A Commercially Available Lab-on-PCB Technology for Affordable, Electronic-Based Point-of-Care Diagnostics

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***Abstract*— In this paper a novel, commercially available Lab-on-Printed Circuit Board (LoPCB) manufacturing technology is demonstrated for the development of low-cost electrochemical biosensors combined with microfluidics for Point-of-Care (PoC) applications. An analysis of the developed PCB architecture is presented, detailing the three development areas of the proposed LoPCB platform, i.e. microfluidics, biosensors and electronics. Design rules and potential fabrication limitations are also discussed, based on the characterization of prototype fabricated systems. Two PCB-based devices have been designed and fabricated, a microfluidic active diluter with a variable and actively controlled dilution ratio and an electrochemical biosensor. Preliminary results obtained demonstrate the feasibility of a complete LoPCB platform, where all three compartments will co-exist and co-operate, providing an electronic-based PoC system for electrochemical biosensing.**

I. Introduction

Modern medical and technological progress has clearly outlined the need for compact and portable devices for specific biochemical analyses. Microfluidic and molecular recognition technologies have advanced rapidly in step with the improved diagnostic and prognostic capabilities that have resulted from a dramatically enhanced understanding of genomic and proteomic systems. Lab-on-a-Chip (LoC) technology suitable for the development of PoC diagnostics has been widely demonstrated [1], however ubiquitous PoC technology remains elusive.

The majority of published LoC devices are fabricated using paper or polymer substrates as these are cost-effective and readily disposed materials [2-5]. However, the physical properties of these materials mean their integration with functional components such as heaters, electrodes, optoelectronics and sensing elements is challenging. It is reasonable to assume that the major driving force behind this situation is cost [6]. The lack of integration of the aforementioned components with the specific substrates in a cost-effective manner, combined with their additional limitations and published issues with sample preparation, sensitivity and specificity [7], results in a more quantitative analysis from these devices.

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In an attempt to incorporate electronic components into LoC platforms and minimize the effective area of the whole diagnostic system, the idea of the LoPCB approach has been introduced. In 1996, the work of Lammerink *et al.* [8] introduced for the first time the concept of forming a mixed circuit board (MCB) by proposing the extension of the conventional PCB into a microfluidic platform. Three years later, Merkel *et al.* [9], proved successfully the aforementioned concept. So it has already been shown that existing PCB technology is suitable for developing microfluidic systems. What has not been shown yet, is the combination of PCBs with electronic components for microfluidic systems control and electrochemical detection in a monolithic manner. Compared to other LoC platforms, PCB demonstrates ideal electronics integration and high fabrication precision, resulting from the several decades of industrial manufacturing accumulated experience [7, 10]. For PoC diagnostic devices, both commercially and scientifically, such properties are of great significance.

Here we demonstrate the application of mainstream PCB manufacturing technologies in the development of fully integrated PoC components, comprising microfluidic networks, electrochemical biosensors and electronics. Design rules for microfluidic and biosensing components are described and illustrated, and limitations of fabrication discussed. We have also demonstrated the further enhanced inherent characteristics of a PCB-based device by rendering the microfluidic network passive, while enabling the autonomous passive flow through the microchannels. Results from various aspects of our LoPCB development and integration work are presented, including a microfluidic active diluter with a variable and actively controlled dilution ratio, comprising a power MOSFET and digital temperature sensors and an electrochemical biosensor, whose functionality is presented by showing that our electronic control board and sensors are capable of detecting product concentrations of a common colorimetric reagent, 3, 3, 5, 5, Tetramethybenzadine (TMB), through amperometric analysis.

II. The Developed Lab-on-PCB Manufacturing Technology

Figure 1 presents the concept of the developed commercially available LoPCB manufacturing technology. The technology combines the standard PCB attributes, such as electrical traces and vertical interconnection accesses (VIAs) with electronic components, microfluidic networks and electrochemical sensors, including gold (Au) and silver

(Ag/AgCl pseudo-reference electrodes) sensing sites [11]. Unlike optical sensing methods, where the reporting system must be converted to an electrical signal for further processing, electrochemical sensing exploits the concept of an immediately available converted electrical signal in the form of electrolyte conductivity, without the need for additional conversion steps. The ability to integrate electronic components on the same platform significantly increases PoC implementation potential [12].

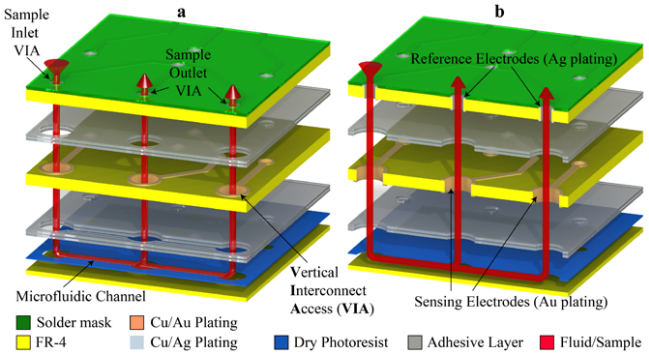

Figure 1: The architecture of the developed LoPCB. a) an exploded view b) a cross sectional view along the microfluidic channel.

The manufacturing process has developed to realize a 3-layer flame retardant class 4 laminate (FR-4) stack, comprising a top, middle and bottom layer. The top layer is silver plated, thus, the VIAs can be employed as pseudo reference electrodes, if pre-chlorinated. The middle layer is gold plated, therefore, the VIAs can be used as sensing electrodes, since enzymes, antibodies, cells and microorganisms can be immobilized onto the gold electrode surface, functionalizing them as biosensors [13]. The bottom layer serves as the microfluidic network, interconnecting the inlet and outlet VIAs of the PCB. The microchannels are formed in dry photoresist, through standard lithography.

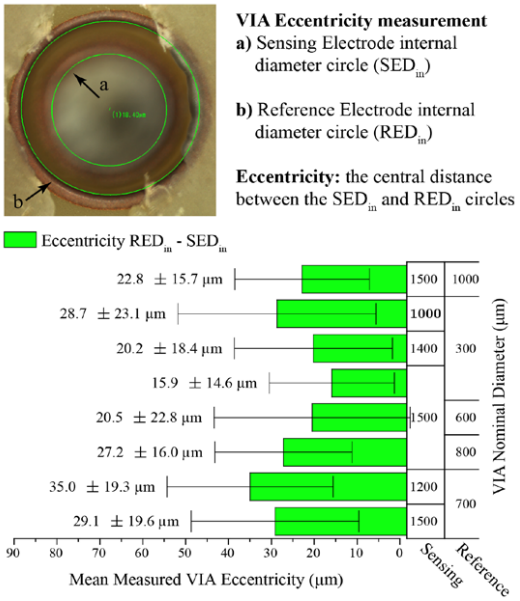

Figure 2: Measurement and statistical analysis of the eccentricity between the internal diameters of sensing and reference electrodes.

Based on Figure 1 architecture, a test design including various reference and sensing VIA geometries (internal diameter and ring width) were fabricated by a PCB manufacturer (Newbury electronics Ltd). In an attempt to fully characterize the limitations of the manufacturing process, the following geometrical process variations were investigated. Figure 2 summarizes the statistical analysis of the measured VIA eccentricity between reference (Top Layer) and sensing (Middle Layer) electrodes. The average eccentricity with a 95% confidence interval against the different nominal design diameters is presented. The worst average measured eccentricity was found to be 35 µm with 19.3 µm variation (again 95% level of confidence). This parameter defines the minimum required design diameter difference between the stacked sensing and reference electrodes, in order to avoid potential short-circuits. Subsequently, similar studies for all the other manufacturing variations (e.g. difference between nominal and the fabricated diameter) were performed and based on the aforementioned statistical analysis, Figure 3 has been produced, summarizing the design rules driving the minimum nominal sensing electrode diameter with respect to the nominal reference electrode diameter. Different lines are drawn for 100, 200, 300 and 400 µm VIA ring width.

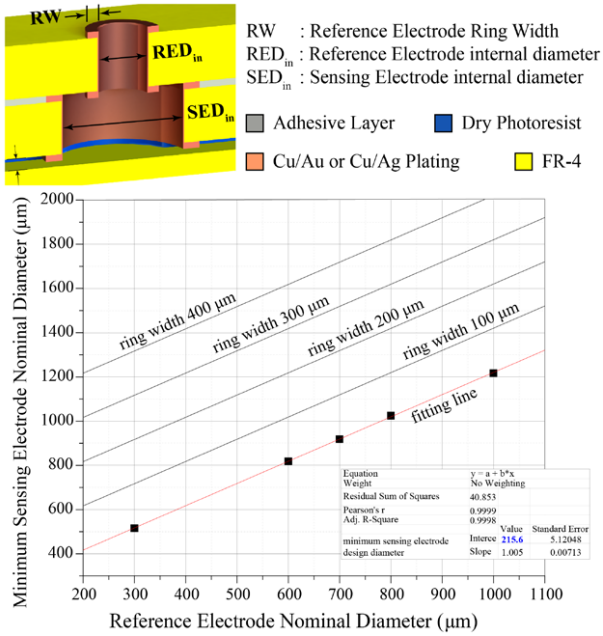

Figure 3: The design rule for the minimum nominal sensing electrode diameter with respect to the nominal reference electrode diameter.

III. Evaluating the Functionality of the Microfluidic Network

Due to the intrinsic hydrophobic properties of the PCB manufacturing materials, filling the device with aqueous solutions requires overpressure. An additional interfacing layer fabricated by PMMA comprising 2.8 mm wide through holes for mitigating capillary phenomena was added on top of the previous commercially fabricated test design. As presented in Figure 4, a 4 mbar overpressure enables the sample flowing through the inlet VIA but cannot brake the capillary barrier formed by the microfluidic channel. Hence,

a 5 mbar overpressure is required for filling the second sensing VIA and subsequently flowing through the second reference VIA. Increasing the inlet overpressure did not result into the filling of the third and final VIA.

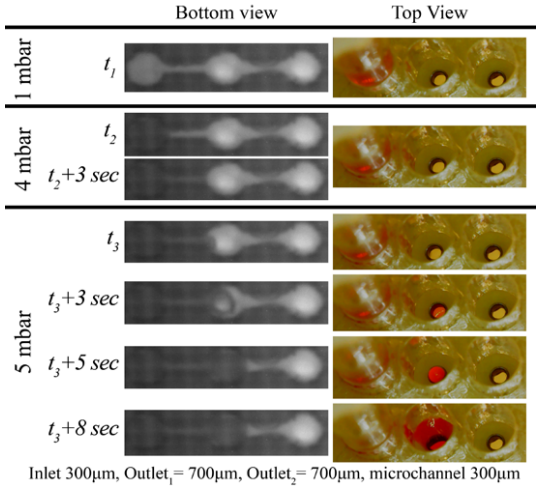

Figure 4: Pressure driven filling of the device.

In an attempt to overcome the previous obstacle, the idea of oxygen plasma treatment of sealed PCB microfluidic structures has been introduced [14], rendering them hydrophilic thus, enabling the autonomous passive filling of the device as shown in Figure 5.

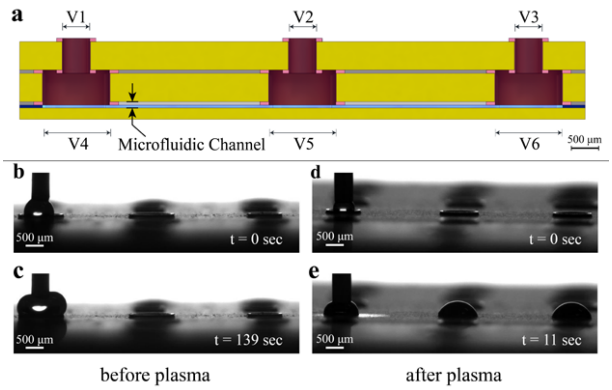

Figure 5: Still images from the DI water-filling experiment on the commercially manufactured microfluidic PCB a) cross-sectional view of the PCB microfluidic structure device. b) and c) filling before plasma treatment, d) and e) after plasma treatment.[14]

A long-lasting hydrophilic FR-4 surface can be achieved under particular O_2 plasma treatment. Thereafter, high-throughput microcapillary pumps based on the recently proposed microchannel integrated micropillar (MIMP) geometries can be developed [15], enhancing further the inherent advantages of the LoPCB technology (high integration ability and low cost-to-footprint ratio, about 0.3 \$/cm^2) by the prominent capabilities of passive microfluidics (no external power source for liquid flow).

IV. Fabricated LoPCB Devices for PoC Diagnostics

A. A Portable PCB-Based Microfluidic Diluter

Figure 6 illustrates a portable microfluidic diluter with a variable and actively controlled dilution ratio suitable for PoC implementations, fabricated entirely using the developed LoPCB manufacturing technology by the same industrial PCB manufacturer. A standard microfluidic network (Figure 6a) comprising two inlets and two outlets has been designed (Figure 6d) and fabricated (Figure 6b, c and e), where the resulting dilution ratio is thermally regulated using a power MOSFET as heating element (Figure 6c).

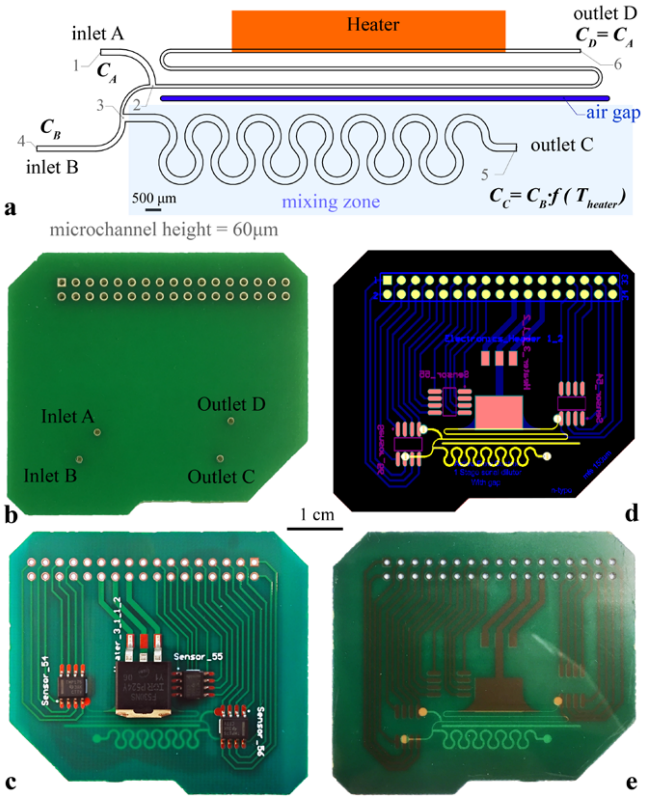

Figure 6: The PCB-based active control diluter. a) the diluter design with the heating element and the thermal insulation air gap b) the top layer of the manufactured prototype comprising inlet and outlet VIAs. c) bottom layer of the manufactured prototype with integrated functional electronics d) a layout representation of the device e) bottom layer of the microfluidic side.

The design relies upon constant flowrate through both inlets, while the flowrate along the microchannel between positions 2 and 6 (see Figure 6a) can be regulated by adjusting the MOSFET's gate voltage (which consequently changes the MOSFETs case temperature). A dedicated digital temperature sensor is monitoring constantly the temperature of the MOSFET. The temperature of the liquid flowing through the microchannel underneath will increase while its dynamic viscosity will decrease, as the temperature of the MOSFET increases, resulting in higher flow rate for the same pressure drop. Hence, the dilution ratio of the device can be controlled dynamically. The design includes two additional temperature sensors for implementing the calorimetric flow sensing method between positions 2 and 6.

The combined system is able to achieve dilution ratio ranges from 2:3 up to 3:4, with a sufficient dilution ratio resolution. Computational results of the aforementioned system can be found in Figure 7. The proposed system illustrates for the first time microfluidics and electronics,

combined in an embedded manner on a standard PCB. The miniaturized and power-efficient properties of the proposed system make it an excellent candidate in PoC diagnostics applications, where both microfluidics and electronics are sought after.

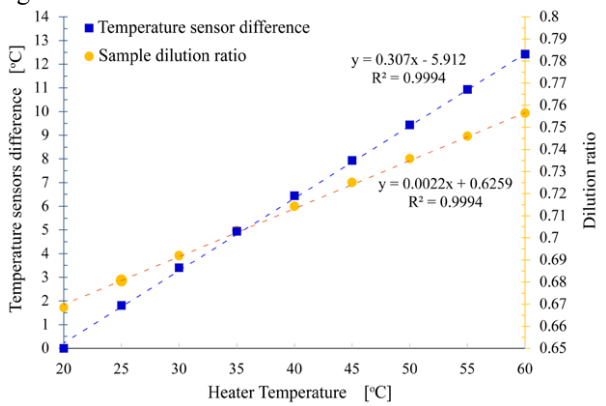

Figure 7: Temperature sensors difference and resulting dilution ratio against heating element temperature.

B. *A PCB-Based Biosensing Platform*

Amperometric sensing has been demonstrated using a range of TMB product concentrations. TMB is a common component of colorimetric detection systems. In the presence of H_2O_2 co-factor TMB is converted by the horseradish peroxidase enzyme (HRP) from a colourless substrate to a blue product, which is also electrochemically active.

A range of TMB product concentrations was prepared by diluting HRP enzyme across 8 points in Log_2 series. 5µL of each HRP dilution was added to 100µL aliquots of TMB substrate solution (Sigma-Aldrich) containing 0.014% H_2O_2 by volume. Enzymatic TMB conversion was allowed to proceed for 10 minutes before the reaction was stopped by addition of 10µL 1M HCl. Amperometric detection was conducted using our in-house electronic analysis system with an applied bias of -300mV. The geometry of the electrodes of the sensing apparatus been employed for this type of experiment can be seen in Figures 1 and 3.

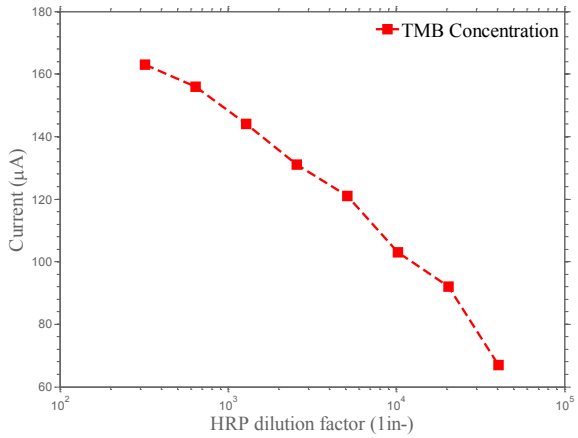

Figure 8: Samples containing a range of TMB product concentrations were assessed amperometrically using the described sensors and electronic control board. A clear linear relationship is observed between TMB product concentration and the resolved electrolyte current in µA.

Between measurements wells were rinsed twice with 2M HCl, then twice with DI water. Figure 8 data shows a clear correlation between TMB product concentration and magnitude of amperometric signal.

V. Conclusion

This paper demonstrates the potential of LoPCB technology produced using existing commercial manufacturing technologies. Discrete components are successfully demonstrated, including controlled microfluidics, electronics, and electrochemical sensing, showing the feasibility of an efficient and integrated low cost LoPCB PoC system.

Acknowledgment

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