Leakage-Free Nucleic Acid Biochip Featuring Bioinert Photocurable Inhibitor

NORSIH RIZAL ALI, MOTASEM H. GHANIM, MOHD ZAID ABDULLAH, AND MOHAMAD ADZHAR MD ZAWAWI
School of Electrical and Electronic Engineering, Universiti Sains Malaysia, Engineering Campus, Nibong Tebal, Penang 14300, Malaysia
Corresponding author: Mohamad Adzhar Md Zawawi (adzhar@usm.my)

This work was supported by Universiti Sains Malaysia under Grant RUI 1001/PELECT/8014048 and Grant 304/PELECT/807007.

ABSTRACT A leakage-free and disposable biochip for deoxyribonucleic acid (DNA) separation and detection is developed in this study. The biochip comprises of 50 mm long polydimethylsiloxane (PDMS) microchannel and copper electrodes engraved on flame retardant (FR-4) printed circuit board (PCB) substrate. An inhibitor made from photocurable diacrylate bisphenol-A polymer (DABA) was used to establish a permanent bonding between PDMS and PCB substrates. Pull-off test experiments resulted in average strength of 287.4 kPa and a standard deviation of ±23.8 kPa. These results are comparable to recent studies on leak-free microfluidic applications. Meanwhile, the leakage test showed that the biochip could withstand a pressure of more than 190 kPa, which is sufficiently high for most DNA measurements. Finally, experiments performed on two different DNA samples indicated that the proposed design could accurately detect DNA fragments with current sensitivity higher than 100 nA, under electric field strength of 20 V/cm. The new design effectively seals the biochip, thus preventing leakage of liquid from the sensor matrix. Together with its biologically and electrochemically inert characteristic, it opens up possibilities in building a truly portable biosensing device.

INDEX TERMS Amperometric, biocompatible, biosensor, circuit-board, copper, disposable, DNA, bioinert, irreversible bonding, leakage, lab on PCB, microfluidic, nucleic acid, PCB, PDMS, polymer.

I. INTRODUCTION
Recent outbreaks of respiratory infections in humans, including MERS-CoV, SARS-CoV-1, and SARS-CoV-2 (COVID-19) viruses, emphasize the importance of rapid process, point-of-care technology (POCT) diagnostic tools that can be deployed in the community. The circuit-board and polymer-based microfluidic devices are the emerging diagnostic tools that offer portable, easy miniaturization, biocompatible and cost-effective materials [1]–[3]. Another significant advantage of the construction is the range of materials that can be utilized to build a leak-free diagnostic tool [1].

Leakage is a common issue in constructing any microfluidic device, where tight sealing is required to manipulate liquid into microfluidic networks properly. Leakless sealing is critical when designing a device with a multi-layer structure, whether utilizing the same or different materials. Hence, bonding can be classified either directly or indirectly, where indirect bonding involves the assistance of an intermediate material like an adhesive. Direct bonding, which includes thermal and solvent bonding, requires high temperature and pressure, implemented primarily for plastic–plastic devices. Each approach has its benefits and limitations, such as some bonding methods may deform the microchannel, alter its dimensions and cause clogging. On the other hand, indirect bonding employed multiple strategies to avoid channel clogging by sealing the two incompatible surfaces with an intermediate substance.

Due to its unique properties, PDMS is a favoured material for fabricating microfluidic devices, offering various advantages over silicon and glass, including flexibility, ease of moulding with soft-lithography, biocompatibility, and optical transparency [4]. The most popular PDMS bonding is the oxygen plasma-treated PDMS to Si-containing substrates, such as glass [5], [6] or PDMS itself [7]. Other PDMS bonding techniques include corona discharge [8], partial curing, cross-linker variation [9] and uncured PDMS adhesive [10].
Alternative materials such as PCB, either rigid or flexible plastic, are gaining popularity because of their high integration prospective, process maturity, biocompatibility, and potential for mass production commercialization [11, 12]. Lab on PCB (LOP) is the future of microfluidics; its globally available equipment and techniques enable the production of inexpensive disposable devices. Among the recent works on PDMS-PCB bonding are; half-cured PDMS technique on rigid PCB for construction of continuous-flow PCR [13], polyimide to PDMS bonding with thin-film SiO$_2$ [14], and surface functionalized PDMS bonding to several plastic substrates [15], [16]. The former device, the continuous-flow PCR [13], used PDMS as a planarization layer to encase the electronics components on the printed circuit board, whereas the latter three are the other indirect bonding methods for flexible plastic PCB-PDMs pair substrates.

Amperometric-based nucleic acid-sensing is among the prevalent method in the research community [17]–[19]. The sensor can be fabricated from two-layer substrates. They are (i) the polydimethylsiloxane (PDMS) substrate through which the microchannel is engraved [20], [21], and (ii) the printed circuit board (PCB) on which the electrodes are etched [22].

One benefit of this structure is that copper electrodes can be manufactured using any conventional PCB processing, where the material has proven outstanding in DNA sensing [22]. Additionally, copper is also inexpensive and can be made disposable. Recently, several copper-based electrodes were used in amperometric sensings, such as; determination of ampicillin using a copper electrode [23], determination of glucose utilizing carbon nanodots (C-dots) and copper oxide (CuO) nanocomposites (CuO-C-dots) [24], detection of nitrate ions in water based on copper-silver bimetallic nanostructures deposited on pencil graphite substrate [25], and hierarchical CuO nanostructure as non-enzymatic glucose sensor [26].

The advantages of these LOP-based sensors include their simplicity, high accuracy, and performance, as well as the relative ease and convenience with which they can be installed in a single cell [27]–[29]. Their primary disadvantage is that most of these devices are prone to leakage, particularly at high pressures [30]–[33]. Even though the DNA fluid can quickly be introduced into a microchannel by injection, it can leak from the matrix through small gaps at the PDMS-PCB interface [34]–[36].

Our previous design partially resolved this issue by temporarily attaching these two substrates with metal clips [22]. Despite their ruggedness and bulky design, the clips were helpful because they enabled us to evaluate the performance of the biochip. However, irreversible bonding is preferred in order to create a portable, leak-proof device.

Uneven PCB surface introduces gaps, mainly due to its rough surface and the thickness of the electrodes following etching. To solve this issue, this design requires a smooth planarization layer on PCB which can tightly seal with PDMS substrate. One significant advantage of a photocurable polymer is its ability to precisely cured with accurate dimensions. Additionally, the dry bonding can fully eliminates microchannel distortion and clogging [1]. We developed a novel technique for permanently attaching PCB to PDMS to address this issue, thereby preventing microfluidic spillage from the sensor. The new bonding is not only biologically [37]–[39] and electrochemically [40]–[42] inert but also exhibits good mechanical properties. This article discusses the fabrication techniques for this biochip as well as its performance.

II. MATERIALS AND METHODS
A. CHEMICALS AND MATERIALS
Negative Resist NR21-20000P (Futurrex, Inc.) was used in photolithographic processing. Meanwhile, Sylgard 184 (Dow Corning Corp.) was employed in preparing the PDMS microchannels. The chemicals used in the fabrication were all prepared based on absolute ethanol 99.8%. This solvent was proven to be an effective developer [43] and was used for preparing the Modified Mould Release Agent (MMRA) [44]. It was also used to prepare the ethanolic solution with (3-aminopropyl) trimethoxysilane, 97% (APTM), and the acetic acid required for bonding. Bonding material comprised of a mixture from diacrylate ester of bisphenol A epoxy resin (Ebecryl®600), 1,6-Hexanediol Diacrylate (HDDA) and 2-Hydroxy-2-methyl-1-phenyl propaneon (ADDITOL®HDMAP) [43].

Cyclic voltammetry (CV) experiments were performed in this work using deoxyguanosine triphosphate (dGTP) samples prepared from a dNTP set (1st BASE dNTP Set, First BASE Laboratories Sdn. Bhd.) and a buffer solution of EDTA. Meanwhile, DNA separation experiments were conducted using two different DNA samples. The first was a single-band DNA generated via polymerase chain reaction (PCR), and the second was a multi-band commercial DNA marker. The separation medium was agarose gel (1st BASE Agarose Biotechnology Laboratories Sdn. Bhd.), and the buffer was EDTA.

B. FABRICATION OF SUBSTRATES
Fig. 1 (a) illustrates the procedures for preparing the microchannel from PDMS, while Fig. 1 (b) illustrates the process of engraving Cu electrodes on FR-4 PCB. Mould for fabricating the PDMS microchannel was made from microscope glass slides. Referring to Fig. 1 (a), the glass was cleaned in turn using acetone, methanol and isopropanol solutions.

The procedures for preparing the microchannel from PDMS are illustrated in Fig. 1 (a), while the engraving of Cu electrodes on FR-4 PCB is illustrated in Fig. 1 (b). The PDMS microchannel was fabricated using microscope glass slides as the mould. The glass was cleaned in sequence using acetone, methanol, and isopropanol solutions before the procedures shown in Fig. 1 (a). It was then rinsed with de-ionized water to remove any remaining alcoholic residue on its surface. Subsequently, the glass was dried using nitrogen gas before spin coating.

Next, the glass was coated with photoresist NR21-20000P using the spin coater (Laurell Technologies Corp.) running
at 800 rpm for 10 s. It was then baked for 600 seconds on a hotplate at 80°C, followed by another 300 seconds of soft baking at 150°C. Patterning was achieved by exposing the mask to UV light at 220 W for 310 s (Light source unit: UVE-251S, Irradiation unit: EL-100, San-Ei Electric Co. Ltd, Japan). The glass was subjected to post-exposure baking by treating at 80°C for 600 s and then dipping into a resist developer. Then, it was immersed for 30 s in MMRA solution to aid in detaching the cured PDMS microchannel from the glass [44]. The mould was immediately dried with nitrogen spray following this step. The PDMS pre-polymer mixture was then poured into the mould and degassed for 20 minutes at -0.1 MPa to remove air bubbles. Hence, the bubble-free PDMS was cured in an oven at 65°C for 1 h. The cured PDMS was peeled off from the mould. Finally, two reservoirs were made at each end of the microchannel using a 3 mm circular puncher.

C. PCB-PDMS BONDING
Bonding between the PCB electrodes and the PDMS microchannel is the final step in the fabrication. The method published in [43], [45] was modified adaptively to suit a different application and improve its bonding performance. It utilizes a photocurable diacylate bisphenol-A polymer as an intermediate or inhibitor layer (DABA).

DABA inhibitor was prepared by mixing the oligomer Ebecryl®600 (Allnex) with the monomer (1,6-Hexanediol Diacylate (HDDA)) and photoinitiator (ADDITOL® HDMAP) like previously proposed in [43]. The overall procedure for establishing the PDMS-DABA-PCB bonding is schematically illustrated in Fig. 2.

The steps began with cleaning the PCB with acetone, followed by isopropanol, and finally with de-ionized water. The chlorides to pattern the electrodes on its substrate. Finally, the PCB was precisely cut to 35 mm × 70 mm dimensions using a CNC machine.

Electrodes were patterned on PCB with semi-automatic procedures as described in Fig. 1 (b). The process began with PCB cleaning with a washer machine (Bungard) to remove the copper oxide layer formed on the upper copper-clad of the prefabricated FR-4 material. The cleaned PCB was then laminated with photopolymer dry film resist (photoresist layer) using a laminating machine. Next, the mask was exposed to UV and consequently developed through a conveyorized photoresist machine. The PCB was then etched with ferric chloride to pattern the electrodes on its substrate. Finally, the PCB was precisely cut to 35 mm × 70 mm dimensions with a CNC machine.
cleaned PCB was dried by blowing with nitrogen gas. Next, it was coated with DABA photo pre-polymer liquid using a brush, ensuring a uniformly spread layer with the same thickness. Subsequently, a piece of PET/PVC film was laid on top of the DABA surface. Then, using a smooth surface utensil, the film’s top surface was pressed firmly outward from the middle area to remove bubbles formed at the middle of the PCB.

After aligning the mask, the DABA coated PCB substrate was first exposed to 180 W UV light for 10 s (Light source unit: UVE-251S Irradiation unit: EL-100, San-Ei Electric Co. Ltd, Japan). Then the unit was dipped into an ethanol solution, forming a thin cured DABA layer. The PDMS microchannel was then bonded onto the PCB via the DABA layer, as illustrated in the last step of Fig. 2.

One issue that arises during the bonding procedures described in Fig. 2 is displacement caused by PDMS shrinkage. It is well-known that when PDMS is cured at a high temperature, the monomer becomes cross-linked, and the total volume decreases. In this case, the shrinkage ratio increases linearly with increasing curing temperature [46]–[48]. As a result, misalignment occurs when shrunken PDMS is assembled with other rigid structures, such as a printed circuit board. One solution is to pre-shrink the PDMS by heating it above the bonding temperature. Because the assembly is carried out at a much lower temperature, additional shrinkage of the PDMS is avoided. The PDMS was heated to 150°C in this experiment, which is a sufficiently high temperature for the applications discussed in this paper.

Before bonding, the PDMS was firstly cleaned thoroughly with ethanol and distilled water to remove any debris, especially dust that might prevent covalent bonding. After blow-drying with nitrogen, the PDMS surface was oxidized in oxygen plasma at 600 W for 20 s (Plasma Preen 11-862, Plasmatic System Inc.). Then it was dipped into an ethanolic solution containing 5% 3-aminopropyl trimethoxysilane (APTMS) and 0.1% acetic acid for 1 h. Next, the DABA and PCB surfaces were again cleaned with ethanol and blow-drying with nitrogen. The silanized PDMS was then brought into contact with the DABA surface, and the microchannel was adjusted to ensure that it was precisely aligned with the copper electrodes.

Finally, the resulting device was placed in an oven at 80°C for 1 h, allowing the covalent reaction. Strong paper clips were used to ensure that both PDMS and DABA surfaces were in close contact during the chemical reaction.

III. EXPERIMENTAL
A. BONDING CHARACTERISTICS
Experiments were conducted to evaluate the biochip’s performance objectively. The bonding properties were initially investigated, followed by DNA experiments. In the first investigation, two different bonding tests were performed. They are (i) the pull-off test and (ii) the leakage test, as illustrated in Fig. 3(a) and Fig. 3(b), respectively.

Referring to Fig. 3(a), the pull-off test was performed using a universal testing machine (Instron, model: 3367) by applying two forces in opposite directions and perpendicular to the PDMS-DABA-PCB structure. The forces were applied continuously at a pull speed rate of 0.02 mm/s until the FR-4 on either side of the substrate is wholly detached from the PDMS. The structure, which measures 20 mm x 20 mm, was bonded in the same manner as previously described. The sensing area on the biochip’s PCB was randomly chosen to contain copper-clad and PCB composite to simulate the actual critical zone on the biochip.

Further, the biochip’s adhesive strength or liquid tightness was assessed employing a leakage test, as shown schematically in Fig. 3(b). As illustrated in this figure, this test was conducted by applying a pressure ramp to the biochip’s reservoirs filled with a dyed solution while observing for any indication of leakage within the device. A fluid pump (Cole-Parmer Instrument Company, Masterflex Console Drive 77390-00) was used to generate the required pressure. As shown in the figure, the output duct of the pump
was connected to one end of the reservoir while the other end was connected to a digital pressure indicator (Druck, DPI 705) via a control valve. A mechanical valve was used to remove air from tubes connected to reservoirs, and the fluid pump was set at a flow rate of 4 ml/min.

**B. DNA SEPARATION AND DETECTION**

Before performing DNA experiments, cyclic voltammetry (CV) was used to determine the optimal redox potential. Three distinct solutions were developed and tested in this manner. CV experiments were performed initially with only EDTA buffer and then with DNA-EDTA mixtures of two different concentrations. In this case, the mixtures were created by mixing dGTP at 100 and 200 mmol/litre concentrations with EDTA buffer. The experiments were carried out with a Metrohm Autolab Type III potentiostat (Metrohm Autolab B.V.) programmed to acquire data at an 80 mV/s scan rate. In those experiments, a three-electrode system was used where: (a) copper wire as the working electrode (WE), (b) Ag/AgCl electrode (a silver wire soaked in silver chloride solution) as the reference electrode (RE), and (c) platinum wire as the counter electrode (CE).

**FIGURE 4.** Standard gel electrophoretic bands of two DNA samples, (a) single DNA fragment and (b) 1 kbp GeneDirex DNA ladders.

The biochip’s separation and detection performance were evaluated using two different DNA samples. The first sample was a single DNA band of 1100 bp extracted from PCR. Meanwhile, the second sample was a 1 kbp DNA ladder manufactured by GeneDirex [49]. The DNA ladder is a 13-band marker with fragment sizes ranging from 250-10,000 base pairs (bp). Fig. 4 shows the electrophoretic bands of these two DNA samples produced using the standard gel electrophoresis. The number printed on the left in each lane of Fig. 4(b) corresponds to each bp size of DNA fragments.

Before running the DNA separation and detection experiments, the biochip was further treated to clean and prepare for agarose gel injection [50]. A high voltage power supply (Bertan Series 230) generated the essential electrical field strength. The electric field of 20 V/cm, corresponding to 100 V separation voltage, was used with an agarose gel percentage of 1.0%. The reference voltage was set to the best reduction potential obtained from CV experiments described previously. This voltage allows redox currents resulting from a chemical reaction between DNA nucleotides and electrodes to be detected with maximum sensitivity. The DNA samples were injected into the sample reservoir using a micropipette.

**IV. RESULTS AND DISCUSSIONS**

**A. BONDING CHARACTERISTICS**

An example of a successfully bonded biochip fabricated using the procedures described previously is shown in Fig. 5. In this design, the biochip contains multiple detection units. Each unit is composed of a working electrode (WE), a reference electrode (RE), and a counter electrode (CE).

**FIGURE 5.** Example of a DNA biochip after bonding. The biochip’s components consist of a microchannel, sample tanks, and biosensing electrodes. The blue-marked region, showing the detection unit, while the red-marked regions are the spare electrodes.

Fig. 6 shows the dimensions of the electrodes of the resulting biochip measured using Zeiss LSM 700 MAT with Axio Imager Z2 Vario confocal laser microscope. Referring to Fig. 6(a), the average width of WE and RE is 273.9 µm. This dimension meets the design specification within some tolerance limit. Meanwhile, the width of decoupler and CE electrodes averaged at 1057.7 µm as evident from Fig. 6(b). This size is also within the design specification with some experimental errors.

Fig. 7 (a) and Fig. 7 (b) show a part of the microchannel captured using a laser microscope, depicting its depth and width, respectively. Analyzing these images, the following approximate dimensions of the microchannel are: depth 80 – 90 µm and width 385.0 ± 5.8 µm. The length is approximately 50 mm. Meanwhile, Fig. 7(c) and Fig. 7(d) show the full-size image of the microchannel engraved on PDMS and electrodes etched on FR-4 PCB, respectively.

**B. BONDING LAYER**

The function of a bonding layer on a circuit board is to create a consistent horizontal and smooth surface planarization.
layer, especially around the sensing area. The layer prevents multiple bounces and having a uniform surface aids in sealing onto a microfluidic structure [1].

In this work, DABA pre-polymer layer was covered with PET film during exposure to avoid oxygen inhibition of the cross-linking reaction after cure [43], [45], [51]. However, the use of PET film resulted in the formation of air bubbles underneath the film. This problem is due to the difference in the surface tension between DABA pre-polymer and other substrates. These bubbles could potentially alter the shape of the microchannel mainly when they are formed closer to it. Therefore, effort must be taken to ensure no air bubbles are formed during curing. Coating the PCB with a thicker DABA layer helped prevent large-sized air bubbles while reducing smaller ones. The latter was removed by firmly pressing the film outwards from the centre of the PCB surface. In addition to PET, the PVC film also was experimented with as an oxygen inhibitor material. Compared to PVC, thinner PET film was much easier to use for controlling DABA photopolymerization. Hence this material was used in all biochips fabricated in this study.

Along with air bubbles, misalignment of PDMS during bonding processes is another significant issue in the fabrication, as previously discussed. The problem is due to the PDMS shrinkage since PDMS-DABA-PCB bonding must be performed at a higher temperature [46]. The bonding temperature, in this case, is 80 °C. This problem was solved by pre-shrink the PDMS before bonding. The already shrunk PDMS would not shrink further if the bonding temperature is lower than the pre-shrink temperature. In this study, the PDMS was pre-shrink at 150 °C, nearly twice as high as the temperature used during bonding. This method also helped prevent shrinkage during DNA experiments since the biochip is usually heated at a high temperature ranging from 70 °C to 100 °C to ensure the smooth flow of the molten agarose gel in the microchannel.
C. BONDING STRENGTH

Although the technique theoretically bonds DABA to both the copper-clad and insulating substrates of the PCB, the adhesion between DABA and copper (Cu) layers is substantially lower than DABA and PCB composite adhesion. The stronger bond is due to the rougher surface of the PCB composite compared to the copper surface.

Fig. 8 illustrates the pull-off test results for five test samples. According to this figure, a minimum tensile strength of 287.4 ± 23.8 kPa was required to break the bonding permanently. The mean standard error of the measurements was 10.6 kPa. The maximum value was measured at approximately 324 kPa. In all PDMS-DABA-PCB samples, failures occurred at the DABA-PCB interface. These values are consistent with those obtained for covalent bonding between PDMS and DABA [45], which has a higher tensile strength than the DABA-PCB interface.

Meanwhile, the leakage test indicated that the biochip was capable of withstanding pressures greater than 190.0 kPa. No experiment was performed beyond this figure since the pressure needed to operate the biochip rarely exceeds 40 kPa. Although a much higher pressure is required to clean and flush the agarose gel from the microchannel, the pressure required is still significantly less than 190 kPa. On average less than 120 kPa is needed for cleansing. As a result, this bonding also satisfies the requirement for creating a leak-free microfluidic device.

Table 1 summarizes several recent bonding studies conducted using various methodologies, including this study, either with direct or indirect bonding. Despite operating at high temperatures, thermal bonding typically yields the most robust bond strength compared to the rest. The following methods with high bond strength are the surface modifications techniques. Although it is not the strongest bond, however the mechanical strength and leak pressure obtained in this study are adequate for most fluidic applications. The bond strength is comparable to the oxygen plasma bonding for PDMS and gold-patterned glass [6] and the half-cured PDMS bonding for PDMS and PCB [13]. Additionally, the dimensional channel accuracy can be easily adjusted by controlling photopolymerization, which is impossible with other technologies. Furthermore, the planarization layer’s shape and its thickness also can be easily customized.

D. DNA EXPERIMENTS

Following the successful completion of leakage and bonding tests, the performance of the biochip was evaluated using actual DNA samples. Before this, CV experiments were conducted to determine the optimal redox potential of dGTP on a copper electrode. Three distinct experiments were conducted. The first experiment used only EDTA buffer, whereas the second and third experiments used a mixture of EDTA and dGTP at two different concentrations. The results are shown in Fig. 9.

Close examination of this figure revealed two important points. All three experiments produced cyclic voltammograms with two distinct anodic (positive) peaks. The first peak is located around 0.4 V, while the second peak is located between 0.8 and 1.0 V. When the dGTP concentration is increased from 100 mmol/litre to 200 mmol/litre, it is possible to observe that the anodic reaction is significantly more intense in the second peak than it is in the first peak. In this case, the anodic current in the first peak increase merely by 8.3 μA compared to 158.3 μA in the second peak with increasing dGTP concentration. As suggested previously, the small and insignificant increase in anodic current during the first peak was primarily due to the chemical reaction between EDTA buffer and copper electrodes [56]. Therefore, this peak can be ignored. Hence, the potential corresponding to the second anodic peak is the best reference potential for optimum sensitivity of DNA detection. Based on this information, the average potential of 0.9 V was selected and used as a reference potential for all experiments described in this paper.

E. DNA ANALYSIS

The biochip essentially performs two functions, i.e., separation and detection. Therefore, both functions were
investigated in this study. Fig. 10(a) and Fig. 10(b) are the electropherograms obtained from the 1100 bp DNA sample and the 1 kbp GeneDirex DNA ladder, respectively. Each number in Fig. 10(b) corresponds to each bp size in Fig. 4(b). Meanwhile, Fig. 10(c) is the close-up view of the selected peaks in Fig. 10(b).

The information provided by traditional gel electrophoresis as shown in Fig. 4 and the manufactured biochip, as shown in Fig. 10, is identical. The single band DNA sample resulted in one single electropherogram pulse, as clearly shown in Fig. 10(a). This data is consistent with the electrophoretic band in Fig. 4(a).

Similarly, the experiment using a 1 kbp GeneDirex DNA ladder produced an electropherogram with 13 distinct pulses, as shown in Fig. 10(b). These pulses are also consistent with the electrophoretic bands in Fig. 4(b). Both sequence and size of each peak in the electropherogram agree with each electrophoretic band’s sequence and size. The experiment revealed that the DNA biochip had separated and identified the DNA fragments successfully. Close analysis of Fig. 10(a) indicated a pulse that appeared roughly around 1100 s is corresponds to the 4th pulse in the electropherogram of Fig. 10(b). The parallelism in terms of time is because the bp size of these two peaks is nearly identical. In this scenario, the single band DNA sample size is 1100 bp, whereas the 4th band of 1 kbp GeneDirex is 1000 bp. This result has again demonstrated the accuracy of the biochip in first detecting and second separating DNA based on their bp sizes.

Comparing these results with our previous work [22], which utilized metal clips to attach the two substrates, the new bonding technique used to fabricate the new biochip has delivered the same performance. Both biochip designs employed in this development and the earlier work [22] can equally separate and detect DNA fragments with excellent detection accuracy and sensitivity. In addition, tightening with screws in the previous design failed to prevent leakage. The information provided by traditional gel electrophoresis as shown in Fig. 4 and the manufactured biochip, as shown in Fig. 10, is identical. The single band DNA sample resulted in one single electropherogram pulse, as clearly shown in Fig. 10(a). This data is consistent with the electrophoretic band in Fig. 4(a).

Similarly, the experiment using a 1 kbp GeneDirex DNA ladder produced an electropherogram with 13 distinct pulses, as shown in Fig. 10(b). These pulses are also consistent with the electrophoretic bands in Fig. 4(b). Both sequence and size of each peak in the electropherogram agree with each electrophoretic band’s sequence and size. The experiment revealed that the DNA biochip had separated and identified the DNA fragments successfully. Close analysis of Fig. 10(a) indicated a pulse that appeared roughly around 1100 s is corresponds to the 4th pulse in the electropherogram of Fig. 10(b). The parallelism in terms of time is because the bp size of these two peaks is nearly identical. In this scenario, the single band DNA sample size is 1100 bp, whereas the 4th band of 1 kbp GeneDirex is 1000 bp. This result has again demonstrated the accuracy of the biochip in first detecting and second separating DNA based on their bp sizes.

Comparing these results with our previous work [22], which utilized metal clips to attach the two substrates, the new bonding technique used to fabricate the new biochip has delivered the same performance. Both biochip designs employed in this development and the earlier work [22] can equally separate and detect DNA fragments with excellent detection accuracy and sensitivity. In addition, tightening with screws in the previous design failed to prevent leakage.

### TABLE 1. Comparative analysis of several bonding procedures using PDMS, various types of glass, and polymers.

| Ref. | Bonding substrate pair | Bonding method | Bonding/Leakage test |
|------|------------------------|----------------|---------------------|
| This work | DABA and FR-4 with copper | Adhesion by FR-4 and Cu rough surface with DABA photopolymer. | Pull-off test: average bond strength of 287.4 ± 23.8 kPa. Leakage test: higher than 190.0 kPa |
| [6] | PDMS and gold-patterned glass | Oxygen plasma | Average leak pressure of 238 ± 22 kPa |
| [13] | Cured PDMS, half-cured PDMS and PCB | Half-cured PDMS | No leakage when injected at maximum flow rate of 950 μL/min. |
| [15] | PDMS and PMMA | PDMS and thermoplastics surface activation using oxygen plasma, followed by surface modification using amino- and epoxy-based silane coupling reagents | No leakage when injected at maximum flow rate of 30 mL/min. |
| | PDMS and PS | | Pull-off test: average bond strength of 274.5 ± 27 kPa |
| | PDMS and PC | | 591.7 ± 44 kPa |
| | PDMS and PET | | 594.7 ± 25 kPa |
| | 510 ± 47 kPa |
| [52] | Fused-silica glasses | Oxygen plasma, pressure, and annealing temperature 200 to 400°C | Bonding energies from 0.33 to 0.48 J/m² |
| | Fused-silica glass and borosilicate glass | Oxygen plasma, pressure, and annealing temperature below 200°C | Pressure resistance higher than 600 kPa |
| [53] | PDMS and crown glass | H₂O plasma | Bonding strength higher than 9 N/cm² (90 kPa) |
| [54] | PC/TPE-hybrid and COC, PS, PC | Thermal bonding, overnight at 60°C | Leakage test (nitrogen gas): higher than 750 kPa |
| | PC/TPE-hybrid and glass | Oxygen plasma and surface functionalization using bis amino silane with thermal bonding, overnight at 60°C | Higher than 750 kPa but failed with hydrolytic test |
| [55] | PDMS-acrylic PSA | PSA adhesive tape and oxygen plasma treatment | No leakage at maximum applied flow rate of 30 mL/min. |
| | PDMS-silicon PSA | | 565.37 kPa |
| | PMMA-acrylic PSA | | 503.317 kPa |
| | PMMA-silicon PSA | | 372.317 kPa |
| | | | 358.527 kPa |
two substrates permanently. One advantage of this technique is simple to fabricate by using only off-the-shelf materials.

The DNA experiments show that the bonding technique is reliable because the new DNA biochips could separate and detect DNA fragments, either single or multiple bands samples. These results also prove that the DNA molecules are entirely bioinert to the materials used for PDMS-PCB bonding which is essential for accurate and reliable sensing.

ACKNOWLEDGMENT

The authors are grateful to Allnex Malaysia Sdn. Bhd. for the supply of bonding materials. They also wish to thank Prof. Nazalan Najimudin and the Ph.D. Scholar Ahmad Faisal Mohamad for discussions and their contributions to the DNA experiments.

REFERENCES

[1] N. R. Ali@Hasim, A. Ahaitouf, and M. Z. Abdullah, “Irreversible bonding techniques for the fabrication of a leakage-free printed circuit board-based lab-on-chip in microfluidic platforms—A review,” Meas. Sci. Technol., vol. 32, no. 5, May 2021, Art. no. 052001, doi: 10.1088/1361-6501/abeb92.
[2] D. Sadighbayan and E. Ghafar-Zadeh, “Portable sensing devices for detection of COVID-19: A review,” IEEE Sensors J., vol. 21, no. 9, pp. 10219–10230, May 2021, doi: 10.1109/JSEN.2021.3059970.
[3] J. Huang, Y. Liu, Z. Tang, X. Shao, and C. Zhang, “A polymer-based microfluidic sensor for biochemical detection,” IEEE Sensors J., vol. 20, no. 12, pp. 6270–6276, Jun. 2020, doi: 10.1109/JSEN.2020.2974544.
[4] I. Martinec, D. Pudis, and M. Chalupova, “Technology for the preparation of PDMS optical fibers and some fiber structures,” IEEE Photon. Technol. Lett., vol. 26, no. 14, pp. 1446–1449, Jul. 15, 2014, doi: 10.1109/LPT.2014.2326695.
[5] S. Bhattacharya, A. Datta, J. M. Berg, and S. Gangopadhayay, “Studies on surface wettability of poly(dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength,” J. Microelectromech. Syst., vol. 14, no. 3, pp. 590–597, Jun. 2005, doi: 10.1109/JMEMS.2005.844746.
[6] C. L. Gonzalez-Gallardo, A. D. Diaz, and J. R. Casanova-Moreno, “Improving plasma bonding of PDMS to gold-patterned glass for electrochemical-microfluidic applications,” Microfluidics Nanofluidics, vol. 25, no. 2, p. 20, Feb. 2021, doi: 10.1007/s10404-021-02420-3.
[7] S. H. Tan, N.-T. Nguyen, Y. C. Chuah, and T. G. Kang, “Oxygen plasma treatment for reducing hydrophobicity of a sealed polydimethylsiloxane (PDMS) microchannel,” Biomicrofluidics, vol. 4, no. 3, Sep. 2010, Art. no. 032204, doi: 10.1063/1.3466882.
[8] S. Ali, D. Maddipatla, B. B. Narakathu, A. A. Chilaihawi, S. Emamian, F. Janabi, B. J. Bazuin, and M. Z. Atashbar, “Flexible capacitive pressure sensor based on PDMS substrate and Ga-In liquid metal,” IEEE Sensors J., vol. 19, no. 1, pp. 97–104, Jan. 2019, doi: 10.1109/JSEN.2018.2877929.
[9] E. A. Shin, S. B. Lee, G. H. Kim, J. Jung, and C. K. Lee, “Enhanced interfacial adhesion of polydimethylsiloxane (PDMS) by control of the crosslink density,” J. Nanosci. Nanotechnol., vol. 20, no. 11, pp. 6768–6775, Nov. 2020, doi: 10.1166/jnn.2020.18804.
[10] M. A. Eddings, M. A. Johnson, and B. K. Gale, “Determining the optimal PDMS–PDMS bonding technique for microfluidic devices,” J. Micromech. Microeng., vol. 18, no. 6, Jun. 2008, Art. no. 067001, doi: 10.1088/0960-1317/18/6/067001.
[11] W. Zhao, S. Tian, L. Huang, K. Liu, and L. Dong, “The review of lab-on-PCB for biomedical application,” Electrophoresis, vol. 41, nos. 16–17, pp. 1433–1445, Sep. 2020, doi: 10.1002/elps.201900444.
[12] D. Moschou and A. Tserepi, “The lab-on-PCB approach: Tackling the µTAS commercial upscaling bottleneck,” Lab Chip, vol. 17, no. 8, pp. 1388–1405, Apr. 2017, doi: 10.1039/c7lc00121e.
[13] Y. Chang and H. You, “Efficient bond of PDMS and printed circuit board with an application on continuous-flow polymerase chain reaction,” BioChip J., vol. 14, no. 4, pp. 349–357, Dec. 2020, doi: 10.1007/s13206-020-4403-0.

FIGURE 10. Electropherograms of (a) Single band DNA sample of 1100 bp, (b) 1 kbp GeneDirex DNA ladder, and (c) Closed-up view of the shaded region.

microchannel’s leakage. The results also indicate that the materials used in the fabrication are biocompatible with the DNA and electrochemically inert as they do not interfere with the redox reactions between DNA molecules and electrodes.

Despite the toxicity of copper for cells and DNAs [57]–[60], copper electrodes are demonstrated safe in this particular setting. Compared to the previous design, the new biochip is more practical and user-friendly. Results from this research could pave the way for designing truly portable biochips for nucleic acid sensing and applications.

V. CONCLUSION

This paper discusses a new technique for designing a disposable and leak-free biochip used for nucleic acid analysis, particularly for a large sensing area device. Since it is practically challenging to attach PDMS onto PCB directly, thus a new technique utilizing a DABA inhibitor was used to bond these
D. Evans, K. I. Papadimitriou, L. Greathead, N. Vasilakis, P. Pantelidis, H. Shamkhalichenar, C. J. Bueche, and J.-W. Choi, "Printed circuit M. H. Ghanim and M. Z. Abdullah, "Design of disposable DNA biosensor W. B. Veloso, G. A. C. Ribeiro, C. Q. da Rocha, A. A. Tanaka, I. S. da Silva, and L. M. F. Dantas, "Flow-through amperometric determination of ampicillin using a copper electrode in a batch injection analysis system," J. Electroanal. Chem., vol. 878, Dec. 2020, Art. no. 114596, doi: 10.1016/j.jelechem.2020.114596.

K. M. Schilly, S. S. Gunawardhana, M. B. Wijesinghe, and S. M. Lunte, "Biological applications of microchip electrophoresis with amperometric detection: In vivo monitoring and cell analysis," Anal. Bioanal. Chem., vol. 412, no. 24, pp. 6101–6119, Sep. 2020, doi: 10.1007/s00216-020-02647-7.

P. Rewatkar, A. Kothuru, and S. Goel, "PDMS-based microfluidic glucose biofuel cell integrated with optimized laser-induced flexible graphene bioelectrodes," IEEE Trans. Electron Devices, vol. 67, no. 4, pp. 1832–1838, Apr. 2020, doi: 10.1109/TED.2020.2971480.

F. Stradolini, A. Tuoheti, T. Kilic, S. L. Ntella, Z. Huang, G. De Micheli, D. Demarchi, and S. Carrara, "An IoT solution for online monitoring of anesthetics in human serum based on an integrated fluidic biotronic system," IEEE Trans. Biomed. Circuits Syst., vol. 12, no. 5, pp. 1066–1069, Oct. 2018, doi: 10.1109/TBCAS.2018.2855048.

M. H. Ghanim and M. Z. Abdullah, "Design of disposable DNA biosensor microchip with amperometric detection featuring PCB substrate," BioChip J., vol. 7, no. 1, pp. 51–56, Mar. 2013, doi: 10.1007/s13206-013-7108-9.

W. B. Veloso, G. A. C. Ribeiro, C. Q. da Rocha, A. A. Tanaka, I. S. da Silva, and L. M. F. Dantas, “Flow-through amperometric determination of ampicillin using a copper electrode in a batch injection analysis system,” J. Electroanal. Chem., vol. 878, Dec. 2020, Art. no. 114596, doi: 10.1016/j.jelechem.2020.114596.

T. Sridara, J. Upan, G. Saianand, A. Tuantranont, C. Karuwan, and J. Jakmunic, "Non-enzymatic amperometric glucose sensor based on carbon nanodots and copper oxide nanocomposites electrode," Sensors, vol. 20, no. 3, p. 808, Feb. 2020. [Online]. Available: https://www.mdpi.com/1424-8220/20/3/808

N. M. Harmeyreddy and T. Simon, "Templated bimetallic copper-silver nanostructures on pencil graphite for amperometric detection of nitrate for aquatic monitoring," J. Electroanal. Chem., vol. 856, Jan. 2020, Art. no. 113660, doi: 10.1016/j.jelechem.2019.113660.

B. Show, S. F. Ahmed, A. Mondal, and N. Mukherjee, "Hierarchical copper oxide as efficient enzymeless amperometric biosensor and promising photocatalyst," J. Environ. Chem. Eng., vol. 9, no. 2, Apr. 2021, Art. no. 104748, doi: 10.1016/j.jece.2020.104748.

Y. Dong, X. Min, and W. S. Kim, "A 3-D-printed integrated PCB-based electrochemical sensor system," IEEE Sensors J., vol. 18, no. 7, pp. 2959–2966, Aug. 2018, doi: 10.1109/JSEN.2018.2810459.

H. Shamkhilichenar, C. J. Bueche, and J.-W. Choi, “Printed circuit board (PCB) technology for electrochemical sensors and sensing platforms," Biosensors, vol. 10, no. 11, p. 159, Oct. 2020. [Online]. Available: https://www.mdpi.com/2079-6374/10/11/159

D. Evans, K. I. Papadimitriou, L. Greathead, N. Vasilakis, P. Pantelidis, P. Kelleher, H. Morgan, and T. Prodomakis, "An assay system for point-of-care diagnosis of tuberculosis using commercially manufactured PCB technology," Sci. Rep., vol. 7, no. 1, p. 685, Apr. 2017, doi: 10.1038/s41598-017-00783-8.

G. Dutta, A. Regoutz, and D. Moschou, "Enzyme-assisted glucose quantification for a painless lab-on-PCB patch implementation," Biosensors Bioelectron., vol. 167, Nov. 2020, Art. no. 112484, doi: 10.1016/jbiosens.2020.112484.
[50] M. H. Ghanim, N. Najimudin, K. Ibrahim, and M. Z. Abdullah, “Low electric field DNA separation and in-channel amperometric detection by microchip capillary electrophoresis,” IET Nanobiotechnol., vol. 8, no. 2, pp. 77–82, Jun. 2014. [Online]. Available: http://digital-library.theiet.org/content/journals/10.1049/iet-nbt.2012.0044

[51] J. Y. Crivello and E. Reichmanis, “Photopolymer materials and processes for advanced technologies,” Chem. Mater., vol. 26, no. 1, pp. 533–548, Jan. 2014. doi: 10.1021/cm402262g.

[52] K. Shoda, M. Tanaka, K. Mino, and Y. Kazoe, “A simple low-temperature glass bonding process with surface activation by oxygen plasma for micro/nanofluidic devices,” Micromachines, vol. 11, no. 9, p. 804, Aug. 2020. [Online]. Available: https://www.mdpi.com/2072-666X/11/9/804

[53] M. Tohnishi and A. Matsutani, “Surface treatment of polydimethylsiloxane and glass using solid-source H2O plasma for fabrication of microfluidic devices,” Sensors Mater., vol. 33, no. 2, pp. 569–574, 2021. doi: 10.18494/SAM.2021.3156.

[54] S. Schneider, E. J. S. Brás, O. Schneider, K. Schlünd, and P. Liskoll, “Facile patterning of thermoplastic elastomers and robust bonding to glass and thermoplastics for microfluidic cell culture and organ-on-chip,” Micromachines, vol. 12, no. 5, p. 575, May 2021. [Online]. Available: https://www.mdpi.com/2072-666X/12/5/575

[55] S. Hassanpour-Tamrin, A. Sanati-Nezhad, and A. Sen, “A simple and low-cost approach for irreversible bonding of polymethylmethacrylate and polydimethylsiloxane at room temperature for high-pressure hybrid microfluidics,” Sci. Rep., vol. 11, no. 1, p. 4821, Dec. 2021. doi: 10.1038/s41598-021-83011-8.

[56] L. Nagy, G. Nagy, and P. Hajós, “Copper electrode based amperometric detector cell for sugar and organic acid measurements,” Sens. Actuators B, Chem., vol. 76, nos. 1–3, pp. 494–499, Jan. 2001. doi: 10.1016/S0925-4005(01)00599-8.

[57] M. C. Cortizo and M. F. L. de Mele, “Cytotoxicity of copper ions released from metal,” Biol. Trace Element Res., vol. 102, no. 1, pp. 129–141, Dec. 2004. doi: 10.1385/BTER:102:1-3:129.

[58] H. L. Karlsson, P. Cronholm, J. Gustafsson, and L. Möller, “Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes,” Chem. Res. Toxicol., vol. 21, no. 9, pp. 1726–1732, Sep. 2008. doi: 10.1021/tx800066j.

[59] A. Le, N. Shibata, S. French, K. Kim, K. Kharbanda, M. Islam, J. LaSalle, C. Halsted, C. Keen, and V. Medic, “Characterization of timed changes in hepatic copper concentrations, methionine metabolism, gene expression, and global DNA methylation in the Jackson toxic milk mouse model of Wilson disease,” Int. J. Mol. Sci., vol. 15, no. 5, pp. 8004–8023, May 2014. [Online]. Available: https://www.mdpi.com/1422-0067/15/5/8004

[60] M. Mazzuferi, R. Bovolenta, M. Bocchi, T. Braun, J. Bauer, E. Jung, B. Jafelice, R. Guerrieri, F. Destro, M. Borgatti, N. Bianchi, M. Simonato, and R. Gambari, “The biocompatibility of materials used in printed circuit board technologies with respect to primary neuronal and K562 cells,” Biomaterials, vol. 31, no. 6, pp. 1045–1054, Feb. 2010. doi: 10.1016/j.biomaterials.2009.10.025.

NORSHARIZAL ALI (HASIM) received the B.Eng. degree (Hons.) in electronic and optoelectronic engineering from The University of Manchester, U.K., in 1998, and the M.Sc. degree in electrical and electronic engineering (sensor and instrumentation) from the University of Science, Malaysia, in 2017, where he is currently pursuing the Ph.D. degree in electrical and electronic engineering. His research interests include biomedical devices, biosensors, optoelectronic, and holds a patent.

MOHAD ADZHAR MD ZAWAWI received the B.Eng. degree (Hons.) in electronic engineering from The University of Sheffield, in 2003, the M.Sc. degree in electronic systems design engineering from Universiti Sains Malaysia (USM), in 2010, and the Ph.D. degree in electrical and electronic engineering from The University of Manchester, in 2015. He is currently a Senior Lecturer with the School of Electrical and Electronic Engineering, USM. Prior to joining USM, he has several years of industrial experience with multinational semiconductor companies in product and test engineering and wafer fabrication. His research interests include modeling, design, and fabrication of compound semiconductor and quantum devices, monolithic microwave integrated circuit (MMIC), THz devices, printable electronics, and sensors.

MOTASEM H. GHANIM was born in Palestine. He received the Ph.D. degree in electrical and electronic engineering (sensor and instrumentation) from the University of Science, Malaysia, in 2013. He was a Lecturer at Arab American University, from 2002 to 2008. From 2014 to 2017, he was a Postdoctoral Research Fellow at the Collaborative Microelectronic Design Excellence Centre, Universiti Sains Malaysia. His research interests include biomedical devices, amperometric detection, and biosensors.

MOHD ZAID ABDULLAH (Member, IEEE) received the B.App.Sc. degree in electronic from Universiti Sains Malaysia (USM), in 1986, and the M.Sc. degree in instrument design and application and the Ph.D. degree in electrical impedance tomography from the Institute of Science and Technology, The University of Manchester, U.K., in 1989 and 1993, respectively. He joined Hitachi Semiconductor, Malaysia, as a Test Engineer. He is currently a Lecturer and a Professor with USM’s School of Electrical and Electronic Engineering. He has published numerous research articles in international journals and conference proceedings. His research interests include microwave tomography, digital image processing, computer vision, and ultra-wide band sensing.

Prof. Abdullah’s one of the papers was awarded The Senior Moulton Medal for the Best Article published by the Institute of Chemical Engineering, in 2002.