.954                                    | 7.947                                           | 0.993                                         |
| 3                 | 6.631                                    | 8.634                                           | 2.003                                         |
| 4                 | 7.122                                    | 7.804                                           | 0.682                                         |
| 5                 | 6.616                                    | 7.670                                           | 1.054                                         |
| 6                 | 6.348                                    | 7.362                                           | 1.014                                         |
| 7                 | 6.735                                    | 7.698                                           | 0.963                                         |
| 8                 | 6.981                                    | 8.490                                           | 1.509                                         |
| 9                 | 7.149                                    | 7.885                                           | 0.736                                         |
| 10                | 7.416                                    | 8.362                                           | 0.946                                         |

The I-V characteristics of the sensing layer before and after the exposure to the different concentrations of \textit{E. coli} bacteria are shown in Table 3. When in contact with the \textit{E. coli} bacteria, the I-V graph shifts to the y-axis which shows the increasing current. When the number of \textit{E. coli} bacteria increases, the increase of current becomes higher as well. This corresponds to research undertaken by Akbari \textit{et al.} which shows that the I-V curve of graphene shifts upwards after increasing the concentration of \textit{E. coli} bacteria [27]. In their research, the charge transfer between the graphene immunosensor and the low concentration of \textit{E. coli} bacteria is between 100 to 105 CFU/ml. A similar result was also shown by Wu \textit{et al.}, who measured the current with the \textit{E. coli} bacteria concentration of 103 to 105 CFU/ml. The result of their study indicates that increasing the number of \textit{E. coli} bacteria leads to an increase in the current [19][28]. Hence, the results show a decreasing graphene resistivity. This is attributed to the negatively charged \textit{E. coli} bacteria inducing holes in the graphene. The linear pattern indicates the metallic nature of the sheets and the formation of ohmic contact between the sheets and the electrode [18]. From the I-V graph, the slope can be used to calculate the resistance of the graphene sensor. The change in resistance gradually increases from 0.04 to 2.62 \(\Omega\) when the number of bacteria increases from 55 to 1200 CFU/ml as shown in Table 4.

Table 4. The change of resistance caused by differences concentrations of \textit{E. coli} bacteria

| Number of Bacteria (CFU/ml) | Change of resistance (\(\Delta R\)) |
|----------------------------|-----------------------------------|
| 55                         | 0.04                              |
| 160                        | 0.13                              |
4. Conclusion
From this experiment, it was observed that graphene material has good biocompatibility toward the *E. coli* antibody which is proved by topological imaging by AFM and the fingerprint by Raman Spectroscopy. Furthermore, the electrical property of graphene was also increased due to the immobilization of the *E. coli* antibody. Increasing the number of *E. coli* results in decreasing the resistivity of the sensing layer (SiO$_2$/Graphene/ PBA-NHS/anti O and K *E. coli* antibodies). It was verified that the graphene sensing layer very promising to detect *E. coli* bacteria.

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