Screen-Printed Carbon Electrode Fabrication Method for Electrochemical Biosensor Application

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Abstract. Screen-printed carbon electrode (SPCE) is one type of electrode commonly used for electrochemical biosensor application. It is fabricated using a screen-printing method, a method in which conductive ink is piled up layer-by-layer using some screen frames. SPCE combines three main electrodes used in the electrochemical sensing method of biosensors: working electrode (WE), counter electrode (CE), and reference electrode (RE). In this study, screen-printed electrodes were made using a simple and affordable screen-printing method on three varied substrate materials: polyethylene terephthalate (PET), polyvinyl chloride (PVC), and FR-4 epoxy of a printed circuit board (PCB). The conductive inks used are silver ink (Ag) for the conductive path, carbon ink (C) for the WE and CE, and silver/silver chloride ink (Ag/AgCl) for the RE. A dielectric ink was also used to isolate the connective pad between the conductive path and the electrodes. The electrochemical performances of the fabricated electrodes were characterized using cyclic voltammetry (CV) test in potassium ferricyanide solution. The tests resulted in characteristic duck-shaped cyclic voltammetry plots, which confirmed that the fabrication had been successfully conducted. The CV results were also compared among the SPCEs, based on the three substrate material variations used. The fabricated SPCE has the potential to be used for future biosensing application.

Keywords: Cyclic Voltammetry · Electrochemical Biosensor · Screen-Printed Carbon Electrodes · Screen-Printing Method

1 Introduction

In the scope of biosensors, there are many types of method used for the means of transducing biological events, one of which is electrochemical (EC) biosensor. Electrochemical biosensor is a type of biosensor that works based on the direct conversion of biological phenomenon to an electronic signal [1]. Nowadays, EC biosensor is quite popular because of its advantages, such as having a fast detection performance, high sensitivity, energy efficiency, and cost-effectiveness [2]. Electrochemical biosensor research

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that currently exists includes the detection of biochemicals like glucose [3], and also microbes such as viruses [4] and bacteria [5]. In terms of EC biosensor, its one of the most important component is electrode [6]. There are many types of electrodes that are often used, such as Glassy Carbon Electrode (GCE) [7], gold electrode (which is usually combined with other elements to strengthen detection) [8], silicon electrodes [9], and other kind of electrodes. These electrodes are commonly used as the standard sensing configuration in laboratory [10]. They are used as static devices stored on the laboratory bench which make them less portable [11].

Recently, a faster and instantaneous detection equipment is very expected. Thus, a sensing device must be able to be carried everywhere (portable), small, and supports flexible point-of care testing. Over the last few years, many other types of electrodes have been developed to overcome this deficiency. One of them is in the form of a small strip electrode, often called as Screen-Printed Electrode (SPE) [12]. SPE is a small electrode that is fabricated through screen-printing process [13]. The basic materials of SPE are various but generally carbon-based which further will be referred also as Screen Printed Carbon Electrode (SPCE) [14].

SPCE generally consist of several parts, namely base substrates, Working Electrode (WE), Reference Electrode (RE), Counter Electrode (CE), and insulator ink materials (dielectric) [15]. SPCE/SPE has been used in many fields of electrochemistry such as for bio and chemical sensor [16–18], energy storage and conversion [17], microelectronics [17], and also supercapacitors [19]. Several researchers have conducted SPE fabrication experiments in the past years by utilizing existing carbon ink [20–22].

In this study, we report a simple and comprehensive SPCE fabrication process. We varied the base substrates of the SPCE on three different materials: PCB, PET, and PVC; and then analyze the difference performances resulted. This study shows that the SPCE has been successfully fabricated using a manual screen-printed method and produces electrochemial biosensing characteristics.

2 Materials and Methods

2.1 Materials

The screen-printed electrode (SPE) fabricated in this study is common carbon-based SPE (also called Screen Printed Carbon Electrode/SPCE) with CERES YT-581 black carbon conductive ink used as the working and counter electrode (WE-CE), LEED-INK DT1201 silver conductive ink used as the conductive path, SANDOZ SAN-C325 silver/silver chloride conductive ink used as the reference electrode (RE), and SEL photoresist anti-etching blue ink used for dielectric. In addition, as the base substrates of the SPE, 0.3 mm polyethylene terephthalate (PET) sheet and 0.3 mm inkjet printing polyvinyl chloride (PVC) sheet were purchased from local stores, while FR-4 epoxy-based 0.6 mm single printed circuit board (PCB) sheet was purchased from JLCPCB. The customized screen-printing mesh was made of monyl/polyester with a mesh size of 300 microns, and its squeegee was used in the screen printing process. Both were purchased from local store. Other tools used were a 50 W ultraviolet lamp obtained from Yee Yang, M3 thinner/reducer agent, and tracing paper as screen-printing photomask film which were all obtained from local stores.

For electrochemical performance testing, measurements were conducted using a potentiostat from Zimmer Peacock and IORodeo. Potassium ferricyanide ($K_3[Fe(CN)_6]$) from Sigma Aldrich and Phosphate Buffered Saline (PBS) from Biogear were purchased as the analyte and electrolyte, respectively. Commercial SPCEs purchased from Zimmer Peacock were also used for comparison. All electrochemical tests in this paper were carried out using distilled water.

2.2 Methods

2.2.1 Fabrication

The fabrication of the Screen-Printed Carbon Electrode (SPCE) was conducted using a low-cost and simple method. SPCE patterns were drawn using computer design software as it can be seen in the Fig. 1-a. There are four layers of the SPCE: silver conductive path layer, carbon working and counter electrode (WE-CE) layer, silver/silver chloride reference electrode (RE) layer, and dielectric layer. Each layer was then multiplied and arranged separately which resulted in four layer patterns. These four patterns were then printed on the four different tracing paper-based screen-printing photomask transparent films (Fig. 1-b). The photomask films were used to prepare the customized screen-printing meshes. In this study, the preparation of the meshes was conducted by a third party (a local screen-printing mesh workshop). Briefly, the process of the mesh preparation began with covering a polyester-based mesh using a negative photoresist emulsion and then letting it dry in room temperature. The previously prepared photomask was then placed horizontally-mirrored (flipped-over) above the mesh. UV exposure was given to the mesh for a couple of minutes to polymerize the negative photoresist emulsion. The mesh was then sprayed with water to remove the unexposed part of the mesh that was covered by the pattern printed on the photomask. The result was a patterned mesh which can be seen in the Fig. 1-c. This procedure was done for each of the SPCE layers patterns. The customized mesh was then ready to be used to screen-print the electrode pattern onto the surface of the substrate.

The screen-printing process of the SPCE was done layer-by-layer with the lowermost layer is Ag conductive path pattern, followed by carbon WE-CE pattern, Ag/AgCl RE pattern, and photoresist ink-based dielectric pattern on top (Fig. 1-h). To begin screen-printing, the patterned mesh should be flipped-over beforehand (Fig. 1-d). The PCB/PET/PVC substrate was then placed under the flipped-over mesh (Fig. 1-e). Conductive ink was applied on the surface of the mesh and then squeegee was pressed and passed along the mesh to print the conductive ink onto the surface of the substrate (Fig. 1-f). This was done for around 5 times or until all of the electrode patterns had been covered with the ink. The conductive ink can penetrate through the uncovered part of the mesh which has the pattern of the SPCE layer. Which patterned mesh and conductive ink used is based on which layer of the SPCE is being screen-printed. After a pattern was succesfully printed on the substrate, the substrate was removed from the mesh (Fig. 1-g). The mesh was then cleaned from the residual ink using M3 reducer.

Meanwhile, the printed ink on the substrate was then dried using a specific method based on the type of the ink and the substrate. For carbon ink, heating using hotplate was conducted at 60 °C for 60 min (for PET and PVC substrate) or at 120 °C for 30 min

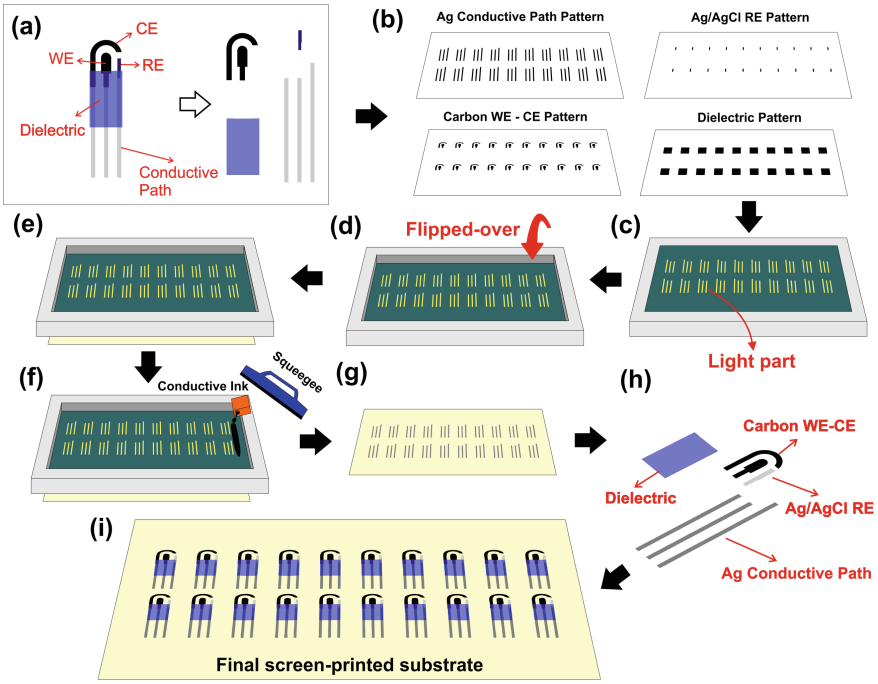

Fig. 1. The graphical step-by-step process of SPCE fabrication: (a) The electrode patterns are drawn using computer design software, each layer of the patterns are then separated and arranged (there are 4 layers); (b) Each layer of the electrode patterns is printed on different screen-printing photomask transparent film using printer; (c) One of the customized screen-printing meshes ready to be used, the electrode pattern on the mesh (the light part) is not covered by photoresist ink, thus conductive ink can be penetrated through this part in the screen-printing proccess; (d) The patterned screen-printing mesh is flipped-over before used for electrode fabrication; (e) PCB/PET/PVC susbtrate is placed under the flipped-over mesh to start screen-printing the electrode pattern on the surface of the substrate; (f) Specific conductive ink is dropped onto the mesh for specific electrode layer pattern, squeegee is then used to print the ink onto the surface of the substrate through the uncovered electrode pattern part of the mesh (light part); (g) The screen-printed substrate is then removed from the mesh and dried using method that is specific for the ink and the substrate type; (h) The other electrode layers are then screen-printed on top of the previous pattern and then dried, with the lowermost layer is Ag conductive path pattern, followed by Carbon WE-CE pattern, Ag/AgCl RE pattern, and dielectric pattern; (i) The final result of the fabricated screen-printed carbon electrodes (SPCEs).

(for PCB substrate). For silver and silver/silver chloride ink, heating using hotplate was conducted at 60 °C for 60 min (for PET and PVC substrate) or at 130 °C for 30 min (for PCB substrate). While, for photoresist blue ink, 50W UV lamp exposure was used to dry the ink for 60 min (for all types of substrates).

After all layers of the SPCEs had been screen-printed on the substrate, the resulting SPCEs would be like the ones displayed in the Fig. 1-i. For each time of fabrication, up

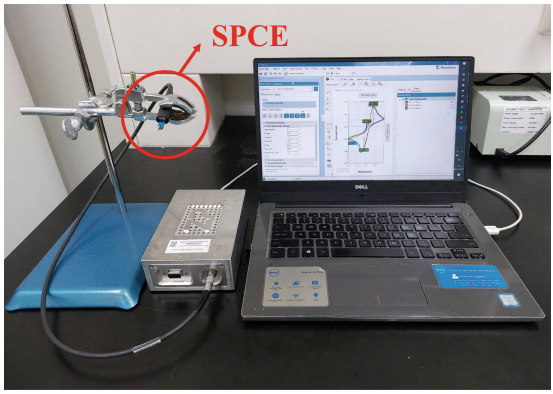

Fig. 2. Electrochemical assays arrengement of the screen-printed electrodes characterization using portable potentiostat and a personal computer.

to 20 SPCEs could be printed on the substrate. This number actually depends on the size of the SPCE, the substrate, and the mesh used.

2.2.2 Electrochemical Characterization

The electrochemical performance of the fabricated SPCE was tested and compared with the commercials. Cyclic Voltammetry (CV) measurement was conducted using $K_3[Fe(CN)_6]$ as a conventional redox probe. The expected result is a duck-shaped current vs potential curve with noticeable oxidation and reduction peak. The detection analyte was prepared using 30 mM $K_3[Fe(CN)_6]$ in 10 mM PBS. A 50 μL of the analyte solution was dropped on top of the electrode area. Subsequently, the electrical potential was cycled from − 1.0 V to 1.0 V *vs* Ag/AgCl with a scan rate of 50 mV/s controlled using a portable potentiostat. In general, the configuration for electrochemical measurements is depicted in Fig. 2.

3 Results and Discussion

3.1 Fabrication Results

The fabrication results of the SPCEs varied among the substrates used. Different factors such as the temperature and duration of drying (depending on the type of substrate), the surface properties of the substrate, as well as the homogeneity of the conductive ink used, play a role in producing these varying results. One batch of fabrication process could take about 3 h for PCB substrates and 4 h for PET and PVC substrates. This is due to the waiting time for the drying process of the inks. Therefore, usually up to three batches of fabrication are carried out in parallel in one day of fabrication to save time.

3.1.1 PCB (FR-4 Epoxy) Substrate

The results of the PCB (FR-4 Epoxy)-based SPCEs can be seen in the Fig. 3. The silver conductive paths of the SPCEs were well-printed on the PCB, while the carbon working

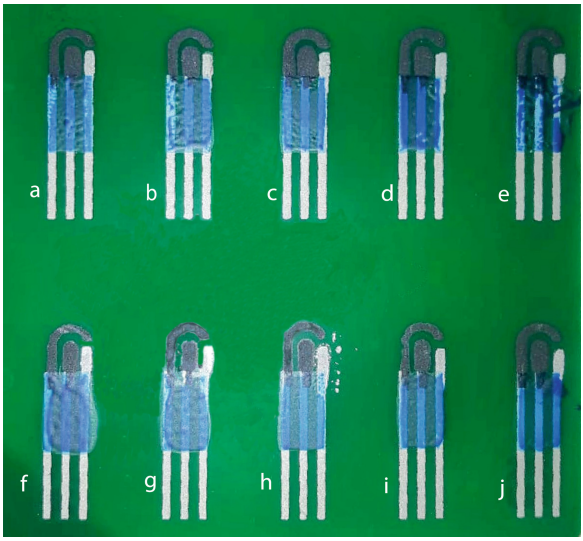

Fig. 3. The fabrication result of PCB-based SPCEs.

and counter electrodes were not printed as homogen as the silver paths. It can be seen that some carbon electrodes are thicker than some others (SPCEs a–e in the Fig. 3 have thicker carbon electrodes than SPCEs f–j), possibly due to the uneven pressure applied when the squeegee was used to spread the ink on the mesh during screen-printing process. This could also be due to the inhomogeneous pattern of the electrodes on the screen-printing mesh. It can also be seen that the Ag/AgCl ink and the dielectric ink were not printed neatly, especially for SPCEs b, e, f, g, h, j (Fig. 3). Both inks have a more fluid structure, making them easier to seep/penetrate through the porous of mesh. The excessive force applied on the ink during the screen printing process on the substrate, resulted in some of the ink leaking out of the mesh pattern. Nevertheless, the conductive inks printed on PCB substrate were given the most suitable drying treatment based on the datasheet. Carbon ink was dried using hotplate at 120 °C for 30 min, while silver and silver/silver chloride ink were heated at 130 °C for 30 min. These should have resulted in the best conductive performances of the electrodes.

In one batch of SPCEs fabrication on PCB substrate (10–14 SPCEs), the percentage number of succesful fabrication physically was around 60–70%. The electrodes in the middle of the substrate usually appears to be physically failing. This is because the conductive ink is applied and screen-printed from the edges of the pattern back and forth, thus it becomes thinner when it reaches the middle of the substrate.

3.1.2 PET Substrate

The results of the PET-based SPCEs can be seen in Fig. 4. From the figure, it can be seen that the electrodes fabrication results on the PET substrate are the best physically. Each layer of the electrodes is imprinted homogeneously and neatly. There is no significant difference between one SPCE result with the others. There are also no Ag/AgCl and

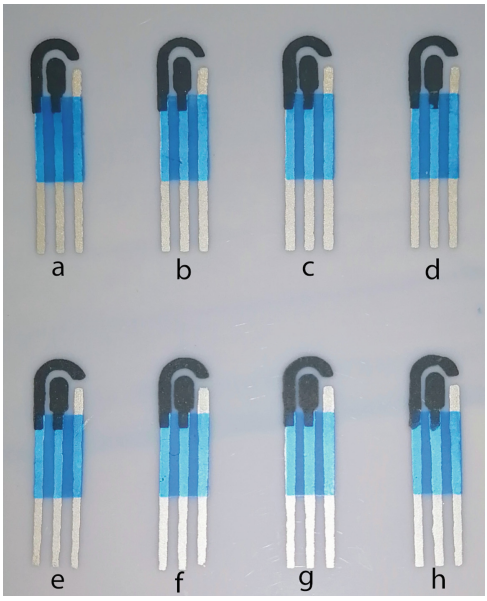

Fig. 4. The fabrication result of PET-based SPCEs.

dielectric ink that were printed outside of the patterns. These good results probably due to a much more even pressure that were given during the screen-printing process. However, the conductive inks printed on this substrate could not be given the proper temperature and duration drying parameter based on the ink data sheet. Carbon ink was heated at 60 °C for 60 min, while silver and silver/silver chloride ink were heated 60 °C for 60 min. The reason is that the PET substrate cannot withstand a high temperature treatment above 60 °C. These could result in suboptimal conductive performances of the electrodes. The percentage number of succesful fabrication on PET substrate physically is around 80–90% from 8–14 SPCEs in a single batch of fabrication. In the Fig. 4, it can be seen that the electrodes in the middle of the substrate still have a very good form. This is because the fabrication process involved only 8 electrodes, thus the conductive inks could be screen-printed evenly to the entire surface of the substrate.

3.1.3 PVC Substrate

The results of the PVC-based SPCEs can be seen in the Fig. 5 a to h. The SPCE fabrication results on the PVC substrate are the worst physically. All of the fabricated SPCEs have defect either on the conductive path or on the electrodes. Defects on the carbon electrodes can be seen clearly in the SPCEs b to h. A small defect on silver conductive path can be seen in the SPCE a. The conductive path become thinner in the middle of the path. Due to the same reasoning, the drying parameters used for PVC substrate were the same as for PET substrates, thus suboptimal conductive performances of the electrodes are also to be expected. The percentage number of succesful fabrication on PVC substrate physically can be said to be less than 10%. The reason for these bad results are probably

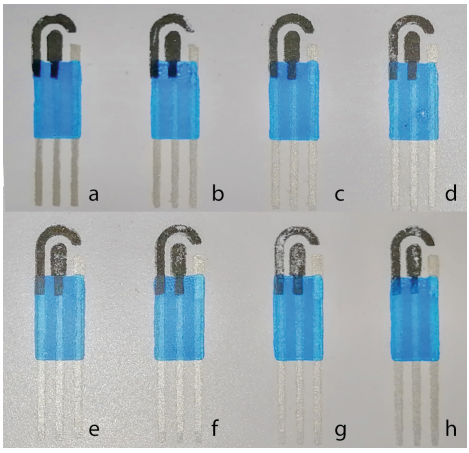

Fig. 5. The fabrication result of PVC-based SPCEs.

due to the surface properties of the PVC sheet used in this fabrication that are not suitable to the conductive inks. It makes the inks could not be spread and adhered evenly onto the surface of the PVC, thus resulted in defects.

3.2 Electrochemical Characterization

The fabricated SPCEs were characterized using cyclic voltammetry (CV) method to test their electrochemical biosensing performance. Commercial SPCEs obtained from Zimmer Peacock were also tested using the exact same parameters to be the benchmark for the fabricated SPCEs. The CV tests were done for each of the SPCE types using 30 mM $K_3[Fe(CN)_6]$ in 10 mM PBS. $K_3[Fe(CN)_6]$ is a common analyte used to characterize the electrochemical performances of biosensor electrodes. Some other studies had also used this analyte [23, 24]. There were two kinds of tests conducted in this session, namely CV measurement to perform redox peak characterization and robustness test by 20 continuous cycles.

The first CV test was conducted using Zimmer Peacock potentiostat. The results can be seen in Fig. 6. Four different kinds of SPCEs were tested, namely the commercial Zimmer Peacock (ZP) SPCE, PCB-based SPCE, PET-based SPCE, and PVC-based SPCE. PCB-based SPCE has the highest oxidation peak current value of 202 μA, followed by ZP SPCE (131 μA), and PET-based SPCE (121 μA). As for PVC-based SPCE, no redox peaks were detected at all. This result shows that the fabricated SPCE, except the PVC substrate SPCE, was successfully developed to probe redox reaction with the ZP SPCE as the reference. The high measured current is indicating a good electron transfer rate between electrode and analyte [25].

PCB-based SPCE has the highest peak among the two other fabricated electrodes. This result is most likely due to the optimal drying parameters that could be given for PCB-based SPCE, thus an optimal electrochemical performance result can be obtained. While the reason for PCB-based SPCE having a higher peak than commercial ZP SPCE is probably due to the larger area of its working electrode. PVC-based SPCE could not

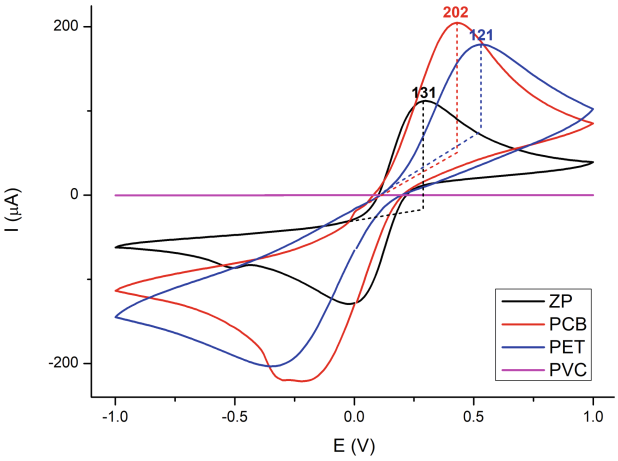

Fig. 6. The cyclic voltammogram of 4 different electrodes using 30 mM $K_3[Fe(CN)_6]$ in 10 mM PBS with the oxidation peak current value following each graph.

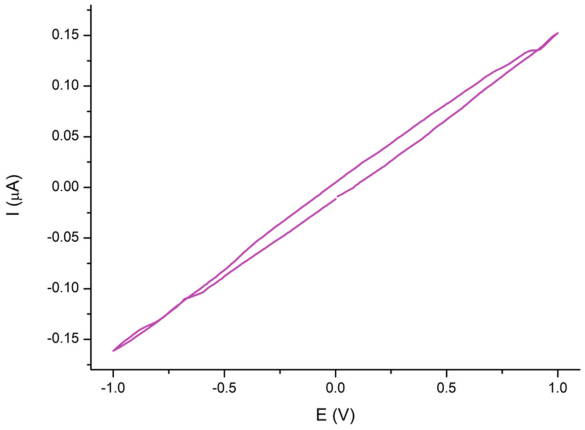

Fig. 7. The cyclic voltammogram of PVC substrate electrodes using 30 mM $K_3[Fe(CN)_6]$ in 10 mM PBS which resulted in no redox peak.

result in any peak, as further clarified in Fig. 7. This is due to the previously discussed electrodes defects effect shown in Fig. 4. These defects cause electricity cannot flow smoothly on the electrodes so that the electrodes cannot work properly.

Figure 8 shows the results of the robustness test of the SPCEs by 20 continuous cycles of CV. In this test, only PET and PCB-based SPCE that were tested. It can be seen in Fig. 8-b that PET-based SPCE could handle the test well by outputting a stable current throughout the 20 cycles of CV with no signs of current signal distortion, or in other words no indication of electrode defects. PCB-based SPCE was also tested to be robust enough to conduct the continuous CV test as it is seen in Fig. 8-c. These robustness test results open the potential of the SPCEs to be modified with other materials as

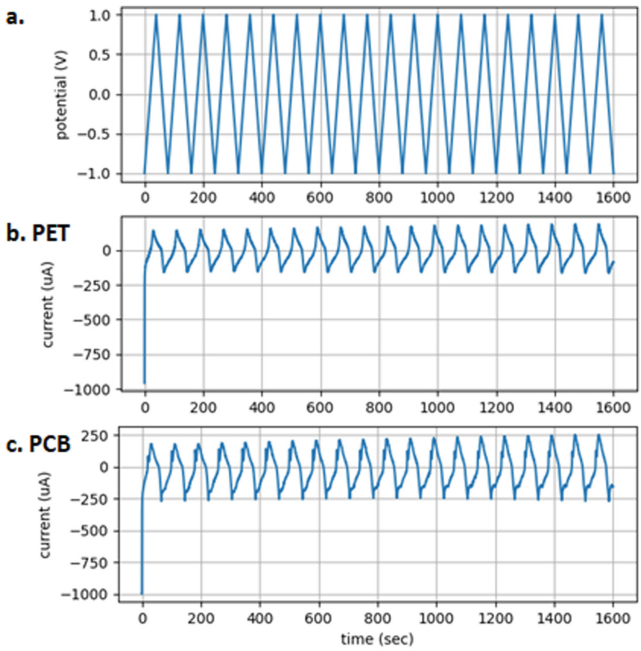

Fig. 8. (a) The potential cycling profile for 20 cycles in cyclic voltammetry measurement, followed by the measured current of PET substrate (b) and PCB substrate (c) based electrodes using 30 mM $K_3[Fe(CN)_6]$ in 10 mM PBS.

signal enhancement through the electrodeposition technique. This type of modification method generally requires a long duration of continuous potentials cycling/scanning of the electrodes [26, 27].

3.3 Recap and Future Potential for Biological Detection Application

Low-cost and home-made SPCEs have been succesfully fabricated in this study using a simple screen-printing method. The SPCEs are made on three varied substrates, namely PCB (FR-4 Epoxy), PET, and PVC. All of the fabricated electrodes and also a commercial SPCE used as comparison were tested using cylic voltammetry. The resulting characteristic duck-shaped cyclic voltammetry plots are displayed with the oxidation peaks of the electrodes are also recorded, especially for the SPCEs fabricated using PCB and PET substrates. The robustness of the SPCEs (PCB and PET-based) were also tested by performing a 20 cycles of CV. The results indicate a stable current output along the 20 cycles, thus a potential application of the SPCE for biosensing application is available. The robustness test result also indicates that in the future the SPCE surface can also be modified using other materials or nanomaterials via electrodeposition technique to not just enhance the electrochemical performance of the electrodes but also to be used as nonenzymatic biosensor.

In this study, the biological detection application of the fabricated SPCEs has not been conducted yet. However, based on the detection performance of the electrodes, such

applications are within reach, therefore some future studies will be conducted. There have been many studies which were succesfully used SPCEs as the electrodes for biological detection analysis. One example is a study by Sorouri et al. [24] which fabricated an immunosensor to detect Botulinum Neurotoxin Serotype A using SPCE modified with gold nanodendrites, chitosan nanoparticles, and BoNT/A polyclonal antibody. Another study show a simpler biosensing application which utilizes glucose oxidase enzyme and gold nanodendrites modified on SPCE's working electrode surface to detect glucose sample [28].

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Authors' Contributions. EA and IAPN performed the fabrication and characterization; EA, IAPN, RRA, and IA analysed and interpreted the data; EA, IAPN, and RRA wrote the paper; IA conceptualized, designed, and supervised the work. All authors read and approved the final manuscript.

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