Selective detection of Gram-negative bacteria by color changing polydiacetylene nanofiber

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Keywords: Polydiacetylene, nanofibers, biosensor, bacteria

Introduction: Pathogenic bacteria is one of the leading causes of human deaths around the world. Every year, millions of children and infants die due to severe bacterial diseases such as diarrhea which is mainly caused by *Escherichia coli* (*E. coli*) bacteria (World Health Organization, 2017). Bacteria can be grouped into two large categories- Gram positive and Gram negative- based on the Gram staining technique developed by the Danish bacteriologist Hans Christian Gram. Although human body contains helpful bacteria, some strains of Gram-negative and Gram-positive bacteria can cause deadly diseases. To prevent high bacterial infection among human, rapid and selective detection of bacteria is necessary. Traditional bacteria detection technique uses plate colony counting method, which is prone to low sensitivity and requires long output time (Qiao et al., 2020). Recent bacteria detection techniques utilize polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), and flow cytometry which are highly sensitive but possess various shortcomings such as high cost, long output time, shortages of antibodies for target strains, and use of expensive laboratory equipment. The use of nanotechnology can offer tremendous advantages in bacteria detection and can help in overcoming the existing drawbacks. Recently, Yapor et al. (2017) and Bhattacharjee et al. (2020) had developed a nanofiber-based biosensor to detect *E. coli*. This promising platform uses polydiacetylene (PDA) macromolecule with a supporting matrix polymer (polyurethane, PU) that can effectively sense *E. coli*. Based on that approach, a PU-PDA nanofiber was developed in this work via electrospinning and photopolymerization. When the blue colored PU-PDA was tested with a Gram-negative bacteria *E. coli*, the blue color of the fiber turned into red within minutes. However, when the fiber was tested with a Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), the fiber color remained unchanged. The Gram-negative bacteria strain produced a 18% colorimetric response in the fiber compared to the 1.7% by the Gram-positive strain. To confirm the selectivity of the detection, another Gram-negative bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) was tested which also changed the color of the blue fiber to red. These results confirmed that PU-PDA nanofibers can selectively detect Gram-negative bacteria via a visible blue-to-red color shift in the fiber.

Materials and methods: 10, 12- pentacosadiynoic acid (PCDA) monomer powder and PU chips were mixed using N, N- dimethylformamide (DMF) via continuous stirring at 55°C until a homogeneous pink

solution was achieved. The solution was used as electrospinning solution and a white fiber mat was collected after 1 hour of electrospinning. The white fiber mat was photopolymerized by 254 nm UV irradiation for 3 minutes which turned the white fiber into blue. Three bacteria strains- *E. coli* (ATCC25922), *P. aeruginosa* (P01), and *S. aureus* (ATCC 6538) were cultured in a nutrient broth. Color changing behavior of the fibers were recorded as photographs when the bacteria cultures were introduced to the fibers. A spectrophotometer was used to collect spectra and calculate CR% of the fibers before and after testing with bacteria strains following the protocol described by Bhattacharjee et al. (2020).

Results and discussions: The PU-PDA nanofiber mats were blue in color after photopolymerization which confirmed successful polymerization of PCDAs into PDAs (Figure 1a). Optical microscopic images of the fibers confirmed continuous fibers without any beads or irregular shapes (Figure 1b). The average diameter of the fibers was calculated as 1400-1700 nm using the optical microscope images and imageJ software.

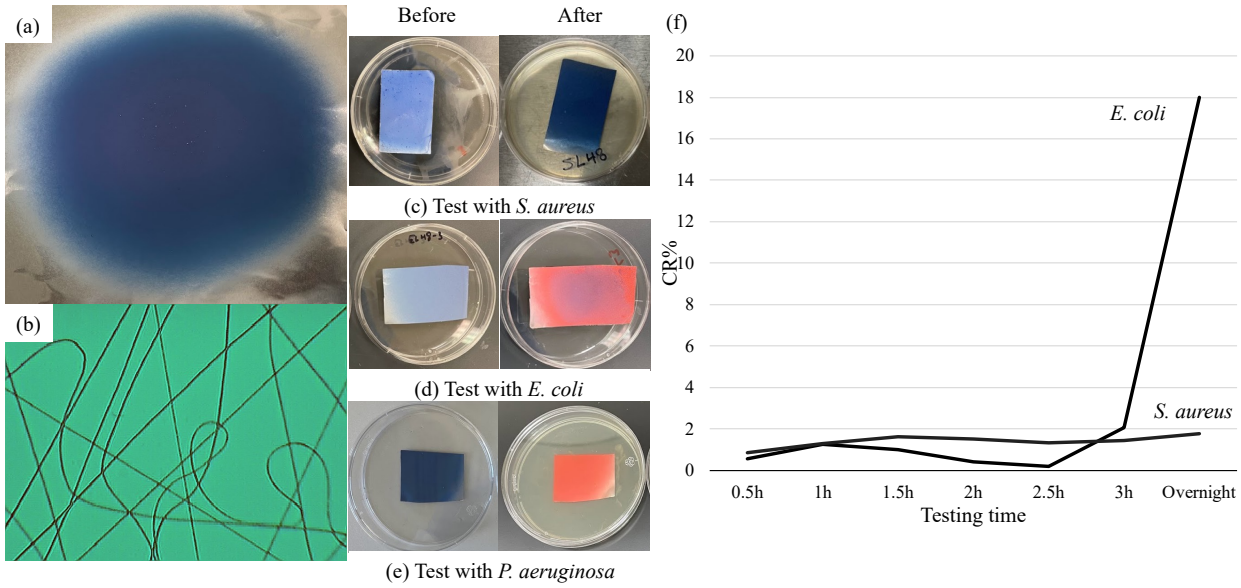

Figure 1. (a) PU-PDA fiber mat, (b) Optical microscopic image of PU-PDA fiber, (c, d, e) PU-PDA fiber before and after image when tested with *S. aureus*, *E. coli*, and *P. aeruginosa* respectively, (f) Colorimetric response (CR%) of PU-PDA fiber when tested with *S. aureus* and *E. coli*.

The concentration of the bacteria culture was calculated by serial dilution and plate colony counting methods. All the bacteria strains yielded a 10^8 CFU/mL concentration after 48h of incubation. When the PU-PDA fiber was introduced to the *S. aureus* culture, no color change was observed for 24 h (Figure 1c). However, when the fiber was introduced to the *E. coli* culture, a shift in the color was observed within

minutes. The red color was prominent after 0.5 h of testing (Figure 1d). Similar result was found for the test with *P. aeruginosa* (Figure 1e). The CR% was calculated for *E. coli* and *S. aureus* strains. *E. coli* introduced a 18% color change in the fiber, whereas *S. aureus* only introduced 1.7% color change which is negligible because the fiber became wet after testing with bacteria culture solution. These results confirmed selective detection of Gram-negative bacteria by the PU-PDA nanofiber.

Conclusion: This is a preliminary study to selectively detect Gram-negative bacteria strains. The PU-PDA biosensor platform can be useful to identify pathogenic Gram-negative strains rapidly and without any need for expensive testing equipment. The vivid color shift can also help in easy identification of bacterial infection in wound dressing, food packaging and point-of-care devices. In future, the amount of live and dead bacteria, protein concentration and nature of the proteins in Gram-negative bacteria culture will be investigated to identify responsible factors that reacts with the PU-PDA fiber to change its color.

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