Observing Behavior of Fluid Flow through Carbon Nanotube Arrays

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Observing Behavior of Fluid Flow through Carbon Nanotube Arrays

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Mechanical Engineering

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Abstract

This work establishes a platform technique for visualizing fluid transport through Anoidisc Alumina Oxide (AAO) membranes, which can be applied to Carbon Nanotube (CNT) arrays, and allow for the testing of the effects of other parameters on flow. Arrays of CNTs have shown significant promise for delivering biomolecules into cells with high efficiency while maintaining cell viability. In these applications, biomolecules flow through CNT arrays manufactured in our lab using Template-Based Chemical Vapor Deposition. By culturing cells on the opposite side of the array, they can be used to transfect biomolecules into cells. In this research, it was discovered that the transfection rate was dependent on the type of biomolecule being delivered into the cells. It was also inferred that the number of CNTs the cells covered would affect the transfection rate. In order to characterize flow through the CNT arrays, an experiment was designed and conducted to test the effect of changing the number of active CNTs. Preliminary testing showed the occurrence of an unknown error in the CNT array manufacturing process which prevented material from flowing through the CNT arrays. As a result, the study was modified to characterize flow through AAO membranes, which serve as the template for the CNTs. To accomplish this, a flow device was developed which restricted flow to a predefined circular area. Three different diameters were tested 6 mm, 4 mm, and 2 mm. Flow data was taken using fluorescent dye, as it diffused through the AAO into a volume of water on the opposite side, fluorescent intensity would increase. This data was plotted against time and used to model flow for the three tested diameters. The results indicated that the total time for diffusion increased as the diameters decreased. However, the relationship between the number of exposed pores and the flow time were not directly related, meaning the amount of flow through one pore changes with the total number of exposed pores. Testing was also conducted regarding the development of a flow device designed with two distinct flow inlets to the CNT array. This was done to determine the future feasibility of administering two distinct solutions into groups of cells. It was concluded that these devices could successfully be used for that purpose.
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NONMENCLATURE

CNT = Carbon Nanotubes

SWNT = Single Wall Nanotube

MWNT = Multi-Wall Nanotubes

TB-CVD = Template-Based Chemical Vapor Deposition

Re = Reynolds Number

Kn = Knudsen Number

ρ = Density

μ = Viscosity

AAO = Anodisc Aluminum Oxide

CO = Carbon monoxide

Fe(CO)_5 = Iron Carbonyl

AFM = Atomic Force Microscopy

SEM = Scanning Electron Microscopy

FDM = Fused Deposition Modeling

PLA = Polylactic Acid

DI = Deionized

ROI = Region of Interest

PBS = Phosphate Buffer Solution

PI = Propidium Iodide
1.0 INTRODUCTION

In recent years, CNTs have become an essential component of nanofluidic research. CNT devices have a variety of applications, the most prominent of which is nanofluidic transport. By design, CNTs possess properties that make them appealing candidates for biomedical and single cell applications. Significant research has been done utilizing CNTs in CNT-tipped devices and probes for use as intercellular delivery devices and sensors. While this research is significant, CNT-tipped devices only allow for the processing of single cells, and lack the ability to process cells in parallel. To overcome the limitations of single CNT-tipped probes, our lab created novel CNT arrays consisting of open ended, vertically aligned CNTs for parallel cell interfacing. Using these CNT arrays, it was shown that tens of thousands of cells could be injected simultaneously and the required injection time was dependent on the type of molecule being injected. In order to facilitate wide spread adoption of the technology, a comprehensive understanding of molecular transport through the CNT array is needed.

1.1 Motivation

In our lab CNT arrays are manufactured using an AAO template. CVD is used to deposit carbon into the pores of the membrane to create arrays of aligned CNTs with an amorphous carbon structure. The template is then removed using reactive ion etching to expose the CNTs. The resulting product is an array of aligned CNTs with an average diameter of 200 nm and an amorphous carbon structure. These arrays have a variety of different uses, mainly, cell injection. The CNT arrays can accomplish the same job as single CNT-tipped devices while injecting thousands of cells at a time, rather than just one. Research in our lab by Golshadi M. et al has demonstrated that cells can be successfully cultured on top of the CNT arrays at the same rate as standard tissue plates [1]. Cells were cultured for 48 hours on both a CNT array and a tissue culture plate of similar size. The number of living cells was then counted and proved to be similar among the two. Subsequent research was also conducted in our lab which demonstrated the viability of culturing cells on the CNT arrays for up to 96 hours.

Once it was proven that cells could be successfully cultured on the arrays, cell injection studies were enacted. Both tetramethylrhodamine (dextran) dye and DNA were tested and produced varied results. A schematic of the injection process is located in Figure 1. 10 µM of
dextran dye was diffused through the CNT array and into HEK293 cells which had been cultured on top of the CNT array for 48 hours. Using fluorescent microscopy, the amount of time for all the dye to diffuse through the CNT array was monitored and resulted in a final injection time of 16 min. Using the same setup which was used to diffuse dextran through the arrays, plasmid DNA was transferred into HEK293 cells cultured on the device, taking two hours to fully diffuse through the array. The viability of the cells was then quantified with a resulting 84% of the cells alive and viable. This research proves that the CNT arrays can successfully and efficiently be used for cell injection without damaging the cells, however, how material flows through the arrays is understudied. The reasons dextran diffuses into the cells in 16 minutes, while DNA takes two hours, are currently unknown. As a result, the vast range of applications for which the device can be used cannot yet be classified.

1.1.1 Flow through Carbon Nanotubes

Typically, there are two categories of nanotubes – SWNT and MWNT. SWNTs are formed from a single layer of carbon and MWNTs are formed from multiple layers of carbon [2]. Current bulk manufacturing methods for these nanotubes tend to produce batches of individual CNTs which result in large inconsistencies in diameter and length. In order to form them into arrays significant post production processing is required to align the CNTs onto a substrate. Our lab has developed a method of CNT array production which produces dimensionally consistent arrays of CNTs capable of sensing electrical signals as well as nanofluidic transport. However, the CNTs which are produced in our lab do not fit into either of the previously mentioned categories. Instead of being formed from layers of carbon, they are created using TB-CVD, producing arrays of amorphous carbon shaped as nanotubes. This differs dramatically from the fullerene structure commonly used in CNTs. However, they can still be used for nanofluidic
transport. Flow through CNTs has been researched in the past. A study by Mujumdar, M. et al analyzed pressure driven flow through an array of MWNTs with an average diameter of 7 nm [3]. Another study done by Whitby, M. et al studied pressure driven flow through arrays of amorphous CNTs with an average diameter of 45 nm [4]. While both these studies yielded results regarding flow, the produced results pertained to slip length, and the overall volumetric flow rate of solution through the CNTs. In addition, no information was gathered regarding the behavior of the flow through the CNTs, or how changing the number of CNTs being testing would affect flow. While the information Whitby et al. and Majumder et al. gathered is valuable, solution was flown through their CNTs using pressure driven flow. Therefore, these results cannot be applied to characterize flow through the CNT arrays produced in our lab due to flow being driven by diffusion, and not an external force. Another factor, is the difference in structure and diameter between the CNTs. The CNTs tested by Majumder et al. were MWNTs with a fullerene structure, which differs dramatically from the amorphous carbon structure of the CNT arrays produced in our lab. The CNTs tested by Whitby et al. did have a fullerene structure, however the CNTs only had a diameter of 45 nm, which is significantly smaller than the 200 nm diameter possessed by our arrays. As a result, a flow study to classify flow through our arrays of CNTs was designed.

1.1.2 Summary

While our lab has created a method for the large-scale manufacturing of dimensionally consistent arrays of CNTs, the fluidic transport properties of the resulting amorphous carbon CNTs are understudied and currently not well understood. Previous research regarding flow through our CNT arrays produced varied results which cannot currently be explained. In order to utilize these arrays for nanofluidic transport, more research must be done to uncover the specifics of the fluid flow. To begin characterizing the flow, experiments have been developed to characterize the impact the number of exposed CNTs has on the behavior of the flow through the arrays, as well as the plausibility of creating multiple, distinct inlets for flow. Once flow is better understood, the arrays’ applications can be further identified.

1.2 Research Goals

Macromolecules, nanoparticles, and genetic material can be delivered with high efficiency into tens of thousands of cells simultaneously through CNT arrays. Little is
understood about how the dimensions of the CNTs and CNT arrays and how or if the flow changes as a function of time, or by the type, size, and concentration of the injectable. Through this study, a set-up will be developed to capture flow behavior through the CNT arrays in real time. A characterization method to compare flow behavior for each experimental trial will also be developed.

1. Flow Observation

In order to observe flow using a set-up similar to those used for cell injection. The membrane will be oriented in a manner that allows for optimal viewing under a microscope. To accomplish this, the membrane will be positioned such that it lies flat, with the top face of the membrane facing upwards. This allows for optimal viewing of the top face under the microscope, and for monitoring the injectable which flows through the CNTs. In order for the injectable to flow through the membrane an apparatus will have to be developed which allows for the injectable to be placed underneath the CNT array, and to ensure that the injectable underneath the array does not mix with the injectable which has flown through the array.

2. Flow Quantification

Flow through the CNT arrays cannot be assumed to be constant. Classifying flow through the array as an average flow rate over the injection period does not allow for the observation of the flow shaped by the fundamentals of diffusion. In order to observe these changes, flow will be observed in real time using fluorescence microscopy. To view the flow, an optical microscope will be used to view the top surface of the membrane. The injectable flowing through the membrane will be observed using a method of viewing, and quantifying the amount of fluorescent injectable which has flown through the array. Once the injectable is placed under the array the amount of the injectable material which has flown through the CNT arrays and gathered on top of the array will increase over time until equilibrium is reached. By capturing this changing intensity data and plotting it against time, trends which occur within the flow can be observed and analyzed.
1.3 Literature Review

1.3.1 Carbon Nanotubes

Since the 1990s, carbon nanotube research has been gaining in popularity. This is due to the variety of ways that CNTs can be utilized, especially in the field of nanofluidics. CNTs, which are microscale cylinders commonly formed by rolling planar sections of hexagonal graphite lattice, historically have fallen into two categories SWNT and MWNT [2]. SWNT have an average diameter of 1-2 nm, while the diameters of MWNT can range anywhere from 2-23 nm. In recent years, another category of nanotubes have appeared which are called amorphous carbon nanotubes. Amorphous carbon is a carbon which has no crystal structure; and as a result, the tubes produced do not have the same mechanical and material properties as the fullerene structures of SWNT and MWNT [5].

CNTs are typically manufactured through four main synthesis techniques: electric arc discharge [6], laser ablation [7], high pressure carbon monoxide disproportionation [8], and CVD [9-11]. Each technique offers its own advantages and produces nanotubes specific to that technique.

Previously, the electric arc technique was only capable of producing small batches of MWNT; however, because of the work of C. Journet et al, the same technique can now be used to produce large quantities of SWNT bundles [6]. Journet’s method employed an arc generated between two electrodes in a reactor under a helium atmosphere. Both the cathode and anode were graphite rods, with the anode having a cavity down the length filled with a mixture of metallic catalyst and graphite powder. This produced fullerene SWNT with an average diameter of 1.4 nm. Electric arc discharge is capable of producing both fullerene structured MWNT and SWNT bundles, however, it offers limited dimensional control over the produced CNTs.

Laser ablation, which entails synthesizing SWNT through vaporizing a mix of carbon and transition metals by laser impinging onto a metal-graphite substrate, is a continuous process which produces SWNTs with a fullerene structure about 1-2 nm in diameter. Laser ablation allows for increased control over growth conditions when compared to the electric arc technique, but can only be used to synthesize SWNT [7].
High pressure carbon monoxide disproportionation, similar to the electric arc and laser ablation techniques, produces large quantities of SWNT. However, unlike the electric arc and laser ablation techniques, high pressure carbon monoxide disproportionation can be used to yield larger quantities of SWNT [8]. This was done by M. J. Bronikowski through the use of CO and Fe(CO)$_5$ [8]. CO mixed with a small amount of Fe(CO)$_5$ is flown through a heated reactor causing thermal decomposition producing gaseous iron cluster which serve as nuclei for the SWNT to grow.

Of all the techniques, CVD is the most economical and simple to regulate, making it an attractive option for both research and industrial applications [9]. CVD has the ability to produce several types of CNTs and is not limited to a single substrate. Various CVD methods can be employed to produce CNTs, including thermally activated CVD [12], plasma enhanced CVD [13], and fluidized-bed CVD [14]. Multiple studies have been conducted utilizing different CVD methods which have successfully produced SWNT, MWNT, and amorphous CNTs [12, 13, 15]. Amorphous carbon is a carbon which has no crystal structure; as a result, the tubes produced do not have the same mechanical and material properties as SWNT and MWNT with a fullerene structure [5]. Template-based manufacturing, an additional CVD process which utilizes thermal decomposition, is another popular CNT fabrication method. Template-based manufacturing utilizes a carbon-carrying precursor gas subjected to high temperature resulting in thermal decomposition [9, 10, 15, 16]. This produces amorphous CNTs and is unique in its ability to offer dimensional control over both length, outer diameter, and wall thickness through the choice of template and synthesis time [10].

The CNTs manufactured here are made from amorphous carbon, which has dramatic structural differences from the fullerenes in traditional SWNT and MWNT. However, they can still be used in nanofluidic transport. Research from M.G.Schrlau et al. utilized carbon nanopipettes for the injection of reagents into cells [17]. The carbon nanopipettes were fabricated through CVD on the inside of quartz pipettes, which were then wet etched to expose the carbon tip. The resulting nanopipettes had an amorphous carbon structure similar to that found in the arrays described above. This demonstrates that while amorphous CNTs have a different structure from their fullerene counterparts, they are capable of performing similar tasks while maintaining the vitality of the cell.
1.3.2 CNT Devices

There are two chief forms of CNT devices, CNT-tipped probes with a single or multiple nanotubes, and arrays of nanotubes. These devices utilize SWNT, MWNT, and amorphous nanotubes.

1.3.2.1 Single-Tipped Devices

CNT-tipped devices have multiple uses. Research has been conducted using SWNT and MWNT probes to deliver chemicals and other materials directly into cells, as well as utilizing them for atomic force microscope probes as tools for high-resolution scanning of nanostructures [18-20].

Wong, S. S. utilized both SWNT and MWNT tips for biological imaging [19] . MWNT and SWNT were attached to the ends of single-crystal cantilever tip assemblies in Figure 2a. These probes were then used to for AFM image amyloid-β 1-40, which is an amino acid involved in Alzheimer’s disease producing images like those in Figure 2b. The images which were obtained from the CNT probes had a higher resolution than those from silicon tips.

Research has also been conducted using amorphous CNT probes. M. G. Schrlau utilized carbon nanopipettes for the injection of reagents into cells [17]. The carbon nanopipettes were fabricated through CVD on the inside of quartz pipettes, which were then wet etched to expose the carbon tip, the process is diagramed in Figure 3. The resulting nanopipettes had an amorphous carbon structure similar to that found in the arrays described above. This demonstrates that while amorphous CNTs have a different structure from their fullerene

Figure 2: A) SEM image of MWNT tip attached to a silicon cantilever assembly B) AFM image of amyloid-β 1-40 produced by CNT probes [17].
counterparts, they are capable of performing similar tasks while maintaining the vitality of the cell.

While important research has gone into the various applications of CNT-tipped probes, and their value in nanofluidic studies cannot be disputed, progress has been limited. While CNT-tipped probes can work with single cells that is all they can do, making any task involving multiple cells a long and tedious process [21]. CNT arrays have the ability to process multiple cells concurrently, while achieving the same results as CNT-tipped devices.

1.3.2.2 Array Devices

The applications of CNT arrays have not been as fully explored as their CNT-tipped probe counterparts. Arrays consist of aligned CNT on a substrate or embedded in a membrane. An array of CNT on a Cr-coated Si substrate can be seen in Figure 4a [22].

To date, their uses have been limited to straightforward electrical measurements, electrodes, and some biosensors. In his work, J. Justin Gooding used arrays for protein electrochemistry by taking advantage of the electrocatalytic properties of the nanotubes. By aligning them normal to an electrode the CNTs will act as molecular wires allowing electrical communication between the electrodes and the redox proteins covalently which are attached to the ends of the SWNTs a schematic of this is in Figure 4b [23].

Arrays of MWNT on platinum substrate were utilized by Sofia S. et al. to develop amperometric biosensors [24]. The opening at the end of CNTs allowed for immobilization of the glucose oxidase, while the platinum provided the direct transduction necessary for signal monitoring. An image is located in Figure 4c.

The limited use of CNT arrays is due, in part, to the complex nature of creating arrays from SWNTs and MWNTs. While the manufacturing methods mentioned above can produce
SWNT and MWNT, forming them into arrays involves a substantial amount of post-production processing. This post-production work is due to the fact that the majority of synthesis techniques producing bundles of CNTs result from the lateral adhesion of the exposed CNTs. In order to arrange them into arrays, they need to be separated and ordered into aligned arrays [22, 25, 26]. While significant research has been done in this field, production remains a time consuming process [27]. In addition, current production methods used to create the tubes often results in inconsistencies in diameter and length among the produced nanotubes [28, 29]. In other words, the arrays which are produced are labor intensive, and often contain inconsistent dimensions among the CNTs.

1.3.3 Amorphous Carbon CNT arrays

A process developed by Golshadi and Schrlau to produce arrays of CNTs addresses the issues noted above, and yields arrays with both diameter and length consistency, while requiring minimal post-production processing [9, 10]. This method yields arrays of amorphous carbon nanotubes through template-based synthesis. Little has been done with arrays of amorphous CNTs, but some research has been done utilizing them in Lithium Ion batteries [30]. Reddy, A. L. M. utilized CNT array electrodes for use in Lithium batteries. Coaxial manganese oxide and CNT arrays were used as cathodes in a lithium battery, due to their increased cyclic stability, this can be seen in Figure 4d [30].

To manufacture the amorphous CNT arrays, template-based manufacturing is utilized. The use of a template offers several advantages over other array production methods. The

![Figure 4: A) SEM images of CNT array scale bars of 10 µm, 5µm and 0.5µm [20], B) CNT array used in electrochemistry of proteins [21] C) Amperometric biosensor from array of MWNT on platinum substrate, D) Lithium Ion battery from CNT arrays of amorphous carbon [30].](image)
template allows for increased dimensional control of both the length and wall thickness through template selection and total synthesis time. The template also provides structural stability to the array of CNTs and eliminates the need for a nanoassembly. This results in a manufacturing method that can be used to produce nanofluidic devices with multiple applications.

The manufacturing method utilized here results in vertical arrays of CNTs through which fluids can flow. The templates used are AAO membranes, which are riddled with pores going through the length of the membrane. These pores serve as the template for the CNTs. CVD is employed to deposit carbon into the pores of the membranes creating the CNT arrays, a schematic of the process is located in Figure 5 [9].

The initial stage of the membrane manufacturing process is annealing the AAO membranes to prevent them from curling during the CVD process. The membranes are placed between two quartz plates located in Figure 6 and annealed in the furnace which can be seen in Figure 6 for 4 hours at 730°C.

The annealed membranes are then placed upright into a quartz boat in Figure 8C and placed in the middle of the furnace’s reactor tube. Argon gas is flown over the membranes at 100 sccm while the temperature of the furnace steadily rises to 705°C. Once the temperature has stabilized, the Argon gas is turned off and a 30% Ethylene/Helium mixture is flown inside the reactor tube at 60 sccm for 5 hours. As a result of this process, the carbon is deposited into the

![Figure 5: Schematic of AAO membranes in template-based synthesis of CNT arrays [9].](image)

![Figure 6: A) Schematic of AAO membranes between quartz plates, B) Image of AAO membranes between quartz plates.](image)
pores of the membrane via CVD. After the 5 hours, the Ethylene/Helium is stopped, and the temperature is turned down to 100°C. The Argon gas is then turned back on and flown over the membranes at the previous speed of 100 sccm. A plot of temperature vs. time for the CVD process is located in Figure 7. Once the furnace has cooled, the Argon gas is shut off and the membranes are removed. The resulting CNTs have an average outer diameter of 200 nm and wall thickness of 25 nm.

1.4 Flow Through CNTs

Considerable research has been done to characterize fluid flow through SWNT, and MWNT. Much of this research encourages the fluid to flow through the membranes by using either an external pressure to create a pressure gradient or through electrical charge [3, 4, 31]. This forces the fluid to flow through the membranes quickly, and flow is then classified through flow rates and the changes which result from altering the diameter of the tubes. In a study by Majumder, M., pressure driven flow through an array of aligned MWNT with an average diameter of 7 nm was examined [3].
The results from the study are located in Table 1. Water, ethanol, iso-propanol, Hexane, and Decane were driven through the array to determine flow rates of different solutions. The study yielded the results in Figure 7 [3]. The results indicated an observed flow of 25 cm/s for water, and no change in flow based on the viscosity of the fluid. This is verified by comparing the observed velocities for water, and hexane, which have two different viscosities, yet very similar flow velocities [3]. This was inferred to have occurred as a result of the reaction between the hydrophobic walls of the CNTs and the water molecules leading to very low friction.

Flow through arrays of amorphous CNTs is understudied, however research conducted by Whitby, M considered the behavior of pressure driven flow through arrays of amorphous CNTs with an inner diameters of 45nm [4]. Water, Decane, and Ethanol were driven through the membrane using a 7.1kPa pressure. Fluid was driven through the arrays using a glass syringe pump with a 50 ml capacity. The syringe pump was set to a fixed flow rate, and the steady state pressure at the given flow rate was measured. This pressure was used in the Hagen-Poiseuile flow equation to obtain the volumetric flow rate through the CNTs. The study found that water, and ethanol had an average flow rate similar to that found by Majumder, M. However, Decane, which had a slower flow velocity in the Majumder, M study was found to have a faster flow velocity. It was concluded that the effect of the frictionless CNTs which results from the hydrophobic walls of the CNTs, reacting with the water molecules was not applicable on this larger scale [4].

While all the research from Majumder, M, and Whitby, M is significant in the field of nanofluidics it cannot be applied to the amorphous CNT arrays. In the work done by Majumder, M, this is due in part to their dramatic structural differences, which prevent conclusive studies involving fullerene structured CNTs from being applied to amorphous CNTs. While the Whitby, M study utilized amorphous CNT, the average diameter of the CNTs was 45nm, which is

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Initial permeability</th>
<th>Observed flow velocity</th>
<th>Expected flow velocity</th>
<th>Slip length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.58</td>
<td>25</td>
<td>0.00057</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>43.9</td>
<td>0.00057</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>9.5</td>
<td>0.00015</td>
<td>39</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.35</td>
<td>4.5</td>
<td>0.00014</td>
<td>28</td>
</tr>
<tr>
<td>iso-Propanol</td>
<td>0.088</td>
<td>1.12</td>
<td>0.000077</td>
<td>13</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.44</td>
<td>5.6</td>
<td>0.00052</td>
<td>9.5</td>
</tr>
<tr>
<td>Decane</td>
<td>0.053</td>
<td>0.67</td>
<td>0.000017</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Units, cm$^2$/per cm$^2$. † Velocities in cm/s.
significantly smaller than the average 200 nm in our arrays. In both cases, the lack of an external force is also a significant difference between prior flow studies and the study which will be conducted regarding flow through our CNT arrays. In our study, fluid is transported through our amorphous CNT arrays using diffusion and does not require any external forces. In order to observe flow through the CNT arrays manufactured in our lab, fluorescent dye is injected under the array. This solution then diffuses through the array into a volume of DI water placed on the top face. As the dye diffuses through the membrane, the fluorescent intensity in the DI will increase, providing a method of flow observation. In order to ensure accurate transient data, the DI water has to form a uniform cross section, to ensure the concentration of the dye is equal throughout the volume. As a result of the vast differences between this observation method and the others described, the outcomes of studies employing external forces cannot be assumed true for our amorphous CNT arrays.

1.4.1 Flow Through CNT Arrays Used in Cell Injection

Currently, an effective method for array fabrication exists, however the specifics of how materials flow through the membrane are unknown. What is known about the flow is that it can be modeled using continuum mechanics. It should be noted that for flow to be modeled as continuum, it must meet three main criteria; the fluid is continuously distributed, the fluid fills the entire space, and the fluid can be divided into infinitesimal elements with the same properties of the bulk material. For the aqueous solutions that will be used to understand flow through the membranes, all of this holds true. This can be proved through the use of the Knudsen number, which in a non-dimensional value representing the number of times a particle will travel its own diameter before hitting another particle [32]. If the Knudsen number is less than 0.1, then flow can be qualified as within the range for continuum mechanics.

\[
Kn = \frac{\text{mean free path}}{\text{Length}} = \frac{\lambda}{L}
\]  

(1.1)

If the fluid modeled is assumed to be water, which is similar to the solutions that will be used \( \lambda \) will equal an average of 0.25nm [32]. The CNT will assumed to be the CNT which are manufactured in our lab resulting in a length of 200nm. The Knudsen number for a single CNT in the array is the following:

\[
Kn = \frac{0.25\text{nm}}{200\text{nm}} = 0.001
\]  

(1.2)
This results in a Knudsen number of 0.001, which is significantly less than 0.1 putting the flow in the continuum regime.

Another question that arises is whether the flow is laminar or turbulent. Due to the small distance, and slow speed the fluid travels through the CNTs it can be observed that the flow is laminar, but to ensure this as true the Reynolds number was calculated [32].

$$Re = \frac{Density \times Velocity \times Diameter}{Viscosity} = \frac{\rho V D}{\mu}$$  \hspace{1cm} (1.3)

Keeping with the assumption the fluid is water at 25°C and estimating a velocity based off of the work of Mujumder et al. results in the following [32]:

$$Re = \frac{997 \text{ kg/m}^3 \times 25 \text{ cm/s} \times 200 \text{ nm}}{8.9 \times 10^{-4} \text{ Pa \cdot s}} = 0.056$$ \hspace{1cm} (1.4)

For flow to be classified as laminar the Reynolds number must be lower than 2,000 [32]. For the flow through the CNT arrays the Reynolds number is 0.056, which is well below 2,000 making the flow laminar. Any slight changes in the values will not result in drastic changes to the Reynolds number keeping the flow well in the laminar range.

By increasing the number of exposed CNTs within the CNT arrays, the flow rate is expected to increase proportionally to that value. By opening up more CNTs the solution has more pathways to flow through, resulting in an overall faster rate of diffusion through the CNT array. For this study, the effects of changing the number of exposed CNTs will be explored. While it is predicted that the rate of diffusion will decrease when more CNTs are exposed, this is not known for certain.
2.0 DEVELOPMENT OF A SETUP TO OBSERVE FLOW

2.1 Introduction

Significant work has been done to develop the array manufacturing method. However, little has been done to observe the behavior of injectable material through the arrays. While some work has been done, it utilizes pressure driven systems which obtain values of flow rate through measured pressure gradients. In order to observe the flow without an external force, a setup has to be constructed which will allow for the membrane to sit slightly above the injectable material to ensure no material passes through the CNTs before data recording has begun. By placing the solution underneath the array, and observing the top face, the membrane will be under conditions similar to those used during cell transfection. The device will also need to form the fluid that flows through the array into a uniform cross section to ensure accurate transient results. After material flows through the membrane, it forms a “dome” on top of the membrane due to surface tension. This results in inconsistencies in the transient data due to the lack of a uniform cross section. In order to fix this, the setup will incorporate a method of forming a uniform cross section.

2.2 Design

2.2.1 Preliminary Flow Imaging Fixture

To inject material underneath the arrays to capture the entire flow period, the apparatus used for flow studies needed to be situated such that there is space underneath the CNT arrays for the injectable, isolate an active area of the membrane, and create a uniform cross section. A well for solution also needs to be created on top of the membrane. In order for solution to flow through the CNT arrays, diffusion needs to occur. Therefore, DI water, or a different solution needs to be placed on top of the membrane providing a means for diffusion for the injectable material. In order to take transient results, the solution placed on top of the membrane needs to form a uniform cross section.

In order to position the array such that solution could be positioned underneath, a single channel was designed where the width of the channel is smaller than that of the array. This allows for the array to sit above the channel, and for solution to be positioned underneath the array using a syringe while concurrently monitoring the top surface of the array. The channel was prototyped utilizing glass slides, and can be seen in Figure 9. It consisted of three glass
slides which had been glued to a fourth slide to form a channel for the injectable material to be placed.

In order to generate a uniform cross section for the material on top of the arrays a second “step” was added to the apparatus in Figure 9. A uniform cross section is needed to ensure that when fluorescent dye diffuses through the CNT array, into the DI water on top, the concentration through the DI water is consistent. This was done by attaching two more glass slides to the sides of the well, creating a gap. The width of this gap is larger than the arrays but small enough that a standard 13 mm X 13 mm cover glass could be placed over it. When DI water, or another solution is placed on top of the CNT array a cover slip can be placed onto the second step, and over the drop, flattening it; creating a uniform cross section. This can be seen in Figure 9c.

When running preliminary flow tests with the device, it was discerned that an area of the CNT would need to be isolated from the array as a whole to allow for testing of the effects of changing the exposed CNT area. In order to isolate the active area from the rest of the array, a well has to be made in order to hold solution on top of the array. This requires that the well have a liquid tight seal with the array to ensure no leakage occurs. Initially the active area was separated utilizing a 4 mm diameter metal washer. The washer was glued onto the array using superglue. This provided a well for the solution to sit on top of the membrane, as well as
containing the active area. An image can be seen in Figure 10. During preliminary testing, it was determined that this method did not work as well as expected. The CNT arrays are extremely fragile, and gluing the washer to them would often result in breakage. It also resulted in issues of obtaining a consistent active area. When gluing on the washer, glue was first applied around the opening, on the underside of the washer. The washer is then attached to the membrane producing a membrane with an active area equal to that of the washer’s inner open area. However, that is not completely accurate. Instead of the active area being the inner open area of the washer, it is the area formed by the glue, causing discrepancies in the active area between experiments. Methods of applying the glue to obtain a more consistent active area among experiments were considered, such as a micromanipulator. However, this did not solve the issue of the membrane fragility.

2.2.2 Finalized Flow Imaging Fixture

While initial prototyping, and testing was done utilizing glass slides due to their availability and economical cost, this did not provide a permanent solution. In the future this setup will be employing biological materials, such as DNA. In order to avoid contamination between trials, the flow channel would need to be thoroughly cleaned, and inspected for traces of biological material after every trial. While this is possible, it would prove tedious, and provide no guarantee that contamination is not occurring. In order to obtain the most accurate results possible, and eliminate the concern of sample contamination, a single use, disposable channel was developed utilizing 3D printing. For these purposes, 3D printing provides an inexpensive method of device fabrication, making it feasible for them to be disposable. 3D printing is the ideal platform for creating multiple copies of the same channel design with minimal variation.
In order to print the channels a CAD model was developed, a dimensioned version can be seen in Figure 11a. The channels were printed using a FDM printer with PLA. PLA was used due to its wide availability, and inexpensive cost. The channels are not subjected to any stress, and therefore do not require the strength, or flexibility of other printer materials. The printer used was a Flashforge Creator Pro. The channels were printed using 15% fill density, a 60 mm/s print speed, and can be seen in Figure 11b.

![Figure 11: A) CAD model of flow channel, all dimensions shown in millimeters, B) 3D printed flow channel](image)

In order to create a consistent active area that was water tight, and provide more structural support to the membrane 3D printing was employed again. This portion of the flow devices is referred to as the outlet cover. The outlet covers were printed on top of the membranes, and were designed with a hole in the middle of varying diameters, exposing the membrane. The outlet covers also incorporated a ledge for a 13 mm x 13 mm coverslip to sit. This provides a method of forming a uniform cross section from the DI water placed on the top face of the CNT array. To minimizes contaminants being introduced into the top well, placing coverslip onto the step creates a semi closed system. The introduction of a second step which rises above the top of the cover slip, creates a seal when the cover slip is in place. This procedure minimizes the risk of contaminants entering the system, and limits the effects of evaporation. If the system were left open, water from the top well would evaporate, skewing the flow results. In order to remove the cover slip after testing is completed, and slot was placed into the edge of the membrane, allowing for a pair of tweezers to get underneath the coverslip, and pull it away. A Printrbot Simple Metal was used to manufacture all the 3D printed devices containing an AAO
membrane or CNT array. The devices were all designed using Solidworks and then exported as STL files. These STL files were then loaded into a slicing program, Cura 15.04.5 by Ultimaker. Devices were printed using 1.75mm PLA from Hatchbox or Printrbot. The nozzle temperature was at 210°C and the bed temperature was at 40°C for all prints. No support or adhesion techniques were necessary. The print layer height was also held constant at 0.15mm for all prints. In order to print onto the membranes, due to their fragile nature, the fill density of the device was set to 100% and the print speed was set to 30mm/s. The increased fill density ensured that the printer nozzle made fewer quick, or jolting motions while printing, decreasing the chance of the membrane cracking. The final produced design can be seen in Figure 12.

2.2.3 Completed Flow Set-up

Assembling the channel and the membrane coupons, to create a single flow setup was done utilizing super glue. A small amount was placed on the outer edges of the channel. The outlet cover was then placed on top of the channel and allowed to dry. Placing the glue on the outer edges of the channel ensured that, when the glue spread out after the coupon was placed on top, no glue would come into contact with the membrane. This would result in a change in the active area leading to discrepancies among the experimental trials. To ensure the membrane outlet cover would be placed in the same place relative to the channel for each device, the channel, and outlet cover were designed to have the same width. Aligning the edges of the coupon to be flush with those of the channel ensured the same placement for each device. A CAD model, and image of the completed flow setup can be seen in Figure 13.

To conduct flow tests utilizing the devices a volume of DI water is placed into the top well (V2) which was then covered with a glass cover slip to produce a consistent cross section in order to assure the collection of accurate transient data. The solution to be tested is then injected into the solution channel (V1) and allowed to diffuse through the membrane into V2. This
process is monitored, and recorded to observe trends in the flow. During testing, no leakage was observed at the seam between the channel, and the outlet cover.

### 2.3 Feasibility of 3D printing on CNT arrays

A major concern regarding 3D printing on the CNT arrayas involved the possibility that the 3D printed material would clog the CNTs. Clogging of the CNTs would result in a loss of the CNT array’s transfection potential. In order to ensure a 3D printing fabrication technique would be a viable option when interfaced with CNT arrays, a CNT array was created using the same technique to create CNTs as outlined by Golshadi et al [9]. The produced CNT array had tubes which were roughly 200 nm in outer diameter protruding 180 nm from the surface. The device was then printed on the array using the techniques, and parameters outlined above. After manufacturing, an SEM was utilized to examine the CNTs. The area of most concern regarding PLA clogging the CNTs was where the PLA met the CNT array. This is due to it being the most likely location for obstruction due to manufacturing to occur. As can be seen in Figure 14 this...
was not the case. Even the CNTs that were very close to the edge of the PLA were not obstructed. Thus, the transfection potential of the CNT array would likely not be altered.

A secondary concern regarding the 3D printing fabrication method was the retraction of the PLA after printing. Once the PLA is printed onto the CNT array it adheres, however while the PLA cools it retracts a small amount resulting in the possibility of micro-cracking in the CNT array. However, the SEM images in Figure 14 show no signs of cracks within the CNT arrays therefore preserving the viability of the integration of CNT arrays with 3D printed manufacturing.

![SEM Images of PLA interface with CNT array. A) 5 µm scale bar, and B) 2 µm scale bar.](image)

Figure 14: SEM Images of PLA interface with CNT array. A) 5 µm scale bar, and B) 2 µm scale bar.
3.0 FLOW CHARACTERIZATION

3.1 Introduction

In order to understand and accurately characterize flow through the CNT arrays, a flow characterization method had to be developed. To obtain results regarding trends which occur in the flow through the CNT arrays, flow was monitored in real time. To capture transient results, the injectable solution utilized needed to demonstrate a change over time. To monitor flow, the fluorescent dye, Dextran was utilized as the injectable solution. As the concentration of the fluorescent dye increases the brightness will increase. As a result, as more dye diffuses through the CNT array and into the solution on top, the concentration of dye will increase resulting in an increase in fluorescent intensity. By capturing this intensity data and plotting it against time, trends which occur within the flow can be observed and analyzed. and a greater amount of space between data points. Because of this, more interpolation would occur, negatively effecting the accuracy of the data.

3.2 Fluorescent Imaging

To capture the flow period, a Zeiss Scope.A1 upright microscope was used paired with an AxioCam MRm pictures of which can be seen in Figure 15. An upright microscope was utilized to guarantee capture of the entire flow period. In order to ensure the most accurate data, the camera was set to take pictures every 0.5 s for the duration of the experimental trial. This is the smallest time increment capable of this setup. To image fluorescent intensity, a fluorescent light was utilized with a Cy3 green-excitation filter. Once an experiment concluded the resulting image sequence was compiled utilizing ImageJ. In ImageJ, a rectangular region of interest (ROI) was set to encapsulate the fluorescent region of the images. This allowed for the maximum area possible to be captured within the ROI. In order to compute fluorescent intensity for each image, ImageJ calculated the intensity of each pixel, and averaged them together, providing a single intensity value. This accounts for the importance in the consistency of the ROI used. For each experiment, to keep the number of pixels consistent, and avoid errors in the data due inconsistencies in the number of pixels being averages the same ROI shape, and area was utilized for each experiment. Once the ROI was set, the Time Series Analyzer V3 Plugin within ImageJ was used to extract the average intensity from each image in the sequence utilizing the same ROI.
To extract intensity data from the image, the analyzed images needed to be grayscale images, containing only black, white and gray. Intensity values were calculated based upon the pixel values within the set ROI. The pixel value, is a single dimensionless value which represents the brightness of the pixel. In a grayscale image, the assigned pixel value is determined by how much white the pixel contains, or the white percentage. For an 8-bit image the pixel values range from 0 – 255, with 0 being pure black or 0% white, and 255 being 100% white. This method of quantifying image brightness is called 8-bit grayscale. An 8-bit grayscale value bar can be seen in Figure 16. Once the fluorescent dye was injected into the channel, dye begins to diffuse through the CNT array and into the top well containing DI water. As more dye diffused through the array, the dye concentration in the top well would increase, resulting in a brighter image. The intensity values for each image could then be plotted against time.
example intensity vs. time plot can be seen in Figure 17. Grayscale images can be obtained be either using a camera which can capture greyscale images, or by using a color camera and converting the images to greyscale. For these experiments the images were captured using a greyscale camera, eliminating the need to convert them. This eliminates an extra step, and ensures no brightness data is lost during the image conversion.

![8-bit grayscale value bar](image)

\[
\begin{array}{ccc}
0\% & 50\% & 100\% \\
0 & 127.5 & 255 \\
\end{array}
\]

*Figure 16: 8-bit grayscale value bar. The values above the image represent white percentage, while the values below represent pixel value.*

### 3.2.2 Exposure

In order to utilize 8-bit grayscale as an image analysis tool, the exposure value of the camera needs to be accounted for in order to assure accurate results. When conducting experiments utilizing fluorescence, the exposure time which should be used is dependent on the molarity of the dye in use. Exposure time, represents the total amount of light which is permitted to reach the camera's sensor when taking a photograph. A lower exposure time permits less light to go through, while a higher exposure time permits more. As a result, a higher molarity dye requires a lower exposure setting than a lower molarity dye. This is due to the higher concentration dye having a higher fluorescent intensity than a lower one. If the wrong exposure setting is used the resulting intensity values will not be accurate.

When setting the exposure time to begin a flow experiment, it should be set to saturate, or show 100% white at the maximum concentration of dye which will be used. This ensures that lower dye concentrations will produce an average pixel value that is between 0 and 255. Therefore, as the dye concentration increases within the top well of the flow device, the intensity
value will climb until diffusing through the membrane stops. However, if the exposure value is set too low such that the camera shows an image with a 255 pixel value at a dye concentration that is lower than the maximum concentration being used, the intensity data will be cut off. A plot of intensity vs. time for a flow experiment with an exposure setting that is too low can be seen in Figure 18. When compared to the plot is Figure 17 above, which shows a steady rise of intensity vs. time until reaching the saturation point. The plot in Figure 18 rises until about 120 s, where the intensity value abruptly reaches saturation. As a result, the data from this experimental trial cannot be used. This is due to the fact that dye concentration in the top well of the flow device, may have continued increase after the 120 s mark. However, this behavior cannot be seen, because the exposure setting was set lower than required for the maximum concentration of dye which could occur in the top well. As a result, every concentration past the saturation

Figure 17: 8-bit gray scale values plotted against time.

Figure 18: Intensity vs. Time Plot. Data collected using incorrect exposure setting.
point will only show the maximum value of 255, resulting in inaccurate data.

To ensure that the correcting exposure setting is used for each experiment, it needs to be measured every time. This is due to changes in the ambient light in the lab where the experiments were being conducted. Setting the exposure time, can only control how much light is allowed in, and as a result this value will change depending on the level of light in the room. Setting the exposure time to the same value every time, can only control how much light is allowed in, as a result the same exposure time can produce images of the same concentration dye with different fluorescent intensity values. This is a result of changing levels of ambient light. When intensity experiments are conducted, the lights in the room are shut off to ensure there is no interference from the fluorescent ceiling lights. In spite of this, the levels of light in the room are not always consistent; the time of day, light from computer screens, and desk lamps all have an effect on the level of light within the room. In order to block out as much ambient light as possible, a piece of UV impenetrable fabric was wrapped around the microscope stage. While this blocks out a significant portion of the light, it is not perfect. In order to account for changes due to exposure, a characterization method was developed which allowed for data from different experimental trials to be compared to one another regardless of exposure settings.

3.3 Increasing Exponential Decay

By analyzing the trend in the data in Figure 17 above, it was observed that the rising intensity values appeared to follow a trend similar to the increasing form of exponential decay. This is not the same as exponential growth. Exponential growth produces a curve that is concave, while the increasing form of exponential decay is convex. By trimming the collected data to eliminate the initial plateau before intensity begins to rise, and transforming it such that the first point has an intensity of zero. The equation for increasing exponential decay can be fit to the data using Equation 3.1. Where \( I(t) \) represents intensity, \( C \) is the maximum intensity, \( t \) is the time in seconds, and \( \tau \) represents the time constant, also with the units of seconds.

\[
I(t) = C \left(1 - e^{-\frac{t}{\tau}}\right)
\]  

(3.1)

The initial rise of the intensity data appears to follow exponential growth, this is due to the dye initially coming into contact with the DI water, and diffusing through the volume. Accounting for this behavior would result in the data following a second order equation with two
values for $\tau$, $\tau_1$, and $\tau_2$. Where $\tau_1$ is the dominant time constant for the portion of the function following the inverse of exponential decay, while $\tau_2$ is the secondary time constant for the initial portion of the function following exponential growth. For these experiments the amount of time required for the volume of DI water to become a homogeneous solution after the dye initially diffuses through the array, was not of importance, but rather the overall amount of time for the intensity to reach its maximum. Therefore, it was assumed to be negligible, and only the increasing exponential decay portion of the function was taken into account resulting in the use of Equation 3.1.

For each experimental trial that was conducted, a different amount of dextran diffused through the AAO membrane. This resulted in differing maximum intensity values for each trial. To make the trials directly comparable to one another, each trial was nondimensionalized using the maximum intensity value for each trial. Increasing exponential decay, has a horizontal asymptote, meaning it will never reach its maximum value. In the case of the intensity curves, this is not essentially true, they do reach their maximum intensity. To account for this during nondimensionalization, the data was nondimensionalized to 99% of the maximum, accounting for the asymptotic behavior of increasing exponential decay. The equation for this is in Equation 3.2 where $I(t)\ast$ is set to 99% of the maximum intensity.

$$I\ast = 1 - e^{-t/\tau}$$

(3.2)

By nondimensionalizing to 99% of the maximum intensity rather, than just the maximum intensity value warrants that the function follows the proper form of exponential decay. As a result, the nondimensionalized function has an asymptote at $I(t)\ast = 1$, meaning it approaches, but never reaches it, properly mimicking the behavior of increasing exponential decay. By fitting the data to increasing exponential growth, a value for $\tau$ can be determined, and used to characterize the flow. $\tau$ represents the time constant which signifies the speed at which the system can respond to change. As the value of $\tau$ increases, the amount of time required for the system to reach maximum intensity increases. Nondimensionalization, does not have an effect on the overall behavior of the intensity vs. time plots, resulting in no change to the value for $\tau$ for each trial. Nondimensionalizing allowed every trial to span the same range of intensity values, making them easily comparable regardless of maximum intensity. Transforming the data in Figure 17,
nondimensionalizing it, and fitting an increasing exponential decay curve results in Equation 3.3. Where $I^*$ is the nondimensionalized form of increasing exponential decay.

$$I^* = 1 - e^{-\frac{t}{72.02}}$$  \hspace{1cm} (3.3)

Looking at Equation 3.3, it can be seen that the curve fit yielded a value for $\tau$ of 72.02 s. A plot of the transformed data compared to the increasing exponential decay curve can be seen in Figure 19. While fitting the experimental trials to increasing exponential decay does not provide a perfect fit, it provides a good reference for rise time. To calculate the overall correlation between the gathered data, and the curve fit, the coefficient of determination, $R^2$ was used. The Equation is located in 3.4 where $y_i$ are the collected intensity data values, $\hat{y}_i$ are the curve fit intensity values, $\bar{y}$ is the average of the collected intensity value.

$$R^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y})^2}$$  \hspace{1cm} (3.4)

This results in a value for $R^2 \leq 1$, where 1 represents a perfect match between the gathered data, and the curve fit. A negative value for $R^2$ means that a linear fit would produce a stronger relation than the one being used. Using the data in Figure 17 above to calculate $R^2$ produces a value of 0.949 which demonstrates that the curve fit does not produce a function identical to the collected data. As a result of this, a secondary method of calculating $\tau$ was also employed. The time constant, $\tau$ represents how long the function takes to reach 99% of its maximum intensity. This can be calculated by using Equation 3.2 to solve for

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Normalized_Data_Curve_Fit.png}
\caption{Intensity vs time plot with transformed data compared to exponential curve fit.}
\end{figure}
\[ \tau = \frac{t}{-\ln(1-t')} \]  

(3.5)

Due to \( \tau \) representing how long the function takes to reach 99% of its maximum intensity, \( I^* \) is equal to 99% resulting in Equation 3.6.

\[ \tau = \frac{t}{\ln(100)} \]  

(3.6)

Using this method to calculate \( \tau \), for the data in Figure 19 results in a value for \( \tau \) of 109.44, which is significantly larger than that achieved through the curve fitting method. Due to the method of mathematically calculating \( \tau \) providing an exact answer, rather than the close approximation gained from curve fitting, the calculated value will be used as the correct value. However, both methods were used to calculate \( \tau \) for each experimental trial.

Calculating \( \tau \) for each trial to obtain a time constant, provides a consistent method of identifying how long it takes for the trial to reach maximum intensity. By comparing these values, flow behavior can be compared over a variety of changing parameters independent of exposure settings.
4.0 FLOW ANALYSIS

4.1 Introduction

While many aspects of the behavior of flow through amorphous CNT arrays are misunderstood, little is known regarding the effect of the number of exposed CNTs on flow. Understanding the effect, the number of exposed CNTs has on flow, will provide valuable insight into the use of the CNT arrays for cell transfection. Characterizing flow through different numbers of CNTs served as a representation for varying numbers of cells being transfected on a CNT array. When cells attach to the surface of a CNT array, they come in contact with varying numbers of CNTs, resulting in a different number of “active” CNTs, or CNTs which are in use. Understanding the effects of different active areas will provide a level of understanding for the flow behavior which will occur when transfecting different numbers of cells.

4.2 Array Manufacturing

In order to conduct flow studies utilizing the CNT arrays, the arrays needed to be manufactured following the process developed by Golshadi et al [9]. The produced arrays were the standard arrays manufactured in the NBIL lab having an inner diameter of 200 nm after CVD. To ensure the procedure was being followed correctly SEM images were taken of an AAO membrane before and after the CVD process. Examining the AAO membrane in Figure 20a and Figure 20c and comparing it the membrane after CVD in Figure 20b and Figure 20d the membrane pores appear to have been coated with carbon, ensuring that the CVD process was being executed correctly. Once it was confirmed that the CVD process was being performed correctly, flow testing could be conducted utilizing the CNT arrays.

4.3 Flow Through CNT Arrays

Significant flow testing was done utilizing the CNT arrays in the single channel devices, however inconsistent results were obtained. It was determined, that due to the possibility of an unknown error occurring in the manufacturing process, the CNT arrays were not allowing for diffusion to occur.

4.3.1 Solution Testing

In order to provide the most accurate simulation of various solutions that may be transfected into cells using the CNT arrays, DI water was used as the aqueous solution to dilute
the dextran. In order to establish whether the lack of diffusion was caused by either the dextran or the DI water tests were run using different aqueous solutions, and different fluorescent markers. PBS, and ethanol were tested as the aqueous solution in addition to DI water, while Quantum dots, PI, and dextran were used as the fluorescent markers. Table 2 below contains the results of the study. Looking at the table shows that the results yielded diffusion, but only when ethanol was used as the aqueous solution. It was predicted that this was related to the highly hydrophilic properties of ethanol, compared to DI water, and PBS which have more hydrophobic behavior. The contact angles of ethanol and DI were taken on a CNT array using a Ramey Hart. The DI water produced an average contact

![Figure 20: SEM images of AAO membrane, 25µm scale bar A) before CVD, B) after CVD. SEM images of AAO membrane, 1µm scale bar C) before CVD, D) after CVD.](image)

<table>
<thead>
<tr>
<th>SOLUTION</th>
<th>DI Water</th>
<th>PBS</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEXTRAN</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>PI</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>QUANTUM DOTS</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 2: Flow study with different aqueous solutions and fluorescent markers utilized with CNT arrays. The "Yes" or "No" indicates whether diffusion occurred.
angle of 152.3°, a contact angle above 90° is considered hydrophobic. However, when an ethanol droplet was placed onto the array, it immediately spread out before contact angle data could be collected, making it extremely hydrophilic.

While ethanol could be used to diffuse through the CNT arrays, it would not serve as an appropriate substitute for cell transfection solutions, due to ethanol resulting in cell death upon injection. Therefore, further research was conducted into the reasons why DI water was not diffusing through the arrays.

**4.3.2 Induced Oxidation for Carbon Removal**

The initially hypothesis for why diffusion was not occurring was the possibility, that carbon deposited into the lattice layer of the membrane was blocking the tubes, and therefore blocking flow. In the AAO membranes used, the pores start at a consistent diameter, but within the last micro of the membrane, each pore spits into multiple pores, creating a lattice like structure. This is referred to as the lattice layer. SEM images and a schematic of this can be seen in Figure 21. While the pores start out at a diameter of around 200 nm, the pores within the lattice layer can get down to diameters as small as 1 nm. As a result, it was inferred that carbon deposited after the CVD process was blocking these pores.

To test this, removal of the carbon in the lattice layer was attempted. In order to complete the CVD process, carbon is deposited onto the membranes at a temperature of 705°C. After which, the system is flooded with argon, while the temperature drops. This is done to ensure that oxidation does not occur, reversing the CVD process. This process is explained in detail in Section 1.3.3. By exposing the system to oxygen, at a temperature above 400°C, oxidation will occur, and the freshly deposited carbon will be stripped from the AAO. This reaction was taken advantage of, and used in an attempt to remove a thin layer of carbon from the surface of the AAO. A batch of 7 annealed, AAO membranes were prematurely exposed to oxygen for 10 minutes at 450°C. Upon inspection, the CNT arrays did not have any noticeable difference in appearance when compared to the standard arrays. Single channel flow results revealed that no diffusion was occurring. A second set of 7 annealed, AAO membranes were exposed to oxygen at 600 °C for about 20 minutes, while the system cooled to 400°C, the resulting arrays had no carbon on the surface, and had been retuned back to their original state of bare AAO. A third batch of 7 annealed membranes were prematurely exposed to oxygen for 10 minutes at 600°C.
Flow testing again revealed that no diffusion was occurring. This demonstrated that while induced oxidation provided a method for carbon removal, it was difficult to control, and did not produce membranes that were susceptible to diffusion.

In a final effort to achieve diffusion using DI water, and a CNT array, an array which had undergone oxygen plasma was tested. Oxygen plasma is the method used to remove carbon from the lattice layer of the membrane in the manufacturing process developed by Golshadi et al [1]. Testing revealed that no diffusion was occurring, resulting in the conclusion that an unknown error was occurring during the manufacturing process.

Due to time constraints, it was decided that the flow studies would be done utilizing plain AAO membranes. While the results gained from AAO would not provide insight into the effects
of CNTs on the flow, it would still provide information regarding flow within the microfluidic spectrum.

4.4 Flow Testing Parameters

In order to use the CNT arrays for cell transfection, the effect of the number of exposed tubes on flow behavior was analyzed using different active areas of the AAO membranes. This was done through changing the diameter of the PLA opening on the outlet covers discussed above in Chapter 2. Three different diameters were tested, 6mm, 4mm, and 2mm. An image of the three devices, with the three separate diameters can be seen in Figure 22. Comparing the overall area of the exposed membranes, and dividing it by the pore density for the AAO membranes, $7.38 \times 10^8$ tubes/cm$^2$ provides a value for the number of exposed tubes for each PLA diameter. All the outlet covers used were printed on either a Printrbot Simple Metal, or a MonoPrice Maker Select 3D Printer v2, both of which have a resolution of 50 µm. This results in a variance in the area of the exposed membrane, and therefore the number of open pores. The results are located in Table 3. The diameter range tested was chosen due to the printer capabilities. As a result of the fragility of the AAO membranes, a diameter greater than 6 mm resulted in the membrane cracking from the force of the printer nozzle. The resolution of the printer prevented it from producing a clean print at a diameter lower than 2mm. A 2mm step between each diameter allowed for the change in the number of exposed nanotubes to be large enough to demonstrate the effect the difference in the number of exposed pores has on flow behavior.

![Image of flow devices with different diameters](image)

**Figure 22: Single channel flow devices with PLA diameters of 6 mm, 4 mm, and 2 mm from left to right.**

**Table 3: AAO active areas with corresponding number of open pores.**

<table>
<thead>
<tr>
<th>DIAMETER (mm)</th>
<th>AREA ± ε (mm$^2$)</th>
<th># OF PORES ± ε (E+7)</th>
<th>Δ # OF PORES (E+7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>28.27 ± 0.017</td>
<td>20.86 ± 0.013</td>
<td>11.59</td>
</tr>
<tr>
<td>4</td>
<td>12.57 ± 0.025</td>
<td>9.28 ± 0.018</td>
<td>6.96</td>
</tr>
<tr>
<td>2</td>
<td>3.14 ± 0.050</td>
<td>2.32 ± 0.037</td>
<td></td>
</tr>
</tbody>
</table>
4.5 Experimental Testing

As a result of using the AAO membranes, the microscope could not be placed such that it was looking directly over the membrane. Due to the translucent properties of the AAO the fluorescence from the Dextran could be viewed through the membrane. As a result, placing the microscope directly over the membrane resulted in the fluorescent intensity from the injected dye eclipsing any changes in the intensity of the DI water that may have been occurring. An image sequence illustrating this effect is Figure 23. To overcome this, the microscope was offset from the membrane, such that it was focused on the DI water that extended beyond the AAO, and onto the PLA. This allowed for changes in the intensity of the DI water due to diffusion to be observed. An image demonstrating the DI water extending beyond the AAO membrane, and the flow device setup under the microscope is located in Figure 24.

![Image sequence](image)

**Figure 23: Portion of image sequence illustrating the translucency of AAO.**

Flow studies were conducted utilizing the AAO membranes within the single channel devices. In order to create a uniform cross section of the DI water, 80 µL of DI water was placed into the top well, and a glass cover slide was placed on top. This quantity of DI water was chosen for the top volume because it allowed for water to extend onto the PLA, allowing for the microscope to view the DI water over the PLA, without the fluorescence of the injected dye being seen through the AAO. Once the DI water was placed on top, and the device was correctly positioned under the microscope, the image capture program was initiated. Then 20 µL of dextran in DI water was injected into the front opening of the channel using a 1 ml syringe. Images were taken every 0.5 seconds using the setup described in Chapter 3. The multidimensional acquisition tool in Axiovision was used to capture images in a 0.5 s time increment, for 15 minutes.
4.6 Exposure

In order to use the proper exposure setting the maximum concentration of dextran possible after diffusion was calculated. This was calculated by using the general dilution equation below, where \( M_i \) and \( V_i \) are the initial molarity and volume, and \( M_f \) and \( V_f \) are the final molarity and volume.

\[
M_i V_i = M_f V_f
\] (4.1)

Applying Equation 4.1 to the single channel flow setup, a schematic of which can be seen in Figure 25 results in the Equation below. Where \( M_1 \) and \( V_1 \) represent the concentration, and volume of the injected dye, \( V_2 \) represents the volume of DI water on the outlet cover, and \( M_2 \) represents the final molarity of diffusion.

\[
M_1 V_1 = M_2 (V_1 + V_2)
\] (4.2)

Solving this Equation for \( M_2 \) results in the following:

\[
M_2 = \left( \frac{M_1 V_1}{V_1 + V_2} \right)
\] (4.3)

Plugging in 2 \( \mu \)M for \( M_1 \), 100 \( \mu \)L for \( V_1 \) and 80 \( \mu \)L for \( V_2 \) results in a maximum value for \( M_2 \) of
1.11 µM. The exposure settings were set using a 1.25 µM dye to be certain that the results would not be cut off due to premature saturation.

4.7 Data Analysis

4.7.1 Flow Behavior

In order to analyze flow behavior, the image sequences from each experimental trial were compiled and analyzed using ImageJ. ImageJ was then used to extract intensity values from each image using the 8-bit greyscale method described in Section 3.2.1. For every image, the average intensity value of the entire image was calculated. Each image sequence contained a portion of bare PLA with no DI water, resulting in no intensity change occurring in that segment causing the average intensity value of the entire image to be lower than that of just the dye. Figure 26 demonstrates this. This did not interfere with the results, because the intensity values themselves were not of interest, but rather the growth of the intensity values. The average intensity of the entire image was taken, rather than just that of the portion with DI water, due to this area being different for each experiment. Using an ROI with the same area for every trial ensured that the same number of pixels were analyzed. Once the intensity values were obtained they were plotted against time. Flow was then characterized using the method described in Section 3.3.

4.8 Amount of Diffused Dye

While conducting flow experiments it was unclear how much of the injected dye was diffusing into the DI water. One way to quantify this is to use spectrophotometry, which can determine how much a chemical substance absorbs light over a certain
wavelength, the amount. This is done by shining a light through a fluorescent sample. The wavelength of the light must match or be close to the excitation wavelength of the sample. The spectrophotometer can then sense how much light was absorbed after passing through the sample. This value can then be used to determine the concentration of a known chemical substance. While spectrophotometry provides a concrete method of determining a solution concentration, it requires that multiple samples be tested at once to ensure the most accurate data. Spectrophotometry requires that after a test is conducted, the solution within the outlet cover needs to be collected and stored until all the experimental trials have been completed. Then spectrophotometry can be done on all the samples concurrently.

4.8.1 Fluorescent Intensity Calibration Curve

In order to avoid the use of a spectrophotometer, a method was developed to attempt to identify solution concentrations without spectrophotometry. In order to do this a curve was created by plotting the fluorescent intensity of known concentrations of dextran against their molarity essentially creating an intensity calibration curve. To do this, the exposure setting on the microscope was set to saturate at the highest concentration of dye tested, which was 1 µM which resulted in an exposure setting of 20 ms. Nine samples of dextran in DI water with a concentration between 0.02 µM and 1 µM were tested along with a drop of DI water, which served as the control and a concentration of 0 µM. To block out ambient light from the room, a piece of UV impenetrable fabric was wrapped around the stage. Fluorescent intensity was analyzed using the 8-bit grayscale method. Plotting the molarity vs. fluorescent intensity for each sample

![Molarity vs. Intensity - Dextran](image)

Figure 27: Molarity vs. Fluorescent intensity of dextran in DI water for samples ranging from 0 µM to 1 µM with exponential trend line.
produced the plot in Figure 27. Looking at the data initially, it appeared that it may follow an exponential growth trend. However, by looking at the exponential growth trend line, it is clear that this is not an accurate representation of the data.

Analyzing the data further revealed that the data points from 0 µM – 0.25 µM followed a linear trend, while the data from 0.5 µM – 1 µM followed a different linear trend. A plot containing the data with the linear trend lines is located in Figure 28. The trend line equations for both data ranges can be seen on the plot, as well as their relative $R^2$ values. These linear equations were utilized as calibration curves, which allowed for a molarity value to be gained from a value for fluorescent intensity. The equations for the upper and lower ranges of data are located below as Equations 4.4 and 4.5. In these equations, $x_L$ and $y_L$ represents fluorescent intensity and molarity in the lower range, while $x_U$ and $y_U$ represents fluorescent intensity and molarity in the upper range.

$$y_L = 0.0016x_L$$ (4.4)

$$y_U = 0.0254x_U - 5.5472$$ (4.5)

After an experiment was conducted, a sample from the outlet cover was immediately taken, and placed onto a black slide. The exposure setting was set to 20 ms, the same setting which was used to take the data for the calibration curve. An image of the sample was taken and saved. The resulting image was then analyzed using imageJ to extract an intensity value. If the intensity was 150 or less, the value could be plugged into the molarity equation for the lower range of molarities, Equation 4.4. If the
intensity was 240 or greater, than the molarity equation for the upper range of data was used, Equation 4.5. Plugging the fluorescent intensity value into the equations above, produced a value for molarity. This value could then be used to determine how much dye diffused through the membrane. This was done by using the diffusion equation in Equation 4.3. In this case \( V_1 \) does not represent the volume of the injected dye, but instead represents how much dye diffused through the membrane, therefore it will be referred to as \( V_D \). The updated equation is Equation 4.6 below. Where \( M_1 \) is the molarity of the injected dye, \( M_2 \) is the final molarity of the outlet cover which was solved for using Equations 4.4 or 4.5, and \( V_2 \) is the volume of DI water on the outlet cover.

\[
M_1 V_D = M_2 (V_D + V_2)
\]  
(4.6)

Solving Equation 4.6 for \( V_D \) results in an expression for \( V_D \) in terms of \( M_1 \), \( M_2 \), and \( V_2 \) located in Equation 4.7.

\[
V_D = \left( \frac{M_2 V_2}{M_1-M_2} \right)
\]  
(4.7)

Plugging into the equation using the value for \( M_2 \), calculated using Equation 4.4 or 4.5, and the other known values results in a value for the volume of water which diffused through the membrane. This value could then be used to calculate the rate dextran diffused through the AAO for each experimental trial.

### 4.9 Results

#### 4.9.1 Relationship Between \( \tau \) and Active Area

Six experimental trials were conducted for each of the three different diameters. For each trial the intensity values were plotted against time, and normalized using the method described in Section 3.3. \( \tau \) was found using the two different method described in Section 3.3 An increasing exponential decay curve was then fit to the data to produce a value for \( \tau \). The data was normalized, and fit using the methods described above in Section 3.3. To calculate the relationship between the curve fit, and the collected data, the determination coefficient, \( R^2 \) was calculated for each function. However, due to the curve fit not exactly matching the data, this value is only an approximation for \( \tau \). In order to obtain an exact value for \( \tau \), it was also calculated using Equation 3.7 above. The plotted intensity data for each trial, along with the increasing exponential decay curves using both values for \( \tau \) are located in Appendix A.1. It can be inferred
that a larger active area, which results in a larger number of exposed nanotubes would allow for faster fluid flow, and therefore produce a smaller time constant. This is backed up by the data. The average value for $\tau$ for each diameter increases as the diameter decreases due to the decreasing number of exposed tubes. The determined values for $\tau$ are dependent on the number of exposed pores, such that it is actually $\frac{\tau}{\text{# of Exposed Pores}}$. By multiplying each value of $\tau$ by the number of exposed pores, this dependence can be counteracted, making them directly comparable to one another, effectively normalizing $\tau$. It is predicted that these values would turn out the same, meaning that the number of exposed pores has a direct effect on flow. However, as can be seen in Table 4 that is not the case.

A chart comparing $\tau$, and normalized $\tau$ to the active diameter tested is located in Figure 29. By looking at the range chart, it can be seen that while the value for $\tau$ are inversely related to diameter overall, there is some overlap amongst the diameters. The values for the 2 mm and 4 mm flow devices are extremely similar compared to those of the 6 mm device. This may be due to the difference in area between the 6 mm and 4 mm devices being twice as large as that of the 4 mm and 2 mm devices. Some of the variation in the values is due to the resolution of the 3D printer. All the outlet covers used were printed on either a Printrbot Simple Metal, or a MonoPrice Maker Select 3D Printer v2. Both of these printers have a resolution of 50 $\mu$m, which results in discrepancies within the active area for each outlet cover as discussed above in Section 4.4. For each of the three tested diameters, there is a maximum and a minimum area possible when the printer resolution is accounted for. These areas can be seen in Table 5. However, by looking at the values, it can be seen that when the uncertainty for area is factored in, the area values do not overlap one another, which does not explain the coinciding values for $\tau$ amongst the different tested diameters. A possibility for this could be the existence of micro-cracks within the AAO membranes. SEM images were taken of a 3D printed flow device, which demonstrates no cracks, however SEM images were not taken for every flow device, allowing for the existence of micro-cracks that were too small to be seen under the upright microscope. This would cause dye to diffuse through the AAO membrane faster than it would without the presence of cracks resulting in a lower time constant. However, this still does not account for the relationship between $\tau$ and the number of exposed pores which was discovered. Figure 29 also demonstrates that the normalized values for $\tau$ are not the same for each diameter, instead it increases with
Table 4: Values for $\tau$, and $R^2$ for each experimental trials. Diameters tested were, 6 mm, 4 mm, and 2 mm.

<table>
<thead>
<tr>
<th>ACTIVE AREA DIAMETER (mm)</th>
<th>CURVE FIT</th>
<th>CALCULATED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment #</td>
<td>$\tau$ (s)</td>
</tr>
<tr>
<td>6</td>
<td>6.1</td>
<td>53.55</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>72.02</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>81.12</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>67.26</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>17.35</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>48.31</td>
</tr>
<tr>
<td></td>
<td>Avg.</td>
<td>56.6</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>20.7</td>
</tr>
<tr>
<td>4</td>
<td>4.1</td>
<td>61.51</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>118.2</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>70.73</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>125.97</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>52.36</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>46.97</td>
</tr>
<tr>
<td></td>
<td>Avg.</td>
<td>79.29</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>31.23</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>105.7</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>179.44</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>113.25</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>165.26</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>159.08</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>167.86</td>
</tr>
<tr>
<td></td>
<td>Avg.</td>
<td>148.43</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>28.28</td>
</tr>
</tbody>
</table>

diameter, indicating that a single pore experiences more flow as the number of exposed pores increases, which is not the relationship that was expected.

When looking at the average value for $\tau$, it does increase as the average value for $\tau$ for each diameter, are compared to one another, the results illustrate that the change in $\tau$ is semi-proportional to the increase in the number of open pores. The difference in the number of pores in the 4 mm diameter device, compared to the 6 mm diameter device is a little less than half. It would therefore be expected that the average value for $\tau$ for the 4 mm diameter would be twice as large as the value for $\tau$ for the 6 mm device. However, when the average values for $\tau$ for both
devices are compared, the value for the 4 mm is not quite double that of the 6 mm. The same goes for the 4 mm and 2 mm diameters. The 4 mm diameter device has 4 times as many pores as the 2 mm diameter device, however the value of $\tau$ for the 2 mm diameter is not four times as large as that of the 4 mm device. This leads to the idea that the relationship between $\tau$ and the number of exposed pores, does not increase at a constant rate. If they did, their relationship would form a power series, where $\tau$ was inversely related to the number of exposed pores. A plot of the average and predicted values for $\tau$ vs. the number of exposed pores can be seen in Figure 30. The predicted values for $\tau$, form a power series, in which the predicted values for $\tau$ increase by the same magnitude that the number of pores decreases. This can be represented by a power series of

\[
\tau \text{ Calculated, and Normalized}
\]

![Graph showing the relationship between $\tau$ and diameter](image)

**Figure 29:** Values for calculated $\tau$, and normalized $\tau$ for 6 mm, 4 mm, and 2 mm diameters. Error bars represent standard deviation, $n = 6$.

<table>
<thead>
<tr>
<th>DIAMETER (mm)</th>
<th>AREA ± ε (mm²)</th>
<th># OF PORES ± ε (E+07)</th>
<th>$\tau ± \sigma$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>28.27 ± 0.017</td>
<td>20.86 ± 0.013</td>
<td>111.23 ± 21.37</td>
</tr>
<tr>
<td>4</td>
<td>12.57 ± 0.025</td>
<td>9.28 ± 0.018</td>
<td>148.67 ± 14.46</td>
</tr>
<tr>
<td>2</td>
<td>3.14 ± 0.050</td>
<td>2.32 ± 0.037</td>
<td>150.07 ± 11.05</td>
</tr>
</tbody>
</table>

**Table 5:** Relationship between minimum and maximum active area diameters, and $\tau$. 


the following Equation 4.8, where \( x \) is the number of exposed pores times ten to the seventh power. 1180.9 is the value of \( \tau \) when \( x \) is one.

\[
\tau = 1180.9x^{-1}
\]  

(4.8)

However, the relationship between the actual values for \( \tau \), and the number of pores does not follow this trend. While the relationship is that of a power series, the value for \( \tau \) increases proportionate to the number of pores risen to the power of 0.122. This equation is located below in Equation 4.9.

\[
\tau = 173.66x^{-0.122}
\]  

(4.9)

This equation leads to the idea that the amount of flow produced by a single pore depends on the total number of exposed pores. However, there is no fundamental reason for this to be occurring. In order to determine what was causing this, the possibility that the fluorescent dye dextran, which was used to conduct the flow experiments was clogging the pores of the AAO prohibiting flow.

To investigate this, tests were run using 0.5 µM dextran, and 10 µM dextran. For each active area, two trials were run at the different concentrations. The time constant \( \tau \), was

Figure 30: Average and predicted values for \( \tau \) vs. the number of exposed pores. The predicted values form a power series due to their changing at a consistent rate.
calculated using the two methods described in Section 3.3. Equation 3.7 was used to calculate \( \tau \) at 99% of the maximum intensity, \( \tau_{99\%} \) as well as at 50% of the maximum intensity, \( \tau_{50\%} \). This was done in order to quantify how quickly the intensity initially increased. The results are located in Table 6. The plots of intensity vs. time, as well as the increasing exponential decay curves are located in Appendix A.2 and A.3. A figure containing the relationship between \( \tau_{99\%} \), molarity, and diameter, as well as the same for \( \tau_{99\%} \), normalized are located in Figure 31. The \( \tau_{99\%} \) By initially looking at the values for \( \tau_{99\%} \), a trend does not seem to be occurring, and the calculated values are within error bars of each other. The normalized values follow the same trend as shown with the 2 \( \mu \)M dye, decreasing with diameter, which does not follow the anticipated trend. However, \( \tau_{99\%} \) quantifies how long the intensity takes to rise to 99% of its maximum value. This can occur in different ways. The data can have a steady rise, as seen in Figure 33 which has a value for \( \tau_{99\%} \) of 135.73, or it can have an initial sharp increase, and then rise steadily, as seen in Figure 32 which has a for \( \tau_{99\%} \) of 137.89. While the values for \( \tau_{99\%} \) only differ by 2.16 s, the behavior of the intensity vs. time varies drastically between the two. In order to identify these differences in behavior the values for \( \tau_{50\%} \) were calculated.

### Table 6: Results for \( \tau \), for 0.5 \( \mu \)M and 10 \( \mu \)M dextran testing

<table>
<thead>
<tr>
<th>ACTIVE AREA DIAMETER (mm)</th>
<th>Molarity (( \mu )M)</th>
<th>Experiment #</th>
<th>( \tau )</th>
<th>( \tau_{99%} ) (s)</th>
<th>( \tau_{50%} ) (s)</th>
<th>( \tau_{99%} ) Normalized</th>
</tr>
</thead>
<tbody>
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44
While Figure 32 and Figure 33 have similar values for $\tau_{99\%}$ they have drastically different values for $\tau_{50\%}$, 8.38, and 2.39 respectively. This illustrates the initial sharp rise of Figure 32 compared to the more gradual rise of Figure 33. The relationship between $\tau_{50\%}$, concentration, and diameter is located in Figure 34. By looking at Figure 34, it initially appears as if there is no relationship between $\tau_{50\%}$ and concentration, however within error bars the value for $\tau_{50\%}$ decreases as the concentration of the dextran increases. This would be caused by the dye initially flowing through the pores of the AAO, but then quickly clogging the pores, resulting in a much slower increase in intensity for the duration of the experimental trial. The presence of such large error bars is due to some outliers in the data, which produce a much higher value for $\tau_{50\%}$ than the other trials. These may be caused by micro-cracks forming within the membranes. When the membranes are 3D printed on, the extruder nozzle exerts a force onto the membrane. The larger the diameter of the flow device, the larger the space between the exerted forces resulting in a higher chance of cracks occurring. This would account for why one 6 mm, and one 4 mm trial at the 10 $\mu$M dye do not follow the expected behavior which results from membrane clogging. If the membrane were to be cracked, the effects of clogging would be minimized, such that the dye could flow through the cracks, surpassing the pores, resulting in a smooth rise of intensity over time, and therefore a larger value for $\tau_{50\%}$.

Figure 31: A) Relationship between $\tau_{99\%}$ and the three tested concentrations of dextran, and diameter. B) Relationship between $\tau_{99\%}$ normalized and the three tested concentrations of dextran, and diameter. For the 0.5 $\mu$M and 10 $\mu$M dye n = 2, for the 2 $\mu$M dye n = 6. Error bars represent standard deviation.
Dextran clogging the pores within the AAO may have also skewed the relationship between area and $\tau_{50\%}$. It is expected that, similar to $\tau_{99\%}$, $\tau_{50\%}$ would increase as the area, or the number of exposed tubes decreased. While this trend does occur within error bars for the 0.5 $\mu$M and 2 $\mu$M concentrations of dextran, it is not true for the 10 $\mu$M dye. This may be a result of the lower number of exposed pores clogging up faster, due to the higher concentration dextran, causing $\tau_{50\%}$ to decrease with the number of exposed pores.

In order to further investigate the hypothesis that dextran was clogging the pores of the AAO membrane, the amount of dye which diffused through the membrane for all the conducted testing was determined.

![Figure 32: 4 mm diameter, 10 $\mu$M concentration dextran, $\tau = 137.89$ s.](image)

![Figure 33: 6mm diameter, 0.5 $\mu$M concentration dextran, $\tau = 135.27$ s.](image)

4.9.2 Volume of Diffused Dye

To determine the amount of diffused dye for each experiment needed to be determined. This was done utilizing the method described above in
Section 4.8.1. However, this method is dependent on exposure. In order to confirm the relationship between concentration and fluorescent intensity, spectrophotometry was also run on the samples. In order to determine the concentration of the samples after testing using spectrophotometry, a calibration curve had to be established. To produce this curve, a set of samples of dextran of known molarities were run through the spectrophotometer to produce a set of absorbance values. The resulting data was then plotted against concentration, and connected with a linear trend line to create the calibration curve. The plotted data is located in Figure 35. The calibration curve produced the trend line in Equation 4.10. Where \( M \) is molarity in \( \mu \text{M} \), and \( y \) is absorbance.

\[
y = 0.0868 \times M + 0.0209
\]  

Once all the experiments had been conducted, all the samples from V1 were tested concurrently to produce an absorbance value for each sample. By solving Equation 4.10, and plugging in the obtained absorbance values, the molarities for each sample could be acquired. Using Equation 4.3, the resulting molarities were used to find the amount of diffused dye from the dye volume.
underneath the array represented by V1 in Figure 25. These values were compared to those found using the fluorescent intensity calibration curve, and are located in Table 7 where the experimental values were found using fluorescent intensity, and the actual values were found using spectrophotometry. The experimental values do not match those obtained from spectrophotometry. As a result, fluorescent intensity does not provide an accurate method for obtaining molarity. This is due to the high dependency of the fluorescent intensity on exposure. The calibration curve which was created in Figure 28, analyzed samples of dextran using an exposure setting of 20 ms. However, exposure is dependent on the amount of ambient light in the room, which is constantly changing. While measures were made to block out as much light as possible, only so much could be done without a designated fluorescent microscopy area. Therefore, when the fluorescent intensity values were collected from samples form the outlet reservoir after experimental trials, the level of light in the room may have varied from that of when the calibration curve was created. As a result, when the intensity values were plugged into Equation 4.4 or 4.5, it would result in a molarity value which is incorrect. However, while the results were not exact, they are relatively correct to one another. Essentially, a sample with a higher fluorescent intensity than another would have experienced a higher volume of diffused dye. However, spectrophotometry should be used to determine the exact volumes of diffused dye.
Table 7: Amount of diffused dye. Actual found using spectrophotometry. Experimental found using fluorescent intensity.

<table>
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<tr>
<th>ACTIVE AREA DIAMETER (mm)</th>
<th>MOLARITY OF M1 (µM)</th>
<th>EXPERIMENT #</th>
<th>MOLARITY OF M2 (µM)</th>
<th>AMOUNT OF DIFFUSED DYE (µL)</th>
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<td>Actual</td>
<td>Exp.</td>
<td>Actual</td>
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<td>6.3</td>
<td>0.003</td>
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<td>0.174</td>
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<td>0.164</td>
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For each trial 100 μL of dextran were placed underneath the array, and allowed to diffuse through the AAO into 80 μL of DI water. If all the dextran were to diffuse through the membrane as expected, it would result in the samples taken from the top volume of the flow device having the maximum value for molarity. However, all of the dye is not diffusing through, resulting in only a fraction of the max molarity being achieved. A chart comparing the average percentage of

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<th>ACTUAL MOLARITY (µM)</th>
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<td>0.324</td>
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</table>
the maximum molarities achieved is located in Figure 36. The achieved molarities compared to the maximum molarities are located in Table 8.

From Figure 36 it becomes clear that the percentage of diffused dye decreases as a function of concentration, confirming the hypothesis that dextran is clogging the pores of the AAO membrane. Significantly more of the 0.5 µM dye diffused through the membranes, than the 10 µM, with the 2 µM lying between the two. Therefore, as concentration increases over time, it never reaches the true maximum concentration, instead it stabilizes at an average of 48.8% for the 0.5 µM dextran, 12.8% for the 2 µM dextran, and 4.9% for the 10 µM dextran. A plot of the percentage of the maximum concentration vs time for each of the tested molarities for the 6 mm diameter is located in Figure 37. The relationship between the molarity of the dextran and the achieved percentage of the maximum concentration, such that the higher the molarity of dextran the lower the achieved concentration percentage supports the hypothesis that the dextran is clogging the pores of the AAO membrane. The higher the molarity of the dextran, the more of

![Percentage of Achieved Maximum Molarity](image)

**Figure 36:** Chart comparing average percentage of the maximum molarity achieved for each tested diameter, and dye concentration. Error bars represent standard deviation. For the 0.5 µM and 10 µM dye n = 2, for the 2 µM dye n = 6.
the dextran molecule it contains, resulting in the occurrence of more clogging of the pores. Due to this clogging, less of the 10 μM and 2 μM dyes have a chance to diffuse through the AAO before the pores clog completely, stopping the rise of the concentration of the top well of DI water. As a result, the higher molarities of dye achieve a constant concentration faster, due to less of the dye having the chance to diffuse through the AAO before clogging occurs. This produces a quicker initial rise in the concentration, and therefore a lower value for τ_{50%}. This is illustrated in Figure 37. As a result of this, the values for τ_{99%} would be effected, explaining why the values for τ do not directly correspond to the number of open pores as expected.

![Percentage of Achieved Concentration - 6 mm](image)

Figure 37: Percentage of the maximum concentration achieved for the 0.5 μM, 2 μM, and 10 μM dextran for the 6 mm diameter active area.

### 4.9.3 Rate of Diffusion

To determine the rate at which dye diffused through the membrane, the rate of diffusion for each experimental trial was calculated. This was done by dividing the volume of diffused dye by the value for τ. The results are located in Table 9. A figure comparing rate of diffusion to the
tested concentrations of dextran and active area diameter is located in Figure 38. By looking at the results, the 6 mm diameter produced the highest rate of diffusion rate, which corresponds to it

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<th>ACTIVE AREA DIAMETER (mm)</th>
<th>MOLARITY (μm)</th>
<th>EXPERIMENT #</th>
<th>τ (s) - CALCULATED</th>
<th>AMOUNT OF DIFFUSED DYE (μl)</th>
<th>RATE OF DIFFUSION (μl/s)</th>
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<td>159.08</td>
<td>5.111</td>
<td>3.21E-02</td>
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<tr>
<td></td>
<td>2.6</td>
<td>167.86</td>
<td>5.111</td>
<td>3.04E-02</td>
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<tr>
<td></td>
<td>Avg.</td>
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<td></td>
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<td>10.2.2</td>
<td>90.92</td>
<td>1.673</td>
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</table>
having the lowest time constant, and fits with the concept that diffusion will occur quicker when there is a larger number of exposed pores. The rate of diffusion also decreased as the dye concentration increased, this corresponds to a lower amount of dye flowing through the membrane, and therefore a slower rate of diffusion.

**Figure 38**: Rate of Diffusion for each dextran concentration compared to diameter of the active area. For the 0.5 µM and 10 µM dye n = 2, for the 2 µM dye n = 6.
5.0 MICROFLUIDIC CHANNELS

5.1 Introduction

Three-dimension (3D) printing is a novel technology whose versatility allows it to be implemented in a multitude of applications. Here in, low-cost 3D printing techniques have been implemented to add resilience to the otherwise fragile CNT arrays, and create a flow device capable of being used for flow quantification experiments. In order to broaden the range of applications for the flow devices, a second flow device was developed which allowed for multiple isolated inlet flows to the arrays. Utilizing this multiple inlet design permits distinct fluids to enter the array disjointedly and allows for the possible future use of utilizing the same CNT array to inject different solutions into separate groups of cells. This technique would prove useful in implementing a CNT array for enhanced drug delivery. Testing was conducted to determine if a 3D printed multiple channel device could be used to flow distinct solutions through a CNT without mixing until after diffusion.

5.2 3D Printed Device Design & Fabrication

Similar to the design for the Single channel flow devices discussed above, the multichannel devices was designed to serve three main purposes; provided channels for the solution to be injected, isolate an active area of the CNT array, and offer a method of producing a uniform cross section.

Unlike the single channel flow devices which were printed in two pieces; the channel, and the outlet cover, and then assembled together, the multichannel devices were printed in a single piece. The channels utilized for these devices, were significantly smaller than those used in the single channel device. As a result, this causes a higher pressure within the channels, and with that a greater risk of the injectable solution breaking through the bond which hold together the top and bottom pieces. In order to create the strongest bond, and ensure that the entire surface area of the outlet cover was in contact with the base. A Printrbot Simple Metal was used to manufacture all the multichannel flow devices. The 3D printed devices were all designed using Solidworks. The files were then exported as STL files, and loaded into a slicing program, Cura 15.04.5 by Ultimaker. The devices were printed using 1.75mm PLA from Hatchbox or Printrbot. The devices were prototyped using Whatman Anodisc 13 mm diameter anodized aluminum oxide (AAO) circular membranes that contained 0.2μm pores and were 50μm thick. Once a
successful design, and print method was determined, the devices were printed to embed a CNT array manufactured in the method described above.

In order to print the devices with the CNT array embedded, the devices were printed in two parts; the base, and the outlet cover. The base consisted of a 33 mm x 33 mm block that was 5mm tall. Two 2 mm x 3 mm troughs were then added and spaced 3 mm apart to act as conduits to introduce the fluid to the bottom of the AAO membrane. The channels were 2mm deep at the ends, and dipped down in the center to a depth of 3 mm. This was done to help promote fluid flow through the channels. Due to the versatility of 3D printing, the size and number of troughs could easily be increased. The only limiting factors being the resolution of the 3D printer in use, and the size of the membrane. Using the Printrbot Simple Metal, and the 13 mm diameter membranes, the maximum number of channels possible to print was six. This was done by decreasing the channel width to 1 mm, and spacing them 1 mm apart.

The upper portion of the flow device, or the outlet cover, was designed to ensure that the flow from the multiple inlets would enter the CNT array distinctly, and mix upon exiting the array. To achieve this a 10 mm x 2 mm section of the CNT array as left exposed to serve at the mixing chamber. The mixing chamber for the multichannel devices was designed as a rectangle, rather than a circle like in the single channel devices, to ensure that the mixing chamber would span both of the channels with minimum area in-between. Like the single channel flow devices, the multichannel devices needed to provide a method of creating a uniform cross section to ensure accurate transient data, and prevent foreign debris from entering the system. The same method that was used for accomplishing this in the single channel devices, was employed for the multichannel devices. The outlet cover consisted of a 23mm x 23mm block that was 3mm thick. A 19mm x 19mm step was then made to hold the glass slide 1mm below the surface. A small section along the side was also removed to the same level of the step to allow easier placement of the glass slide using tweezers. A 17 mm x 17 mm well that was 2mm below the surface was then added to contain any fluid that spilled over from the membrane outlet.

In order to print the devices on the CNT arrays without damaging them, the parts were printed in two steps. The nozzle temperature was set to 210°C and the bed temperature was set to 40°C for all prints. No support or adhesion techniques were necessary. The print layer height was held constant at 0.15mm for all prints. The base was printed first using an 18% fill density at 60 mm/s. Once the base was completed, the CNT array was secured to the center of the base, above
the channels. The outlet cover was then printed over the base and array by adjusting the z-offset of the printer nozzle by 5.05mm. Due to the fragile nature of the CNT array, the fill density of the outlet cover was increased to 100% and the print speed was lowered to 30mm/s. The increased fill density produced less quick and jarring motions while printing, and reduced the risk of cracking the array. A model of the completed device can be seen in Figure 39.

5.3 Flow Through the Microfluidic Channels

Flow through the CNT arrays was facilitated through diffusion; DI water was placed into the mixing chamber on top of the CNT array before injecting solution into the channels. In order to monitor diffusion through the CNT array, the fluorescent dye, dextran was used. Flow was observed in real time using a Zeiss Scope.A1 upright microscope paired with an Axiocam Mrm, the same as was used for the single channel experiments. In order to ensure that the multichannel device would not fluoresce under the microscope, interfering with the fluorescence from the dye, all the devices were printed using black colored PLA. The one shown in Figure 39 was printed in green in brown to make the geometry more visible.

Testing was conducted utilizing dextran in ethanol as the injectable solution in the multichannel flow devices due to this being a proof of concept experiment. The goal of these experiments were to see if 3D printed channels could be used to keep injected solutions separate.
until after diffusion through a CNT array. This differs from the single channel experiments, the reason for this being, the single channel devices were being tested to quantify flow behavior, with the future hope of using them for cell transfection. Ethanol could not be used in this case because it does not provide an accurate substitute of a solution that would be injected. Utilizing ethanol with the multichannel flow devices allows for testing to be conducted with CNT arrays, rather than plain AAO membranes. Because of the opacity the CNT arrays possess, the camera did not need to be offset from the array, but could be placed directly above it, due to their being no concern of the fluorescence from the injected dye interfering with the results before diffusion occurred.

To observe flow through the microchannels 40 µL of DI water was placed into the mixing chamber, and a cover slip was placed onto the inner ledge of the outlet cover flattening the dome created by the DI water. The setup was then placed onto the microscope stage, and placed such that both channels were in the field of view of the microscope. The 2.5x lens was used due to it being the only one with the capability of capturing both of the channels at once. Once the microscope was properly focused, and the fluorescent light was turned on, the camera was set to take pictures in a 0.5 s time increment for 30 minutes. 20 µl of 2 µM dextran in ethanol was then injected into each channel. The dextran was injected into the channels after the camera started capturing images to guarantee that the entire flow period would be captured. A schematic, and image of the experimental setup is located in Figure 40.

5.4 Channel Breakthrough Testing

To confirm that the solution injected into the devices channels were not mixing before diffusing through the CNT array experiments were conducted utilizing the multi-channel devices
interfaced with AAO membrane. Due to the opacity of the carbon in the CNT arrays it could not be assured that the fluids injected into the device were not mixing before diffusion. AAO membranes offer a level of translucency. This allowed for the injected solution to be visible through the membrane before diffusion. Experiments were conducted utilizing the multi-channel devices interfaced with the AAO membranes, and Dextran in ethanol as the injected solution. Images were taken every half second with a fluorescent light paired with a Cy 3 filter. Image sequences conducted flow studies where breakthrough did, and did not occur are located in Figure 41A and Figure 41B. In the trial where breakthrough occurred, 30 μL of ethanol was injected into each channel, at a flow rate of 60 μL/s. By looking at the image sequence in Figure 41A, it can be seen that dye is initially injected into channel A at Bii. In comparison with the next image, Biii, it can be seen that the dextran which was injected into channel B has broken through the PLA barrier and began to migrate over into Channel B. As a result, when the dye was injected into channel B at Bv, it immediately mixed with the dye from channel A before diffusion. Comparing this to the image sequence in Figure 41B, it can clearly be seen that the dye injected into channel A at Bii does not break through the barrier, and instead stays in the channel until diffusing through the AAO membrane. Channel B, is injected at Biv, and like channel A, does not break through the barrier, therefore the solution from both channels does not mix until after diffusing through the membrane.

In total, 20 different flow rates, and volume combinations were tested utilizing different volumes of ethanol injected into each individual channel at different flow rates to observe whether channel breakthrough occurred. Each combination was tested three times. The results are located in Table 10 below. The results yielded that both flow rate, and injected fluid volume have an effect on whether the injected solution breaks through the PLA channel barrier before diffusion. Injecting any solution volume into the channels with a volume fraction of 0.06 or below can be done at any flow rate without breaking through the barrier. However, injecting any volume of solution with a volume fraction of 0.28 or above will break through the PLA barrier regardless of flow rate. Injected volumes with a volume fraction between 0.06 and 0.28 will break through the barrier depending on flow rate. Flow rates at 40 μL/s or below will not break through the barrier as long as the solution volume fraction is between the above stated range. It is predicted that this is a result of an increased pressure gradient in the channel due to the higher volume and flow rate.
Figure 41: Time sequence of dextran injected into flow device. a) Bright field image. b) Fluorescent images, Cy3 filter. A) Breakthrough occurred, B) No breakthrough.
Table 10: Effect of different volumes and flow rates of DI water injected into the channels on the breakthrough of the PLA barrier between channels.

<table>
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<tr>
<th>INJECTED VOLUME (µL)</th>
<th>VOLUME FRACTION (INJECTED/TOTAL CHANNEL VOLUME)</th>
<th>TIME (s)</th>
<th>FLOW RATE (µL/s)</th>
<th>BREAKTHROUGH (YES/NO)</th>
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</tr>
<tr>
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<td>No</td>
</tr>
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<td>10</td>
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</tr>
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<td>5</td>
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5.5 Flow Study

Once the parameters for channel breakthrough were determined, and it was proven that under the proper injection conditions, the injected solutions would remain separate until after diffusion experiments could conducted utilizing the CNT arrays in the multichannel devices.

Six experiments were completed using the multichannel flow device interfaced with the CNT arrays, and ethanol as the injectable. Each experiment was run for 30 minutes, using the setup described above. Images were taken every 0.5 s. The multichannel experiments ran for 30 minutes, rather than the 15 minutes used in the single channel trials, owing to ethanol taking longer to diffuse through CNT arrays than DI water takes to diffuse through AAO membranes. Once a trial is complete the resulting image sequence was then compiled, and analyzed using...
Image J. Fluorescent intensity was analyzed using the same 8-bit grayscale method that was used for the single channel flow experiments. To extract intensity data from the images the Time Series Analyzer plugin was employed paired with an ROI. The ROI used was set to encaptured the entire region of DI water on the outlet cover. An image of an example ROI is located in Figure 42. The region of interest can be seen in white. The outer edge of the DI water is not included, due to this fluorescing brighter than the rest of the DI water in the mixing chamber. Once intensity values were obtained, they were plotted against time. The result was a curve which resembled the increasing form of exponential decay, similar to the single channel trials.

![Figure 42: ROI used for multichannel flow experiments.](image)

5.5.1 Results

For each conducted trial, the intensity vs time curve was plotted, and fit to the increasing form of exponential decay using the same process described above in Section 3.3, to obtain a value for $\tau$. $\tau$ was also calculated at 99% of the maximum intensity using Equation 3.7. The calculated value for $\tau$ provides the most accurate representation of the fluid behavior, therefore conclusions regarding flow behavior were based off of the calculated values for $\tau$. The plots of intensity vs. time for each of the six conducted trials can be seen in Appendix A.4. The results for $\tau$ for the six trials are located in Table 11. Like the single channel results, the values for $\tau$ for the multi-channel devices have a significant amount of variation. This could be related to the resolution of the 3D printer used. The 3D printer used for manufacturing has a resolution of 50 $\mu$M, resulting in a total uncertainty of the mixing chamber of 5.1 x 10^5 $\mu$M^2. This results in maximum possible variation of the number of exposed CNTs of 8.86 x 10^6, which could impact
the speed at which dye diffused through the CNT. Another reason for variation could be due to micro-cracks within the arryas. Like the single channel devices SEM images were not taken for every flow device, allowing for the existence of micro-cracks that were too small to be seen under the upright microscope, causing the dye to diffuse through the AAO membrane faster than it otherwise would.

The average value for $\tau$ for the multi-channel devices was 232.73, which is significantly higher than any of the values for $\tau$ determined from the single-channel experiments which are listed in Table 4. The active area of the mixing chamber was 20 mm$^2$ which lies inbetween the areas of the 6 mm – 28.27 mm$^2$ and 4 mm – 12.57 mm$^2$, diameter single channel devices. Therefore, it would be predicted that the values for $\tau$ for the multi-channel devices would fall inbetween those of the 6 mm and 4 mm devices. However, the produced values for $\tau$ are significantly larger, meaning the rate of diffusion was much slower. This could be related to the use of ethanol rather than the DI water used in the single channel devices. Another cause could be the smaller diameters of the CNTs within the CNT array. The pores within the AAO membrane have a diameter of 200 nm, however after the CVD process this shrinks to 150 nm, which may have slowed down diffusion through the membrane. Once the devices are manufactured such that DI water can diffuse through them, further testing of the devices should be conducted.

**Table 11: Results for tau for multi-channel flow devices.**

<table>
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<tr>
<th>EXPERIMENT #</th>
<th>$\tau$(s) – Curve Fit</th>
<th>$R^2$</th>
<th>$\tau$(s)–Calculated</th>
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<td>0.999</td>
<td>233.87</td>
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<td>M.2</td>
<td>365.42</td>
<td>0.986</td>
<td>311.28</td>
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<tr>
<td>M.3</td>
<td>508.38</td>
<td>0.986</td>
<td>331.04</td>
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<td>M.4</td>
<td>310.89</td>
<td>0.979</td>
<td>211.83</td>
</tr>
<tr>
<td>M.5</td>
<td>188.13</td>
<td>0.998</td>
<td>199.02</td>
</tr>
<tr>
<td>M.6</td>
<td>157.05</td>
<td>0.991</td>
<td>109.33</td>
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<tr>
<td><strong>AVERAGE</strong></td>
<td><strong>308.73</strong></td>
<td><strong>0.990</strong></td>
<td><strong>232.73</strong></td>
</tr>
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<td><strong>STANDARD DEVIATION</strong></td>
<td><strong>116.06</strong></td>
<td><strong>0.007</strong></td>
<td><strong>68.28</strong></td>
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</tbody>
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6.0 CONCLUSIONS

This research culminated in an effective method for observing, and characterizing flow through AAO membranes in real time. This information can later be applied to characterize flow through arrays of amorphous carbon CNTs.

6.1 Setup to Observe Flow

While previous research regarding flow through arrays of CNTs has been conducted, it has involved pressure driven flow. For the arrays produced in our lab, flow is facilitated by diffusion, and does not require an external force. Due to this change in production, results from previous studies could not be applied to these CNT arrays. In order to observe flow in real time and to accurately characterize its behavior, a set-up procedure was developed which allowed for a solution to be placed under the CNT array into a channel, while the opposite surface was monitored using an upright microscope.

While designing the flow set-up, the need for a disposable device became apparent. In the future, the flow devices will be used with biological components, such as cells, and DNA. A disposable device, ensures that no cross contamination will occur.

3D printing through FDM provided the best method for constructing the devices. Utilizing PLA provided a quick and inexpensive method for producing multiple copies of the devices. In addition to efficiency, 3D printing also provided a method of isolating a specific area of the CNT area. 3D printing directly onto the arrays, allowed for different diameter circular areas of the CNT to be left bare, therefore changing the number of exposed CNTs solution could then flow through. SEM images showed that 3D printing onto the arrays, did not cause damage or cracking.

6.2 Flow Observation and Characterization

To observe flow, the fluorescent dye, dextran was utilized. By placing DI water on top of the CNT array, and dextran underneath, the dextran would diffuse through the membrane increasing the fluorescent intensity of the volume of DI water. Monitoring this with an upright microscope, paired with a camera taking pictures every 0.5 s provided an image sequence which
could be analyzed to obtain the fluorescent intensity values. These values were then plotted against time, creating a curve which demonstrated behavior similar to that of the increasing form of exponential decay. By fitting the experimental curves to the increasing form of exponential decay each experiment could be characterized by a time constant, $\tau$, which represents the amount of time required for the curve to reach its maximum fluorescent intensity. While this provided a value for $\tau$, it was not exact. Therefore, it was also calculated using the amount of time required to reach 99% of the maximum intensity.

6.2 Flow Analysis

Flow through three different areas of AAO membranes was analyzed. Testing was not conducted utilizing CNTs as initially planned, due to an unknown error in the manufacturing process prohibiting the diffusion of DI water through the array. As a result, AAO membranes which serve as the template for the CNT arrays were tested.

Three different areas were tested of 2 mm, 4 mm, and 6 mm diameter in order to understand the effects of changing the number of open pores on flow. For each diameter, trials were run. Each trial was fit to increasing exponential decay to produce a value for $\tau$. While a trend did appear showing that the average value for $\tau$ increased as the diameter decreased, additional testing needs to be conducted to confirm the relationship. The range of values for $\tau$ for each diameter all had a large range. Their variance is due in part to the resolution of the printer which causes slight changes in the number of exposed tubes for each diameter. However, another reason for this is that the dextran which is used to observe flow is clogging the membranes. This was verified by testing different molarities of dextran. It was concluded that significantly less of the higher concentration dextran was diffusing through the AAO membranes, confirming the idea the pores of the AAO membrane were clogging prohibiting the diffusion of dextran. This would drastically effect the values for $\tau$ causing flow to not be directly dependent on the number of exposed CNTs. In order to verify this, future testing should be conducted with a wider range of diameters, and different fluorescent trackers to observe if clogging is occurring.

To characterize the amount of dye which diffused through the CNT arrays, a calibration curve which related fluorescent intensity to molarity was developed. The results from this method were compared to those using spectrophotometry. This method proved unsuccessful at providing the volume of dye which diffused through the membrane. This is due to the sensitivity
of fluorescent intensity to exposure, which is dependent on level of light in the room. It is believed that this method would work, if ideal fluorescent microscopy conditions were used. To look into this further, future work should be conducted using an intended fluorescent microscopy set-up, which would eliminate any issues with changing levels of light, and their relation to exposure. Future work should also be done utilizing the CNT arrays, once the issue prohibiting flow is discovered. Utilizing the flow device, testing should be conducted with different concentrations, and particle sizes in order to obtain the effects they have on flow through the arrays.

6.3 Microfluidic Channels

In addition to 3D printing devices with a single channel, research was conducted to develop multi-channel devices. These devices allowed for solution to be placed underneath the array in two distinct inlets, which did not mix until after diffusion through the CNT array. Testing was conducted utilizing CNT arrays and ethanol, due to it being a proof-of-concept experiment. The results indicated that 3D printing could be used to successfully create a device allowing for distinct inlets to the CNT array, however the conditions in which fluid is injected into the channel need to be monitored. If too much solution is injected, at too high of a flow rate, the solution will break through the channel walls and mix before diffusion.

Flow was characterized using increasing fluorescent intensity, in the same method employed for the single-channel devices. Ethanol took considerably longer to diffuse through the arrays. This could be due to the smaller diameter of the CNTs compared to that of the pores of the AAO membranes. Future testing should be done using DI water as the injectable, and the possibility of adding more channels should be investigated.

Ultimately, the developed single channel, and multi-channel flow devices, and characterization method, lend themselves well to future testing endeavors.
APPENDIX A

Intensity vs. Time plots from the flow devices

All plots are normalized to 99% of I*  

A.1 Single Channel Flow Devices - 2 μM Dextran

A.1.1 6 mm Devices

Figure A.1: Experiment 6.1.

\[ I^* = (1 - e^{-t/\tau}) \]

\[ R^2 = 0.861 \]

\[ I^* = (1 - e^{-t/143.10}) \]

\[ R^2 = -0.0945 \]
Figure A.2: Experiment 6.2.

\[ I' = \left(1 - e^{-t/72.02}\right) \]
\[ R^2 = 0.949 \]

Figure A.3: Experiment 6.3.

\[ I' = \left(1 - e^{-t/81.12}\right) \]
\[ R^2 = 0.978 \]

\[ I' = \left(1 - e^{-t/114.87}\right) \]
\[ R^2 = 0.896 \]
Figure A.4: Experiment 6.4.

Figure A.5: Experiment 6.5.
Figure A.6: Experiment 6.6.

A.1.2 4 mm Devices

Figure A.7: Experiment 4.1.
$I^* = (1 - e^{-t/118.2})$  
$R^2 = 0.932$

$I^* = (1 - e^{-t/139.52})$  
$R^2 = 0.889$

$I^* = (1 - e^{-t/130.07})$  
$R^2 = 0.644$

Figure A.8: Experiment 4.2.

Figure A.9: Experiment 4.3.
Figure A.10: Experiment 4.4.

\[ I_\ast = \left(1 - e^{-t / 125.97}\right) \]
\[ R^2 = 0.850 \]

Figure A.11: Experiment 4.5.

\[ I_\ast = \left(1 - e^{-t / 52.36}\right) \]
\[ R^2 = 0.988 \]
A.1.3 2 mm Devices

Figure A.12: Experiment 4.6.

Figure A.13: Experiment 2.1.
Figure A.14: Experiment 2.2.

\[ I^* = \left(1 - e^{-t/179.44}\right) \]
\[ R^2 = 0.999 \]

Figure A.15: Experiment 2.3.

\[ I^* = \left(1 - e^{-t/113.25}\right) \]
\[ R^2 = 0.925 \]
Figure A.16: Experiment 2.4.

\[
I^* = \left(1 - e^{-t/165.26}\right)
\]

\[R^2 = 0.878\]

Figure A.17: Experiment 2.5.

\[
I^* = \left(1 - e^{-t/159.08}\right)
\]

\[R^2 = 0.816\]
A.2 Single Channel Flow Devices – 0.5 μM Dextran

A.2.1 6 mm Devices

Figure A.18: Experiment 2.6.

Figure A.19: Experiment 0.5.6.1.
A.2.2 4 mm Devices

Figure A.20: Experiment 0.5.6.2.

\[ I^* = \left( 1 - e^{-\frac{t}{149.72}} \right) \]
\[ R^2 = 0.999 \]

\[ I^* = \left( 1 - e^{-\frac{t}{125.84}} \right) \]
\[ R^2 = 0.969 \]

Figure A.21: Experiment 0.5.4.1.

\[ I^* = \left( 1 - e^{-\frac{t}{213.83}} \right) \]
\[ R^2 = 0.873 \]

\[ I^* = \left( 1 - e^{-\frac{t}{168.61}} \right) \]
\[ R^2 = 0.881 \]
A.2.3 2 mm Devices

Figure A.22: Experiment 0.5.4.2.

Figure A.23: Experiment 0.5.2.1.
A.3 Single Channel Flow Devices – 10 \( \mu \)M Dextran

A.3.1 6 mm Devices

Figure A.24: Experiment 0.5.2.2.

\[
I^* = \left( 1 - e^{-t/249.79} \right) \\
R^2 = 0.912
\]

\[
I^* = \left( 1 - e^{-t/93.26} \right) \\
R^2 = -0.013
\]

Figure A.25: Experiment 10.6.1.

\[
I^* = \left( 1 - e^{-t/154.60} \right) \\
R^2 = 0.958
\]

\[
I^* = \left( 1 - e^{-t/127.57} \right) \\
R^2 = -0.767
\]
A.3.2 4 mm Devices

Figure A.26: Experiment 10.6.2.

\begin{equation*}
I^* = (1 - e^{-t/287.76})
\end{equation*}

\begin{equation*}
R^2 = 0.998
\end{equation*}

Figure A.27: Experiment 10.4.1.

\begin{equation*}
I^* = (1 - e^{-t/159.82})
\end{equation*}

\begin{equation*}
R^2 = 0.937
\end{equation*}

\begin{equation*}
I^* = (1 - e^{-t/137.89})
\end{equation*}

\begin{equation*}
R^2 = -1.535
\end{equation*}
A.3.3 2 mm Devices

Figure A.27: Experiment 10.4.2.

Figure A.28: Experiment 10.2.1.
A.4 Multi-Channel Flow Devices

Figure A.29: Experiment 10.2.2.

Figure A.30: Experiment M.1.
Figure A.31: Experiment M.2.

\[
I^* = \left( 1 - e^{-t/311.28} \right)
\]
\[R^2 = 0.988\]

Figure A.32: Experiment M.3.

\[
I^* = \left( 1 - e^{-t/331.04} \right)
\]
\[R^2 = 0.9858\]
Figure A.33: Experiment M.4.

Figure A.34: Experiment M.5.
Figure A.35: Experiment M.6.

\[ I^* = (1 - e^{-t/157.05}) \]

\[ R^2 = 0.990 \]

\[ I^* = (1 - e^{-t/109.33}) \]

\[ R^2 = 0.904 \]
REFERENCES


