String Microfluidics

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August 15, 2014

A thesis submitted to McGill University in partial fulfillment
of the requirements of the degree Master of Engineering.

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Abstract

Microfluidics enables the manipulation and analysis of very low volume samples, opening doors in basic research where new phenomena can be studied, and improving diagnostics by boosting sensitivity and lowering costs. Two recent developments include digitization, where discrete droplets are created and individually manipulated using complex systems, and low-cost porous materials, such as paper and thread that were proposed for global health applications. Here we propose string microfluidics that are based on (i) discrete droplets, (ii) simple setups and low cost materials, and (iii) operate under a different paradigm as the fluid is transported not by flow, but by the movement of the carrier string. Strings are patterned with hydrophilic reservoirs and hydrophobic barriers and manipulated manually or with simple mechanical setups to perform basic lab-on-a-chip fluid manipulation such as droplet creation, transport, splitting, and merging. Droplets can be rapidly mixed by repeated stretching, and arrays of reagents on a string can be rapidly transferred to one or multiple other strings, effectively copying the original droplet distribution, for performing chemical reactions or multiplexed analysis. Various microfluidic operations are demonstrated with string microfluidics, the reproducibility is measured, and the strengths and weaknesses of the platform are discussed. String microfluidics were then used for a glucose test and shown to replicate the results obtained by conventional analysis. String microfluidics has the potential to be developed further, and to be optimized for conducting chemical reactions or diagnostic assays.

Résumé

La microfluidique permet la manipulation et l'analyse d'échantillons très petits, amenant avec elle la possibilité de faire de la recherche sur des phénomènes précédemment impossibles à étudier, et améliorant la sensibilité et diminuant les coûts des méthodes de diagnostique. Parmi les développements récents, on compte la digitalisation, où des gouttes sont crées et manipulées individuellement en utilisant des systèmes complexes, ainsi que l'utilisation de matières poreuse comme le papier et les cordes qui ont été proposées dans le contexte de la sante publique mondiale. Nous proposons la microfluidique des cordes qui est basée sur (i) des gouttes discrètes, (ii) une organisation simple avec des matériaux abordable, et (iii) une nouvelle approche à la microfluidique qui consiste de transporter le fluide avec le mouvement de la corde plutôt qu'avec l'écoulement du fluide. Les cordes sont fonctionnalisées avec des réservoirs hydrophiles et des barrières hydrophobes, et manipulées à la main ou avec une installation simple pour reproduire les opérations basiques de « lab-on-a-chip » telle que la création, le transport, la division, et la fusion de gouttelettes de réactifs. Les gouttelettes peuvent être rapidement mélangées en étirant la corde plusieurs fois et un motif de réactifs peut être rapidement copié sur une ou plusieurs cordes en calquant sur une corde source dans le but d'accomplir des réactions chimiques ou faire multiples essaies. Plusieurs opérations microfluidique sont démontrées avec les cordes microfluidiques, la reproductibilité est mesurée, et les atouts et les points faibles du système sont discutés. Les cordes microfluidiques sont ensuite utilisées pour un test de glucose, montrant des résultats comparables aux méthodes conventionnels. Les cordes microfluidiques ont le potentiel d'être développé encore plus et optimisée pour des réactions chimiques ou des applications comme les diagnostiques médicaux.

Acknowledgements

I would like to thank David Juncker for his guidance throughout the project. In addition, I would like to thank the DJ group for providing a good environment to work along with valuable discussions and in particular Kwi-Nam Han and Mengying Fang for working with me on parts of this project. I would also like to thank my parents for their never-ending support and my sister for the inspiration she provides. Last but not least, I would like to thank the McGill University Biomedical Engineering Department, Grand Challenges Canada, and the National Science and Engineering Research Council of Canada for their financial support.

1.0 Introduction

Two recent trends in microfluidics include the use of porous materials such as paper and thread for low-cost fluid manipulation and digitization where discrete droplets can handle many assays in parallel. In digital microfluidics, a sample is broken up into discrete droplets, each acting as individual reaction wells, entirely independent from the other droplets. This is in contrast to continuous flow microfluidics where the entire channel from inlet to outlet must go through the same path carrying out the same assay. This digitization allows for greater multiplexing of assays, more efficient use of sample volume, and the ability to perform more complex assays requiring multiple different reagents.

Paper and thread devices have generally followed a continuous flow approach to fluid handling. In the next sections, we will present the fabrication and applications of thread and paper microfluidics, highlighting steps towards digitization as a background for our string microfluidics. We will then conclude the introduction with a discussion of the specific aims of the project as we bring some of the advantages of digital microfluidics to thread microfluidics.

1.1 Low-cost paper microfluidics

The push for appropriate technology for global public health led to the resurgence of paper microfluidic devices, often called μPADs (microfluidic paper-based analytical devices). μPADs can surpass many other devices according to the ASSURED criteria issued by the World Health Organization describing the ideal point of care device to be used in a resource-poor setting[1]. According to this criteria, an ideal test is Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Delivered to

end-users. Paper provides a medium that is readily affordable and can be designed to create devices that are simple and effective without the need for complex equipment or manipulations. The simplest of tests gives a rapid colorimetric readout, but can also include a handheld reader for better sensitivity and specificity. Importantly, paper devices are autonomous, running on their own power once the sample is added.

It is common knowledge that water will spread on a piece of paper, wetting the whole sheet if there is enough water. Paper microfluidics uses this phenomenon to flow reagents together and perform reactions. With carefully designed channels defined and functionalized in paper or composites of nitrocellulose and glass fibers on a polymer backing as in many lateral flow assays[2], many fluidic operations and assays can be performed. The main challenges in the field have included increasing the range of assays that can be performed with higher sensitivity and specificity, providing a quantitative readout, and integrating sample preparation in the same device.

From the beginning of its recent resurgence, paper microfluidics was marketed as the ideal medium for point of care diagnostics where the first paper demonstrated a urine glucose and protein test[3]. Another example is a blood typing device that takes 20 uL of blood to determine blood group[4]. In addition to medical diagnostics, paper microfluidics has been used for environmental testing[5], and food quality testing[6]. Other devices have been made such as batteries that can be incorporated into testing devices to power the readout or sensing mechanisms[7]. Many reviews have come out on the subject of paper microfluidics so more details on applications and sensor types can be found there[8,9]. This section will cover the main fabrication methods and fluid handling capabilities of paper

microfluidics, providing the context for the development of thread microfluidics without being comprehensive.

1.1.1 Creating Channels and Barriers in Paper

Because fluids will wick in every direction on paper, it is necessary to create channels to guide the liquid. The ways that have been developed to create channels can be categorized as either chemical blocking or physical blocking. Chemical blocking involves changing the surface chemistry of the paper such that the contact angle of water increases until the paper no longer wicks. Physical blocking consists of filling up the pores in the paper until there is no space for water to go through. While a physical method may also involve a chemical method if the blocking material has a higher contact angle, methods where the pores are still open will be categorized as chemical whereas any method where the pores are clogged will be categorized as physical blocking. As we compare these different methods, it is important to note that none of them are universal barriers as organic solvents or harsh cell-lysing conditions will cause barriers to leak[10].

The first chemical blocking methods involved making the entire paper hydrophobic, followed by making selected areas hydrophilic again. These methods have the advantage of maintaining the flexibility of paper devices and not being affected by mechanical deformation, which can crack physical barriers. Early work towards chemical pattern formation involved using paper additives to make the paper hydrophobic and creating a pattern with plasma treatment[11]. Alkyl-ketene-dimer (AKD), a cellulose-reactive chemical used to make the paper hydrophobic, was soaked into the paper and dried. A pattern cut into a stainless steel sheet was then transferred to the

paper using plasma treatment to make those sections hydrophilic. While this approach is successful at creating reproducible channels, it requires a plasma chamber.

Another method to make the paper hydrophobic is by coating it with poly(styrene)[12]. To etch channels into this paper, an inkjet printer was used to apply toluene, dissolving the poly(styrene) and re-exposing the hydrophilic paper. A drawback of this method is that the hydrophilic patterns need to be printed 10 times or more in order for the channels to wick water. Even so, this method only requires an inkjet printer, which is a less specialized setup.

More recently an inkjet printer was modified in order to print AKD directly into the desired hydrophobic patterns without needing to make the entire paper hydrophobic[13]. After printing, the AKD is cured to the cellulose at 100°C for 8 minutes and the devices are ready to be functionalized with reagents. Plasma activation is not needed because the channels that were not exposed to AKD remain hydrophilic. This technique has fewer and less complicated steps that could be adapted for low cost high throughput manufacturing.

Physical blocking methods are more common and more varied. The revival of paper microfluidics was started by using standard photolithography techniques to pattern channels in paper[3]. Instead of applying photoresist on silicon wafers, the photoresist is soaked into paper and exposed to create channels. After developing and washing, the device needs to be plasma treated to make it hydrophilic enough to wick reagents. While this method demonstrated the benefits of paper devices, it is not scalable, affordable, or easy to make. As a result, many other methods were developed to improve the fabrication process, only some of which will be covered here.

Cutting out the pattern for the device is sufficient for channeling fluids in the desired way. To create intricate patterns reproducibly, Fenton et al. used a knife plotter to cut out devices, which were then laminated to make them easier to handle[14]. In addition to patterning channels, the cutter was used to cut out information about the test directly onto the assay, providing greater ease of use.

The whitesides group developed another method to pattern paper involving the printing of a polydimethylsiloxane (PDMS) ink[15]. PDMS was dissolved in hexane and dispensed using an x-y plotter in order to print the channel walls. While this technique had the advantage of maintaining some of the paper's flexibility, which the photoresist does not do, the printing resolution is limited due to the creeping flow of the PDMS ink before it fully cures.

Wax patterning was among the first methods to define channels in a paper device[16]. To define the channels of their chromatography setup, Müller and Clegg transferred paraffin wax into the pores of a filter paper. Wax is still one of the most common materials used for defining channels in μPADs with the first recent description of the patterning technique by Lu et al. In addition to manually applying wax, they demonstrated using a wax printer to produce their devices[17]. After printing with the wax printer, the paper is heated at 135°C in an oven for 30 seconds in order to melt the wax into the paper. While this process reduces the resolution of the channels, it is possible to predict the spread of the wax in order to correctly design channels. Carrilho et al. reproduced the wax printing on Whatman no. 1 chromatography paper and melted the wax on a hotplate at 150°C for 120 seconds. It was found that a 300 μm line of wax was necessary in order to reliably get functional barriers and these would spread to a width of

850 μm on average. In addition, they showed that the edges of the barriers were uneven with a root-mean-square roughness of 57 μm for a 300 μm printed line[18]. However, the coarse nature of wax printing does not preclude it from being one of the most cost-effective ways to prototype μPADs as the materials are affordable and the process is very simple.

While the techniques discussed above are generally successful for rapid prototyping, they need to be adapted to be mass-produced in printing houses. Some techniques have been developed using flexographic printing that allows for high throughput printing. Flexographic printing is a contact printing method used by printing houses for high throughput production. Flexible printing plates are placed on rollers and transfer the inked pattern by direct contact. Various inks have been used such as a polystyrene solution[19], or a rapid curing PDMS ink[20]. While these techniques require the fabrication of printing plates and are not amenable to rapid prototyping, they do not require heat treatment as in the case of wax barriers and can be used for high throughput roll-to-roll fabrication of μPADs.

1.1.2 Flow Control in Paper Devices

In order to have a functional device, reagent flow must be controlled to have specific volumes coming into contact at specific times. In order to design devices, it is necessary to understand the physics governing flow along paper devices. This section will cover the basics of fluid flow along paper and the various methods that were developed to control the volume, mixing, and timing of fluidic circuits on paper.

The fundamental governing equation in the low Reynolds number stokes' flow in a porous medium is Darcy's law[21]:

$$q = \frac{\kappa WH}{\mu L}(\Delta P)$$

This equation describes the volumetric flow rate q as a function of κ, the average permeability of the paper, μ, the average viscosity, and ΔP being the change in pressure over the length L. The cross-sectional width and height are represented by W and H respectively. This equation can be used to determine the effect of varying the geometry of paper devices on the volumetric flow rate.

The time dependent transport of fluid along a paper strip is described by the Washburn equation[22]:

$$L = \sqrt{\frac{\gamma \cos\theta \, Dt}{4\mu}}$$

where L is the length along the strip of paper, γ is the surface tension, θ is the contact angle, D is the average pore diameter, t is time, and μ is the viscosity of the fluid. The characteristic behavior of washburn flow is that $L \propto \sqrt{t}$. This means that the flow rate decreases with time as the length of the wetted channel and the resistance increases. It illustrates the fact that a paper device has a limited functional length along which every operation needs to be completed before the flow is effectively brought to a standstill by the high flow resistance.

Qualitatively, thin film flow through small inter-fiber space is the primary mechanism of transport in unsized paper based on cryogenic SEM[23]. Based on this model, the fluid does not have a uniform flow front, but advances preferentially along the fibers. This rapid flow along the small interfiber spaces leaves air gaps in the larger pores between the fibers that are gradually filled in as the fluid is pulled further along the tight interfiber gaps.

Geometry and surface functionalization have been successfully used to time reagent progression through a circuit. The simplest technique to increase the time it will take for a fluid to arrive at a reaction zone is to make a longer channel for it. This increases the volume of paper that the fluid needs to saturate, and increases the flow resistance by increasing the *L* term. This method was used to sequentially deliver three reagents to a test zone[24]. Another way to delay flow is by having a widening of the channel. A device with a narrow channel opening into a wider channel will have slower flow than a device with a narrow channel of the same length. In this case, the wider channel requires more volume to fill, slowing down the fluid front while the straight channel does not have to fill in the extra volume. The third way to slow down a fluid front is to increase the viscosity, changing μ. By drying trehalose onto paper strips, the Yager group has successfully demonstrated slowing down water flow and timing the arrival of fluid[24]. This method has the advantage of not increasing the volume required to fill the channel and therefore does not increase the sample volume required.

The volume of reagents used in paper devices can be measured passively by the pumping action of a fixed volume of paper. For example, glucose and protein tests were performed on a set volume of 5 μL that were wicked up through a paper device. In order to measure this volume reproducibly, the device was placed in 10 μL of sample and allowed to wick the fluid up until the end of the channels when it was pulled out. Because of the constant surface area in each device, the volume wicked into the device was reproducible[25]. While this is possible for a single sample volume in a dead end channel, it cannot be done for mixing multiple reagents. In those cases, it is generally required to add the required volume into the device.

Stacking and folding paper opens up the realm of 3D devices where arbitrary timing of fluid delivery can be accomplished. First, passive devices were fabricated layered with double sided tape and paper to create complex channels crossing each other without mixing[26]. In order to create 3D networks from a single sheet of paper, Liu and Crooks adapted the concept of origami to make devices that were functional simply by folding them by hand[27]. These 3D networks have added flexibility in the design for many devices, allowing them to perform more complex operations. This concept was then further expanded, moving towards digitization with the slipPAD where a top layer was slipped along a bottom layer to reconfigure channels, allowing for greater multiplexing[28]. This device was a paper analog to the SlipChip[29] providing some level of digitization by introducing test reagents in individual reservoirs that are then slipped onto the sample channel in order to test for multiple analytes. Recently, a digital microfluidic platform was made on paper where silver electrodes were printed using an inkjet printer on hydrophobic paper[30]. This platform was able of performing similar functions as conventional glass digital microfluidic platforms but fabricated at a fraction of the cost and time.

1.2 Thread Microfluidics

Thread and textile microfluidics has been through many of the same developments as paper microfluidics. Three groups developed thread microfluidics in parallel, showing its similarity to paper microfluidics and some possible applications it could be used for[31–33]. There has also been a lot of interest in developing thread and textile outside of the microfluidics domain, in particular in the fields of wearable sensors and smart fabrics. Textile microfluidics is similar to paper microfluidics in that it is

planar, and requires patterning to define circuits. Thread microfluidics differs from paper and textile by having ready-made 1D channels that need to be assembled to create a circuit. However, textile and thread microfluidics are grouped together because their properties are similar. This section will cover the wide range of materials that have been used to develop thread and textile microfluidics along with the fluid operations that can be performed and their applications.

1.2.1 Materials Used in Thread Microfluidics

An important step when designing a thread microfluidic device is the choice of the materials. A great advantage of thread and textile microfluidics the wide range of fiber materials available. These different commercial threads and fabrics will significantly affect reagent transport rates by affecting the bulk flow rate and the specific interaction between the fibers and the solutes. The most common thread material used is cotton because it is white, can easily be sewn, and will adhere to common adhesives. In addition, mercerized cotton does not need to be plasma activated because it is already sufficiently hydrophilic for rapid flow rates[32]. Reches et al. studied the flow along various materials such as rayon, hemp, cotton, nylon, polyester, wool, acrylic, and natural silk before and after plasma activation. Materials such as wool, acrylic, and silk did not wick without plasma activation, while rayon, and mercerized cotton wicked water rapidly before treatment. Plasma treatment increased the wicking rate across the board. Materials such as hemp were not preferable because the brown color of the fibers is a challenge for colorimetric assays.

Fiber blends can be used to obtain the properties of multiple different materials in both thread and textile microfluidics. Some materials such as nylon fiber bundles

appeared to have non-fouling properties ideal for antibody release whereas antibodies adhere to cotton threads[34]. In the case of textile microfluidics, blends of polypropylene and poly (ethylene terephthalate) (PET)[35] as well as blends of hydrophilic silk and brass-coated hydrophobic silk have been used to control fluid flow[36].

In addition to the fiber material varieties, each of these materials can be further functionalized or modified for specific purposes. Plasma treatment is the most common modification that is used to make the surface more hydrophobic and increase flow rate. Processing steps such as mercerization can be used to increase the hydrophilicity of the thread. Bhandari et al. also showed that yarns with different numbers of twists per inch for optimized absorbance and wicking rate can be integrated together to form different sections of a device. Chemical functional groups can be added to fibers in order to give them more intrinsic properties. For instance, PET can be selectively modified with poly (2-vinylpyridine) (P2VP) or polyacrylic acid (PAA), achieving pH dependent wetting[37].

Another important material consideration is the type of support used to maintain the thread device's shape. Threads can be supported by polymer films[34,38], sewn into bandages [32], or integrated into fabrics integrating multiple types of thread[39]. In some cases, the support is secondary, such as in the case of the polymer film, where the support was only required to maintain its shape. In other cases, the support is integrated with the functionality of the device, such as when it is integrated into bandages. Recently, the trend has been to include sensors into wearable technology, pushing the integration of new microfluidic sensors with everyday apparel[40,41].

1.2.2 Flow Control in Thread Devices

It is generally well known that water will wick along the fibers of threads and textiles. While the morphology of the pores is different from those in paper, the flow follows the same general equations. In general, the fluid front will wick a distance *L* proportional to the square root of time *t*, following the Washburn equation just like paper devices[42]. This is because thread is also a porous medium that can be approximated as a group of capillaries in parallel and just as in paper devices, the resistance to flow increases as wetted length increases. However, the specific rate and dynamics of this fluid flow depends on the material that the thread is made of as well as the surface treatment.

Flow control using thread microfluidics takes advantage of the existing channels and flexibility offered by the thread. The thread offers ready-made channels that only need to be assembled in order to create functional networks. Depending on how the threads are assembled, different operations can be performed. The most common flow control feature is joining or tying threads together to create flow mixers or flow separators depending on the fluid flow direction. Safavieh et al. demonstrated that different knots joining two threads would determine the mixing ratio of the fluids being mixed[42]. These knots modulate the flow resistance of the threads, varying the flow rates based on the tightening tension. In addition, they demonstrated a gradient generator based on a knotted web or net of threads. In order to introduce real-time control on fluid flow, Ballerini et al. blocked a section of thread using glue to create on-off valves and flow selectors[43]. The thread was moved to bypass a blocked region to turn on the flow in on-off switches. In flow selector switches, the outlet was originally on the blocked

region of thread and either the left or the right inlet could be selected to connect with the outlet. While these are interesting concepts, none of these functional networks have been demonstrated in a practical application.

Textile devices must feature ways to direct liquid flow just like in paper devices otherwise the fluid will wick isotropically. Fabrics with different fiber blends and weave patterns can be used to direct flow as was shown by the anisotropic wicking of certain fabrics[44]. Owens et al. created fabrics with different weave patterns of a blend of hydrophobic polypropylene and hydrophilic PET, showing different fluid flow patterns as a result. In addition, they propose this platform as an efficient liquid-liquid extractor, as they demonstrate successful co-flow of water and dodecane which are immiscible[35]. Vatansever et al. also used a fabric blend of PP and PET and selectively modified the PET with P2VP and PAA in order to create a pH sensitive fluid flow direction. At neutral pH, water flowed mainly towards the PAA functionalized side, and at acidic pH, water flowed both towards the P2VP and PAA functionalized sides. While there remained a large amount of leaking because the contact angle change is not significant enough to completely stop fluid flow, this is a demonstration of what smart fabrics could do[37]. Another way to pattern a cloth is to melt a wax paper pattern into the fabric[45]. The wax pattern made using this method created well-defined 3D channel geometries by folding the cloth onto itself.

Xing et al. have recently developed the micropatterned superhydrophobic textile (MST) platform that is a thread microfluidics system that relies on Laplace pressure for discrete or continuous fluid flow. This platform consists of sewing a hydrophilic pattern into a hydrophobic fabric (contact angle of $140 \pm 3°$). The inlet pattern has a smaller

radius than the outlet pattern, creating the differential Laplace pressure resulting in fluid flow from the inlet to the outlet. The hydrophobic fabric is cotton coated with a perfluorinated polymer that was sprayed on while the hydrophilic thread is mercerized cotton washed in boiling water with soap and sodium carbonate. The resulting composite fabric can rapidly transport liquids from one side of the fabric to the other and concentrate it to a single outlet. In addition, the fabric remains highly gas permeable, allowing for gas exchange even in wet conditions [39].

Thread and textile fluid manipulation techniques developed thus far have taken advantage of the range of materials available but only involve continuous flow. While on/off switches were demonstrated, there has been no digitization of fluid manipulation like the slipPAD or paper digital microfluidics. This limits the potential for multiplexing and parallel operations. Longer branched devices for parallel assays are impractical due to the larger dead volume, greater flow resistance, and increased evaporation all becoming limiting factors.

1.2.3 Applications of Thread Microfluidics

Color-Based Assays

A variety of diagnostic assays have been performed on thread to demonstrate its compatibility with biological reagents. Most assays have a colorimetric readout that can be estimated visually or quantified by a scanner or phone camera. Simple chemical assays can be performed to detect protein, nitrites, ketones[32], and uric acid[38]. These assays require very small sample volumes because of the small volume of the thread used. For example, Li et al. used 0.1 μL of sample for a single nitrite assay. In addition, enzymatic assays can be done to detect levels of glucose and alkaline phosphatase[32].

Diagnostic assays can also be performed based on a color length change. These assays take advantage of the interaction between the fibers and the analyte in order to create or accentuate a color change along the length of the thread. The first example of this is a blood typing test[46] that adapted a previous paper-based assay to threads[4]. Polyester threads are coated with antibodies specific for each blood group and a blood sample is added to the center of the thread. If the corresponding antigen is present in the blood, the red blood cells will agglutinate and remain trapped in the thread as the blood wicks through the dried antibodies. This will result in a chromatographic separation with the red blood cells stopping before the serum fluid front in the presence of the antigen. Without the antigen, the red blood cells and the serum to not separate. The group also used a needle eye for blood dosing with a reproducible volume between 0.6 and 1 μL.

More recently, the same group adapted this color length measurement technique for other assays based on a chromatographic separation of the analyte and solvent[47]. The assay on thread was demonstrated for nickel in water as well as for protein and nitrite in simulated urine. This technique consists of coating the entire thread in reagent solution and applying the sample at the center of the thread. As the sample wicks along the thread, the analyte will react with the reagents on the thread and change color until all analyte is consumed. If the resulting product has a higher affinity for the thread fibers than for the solvent, it will stop before the fluid front at a distance from the origin that is proportional to the total amount of analyte in the sample. The variety of fiber materials and the different methods they can be treated is a great advantage for this technique as the affinity can be tailored to the colored product. This was an adaptation of earlier work on

paper which introduced the idea of measuring the length of a color change in order to quantify the amount of analyte in a solution[48].

Thread and fabric devices have also been used to perform sandwich immunoassays. Bhandari et al. created an immunoassay on a textile device[36] while Zhou et al. performed it on a single thread called the immunochromatographic assay on thread (ICAT)[34]. In both cases, the sample with the analyte flows through the detection antibody zone and brings it to both a capture zone and a control zone. The colorimetric readout is based on gold nanoparticles that give a red tint when bound in a large concentration to the fabric. The thread device used nylon fiber bundles to ensure the release of the detection antibody and cotton for the adherence of the capture antibody. In contrast, the textile device was all made of silk, but with few TPI in the detection antibody zone to maximize release and more in the capture antibody zone to maximize capture. In addition, the detection antibody zone was treated with surfactant and polyethylene glycol for better release. The ICAT demonstrated a better limit of detection of 9.82 ng/mL for C-reactive protein, which is comparable to many commercial and academic devices[34].

While most thread microfluidics work has been geared towards diagnostics, some work has been done to demonstrate its use in general purpose chemistry. Banerjee et al. demonstrated chemical synthesis of both an organic and a non-organic compound on cotton and silk threads[53]. Simple Y-channel devices were used to combine reagents to form ferric hydroxide and 2,4-dichloro-N-(2-morpholinoethyl) benzamide with a yield only slightly below that of a conventional test tube reaction.

Non-Color-Based Assays

While most thread and textile microfluidics have a colorimetric readout for ease of use and portability, a few designs have featured other readout methods. Electrophoretic separation and electrochemical detection was reported for the detection of ions[50]. Br^-, I^-, and Cl^-, was separated and detected on polyester threads in a carrier buffer. More recently, surface-enhanced raman scattering (SERS) using gold nanoparticles was adapted to a thread platform[51]. Cotton thread was coated with cationic polyacrylamide (CPAM) and gold nanoparticles in order to create SERS hotspots that were used to detect 4-ATP, ninhydrin, and phenolphthalein. The functionalization of the thread showed increased SERS activity, but due to the random clustering of the gold nanoparticles and the variation in local environment, the variation in signal intensity was a limiting factor for practical applications. More recently, thread-based wearable sensors have demonstrated potentiometric measurements of pH, sodium, and ammonium[52] without being significantly affected by being embedded in a fabric. Cotton threads were soaked in carbon nanotube ink and coated with an ion-selective membrane in order to be sensitive to a single ion. The functionalized thread electrode could then be integrated in a Band-Aid and added to a textile without affecting the response of the electrode to solutions of different potassium concentrations.

While new measurement modalities for thread open up new possibilities, most are similarly limited in reproducibility or portability when compared to colorimetric measurements. Random variations in fiber size and orientation within the thread are impossible to account for and will affect both SERS and electrochemistry. Guinovart et al. appear to have limited the variation caused by mechanical deformation of the thread by using carbon nanotubes that will maintain their conductivity while deforming with the

thread[52]. However, the setup is currently not fully wearable as there is still the challenge of integrating a reference electrode. As a result, along with SERS and electrophoresis on thread, it is not yet portable. SERS requires an extensive optical setup and electrophoresis requires a power supply, reducing portability and ease-of-use.

Wearable Sensors

Thread and textile microfluidics can help with the integration of sensors monitoring health and performance in our daily lives. There are many electronic devices that can be used to track movement and physical activity, but chemical analysis remains a challenge. Textile and thread microfluidics can tackle some of the challenges by providing an integrated sampling and analyzing platform that can be worn throughout the day or during exercise. Sweat is a readily available body fluid that may provide biomarkers for many different conditions and can be readily sampled and analyzed by an integrated sensor. For example, real-time sweat pH analysis can be carried out by a composite microfluidic device that collects sweat through a textile patch and channels it via a laminated thread to a colorimetric detection area [49]. The color is measured by paired emitter-detector LEDs and relayed wirelessly to a computer achieving a run time of about 1 hour. Challenges that are still present in the field of wearable biochemical sensors include the lack of reliable biomarkers and limited autonomy of the device that cannot run for very long and is not very robust.

The MST demonstrated an improved sweat collection system by continuously drawing fluid from a specific area to a collection reservoir. The composite fabric made of hydrophobic fabric and hydrophilic thread has high gas permeability even when wet,

which increases comfort of skin areas experiencing heavy perspiration and could be used for controlled biofluid removal from specific areas.

1.3 Project Rationale and Description

The goal of the project is to bring digital microfluidic functionality to thread microfluidics to improve the ability of threads to perform multiplexed and multistep assays. It is clear from the quick review on thread microfluidics above that the unique capabilities of thread have not yet been harnessed for microfluidics. Most devices mirror paper microfluidics in form and function without providing the significant advantages that would justify adapting to the new material. In particular, digitization has not been developed in thread devices. Recently, some interest has been shown in the field of multidimensional microreactors[54] which could be addressed by digitized fluid handling on threads. Therefore, the aim was to develop techniques to digitize fluid manipulation on thread in order to mirror the capabilities of digital microfluidics.

In a typical digital microfluidic platform, the water droplets are manipulated between two parallel insulator plates. Electrodes embedded in a dielectric material actuate droplets by changing their contact angle via electric fields in a method called electrowetting on dielectric (EWOD). This allows for the individual manipulation of many discrete droplets. The first operations in digital microfluidics that were considered the building blocks of a lab on a chip included creating, transporting, splitting, and merging droplets[55]. To accomplish these operations on thread without electric activation, many challenges need to be addressed. First, reservoirs need to be defined on the thread such that samples do not flow along the thread and mix. Then, discrete droplets need to be formed by loading the reservoirs with fluid. Then, transfers between full reservoirs as

well as between a full reservoir and an empty one need to be demonstrated using only mechanical forces for droplet merging and splitting. Transport is be accomplished by moving the thread to the desired position in order to merge, split, or load the right droplet. In summary, the goal of the project was to develop a platform called string microfluidics that can create, transport, merge, and split individual droplets of fluid and demonstrate biological assays with it.

In this thesis, we will first cover the choice of threads and the methods used to create discrete reservoirs on thread. Once leak-proof reservoirs are demonstrated, we will describe the operations that can be done using these reservoirs, including splitting droplets, one-way transfers, mixing, and various copying methods. Next, we will describe proof of concept biological assays. Finally, we will delve into a quantitative analysis of the fluid operations and discuss the strengths and weaknesses of the platform.

2.0 Materials and Methods

There are many different materials that threads can be sourced from. Natural fibers include plant fibers such as cotton and hemp, and animal fibers such as wool and silk. Synthetic fibers are generally made from petrochemicals, such as nylon and polyester. Depending on how these fibers are made and processed, they can have many different properties. Qualitative testing of a large range of threads was done in order to find those with the most promising qualities.

The first parameters of interest were hydrophilicity or hydrophobicity. To test for this, a drop of dyed water was applied to the thread and the flow behavior was observed with the naked eye and the inspection microscope. Other parameters became interesting

later on, such as the stretch of the thread, the change in volume with tension, and the volume of the thread. Adding a droplet of water on the thread and stretching it was a rapid test to tell whether the thread stretched and decreased in volume under tension. By performing this rapid qualitative test, two threads, Oral-B ultrafloss and woolly nylon overlock thread were selected for more in depth testing because of their unique properties.

The Oral-B ultrafloss is coated with lubricants, scented oils, and dye that had to be washed off. Various methods were tried, but in the end, sonicating with 70% ethanol for 10 minutes was the preferred method to wash the threads. The threads were then rinsed in DI water and dried with a nitrogen gun. While it is not apparent that the woolly nylon overlock thread has any coatings, the same washing procedure was followed to normalize the treatment between the two threads.

In order to verify the level of autofluorescence in the polymer forming the thread, the Oral-B ultrafloss was observed under the Nikon Eclipse TE2000-E inverted fluorescent microscope. Both the original thread and the hydrolyzed thread treated with 10% hydrochloric acid were observed with multiple excitation wavelengths in order to detect any trends in autofluorescence. Fluorescence intensity was measured using ImageJ.

2.1 Creating Reservoirs on Strings

To create reservoirs for string microfluidics, it is necessary to create barriers to fluid flow that will work throughout tension cycles. This is a challenge because when the thread is under tension, the fluid in the reservoir is pushed out by the fibers coming closer

together, adding to the fibers' natural tendency to wick fluid along the thread. In addition, the barrier needs to withstand the mechanical stress from the tension and relaxation cycles that occur repeatedly. The ideal barrier is low-cost, storable, does not impede the ability of the thread to roll up in a bobbin, creates reproducible reservoir volumes, and is easy to fabricate in large quantities. Fabrication details of each method can be found in the next two sections where they are broken down into mainly physical blocking methods or chemical blocking methods.

2.1.1 Creating Reservoirs on Strings by Physical Blocking

Because wax printing is currently one of the main methods used to block paper channels, various waxes were tried for threads. Paraffin wax was powdered and applied to specific locations along a thread. Using a block of aluminum at 60°C, the paraffin wax powder was melted into the thread to form a solid barrier. Another wax patterning method used Logitech thin film bonding wax 0CON-193 because of its low melting temperature and its solubility in an ethanol solution. This wax was dissolved in 70% ethanol at 55°C to create a wax tincture. This tincture was then applied to the thread using a pipette when it was hot as well as when it was cold. When the ethanol evaporated, only the wax was left, coating the threads.

Various glues and adhesives were tested to create barriers because of their ability to fill the pores. These included epoxy, hot glue, a photocurable polymer (Norland Optical Adhesive, NOA), and nail polish. A two part rapid curing epoxy was premixed and dabbed onto the thread at the desired location using a toothpick. Hot glue was applied directly to the thread using a glue gun with the help of a toothpick. NOA was applied to the thread using various patterning techniques and then exposed to UV light in

order to cure. Patterning techniques were required in order to increase the throughput of the technique and ensure that the NOA did not wick along the thread, creating irregular sized wells. These techniques included masking the reservoirs with black electrical tape such that only the barrier would be exposed to UV light and therefore only the barrier would be cured. Another technique involved displacing the NOA with mineral oil such that it would only be localized in the barrier. After curing under UV light, the thread was then washed under running water and with a 1% Nalgene solution in order to remove the oil. Finally, clear nail polish was used as a barrier as well. While the first formulations of the barrier were made only with nail polish, it was easier to pipette for a longer period of time if it was diluted with 50% acetone by volume. A few μL could be used to block the thread, with the size of the barrier increasing with the volume of nail polish solution added.

Melting the thread or heating it up to a more plastic state could change the texture of the thread. By doing so, the thread fibers could be straightened, reducing their ability to stretch. While this was not sufficient to stop fluid flow on its own, it was combined with other methods in order to increase the barrier's resistance to fluid flow.

Just like water in oil droplet microfluidics, oil was tried as a blocking agent to form barriers between reservoirs. However, mineral oil had a tendency to flow along the thread. Therefore a mixture of silicone oil and silicone grease was created that would not flow along the thread like the oil and that would not break apart like the grease. This mixture was applied to the thread using a syringe and was worked into the thread to fill in all the pores using tweezers.

The final physical blocking method that was tested was Cytop CTL 809M. This fluoropolymer binds to a surface like a silane and creates both a physical barrier and a hydrophobic coating. Prior to application, the thread was sonicated in 70% ethanol and 10% HCl for 10 minutes each, rinsed in deionized (DI) water, and dried using a nitrogen gun. 5 uL of the 9% solution was then pipetted onto the thread and placed in an 80°C oven for two hours and a half.

2.1.2 Creating Reservoirs on Strings by Chemical Blocking

Chemical functionalization of the nylon was also explored in order to create reservoirs along the thread. In a first attempt, Neverwet®, a commercial superhydrophobic coating was applied to the thread. The product involves a primer coat that adds roughness to the surface and a hydrophobic coat that are both applied using an aerosol can. Due to the solvents in the coats, it was challenging to limit the spread of the coats once it was on the thread. Various masking techniques were tested, such as slits in paper, but the Neverwet® would spread once it was on the thread.

Another chemical treatment that was tested was silanization of the nylon thread. Because of its hydrophobic nature, trichloro(1H,1H,2H,2H-perfluorooctyl)silane was used. The first time this treatment was tried, there was no effect on the hydrophobicity of the thread. However, after washing the thread and activating the surface with 10% hydrochloric acid, the thread was made hydrophobic by silanization. After trial and error, the final protocol for silanizing thread is as follows. First the thread is sonicated in 70% ethanol for 10 minutes, rinsed in DI water, and sonicated 10% hydrochloric acid for another 10 minutes. The thread is then rinsed in DI water, dried with a nitrogen gun, and placed in the desiccator for an hour with three drops of the silane. After two hours, the

thread is hydrophobic along its entire length. Adding surfactants creates the reservoirs. After trial and error, the best reservoir solution was adding a total of 4 μL of 0.05% tween 20 in 1x Phosphate Buffered Saline (PBS) to the thread at the desired interval. Due to the hydrophobicity of the thread, it helps to tweeze 2 μL of the solution into the thread first, then add the remaining 2 μL and stretch the thread a few times to straighten out the fibers again.

2.2 Protein Assay on String

A tetrabromophenol blue protein assay was performed according to the protocol described in the ground-breaking paper microfluidics paper by Martinez et al. [3]. The assay was reproduced as in this original paper. The assay reagents are prepared as a primer solution and an indicator solution. The primer solution consists of a 92% water and 8% ethanol solution by volume with a 250mM citrate buffer at a pH of 1.8. The indicator solution is 95% water and 5% ethanol by volume with 9mM tetrabromophenol blue.

To perform the assay, the reservoirs are prepared with a 3 uL 0.1% tween 20 solution in 1x PBS on silanized Oral-B Ultrafloss. 0.5 uL of primer solution is added to the reservoir and allowed to dry. 0.5 uL of the indicator solution is then added to the primed reservoirs and allowed to dry. A serial dilution of Bovine serum Albumin (BSA) in 1x PBS was prepared and 5 uL of each BSA solution is added to a reservoir and mixed by tension cycles. The assay is then scanned after 20 minutes on a desktop scanner. The assay was then repeated on woolly nylon with the same conditions except the concentration of tween 20 was brought down to 0.05% in order to reduce potential interference with the assay.

Colorimetric analysis was used to measure the BSA concentration. Following the technique established by Martinez et al., the image was converted to CMYK color using Photoshop and the cyan intensity was measured in each reservoir. Each cyan intensity measurement was then subtracted from the cyan intensity at 0 μM BSA concentration.

A commercial Bradford assay kit was used as a total protein assay to verify the results of the tetrabromophenol blue assay. The coomassie plus reagent for the Bradford assay was purchased from thermo scientific and was used according to their standard protocols both to verify the concentration of the serial dilution of BSA and to test the compatibility of thread with the assay. The protocol for microplates was followed for the BSA standard. In short, 1 μL of BSA solution was added to 30 μL of coomassie plus reagent and mixed by pipetting. The absorbance at 595nm was then measured after a 10-minute incubation period at room temperature.

The Bradford assay was also performed on woolly nylon reservoirs created with a 0.05% tween 20 solution in order to stay within the upper limits of surfactant concentration. In short 6 uL of coomassie plus solution are added to the reservoir followed by 0.2 uL of a BSA solution. The assay is mixed by stretching and incubated for 10 minutes before transferring the solution to the spectrophotometer for an absorbance measurement at 595nm.

2.3 Glucose Assay on String

The glucose assay that is commonly used involves the oxidation of iodide to iodine resulting in a brown color. Iodide is oxidized with the help of horseradish peroxidase, using hydrogen peroxide as the oxidizing agent. The hydrogen peroxide is

one of the products (along with D-glucono- δ -lactone) of the oxidation of glucose by glucose oxidase.

The glucose assay solution consist of a 5:1 solution of glucose oxidase to horseradish peroxidase (150 units of glucose oxidase to 30 units of horseradish peroxidase), 0.6 M potassium iodide, and 0.3 M trehalose in a pH 6 buffer in DI water. A glucose serial dilution was prepared in 1x PBS and a standard curve in a test tube was created by adding 5 μL of glucose solution to 2 μL of glucose test solution and incubating for 10 minutes. While the results of this assay are usually determined by color intensity, it the color was measured by absorbance at 440nm in this case.

To perform the assay on thread and to avoid interaction between the hydrophobic threads and the enzymes involved in the reaction, it was necessary to block the thread. BSA, a common blocking agent was added in the formation of the reservoirs. The reservoir solution was therefore 1% BSA and 0.05% tween 20 in 1x PBS. 2 μL of the solution were added to the tip of tweezers in order to press the drop in to the hydrophobic thread. Then, an additional 2 μL were added in order to fill out the reservoir. 1 μL of the glucose test solution was directly added to the reservoir and the thread was allowed to dry in the dark. 5 μL of glucose test solution were then added to the reservoir once dry, mixed, and allowed to incubate at room temperature for 10 minutes. A droplet was then transferred to the nanodrop spectrophotometer for an absorbance measurement at 440nm. The colorimetric analysis was also carried out by imaging the reservoirs and measuring the mean RGB color intensity. The mean intensity value was then subtracted from the 0 mM glucose sample.

2.4 Quantitative Fluid Flow Tests

While qualitative demonstrations of fluidic functions were the first step, it became important to quantify these operations to determine how reproducible and reliable they were. To measure the amount of fluid loaded into a reservoir, a 500 μL droplet of water was placed on a weigh boat and massed. The patterned thread was placed on the bilateral tension setup to keep the reservoirs from moving throughout tensioning and loosening. The sample was then applied to the thread, and then massed again after the loading procedure. By subtracting the final weight from the initial weight, the weight of water loaded into the reservoir could be measured. This was done at constant time intervals to correct for evaporation during that time period. Series of evaporation measurements were conducted before and after measurement period and periodically in between. For these assays, the reservoirs were made in silanized ultrafloss using 0.1% pluronic instead of Tween 20.

Fluid transfers were also characterized quantitatively to see how reproducible multiplexed and multistep assays could be. Single transfers from one reservoir to another can be measured with the change in mass of the reservoirs. A known volume of water is added to the donor reservoir and transferred to the receiving reservoir on a weigh boat that is massed before and after the transfer. Evaporation is taken into account by measuring the average evaporation rate over the time interval that the change in mass of the receiving reservoir is measured in the same way that it was accounted for during the loading trials. Serial transfers were tested with a single donor and many receiving reservoirs on weigh boats, measuring the mass before and after of each receiving reservoir as they received their droplet.

3.0 Results and Discussion

Developing digital microfluidic functionality on thread involved multiple steps. First, discrete droplet reservoirs had to be defined in thread with hydrophilic reservoirs and hydrophobic barriers as seen in Figure 1 and section 3.2. These patterned threads could then be used to perform a range of operations like copying, transferring, and mixing as shown in Figure 1a-d and section 3.3.

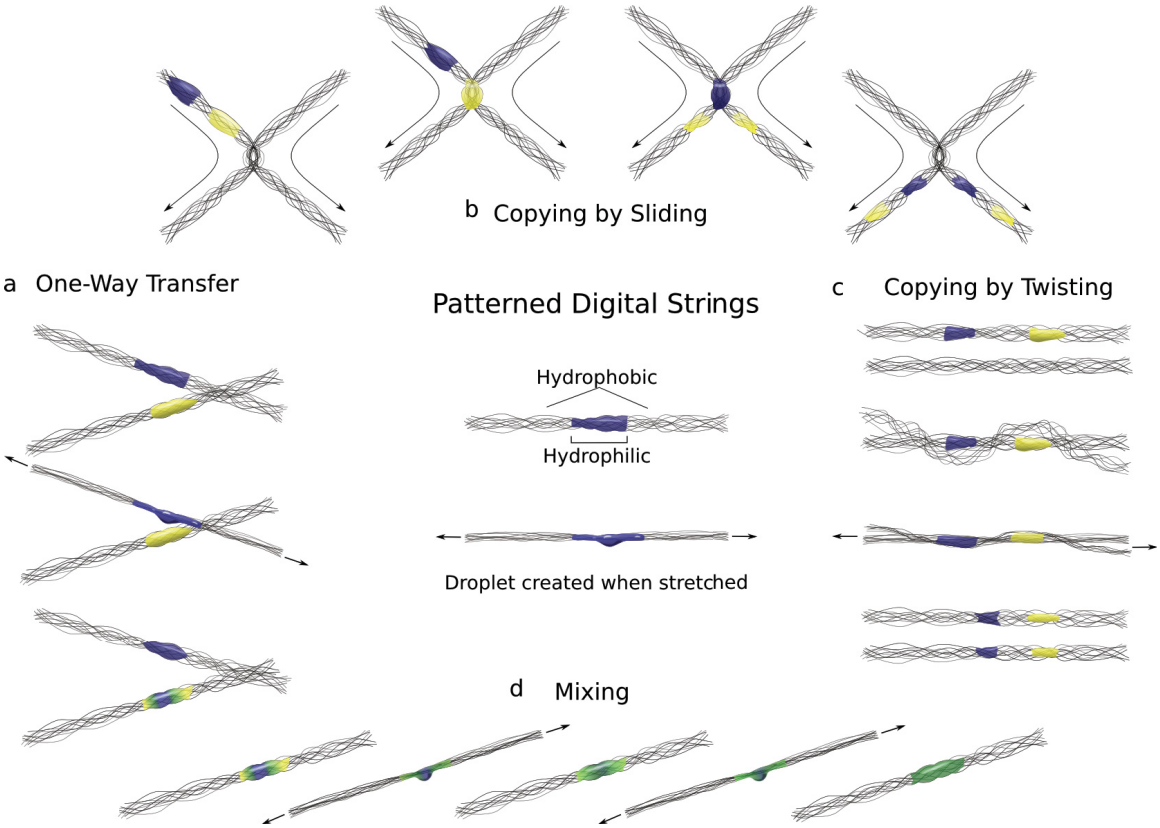

Figure 1: Summary of string microfluidic operations. *The patterned digital strings have hydrophilic reservoirs separated by hydrophobic barriers that create a droplet when the string is stretched as seen in the center. a) This allows for one-way transfers between two filled reservoirs as the fluid in the stretched reservoir is extruded out and transferred. b) Copying a pattern of reagents on one string can be rapidly accomplished by sliding another thread along the patterned string. c) Twisting and stretching can copy a loaded string to multiple blank strings. d) After transferring, the solution in the reservoir can be rapidly mixed by stretching cycles.*

3.1 Fluid Flow in String

Studying the flow properties of different threads showed that there was a range of hydrophobicity and hydrophilicity. Most threads exhibited generally uniform flow behavior under tension and along its entire length. However, textured nylon threads demonstrated drastically different flow characteristics under different tension and these were selected for further study.

Woolly nylon overlock thread and Oral-B Ultrafloss are both textured synthetic threads with highly crimped fibers that gives the thread extra volume. The crimped or curvy fibers allow the thread to contain a large volume when loose and a much smaller volume when taut as seen in figure 2.

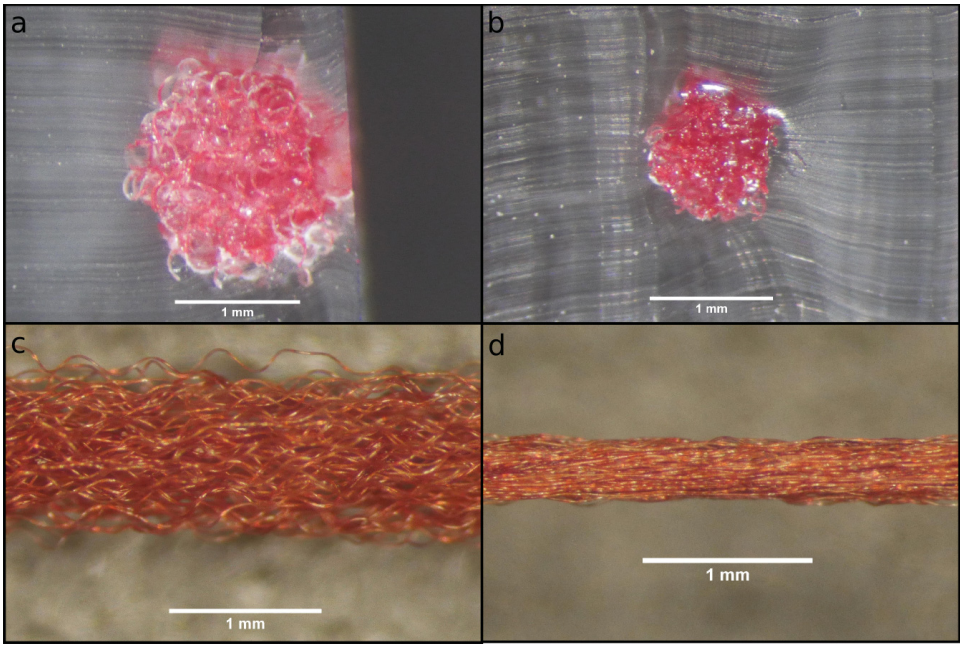

Figure 2: Ultrafloss side view and cross section when loose and taut. *All threads were dyed red for better visualization. (a) Cross section of loose ultrafloss. (b) Cross-section of taut ultrafloss. (c) Side-view of loose ultrafloss. (d) Side-view of taut ultrafloss. All scale bars are 1mm.*

When looking at the flow front under a microscope, as in figure 3, it becomes apparent that the fluid does not move uniformly along the cross section of the thread and

in fact, a fluid front is hard to define. The fluid will rapidly advance along fibers that are close packed, advancing ahead of the rest of the fluid front. If the fibers get farther apart, the fluid flow slows down until enough fluid builds up to jump the gap to another close packed bundle of fibers. From there, the fluid can flow both forwards, advancing the fluid front, and backwards, filling in the gaps left behind by the rapidly advancing films of water. In this way, the thread may not be saturated even if the fluid front is multiple centimeters further. When the thread is not taut enough, or a group of fibers is twisted away from the rest, gaps form that are too large for the fluid to bridge, and pockets of unsaturated thread can remain within an otherwise saturated thread. This fluid flow behavior matches that of thin film wetting of paper substrates where thin films of liquid rush along the very tight inter-fiber junctions before filling in larger pores [23].

This non-uniform flow may have important consequences for timing operations, volume measurements, and mixing. The chaotic flow front means that the fluid may be rapidly mixed by flowing along the thread, but it also means that a sample may be dispersed and have a lower effective concentration as it flows along a thread. The non-uniform filling of the thread adds challenges for specific volume measurements and timing, as it is hard to predict the arrangement of the fibers and small differences between similarly fabricated threads will cause timing and volume differences.

Another important observation when looking at the fluid flow in figure 3 is that flow along the thread is much faster than flow across the thread. It takes 1.4 seconds to saturate the entire cross section of the thread at the point of contact with the reservoir, whereas the fluid has moved at least an order of magnitude further along the thread.

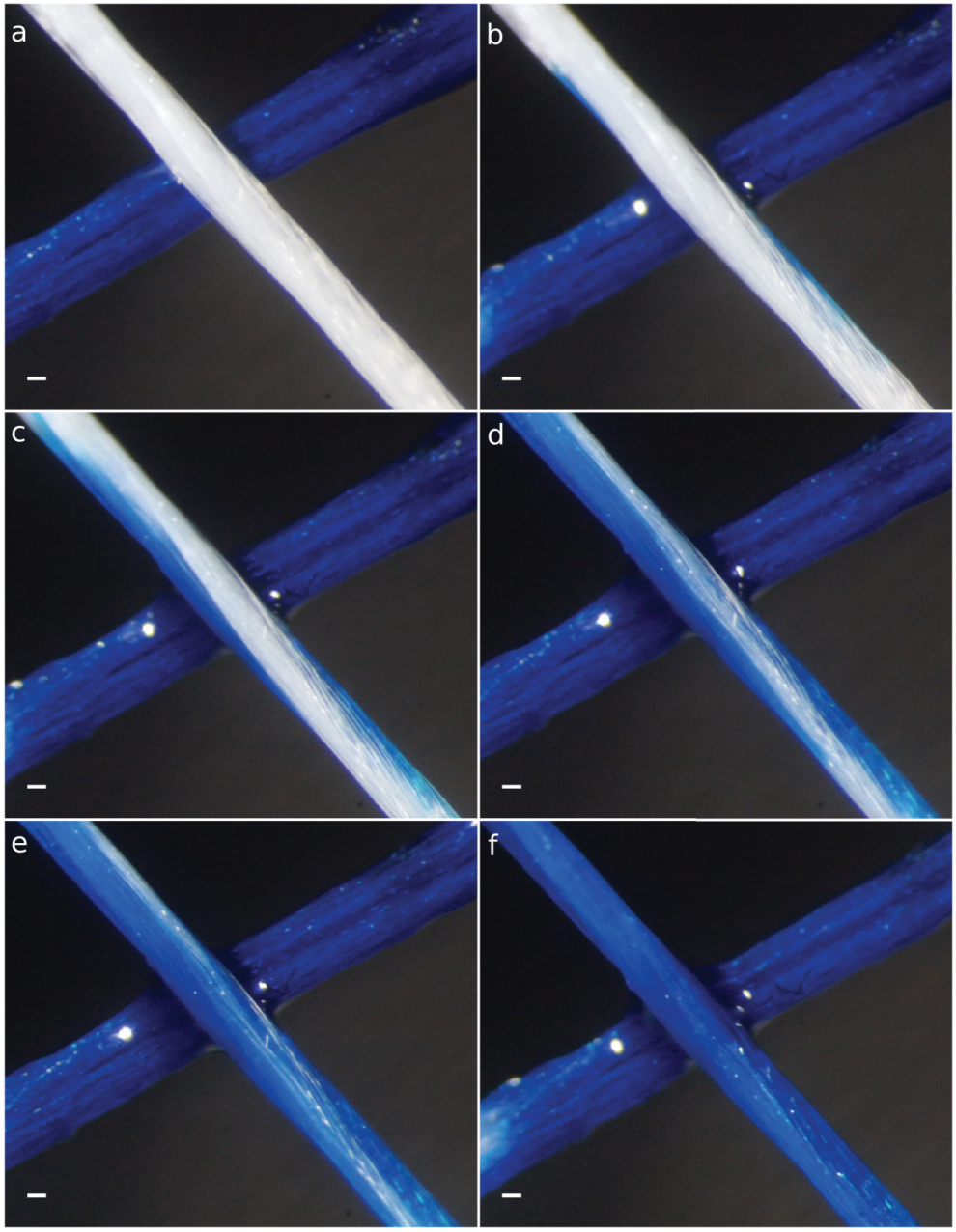

Figure 3: Fluid flow along Ultrafloss. *(a) Initial contact between the white thread and the reservoir of blue dye. T = 0s. (b-f) progression of fluid flow along the Ultrafloss at time (b) t = 0.27s, (c) t = 0.47s, (d) t = 0.80s, (e) t = 1.00s, and (f) t = 1.4s. Scale bars are 100 μm.*

The fluid flow's dependence on inter-fiber spacing allows for tension-triggered flow. When the thread is loose, it does not wick because the fluid rapidly runs into discontinuities and large fiber spacing. However, as the string is stretched, the inter-fiber spacing is decreased, increasing the capillary pressure, and the number of discontinuities

drops. This results in tension-triggered flow where the flow can be started and stopped by applying tension to the thread. A loose thread with large pores will pin the fluid and stop fluid flow as seen in figure 4a-b. Stretching the string will decrease the pore size and the fluid will flow, figure 4c-d. It is important to note that the tension does not in fact deform the fibers themselves but simply aligns them by reducing the kinks and curves. In this regime, when the thread is loosened, it will regain its original conformation within a few seconds. However, if the thread is put under too much tension, inelastic deformation will occur and the thread will not return to its original conformation.

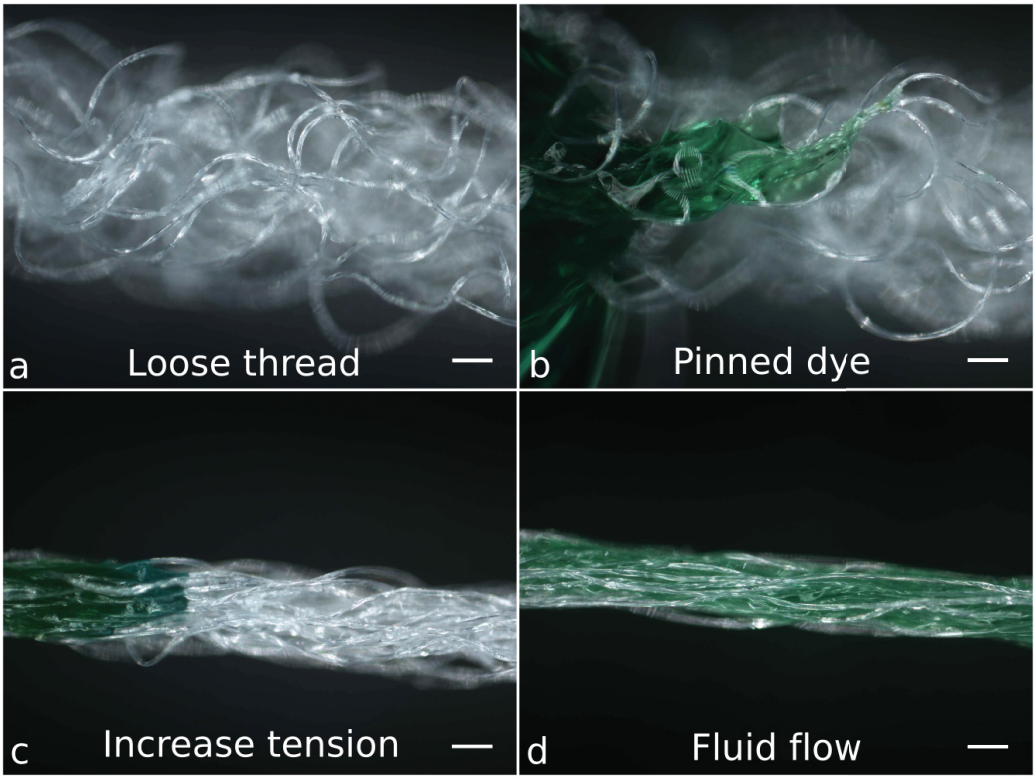

Figure 4: Tension triggered flow. *(a) Loose woolly nylon thread. (b) Addition of green dye, but no flow happens because it is pinned. (c) Increase in tension to the point just before fluid flow. (d) Taut thread resulting in fluid flow. Scale bar is 100 μm*

3.2 Reservoirs on String

While tension-triggered flow is an interesting research topic to pursue, our goal was to perform combinatorial operations, requiring distinct reservoirs on the thread. This required creating hydrophobic and hydrophilic zones that broke the flow along the thread. As discussed in the materials and methods section, many different ways to do this were investigated and they are summarized in table 1.

Table 1: Strategies that were tested to create reservoirs on thread.

Blocking strategy	Notes	Results
Paraffin wax	Powder on the thread and melt at 60°C	Blocks fluid flow when loose, but crumbles with tension cycles
Logitech thin film bonding wax 0CON-193	Dissolved in 70% ethanol at 55°C and applied when hot or at room temperature	Works best when tincture is applied hot, but crumbles with tension cycles
Epoxy	Mix two parts and apply to area to block	Hard to apply and fluid accumulates on the barrier
Hot glue	Apply with a glue gun to the thread	Hard to apply and fluid accumulates on the barrier
Norland Optical Adhesive	Apply to area to block and expose to UV light	Hard to apply and fluid tends to wick along the barrier
Nail Polish	50% nail polish and 50% Acetone by volume to make it easier to dispense	Easy to apply and blocks fluid flow well, even with tension cycles
Melting thread	Use a hot soldering iron at 200°C	Can be a minor help to block fluid flow but is hard to apply
Silicone grease and oil	Mix to a good consistency	Good blocking but hard to

	and tweeze onto the thread	work with
Cytop CTL 809M	Apply to thread and cure in 80°C oven for 2.5 hours	Equivalent results to nail polish, but more complicated fabrication
Silane	Clean and hydrolyze thread before silanizing with trichloro(1H,1H,2H,2H-perfluorooctyl)silane	Long fabrication, but reliable blocking and easily stored. Requires surfactant
Neverwet®	Apply both coats multiple times	Reasonable blocking, but the barriers will fail with tension cycles and patterning is a challenge

The wax patterning methods generally worked for static applications, but once the threads were stretched, the barriers began to leak. Applying paraffin wax to a specific location on thread also proved to be an unwieldy process, which could not be easily done by a wax printer because of the thread's 3D nature. The resulting barriers were very irregular as a result as seen in figure 5d. The wax tincture method worked best when the solution was hot and most of the thin film bonding wax was in solution. As the solution cooled down, most of the wax would precipitate resulting in very thin barriers when applied to the thread. However, the main issue with the different wax solutions is that the final barrier had a tendency to crumble through the tension cycles, and once it was cracked, it was not hydrophobic enough to keep the fluid in the reservoir from spreading.

The epoxy, hot glue, and NOA all leaked due to the liquid flowing on the surface of the barrier. This may be due in part because it was hard to apply a thin layer of the adhesive, and the resulting large barrier created a low point where water would collect by

gravity. In addition, the surface was not hydrophobic, so in the cases where the barrier was smaller, the fluid still wicked on the surface. It was thought that this might be from the surface texture caused by the texture of the thread itself. However, even after retexturing or melting the thread with a heated soldering iron, leakage could occur over the glue barrier. In addition, the difficulty in applying these adhesives to the thread meant that they were not suitable for creating many reservoirs.

The silicon oil and grease mixture was successful at blocking fluids from wicking along the thread when properly applied in the right proportions. When the mixture had too much grease, the barrier would crumble under tension cycles. When the mixture had too much oil, the barrier would spread and emulsions would be formed with the fluid in the reservoir during tension cycles. However, under the right conditions, the barrier was solid and was the first method used to develop digital thread microfluidic functions as seen in figure 5c. The main drawbacks of the method are the serial fabrication and the difficulties in storing and manipulating the strings once the reservoirs are defined. Having to tweeze each individual barrier requires a large amount of time and they cannot be made in advance because they cannot be stored without smearing the grease. In addition, because the barriers cannot be touched at the risk of spreading silicone grease everywhere, the manipulation is delicate. Thus, this method was a good proof-of-concept for string microfluidics, it cannot easily be used with mechanized platforms that use thread bobbins to automate the operations and therefore had to be replaced.

Cytop successfully blocked fluid flowing along the thread, but was not an improvement on nail polish, and was therefore not pursued any further. The process to

fabricate the barriers was much longer and more expensive for no noticeably different results.

The nail polish solution in acetone was much easier to apply to the thread and much more successful at blocking fluid flow. This solution could fill in all the pores of the thread without creating a large barrier onto which water would accumulate. In addition, it was strong enough to resist the stresses caused by the tension cycles without cracking. This solution had the big advantage that it could be stored on a thread bobbin and used later even if it introduced rigid kinks in the bobbin. The nail polish barrier could resist leaking for a large number of tension cycles and was further investigated for its liquid handling ability.

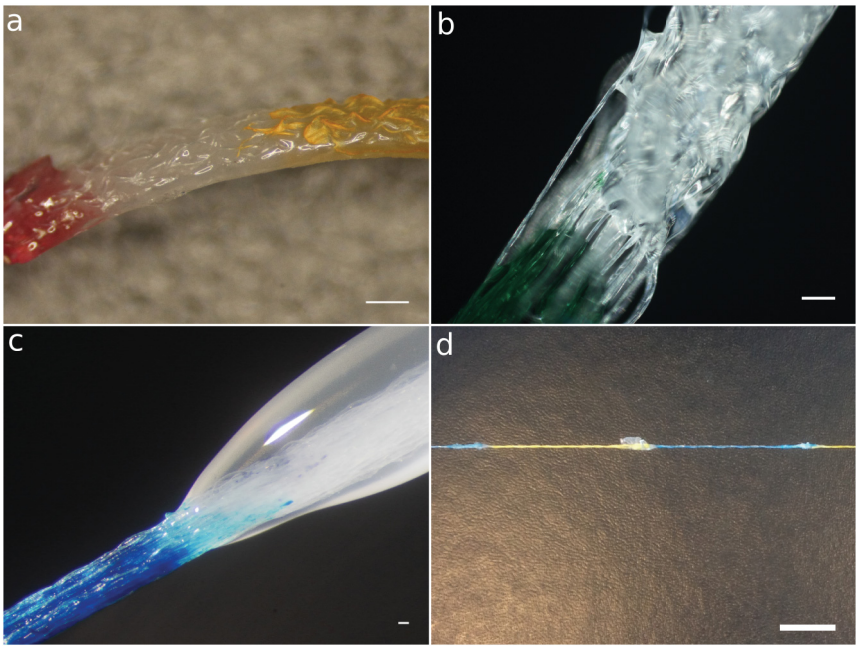

Figure 5: Physical blocking methods. *(a) NOA barrier on ultrafloss. (b) Nail polish barrier on woolly nylon thread. (c) Silicone oil and grease barrier on ultrafloss. (d) Paraffin wax barriers on polyester thread.*

Based on further experimentation with the nail polish blocking, it rapidly became apparent that physical barriers that impeded the stretching of the nylon could not be used.

As shown in figure 6a, when a nail polish reservoir is stretched, the fluid forms a dumbbell shape with droplets on the edges of the reservoir. This is due to the fact that the fibers close to the nail polish remain far apart even when under tension. This configuration of droplets makes it very hard to align reservoirs for transfers and only half of the reservoir can be transferred at a time. In addition, mixing efficiency is significantly reduced, as the left half of the reservoir will always form the left-hand droplet whereas the right half of the reservoir will always form the right-hand droplet. Attempts to make the reservoir smaller to merge the two droplets together in the center of the reservoir makes the reservoir impossible to stretch and create a droplet as all the fibers are held apart by the nail polish barriers. It was therefore decided that a chemical barrier was the only way to create droplets as in figure 6b where all of the fluid joins into the droplet at the center of the reservoir.

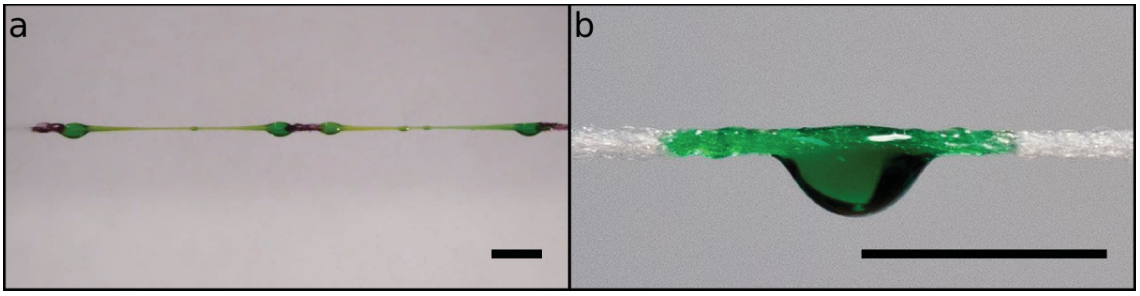

Figure 6: Physical blocking issues. *(a) Nail polish (dyed black) defines the reservoirs. Under tension, the fluid forms a dumbbell with droplets accumulating on the edges of the reservoir. (b) A chemical barrier allows the thread to stretch and the droplet remains at the center of the well. Scale bars are 5mm.*

The Neverwet® barrier was extremely resistant to water droplets entering the thread. However, once the droplet was in the thread, it could still spread with tension cycles. As the application process involves spraying an aerosol of each solution onto the thread, it is possible that even though the coats were applied until the thread was saturated, mostly the outside was coated in Neverwet without affecting the inside of the

thread. This is likely to have left channels in the middle of the thread that allowed the water to leak through. The Neverwet® coating also highlighted the challenge that fluids will travel along the thread much faster than they will cross the thread. This implies that while a thread might be difficult to wet in the first place to get liquid into the reservoir, once it is wet, it will have a tendency to flow.

The reservoirs created from the silanization protocol readily wick fluid into them and do not spread during tension cycles. These reservoirs can be made beforehand and stored until future use. They can also be easily manipulated and wrapped around a bobbin because the treatment does not change the flexibility of the thread and there is no risk of smearing the barrier.

A few downsides to the method include the need for surfactants and the length of the fabrication. Following this protocol, the nylon thread is made hydrophobic along its entire length. While silanizing only the barriers would have been ideal, no easy way to do this was found. In addition, the woolly nylon thread is not hydrophilic enough for water to easily enter the untreated reservoirs. As a result, it is necessary to help the process with a small amount of surfactant. Because the surfactant is already useful to get the fluid to enter the thread before it is treated, it is acceptable to have the entire thread silanized, and still use surfactant to define the reservoirs along the thread. Tween 20 is used as a surfactant because it is commonly used in biological assays.

Finally, the silanization protocol was chosen as the preferred method to create reservoirs on thread. This method created reservoirs that were very regular and most reliable as seen in figure 7a and c. Leaking does not occur and the reservoirs have clearly defined edges. While the time it takes to fabricate is a drawback, the results shown in

figure 7 were good enough that this method was used for developing digital microfluidic threads.

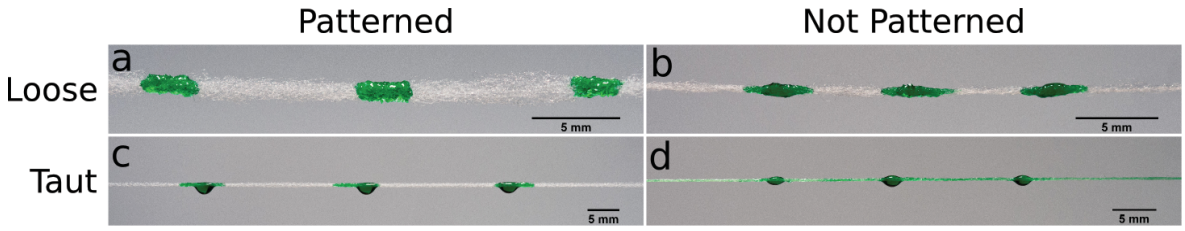

Figure 7: Comparison between patterned and not patterned woolly nylon thread. *The patterned thread wicks the fluid into the reservoirs when loose (a) and maintains the reservoir integrity when taut (c). The thread that is not patterned will accept fluid when loose (b) but will allow the fluid to flow when taut (d). Scale bars are 5mm.*

3.3 Digital String Microfluidic Functions

The functionalization of the thread as described in the previous chapter created a tool that we call digital string microfluidics with many different functions. The name came from digital microfluidics which was created by defining four functions that were considered to be the basic building blocks of a lab on a chip[55]. By combining droplet creation, transport, cutting, and merging, it was argued that most fluidic operations required for laboratory assays could be accomplished. These functions can now be recreated on thread, leading to a new fluid handling method.

The foundation of digital thread microfluidics consists of four functions: droplet creation, transport, cutting, and merging. By forming reservoirs on textured thread, these functions were replicated and used to accomplish a variety of operations including one-way transfers, mixing, and copying. This ability stems from the tension-controlled volume contained within the thread. As the thread is tensioned, the fibers come together and the pore volume decreases. If the reservoir is full, the fluid is extruded out of the reservoir into an outer fluid layer, held to the thread by surface tension. Releasing the

tension causes the fluid to recede back to the middle of the thread. This simple concept is used to build all of the digital thread microfluidic functionality.

3.3.1 Droplet Creation

Droplet creation is accomplished by wicking liquid into the reservoir. This can be done passively by simply contacting a solution of interest to the reservoir. However, in order to increase reproducibility, loading the reservoir is generally done with a tension cycle, that breaks any discontinuities that the liquid could be pinned by when attempting to enter the reservoir. While this loading is a time-dependent process, it is easiest to create different droplet volumes by varying the volume of the reservoir itself. The volume of the reservoir can be varied by increasing the length along the thread or by using a thread with a larger diameter. The woolly nylon has a lower volume per unit length than the Oral-B Ultrafloss.

3.3.2 Droplet Transport

Droplet transport is accomplished by moving the carrier thread. While it is possible to perform the operations manually, it is much more reproducible if it is performed with an automated setup. We have the advantage of being able to look towards the textile industry to design the apparatus that could be used for fast and high throughput manipulation. As proof-of-concepts in the lab, transport was performed with threads loaded on bobbins as in figure 12a or with threads wrapped around a stepper motor shaft as in figure 12c.

The bobbins were set up to create a grid of threads, and could be activated manually by wrapping the thread around one bobbin and releasing it from the other side. This simulated a combinatorial operation in a 3 by 3 grid. While it was a good device to

rapidly show that it could be done, manipulating each bobbin by hand is time consuming and hard to do. One challenge is to maintain the same tension throughout the transport. Not only do both bobbins need to turn at the same time, but as the thread wraps around a bobbin, the diameter increases. Thus, for every turn that bobbin completes, it pulls in more thread than the one letting the thread out.

Automating the system with a stepper motor allows for better tension control without the variations imposed by manual transport. However, only a single thread could be actuated at a time with the stepper motor, so the range of movements that could be performed was smaller. Manually turning a bobbin takes a long time, shakes the thread, and stretches the thread a different amount each time. The stepper motor system controlled from a command line prompt eliminates the change in tension between each stretch and can be done very quickly. The only source of variability between threads is the tension at which the threads are attached to the stepper motor. This tension was roughly controlled by attempting to tension it by hand to the point where there was no longer a noticeable sag along the string. This was only an approximation of the tension, and could have caused some variation in the volumes of different strings.

3.3.3 Droplet Splitting

Droplet cutting or splitting can be done by transferring liquid from one reservoir to an empty reservoir. By doing so, the droplet is split between the two reservoirs. It is important to note that this is the same concept as droplet merging and one-way transferring in the next section but the transfer occurs from a full reservoir to an empty reservoir instead of between two full reservoirs. In order to transfer a part of the droplet, the donor reservoir is tensioned and put in contact with the loose or slightly tensioned

receiving reservoir. The volume transferred is dependent on the tension of the donor reservoir, the initial volume in the donor reservoir, and the contact time.

3.3.4 Droplet Merging – One Way Transfers

Droplet merging is similar to droplet splitting, however, the donor reservoir transfers the fluid to a receiver reservoir with fluid already in it. In this way, the two reservoirs can contain both solutions. Thread has the added functionality that one-way transfers can be accomplished where the donor thread does not receive any of the receiver thread fluid as seen in figure 8. This avoids cross-contamination and means that a donor thread can be used over and over again to transfer a fluid to multiple reservoirs. The one-way transfer occurs when the receiving reservoir is not full such that it still has space to wick extra fluid into its pores. As the receiver comes into contact with the extruded droplet from the donor reservoir, the liquid rapidly wicks into the available pore spaces in the receiver reservoir. As will be further discussed in the quantitative investigation in section 3.5, the volume transferred depends on multiple parameters including the tension of the donor thread, the volume in the donor reservoir, the volume in the receiver reservoir, and the contact time.

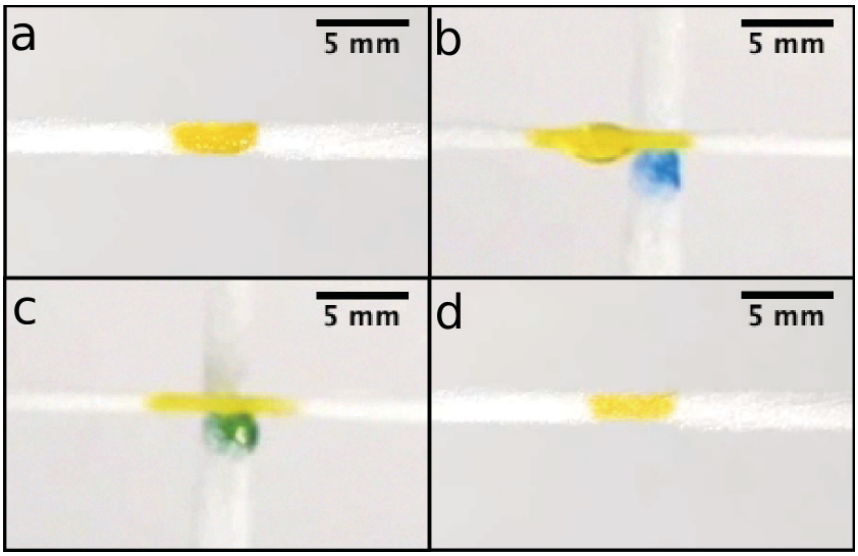

Figure 8: One-way transfer. *(a) The loose donor reservoir filled with yellow dye. (b) The donor reservoir is tensioned, extruding a droplet. (c) The taut donor reservoir comes into brief contact with the loose receiver reservoir to transfer the extruded liquid. (d) The loose donor reservoir after the transfer.*

3.3.5 Additional Droplet Functions

Creating, transporting, splitting, and merging droplets allows us to easily execute additional functions as well. These include rapidly mixing two merged droplets and copying reagents from one string to another.

It is not trivial to mix two fluids in a microfluidic system because of the lack of turbulence. For most reactions, mixing is a critical step and therefore it is important to be able to rapidly mix two solutions. The droplets in digital thread microfluidics can be rapidly mixed by tension cycles. As the fluid is extruded out of the pores and brought back in, the solution is rapidly homogenized. As seen in figure 9, a fully mixed reservoir can be obtained in less than 10 stretching cycles.

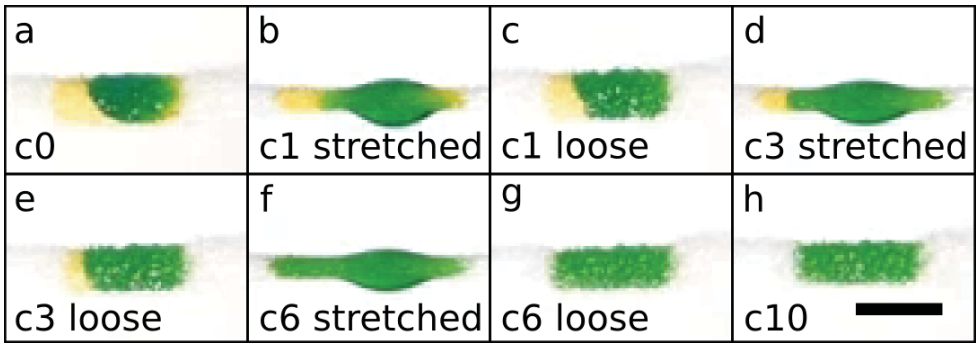

Figure 9: Mixing by stretching cycles. *(a) Blue dye is added to a yellow dye reservoir. (b) The string is stretched and (c) loosened in the first cycle. The string is repeatedly stretched and loosened with cycle 3 shown in (d-e) and cycle 6 shown in (f-g) until it is fully mixed after 10 cycles in (h). Scale bar is 3 mm.*

Reproducing the same series of reagents on multiple threads can lead to increased multiplexing or throughput. Two different ways to rapidly copy reagents were investigated. If the aim is to reproduce a pattern onto a single other thread, a rapid way to do this is to draw one thread across the other, with each thread moving at the same rate. When the threads are crossed as seen in figure 10 and rapidly pulled under tension, they slip past each other transfer the fluid in the reservoirs at the point of contact. While this was done by hand at the rate of approximately one reservoir per second, it is believed that an automated setup could accelerate the process.

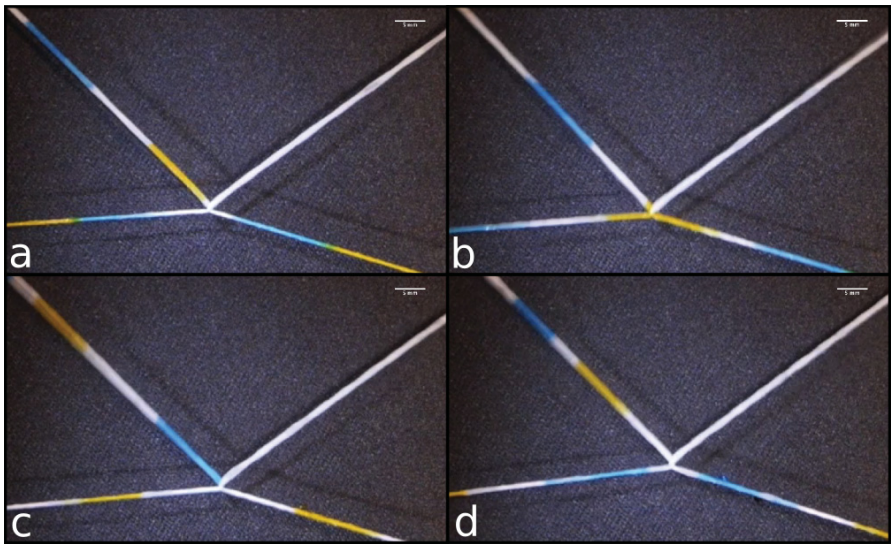

Figure 10: Fast serial copying. *(a) The pattern is originally only on the top side of the left thread, while the top right thread is blank, t = 0s. (b) As the threads are pulled down at the same rate, they slide along each other, transferring the yellow reservoir to the lower section of the right thread. T = 0.25s. (c) Beginning of the blue reservoir transfer. T = 1.1s. (d) End of the blue reservoir transfer. T = 2.05s. Scale bar is 5mm.*

If the aim is to reproduce the pattern of reagents on many threads, a batch method called twist copying can be employed instead of a serial method. To do this, a bundle of blank threads is lined up with the template thread and twisted and stretched together. As the bundle twists and stretches, the fluid is extruded from the template thread and distributed throughout the hydrophilic reservoirs. This method can pattern multiple strings at once as seen in figure 11, but the volume of the template string limits the total number of copies. If there are too many strings in the bundle, the fluid will not be well distributed between each string.

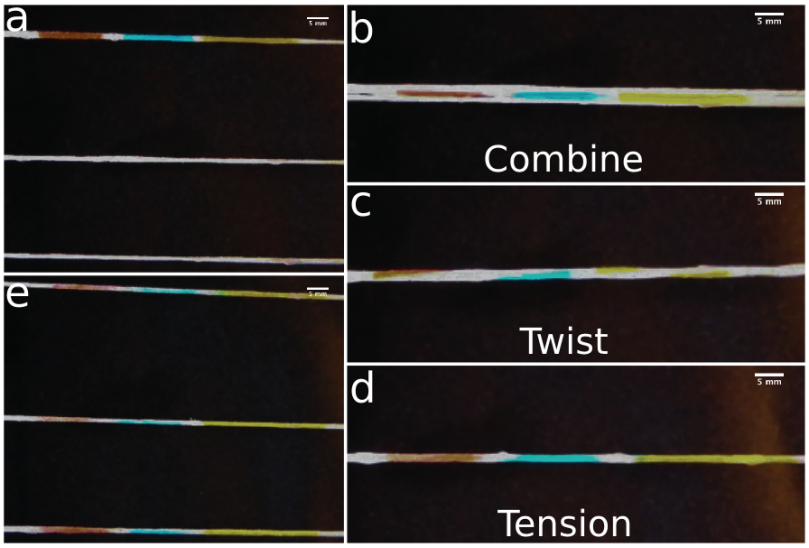

Figure 11: Twist copying. *The pattern found only on a single thread in (a) is replicated onto the two other threads such that they all have the pattern as in (e). To do so, the threads are first brought together (b), then twisted (c), and finally pulled under tension (d) before being separated. Scale bar is 5mm.*

3.4 Physical Support for String Microfluidics

While thread is appealing because of its flexibility and ability to create arbitrary shapes, it also requires a physical support to make the handling easier. Various supports were used for different applications, from the simple petri dish and tape holder for rapid tests to a motorized setup for controlled liquid manipulation.

Many initial tests were setup simply by taping the thread across a petri dish. All protein and glucose assays were also performed when the thread was taped on a glass slide holder. However, more complicated rigs were made to measure fluid flow and recreate a high throughput system. In order to reproduce combinatorial operations along a 3 by 3 matrix, a wooden frame was built to hold up 4 axles in a square as seen in figure 12a. The threads were mounted on bobbins that were slipped onto the axle and lined up with the opposite bobbin on the opposite axle. This setup allowed for multiple threads to

be held in place and to easily actuate each one by turning the bobbins on the axles. This provided a manual demonstration of a small-scale combinatorial setup. However, everything had to be done by hand and as a result, it was not a great platform to quantify fluid transfers and mixing. In addition, the time required to actuate each thread by hand limited the size of the combinatorial matrix to approximately a 3 by 3 because of evaporation.

A new motorized platform was built to perform the quantitative studies as pictured in figure 12b-g. The design of the platform is shown in figure 12e and the device as built is seen in figure 12c. The platform consists of white plastic plates connected by four aluminum rods. The largest plastic plate is used to mount a 6V 1.2A unipolar stepper motor and an arduino board and motor shield to control it. The stepper motor drives the rotation of the white plastic wheel onto which threads are mounted. A command line program was written to easily carry out steps such as tensioning, loosening, mixing, loading, and transferring fluid from reservoirs on the thread.

This device can be used in multiple different ways based on how you attach the thread to the rotating wheel. The most common use for the platform is seen in figure 12b where the thread is attached to the wheel on one side for unilateral tensioning and in figure 12d where the thread is attached to the wheel on both sides for bilateral tensioning. The first case was used to study how the flow along the thread was affected by tension. The second case was used to perform loading and transfer operations as it reduced the movement of the thread making alignment much easier.

The device was originally designed to be used as a multistep transfer timing system as shown in figure 12f-g and will briefly be described here even though it was not

extensively tested in this mode. In these images, the threads are attached to holes in the wheel and threaded across the gap to the corresponding hole in the square plastic plate. Multiple threads are threaded at the same time in the correct order and with the proper spacing such that as the wheel rotates, the first thread will first contact the second one, then the first and second thread will contact the third, and so on and so forth. In addition to contacting one additional thread at a time, the wheel stretches the first thread more than the following threads, causing a general flow towards the newly added thread. The resulting operation is a sequential addition of reagents as the wheel is twisted, which can be done at any arbitrary time that is pre-programmed by the thread distance and the rotating speed. This device replaces a complicated movement such as stretching and translating a thread in 3 dimensions to a simple rotation that accomplishes the same effect. Figure 12f shows the initial setup with three threads, the first being yellow, the second blue, and the third empty. Figure 12g shows the final result after twisting the wheel, with the first thread still yellow, the second thread with a green core and blue outside, and the final thread that is entirely green.

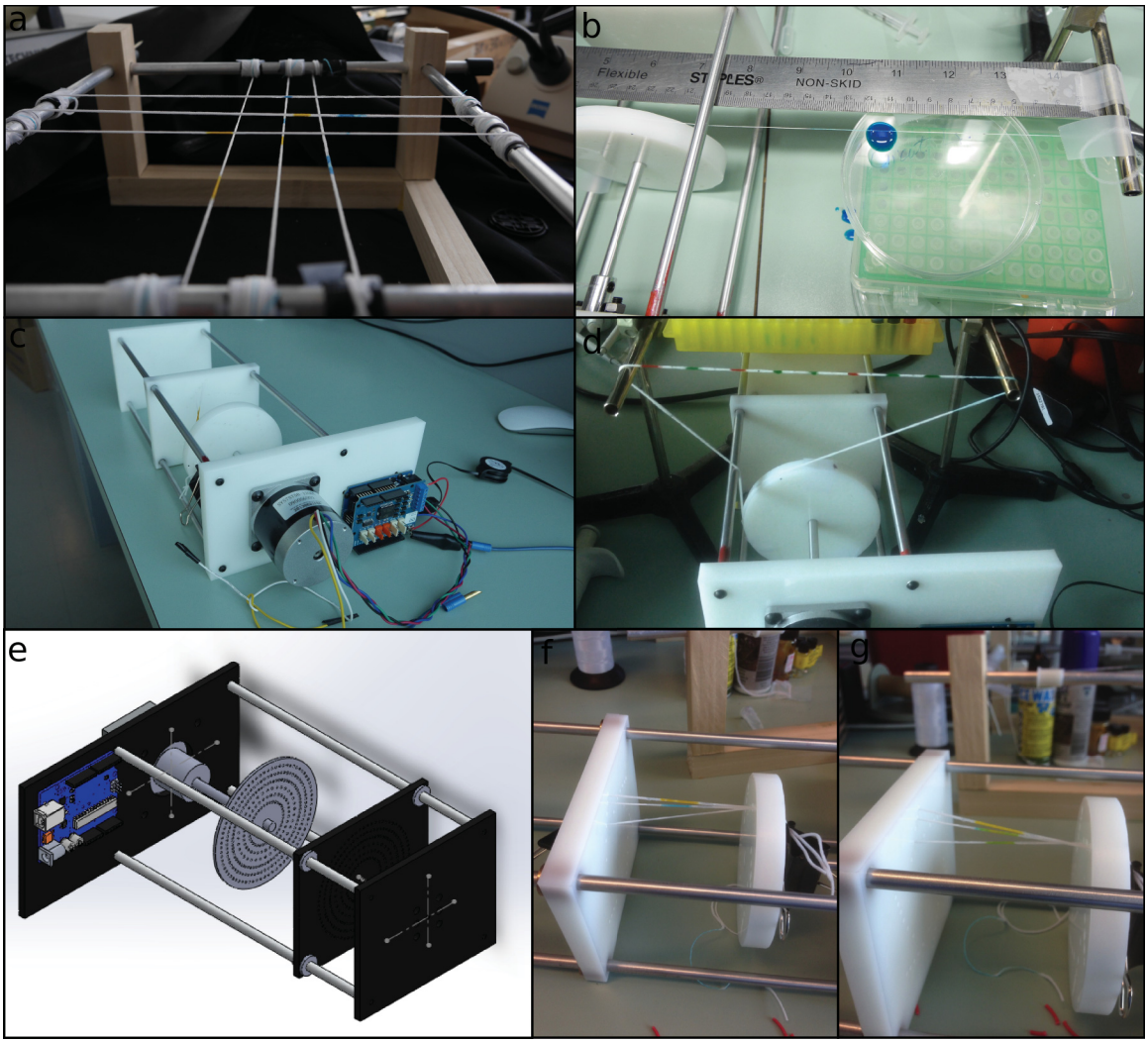

Figure 12: Physical supports for thread manipulations. *(a) A frame and bobbin setup for digital microfluidics. (b) One direction tensioning setup for flow testing. (c) Stepper motor setup for standardizing tension. (d) Bi-directional tension setup for testing. (e) CAD design of the motorized setup. Proof of concept transfer using the motorized setup where (f) is before the transfer and (g) is after the transfer.*

3.5 Proof of Concept Assays

When developing a new medium on which assays can be performed, it is important to determine if the material is compatible with the assay type. Many specific and non-specific interactions can occur between the material and the reagents, affecting assay results. It is therefore common in paper and thread microfluidics to complete

proof-of-concept assays such as measuring BSA, glucose, and other chemicals to demonstrate the applicability of the new technique. These well-characterized assays allow for the detection of any interactions that would cause problems with other assays.

A common challenge when using polymer substrates for diagnostic assays is the high level of autofluorescence limiting the readout modalities for tests. The polymer making up this thread is no exception as seen in figure 13. The level of fluorescence is very high for all excitation wavelengths even if the exposure time is very short (a second or less) in all cases. It appears that hydrolyzing the polymer in hydrochloric acid slightly reduces the autofluorescence at short wavelength excitation, but greatly increases it at longer wavelength excitation light. As a result, there does not appear to be a way to avoid autofluorescence and therefore fluorescent assays were not attempted on the platform.

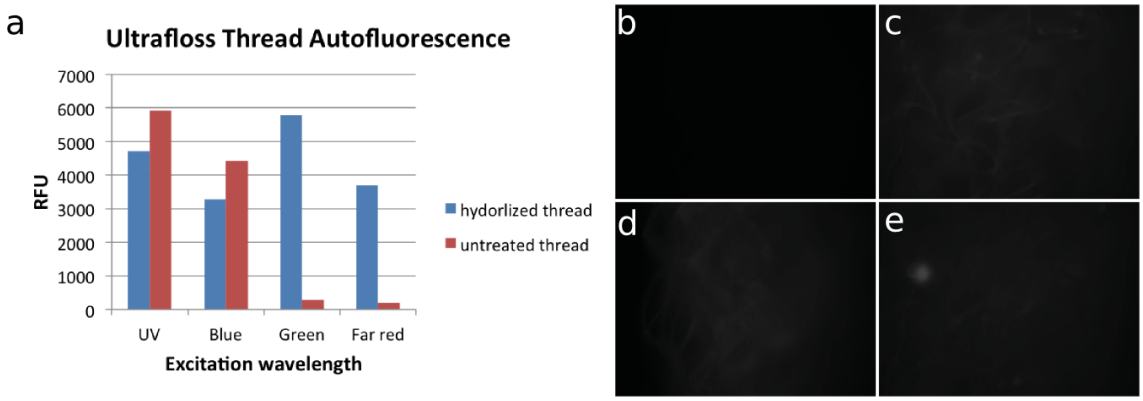

Figure 13: Ultrafloss thread autofluorescence. *(a) Comparison of fluorescence between untreated ultrafloss and hydrolyzed ultrafloss. Exposure times are of 500ms, 1s, 100ms, and 100ms for UV, blue, green, and far red respectively. (b) Green excitation on untreated thread (100ms). (c) Green excitation on hydrolyzed thread (100ms). (d) UV excitation of untreated thread (500ms). (e) UV excitation of hydrolyzed thread (500ms).*

In order to demonstrate the compatibility of the digital threads with biological and chemical assays, the reservoirs were used to perform a BSA assay and a glucose assay.

While the BSA assay was not successful due to non-specific interactions, the glucose assay showed results comparable to those measured in a test tube.

3.5.1 Protein Assay on String

There are many different types of total protein assays. Many of these assays rely on the dye-binding method that results in a pH indicator error in the presence of a protein. The pH transition range for colorimetric indicators changes based on the concentration of protein. The indicator is added to a buffered solution at a pH that is very close to its transition pH. If protein is added, the indicator will change color. Different indicators can be used, that will be more or less sensitive to certain proteins. Martinez et al. used tetrabromophenol blue in a citrate buffer solution for their ground breaking paper and this assay was subsequently repeated as a proof-of-concept for many variations on the paper and thread platforms[3]. This assay was reproduced as described in the materials and methods section.

The tetrabromophenol blue protein assay was first performed on ultrafloss thread. The initial results were not promising, with very little color change or without a consistent trend based on BSA concentration. This experiment was not performed in a test tube to verify its efficacy because the protocol to do so was not described. To improve the result, the woolly nylon was used instead of the ultrafloss because it has a smaller volume and is more appropriate for handling very small quantities of fluid. The concentration of surfactant forming the reservoir was also decreased to reduce the affect it could have by binding to the dye or the protein. However, the inconsistent results still persisted as seen in figure 14b. In addition to variable results, there was also a very low signal from all of the reservoirs as seen in figure 14d where the changes are not visible by

eye. This meant that very small variations within the reservoir could result in misinterpretation of the protein concentration, as seen in figure 14c. Here, a 60 µM BSA solution has a reading between 8 and 18 mean cyan intensity depending on the region of the reservoir, overlapping with the 40 µM intensity reading. This means that the protocol that involved mixing cycles being done until the reservoir was visually uniform was lacking. However, the exact number of mixing cycles was not standardized.

The Bradford assay was used to verify the protein concentrations because of the inconsistency of the tetrabromophenol blue assay. When performed in a test tube, the Bradford assay confirmed the proper concentration of the BSA dilution series. However, the assay remained inconsistent when performed on woolly nylon thread, even with low concentrations of surfactant within the operating limits of the assay as seen in figure 14a.

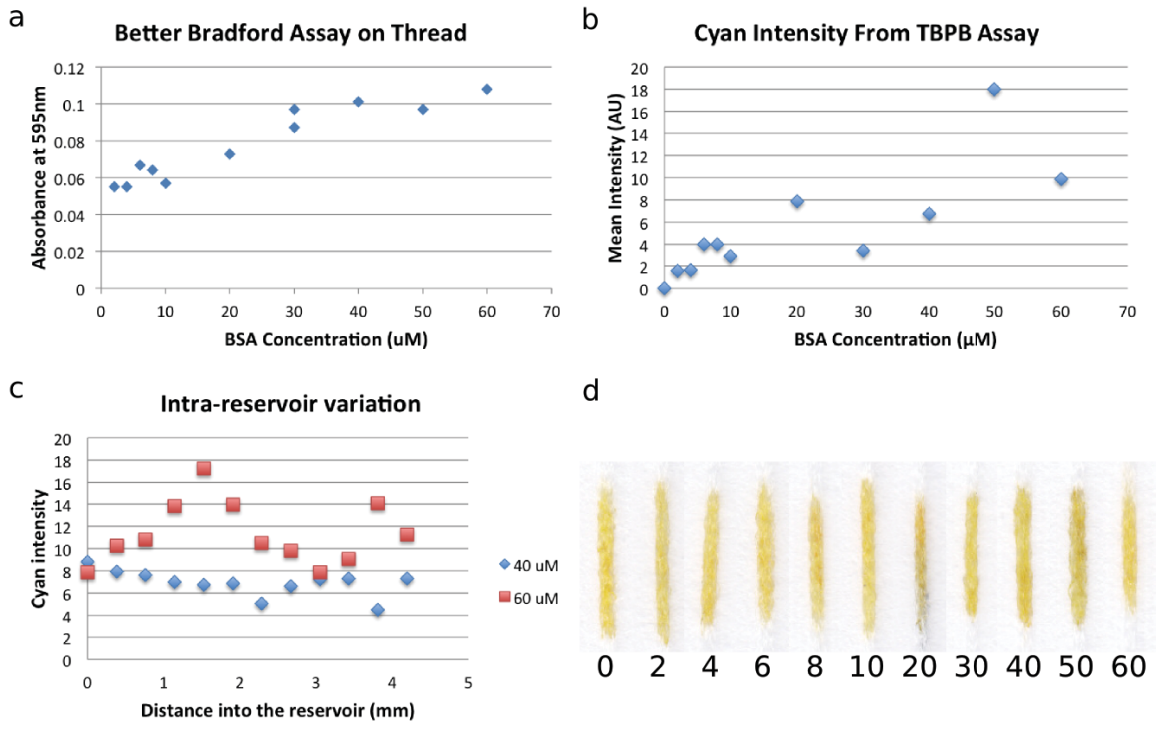

Figure 14: Protein assays on digital threads. *(a) Bradford assay on woolly nylon thread. (b) Tetrabromophenol blue assay on woolly nylon thread. (c) Intra-reservoir variation of*

the tetrabromophenol blue assay on woolly nylon thread. (d) Scanned images of the tetrabromophenol blue assay reservoirs.

This was attributed to hydrophobic interactions between the thread fiber and the proteins and dye. Both dyes have hydrophobic regions that will readily interact with the hydrophobic thread, even if it has been coated with a surfactant. This is shown by the fact that when a thread section patterned with a reservoir is added to a Bradford assay in a test tube, the absorbance of the solution drops in the first 10 minutes before settling at a new value as seen in figure 15a. This is thought to be caused by the fact that in time, the dye molecules in solution reach equilibrium with those adhered to the surface of the thread, slowly reducing the effective concentration in solution. Dyes will generally have hydrophobic regions, therefore most colorimetric readouts will suffer from interaction with an unblocked hydrophobic thread. A hydrophobic section of ultrafloss was dropped into a solution of blue food dye and incubated at room temperature for 12 hours, and the final result pictured in figure 15b shows that the dye will readily adhere to the fibers. The concentration of molecules in solution is therefore not indicative of the original concentration because part of it is adhered to the reservoir fibers. In addition to adhering to the dye molecules, the fibers are likely to interact with the BSA itself by hydrophobic interactions, reducing the apparent solution concentration and therefore causing the irregularities seen in the tetrabromophenol blue assay.

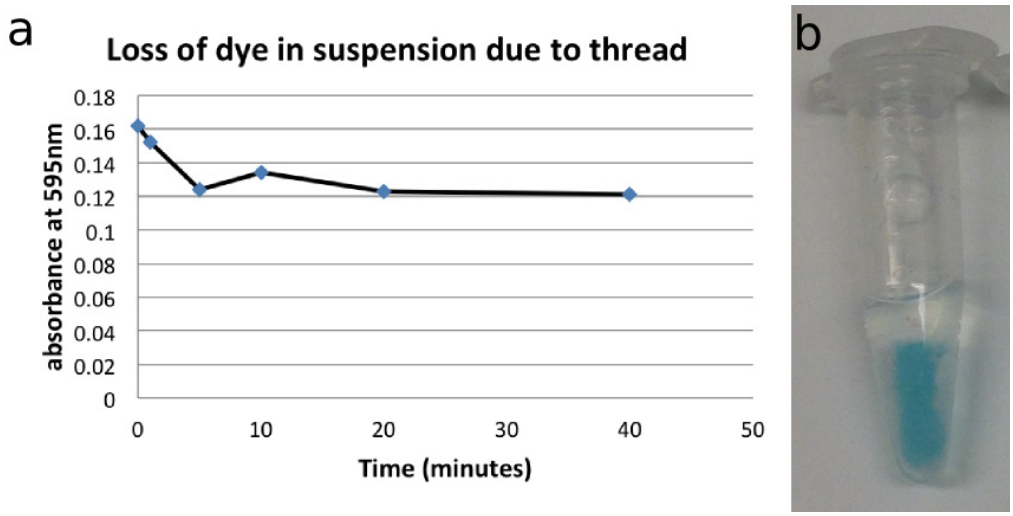

Figure 15: Loss of dye in suspension due to thread. *(a) Time course of Bradford assay dye in contact with woolly nylon thread. (b) Blue food dye binding to ultrafloss. The originally blue solution became clear as the thread became blue over the course of 12 hours.*

The problem with hydrophobic interactions affects two critical functions of string microfluidics. First, as the concentration of an analyte is not representative in the solution, colorimetric changes will generally underrepresent the concentration of the sample. Second, when transferring the fluid from a reservoir to another analysis platform such as the spectrophotometer, some of the analytes are left behind on the thread. While there are methods to avoid hydrophobic interactions between reagents and the surface of the reservoirs, they generally involve blocking the surface with a protein, which would affect the assay results. It was therefore concluded that due to the hydrophobic interactions, digital thread microfluidics is not the appropriate platform for a total protein measurement. However, it is possible to block the surface for other assays to be performed on the thread.

3.5.2 Glucose Assay on String

A glucose assay was selected as the next proof-of-concept because it would be possible to block the surface of the thread without interfering with the results, and the colorimetric reporter, iodine, is a small molecule that should have little hydrophobic interaction with the thread. While the assay does involve enzymes that could interact with the hydrophobic fibers of the digital thread microfluidics, it is now possible to block the surface to avoid interactions without affect assay results.

As discussed in the methods section, the reservoirs were created with a solution of surfactant and BSA in PBS and the glucose test solution was immediately added to the reservoir before drying. This was shown to be the most effective way of optimizing the enzyme activity by assays performed on glass slides and threads. Various parameters were tested to see if it could have a consistent effect on results such as temperature of the reservoir solutions, temperature of the glucose solution, concentration of surfactant, concentration of salt, and drying time. While it was shown that there was much ore enzyme activity when the solutions were not dried, this is not a practical way to prepare the assay, and therefore the solutions were dried onto the thread. The temperature of the solutions as well as the surfactant and salt concentrations did not appear to have any consistent effect on the results. The color change was measured by absorbance at 440nm because of the convenience of measuring a solution's absorbance. The wavelength was chosen because the solution had a large change in absorbance at that wavelength without being saturated as fast as the shorter wavelengths. Colorimetric analysis could be performed once the threads were dry and could be scanned, and unlike the tetrabromophenol blue assay, the color did not change rapidly in ambient air after drying.

Once the assay conditions were optimized, the assay was reproduced three times in test tubes and three times on thread. As seen in figure 16a, there is not an exact match between the two assay results, but as seen from the error bars, each condition is fairly reproducible. Above 10 mM glucose, the color change was large enough that the spectrophotometer was saturated, therefore the error bars are very large. The difference between the test tubes and the threads was expected, as the conditions are not the same for both assays. The test tube solutions only contained the assay reagents and the glucose solution whereas the thread solutions contained extra salt, BSA, and surfactant that would be expected to affect the assay. In the smaller concentration range, it is apparent that the extra solutes in the thread solution reduced the enzymatic activity or there was still some non-specific interaction between the thread and the analyte.

The colorimetric measurement of glucose from a scanned image allowed for better throughput as many assays could be processed at once. It was therefore interesting to see if the assay reservoirs themselves could be produced at the same time, using the twist copying method in order to reduce the work required to make the devices. To do so the amount of reagents required for the number of threads used was pipetted onto a thread and then copied across the bundle. The threads could then be separated before or after drying. As seen in figure 16b, the fabrication of the glucose test reservoirs did not introduce large error in the glucose measurement. The success of the assay on thread is apparent in figure 16c where we can see the color change is very apparent based on the glucose concentration. This is in contrast to figure 14d where the protein concentration barely affected the color on thread.

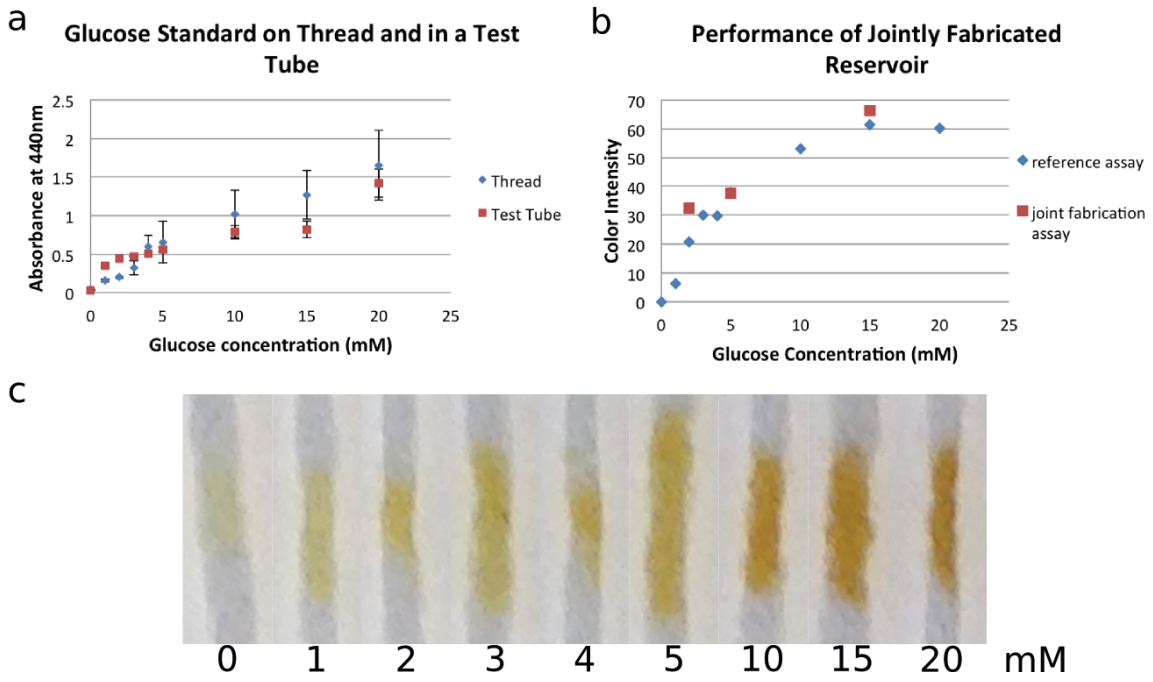

Figure 16: Glucose assay. *(a) Triplicate assays for glucose in test tubes and on thread when measured by absorbance using a spectrophotometer. Above 10 mM, the spectrophotometer is saturated, but the data points are left in the graph for reference. The error bars represent 1 standard deviation. (b) Performance of the glucose assay on thread when the reservoirs are created together. Measurement is performed by RGB color intensity. (c) Typical color change for the glucose assay on thread after drying.*

3.6 Quantitative Reproducibility of Fluidic Functions

In order for the platform to be useful on a standalone basis, it is important for the fluidic functions to be reproducible. Many assays report the total amount of an analyte present, rather than the concentration. It is therefore important to be able to reproducibly aliquot and transfer a specific volume. In this section, the reproducibility of different loading and transferring modalities is assessed.

3.6.1 Loading Reservoirs

Loading a sample into a reservoir is necessary for all assays. Only once the sample is on the thread can it be manipulated and tested. While it is trivial to do this with

a pipette, it would be greatly advantageous to do this reproducibly without a pipette. The most straightforward way to load a sample into the reservoir is simply to dip the reservoir into the sample and allow it to wick. After a specific amount of time, the reservoir is withdrawn from the sample. In an ideal case where each reservoir was identical, this would result in the same amount of fluid being drawn into the reservoir each time. However, this is not the case as there are often irregularities in the thread that cause differences in wicking rates.

The factors that can affect loading volume include tension on the thread, pressure applied on the sample drop, contact time, the rate at which tension is applied or released, and the number of tension cycles. To avoid having to optimize every parameter, some were adjusted for convenience. For instance, the pressure applied on the drop was limited to simply touching the thread to the sample droplet. The other parameters were used to minimize the variation in loading volume.

It was common to get very little to no loading because the sample was pinned at a discontinuity in the fibers, and was never wicked any further. This is once again the challenge that fluid will penetrate along the fibers much more readily than across the fibers. It was found that by tensioning the thread at the same time as contacting the reservoir to the sample, the differences in wicking rate between threads could be reduced. Tensioning the thread reduces the chances that a large discontinuity between the fibers will pin the fluid and stop the reservoir from loading. It also made it easier to break through the surface tension of the sample droplet, which sometimes stopped the thread from wicking any sample. As a result, this reduced the occurrence of catastrophic failures where no fluid would enter the reservoir.

If the thread remains taut throughout the loading, very little fluid will enter the reservoir. The taut reservoir has very little volume; therefore it is important to release the tension in order to allow fluid to enter the reservoir. This means that a full tension cycle needs to be performed in order to load a reservoir. First, tensioning the thread helps it break through the surface tension of the water droplet. Then, as the tension is released, the water enters the pores of the thread and fills the reservoir. However, if the tension is released too quickly, the water does not enter the thread properly. It is therefore important to induce progressive tension cycles, allowing time for the fluid to move into the reservoir.

While the protocol for loading fluid into the reservoirs improved, there still remains a significant amount of variation in the loading volumes. The final loading program was to tension the thread fully at the beginning, and make contact with the sample droplet. After a pause, the thread tension would be released to 25% of its fully stretched state. After another pause, the thread was tensioned again to 75% of its fully stretched stated. Finally, the thread was completely released. The results for 4 different threads from 2 different batches are shown in figure 17. The threads in batch 1 had 2-second pauses between each change in tension whereas the threads in batch 2 had 1-second pauses.

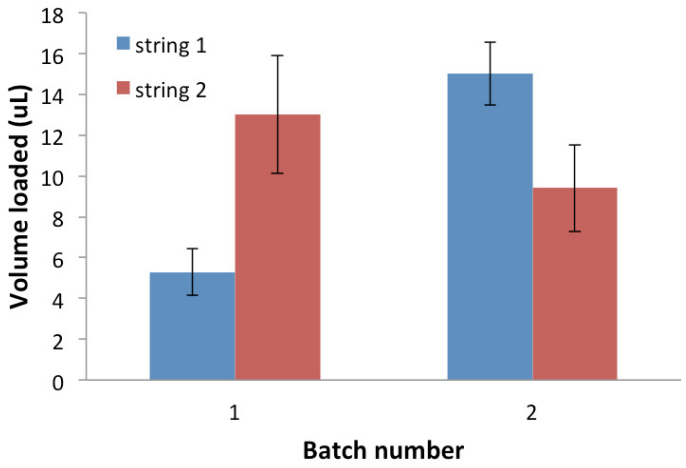

Figure 17: Volume loaded in string reservoirs. *Two different threads are shown for two different batches. Both batches were prepared the same way and the threads in each batch were prepared at the same time. Batch 1 threads were in contact with the sample droplet for twice the time as those in batch 2. Error bars represent 1 standard deviation. $N > 5$.*

It is clear from figure 17 that there are issues with reproducibility when loading reservoirs. While each string has a low level of variation between all of its reservoirs, there is large variation between threads. It is not yet entirely clear where all of the variation stems from but they appear to be thread-specific and not related to the batch the thread was processed in. While there are variations coming from differences in the contact angle of the thread it is thought that the majority of the differences stems from the physical manipulation that affects the pore structure. As the threads are prepared, any compression will result in smaller or lopsided reservoirs that are hard to fill and have smaller volume.

3.6.2 Transferring and Splitting Reservoirs

Droplet transfers and splitting allows for multistep and multiplexed assays on string. Various modes of transfer can be interesting for an assay. A sample can be transferred from one reservoir to multiple test reservoirs for a multiplexed assay. These

transfers were called serial transfers because a single donor reservoir is transferred to many receiver reservoirs in a serial fashion. Other types of transfers can be done such as transfers from multiple donors to a single receiver reservoir in the case of a reaction needing multiple reagents.

Serial transfers can be performed to create a serial dilution or many aliquots of the same sample. The change in tension controls the volume of fluid that is readily accessible outside of the fiber bundle ready to be transferred. If each tension step between transfers is too small, very little fluid forms a droplet and the transfers are very small after the initial transfer as seen in figure 18a. However, with a larger change in tension between transfers, it is possible to increase the volume of the subsequent transfer. This is limited by the maximum stretch that the thread can achieve. With the setup used to test the transfers, the maximum stretching was between 800 and 1000 motor steps, resulting in a tight bundle of fibers with very little interstitial space between the fibers. Using 800 as the maximum stretch, two transfers were performed with the second one being approximately half of the first one as seen in figure 18b. In this transfer, the initial volume of the donor was 15μL, with the first transfer averaging 7.6 μL and the second 4.4 μL. This means that on average, the dead volume of the reservoir was 3 μL. While the thread could be stretched more on average, it was not done to avoid any setup failure.

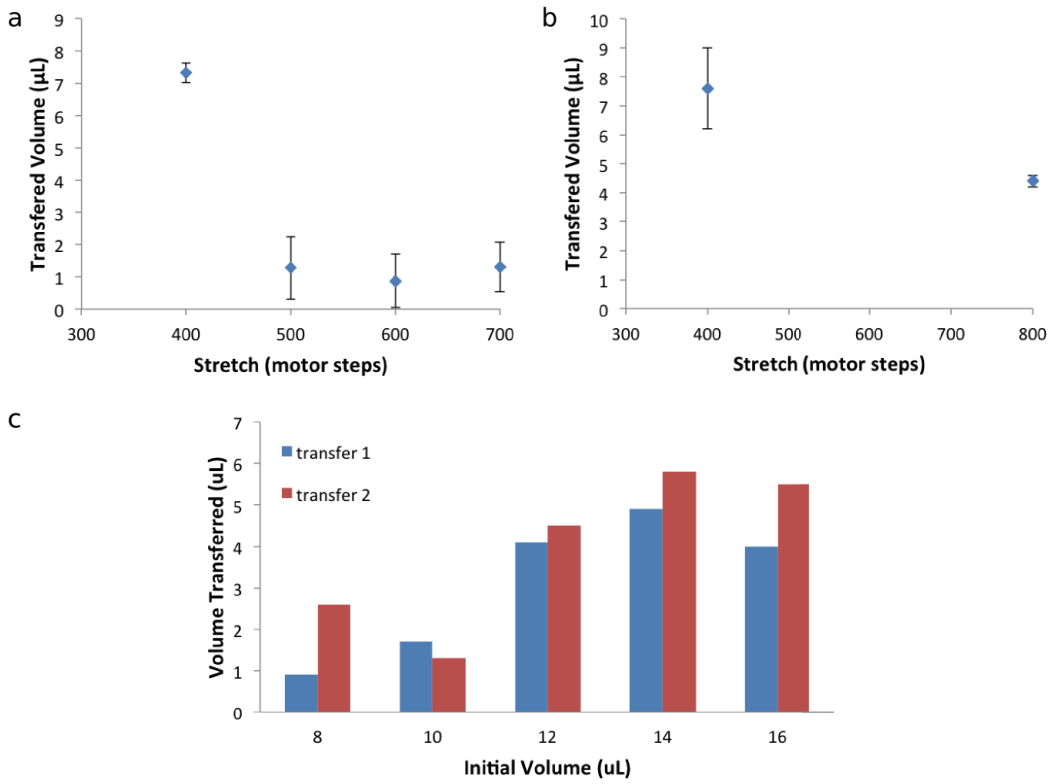

Figure 18: Reproducibility of transfers. *(a) Four serial transfers with small tension intervals. (b) Two serial transfers with a larger tension interval showing the possibility of a serial dilution. (c) Initial volume effect on 2 serial transfers. (a) and (b) Error bars represent 1 standard deviation, n = 3 and 2 respectively.*

The effect of the initial volume in the reservoir was also investigated. It was thought that the transferred volume may be proportional to the fluid remaining in the reservoir and therefore smaller transfers would occur when less fluid was in the reservoir. This was tested by performing 2 serial transfers at the same tension for reservoirs loaded with different volumes, as seen in figure 18c. Based on this single trial, it appears that there is a threshold above which the initial volume has a minimal effect on the transfer volumes. While this needs to be verified with further trials, it could potentially be useful for loading reproducible volumes. This experiment also shows the possibility of aliquoting similar volumes using transfers as both transfer 1 and 2 have similar volumes in all cases.

4.0 Conclusion

4.1 Summary

We propose a new fluid manipulation platform called string microfluidics consisting of textured synthetic strings with defined hydrophilic reservoirs. Using these functionalized strings, basic fluid operations required in a lab on a chip such as creating, transporting, splitting, and uni-directionally transferring droplets was performed. Additional manipulations can be easily accomplished such as mixing a reservoir and copying a series of reagents from one thread to others. This was made possible by the unique stretch of textured synthetic strings and the surface functionalization to create hydrophilic reservoirs and hydrophobic barriers. By developing these operations on thread, a new fluid manipulation paradigm was introduced where fluid is moved by the underlying substrate and not by the fluid itself moving. This is a fluid manipulation concept that reduces sample dispersion and lends itself to a new type of automation for fluidic manipulations.

While many different methods to create these reservoirs were investigated, in the final design, the strings are functionalized with a perfluorinated silane to create hydrophobic barriers and surfactant to create hydrophilic reservoirs. In this way, the string can easily be stored and manipulated without affecting the barriers. The strings were then demonstrated to be compatible with biological assays with some modifications such as blocking with BSA to avoid non-specific interactions. Finally, the fluid operations were studied quantitatively, showing that while there are some irregularities in transfer and loading volumes, there are steps that can be taken to minimize this variation.

4.2 Future Work

The platform that has been developed can now be expanded to explore many more opportunities. New functionality can be developed while existing functionality can be packaged into easy to use devices. The majority of the work to date was focused on demonstrating the potential of the string microfluidic concept and forming hydrophobic barriers. Now that this has been done, further work can concentrate on assay development and device design for ease of use and reproducibility. In order to make the presented concepts useful, string microfluidics needs to be applied to a specific assay and developed into a user-friendly platform. An assay appropriate for the capabilities of the platform should be identified and optimized on the platform. This will have the effect of making the results more reliable with a streamlined fluid manipulation device and making the technology easier to adopt.

Developing a user-friendly system goes hand in hand with reducing the sources of variability in transfers and loading as well as increasing the range of reproducible actions. Different avenues can be explored to increase the reproducibility. A platform that is easy to operate will have a small learning curve and can be operated by different people with little variation. In addition, various methods for loading can be further tested experimentally. For example, it may be possible to reliably load reservoirs by doing sacrificial transfers that regulate the final loading volume. A theoretical approach such as modeling the fluid dynamics of the liquid as the thread is tensioned would help in defining the criteria for reproducibility as well. Exploring different thread manufacturing methods could address some of the irregularities that are inherent to the threads and impossible to control after fabrication. This research would make the existing transfers

and loadings more reproducible, and would increase the capabilities, such as adding the possibility to reliably make a serial dilution.

Finally, string microfluidics can also be developed for greater functionality to see what possibilities are still unexplored. For example, liquid-liquid extraction methods could be developed using digital string microfluidics and used as a sample extraction step for an assay. Another remaining question is how the string interacts with blood and other biological fluids. It could also be advantageous to look into tension-triggered flow device designs to see what else these unique strings can do.

As the functionality issues are being further researched and resolved, it is also important not to loose sight of the low-cost fabrication of the device. In order to make the platform accessible, high-throughput fabrication of these devices needs to be further researched in parallel with the development of new functions. Many promising proof-of-concept microfluidic platforms have never made it to the users because of a lack of scalable fabrication processes. In the case of string microfluidics, this could involve adapting existing textile machinery to pattern threads with the required reagents. However, many hurdles will have to be overcome to operate this type of machinery with the precision required to pattern individual threads on the millimeter scale. Further research will therefore be needed to fabricate string microfluidic devices and deliver on the promising functions and concepts that have been presented.

Abbreviations

BSA	Bovine Serum Albumin
DI	DeIonized
ELISA	Enzyme-Linked Immunosorbent Assay
NOA	Norland Optical Adhesive
PBS	Phosphate Buffered Saline
PDMS	Polydimethylsiloxane
TBPB	Tetrabromophenol blue
μPADs	Microfluidic Paper-based Analytical Devices

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