A Novel Encapsulation Device for Mouse Neural Stem Cells

A Capstone Technical Report Submitted to the

Department of Biomedical Engineering
School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements of the Degree
Bachelor of Science, School of Engineering

Spring 2020

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Words: 4,978

Number of Figures: 6

Number of Tables: 0

Number of Equations: 0

Number of Supplements: 1

Number of References: 21

A Novel Encapsulation Device for Mouse Neural Stem Cells

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Abstract

Cell encapsulation—the process of coating cells with a semipermeable polymeric matrix—is of critical importance for many biomedical research applications including stem cell technologies, as the coating that encapsulates the cell is necessary to prevent immune rejection in cell transplantations. Using the layer-by-layer technique to encapsulate cells can provide several advantages to researchers, however, the current manual layer-by-layer process can be time-consuming and tedious, create difficulties in saving excess unbound material, and require cells to be kept out of an incubator for long periods of time during the encapsulation procedure. Therefore, a novel cell encapsulation device was designed to address these limitations. Through an iterative design process, an optimized prototype for this cell encapsulation device was developed specifically to aid stem cell researchers by providing improvements over the traditional layer-by-layer process. While lab closures due to the COVID-19 pandemic prevented complete assembly and testing of the final design iteration, it is expected that the outlined design validation process will prove the device capable of reducing the time necessary to complete the encapsulation procedure, reducing the amount of material lost during the procedure, as well as increasing the number of viable cells encapsulated during the layer-by-layer coating process.

Keywords: layer-by-layer, cell encapsulation, mouse neural stem cells

Introduction

For many labs conducting biological research on the cellular level, cell encapsulation is a critical process required for effective cell delivery into a target medium[1-3]. Specifically, cell encapsulation refers to the technique of enclosing the biological active material within a polymeric matrix by a semipermeable membrane[1]. The encapsulation process has been proven to be particularly useful for researchers as it maintains cell viability and functionality by offering efficient transfer of oxygen, nutrients, and cell metabolite by-products[4]. Encapsulation is typically used for cell-based therapeutic purposes as well as cell transplantation and organ replacement[4] as it can provide mechanical protection for the enclosed cells[5]. Additionally, encapsulation prevents immune reactions that arise from cell injection, therefore reducing the need for use of immunosuppressants[6].

Since the beginnings of cell encapsulation, which began when Chang et al. first reported encapsulating human erythrocytes in nylon microspheres[7], a variety of methods for cell encapsulation have been designed and developed over the years[8-11]. Depending on the purpose of the experiment, a different type of cell encapsulation may be used. Cell encapsulations may be classified into two main categories: macroencapsulation, which typically refers to the encapsulation of multiple cells within a larger bulk hydrogel using macroscale devices[12], and microencapsulation, which refers to encapsulating cells into a semipermeable polymer matrix (microsphere, microbead) or membrane (microcapsule) at the micrometer scale[6,12]. While macroencapsulations can be useful in providing cells with a 3D hydrogel scaffolding that can model the body[13], the density of cells within the hydrogel is much lower than in microencapsulations. Therefore, the Highley Lab is using a microencapsulation method to provide a protective cell

coating with only a few layers of polymer, thus achieving a high cell density encapsulation.

Specifically, a multi-layer biointerface method for the microencapsulation of mouse neural stem cells (mNSC) is being developed by graduate student Jack Whitewolf, who conducts research in the Highley lab. In this layer-by-layer approach, the first coating layer, which is made up of a lipid-and-thiol-modified hyaluronic acid (HA), is attached to the cell membrane through lipid-insertion, and subsequent layers (also modified forms of HA) are bound to the previous layer. While other layer-by-layer cell microencapsulation methods have been developed[14-17], this method of using lipid-insertion to develop a multi-layer cell coating is a novel approach, allowing for the outer polymer layers after the initial layer to be easily modified. These modifications to the HA polymers allow for control over the thickness and mechanical properties of the coating, as well as the interchanging of degradable and non-degradable layers.

However, the current layer-by-layer cell encapsulation technique can be tedious and time consuming for researchers. The process requires numerous steps in which cells must be repeatedly moved between various solutions with centrifugation and washes required between each step of the process. Encapsulating cells with just two thin (<1 μm) layers takes approximately 30 minutes, but this coating procedure could take much longer if coatings of significant thickness are required. Due to this long encapsulation process, the cells must be kept out of their incubator for a long period of time. Finally, in the current layer-by-layer encapsulation process, it can be difficult to save all of the excess unbound polymer solution, which can be quite costly to produce. This is due to the fact that, following the centrifugation process, any excess solution must be carefully pulled off the top of a pellet of cells without removing any cells or leaving too much fluid behind, which can be difficult to do by hand with a micropipette. Therefore, our capstone group has developed a novel device design for encapsulating mNSCs to address these stated issues with the layer-by-layer technique.

Device Design and Optimization

In order to address the aforementioned issues associated with the manual coating process, our capstone team has developed a device design with the purpose improving the current layer-by-layer cell encapsulation process. The encapsulation device is designed to provide a more efficient process for encapsulating cells and prioritize ease-of-use for the researcher. To conduct the device design and optimization process, our work was focused around three specific aims, as outlined in the initial capstone proposal. These aims were to: determine the most effective porous membrane filter for the device, design and construct a prototype device that incorporates the membrane, and implement a control system for the device.

Membrane Selection and Testing

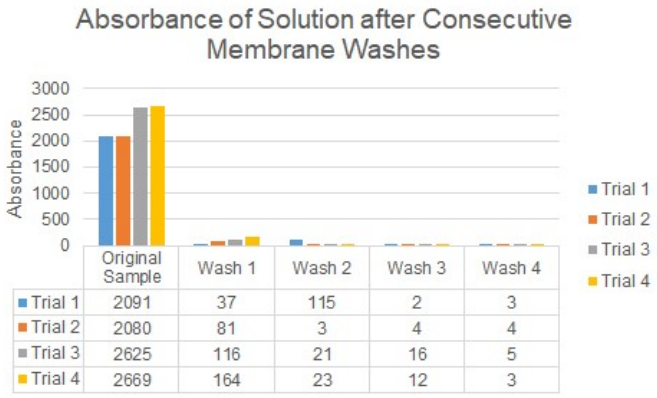
Selecting the Membrane

For our first aim, *determining the most effective porous membrane filter for the device*, we had selected a 0.65μm Durapore porous membrane to begin testing on. The 0.65μm Durapore membrane was specifically selected because we expect the membrane will allow for proper filtration in our device; the 0.65μm pores are large enough to allow unbound polymers to wash out but small enough to keep the cells from passing through the membrane without rupturing. Furthermore, the 0.65μm Durapore membrane has been used in the Highley lab previously and has achieved favorable results during experimentation.

Testing the Membrane

After our selection had been made and the membranes were ordered for the lab, we began the testing of the membrane to determine if solution could properly travel through and if so, how many washes are necessary to clean out the membrane. It is critical to clean out the membrane between each step because if an initial polymer coating solution is still present in the membrane when a secondary polymer coating solution is added, the two polymers coating may bind together in the membrane, thus clogging the membrane filter.

To conduct this membrane testing, we used a solution containing fluorescent dextran in order to model the hyaluronic acid solution that is used during the cell coating process. Fluorescent dextran was chosen specifically because of its similar structure and molecular weight to HA as well as having a fluorescent property that allows it to be detected by a plate reader. The solution containing the fluorescent dextran was run through the membrane and collected in a microtube. Subsequently, a phosphate-buffered saline (PBS) solution was run through the same membrane four times and the solution from each wash was collected in separate microtubes. Four trials of this procedure were performed on the membrane and then all samples were plated and placed into a plate reader.



	Original Sample	Wash 1	Wash 2	Wash 3	Wash 4
Trial 1	2091	37	115	2	3
Trial 2	2080	81	3	4	4
Trial 3	2625	116	21	16	5
Trial 4	2669	164	23	12	3

Fig. 1. Absorbance of Solution after Consecutive Membrane Washes. A solution containing a fluorescent dextran solution was run through the membrane followed four consecutive PBS washes of the membrane. Absorbance values for four trials are recorded in the table and displayed in corresponding the clustered column chart.

The relative absorbance of each sample solution is shown in Figure 1, where higher absorbance corresponds to a higher concentration of fluorescent dextran present in the solution. After the first membrane wash, the relative absorbance of the sample solution collected is much lower (average absorbance of 99.5) than the original sample of fluorescent dextran solution (avg. 2366.25), however this indicates that some fluorescent dextran is still present in the membrane. After the second membrane wash, the corresponding solution contained even less fluorescent dextran, and by the third wash, the membrane contained an insignificant concentration of fluorescent dextran. Thus, we were able to conclude that two to three washes would be significant to thoroughly cleanse our porous membrane filter in between each step of the cell encapsulation process. However, as only one experimental trial was able to be run before COVID-19 complications, further testing would be necessary to validate these results.

Design and Optimization of the Prototype Device

To address our second project aim, *designing and constructing a prototype device that incorporates the membrane*, we began with the initial design schematic submitted for our capstone proposal and then developed an optimized version of our device through several design iterations over the course of the year.

Initial Device Design

The schematic for the initial device design is shown in Figure 2. This initial design consisted of a main chamber with four tubes inserted into openings in the both the top and bottom of the chamber. Additionally, this design utilized three syringe pumps and a vacuum pump which were connected to tubing that would be inserted into the openings in the main chamber. An Arduino microcontroller would be programmed to control stepper motors which would in turn control syringe pumps. Near the bottom of this chamber, a support structure was designed to hold the porous membrane filter.

In the first step of this coating process, the mNSCs would be placed on top of the membrane and then syringe pump 3 would pump a PBS solution upwards, causing the cells to rise up off of the membrane filter and suspend in the solution as it fills up the main chamber. Following this step, syringe pump 1 would dispense the first polymer coating

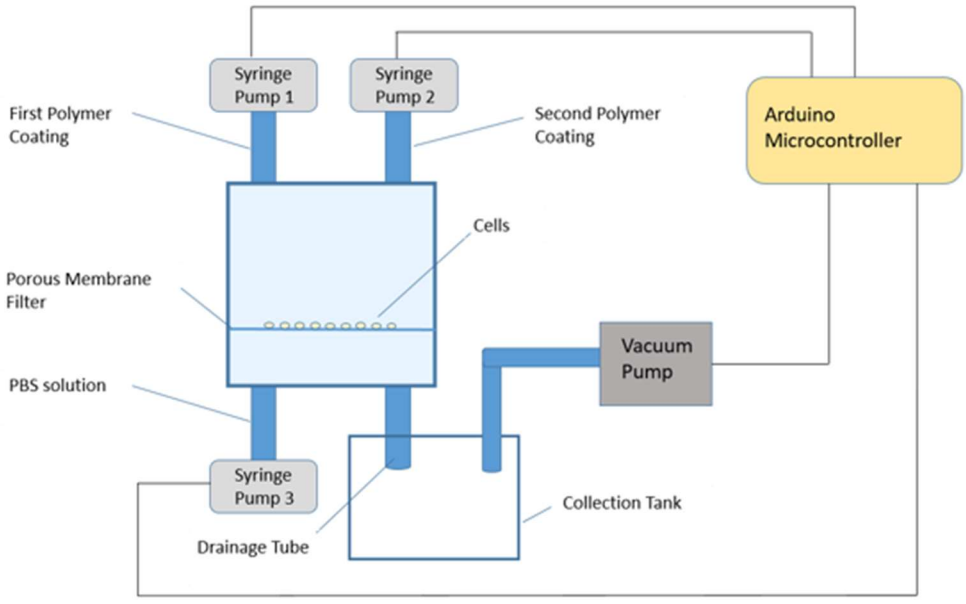


Fig. 2. Initial Design Schematic for the Encapsulation Device. The preliminary design for our purposed mNSC encapsulation device features a main chamber containing a porous membrane filter, three syringe pumps, a vacuum pump, a collection tank, several sections of tubing, and an Arduino microcontroller. The Arduino microcontroller is used to control several stepper motors which in turn control the syringe pumps to dispense the polymer and PBS solutions into the main chamber.

solution into the main chamber. The polymer contained within the first coating solution would then bind to the cells. After sufficient time was given for the binding of the primary polymers, any excess polymer solution would then be suctioned downward through the porous membrane filter into a collection tank using the vacuum pump. Once all of the fluids had been drained out of the main chamber, the cells would again be resting on the porous membrane filter, thus completing the first layer coating of the mNSCs.

To resuspend the cells in PBS solution, syringe pump 3 would again utilized to pump PBS upward into the main chamber. Subsequently, syringe pump 2 would dispense the second polymer coating solution into the main chamber to bind with the first layer polymer coating on the mNSCs. Once again, any excess fluid would be drained out into the collection tube for future use, thus completing a two layer encapsulation of the mNSCs.

To begin developing the prototype device for our initial design, we created a 3D CAD model of the main chamber using Autodesk Fusion 360 (Figure 3A). The first CAD model of the main chamber featured a removable lid, an internal support structure near the bottom of the chamber to hold the porous membrane (Figure 3B), and two openings on both the bottom and lid of the chamber, allowing for the insertion of tubing that leads to the syringe pumps.

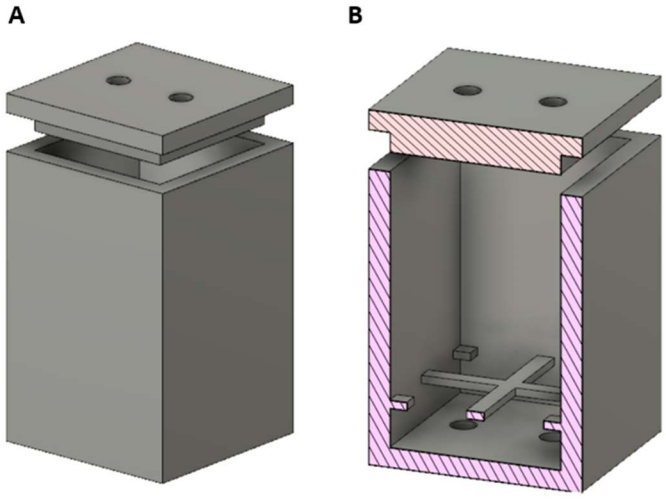


Fig. 3. Initial CAD Model for the Encapsulation Device. Using Autodesk Fusion 360, a 3D CAD model was created based on the initial design schematic (A). A cross section reveals the inside of the chamber containing a support structure for the membrane filter (B).

Drawbacks of the Initial Design

While the initial design proved to be useful starting point, it contained several drawbacks and potential points of failure that needed to be addressed in future design iterations. First, the porous membrane filter was not properly secured in place in our initial device design. While there was a support structure in place to hold the membrane above the bottom of the chamber, this structure would not be able properly stabilize this membrane when solutions began flowing into the chamber during the encapsulation process, resulting in the free movement of the membrane around the chamber, and subsequent failure of the encapsulation process. Thus, a future design iteration necessitated a method for securing this membrane in place to ensure the proper filtration of solutions during the encapsulation process.

Another drawback of the initial design was the lack of proper sealing between the main chamber and the lid, which could result in leakage of the polymer solutions. Furthermore, having two small openings on the bottom of the main chamber and two more openings on the lid added unnecessary complexity to our design, requiring a system of several valves to be turned on and off throughout the process. While this would have been possible to create, our capstone team determined it would be more efficient to reduce the number of total openings and therefore we planned to address this issue in our next design iteration.

Updated Design Schematic

To account for the aforementioned drawbacks of the initial design, our capstone team updated our design schematic as shown in Figure 4, thus providing several improvements to the prototype device. First, the main chamber of the device was redesigned as a cylindrical three part chamber, including a bottom section, top section, and lid. By splitting the main chamber into separate top and bottom sections, the membrane filter was able to be properly secured as it could now be sandwiched between the two sections. Additionally, by making the chamber cylindrical in our updated design, O-rings were able to be installed in between the sections of the device. This solved the anticipated problem of fluid leakage by providing a means to properly seal the device.

Furthermore, the device design was simplified by reducing the two separate openings on the bottom of the device to a single opening in which the inserted tube can be directed to either a collection vial or a vial containing PBS solution (Figure 4). The lid design was also updated to have a single opening, allowing for the insertion of tubing. When the lid is placed onto the main chamber, and the inserted tubing directed to a collection vial, PBS solution can be suctioned upward from the opening in the bottom of the chamber through the porous membrane in order to resuspend mNSCs in PBS solution (Figure 4C). In addition,

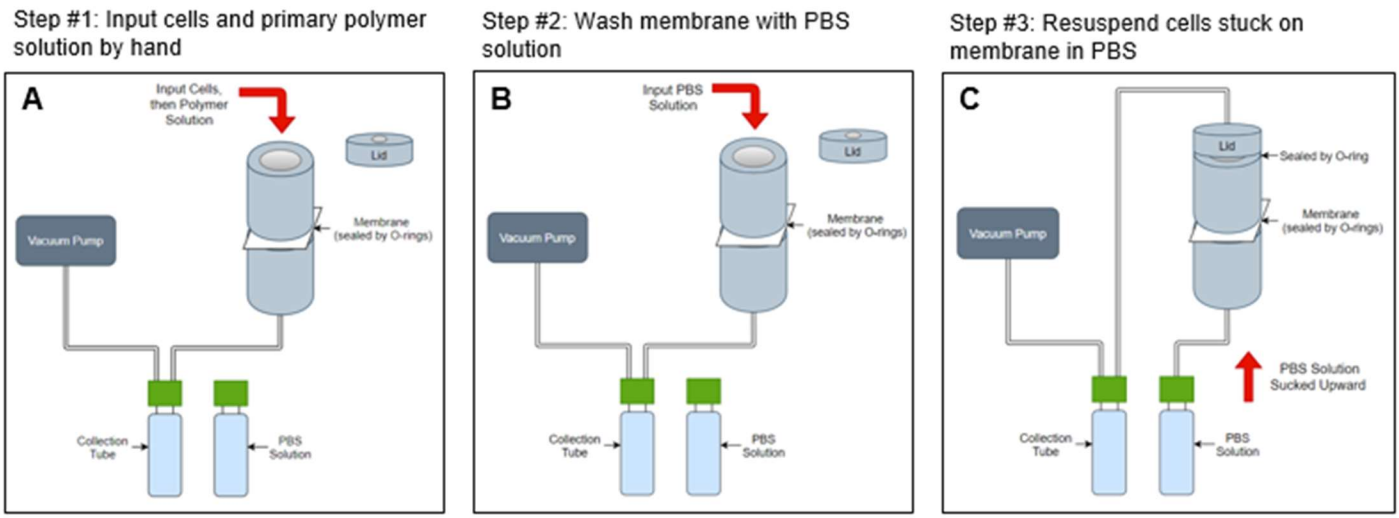


Fig. 4. Updated Design Schematic for the Encapsulation Device. In this updated version of the initial design schematic, the three step process for encapsulating mNSCs with a single layer coating is described (A,B,C). For any additional layers, the three steps should be repeated.

the lid can be removed in order to input the cells and the coating solutions by hand using a micropipette (Figure 4A).

In this updated design, adding each layer to the encapsulation follows a simple three-step process. First, after the mNSCs have been add to the chamber in a PBS solution, the primary polymer coating solution (lipid-and-thiol-modified HA) is added into the top of chamber as illustrated in step 1 (Figure 4A). After this polymer coating has bound to the mNSCs, any excess solution will then be suctioned down through the membrane and tubing into the collection vial, to be used for future encapsulation procedures. Following this step, the membrane should then be washed by running a PBS solution through the membrane twice in order to remove any excess polymer trapped in the membrane as demonstrated in step 2 (Figure 4B). Finally, the tubing from the lid should be inserted into the collection vial, the lid placed on top of the chamber, and the tubing on the bottom of the chamber directed to the PBS solution as shown in step 3 (Figure 4C). This will allow the PBS solution contained in the vial to be suctioned upward through the membrane, thus resuspending the mNSCs that had been resting on the membrane in a PBS solution as well as completing the process for the first layer of the cell encapsulation. To add the second layer and any additional layers, steps one through three can be repeated except with

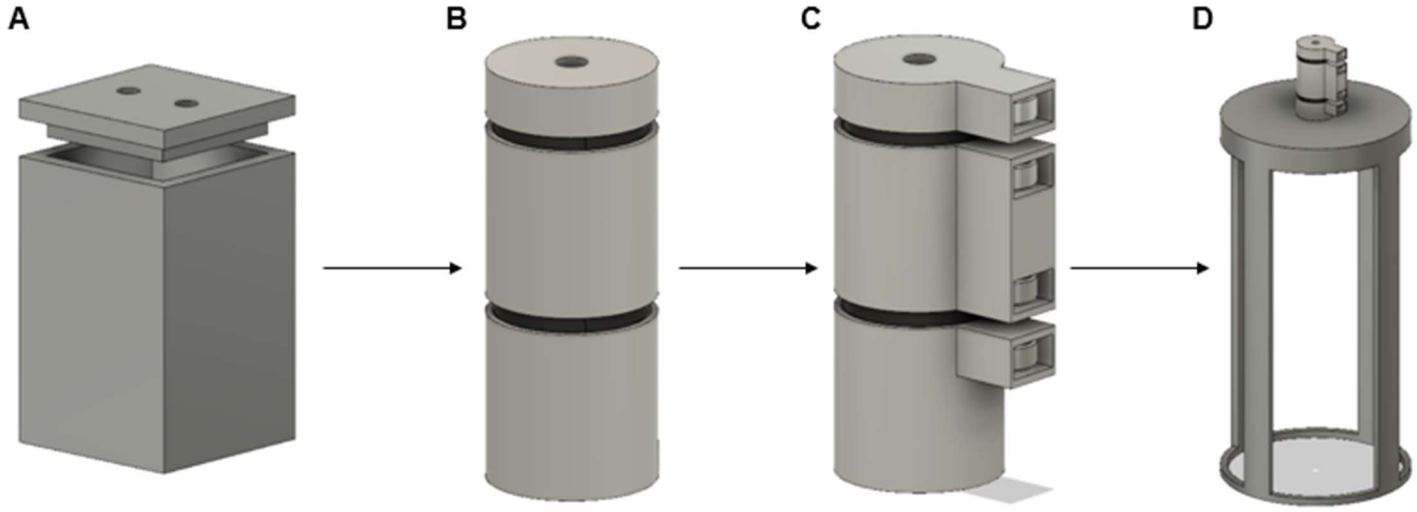


Fig. 5. Iterations of the Device Design. The four iterations of the CAD design for the device prototype are illustrated including: the initial design (A), the updated design (B), the updated design with magnetic clasps (C), and a support stand to hold the final prototype design (D).

a secondary polymer solution (maleimide-modified HA) and without the addition of any more cells in Step 1.

Iterations of the Prototype Design

In accordance with the updated design schematic, several iterations were able to be made on the initial CAD model for the device (Figure 5). Following the first CAD design (Figure 5A), an updated CAD model (Figure 5B) was created in Fusion 360 to account for all of the changes made to the initial design, including the cylindrical two-part chamber and lid, O-rings seals, and a single opening in both the bottom section and lid of the chamber. In the next iteration of design, magnetic clasps were added to the side of the chamber to hold the sections of the prototype device together using small neodymium magnets (Figure 5C). These magnetic clasps were added to securely connect each section of the device during the encapsulation procedure, while also allowing the researcher to easily take the sections of the device apart for either removal of the lid for pipette access or removal of the top section of the chamber for membrane filter replacement.

In the final design iteration, a supporting stand was created to hold the encapsulation chamber securely above the surface of a lab table (Figure 5D). This stand functions as supporting structure to stabilize the device during the encapsulation process as well as holding the encapsulation chamber above the lab table to give space for tubing to be run from the bottom section of the chamber into a collection or solution vial. In Figure 6, a 3D model of the final device prototype is shown fully assembled with tubing, O-rings, a collection vial, and a luer-lock blunt needle tip, to illustrate the complete experimental set-up for our device.

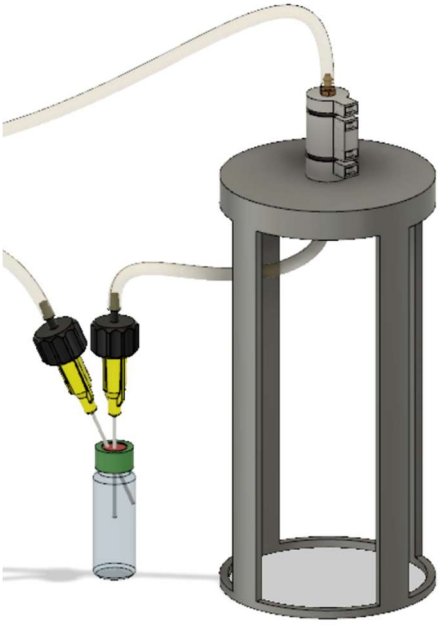


Fig. 6. Fully Assembled Prototype of Final Design. This fully assembled final design illustrates what the experimental set up would look like in a lab setting.

Control System for the Device

As per the initial design specifications of our design, the idea of using an Arduino microcontroller to control a series of stepper motors was explored. The original plan was to set each pin on the Arduino board to a different output that would be connected to a stepper motor. Each stepper motor would then control a different syringe pump. After the specific flow rates and desired volumes of each solution had been programmed in, the Arduino would be instructed to turn on the stepper motors for a specified portion of time that would allow for the exact amount of each solution to be dispensed. Furthermore, the stepper motors would be turned on and off in a predetermined order to completely automate the process for the researcher, therefore reducing the human error that could result from pipetting each volume individually.

After further consideration, however, we became aware of inaccuracies that could arise from the Arduino board itself. The amount of each solution required was on a microliter scale, so in order to dispense the exact amounts specified each motor would be on for a very short period of time. Due to this small scale of volumes that our device would be dispensing, we believed that using the Arduino microcontroller in this manner would create difficulties in applying accurate solution volumes and would likely dispense more solution than necessary. This would defeat the purpose of the device, as it would not reduce potential human error and could increase the amount of expensive material that was lost in the process.

As the design of our device was updated, the specifications for the Arduino code simplified. It was decided that the microcontroller would be used only to control a valve to allow a vacuum to suction out any excess solution or to suction PBS solution into the device chamber. This vacuum is opened and closed by a solenoid valve which can be easily controlled with an Arduino. Our proposed code sets a switch on the Arduino board as an input and a pin as an output (S1). Once the switch is activated, the pin which is to be connected to a solenoid valve would also be activated and the valve would open, turning on the vacuum. After the excess solution is vacuumed out of the device, the user can activate the switch again which will close the solenoid valve. An iteration of our code can be seen in Supplement 1.

Design Validation

In order to validate the effectiveness of our final device design, we had planned on testing three key areas to determine if our device demonstrated any advantages over the current layer-by-layer coating technique utilized by stem cell researchers. While our capstone team was unable to perform the necessary testing on our device—as lab access has been restricted due to the COVID-19 pandemic—we propose the following three research questions for future work in order to validate the device design:

1. How does the cell viability in the encapsulation device compare to the cell viability when performing the traditional layer-by-layer method?
2. How does the material loss in the encapsulation device compare to the material loss while performing the traditional layer-by-layer method?
3. How does the time necessary to carry out the final process/procedure compare to traditional layer-by-layer method?

Testing Cell Viability

To determine the capability of the device to effectively coat viable mNSCs without significant cell death, the following experimental procedure should be conducted. First, the device prototype should be used to coat a sample of mNSCs with a two layer polymer coating (lipid-and-thiol-modified HA & maleimide-modified HA) as stated previously. After the two coatings are applied to the mNSCs and the unbound polymers and excess fluid is vacuumed out, the encapsulated mNSCs will be left. Flow cytometry will now be used to with the sample of encapsulated cells in order to distinguish viable, coated cells from those that have ruptured, because the diameter of the ruptured cells is smaller than the diameter of a living, undamaged cell. Thus, from the cytometry data, the number of coated, viable cells can be quantified.

Additionally, the traditional layer-by-layer coating technique should be performed, again encapsulating mNSCs with a two layer polymer coating (lipid-and-thiol-modified HA & maleimide-modified HA). Another flow cytometry test can then be performed on these encapsulated mNSCs to determine the total number of viable cells encapsulated by utilizing the traditional layer-by-layer approach.

Statistical Analysis

In order to demonstrate that our device is an effective option for automating the cell coating process, it must be statistically proven that the number of viable cells successfully coated will be the same or better than if the experiment was run manually. Thus, we will compare two test groups: the control group, where the layer-by-layer cell encapsulation procedure is run manually, and the experimental group, where the cell encapsulation is completed using our prototype. Using flow cytometry as mentioned above, the number of coated, viable cells can be determined for both test groups. After running several experimental trials, a one-tailed, unpaired t-test will be used to calculate the p-value and therefore help us to conclude whether or not a statistically significant claim can be made that the device has the capability to produce an equal or greater number of viable encapsulated cells compared to the manual cell coating process.

Testing Material Loss

To determine the capability of the device to reduce the amount of material loss during the encapsulation process, the following experimental procedure should be conducted. First, exactly 100µL of HA should be pipetted into the top of the device and then suctioned through the membrane filter and tubing into the collection vial. The total volume of HA contained in the collection vial should then be measured. This final HA volume should be divided by the initial 100µL volume pipetted into the device, then subtracted from 1, and finally multiplied by 100 to calculate what percentage of the material was lost in the device. Similarly, the material loss for the traditional layer-by-layer cell encapsulation process should be determined by comparing the final and initial volumes of HA solution to calculate the percentage of material lost during the manual process.

Statistical Analysis

To give statistical significance to these results, several trials of this experiment procedure must be conducted. We will again compare the two test groups: the control group, where the layer-by-layer cell encapsulation procedure is run manually, and the experimental group, where the cell encapsulation is completed using our prototype. Using the procedure as described above, the percentage of material loss can be determined for both test groups. After running several experimental trials, a one-tailed, unpaired t-test will again be used to calculate the p-value and therefore help us to conclude whether or not a statistically significant claim can be made that utilizing this encapsulation device reduces the amount of material loss as compared with the traditional layer-by-layer coating technique.

Total Procedure Time

To determine the total procedure time necessary for completing the cell encapsulation with our device, the cell encapsulation process will be carried out as previously described in order to provide a two-layer coating for a sample of mNSCs. This process will be timed from start to finish and the time recorded. After repeating this procedure several times, the times should be averaged to determine the average time necessary to carry out the cell coating process with the encapsulation device. Similarly, the traditional cell coating process should be performed several times and the procedure times recorded to determine the average time necessary to complete the manual cell encapsulation process. These two times can then be compared to determine whether our device demonstrates a statistically significant reduction in the total procedure time needed to encapsulate mNSCs in a two-layer coating.

Discussion

Over the past few decades, the encapsulation of living cells in a polymeric matrix has been heavily studied, resulting in the rapid development of numerous biomedical applications such as cell-based transplantation therapy, drug delivery, and tissue engineering[18-21]. The microencapsulation process has proven to be a valuable tool for researchers as the encapsulation shell can protect cells from harsh environments as well as act as an immunoisolation barrier to stop an immune reaction from occurring[19]. While several techniques have been developed for enclosing cells in a microencapsulation, the layer-by-layer technique utilized in the Highley lab provides advantages over other methods as each polymer coating can be modified in order to control the thickness and mechanical properties of that specific layer. However, the layer-by-layer approach can be time-consuming and tedious, create difficulties in saving excess unbound material, and require cells to be kept out of incubation for long periods of time during the encapsulation process.

The novel device for encapsulating mNSCs described in this paper was designed to specifically to address these limitations of the layer-by-layer technique. This device design was developed over the course of the last year by conducting research based around three specific aims. First, a 0.65µm Durapore porous membrane was selected and tested with a fluorescent dextran solution, showing that the membrane can be cleansed in two to three washes. Additionally, an initial device design schematic was created and then put through iterative design process. During this design process, the drawbacks of the initial design were identified and specifically addressed in order to optimize the final design for the prototype cell encapsulation device. Finally, an initial plan for an Arduino microcontroller design system was formulated and then updated to account for changes in the device design.

After the design was optimized, three research questions regarding cell viability, material loss and total procedure time were purposed to begin validating the effectiveness of the device design compared with the traditional layer-by-layer cell encapsulation method. And while lab closures prevented the complete assembly and testing of the final design iteration, it is expected that the outlined design validation process will prove the device capable of reducing the time necessary to complete the encapsulation procedure, reducing the amount of material lost during the procedure, as well as increasing the number of viable cells encapsulated during the layer-by-layer coating process.

As the design and its expected outcomes have not been validated yet, future work on this encapsulation device design should be directed toward running the experimental procedures outlined in the design validation section of this paper. Further research could also be focused on fully automating the cell encapsulation device after validating the current design. Finally, if the device is developed even further, it may have the potential to be used in a clinical setting for various stem cell therapies in the future.

End Matter

Author Contributions and Notes

C.B.H, N.B.V, M.K, and G.V.P. designed research, N.B.V, M.K, and G.V.P. performed research, G.V.P. wrote Arduino code, N.B.V. performed CAD modeling, N.B.V. analyzed data; and N.B.V. wrote the paper.
The authors declare no conflict of interest.

Acknowledgments

We would like to thank Dr. Chris Highley and Dr. Barker for their advising.

References

1. *Principles of Regenerative Medicine*. (Elsevier, 2008). doi:10.1016/B978-0-12-369410-2.X5001-3.
2. Prakash, S., Bhathena, J. R. & Chen, H. 1 - Introduction to artificial cells: concept, history, design, current status and future. in *Artificial Cells, Cell Engineering and Therapy* (ed. Prakash, S.) 3–41 (Woodhead Publishing, 2007). doi:10.1533/9781845693077.1.3.
3. Murua, A. *et al.* Cell microencapsulation technology: Towards clinical application. *Journal of Controlled Release* **132**, 76–83 (2008).

4. Farina, M., Alexander, J. F., Thekkedath, U., Ferrari, M. & Grattoni, A. Cell encapsulation: Overcoming barriers in cell transplantation in diabetes and beyond. *Advanced Drug Delivery Reviews* **139**, 92–115 (2019).
5. Uludag, H., De Vos, P. & Tresco, P. A. Technology of mammalian cell encapsulation. *Advanced Drug Delivery Reviews* **42**, 29–64 (2000).
6. Olabisi, R. M. Cell microencapsulation with synthetic polymers. *Journal of Biomedical Materials Research Part A* **103**, 846–859 (2015).
7. Chang, T. M., MacIntosh, F. C. & Mason, S. G. Semipermeable aqueous microcapsules. I. Preparation and properties. *Can. J. Physiol. Pharmacol.* **44**, 115–128 (1966).
8. Orive, G. *et al.* Cell encapsulation: promise and progress. *Nat. Med.* **9**, 104–107 (2003).
9. Batorsky, A., Liao, J., Lund, A. W., Plopper, G. E. & Stegemann, J. P. Encapsulation of adult human mesenchymal stem cells within collagen-agarose microenvironments. *Biotechnology and Bioengineering* **92**, 492–500 (2005).
10. Peirone, M., Ross, C. J. D., Hortelano, G., Brash, J. L. & Chang, P. L. Encapsulation of various recombinant mammalian cell types in different alginate microcapsules. *Journal of Biomedical Materials Research* **42**, 587–596 (1998).
11. Kampf, N. The use of polymers for coating of cells. *Polymers for Advanced Technologies* **13**, 895–904 (2002).
12. Kang, A., Park, J., Ju, J., Jeong, G. S. & Lee, S.-H. Cell encapsulation via microtechnologies. *Biomaterials* **35**, 2651–2663 (2014).
13. Wang, K. *et al.* From Micro to Macro: The Hierarchical Design in a Micropatterned Scaffold for Cell Assembling and Transplantation. *Advanced Materials* **29**, 1604600 (2017).
14. Veerabadran, N. G., Goli, P. L., Stewart-Clark, S. S., Lvov, Y. M. & Mills, D. K. Nanoencapsulation of Stem Cells within Polyelectrolyte Multilayer Shells. *Macromolecular Bioscience* **7**, 877–882 (2007).
15. Sukhorukov, G. B. *et al.* Layer-by-layer self assembly of polyelectrolytes on colloidal particles. *Colloid Surf. A-Physicochem. Eng. Asp.* **137**, 253–266 (1998).
16. Caruso, D. Limits of the classic method: Positive action in the European Union after the new equality directives. *Harv. Int. Law J.* **44**, 331–386 (2003).
17. Lvov, Y., Antipov, A. A., Mamedov, A., Mohwald, H. & Sukhorukov, G. B. Urease encapsulation in nanoorganized microshells. *Nano Lett.* **1**, 125–128 (2001).
18. Alkayyali, T., Cameron, T., Haltli, B., Kerr, R. G. & Ahmadi, A. Microfluidic and cross-linking methods for encapsulation of living cells and bacteria - A review. *Analytica Chimica Acta* **1053**, 1–21 (2019).
19. Liu, T. *et al.* Biomedical Applications of Layer-by-Layer Self-Assembly for Cell Encapsulation: Current Status and Future Perspectives. *Advanced Healthcare Materials* **8**, 1800939 (2019).
20. Orive, G. *et al.* Cell encapsulation: technical and clinical advances. *Trends Pharmacol. Sci.* **36**, 537–546 (2015).
21. Kim, B. J., Cho, H., Park, J. H., Mano, J. F. & Choi, I. S. Strategic Advances in Formation of Cell-in-Shell Structures: From Syntheses to Applications. *Advanced Materials* **30**, 1706063 (2018).

Supplementary Material

Supplement 1: Potential Arduino Code for opening and closing a solenoid valve

```
int pin_SOLENOID = 10;
int pin_switch = 2;

void setup() {
  // put your setup code here, to run once:
  pinMode(pin_SOLENOID, OUTPUT);
  digitalWrite(pin_SOLENOID,LOW);

  pinMode(pin_switch, INPUT);
}

void loop() {
  // put your main code here, to run repeatedly:
    if ( digitalRead(pin_switch) == HIGH)
    {
      digitalWrite(pin_SOLENOID, HIGH);
    }
    else
    {
      digitalWrite(pin_SOLENOID, LOW);
    }
}
```