

DEVELOPMENT OF 3D PRINTED MICROFLUIDIC DEVICE FOR BLOOD TYPING

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biomedical Engineering

by

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DEPARTMENT OF BIOMEDICAL ENGINEERING

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

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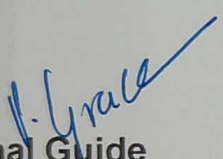
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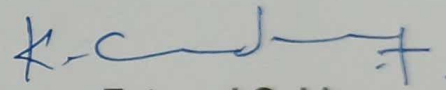
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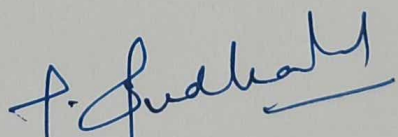
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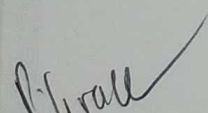

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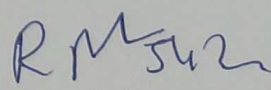
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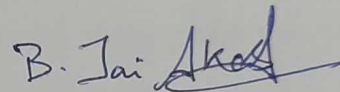
DECLARATION

We **Jai Akash. B (40240011)** and **Srivari Mukunth. S (40240038)** hereby declare that the Project Report entitled "**DEVELOPMENT OF 3D PRINTED MICROFLUIDIC DEVICE FOR BLOOD TYPING**" done by us under the guidance of **Dr. Grace Kanmani Prince M.E., Ph.D** (Internal Guide) and **Dr. K. Gobi Saravanan M.sc., M.Tech., Ph.D**(External Guide) at **Makerspace Lab** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biomedical Engineering.

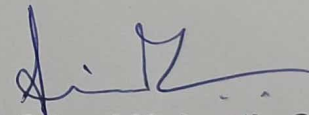
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ABSTRACT

Blood typing is a crucial diagnostic procedure with wide-ranging clinical applications, from blood transfusions to prenatal care. This research describes a unique method for blood typing that combines 3D printing with paper-based microfluidics. 3D printing makes it easier to create specialized, precisely constructed microfluidic devices, which makes it possible to manipulate blood samples and chemicals under control. To precisely identify blood types, a variety of detection methods—including optical, colorimetric, electrochemical, immunofluorescence, and chemiluminescence—are used in conjunction with 3D-printed parts. With potential applications in settings with limited resources and point-of-care diagnostics, these cutting-edge devices provide affordable, portable, and user-friendly alternatives for blood type. The manufacturing, testing and design of 3D-printed paper-based microfluidic platforms for blood type are covered in this article, with an emphasis on how they may improve the usability and effectiveness of blood testing.

Keywords: Blood typing; 3D printing; Microfluidic device; Paper based sensors

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
ABS	ACRYLONITRILE BUTADIENE STYRENE
3D	3 DIMENSIONAL
FFF	FUSED FILAMENT FABRICATION
i3DP	INTEGRATED 3D PRINT
POC	POINT OF CARE
POCT	POINT OF CARE TESTING
RBC	RED BLOOD CELL
PMMA	POLYMETHYL METHACRYLATE
PAE	POLYAMIDE EPICHLOROHYDRIN
PDMS	POLYDIMETHYLSILOXANE

CHAPTER 1

INTRODUCTION

1.1 BLOOD TYPING

Blood typing is a fundamental diagnostic procedure with widespread clinical significance, serving as the cornerstone of various medical interventions, including blood transfusions, organ transplantation, and prenatal care. The accurate identification of blood types, characterized by the presence or absence of specific antigens on the surface of red blood cells, is essential for ensuring compatibility between donor and recipient blood samples, thereby mitigating the risk of adverse transfusion reactions and improving patient outcomes.

Traditionally, blood typing has relied on labor-intensive and time-consuming laboratory techniques, such as agglutination assays performed on glass slides or in test tubes. While these methods are effective in providing accurate blood typing results, they often require specialized equipment, trained personnel, and controlled laboratory environments, limiting their accessibility and applicability in resource-limited settings or remote areas.

In recent years, there has been a growing interest in the development of paper-based microfluidic devices as a promising alternative for blood typing assays. Leveraging the principles of microfluidics and the unique properties of paper substrates, these miniaturized diagnostic platforms offer numerous advantages, including simplicity, portability, cost-effectiveness, and rapid turnaround times.

The integration of reagents onto paper substrates enables the creation of intricate microfluidic channels and detection zones, facilitating the controlled manipulation and

analysis of blood samples with minimal sample volume requirements. Moreover, the porous nature of paper provides an ideal matrix for immobilizing reagents, ensuring stable and long-lasting interactions with target analytes. Despite the significant progress made in the field of paper-based microfluidics for blood typing, several challenges remain to be addressed. These challenges include optimizing the performance and stability of immobilized reagents, enhancing the sensitivity and specificity of detection methods, and overcoming barriers to widespread adoption in clinical practice.

In this thesis, we aim to address these challenges by exploring novel approaches for the design, fabrication, and characterization of paper-based microfluidic devices for blood typing applications. By leveraging advancements in materials science, microfabrication techniques, and assay development strategies, we seek to develop innovative solutions that offer improved performance, reliability, and usability in diverse healthcare settings.

Through a combination of experimental investigations, computational modeling, and theoretical analyses, we endeavor to elucidate the underlying principles governing the behavior of paper-based microfluidic systems and optimize their functionality for practical implementation in clinical diagnostics.

By advancing our understanding of paper-based microfluidics for blood typing, this thesis aims to contribute to the ongoing efforts to democratize access to diagnostic testing, improve patient care outcomes, and address global healthcare challenges. Through interdisciplinary collaboration and innovation, we aspire to pave the way for the development of next-generation diagnostic technologies that are accessible, affordable, and adaptable to the needs of diverse populations worldwide.

Blood typing is a critical procedure in healthcare, transfusion medicine, and emergency situations, as it determines an individual's blood group and Rh factor. Accurate and rapid blood typing is essential to ensure patient safety during transfusions and to guide medical treatment decisions. Conventional blood typing methods are often time-consuming, require specialized equipment, and may not be readily available when resources are scarce (**wang et al., 2020**). To address these challenges and revolutionize blood typing, researchers have turned to the emerging field of microfluidics and additive manufacturing techniques, particularly 3D printing(**Gonzalez et al., 2022**). Microfluidic devices have attracted a lot of interest in recent years due to their ability to control small amounts of liquid with precision, providing a variety of diagnostic applications(**Zhong et al., 2019**). Among these, paper-based microfluidic devices have developed into a promising POC platform in blood typing due to their portability, affordability and simplicity(**Faustino et al., 2016**). These devices utilize the natural wicking properties of paper to transport and mix reagents and blood samples within microscale channels, facilitating rapid and reliable blood typing. This paper aims to explore the fusion of two cutting-edge technologies: paper-based microfluidic devices and 3D printing, in developing a novel approach for blood typing(**Chan et al., 2017**).

In this context, we will delve into the fundamental principles of blood typing, the advantages and limitations of existing methods, and the recent advancements in paper-based microfluidics and 3D printing technologies. We will also discuss the potential impact of this innovative approach on blood typing in various healthcare settings, including remote or resource-constrained environments, emergency response situations, and routine clinical practice. By harnessing the synergy between paper-based microfluidic devices and 3D printing, this paper sets the stage for a paradigm shift in blood typing, offering faster, cost-effective, and more accessible solutions that have the potential to save lives and enhance healthcare delivery worldwide.

CHAPTER 2

REVIEW OF LITERATURE

Anushka *et al.*, (2022) This assessment places special emphasis on the technology's use in the fields of energy production, food quality control, environmental monitoring, and medical diagnosis. Theoretical fluid flow analysis in porous media is also presented in this review for effective fluid management and control. With an emphasis on the worries over the transition of such devices from the laboratory to the user, the limitations of PAD have also been highlighted.

Chan *et al.*, (2017) The authors of this review have concentrated on presenting recent research that uses 3D printing to construct POCT devices, emphasizing its usefulness across all analytical processes. Additionally, we go over the major benefits of incorporating 3D printing into the device development process and pinpoint exciting prospects for 3D printing applications that can improve applications for global health.

Chang *et al.*, (2018) In order to assess the level of red blood cell (RBC) agglutination, a small amount of blood sample is used in this study to demonstrate a microfluidic blood typing system. Impedimetric measurement and electroanalytical measurement were the two suggested measurement techniques. The power parameter in the electroanalytical measurement and the charge transfer resistance in the impedimetric measurement were both employed to analyze the agglutination level. Both measurement techniques produce quantitative data based on the experimental findings, and the parameters are directly and monotonically related to the level of RBC agglutination.

Das *et al.*, (2022) The fundamental concepts and elements involved in the design of paper-based devices were then succinctly explained in the review. Additionally, numerous applications from diverse sectors were also assembled in text form under

Various sections and tables. Paper-based analytical devices may be a great tool for a range of analytical tasks because these methods are easy, quick, affordable, and demand little in the way of human effort or special training. They are widely used in the examination of biological fluids, medications, foods, colorants, biomarkers, and many other things.

Duong *et al.*,(2019) To test the effectiveness of the suggested plan, the authors constructed a microfluidic chip with an S-microchannel to characterize the bonding protocol and two application-oriented microfluidic chips, a 3D split-and-recombine-based passive micromixer and an integrated microchip for mixing two streams of liquid before the formation of double-emulsion droplets. It was confirmed that using this bonding technique, it is possible to create optically detectable 3D-printed microfluidic devices with a bonding strength of at least eight bars between PMMA/ABS substrates.

Espinosa et al.,(2022) Through the use of wax filament and 3D printing, the authors of this work have created an alternative manufacturing process for paper-based microfluidics. Microfluidic paper-based analytical devices (PADs) are inexpensive, simple to make, and utilized for a variety of chemical and biological assays and investigations. Wax-based microfluidics on paper have been rare because most wax printing equipment has been retired by the makers. They want to create an affordable manufacturing process that can take the place of traditional wax-on-paper-based microfluidic manufacturing techniques. They have produced hydrophobic wax barriers on the surface of various paper kinds using widely accessible commercial 3D printing technology and wax filament.

Gonzalez *et al.*, (2022) By bringing quicker and less expensive methods of producing microdevices, polymeric 3D printing is a promising technology that has the potential to completely change the microfluidic area. These technologies, particularly FFF, i3Dp, and VP-3D printing, are also creating new domains of use, commercialization tactics, and production techniques for such devices. Despite 3D printing's anticipated promise, there are a few drawbacks that must be resolved before it can compete with established technologies.

Shangguan *et al.*, (2017) The authors of this work looked into how adding the wet strength agent PAE affected paper's ability to function as a bioactive substrate for a paper-based blood-typing device. The apparent thickness of hardwood and softwood sheets produced with 0.5–2% PAE increased. These alterations might be brought on by the fibers potential flocculation with the addition of PAE. The study also revealed an unexpected benefit of using PAE: a slight delay in the time required for water to penetrate the paper delays the drying of the blood sample at the drop's edge, improving the clarity of negative assays by lessening the development of ring-shaped patterns.

He *et al.*, (2016) The μ 3DPAD, a novel capillary-driven microfluidic analytical device, is introduced in this paper as a useful complement to the μ PAD in many applications thanks to its benefits in flow speed control, ease of construction, and ease of encapsulation. In order to achieve flow rate programming, it was necessary to investigate the relationship between channel depth and flow time. The results of this inquiry showed that it is simple to control the flow speed within a particular range using this method.

Hiltunen *et al.*, (2018) In this study, paper with an aluminium (Al) coating served as a stable platform for R2R processing. The paper carrier was treated with an R2R atmospheric corona unit and

R2R gravure coated with an adhesion promoter prior to the creation of PDMS structures. To create a continuous coating of uncured PDMS, uncured PDMS was dispensed over the running web and the meandering dispense pattern was leveled with a spiral bar.

Huet *et al.*, (2018) The authors of this work have discussed the creation of an RBC agglutination assay for point-of-care blood type. Anti-A or anti-B dried reagents were placed inside the microchannel of an injection molded microfluidic chip that was created to improve capillary flow. The single blood handling step in the test methodology involved placing a blood drop at the biochip's tip, after which imaging was possible. The ability of the implanted reagents to initiate RBC agglutination in-situ allowed us to see the entire procedure in real time. To calculate a real-time agglutination indicator using diluted blood, an image processing method was created and afterwards confirmed using undiluted blood.

Lim *et al.*, (2019) The authors of this paper have covered a variety of 2D and 3D construction techniques, flow control strategies, and applications of PADs, which exhibit a number of benefits like affordability, rapid response utilizing little volume, simplicity, and biodegradability. Parallel to the development of several microfabrication techniques, the fabrication of PADs has evolved into 3D procedures, which are capable of complicated problem-solving for numerous sequential processes. Increasing reaction speed and sequential administration of reagents with free direction are two additional benefits of 3D PADs. Stacking, folding, and a combination of 2D production methods are required for the straightforward fabrication procedures.

Lin *et al.*, (2020) A multipurpose, portable, and disposable microfluidic system for blood type and initial screening of blood disorders has been demonstrated by the authors in this work. Injection of whole blood cells interacts with preloaded antibodies (anti-A, anti-B, and anti-D) to trigger an agglutination reaction that plugs a microslit in the microfluidic channel, causing red blood cells to collect and produce a visible red line that can be quickly read to establish the blood type.

Morbioli *et al.*, (2017) This article presents a critical review of recent developments and challenges of colorimetric detection on microfluidic paper-based analytical devices (μ PADs), and present thoughts and insights towards future perspectives in the area to improve the use of colorimetric readouts in conjunction with μ PADs. here are also multiple variables that impact the analysis of colorimetric reactions in microfluidic paper-based systems, including color homogeneity, image capture methods, and the data handling itself.

Nishat *et al.*, (2021) This review provides an overview of current methods of fabricating paper-based microfluidic devices, examples of applications of these devices, a discussion of their current limitations, and an outlook on their future. The creation of point-of-care (POC) diagnostic devices, which potentially do away with the need for expensive and time-consuming laboratory-based analytical procedures, is the most common application of paper-based microfluidic devices.

Noviana *et al.*, (2020) This study seeks to describe the distinctive properties of microfluidic paper-based analytical devices (PADs), which are advantageous for pharmaceutical analysis, and to assess the cutting-edge technologies and uses of the platform for drug analysis in both academic and practical contexts. The field's present difficulties and potential future prospects are also mentioned. Over the past ten years, there has been an increase in interest in using paper microfluidics to conduct chemical assays for diverse analytical purposes.

The qualitative and quantitative analysis of pharmaceuticals is one of the platforms' expanding applications. The development of analytical tools capable of on-site drug screening is attracted by the low cost and self-pumping capabilities of paper microfluidics.

Preetam *et al.*, (2022) The developments in microfluidics for biomedical engineering, including its use in bioassays, bio-fabrication, and drug delivery, put the spotlight on the fundamental concepts and emerging research directions in the discipline. To determine whether novel microfluidic techniques for clinical research offer valuable capabilities, they should be compared to more established techniques. New real-world applications will be made possible by the developing microfluidics technologies, including the use of cutting-edge industrial procedures and customized medicines and diagnostics for med-tech. Although microfluidics has made great progress, there are still several obstacles to the efficient and thorough integration of such frameworks into applications, such as routine clinical diagnosis.

Quraishy *et al.*, (2016) Molecular testing for blood group antigens has improved our understanding of red cell alloimmunization, according to the review's findings. This knowledge has also been applied to patient care, particularly in the management of chronic transfusion therapy. Phenotype matching of transfused RBC units is made possible by molecular testing of blood donors and recipients, and the idea of allele-matched transfusions for some patients is not implausible. The advancements made so far could be considered the beginning of a revolution in clinical transfusion medicine. Technological advancements must be coupled with initiatives to boost blood donation, particularly among minority populations, and to ensure consistent, coordinated patient care across institutions if this revolution is to continue.

Shahrubudin *et al.*, (2019) The overview of the various 3D printing technologies, applications of the technology, and materials utilized in the manufacturing business are all covered in this article. By gradually adding materials, digital fabrication technology—also known as 3D printing or additive manufacturing—creates tangible items from a geometric representation. A rapidly developing technology is 3D printing. The use of 3D printing is very common these days. In the fields of agricultural, healthcare, the automotive, locomotive, and aviation sectors, 3D printing technology is increasingly employed for mass modification and fabrication of any kinds of open source designs. Layer by layer, material can be deposited to create an object using 3D printing technology and a computer-aided design (CAD) model.

Shah *et al.*, (2021) With an emphasis on recent publications, this study examines inkjet printing technologies' classifications and uses. Several inkjet printing techniques are covered, along with the various design strategies, applications, and research advancements. Due to its dependability and capacity to work with a wide variety of inks, the piezoelectric inkjet printing technique is the one that receives the most attention among them. Applying a voltage waveform to a piezoelectric membrane causes it to move, activating a piezo-driven inkjet printhead. The waveform makes sure that the intended droplet and a stable jet form. Recent studies answers to the problems of printing speed, stability, and quality are reviewed. The development of high-viscosity inkjet printing processes is examined, and the uses of inkjet printing for textile, displays, and wearable technologies are discussed.

Farhan, M. A. (2020) This article discusses the colorimetric detection of metal ions and nitrites from diverse sources utilizing a straightforward filter paper (Whatman®) as a "PAD." Since filter paper is white in color, the potential for liquid movement by capillary action, and high surface area enables good detection efficiency. An attempt has been made here to critically analyze the current state and potential of this technique in light of its significance as an analytical tool.

Strong *et al.*, (2019) The current work illustrates that wax-printed paper can be reduced in size by using periodate to create microPADs with improved fidelity and resolution. MicroPADs were submerged in various concentrations of aqueous sodium periodate (NaIO_4) for varying amounts of time in order to discover the ideal miniaturization parameters. Depending on the amount of periodate present and how long it took for the reaction to take place, this treatment reduced the surface area of microPADs by up to 80%. MicroPADs were reduced in size by 78% after being submerged in 0.5-M NaIO_4 for 48 hours. This process made it possible to create functional channels with a width of as little as 301 μm and hydrophobic barriers with a width as tiny as 387 μm . The compact devices have been demonstrated to work with redox-based colorimetric tests and enzymatic reactions.

Yang *et al.*, (2022) The authors have examined current developments in the field, such as ink characteristics, structural resolutions, and hierarchical degrees of integration with functional platforms, and summarized the significant figures of merit of 3D printed microfluidics in this review. They have specifically emphasized improvements in printing techniques with higher levels of automation and precision, direct printing of microfluidics on diverse 3D surfaces, and microfluidic devices printed with thermosetting elastomers. A future that is rapidly approaching in which these adaptable devices might be released from microfabrication facilities and made on demand by users in random settings with little to no training is suggested by the significant improvements in performance and multifunctionality of 3D printed microfluidics.

Thaweskulchai *et al.*, (2021) In this study, the feasibility of employing a low-cost 3-in-1 3D printer for the production of microfluidic devices was investigated. For the formation of microchannel chips and base plates, each of the three accessible functions—3D printing, CNC milling, and laser engraving—was used individually. There is proof that all three tasks can be implemented using functional microfluidics, albeit with varied degrees of effectiveness.

The availability of three micromachining techniques in a low-cost workstation has benefits but necessitates trade-offs.

Faustino *et al.*, (2016) The achievements and difficulties of each component, including sample collection/pretreatment, signal transduction and amplification, and results output, are highlighted in this review, with a focus on the most recent advancements of integrated PADs. In addition, we provide a summary of the emerging trend of unique integrated PADs and examine current difficulties as well as future perspectives for integrated PADs for point-of-care testing (POCT).

CHAPTER 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

The aim is to fabricate a cost-effective, portable, and easy-to-use microfluidic device for blood typing using 3D printing technology.

The device would be designed to use small amounts of blood and would be able to process the sample rapidly and accurately.

Design and fabrication of the microfluidic device includes the use of 3D printing techniques to create the necessary channels and features on the device. It would also discuss the selection of appropriate reagents and antibodies for use in the blood typing process, as well as the development of a reliable detection system.

This report would also involve testing the performance of the device, including its accuracy and sensitivity compared to traditional blood typing methods. The potential for the device to be used in point-of-care settings or in resource-limited environments would also be explored in future studies.

Overall, the aim and scope of this research work is to present a novel approach to blood typing using a 3D-printed, microfluidic device that offers a low-cost, portable, and easy-to-use alternative to traditional methods.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS:

- Blood Samples
- Reagents used for blood typing (anti-A, anti-B and anti-D)
- Whatman Filter paper
- 3D printer material - Acrylonitrile Butadiene Styrene (ABS), QSR support
- Micropipettes

4.2 METHODS:

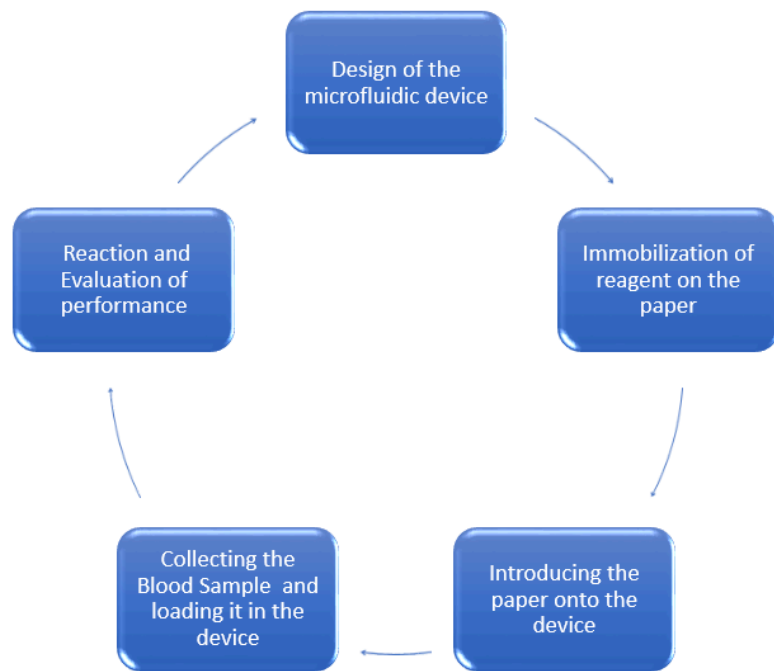


Figure 4.1 Block diagram of methodology

4.2.1 Design of the microfluidic device:

- Design the device layout using computer-aided design software called CATIA V6 3D EXPERIENCE.

- The device is printed using a biocompatible material called Acrylonitrile Butadiene Styrene (ABS)
- Fabrication of the microfluidic device is done using STRATASYS F370 3D printer which is shown in the Figure 4.2



Figure 4.2 STRATASYS F370 3D printer

4.2.2 Immobilization of reagent on the paper:

- Dilute the blood typing reagents to the desired concentration in a suitable buffer solution (e.g., PBS).
- Apply a small volume (typically a few microliters) of each reagent onto specific spots on the paper strip (Whatman Filter paper).
- Allow the applied reagents to air-dry on the paper. This will create spots of dried reagents on the paper.

- Place the paper strips with the immobilized reagents into the freeze-dryer.
- The process involves freezing the samples and then subjecting them to a vacuum to remove moisture under low temperature conditions.

4.2.3 Introducing the paper onto the device:

- Cut the reagent immobilized Whatman paper with the desired size and shape for the device.
- This device will be the carrier for the immobilized reagents.

4.2.4 Collecting the blood sample and loading it in the device:

- The blood samples are collected from the volunteers having different blood groups.
- The collected blood samples are loaded onto the device.

4.2.5 Reaction and Evaluation of the performance:

- The blood flows through the microfluidic channel and reacts with the reagent immobilized Whatman paper.
- The reaction which occurs is agglutination which helps in determining the blood type of the individual.

4.2.6 Immobilization beads by sodium alginate:

- For the preparation of beads ,
- sodium alginate 0.5g was weighed and mixed with 5 mL distilled water until it dissolved.
- 2mL of the sample was dissolved in 5 mL sodium alginate solution.
- In a separate beaker, 0.5 g of calcium chloride was dissolved in 5mL distilled water.
- Sodium alginate which consisted of the dissolved sample was poured into calcium chloride dropwise beads were formed.

CHAPTER 5

RESULT AND DISCUSSION

5.1 DESIGN OF THE MICROFLUIDIC DEVICE:

5.1.1 Challenges in Model 1:

In order to optimize the ideal 3D prototyping microfluidic channel, the microchannel diameter was set at 1mm, wherein, an unexpected obstruction was observed in the microfluidic channel caused by the support material which was very tedious to remove by post processing process. Here's a detailed explanation of the issue and its consequences:

- **Microchannel Diameter:** The microchannel is a crucial component of the microfluidic chip, responsible for guiding the flow of fluids, such as blood samples, through the device. In this case, the microchannel was designed with a diameter of 1mm, presumably to accommodate the intended flow of blood samples.
- **Support Material Struck in Microchannel:** During the 3D printing process, support material is often used to provide structural integrity for overhanging features or intricate geometries. However, in this instance, the support material became stuck within the microchannel, obstructing the passage of fluids.
- **Obstruction Consequences:** The presence of support material within the microchannel prevented the intended flow of blood samples through the device. As a result, the functionality of the microfluidic chip was compromised, rendering it unable to perform its intended purpose of guiding and manipulating blood samples for analysis.
- **Flow Impediment:** The presence of the support material created a physical barrier within the microchannel, effectively blocking the flow of fluids. This impediment disrupted the fluid dynamics within the microfluidic chip, preventing the accurate execution of blood typing assays or other diagnostic procedures.
- **Resolution and Mitigation:** To address this issue, it may be necessary to redesign the microfluidic chip, considering factors such as the size and

placement of microchannels relative to the support structures. Alternatively, adjusting the printing parameters or using different support materials that are easier to remove or less likely to interfere with the microchannels could help mitigate this problem in future iterations of the design. The fabricated microfluidic device is shown in Figure 5.1.

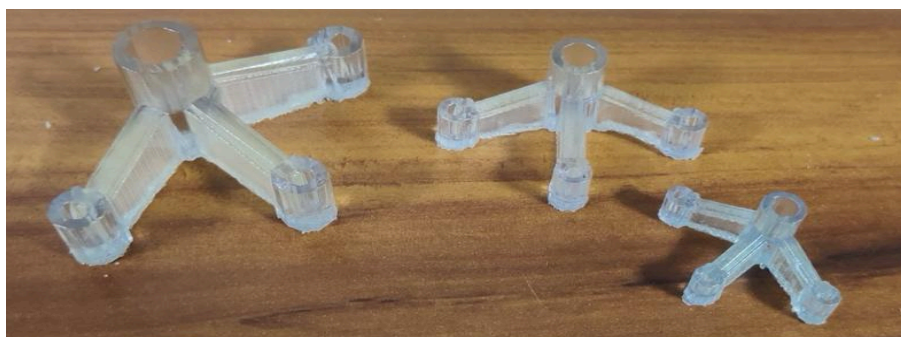


Figure 5.1 Model 1

In summary, the inadvertent obstruction of the microchannel by support material during the 3D printing process represents a significant design flaw that hinders the functionality of the microfluidic chip. Addressing this issue requires careful consideration of design parameters and printing conditions to ensure unimpeded fluid flow and optimal performance of the device.

5.1.2 Challenges in Model 2:

The issues encountered in the second model are primarily related to the design parameters and material properties, leading to difficulties in observing blood agglutination and rendering the model impractical for use (as shown in Figure 5.2). Let's elaborate on these problems:

- **Inadequate Well Diameter:** The small diameter (3mm) of the surrounding wells may have limited the diffusion of blood samples and reagents, impacting the visibility and efficacy of the agglutination reaction. Insufficient space within the wells can hinder proper mixing and interaction between the components, resulting in reduced sensitivity and accuracy of the blood typing assay.

- **Visibility of Agglutination:** The limited size of the wells may have made it challenging to observe the agglutination reaction clearly. Proper visualization is essential for accurate interpretation of the results, and the small diameter may have hindered the ability to discern subtle changes indicative of agglutination.
- **Material Porosity:** The porosity of the material used to fabricate the model presents another challenge. Porous materials can absorb fluids, leading to unpredictable interactions and potentially affecting the performance of the device as shown in Figure 5.3. In the context of microfluidic devices, material porosity may interfere with fluid flow dynamics, compromising the reliability and reproducibility of experimental results.
- **Practical Usability:** The combination of small well diameter and material porosity renders the model impractical for real-world applications. The challenges associated with observing agglutination and potential interference from material properties undermine the usability and reliability of the device in clinical or research settings.

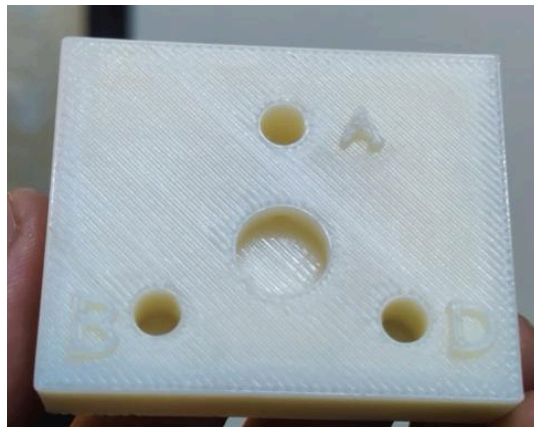


Figure 5.2 Model 2

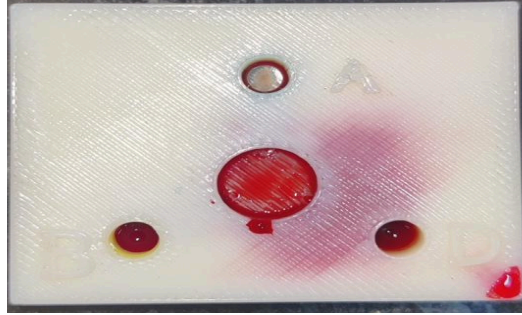


Figure 5.3 Blood leakage inside the device due to porosity

5.1.3 Challenges in Model 3:

The improvements made in the third model address the issues encountered in the previous iterations, enhancing the functionality and usability of the microfluidic chip for blood typing assays.

- **Increased Well Diameter:** By increasing the diameter of the surrounding wells, we have provided more space for fluid samples and reagents, facilitating better mixing and interaction during the blood typing process. This enhancement allows for improved visibility of agglutination reactions, making it easier to observe and interpret results accurately.
- **Reduced Material Porosity:** Minimizing the porosity of the material used to fabricate the microfluidic chip helps prevent unwanted fluid absorption and interference, ensuring consistent and reliable performance. Non-porous materials maintain the integrity of the microfluidic channels and wells, preserving fluid flow dynamics and minimizing the risk of experimental artifacts.
- **Optimized Well Depths:** The differential depths of the main well (5mm) and the surrounding wells (8mm) contribute to efficient fluid flow throughout the microfluidic chip. The deeper main well provides a reservoir for the blood sample, while the shallower surrounding wells allow for controlled distribution

of the sample to multiple testing regions. This design feature ensures uniform access to the surrounding wells, facilitating comprehensive blood typing analysis.

- **Enhanced Fluid Flow:** The optimized well depths and increased well diameters promote proper fluid flow throughout the microfluidic chip, enabling efficient transport of blood samples and reagents. This design improvement minimizes the risk of sample cross-contamination and ensures accurate delivery of fluids to each testing region, enhancing the reliability and reproducibility of blood typing assays.
- **Improved Usability:** By addressing the shortcomings of the previous models, the third iteration of the microfluidic chip offers enhanced usability and practicality for blood typing applications. The optimized design parameters and material properties contribute to a more robust and user-friendly device, facilitating seamless integration into clinical and research workflows.

Overall, the enhancements made in the third model represent significant advancements in microfluidic chip design, enabling more accurate, reliable, and efficient blood typing assays as shown in Figure 5.4.



Figure 5.4 Model 3

5.2 IMMOBILIZATION OF REAGENT ON THE PAPER:

Immobilizing blood typing reagents on Whatman paper is a crucial step in the development of paper-based microfluidic devices for blood typing assays. Here's an elaboration on the process of immobilizing blood typing reagents on Whatman paper:

- **Selection of Blood Typing Reagents:** The first step is to select the appropriate blood typing reagents for the specific blood typing assay being performed. These reagents typically include antibodies specific to blood group antigens, such as anti-A, anti-B, and anti-Rh(D) antibodies.
- **Activation of Whatman Paper Surface:** The surface of the Whatman paper needs to be activated to facilitate the binding of blood typing reagents. Activation can be achieved through various methods, including chemical treatments or physical processes such as plasma treatment. Activation creates functional groups on the paper surface, such as carboxyl or amino groups, which can covalently bind with the reagents.
- **Preparation of Blood Typing Reagents:** The selected blood typing reagents are prepared for immobilization. This may involve conjugating the antibodies with labeling molecules or functional groups that facilitate binding to the activated paper surface.
- **Immobilization Method:**
 - Direct Adsorption: Blood typing reagents can be directly applied to the activated paper surface and allowed to adsorb through physical interactions such as van der Waals forces or hydrogen bonding as shown in Figure 5.5.
- **Optimization:** The immobilization process may require optimization to ensure efficient binding of blood typing reagents while maintaining their activity and specificity. Factors such as reagent concentration, incubation time, and temperature may need to be optimized through experimental testing.

- **Drying and Stabilization:** After immobilization, the Whatman paper is typically dried to remove excess solvent and stabilize the immobilized blood typing reagents. Care must be taken to avoid denaturation or loss of activity during the drying process.
- **Storage and Handling:** Proper storage conditions, such as humidity control and protection from light, should be implemented to preserve the stability and functionality of the immobilized blood typing reagents.
- **Quality Control:** Immobilized blood typing reagents should undergo quality control testing to ensure their stability, specificity, and activity before use in blood typing assays.

By carefully controlling the immobilization process, Whatman paper can serve as an effective substrate for immobilizing blood typing reagents in paper-based microfluidic devices, enabling rapid and reliable blood typing assays.

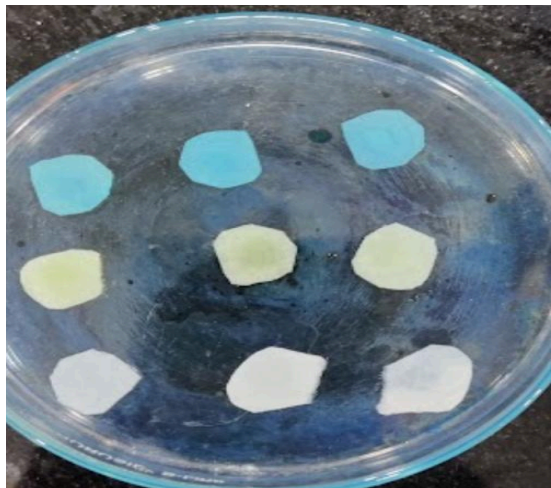


Figure 5.5 Reagent immobilized whatman paper

5.3 INTRODUCING THE PAPER ONTO THE DEVICE:

- Preparation of Reagent-Immobilized Whatman Paper:

The Whatman paper, carefully selected for its absorbent properties and compatibility with blood typing reagents, is immobilized with specific antibodies tailored for blood typing. Anti-A, anti-B, and anti-Rh(D) antibodies are meticulously applied onto the paper surface using optimized immobilization techniques.

The immobilized Whatman paper undergoes thorough drying and conditioning to maintain the stability and activity of the immobilized reagents, ensuring optimal performance during blood typing assays.

- Preparation of Microfluidic Device:

The microfluidic device, meticulously crafted according to design specifications, awaits the integration of functional components for blood typing assays. The substrate layers, fluidic channels, and detection zones are meticulously assembled to ensure precision and accuracy in sample processing.

- Placement of Reagent-Immobilized Whatman Paper:

With utmost care and precision, the reagent-immobilized Whatman paper is delicately positioned onto the designated region of the microfluidic device. Aligning the paper with the fluidic channels and detection zones is crucial for optimal sample processing and accurate analysis.

- Securement and Integration:

Using specialized adhesive tapes designed for microfluidic applications, the reagent-immobilized Whatman paper is securely affixed onto the microfluidic device. The integration is meticulously executed to ensure stability and prevent displacement during subsequent handling and operation.

- **Verification of Integration:**

A meticulous visual inspection is performed to verify the secure integration of the reagent-immobilized Whatman paper onto the microfluidic device. Functional testing is conducted to confirm proper alignment and functionality, ensuring readiness for blood typing assays.

- **Validation and Calibration:**

Prior to conducting blood typing assays, rigorous validation and calibration procedures are undertaken to ensure the integrity and accuracy of the integrated reagent-immobilized Whatman paper. Control experiments and calibration tests are meticulously performed to validate the functionality and reliability of the immobilized reagents as shown in Figure 5.6.

With the successful integration of reagent-immobilized Whatman paper onto the microfluidic device, the stage is set for accurate and reliable blood typing assays. The meticulous execution of each step ensures optimal performance and enhances the utility of the microfluidic device in diagnostic applications.

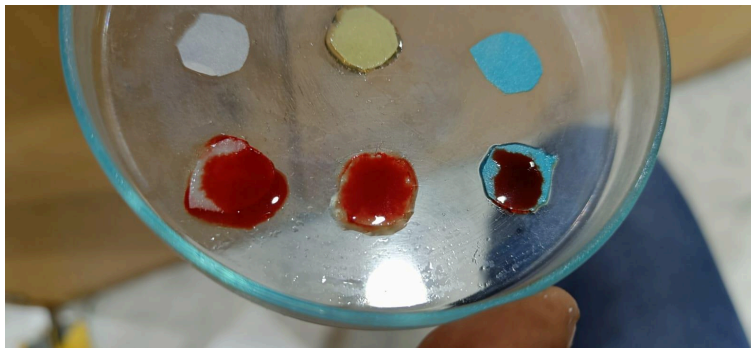


Figure 5.6 reagent immobilized paper

5.4 Reaction and Evaluation of the performance:

- Sample Introduction:

The blood sample, obtained from a donor or patient, is introduced into the microfluidic device through the designated inlet port. Care is taken to ensure precise loading of the sample into the microfluidic channels to avoid spillage or cross-contamination.

- Flow through Microchannels:

As the blood sample flows through the microchannels of the microfluidic device, it encounters the immobilized reagents on the Whatman paper. These reagents include antibodies specific to blood group antigens (anti-A, anti-B, anti-Rh(D)).

- Agglutination Reactions:

Upon encountering the immobilized antibodies, the blood sample undergoes agglutination reactions if it contains the corresponding antigens.

For example:

If the blood sample contains antigen A, it will agglutinate when it encounters immobilized anti-A antibodies.

Similarly, blood samples containing antigen B or Rh(D) will agglutinate when they encounter immobilized anti-B or anti-Rh(D) antibodies, respectively.

- Observation of Agglutination:

The agglutination reactions manifest as visible clumping or aggregation of red blood cells within the microfluidic channels. These reactions are observed visually under a microscope or with the naked eye, depending on the sensitivity of the assay and the equipment available.

- Evaluation of Results:

The presence or absence of agglutination in the microfluidic channels is carefully evaluated to determine the blood type of the sample. Positive

agglutination indicates the presence of specific blood group antigens, while negative agglutination suggests their absence.

By comparing the observed agglutination patterns with established blood typing standards, the blood type of the sample (e.g., A, B, AB, O) and its Rh factor (positive or negative) can be accurately determined as shown in Figure 5.7.

- **Quality Control and Validation:**

Rigorous quality control measures are implemented to ensure the accuracy and reliability of the blood typing assay. Control experiments with known blood samples of different types are performed to validate the performance of the microfluidic device and confirm the specificity of the immobilized reagents.

Any deviations or inconsistencies in the agglutination patterns are carefully investigated, and corrective actions are taken to address potential issues such as improper sample handling or device malfunction.

- **Data Analysis and Interpretation:**

The observed agglutination patterns are documented and recorded for further analysis. Data analysis may involve quantifying the extent of agglutination or comparing the results with those obtained from conventional blood typing methods.

The interpreted blood typing results are communicated to relevant healthcare professionals or personnel for further clinical or research purposes, contributing to informed medical decision-making and patient care.



Figure 5.7 Reaction of blood showing the result for B+ve group

5.5 IMMOBILIZATION BEADS:



Figure 5.8 Preparation of beads

- **Bead Selection and Activation:**
Porous beads with suitable size and porosity are selected based on compatibility with the reagents and their ability to retain immobilized molecules. Beads are activated by treating them with appropriate chemicals or plasma to introduce functional groups on their surface, facilitating reagent binding.
- **Preparation of Reagents:**
Blood typing reagents, such as anti-A, anti-B, and anti-Rh(D) antibodies, are prepared in appropriate concentrations and buffer solutions.
- **Immobilization of Reagents:**
The activated beads are mixed with the prepared reagent solutions, allowing the reagents to bind to the surface of the beads.
The mixture is incubated under controlled conditions (e.g., temperature, pH) to promote efficient binding of the reagents to the beads.
- **Storage and Stability Assessment:**
Immobilized beads are stored under appropriate conditions to maintain the stability and activity of the immobilized reagents.
- **Integration into Blood Typing Assays:**
Immobilized beads are integrated into microfluidic devices or assay platforms designed for blood typing applications and preparation of beads is shown in Figure 5.8.
During blood typing assays, the beads are introduced into the assay system, where they interact with blood samples and facilitate agglutination reactions.
- **Evaluation of Blood Typing Results:**
Agglutination reactions observed in the presence of immobilized beads are evaluated to determine the blood type of the sample as shown in Figure 5.9.
The efficiency and specificity of the immobilized reagents contribute to accurate and reliable blood typing results.

- Optimization and Iterative Improvement:

The immobilization procedure may be optimized through iterative refinement based on feedback and performance evaluations.

Continuous improvement ensures the reliability, accuracy, and usability of the immobilized beads for blood typing and other diagnostic applications.



Figure 5.9 Reaction of blood with immobilized beads showing B+ve blood group

CHAPTER 6

SUMMARY AND CONCLUSION

Through our project, we have observed significant differences in the duration of agglutination between paper-based microfluidic chips and immobilized beads for blood typing assays.

In the paper-based microfluidic chips, we found that agglutination remained visible for up to 7 days, indicating the prolonged stability and durability of the immobilized reagents within the paper matrix. This extended duration of agglutination suggests the potential for reliable and long-lasting results, making paper-based microfluidic chips suitable for applications requiring delayed or remote testing scenarios.

On the other hand, in the case of immobilized beads, agglutination was visible for only up to three days. Despite the advantages offered by immobilized beads, such as higher reagent loading capacity and controlled release kinetics, the shorter duration of agglutination limits their practical utility in scenarios necessitating prolonged stability of test results.

In conclusion, our findings highlight the superiority of paper-based microfluidic chips over immobilized beads in terms of prolonged stability and durability of agglutination reactions for blood typing assays. Paper-based microfluidic chips offer a cost-effective, portable, and user-friendly alternative for blood typing, particularly in resource-limited settings or point-of-care diagnostics.

Moving forward, further research and development in paper-based microfluidic technology hold great promise for revolutionizing diagnostic testing, offering rapid and reliable solutions for a wide range of clinical applications.

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