Design And Characterization Of A Microfluidic Device For A Preliminary Study On The Origins Of Life

BY

Felix Leonardo Morales Caballero
B.S., University of Illinois at Chicago, Chicago, 2015

THESIS

Submitted as partial fulfillment of the requirements for the degree of Master of Science in Bioengineering in the Graduate College of the University of Illinois at Chicago, 2017

Chicago, Illinois

Defense Committee:
Dr. David Eddington, Chair and Advisor
Dr. Richard Magin
Dr. Jie Xu, Mechanical and Industrial Engineering
Para mami
ACKNOWLEDGEMENTS

I want to thank my advisor Dr. David Eddington for the opportunity to work at the start of a very exciting journey towards a better understanding of the origins of life. I would have not believed I was going to do this when I started as a freshman in 2011.

Deep thanks to Gerry Smith! This would not exist if you didn’t give me a chance as a Graduate Assistant.

Thanks to Martin Brennan, whose suggestions at the beginning of this project were tremendously helpful; Gerardo Mauleon, for my training during my undergraduate years; and Carlos Ng, for opening the door to this laboratory.

I also want to acknowledge Daniel Gray for his assistance in the creation of the fabrication schematics.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>A. Microfluidics</td>
<td>1</td>
</tr>
<tr>
<td>B. Origins of life</td>
<td>6</td>
</tr>
<tr>
<td>C. Research Purpose</td>
<td>9</td>
</tr>
<tr>
<td>II. MATERIALS AND METHODS</td>
<td>11</td>
</tr>
<tr>
<td>A. Device design and fabrication</td>
<td>11</td>
</tr>
<tr>
<td>a. Mold fabrication using a photomask</td>
<td>11</td>
</tr>
<tr>
<td>b. Maskless mold fabrication</td>
<td>13</td>
</tr>
<tr>
<td>c. Soft Lithography</td>
<td>13</td>
</tr>
<tr>
<td>d. Assembly</td>
<td>14</td>
</tr>
<tr>
<td>B. Characterization experiments</td>
<td>15</td>
</tr>
<tr>
<td>a. Sweep protocol experiments</td>
<td>15</td>
</tr>
<tr>
<td>b. Mixing</td>
<td>17</td>
</tr>
<tr>
<td>c. Imaging</td>
<td>17</td>
</tr>
<tr>
<td>C. Data analysis</td>
<td>17</td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>19</td>
</tr>
<tr>
<td>A. Discrete chamber devices</td>
<td>19</td>
</tr>
<tr>
<td>B. Hydrodynamic focusing and mixing devices</td>
<td>21</td>
</tr>
<tr>
<td>a. Optimization of the central pump’s injection rate</td>
<td>21</td>
</tr>
<tr>
<td>b. Mixing effectiveness</td>
<td>26</td>
</tr>
<tr>
<td>c. Sweep protocol optimization</td>
<td>29</td>
</tr>
<tr>
<td>d. Control experiment protocol</td>
<td>35</td>
</tr>
<tr>
<td>IV. DISCUSSION</td>
<td>39</td>
</tr>
<tr>
<td>A. Discrete-chamber design</td>
<td>39</td>
</tr>
<tr>
<td>B. Mixing performance</td>
<td>39</td>
</tr>
<tr>
<td>C. Sweep</td>
<td>40</td>
</tr>
<tr>
<td>D. Control</td>
<td>41</td>
</tr>
<tr>
<td>V. CONCLUSION</td>
<td>43</td>
</tr>
<tr>
<td>CITED LITERATURE</td>
<td>44</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>48</td>
</tr>
<tr>
<td>VITA</td>
<td>49</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE I. FIRST SWEEPING PROTOCOL</td>
<td>30</td>
</tr>
<tr>
<td>TABLE II. FINAL SWEEPING PROTOCOL</td>
<td>32</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>Figure 1. Overview of the mold fabrication process</td>
<td>12</td>
</tr>
<tr>
<td>Figure 2. View of the assembled microfluidic device</td>
<td>14</td>
</tr>
<tr>
<td>Figure 3. Illustration of the experimental setup</td>
<td>16</td>
</tr>
<tr>
<td>Figure 4. Example of an initial design based on discrete chambers</td>
<td>19</td>
</tr>
<tr>
<td>Figure 5. Ineffective discrete chamber devices</td>
<td>20</td>
</tr>
<tr>
<td>Figure 6. First iterations of the hydrodynamic focusing design</td>
<td>22</td>
</tr>
<tr>
<td>Figure 7. Sharpening of stream boundaries with increased injection rates</td>
<td>23</td>
</tr>
<tr>
<td>Figure 8. Stream definition change due to decreased chamber height</td>
<td>24</td>
</tr>
<tr>
<td>Figure 9. First attempt at minimizing the central pump’s injection rate</td>
<td>25</td>
</tr>
<tr>
<td>Figure 10. Illustration of the final microfluidic device design</td>
<td>26</td>
</tr>
<tr>
<td>Figure 11. Mixing effectiveness of FITC and water at different injection rates</td>
<td>27</td>
</tr>
<tr>
<td>Figure 12. Mixing of FITC and water at an injection rate of 0.5 µL/min</td>
<td>28</td>
</tr>
<tr>
<td>Figure 13. Adjustment of central stream widths by switching injection rates</td>
<td>29</td>
</tr>
<tr>
<td>Figure 14. Results of the initial sweep protocol</td>
<td>31</td>
</tr>
<tr>
<td>Figure 15. Results of the reagent-saving sweep protocol</td>
<td>33</td>
</tr>
<tr>
<td>Figure 16. Results of the sweep using high-volumetric capacity syringes</td>
<td>35</td>
</tr>
<tr>
<td>Figure 17. Course of events of typical control procedure</td>
<td>37</td>
</tr>
<tr>
<td>Figure 18. Final position of generations 2, 6, and 10 after the control procedure</td>
<td>38</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometers</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width half max</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometers</td>
</tr>
<tr>
<td>μL</td>
<td>Microliters</td>
</tr>
<tr>
<td>L</td>
<td>Liters</td>
</tr>
<tr>
<td>μM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeters</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>d</td>
<td>Days</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeters</td>
</tr>
<tr>
<td>cm/s</td>
<td>Centimeters per second</td>
</tr>
<tr>
<td>cm²</td>
<td>Centimeters Squared</td>
</tr>
<tr>
<td>mg</td>
<td>Miligrams</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein Isothiocyanate</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropanol</td>
</tr>
</tbody>
</table>
SUMMARY

How did life on Earth begin? It has been recently proposed that chemical entities that formed autocatalytic sets in mineral surfaces could have increased their fitness/complexity over time through neighborhood selection. To test this idea, a mineral surface that is swept with a “prebiotic mix” should exhibit growth of these autocatalytic sets. If non-overlapping areas of the surface are exposed, then each area should exhibit similar levels of attachment without apparent growth. A simple microfluidic device is thus proposed as an experimental model for this hypothesis. It uses a laminar-flow technique known as hydrodynamic focusing to expose a given surface to a prebiotic mix stream in a highly controlled manner.

In this project, two surface-exposure protocols were developed using a 20 µM-solution of fluorescein isothiocyanate (FITC): a sweep protocol and a control experiment protocol. The sweep protocol was optimized to accommodate a non-stop 14-day experiment by injecting FITC at 0.5 µL/min, which consumes only 10 mL of reagent. Coupled with a total flanking injection rate of 6 µL/min, the FITC stream had an average width of 264±22.1 µm inside the reaction chamber. For a chamber 2-mm wide, this stream width allowed 11 overlapping stream positions within the chamber.

In addition, the control protocol was developed to achieve placement of the FITC stream in non-overlapping positions within the chamber without sweeping. It did so through temporarily increasing the injection rates by two orders of magnitude. Despite this, the stream width obtained was consistent with the sweep protocol’s stream width (262±19.8 µm).

The ability of microfluidic devices to provide a suitable experimental model for the origins of life question is thus shown. Using the inherent advantages of microscale flow, the proposed device constitutes the first of many prototypes that aim to test different ramifications of the neighborhood selection hypothesis.
CHAPTER I: INTRODUCTION

A. Microfluidics

The field of microfluidics is accurately defined as the “the science and technology of systems that process or manipulate small \((10^{-9} \text{ to } 10^{-18} \text{ litres})\) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres” [1].

Its popularity hinges around the delivery of improved techniques for analysis, primarily in molecular biology, and its ability to provide enhanced experimental models for a variety of empirical investigations. The augmented performance of devices using microscale flow is explained by the inherent advantages of miniaturization: low reagent consumption, higher sensitivity and resolution, more precise control of processes, short analysis runtimes, among others [1], [2].

Microfluidic applications have found their most prolific setting in academic research laboratories, mainly consisting in proof-of-concept projects ranging from protein crystallization condition screening to isoelectric focusing for pH gradient generation [1], [3]–[5]. Most of these applications involve the handling of liquids, but they also include the handling of gases, most notably in the control of oxygen gradients for biological studies [6], [7].

Key to the spread of these applications has been the development of microfabrication techniques based on silicon, which started in the late 1970s [8]. From that time, further refinements in the process have allowed time and cost-effective fabrication schemes, which typically involve two main stages: mold fabrication, and replica molding.

The process of mold fabrication is achieved through photolithography, a technique capable generating molds with feature sizes well within the microscale. This is achieved through the exposure of a photosensitive epoxy to light. Specifically, this “photoresist” is spin-coated onto a suitable substrate, with the coating thickness determined by the spin velocity and the photoresist’s viscosity. Then, the photoresist is exposed to either light, X-rays, electron beams, or proton beams
After exposure, the substrate along with the photoresist is immersed in a developer solution, which either dilutes away the exposed or unexposed areas: in the former case, the photoresist is considered a positive photoresist, and the latter a negative photoresist.

In microfluidics, the most commonly used photoresist for mold fabrication is the negative photoresist known as SU-8. Formulated by IBM scientists, SU-8 has high aspect ratio capability, and can be used to create molds with heights of 1-300 µm using a single spin-coating step [9], [10]. Given its excellent adhesion to a silicon substrate, silicon wafers are commonly used as the spinning substrate for microfluidic mold fabrication [9].

Replica molding is then used to cast the pattern contained in the molds onto a “positive stamp”, in a process better known as soft lithography. In this process, an elastomer is mixed with a curing agent -usually in a 10:1 mass ratio- and this mixture is poured onto the previously fabricated mold, which is then heated to allow the curing agent to crosslink the elastomeric chains. This eventually results in a hardened elastomer, at which point it is removed from the mold [11].

Soft-lithography allows for relatively short fabrication times compared to other microfabrication techniques, and this is further facilitated by polydimethylsiloxane (PDMS). The properties of this elastomer have popularized soft lithography as the easiest fabrication method choice for proof-of-concept experiments [12]. These properties include: Optical transparency, tunable elastic modulus, low surface energy, gas permeability, impermeability to liquid water, and most importantly, high pattern-transfer fidelity [12], [13].

However, aside from these enabling microfabrication techniques, what is the fundamental nature of microscale flow that makes microfluidic devices so prolific in applications?

Flow at the microscale brings about conditions which allow simple analytical solutions to the Navier-Stokes equations, due to its predominantly laminar nature. This is conceptually defined through the Reynolds number:
\[ \text{Re} = \frac{\rho VL}{\eta} \quad \text{Equation 1} \]

Where \( \rho \) represents fluid density, \( \eta \) the fluid’s dynamic viscosity, \( V \) the characteristic velocity scale and \( L \) the characteristic length [14].

The Reynolds number is a dimensionless number given by the ratio of inertial to viscous forces. Whenever viscous forces predominate, Reynolds number is low, and flow is considered laminar (\( \text{Re} \ll 2300 \) for Hagen-Poiseuille flow) [14]. In relatively big microchannels, the characteristic length varies between 1 \( \mu \)m to 100 \( \mu \)m. Using water as the fluid of choice, where density is \( \rho = 1000 \text{ kg/m}^3 \) and viscosity is \( \eta = 1 \text{ Pa}\cdot\text{s} \), and a characteristic velocity ranging between 1 \( \mu \)m/s to 1 cm/s, the Reynolds number oscillates between \( \text{Re} = 10^{-6} \) to \( \text{Re} = 10 \) [15]. This is well within the laminar flow regime.

A consequence of this flow regime is the ability to model flow in microfluidic devices in a manner analogous to electrical circuit analysis. Termed hydraulic circuit analysis, it is based on the approximation of infinitely large tubes, which can be safely assumed for microfluidic devices whenever \( \frac{R}{L} \ll \frac{1}{\text{Re}} \), which is the case for most channels designed in this project [14]. The approximation allows the assumption of a uniform pressure gradient along the length of the channel, and the resultant Hagen-Poiseuille law is:

\[ Q = \frac{AR^2}{8\eta L} \Delta P \quad \text{Equation 2} \]

\[ Q = \frac{\Delta P}{R_h} \quad \text{Equation 3} \]

Where the hydraulic resistance is \( R_h = \frac{8\eta L}{AR^2} \), and \( A \) is the cross-sectional area of the channel. The ability to perform hydraulic circuit analysis stems from the resemblance of Hagen-Poiseuille law to Ohm’s law \( I = \frac{\Delta V}{R} \) [14].
Because the typical cross section of microfluidic channels is rectangular, rather than the circular cross-section assumed by the Hagen-Poiseuille law, the hydraulic resistance is approximated by using the hydraulic radius \( r_h \) instead of \( R \),

\[
R_h \approx \frac{8\eta L}{A r_h^2} \quad \text{Equation 4}
\]

\[
r_h = \frac{2A}{p} \quad \text{Equation 5}
\]

Where \( p \) is the perimeter of the channel’s cross-section [14].

The relative simplicity of microscale flow has yielded two important and interrelated techniques in microfluidics: Hydrodynamic focusing and mixing. In hydrodynamic focusing, which is also known as flow focusing or laminar flow patterning, a central stream of fluid is focused by two flanking streams. Precisely because of the laminar flow regime, the three streams flow together without mixing in a turbulent fashion (although diffusive mixing still occurs). Most importantly, the technique allows precise flow control, since the width of the central stream depends on the ratio of the flanking to central stream flow rates and the position of the central stream can be tuned through a flanking flow rate ratio [16], [17].

Previous reports indicate that for rectangular channels with an aspect ratio (height/width) close to zero, the dependence of the width and position of the central stream on flow rate ratios is respectively given by,

\[
\frac{w_f}{w_o} = \frac{Q_c}{Q_c + Q_{f1} + Q_{f2}} \quad \text{Equation 6}
\]

\[
\frac{Y'}{w_o} = \frac{Q_{f2}}{Q_c + Q_{f1} + Q_{f2}} \quad \text{Equation 7}
\]
Where \( w_f \) is the width of the focused central stream, \( w_0 \) is the width of the focusing channel, \( Q_c \) is the central stream flow rate, \( Q_{f1} \) and \( Q_{f2} \) are the flanking flow rates 1 and 2, and \( Y' \) is the distance of one edge of the focusing channel to the closest edge of the focused central stream [17].

The hydrodynamic focusing technique has found extensive application in industry and research settings. It is the principle behind flow cytometry, and it has also been applied in substrate patterning, droplet and bubble formation, flow switchers, and most notably, mixers [16], [17].

The mixing application is particularly relevant, given the relative sluggishness of mixing at the laminar flow regime due to diffusion serving as the only driving mechanism [14], [18]. For the limit of very low aspect ratios, a simple 1-D diffusion version of the passive scalar convection-diffusion equation is:

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad \text{Equation 8}
\]

Where \( c \) is the species concentration, and \( D \) is the diffusion constant. This equation has the following solution, with boundary conditions \( c(x < 0, t = 0) = c_0 \) and \( c(x > 0, t = 0) = 0 \):

\[
c = \frac{1}{2} c_0 \text{erfc} \left( \frac{x}{2\sqrt{Dt}} \right) \quad \text{Equation 9}
\]

Where the term \( \sqrt{Dt} \) is known as the diffusion length scale \( l \), which is the distance the species has diffused into the \( c = 0 \) region in time \( t \) [14].

An examination of the diffusion length scale reveals the relatively long diffusion times required for microfluidic mixing. This is further evidenced by a dimensionless number called the Péclet number:

\[
\text{Pe} = \frac{Vl}{D} \quad \text{Equation 10}
\]

This dimensionless number indicates whether the scalar transport is mostly due to convection or diffusion, with higher Péclet numbers indicating the prevalence of convective transport. Whenever
the diffusion length scale $l$ is taken to be the width of a microfluidic channel, the Péclet number is also an indication of how many channel widths are needed for complete mixing [14], [15].

Many passive mixing approaches have been reported that attempt to reduce the diffusion length scale - hence the Péclet number. One of these approaches consists in narrowly focusing a stream using hydrodynamic focusing, thereby reducing the diffusion length scale [18]. In another approach, side pressures - normal to the flow direction- are generated by the anisotropic resistance given by grooves in the surface of microchannels [19]. The generation of side pressures adds convection to diffusive mixing via “chaotic advection”, which reduces the diffusion length scale dramatically. Other approaches include patterning of channels intersecting serpentines, 3D serpentines, and embedding barriers in microchannels [20].

The mixing phenomena, along with laminar-flow patterning, constitute the basis the preliminary experimental model developed in this project. As mentioned before, hydrodynamic focusing allows precise flow control, and a myriad of approaches have been pursued to enhance passive microfluidic mixing. Based on a plausible hypothesis of the origins of life, the proposed experimental model has been designed to enable the sequential exposure of a surface to an aqueous mixture of organic compounds.

B. Origins of life

How did life arise? This question remains one of the great unsolved mysteries of the scientific enterprise. For all the advancements in explaining the current diversity of life, elucidating how life might have started has been difficult.

A generally accepted idea bridging the gap from inanimate matter to life is the notion of chemical evolution, which was first suggested by Aleksandr Oparin and J.B.S. Haldane in the “Oparin-Haldane hypothesis” [21]. The hypothesis was then experimentally validated through the famous Miller-Urey experiment, in which various amino acids were obtained by sparking a solution of simple compounds - ammonia, water, methane, etc.- within a cyclic apparatus. Since then, the
experiment has been successfully replicated with varying conditions thought to have existed on the primitive Earth, cementing the plausibility of organic monomers spontaneously forming from simple compounds [2].

What has been more elusive is to bridge the gap between complex aqueous mixtures of organic polymers and the first “cell”. Two apparently competing sets of hypotheses attempt to bridge this gap: “replication-first hypotheses”, best embodied by the RNA world hypothesis, and the “metabolism-first hypotheses”, with the Iron-Sulfur world being its flagship idea.

The RNA world hypothesis suggests that “current” life most likely arose from molecules capable of both of information storage and autocatalysis, although it could be recognized that the “first” life forms may not have been RNA-based [22]. The hypothesis is plausible given the dual function of RNA as a catalyst and information carrier, which has not been observed in other biomolecules [23]. However, the hypothesis struggles to explain how organic polymers could have given rise to RNA molecules capable of faithful information storage and catalysis. Moreover, it remains obscure how exactly this information storage molecule could have coded for an enclosed compartment leading to the first cell, or how a conglomerate of cooperating RNA molecules could have replicated without growing -i.e. harnessing energy sources [24].

The iron-sulfur world hypothesis championed by Günther Wächtershäuser in contrast claims a gradual development of ancient biochemical pathways on mineral surfaces prevalent in hydrothermal vents -specifically, iron pyrite, FeS₂-, before the advent of any functionally complex molecule such as RNA [25]. Energy and inorganic nutrients were churned out by the hydrothermal vents, and the mineral surfaces served as transition-metal catalysts for carbon fixation. This process synthesized organic compounds that became ligands for the transition-metal surfaces, with the ionic bonding strength of the ligands becoming a selective trait in the face of continuous flow from the hydrothermal vents. In turn, these organic ligands favored the synthesis of similar organic ligands onto the surface -i.e. became autocatalytic-, developing a primitive version of the tricarboxylic acid cycle as the carbon fixation pathway [25]–[27].
Both the RNA-world and the iron-sulfur world hypotheses are very detailed in terms of the chemical reaction schemes at each step of the life-originating process. Presumably, this would allow for rich empirical testing of the hypotheses, which has been done in various ways [28]–[32]. However, they do not propose a consistent guiding mechanism that would result in the first life form. They rather attempt to explain in excruciating detail how each step might have happened, assuming it is known what steps or how many of them are required to generate life. While iron-sulfur invokes selection to explain why metabolic pathways would form on a mineral surface, this is not enough to guide the process towards life [24].

This gap was recently addressed through what was termed “neighborhood selection” [24]. Neighborhood selection is based on the concept of group selection, which favors the development of cooperation among individual entities within a group provided this increases the fitness of the group. It was further argued that such mechanism does not need bounded entities to operate, which can be readily attested by the cooperation exhibited by chemical entities present in current cells. Because of this, it is reasonable to infer that increased levels of cooperation would be selectively favored in non-discrete autocatalytic sets [24].

The key implication of this idea is that it obviates the need to develop sophisticated reaction schemes a priori for a coherent theory of the origins of life. Because of the vast amount of theoretical and empirical research, there are many reaction schemes and chemical building blocks demonstrated to exist on the early Earth. With an underlying process hypothesized to have driven chemistry to become biology, empirical testing can now deviate from proving reaction schemes, and focus on finding which chemistries can form autocatalytic groups. Furthermore, imposed selective pressures may allow researchers to find ways in which the groups would synthesize an enclosing membrane, at which point further selective pressures could be tested for the development of the currently known cellular machineries.

The selection-first idea suggests that the iron-sulfur world hypothesis is the most plausible starting point for empirical investigation [24]. The hypothesis does not invoke the spontaneous
increase in complexity claimed by the RNA world hypothesis, and it already incorporates the notion of autocatalytic entities, thus allowing selection to explain the development of life-like properties [24], [27].

The synthesis of the iron-sulfur proposal along with neighborhood selection guiding the growth of autocatalytic sets can be readily tested as follows: An autocatalytic set may nucleate on a mineral surface if such surface is continually exposed to a mixture of chemical entities -the prebiotic mix-, capable of forming such set. If the surface surrounding the autocatalytic set is gradually swept with the prebiotic mix, the set should grow from the initially exposed area to the next. This will happen because the already present autocatalytic set would facilitate the synthesis of more of its own on the newly-exposed areas, with building blocks and chemical energy provided by the prebiotic mix stream. Furthermore, neighborhood selection would favor the synthesis of autocatalytic sets that better adhere to newly-exposed surfaces, which would be evidenced by a gradual change in the composition and increased concentration of adhered autocatalytic sets in the direction of the sweep. This idea suggests a control experiment in which a surface that is not swept with the prebiotic mix, but rather exposed in non-overlapping areas, would not be expected to show increased adhesion of autocatalytic sets or consistent changes in set composition compared to the positive experiment.

C. Research Purpose

As mentioned before, the iron-sulfur world hypothesis has generated various empirical investigations. However, most of these experiments involved complicated equipment designed to meet the constraints imposed by the reaction schemes they attempted to test [28], [29]. To the best of our knowledge, microfluidic devices have not been employed as experimental models for the iron-sulfur world hypothesis. While the well-known Whitesides group is pursuing the origins-of-life question, they are not employing microfabricated devices to further their research agenda [33], [34].
The capabilities afforded by microfluidic techniques yield themselves to the question of neighborhood selection. Thanks to hydrodynamic focusing, the prebiotic mix can be flowed into a microfluidic channel along with two inert flanking streams, with the position and width of the prebiotic mix stream determined by flow rate ratios. In addition, microfluidics enables the *in-situ* mixing of the prebiotic mix with a solution serving as a source of chemical energy -called “energy mix”-, which would be necessary for a nucleation event to occur.

Hence, this project aimed to design and characterize a microfluidic device design enabling the sequential exposure of a surface to a stream of flow using hydrodynamic focusing. The proposed design has a modified T-shape geometry, and it incorporates a serpentine channel to attain binary diffusive mixing. Lastly, the project aimed to demonstrate a control procedure whereby the central stream was placed in non-overlapping positions in a discontinuous manner.

The flow-focused stream was moved in discrete positional steps -called “generations”-, from one edge of a reaction channel -known as “chamber”- to the next, which constituted a “sweep”. This project also aimed to optimize the parameters of the sweep considering the limited supply of the prebiotic mix, and the extended experiment runtime.
CHAPTER II: MATERIALS AND METHODS

A. Device design and fabrication

Device designs were conceived using the computer-aided design (CAD) software AutoCAD (Autodesk, Inc.), which were then either commercially printed on a high-resolution transparency film (Fineline Imaging), or saved as a drawing file and used as an input for maskless photolithography.

Devices made for this project were made through the following procedure: (1) Mold fabrication through photolithography, which either used a mask for photoresist-patterning, or a mask-less patterning approach, (2) fabrication of the positive stamp using soft-lithography, and (3) assembly of the individual components of the microfluidic device.

a. Mold fabrication using a photomask

Photolithography through a photomask was done by first plasma treating a 4-inch silicon wafer (UniversityWafer, Inc.) using a corona discharge (Electro-Technic Products, Inc.). The wafer was then sequentially rinsed using acetone, isopropanol (IPA), water, and again IPA. Next, the cleaned wafer was blow-dried using an air gun, and placed in a hot plate with temperatures between 85-100 °C (Figure 1a).

After these preparation steps, the wafer was spun-coated with a negative photoresist (SU-8 2015, MicroChem Corporation) to a coating thickness of 20 µm. This was achieved by setting the spin coater (Laurell Technologies Corporation) to a pre-coating speed of 500 RPM for 10 s, and a final coating speed of 2125 RPM for 30 s (Figure 1b).

After the coating step, the wafer was placed on a hot plate to be soft-baked at 95 °C for 3 min. It was then exposed to UV-light through a photomask for a time prescribed by the measured power output of a UV light source (EXFO Inc.), and by the following conversion:

\[
2 \left( \frac{\text{wafer area (cm}^2\text{)} \times \text{exposure energy (mJ/cm}^2\text{)}}{\text{power output (mJ/s)}} \right) \quad \text{Equation 11}
\]
Figure 1. Overview of the mold fabrication process. a) A silicon wafer which is 4 inches in diameter is cleaned as described in the text, and exposed to a corona discharge, b) the wafer is then spin-coated with SU-8 with a layer thickness determined by the spinning parameters and the SU-8 viscosity, c) the SU-8 is exposed to UV light through a photomask containing the intended design, after which d) the wafer is developed, washing away unexposed areas.

Dimensional analysis reveals that the conversion results in a unit of time. All wafers had a diameter of 4 in, resulting in an area of ~81.07 cm², with the exposure energy for a 20 µm-thick coating in the range of 140-150 mJ/cm². Usual power outputs were ~700 mW, for a calculated time of ~36 s, which was always rounded up to the greatest multiple of five; this resulted in an exposure time of 40 s (Figure 1c). The factor of two in the conversion prevents exposed photoresist from being dissolved too early in the developing step.

Following exposure to UV light, the wafer was then placed again in a hot plate for the post-exposure bake at 95 °C for 3 min. The post-exposure bake was followed by a developing step, in which the wafer was immersed in an SU-8 developer solution (MicroChem Corporation), and
shaken by titer plate shaker (Lab-Line Instruments, Inc.) for 3 min. Finally, the wafer was cleaned using the same cleaning procedure described above (Figure 1d).

b. Maskless mold fabrication

Exposure of the photoresist without using a printed photomask followed a very similar photolithographic procedure, with the exception that exposure was done using a direct write micropattern generator (µPG 101, Heidelberg Instruments Mikrotechnik GmbH), rather than a UV-light source.

However, maskless photolithography involved some different considerations. During design conception, it required centering the drawing around the Cartesian coordinates, and processing of the design file through LinkCAD (Bay Technology). During fabrication, it required the coating of an SU-8 base layer which was 12-µm thick. This base layer was then directly exposed to UV light for 20 s.

Then, the spinning steps to obtain a 20-µm SU-8 film were performed as described previously, followed by another soft bake. The exposure was then done through the direct write micropattern generator. Just like mask photolithography, the exposure parameters depend upon the coating thickness, which was 20 µm for the top layer. Thus, the wafer was exposed twice, with the first exposure set at a laser exposure power of 18 mW, a pixel pulse duration of 97%, and an energy factor of x4. The second exposure had these parameters halved.

As an optional step, the newly-made masters or molds were silanized by placing them inside a vacuum chamber, pouring 200 µL of (Tridecafluoro 1, 2, 2-tetrahydroctyl)-1-trichlorosilane on a glass slide adjacent to the mold, and turning on the vacuum for a minimum of two hours.

c. Soft Lithography

Fabrication of the positive PDMS stamps started with the mixing of a PDMS base with PDMS curing agent (Sylgard® 184 silicone elastomeric kit, Dow Corning Corporation) in a 10:1 mass ratio, using a planetary centrifugal mixer (AR-100, Thinky Corporation). The mixed PDMS was then
poured on a polystyrene square Petri dish containing the negative mold, and degassed in a vacuum chamber for approximately 2 h to eliminate bubbles.

Once bubbles were removed from the poured mixture, the dish was placed on a hot plate at 85 °C for 24 h. After baking, a square piece of PDMS was cut out of the mold, with each stamp later cut from the square piece. Lastly, inlet holes were punched on the stamps using a pre-cut 13-gauge syringe needle.

d. Assembly

To assemble the devices, PDMS stamps and standard microscope glass slides (Fisherbrand, Thermo Fisher Scientific, Inc.) were exposed to a corona discharge with the PDMS stamp being exposed on the patterned side. The two components were immediately put together after plasma treatment, and baked at 85 °C for around 1 min with a metal weight on top. An assembled device is shown in Figure 2.

Figure 2. View of the assembled microfluidic device. The PDMS stamp is bonded to a standard size microscope glass slide (75 mm by 25 mm). The microfluidic channels have been filled with food dye to illustrate them.
B. Characterization experiments

Before the experiments, the microfluidic devices were placed in a plasma cleaner (PE-50, Plasma Etch, Inc.) for 8 min to enhance their loading with water using a 1-mL syringe (Luer-Lok, Becton, Dickinson and Company). After loading with water, the microfluidic devices were placed in an inverted fluorescence microscope (IX51, Olympus Corporation of the Americas).

Tygon tubing was used to connect the inlets and outlets of the microfluidic devices (Cole-Parmer), as shown in Figure 3a. Each of the inlet tubes was connected to syringes through pre-cut 25-gauge needles (PrecisionGlide, Becton, Dickinson and Company), with the syringes placed in syringe pumps for actuation (Figure 3b).

a. Sweep protocol experiments

To test for optimal sweep protocols, the two syringes serving the central inlets contained a 20-µM solution of fluorescein isothiocyanate (FITC Isomer I, Sigma-Aldrich Co. LLC.), while the two syringes serving the flanking inlets contained distilled water. The flanking syringe pumps were of lower motor step resolution (NE-300, New Era Pump Systems, Inc.) than the central syringe pump (Fusion 100, Chemyx). Setting the intended syringe pump flow rates required prior specification of the syringe-barrel diameter.
Figure 3. Illustration of the experimental setup. a) A microfluidic device positioned in the microscope, with the tubing attached. b) View of the overall setup. The outlet tube is connected to a cup to prevent spilling. Notice that the central pump would inject both of its syringes at the same flow rate.
b. Mixing

The mixing performance of the serpentine channel was characterized through a setup like the sweep protocol experiments. However, only one of the two central syringes contained the FITC solution, while the other contained distilled water. The central syringe pump had its injection flow rate sequentially switched from 0.5 µL/min to 1, 2, and 3 µL/min, and images were taken at the beginning and end of the serpentine for each flow rate. The flanking pumps had their injection flow rates constant at 3 µL/min throughout the mixing experiments.

c. Imaging

Images and time lapses of the experiments were taken by a CCD camera attached to the inverted fluorescence microscope, using the 4x objective. Image acquisition was performed through image acquisition and analysis software (MetaMorph Premier, Molecular Devices, LLC.). This software also allowed for microscope stage control, as well as focusing of the live images.

To acquire fluorescence intensity values, an image analysis software (ImageJ, National Institutes of Health) was used to collect line scans that spanned the width of the imaged microfluidic chamber or channel, depending on the experiment performed. The line scans consisted of averaged intensity values, each value being the average of 300 pixels. Each line was converted from length in pixels to length in mm by inputting the actual width of the microfluidic channel/chamber.

Time lapses were captured using a 2000 ms exposure time, resulting in one frame collected every 2 s.

C. Data analysis

Microsoft Excel was used to normalize intensity values and perform statistical analysis. Each experiment (mixing, sweep, and control procedure) was repeated three times.

Stream widths were calculated using full width half max considerations -FWHM. Briefly, the position values -x-values- corresponding to the two points of half-intensity were subtracted for each of the Gaussian-like curves, which represent each generation. Whenever intensities did not
correspond to 0.50, either directly or through rounding, an average between the x-values of the closest intensity points was taken (e.g. 0.491 and 0.507). The one-way ANOVA test was done to statistically determine differences in stream width due either to protocol, or to devices. It was also used to assess differences in stream position, which was obtained through the x-value of the maximum intensity data point (normalized intensity=1).

A custom MATLAB script was developed to obtain the time for equilibration of the central stream. This script first captures an AVI video, then reads the intensity of pixels along a line, averages the pixel indices corresponding to the top 91% in intensity, and fits an exponential function to the plot of indices (i.e. positions) vs. time. Whenever slope is zero, or reaches a minimum, it corresponds to the equilibration time. The script is shown in the Appendix.
CHAPTER III: RESULTS

A. Discrete-chamber devices

In the beginning stages of the project, the proposed designs did not exploit hydrodynamic focusing, but rather consisted of a discrete number of chambers. These chambers had closely-packed pillars at their interface, which caused high fluidic resistance between them (Figure 4).

Figure 4. Example of an initial design based on discrete chambers. The fluidic resistance was provided by pillars, which created an interface between chambers.
Devices designed this way were to be operated by having one inlet and one outlet open to flow while the remaining inlets and outlets would remain closed. For instance, the prebiotic mix would be injected through inlet 1, expected to flow through chambers 1 and 2, and exit through outlet 1. Then, inlet and outlet 1 would be shut off to open inlet and outlet 2, having the primordial soup flowing through chambers 2 and 3. This basic operation was to be repeated until reaching the last set of inlets/outlets.

However, the operation described did not come to fruition. Not only was it burdensome to shut off inlets and outlets, but the food dye employed to test flow in these devices always flowed towards the four chambers before exiting the device (Figure 5a). In addition, many devices suffered from leaks due to certain channels being insufficiently separated from each other, which highlighted the limited resolution of photomask photolithography.

Figure 5. Ineffective discrete chamber devices. a) Food dye injected onto the devices invariably flowed through every chamber before exiting the device, b) Despite the usage of vacuum rather than pumping, flow control was still difficult.
The issues were addressed with improved designs (Figure 4 above), direct-write photolithography, tighter packing of pillars, and negative-pressure actuation. Nevertheless, the main issue of fluid flow through unintended areas of the device persisted (Figure 5b).

B. Hydrodynamic focusing and mixing devices

The continued performance issues lead to the switch to hydrodynamic focusing as the fundamental operational principle, which allowed for easier fabrication, operation, and flow control. Furthermore, it made possible the continuous or gradual exposure of a surface to the primordial soup, as opposed to the discrete exposure allowed by the discrete-chamber devices.

Experiments were done to fine-tune the operational protocol of devices using hydrodynamic focusing for eventual testing of the neighborhood selection premise. In terms of the sweeping and mixing protocols, the performance criteria selected for testing included the injection rates of the central and flanking pumps. These flow rates were to yield a continuous sweep with consistent widths. In addition, the devices were tested for their ability to support a control procedure.

a. Optimization of the central pump’s injection rate

The premise of hydrodynamic focusing was first evaluated on preliminary designs like the ones shown in Figure 6. The T-shape designs consisted of three inlets with their respective three channels converging to a central “chamber”. The chamber would lead to the only outlet of the device. This basic modified T-shape design was iteratively refined due to the experiments here described.

Initially, determining the best rate of injection of the central pump was done to prevent extensive diffusion of FITC into the water streams, and vice versa. Empirically, this implied sharpening the stream boundaries. To assess the effect of injection rates in sharpening stream boundaries, all pumps were set to inject at 2, 20, and 200 µL/min, respectively. As expected, the stream boundaries became sharpened with increased injection rates (Figure 7).
This can be better appreciated by comparing the plots of gray values, which show steeper increases in pixel intensity corresponding to sharper boundaries.

However, sharply defined stream boundaries were achieved using relatively high injection rates, which could result in rapid depletion of the available prebiotic mix. Assuming a two-week uninterrupted origins of life experiment, an injection rate of 200 µL/min would consume approximately 4 L of prebiotic mix solution, whereas 2 µL/min would consume 40 mL. Figure 7 below shows that at 2 µL/min the stream definition was sufficient to assume FITC would not diffuse appreciably towards the water streams, given the chamber length.

The height of the microfluidic channels was then reduced from 100 µm to 20 µm in the hopes of achieving better stream definition at low injection rates. Figure 8 shows that such improvement was not evident. The average slope from both graphs revolve around 0.01 units of intensity/µm, with the slope for the 20-µm-tall channel being slightly higher than the slope of the 100-µm-tall device (0.012 vs. 0.009 units of intensity/µm, respectively). Because such a small change in slope was observed for an 80% reduction in microchannel height, and shorter heights would complicate device fabrication, the subsequent height of the microfluidic devices was kept at 20 µm. This new height decreased the aspect ratio of the microfluidic channels, which made Equations 6 and 7 more valid to these designs.
Figure 7. Sharpening of stream boundaries with increased injection rates. a) 2 µL/min, b) 20 µL/min, c) 200 µL/min. The lines on each device picture illustrate the line scan method, which measures the gray scale intensity along the drawn line. The graphs in turn depict gray scale (fluorescence) intensity vs. distance along chamber. Note the sharper increase in gray values as the injection rates increase.
Figure 8. Stream definition change due to decreased chamber height. a) Height=20 µm, b) Height=100 µm. Changing the height of the microfluidic channels did not affect stream definition appreciably.

With the newly defined channel height, the following tests attempted to elucidate the lowest injection rate possible with the current devices. If the goal was to reduce consumption of the primordial soup solution to less than 15-20 mL after a 14-day experiment, the maximum FITC injection rate had to be 1 µL/min. The first injection rate tested was 30 µL/h (0.5 µL/min), which exhibited flow instability, and diffusion of the FITC solution towards the buffer streams (Figure 9).

It quickly became apparent that the pumps used to actuate the central FITC stream had very limited motor step sizes (low pumping resolution). Until this point, all the pumps employed had a resolution of 0.21 µm/step. Once the central syringe pump was replaced with a more precise one (0.098 µm/step), the flow instability issue was resolved.
Figure 9. First attempt at minimizing the central pump’s injection rate. Top: Individual frames from a time lapse. Each frame is 1 s apart, and they show how the FITC stream was not steady and rather oscillated, resembling the lateral undulation motion exhibited by many snakes. Bottom: FITC diffusion towards the water streams right before the end of the chamber.
Given that an injection rate of 0.5 µL/min consumed approximately 10 mL in 14 d, the central pump's injection rate was finally set at 0.5 µL/min for the remaining experiments. This resulted in a net central flow rate of \( Q_c = 1 \) µL/min, as indicated by mass conservation and hydraulic circuit analysis.

b. Mixing effectiveness

Despite the many mixing approaches available, a simple mixing geometry was materialized through a serpentine channel that would connect two new inlets to the device chamber; this channel geometry constituted the final design iteration (Figure 10).

Figure 10. Illustration of the final microfluidic device design. The channel architecture comprises two inlet channels that converge into a serpentine channel, and two flanking inlet channels. All inlet channels are 250-µm (0.25 mm) wide, with a flanking-channel length of ~11.5 mm, and total serpentine channel length (from points of convergence, or stars) of ~194 mm. The channels then converge together into a “chamber” which is 8.9 mm long and 2-mm wide.
The relatively long serpentine channel allows for diffusive mixing to occur in the time FITC and water take to transit through the serpentine. The serpentine channel was first tested using 0.5, 1, 2, and 3 µL/min as the central pump injection rates, which pumped one syringe containing 20 µM-FITC solution, and another syringe water. The flanking pumps’ injection rates were kept at 3 µL/min throughout the experiment. The lowest injection rate (0.5 µL/min) demonstrated the best mixing performance (Figure 11).

Figure 11. Mixing effectiveness of FITC and water at different injection rates. The line colors correspond to their plot colors in the graph to the right. Vertical bars represent standard deviation (n=3).
Figure 12 further shows that at an injection rate of 0.5 µL/min, mixing of FITC and water is optimal, but it does not follow a perfectly flat line (slope of zero).

Figure 12. Mixing of FITC and water at an injection rate of 0.5 µL/min. Line scans were collected at the beginning and end of the serpentine for three different devices. Vertical bars represent standard deviation (n=3).
c. Sweep protocol optimization

Another important performance parameter revolved around the controllability of stream widths, which is given by Equation 6 for channels of low aspect ratio. Because of the ability to control at least two distinct flow rates, a myriad of injection-rate combinations could generate one central stream width (Figure 13).

Figure 13. Adjustment of central stream widths by switching injection rates. Initially, the ability to control stream width was tested by keeping the flanking injection rates consistent, while changing the central pump’s injection rate. Legend depicts “flanking-central-flanking” injection rates in µL/min.
Stream width was a parameter of interest given the need of a continuous sweep through the width of the chamber. Because a strictly continuous sweep required programmable syringe pumps (which were not available), the sweep was done sequentially through discrete positions of the central stream with each position kept for a certain time. To attain this pseudo-continuous exposure, stream positions had to overlap with each other.

With these constraints, stream width became important so long each stream position overlapped with each other. In turn, the initially proposed sweep protocol kept the central and total flanking injection rates constant (which per Equation 6, ensured a consistent stream width), but alternated the individual flanking pumps’ injection rates to move the central stream (per Equation 7). This initial procedure is shown in Table 1, which shows that the amount of central stream positions available would depend on the injection rate stepping for the sweep.

<table>
<thead>
<tr>
<th>Generation #</th>
<th>Flanking pump 1 injection rate (µL/min)</th>
<th>Flanking pump 2 injection rate (µL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td>1</td>
</tr>
</tbody>
</table>
The first attempt to perform a sweep using this protocol is illustrated in Figure 14. It can be observed that the criteria of having stream positions overlap with each other was satisfied. Using a stepping of 1 µL/min and a total flanking flow rate of 20 µL/min, 19 positions (alternatively known as “generations”) were possible with this protocol.

![Figure 14. Results of the initial sweep protocol. The five pictures illustrate how the central stream moved up and/or down (left and/or right) depending on the two flanking injection rates. Top and bottom pictures of the chamber show the central stream reaching the edges of the chamber; a result achieved by setting the flanking flow rates 1-19 and 19-1 µL/min, respectively (note the legend in graph to the right). The middle picture corresponds to a flanking injection rate of 10-10 µL/min. At all positions, the total flanking flow rate adds to 20 µL/min.](image-url)
Once a satisfactory way to perform a sweep was attained, it became relevant to consider the rate at which water was being depleted. If two 60-mL syringes were to be used to hold the water required for the flanking streams, and both would inject at a total rate of 20 µL/min, each generation was to last approximately 5 h. Furthermore, the experiment would have only lasted 4 d, rather than the assumed 14 d.

A new protocol was then devised which considered the need to accommodate a 14-day experiment using the biggest available syringes for the flanking streams, a 10-mL prebiotic mix consumption limit, and an easier manual operation (generation runtime above 24 h). It involved a total flanking injection rate of 6 µL/min, with steps of 0.5 µL/min, to obtain 11 generations. This protocol would consume 59.4 mL of water and 9.99 mL of prebiotic mix/energy solution after 14 d, and allow for a generation runtime of around 30 h (Table 2).

<table>
<thead>
<tr>
<th>Generation #</th>
<th>Flanking pump 1 injection rate (µL/min)</th>
<th>Flanking pump 2 injection rate (µL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>5.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The protocol was first tested using relatively small syringes: two 10-mL syringes for the flanking streams, and two 1-mL syringes for the central stream. Rather than executing the protocol to emulate the 14-day experiment, the central stream was allowed to stabilize in its target position for
5 min, and then imaged. The results are shown in Figure 15. As expected, the stream widths increased given the lower total flanking flow rate. Most importantly, each generation overlapped with each other.

Figure 15. Results of the reagent-saving sweep protocol. The top three pictures depict the position of the central stream corresponding to the black plots (Generation 2, 6, and 10). Horizontal bars portray the standard deviation of the positions (n=3).
To statistically determine the consistency of stream widths, a one-way ANOVA test was performed on stream widths. The ANOVA tests were done having either each of the 11 generations or the 3 devices used, as treatments.

When using one-way ANOVA with generations as treatments, no statistically significant difference was found between central stream widths (F(10,22)=0.208, p=0.993). However, one-way ANOVA with devices as treatments yielded a statistically significant difference (F(2,30)=7.72 p=0.00197). Each device had, respectively, the following stream widths: 0.274±0.013 mm, 0.246±0.024 mm, and 0.272±0.017 mm. These results suggest that stream width is consistent during a sweep, but not across devices. However, the overall average stream width was 0.264±0.022 mm.

Once the protocol was validated with small syringes, and the consistency of stream widths statistically demonstrated, the protocol was tested using syringes of higher volumetric capacity. Because of the calculated reagent consumption, which was based on the available syringes, 60-mL syringes were chosen for the flanking streams, and 10-mL syringes were chosen to contain the prebiotic mix/energy mix solution. As Figure 16 shows, the sweep was also possible using syringes of higher volumetric capacity. However, the generation overlap did not appear as uniform as the overlap attained with smaller syringes.

Statistical analysis of the stream width consistency showed similar results to the width consistency of small syringes. Namely, when generations were used as treatments, no statistically significant difference was found between stream widths throughout generations (F(10,21)=0.420, p=0.921). However, there was a statistically significant difference in widths across devices, which was more pronounced than the case with small syringes (F(2,29)=27.5, p=1.99e-7). The average stream width in this case was 0.210±0.048 mm.

The main difference between the sweep performed with both syringe-size cases was the time required for each generation to reach an equilibrium position. While generations could equilibrate within 10 min in the small syringe sweep (6.37±3.39 min), the big syringe case took an average of
28.5±19.5 min to equilibrate in position. Furthermore, the central stream did not take a significantly higher time to equilibrate when going from generation 6 to 1 (32.7±4.97 min).

Figure 16. Results of the sweep using high-volumetric capacity syringes. The color scheme remains the same as before. It is apparent that controllability was more difficult in this scenario.
d. Control experiment protocol

The last performance parameter of interest was the ability to perform an origins-of-life control experiment. Since the purpose of the sweep is to gradually expose a surface to a fluid stream, the control protocol would have to place the central stream in non-overlapping positions within the chamber without sweeping it from one position to the next.

The proposed control protocol involved shutting off the central stream by overwhelming it with the flanking streams. Specifically, the central pump was first turned off and the total flanking injection rate was increased two orders of magnitude (from 6 µL/min to 600 µL/min). This would pinch off the central stream and make it disappear (Figure 17a). Once the central stream was observed to recede up to the beginning of the serpentine, the flanking pumps had their injection rates readjusted to correspond to the ratio of injection rates of the target generation (Figure 17b). This allowed the central FITC stream to return to the chamber thanks to its residual pressure. Finally, at the sight of the stream returning to the chamber, the total injection rate of the flanking pumps was set back to 6 µL/min, and the central pump was turned on (Figure 17c). This procedure was repeated to drive the stream from generation 2 to 6, and from generation 6 to 10 (Figure 18).

The average time for pinch off, with \( t=0 \) at the point of pump adjustment, was 6±2 s, while stream retreat clocked at 72±29 s. Furthermore, from the start of the protocol, the stream took 169±25 s to return to the chamber.

The control procedure yielded stream widths and positions close to those obtained by the sweep protocol (Figure 18). For generation 2 and 10, there was no statistically significant difference between the positions obtained through both protocols (\( F(2,5)=2.74, p=0.157 \), and \( F(2,6)=2.56, p=0.157 \), respectively). However, a difference was observed for generation 6 (\( F(2,6)=10.4, p=0.0112 \)). In terms of the width, there was also a statistically significant difference between protocols (\( F(2,6)=13.2, p=0.00637 \)), which was due to the inconsistency observed for the sweep with high-volumetric capacity syringes.
Figure 17. Illustration of a course of events of the control procedure to move the central stream from Generation 6 to Generation 2. a) The central pump was turned off, and the flanking pumps’ flow rates were increased from 3-3 µL/min to 300-300 µL/min. At t=10 s, the adjustments to the pumps were completed, after which it can be seen how the central FITC stream was completely pinched-off within 6 s of completing the adjustments. b) Once the FITC stream was observed to retreat at the beginning of the serpentine (after t=74 s), the flanking pumps had their flow rates adjusted to 10-50 µL/min. c) At t=154 s (2 min and 34 s) the FITC stream was seen to return to the chamber. The flanking pumps were finally adjusted to 1-5 µL/min, and the central pump was turned on.

Comparing the small syringe sweep width (0.261±0.004 mm) with the control procedure width (0.262±0.015 mm) using an independent-samples t-test reveals no significant difference; t(2)=−0.00345, p=0.976. However, the same test reveals a difference between big syringe sweep (0.197±0.026 mm) and control procedure (0.262±0.015 mm); t(3)=3.67, p=0.0351.

One-way ANOVA did not reveal differences in stream width due to generations or devices. Specifically, no statistically significant difference was observed using generations as treatments (F(2,6)=2.47, p=0.165), or devices as treatments (F(2,6)=1.74, p=0.251).
Figure 18. Final position of generations 2, 6, and 10 after the control procedure. It can be observed that the non-overlap was achieved.
CHAPTER IV: DISCUSSION

A. Discrete-chamber design

Given the need to sequentially expose a surface to a solution, the discrete-chamber design was conceived to achieve step-by-step exposure. Most importantly, the design predetermined the number of samples to be collected by defining at least four distinct areas of exposure. However, considering the theoretical treatment of microfluidic flow, the design premise had the basic issue of considerably lower resistance through the chambers than through the outlet channels. It is no surprise then that flow invariably preferred to go through the chambers before exiting the device, despite the presence of pillars.

A possible way to make the discrete-chamber devices functional is to incorporate valves that would prevent flow toward unintended areas. Specifically, the proposed valves would consist of a bottom layer through which flow would circulate and a top layer containing pneumatically-actuated valves [35]. However, laminar flow at the microscale immediately appeared to solve the flow-control issue of discrete-chamber devices, without resorting to the increased operational complexity of pneumatic actuation on multilayer devices.

B. Mixing performance

The serpentine channel showed excellent results despite what basic theoretical considerations would predict. Using a flow rate of 1 μL/min to estimate the velocity, a channel width of 250 μm, and 4.9x10^{-10} m^2/s as the diffusion constant of FITC in water [36], the Péclet number is approximately 1700. This means that the minimum serpentine channel length should be approximately 425 mm, which is around two-times longer than the designed serpentine length of 194 mm. This points to further enhancements in the design of the mixing stage, albeit marginal given the results of the current serpentine.
The above calculations are accurate for the prebiotic mix provided the diffusion constant remains similar. Because the diffusion constant of the prebiotic mix and energy source may be unknown a priori, microfluidic mixing design should assume very high Péclet numbers.

One approach in terms of the current device would be to decrease the central pump’s injection rate to 0.25 μL/min, which would result in a minimum serpentine length of around 213 mm. However, the length of the serpentine can be further reduced by incorporating a Venturi-like junction at the beginning of the serpentine. This design has shown a 75% reduction in mixing length compared to a standard Y-type junction similar to the one employed for this project [37]. Incorporation of this feature probably works by transiently reducing the diffusion length scale, but it may not significantly reduce the hydraulic resistance because of the inverse square dependence of the resistance on the hydrodynamic radius given by Equation 4. Therefore, this change is recommended whenever a bulky serpentine channel is undesirable.

C. Sweep

Equations 6 and 7 state that the width and position of the central stream is dependent upon flow rate ratios. This behavior was observed in the devices made for this project, for which stream width of 0.286 mm is predicted through Equation 6. This stream width is close to the observed widths in the actual devices (0.264, 0.262, etc.).

The inconsistency of stream widths across devices is readily explained by the introduction of human error. This may have started during device fabrication, in which the skill involved likely played a role in the consistency of device specifications. Furthermore, the experimental setup required careful handling during device loading with water, and careful insertion of tubing into the microfluidic device. Once the devices were loaded, and the pumps operated on the device, human error was decreased. This accounts for the consistency of stream widths during each sweep. However, stream width in the context of the sweep may not be a design target if the sweep is truly continuous, which can be attained by programmable syringe pumps.
It is also worth discussing the delay in flow stabilization when using high volumetric capacity syringes. Microfluidic devices fabricated on PDMS will invariably deform upon the application of pressure from a syringe pump, which is known as fluidic capacitance or compliance. Electrical circuit analysis reveals that pressure switches on a constant resistance circuit would immediately result in current changes. This would be the case in microfluidic devices if the net resistance is low, and the capacitance is close to zero (i.e. a rigid material). However, the elasticity of PDMS makes for the response times observed in the experiments, which were more pronounced using larger syringes. This response is independent of the flow rate switch, which agrees with the observed behavior of the devices in this project, and the behavior expected from an RC circuit. In addition, it is known that whenever a syringe pump has low-precision motor steps, using high volumetric capacity syringes with low injection rates creates conditions that push the syringe pump’s specifications to its limits.

A positive consequence of this slow response time is that it dampens the oscillations caused by a low motor-step resolution pump, which is heightened by the increased elasticity of the microfluidic device. If a faster response time is still needed, any other rigid material well-known within the microfluidics community may be employed, such as PMMA, glass, or polycarbonate. In addition, the tubing used to serve the device may have to be rigid as well to minimize the system’s overall capacitance. As mentioned before, this will increase the propensity of the system to pump oscillations, for which pumps with higher motor-resolution may be required.

D. Control

The control procedure demonstrated how to employ the proposed device design to place the central stream in non-overlapping positions. It requires a fast response time, which is not a drawback considering that high-volumetric-capacity syringes are not needed for a long-term experiment. Stream widths could be reduced either by decreasing the central pump’s injection rate
(as recommended for mixing), or increasing the total flanking injection rate. In this fashion, more than three non-overlapping positions may be sampled, if so required.

With the times obtained through experiments, it is possible to devise a control protocol as follows: (1) Turn off central pump, and increase the injection rate of each flanking pump by two orders of magnitude (this would be t=0), (2) Right at 43 s, decrease total flanking injection rate by one order of magnitude, to the corresponding generation/position flanking injection rate ratio, (3) After 144 s (2 min and 24 s), turn on the central pump, (4) After 194 s (3 min and 14 s) return the total flanking flow rate to its original value, decreasing it one order of magnitude.

The drawback of this procedure is its complex manual operation, which will increase human error. Thus, it requires programmable syringe pumps in the same way they are needed for a truly continuous swipe and improved flow stability.
CHAPTER V: CONCLUSION

This project constitutes the first step towards what would hopefully be the creation of life from inanimate matter. It does so by proposing an experimental model that uses hydrodynamic focusing to test for the ability of prebiotic mix to colonize a mineral surface, effectively imposing a selective pressure inside microfluidic devices.

A sweep protocol was optimized to obtain the greatest number of overlapping generations, on an extended experiment runtime, while minimizing reagent consumption. The protocol showed successful results independent of the volumetric capacity of the syringes, suggesting further improvements in experiment runtime. However, as the volumetric capacity of the syringes increases, the responsiveness of elastomeric PDMS devices decreases.

Lastly, a protocol for a control experiment involving the pinching of the central stream was proposed. This protocol, while requiring precise syringe pump operation, was effective in placing the central stream in non-overlapping positions in a discontinuous manner, without requiring more complex device designs.
CITED LITERATURE


APPENDIX

The following MATLAB algorithm computes the time it takes for the central stream to reach an equilibrium position. It does so by reading a video file, and doing the following for each frame:

1. Select an area of the frame which is one pixel wide, and n pixels long (effectively like drawing a line).

2. Average the indices corresponding to pixel intensities above 90% of maximum intensity.

3. Store the indices, and the time.

4. Fit an exponential function to indices as a function of time (so indices are a proxy for position). The exponential model corresponds to the solution of an overdamped oscillator, which closely resembles the movement of the stream from one position to the next. If MATLAB cannot fit an exponential function properly, use a 9th degree polynomial.

5. Obtain the time from these cases: a) Whenever the slope of the fit switches sign, or b) Right when it reaches its minimum value.

clear all;
close all;
clc;

filename=input('Which video? ', 's'); %Ask the user for the video to be analyzed
v=VideoReader(filename); %Load the video onto an object
% v.CurrentTime=6.8; %Start video play/manipulation at a specific time
k=1;
while hasFrame(v) %As long as there are frames in the video
    vidFrame = readFrame(v); %Read the frame
    image(vidFrame);
    region=vidFrame(:,1310); %Define the line of interest
    %image(region);
    indices=find(region>(0.91*max(region)));
    p(k)=mean(indices); %Store the mean of the indices in a "position" variable.
    time(k)=(k*2); %Each frame was collected every two seconds
    k = k+1;
end
damp=fit(time',p','exp2','normalize','on'); %No scaling
% damp=fit(time',p','poly9','normalize','on'); %Just in case
realtime=(0:1:10000)';
slope=differentiate(damp,realtime);
[value t]=min(slope(slope>0)); %Get and locate minimum slope
subplot(2,1,1)
plot(damp,time,p)
grid on
subplot(2,1,2)
plot(realtime,slope)
grid on
VITA

Félix Leonardo Morales Caballero

EDUCATION
Master of Science, Bioengineering, expected May 2017
University of Illinois at Chicago, Chicago, IL
GPA: 3.42/4.00
Advisor: David Eddington, PhD

Bachelor of Science, Bioengineering, May 2015
University of Illinois at Chicago, Chicago, IL
Cumulative GPA: 3.69/4.00 Cum Laude
Senior Design Project: Automated Assessment of Human Islet Samples
Supervisor: Yong Wang, MD

RESEARCH EXPERIENCE
Undergraduate Research Assistant/M.S. student (August 2013-Present)
Biological Microsystems Laboratory
Principal Investigator: David Eddington, PhD
University of Illinois at Chicago, Department of Bioengineering

TEACHING EXPERIENCE
Pre-Calculus Instructor (June-July 2015 and 2016)
University of Illinois at Chicago
Minority Engineering Recruitment and Retention Program
Supervisor: Gerry Smith

Guest Lecturer (March 2016)
University of Illinois at Chicago
Department of Bioengineering
Profesor: Daniela Valdez-Jasso, PhD
Course: BIOE 102: Bioengineering Freshman Seminar

Tutor for CHEM 114: General Chemistry II (October 2013-April 2014)
University of Illinois at Chicago
Minority Engineering Recruitment and Retention Program
Supervisor: Gerry Smith

Mentor (September 2013-April 2015)
University of Illinois at Chicago
Society of Hispanic Profesional Engineers
Students: Michael Godoy, Ernesto Berrum
PUBLICATIONS


CONFERENCE PRESENTATIONS

AFFILIATIONS
Tau Beta Pi, January 2014
Alpha Eta Mu Beta, November 2013
Society of Hispanic Professional Engineers, September 2011
  Graduate Ambassador, 2015-2016
  Academics Chair, 2014
  Academics Co-Chair 2013-2014
Biomedical Engineering Society, September 2011
  Stem Cell Society Director, 2012-2013

HONORS (2012-2015)
Xerox Scholarship, 2012-2013 and 2014-2015
Dean’s List, every semester except Spring 2014
SHPE Foundation General Scholarship, 2013-2014
SHPE Chicago Scholarship, 2012 and 2013
Chancellor’s Student Service Award, April 2013