Fabrication and characterization of colorimetric polymer based novel nanofibers for sensing and blocking of bacterial

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Keywords: biosensor, blocking and sensing of bacteria, nanofibers, polydiacetylene(PDA)

Abstract

In this study, novel and innovative nanofibers were fabricated using polydiacetylene(PDA) which have colorimetric properties. The resultant nanofibers were evaluated against the bacteria detection and blocking properties of nanofibers. PDA/PU nanofibers effectively detected both gram-positive and negative bacteria through color change and as per evaluation of the bacterial blocking properties of the nanofibers, it was confirmed that the nanofibers were kept in blue color because the bacteria were not transmitted. In conclusion, the PDA/PU nanoweb detects bacteria and changes its color from blue to red, and quantitative analysis is possible through the degree of color change. The nanofibers developed through this study can be used as fiber-type biosensors because it can detect quantitatively the bacteria through rapid colorimetric reaction and confirm the bacterial infection of fiber materials and foods. In addition, it can be used as a protective clothing material such as a mask and a filter because it can block bacteria.

1. Introduction

More than 20,000 children die every year from bacterial infections [1]. These bacterial infections are mainly caused by ingestion of the spoiled foods, it’s causing diseases such as food poisoning and sepsis. Staphylococcus aureus, a typical food poisoning germ, is a gram-positive, facultative anaerobic bacteria in the form of coccus. This is one of the major foodborne pathogens and the route of contamination is very diverse. In addition, infections in food are frequent because they are put on the skin of people and animals. This Staphylococcus aureus produces enterotoxins and causes poisonous food poisoning. Symptoms include vomiting, diarrhea, and gastro-spasms [2–5]. Escherichia coli is a gram-negative, anaerobic bacteria in the form of bacillus that produces verotoxin, which causes colitis and intestinal bleeding. It is mainly infected through various routes such as less cooked meat, dairy products, and water [6–8]. Therefore, there is a growing interest in sensors that can detect bacteria, and various methods are being studied accordingly. Recently, the trend of technology development is being developed in a form capable of detecting various components at the same time. However, due to the combination of electrical and optical devices for detection, the price is high, and there are various problems such as severe change of sensitivity due to electric force, specificity of detection of a substance to be analyzed or chemical component detection. To solve these problems, many studies have been conducted to achieve stability and reliability, and easy measurement methods. In particular, researches on nanomaterials such as carbon nanotubes and graphene have been actively studied [9–13].
Polydiacetylene (PDA), as a conjugated polymer, can be easily synthesized by polymerizing diacetylene monomers through UV light irradiation or plasma treatment [14, 15]. This photo-polymerized polydiacetylene is a blue material with the maximum absorption wavelength at 650 nm. The blue PDA is reddish when the external environmental factors (temperature, pH, chemical, or bacteria) are approached or combined [14, 16, 17]. In the field of biosensor application research using these colorimetric properties, they are studied in the form of solutions, liposomes, and films etc. It has been studied for the color change by cultivated together bacterial [15, 18]. Nanofibers have been used in various sensor studies because they have a large specific surface area and a high reaction rate [19, 20]. Therefore, when PDA is applied to nanofibers, the presence of bacteria can be visually detected through colorimetric, and the reaction rate can be improved by improving the specific surface area. The degree of color change can be used to examine the possibility of quantitative analysis. In this study, we fabricated nanofibers according to the content of PDA by using polydiacetylene which has colorimetric characteristics and can be used as a sensor material. And to provide basic data that can be applied to a mask, a filter, and a biosensor by evaluating the bacterium detection and blocking property of the produced nanofiber.

In this report novel nanofibers as the sensor of detection and blocking of bacteria were fabricated and resultant nanofibers were characterized by SEM, imageJ, colorimeter and solution antibacterial test for the evaluation of its properties.

2. Experimental

2.1. Materials
We used 10, 12-pentacosadiynoic acid (PCDA) from Sigma Aldrich (Poland). And Polyurethane (PU, Lubrizol) with a molecular weight of 80,000 g/mol was used for the preparation of nanofibers using electrospinning. *Staphylococcus aureus* ATCC 6538, gram-positive, and *Escherichia coli* ATCC 25922, gram-negative, were used for bacterial sensing experiments.

2.2. Fabrication of PDA/PU nanofiber
12% nanofiber spinning solution was prepared by dissolving PU and PCDA in N,N-dimethylformamide (DMF) at the ratio of 4:1 ∼ 6:1. The spinning conditions were fixed at a voltage of 12 kV, a Tip to Collector distance of 15 cm, and a fluid velocity of 0.6 ml/h. The weight of the spun nanofibers was adjusted to be 10 g m\(^{-2}\). The prepared nanofibers were photo-polymerized by treatment with a UV lamp (UVC: SANKYO DENKI, model-G40T10) at a distance of 10 cm for 5 s. The morphology of nanofibers prepared using a scanning electron microscope (S-4800, Hitachi, Japan) was observed.

2.3. Film Manufacturing of PDA/PU film
In order to compare the reaction rate with nanofibers, 12 wt% of spinning solution with a ratio of PU and PCDA of 6:1 was applied uniformly coated with a squeeze on the polyester film. The weight of the Film was adjusted to be 10 g m\(^{-2}\). The coated solution was dried at room temperature for 24 h to prepare a film, which was then photo-polymerized.

2.4. Bacteria sensing properties
The sensing of *Staphylococcus aureus* and *Escherichia coli* in the prepared nanofibers and films was examined through color change. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were diluted in BHI broth and LB broth to a concentration of 5 × 10\(^2\) CFU ml\(^{-1}\), respectively. The diluted bacterial solution was incubated at 37 °C for 2, 4, 6, 8, 10, 12 h, and then the concentration of the bacteria was quantified using an absorbance calibration curve. 1 ml of the cultured and quantified bacterial culture for a period of time was exposed to nanofibers and films, and the color change of the exposed surface after 10 s was measured with a colorimeter (JS-55S, Color Techno System. co. Ltd, Japan). The color change was measured by measuring the a* and b* values of the CIE Lab colorimeter, and a* and b* represent redness-greenness and yellowness-blueueness, respectively. The larger the positive a* value, the stronger the redness, and the greater the negative b* value, the stronger the blueness. All tests were repeated 3 times.

2.5. Bacteria blocking properties
For the evaluate the bacterial blocking properties of the manufactured nanofibers, *S. aureus* and *E. coli* were diluted to a bacterial count of 5 × 10\(^5\) CFU ml\(^{-1}\) and inoculated on an agar medium. Two-layer nanofibers web were contacted with the agar medium inoculated with the bacteria and maintained at 37 °C. The change in color of the first-layer contact surface (figure 1(a)) directly exposed to bacteria at 2-hour intervals and the contact surface of the 1st-layer and 2nd-layer (figure 1(b)) was observed. The schematic for this is shown in figure 1.
3. Results and discussion

3.1. Morphology analysis of nanofibers
The nanofibers prepared by electrospinning were polymerized by ultraviolet radiation. Also, photo-polymerization of nanofibers resulted in the conversion of white to blue, which confirmed that photo-polymerization occurred well on the PDA. In order to investigate the morphology of the resultant nanofibers, SEM images were studied as shown in figure 2. The nanofibers produced were smooth without beads and ranged in diameter from 100 to 400 nm. The diameter of nanofibers was measured by imageJ software, where 50 different nanofibers diameter was measured 225 ± 30, 280 ± 35 & 250 ± 20 nm of PU to PCDA ratio 4:1, 5:1 & 6:1, respectively. It means the ratio of polymers did not affect the morphology and diameter of the nanofibers as shown in the figure 2.

3.2. Bacteria sensing properties
In order to investigate the bacterial sensing properties, colorimeter was used as shown in the figures 3, 4 which confirmed the color change and a‘ value change of the prepared nanofibers and film exposed to bacteria. PDA/PU nanofibers were exposed to bacteria, and as the number of bacteria increased, the surface color changed to blue in red and a‘ value increased. In addition, it was confirmed that colorimetric effect by bacteria was sufficiently achieved even in a 6:1 ratio nanofiber having a small content of PCDA. This means that the content of PDA is not significantly affected, and a small amount of PDA is sufficient to detect bacteria. In figure 4, when comparing the a‘ value change of the nanofiber and the film, it can be observed that the PDA/PU nanofiber starts to change color in bacteria with a lower concentration than the film. The red arrow marks the initial point where the a‘ value changes. These results indicate that the nanosized fiber strands have a multi-layered structure, and that the specific surface area of the nanofibers is larger than that of the film, and the bacteria react with the respective nanofibers [19, 20]. It has been reported that nanofibers with a large specific surface area have a significantly higher reaction rate than films [21]. Nanofibers exposed to S. aureus, gram - positive, began to show color change when the number of bacteria was about 45 × 10^2 CFU ml^-1. When exposed to E. coli, gram-negative, the color change was observed at number of bacteria was about 5 × 10^2 CFU ml^-1. The change in color of the nanofibers is thought to be due to the reaction of the phospholipid and PDA, resulting in shortening of the main chain of the PDA and conversion of the binding from the in-plain mode to the twist mode [22]. PDA/PU nanofibers responded to smaller amounts of bacteria when exposed to E. coli (Gram-negative) than S. aureus (Gram-positive). This is considered to be influenced by the thickness of the peptidoglycan layer which is the outer wall layer of bacteria. The peptidoglycan layer thickness is E. coli, gram negative, is thinner and weaker than S. aureus which is gram positive. The peptidoglycan cell wall is mechanically and chemically strong.

![Figure 1](image1.png)

**Figure 1.** Schematic diagram of the bacteria detection method; (a) contact surface with bacteria, (b) contact surface with first layer.

![Figure 2](image2.png)

**Figure 2.** SEM image of nanofiber according to polymer mixed ratio of PU to PDA (a) 4:1 (b) 5:1 (c) 6:1.
in order to protect the bacterial cell, and the cell walls of gram-positive bacteria are more resistant to mechanical or chemical stresses than those of gram-negative bacteria [23, 24].

Figure 5 shows the regression equation for the change of $a^*$ value and $b^*$ value of PDA nano fiber according to the number of bacteria. As shown in the figure, it is possible to predict the exposure of bacteria by $a^*$ and $b^*$ values. S. aureus showed higher $R^2$ in both $a^*$ and $b^*$ values than E. coli. S. aureus had a higher $R^2$ value for $a^*$ value indicating redness. In the case of E. coli, the $R^2$ value for $b^*$ indicating the blue phase was large. Therefore, it can be concluded that the quantitative analysis is possible through the change of $a^*$ value and $b^*$ value of nano fiber when exposed to bacteria.

3.3. Bacteria blocking effect of PDA/PU nanofiber
To examine whether bacteria pass through the nano fiber web, the color change of the nanofibers was observed after exposure of the two-ply nano fiber web to the bacteria. figures 6 and 7 show the color change and $a^*$, $b^*$.
values of the first layer nanofiber (figure 1 (a)) and the second layer nanofiber (figure 1 (b)), respectively. Shows The first layer is directly exposed to S. aureus and E. coli, and the second layer is overlying the first layer. As shown in the figure, the colorimetric polymer-based nanofibers showed color change when exposed directly to bacteria. The nanofiber of the first layer was exposed to bacteria, and as the inoculation time increased, the surface color changed to red to blue, and the a* and b* values increased. However, in the case of the 2nd-layer, there was no color change, so the a* and b* values did not change. It was meaning that bacteria is not transmitted. These
results indicate that the bacteria of several micrometers are difficult to infiltrate into nanofibers in size because the pores between the fibers are nano-sized. The bacteria’s permeability was due to the nanostructure, so it was not affected by the PDA content.

4. Conclusion

The PDA/PU nanofibers developed through this study were detect effectively of gram positive and negative bacterial. Bacteria could be detected even if the content of PDA in nanofiber was low. And the color changed depending on the amount of bacteria, so it is possible to detect quantitatively. In addition, it can be used as a fiber type biosensor capable of detecting bacterial infection such as mask, filter, etc, which can block and detect bacteria due to nano-sized pores, which is a structural advantage of nanofiber.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by Ministry of Science, ICT & Future Planning of the Korea government. (NRF-2017R1A2B4009315).

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