

DESIGN AND EVALUATION OF AN ADDITIVE-MANUFACTURED MICROFLUIDIC
DEVICE FOR OBSERVING INITIAL BIOFILM ATTACHMENT
ON COPPER SURFACES

by

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DEDICATION

This thesis is dedicated to my family, whose unwavering support, encouragement, and belief in me have been my greatest strength. Their love and constant faith in my journey have made this work possible, and for that, I am endlessly grateful.

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ABSTRACT

The growing interest in microfluidic devices has highlighted the need for improved manufacturing methods that overcome the limitations of traditional methods. One challenge is integrating copper and other metal surfaces into microchannels, as achieving strong and stable bonding is complex. Reliable bonding requires thorough control over surface and bonding quality. This study presents an alternative fabrication approach using double-sided pressure-sensitive adhesive (PSA) tape. Traditional methods are often expensive, time-consuming, and complex, whereas this approach enables the integration of copper surfaces while maintaining cost-effectiveness, repeatability, optical transparency, and compatibility with confocal laser scanning microscopy (CLSM) to facilitate bacterial adhesion observation.

A straight 80 μm -thick microchannel was selected as the optimal design among three designs for biofilm studies. The impact of flow rate, surface roughness, hydrophobicity, and salt concentration on bonding quality was examined. COMSOL Multiphysics® was used to visualize flow profiles and shear stress distributions across the channel geometry. We developed a low-cost microfluidic device using PSA tape, designed to support the observation of bacterial attachment under controlled conditions. Although biofilm formation was not the main focus, the device is CLSM-compatible and shows potential for future biofilm studies. Additionally, Saffman-Taylor fingering was used as an assessment tool to validate bonding quality. Experimental tests examined how salt concentration, flow rate, and surface finish influenced instability patterns. The fabricated device was tested under controlled flow conditions with *Pseudomonas fluorescens* N2E2 to evaluate bacterial adhesion.

The PSA copper microfluidic device was optimized to have smoother surfaces, reduced STF, and negligible leakage. Contact angle measurements confirmed that smoother surfaces became more hydrophobic, which may impact cell behavior. Higher flow rates and increased salt concentration promote instability, reinforcing the role of viscosity in fluid behavior. However, cell adhesion was not observed on the metal surface. Instead, cells adhered to the cover glass, suggesting that device orientation and gravitational forces played a role. Moreover, since the surface preference of the bacterial strain remains unclear, future work should include testing alternative bacterial strains.

Overall, this study demonstrates a reproducible, low-cost fabrication method that supports biological integration and provides an optically transparent platform compatible with CLSM for high-resolution microscopy.

CHAPTER ONE

INTRODUCTION

Scope of The Thesis

This research focuses on the design and characterization of a low-cost microfluidic device that integrates a copper metal surface within its channel to observe initial biofilm attachment. This research seeks to overcome the limitations of traditional fabrication techniques, such as lack of optical transparency, high cost, poor sealing, and limited compatibility with confocal laser scanning microscopy (CLSM), by applying a double-sided pressure sensitive adhesive tape method while incorporating computational modeling for effective device characterization. Experimental validation included evaluation of Saffman-Taylor fingering (STF), testing with media at different salt concentrations, and analysis of surface and bonding quality. The results contribute to the design of microfluidic systems by providing insights into device characterization and the initial attachment of biofilms on metal surfaces.

Chapter 1: This chapter presents background information about copper's role in biofilm formation, the fundamentals of microfluidics, and the microscale behavior of fluids. It also covers computational modelling, traditional fabrication methods for microfluidic devices, a double-sided pressure-sensitive adhesive (PSA) tape method, and the limitations of three-dimensional (3D) printing in this context. Finally, this study introduces the concept of Saffman-Taylor fingering and its relevance to microfluidic systems.

Chapter 2: This chapter outlines the device design and fabrication steps of the microfluidic device. This study overcomes the limitations of traditional manufacturing methods

by utilizing the double-sided-pressure-adhesive tape technique and ensuring device characterization through computational modeling. The chapter also describes the incorporation of metal in the device channels and the preparation of surfaces for the leak tests under controlled flow conditions.

Microfluidic device quality was assessed by the extent of Saffman-Taylor fingering and validated by experimental results using media with three different salt concentrations. This chapter also includes assessments of surface and bonding quality. It also includes the results of biotic experiment carried out in the media determined as a result of abiotic experiments.

Chapter 3: This thesis summarizes the key results of Chapter 2 while providing recommendations for future research on microfluidic system development.

Background

Copper

Copper, was the first metal that human civilizations used because it naturally exists in a metallic state. It plays a crucial role as a trace element in almost all living organisms and is found in major body tissues (Elguindi et al., 2011). Its versatility and antimicrobial activity have led to its widespread use worldwide. For example, copper exhibits excellent antibacterial activity against *Escherichia coli* (*E. coli*) (Woźniak-Budych et al., 2023); making it an effective choice in various fields such as water treatment, plumbing, and cooling (Gomes et al., 2020). The formation of biofilms in water distribution presents a significant challenge for industries, mainly because it can harbor harmful microorganisms that threaten public health (Elguindi et al., 2011). Copper and its alloys are commonly used in these systems because they can resist corrosion and have strong antibacterial properties. Copper surfaces can reduce biofilm formation, but long-term

studies suggest their ability to prevent biofilm buildup may not always be consistent or reliable (Gomes et al., 2020). Understanding the mechanisms of initial biofilm attachment and developing strategies to control biofilm formation on copper surfaces are essential for maximizing the benefits of copper in various applications.

Biofilm

Biofilms are generally understood as clusters of bacteria embedded in a self-produced extracellular polymeric substance (EPS) matrix, composed of substances like extracellular DNA (eDNA), proteins, and polysaccharides (Vestby et al., 2020). Interfaces such as solid-liquid, liquid-liquid, liquid-gas, and solid-gas are sufficient for the formation of biofilms because they provide conditions that support microbial attachment and aggregation. (Flemming et al., 2019).

Biofilm development is observed as a consequence of chemical, physical, and biological processes such as initial attachment, irreversible attachment, microcolony formation, maturation, and dispersion (Flemming et al., 2016). Figure 1.1 shows the stages of biofilm formation. The initial step in the surface colonization of microorganisms involves the transportation of organic compounds and microbial cells to the surface. Subsequent to their transport, microbial cells attach to the surface and then begin to proliferate and aggregate within the extracellular polymeric substances (EPS) they produce (Rather et al., 2021). Mature biofilms exhibit a variety of different structures, typically upon reaching a certain stage, portions of it detach and disperse to make a new biofilm (Rabin et al., 2015). Monitoring and examining these stages can help in developing strategies to control or prevent biofilm formation in diverse environments, from industrial processes to infections associated with medical devices.

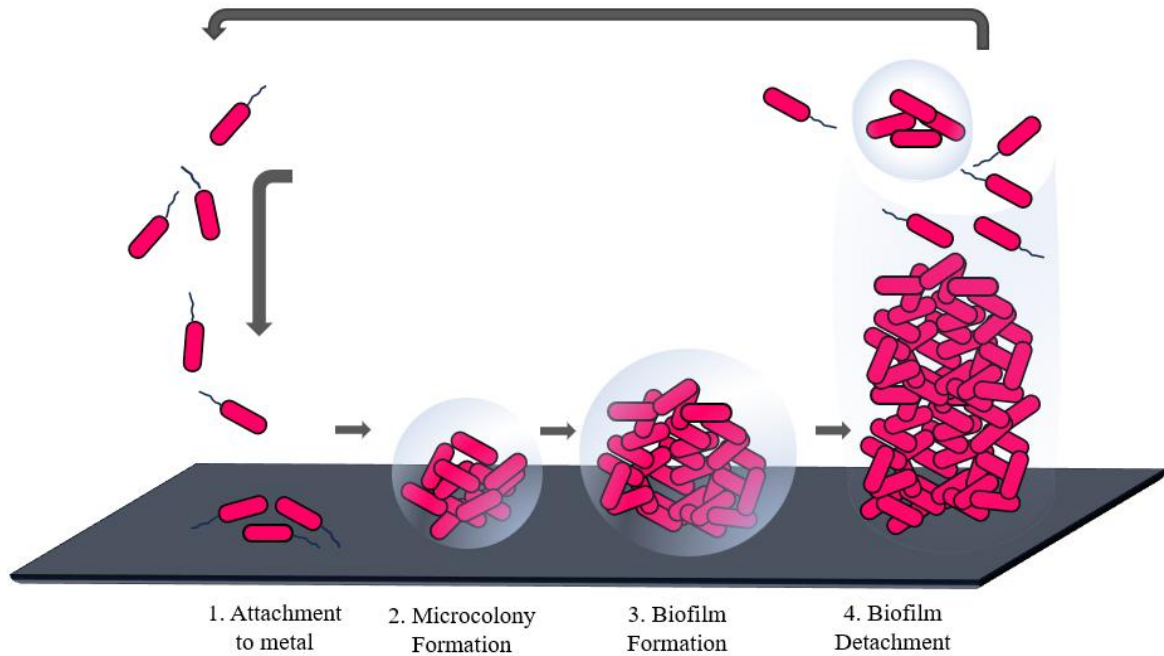


Figure 1.1 Key stages of biofilm development and life cycle: (1) Adhesion, (2) Proliferation, (3) Maturation, (4) Dispersion. Image adapted from (Center for Biofilm Engineering).

Interfaces serve as surfaces or boundaries to which microorganisms can adhere. For example, at solid-liquid interfaces, microbes can adhere to solid surfaces such as pipes, initiating biofilm formation. Adhesion is mediated by surface properties such as hydrophobicity and surface roughness, which influence microbial adhesion (William Costerton et al., 1995; Donlan, 2002). Physicochemical properties of interfaces (e.g., hydrophobic interactions and van der Waals forces) promote microbial aggregation. This aggregation facilitates biofilm development by promoting cell-cell signaling and EPS production, key elements in biofilm stability (Donlan, 2002). Hydrophobic forces and van der Waals interactions help microorganisms stick to surfaces and each other by overcoming naturally occurring repulsion (Israelachvili, 2011). This repulsion force arises from electrostatic interactions since microbial cells generally carry a negative charge due to some functional groups found on their outer membranes (e.g., carboxyl, and phosphate)

(Hermansson, 1999; Van Loosdrecht et al., 1987). Since same charges repel each other, this creates an electrostatic barrier that must be broken for microbes to cluster. In aqueous environments, hydration forces add another layer of resistance by forming structured water layers around the cells, keeping them from direct contact. However, hydrophilic interactions reverse this, driving nonpolar cell membranes together by reducing their exposure to water. Van der Waals forces that are weak and act over shorter distances to help cell-cell and cell-surface adhesion (Marshall et al., 1971). The balance between these forces determines the extent and stability of microbial aggregation in different environments.

Also, the adhesion and movement of bacteria are fundamentally influenced by surface characteristics, including the chemical configuration, which refers to the arrangement of molecules and functional groups on a surface, the degree of wettability, and surface roughness (Díaz et al., 2007). Bacteria adhere to surfaces through various mechanisms that involve physical, chemical, and biological interactions [e.g., hydrophobic interactions (Bruinsma et al., 2001), surface properties (Terada et al., 2012), and environmental factors], leading to colonization as a result of their initial attachment (Kreve & Reis, 2021). For that reason, the role of initial attachment in biofilm maturation is pivotal, and examining this early stage of biofilm development is one of the tasks of this study. The strength and stability of this initial attachment can influence the resilience and mechanisms of the mature biofilm. The analysis of initial attachment encompasses investigating microbial cells' surface properties and the relevant substrate's physicochemical properties.

Imaging and sensing serve distinct purposes in analytical processes. Imaging primarily involves creating visual representations of a subject but can also be used to extract quantitative

properties such as size, shape, and volume; in contrast, sensing focuses on the direct quantitative assessment of specific attributes or properties of the subject or system (Saccomano et al., 2021). Microscopy techniques are commonly employed to acquire imaging data for biofilms. Some popular microscopical methods are confocal laser scanning microscopy (CLSM) for high-resolution 3D imaging, scanning electron microscopy (SEM) for surface morphology imaging, transmission electron microscopy (TEM) for detailed internal structure visualization, and atomic force microscopy (AFM) for surface topography and properties (van Hoogstraten et al., 2023; Jarmar et al., 2008).

Sensors are utilized to study the growth of biofilms and the dynamics of cells within the biofilm, such as detecting metabolic activity, monitoring gene expression, tracking cell motility, and structural changes in the biofilm (Stewart et al., 2008). After measuring the initial attachment, sensors can be employed to analyze how chemical or physical events (e.g., pH, temperature, and oxygen level) influence the growth process of the biofilm (Funari et al., 2022). Understanding biofilm monitoring techniques is crucial for formulating effective strategies to manage or eliminate biofilms. These techniques can be broadly categorized into sensing devices and sensor-free methods (Funari et al., 2022). Sensing devices include optical sensors, electrochemical sensors, and acoustic sensors. On the other hand, examples of sensor-free methods are flow cells and microfluidics, which rely on imaging techniques like microscopy to capture biofilm development and provide precise control over experimental conditions. This makes them invaluable for studying biofilm formation and dynamics in controlled environments (Funari et al., 2022). In this study, a sensor-free approach, combined with microscopy, is

employed to monitor the initial attachment of biofilms, providing important insights into the effects of salt concentration on initial biofilm attachment.

Table 1.1 presents a selection of studies that investigated bacterial adhesion and biofilm growth using microfluidic devices. Each study used different materials, methods, and bacterial strains. The channel height and shear stress values reported in these studies also varied widely. Most of the research used polydimethylsiloxane (PDMS) and glass as base materials in combination with different microfabrication techniques. Some studies such as Salta et al. (2013), opted for micro milling to work with alternative materials like polymethyl methacrylate (PMMA). The range of the shear stresses, from micro-Pascal levels to several Pascals, reflects the diversity of flow conditions under which initial bacterial attachment and biofilm development can be analyzed. This comparative overview provides insight into how channel geometry and shear stress impact the design of microfluidic systems for biofilm studies and it supports the rationale behind the device configuration, choice of bacterial species, and shear stress analysis employed in the present work.

Table 1.1 Overview of microfluidic device fabrication for studying bacterial adhesion and biofilm formation.

Authors	Organism	Materials	Channel Height (μm)	Shear Stress (Pa)	Methods
(Salta et al., 2013)	<i>Cobetia marina</i>	PMMA (polymethyl methacrylate), glass and PDMS (polydimethylsiloxane)	120-1000	0.03-4.3	Micro milling

Table 1.1 Continued.

Authors	Organism	Materials	Channel Height (μm)	Shear Stress (Pa)	Methods
(Janakiraman et al., 2009)	<i>Pseudomonas aeruginosa</i>	PDMS and glass	250	6×10^6	Photolithography and Soft lithography
(J. Kim et al., 2013)	<i>Pseudomonas aeruginosa</i>	PDMS and glass	100	70	Photolithography and Soft lithography
(Cheah et al., 2023)	<i>Pseudomonas aeruginosa</i>	PDMS and PET (polyethylene terephthalate)	100	8.4×10^{-7}	Photolithography and Soft lithography
(Lecuyer et al., 2011)	<i>Pseudomonas aeruginosa</i>	PDMS and glass	30-65	0.05-10	Photolithography and Soft lithography
(Wright et al., 2015)	<i>Pseudomonas aeruginosa</i>	PDMS and glass	500	5×10^5 $- 8 \times 10^5$	Photolithography and Soft lithography

Microfluidics

The impact of microfluidic technologies is increasingly significant, both industrially and academically worldwide. To observe the growth or formation of biofilms, micro- and macro-scale reactors equipped with flow cells are utilized to mimic real environments or in vivo conditions (Funari et al., 2022). Microfluidics is the science and technology of systems that process or manipulate small amounts of fluids using channels with dimensions of tens to hundreds of micrometers (Whitesides, 2006). In this thesis, the designed flow cell can be

considered microfluidic and consists of a physical channel with inlets and outlets where experimental measurements are conducted. Flow cells are commonly categorized into single channel, and multiple channel designs. In single-channel designs, reactions take place within a single fluid stream, which is why they are referred to as single-stream flow cells. In contrast, multichannel designs involve multiple entry channels (two or more) that merge into a single physical channel, forming a more complex flow system (Brewer et al., 2008).

Microscale Behavior of Fluids

Microfluidics manipulates nano- to microliter flow volumes (Maged et al., 2022). Among the various dimensionless numbers used to describe fluid flow, the Reynolds number stands out as one of the most important (Weibel et al., 2006). It determines whether the flow will be laminar, transitional, or turbulent, which plays a significant role in engineering design considerations. Osborne Reynolds first demonstrated that a relationship between spatial dimensions and velocity links the distinction between regular and turbulent movement (Eckhardt, 2009). This dimensionless relationship is known as the Reynolds number.

It characterizes the behavior of fluid flow and is defined as follows (Convery & Gadegaard, 2019):

$$Re = \frac{\rho \times u \times D_h}{\mu} \quad (1.1)$$

with ρ (kg/m³) represents the fluid's density; u (m/s) is a characteristic velocity of the flow; D_h (m) is the hydraulic diameter of the channel; and μ (Pa×s) is the fluid's dynamic viscosity, respectively. The hydraulic diameter of the circular channel is calculated as:

$$h = 4 \times \frac{A}{P} \quad (1.2)$$

where A represents the cross-sectional area of the flow, and P is the perimeter of the cross-section. In the case of a circular channel, the hydraulic diameter D_h (m) is simply equal to the pipe diameter D (m). For rectangular channels, the hydraulic diameter is:

$$D_h = \frac{4hw}{2(h+w)} = \frac{2hw}{h+w} \quad (1.3)$$

where h is the channel height, and w is the width.

The Reynolds number is the ratio of inertial to viscous forces. This ratio provides insight into whether the flow is laminar, turbulent, or in transition between the two. Turbulent flow refers to a state where irregular fluctuations occur, while laminar flow describes a condition where the fluid moves smoothly without noticeable disturbances. Turbulent flow is associated with high Reynolds numbers, while laminar flow corresponds to lower values (Eckhardt, 2009). Transition flow occurs at intermediate Reynolds numbers, where the flow is neither fully orderly nor chaotic (Sengupta, 2012). When fluid moves through a narrow channel at low speeds, it typically flows in a smooth manner, known as laminar flow. This happens because the channel's confinement and viscous forces dominate over inertia. For instance, blood flow through capillaries is typically laminar. In the laminar flow regime, fluid particles move in parallel layers with minimal disruption between them, maintaining distinct flow paths. In circular pipes, laminar flow occurs when the Reynolds number is below 2300, indicating low values, and as the Reynolds number increases, the dominance of inertial forces over viscous forces grows. The flow eventually becomes turbulent at high Reynolds numbers, as fluid particles tend to exchange positions between streamlines, characterized by irregular fluid motion and mixing. A high Reynolds number is defined as 4000 or greater, with flows in the intermediate range ($2300 < Re < 4000$) referred to as transitional, these values are specific to cylindrical geometries (Saldana et

al., 2024). In this thesis, non-circular rectangular microchannels were used to study flow behavior. The transition to turbulence in rectangular channels occurs at different Reynolds numbers, which depend on the aspect ratio of the channel (Van Dinther et al., 2012; Duan & Ma, 2020).

Computational Modelling

Computational fluid dynamics (CFD) is a methodology that employs numerical simulations to examine and resolve issues related to fluid dynamics (Raman et al., 2018). COMSOL Multiphysics® software is used for shear stress analysis in different fields, such as biomedical engineering, microfluidics, chemical engineering, and mechanical systems. However, the main purpose of using CFD is accurate prediction of fluid flow patterns in complex geometries, visualization of shear stress distribution along surfaces, and optimization of device designs to either reduce or control biofilm formation. CFD modeling with COMSOL Multiphysics® provides essential insights into how shear stress influences initial biofilm attachment on metallic surfaces in this research. Also, CFD solves the Navier-Stokes equations for fluid flow, which describes how the velocity of a fluid changes due to forces like pressure, viscosity, and external influences. Here is the general form of the incompressible Navier-Stokes equations (Nairn et al., 2024):

$$\rho (\vec{u} \cdot \nabla) \vec{u} = \nabla \cdot [-p\mathbf{I} + \mathbf{K}] + \vec{F} \quad (1.4)$$

where ρ (kg/m³), the density; u (m/s), the velocity vector; p (Pa), the dependent pressure variable from the Navier-Stokes; $\mathbf{I}$ (dimensionless), the unit tensor, which is an identity matrix; $\mathbf{K}$ (dimensionless), the symmetric deformation tensor; F (N/m³), body force.

The equations of fluid motion in the convective form:

$$\rho \nabla \times \vec{u} = 0 \quad (1.5)$$

where p (Pa), the pressure; ρ (kg/m³), the density; $\vec{u}$ (m/s), the velocity vector.

The deformation tensor is defined as:

$$\mathbf{K} = \mu(\nabla \vec{u} + (\nabla \vec{u})^T) \quad (1.6)$$

where μ (Pa $\times$ s), fluid viscosity; $\vec{u}$ (m/s), the velocity vector. This vector is important in describing how a fluid element deforms under shear and normal stresses (Ferziger & Peric, 2002). By incorporating both the velocity gradient and its transpose, Equation 1.6 describes both elongational and shear deformations while maintaining stress symmetry. It represents how internal stress distributes within the fluid as it moves.

In an incompressible fluid, the divergence of velocity is zero, meaning the fluid does not expand or compress. This condition ensures that the overall density of the fluid stays constant over time (Batchelor, 2010).

The no-slip velocity boundary condition applied by assuming that the velocity of the fluid layer in direct contact with the wall is equal to the velocity of the wall, which is zero in this study, and is defined as:

$$\vec{u} = 0 \quad (1.7)$$

This study defined the boundary condition at the inlet as a fully developed flow. The fully developed flow condition ensures that the velocity profile is constant along the flow direction, meaning that no significant variation in velocity occurs further along the channel. This condition was applied separately to each disjoint inlet region, ensuring independent treatment of each segment.

The equations governing the inlet velocity boundary condition are as follows:

$$\vec{u} \cdot \vec{t} = 0 \quad (1.8)$$

$$V_0 = - \int_{\partial\Omega_{inl}} \vec{u} \cdot \vec{n} dS \quad (1.9)$$

where, $\vec{u}$ (m/s), the velocity vector; $\vec{t}$ (dimensionless) denotes a tangential unit vector along the boundary. V_0 (m³/s) is the volumetric flow rate across the inlet boundary. $\partial\Omega_{inl}$ (m²) refers to the inlet boundary, which is the surface area through which fluid enters the system. $\vec{n}$ (dimensionless) is the normal vector perpendicular to the surface of the boundary, and dS (m²), the differential element of the inlet surface. The term $\vec{u} \cdot \vec{n}$ (m/s) is the dot product of velocity and the normal vector, representing the component perpendicular to the surface at the inlet surface. This formulation assumes that the outlet pressure is set to zero (gauge pressure) to define the pressure difference across the system.

As outlet condition pressure is defined as:

$$[-p\mathbf{I} + \mathbf{K}]\vec{n} = -p_0\vec{n} \quad (1.10)$$

where p (Pa) the pressure; $\mathbf{I}$ (dimensionless), the unit tensor; $\mathbf{K}$ (dimensionless), the symmetric deformation tensor; p_0 (Pa), outlet pressure. The flow model within the microfluidic device was determined based on the properties of water with a dynamic viscosity is 10^{-3} Ns/m², and the density is 1000 kg/m³.

In shear stress analysis, particularly for biofilm attachment, the primary focus is wall shear stress (WSS), the tangential force per unit area exerted by the fluid on the surface. The effects of shear stress on biofilm formation have been studied in various systems, and shear stress plays a crucial role in forming biofilm characteristics (Tsagkari et al., 2022a). Adjusting the flow rate and microchannel diameters allows for applying various flow shear stresses. Park et

al. (2011) reported that the optimal shear stress for stable biofilm formation was approximately 0.017 Pa (0.170 dyn·s/cm²); deviations toward lower or higher shear stresses hindered biofilm development by either insufficient stimulation or excessive detachment of cells. It is important to note that the threshold shear stress for promoting or inhibiting biofilm formation can vary depending on physicochemical surface properties, temperature, bacterial species, and flow conditions.

In the conventional conceptual model of biofilm formation, it is thought that steady flow regimes with higher wall shear stresses would have less biomass due to increased sloughing (Tsagkari et al., 2022b; Arrojo et al., 2006; Klapper et al., 2002). Regions of low shear stress can lead to biofilm attachment (Duddridge et al., 1982). In contrast, high shear stress may not promote biofilm growth or cause detachment. Thus, the low shear stress conditions are preferable for initial attachment studies. To further understand the influence of shear stress on biofilm formation, computational fluid dynamics (CFD) analysis was performed to visualize the flow profiles and wall shear stresses within the microfluidic device using COMSOL Multiphysics® Version 6.0. The relationship between shear stress and flow rate was established using the finite element method based on the designed flow channels. Flow channels, originally designed as 3D models, were imported into COMSOL through the computer-aided design, CAD import module, followed by the section of Laminar flow physics for simulation. Various parameters are defined in COMSOL Multiphysics® to create the virtual environment for analysis, such as fluid density, viscosity, and flow velocity. A physics-controlled extra coarse mesh size was used for simulation. Incompressible and steady-state flow conditions were simulated using the experimental flow rate of 0.1 µl/min. In this study, focusing on computational simulations of shear stress (using varying

flow rates and modified surface properties) through COMSOL modeling aided in selecting the appropriate channel design.

Saffman-Taylor Fingering

Saffman-Taylor fingering is a fundamental phenomenon in fluid dynamics. In microfluidic devices, understanding this instability provides insight into how immiscible fluids interact under different conditions. In this study, the effect of low and high flow rates and different salt concentrations on Saffman-Taylor fingering is used as a diagnostic tool to evaluate how reliable the device is under changing conditions for future experimental applications.

In a study by Saffman and Taylor, fingering is described as follows: Suppose two different viscous liquids lie horizontally in a porous medium or in a Hele-Shaw cell (see Figure 1.3 and 1.4). There is a sharp interface between the bottom liquid with viscosity μ_1 and the top liquid with viscosity μ_2 and this interface forms a horizontal plane. The lower liquid is moved upwards at a constant, steady flow rate, so the upper liquid is pushed upwards by the lower liquid. So, when a less viscous liquid pushes a higher viscous liquid, the distortions at the interface become larger and unstable and form finger-like structures (Figure 1.4). In a Hele-Shaw cell with height h and width W , the flow of two fluids with surface tension σ is governed by Darcy's Law. This law is derivated from the incompressible Navier-Stokes equation (Bird et al., 1924):

$$\frac{\partial \vec{u}}{\partial t} + (\vec{u} \cdot \nabla) \vec{u} = -\frac{1}{\rho} \nabla P + \nu \nabla^2 \vec{u} + \vec{g} \quad (1.11)$$

where ρ is the fluid density, $\vec{u}$ is the velocity field, p is the pressure, and ν is the kinematic viscosity. This equation is essential for representing the inertial and viscous effects in fluid

dynamics and interfacial motion (Saffman & Taylor, 1958). The following assumptions are made: a no-slip boundary condition is applied at the surfaces, the flow is considered uniform across the channel width, the gap thickness (h) is much smaller than the channel length (L) ($h \ll L$), and gravitational effects are neglected. Under these conditions, the incompressible Navier-Stokes equation simplifies, leading to a modified Darcy's law describing the flow between two parallel plates:

$$u = -\frac{h^2}{12\mu} \nabla P \quad (1.12)$$

here, u represents the average velocity of the fluid, h is the gap between two plates, μ is the dynamic viscosity of the fluid and P is the flow pumping pressure from the microchannel. In this research the term $\frac{h^2}{12}$ represents hydraulic permeability of the narrow microchannel gap and originates from the Hele-Shaw approximation. This expression describes pressure-driven, laminar flow between two parallel plates separated by a small gap, h , and it captures the resistance to flow caused by the confined geometry. This permeability is purely mechanical and does not involve membrane transport, thermal gradients, or electric fields, it is related to viscous effects and channel geometry under low Reynolds number conditions, where the flow is governed by the balance between pressure and viscous forces.

In this study, the PSA tape is treated as viscous liquid due to its viscoelastic properties (Fuensanta et al., 2019), allowing it to behave like a second fluid in the Saffman-Taylor fingering. To better understand how Saffman-Taylor fingering behaves, researchers rely on a controlled experimental setup using a Hele-Shaw cell to create an ideal setting for studying interfacial flow dynamics (Park et al., 1984). The simple design of the Hele-Shaw cell makes it easier to closely observe how instability forms and progresses (Kale et al., 2023). Thus, it is

widely used to study viscous fingering and flow instabilities in confined systems. A Hele-Shaw cell is an experimental setup used to study viscous fluid flow between two glass plates, which are positioned parallel to one another with a narrow, uniform gap between them to allow fluid passage (Shafir & Kessler, 2020). Initially, the gap is occupied by water, and air is subsequently introduced to displace the water (Shafir & Kessler, 2020). The emphasis is on the development of the air-water interface.

Although the volumetric flow rate Q does not appear explicitly in Equation 1.12, it is inherently linked to the fluid velocity u through the relation

$$Q = u \cdot A \quad (1.13)$$

where A is the cross-sectional area of the channel. Therefore, an increase in flow rate implies an increase in the pressure gradient (∇P) required to sustain that flow, particularly under low Reynolds number conditions. This relationship is important when considering the onset of Saffman-Taylor fingering, which arises when a less viscous fluid displaces a more viscous fluid (Yazdi & Norouzi, 2018). As the flow rate increases, the fluid moves faster, and the viscous forces acting at the fluid-fluid interface increase. When viscous forces dominate over surface tension forces, the interface becomes unstable, leading to the development of fingering patterns.

The balance between viscous forces and surface tension is described by the dimensionless Capillary number (Ca) (Park et al., 1984):

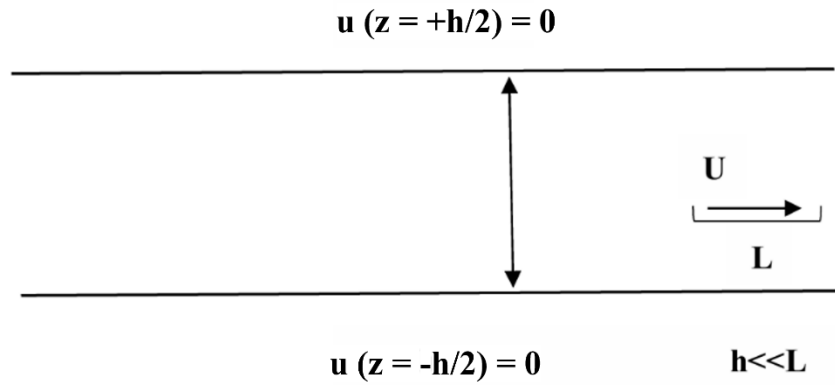
$$Ca = \frac{\mu u}{\sigma} \quad (1.14)$$

where μ is viscosity of the displaced fluid (the PSA layer), u is the average velocity, and σ is the surface tension between the two fluids. A higher Capillary number indicates that viscous

forces dominate over surface tension, leading to destabilization of the interface and the onset of Saffman-Taylor fingering pattern (Park et al., 1984; Yazdi & Norouzi, 2018).

In this research, the primary experimental parameters controlled are the flow rate, and the viscosity difference between the displacing fluid and the PSA tape. Surface tension is assumed approximately constant across all experiments because the variations introduced by different salt concentrations are relatively small compared to the effects of viscosity contrast and flow rate on fingering behavior (Weissenborn & Pugh, 1996; Li & Sadeghi et al., 2010). The PSA tape, due to its viscoelastic properties (Fuensanta et al., 2019), is treated as a viscous material under the different flow conditions studied, allowing elastic effects to be neglected. Consequently, the analysis focuses on the interplay between flow rate, and viscosity contrast in controlling the development of fingering formations.

a)



b)

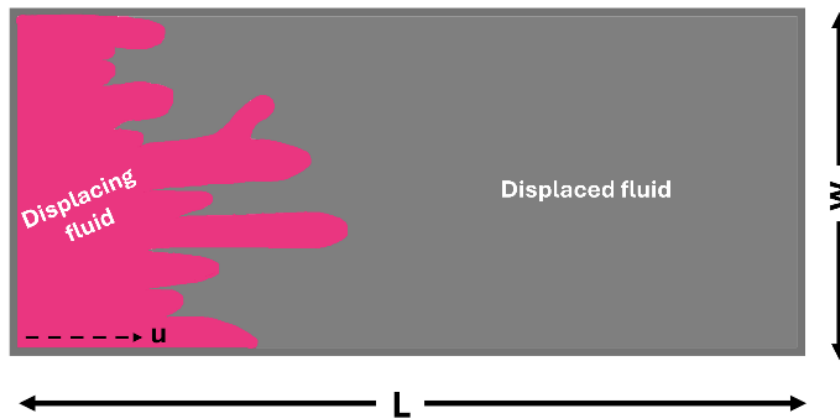


Figure 1.2 (a) Schematic of the mechanism of Saffman-Taylor fingering, where u is the velocity, z is the vertical position, and L is the length scale of the flow cell. (b) Top view schematic of Hele-Shaw cell. The microchannel has a width w , and length L , with a small gap height h between top and bottom plates. Image adapted and modified from (Yazdi & Norouzi, 2018).

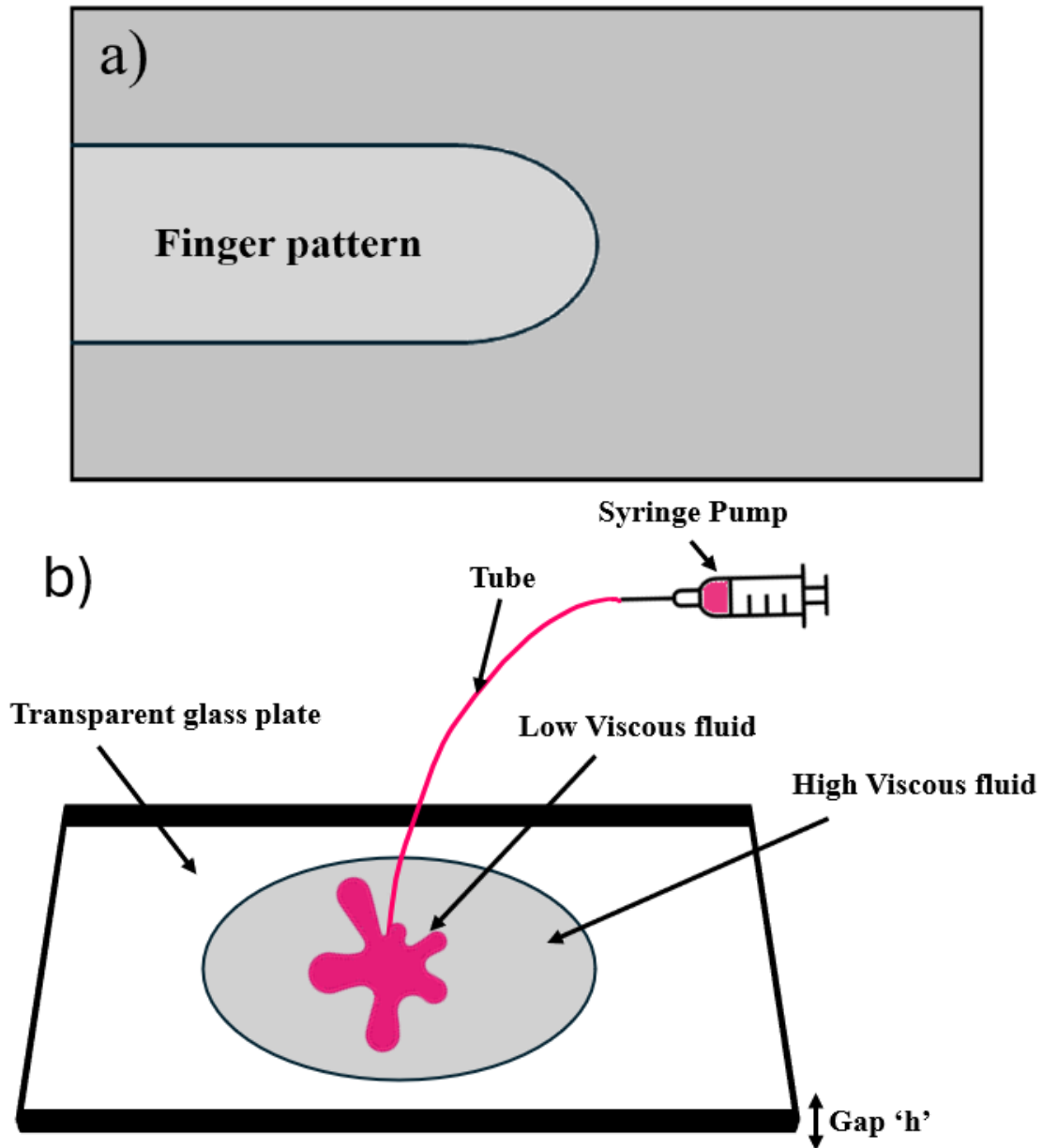


Figure 1.3 Schematic of (a) finger patterns in Hele-Shaw cell, and (b) fingering patterns in Radial Hele-Shaw cell. Image adapted from (Singh et al., 2020; Tabeling P., 1988).

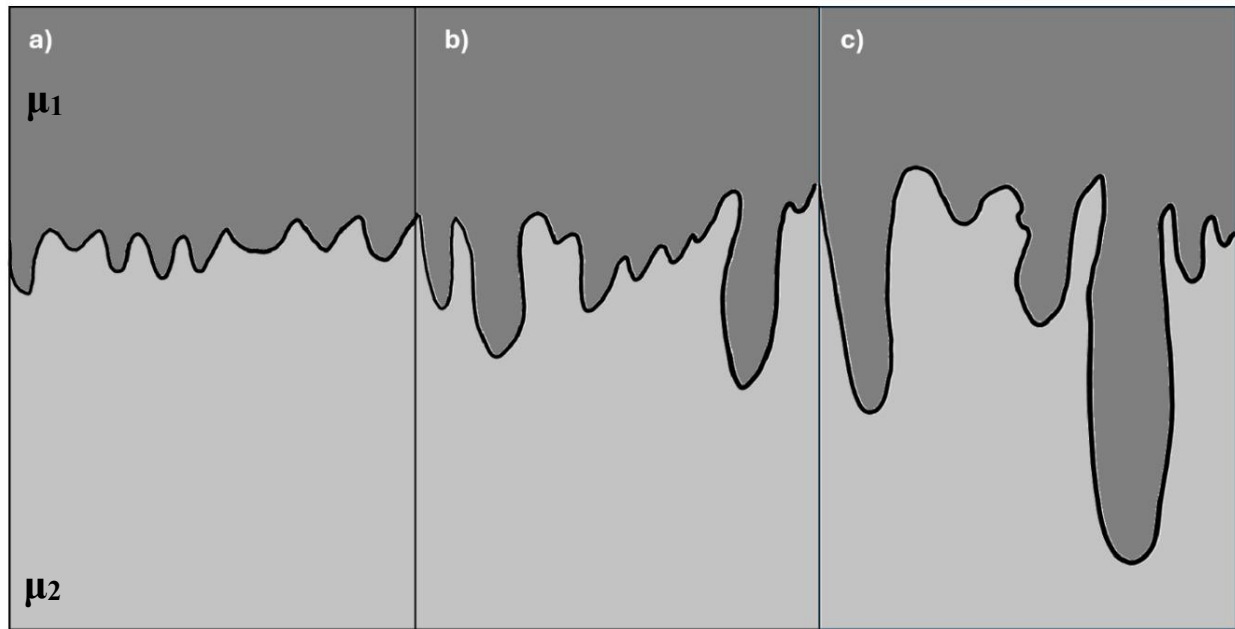


Figure 1.4 (a) Interface between less viscous fluid (μ_1) and a more viscous fluid (μ_2) during the initial phase of the instability, (b) Development of instability (fingering patterns), and (c) Suppressive influence of a finger that advances beyond its adjacent counterpart (beginning of a single propagating finger). Image adapted from (Saffman and Taylor, 1958).

Traditional Microfabrication Techniques for Microfluidic Device Fabrication

For the fabrication of microfluidic devices, traditional techniques such as micromachining, injection molding, and soft lithography are used. Micromachining involves bulk micromachining, such as wet etching and dry etching as well as surface micromachining techniques like photolithography, and chemical vapor deposition (Verpoorte & De Rooij, 2003). Photolithography works by coating a substrate with a light-sensitive material (photoresist) and then exposing it to light (Ma et al., 2010). This exposure allows specific areas to be either protected or modified in later steps like etching or material deposition (Ma et al., 2010). Injection molding is a dynamic and cyclic process that injects heated plastic material into a mold (Agrawal

et al., 1987). Soft lithography emerged as an alternative to photolithography, offering a different approach to fabricating microstructures because the technique replicates a master using flexible organic materials (e.g., polymers, gels rather than silicon and glass) rather than rigid materials (Xia & Whitesides, 1998; Weibel et al., 2007). Microfluidic devices are commonly fabricated from polydimethylsiloxane (PDMS) with a soft lithography technique, which requires a cleanroom to create the master that might not be cost-effective (Faustino et al., 2016). Also, this fabrication method involves designing microstructures using computer-aided design (CAD) program, followed by fabrication of a master mold through photolithography, and subsequent replica molding of PDMS from the master (Faustino et al., 2016). However, traditional fabrication techniques have some disadvantages compared to additive manufacturing, such as low level of design freedom, complexity, slow processing, expensive tooling, and requirement of clean room (Pereira et al., 2019). These requirements make the design of complex 3D structures challenging. In this study, we propose an alternative microfluidic fabrication method that addresses key limitations of traditional manufacturing, including high cost, limited material compatibility, complex fabrication steps, lack of optical transparency and CLSM integration deficiency. By using double-sided pressure-sensitive adhesive tape combined with laser cutting and metal integration, our approach enables rapid prototyping with minimal equipment, expands material selection to include metals like copper, eliminates the need for cleanroom facilities or multi-step lithography processes, provide optical transparency, and meets requirements for CLSM imaging.

3D Printing Technologies in Fabrication of Microfluidic Devices and Limitations

Microfluidic devices have played a critical role in various applications, such as chemical and biological applications (Mehta & Rath, 2021). In recent years, 3D printing has become an alternative method for fabricating microfluidic devices. 3D printing is a process that converts 3D computer designs into physical objects by adding material layer by layer, which enables rapid prototyping (RP) (Amin et al., 2016). Additive manufacturing (AM) and 3D printing include creating 3D designs with computer-aided design (CAD) (e.g., AutoCAD, SolidWorks) that can describe the geometry and dimensions of the prototype within an STL file (Monia Kabandana et al., 2022). After that, 3D printers can select the STL file format and start to build parts layer-by-layer from various materials such as curable resin material, thermoplastic filaments, etc.

At the beginning of this research, microfluidic devices were fabricated by 3D printing. However, the printing process encountered some limitations, such as surface properties for sealing, resolution, compatibility, and transparency issues for microscope analysis. For example, microchannels fabricated by 3D printing exhibited height variations between 140-200 μm , which exceeded the optimal thickness range required for high-resolution confocal laser scanning microscopy. CLSM typically requires microchannels with heights less than approximately 100 μm to ensure sufficient optical sectioning, minimize out-of-focus light, and maintain image clarity (Pawley, 2006; Schneckenburger, 2007). Therefore, it was necessary to reduce the channel height to meet the imaging requirements for CLSM analysis.

Additionally, the limited resolution of 3D printing process led to thicker layer deposition and visible surface irregularities, resulting in increased roughness and poor sealing performance. The composition of the resins and plastics used in the 3D printing process is not always known,

and material biocompatibility is crucial for some applications (Bhattacharjee et al., 2016a). For instance, flow issues may arise from certain 3D-printing materials swelling in aqueous systems (C. Chen et al., 2016). To overcome these challenges of 3D printing, a method using double-sided pressure adhesive tape was implemented for assembling microfluidic devices. When compared to traditional techniques, this approach to microchannel sealing is the most basic, reliable, high-throughput, and economical (Tsao & Syu, 2020a).

First and foremost, a device must have strong bonding quality and no leakage issues. There are two types of bonding techniques in microfluidics: direct and indirect techniques (Giri & Tsao, 2022). Direct bonding involves joining thermoplastic materials directly without the need for additional substances such as reagents or supplementary layers at the bonding interface. According to Giri and Tsao (2022), techniques such as thermal fusion, solvent bonding, surface modification, and ultrasonic or laser welding are all forms of direct bonding. In contrast, indirect bonding uses additional materials, such as epoxies or adhesive tapes to aid the process. Among these, adhesive bonding is the predominant indirect bonding technique in microfluidics (Tsao & Syu, 2020a). Adhesive bonding can be classified as “wet” or “dry.” Wet adhesive bonding often employs wax, SU-8, or UV-curable epoxy for surface adhesion (Giri & Tsao, 2022). Dry adhesive bonding utilizes tapes for the adhesion of microfluidic devices (Tsao & Syu, 2020a). While various adhesive tapes (e.g., single-sided, double-sided, and pressure-sensitive tapes) are available on the market for the large-scale production of microfluidic devices, Tsao and Syu (2020) emphasize that double-faced tape offers strong bonding capabilities, which results from its uniform adhesive layer and compatibility with a wide range of materials. The bonding strength should be substantial to prevent leakage during microfluidic device flow operation

procedures. This may result in operations transitioning from low flow rates to high flow rates. Particularly, research that includes the observation of cells or fluorescent images under a microscope necessitates reliable and optical transparent microfluidic devices (Giri & Tsao, 2022). The materials utilized in flow cell construction are crucial for their efficacy. Ideal materials for microfluidic channels should be clear, thin, flexible, and impervious to organic solvents. This research employs a double-sided pressure-sensitive adhesive tape method to create flawlessly bonded microchannels with optically clear acrylic sheets, thereby manufacturing a leak-proof interface for microfluidic applications. The bonding quality is measured through Saffman-Taylor fingering observation, and the compatibility of the concept for the production of next-generation microfluidic devices is evaluated by assessing the initial attachment of cells on a metallic surface.

Double-sided pressure-sensitive adhesive tape (PSA)

The double-sided pressure-sensitive adhesive (PSA) tape technique has emerged as a rapid and efficient approach for fabricating microfluidic devices, offering an alternative to traditional bonding methods (Smith et al., 2024). This method involves using a thin layer of acrylic-based adhesive tape, which is commercially available and widely utilized for assembling microfluidic channels in diverse substrates, including PDMS, PMMA, polycarbonate (PC), cyclic olefin copolymer (COC), glass, and 3D-printed resins (Serra et al., 2017; Kratz et al., 2019). Unlike conventional bonding techniques that often require chemical or thermal treatments, PSA tape enables simple, reliable, and cost-effective assembly without complex processing steps (Patko et al., 2014; You et al., 2020). Laser cutting is a widely used technique in microfluidic fabrication for creating channel patterns with high precision in various materials,

including PSA tapes, polymers, and even thin glass sheets. By applying laser cutting to PSA tapes, microchannel patterns can be precisely tailored to desired dimensions and geometries, allowing for rapid customization (Smith et al., 2024). The adhesive layer forms a strong seal between substrates, ensuring leak-free bonding while preserving optical transparency, which is particularly advantageous for brightfield microscopy (Thompson & Abate, 2013).

PSA tape typically comes in two structural configurations (Figure 1.2): single-layer adhesive tape and double-layer PSA tape with an intermediate carrier, which enhances adhesion across different material surface. The release liners prevent premature sticking before assembly, while the adhesive layer enables durable bonding upon application (Islam et al., 2015; Serra et al., 2017). The total thickness of the tape can change brand by brand and it can also determine the height of the microfluidic channel and can be cut with laser cutting. Given these advantages, PSA tape has gained popularity in microfluidic research, particularly for applications that require rapid assembly and flexible prototyping without compromising material integrity. For instance, in a study of bioanalytical wearable devices, several layers of double-sided adhesive tape were used to construct fluidic networks for sweat analysis, demonstrating the potential of PSA-based fabrication in wearable biosensors (Lin et al., 2019). Similarly, another study utilized double-sided biocompatible adhesive to seal microfluidic channels between different materials, such as glass, cover glass, and polyester to fabricate microfluidic devices for in vitro assays (Ma et al., 2021). Additionally, a low-cost fabrication approach for microfluidic-based electrochemical sensors was demonstrated by designing and cutting micro-patterns directly from double-sided adhesive tape, which was then used to bond electrodes on a glass layer with COC slide to form microfluidic channels (Khashayar et al., 2017). Furthermore, another study employed double-

sided PSA tape to bond different 3D-printed microchannel designs onto PMMA sheets, creating inertial microfluidic devices, demonstrating the technique's effectiveness in integrating 3D-printed microfluidics with transparent substrates (Razavi Bazaz et al., 2020). Their approach involved bonding three separately printed microfluidic devices onto a PMMA sheet via adhesive tape, further emphasizing the versatility of PSA tape in microfluidic device fabrication.

In this study, ARcare® 90445Q acrylic adhesive tape was selected due to its strong bonding performance, compatibility with various substrates, and ability to form well-defined microchannels of tunable height, dictated by the tape thickness. This technique eliminates the need for additional post-processing steps, making it a practical and efficient solution for microfluidic device fabrication (Smith et al., 2024).

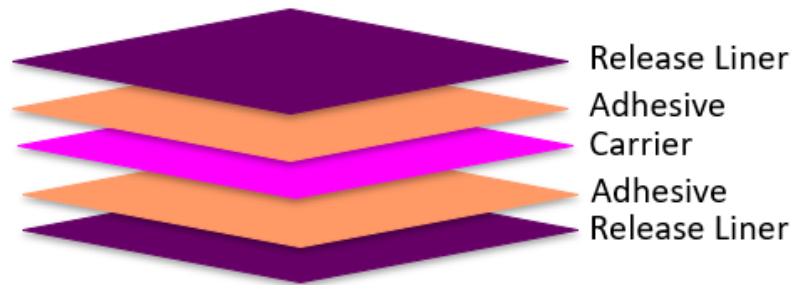


Figure 1.5 Schematic design of the double-sided pressure-sensitive adhesive tape.

Research Aim

Although traditional methods (e.g., photolithography and soft lithography) are popular for manufacturing a reliable microfluidic device, they present challenges related to component cost, customizability, scalability, and longer processing times. To address these limitations, this study aims to design and fabricate a reliable and cost-effective microfluidic device that incorporates metallic surfaces. The focus of this study is to investigate how different salt-

concentrated media affect Saffman-Taylor fingering (STF) in confined microfluidic system by analyzing this instability. There are two hypotheses for this work with their associated objectives:

Hypothesis 1: Microfluidic devices fabricated using the double-sided pressure sensitive adhesive tape method will reduce fabrication complexity and cost while achieving bonding integrity, optical transparency, and CLSM-compatible prototyping compared to traditionally manufactured microfluidic systems. Computational modelling of flow over the metal surface will provide insights into the shear stress distribution in different designs for future biofilm research.

Objective 1: The goal is to overcome the limitations of traditional manufacturing methods by utilizing the double-sided pressure adhesive tape technique and characterizing the device through computational modeling using COMSOL Multiphysics®. To achieve this goal, the fabrication of conventional microfluidic systems was investigated, and the fabrication process was simplified. More cost-effective materials and methods were selected, and costs were reduced. An additional task is to optimize flow conditions before assembling the device and conducting physical experiments. This is achieved by modeling shear stress on the metal surface, ensuring effective design characterization for future biofilm studies. This part of the research provided insight into the device fabrication before expensive and time-consuming experiments.

Hypothesis 2: The surface and bonding qualities of the device will be directly related to surface roughness, contact angle measurement, flatness measurement, and Saffman-Taylor fingering analyses. A well-bonded device will be sufficient to conduct controlled flow experiments without major leaking problems or Saffman-Taylor fingering, provided that a consistent fabrication protocol is followed during assembly for biofilm studies. Saffman-Taylor fingering will serve as a diagnostic tool to identify weak bonding points, and potential leakage

problems, indicating adhesive failures. It is expected that initial attachment will be observed on the metal surface (copper) in devices that assembled appropriately.

Objective 2: Assessing and validating the quality of the microfluidic device. Assessing bonding quality is crucial for developing reliable microfluidic devices and biofilm experiments. Robust bonding can therefore maintain microchannel integrity, prevent contamination and deliver accurate results. This involves assessing surface quality by measuring surface roughness and contact angle to ensure a well adhesion. The use of a coordinate measuring machine (CMM) verifies alignment of the devices. Finally, the Saffman-Taylor fingering criterion meets with bonding quality rather than surface quality and helps identify potential weak points in adhesive bonds under fluid flow conditions in different salt concentration. Thus, it plays a role in determining the appropriate media for the biotic experimental setup.

CHAPTER TWO

DESIGN AND EVALUATION OF AN ADDITIVE
MANUFACTURED MICROFLUIDIC DEVICE FOR
OBSERVING INITIAL BIOFILM ATTACHMENT ON COPPER
SURFACES

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

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Acquisition, Writing – Review & Editing

Manuscript Information

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Abstract

Microfluidic devices play an important role in environmental research, biomedicine and pharmaceuticals. However, fabrication of these devices comes with some challenges such as high cost, complexity, and reliability concerns. Traditional fabrication techniques such as photolithography and soft lithography rely on expensive materials and intricate processes. This study provides an alternative method using double-sided pressure-sensitive adhesive (PSA) tape to create a microfluidic device more efficiently and affordably to observe initial biofilm attachment on copper surfaces. Unlike conventional techniques, PSA tape allows for quick assembly without the need for chemical or thermal bonding. Since shear stress plays a role in biofilm research, to analyze fluid dynamics and shear stress on copper surface, computational modeling was conducted using COMSOL Multiphysics®. Experiments assessed various aspects of the devices, including bonding quality, surface quality, and Saffman-Taylor fingering (STF) effect in different salt concentrations. The findings suggest that low flow rates, low salt concentrated media and smoother surfaces help suppress fingering formation, resulting in more stable flow patterns. Additionally, bacterial adhesion tests using *Pseudomonas fluorescens* N2E2 highlighted the impact of surface properties and the device orientation on bacterial adhesion. Findings suggest that hydrophobicity, variations in bacterial strains and gravity impact microbial attachment, and emphasize the need to repeat the experimental designs with new optimizations. Overall, this research demonstrates that PSA tape-based microfluidic devices offer a practical and low-cost alternative for future biofilm studies.

Introduction

Microfluidic systems are small devices that manipulate and analyze fluid dynamics at the microscale (Vera Faustino et al., 2015). Globally, microfluidic technologies are having an increasingly significant impact on academia and industry. There are several uses for microfluidics, such as biomedicine, environmental research, pharmaceuticals, and energy (Tsao & Syu, 2020b). Despite their widespread use, the manufacture of microfluidic devices continues to present significant challenges, particularly in terms of material compatibility, manufacturing complexity, and maintaining reliability in real-world conditions (Bhattacharjee et al., 2016b).

Conventional manufacturing methods, such as 3D printing and soft lithography, often face challenges related to manufacturability and device reliability, such as leakage and material limitations (Bhattacharjee et al., 2016b). Compared to traditional methods (see Table 2.1, which summarizes fabrication techniques and their key difference), the double-sided PSA tape fabrication method for microfluidic devices offers a faster, lower-cost alternative. PSA tape is a self-adhesive material that bonds surfaces through light pressure without requiring heat or chemical (Patko et al., 2014). It comes in two main forms: one with a single adhesive layer between release liners and another with two adhesive layers separated by a carrier layer. The release liners protect the adhesive before use, while a carrier layer maintains durability and compatibility with different materials (Kratz et al., 2019). Unlike micromachining or injection molding, which require expensive materials and prolonged fabrication times, this approach enables rapid, low-cost assembly using flexible substrates and adhesive bonding (Smith et al., 2024; Thompson & Abate, 2013). It should be noted that while injection molding is cost-effective for large-scale manufacturing, the [PSA-tape method offers advantages in terms of

simplicity, and speed for small-batch or research-focused microfluidic fabrication. Similarly, while methods like photolithography and soft lithography have complex material processing, using PSA tape provides a much simpler and more accessible option for microfluidic applications (Serra et al., 2017). Table 2.1 summarizes common fabrication methods used in microfluidic device development, including photolithography, soft lithography, injection molding, and micromachining. These techniques vary widely in terms of pattern definition, materials used, fabrication time, and cost-effectiveness. These limitations highlight the need for alternative, more accessible approaches to microfluidic fabrication.

Table 2.1 Comparison of fabrication methods.

Fabrication Method	Definition of Patterns	Materials	Time to Fabricate	Cost-Effectiveness	References
Photolithography	Use light to imprint patterns from a photomask onto a light-sensitive coating (photoresist) on a substrate.	Silicon, Glass, Polymers.	Moderate	Moderate to Expensive	(Ma et al., 2010; Scott & Ali, 2021; Gale et al., 2018a).
Soft Lithography	Elastomeric molds or stamps transfer microscale/nanoscale patterns.	Silicone, Photoresists, and Polymers.	Slow to Moderate	Moderate	(Xia & Whitesides, 1998; Gale et al., 2018 X. Liu et al., 2024; Scott & Ali, 2021; Weibel et al., 2007).
Injection Molding	Injection of molten material into a mold to form parts.	Thermoplastics, Glasses, Metals, and Polymers.	Slow to Moderate	Expensive	(Piotter et al., 1997 Gale et al., 2018; X. Liu et al., 2024; Scott & Ali, 2021).

Table 2.1 Continued.

Fabrication Method	Definition of Patterns	Materials	Time to Fabricate	Cost-Effectiveness	References
Micromachining	Subtractive (e.g., etching, laser) or mechanical processing of microstructures.	Metals, Ceramics, Glasses, and Polymers.	Slow	Expensive	(Verpoorte & De Rooij, 2003; Quero et al., 2018; Gentili et al., 2005; X. Liu et al., 2024; Scott & Ali, 2021).
Layered Assembly Using Double-Sided Adhesive Tape (The method of this study.)	Layer-by-layer material deposition, bonded using adhesive tape instead of thermal/chemical adhesion.	Polymers, Composites, Flexible substrates.	Fast	Low	(Razavi Bazaz et al., 2020; Xia & Whitesides, 1998; Nath et al., 2010).

Microfluidic devices are useful tools for studying microbial behavior and biofilm formation under controlled flow conditions. Biofilms are surface-associated microbial communities embedded in extracellular polymeric substances, playing a role in environmental contamination, chronic infections, and industrial fouling (William Costerton et al., 1995; Klapper et al., 2002). Understanding the initial bacterial attachment, the earliest stage of biofilm formation, is important for developing materials and systems that can either inhibit or support microbial colonization, depending on the intended application (Kreve & Reis, 2021).

The integration of copper as the base surface material offers a relevant substrate for biofilm research due to copper's antimicrobial properties (Vincent et al., 2016). Although copper surfaces can help to inhibit biofilm development, long-term research points to their possible inconsistent or unreliable capabilities (Gomes et al., 2020). Investigating initial biofilm attachment on metallic surfaces and improving methods to manage biofilm growth on copper surfaces is crucial to optimizing its use across different applications.

A common limitation in biofilm studies is confocal laser scanning microscopy (CLSM) compatibility. Many traditional devices, such as thick polydimethylsiloxane (PDMS) layers or significant surface roughness because of the fabrication process, produce non-optically transparent devices, making bacterial imaging difficult (Berthier et al., 2012). The PSA tape-based fabrication method developed in this study addresses this issue by enabling optical clarity and compatibility with CLSM, allowing for high-resolution imaging of bacterial attachment on copper surfaces within the device.

Furthermore, computational modeling using COMSOL Multiphysics® plays an essential role in the design and validation of the device. This study uses simulations to examine the behavior of fluids through microchannels and observes shear stress on copper surfaces for biofilm studies using computational simulations. Shear stress analysis using computational modeling is crucial to the goal of improving a microfluidic device that allows for the observation of biofilm studies on a metallic surface. Since higher shear stress makes cells more prone to detaching and interferes with cell-to-cell connections, maintaining low shear stress is critical for initial attachment. A flow rate of 0.1 $\mu\text{L}/\text{min}$ corresponded to a shear stress of approximately 0.170 $\text{dyn}\cdot\text{s}/\text{cm}^2$ (0.017 Pa) (Park et al., 2011), which is considered low; however, even slight increases in flow rate or shear stress can significantly impact the stability of initial microbial attachment. The performance of microfluidic devices developed in this study were validated through experimental leakage tests under controlled flow conditions by monitoring Saffman-Taylor fingering (STF) formation in media with varying salt concentrations. This study offers a comprehensive analysis of the microfluidic device's performance, paving the way for future developments in biofilm research and microfluidic technology. The impact of different salt

concentrations on the Saffman-Taylor fingering in microfluidic devices is important, especially in experimental setups that incorporate various medium characteristics. As a basic concept, when a less viscous fluid pushes a more viscous fluid, it creates more Saffman-Taylor fingering patterns at the interface (Yazdi & Norouzi). Initially Hill (S. Hill et al., 1952) and Chouke (Chouke et al., 1959) worked on this phenomenon for macroscopic instabilities at the interface between two fluids. Nonetheless, it was Saffman and Taylor that systematically investigated the viscous fingering instability. Their study yielded conclusions so fundamental that this phenomenon is currently referred to as Saffman–Taylor fingering (P.G. Saffman & G.I. Taylor, 1958; Saffman, 1986)]. Variations in salt concentration can have an impact on the development interfacial instabilities in systems where a less viscous fluid displaces a deformable material. As the salt concentration of a fluid increases, the viscosity has been observed to increase (Drecun et al., 2021). This occurs because dissolved ions interact with water molecules and limit their movement, making the fluid more resistant to flow. These enhanced ion-solvent interactions reduce molecular mobility, leading to higher viscosity (Jones & Dole, 1929). In experimental systems, changes in surface tension are often considered secondary compared to viscosity effects. Therefore, the primary factor considered in this study is the change in the viscosity of the displacing fluid. However, although the aqueous phase viscosity increases slightly with higher salt concentrations, it remains much lower than the effective viscosity, or viscoelastic response of soft layers such as adhesives, maintaining a significant viscosity contrast. In addition, it is important to recognize that salt concentration may also subtly influence other system properties, such as surface tension or the mechanical behavior of the substrate, although these effects are typically less dominant at low to moderate ionic strengths.

This change in fluid characteristics can affect the behavior of Saffman-Taylor fingering. This paper presents theoretical and experimental evidence of the occurrence of Saffman-Taylor fingering in the displacement of different salt-concentrated media by double-sided pressure adhesive tape, which introduces another viscous interface into the system, and to compare available experimental data with some predictions of a theory of instability developed.

Materials and Methods

Fabrication of Microfluidic Device

In this study, microfluidic channels were designed with AutoDesk® Inventor. The desired microfluidic device is first drafted using commercial CAD drawing software (AutoDesk® Inventor Professional 2021) and imported to the COMSOL Multiphysics® computational modeling in IPT format. The study involved the analysis of three proposed channel designs (Appendix A) for a future microfluidic device using the COMSOL Multiphysics® software. Computational simulations compared shear stress graphs in different microchannel designs and examined how each design would behave in a virtual environment. The straight microfluidic device channel (Figure 2.1) was fabricated using the double-sided pressure adhesive tape featuring $\sim 80\ \mu\text{m}$ channel thickness. The device (Figure 2.2) contained a straight tape channel and copper coupon with a diameter of 12.7 mm, which was sealed within a cavity inside an acrylic block by Loctite® Clear Silicone (Waterproof sealant, transparent). The device was completed with a cover glass of the same dimensions (24 mm x 50 mm, No. 1,5; thickness: 200 μm) as the acrylic block. To ensure compatibility with high-resolution inverted CLSM imaging using 63x objective lens (with a working distance of $\sim 140\ \mu\text{m}$), a #1.5 cover glass was selected. This thickness matches the optical correction of the objective lens. Standard microscope slides ($\sim$

1 mm thick) were avoided, as their greater thickness would exceed the optical working distance and result in poor focusing and image aberrations. The device features a single inlet and a single outlet, both designed to form a compressive seal with the tubing (TYGON® S3 E-Lab, E-3603, 1/32" ID). A copper coupon is incorporated into the device, allowing cells to attach and be observed microscope. The average gap between the acrylic block and copper coupon is 200 μm .

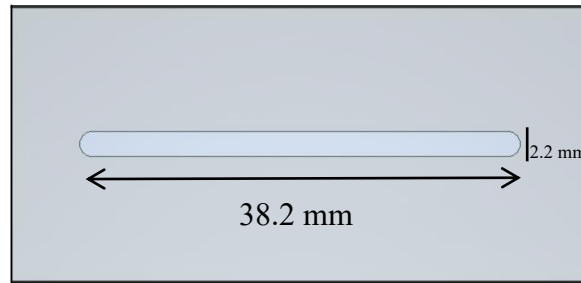


Figure 2.1 Channel design of microfluidic devices created using AutoDesk® Inventor and modeled in COMSOL Multiphysics®. The channel dimensions are 38.2 mm length, 2.2 mm width, and 80 μm depth. The tape aligns with cover glass dimensions (24 mm x 50 mm).

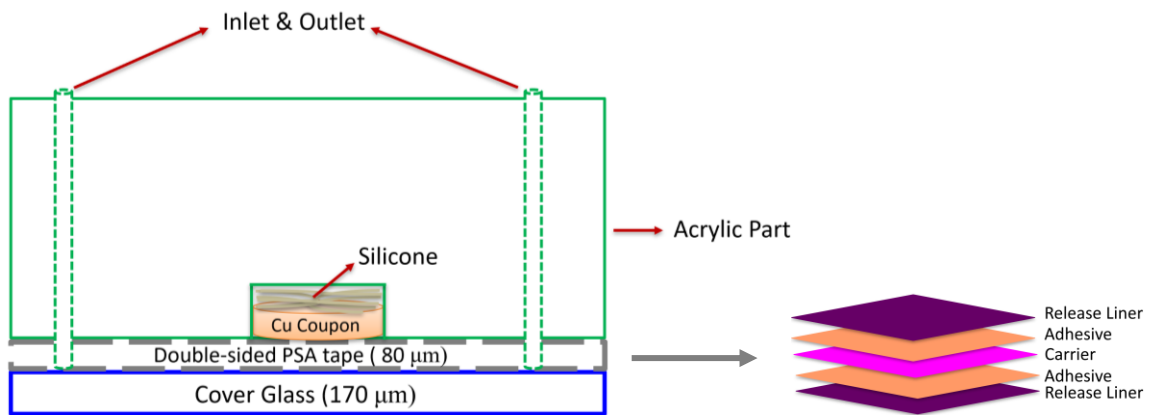


Figure 2.2 Schematic design of the microfluidic device. The acrylic (green, thickness: 0.177" or 4496 μm) is designed with a depth area that safely fits the copper (Cu) coupon (orange, thickness 0.063" or 1600 μm) while also incorporating the inlet and outlet. The Cu-coupon is sealed to acrylic using silicone. A transparent double-sided pressure-sensitive adhesive tape (PSA) (grey dashed outline) is adhered to the acrylic part. PSA tape is not only an adhesive but also plays a role in defining the height of the microchannel, hence creating its structure. Lastly, cover glass (blue) is assembled to the device. It covers PSA tape while providing a clear surface for imaging.

A step-by-step description of the fabrication process follows, beginning with the preparation of copper coupons and acrylic parts, followed by the formation of microfluidic channels and the assessment of surface and bonding quality.

Preparation of Copper Coupons

C110 Copper (99.9% Cu) coupons with a diameter of 0.5 inches and a thickness of 0.063 inches were used in all the experiments. Copper coupon edges were filed and straightened by a vise. Each copper coupon was polished on one side using a combination of different grit sequences to achieve two distinct surface finishes. The polishing process followed three different methods: first, applying 320 US grit followed by 400 US grit; second, using a combination of 320 US grit and 600 US grit; and third, applying 400 US grit followed by 600 US grit using metallographic procedures with an Allied High Tech MetPrep 3 Grinder/Polisher equipped with a power head. To remove any polishing residue, ultrasonic cleaning was performed in a two-step process with 100% Acetone and 99.9% Isopropyl Alcohol (IPA) for 5 minutes each. Before abiotic experiments, all coupons were dipped 10 times in A144C-122 hydrochloric acid (HCl) (37%) in a biosafety cabinet to prevent surface oxidation and then washed with distilled water. This step was followed by wiping each side of the coupon during drying in a biosafety cabinet. Afterward, images were taken using an AmScope (MU503) LED light microscope equipped with top illumination to visually observe surface gloss differences between HCl-treated and non-HCl-treated copper coupons. Images were captured at 0.7x magnification.

Preparation of Acrylic Components

Acrylic was chosen for its optical transparency, cost-effectiveness, and compatibility with laser-cutting technology, which allows for extremely precise shapes (Pawar, 2016). However, it

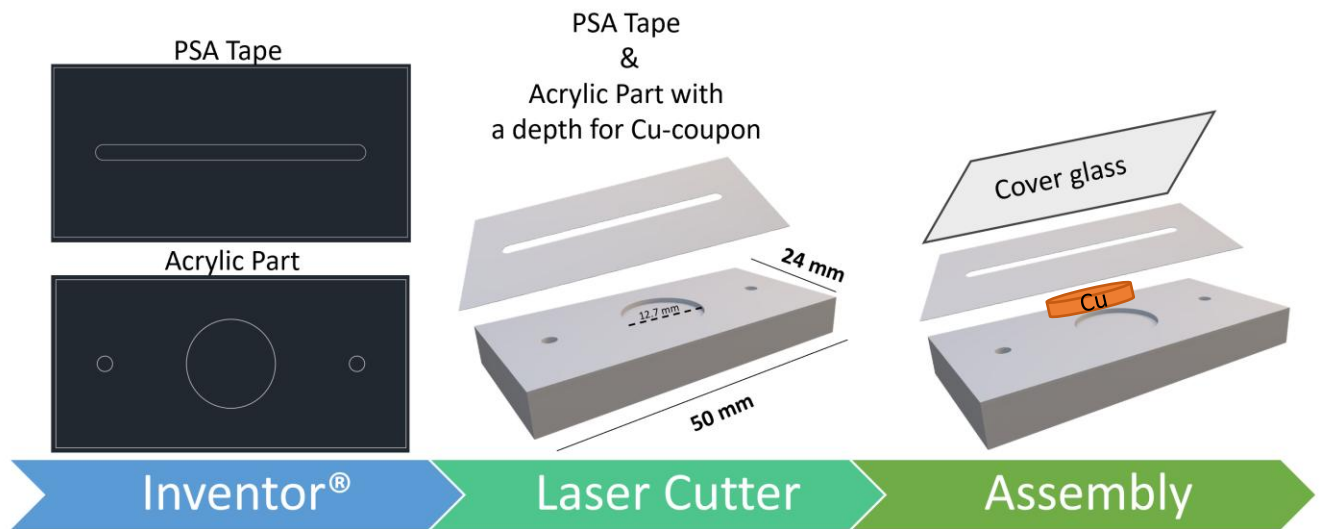
is prone to cracking, dirt accumulation, and scratches. To achieve the required dimensions with high precision, the design was first created in Autodesk Inventor and then fabricated using laser cutting (Figure 2.3). The laser cutter (Boss LS1420, 70W) cut a 4.5 mm thick clear acrylic plexiglass sheet to the desired size (50 mm x 24 mm). The cutting process, RDWorksV8 software set the power and speed parameters to $80 \pm 10\text{-}15\%$ power and 8 (mm/s) speed settings for the cutting and $80 \pm 15\text{-}20\%$ power and 65 $\pm 10\text{-}15\%$ (mm/s) speed for the scanning. After laser cutting, peeling off the acrylic protective liners left residues and before bonding to the cover glass, the acrylic part was cleaned using the Novus 7136 plastic polish kit to remove air pockets. Heavy scratch remover, fine scratch remover, and plastic clean & shine were applied, respectively, to ensure a smooth surface.

Preparation of Microfluidic Channels

ARcare® 90445Q (Adhesives Research Inc.) clear polyester double sided pressure sensitive tape with As-110 acrylic medical grade adhesive coatings were used and had a total thickness of 81 μm . This tape was selected for its optical clarity, and flexibility, which enables clear reflection microscopy images, reduce device deformation on flexible cover glass, and minimize leakage or failure. Additionally, it is cost-effective and easily cut with a laser cutter, making it an ideal choice for the application. The laser cutter was employed to cut this PSA tape to the desired size with 15% power and 15 (mm/s) speed settings. Three different channel designs were imported to the RDWorksV8 software, and the same power and speed settings were determined for all microchannels. The PSA tape is designed with protective layers, known as release liners, covering both sides to protect the adhesive before use. Before the final assembly

of the structured tapes, the release liners were removed to expose the adhesive layers, allowing for proper bonding between surfaces.

a)



b)

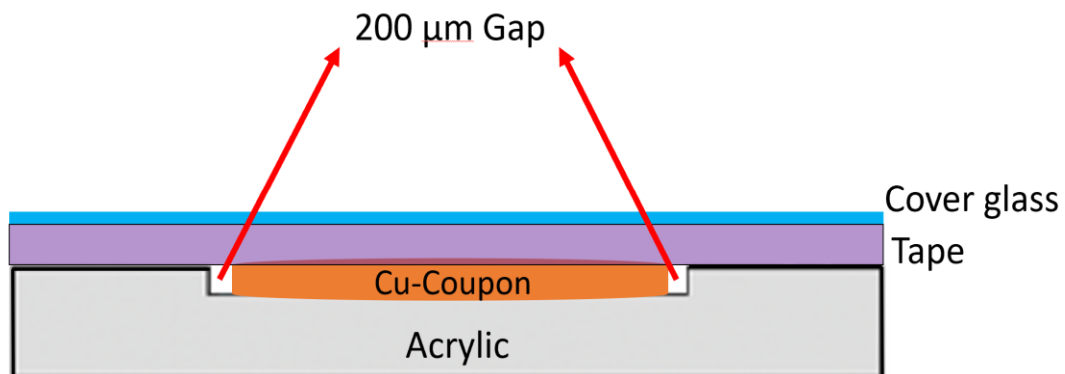


Figure 2.3 (a) Schematic of the entire process flow diagram of the microfluidic device. (b) Cross-sectional side view showing the layered structure. A 200 μm gap is present between the copper coupon and the surrounding acrylic pocket, resulting from the laser cutting failures during device fabrication.

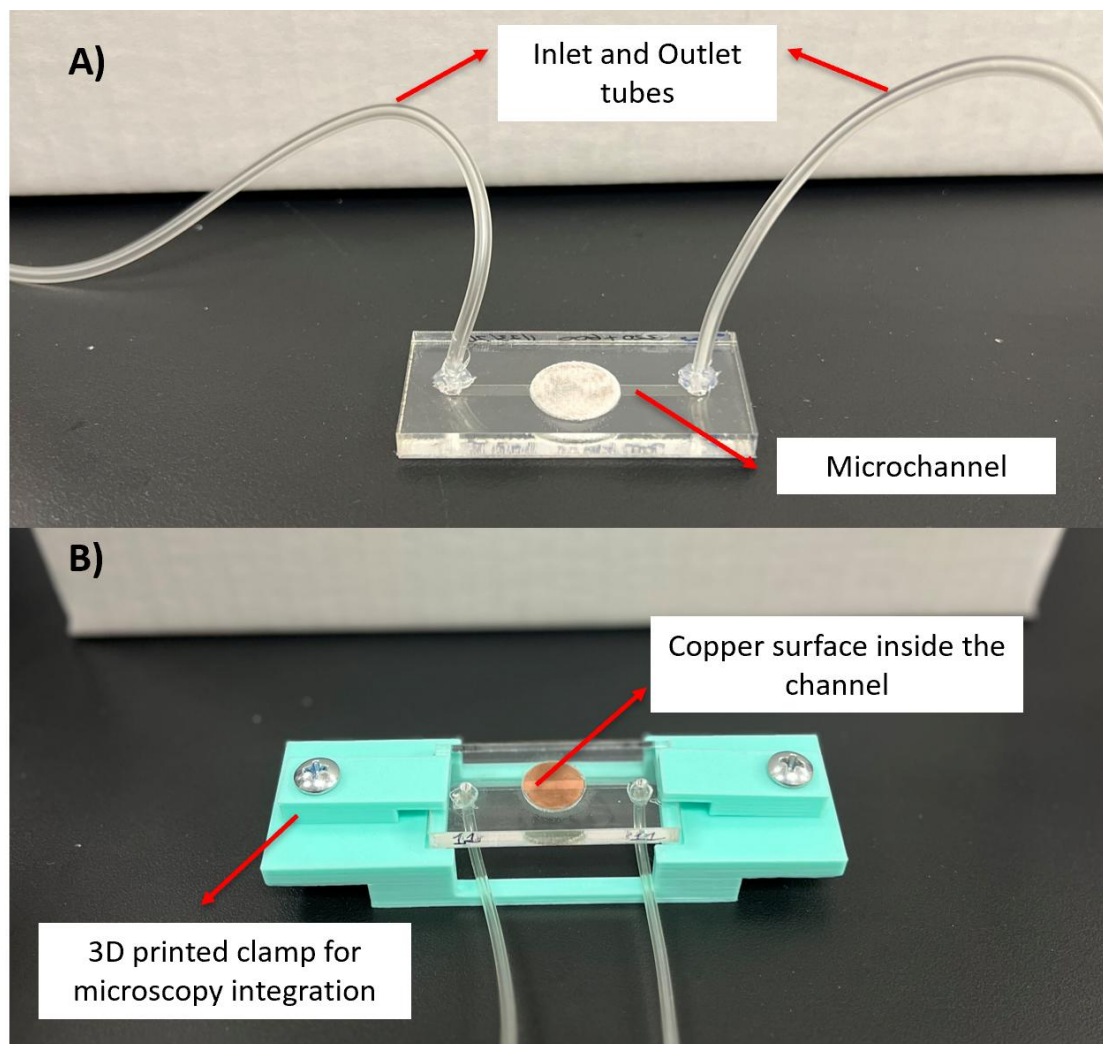


Figure 2.4 Assembled microfluidic device showing the straight channel, copper coupon surface, and inlet and outlet tubes.

Surface Quality

Surface quality of the copper coupons was evaluated using three techniques. The first was surface roughness measurement conducted in a clean room using a White Light Interferometer (WLI) with the profilometry tool. Surface roughness was quantified using the root mean square roughness (S_q), which is defined as the standard deviation of the surface height deviations relative to the mean plane (Whitehouse, 1997). S_q provides a more sensitive measure of surface

variability than the arithmetic mean roughness (S_q), because it places greater weight on larger peaks, and valleys in the surface profile (de Groot, 2015). Given that WLI measures surface heights over an area with high sensitivity to small topographical variations, S_q was chosen over S_a to better capture subtle differences in surface texture critical for device fabrication performance. Surface and mechanical properties are indispensable for material characterization, especially in device fabrication. White WLI is a widely utilized optical measurement technique that enables rapid, straightforward, and precise assessment of surface roughness, an essential indicator of product quality (Bakhtazad & Chowdhury, 2019; De Groot & Deck, 1995; Makadia & Nanavati, 2013). WLI is a non-physical interaction technique while stylus profilometry is a contact-based technique that measures surface properties by tracing a physical stylus across the surface (Blunt et al., 2003). Although stylus techniques are quite accurate and widely used, the risk of sample damage and limited applicability to soft materials makes optical interferometry more suitable.

A total of nine data points were collected from each copper coupon, with three measurements taken from the center, midsection, and edge. After surface roughness measurements, contact angle measurements were performed for each coupon. To assess the wettability of the surface, the video contact angle system (VCA 2500XE) was used. The sessile drop technique was applied using 5 μL of distilled water, precisely measured with a micro syringe, and placed onto the copper surface to analyze the right and left contact angles by the VCA Optima XE software. This software automatically calculates the left and right angles. For each sample, three droplets were captured, with measurements taken from the center, midsection, and edge of the surface. The average contact angle was calculated from these measurements.

Lastly, the flatness of the coupons was measured by the ZEISS VAST XXT Coordinate Measuring Machine (CMM). Stylus placed fifteen points on the copper coupon surface to measure flatness and the data collected is used to create a three-dimensional model of the surface, which is then compared to the ideal flat plane to determine flatness.

Bonding Quality

Experimental Setup for Leak Tests with Tap Water and Abiotic Experiments. The microfluidic device was connected to a Kent Scientific Corporation Genie Touch™ syringe pump via BD Plastipak syringes and tested under different conditions to evaluate the formation of Saffman-Taylor fingering. For leak tests with tap water, a 20 ml BD Plastipak syringe containing tap water was used at two different flow rates: a low flow rate of 7 $\mu\text{l}/\text{min}$ and a high flow rate of 17 $\mu\text{l}/\text{min}$. The flow rates were intentionally selected for practical reasons. Since the actual experimental flow rate used in biological studies was much lower than (0.1 $\mu\text{l}/\text{min}$), these higher values were chosen to simulate more demanding conditions and demonstrate the device's mechanical integrity under increased flow. Additionally, they allowed the syringe volume to be consumed within approximately 24 hours, making it easier to monitor leakage and ensure a complete test cycle within a manageable time frame.

During leak tests, the channel area of the device was imaged using an Olympus BX41 upright microscope equipped with an AmScope (MU503) camera. Brightfield imaging was performed at 20x magnification to visually assess STF formation near potential bonding imperfections. Images were captured periodically throughout the approximately 24-hour experiments to track STF development over time.

For abiotic experiments, a 1 ml BD Plastipak syringe was used to introduce media with varying salt concentrations: low (0.05% NaCl in MilliQ water), medium (1% NaCl in MilliQ water), and high (3.5% NaCl in MilliQ water) saline solutions at a low flow rate of 0.1 $\mu\text{L}/\text{min}$.

All saline solutions were prepared by weighing fine crystalline sodium chloride (analytical grade, Fisher Scientific), and dissolving it into MilliQ water to achieve 0.05%, 1%, and 3.5% NaCl concentrations. Each salt concentration was tested independently for approximately 24 hours, and STF formation was again monitored via brightfield microscopy as described above.

In both cases, experiments were performed for 24 hours, after which the device channels were examined under a microscope (Olympus BX41) at 20x magnification to assess the presence of Saffman-Taylor fingers.

Bacterial Cultures and Microfluidic Experiment

Similar to other microfluidic investigations, *Pseudomonas fluorescens* N2E2 strain harboring the RFP-expressing pGinger suite plasmid was obtained from Dr. A. Deutschbauer (Lawrence Berkeley National Laboratory). To grow the organism, a single colony was removed from a tryptic soy broth (TSB) agar plate and transferred to 10 mL of liquid R2A broth. R2A (Reasoner's 2A) is a low-salt, rich medium suitable for the cultivation of slow-growing bacteria, it contains yeast extract, proteose peptone, casamino acids, glucose, starch, sodium pyruvate, dipotassium phosphate, and magnesium sulfate (Reasoner & Geldreich, 1985). Both the agar and broth contained 60 $\mu\text{g}/\text{mL}$ kanamycin to maintain antibiotic selection. The culture was incubated overnight at 30°C with shaking at 72 rpm. The suspension was then centrifuged at 5,000 $\times g$ for five minutes and the resulting pellet was resuspended in R2A medium and diluted to a final

optical density of 0.1. The optical density was measured using a Thermo Scientific, Genesys 10S UV-VIS Spectrophotometer at the 600 nm wavelength range.

For the microfluidic flow experiment, 1 mL of the prepared bacterial suspension was transferred into a 1 mL syringe and mounted onto a syringe pump. Before connecting it to the device, the flow line was primed, ensuring smooth operation. The flow was then set to a steady rate of 0.1 $\mu\text{L}/\text{min}$. After running continuously overnight, the flow was stopped, and the device was brought to the Center for Biofilm Engineering (CBE) Bioimaging facility. Inverted confocal laser scanning microscopy (CLSM) imaging was performed using a 63x oil immersion objective.

Computational Model

Computational fluid dynamics simulations were performed to determine the optimal channel shape and width for efficient shear stress on copper coupon. First, a three-dimensional (3D) model was designed using AutoDesk® Inventor program and then imported into COMSOL Multiphysics®, a computational fluid dynamics (CFD) simulation software to simulate fluid flow within a microfluidic device. The model was conducted under laminar flow physics with a stationary study and solves the incompressible steady-state Navier Stokes equations to characterize fluid behavior within channel (Nairn et al., 2024). The model simulated the flow within a 38.2 mm long section of channel. The fluid was modeled as water at room temperature. The boundary conditions were defined as a fully developed laminar flow profile was imposed at the inlet, while the outlet was set to zero pascal pressure to allow for natural flow development. No-slip conditions were used for the channel. The velocity at the inlet was defined as a function of the imposed flow rate, ensuring consistency with experimental conditions. Shear stress on copper surface along the channel were computed (See Appendix A to examine the other

channels.). From the Navier-Stokes equation, it is possible to express the wall shear stress along a rectangular cross-section microchannel as (Pisapia et al., 2022):

$$\tau = \frac{6\mu Q}{wh^2} \quad (2.1)$$

where μ is the dynamic viscosity of the fluid (Pa·s), Q is the volumetric flow rate (m^3/s), w is the channel width (m). and h is the channel height (m).

Statistical Analysis

Surface quality measurements, including surface roughness, contact angle, and flatness, were collected across multiple copper coupons. For surface roughness analysis of the 400 and 600 US grit polished level copper coupons, nine measurements per sample were obtained per sample by collecting three data points from the center, midsection, resulting in a total 135 data points across 15 samples. Additionally, 18 roughness measurements were collected to assess the effect of different polishing grit combinations on STF formation. Specifically, three devices were prepared using 320 and 400 US, three devices using 320 and 600, and 400 and 600 US grit polishing; each device was evaluated under both low (7 $\mu\text{l/min}$) and high flow rate (17 $\mu\text{l/min}$) conditions. For the contact angle measurements of the 400 and 600 US grit polished level copper coupons, three measurements were taken per coupon (from center, midsection and edge), for a total of 45 data points across 15 samples. Flatness measurements were based on fifteen individual point measurements per coupon, but one overall flatness values was calculated per coupon, resulting in 15 total flatness results. Bonding quality was assessed by conducting leak tests with tap water using 100 devices across low (7 $\mu\text{l/min}$) and high flow rates (17 $\mu\text{l/min}$), and saline solution leak tests were performed using 12 devices with different salt concentrations (0.05%, 1%, and 3.5% NaCl).

All experimental comparisons were analyzed using a two-sample-t-test assuming unequal variances to compare groups. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using Microsoft Excel.

Results and Discussion

Computational Modeling – COMSOL Multiphysics

Computational modeling results indicate that the liquid in the microfluidic channel exhibits a laminar flow regime across all three channel designs (see Appendix A for other channel designs), as confirmed by the low Reynolds number (Re) obtained from simulations (Saldana et al., 2024). The single channel design with a linear structure, had a Reynolds number of 0.001462, confirming that the flow remains well within the laminar regime, as expected in microfluidic systems due to the small characteristics length scales and low flow velocities (Eckhardt, 2009).

In this study, the straight channel was designed to investigate the shear stress distribution on the middle of the copper coupon within the microfluidic channel and was analyzed using COMSOL Multiphysics. Figure 2.5 A shows a streamline plot of the velocity field within the microchannel. The color bar represents the velocity magnitude, ranging from $1 \times 10^{-5} \mu\text{m/s}$ (blue) to $20 \mu\text{m/s}$ (red). The streamlines illustrate the direction and magnitude of fluid flow, with higher velocities observed along the channel centerline and lower velocities near the channel walls, consistent with laminar flow behavior. Additionally, the streamline model shows that fluid moves parallel to the walls within the microfluidic channel, reaching the highest velocity magnitude in the center of the channel and a minimum velocity at the walls due to the no-slip boundary condition. The velocity streamline analysis reveals a smooth and predictable flow

profile. Gradually color changes from the center (high velocity) to the wall (low velocity) forming a parabolic shape, which is characteristics of laminar flow through confined microchannels. No significant recirculation or vortex formation was observed, further supporting the stability of the designed flow conditions. This is one reason why maintaining a steady flow velocity and shear stress within the channel can support cell adhesion (Pisapia et al., 2022).

Studies suggest that channel geometry can also play a role in cell adhesion because of a uniform velocity profile; and this indicates that the shear stress within the device is also uniform that is why this design supports cell adhesion (Green et al., 2009; Pisapia et al., 2022). In this study we selected a confined single straight, narrow microchannel design (38.2 mm length, 2.2 mm width). Since the channel width remains constant in this study, the shear stress distribution should stay uniform along its length, allowing for a controlled evaluation of its impact on cell attachment and flow behavior.

Additionally, the shear stress simulation distribution within the channel, particularly at the center of the copper coupon, is visualized in Figure 2.5 B. The maximum modeled shear stress was 674 μPa and minimum shear stress was 362 μPa . The color gradient represents the magnitude of shear stress across the surface, with variations depicted using a color scale ranging from blue (low shear stress) to red (high shear stress). The observed distribution shows that the majority of the surface exhibits orange-red color, indicating regions of moderate to high shear stress. The absence of abrupt color changes suggests that shear stress is relatively uniform across the copper coupon, reinforcing the expected laminar nature of the flow. The uniform shear stress within the device should support homogenous cell adhesion within the channel (Green et al., 2009; Pisapia et al., 2022).

The observed trends in shear stress align well with previous studies on laminar flow in microfluidic systems (Yu et al., 2014; Yoshinaga et al., 2014). Similar studies have reported that microchannels with dimensions on the order of tens of micrometers exhibit shear stresses in the range of hundreds to thousands of μPa , depending on flow rate conditions (Janakiraman et al., 2009; Kim et al., 2013; Lecuyer et al., 2011; Wright et al., 2015).

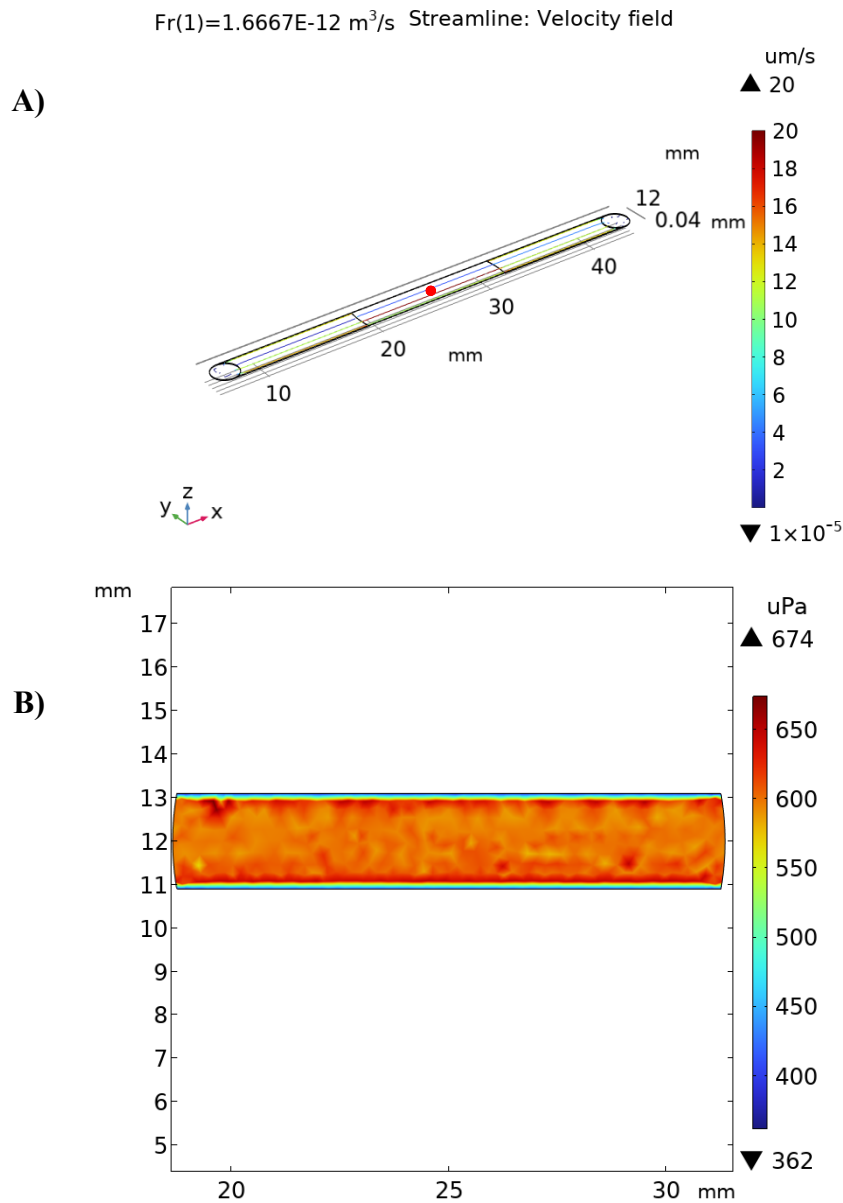


Figure 2.5 Streamline velocity field of the straight microfluidic channel at 0.1 $\mu\text{L}/\text{min}$ (A). The color gradient represents velocity magnitude ($\mu\text{m}/\text{s}$), with the highest velocity observed at the channel center and the lowest at the walls due to the no-slip boundary condition. The flow remains laminar, as indicated by the smooth parallel streamlines. Shear stress on copper coupon surface within the microfluidic channel (B). The color gradient represents the shear stress magnitude (μPa), with higher shear stress concentrated along the surface while maintaining a uniform distribution. The absence of abrupt changes confirms stable laminar flow conditions. Red dot represents center (mid) of the copper coupon surface for shear stress analysis.

While the initial modeling focused on idealized channel conditions without structural defects, real-world fabrication process may introduce imperfections that alter the flow profile and shear stress distribution. Therefore, additional simulations were performed to assess the effects of assembly-related gaps between the copper coupon and acrylic part (Figure 2.6 A). This study demonstrates that in the straight channel, the presence of a 200 μm gap between the copper coupon and acrylic part creates an expanded region where fluid can flow. This expansion reduces the velocity gradient near the surface, leading to lower shear stress values in the ‘with gap’ case compared to the ‘without gap’ case. Despite the gap, the flow remains laminar without significant vortex formation. In order to understand the influence of this gap on shear stress distribution, simulations were conducted for both gap and no-gap conditions.

A fundamental principle in fluid mechanics is that shear stress (τ) in a Newtonian fluid is directly proportional to the velocity gradient (du/dy) perpendicular to the flow direction (Yoshinaga et al., 2014):

$$\tau = \mu \frac{du}{dy} \quad (2.2)$$

where μ is the dynamic viscosity. Shear stress is also defined as force per area (Lacombe & Zenhausern, 2022). In Figure 2.6 B, the maximum shear stress was 80.1 μPa and minimum shear stress was 27.5 μPa . Also shear stress is proportional to the velocity gradient near the wall and inversely proportional with cross-sectional area. When the fluid spreads into the gap, the velocity gradient decreases, resulting in reduced shear stress on the surface (White, 2009; Yoshinaga et al., 2014). Our simulation results confirm this effect, showing a significant decrease in shear stress from 604.4 μPa (no-gap) to 63.8 μPa (with gap) at the mid-coupon region.

The streamline analysis shows that, in the baseline (no-gap) case, streamlines remain tightly confined, and velocity distribution is more uniform along the channel while in the ‘with gap’ case (Figure 2.6 A), streamlines spread slightly near the copper region, indicating that the gap allows some flow redistribution. The color gradient suggests that velocity is slightly lower around the copper coupon edge walls (gap region), supporting the idea that velocity gradients near the surface are reduced. These findings show the importance of streamline behavior and flow confinement effect when designing microfluidic systems.

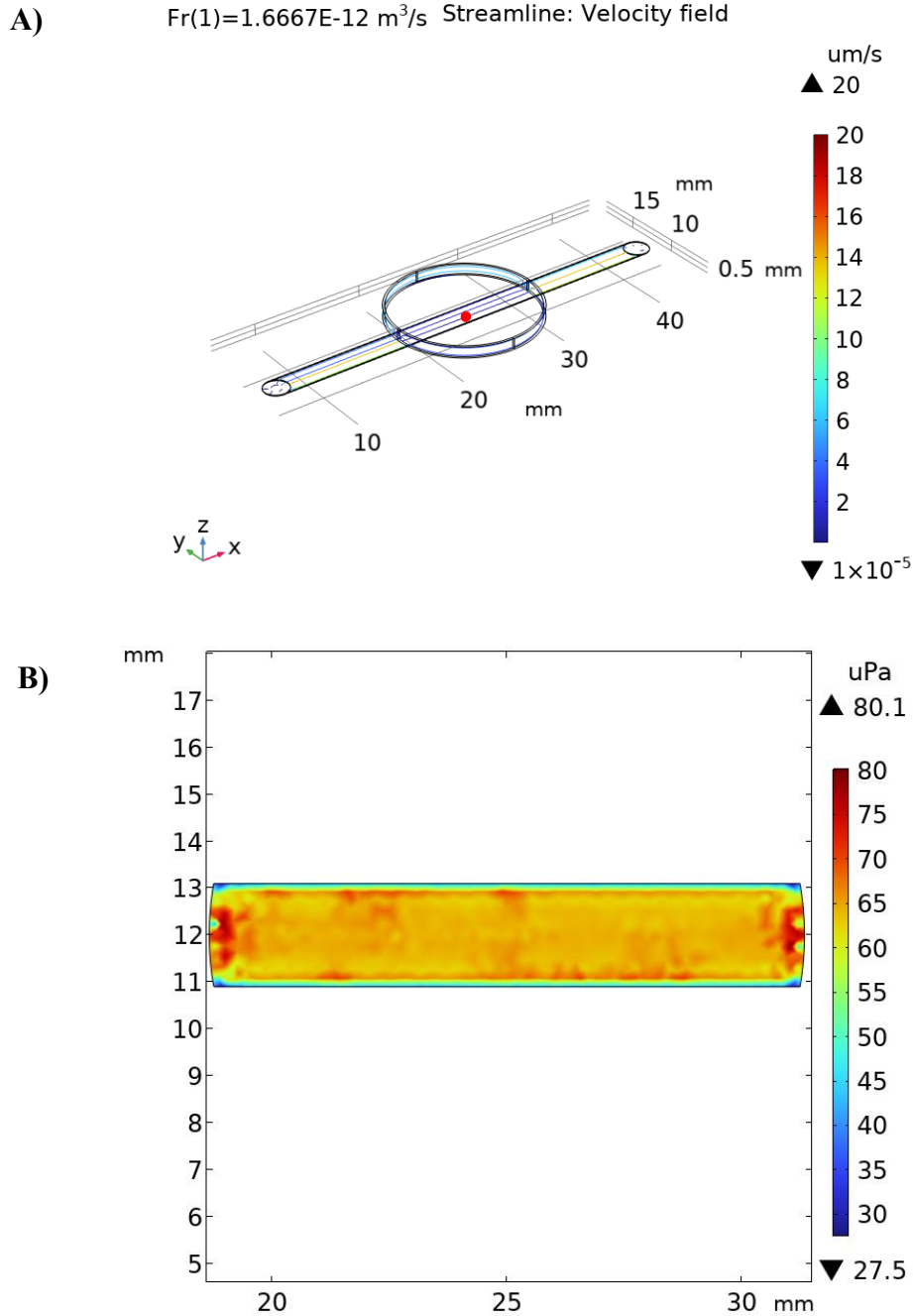


Figure 2.6 Streamline velocity field (A) shows flow redistribution due to the presence of the gap at $0.1 \mu\text{L}/\text{min}$. These streamlines expand slightly around the copper coupon, indicating a change in velocity gradients. The velocity magnitude is highest at the channel center and decreases towards the walls, with no significant vortex formation observed. Shear stress on the copper coupon surface (B); compared to the no-gap condition, shear stress is significantly reduced, with values ranging from 27.5 to $80.1 \mu\text{Pa}$. Red dot represents center (mid) of the copper coupon surface for shear stress analysis.

Furthermore, the shear stress values were calculated for five flow rates (0.1 $\mu\text{L}/\text{min}$, 0.33 $\mu\text{L}/\text{min}$, 0.55 $\mu\text{L}/\text{min}$, 0.78 $\mu\text{L}/\text{min}$, and 1 $\mu\text{L}/\text{min}$, respectively) using Equation (2.1). The shear stress vs. flow rate plot (Figure 2.7 – blue and red line) shows a linear relationship. The blue line represents the without gap around the coupon condition, with modeled shear stresses of 604.4 μPa , 1964.21 μPa , 3324.05 μPa , 4683.90 μPa , and 6043.74 μPa , respectively; the red line represents the with gap around the coupon condition, with modeled shear stresses of 63.80 μPa , 207.36 μPa , 350.92 μPa , 494.48 μPa , and 638.05 μPa , respectively. This confirms that shear stress increases proportionally with flow rate, thus we only need to simulate one flow rate (0.1 $\mu\text{L}/\text{min}$). This correlation aligns with theoretical expectations, as equation (2.1) indicates that increasing flow rate leads to a proportional increase in shear stress, assuming all other factors remain constant (Marquet, 2023).

Overall, the results highlight the role of channel geometry in controlling shear stress and velocity distributions, which are critical factors for initial biofilm attachment. Fine-tuning flow conditions and adjusting geometric constraints with precision can help shape biological interactions in a controlled way. These findings are valuable for developing more effective microfluidic devices in the future.

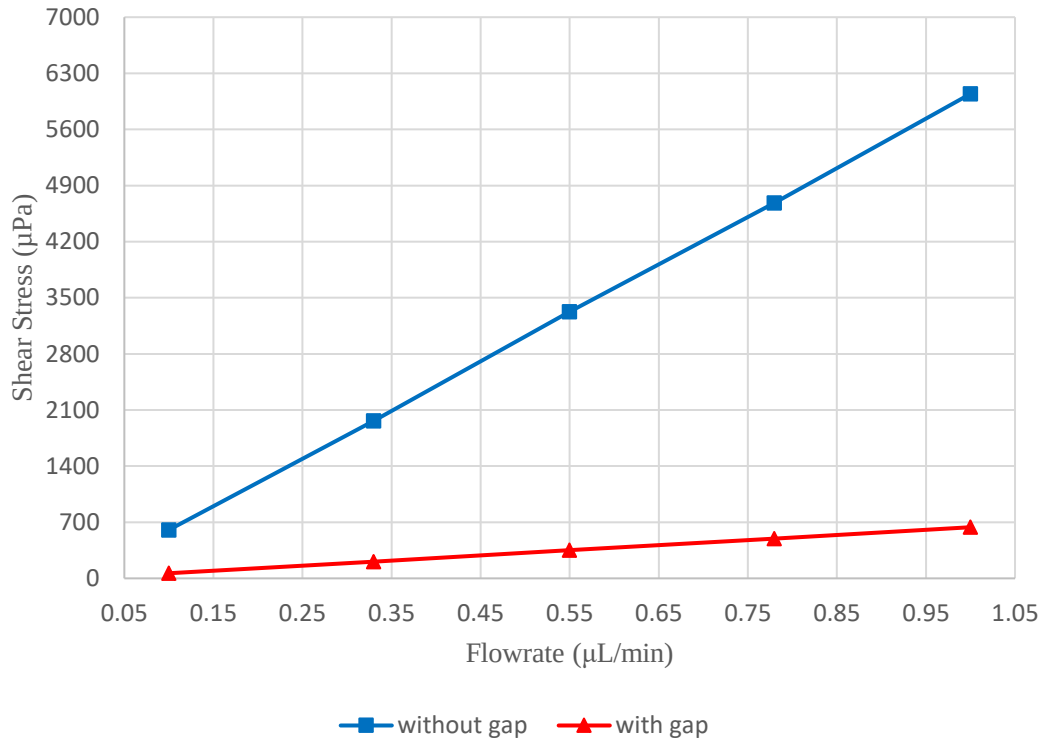


Figure 2.7 This graph represents the shear stress versus flow rate (0.1, 0.33, 0.55, 0.78, 1 $\mu\text{L}/\text{min}$, respectively) for the microfluidic channel with (blue) and without (red) a 200 μm gap between the copper coupon and acrylic part. Shear stress values were measured at the center region of the copper coupon surface using COMSOL Multiphysics®. The blue line represents shear stress in the without gap condition, showing a strong linear increase with flow rate. The red line represents the with gap condition, where shear stress remains significantly lower across all flow rates. This result confirms that the presence of gap reduces shear stress due to increased flow area and redistribution, leading to a lower velocity gradient near the coupon surface.

Surface Quality

Profilometer. The topography of copper surfaces was assessed using White Light Interferometry (WLI) to evaluate surface roughness, a critical parameter for device fabrication that also provides insight into cell attachment studies. Surface roughness can either increase or decrease the contact area between bacteria and the surface, thereby influencing their initial adhesion (Yang et al., 2022). Some studies suggest that acid treatment can increase the roughness of the surface leading to more cell attachment (Zareidoost et al., 2012). Similarly,

while some research suggest that increased surface roughness promotes bacterial adhesion (Renner & Weibel, 2011); Rühs et al., 2020), others suggest the opposite (Mitik-Dineva et al., 2008a); (Može et al., 2021). Given these findings, evaluating surface roughness was necessary to understand its potential impact on bacterial adhesion studies as well. Additionally, no studies directly focus on the effect of surface roughness on Saffman-Taylor fingering and bonding. This study takes a closer look at this aspect and explains the choice of surface treatment methods.

In this work, the polishing sequences employed included 320 and 400, 320 and 600, and 400 and 600 US grit. Surface roughness presented in Table 2.2 were also measured using WLI on a few representative copper coupons to illustrate the effect of different polishing sequences. The unpolished copper coupon exhibited a rougher surface ($1.098\ \mu\text{m}$) compared to the polished variants, as anticipated due to the manufacturing processes (Figure 2.8. A-a). Analysis of the polished surfaces revealed a homogenous distribution of grinding marks for each grit combination (Figure 2.8 B-D). As the grit number increased, the observed surface roughness decreased progressively. Specifically, the highest roughness measured approximately $1.1\ \mu\text{m}$ with unpolished, which was reduced to roughly one-fourth ($0.29\ \mu\text{m}$) of this value after 400 and 600 US grit polishing. Additionally, the surface roughness was measured as $0.53\ \mu\text{m}$ after polishing with 320 and 400 US grit, while it decreased to $0.37\ \mu\text{m}$ when 320 and 600 US Grit were used.

Table 2.2 Surface roughness of copper samples prepared with different surface finish.

Surface Finishes	Mean Roughness (μm)
Unpolished	1.098
320 & 400 US grit	0.53

Table 2.2 Continued.

Surface Finishes	Mean Roughness (μm)
320 & 600 US grit	0.37
400 & 600 US grit	0.29

Although surface finishes appeared similar, slight roughness differences were observed between them. However no statistical analysis was performed between these groups due to limited sample size. These observations suggest that surface roughness is influenced not only by final grit but also by the initial one (Aydin et al., 2020). That is why the surface roughness might be enhanced by the polishing procedure. The surface was rougher with smaller grit sizes (Q. Liu et al., 2020). Starting with a 320 US grit creates deeper scratches, so even after polishing with 600 US grit, the surface remains rougher than if it had started with 400 US grit. Therefore, the order of grit application plays a key role in achieving a smooth finish.

For full statistical evaluation, a total of one hundred thirty-five roughness measurements were obtained using a WLI, with three measurements conducted on each copper coupon, resulting in nine measurements per device across fifteen device designs. The results showed no significant variations in surface roughness ($p > 0.05$, two-sample t-test assuming unequal variances), confirming the uniformity of the copper surface preparation (Figure 2.9). This uniformity indicates that the optical interferometric methods adequately met the requirements for profiling the copper coupon surfaces necessary for this study.

In terms of fluid flow characteristics, Saffman-Taylor fingering was observed on rougher surfaces; however, no instability was noted at either low or high flow rates on surface polished with 400 and 600 US grit (Figure 2.10). Although surface roughness levels did not present

drastic difference, the observed stability suggests that smoother surfaces contribute positively to reliable device design.

Figure 2.10 presents the average Saffman-Taylor fingering length under different polishing conditions, with error bars representing the standard deviation. Consistent with the stability observed on smoother surface, the results show that polishing with 320 and 400 US grit produced the longest STF formations, with mean lengths of 70.5 μm at low flow rate and 92.06 μm at high flow rate. When the polishing condition was modified to 320 and 600 US grit, STF lengths decreased to 43.47 μm at low flow rate and 51.22 μm at high flow rate. Notably, no Saffman-Taylor fingering was observed on surfaces polished with 400 and 600 US grit, regardless of flow rate.

This trend suggests that higher US grit polishing inhibits STF formation, likely due to the reduced surface roughness. These findings reinforce the role of surface roughness in STF behavior, where rougher surfaces promote larger STF structures, while smoother surfaces, such as those polished with 400 and 600 US grit, suppress STF formation. The absence of STF on these surfaces further supports their suitability for leak-free device performance.

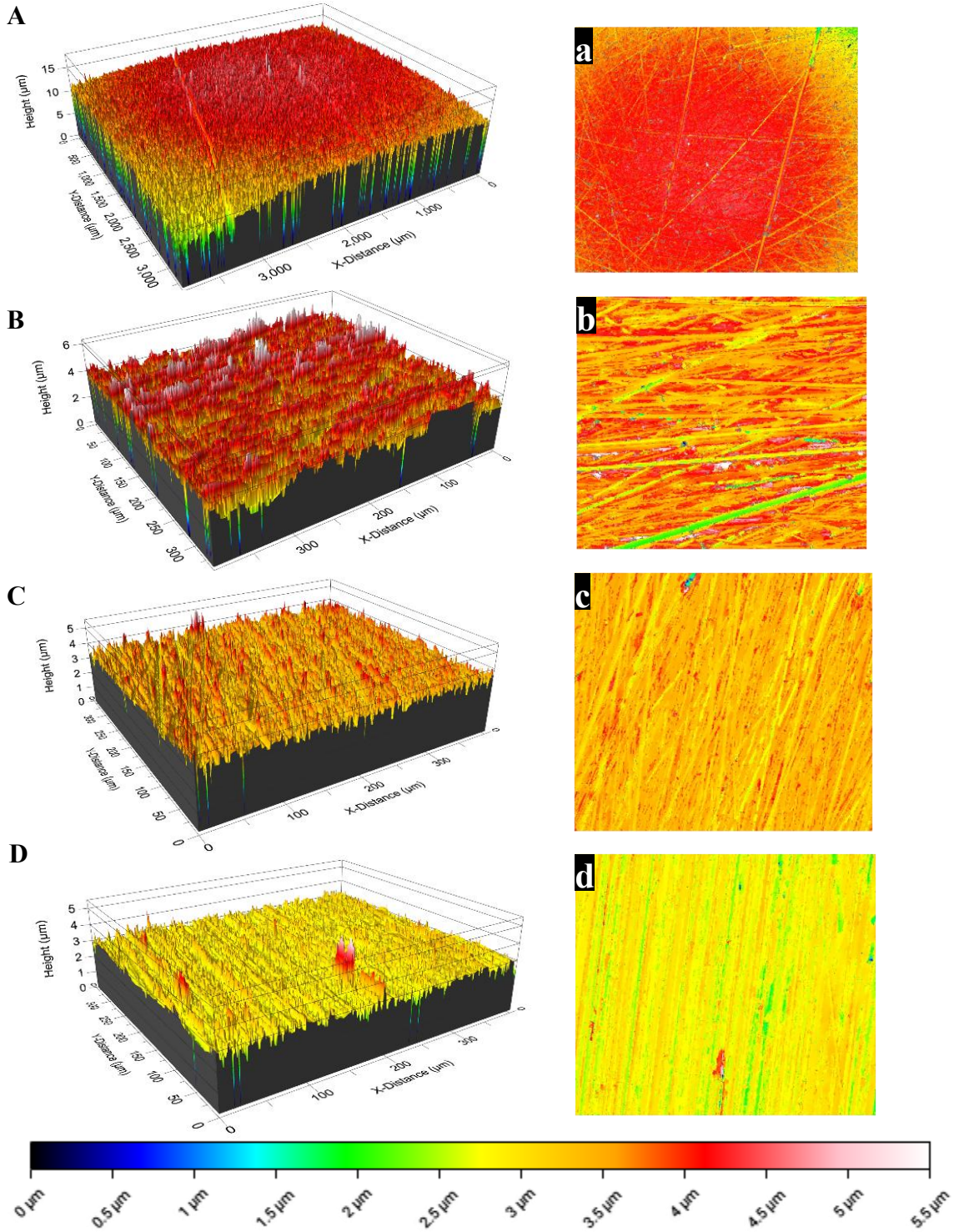


Figure 2.8 3D topography of copper coupons prepared with different polishing and the corresponding distribution of roughness: (A-a) unpolished, (B-b) 320 and 400 US grit, (C-c) 320 and 600 US grit, (D-d) 400 and 600 US grit, respectively. The right column represents the top view.

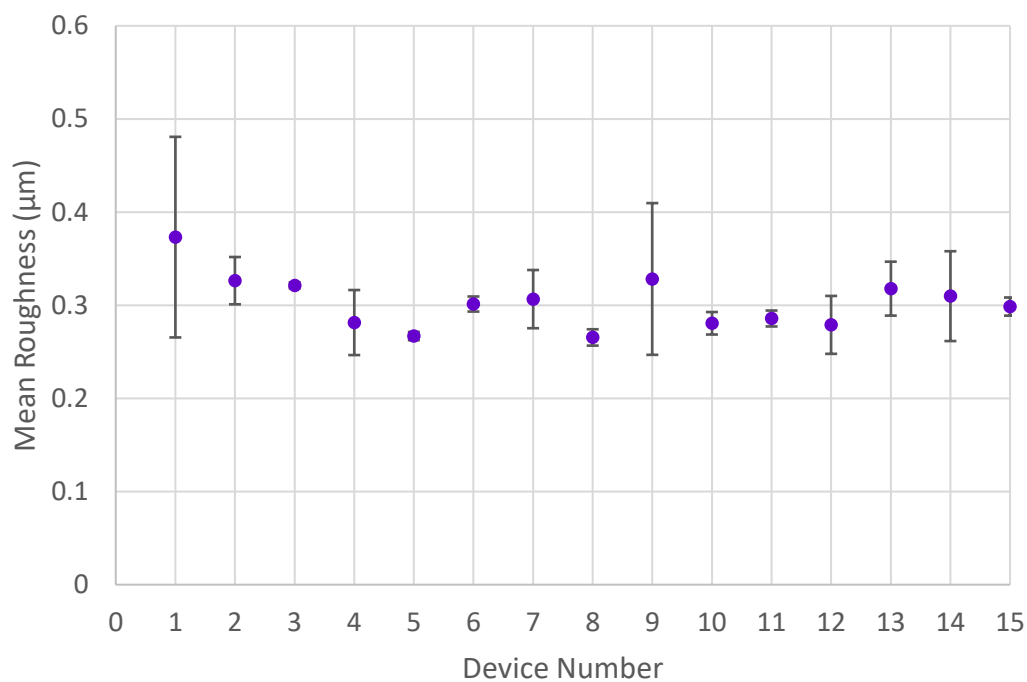


Figure 2.9 Mean surface roughness values of copper coupons measured using profilometry. Nine roughness data points were collected from each coupon (400 and 600 US Grit), resulting in a total of one hundred thirty-five roughness data points. Error bars represent $\pm$ standard deviation (SD), indicating the variability in surface roughness measurements.

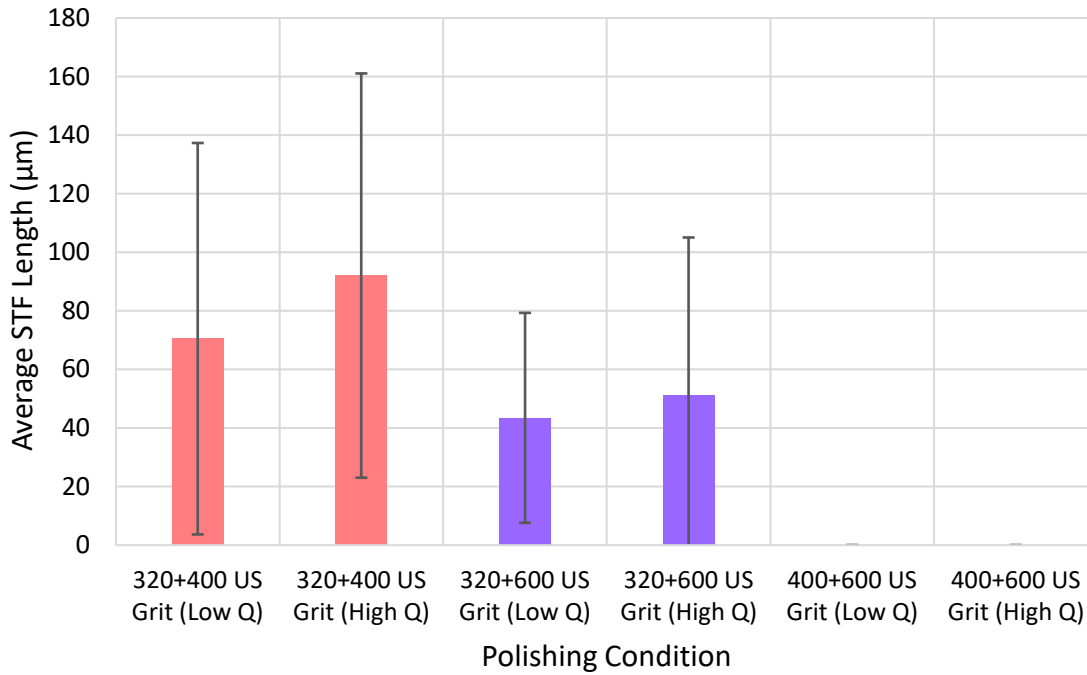


Figure 2.10 Effect of surface roughness and flow rate on Saffman-Taylor fingering. Error bars represent standard deviations. Average STF length (μm) for different polishing conditions was tested with six devices for each polishing, totaling eighteen across all conditions. While greater roughness enhanced instability formation, surface polished with 400 and 600 US grit showed no instability at both low ($7 \mu\text{l/min}$) and high ($17 \mu\text{l/min}$) flow rates, suggesting a suppression of interfacial perturbations at these smoothness levels.

Contact Angle Measurement. Contact angle measurement was used to determine surface wettability, providing insights into surface energy and hydrophobic or hydrophilic behavior of the copper samples. This technique analyzes the angle formed between a liquid droplet and the surface, providing direct assessment of material-liquid interactions and ensuring compatibility with device manufacturing processes (D.Y. Kwok & A.W. Neumann, 1999). The contact angle measuring instrument (VCA 25500XE) was used to test the wetting behavior of HCl-treated copper coupons polished with 320 and 400, and 320 and 600, and 400 and 600 US grit sandpapers. The results showed that the mean contact angles increased after polishing with

values ranging from approximately (59° , 61°) to (101° , 101°). According to wettability classification, surfaces with a contact angle greater than 90° are hydrophobic, while those below 90° are hydrophilic (Han et al., 2022). Lower contact angles correspond to rougher surfaces (Figure 2.11), suggesting that increased surface roughness enhanced hydrophilicity compared to smoother, more hydrophobic surfaces (Qin et al., 2014). Additionally, typically, copper has a wettability greater than 70 degrees, and the oxide layer that forms on its surface makes it even more wettable (Može et al., 2021). However, in this study, the surface underwent HCl treatment, which increased its hydrophobicity, making it more resistant to water.

Before the HCl treatment, oxidation was present on the copper coupons, leading to the accumulation of oxide particles over time, which resulted in surface color change (Figure 2.12) and loss of luster (A. Takemura et al., 2017). To remove these oxide layers, the copper coupons were treated with 37% hydrochloric acid (HCl), a widely used strong acid with high chemical reactivity (Tran et al., 2019). The copper samples were submerged in HCl at the final stage of preparation, primarily to eliminate organic contaminants and oxide residues (Li et al., 2022; Tong et al., 2021). The results in Figure 2.13 show that the HCl-treated surface had a higher contact angle than the untreated surface, confirming that oxidized copper surfaces are typically more hydrophilic than freshly cleaned copper surfaces (Hong et al., 1994). This suggests that HCl treatment raises hydrophobicity, consistent with recent studies reporting that copper surfaces can exhibit strong hydrophobic behavior (Korczeniewski et al., 2021). HCl treatment was primarily used to remove surface oxides from copper; however, it also resulted in increased surface hydrophobicity. Whether this outcome is beneficial depends on the specific biological application. While this may be advantageous for studying bacterial species that favor

hydrophobic surfaces, it could pose a limitation when working with bacteria that require hydrophilic environments for attachment. This could be noted as consideration when selecting surface treatments for future biofilm studies.

To further characterize the surface wettability, a total of forty-five contact angle measurements were obtained using the sessile drop technique, with three measurements per coupon across fifteen coupons. The coupons in Figure 2.14 were polished using the 400 and 600 US grit combinations. The mean values mostly clustered around 80°-90°, with minor deviations but no clear increasing or decreasing trend. The results showed no significant variations in contact angle measurement ($p > 0.05$, two-sample t-test assuming unequal variances), confirming high reproducibility of the copper surface preparation. This consistency demonstrates that the sessile drop technique met sufficiently the criteria for profiling the copper coupon surfaces for this study.

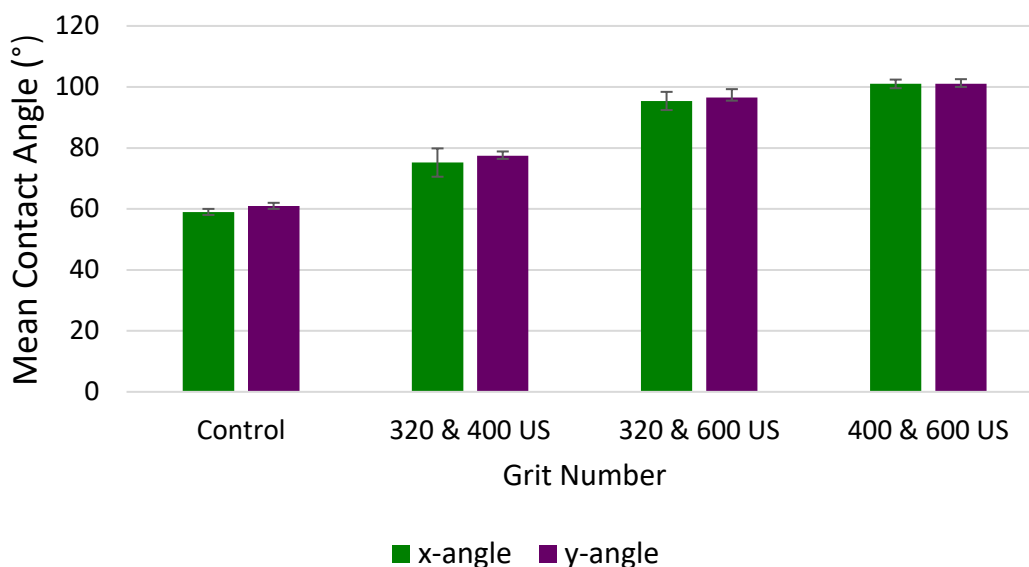


Figure 2.11 Mean contact angle measurements of HCl-treated copper surfaces polished with different grit combinations. Three replicate measurements were used to calculate the standard deviation for each condition and each angle (x and y).

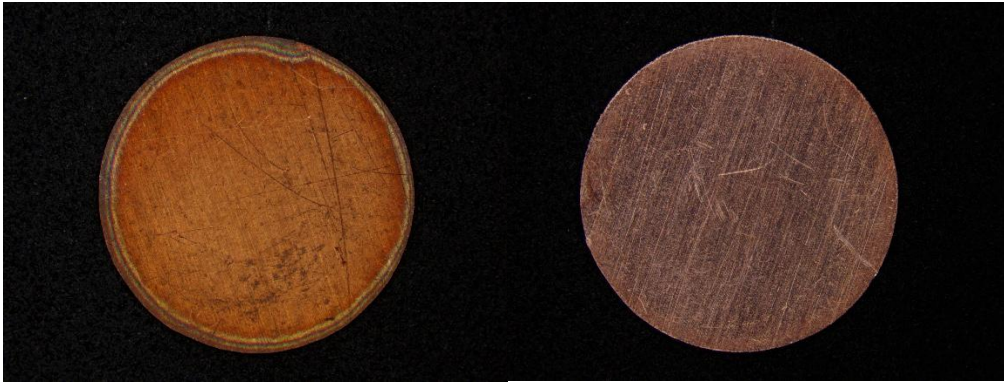


Figure 2.12 Copper specimens under AmScope LED light microscope. (Left) non-HCl treated unpolished copper coupon; and (Right) HCl-treated unpolished coupon (0.063" thickness, 12.7 mm diameter).

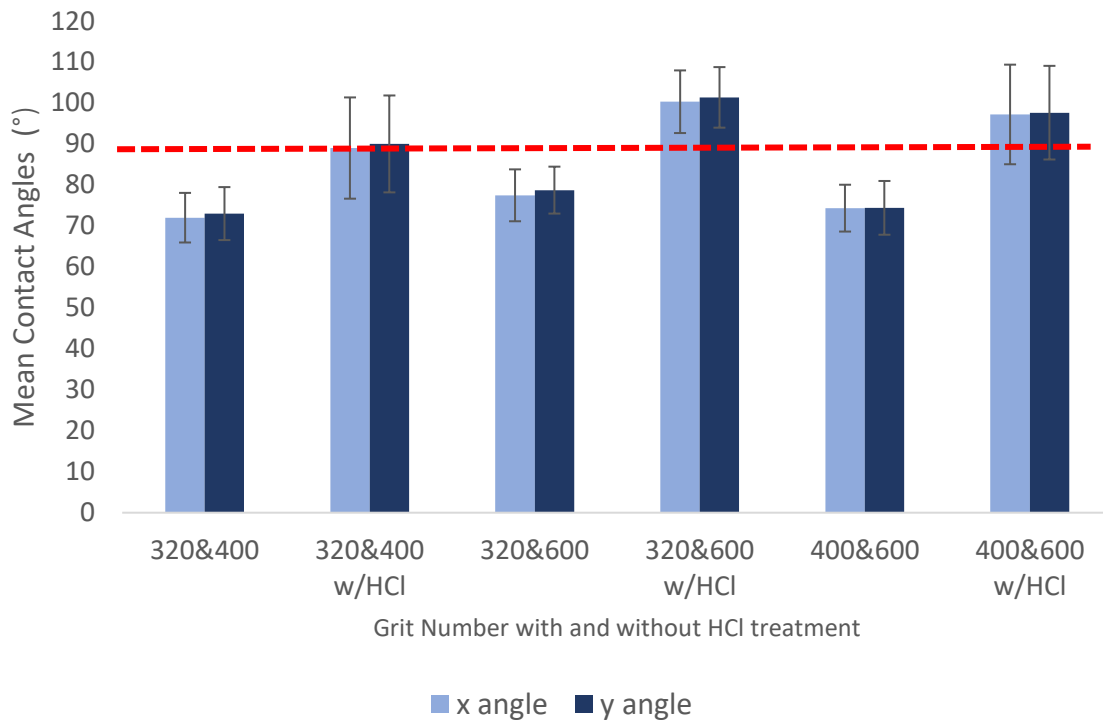


Figure 2.13 Mean contact angle measurements for different grit numbers with and without HCl treatment. The data compares the x and y contact angles for copper surfaces polished with different grit numbers (320 and 400; 320 and 600, and 400 and 600) before and after HCl treatment. HCl-treated coupons generally show higher contact angles, indicating hydrophobicity ($> 90^\circ$). The red dashed line represents 90° hydrophobic thresholds. The x and y measurements show consistent trends across all conditions. Each average and standard deviation was calculated based on three independent replicate measurements per condition and angle (x and y).

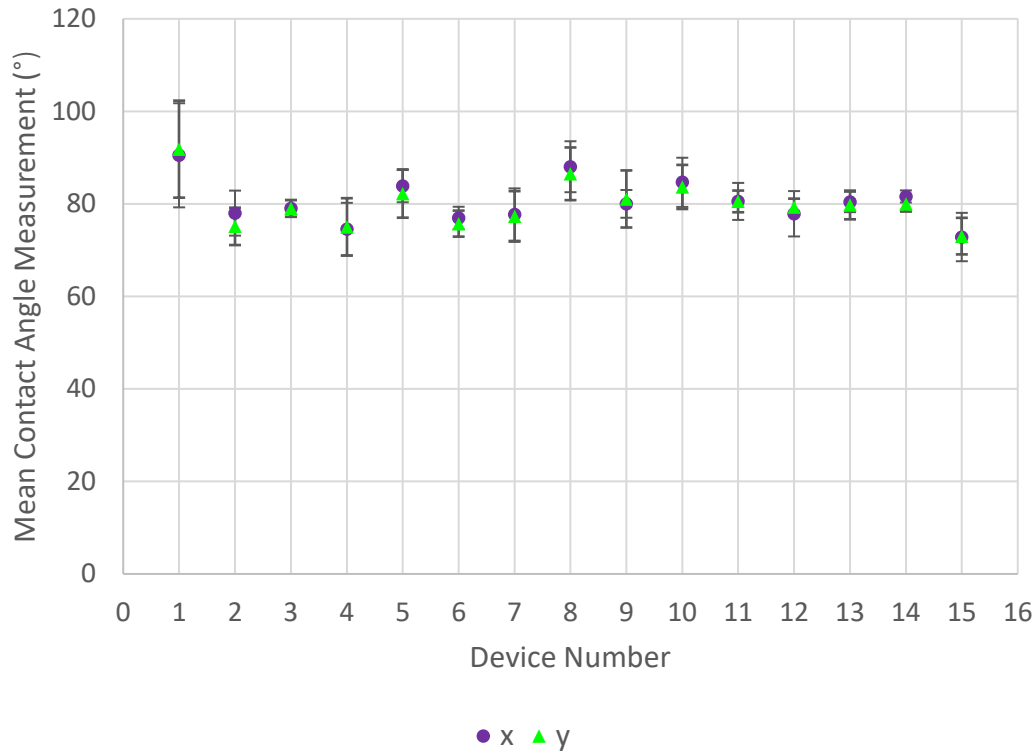


Figure 2.14 Mean contact angle measurements of copper coupons polished with 400 and 600 US grit and treated with HCl, obtained by sessile drop method. Both x-angle and y-angle measurements were collected at the center, midsection, and edge of each coupon (three data points per region). Error bars represent $\pm$ standard deviation (SD), indicating the variability in contact angle measurements.

Coordinate Measuring Machine (CMM). Ensuring flatness in sealing surfaces is important, as insufficient flatness may lead to leaks in the device. To evaluate the dimensional properties of the copper coupons, a Coordinate Measuring Machine (CMM) was used. The CMM is a contact-based measurement tool that tracks a probe over the surface of the copper coupon to measure its geometry with high accuracy. This technique is widely used in engineering areas due to its ability to evaluate complex shapes and dimensions with micron level accuracy (Schwenke et al., 2008). Compared to non-contact optical measurement systems (e.g., Optical Measuring Machine (OMM)), the CMM offers higher accuracy for rigid and stable materials.

However, given the rigidity of the copper coupons, its contact-based character may cause damage on soft materials, which was not a factor of concern in this work. To maintain measurement consistency and prevent impact on copper surface, the probe speed and contact force were calibrated. The high sensitivity of this technique combines with its suitability for rigid materials, makes it a reliable tool for verifying the structural integrity of microfluidic devices.

The flatness of the copper coupon was measured $7.7 \pm 1.4 \mu\text{m}$ across all devices. Minor variations were observed, likely due to the manufacturing process; however, the small standard deviation ($p > 0.05$) confirmed that the measurements were highly repeatable and that no significant flatness differences existed among the devices.

Bonding Quality

Leak Test with Tap Water. A preliminary leak test was conducted using tap water to evaluate the mechanical robustness of the device. The reason tap water is preferred over deionized (DI) water is that the minerals in tap water help form a protective oxide layer on copper surfaces (Quispe et al., 2022). In contrast, studies have shown that DI water, which lacks these ions, can cause corrosion and pitting on copper surfaces (Szakálos et al., 2007).

The leak test involved injecting tap water at low ($7 \mu\text{L}/\text{min}$) and at high ($17 \mu\text{L}/\text{min}$) flow rates and monitoring for leaks along the bonded interfaces and around inlet/outlet ports. Results indicated that at low flow rates, no leakage was observed, confirming the effectiveness of the bonding process. This validation allows for the use of even lower flow rates (e.g., $0.1 \mu\text{L}/\text{min}$ in a biotic test), ensuring stable operation under experimental conditions. Additionally, for flow rates lower than $7 \mu\text{L}/\text{min}$, the Saffman-Taylor fingering does not appear. This suggests that variations in flow rate play a significant role in instability formation, as higher flow rates

promote viscous fingering at the interface, whereas lower flow rates lead to more stable flow along the deformable PSA surface without additional interface deformation (Razavi Bazaz et al., 2020) (Figure 2.15). In this system, the PSA remains fixed and is not displaced by flowing fluid; rather, the water flows along a boundary that can slightly deform under flow conditions.

However, at a higher flow rate of 17 $\mu\text{L}/\text{min}$, STF patterns became more visible, further supporting the idea that flow dynamics contribute to fingering. This observation aligns with previous studies, which also observed that instability increases with higher flow rates (Den Chen, 1989; Razavi Bazaz et al., 2020). Unlike findings by Singh et al. (2021), where STF patterns appeared even at ultra-low flow rates, our results indicate that flow rate thresholds may vary depending on channel surface properties. Additionally, as seen in Figure 2.16, the plot shows the length of Saffman-Taylor fingering at two flow rates: 7 $\mu\text{L}/\text{min}$ and 17 $\mu\text{L}/\text{min}$. At the lower rate, finger lengths are shorter and more consistent. At the higher rate, they are longer and more variable, including one clear outlier. This confirms that higher flow promotes more pronounced fingering due to increased interface instability. Overall, these results support using Saffman-Taylor fingering to assess bonding quality, especially under higher flow conditions where defects become more visible.

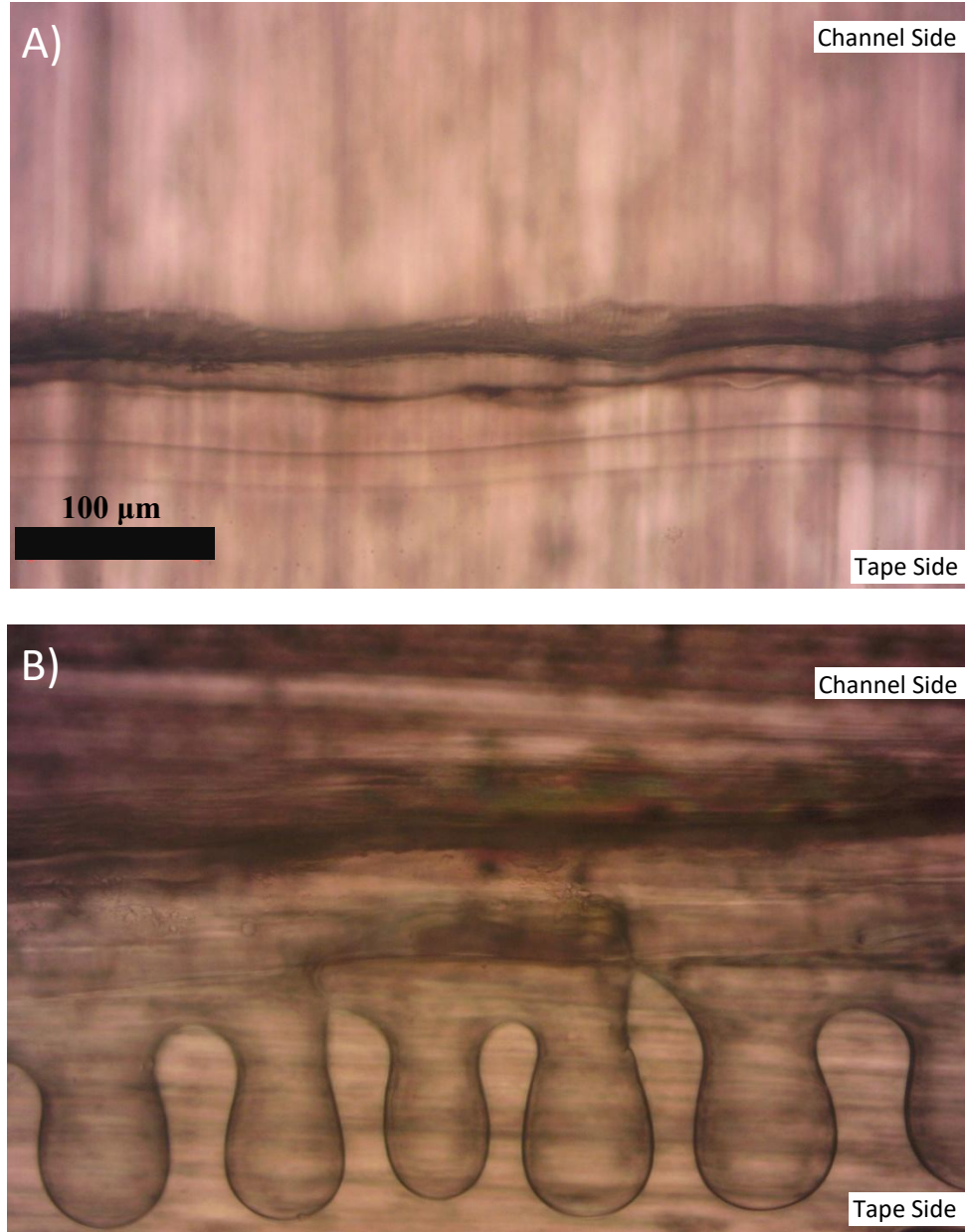


Figure 2.15 Comparison of Saffman-Taylor fingering at different flow rates. The top image (A) represents a stable interface at a low flow rate, where no fingering patterns are observed. The bottom image (B) shows the development of fingering at a higher flow rate, highlighting the effect of flow dynamics on fingering formation. The scale bar represents 100 μm .

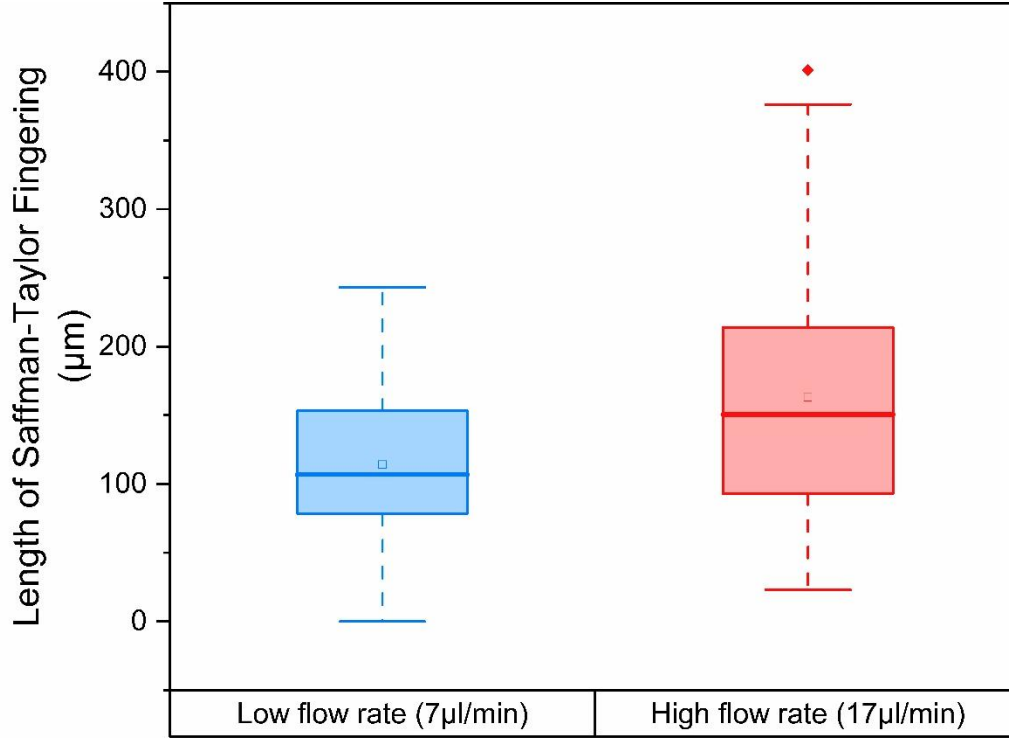


Figure 2.16 The lengths of Saffman-Taylor fingering patterns formed at low (7 $\mu\text{L}/\text{min}$) and high flow (17 $\mu\text{L}/\text{min}$) rates were measured among 100 samples. Boxplots show the median (line), mean (square), and outlier (diamond).

Saline Solutions. The Saffman-Taylor fingering occurs when a less viscous fluid displaces a more viscous one in confined geometries such as microfluidic channels (Singh et al., 2021; Coussot, 1999). In this study, a microfluidic channel was fabricated using double-sided PSA tape, which has viscoelastic properties, meaning it shows viscous characteristics (Fuensanta et al., 2019; Fuensanta & Martín-Martínez, 2020). When fluid flows through the channel, the adhesive layer can deform, potentially developing the formation of fingering patterns. The PSA tape serves as a more viscous and deformable medium compared to the injected fluid, potentially altering the characteristic STF patterns by contributing to interface deformation in addition to fluid viscosity contrast.

Unlike conventional STF studies, where instability occurs between two immiscible fluids with distinct viscosities, our system introduces a case where the instability is observed at the bonding interface which also serves as the microfluidic channel itself.

An increasing gradient of saline solutions were used in abiotic experiments to simulate biologically relevant conditions and to investigate the effect of salt concentration on the development of fingering patterns. The formation of Saffman-Taylor fingering is strongly influenced by variations in salt concentration, which alters the viscosity of the solution. Figures 2.17 and 2.18 demonstrate the influence of salt concentration on STF patterns. Figure 2.17 provides quantitative measurements showing how fingering length changes with varying salt concentrations, while Figure 2.18 visually captures the corresponding instability patterns. Device numbers one to four exhibited no observable fingering at 0.05% salt concentration, suggesting that fingering instability did not occur below this threshold (Figure 2.17). This observation correlates with Figure 2.18, where the interface remains smooth, indicating that low salt concentration contrast prevents finger formation. Devices five to seven began to show fingering instabilities, with 1% salt concentration. This suggests that a moderate increase in salt concentration can cause instability but not as much as the 3.50% salt concentration because the patterns remained relatively contained (see Figure 2.18). Devices eight to twelve show noticeably longer fingering patterns. Compared to the 1% saline solution, the average length at 3.50% NaCl is approximately twice as large, further supporting the hypothesis that increasing salt concentration enhances instability development. This corresponds directly with Figure 2.18, where elongated fingering structures are clearly visible.

This finding aligns with theoretical predictions which suggest that increasing viscosity contrast enhances instability growth (Saffman & Taylor, 1958 Tabeling et al., 1987). Unlike Saffman-Taylor fingering studies, where the instability occurs at the boundary between two immiscible fluids, our system introduces a viscoelastic bonding layer (PSA) that may influence instability formation through its deformability, and ionic interaction potential. While PSA tape was treated as a primarily viscous material in earlier analysis, ionic environments may locally alter its mechanical properties, introducing minor viscoelastic contributions under certain conditions (e.g. long exposure time to saline solutions, high flow rate, ionic strength) (Benedek & Feldstein, 2009). At higher salt concentrations, not only does the viscosity of the aqueous phase increase slightly, but the PSA material may also undergo ionic-induced softening or changes in local viscoelasticity, which could enhance interface deformability and finger propagation. Moreover, while surface tension was assumed constant in earlier, electrolytes like NaCl are known to slightly modify the interfacial tension of aqueous solutions depending on concentration (Weissenborn & Pugh, 1996; Dutcher et al., 2010). Although these changes are relatively small, they may still affect pattern formation, especially in soft or deformable boundary systems. Even small changes in tension could alter the fingering formation in soft-material-based-devices. Therefore, the enhanced instability observed at higher salt concentrations may be the result of a coupled effect involving increased viscous fingering, modified surface tension, and ion-induced changes in the PSA-tape's mechanical response.

While previous studies mainly focused on rigid microchannel layers, fabricated with different techniques such as microchannel milling or 3D printed substrates; double-sided adhesive tape was primarily used as a cover layer to bond components together (Tsao & Syu,

2020a; Razavi Bazaz et al., 2020). But there are the other studies that have taken a different approach by directly using double-sided adhesive tape as the microchannel itself (Yuen & Goral, 2010; Khashayar et al., 2017). Despite this, these studies do not concentrate on checking bonding quality, or they checked it in different ways (Lu et al., 2024; Khashayar et al., 2017). However, in this study Saffman-Taylor fingering phenomenon serves as a diagnostic tool to detect weak points and adhesive failures.

The data suggests that higher saline concentrations amplify the Saffman-Taylor fingering, resulting in broader and more irregular patterns. These results align with theoretical predictions, showing that salt concentration can trigger instabilities and further influence pattern formation (Liu et al., 2019).

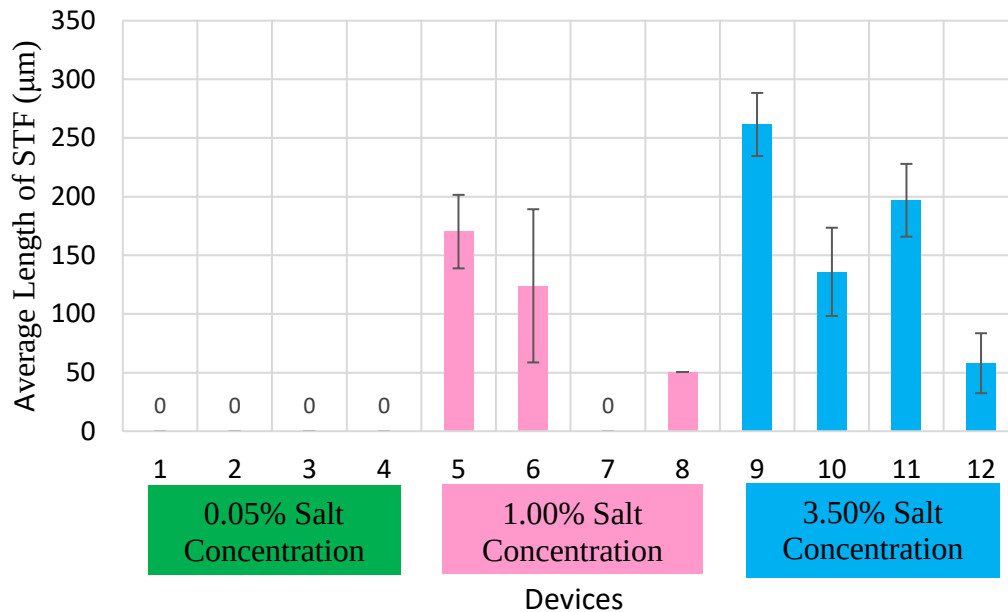


Figure 2.17 Averaged measured STF lengths (μm) observed across different devices under varying salt concentrations (0.05%, 1.00%, and 3.50%). Devices exposed to low salt concentration (0.05%, green) showed no observable fingering, supporting that instability did not occur below this threshold. At 1% salt concentration (pink), initial fingering patterns were observed, suggesting the onset of instability around this concentration. The most pronounced fingering occurs at 3.50% salt concentration (blue), demonstrating higher viscosity contrast in amplifies Saffman-Taylor fingering behavior.

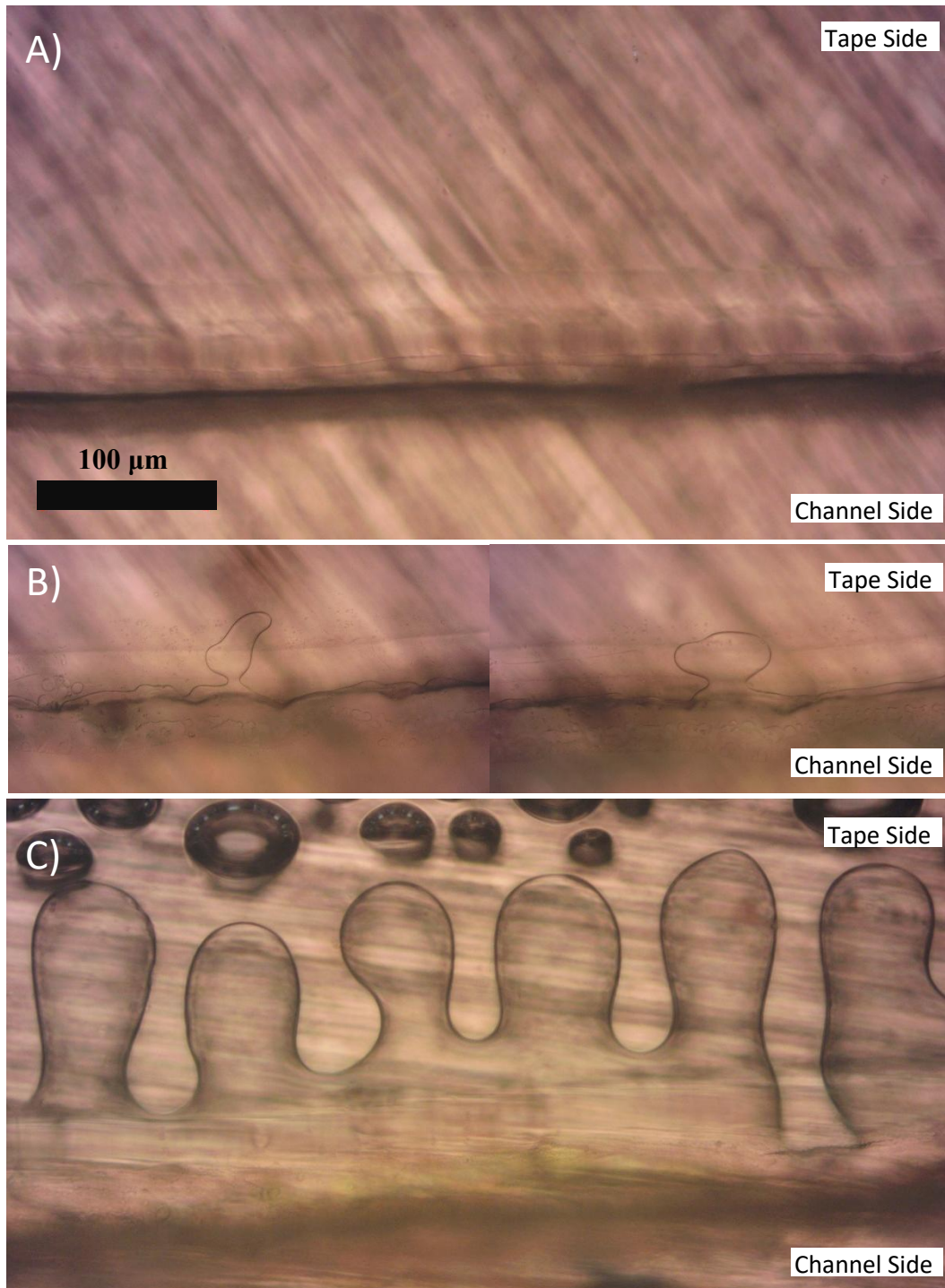


Figure 2.18 Microscopic images of Saffman-Taylor fingering pattern at different salt concentrations (20x magnification). Top image (A) 0.05% salt solution with no fingering patterns observed, indicating a smooth interface. Middle images (B) initial signs of instability are visible, but fingers are less pronounced and contained. Bottom image (C) The interface shows large, irregular and elongated fingering structures, characteristics of high viscosity contrast.

Biotic Experiment

Pseudomonas fluorescens is a common environmental bacterium, capable of contaminating water sources (Vilas Boas et al., 2024). This study investigated the attachment of *Pseudomonas fluorescens* N2E2 under continuous flow conditions in a microfluidic device incorporating a copper surface. Since higher concentration saline solutions triggered STF during abiotic experiments R2A was selected as a growth media (0.06% salt concentration) to minimize this effect. Cells were expected to adhere to the copper surface after an overnight flow at 0.1 $\mu\text{L}/\text{min}$. However, CLSM imaging revealed that the cells are predominantly attached to the cover glass rather than the copper surface inside the channel (Figure 2.19). These findings suggest that device positioning (gravity) and physical properties such as roughness and wettability of surfaces may have influenced bacterial adhesion patterns. To better visualize and analyze bacterial adhesion, CLSM was chosen because it is commonly used to analyze key biofilm characteristics such as surface coverage, volume, and thickness (Yuan et al., 2023). In CLSM, the limited diffusion of fluorescent probes into the deeper regions of the biofilm can restrict observation depth; however, thanks to signals from fluorescently labeled bacteria or staining probes, CLSM can also be used as a non-destructive, in-situ measurement technique (Yuan et al., 2023).

Several studies have investigated the effects of surface roughness on cell attachment (Yang et al., 2022; Song et al., 2015; Mitik-Dineva et al., 2008; Renner & Weibel, 2011). Surface roughness plays an important role in bacterial adhesion to surfaces. A previous study suggested that surface roughness lowers the shear force on bacterial cells placed in flowing liquids; this phenomenon is especially important for biofilms as these structures commonly arise where fluids are flowing, at high rates such as in water pipelines in industrial facilities (Renner & Weibel, 2011).

Surface roughness promotes the attachment of bacteria to the surface during the initial step of colonization as it ensures more surface area for cell attachment (Renner & Weibel, 2011). However, in this study, when we increased the polishing US grit number to prevent STF formation the surfaces were made smoother and cell attachment at the copper surface was not observed.

Contrary, to common belief that the smoother surfaces create a more resistant environment against bacteria (Bächle et al., 2023), another study argues that there is no clear evidence supporting the idea that smooth surfaces reduce bacterial adhesion (Frank Riedewald, 2006). Furthermore, work suggesting that bacteria may be sensitive to surface roughness even at the nanometer scale questions this conventional wisdom (Mitik-Dineva et al., 2008; Bohinc et al., 2014). Therefore, drawing a definitive conclusion based on a single experiment in this study is not possible. To gain a clearer understanding, future studies could involve experiments using devices polished with different grit levels for comparison.

Alternatively, due to hydrophobicity of the surface, we can focus on examining surface wettability and its interaction with cells since the hydrophobic properties of the bacteria can show variability. Hydrophobic bacteria tend to adhere more readily to hydrophobic surface, the same principle applies in reverse (Berne et al., 2018). For copper, the contact angle is typically around 70°, and its oxide layer makes it even more wettable (Može et al., 2021). However, in this study, HCl treatment was applied to remove the oxide layer and increase the contact angle which resulting in a more hydrophobic surface. As a result, the surface became more hydrophobic.

A study investigating the effect of hydrophobic and hydrophilic surfaces on *P. fluorescens* found that the *P. fluorescens* cells exhibited lower adhesion on hydrophilic surfaces such as glass,

and stainless steel; in contrast, higher adhesion was observed on hydrophobic surfaces such as PVC (Vilas Boas et al., 2024). Another study suggests that *P. fluorescens* exhibits hydrophobic surface properties and tends to grow at relatively slower rate (Mirani et al., 2018). This finding supports the idea that *P. fluorescens* may take longer to form a biofilm. Due to its slow growth rate, researchers believe it can develop stronger and more persistent biofilms over time (Mirani et al., 2018).

In this work, the copper coupon surface integrated within the microfluidic device was expected to facilitate *P. fluorescens* adhesion, as the coupon showed hydrophobic properties. However, during the biotic experiment the device was positioned with the copper surface facing upward and the cover glass facing downward. Since gravity inherently influences the cell adhesion process (Marmur & Ruckenstein, 1986), it is believed that the device's positioning may have played a role in the lack of bacterial attachment observed on the copper surface.

A study in the literature suggests that gravity plays an important role in bacterial inoculation and initial attachment (Korber et al., 1990). Additionally, in their flow cell design, researchers observed that colonization occurred more extensively on the lower surface (such as our device's cover glass side) compared to the upper surface, supporting the idea that gravity affects bacterial attachment (Korber et al., 1990). Therefore, for future experiments, it is recommended to invert the device (upside-down) to assess whether gravity influences bacterial attachment to the copper surface. While this study provides initial insights into bacterial adhesion under flow conditions, further biotic experiments are needed to validate these observations. Since some *P. fluorescens* strains exhibit hydrophobic characteristics while others are more hydrophilic, adhesion behavior can vary depending on the specific strain (Azelmad et al., 2018).

Therefore, experimental evaluation of the hydrophobicity of *P. fluorescens* N2E2 is recommended to better interpret its adhesion behavior.

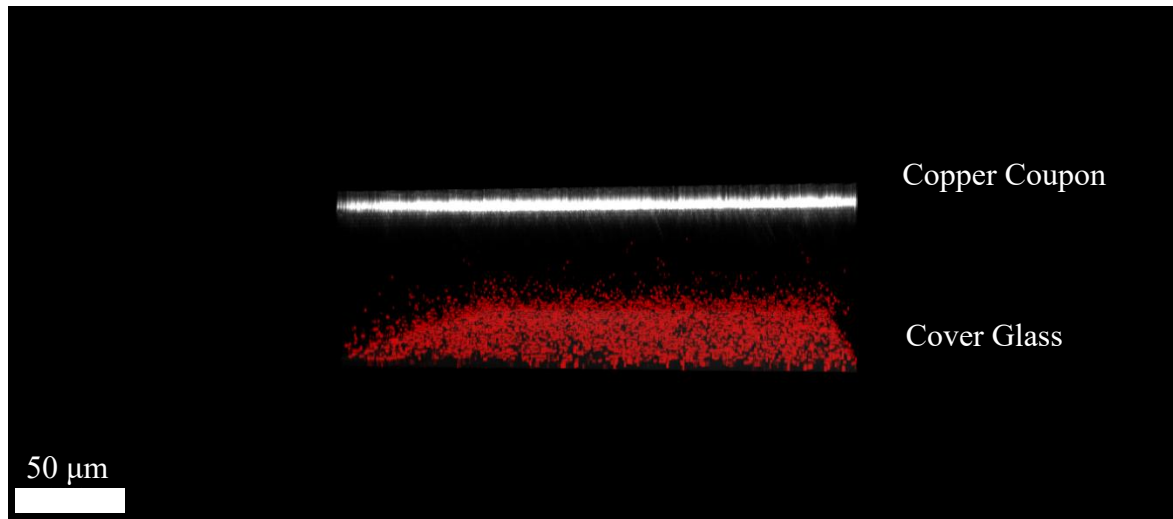


Figure 2.19 A confocal laser scanning microscope (CLSM) with 63x oil immersion magnification was used to observe *P. fluorescens* N2E2 after an overnight flow at 0.1 $\mu\text{l}/\text{min}$. The red-labeled cells adhered to the lower surface (cover glass), while no attachment was detected on the copper coupon (upper surface). The scale bar measures 50 μm .

Conclusions

This study demonstrated that PSA tape-based microfluidic devices provide an efficient and cost-effective alternative to conventional fabrication techniques. The cost per device was calculated based on material usage, including PSA tape, acrylic sheet, copper coupon, cover glass, and inlet and outlet tubes. The total cost of the device was \$2.08. This study confirms their reliability in preventing leakage while enabling precise fluid control. Computational modeling validates shear stress analysis, and the experimental tests show that surface roughness affects Saffman-Taylor fingering, with smoother surfaces enhancing stability. Biofilm research reveals

that *P. fluorescens* N2E2 preferentially adheres to the cover glass rather than the copper surface, suggesting that surface properties and gravity influence microbial attachment.

Although this outcome was specific to the conditions tested, it highlights the need for further biotic experiments to fully evaluate biofilm formation on copper. Future work should also explore different bacterial strains. Additionally, these findings emphasize the importance of optimizing surface roughness, hydrophobicity, and device orientation for future biofilm studies. Overall, by simplifying fabrication and maintaining structural integrity, PSA tape-based microfluidic devices present a promising solution for research and industrial applications, with potential advancements in material selection and microchannel design further enhancing their functionality.

Acknowledgements

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CHAPTER THREE

CONCLUSION AND SUGGESTIONS FOR FUTURE WORK

This study demonstrates that the double-sided pressure-sensitive adhesive tape method can be a useful approach for reliable fabrication of microfluidic devices, which can observe initial biofilm attachment on metallic surfaces. The developed methodology enhances leak-free device assembly while maintaining high reproducibility, and optical transparency.

In this work, three different microfluidic channel designs were created using AutoDesk® Inventor program and evaluated in COMSOL Multiphysics® by incorporating a fluid model to determine shear stress distributions on copper surfaces (Appendix A). Based on the shear stress analysis, the straight-channel design (number 3) was selected for fabrication. A microfluidic channel, with an 80 μm thickness, 38.2 mm length, and 2.2 mm width, was successfully fabricated. The cost of the device is \$2.08 based on the material usage.

Although the fabricated device was durable, initial biofilm attachment was not seen, suggesting that other factors affect bacterial adhesion in microfluidic systems. This indicated that the orientation of the device affects initial cell attachment, with gravity playing a significant role in observed attachment pattern. Cells were mostly attached to the cover glass surface when the cover glass was placed at the bottom in the experimental set-up, suggesting that gravity affected attachment distribution even under flow conditions. Future studies of this phenomenon can be carried out by orienting the device upside down to observe whether adhesion occurs on the copper surface. This can help determine whether gravity plays a significant role in the observed adhesion patterns.

Another key finding of this research was the relationship between surface properties and device performance. It was observed that the smoother surfaces were associated with a decrease in Saffman-Taylor fingering pattern visibility, thereby highlighting the significance of surface quality in achieving leak-free device performance. When polishing grits 400 and 600 US were used, there was no noticeable STF formation. This means that the way the device was made provides repeatability of the fabrication approach. Furthermore, an inverse relationship was found between contact angle measurements and surface roughness of the copper surface. As the roughness of the surface increased, the contact angle decreased, suggesting a shift toward a more hydrophilic surface.

Hydrochloric acid (HCl) treatment was also used for its effect on copper coupon surfaces. Using HCl raised the hydrophobicity of the copper surface by removing the oxide layer, hence smoothing the surface. The relationship between oxide layer removal and hydrophobicity implies that surface chemistry is rather important in defining wettability and adhesion properties. Since the hydrophobic properties of bacteria can vary, future studies should consider testing different bacteria strains that align better with surface hydrophobicity or hydrophilicity; as hydrophobic bacteria tend to adhere more readily to hydrophobic surfaces, while hydrophilic bacteria interact more with hydrophilic surfaces (Berne et al., 2018). Since the hydrophobic nature of the copper surface after HCl treatment may have reduced bacterial attachment, *P. fluorescens* N2E2 may prefer more hydrophilic surfaces. Future studies should explore alternative bacterial strains with different hydrophobicity. Additionally, longer experimental durations may be considered to determine cell attachment over time.

Measurements of flatness were also performed with minor variations, with no statistically significant differences, ensuring consistent surface preparation across samples. This result suggests that although surface roughness and chemistry affect device performance, general flatness might not be a deciding factor in microfluidic flow behavior.

Tap water was used for abiotic experiments to evaluate bonding quality; devices were tested under both low (7 $\mu\text{L}/\text{min}$) and high flow rates (17 $\mu\text{L}/\text{min}$) to look at STF formations. Higher flow rates in devices where STF was present were shown to produce more STF formations than lower flow rates. The result underlines the importance of flow rate in the expression of Saffman-Taylor fingering and shows the efficiency of the bonding techniques in maintaining controlled fluid flow. Additionally, an abiotic experiment incorporating salt concentration variations was conducted to mimic real biological media conditions. STF formation increased with rising salt concentration. These findings establish a foundation for future research on STF formation and cell attachment on metallic surfaces in controlled microenvironments.

Overall, this work presents an alternative microfluidic device fabrication technique for observing initial biofilm attachment on metallic (copper) surfaces via a pressure-sensitive adhesive tape approach. The results offer a reproducible and efficient method for microfluidic applications by highlighting the relationships between surface properties, bonding efficiency, and fluid dynamics. However, the lack of observed biofilm on the copper surface in this study underlines the complexity of microbial attachment in engineered microsystems. To realize the full potential of this device for biofilm research, further biological experiments are necessary, not only to test different bacterial strains, and evaluate the hydrophobic behavior of strain used, but

also to optimize experimental parameters such as device orientation. This study introduces a cost-effective microfluidic device fabrication method that is suitable for CLSM imaging and supports biological integration. The pressure-sensitive adhesive bonding method enables reliable assembly while maintaining compatibility with experiments involving metallic surfaces. These findings highlight the potential of this approach for advancing microfluidic systems designed to study early biofilm formation and other biology-related applications.

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APPENDICES

APPENDIX A

COMSOL MULTIPHYSICS®

The study accomplished in laminar flow physics and stationary study when field variables do not change over time. Any 3D design can be imported into the COMSOL Multiphysics® program by navigating to the “Geometry” section and using the “Import” function. After importing the file, parameters can be determined. The study accomplished in laminar flow physics and stationary study when field variables do not change over time. Any 3D design can be imported into the COMSOL Multiphysics® program by navigating to the “Geometry” section and using the “Import” function. After importing the file, global definitions such as parameters and materials can be determined. Under the section titled “Laminar Flow”, the fluid properties, initial values, wall, inlet, and outlet conditions are specified. Finally, an appropriate mesh is generated, the study is run, and the results can be exported from the “Results” tab. This appendix A presents the parameters of the three-channel design in a table format and illustrates the results using simulation figures.

The center of the channel was used to calculate the Reynolds number (Re), and all channel flows were categorized as laminar flow regimes because Re number were 1.9865×10^{-4} , 2.6082×10^{-4} and 1.462×10^{-3} respectively. The differences in Reynolds numbers between the channel geometries suggest that variations in channel shape influence the flow characteristics, which may have implication for fluid transport efficiency, mixing, and shear stress distribution. These findings provide valuable insight for optimizing microfluidic device design, particularly in applications requiring controlled fluid dynamics

From the Navier-Stokes equation, it is possible to express the wall shear stress along a rectangular cross-section microchannel as (Pisapia et al., 2022):

$$\tau = \frac{6\mu Q}{wh^2} \quad (\text{A. 1})$$

where μ is the dynamic viscosity of the fluid (Pa·s), Q is the volumetric flow rate (m^3/s), w is the channel width (m). and h is the channel height (m).

A fundamental principle in fluid mechanics is that shear stress (τ) in a Newtonian fluid is directly proportional to the velocity gradient (du/dy) perpendicular to the flow direction (Yoshinaga et al., 2014):

$$\tau = \mu \frac{du}{dy} \quad (\text{A. 2})$$

where μ is the dynamic viscosity. Shear stress is also defined as force per area (Lacombe & Zenhausern, 2022).

Based on the equation A.1 when microchannel get narrower, the shear stress as well as the velocity profile increases. This suggests that reducing the channel width increases shear stress; while increasing it decreases shear stress (Kim et al., 2018). As expected, the simulations predict a rise in shear stress when the channel width is reduced (Kim et al., 2018).

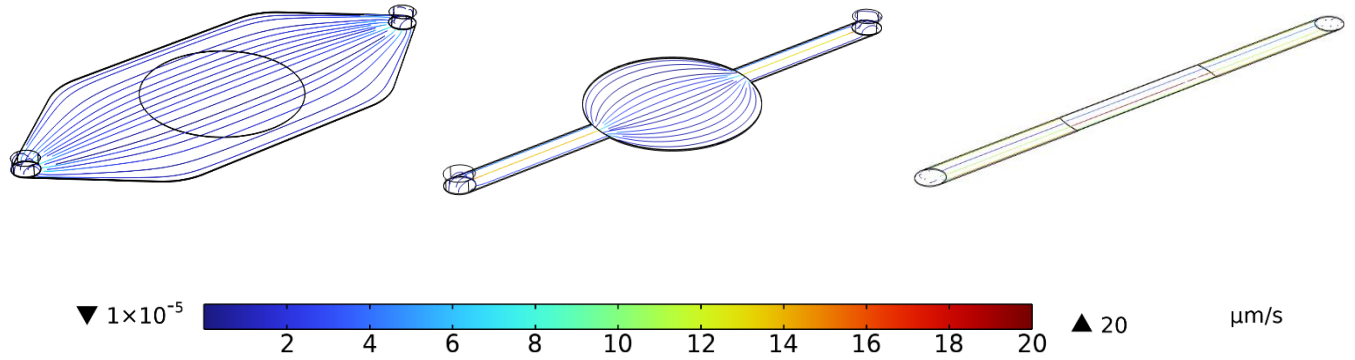


Figure A 1. Streamline velocity field model for three different microchannel designs without a gap at $0.1 \mu\text{L}/\text{min}$ flow rate.

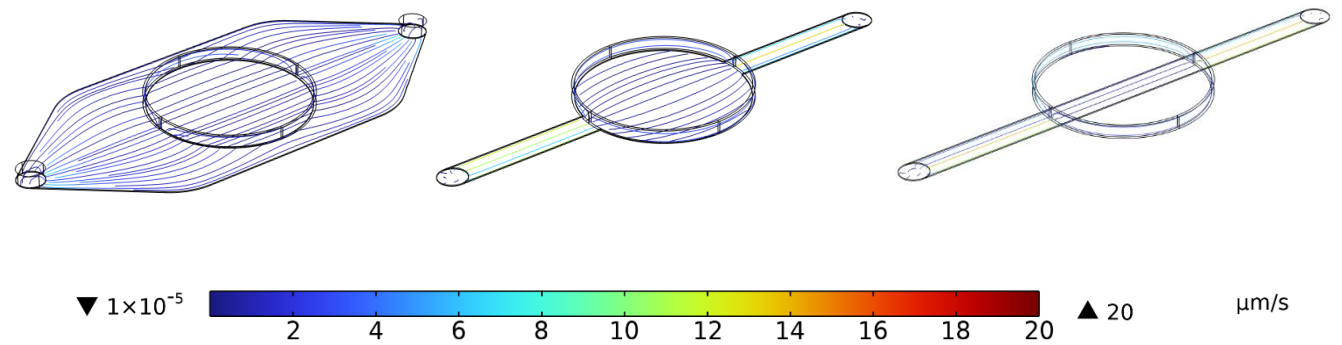


Figure A 2. Streamline velocity field model for three different microchannel designs with a gap at $0.1 \mu\text{L}/\text{min}$ flow rate.

Table A 1. First channel's parameters.

Name	Expression	Value	Description
U	1.25e-6 [m/s]	1.25E-6 m/s	Inlet mean velocity
R	12.7 [mm]	0.0127 m	Coupon radius
c	0.04 [mm]	4E-5 m	Height of coupon
L	38.2 [mm]	0.0382 m	Length
W	16.7 [mm]	0.0167 m	Width
H	0.08 [mm]	8E-5 m	Height of channel
rho	1000 [kg/m ³]	1000 kg/m ³	Density
mu	10 ⁻³ [kg/m/s]	0.001 kg/(m·s)	Dynamic viscosity
g	9.8 [m/s ²]	9.8 m/s ²	Gravity
wss	93.6 [μPa]	9.36E-5 Pa	Wall shear stress
p	9.45e-4 [mbar]	0.0945 Pa	Pressure
Re	1.99e-4	1.99E-4	Reynolds number
Fr	0.1 [μl/min]	1.6667E-12 m ³ /s	Flow rate
width_c	38.2 [mm]	0.0382 m	Thin channel width
Area_c	width_c*H	3.056E-6 m ²	Thin channel area
Perimeter_c	2*(width_c+H)	0.07656 m	Thin channel perimeter
Dh	4*Area_c/Perimeter_c	1.5967E-4 m	Hydraulic diameter
V_avg	Fr/Area_c	5.4538E-7 m/s	Thin channel average velocity
Reynolds	rho*v_avg*Dh/mu	1.9865E-4	Re# of thin channel

Table A 2. The material contents of water which is the fluid in microfluidic device.

Property	Variable	Value	Unit	Property group
Dynamic viscosity	mu	1e-3	Pa.s	Basic
Density	rho	1000	kg/m ³	Basic

Table A 3. Physical and boundary conditions for laminar flow simulation.

Laminar Flow	
Physical Model, Compressibility	Incompressible Flow
Reference pressure level, Pref	1 atm
Reference temperature, Tref	293.15 K
Fluid Properties	
Density, rho	From material
Dynamic viscosity, mu	From material
Initial Values	
Velocity field, u(x,y,z)	0 m/s
Pressure, p	0 Pa
Wall	
Boundary Condition, Wall Condition	No Slip
Inlet	
Boundary Condition	Fully developed flow (Apply condition on each disjoint selection separately.)
Fully Developed Flow	Flow rate, V0=Fr

Table A.3 Continued.

Outlet	
Boundary Condition, Pressure	Static, $p_0 = 0$ Pa (Suppress backflow)
Mesh	Physics-controlled mesh
Element size	Extra coarse

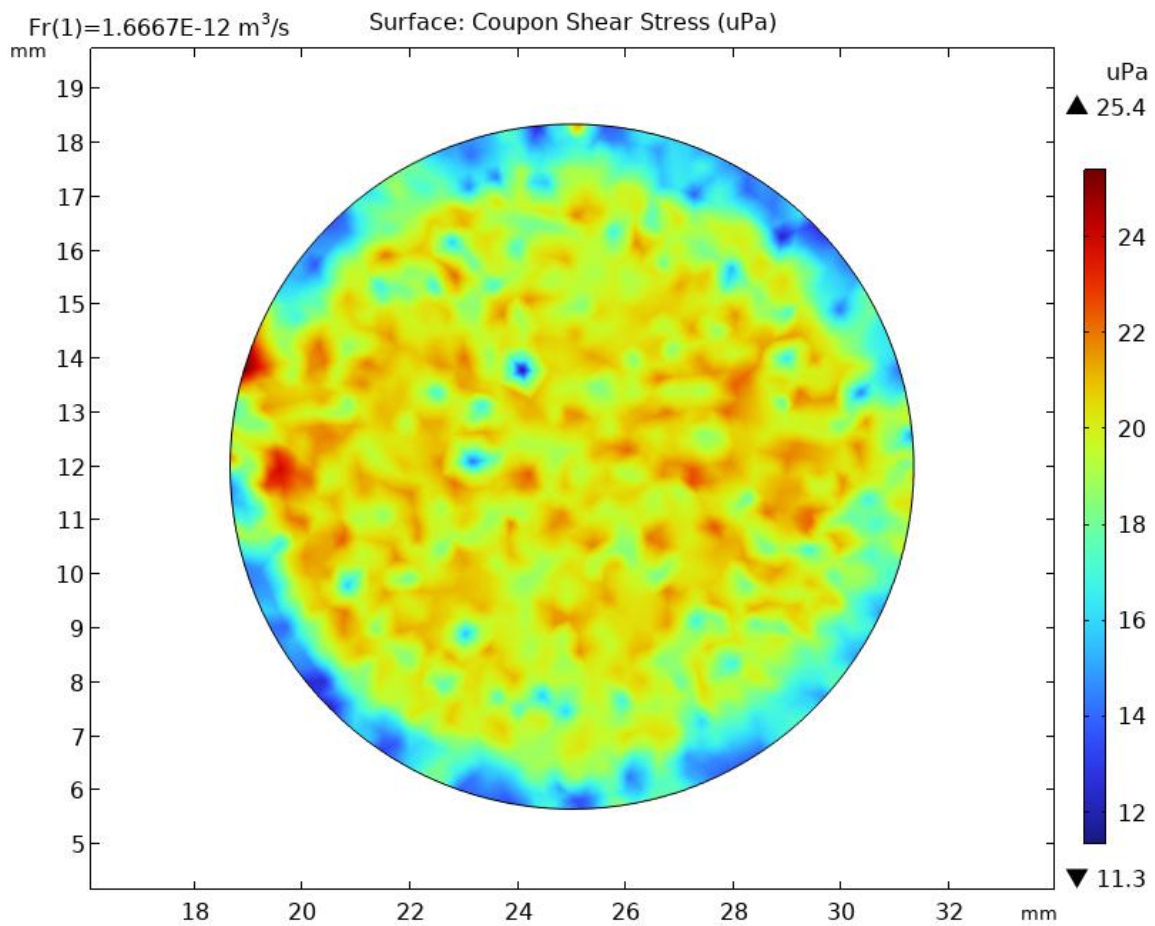


Figure A 3. Shear stress on the center of the copper coupon inside the first microchannel design without gap.

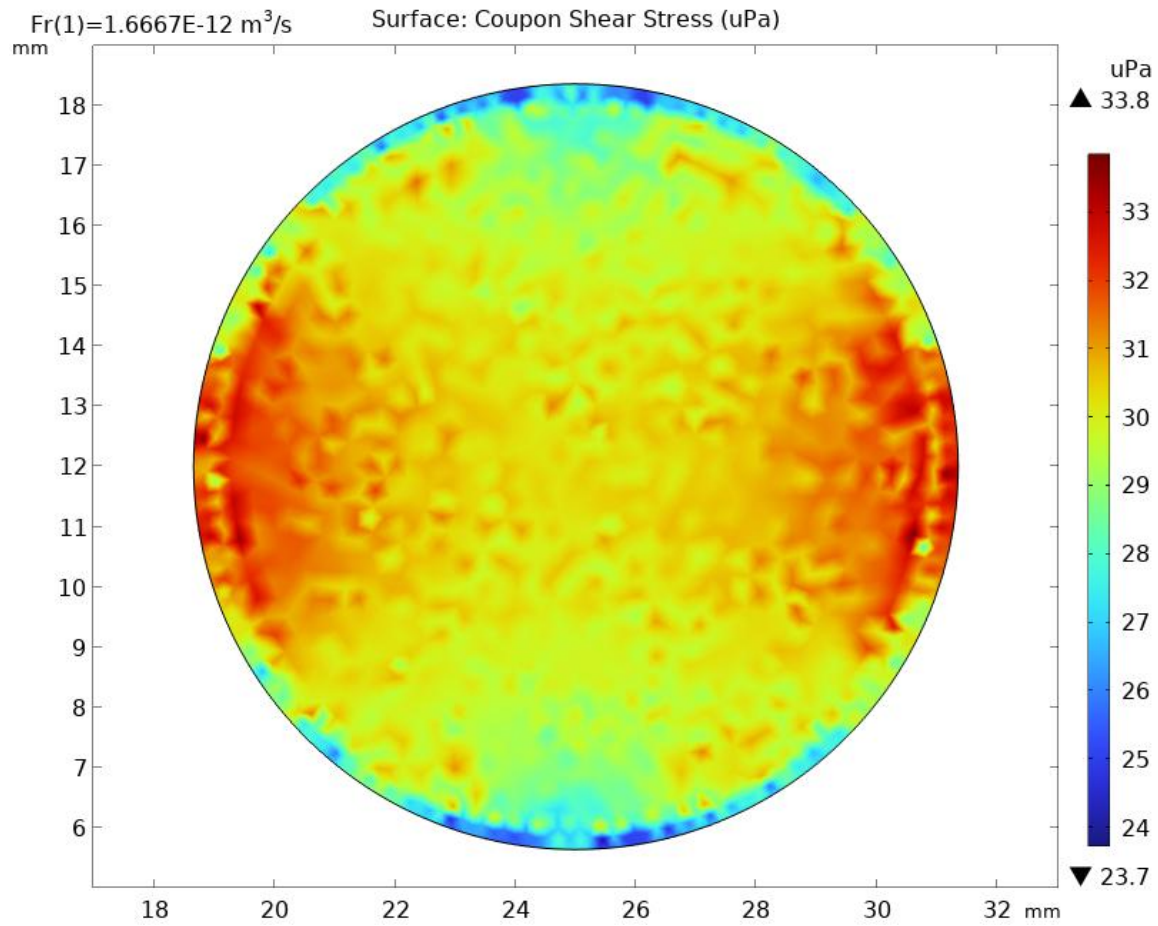


Figure A 4. Shear stress on the center of the copper coupon inside the first microchannel design with gap.

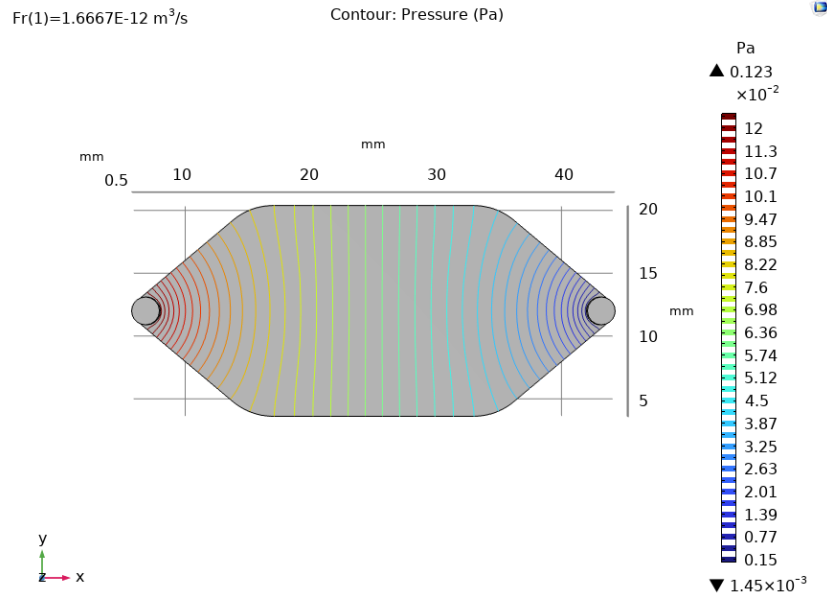


Figure A 5. Pressure inside the first microchannel without gap.

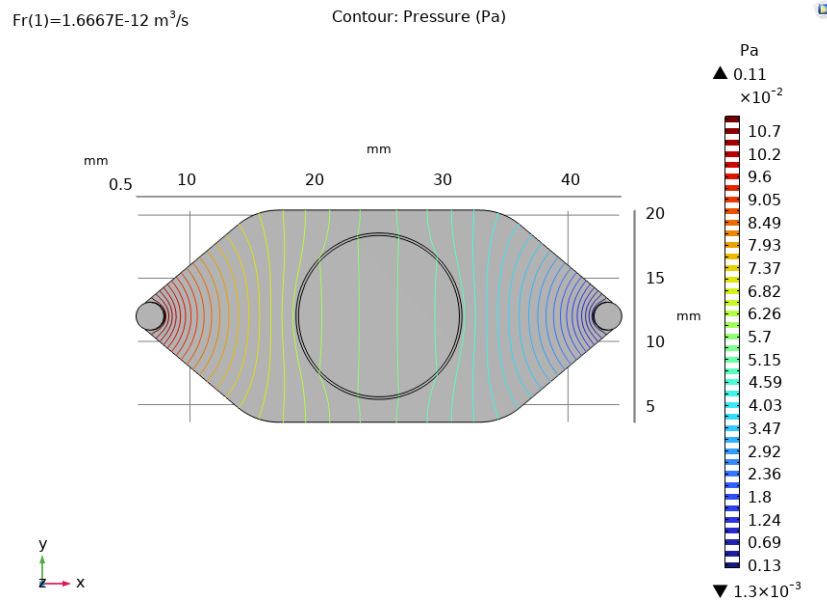


Figure A 6. Pressure inside the first microchannel with gap.

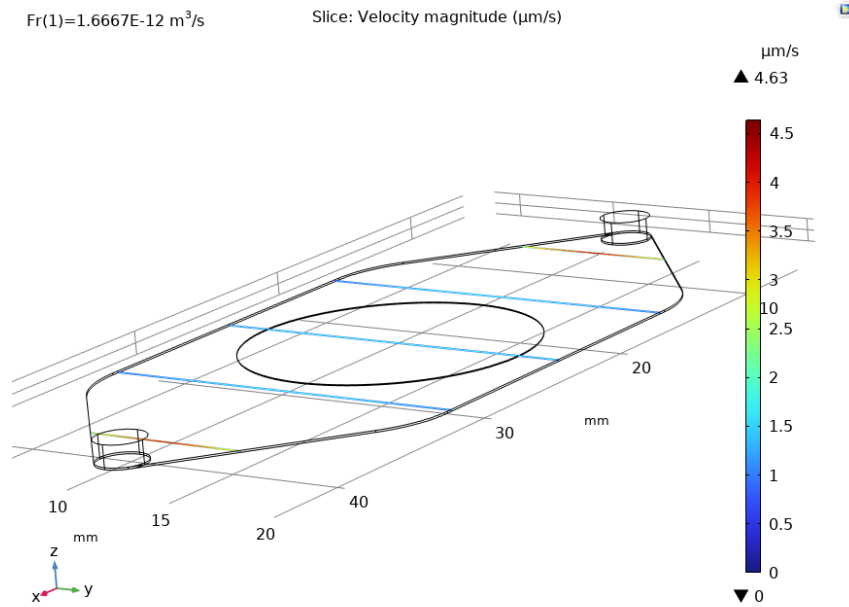


Figure A 7. Velocity magnitude inside the first microchannel without gap.

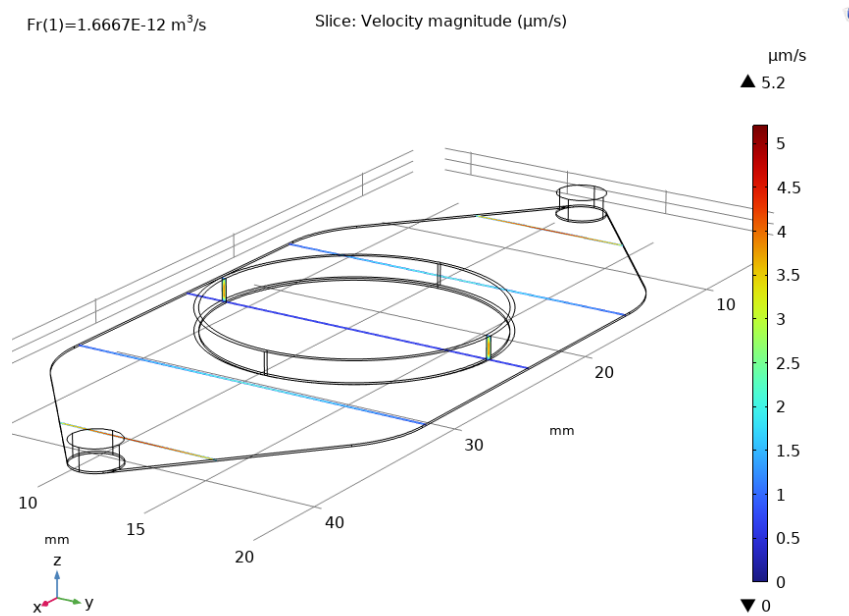


Figure A 8. Velocity magnitude inside the first microchannel with gap.

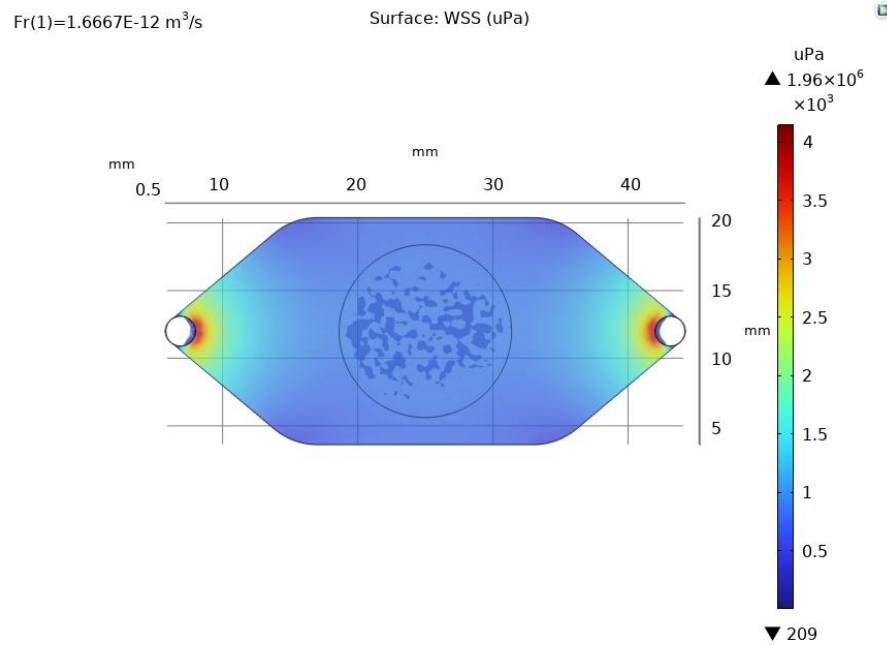


Figure A 9. Wall shear stress inside the first microchannel without gap.

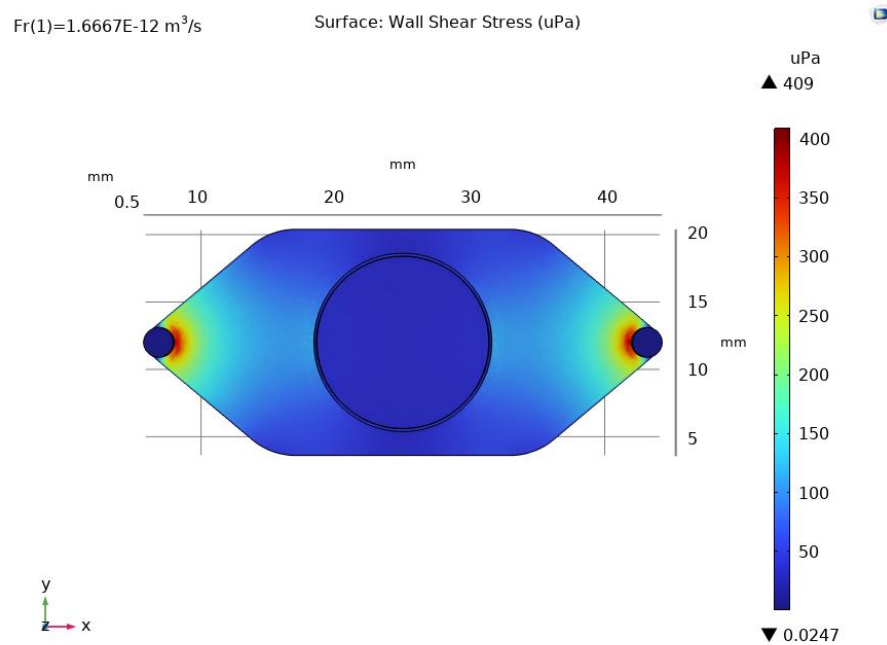


Figure A 10. Wall shear stress inside the first microchannel with gap.

Table A 4. Second channel's parameters.

Name	Expression	Value	Description
U	1.25e-6 [m/s]	1.25E-6 m/s	Inlet mean velocity
R	12.7 [mm]	0.0127 m	Coupon radius
c	0.04 [mm]	4E-5 m	Height of coupon
L	38.2 [mm]	0.0382 m	Length
W	12.7 [mm]	0.0127 m	Width
H	0.08 [mm]	8E-5 m	Height of channel
rho	1000 [kg/m ³]	1000 kg/m ³	Density
mu	10 ⁻³ [kg/m/s]	0.001 kg/(m·s)	Dynamic viscosity
g	9.8 [m/s ²]	9.8 m/s ²	Gravity
wss	93.6 [μPa]	9.36E-5 Pa	Wall shear stress
p	9.45e-4 [mbar]	0.0945 Pa	Pressure
Re	1.99e-4	1.99E-4	Reynolds number
Fr	0.1 [μl/min]	1.6667E-12 m ³ /s	Flow rate
width_c	2.2 [mm]	0.0022 m	Thin channel width
Area_c	width_c*H	1.76E-7 m ²	Thin channel area
Perimeter_c	2*(width_c+H)	0.00456 m	Thin channel perimeter
Dh	4*Area_c/Perimeter_c	1.5439E-4 m	Hydraulic diameter
V_avg	Fr/Area_c	9.4697E-6 m/s	Thin channel average velocity
Reynolds	rho*v_avg*Dh/mu	2.6082E-4	Re# of thin channel

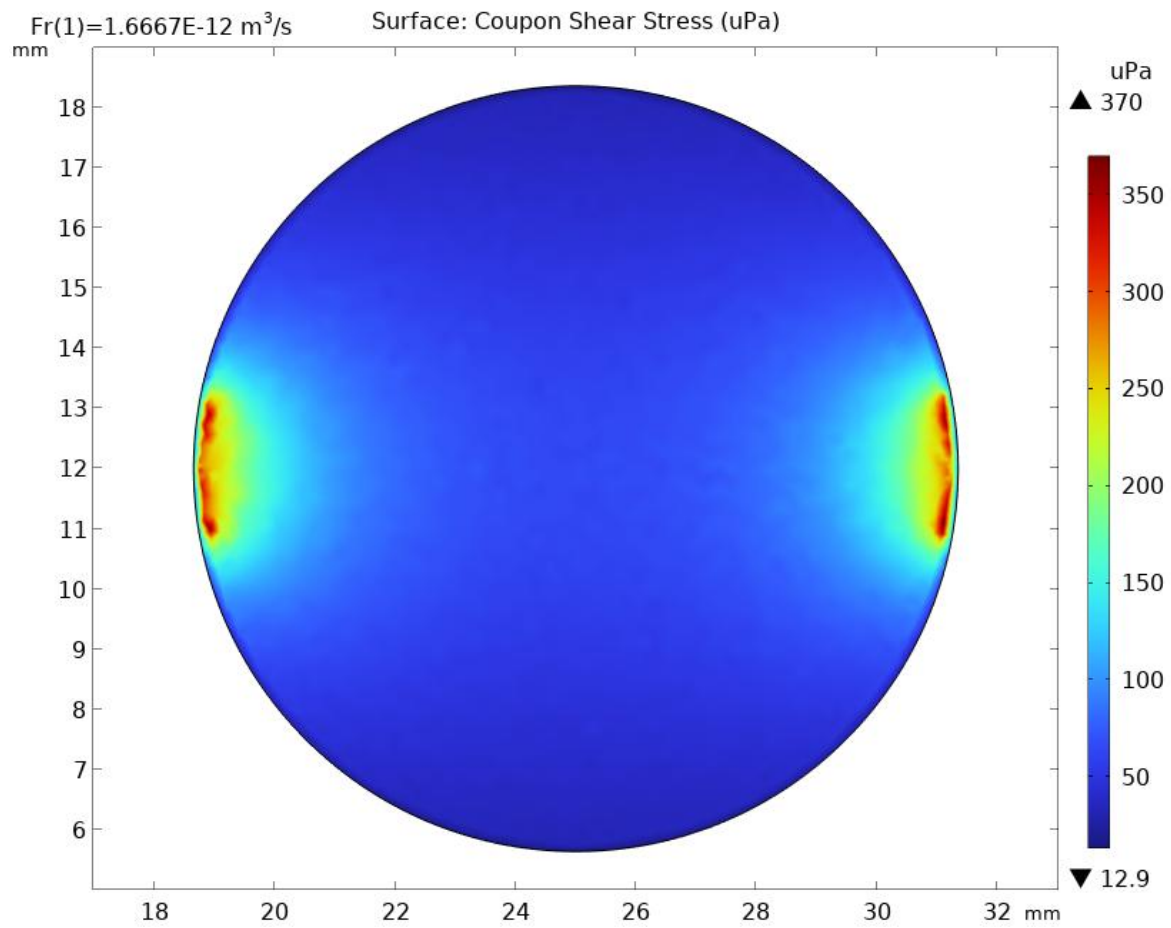


Figure A 11. Shear stress on the center of the copper coupon inside the second microchannel design without gap.

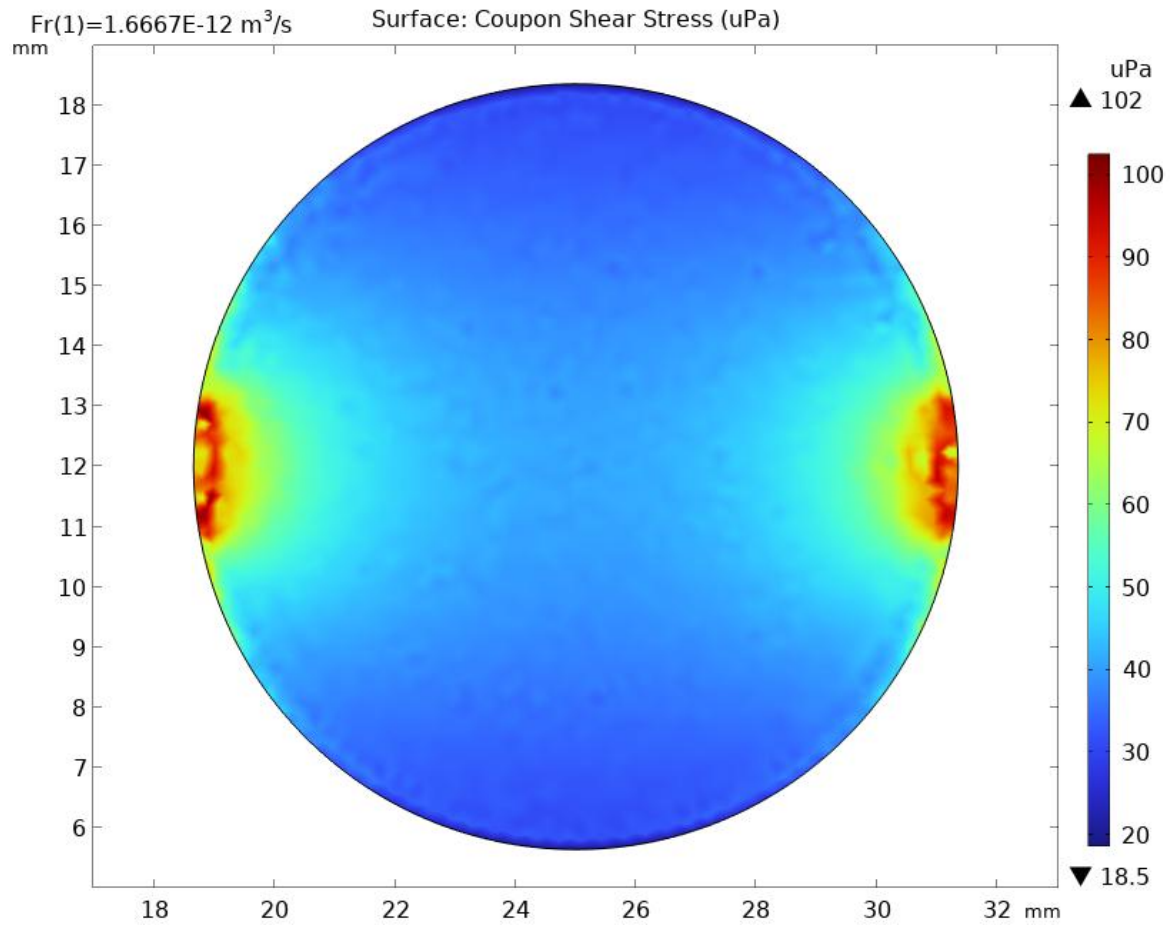


Figure A 12. Shear stress on the center of the copper coupon inside the second microchannel design with gap.

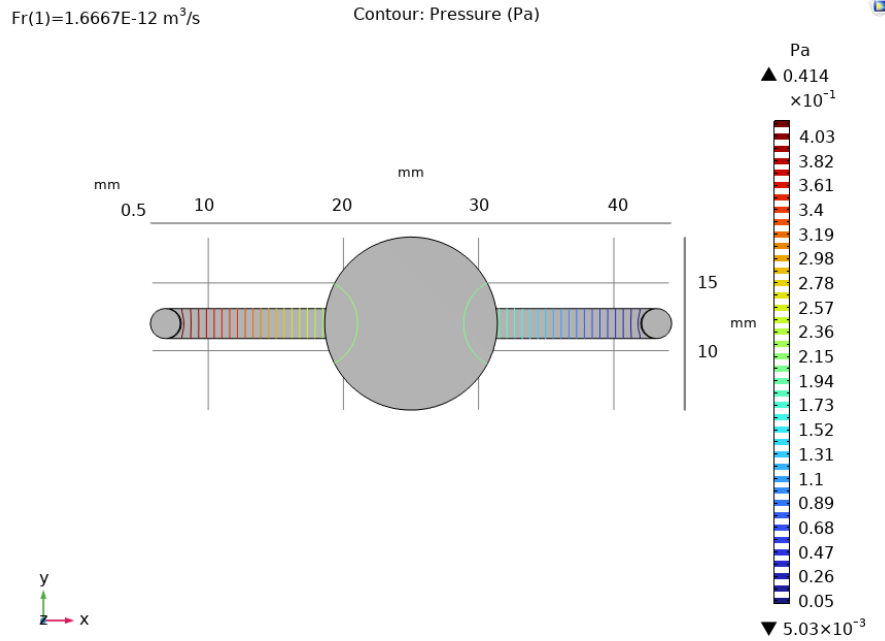


Figure A 13. Pressure inside the second microchannel without gap.

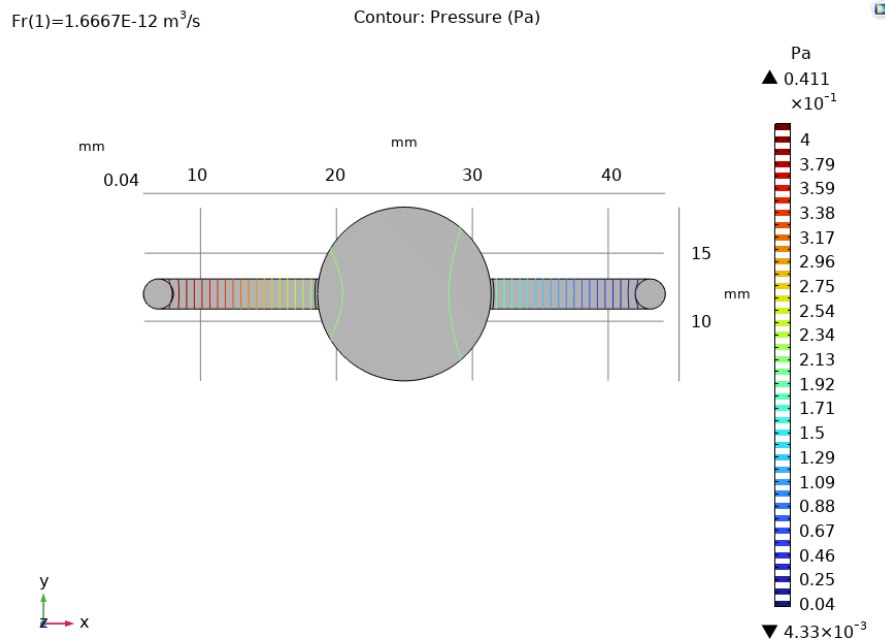


Figure A 14. Pressure inside the second microchannel with gap.

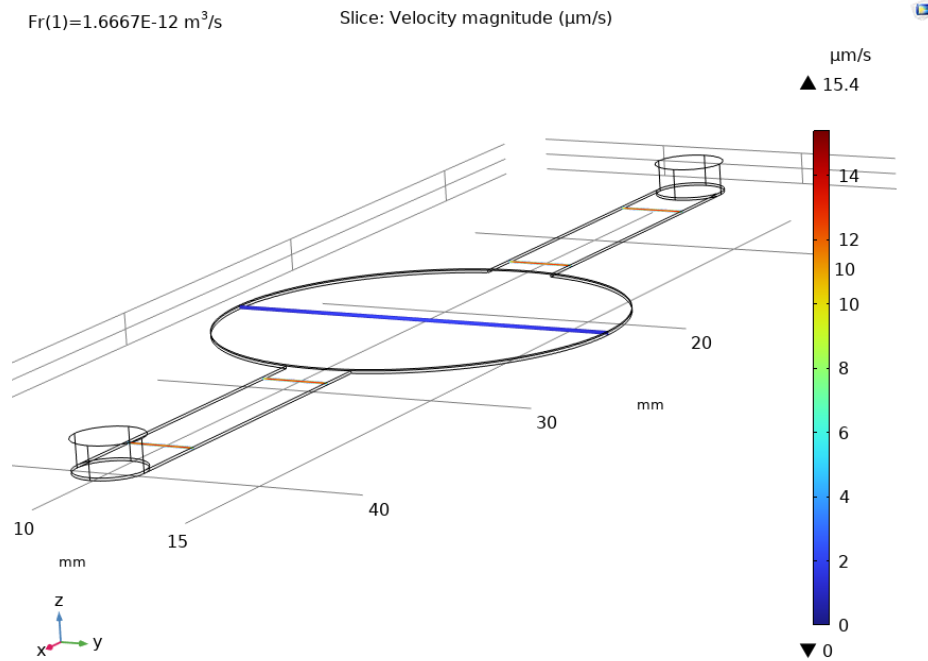


Figure A 15. Velocity magnitude inside the second microchannel without gap.

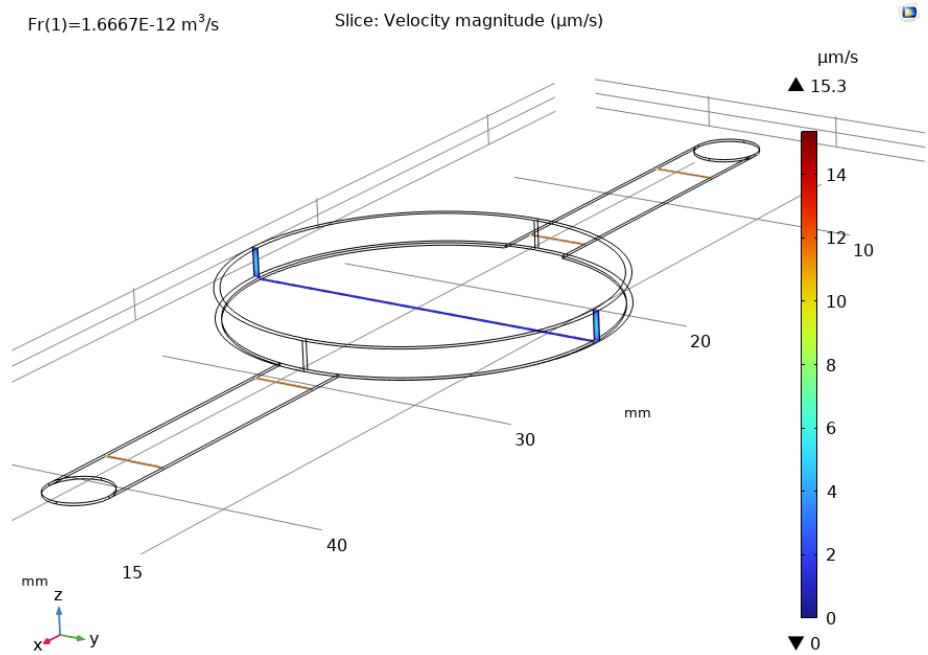


Figure A 16. Velocity magnitude inside the second microchannel with gap.

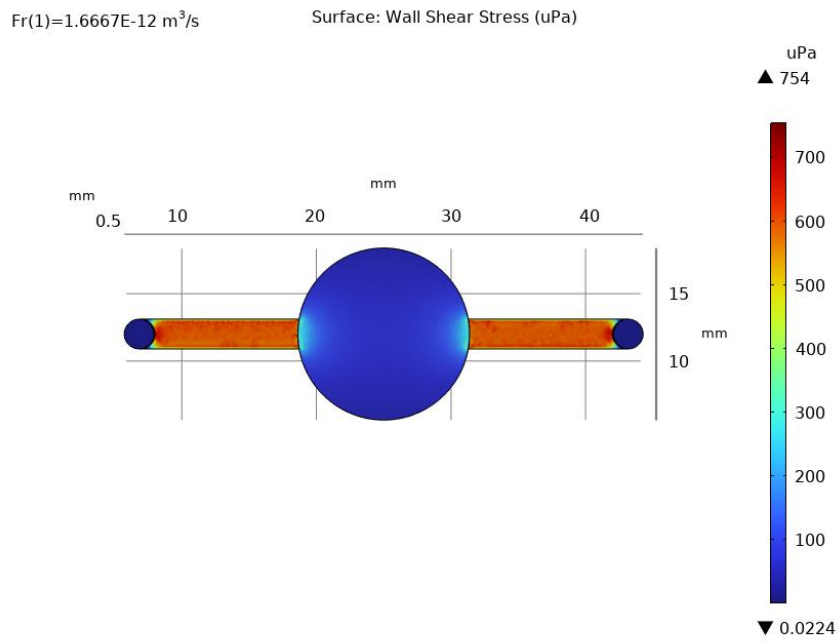


Figure A 17. Wall shear stress magnitude inside the second microchannel without gap.

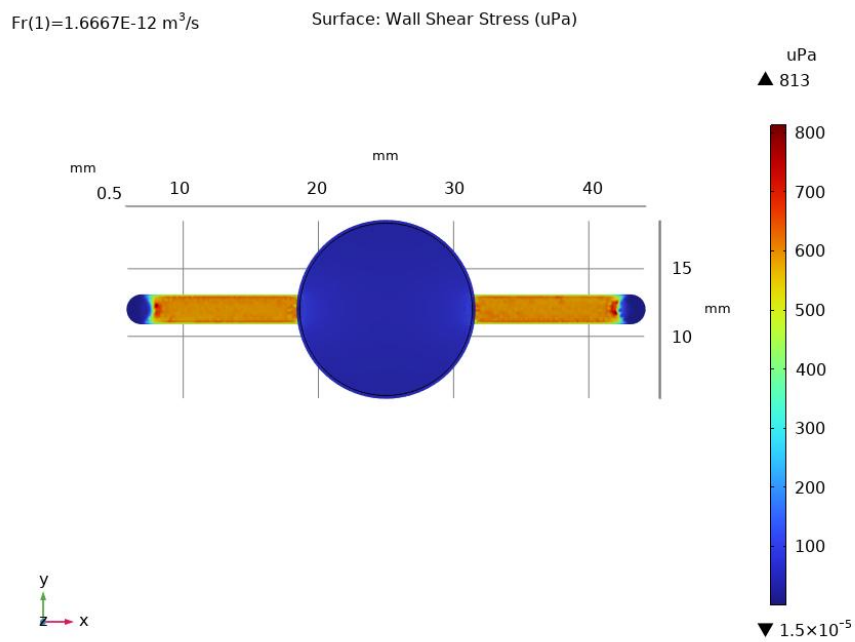


Figure A 18. Wall shear stress magnitude inside the second microchannel with gap.

Table A 5. Third channel's (straight) parameters.

Name	Expression	Value	Description
U	1.25e-6 [m/s]	1.25E-6 m/s	Inlet mean velocity
R	12.7 [mm]	0.0127 m	Coupon radius
c	0.04 [mm]	4E-5 m	Height of coupon
L	38.2 [mm]	0.0382 m	Length
W	2.2 [mm]	0.0022 m	Width
H	0.08 [mm]	8E-5 m	Height of channel
rho	1000 [kg/m ³]	1000 kg/m ³	Density
mu	10 ⁻³ [kg/m/s]	0.001 kg/(m·s)	Dynamic viscosity
g	9.8 [m/s ²]	9.8 m/s ²	Gravity
wss	93.6 [μPa]	9.36E-5 Pa	Wall shear stress
p	9.45e-4 [mbar]	0.0945 Pa	Pressure
Re	1.99e-4	1.99E-4	Reynolds number
Fr	0.1 [μl/min]	1.6667E-12 m ³ /s	Flow rate
width_c	2.2 [mm]	0.0022 m	Thin channel width
Area_c	width_c*H	1.76E-7 m ²	Thin channel area
Perimeter_c	2*(width_c+H)	0.00456 m	Thin channel perimeter
Dh	4*Area_c/Perimeter_c	1.5439E-4 m	Hydraulic diameter
V_avg	Fr/Area_c	9.4697E-6 m/s	Thin channel average velocity
Reynolds	rho*v_avg*Dh/mu	0.001462	Re# of thin channel

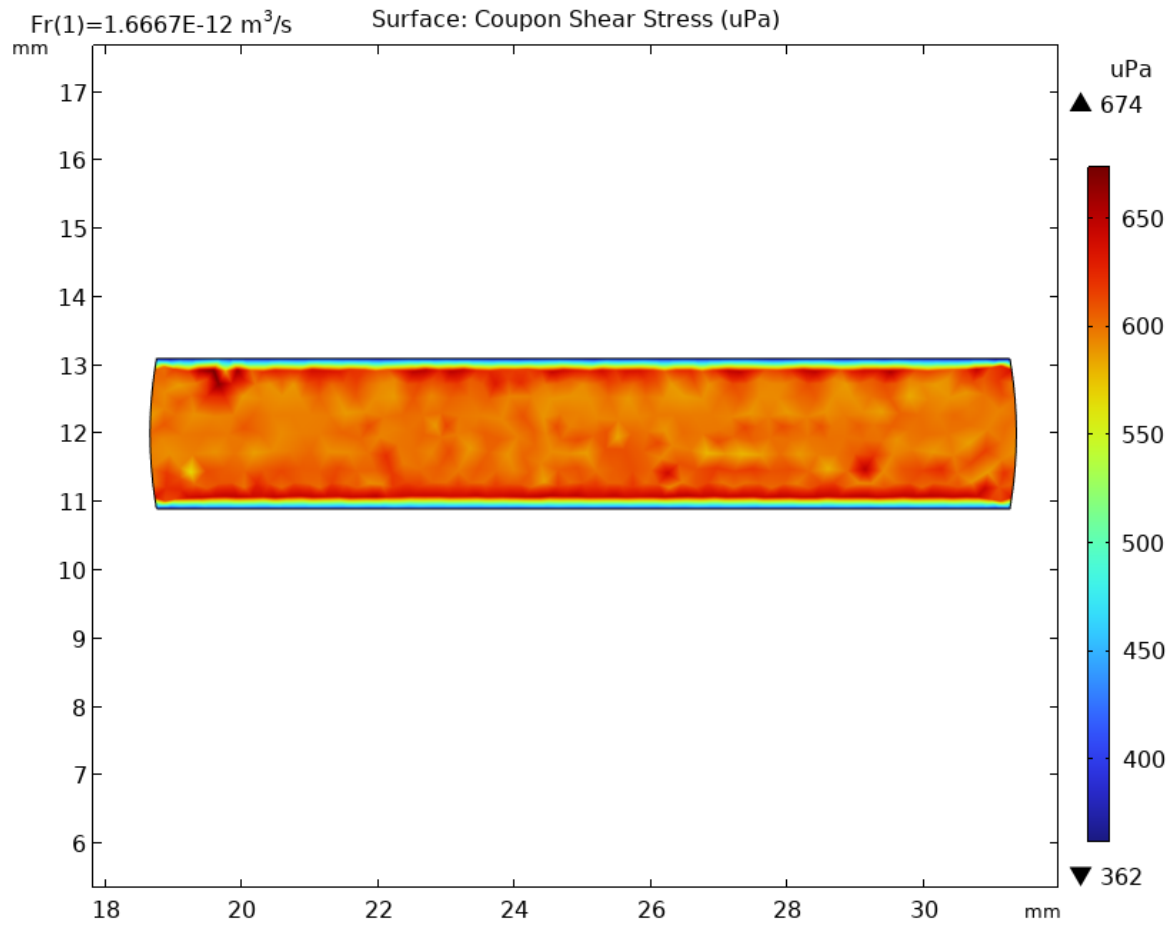


Figure A 19. Shear stress on the center of the copper coupon inside the third (straight) microchannel design without gap.

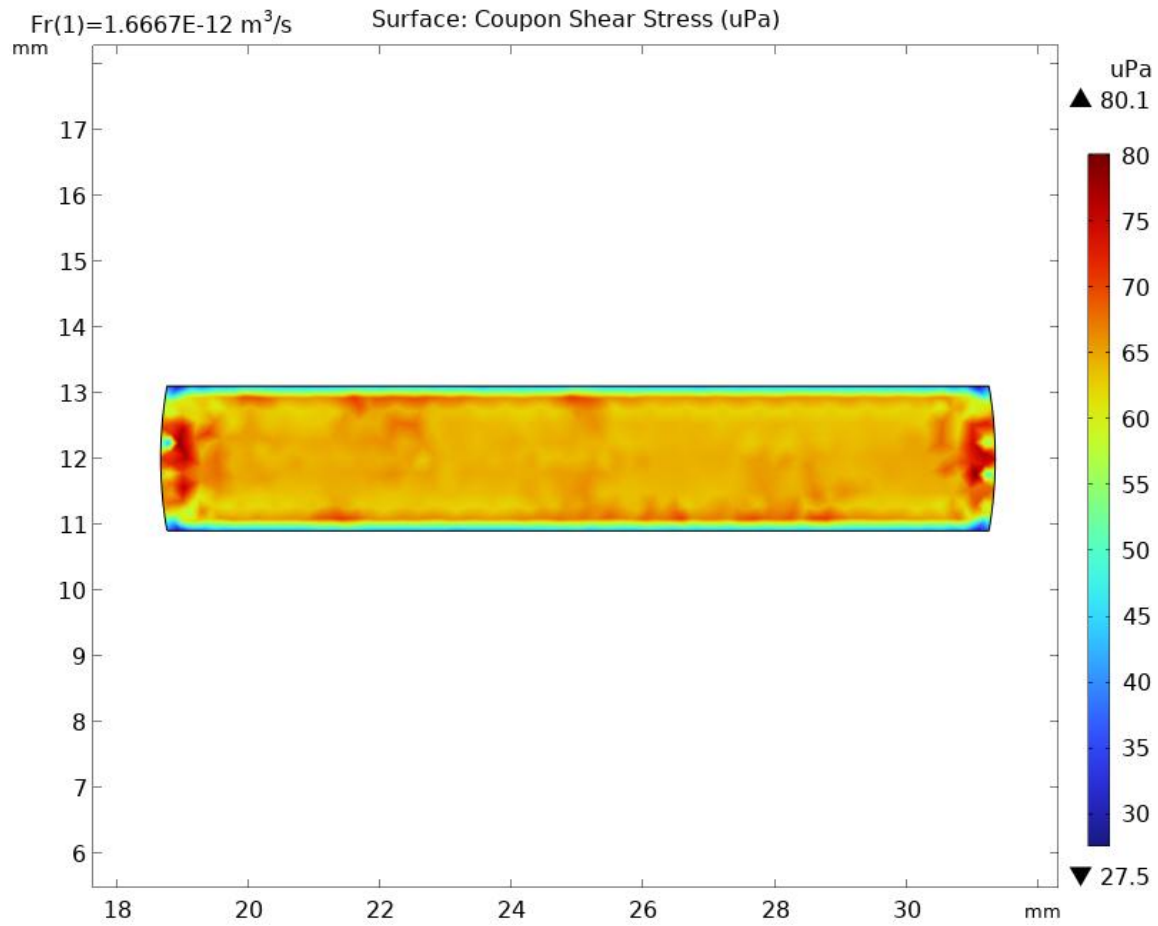


Figure A 20. Shear stress on the center of the copper coupon inside the third (straight) microchannel design with gap.

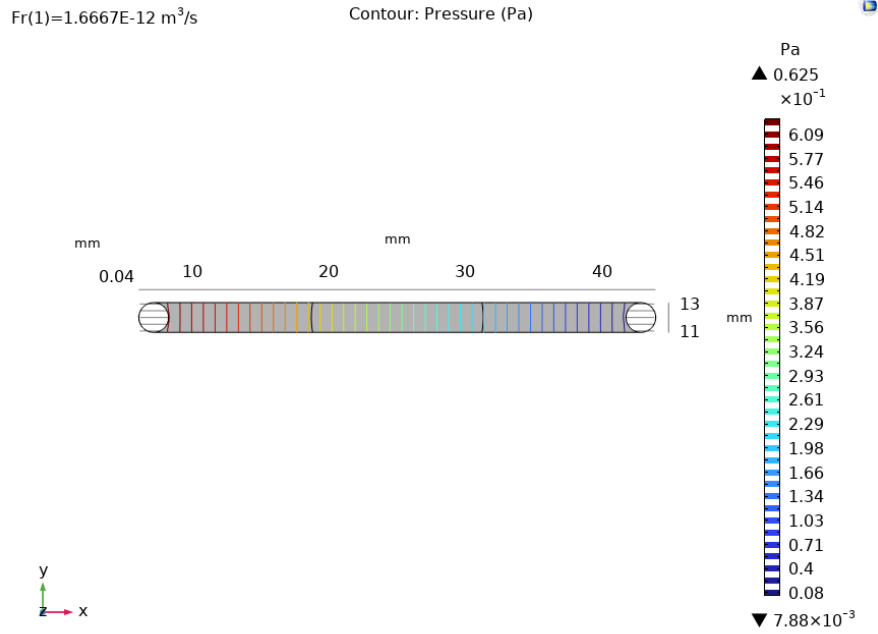


Figure A 21. Pressure inside the third (straight) microchannel design without gap.

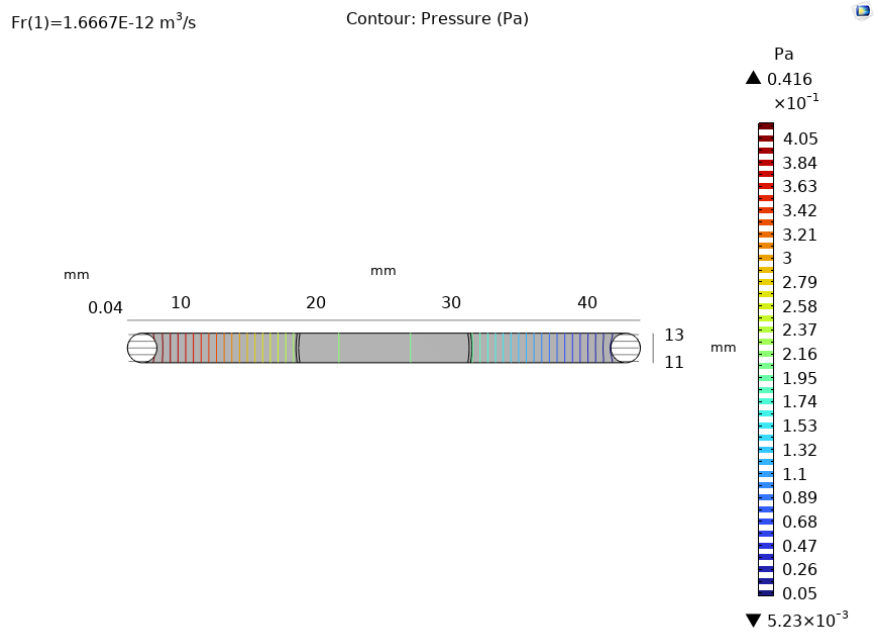


Figure A 22. Pressure inside the third (straight) microchannel design with gap.

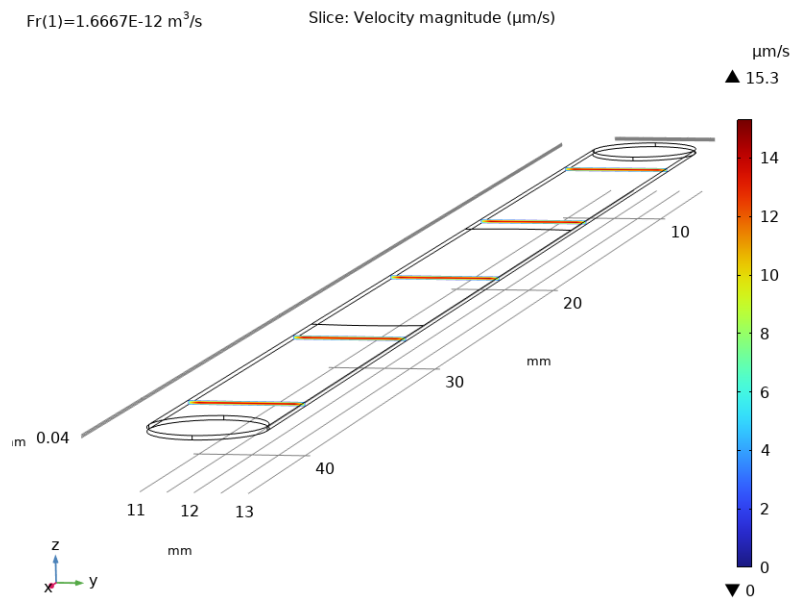


Figure A 23. Velocity magnitude inside the third (straight) microchannel design without gap.

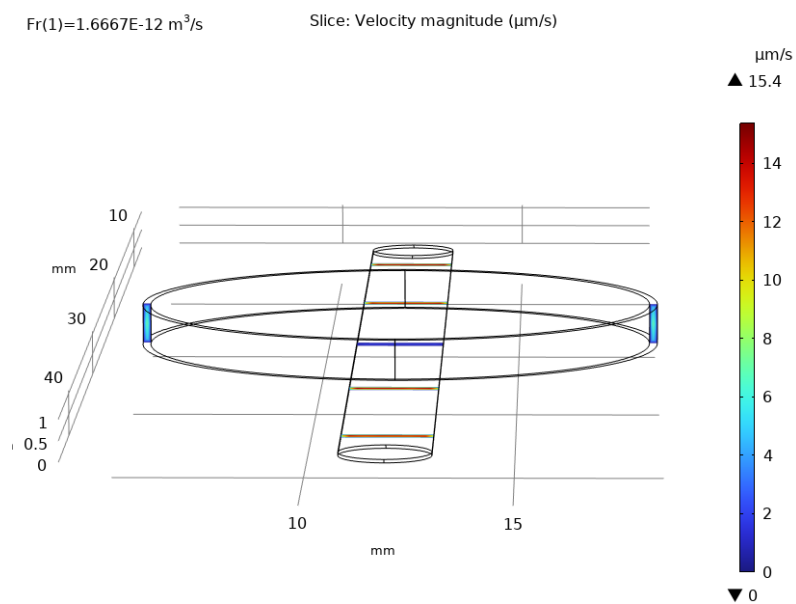


Figure A 24. Velocity magnitude inside the third (straight) microchannel design with gap.

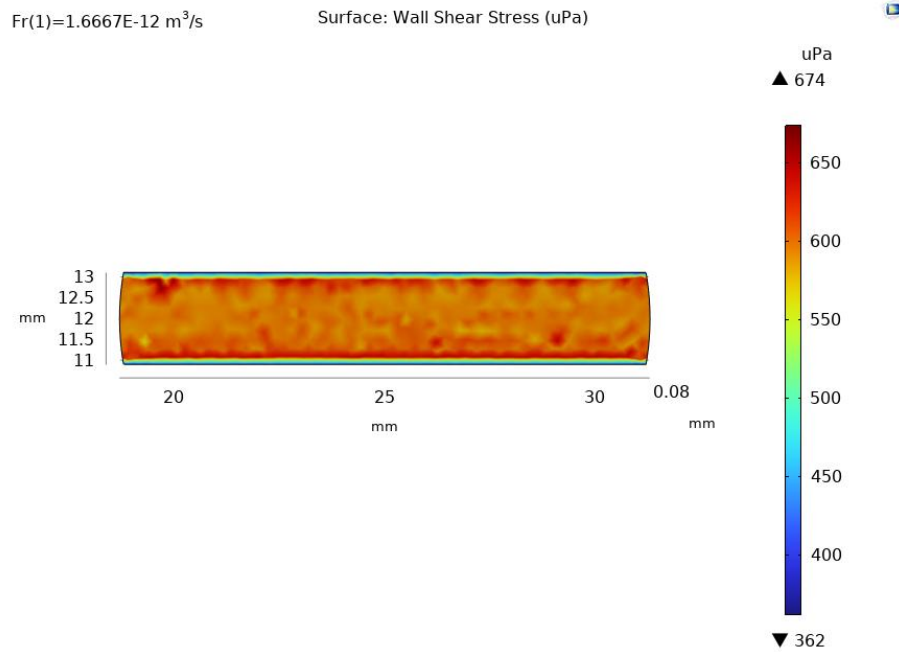


Figure A 25. Wall shear stress inside the third (straight) microchannel design without gap.

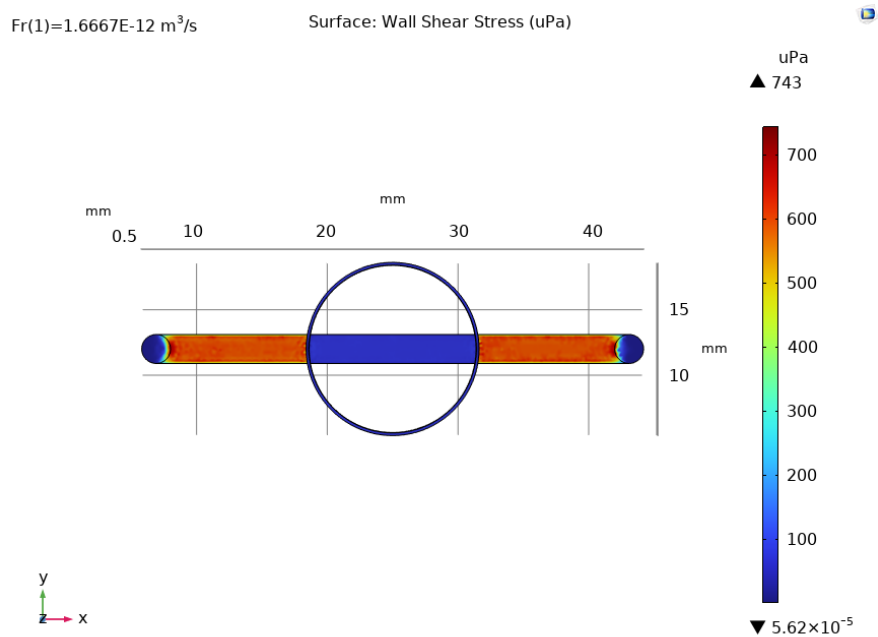
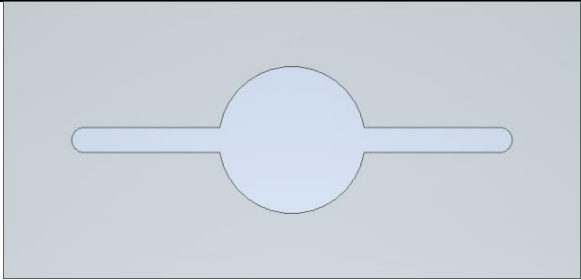


Figure A 26. Wall shear stress inside the third (straight) microchannel design with gap.

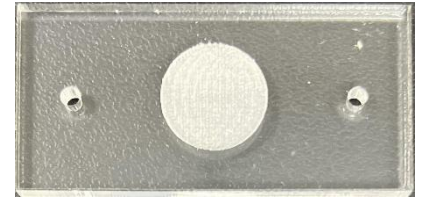
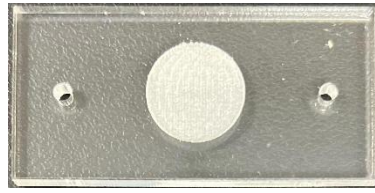
Table A 6. Channel designs of microfluidic devices created using AutoDesk® Inventor and modeled in COMSOL Multiphysics®.

Device Design Number	Channel Geometry
1	
2	
3	

APPENDIX B

EXPERIMENTAL SET UP

- a) Initial condition
(After peeling)



- b) Application of Heavy
Scratch Remover



- c) Application of Fine
Scratch Remover



- d) Application of Plastic
Clean & Shine



Figure B 1. Acrylic polish kit (Novus 7136) was applied to clean acrylic surfaces. A smooth surface was obtained by applying heavy scratch, fine scratch remover and plastic clean and shine, respectively.

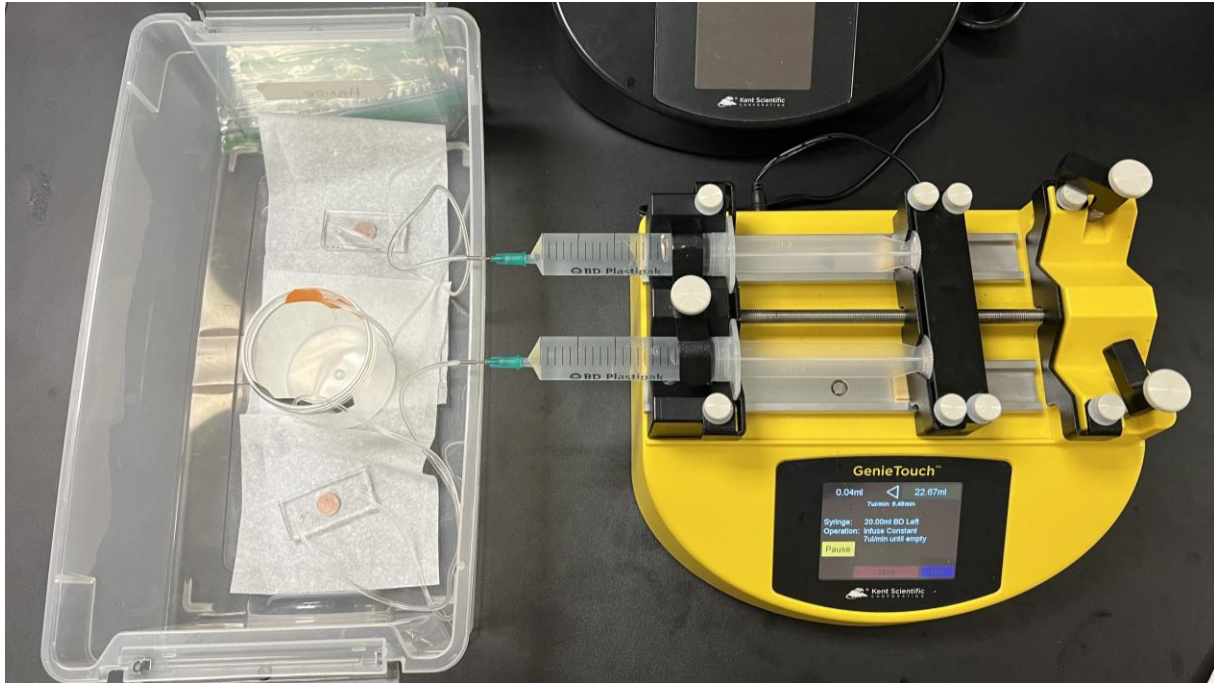


Figure B 2. Experimental setup for abiotic tests: A dual-syringe pump system (GenieTouch®) is used to deliver controlled flow to microfluidic channels at desired flow rate.