

3D printed microfluidic chip design for diagnostic studies

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Abstract. In this study, additive manufacturing (3D printing) is utilised to fabricate lateral flow microfluidic chips (LFMC). Our chips were designed using Autodesk design software and printed using a Formlabs 3D printer. They are printed using Formlabs V4 resin polymer. In this work, the design process is highlighted in detail and shows an LFMC design that is made for potential applications in diagnostics studies. Our study also tested the performance of one of the chip designs in actual diagnostics experiment on an optical transmittance setup with a peristaltic pump. The LFMC was integrated onto a custom-built transmittance optical biosensor to measure the transmission intensity. A real-time kinetic study was conducted using an HIV-1 oligonucleotide probe. The study involved performing real-time transmittance analysis by pumping the HIV-1 oligonucleotide probe at different flow rates, ranging from 9.5 $\mu\text{m}/\text{min}$ to 13 $\mu\text{m}/\text{min}$ with intervals of 0.5 $\mu\text{m}/\text{min}$. During the experiment, transmission intensity or transmitted light was measured in real time as the oligonucleotide HIV probe bound to neutravidin immobilised on the Au metal surface. These measurements were recorded using a USB400 spectrometer, with a broadband UV light source that emits wavelengths ranging from 400 to 800 nm. The study underscored the significance of microfluidic chips as devices capable of enhancing the performance of biosensors as well as the use of 3D printing in the design and manufacture of these microfluidic chips.

Keywords: Additive manufacturing, microfluidic chip, optical biosensor, oligonucleotide HIV probe, and kinetic study.

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1 Introduction

Microfluidics is the study of systems, tools, and techniques that manipulate fluids with small sizes ranging from 10^{-9} L to 10^{-18} L [1]. Microfluidic technology allows the creation of devices known as microfluidic chips, which control fluids through microreactors and microchannels [2]. Microfluidic chips are fabricated using different methods, such as 3D printing, etching, lithography, etc. [3]. In this study, 3D printing (additive manufacturing) is used to fabricate microfluidic chips for diagnostic applications [4]. The study underscored the significance of microfluidic chips as devices capable of enhancing the performance of biosensors as well as the use of 3D printing in the design and manufacture of these microfluidic chips. Microfluidic chips offer advantages such as automation, minimal sample consumption, and improved selectivity and sensitivity, contributing thus to advances in biosensor technology [5]. Having a reliable manufacturing process such as 3D printing can help facilitate the manufacture and design of these chips, particularly when looking at point of care applications [6].

Additive manufacturing, also known as 3D printing, fabricates three-dimensional objects layer by layer by deposition of the material [7]. A 3D printing methods became widely used in a variety of industries, including aerospace, automotive and medical science. Using 3D printing is advantageous because it is a simple process that can quickly produce complicated objects. While there are numerous advantages to additive manufacturing, such as stereolithography and materials extrusion (fused deposition modelling), there are also disadvantages, such as gaps in the top layers, mismatched layers, over-extrusion, and restricted item sizes. Depending on the complexity of the construction, a variety of techniques have been used to print 3D structures, including fused deposition modelling, direct ink writing, digital light processing, and stereolithography. The study uses a stereolithography technique that utilises a vat photopolymer to produce models with complicated structures, complex geometry, and micro-sized structures. The models include devices such as microfluidic chips, which are extensively used in computer hardware, biosensing, and cell culture [8]. Stereolithography offers the fastest printing with different resolutions ranging up to 0.001 mm and prints accurate models, smooth surfaces, and the sharpest details on the surface [9]. Stereolithography uses epoxy photopolymer resin to fabricate 3 dimensional objects. Epoxy resin is a biocompatible material used in industries and in research for different functions due to its strong adhesion, thermal stability, chemical resistance, and UV resistance [10].

To date, several materials are used to print 3D structures, including silicon, metals, thermoplastics, carbon-based composites, ceramics, and even living cells, and the plethora of these materials has brought 3D printing to the era of technological advancement and diverse applications [11], [12]. Although 3D printing uses a wide variety of materials, the temperature stability makes some of them difficult to manage or use, which has led to restrictions on the materials that may be used [13], [14]. The study used 3D printing to print lateral flow microfluidic chips (LFMCs) for application in diagnostic setup used for disease detection and monitoring. Microfluidic technology is flexible enough to provide functions that can process biomedical samples with small volumes and many traits. Microfluidic chips can improve the performance of the setup with which they are integrated [15], [16]. For instance, the integration of microfluidics into an optical setup improves sensitivity and reduces noise measurement due to unstable motion of the sample, especially for real-time experiments. Furthermore, the use of microfluidic chips introduces automation and the use

of small samples, which has improved biological instrumentation to handle very small samples [17]. 3D printing is employed to fabricate microfluidic chip for integration onto optical biosensors; the aim is to show the importance of 3D printing and the use of microfluidic chip for diagnostic purposes. The transmittance-based optical setup is used to study the impact of flow rate, the rate at which the fluids travel within the walls of the chip towards the reaction chamber, and the impact on the transmitted light. Furthermore, the obtained measurements help to extract the kinetic parameter, which shows the rate of reaction between the molecules. An Au-coated glass slide functionalised is used to immobilise the neutravidin protein. Neutravidin is a protein that specifically binds to the probe and is used mainly for biosensing and immunological responses. Neutravidin protein was used to capture the HIV-1 oligonucleotide probe. The lateral microfluidic chip is designed to facilitate the whole process of molecular interaction with light and to obtain accurate measurements. The study investigates the significant of diagnostics devices that can improve the performance (automation, low cross-contamination of samples, and sensitivity) of biosensors. Microfluidic devices will enable rapid detection of disease and allow portability of the biosensors which can be used at the point of care setting. The device is also designed to solve insufficient instrumentation for real-time detection and diagnosis of diseases at point of care. To achieve this, 3D printing is used as a mechanism to fabricate these microfluidic so that they can be integrated into biosensors and tested with pseudovirus.

2 Methods and materials

2.1 Design and fabrication of lateral microfluidic chip

The microfluidic chip was designed using Autodesk Fusion 360. A square-shaped chip was designed with a width x breadth of 26 mm x 26 mm and an inlet and outlet (**Figure 1**). The inlet and outlet have an opening diameter of 3 mm. The chip has channels that transport the fluid to the reaction well. The channels are circular with inner diameter of 1 mm and length of 7 mm. The chip is shown in **Figure 1** where (a) shows a 1- dimensional design of the chip, and (b) shows 3-dimesional structure of the chip, (c) and (d) shows the sideview and front view of the printed chip. The bottom of the chip is designed to mount the thin film Au-coated slide. The chip is designed to hold 150 μ L fluids, and the complete design is saved as .stl files and loaded into a 3D printer. Formlab 3B⁺ printer was used to fabricate the designed microfluidic chip. The printer uses Formlabs photopolymer clear resin v4 and it takes 30 minutes to print at a resolution of 0.001 mm and a layer of 300 μ m . After printing, a Lasec peristaltic pump was used to open the channel and remove the uncured resin inside the channel. Once the channels were open; the chip inside was put into the FormWash machine to wash, clean the chip, and remove any resin that was left uncured during printing. The washing process was done for 15 minutes. After washing, the support structures were removed, and the chip was cured inside FormCure for 10 minutes at a temperature of 65 ° C.

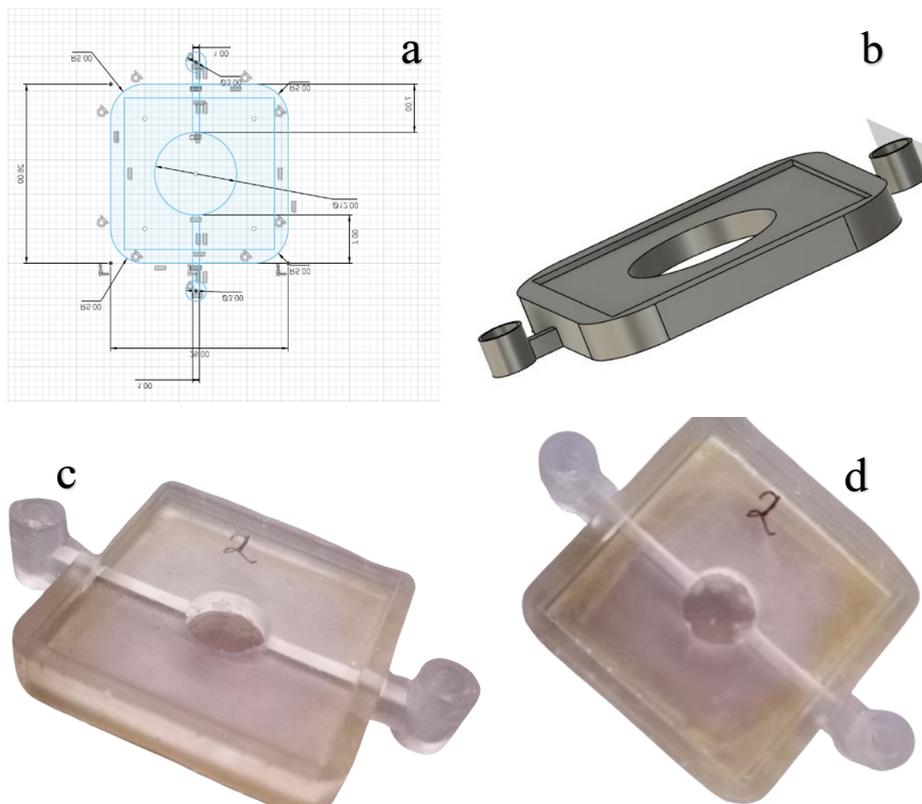


Fig. 1: Design of lateral flow microfluidic chip, (a) 1-dimensional sketch of LFMC in mm, (b) 3-dimensional LFMC extruded from 1D sketch, (c) side view of printed lateral microfluidic chip, (d) front view of printed microfluidic chip

2.2 Integration of the lateral flow microfluidic chip into optical biosensor

The coated thin film slide was mounted to the microfluidic chip using silicon glue and left to dry. After 24 hours the chip mounted with the slide was treated with 75% ethanol, acetone, and water to remove biological contamination [18]. After the chip was pretreated, saline was placed on the surface to prepare the chip for immobilisation of Neutravidin. After immobilisation, the chip was mounted to the transmission optical biosensor using the clamps stage. **Figure 2** displays the steps of the integration of LFMC into the optical biosensor stage for data collection. To flow the fluid inside the chip a peristaltic pressure pump was used to insert the fluid, the fluid was flown at different speeds to find the stable flow rate for reliable results.

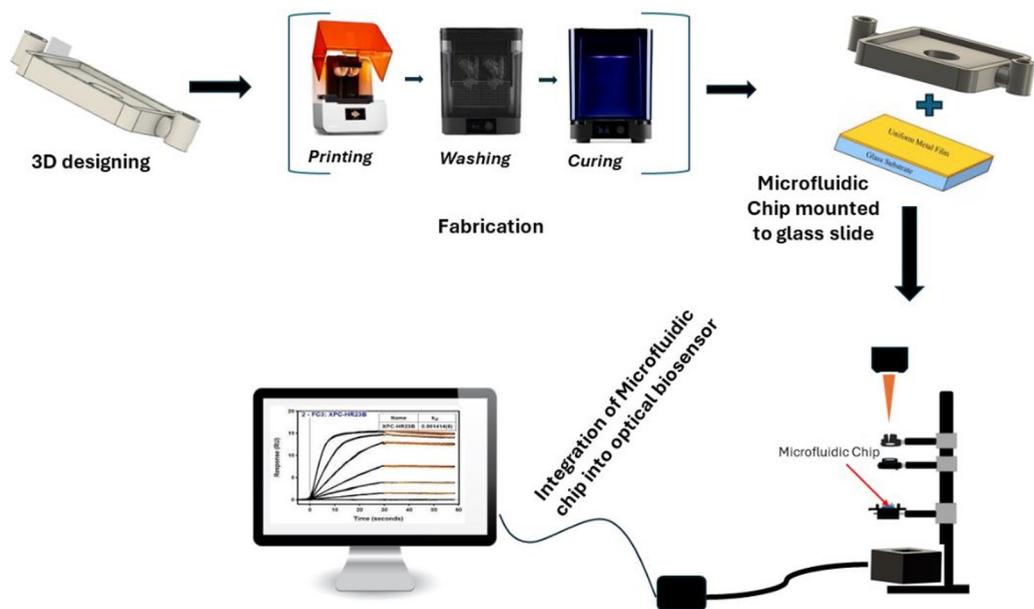


Fig. 2: Integration of the microfluidic chip into the optical biosensor

3 Results and discussion

In this work, a 3D printed lateral flow microfluidic chip was used to perform the transmittance of light on an optical setup. First, the experiment occurred by stabilizing the flow rate using the phosphate buffered saline (PBS). To establish a stable flow rate (one that does not introduce unstable flow and does not introduce much measurement noise) for the transmittance experiment, first, the 150 μL PBS was flowed using a Lasec peristaltic pump, the PBS was pumped at flow rates ranging from 9.5 mL/min to 13 mL/min with intervals of 0.5 mL/min. The PBS was allowed to flow through a range of different flow rates to find the stable flow rate for accurate and stable data acquisition. **Figure 3** shows different results collected at various flow rates ranging from 9.5 to 13.0 mL/min, and the profile indicates stable measurements at 10.5 mL/min. At 10.5 mL/min, there is stable, less noise of transmission of light compared to other flow rates. Next, the transmitted light was investigated by introducing the HIV-1 oligonucleotide probe using a conventional static pump at a flow rate of 10.5 mL/min after determining the steady flow rate, which is at 10.5 mL/min.

The study demonstrated a real-time transmittance-based biosensing experiment using immobilised neutravidin on a glass surface and HIV-1 oligonucleotide probe (**Figure 2**). An HIV-1 oligonucleotide probe solution flows onto a LFMC mounted to a 50-nm Au-coated glass slide functionalised with neutravidin. The mounted glass slide with a layer of gold was part of a transmittance experiment and was used to see if there would be useful diagnostic information or plasmonic effects that could be used. In the experiment, the researchers used PBS solution to baseline the functionalised surface and then let the HIV-1 oligonucleotide diluted in PBS pass through, while tracking the intensity of transmittance in real time. In this

experiment, the data was collected using USB400 spectrometer, and the light source used is broadband white light.

Upon finding the stable flow rate, which is at 10.5 mL/min, the transmission of light was measured by flowing the HIV-1 oligonucleotide probe using a Lasec peristaltic pump at a flow rate of 10.5 mL/min. The HIV-1 oligonucleotide probe binds to Neutravidin immobilised on the surface of the chip. Neutravidin is a good agent for detecting the antigens (HIV-1) because it is flexible to adjust to the density of the antigen. Neutravidin has strong affinity and specificity; HIV-1 oligonucleotide probe binds itself to neutravidin and the binding is studied through the optical properties of light. The optical property used in the study is transmitted light which is recorded when the probe flows through the surface immobilised with neutravidin.

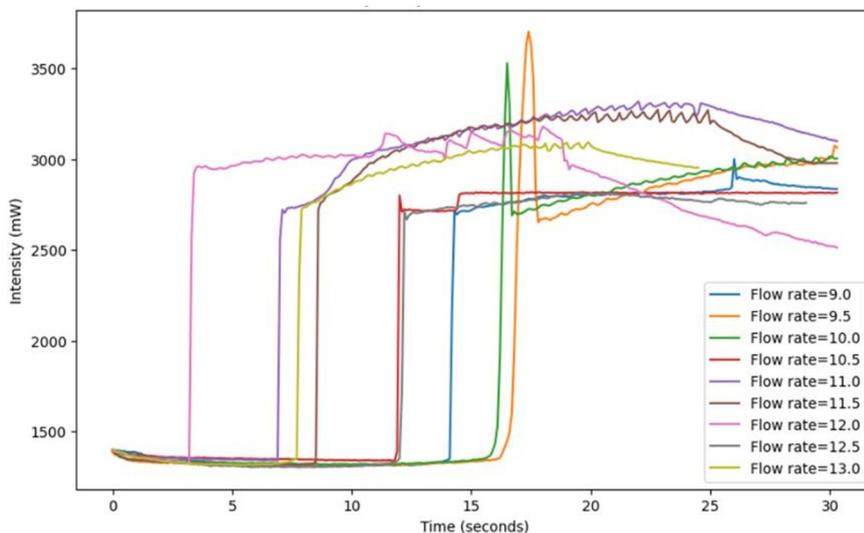


Fig. 3: Stability analysis for different PBS flow rates ranging from 9.0 mL/min to 13.0 mL/min.

Figure 4 shows a transmission profile of intensity vs. time of the 80/20 μL PBS/Probe dilutions, indicating a deep sharp curve. A 100 μL solution containing 80 μL PBS and 20 μL probe. The results depict the occurrence between neutravidin and the 80/20 μL PBS/Probe dilutions when a broadband light of wavelength is exposed; the occurrence is measured as transmitted light which indicates biological interaction as a result of 2 two mediums (the neutravidin the 80/20 μL PBS/Probe). **Figure 4** shows the curve of intensity over time; the result shows a decrease in the intensity of transmitted light when the probe diluted with 80 μL PBS was introduced. Second, the curve is stable, sharp, and deep with less noise; the stable and less noisy results depend on the flow rate and the reaction between the neutravidin/ probe and light.

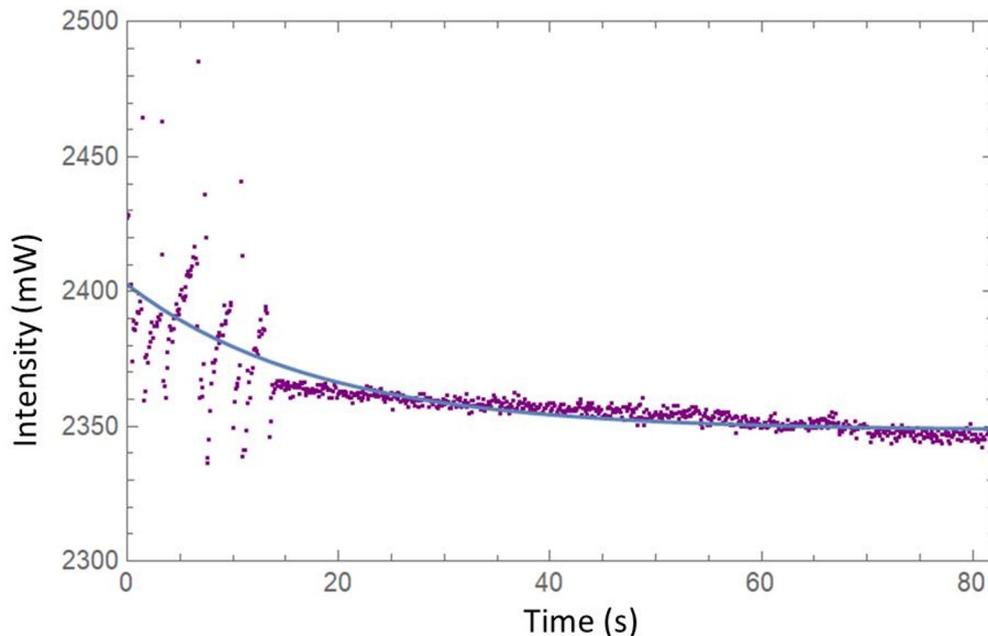


Fig. 4: Transmittance intensity vs time profile of the 80/20 μL PBS/Probe

In an effort to standardise the working operation of the chip, the authors also performed a kinetic interaction analysis, which involved Monte Carlo simulations for data generation and statistical value analysis of the kinetic parameters. The transmission kinetic parameter, which tells us about the interaction between HIV and neutravidin, was extracted from the data. The kinetic parameter is also dependent on the flow rate, and it is a critical parameter for establishing the consistency and repeatability of the experiment. The measured kinetic parameters determine the rate at which the probe was captured immediately when introduced to the functionalised surface. The intensity vs time sensorgram was fitted using the equation $2348.44 + 54.0936 e^{-0.0556033t}$ (an exponential decay fit) and obtained an *r-squared* value of, $R^2 = 0.999$ (recorded in **Table 1**). This tells us that the fit was a good fit. To establish the error in the estimation of the kinetic parameters, Monte Carlo simulations of the sensorgram data were performed and the error was measured as 1.4524410^{-5} .

Table 1: Kinetic parameter fit: Transmittance intensity vs time profile of the 80/20 μL PBS/Probe profile.

Parameter	Value
Kinematic parameters	0.0556033
Coefficient of determination	0.999

4 Conclusions

A transmittance-based experiment was performed using a 3D-printed microfluidic chip integrated onto an optical transmittance setup. A real-time transmittance-based biosensing experiment was demonstrated using immobilised neutravidin and an HIV-1 oligonucleotide probe that can potentially be useful in the diagnosis of HIV. The kinetic study experiment was performed and extracted a transmission intensity's kinetic parameter which is useful for better understanding of the dynamic interactions between HIV and neutravidin on the biosensor surface. A key objective/motivation of this work is to develop affordable and practical point of care diagnostic devices using technologies such as additive manufacturing. The study demonstrated the development of cheaper (compared to commercially available) microfluidic devices (LFMC) which can be useful using additive manufacturing/3D-printing. The lateral microfluidic chip designed in this work could be useful for application in the diagnosis of a variety of other diseases such as HIV-AIDS, and tuberculosis. The results obtained indicate that the device is functioning and performs well on the optical setup. The tested device displays the improvement of the biosensor performance and low noise in data acquisition. This work demonstrates the use of additive manufacturing in developing microfluidic technologies and provides a case study example of the application of such a chip. The authors hope that this work will form building blocks for the development of a simplistic and relatively cheap biosensing setup that can be used in point-of-care setups for rapid disease diagnosis.

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