Study of Bacterial Components Activating a Colorimetric Transition in Bacteria-Detecting Nanofiber Wound Dressing Applications

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Introduction: Polydiacetylene (PDA) demonstrates blue-to-red color transition behavior due to a conformational change in π-conjugated backbone structure of PDA macromolecule at external stimuli of bacteria. Yapor *et al.* (2017) incorporated PDA in polyurethane (PU), resulting in PU-PDA nanofiber composite that demonstrated rapid blue-to-red color transition responding to bacteria. This work introduced huge potential of PU-PDA nanofibers in biosensor applications, especially in developing smart wound dressings that can be used to detect bacterial infection during wound care and management. The color-changing PU-PDA nanofiber can dramatically change the horizon of wound care for public health. In developing a bacteria-detecting smart wound dressing, the bacterial culture components activating colorimetric transition in PDAs is still undetermined due to the complexity of a bacterium system composing of bacterial cells and secretions that the bacteria produce during growth phase. Bacterial secretion components also depend on the nutrient bacteria receives from the culture medium. In this study, PU-PDA nanofibers were prepared and tested for color change with different bacterial culture components including bacterial cell pellets, supernatant fluids and extra-cellular polymeric substances (EPS). The critical bacterial concentration (CBC) to initiate color change in the nanofibers was determined to justify the use of PDA biosensor in wound dressing applications.

Materials and Experiments: 10, 12- pentacosadiynoic (PCDA) was used as a monomer of PDA. Solid PU and PCDA were mixed with a binary solvent of tetrahydrofuran (THF) and N, N-dimethyl formamide (DMF) and used as electrospinning solution. A white nanofiber mat of PU-PCDA was collected after electrospinning and irradiated with UV light to polymerize PCDA into PDA, thus producing blue PU-PDA nanofiber composite mat. *Escherichia coli* (*E. coli*) ATCC25922 was cultured in a Luria Bertani broth (LB) and M9 minimal media. Bacterial cell pellets and supernatant fluids were extracted by centrifugation and filtration methods. One batch of supernatant fluids and bacterial cell pellets were autoclaved. Free EPS was extracted from both autoclaved and non-autoclaved supernatant fluids following a procedure described by Eboigbodin and Biggs (2008). Colorimetric properties of the PU-PDA nanofiber mats were studied when the nanofibers were tested with autoclaved and non-autoclaved bacterial cell pellets and supernatant fluids, free EPS, and 12 common organic solvents. The colorimetric response (CR%) of PU-PDA nanofibers were calculated using a method described by Yapor *et al.* (2017)

to determine the color-changing components in bacteria, the critical bacterial concentration (CBC), and the critical polarity index of organic solvents. Additionally, nanofiber morphology was studied before and after test with bacterial components via a scanning electron microscope (SEM).

Results and Discussions: A critical bacterial concentration (CBC) of ~9×10^8 CFU/mL was found to initiate color change in PU-PDA nanofibers (Figure 1A). The number of bacteria required to cause infection in wound varies between 10^5 to 10^9 or more per gram of wound tissue (Robson, Mannari, Smith, and Payne, 1999; Freshwater and Su, 1980). Therefore, the PU-PDA nanofiber appears to be sensitive enough for practical bacteria detection purposes in smart wound dressing applications. In addition, the sensitivity of the PU-PDA nanofiber was evaluated against common organic solvents since this is a well-studied platform for evaluating sensing capability of PDA based materials. All the common organic solvents except hexane and methanol were able to change the color of nanofibers, confirming superior sensitivity of the PU-PDA nanofiber. Interestingly, the CR% induced by the organic solvents was unique for each solvent, thus opening a new application of PU-PDA nanofiber as a fingerprinting sensor for organic solvents. SEM images of the nanofibers suggested that no significant changes in PU-PDA nanofiber morphology was present after blue-to-red color change due to bacterial culture components, suggesting a great serviceability of the PU-PDA nanofibers in wound dressing application (Figure 1- B1 and B2). The colorimetric responses to different bacterial components indicated that the color changing substances (CCS) were found in supernatant fluids rather than bacterial cells (Figure 1- C1, C2, and C3). The blue-to-red color change due to autoclaved supernatant fluid was substantially high, suggesting that the CCS were able to survive extreme heat and pressure from autoclaving process. The results imply that the CCS were not proteins, DNA or RNA because they were generally denatured during autoclaving. Free EPS from supernatant and autoclaved supernatant fluids changed the color of PU-PDA nanofibers, thus confirming free EPS is the responsible CCS in a bacteria culture. Additional experiments were conducted to understanding the nature of CCS. When the supernatant fluids containing free EPS was stored for an extended period in room temperature, the colorimetric transition reduced significantly, confirming a time-dependent decay of CCS. Also, when the bacteria were cultured in a low nutrition media (M9 minimal media), no colorimetric transition was observed in PU-PDA nanofibers, indicating that the responsible CCS was not produced by the bacteria in a low nutritious culture.

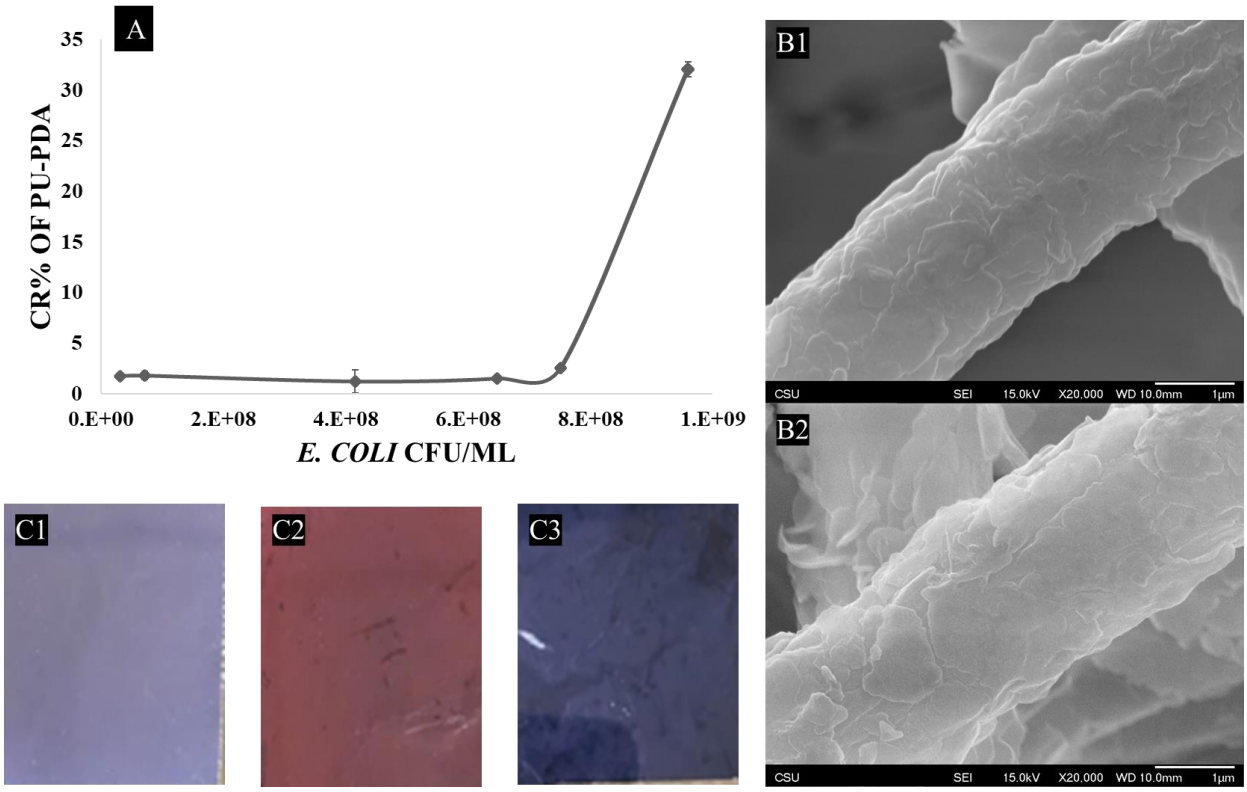

Figure 1 *CR% of PU-PDA nanofiber with E. coli CFU/mL (A); SEM images of PU-PDA nanofiber before (B1) and after testing (B2) with bacterial supernatant fluid; images of PU-PDA nanofiber composite mat before testing (C1), after testing with supernatant fluid (C2), and after testing with bacterial cell pellet (C3).*

Future research: Bacterial EPS is a major component in bacterial biofilm which is responsible for many diseases in human. EPS contain numerous biomolecules, including proteins, polysaccharides, nucleic acids and many more. The study of color-changing behavior of PU-PDA nanofiber against the components of complex EPS system will provide important guidance for preparing wound dressing using PDA. This study found that the production of CCS by bacteria depends on the nutrition and surrounding environment. Therefore, an *in vivo* wound dressing study model can be conducted in future. Additionally, electrospinning parameters can be evaluated to control the sensitivity of the PDA based nanofiber composite for wound dressing applications.

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