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Cover Page Footnote

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Lipid Membrane Self-Assembly and Synthesis in the Lab by Rapid, Low-Cost Microfluidics Approaches

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Abstract

Lipid membranes, which self-assemble in nature and under laboratory conditions, can form vesicles called liposomes. The main uses for liposomes are in drug delivery systems and as animal cell models. From the initial research into lipid membrane science, to the systematic literature review using Web of Science, to refining the project scope, to the description of the method, this project follows the process of developing a method for lipid membrane self-assembly and synthesis in the lab. The result was a rapid prototyping method for fabricating microfluidic devices to test different designs for channels, for use with a liposome synthesis protocol. During development, a simple liposome synthesis protocol was followed, using 1,2 Disteraroyl-sn-glycero-3-phosphocholine (DSPC) to 10 mg/mL in methanol as the lipid solution and 0.9% saline buffer as the aqueous solution. Shrink-plastic (polystyrene) was used as the build material for the microfluidic chip—to construct the design on a larger scale before shrinking to its final size. This method has the advantage of using low cost and easily accessible materials and equipment.

Although the development process for this project was at first limited by resource accessibility, this limitation was ultimately embraced. The materials used to fabricate the microfluidic device can be found in craft stores (shrink-plastic sheets), hardware stores (silicone sealant), and aquarium supply stores (thin tubing). During initial prototyping, it is beneficial to quickly fabricate microfluidic devices of different designs. The method developed in this paper lowers the barrier for entry into these preliminary research activities and encourages experimentation.

Keywords: liposome, DSPC, microfluidics, prototyping, shrink plastic

1. Introduction

In the field of health science, liposomes can be used in drug delivery, bestowing the barrier of a closed membrane. When encapsulated in this manner, the potentially toxic drug product is not released until it reaches its specific destination (El-Beyrouthy and Freeman, 2021). Once at the intended site, the liposome is triggered to leak its contents by a mechanism designed into the properties of the lipid membrane to make it specifically sensitive, for example, to a certain temperature or enzyme (Mouritsen, 2011). As cell membrane models, liposomes can be used to test solutes, drugs, and enzymes to predict interactions and permeation through the membrane (Mouritsen, 2011). Lipid membrane self-assembly also lends insight into the origins of life on Earth. The formation of cell membranes is thought to have been one of the integral steps from prebiotic chemistry towards biology (Deamer, 2017).

Liposomes may be generated in a range of sizes. Giant unilamellar vesicles (GUVs) are of a size comparable to the lower range for animal cells, around 10 µm in diameter (Yu *et al.*, 2009). GUVs are therefore suited for use as animal cell models. They are also visible using light microscopy, which streamlines the detection process (Mouritsen, 2011). Nano-sized liposomes are suited for drug delivery as the small size will increase clearance rate over GUVs. However, the curvature of the liposome increases as the size of the liposome decreases and there is a maximum level of stress that can be endured by the bending of the membrane which limits the minimum possible size (Patil and Jadhav, 2014). Additionally, permeability changes with curvature; variation in liposome size in the final product could influence the results (Mouritsen, 2011).

To confirm the successful generation of liposomes and to characterize them, the samples must be analysed. The advantage of light microscopy is the speed of obtaining a visualization of a sample. This approach uses a standard glass microscope slide and coverslip. It is simple, quick, and inexpensive (Bibi *et al.*, 2011). Light microscopes are readily available in laboratories, which is advantageous when access to instruments such as electron microscopes is not guaranteed. Developing a method that makes use of standard rather than specialized equipment will improve accessibility. Electron microscopy is more powerful and can view small unilamellar vesicles (SUVs). The resolution limit for light microscopy is approximately 300 nm. GUVs are above this limit—over 5 µm in diameter—while SUVs are below it—less than 25 nm in diameter. Information on the 3D structure of liposomes cannot be provided by light microscopy alone (Bagatolli, 2009). Light microscopy is not ideal for information on liposomes, but the size and shape of GUVs can be observed (Robson *et al.*, 2018).

The size of liposomes generated using microfluidics is impacted by the buffer flow rate and the lipid solution flow rate. A relatively fast buffer flow will reduce the size of the liposomes while a relatively slow buffer flow will increase the size of the liposomes (Patil and Jadhav, 2014). As GUVs have a similar size to cells, experiments can be completed on the same scale. This is particularly crucial in cell model studies (Bagatolli, 2009). By controlling the flow rate, the results can be customized. In this case, GUVs are advantageous to simplify visualization.

The goal of this project is to develop a method for fabricating microfluidic devices, which are intended as prototypes of designs for the optimization of a liposome synthesis protocol. By using shrink-plastic as the main material for the device, it can be fabricated quickly and independently of advanced equipment. This avoids barriers such as learning to use 3D modelling software and accessing specialized technology. The other advantages of this material are its thermoplastic properties, low cost, and availability. As the plastic shrinks, the x-y dimensions decrease but retain their proportions, while increasing along the z-axis in height (Yu *et al.*, 2009). When creating a handmade microfluidic prototype, manual dexterity is a limitation, and the ability to fabricate the device design at a larger scale, before shrinking to its final size, is valuable to overcoming this limitation. Proportionality is important for maintaining the microfluidic channel design. For example, pinch-flow fractionation can be used to separate particles by size after they pass through a certain “pinched” section of the channel (Yamada *et al.*, 2004). Distortion of this critical section would impact the intended result. Functionality depends on form.

This method has the advantages of simplifying the project into crude prototypes using cheap materials and easy methods as well as the repetitive trials and result analysis. This method focuses on the impact of variations in channel patterns on lipid bilayer synthesis. Using microfluidics, the simple procedure is readily quantifiable and easily translated into results that are also reliably comparable between trials. Compared to conventional methods, microfluidics balances experiment control over the variables—such as buffer flow rate—with repeatability over the trials. Liposomes are generated at an intersection between the aqueous solution and the phospholipid solution, which are injected through inlets into the system (Patil and Jadhav, 2014). The designs for the new prototypes will be based on existing microfluidic channels for lipid bilayer synthesis—for example, the five-inlet/three-outlet channel design (Yu *et al.*, 2009). Other

sources of design inspiration are from research papers in the medical applications of microfluidics, such as a spiral design for the separation of blood cells by size—a principle that could possibly be adapted for separating unilamellar vesicles by size (Warkiani *et al.*, 2015). The benefit of quick prototyping is that the design can be adapted almost as quickly as the idea is conceived.

2. Materials and Methods

As seen in Figure 1, the three stages for method formation are: A) Assembly, B) Synthesis, and C) Analysis. First, the microfluidic device was assembled, then used to synthesize the liposomes, then collected samples were analysed. A complete overview of the laboratory activities is displayed in the Appendix.

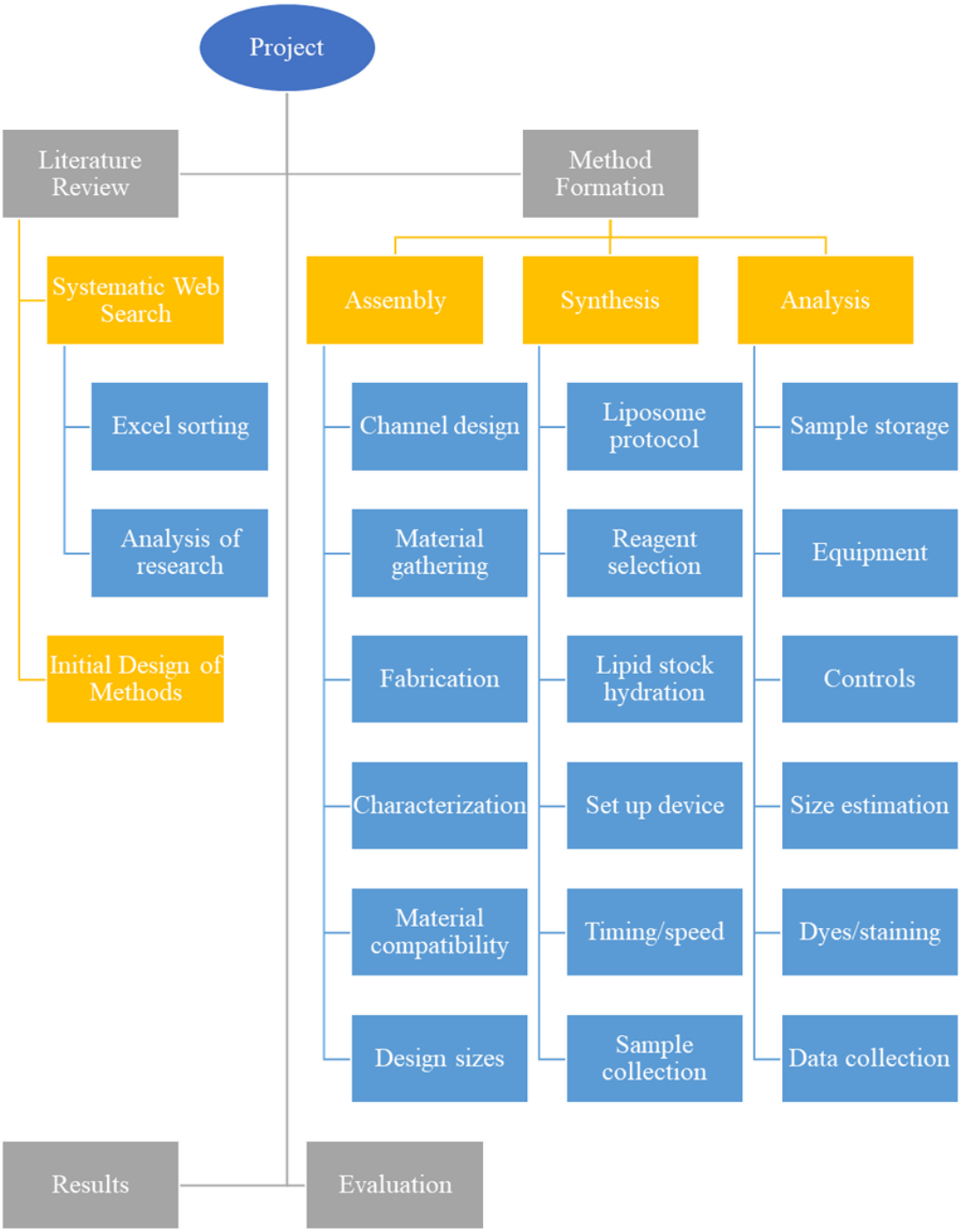

Figure 1: Overview of Stages for Project Method Formation with Considerations (in blue) required for refinement within each stage of method formation

Each of these stages required testing to develop the method. The assembly stage involved investigation of microfluidic channel designs, the project materials, device fabrication, characterization of the chips, material compatibility, and the selection of the device size. In the synthesis stage, the liposome formation protocol was reviewed, along with the reagent selection, lipid hydration, device and sample run setup, and the process of sample collection. Within analysis, the sample storage was explored as well as the equipment, experimental controls, size estimation, dyes, and data collection.

Assembly Method Formation

To design microfluidic channels, existing designs were researched. For example, a spiral microfluidic channel designed for blood cell separation could have potential applications in liposome synthesis and sorting GUVs from SUVs (Warkiani *et al.*, 2015). Similarly, the concept of pinch-flow fractionation—size separation by microfluidic channel—could be adapted into a design (Tivony *et al.*, 2021). From these concepts, composite designs were created that incorporated elements from pre-existing liposome synthesis methods and from designs for other microfluidic applications. These were roughly sketched on paper with a brief caption that described the channel design features. These sketches were used to make digital images with specific dimensions. The website TinkerCAD was used to make 3D models of the microfluidic chips. After amassing a collection of designs, the simplest was chosen—the Basic #1 design—for the initial refinement of the rapid prototyping method.

For the handmade fabrication, three different sizes of the Basic #1 design were printed from the digital image and traced onto the thermoplastic sheets (Shrinky-Dinks® Crystal Clear Creative Pack™). This channel design was cut into one sheet and cut to size into squares—wafer layers that would become part of the microfluidic chips. One aspect of this design size trial was to investigate the best technique for cutting the plastic—scissors, utility knives, flamed needle, or soldering iron. Another wafer was cut to match without the channels, for the top layer. The holes marked on the design needed to align between these two wafers, and the same cutting implements were tested for their ease of use here. The next step was to shrink the plastic in a conventional kitchen oven. The manufacturer's instructions for shrinking the thermoplastic were followed, unless otherwise noted: 170˚C for 2.5 minutes. The characterization of these wafers required a microscope for visualization of the channel cuts, port openings, and heat shrinkage. For shrinkage, the formula for calculation is: shrinkage = $\frac{\text{original size} - \text{shrunk size}}{\text{original size}} \times 100\%$. To complete fabrication activities, the design used here required a base for these layers. Different materials and techniques were attempted: melting to a third shrink-plastic layer, melting to a glass microscope slide, and applying with sealant to glass.

The compatibility of the materials with each other was investigated to ensure that the chip would function as required. The important considerations at this step were examining the sealing, leaching, channel blockage, and water testing for flow. As unwanted reactions between adhesive and plastics can occur, the labelling on the adhesive container should be checked as it will list the proper materials for use. The main activity was a trial with water—a mock-run set up with the device in its near-final configuration. Water was injected into the system to assess it for clogs or leaks. Between the different sizes of chips, it was also important to check the compatibility of the fittings for the device setup—tips, needles, syringes, and tubing. This included different syringes (Omnifix Luer Solo, 5 mL and 10 mL; Omnifix F Tuberculin, 1mL), tubing (5mm diameter, 2mm diameter), and needles (Braun, 23 G x 1" and 18 G x 1½"; BD Microlance, 30 G x ½" and 21 G x 1½"). Both disposable pipette tips (p20, p200, and p1000) and disposable pipettes (1 mL and 5 ml) were also tested for fit in the ports of the fabricated microfluidic chips.

The other aspects of the project investigated in the design size trial were related to feasibility—for fabrication and for synthesis. For the feasibility of device fabrication, the design

size needed to be large enough for the user to handle the chip during assembly. For the feasibility of liposome synthesis, the design size, specifically the channel width, was integral to the result of the microfluidic mixing of solutions within the chip. The latter aspect was explored further in the synthesis stage of method formation.

Synthesis Method Formation

The protocol for liposome synthesis drafted was guided by availability of resources. The lipid used was DSPC (Bachem, 1,2 Disteraroyl-sn-glycero-3-phosphocholine, 1 g). The solvent for the lipid was methanol (VWR, HiPERSolv). The stock lipid solution was made to 10 mg/mL in methanol. This was inverted to mix, then vortexed until the lipid dissolved. This stock solution was stored in a freezer and aliquots removed for working stocks, stored in the fridge. It was tested whether methanol would dissolve the lipid powder or if a stronger solvent was necessary. The buffer solution for the liposome synthesis was 0.9% w/v saline in distilled water. It was expected that testing the prototyping method would lead to unexpected exposure to these reagents; therefore, these choices were made for safety and practicality.

The device setup for the synthesis stage of method formation needed to replicate the assembly trial configuration, now with reagents instead of the previous water trials. The fit of the pipette tips in the ports had to be tested once more for secureness and water-tightness. A strategy for balancing the pipette guns while loading a third had to be developed.

Additionally, the setup of the device with different injection methods was investigated. These choices were reaffirmed from the water testing completed in the assembly stage of the method formation. The three speed settings (G, ♦, ♦♦♦; gravity, reduced speed and full speed, respectively) on the pipette guns (VWR Accurpette) were tested for injection of solutions into the input ports. The channels needed to be primed—pre-wet with buffer. During the previous water trials, coincidental injection of all three inputs was not a factor. When running the liposome synthesis trials on the microfluidic chip, different combinations of pipette sizes and pipette gun speeds required experimentation. A smaller pipette will inject liquid faster than a larger pipette, even when on the same speed setting. When the injection speeds/volumes between inputs were not balanced, there would be backflow through the chip. The battery charge level on the pipette guns was also considered for its impact on speed. Sample collection was set up using tubing and an Eppendorf tube for the output.

Analysis Method Formation

Testing was conducted towards the formation of an analysis method. This is required to evaluate the samples generated by prototyped devices, in aid of optimizing the microfluidic design. The imaging instruments used for this project were an automatic cell counter (Countess II), an inverted microscope (Olympus CKX53), and a light microscope (Olympus CX22). Samples were viewed on the automated cell counter to attempt rapid quantification. Samples were viewed under the inverted microscope at 100X and 400X total magnification. Under the light microscope, samples were viewed under 100X, 400X, and 1000X total magnification. Common dyes also used in haematology labs for analysis of biological samples—crystal violet, haematoxylin, and Leishman—were also used to develop a strategy for analysis. Size estimation was attempted using the built-in function on the automatic cell counter. The software (TCapture) connected to the inverted microscope also had measurement tools for microscope images. Samples were stored in a refrigerator (at around 5°C) between laboratory activities.

3. Results

The development of a microfluidic prototyping method for liposome synthesis protocols began with the design of possible channel patterns. Seventeen initial channel designs were

produced, but one design was fabricated and tested. The basic structure can be seen in Figure 2 below. Digital versions of the basic designs were created in TinkerCAD, as seen in Figure 3.

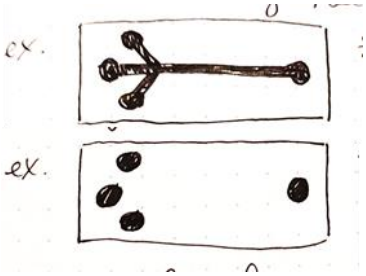

Figure 2: Initial sketch for basic microfluidic design as two-layer chip, one channel layer and one with ports

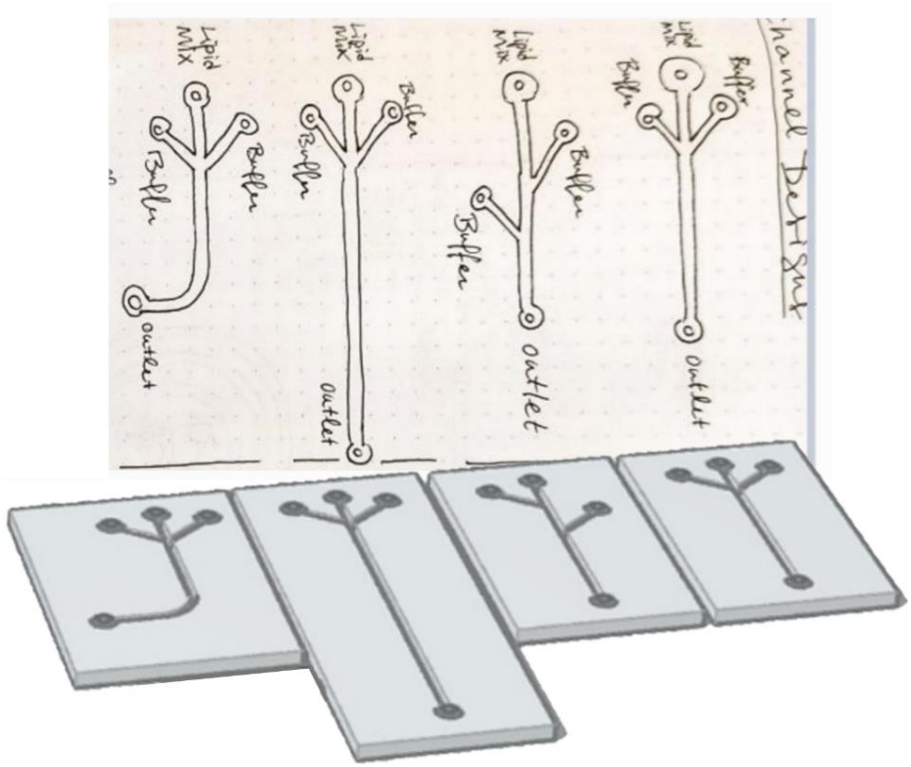

Figure 3: Adaption of sketched channel designs into 3D designs using TinkerCAD website

The “Basic #1” design was printed from the digital image into three different template sizes, measured from length of centre channel and breadth of ports: large (3.7x1.7 cm), medium (3.2x1.5 cm), and small (2.5x1.2 cm). These were traced onto the plastic sheets with a black permanent marker. The wafer was best cut from the sheet with scissors and the channel with a small utility knife. The ports were best opened by using a soldering iron.

The thermoplastic manufacturer estimated that the material used had a shrinkage of two-thirds. For greater precision, a ruler was traced onto the unshrunk plastic. After shrinking in the oven with the microfluidic chips, it measured 6 cm. This was 8 cm of shrinkage, or 57%.

After shrinkage, the wafers in the size trials were examined under an inverted microscope at 100X total magnification to evaluate the cut channels and holes for ports. From the large size, one had separated into its layers and had a closed channel while the other was of decent quality except for some asymmetry of the side channel widths. From the medium size, one had separated into its layers and had warped in the middle, causing misalignment of the channel edges along the z-axis. The other was partially closed along the channel. From the small size, one had rough edges. The replicates that remained in two layers were the ones that had been made by aligning the wafers before melting through to bore the ports. Most of the ports had closed after shrinking,

though the microscope images were difficult to interpret. The sizes of the small wafer after shrinking is shown in Figure 4, with a comparison in Figure 5, and the estimated interior measurements are shown in Figure 6. In this project, the port holes were reopened by soldering iron post-shrinking and a regular diameter was achieved that tightly fit the blue (p1000) pipette tips.

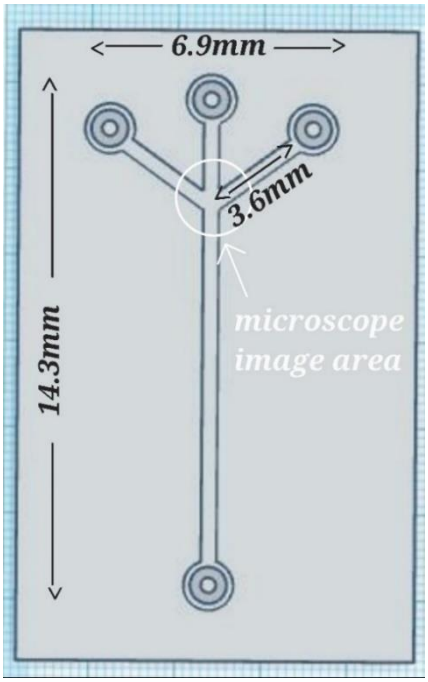

Figure 4: Calculated sizing of microfluidic chip after shrinking, for the small-sized chip (originally 2.5x1.2 cm)

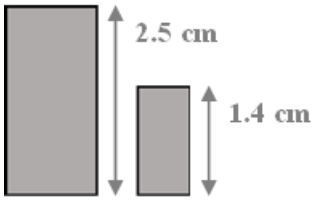

Figure 5: Representation of small wafer in size trial, pre- and post-shrinkage (actual size)

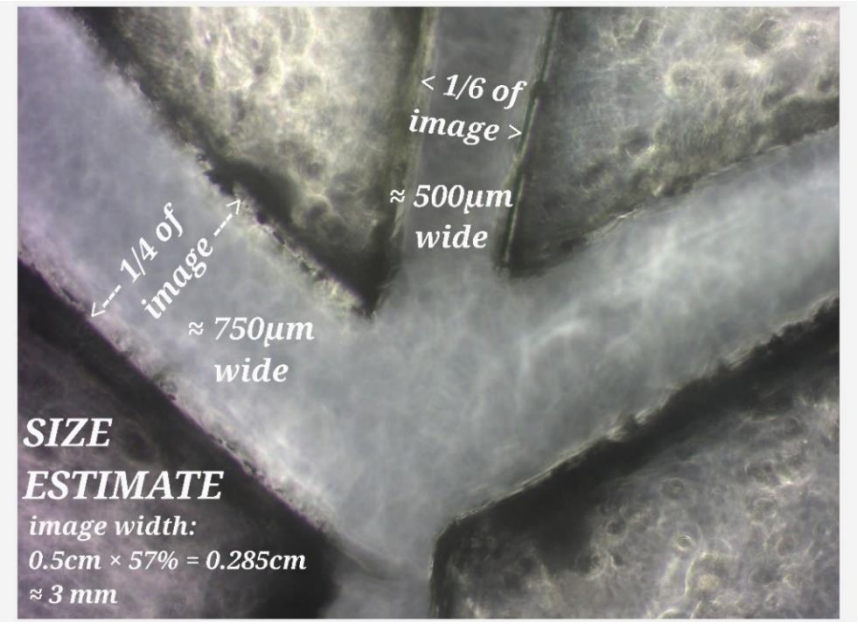

Figure 6: Calculated sizing within channel fork of Basic Small 2 replicate

The device setup for a microfluidic run is shown in Figure 7. Referencing photos of the setup, this figure was drawn digitally (stylus, Corel Painter program) in attempt to simplify the visualization and aid in understanding the different stages of the microfluidic run. Three pipette guns were attached to 1 mL disposable pipettes, attached to p1000 pipette tips. The solutions were drawn into the pipettes. (For the purposes of the illustration, this was colourized as blue for the lipid solution and green for the buffer solution.) The pipettes were fitted into the ports. As the "down" buttons on the pipette guns were depressed, the liquid was injected from the pipettes into the microfluidic device, through the channels, out the output port, through the tubing, and into the collection tube. As more of the solutions left the pipettes, more of the resulting mixture (colourized turquoise for the purposes of the illustration) filled the collection tube, and the tubing that connected it to the device began to bend with the weight. When the run finished, the collection tube was removed and stored with the sample inside it. The device was flushed with distilled water, then buffer, before reusing.

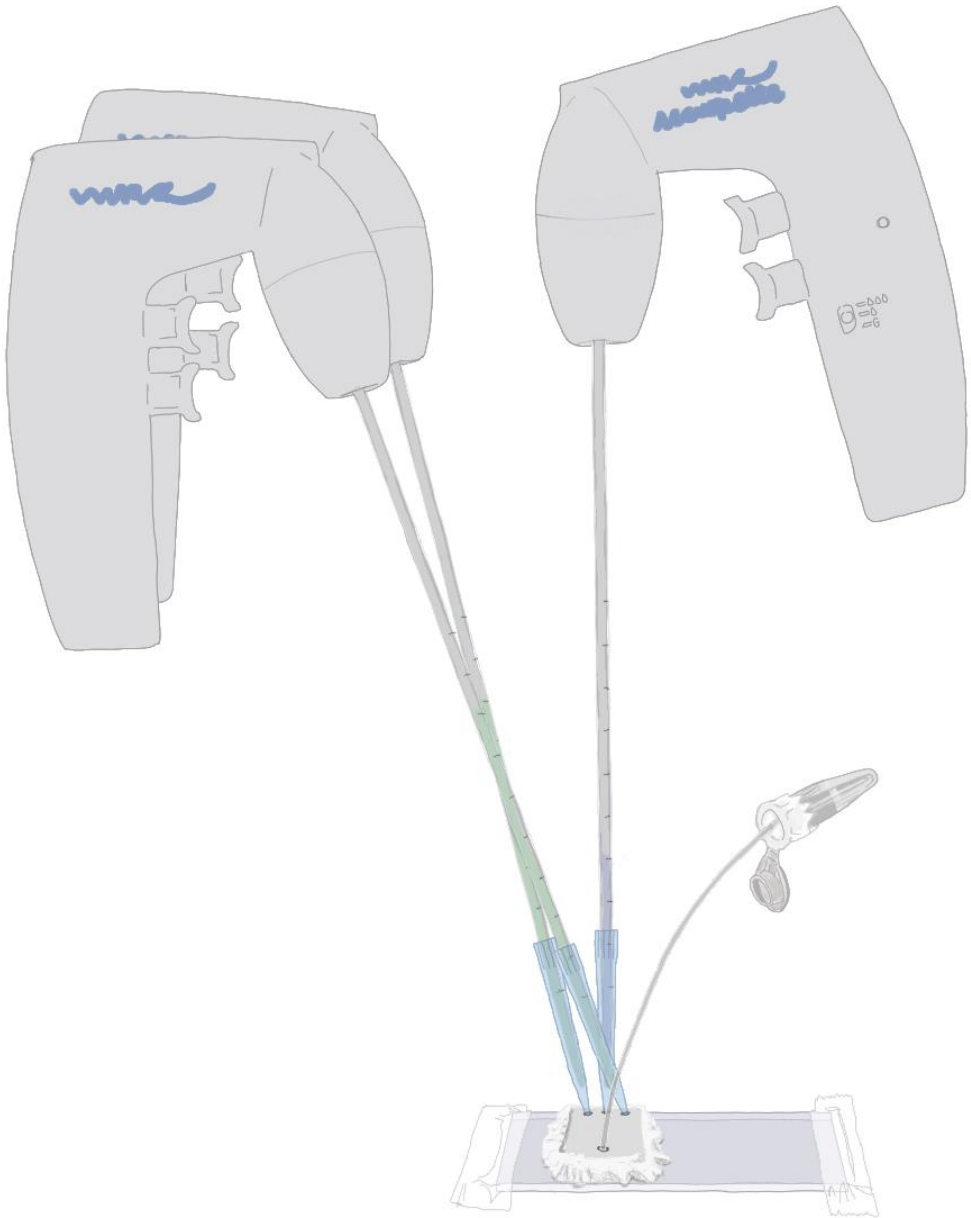

Figure 7: Microfluidic device setup before start of run, digital drawing; aqueous solution represented by green liquid in two pipettes; phospholipid solution represented by blue liquid in one pipette; the view before solutions are injected into the microfluidic chip and collected in the sample Eppendorf

The resulting samples were visualized using the automatic cell counter (Countess II) with the specific slides for the instrument. As seen in Figure 8, ring-shaped spheres were observed in the samples collected at the end of the microfluidic run. The samples were also viewed under an inverted microscope, which had a USB connection for direct image capture. An example of a sphere observed using the inverted microscope is shown in Figure 9. Initially considered anomalous, changes in the sample over time are recorded in Figure 10, showing spheres expanding to double the original diameter over half an hour of observation.

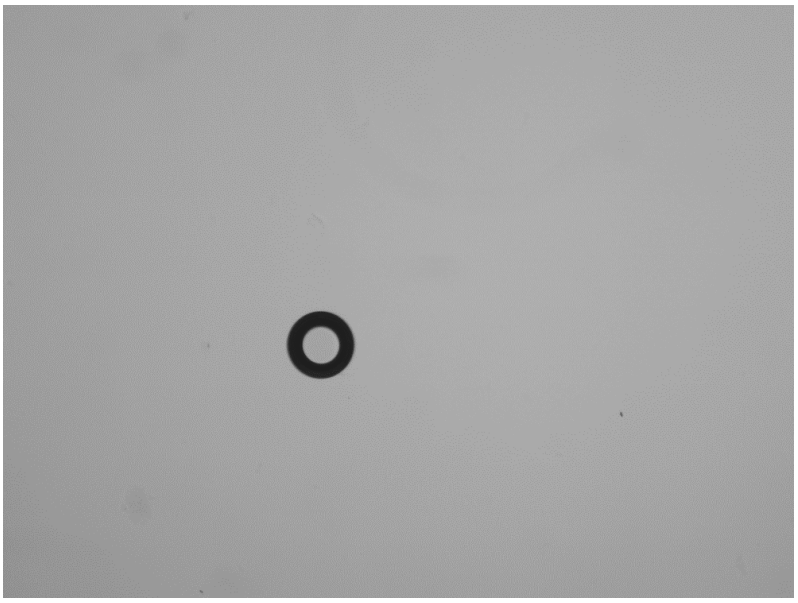

Figure 8: Image captured from Countess II (automatic cell counter) of sample generated using prototype microfluidic device, displaying ring-shaped sphere.

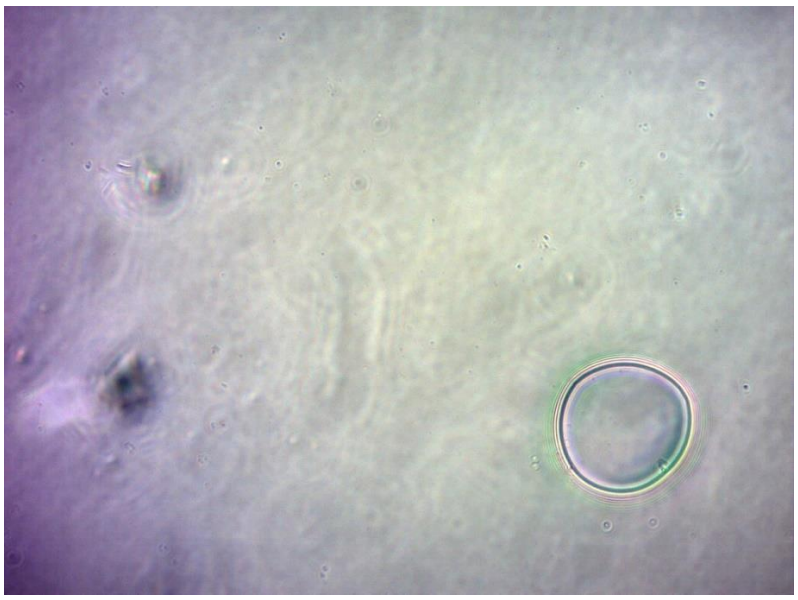

Figure 9: Image captured of sample run on microfluidic device for liposome synthesis, viewed on an inverted microscope at 400X total magnification

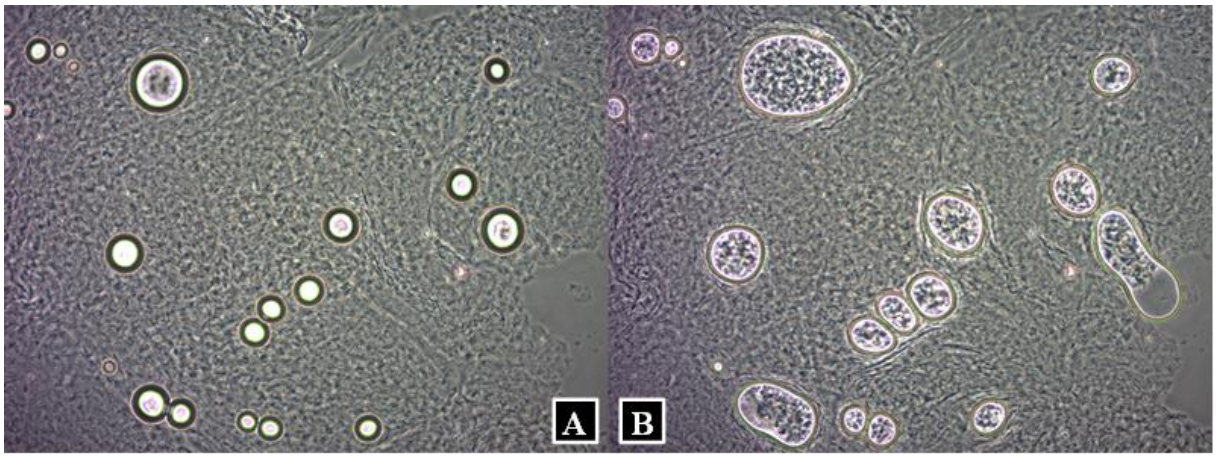

Figure 10: Expanding spheres in control lipid solution with saline buffer, vortexed 30 seconds, visualized under 100X total magnification, at t = 0 minutes and t = 36 minutes.

Resulting Method for Microfluidic Prototyping

The method developed for this project is described in Figure 11. The transparent thermoplastic was overlaid on a digital microfluidic design and traced with a permanent marker. The plastic was cut with scissors on the edges of the chip. A small craft knife was used to cut the channels. Cardboard or a self-healing mat underneath the plastic will protect the work surface. Once each side of the channel was cut, the excess plastic between was gently removed. A matching wafer of shrink-plastic, without the channels, was cut to size and placed over the initial piece. Next, the port holes were opened in the plastic, using either a flamed needle or, briefly, a soldering iron, melted through both pieces at the same time. The wafer layers, still together, were then shrunk in a preheated oven at 170˚C for two and a half minutes, on a foil-lined oven tray. This was then gently flattened by another tray. Silicone sealant was spread thinly on the underside of the chip which was fixed into place on a glass microscope slide—channel-side down, port-side up—using more sealant around the edges of the chip and left to dry for 24 hours.

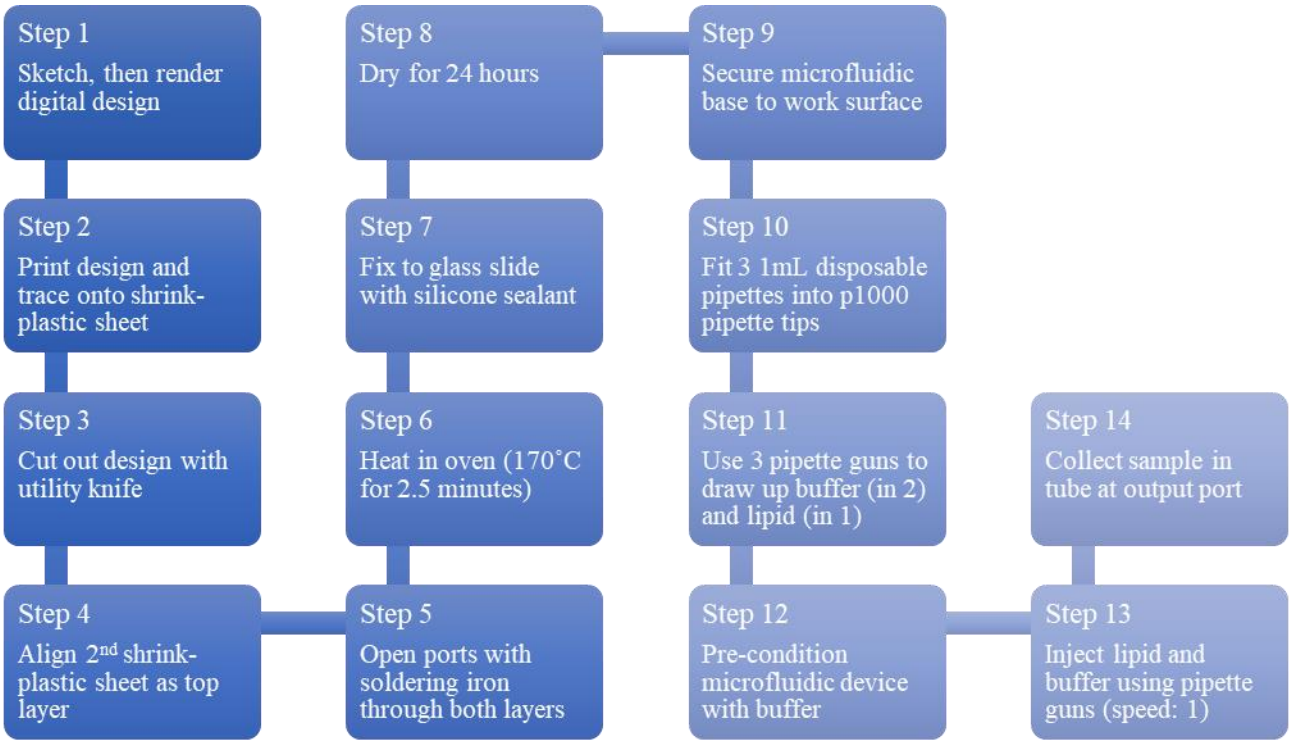

Figure 11: Resulting Microfluidic Prototyping Steps for fabrication of shrink-plastic chips and synthesis of liposomes; Flowchart of the Final, Resulting Method developed from the project

The stock lipid solution was made with 1,2 Disteraroyl-sn-glycero-3-phosphocholine (DSPC) to 10 mg/mL in methanol. It was dissolved by inverting and vortexing. This was stored in the freezer, in a 20 mL centrifuge tube, and aliquots were removed for the working solution. The stock saline buffer was made with sodium chloride to 0.9% in distilled water. It was stored at room temperature in a 500 mL stoppered glass bottle.

After the sealant had set, the device was set up for a run with the sample, as shown in Figure 12. First, the ends of the device base were secured to the counter with tape. A short length of plastic tubing was fitted into the output port. Three blue pipette tips were added to the ends of three disposable 1 mL pipettes. These were attached to the three pipette guns. Buffer was injected into the system to pre-condition the channels. Then, a 1.5 mL Eppendorf tube, with the mouth wrapped in parafilm, was attached to the output tubing by piercing the parafilm with the end of the tubing. One pipette gun was used to draw 0.4 µL of lipid solution. The remaining two pipette guns were used to draw 0.8 µL of buffer. Once filled, the tips were secured in the buffer input ports. Using the pipette guns, with speed set to 1 (♦), the solutions were injected into the device, through the channel, out the tubing, and into the collection tube. The samples were stored in the fridge until analysis.

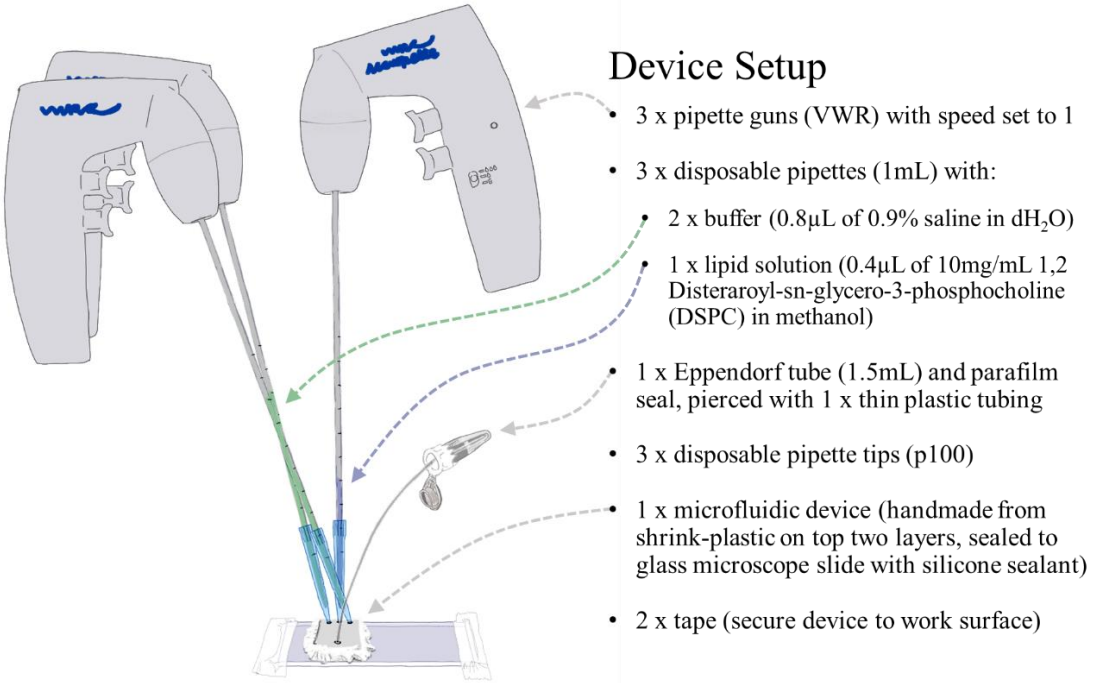

Figure 12: Diagram of Device Setup for Microfluidic Generation of Liposomes

4. Discussion

Microfluidics can be used as a method to synthesize liposomes—the vesicular forms of lipid membranes. Different experimental conditions will have an impact on their synthesis, including ratio of lipid to buffer solution within the microfluidic channels (Patil and Jadhav, 2014). The channel pattern also influences liposome synthesis, as seen in spiral separation of blood cells by size (Warkiani *et al.*, 2015). Therefore, the channel pattern is one of the factors that must be optimized for microfluidic liposome synthesis. To optimise this, many different channel patterns must be tested, and the results compared. To save on resources, rough prototypes can first be fabricated of these different designs. This project, on the topic of Lipid Membrane Self-Assembly and Synthesis in the Lab, resulted in a method for the fabrication of shrink-plastic microfluidic devices. The devices are intended to test different channel designs and their impact on the samples run following a liposome synthesis protocol.

A CNC router would improve the fabrication of the microfluidic chip as it would etch the design into the plastic, eliminating manual limitations (The Thought Emporium, 2019). It would also simplify the design to two layers as the channel is not cut through its layer completely and thus replaces the base layer from this project. Figure 13 shows where this could be integrated into the existing method. However, as this project emphasizes accessibility in equipment and materials, it is acknowledged that a CNC router is not necessary. To improve the three-layer design, another attempt should be made to use shrink-plastic as the base material. If the top layers can adhere to the plastic base, using the oven instead of requiring sealant and setting, this will reduce the time required for fabrication—lending further credence to the "rapid" prototyping claim—and will eliminate a component with the potential for complication of the liposome protocol through contamination.

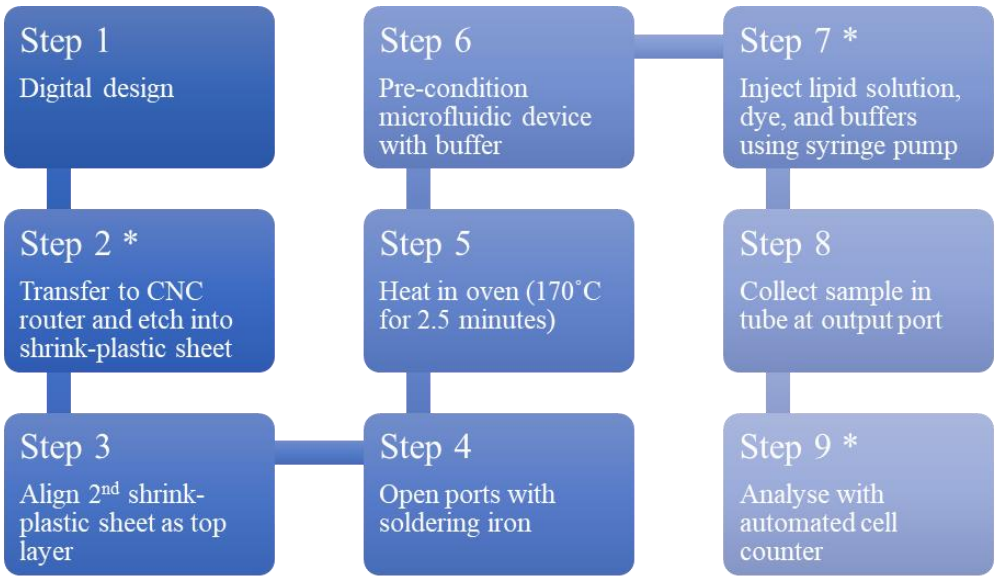

Figure 13: Flowchart of Proposed Method Improvements (asterisks mark changes from developed method)

An improved technique of controlling the flow or pressure in the microfluidic system would increase the reproducibility between trials. Thurgood *et al*. (2019) designed a pump from a reinforced balloon. If this were adopted, it would be in keeping with this project's theme of low-cost, accessible materials. Alternatively, a commercial microfluidic flow controller or syringe pump could be adapted into the protocol. In this project, the flow was regulated by the three speed settings on the pipette guns—G (gravity), 1, and 2. Not only does this imprecision convolute the potential for duplication of the experiment in other laboratories with other equipment, but the issues with variability of speed between different pipette guns—of the same model, within the same laboratory—make this an impractical setup. This effect was only moderately controlled by ensuring that all pipette guns started with a full battery charge. With only three settings, fine-tuning of the speed of injection was not possible. There was a large change in speed between each of the three settings. The exact difference between these could not be measured as no pressure gauge was available, although, this is another essential tool for reproducibility of a microfluidics method.

The analysis strategy for the generated samples/liposomes was not optimised in this project. The initial plan was to use an automated cell counter to rapidly generate the concentration and size distribution of liposomes in the samples—as giant unilamellar vesicles (GUVs) are comparable to mammalian cells in size. The issue with this strategy was the equipment available. The Countess II Automated cell counter has a size range limited to 5–70 µm diameter cells. Larger spheres in the samples could not be accurately quantified by this equipment, and smaller particles interfered with the results. Additionally, the spheres observed in the samples grew in diameter over time due to an unknown mechanism (Figure 10). This was not a function of temperature

(from samples reaching room temperature after removed from the fridge) but would begin when the samples were added into the sample slide. The spheres showed a dramatic increase in size over a span of five minutes. Although the plan was to only use the automated counter for sample analysis, this effect needed to be investigated further using the other microscopes available.

From the details observed using the inverted microscope in Figure 10, the sample matrix exhibits change over time as well. The sample, once applied to a microscope slide, dries over ten to fifteen minutes. After an aliquot is removed from the sample Eppendorf tube, it is exposed to air. The methanol—used as a solvent for the lipid solution—would dry out under these conditions. After an extended period, the spheres in the samples were observed to distort, filling the spaces left as the matrix dried (Figure 10). The boundaries of these spheres remained, which is unexpected if these are air bubbles, though the possibility cannot be eliminated without further testing. This method of visualization with a standard microscope slide and coverslip has previously been noted to result in rapid drying of the sample, and this effect could be reduced by using a slide with a frame seal chamber (Bibi *et al.*, 2011).

Within the samples, differentiation between lipid "bubbles" (the intended liposomes) and air bubbles was more difficult than expected. Further testing is necessary. Stability could be increased by varying the composition of the lipid mix (Deamer, 2017). It could be that liposomes present in the samples remained undetected by the optical techniques attempted in this project. The generated vesicles could also be smaller than the detectable size for the microscope (Robson *et al.*, 2018). With additional resources, dynamic light scattering or transition electron microscopy can be used to determine liposome size (Sturm and Poklar Ulrih, 2021). Monitoring the liposome formation could also aid in identification of the spheres. It would be possible to run the microfluidic device while secured to the platform of the inverted microscope. The digital capture from this microscope would show real-time interactions between the lipid and buffer solutions. However, the microscope image capture software (TCapture) used in this project was not optimized for this purpose, in terms of frame rate. If a lipophilic dye such as Oil Red O was incorporated into the experiment procedure, this could confirm the presence of giant unilamellar vesicles in the samples, even if using light microscopy (Bibi *et al.*, 2011).

5. Conclusions

The aim of this project was to develop a method for rapid prototyping of microfluidic designs for their optimization to liposome synthesis protocols. This was accomplished, with caveats. Due to time limitations and restrictions on materials and equipment, only one design was tested. This basic microfluidic channel design was chosen as its simplicity also simplified the process for testing different fabrication and assembly strategies. Once these strategies were refined, the plan had been to execute the resulting method using the advanced microfluidic designs, running the same liposome synthesis protocol using the different designs and analysing the samples that this would yield. By evaluating the performance of the different designs, a microfluidic device could be produced that would be optimized for that specific liposome synthesis protocol. After the optimal channel design was confirmed, the final device could be fabricated using professional fabrication techniques for microfluidic chips.

Within the resulting method from this project, the protocol for generating the samples could be modified for the purposes of different experiments. This includes the lipids used, the solvent, buffer, and the injection volumes or timing of injections. The intended use of this method is to make prototypes to test the impact of different channel designs on a single microfluidic protocol, but the exact liposome synthesis protocol does not need to be as described.

Initially, this project was intended to develop a method for prototyping microfluidic devices. This progressed into using common materials at low cost to increase the accessibility of research and development activities. A rapid, low-tech prototyping method using low-cost materials improves the accessibility for the development of new devices which might not

otherwise be possible, that is, without constant access to the technical equipment required for industry-standard fabrication.

6. Future Work

One of the deficiencies in the experimental design is regarding sample analysis. Visualization of liposomes in the samples that were run on the microfluidic device was not definitively confirmed. Certainly, there is evidence of their synthesis in the process, from the self-assembling nature of lipid membranes, to the recorded explanation of the matrix-drying effect for the initially inexplicable “growing spheres” phenomenon. These “spheres” described in the samples are very possibly liposomes, yet, as long as doubt remains, they will not be labelled as such. Further testing with reverse-phase microscopy, fluorescence microscopy, light-scattering, or electron microscopy would be required to confirm this. Although the incontestable presence of liposomes in the samples would provide proof of concept for the prototyping method, sample synthesis and analysis were secondary to the assembly stage of the project, which necessarily preluded the other activities.

The suggested alterations to the method developed for rapid prototyping of microfluidic chips include the addition of a CNC router, lipophilic dye, syringe pump, and automated cell counter to the workflow. Though only slightly more advanced, it is possible that these would greatly improve on the reproducibility and speed of the prototyping method. Further testing would be required to support their inclusion.

7. Acknowledgements

[DO NOT COMPLETE.]

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9. Appendix

As seen in Figure A.1 below, the main phases of this project (grey) were the literature review, the method formation, results, and evaluation. The stages of the literature review (yellow) were the systematic web search and the initial design of methods. The literature review phase lasted three months. The stages of the method formation (yellow) were assembly, synthesis, and analysis. These were completed in the laboratory over three months, and the time spent in the lab (with approximately five hours per day) was 10 days in the assembly stage, 2.5 days in the synthesis stage, and 2.5 days in the analysis stage of method formation. In each stage, there were considerations (blue) that were required to investigate, shown by the different activities (green) that were completed.

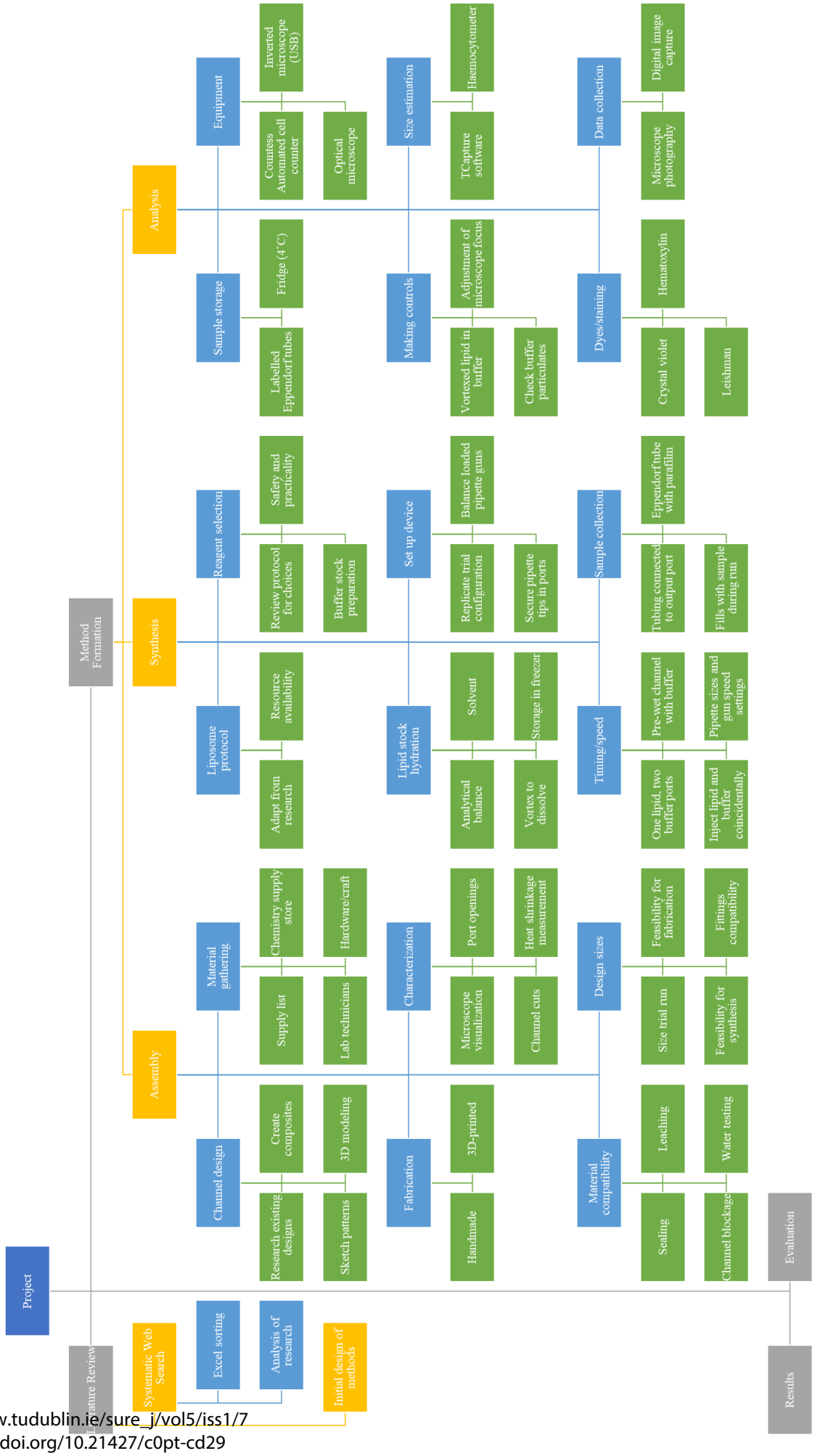

Figure A.1: Complete overview of project activities

Figure A.2: Photograph of shrink-plastic microfluidic chip attached with silicone sealant to glass slide base, with tubing and pipette tip in ports