Design and Development of Two Component Hydrogel Ejector for Three-Dimensional Cell Growth

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Final Report
Design and Development of Two Component Hydrogel Ejector for Three-Dimensional Cell Growth

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0.1. Abstract

Hydrogels are useful in wound healing, drug delivery, and tissue engineering applications, but the available methods of injecting them quickly and noninvasively are limited. The current medical industry does not yet have access to an all-purpose device that can quickly synthesize hydrogels of different shapes and sizes. Many synthesis procedures that have been developed result in the formation of amorphous hydrogels. While generally useful, amorphous hydrogels exhibit limited capability in tissue engineering applications, especially because of their viscous properties. [1]

This endeavor aims to modulate the appropriate gelation parameters, optimize the injection process, and create a prototype that allows for the extrusion of uniformly mixed precursor gelation solutions. It involves the designing and testing of a hydrogel applicator that is capable of quickly dispensing hydrogels of predefined shapes onto a wound for enhanced healing. Said hydrogels can also be used in applications such as tissue engineering, regenerative medicine, and drug discovery. [5]

The hydrogels synthesized by this applicator will be created through the mixing of a solution containing horseradish peroxidase (HRP) and a solution containing hydrogen peroxide ($\text{H}_2\text{O}_2$). Individually, these two solutions both exhibit viscosities similar to that of water. However, mixing of both solutions leads to a gelatinous form within 10 seconds. This hydrogel creation technique is well known and documented. [2][3][4]

The fast formation of HRP-$\text{H}_2\text{O}_2$ hydrogels is convenient in the sense that there is no prolonged mixing process; however it poses a significant challenge in the design of our hydrogel applicator. The device must be designed such that the hydrogel will not clog the chamber in which it is mixed before exiting. Simultaneously, the HRP and $\text{H}_2\text{O}_2$ must be mixed uniformly to ensure maximum hydrogel formation.

In order to resolve the issue of uniform mixing, a mini motor will be introduced into the design of the prototype to aid in the gelation process. This mini motor will be programmed to vibrate during the gel formation progression resulting in more evenly distributed molecules and reducing chance of improper diffusion. Ideally, the motor will be connected to a microcontroller which will allow the user to automate the process by simply pushing a button. To monitor the mixing process and to ensure that the gelation is uniform, different colored fluorophores will be used in testing the consistency of the hydrogel.

Ultimately, the hydrogel will be coated in a mat of nanofibers. These nanofibers will be composed of a hydrogel material (possibly the same material as the HRP-$\text{H}_2\text{O}_2$ hydrogel). The nanofibers will serve two purposes; protecting the body and hydrogel from pathogen access, and improving the regulation of water and oxygen between the body and surrounding environment. This will help to prevent dehydration of the wound site and improve the body’s natural healing response. [10]
1. Introduction

This report details the design and development of a two-component hydrogel ejector, capable of forming hydrogel ribbons that may be used for wound healing, drug delivery, and tissue engineering purposes.

1.1. Background

This project will be overseen by Dr. Lakshmi S. Nair, an Assistant Professor at the UConn Health Center in Farmington, CT, who specializes in studying the interactions of cells and biomaterials. Dr. Nair believes that the project is a worthwhile investment because the hydrogels created by the device will be useful in studies related to tissue engineering—the device provides a means of quickly creating hydrogels that can be inserted into the body and studied in vivo. Additionally, HRP/H2O2 hydrogels have been shown to be effective in wound healing applications. [2][9][16]

1.2. Purpose of the Project

The purpose of this project is to design a hydrogel applicator that can quickly create hydrogels through the reaction of horseradish peroxidase (HRP) and hydrogen peroxide (H2O2). Initially, solutions containing only one of these reactants exist in a liquid phase that is only slightly more viscous than water. However, when solutions of these two reactants are combined, the liquid phases transform into a gelatious phase in a matter of only 5 to 10 seconds. The mechanical properties of the resultant hydrogel can vary depending on several gelation parameters (viscosity, concentration, time, etc.).

Therefore, the present challenge is to design a device in which HRP and H2O2 can be mixed and extruded as a hydrogel without clogging or damaging the device. In addition, the two solutions must be mixed uniformly in order to properly form the bulk hydrogel. Finally, the proper gelation parameters must be determined for optimal hydrogel output. The optimal characteristics of the hydrogel are dependent on the desired application. This project is undertaken with wound healing, drug delivery, and tissue engineering as the primary application focuses.

Finally, the project also encompasses a focus on the encapsulation of live cells within the hydrogel. This final goal is undertaken with the application of tissue engineering in mind. Live cells will be included in the input solutions (HRP and H2O2), and remain viable in the resultant hydrogel. These hydrogels can be extruded onto strips of a PCL nanofiber mat, which can then be rolled up into a “sushi-roll” shaped scaffold and used as a cartilage plug.

1.3. Previous Work

This project is a spiritual successor to another project overseen by Dr. Nair, in which Colberg, Hennessey, and Nowak created a hydrogel ejector to be used in combination with a dual syringe injector and a pore stamper. Their project was successful in establishing an innovative method of forming porous hydrogels to be used for tissue engineering scaffolds or wound dressings. [4]
However, they implemented their design under the assumption that the hydrogel ejector would routinely become clogged and require cleaning. Additionally, the hydrogels formed exhibited a relatively low level of homogeneity. In the present project, the design team aims to reinvent the hydrogel ejector so as to completely avoid blockage and ensure the creation of hydrogels that are homogenous. [4]

Hu et. al. has worked on a similar project in which hydrogel fibers were created by extruding HRP and H$_2$O$_2$ from concentric chambers. Their experiments involved altering the number of concentric layers, creating both hollow and filled fibers, and creating fibers that included live cells. All of their fibers were meant to mimic the qualities of the body’s natural extracellular matrix. [1]

1.3.1. Products

The idea of a hydrogel ejector--i.e. a simple, usually handheld, device that creates hydrogels, is not new in and of itself. A quick internet search brings up dozens of different hydrogel ejectors from different companies. For example, Coloplast is a Minneapolis-based company that markets a hydrogel for wound healing purposes called Purilon® Gel, which is sold in an accordion-style ejector. The ejector is pictured in Fig. 1. [5][21]

![Figure 1](image)

Figure 1: Coloplast’s accordion-style hydrogel ejector containing its Purilon® Gel. The hydrogel is ejected from the nozzle when the device is squeezed.

An even simpler example of a hydrogel ejector is a squeezable tube that ejects the hydrogel, in the same manner as a tube of toothpaste. For example, ConvaTec is a company that markets a wound healing hydrogel called SAF-Gel$^\text{TM}$, which is sodium alginate based. SAF-Gel$^\text{TM}$ comes in a squeeze-tube applicator, which is pictured in Fig. 2.
Products such as Purilon® Gel’s accordion-style ejector and SAF-Gel™’s squeeze-tube ejector are perfect examples of existing hydrogel ejectors that serve a specific purpose (in this case, wound healing) effectively. However, these ejectors are limited to the production of amorphous hydrogels--i.e. hydrogels with no predetermined size or shape. Such hydrogels are not necessarily useful for tissue engineering or drug delivery purposes. These devices are also not capable of creating hydrogels already encapsulating live cells.

1.3.2. Patents

John Wick was granted a patent for a hydrogel applicator in October, 1993. This applicator created a hydrogel that sat on a rectangular plate. The plate and hydrogel could be removed together. The hydrogel could then be applied to a wound by simply pressing against it, much like a stamp. This is a novel approach to hydrogel formation, and it does result in hydrogels of a specific block shape. However, the current design team believes that their proposed design offers a more convenient method of hydrogel formation that is better suited for tissue engineering and drug delivery applications. [2]

1.4. Report Map

The remainder of this report is summarized as follows. Section 2 will cover the project design phase in detail, taking into consideration each of the different designs that were implemented, and each individual subunit of the chosen optimal design. Section 3 will cover the realistic constraints of the project in detail, including how the project conforms to engineering standards, and how the project is constrained by economic, environmental, sustainability, and other factors. Section 4 will address the safety issues and concerns associated with the project. Section 5 will discuss the impact of the project on engineering standards at a global level. Section 6 will discuss the life-long skills that the design team has earned in working on the project. Section 7 will cover the budget of the project in detail. Section 8 will cover each individual team member’s contributions to the project. Finally, the report will conclude, followed by the references, acknowledgements, and appendix with any relevant information.
2. Project Design

2.1. Introduction

This section will cover the project design process in detail, beginning with presentations of each design that was initially considered and developed to incorporate the ideas of using a vibrational motor to aid in the uniformity of hydrogel creation. Each of the designs described in the sections below were all created to the project specifications and tested to compare results in order to select the optimal prototype design. The decided optimal design will then be presented in detail, taking each of its component subunits into consideration.

2.1.1. Alternating Collection Chamber Design

The idea of this alternating collection chamber design was to simulate a syringe-like method of ejecting the hydrogel. Since one of the main issues was uniformity of the gelation process, the alternating channels would allow the two solutions to be separated until the point of ejection, where they will finally come in contact for the cross-linking process to initiate seconds prior to exiting the device. A collection chamber was designed so that the channels could be filled evenly to ideally optimize the uniformity of the hydrogel formation. In order for the channels to be filled, a blocking piece has been designed, as seen in Fig. 7, where it will also allow the channels to be opened once the collection chamber has been evenly filled. Once the channels have been opened, the two solutions will continue to flow through the device into the exit tip, as seen in Fig. 6. In order to optimize the gelation process, multiple vibration motors will be attached to the exit tip, since that is area where the two solutions will come into contact. Figs. 3-8 show the different components that make up the hydrogel ejector. They will be assembled with silicon glue to create the final product.
Figure 3: The base piece of the collection chamber. There is a slit in the slide wall so that the middle compartment (see Fig. 4) can be slid in for the other set of channels to make the alternating channel pattern. There is also an area at the bottom of the compartment which will allow the blocking piece to be inserted.

Figure 4: The middle compartment of the collection chamber, where the separating blocks are alternating.
Figure 5: The top piece of the collection chamber, which encloses the device.

Figure 6: The exit tip of the device. The blocking piece will be inserted in between the top of this compartment and the bottom of the collection chamber. It has been designed so that the solutions will flow into the adjacent channels and at the rectangular prism exit tip, the dividing channels will end so that the solution will come into contact for the gelation process prior to ejection.
2.1.2. Array Design

The array design was conceived by brainstorming methods to achieve optimal gelation conditions. A previous HRP/H₂O₂ hydrogel ejector showed some success, but had a notable tendency for only partial gelation. Testing of the device frequently yielded the result of a thin strip of properly formed hydrogel, with large deposits of unmixed HRP and H₂O₂ on each side. [4]

With the idea of achieving maximum gelation in mind, it was decided that the HRP and H₂O₂ solutions should be rearranged to a checkerboard array pattern inside the device. Such a pattern achieves the maximum amount of direct contact of the two solutions. The amount of direct contact increases as the number of squares (or “elements”) in the board increases, and as these elements decrease in size.

The array design consists of ten subunits, each of which are printed using a Makerbot Replicator 2 3D printer. Figure 9 presents all ten subunits arranged sequentially in SketchUp, while Figure 10 is a photograph of the completed design.
**Figure 9:** All of the subunits of the array design, arranged sequentially in SketchUp. The HRP and H$_2$O$_2$ solutions generally progress through the device from top to bottom.

**Figure 10:** The completed design after all subunits are printed. In this photograph, the subunits have not yet been glued together properly.

The general idea of the array design is that the HRP and H$_2$O$_2$ solutions are injected into separate chambers at the top of the device, and are then rearranged into a checkerboard pattern before being mixed to form a ribbon shaped hydrogel. This will be helpful in the long run, especially since it will allow the molecules of the two different solutions to cross-link uniformly as this device allows the two solutions to form a pattern.
2.2. Prototype

The final design of the prototype is a two component hydrogel ejector, built with a disposable component, in which the two solutions will be injected, and a reusable component, which will consist of the electrical components that power the vibrational motors. This is based on the optimal design, where the prototype is built with alternating channels. These alternating channels will allow the two different solutions to flow through the device separately, prior to ejection, and then at the ejection tip, the two solutions will come into contact for the cross-linking to initiate the hydrogel formation. The vibration motors will be placed near the ejection tip to aid in molecule distribution where the shaking of the device will cause the molecules of the two different solutions to collide, thus forming a uniform strip of hydrogel.

After many trials of testing and results analysis, the alternating channel design was selected to be the optimal design. This prototype was selected after careful consideration, as it provided the best results during the initial rounds of testing. It also had the least error, as the alternating collection chamber design had a large amount of leakage during every single trial of testing, and the array design yielded well-mixed hydrogels, but was too complicated and time-consuming to manufacture. In addition, there was a noted tendency of the input solutions to become stuck in between components of the array design.

The distinguishing feature of this prototype was a series of alternating channels. This prototype was designed to dispense a ribbon gel that is 7mm wide with a height of 2mm. The length of the ribbon would be dependent on the amount of solution injected into the device. The dimensions of each channel were 2mm x 1mm. All of the channels are the same length because the solutions need to be dispensed at the same time. This parameter needs to be kept constant to ensure uniform mixing—otherwise there would be pressure variations associated with each channel, influencing the speed at which the solutions dispense.

2.2.1. Objective

In choosing an optimal design, factors to consider included:

- Gelation time
- Homogeneity of mixture
- 3D printing time/material required per unit
- Ease of use

The alternating channel prototype was chosen as the optimal design because it offers the best mix of functionality (solution homogeneity, gelation time) with efficiency and convenience (small size, simple but effective design). Several rounds of testing at the UConn Health Center proved the prototype’s efficacy in mixing hydrogels, especially after the battery pack with vibration motors was implemented. Finally, the alternating channel design involves the least 3D printing time per unit out of all of the considered designs. Overall, it is generally the most efficient and cost-effective design of the three.
The array prototype was discontinued because its design was too complex. While its mixture homogeneity was superior to the alternating channel design, it featured too many components and therefore took too long to print and assemble each unit. In addition, there was no easy way to remove the hydrogel from the device after it was mixed—a flaw that the alternating channel design does not suffer from.

The alternating collection chamber design was similar to the alternating channel design, but it was larger and more complex. This extra printing time was the justification for discontinuing the alternating collection chamber design.

2.2.2. Subunits

This prototype has three components: the extruder, a 3D printed component through which the solutions are pumped and mixed, an electric circuit that powers two vibration motors to aid in mixing, and the battery pack, a 3D printed component that houses the motors and the circuit.

2.2.2.1. Extruder

The extruder is the disposable component of the hydrogel ejector, shown in Fig. 11. It is based on a 3D printed model (seen in Fig. 12), and contains four channels of equal length that are composed to rearrange the input HRP and H₂O₂ solutions into an alternating configuration at the exit tip.

![Figure 11: The extruder.](image-url)
Figure 12: The 3D model for the extruder, as seen from several perspectives in SketchUp. In the top-left and bottom-right pictures, walls of the extruder have been removed to expose the configuration of the alternating channels inside.

The top portion of the device has two holes for the syringes holding each solution (Figs. 13 and 14) and a chamber where the solutions are dispensed into. Once assembled, each chamber lies above the openings to the alternating channels, helping to easily and efficiently separate a single solution into each of the channels. There are two sets of two evenly distributed channels. These two sets of channels are evenly spaced so that the two syringes can be used easily. From a design perspective, this also allows the alternating channels to come together at the bottom of the device efficiently.

Figure 13: A frontal view of the side of the extruder bearing the syringe holes.
Figure 14: A view of the inside of the alternating channels of the extruder. Perspective is from the syringes (input side).

The extruder contains the alternating channels (Figs. 15 and 16). These channels come together at the exit site. The alternating channels and the spacing between them helps to ensure uniform mixing. The extended extrusion tip placed just below where the alternating channels mix will help to increase the amount of mixing that occurs (see Figs. 17-19). It would also help with the precision of the application process.
**Figure 15:** A view of the inside of the alternating channels of the extruder. Perspective is from the wide side of the device.
Figure 16: A view of the inside of the alternating channels of the extruder. Perspective is from the narrow side of the device.

Figure 17: Frontal view of the exit tip of the extruder.
**Figure 18:** A view of the inside of the alternating channels of the extruder. Perspective is from the extrusion tip (output side).

**Figure 19:** Isometric view of the exit tip of the extruder.
The two solutions will only be in contact once they exit the alternating channels of the device. There are two advantages to this—more uniform mixing and less waste associated with the mixing process. Gelation occurs almost instantaneously once the two solutions come into contact. If there was any point in the extrusion process where the solutions touched before exiting, the viscosity of the gel would cause clogging within the device and loss of material, as well as possible failure to produce a hydrogel. The length of the extrusion tip was increased to 1 cm to enhance the uniformity of the shape of the gel ribbon. This is because the longer length gives the two solutions more time to mix together in a confined area.

This design can easily be scaled up. If the width of the ribbon desired is wider, then the only thing that needs to be changed is the number of channels present within the device. The length of the ribbon can also easily be adjusted by increasing the volume of each solution. The extruder is reasonably small, simple to assemble, and simple to use. The user only needs to insert the two syringes in the top and apply hand pressure to extrude the gel. Figs. 20 and 21 show the syringe hole portion of the device in greater detail.

**Figure 20:** Depicts what the chamber part of the syringe insertion component would look like if it wasn’t modified to have a sloped pyramidal shape within it to more efficiently propel solution to the alternating channels.

**Figure 21:** Depicts the optimized chamber shape for solution delivery to the channels.

The extruder’s alternating channels help to improve the homogeneity of the mixture of the input solutions, this leading to better gelation parameters. This is accomplished by distributing the input solutions into an alternating pattern. This effect is demonstrated with blue and orange cake frosting in Fig. 22.
Figure 22: The alternating channel effect is illustrated using blue and orange cake frosting as input solutions.

2.2.2.2. Battery Pack

The battery pack is the nondisposable component of the hydrogel ejector. It is based on a 3D printed model (seen in Fig. 23), and has been fitted with two vibration motors and a battery-powered circuit to control them. The battery pack also has a switch on the side which turns the vibration motors on or off. The complete battery pack is shown in Fig. 24.

Figure 23: The 3D model of the battery pack, as viewed from several perspectives in SketchUp.

Figure 24: The battery pack—front (left) and back (right).
2.2.2.3. Vibration Motor Circuit

After multiple rounds of designing and troubleshooting, it was found that a two motor circuit with a single 6V battery source would be ideal for assembling the electrical components to power the vibrational motors. Each of the motors has a 2.7V - 3.3V voltage range, where anything lower than 2.7V per motor will not be sufficient enough to turn it on, and anything above 3.3V will destroy the motor. The schematic for the vibration motor circuit is shown in Fig. 25.

![Figure 25: The schematic for the vibration circuit. ‘M’ is representative of the vibration motors.](image_url)
3. Realistic Constraints

3.1. Engineering Standards

The alternating channel prototype of the device was originally designed in SketchUp. The prototype is a primarily architectural design on the centimeter scale—the extruder measures approximately 3.5x1x4cm. This relatively small scale design makes the prototype ideal for both manufacture and application, as smaller designs are generally better for 3D printers, tissue engineering scaffolds, and wounds. As shown in the description of the extruder of Section 2.2.2.1., the design was normalized to be compatible with previous designs of similar devices.

The alternating channel prototype was designed for ease of manufacture. Its components can be printed by relatively inexpensive home 3D printers. For the initial testing phase, the design team used the MakerBot Replicator 2 printer. Once printed, the battery pack is fitted with the vibration motor circuit using non-conductive silicon glue.

3.2. Constraints by Category

The economic constraints on this project are minimal. The most significant expenses incurred resulted from the purchase of two different fluorophores and cells. Additional costs included 3D printing materials, syringes, raw materials and reagents, motors, and microcomponents. In order to reduce costs, the device is reusable, with only the extruder being disposable. This is because it is the only component that comes into contact with the HRP and H$_2$O$_2$ solutions and the finished hydrogel. Finally, the costs of the crucial reagents (MES buffer solution, HRP, H$_2$O$_2$) were considered.

There were two major environmental constraints to consider. First, the assumption is made that the device will only be used at room temperature. This assumption is made to simplify theoretical analysis and the design process. Second, it is important to consider the device’s environmental footprint. Since the extruder is disposable, the device (or at least this component) should ideally be composed only of biodegradable plastics. Finally, the use of syringes must be considered as an environmental constraint. It is likely that the use of the alternating channel prototype will go hand in hand with the use of many disposable syringes.

Since the hydrogels produced are to be used in vivo, there are significant health and safety constraints to consider. The device and the hydrogels it produces should be sterile at time of use. The device should be minimally invasive—application of a hydrogel to the wound site should not require any injection. The hydrogels’ constituent materials are all biocompatible, biodegradable, nontumorigenic, and viable to cells. Gelation can be triggered by temperature, pH, or enzymatic cross-linking. It is important that the precursor solutions require only mild conditions for gelation to occur. Once the hydrogel is formed, it needs to exhibit mechanical properties consistent with the structural support of living cells. Ideally, the hydrogels are porous to allow space for cell growth. The incorporation of an electrospun nanofiber PCL mat increases the functionality of the final hydrogel product. There needs to be a barrier separating the hydrogel and damaged tissue from the outside environment. This layer allows for enhanced healing because it acts as a control mechanism for oxygen and water exchange. It also provides protection from pathogens. [11]
Finally, the physical method of hydrogel application must be considered. Hydrogels must be extruded from the device in a usable state without experiencing a loss of sterility. After testing of the prototype, it has been concluded that this goal was achieved with the current design. Hydrogels can easily be distributed directly onto a nanofiber PCL ribbon.

The ideal use of this product is for a medical environment where the hydrogel encapsulates live cell cultures. This sets many sustainability constraints since the hydrogel needs to be created in a sterile environment each time. Making the extruder disposable is extremely cost efficient, as it disregards the complicated sterilization technique of completely removing any bacteria, cell culture traces, and hydrogel residues. With the product being disposable, there is also a constraint set on the material requirements. The product should be as cost effective as possible, thus the concept of 3D printing is ideal.

It is assumed that precursor solutions and resultant hydrogels will never come into contact with the battery pack and vibration motor circuit. Proper instructions for use of the device are provided in the operator’s manual. This way, these two components of the device can be reusable in order to maximize cost-efficiency.

Additionally, the live cell cultures are sensitive and cannot withstand great forces--hence, there are not any moving components (e.g. motors) in direct contact with the hydrogel during mixing. Such a design would involve cells contained in the hydrogel being killed. [11]

Finally, manufacturability constraints must be considered. Since the devices are being 3D printed, there is a significant printing time associated with each one. If it is decided that the device should be disposable, it must be properly sterilized and packaged prior to distribution. [9]

4. Safety Issues

Existing literature on hydrogels formed by HRP and H₂O₂ shows that both chemicals and the resulting hydrogels are safe for contact with humans. The hydrogels formed are biocompatible and biodegradable, with a controllable degradation rate of 4 to 21 days. With this knowledge in hand, the design team is operating under the assumption that the device will only be used to produce hydrogels of these solutions. Theoretically, it is entirely possible that the alternating channel prototype can be used to mix any two solutions together for any reason. However, using the device for other purposes warrants further research. For the time being, the array prototype will be manufactured and marketed solely for the purpose of producing HRP/H₂O₂ hydrogels. However, it is one noted advantage of the design that it may be adapted for future use with different input solutions for other applications. [9]

The electrical components incorporated into the array prototype all operate in the safe range of 3-6 V. Hence, even if the end user comes into direct contact with or causes a short circuit between any of the electrical components, he or she will not be in danger of any harm. Regardless, all of the electrical components except for the switch will be housed inside the device, underneath a protective 3D printed compartment. This compartment will be removable in the event that the battery must be changed. Changing the battery will simply involve removing the old battery from the battery holder and replacing it with a new battery. This functionality has yet to be implemented. [19][20][21]
Mechanical safety issues will be minimal, as the device includes only a few moving components and a simple vibration mechanism. Currently, the design may be improved to include fillets so that the components do not have as many sharp corners or edges. Such design changes fall into the realm of minor tweaks which may be made after it is determined that the alternating channel prototype is effective in delivering a uniformly mixed hydrogel ribbon. It is also possible that these changes may decrease the chance of residual traces of HRP or H2O2 solutions being left behind in components.

There are no thermal safety hazards present in the use of the alternating channel prototype. It is assumed that the device and associated solutions will be used only under mild conditions (i.e. at room temperature).

The greatest safety hazard present in the array prototype is the possibility of infection due to loss of sterility during transfer of the hydrogel ribbon. The alternating channel prototype allows for easy deposition of the resultant hydrogel onto a nanofiber PCL mat, or directly into a wound. It has been shown through testing and experimentation that the device can be used for its intended purposes without a significant risk of infection.

5. Impact of Engineering Solutions

Hydrogels have found use in a staggeringly wide spectrum of biomedical applications. This is mainly due to the fact that they are mostly water by weight, much like the human body. The value of hydrogels in biomedical engineering is not in their chemical structure, but rather in their mechanical structure—they are powerful tools for the encapsulation of useful items such as cells, drugs, and growth factors. Additionally, hydrogels provide a barrier to protect these items from the external environment. Finally, most hydrogels are biodegradable, meaning that they can gradually deliver their contents in situ. [1][4][7][10][11][14][16]

It is true that the formation of hydrogels through the reaction of HRP and H2O2 is just one of many hydrogel formation mechanisms identified. However, the HRP/H2O2 reaction is useful because it is relatively fast. Therefore, building devices that take advantage of this mechanism is in the best interest of the biomedical engineering community and industries. [10]

When equipped with such a device, researchers and engineers working in the field of tissue engineering can have access to hydrogel ribbons or sushi-roll scaffolds almost immediately, and practically in any number. The device is capable of producing hydrogel ribbons that already contain live cell cultures. The value of this immediacy in the availability of cell cultures to workers in the field of tissue engineering cannot be understated.

The two-component hydrogel ejector is also useful in wound healing applications. It has been shown that hydrogels can aid in wound healing because they can deliver regenerative factors and cells to a wound site. Additionally, they help to maintain a hydration gradient at the wound site--as the wound site becomes dehydrated during the body’s natural healing process, water from the hydrogel gradually diffuses across the hydrogel membrane into the body. Finally, the outer membrane of the hydrogel serves to protect the wound site from entry by pathogens. Clinicians who are equipped with the two-component hydrogel ejector will be able to quickly apply
hydrogels to patients who wounded in order to aid the body in its natural healing process. [10][11]

In its current state, the hydrogel ejector is designed only to eject hydrogels in the shape of rectangular ribbons. This shape has been accepted as generally useful by the medical and research communities. However, it should be noted that other shapes may also be useful. For example, cubes can be used in tissue engineering, while spheres may be useful in drug delivery applications. Furthermore, hydrogel fibers have a wide variety of applications, and are often used in accessory to hydrogels of other shapes (e.g. in this project, as a mat that supports the initially formed hydrogel ribbon). It would be useful to design and develop devices that can eject hydrogels of different shapes, or even to modify the current project to produce hydrogels of different shapes, simply by switching to different components.

6. Life-Long Learning

The 3D modeling, 3D printing, and laboratory techniques learned in this project can all be applied in future work. The 3D modeling work was done in SketchUp and printing was done using a MakerBot Replicator 2. Knowledge of how a single 3D modeling interface works can be helpful when it comes to learning a new interface. The principles are essentially the same. 3D printing involves taking a programmed design, converting it into language that the printer will understand, and then turning that design into a 3D prototype. It can do so quickly and cheaply. We took advantage of its rapid prototyping ability to create our design. Important considerations that needed to be made included safety and practicality of our devices. These are two major components involved in the designing of any prototype that will be used by people.

In the lab, proper safety techniques, titration, gelation processes, dialysis, lyophilization, re-acetylation, electrospinning, and different characterization methods were learned. The working principles here involved understanding how to use a phase diagram, sublimation (what it is and why it’s important), chemical gradients and diffusion, re-acetylation and its role in degradation, buffers, and hydrogels.

Lab safety varies from place to place but the protocol remains relatively the same. Lab goggles need to be worn at all times as well as gloves and a lab coat. Spills should be cleaned up immediately and all items used should be washed and put away. All chemicals should be labeled with no abbreviations, with exact composition, date, initials and amount. Disposal of glassware, hazardous waste, and waste chemicals should be done so accordingly. Having a general knowledge of lab safety is important for your safety and for the safety those around you in the workplace.

A hydrogel is a water swollen covalently bonded cross-linked matrix. It has shown promise for drug delivery and wound healing. A hydrogel ribbon could be applied topically to a wound in combination with an electrospun nanofiber matrix. The nanofiber matrix would help to prevent contamination and bacteria growth and control oxygen and nutrient diffusion with the surrounding environment. The ribbon and nanofiber matrix could also be rolled up and placed into bone defects to help promote new bone growth.
The gelation process requires two polymer-based solutions. Each of these solutions requires the same polymeric base--HPP modified glycol chitosan. To make this polymer a MES buffer that has a pH of 5.5 was needed. This required knowledge of the pH process and the principles behind a pH meter. A buffer is a solution that resists change, in this case to pH. When reactions are taking place like in the case of degradation the byproducts can create an acidic environment. This can be inhibitory to the healing process so we want to keep the pH as stable as possible.

The MES buffer was then used to dissolve glycol chitosan and EDH, NHS, and HPP separately. Once dissolved the two solutions were mixed and left to react. After one hour, dialysis needed to take place immediately to stop the reaction from proceeding further (Figures 26-29). This was accomplished by using special dialysis bags and repeated water changes. The bags needed to be kept in a buffer solution to ensure the bag didn’t dry out which would affect the diffusion of waste by-products into the surrounding water. The dialysis process works by chemical gradients (Figure 30). The molecules want to diffuse from an area of high concentration to low concentration. There are microscopic pores present on the membrane of the tubing that only allows molecules Dalton range to pass through. The cross-linked HPP modified glycol chitosan molecules have a high molecular weight and the byproducts of the reaction weigh much less. So the pores prevented the heavier molecules from leaving and let the waste products diffuse out of the solution, resulting in a chemically pure solution of HPP modified glycol chitosan. The water changes were done to keep the chemical gradient high to drive the diffusion of waste products.
After dialysis the samples are separated into vials and frozen for lyophilization. Lyophilization or freeze-drying is a process that requires that you understand phase diagrams. Particularly what happens during sublimation. For sublimation to occur you keep the temperature constant and drop the pressure so that the sample goes from a solid state to a gas state. This requires working quickly because for the lyophilization, or freeze-drying, process to work the solution needs to remain a solid. To prepare the vials, they needed to be uncapped, covered with a kimwipe, and secured with elastic bands. The vials were then placed in special glassware meant for the lyophilization process. This glassware was then attached to the lyophilization machine using special piping and rubber caps. This machine created a vacuum in the chamber so that the pressure could be dropped, resulting in the frozen water vaporizing.
Re-acetylation is a process that results in the attachment of acetyl groups to the polymer. The amount of re-acetylation that occurs controls the degradation rate of the hydrogel. If no re-acetylation occurs then the polymer won’t degrade and the hydrogel wouldn’t be bioactive or biocompatible, which isn’t what we want. With this knowledge the parameters can be controlled to control degradation rate.

Electrospinning involves charging a solution and driving it from a needle onto a grounded collection plate by using an electric field. Needle size, distance from the needle tip to the collection plate, and voltage are all parameters that can control the diameter of the nanofibers and play a role in controlling porosity. This method is how we created the nanofiber matrix that will be placed underneath the hydrogel.

The nanofiber PCL mats used in this project can be characterized by tensile testing. Testing devices, including Universal Testing Machines (UTMs), can provide data describing the mechanical properties of the material. Tensile testing requires that the PCL mat first be cut into a “dogbone” shape (see Fig. 32). The sample can then be loaded into the clamps of the tensile testing device (see Fig. 33). Sample tensile testing data is presented in the Appendix (see Section 12.3.).
A rheometer measures viscosity and flow rate. If we know the viscosity of each solution and the viscosity of the final gel, the parameters can be more finely tuned and optimized. The hydrogel is shown in a rheometer in Fig. 34. Sample rheology data is given for the HRP/H2O2 hydrogels in the Appendix (see Section 12.2.).
7. Budget

For the design and development of the hydrogel ejector, the team was given a budget of $1,000. At the current point in the design phase, the team has assembled the list of necessary resources presented in Table 1. The total cost is estimated based on the number of prototypes that will potentially be assembled and tested.
### Table 1: The estimated total cost of the project.

<table>
<thead>
<tr>
<th>Item</th>
<th>Approximate Cost (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D Printing Filament</td>
<td>$50</td>
</tr>
<tr>
<td>Vibrational Motor Components</td>
<td>$10/prototype</td>
</tr>
<tr>
<td>Polymer</td>
<td>$200</td>
</tr>
<tr>
<td>Fluorophore dyes</td>
<td>$100</td>
</tr>
<tr>
<td>MES Buffer solution</td>
<td>$60</td>
</tr>
<tr>
<td>Silicon Glue</td>
<td>$10.71</td>
</tr>
<tr>
<td>Arduino Nano</td>
<td>$35</td>
</tr>
<tr>
<td>Additional batteries and switches</td>
<td>$90</td>
</tr>
<tr>
<td>NEBEC Registration</td>
<td>$390</td>
</tr>
<tr>
<td><strong>Total Cost</strong></td>
<td><strong>$965-1125</strong></td>
</tr>
</tbody>
</table>

8. Ethics

As is the case in any engineering project, it is imperative that the design team consider ethical concerns as they arise. The purpose of the hydrogel ejector is to provide a simple means of producing hydrogels for clinical and research applications, thereby directly simplifying research and indirectly improving patient quality of life. However, these benefits are dependent on the ability of the design team to successfully build and deliver the product through ethical means, without taking any risks or shortcuts that might cause accidental injury or death. Such a case would completely defeat the purpose of the project.
Luckily, the hydrogel ejector is not an inherently dangerous product. There are not many ethical concerns to address in its design. The most important ethical issue is that of safety concerns, followed by environmental footprint.

In terms of safety concerns, the most important consideration is that of patients becoming infected with pathogens. This can happen if a non-sterile hydrogel ejector is used to create a hydrogel ribbon or sushi-roll that is injected into a patient or applied to their skin. For example, if the end user chooses to use an extruder twice, there may be residual hydrogel or precursor solutions remaining in the extruder after its first use. These fluids can harbor bacteria and other pathogens, which will enter the patient’s body and potentially cause an infection if the same extruder is used again.

The extruder was designed to be disposable after each use, in order to minimize concerns of non-sterility. The battery pack is reusable, but must be sterilized with ethanol between each use. The operator’s manual makes these instructions explicitly clear, with several messages in bold-faced font throughout to warn the end user of the non-sterility hazard.

Finally, prior literature confirms that all of the compounds which will come into contact with the patient’s body (HRP, H₂O₂, HPPGC, and PCL) are safe for human contact. [1][4][6][10]

All of the electrical components incorporated into the battery pack are designed to deliver currents and voltages that are relatively small and not dangerous, even when entering the human body. The maximum voltage is 6V, provided by the battery. The current running around the loop is approximately 37mA. Therefore, even in the event of an electrical malfunction (short, open circuit, etc.), the chances of human electrical injury are negligible.

The device’s environmental footprint must also be considered. The device contains two components that are 3D printed from (poly)lactic acid (PLA), a relatively popular 3D printing material. Unfortunately, there are no convenient recycling programs in place for PLA. However, it can easily be melted down and reused.

The chosen design for the extruder is the smallest design of the three considered, so as to minimize 3D printing time and environmental waste. The effect of mass use of the disposable components and syringes must be considered in greater detail before continuing with the project. It may be possible to set up a PLA recycling system where users can return their extruders in bulk to have them melted down and formed into new extruders.

The design decision to make the battery pack reusable was made in order to minimize the environmental footprint in addition minimizing the cost of electrical components.

9. Team Members’ Contributions to the Project

9.1. Tom

As a student focusing on bioinformatics, Tom has assumed most of the roles involving computer and information expertise. Tom led the team in learning to use SolidWorks and SketchUp as
software tools for 3D modelling and printing. He also learned and instructed the rest of the team on how to use the MakerBot Replicator 2 and Flashforge Creator Pro printers to print the models. For the final prototype, Tom was the lead designer for the 3D model of the battery pack.

Tom also worked on several electrical projects throughout the design and development phases. He built a circuit in which an Arduino Nano microcontroller maintained automated control of the vibration motors. This functionality can be implemented in future versions of the hydrogel ejector to provide automated vibration that begins as soon as the user begins injecting the input solutions.

Tom has also exercised his time management and managerial skills to keep the group on task and make sure that all deadlines were met on time with work of exceptionally high quality.

9.2. Jess

Jess primarily concentrated on the biomaterials aspect of this project. She synthesized HPP modified glycol chitosan. This was the base polymer used for both precursor solutions. She was in charge of calculating and preparing the concentrations of HRP and H$_2$O$_2$ necessary for gelling to occur. Jess utilized her organizational skills to create the testing protocol and made sure the team had all of the supplies needed for the experimental process. She led the testing of the prototypes at the Health center by instructing the team on how to prepare and use the HRP and H$_2$O$_2$ solutions to create a hydrogel. Jess also qualitatively assessed the mixing that occurred by incorporating fluorescent microspheres into the two solutions and determined that the microspheres inaccurately depicted the gelation that was occurring. To remedy this she researched different dye alternatives that were not charged and came up with a standardized protocol for adding dye. The alternative dyes better reflected the mixing that was actually occurring. Jess learned how to electrospin and optimized the electrospinning process for 7cm x 7cm nanofiber mats. She led the team in testing the tensile properties of the nanofiber mats and in testing the storage modulus of the optimized hydrogel. She took on the responsibility of completing the cell work required to test cell viability with and without vibration. Jess also arranged for the SEM images of the nanofiber mats and the confocal images of the cells encapsulated in HPPGC post vibration.

Jess also designed the alternating channel piece to be used as the ejection device for this project and repeatedly made modifications and 3D printed to assess whether or not those modifications were useful in helping to create a uniformly mixed hydrogel. She did this by going to the health center and testing with the optimized gelation parameters. To determine the optimized parameters Jess calculated the appropriate concentrations and then with the team instructed them on how to test for gelation times. After choosing the concentrations with the best gelation times she then tested the new concentrations against the old concentrations with the ejection prototypes to ultimately conclude which ones were better.

9.3. Connie

Connie was mainly responsible for the instrumentation and electrical portion of this design project. She exercised her creativity skills and brainstormed the idea of incorporating vibrational motors to aid in the cross-linking process during the hydrogel ejection. The electrical
components were researched and selected by her during the ordering process. These components were selected based on need, dimensions, and durability. The rest of the budget was also organized by Connie, as she was responsible for keeping track of all the order form submissions and component research. She also invested a large portion of her time in designing the prototype that she believed would optimize the hydrogel ribbon formation. Once the prototype was designed in SketchUp, the device was printed via the MakerBot 2.

In finalizing the prototype, Connie worked with Tom in designing a circuit that would be able to efficiently power two vibrational motors with a single switch and battery. Once the circuit was deemed optimal, she manufactured all the protoboards for the battery pack, where she soldered all the components of the vibrational motor circuit. She also assembled the battery pack with the screws so that the device was a whole with the electrical components.

10. Conclusion

The objective of this project is to design and develop a device and a method for a dual solution hydrogel ejection. The end result is a dual syringe injection chamber which ejects a hydrogel ribbon that can encapsulate live cell cultures ideal for tissue regeneration. These ejected hydrogels can also be used as a wound dressing.

Previous designs of similar devices have not been successful in a sense that the hydrogels were not mixing uniformly or ejected quickly. This design takes into consideration the viscosity of the two solutions used (horseradish peroxidase, HRP, and hydrogen peroxide, H$_2$O$_2$), as well as the resulting hydrogel. The viscosity of the solutions were tested and determined in the lab using a rheometer. The flow rate was measured to ensure that the distance that the hydrogel travels is optimal in order to prevent the hydrogel from getting lodged within the device.

To improve uniformity, a vibration motor compartment was included in the design to aid in the mixing process. Multiple mini motors were also tested in order to select the most ideal compartments to incorporate into the design. The motors were powered by a battery housed inside the same compartment within the device.

Since the hydrogels produced by this device are to be used in vivo, it is preferable that the hydrogel mixing component of the device be disposable. If that specific compartment was reusable, costly sterilization processes would be necessary in between uses to ensure that no residual polymer, cells, or bacteria remained inside. The design incorporated multiple pieces that do not require assembly. This method optimized cost since a separate compartment was designed to house the electrical components so that the vibrational motors will be reusable.

The current optimal prototype involves separation of HRP and H$_2$O$_2$ using alternating channels. Channels were arranged in different orientations with the goal of producing a hydrogel that is mixed as uniformly as possible. This design is influenced by the idea that greater uniformity will be achieved by using a greater number of smaller channels. Other prototypes mentioned here involve the hydrogel ribbon being mixed and extruded simultaneously, and or involve the hydrogel being deposited into a mixing chamber and being extruded after mixing--a process which is aided by vibrational motors.
The primary advantage of this design approach is that the end user is able to create hydrogels supporting cell cultures which may be used in tissue engineering or drug delivery, or used immediately as a wound dressing. This innovation will further promote the use of hydrogels in all of these fields.

The success of this device may also lead to the development of similar devices that create hydrogels of different shapes or sizes (e.g. blocks, fibers, etc.). Theoretically, a block can be created by layering several ribbons on top of each other. Fibers may be created by cutting ribbons into thinner segments. The functionality of the hydrogel ribbon can also be increased by placing an electrospun nanofiber mat on top of it. This would act as a barrier for pathogens and control the diffusion of water and nutrients between the affected area and the outside environment.
11. References


http://www.medline.com/jump/product/x/Z05-PF4298


We would like to thank…

- Our advisor and client, Dr. Lakshmi S. Nair, and our teaching assistant, Shruti Kuzhippat, for all of their assistance and guidance.
- Dr. Guoan Zheng, Dr. Patrick Kumavor, and Dr. Chen Xu of the UConn BME department for their help with 3D printing and electrical work.
- The staff of the Nair Lab at the UConn Health Center, for their guidance in many of the lab tests performed for this project.
- The UConn Biomedical Engineering Department for sponsoring our trip to the Northeast Bioengineering Conference (NEBEC).
13. Appendix

13.1 Specifications

The device must create a uniformly mixed hydrogel, which will be biocompatible and useful in the field of tissue regeneration. This must happen without any of its internal chambers becoming blocked--i.e., the gel solution must not undergo gelation prior to exiting the device. The hydrogel produced must be ideal for tissue regeneration; uniformly mixed, biocompatible, and biodegradable. The ideal time between mixing of the HRP and $\text{H}_2\text{O}_2$ solutions and injection of the hydrogel must be determined as part of the testing process, and the device must be engineered to deliver the hydrogel from the device to the body in that time. The completed hydrogel must be fitted with a nanofiber mat cover, which will regulate water and shield the hydrogel from pathogens. The mat may be applied by the same device or an accessory device.
### Table A1: Technical specifications by category.

<table>
<thead>
<tr>
<th>Category</th>
<th>Technical Specifications</th>
<th>Reasoning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>- device will be disposable</td>
<td>- having a disposable device will help ensure the sterility of the device and make it more user friendly, cleaning and sterilizing the small compartments and tubes will be difficult post-gelation - re-useable battery and electrical component back will make our device cheaper and allow for battery changes</td>
</tr>
<tr>
<td></td>
<td>- battery pack will be re-useable</td>
<td></td>
</tr>
<tr>
<td>Physical</td>
<td>- where to place vibrational motor</td>
<td>- placement on the device can heavily influence the efficiency of the mixing</td>
</tr>
<tr>
<td></td>
<td>- where to put the battery pack</td>
<td>- battery pack needs to be easily accessible so that batteries can be replaced</td>
</tr>
<tr>
<td></td>
<td>- collection chambers vs alternating tubes</td>
<td>- designs need to consider the benefits and drawbacks to collection chambers and alternating tubes</td>
</tr>
<tr>
<td></td>
<td>- viscosity of solutions</td>
<td>- viscosity of the solutions influences tube diameter and speed they travel down the tubes</td>
</tr>
<tr>
<td></td>
<td>- viscosity of gel</td>
<td>- viscosity of the gel can give more insight into the tube size needed, size of ribbon that can be made, and pressure needed to push the gel out</td>
</tr>
<tr>
<td></td>
<td>- pressure required to push solutions out of syringes</td>
<td>- device should be able to operate with only hand pressure applied to the syringes</td>
</tr>
<tr>
<td></td>
<td>- time required for gelation</td>
<td>- time required for gelation influences the design of the device, which needs to compensate for the change in viscosity</td>
</tr>
<tr>
<td>Mechanical</td>
<td>- moving components can’t be in contact with cells</td>
<td>- moving components will kill cells</td>
</tr>
<tr>
<td></td>
<td>- tubes and mixing chamber can’t be too long</td>
<td>- gelation occurs almost instantaneously, will cause clogging and increased required force to eject gel</td>
</tr>
<tr>
<td></td>
<td>- uniform mixing</td>
<td>- gel needs to be uniformly mixed to be useful</td>
</tr>
<tr>
<td></td>
<td>- device must be structurally sound</td>
<td>- device can’t break apart when in use, don’t want to inject particle of PCL into patient wound</td>
</tr>
<tr>
<td>Safety</td>
<td>Electrical</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>-hydrogel needs to be biodegradable</td>
<td>-vibration motor can’t be too strong</td>
<td></td>
</tr>
<tr>
<td>-hydrogel needs to be biocompatible</td>
<td>-number of motors</td>
<td></td>
</tr>
<tr>
<td>-amount of pressure required to eject the hydrogel needs to be</td>
<td>-wiring</td>
<td></td>
</tr>
<tr>
<td>consistent</td>
<td>-switch to activate motor</td>
<td></td>
</tr>
<tr>
<td>-nanofiber mat</td>
<td>-it’s too strong it will kill the cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-will more motors allow for more mixing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-wiring</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-it won’t degrade allowing for complete tissue regrowth if it’s not</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-application of the gel will cause inflammation and impede the healing process</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-if pressure isn’t consistent this can lead to forcing the syringe tips to eject solution,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>resulting in syringe breakage and accidental injury</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-will provide protection from pathogens and enhance wound healing because it will control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>oxygen and nutrient diffusion between the wound and the environment</td>
<td></td>
</tr>
</tbody>
</table>

13.2. Rheology Sample Data

![Figure A1](image.png)

**Figure A1**: The storage modulus (Pa) of the hydrogel, as measured by a rheometer. The storage modulus describes the material’s elastic properties.
13.3. Tensile Testing Sample Data

**Figure A2:** The tensile stress at break of samples of the PCL mat.

**Figure A3:** The tensile extension at maximum load of samples of the PCL mat.

**Figure A4:** The tensile strain at break of samples of the PCL mat.
**Figure A5:** The Young’s Modulus of samples of the PCL mat.

**Table A2:** The mean and standard deviation of all trials (n = 5) presented in Figs. A2-A5.

<table>
<thead>
<tr>
<th></th>
<th>Tensile Stress at Break (MPa)</th>
<th>Tensile Strain (Extension) at Break (mm/mm)</th>
<th>Tensile Extension at Maximum Load (mm)</th>
<th>Young’s Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>2.686208</td>
<td>0.393304</td>
<td>15.206832</td>
<td>10.153322</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>0.445550151</td>
<td>0.089960898</td>
<td>3.149661923</td>
<td>2.099942817</td>
</tr>
</tbody>
</table>

**13.4 Additional Information**

To create the two solutions the HPP modified glycol chitosan must be aliquoted into 2mL tubes. The amount of polymer designates the viscosity 10mg/mL will produce a less viscous solution than a concentration of 20mg/mL. The polymer must then be dissolved in MEM media. To create the HRP solution 20uL/mL of HRP at a 1 to 1000 dilution needed to be added to each tube. To create solution B the H2O2 containing solution H2O2 was used at a .25% concentration. 25uL of extra polymer solution needed to be added to each tube to account for loss during mixing. Then H2O2 was added at a concentration of 1uL per 10uL. Solution B needed to be created at the time of testing because H2O2 quickly loses its reactivity once placed in the polymer solution.