# Screen-Printed Microfluidic Channel with Hydrophobic-Hydrophilic Treatments for Air Bubble Prevention

Tashfia Ahmed[1], Enayet Rahman[1], Matt Bryan[2], Michael B. Powner[3], Iasonas F. Triantis[1]
[1] Research Centre for Biomedical Engineering, School of Science and Technology, City, University of London, EC1V 0HB
[2] SmartCare Medical Limited, Tring, Herts, HP23 4JY
[3] Centre for Applied Vision Research, School of Health and Psychological Sciences, City, University of London, EC1V 0HB

*Abstract*— The efficacy of a developed microfluidic test strip is investigated in comparison to conventional blood glucose test strips. Utilising a combination of hydrophobic and hydrophilic treatments, the developed test strip showcases significant reduction of air bubble accumulation within the sample chamber. The findings of this study demonstrate the potential of hydrophobic-hydrophilic treatments on microfluidic channels, to address one of the major challenges in microfluidic channel development i.e. air bubble formation.

***Keywords*— microfluidic channels; surface modification; hydrophobic; hydrophilic; biological samples**

## I. INTRODUCTION

Microfluidic channels facilitate the interrogation of samples of small volumes, which is especially significant in the evaluation of biological fluids, such as sweat, urine and blood[1]. The use of microfluidic channels in biosensors, such as those integrated with electrodes for impedimetric measurement, also enables rapid analysis capabilities, aiding real-time analysis of biological samples of interest, for point-of-care applications [2].

One of the most established examples of electrochemistry-based microfluidic channels is the standard blood glucose test strip (Figure 1) [3]. A typical blood glucose test strip comprises of layers of hydrophilic material, commonly referred to as 'hydrophilic treatment', adhesives and spacer layers, an enzymatic layer, and the electrodes [3]. The purpose of the hydrophilic layers is to promote fluid flow from the entrance of the chamber, towards the electrodes for blood glucose measurement and sample interrogation. Moreover, adhesive and spacer layers are present for structural stability, as well as elevation in the capillary chamber to ensure the chamber is filled with the required sample volume for accurate blood glucose measurement. The enzymatic layer covers the microfluidic chamber and utilises the glucose present in the blood sample to generate an electrical current, for measurement via the electrodes.

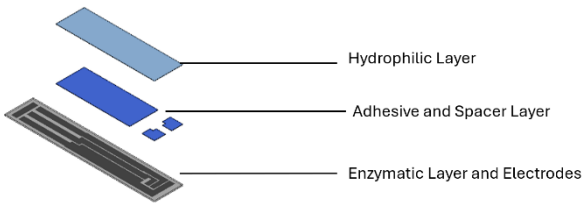


Figure 1 - CAD Drawing of typical Blood Glucose Test Strip showcasing common configuration of hydrophilic, adhesive/spacer, and enzymatic layers.

A major challenge associated with the utilisation of microfluidic channels is the undesired formation of air bubbles. Air bubbles can block fluid flow, interfere with sensor performance, as well as impair function and viability of biological samples [4], [5]. Therefore, the surface characteristics and geometry of microfluidic channels should be considered for the prevention of air bubble formation. Primarily, the hydrophilicity of the sample which flows through the channel and its interactions with the surface of the microfluidic channels gives rise to the formation of air bubbles. This is typically eradicated by the hydrophilic treatment of the channel surfaces, prior to sample loading [6]. Reliable and repeatable hydrophilic surface treatment methods have not yet been developed for mass production. Instead, most studies have highlighted the removal of air bubbles after formation in microfluidic chambers, through means of bubble traps and application of pressure pulses [4], [6], [7], [8], [9]. However, these techniques for air bubble removal are not appropriate when investigating biological liquid samples because it poses the risk of damaging samples e.g. cells rupturing due to increased wall shear stress within the channel pathway [5], [7].

The development of a simple microfluidic channel with stable and homogenous hydrophilic treatment prevents the development of air bubbles within the sample chamber, aiding the rapid and sensitive interrogation of biological samples of interest, in a non-destructive manner. The current study aims to develop a screen-printed test strip comprising of a microfluidic channel with hydrophobic and hydrophilic treatmentss, towards the development of an electrochemical biosensor for use on biological samples of interest.

Surface modification, such as a combination of hydrophilic and hydrophobic treatments within a test strip, promotes the filling of the microfluidic chamber and prevents the formation of air bubbles upon sample loading. In common test strip design, the underside of the top layer (sample chamber cover) is often treated with a hydrophilic layer to encourage fluid flow into the sample chamber [1]. However, the consistency of the coating is of utmost significance, as variations and imperfections in the hydrophilic layers can lead to erroneous results and contribute to measurement inaccuracies [1]. Furthermore, the use of physical boundaries within the sample chamber, such as those present in blood

glucose test strips does not mitigate the potential of air bubbles, which could lead to further system inaccuracies.

Air bubbles often form in microfluidic chambers due to the presence of sharp contact angles between the chamber boundaries and the fluid sample (Figure 2) [6]. Furthermore, microwells where the fluid passes over injection-moulded wells, gives rise to the formation of air bubbles. Moreover, in test trips with contact regions between joining parts, or dead areas, the formation of air bubbles is highly likely. Therefore making the presence of air bubbles within microfluidic sample chambers inevitable. Thus, the correction of this issue is imperative as air bubbles within the sample chamber prevents the correct functionality of biosensors [10].

## II. METHODS

### A. *Test Strip Fabrication*

For the design and development of the current test strip, a hydrophobic boundary was implemented using screen-printing techniques, which are low-cost, accurate and suitable for high throughput test strip manufacturing. Printing of hydrophobic boundaries as opposed to the implementation of physical boundaries mitigates the issues of air bubble formation between wall interfaces, which is a common issue in commercial microfluidic test strips [11]. The lack of physical boundaries ensures that air can escape from the boundaries of the chamber without obstruction. The top layer of the test strip is coated on the underside with a hydrophilic treatment. After this, a hydrophobic pattern is screen-printed onto the hydrophilic surface of the cover layer. Subsequently, a spacer layer of adhesive polymer film of 100µm thickness is placed. The spacer layer includes a cut-out boundary of the hydrophobic pattern. Each hydrophobic pattern layer is of a thickness of 40µm. The hydrophobic layers sandwiching the spacer layer therefore create a 20µm air gap through which air bubbles can escape. After this, a replication of the hydrophobic boundary pattern is screen-printed on the top of the electrode layer. CAD drawings of the strip configuration can be seen in Figure 3.

Figure 2 – Conventional Blood Glucose (BG) test strip with visualization of formation of air bubbles, upon loading of sample, due to sharp contact angles.

The sample is loaded onto the test strip and remains within the implemented hydrophobic boundaries. Capillary action is aided by the hydrophilic coating, to promote filling of the chamber with the sample fluid. Notably, the hydrophobic boundary comprises of a pattern with smooth edges, to further mitigate the formation of air bubbles. As previously mentioned, the test strip is not bound by physical walls/boundaries, which ensures that air bubbles can escape from the chamber through the edges of the test strip, upon sample loading. This process is visualised in Figure 4.

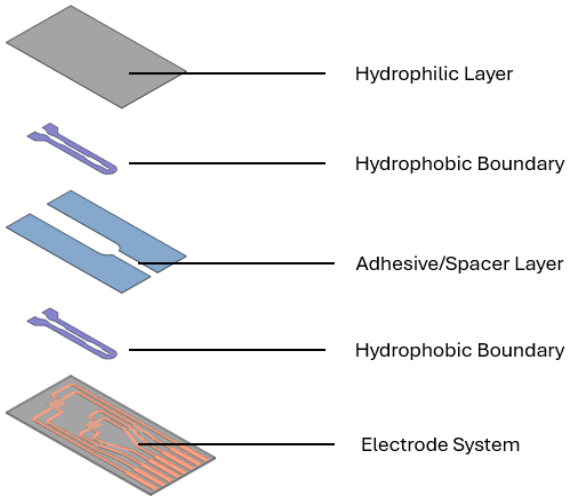


Figure 3 – CAD drawing of proprietary test strip fabrication process demonstrating layers of hydrophobic and hydrophilic treatment and an adhesive/spacer layer for structural stability.

### B. *Air Bubble Formation*

To evaluate the efficacy of the hydrophobic/hydrophilic treated test strip versus the commercially available hydrophilic treated test strip, the following experiment was designed. Comparisons were made between the developed test strips and commercially available blood glucose (BG) test strips. Samples of saline solution (0.9% NaCl concentration in distilled water) were injected into the sample chamber for both the proprietary test strip and the BG strip. Following this, air bubbles were deliberately introduced into the sample chambers. Images were then taken with a desktop handheld microscopic camera to qualitatively evaluate the magnitude of air bubbles visibly present in the sample chamber of both the proprietary test strips and the conventional BG test strips.

A total of 5 proprietary test strips and 5 commercial BG test strips were tested for the duration of this study. To enhance visibility of the introduced air bubbles, the saline solution was dyed yellow. This ensured that any air bubbles present in the sample chamber could be easily discerned using the HT-60S Handheld USB Digital Microscope. Each test strip was loaded with 2.7µl of the dyed saline solution via the sample chamber using an Eppendorf micropipette. The introduction of air bubbles into the test strip was executed using a Hamilton microlitre syringe equipped with a 30G needle, to allow for precise control of air bubbles. Following the introduction of air bubbles into sample chambers, both sets

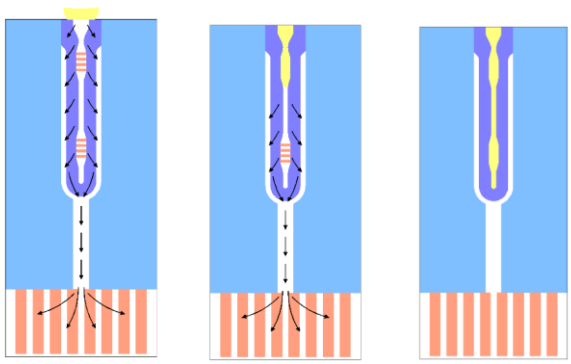

Figure 4 - Visualisation of air pathways within proprietary test strip. Showcases pathways by which air bubbles can escape upon sample loading.

of test strips were placed on the lab benchtop to settle for a duration of 30s before imaging with the handheld microscope.

## III. Results

As demonstrated in Figures 5 and 6, the proprietary test strip is successful in the mitigation of air bubbles, compared to the BG test strips. Upon comparison with the conventional BG test strip, it is evident that the proprietary test strip is more effective in reducing the magnitude of air bubbles in the sample chamber. A larger number of air bubbles can be seen in the BG test strips, compared to the proprietary test strips in Figures 5 and 6. It is apparent that the introduction of air bubbles into the sample chamber of the BG test strips led to the residual presence of air bubbles in the channel, after the 30s settling time, which was not commonly observed in the proprietary test strips. Across the 5 trials that were conducted for both the BG test strip and the proprietary test strips, it is evident that the proprietary test strips were successful and consistent at mitigating the introduction of air bubbles into the sample chamber, compared to the conventional BG test strips.

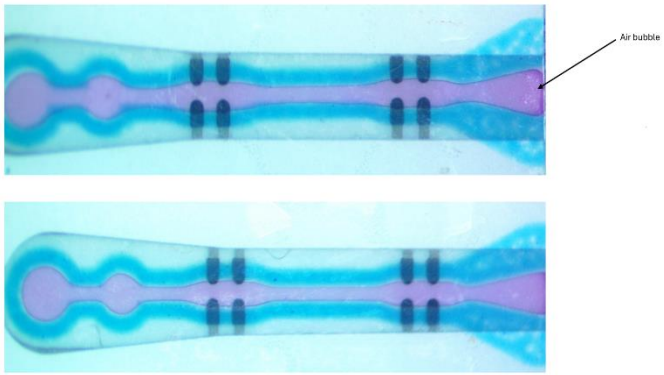


Figure 5 - Images of 2 proprietary test strips, after sample loading and deliberate insertion of air bubbles. Top strip shows a small air bubble, bottom strip shows no air bubbles.

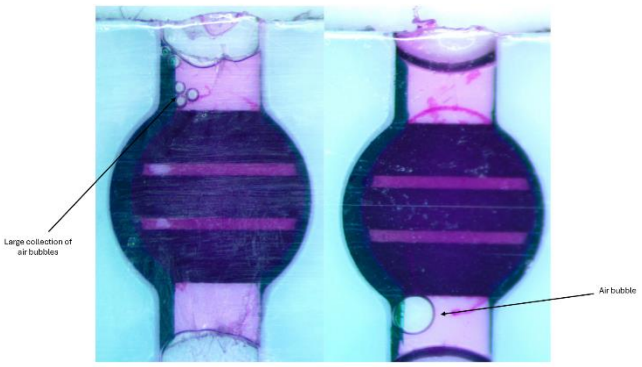


Figure 6 - Images of 2 conventional BG test strips, after sample loading and deliberate insertion of air bubbles. Showcases clear presence of large collections of air bubbles.

Evidently, across the 5 proprietary test strips, after sample loading and air bubble insertion, air bubbles remained in 20% of the test strips, compared to 100% of the conventional BG test strips. Furthermore, singular air bubbles were present in the proprietary test strips, compared to large collections of air bubbles in the BG test strips, which accounted for 25-75% of the total volume of the BG test strips.

## IV. Discussion

The findings showcase that the developed proprietary test strip, which incorporates a combination of hydrophilic and hydrophobic treated layers, significantly reduced the formation of air bubbles within the sample chamber, compared with conventional BG test strips. This improvement is crucial as the presence of air bubbles within the sample chamber of microfluidic channels significantly impacts accuracy in biosensors [1], [5], [10].

The successful mitigation of air bubbles in the sample chamber of the test strip is attributed to several factors. These include the implementation of identical hydrophobic boundary layers on either side of the adhesive/spacer layer to minimise entrapment of air bubbles between wall interfaces, which is a common challenge in microfluidic chambers. This ensures efficient fluid flow within the chamber. Furthermore, the absence of physical wall boundaries ensures that any air bubbles that are formed, or deliberately injected, can escape from the edges of the test strip [11]. Thus, preventing accumulation of air bubbles and potential interference with sensor performance.

An alternative method to prevent air bubble formation, compared to existing methods such as pressure pulses and bubble trap implementation has been introduced [4], [6], [8]. The developed test strip addresses the challenge of air bubble formation without the need for intervention post-sample loading. This is ideal especially for the interrogation of biological samples, as the use of pressure pulses may lead to sample interference or damage [5], [7]. Despite the promising results showcased in this study, there are several limitations that should be acknowledged. The experimental setup focused solely on saline samples, therefore further validation using biological samples is necessary to assess real-world applicability. Furthermore, while the proprietary test strips showcased efficacy in mitigating air bubble formation, some microbubbles remained, possibly due to strip-to-strip variations in manufacturing. Therefore, repeatability testing, as well as durability and stability testing of the fabricated test strip is essential prior to practical implementation.

## V. Conclusion

In conclusion, the current study highlights the potential of utilising a combination of hydrophobic and hydrophilic treatments for the development of microfluidic channels, to effectively address the formation of air bubbles. Through the development of the proprietary test strip, significant improvements in air bubble mitigation have been demonstrated, compared to conventional BG test strips. This advancement showcases the potential in enhancing the accuracy and reliability of biosensors that make use of microfluidic channels, particularly within the field of point-of-care diagnostics, where precise measurement of biological samples is essential. Further research will focus on optimisation of the fabrication process and, repeatability and durability testing of the test strips to facilitate practical implementation of the test strips into an innovative microfluidic biosensor.

## REFERENCES


[1] A. G. Niculescu, C. Chircov, A. C. Bîrcă, and A. M. Grumezescu, "Fabrication and applications of microfluidic devices: A review," *Int J Mol Sci*, vol. 22, no. 4, pp. 1–26, Feb. 2021, doi: 10.3390/IJMS22042011.

[2] V. F. Annese and C. Hu, "Integrating Microfluidics and Electronics in Point-of-Care Diagnostics: Current and Future Challenges," *Micromachines 2022, Vol. 13, Page 1923*, vol. 13, no. 11, p. 1923, Nov. 2022, doi: 10.3390/MI13111923.

[3] S. Liu, W. Su, and X. Ding, "A Review on Microfluidic Paper-Based Analytical Devices for Glucose Detection," *Sensors (Basel)*, vol. 16, no. 12, pp. 1–17, Dec. 2016, doi: 10.3390/S16122086.

[4] C. Huang, J. A. Wippold, D. Stratis-Cullum, and A. Han, "Eliminating air bubble in microfluidic systems utilizing integrated in-line sloped microstructures," *Biomed Microdevices*, vol. 22, no. 4, Dec. 2020, doi: 10.1007/S10544-020-00529-W.

[5] L. Guo *et al.*, "A bioinspired bubble removal method in microchannels based on angiosperm xylem embolism repair," *Microsystems & Nanoengineering 2022 8:1*, vol. 8, no. 1, pp. 1–11, Mar. 2022, doi: 10.1038/s41378-022-00367-1.

[6] Y. Wang *et al.*, "Systematic prevention of bubble formation and accumulation for long-term culture of pancreatic islet cells in microfluidic device," *Biomed Microdevices*, vol. 14, no. 2, pp. 419–426, Apr. 2012, doi: 10.1007/S10544-011-9618-3.

[7] C. Lochovsky, S. Yasotharan, and A. Günther, "Bubbles no more: in-plane trapping and removal of bubbles in microfluidic devices," *Lab Chip*, vol. 12, no. 3, pp. 595–601, Jan. 2012, doi: 10.1039/C1LC20817A.

[8] Y. Tokuoka and T. Ishida, "Local Microbubble Removal in Polydimethylsiloxane Microchannel by Balancing Negative and Atmospheric Pressures," *Micromachines 2024, Vol. 15, Page 37*, vol. 15, no. 1, p. 37, Dec. 2023, doi: 10.3390/MI15010037.

[9] W. Zheng, Z. Wang, W. Zhang, and X. Jiang, "A simple PDMS-based microfluidic channel design that removes bubbles for long-term on-chip culture of mammalian cells," *Lab Chip*, vol. 10, no. 21, pp. 2906–2910, Nov. 2010, doi: 10.1039/C005274D.

[10] X. He, B. Wang, J. Meng, S. Zhang, and S. Wang, "How to Prevent Bubbles in Microfluidic Channels," *Langmuir*, vol. 37, no. 6, pp. 2187–2194, Feb. 2021, doi: 10.1021/ACS.LANGMUIR.0C03514/SUPPL_FILE/LA0C03514_SI_005.MP4.

[11] T. T. Huang *et al.*, "Surface-directed boundary flow in microfluidic channels," *Langmuir*, vol. 22, no. 14, pp. 6429–6437, Jul. 2006, doi: 10.1021/LA053465H/SUPPL_FILE/LA053465HSI20060324_104950.PDF.