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Identification of *Leptospira* in water by Fe-Pd-doped polyaniline nanocomposite thin film

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Abstract

Leptospirosis disease was caused by rat urine which contains the genus *Leptospira* bacteria. In this study, the fabrication of Pd-Fe-doped polyaniline nanocomposite thin films for the determination of the genus *Leptospira* bacteria thin films has been investigated. Pd-Fe-doped polyaniline nanocomposite thin films were fabricated by sol–gel spin coating method. The electrode sensors were immersed in the *Leptospira* solution. The resulting materials were investigated using field-emission scanning electron microscopy, atomic force microscopy, transmission electron microscopy, and current–voltage measurement. The atomic force microscopy images show the specific morphology films' structure for *Leptospira* detection, whereas the field-emission scanning electron microscopy image shows the irregularity of clump nanoparticles in thin film surfaces. Transmission electron microscopy result shows that metal alloy (Fe-Pd) embedded in the polymer matrix. Current–voltage measurement with and without incubation of the thin film into *Leptospira* solution was done to show the relationship between concentration bacteria versus current. The result shows that polyaniline-$Fe_{0.4}$-$Pd_{0.6}$ nanocomposite thin film has higher sensitivity in detecting *Leptospira*, where it has performed with the highest percentage of the sensitivity of 16.9%. Besides that, selectivity tests were conducted to distinguish the existence of *Leptospira*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* bacteria. These results confirm the potentials of polyaniline metal alloys' nanocomposite thin films to be used for *Leptospira* bacteria detection in water.

Keywords

Leptospira, polyaniline, I-V measurement, thin film, sensitivity

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Introduction

Leptospirosis is a zoonotic disease caused by the genus *Leptospira* that consists of saprophytic and pathogenic *Leptospira*. Pathogenic strain can be detrimental to both animals and humans.[1] These bacteria are carried by rodents or domestic animals, which are considered an immediate host. Animals and humans have known to be infected through direct contact with urine, polluted soil, and water sample. *Leptospira* bacteria are contagious in humid tropical countries because this is their method of adaptation. This disease has been reported to be threatening to various groups of people especially those working as veterinarians, farmers,

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abattoir workers, meat inspectors, and workers in rodent control sectors.[2]

Bacterial detection of nanocomposites can be made possible by converting the electrochemical signal into a quantity of electrical response. The electrical signals generated through the biochemical reaction between the thin active surface and the target bacteria, can be quantified, and measured for a reliable bacterial detection method. Currently, the detection of these bacteria is extremely time-consuming. For instance, the Microscopic Agglutination Test (MAT) needs 8 days or more to provide positive result in regards to the diagnosis of these bacteria.[3] Enzyme-linked immunosorbent assay (ELISA) method is considered inadequate and needs time to stabilize for the detection of *Leptospira*.[4] Polymerase chain reaction (PCR) method uses both urine and clinical blood samples in a test which could lead to contamination.[5,6] Difficulties in detecting Leptospirosis occur at the initial stages of this disease because it needs specific instruments, specific and sensitive samples, a large laboratory area, and highly skilled experts. Due to the problems stated above, there is a rising interest in using nanocomposite thin film as a detector. We are fabricating metal alloys–polymer thin film lab-on-a chip for a portable *Leptospira* biosensor that may take 1–2 days of detection. Polyvinyl alcohol (PVA) can be used as stabilizers in the chemical synthesis for particle size control of polyaniline (PANI) where it acts as a cross-linking agent to enhance the properties of PANI.[7] PVA is preferred due to being a good material for metal nanoparticle production, high thermal stability, chemical resistance, electrical conductivity, water solubility, and high mechanical strength.[8] This is the main reason to incorporate metal nanoparticles into PVA where the concentration and distribution of metals can be controlled in the polymer matrix. The incorporation of metal alloys' nanoparticles into a polymer matrix will make this sensor much more effective and carries advantages in terms of usage in hazardous and remote places. This sensor can also be used in conditions where the number of bacteria varies constantly and thus needing highly sensitive and accurate detector.[9] Previous studies have utilized the use of metal oxide, such as ZnO and TiO_2, which shows to exhibit anticorrosive and self-cleaning functionalities and was synthesized using a green chemistry approach.[10,11] PANI is a promising substitute for these materials as it has better conductivity, high cycle stability, and cleanliness, making it a reliable candidate to use as bacterial detection.

This study demonstrates the application of PANI-Fe-Pd nanocomposites thin films as the active sensing material for the detection of saprophytic and pathogenic *Leptospira* in the 10^8 CFU mL^{-1} in water. The morphological structures of the thin films were analyzed using atomic force microscopy (AFM) and field-emission scanning electron microscopy (FESEM). This study aims to help researchers detect *Leptospira* in contaminated waters without the inherent disadvantages of conventional detection methods such as MAT, ELISA, and PCR. To achieve that goal, this new material that incorporates metal alloys in PANI is designed to obtain fast and highly sensitive results for in situ *Leptospira* detection that can serve as an effective public preventive measure against Leptospirosis disease.

Experimental

Materials

The materials used were iron (III) nitrate $Fe(NO_3)_3.9H_2O$, palladium (II) nitrate dehydrate $Pd(NO_3)_2.2H_2O$, PVA (99% hydrolysis and molar weight 85,000–124,000 g mol^{-1}), nitric acid, and aniline monomer ($C_6H_5NH_2$). All the above materials were purchased from Sigma-Aldrich Chemicals, Selangor, Malaysia.

Synthesis of samples

About 2.5 g of PVA solution was added into 40 mL of deionized (DI) water and heated to 90°C. About 0.5 g of iron nitrate and palladium nitrate was added into the PVA solution following the formula of $Fe_{(1-x)}$-$Pd_{(1-x)}$ with x representing the weight ratios of Fe and Pd (x = 1, 0.8, 0.6, 0.4, 0.2, and 0.0). About 1.25 mL of aniline and 1.0 M of nitric acid (HNO_3) were added to each of these solutions. All the solutions were mixed at 90°C 24 h^{-1} until it changes to a dark brown color indicating that the solution has become PANI-Fe-Pd nanocomposite. The nanocomposite solution was spin-coated on glass substrates at the speed of 1800–2000 r min^{-1} for 18–20 s using a spin coater model WS-400BX from the brand Laurell Technologies Corporation, USA. The glass substrates were heated for 10–15 min after the spin coat. These processes were repeated for four to five times until the thin film was obtained. Finally, the thin films were annealed in a furnace at 250°C 24 h^{-1}.

Fabrication of the sensor

A comb structure of silver electrode was sputtered on the thin film to obtain a thickness of 1000 Å using RF magnetron sputtering equipment manufactured by T-M Vacuum Products, Inc. USA . Basically, dimensions of the used thin film size were 20 mm × 25 mm, the measured width of a silver layer was 2.2 mm, and separations between three combs were 1.2 mm. Finally, a copper (Cu) wire was soldered onto the silver layer as a terminal electrode. It connects to the GAMRY-Physical Electrochemistry (GAMRY Instruments, USA) for the sensor's performance measurement as shown in Figure 1.

Preparation of bacteria samples

The *Leptospira* bacteria samples were categorized based on the concentration of 10^8 CFU mL^{-1} (colony forming units per milliliter). All the wet samples were obtained from the

Department of Veterinary Pathology & Microbiology, Faculty of Veterinary, Universiti Putra Malaysia.

Sensor performance

The sensor performance was tested using GAMRY Physical Electrochemistry Instrument G-300 as shown in Figure 2. I-V measurement was recorded by the GAMRY instrument when the thin films were immersed in DI water and *Leptospira* solution. The thin films were exposed to *Leptospira* solution: 10^8 CFU mL^{-1} for 4–5 min. These measurements were repeated for five times. For safety purposes, all materials, tubes, plates, needles, pipettes, and other items used in the experiment should be soaked with a bleach solution for a minimum of 1 h.

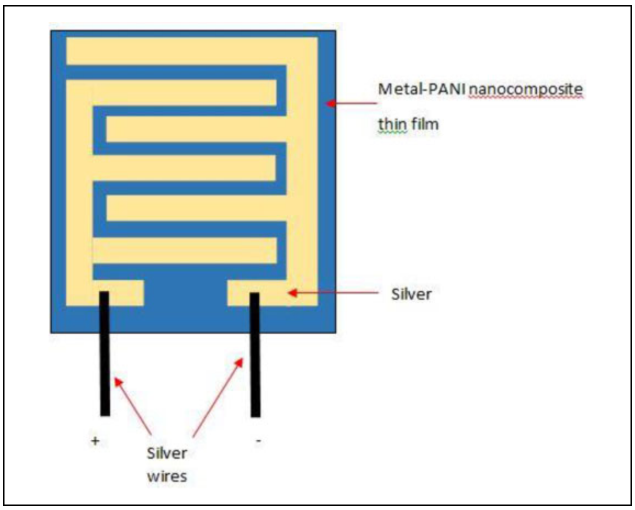

Figure 1. The design of comb-structured silver electrode.

Result and discussion

Morphological studies

FESEM image in Figure 3 shows the distribution of nanoparticles ranging from 80 nm to 100 nm. The arrangement of the thin film presents an irregularity pattern where the metal alloys' nanoparticles are surrounded by polymer on the thin films' surfaces.[12] The particle showed that the thin film has agglomerated.[13] This condition appears after the addition of the doping aniline with PVA during the chemical oxidation process.[14,15] It also happens due to the characteristic of PVA. Besides that, by coating the metal alloys, the sensors become highly beneficial for electrochemical sensing applications. This platform increases the reaction between the bacteria and films' surface via absorption of the gram-negative charged microbes within the film structure.[9,16]

TEM images in Figure 4 show the morphology of PANI-Fe-Pd nanocomposites particles. The metal alloys' (Fe and Pd) nanoparticles are in a spherical pattern with the diameter size of around 10–40 nm. Incorporation of the PANI and metal alloys (Fe and Pd) forms nanocomposites, and it is clearly embedded in the polymer matrix. Meanwhile, the metal alloys-coated PANI is encapsulated and agglomerated.[17,18]

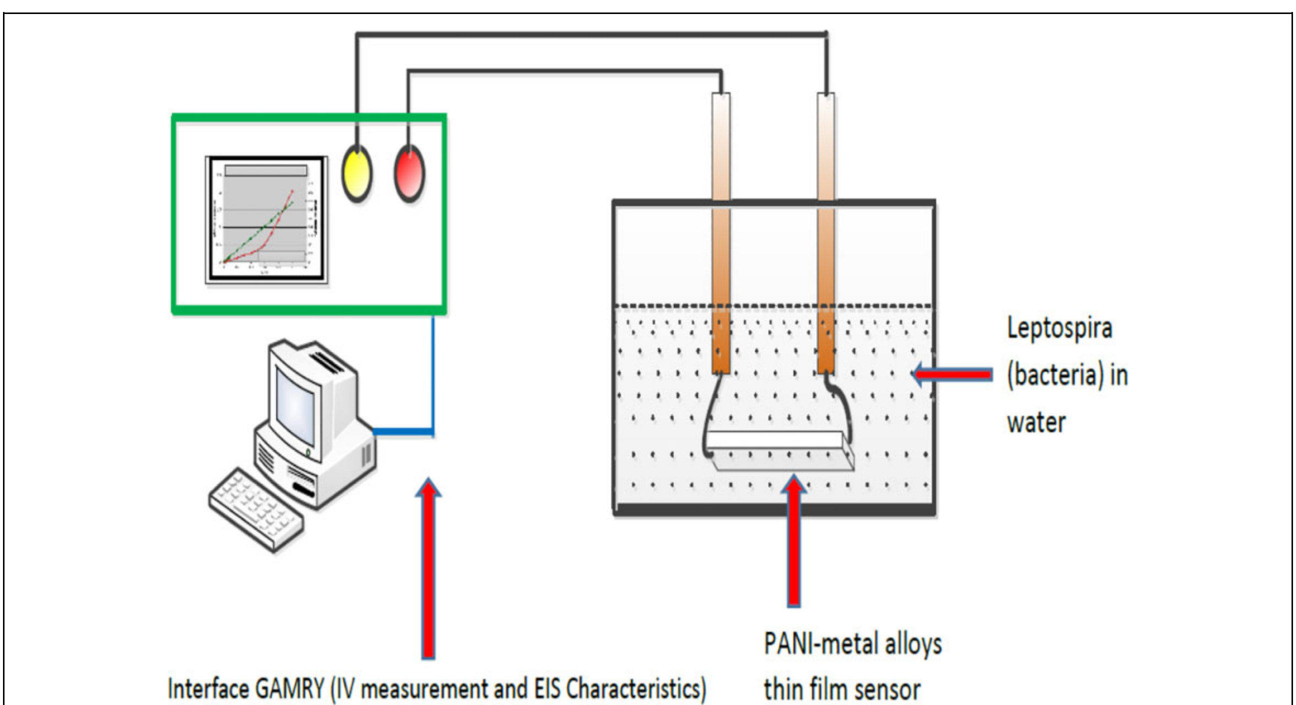

Figure 2. Experimental setup.

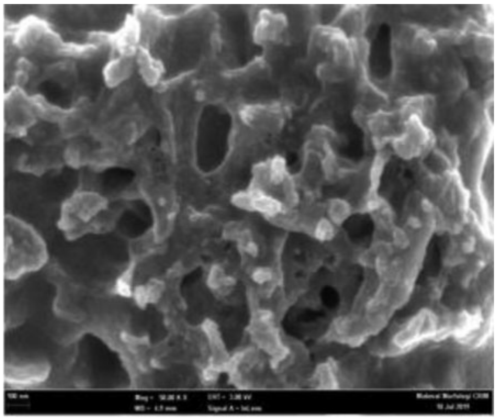

Figure 3. FESEM image of PANI-Fe-Pd. FESEM: field-emission scanning electron microscopy; PANI: polyaniline.

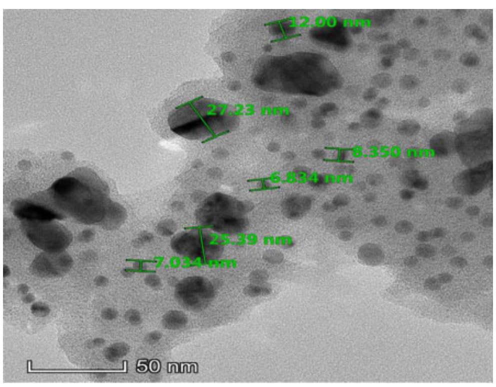

Figure 4. TEM image of PANI-Fe-Pd nanocomposite thin film. TEM: transmission electron microscopy; PANI: polyaniline.

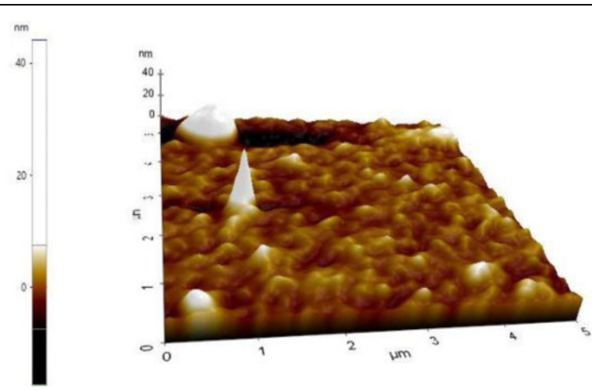

Figure 5. AFM image of PANI-Fe-Pd nanocomposite thin film. AFM: atomic force microscopy; PANI: polyaniline.

AFM image in Figure 5 shows the morphology of the thin films, which contains metal alloys (Fe and Pd) on the polymer substrates. The thin films' surfaces look smooth and homogeneous, which produces high sensitivity for

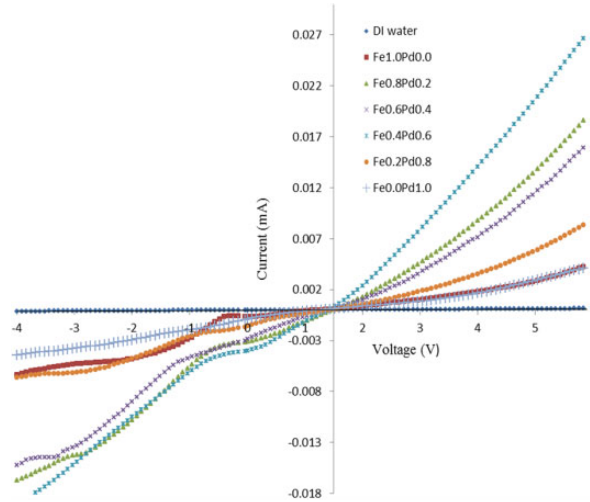

Figure 6. I-V measurement of PANI-Fe-Pd nanocomposite thin film with pathogenic *Leptospira* concentration: 108 CFU mL^{-1}. I-V: current–voltage; PANI: polyaniline.

Leptospira detection.[19] The AFM images show the incorporation of PANI-Fe-Pd on the film causing their morphology to bind to the *Leptospira* bacteria.[20,21] The average roughness and grain size of the thin film were obtained at the range of 2.138 nm and 43.72 nm, respectively.

Current–voltage and sensitivity measurement

The I-V curve shows the current that is flowing through a film when a voltage is applied to the electrode. These results can be used to differentiate which of the sensor is immersed in a bacteria solution or clean water. The sensors were immersed in pathogenic *Leptospira* solution in the 10^8 CFU mL^{-1} range. Figure 6 shows the current measured across the thin film that was exposed to DI water and *Leptospira* concentration range of 10^8 CFU mL^{-1}. PANI-$Fe_{0.4}$-$Pd_{0.6}$ shows the highest curve compared to the others. An interaction between the thin films and *Leptospira* showed the presence of conductivity due to the incorporation of PANI and metal alloys.[22] The result indicated that the cell wall of *Leptospira* is gram-negative bacteria.[23]

The corresponding calibration curve presents a linear relationship between the peak current and the value of *Leptospira* concentration. In brief, the PANI-$Fe_{1.0}$-$Pd_{0.0}$ linear regression equations is expressed as $y = 0.0009x - 0.0016$ with a correlation coefficient of 0.9103, PANI-$Fe_{0.8}$-$Pd_{0.2}$ as $y = 0.0033x - 0.0039$ with a correlation coefficient of 0.9482, PANI-$Fe_{0.6}$-$Pd_{0.4}$ as $y = 0.0028x - 0.0033$ with a correlation coefficient of 0.9447, PANI-$Fe_{0.4}$-$Pd_{0.6}$ as $y = 0.0045x - 0.0041$ with a correlation coefficient of 0.9401, PANI-$Fe_{0.2}$-$Pd_{0.8}$ as $y = 0.0014x - 0.0018$ with a correlation coefficient of 0.9437, and PANI-$Fe_{0.0}$-$Pd_{1.0}$ as $y = 0.0008x - 0.012$ with a correlation coefficient of 0.9544, respectively.

The efficiency of PANI-Fe-Pd nanocomposites thin film in detecting *Leptospira* is determined by tabulating the sensitivity (S), where the ratio of the response volume on imposing to bacteria I_e is being compared to impose without bacteria I_o, using the following formula[23]

$$S = (I_e - I_o/I_o) \times 100\% \quad (1)$$

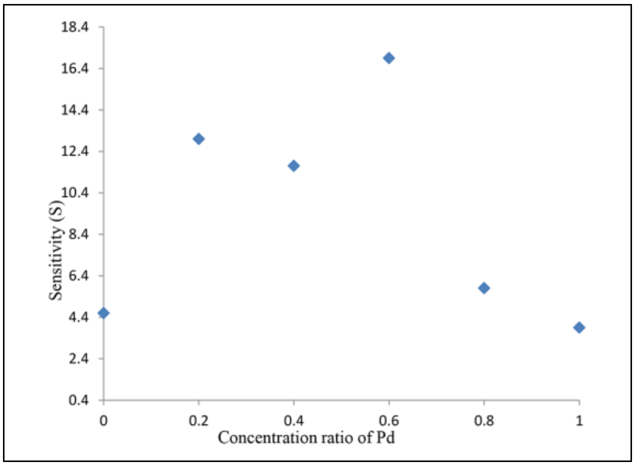

Figure 7. Sensitivity of PANI-Fe-Pd nanocomposite thin film with pathogenic *Leptospira* concentration: 10^8 CFU mL^{-1}. PANI: polyaniline.

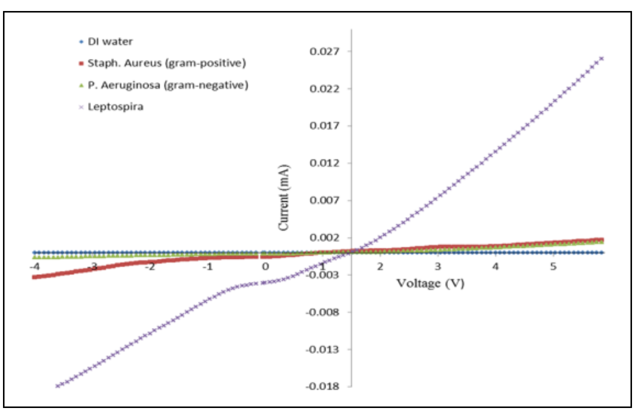

Figure 8. Selectivity test of PANI-Fe-Pd thin films for various concentrations (DI water, *Staphylococcus aureus* (gram-positive), *Pseudomonas aeruginosa* (gram-negative), and *Leptospira*). PANI: polyaniline.

Figure 7 shows the sensitivity of PANI-Fe-Pd nanocomposites thin films toward the *Leptospira* concentration. It has been found that PANI-$Fe_{0.4}$-$Pd_{0.6}$ nanocomposites thin film gave a higher value. The samples of PANI-$Fe_{0.4}$-$Pd_{0.6}$ performed the highest percentage of sensitivity of 16.9%, whereas PANI-$Fe_{0.0}$-$Co_{1.0}$ only performed 3.9% of sensitivity toward *Leptospira* bacteria.[24] These results show that the sensor can detect *Leptospira* for the concentration range of 10^8 CFU mL^{-1}. It is proven that high selectivity of the thin film is achieved by doping it with PANI metal alloys during the sensor preparation.

Selectivity test and error analysis

The selectivity test is used to determine other bacterial signals in comparison with *Leptospira*. In this test, we used both the *Pseudomonas aeruginosa* (gram-positive) bacteria and *Staphylococcus aureus* (gram negative) to see the tendency of thin films toward these bacteria.[25] Figure 8 shows the selectivity test of PANI-$Fe_{0.4}$-$Pd_{0.6}$ curve against DI water, *Leptospira*, *P. aeruginosa*, and *S. aureus* bacteria. The current of *P. aeruginosa* and *S. aureus* bacteria slightly increase in association with DI water but are lower under *Leptospira*. The *Leptospira* linear regression equations are obtained, expressed as $y = 0.0009x - 0.0016$ with a correlation coefficient of 0.9103. *P. aeruginosa* linear regression equations is expressed as $y = 0.0009x - 0.0016$ with a correlation coefficient of 0.9103, whereas the *S. aureus* linear regression equations is expressed as $y = 0.0009x - 0.0016$ with a correlation coefficient of 0.9103. These demonstrate the high ability of thin film to detect *Leptospira* bacteria in comparison to other bacteria.[26,27]

Figure 9 shows the error bar of current–voltage measurements and Table 1 tabulates the error bars value for cycles of I-V measurements. The sensors are regarded as having high linearity if the error bars value obtained is still small after recording the measurement five times (15 s intervals with a total of 75 s). The value of the error bar changes around 5% after several measurements. The testing protocols are used to demonstrate the stability of these sensors. The standard deviation for each sensor was estimated under zero condition after calibration. Based on the above results, the sensors' linearity response to the environment is very stable.[28,29] Sensor performance error

Table 1. Error bar of I-V measurements.

	Cycle of I-V measurements						
Concentration of PANI-Fe-Pd	15 s	30 s	45 s	60 s	75 s	Average	Standard deviations
Fe_1Pd_0	0.000928	0.001132	0.001175	0.001030	0.000984	0.001050	0.000102
$Fe_{0.8}Pd_{0.2}$	0.000796	0.001755	0.002285	0.002459	0.002460	0.001951	0.000707
$Fe_{0.6}Pd_{0.4}$	0.000901	0.001601	0.001726	0.001909	0.001868	0.001601	0.000410
$Fe_{0.4}Pd_{0.6}$	0.000894	0.000092	0.000481	0.000666	0.000763	0.000221	0.000675
$Fe_{0.2}Pd_{0.8}$	0.000874	0.000817	0.000749	0.000714	0.000626	0.000756	0.000095
Fe_0Pd_1	0.000173	0.000472	0.000625	0.000716	0.000739	0.000545	0.000232

I-V: current–voltage; PANI: polyaniline.

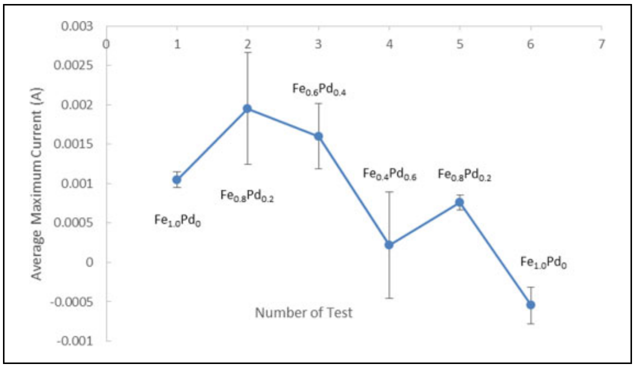

Figure 9. The error bars and the standard deviations ($n = 5$) of I-V measurement. I-V: current–voltage.

analysis shows that the film sensor for *Leptospira* has high stability.

Conclusion

In this study, we successfully synthesized and fabricated PANI-Fe-Pd nanocomposite thin films via the sol–gel method to be utilized as a *Leptospira* microbial sensor. The metal alloys are successfully incorporated into the PANI matrix to increase the sensitivity of the thin film surface, making it extremely effective for *Leptospira* detection. In addition, the AFM images show the roughness and grain size of the film that could bind the cell of microbes. Besides that, the in-depth images of FESEM show irregularity of nanoparticles on the thin film with the deposition of metal alloy, which is beneficial for electrochemical sensing. TEM result shows that metal alloy (Fe-Pd) is embedded in the polymer matrix. The I-V measurement can be used to evaluate the sensitivity of each pathogenic *Leptospira* concentration. The samples of PANI-$Fe_{0.4}$-$Pd_{0.6}$ gave the highest percentage of sensitivity where it about 16.9%. The lab-on-a-chip method is used to detect *Leptospira* bacteria because this method is very fast and highly sensitive. A lab-on-a-chip biosensor is a compatible platform for the portable detection of *Leptospira* bacteria.

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Declaration of conflicting interests

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References

1. Lehmann JS, Matthias MA, Vinetz JM, et al. Leptospiral pathogenomics. *Pathogens* 2014; 3(2): 280–308.
2. Thayaparan S, Robertson ID, Fairuz A, et al. Leptospirosis, an emerging zoonotic disease in Malaysia. *Malays J Pathol* 2013; 35(2): 123–132.
3. Villumsen S, Pedersen R, Borre MB, et al. Novel TaqMan ® PCR for detection of *Leptospira* species in urine and blood: pit-falls of in silico validation. *J Microbiol Method* 2012; 91(1): 184–190.
4. Chalayon P, Chanket P, Boonchawalit T, et al. Transactions of the royal society of tropical medicine and hygiene leptospirosis serodiagnosis by ELISA based on recombinant outer membrane protein. *Trans R Soc Trop Med Hyg* 2011; 105(5): 289–297.
5. Branch T. Isolation and identification of pathogenic and saprophytic *Leptospira* spp. from the rice fields of Tonekabon Township using culture and PCR technique. *Ann Biol Res* 2013; 4(11): 123–128.
6. Yasouri SR, Moghadam RG, and Ghane M. Identification of pathogenic and saprophytic *Leptospira* spp from the rice fields of Tonekabon Township using PCR technique. *Adv Stud Biol* 2013; 5(10): 437–445.
7. Dhand C, Dwivedi N, Mishra S, et al. Polyaniline-based biosensors. *Nanobiosens Dis Diagn* 2015; 2015(4): 25–46.
8. Mahapatra SS, Shekhar S, Thakur BK, et al. Synthesis and characterization of electrodeposited C-PANI-Pd-Ni composite electrocatalyst for methanol oxidation. *Int J Electrochem* 2014; 2014. Article ID 383892. DOI: 10.1155/2014/383892.
9. Abdullah H, Mohammad Naim N, Noor Azmy NA, et al. PANI-Ag-Cu nanocomposite thin films based impedimetric microbial sensor for detection of *E. coli* bacteria. *J Nanomater* 2014; 2014. DOI: 10.1155/2014/951640.
10. de Peres ML, Delucis RD, Amico SC, et al. Zinc oxide nanoparticles from microwave-assisted solvothermal process: photocatalytic performance and use for wood protection against xylophagous fungus. *Nanomater Nanotechnol* 2019; 9: 1847980419876201.
11. Solano R, Patiño-Ruiz D, and Herrera A. Preparation of modified paints with nano-structured additives and its potential applications. *Nanomater Nanotechnol* 2020; 10: 184798042090918.
12. Talib RA, Abdullah MJ, Mohammad SM, et al. Effect of substrate on the photodetection characteristics of ZnO-PANI composites. *ECS J Solid State Sci Technol* 2016; 5(80): 305–308.
13. Kim H-S, Lee S-H, Eun C-J, et al. Dispersion of chitosan nanoparticles stable over a wide pH range by adsorption of polyglycerol monostearate. *Nanomater Nanotechnol* 2020; 10: 1847980420917260.

14. Sowmiya G, Velraj G, and Shanmugapriya C. Investigation of enhanced A.C electrical conductivity of nano aluminium particles doped with polymer composite by in situ chemical oxidation polymerization method. *Adv Nat Appl Sci* 2017; 11(8): 168–172.
15. Elfalaky A, Mansur AF, and Abdel Maged F. Polyaniline-CdS nanocomposite synthesis, structural, thermal and spectroscopic analysis. *IOSR J Appl Phys (IOSR-JAP)* 2015; 7(3): 92–100.
16. Naim NM, Abdullah H, Umar UU, et al. Thermal annealing effect on structural, morphological, and sensor performance of PANI-Ag-Fe based electrochemical *E. coli* sensor for environmental monitoring. *Sci World J* 2015; 2015. DOI: 10.1155/2015/696521.
17. Ebrahimiasl S and Zakaria A. Electrochemical synthesis, characterization and gas sensing properties of hybrid Ppy/CS coated ZnO nanospheres. *Int J Electrochem Sci* 2016; 11(12): 9902–9916.
18. Belay A, Mekuria M, and Adam G. Incorporation of zinc oxide nanoparticles in cotton textiles for ultraviolet light protection and antibacterial activities. *Nanomater Nanotechnol* 2020; 10: 1847980420970052.
19. Maruthupandy M, Anand M, Maduraiveeran G, et al. Investigation on the electrical conductivity of ZnO nanoparticles-decorated bacterial nanowires. *Adv Nat Sci Nanosci Nanotechnol* 2016; 7(4): 045011.
20. Abbasian F, Ghafar-Zadeh E, and Magierowski S. Microbiological sensing technologies: a review. *Bioengineering (Basel)* 2018; 5(1): 20.
21. Maruthapandi M, Saravanan A, Luong JHT, et al. Antimicrobial properties of polyaniline and polypyrrole decorated with zinc-doped copper oxide microparticles. *Polymers* 2020; 12: 1286.
22. Li Y, Afrasiabi R, Fathi F, et al. Impedance based detection of pathogenic *E. coli* O157: H7 using a ferrocene-antimicrobial peptide modified biosensor. *Biosens Bioelectron* 2014; 58: 193–199.
23. Jurait J, Abdullah H, Yahya I, et al. Characterization of expeditious *Leptospira* bacteria detection using PANI–Fe–Ni nanocomposite thin film. *Polym Bull* 2020; 77: 3969–3987.
24. Amlil A, Koffi OEAB, Bile BEA, et al. Electrochemical biosensor for the immediate detection of bacteria. *Biosens Bioelectron* 2016; 7(1): 1–4.
25. Abu-ali H, Nabok A, Smith T, et al. Sensing and bio-sensing research development of electrochemical inhibition biosensor based on bacteria for detection of environmental pollutants. *Sens Bio-Sens Res* 2017; 13: 109–114.
26. Ashraf PM, Lalitha KV, and Edwin L. Chemical synthesis of polyaniline hybrid composite: a new and efficient sensor for the detection of total volatile basic nitrogen molecules. *Sens Actuators B Chem* 2015; 208: 369–378.
27. Akbari E, Buntat Z, Afroozeh A, et al. Escherichia coli bacteria detection by using graphene-based biosensor. *IET Nanobiotechnol* 2015; 9(5): 273–279.
28. Massoumi B, Fathalipour S, Massoudi A, et al. Ag/polyaniline nanocomposites: synthesize, characterization, and application to the detection of dopamine and tyrosine. *Appl Polym Sci* 2013; 130: 2780–2789.
29. Thakur B, Amarnath CA, Mangoli SH, et al. Polyaniline nanoparticle based colorimetric sensor for monitoring bacterial growth. *Sens Actuators B Chem* 2015; 207: 262–268.