Development of a Modular Silk Processing System

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Abstract:
In any biomedical engineering lab or chemical engineering lab, there are not many pieces of lab equipment that aren’t automated. Incubators, refrigerators, autoclaves, bioreactors, chemical hoods, and confocal microscopes all have gradations of automation associated with them. This project aims to automate the silk processing protocol so that making silk solutions becomes as routine as operating the aforementioned equipment.

Silk is an innovative biomaterial that has applications in many fields of engineering. Due to its simple composition, silk has emerged as a modular building block for processes both large and small. Silk has the innate ability to self-assemble from the bottom up because it is comprised of only protein and water. By reprogramming its self-assembly process, researchers can make silk into a variety of things including optical fibers, diffraction gratings, disposable cups, microneedle arrays, and artificial cornea (Lawrence et. al. 2009). The applications of silk as a new material are exciting because they turn conventional materials into biomaterials. While silk is an emerging panacea to problems in the biomedical engineering field, the fibroin solution is tedious to produce in large quantities.

This project aims to create an automated silk processing system. Inputs to this system would be raw silk, water, and salts; the outputs of this system would be a fibroin solution that matches the user’s specifications exactly. This machine would remove human variability in the creation of fibroin solutions, and it would allow for researchers to perform high throughput testing of their experimental setups.

As tissue engineering and silk production increases in popularity, this automatic silk processor will alleviate human production of fibroin solutions. This technology will not only empower Tufts labs to increase experimental results, but it will also encourage researchers at other institutions to investigate silk as a novel material.

Keywords: silk-fibroin solution, automation, systems engineering, rapid prototyping
ELEMENTS OF ENGINEERING DESIGN

Design
Researchers designed and constructed a laboratory grade automated silk processing system made from consumer materials and components. This system would effectively allow researchers to create high quality silk-fibroin solutions of wide variability based on a small range of process inputs.

Objectives
This project aimed to break down the silk engineering process into discrete steps and implement those steps separately in individual systems before they were serially connected into the complete system. Success of this design process was gauged by the quality of solution produced by the system, and by how many resources it saved the lab in which it was implemented.

Science, Math, and Engineering applications
Several science, math, and engineering principles were applied to this project to ensure its success and improve the design of the overall system. Some large ideas included abstraction and modularity, but more specific ideas included PID control, PWM, and related rates.

Testing and Evaluation
Tests for efficacy of action will determine the success of this project. Specific quantifiers related to silk engineering such as molecular weight of fibroin in solution, presence of adulterants, and mechanical tests of solids made from solution will dictate the performance of the system. Endurance testing of the system will also determine the long term behavior, and indicate which components serve as most likely modes of failure.

Constraints
Safety, size, power requirements, chemical effects, heat resistance, run time, and cost were all constraints that dictated design decisions.

Alternative Solutions
Reverse engineering was considered in this project. Since the silk processing procedure is similar to the action of washing clothes, alternative designs were centered on altering an already functional washing machine.

Final Result
A prototype that is able to degum, rinse, dry, dissolve, and dialyze silk cocoons with significantly low oversight from researchers. This machine meets a large percentage of the objectives that were set out however there is still significant work to do before this machine is able to fully accommodate the silk production of a researcher.
Design Flowchart:

Figure 1: A design flowchart describing the design objectives of creating an automated silk processor
Figure Created by Grant Pemberton
Introduction

Background

Silk Engineering

Silk is a widely investigated material at Tufts University. In various BME labs, silk is investigated for its material properties, mechanical properties, chemical properties, and potential as a tissue engineering platform. Silk is an excellent biomaterial because it is comprised of only protein and water in its native state. This protein is an excellent biodegradable building block for mechanical structures and cells alike. (Lawrence et al., 2009)

In a biomaterials context, silk is an attractive material to study because of its simple formulation. Silk fibroin solution has a high casting resolution, but it also has tensile strengths similar to most consumer plastics. Depending on the way that it is processed, fibroin products can take on a variety of mechanical characteristics. Fibroin is also a tunably biodegradable material, so it can be used in an environmental context to create environmentally conscious building materials to replace plastics (Lawrence et al., 2009).

From a tissue engineering perspective, silk is also an excellent material on which researchers can grow cells. By manipulating the structure and formulation of fibroin, researchers can create a variety of geometries that can house cells effectively. Silk is an exciting biomaterial that holds great promise in the realm of tissue engineering. This protein rich material has amazing properties that make it an amazing biocompatible implement for biomedical engineering applications. Silk can be made into sponges, electrospun into fiber bundles, or integrated into hydrogels to create scaffolds for tissue engineering (Helrich, 2015).

Properties and Characteristics of Silk Fibroin Solution

Variance between silk solutions depends on a wide range of processing techniques. Boiling temperature, boiling time, concentration of salt in dissolving solution, and hydration of silk solution play pivotal roles in determining the characteristics of the output fibroin solution. Among the indicators of these variables are the molecular weight of fibroin chains in the product, the concentration of silk-fibroin in aqueous solution, the crystallization properties of the solution, presence of adulterants in solution (Lawrence et al., 2009).

Batch Processing

The biomolecule and drug development industry utilize large batch processing for many production lines. Similarly, many fermented food and drink products are also batch processed at various scales. Batches as large as 3000 gallons are produced at commercial plants in stainless steel reaction vessels. While these batches are enormous, they can also be scaled down to lab dimensions with batches that are in the single gallon range. Scaling of these processes is sometimes linear and volume dependent, however the central concepts remain the same throughout each system. Addition of different chemicals, heating, cooling, and agitation are involved at different steps in each production line. The combination of these various actions can produce a multitude of effects within the final product.
Discrete vs Continuous Processing

In processing biomaterials or other biological components, it is important to consider the sequence of events in the processing timeline. These can be organized in series or in parallel, however common schemes for bio-production are discrete systems or continuous systems. In discrete bio-production, the entire product is assembled in one cycle of the machine. This differs from continuous production because in continuous production each component of the system is designed around completing a certain task and then moving the product to the next stage in the assembly line. While these two schemes are widely used in industry, there are tradeoffs for using either strategy.

The choice to use continuous processing or discrete processing significantly depends on the context in which the system is being used. Discrete processing takes more time and resources to produce at the same rate as a continuous system. Since continuous systems are focused on producing large volumes of product with a pre-specified formulation, they are not flexible systems. Discrete processing machines are much better at taking inputs and producing a variety of formulations. This is largely due to the integration of all systems in a discrete system. This also works better in a setting where there are multiple users for the system.

Control Schemes

Methods for controlling dynamic systems usually focus on two technologies: PID (Proportional Integrative and Derivative) and PWM (Pulse Width Modulation) control. These two control schemes can be applied to a variety of systems to produce desired results. Advantages of these systems are that they are able to respond to various dynamic inputs to produce a stable behavior within a system. PID control provides compliance and dampening to system fluctuations, allowing steady state behavior to be established effectively.

PID control measures deviations from a set-point metric and feeds that back into an effector that works to mitigate the deviations. The proportional part of a PID controller measures the error from the setpoint and multiplies that error by a constant, Kp. This combined term is then fed into the input power of the system. This proportional behavior produces large feedback for large deviations and small feedback for small deviations. The behavior of a proportional controller is similar to a damping system on a harmonic oscillator (figure 2). By tuning the Kp term, one can establish am underdamped, critically damped, or overdamped response to external stimuli. The integrative term in the PID controller allows for corrections to steady state error. Over a period of time, the difference between the set-point and the current point are measured and summed. This serves as a rolling average error over a given time period which is multiplied by the constant, Ki. This part of the controller compounds small deviations in the system and eventually corrects them. This control scheme is most useful in systems that require high precision because steady state errors can produce negative effects. Finally the derivative portion of the PID controller measures the rate of change in the state of the system and feeds that back into the output of the controller. The rate of change metric is multiplied by a constant, Kd, which is then applied to the power of the controller along with the P and I terms before it. The derivative part of the controller works to smooth changes in the state of the system by limiting the rate at which the system can change (National Instruments).
PWM control is the method by which constant power components of a system can become dynamic power components. The presence of a duty cycle within the control of these components changes the component from a binary operator into a variable operator. If a resistive heater outputs 1,100 W, then it can be cycled over a period of time to produce a fraction of its maximum power. By using a duty cycle, \[ \text{Duty Cycle} = \frac{PW}{T} \times 100\% \] where PW is pulse width and T is time for one cycle, one can control how much power is output by the system. This concept is usually presented in terms of a square wave form representing the instantaneous ON/OFF behavior of the binary component (figure 3). Dimensional analysis of the duty cycle multiplied by max power shows that one can produce fractional power from the binary component in a highly controllable way. As seen in figure 3, PWM can provide fractional power to components that are not able to be powered at a variable voltage. PWM control can give variability to component properties such as LED brightness, resistive heating element outputs, and magnetic element controls. (National Instruments)

Other Biomaterials

Common biomaterials that are investigated in line with silk are polysaccharide based. Chitin, cellulose, and alginate are biomaterials that researchers have studied for their biocompatible properties. These biomaterials have advantageous properties in biomedical, mechanical, civil, environmental, and chemical settings, however they require different handling protocols than silk. The protocols to process these biomaterials have similar abstract inputs and
outputs such as water, solvents, heat, and dissolution. It is likely that systems developed to process silk could be adapted to also process these biomaterials. (Younes et. al., 2015)

**Significance**

**Clinical Need**

*Spread the silk revolution*

Silk is clearly a novel biomaterial that has requires more research. The potential for silk to become a standard in healthcare is clear, however silk research has not seen the same popularity that other biomaterials such as chitin have. If silk research were able to spread to other universities and medical institutions, then discoveries would happen at a likely faster rate.

By introducing a machine that is able to create consistent fibroin solution, not only other labs at Tufts University would be able to easily implement silk in their research, but universities and companies across the world would be able to further silk research.

*Higher Production*

By allowing researchers to produce larger amounts of silk fibroin solution, this silk processing machine would allow researchers to conduct more varied experiments in their research of silk as a novel material. Higher production would increase the sample space of experiments that use silk as a tissue culture medium and also as a biomaterial. This would allow researchers to more thoroughly find the optimal characteristics of the silk material that fits their experiment.

Higher production would also allow these researchers to take a more statistical approach to their work. A sample size of above 15 is usually deemed significant in a t-test metric, however to create 15 batches of identical silk fibroin solutions, a researcher would have to spend 60 days of their own time. Even with this time spent making the solutions, the confounding variables in the production of these silk solutions would cause small extraneous variance between these solutions. Simply put, humans are not algorithmic machines, so not each batch of silk solution that they make is perfect. An automated machine that is able to consistently produce silk fibroin solutions with a range of variability is the optimal solution to solving this problem because it eliminates human error from this research step. Consistency and repeatability are key in scientific research, so having a system that governs the fibroin production process would increase the validity of any experiments that are done with silk.

*Popularizing silk in a non-textile context*

Silk as a material clearly has numerous applications. By elevating its popularity among research institutions and in industry, it will be possible to introduce silk to the general public as more than just a textile good. Silk has properties that make it a valuable alternative to plastics in many arenas. The structure and function of silk biomaterials also can be tuned using different chemicals or processing techniques. These functions have relevance to environmental engineering, biological engineering, civil engineering, and mechanical engineering.
The environmental impact of silk is immense. As a tunably biodegradable material, silk could be used to replace various packaging products, but still be compostable. As a protein, silk is biodegradable, however the rate of its biodegradability can be controlled by controlling the crystallinity and microscopic structure of the fibroin building blocks (Lawrence et. al, 2009). A directionally biodegradable silk packaging material could be made from silk fibroin that is designed to degrade in the presence of water, however one side of this material could be textured to be hydrophobic while the other side is hydrophilic. By constructing packaging out of this type of material, environmental engineers could create an array of water degradable packaging that could be used to store liquids. As seen in figure 4 (left), a cup could be made from this type of biomaterial, and only degrade once it is composted. This directional biodegradability is one of the many tools that silk offers environmental engineers, however there are other applications of silk that have environmental impact.

Figure 4: A silk cup (left) has the potential to be a biodegradable alternative to plastic cups. (Right) silk biomaterials combined with specific enzymes indicate the presence of a contamination with a noticeable color change.

Silk fibroin solutions can also serve as building materials. The mechanical strength of silk biomaterials is similar to that of plastics, however the environmental footprint of silk is much smaller than their plastic counterparts. Silk also has the unique property of being able to house biomolecules that can perform several functions relevant to human health. An example of silk being used as a biomaterial in a civil engineering context would be air pollution monitoring tiles. These tiles, made from silk fibroin solution, could house enzymes that induce a color change when introduced to carbon monoxide gas similar to how the glove in figure 4 reacts to contaminations by changing color. These tiles could be placed in homes as passive indicators of carbon monoxide leaks. This technology could help save lives, but it would also usher in a new era of smart building materials.

As stated above, silk solutions have immense applications in the health and medical field. From sutures to tissue engineering scaffolds, silk presents a unique and powerful solution to various biomedical engineering challenges. As a biomaterial, silk can be used to implant electronics within the body. These electronics can be diagnostic or therapeutic, offering health professionals a new toolkit in diagnosing patients and treating illnesses. Silk can also be used to create new forms of medical treatment. Microneedle arrays are among these new forms of medical treatment. The microneedle arrays provide a sustained release of injectable drug into the patient over a wide surface area. This works as a great alternative to needles because it reduces
the invasiveness of the injection procedure, and it allows for more controlled delivery of drugs into the system (Baryshyan et. al, 2012). Fibroin’s tunable biodegradability also plays a significant part in its role as a medical tool. Silk can become bi-inert, bio-active, or bio-resorbable depending on its formulation. This offers health professionals a new way to deliver treatments to their patients.

Apart from silk’s applications as a biomaterial, it is also a novel platform for two dimensional and three dimensional cell culture. The various shapes and geometries that can be created from the silk solution offer a multitude of tissue engineering platforms for researchers. Since the fibroin solution is comprised of protein and water, cells view the silk scaffold as a pseudo extra-cellular matrix (Baryshyan et. al, 2012). This characteristic of silk scaffolds encourages cells to grow into the scaffolds, creating colonies of thriving cells that are capable of sustaining themselves as tissues. This area of biomedical engineering is still young however it is a promising area, and early results indicate that this strategy for generating living tissue could be the first step in organ replication.

Silk is also relevant to the mechanical engineering field. As a rapid prototyping material, silk can be used in construction paradigms such as 3D printing, laser cutting, milling, molding and casting. The various conformations of silk as a material present a unique new building material for mechanical engineers. Apart from its mechanical properties, silk also has chemical properties that make it an attractive material. Biodegradability is a growing concern in robotic design especially as components become more inexpensive. As robots and computers decrease in price, a new concept has emerged in the development field: disposable robotics. These robots would likely serve one purpose and then simply be discarded. With this new frame of mind, it is important to be mindful of costs and environmental impact. Silk provides a good solution to both of these issues because it could become a low cost alternative to expensive materials, and it is an environmentally friendly alternative to plastics. A possible use case for these silk biomaterials in a mechanical engineering context would be using silk in the body of a biodegradable drone. Biodegradable materials being used for drones has already seen success. A drone, seen in figure 5, built by a NASA team of engineers featured a fungus derived cellulose body meant to decompose after the drone has completed its mission (Shadbolt, 2014).

Figure 5: A NASA developed biodegradable drone made from fungus derived cellulose
### Table 1: Innovations of an Automated Silk processing system

<table>
<thead>
<tr>
<th>Conventional silk processing</th>
<th>Automated Silk processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takes over 4 days to process 10g of raw silk</td>
<td>Can process 10g of raw silk in under 2 days</td>
</tr>
<tr>
<td>Limited to glass reaction vessel volume (~4L)</td>
<td>Highly scalable, with high vessel capacity up to 3000 liters (commercial drug development)</td>
</tr>
<tr>
<td>Experienced researchers are required to train new researchers before they can process silk</td>
<td>Users must only read an instruction manual to understand how to use the machine</td>
</tr>
<tr>
<td>Variability of solution formulation between different researchers (low reproducibility)</td>
<td>Consistent solutions independent of which user is operating the system</td>
</tr>
<tr>
<td>No records of solution characteristics</td>
<td>Records and diagnostics of each operation can be filed and analyzed by lab leaders</td>
</tr>
<tr>
<td>No temperature control in the boiling step</td>
<td>Temperature and time control in all steps of the process (Boiling, Rinsing, Dissolution, Dialysis)</td>
</tr>
<tr>
<td>Unable to be completed in a completely aseptic environment</td>
<td>Small footprint of system allows it to operate within a biological fume hood, increased sterility</td>
</tr>
<tr>
<td>Designed specifically for silk processing</td>
<td>Can be applied to other biomaterial processing protocols</td>
</tr>
</tbody>
</table>

### Specific Aims

The goal of this project is to create a system that makes creating a silk solution a faster and easier process for researchers. The overarching idea is to create a system in which we may completely abstract the process for boiling, dissolving, and dialyzing silk. This will not only save time for researchers, but it will also allow for researchers to develop a higher sample size for their experiments without wasting time on the lengthy task of processing raw silk into a fibroin solution.

**Aim 1: Breakdown the silk processing procedure into a flow diagram of discrete steps that can be analyzed independently**

The process of creating a fibroin solution from raw silk, water, and salts can be broken down into five steps: degumming, rinsing, dissolution, dialysis, and centrifugation. Automation of each of these steps is possible, although the interface between steps may require human intervention.

**Aim 2: Implement each step of the flow diagram using computer controlled devices, then combine these devices to create the full system**

To automate silk processing, we will need to use a computer controlled system to carry out each step of the protocol. This will require a computer (Raspberry Pi, Arduino or similar...
microcontroller), software to control each step of the procedure, and equipment used in each step of the protocol (hot plate, beaker, dialysis cassettes, etc.). These implementations of items in the flowchart will then be combined to form the prototype automated silk processor.

**Aim 3: Test the prototype for completion of the task and long term use**

We will compare the quality of the silk solution produced by our machine to a solution that is produced by a researcher. High throughput testing of the system will also be performed to understand the weak points of the prototype design. Taking into consideration both of these results, the system will be redesigned to create a higher functioning system.

**Methods**

**Design of systems**

Abstract Inputs and Outputs

To accomplish the first specific aim of this project it is necessary to abstract the components of the silk processing procedure. The system can be designed by addressing these abstract inputs and outputs independently and then connecting the constituent systems in series. The silk processing procedure deals with a small set of consumables: Deionized water, Sodium Carbonate, silkworm cocoons, and Lithium Bromide. Materials necessary for silk processing include a boiling vessel (metal or glass), a dissolving vessel (glass or plastic), and dialysis membrane (cassette, tubing, or separate machine). Components of the system also include a water transfer system, a heating system, and a silk solution transfer system. With this small set of abstract components, we are able to complete each step in the silk processing procedure as seen in table 2. By combining these processes serially, the whole system accomplishes the goal of taking silk cocoons and turning them into a usable silk fibroin solution.

**Table 2: Abstract Task analysis**

<table>
<thead>
<tr>
<th>Task</th>
<th>Abstract inputs</th>
<th>Abstract Outputs (Waste)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degumming</td>
<td>Silk Cocoons, Sodium Carbonate, Deionized Water, Heat (90°C)</td>
<td>Fibroin Bundle, Sericin Solution</td>
</tr>
<tr>
<td>Rinsing</td>
<td>Fibroin Bundle, Deionized Water</td>
<td>Rinsed Fibroin Bundle, Dilute Sericin Solution</td>
</tr>
<tr>
<td>Drying</td>
<td>Rinsed Fibroin Bundle, Heat, Air Circulation</td>
<td>Dry Fibroin Bundle</td>
</tr>
<tr>
<td>Dissolving</td>
<td>Dry Fibroin Bundle, LiBr, Deionized Water</td>
<td>Fibroin-LiBr Solution</td>
</tr>
<tr>
<td>Dialyzing</td>
<td>Fibroin-LiBr Solution, Dionized Water, Dialysis Cassette</td>
<td>Dialyzed Aqueous Fibroin Solution, Dilute LiBr Solution</td>
</tr>
</tbody>
</table>
Design of Systems

Degumming

The degumming process is the first step in the silk processing procedure. It uses a sericin dissolving salt, Sodium Carbonate, to strip the adhesive protein from the silk fibers. The way this is accomplished conventionally is by boiling the silk in a Sodium Carbonate solution for 30 minutes. To accomplish this in an automated fashion, a water inlet, waste outlet, and a heater were needed. The water inlet was created by connecting a DI water compatible pump to a DI water source and feeding it into the boiling vessel. The waste outlet was constructed in a similar way, however it required the use of a valve to prevent a siphon from forming within the vessel. A filter was added to the waste outlet to prevent solid material from jamming the pump. The outlet pump was required to be temperature resistant so that it could handle the waste of the boiling procedure. The sequence of events in this process was: pump water in, heat water to boiling temperature, add sodium bicarbonate, add silk cocoons, boil for input time at input temperature, and pump waste out. A schematic of the degumming procedure can be seen in figure 6.

Rinsing

Rinsing is a similar process to degumming, however it does not involve heat, and it is run in cycles. Each time water flows in, it washes away extra sericin from the fibroin bundle. In the human controlled protocol, the fibroin bundle is usually wrung to increase the amount of sericin that leaves the fiber bundle, however the same effect could be accomplished through rinsing the fiber bundle several more times.

Drying

The drying cycle usually occurs in a fume hood at ambient temperature, however heat could increase the rate of drying. This would significantly expedite the process. While it is not known exactly what heat should be used in this process, a temperature of 50-60°C would be adequate for drying the fibers because this is the temperature that conventional dryers use to dry clothes. Temperature regulation through the PWM of the hotplate is crucial in this step to avoid burning the fiber bundle.

Dissolving

The introduction of a protective vessel allows for the addition of LiBr solution to the boiling vessel. Due to its corrosive nature, LiBr should not come in contact with the surface of the metal boiling vessel, nor with any other metal components in the system. This part of the process is very similar to the Drying process, thus allowing many of the same control principles to be recycled from the Drying step. Human intervention in this step of the process will be necessary in this prototype to manipulate the fiber bundle and also measure the proper quantity of chemicals used. Future prototypes will include a scale to determine the correct amount of solvent to be used.
Dialyzing

A researcher will load the fibroin-LiBr solution into dialysis material (tubing, cassette, or machine) and place it in the boiling vessel. The machine will then conduct a modified rinsing cycle in which deionized water is continually flowed over the surface of the membrane. This continuous flow will severely expedite the dialysis step. Once this step is complete, the aqueous fibroin solution must be centrifuged by a researcher to yield the final solution.

![Dialyzing Diagram](image)

**Figure 6:** A control flow diagram of the automated silk processing procedure featuring abstract inputs and outputs of each constituent system.

Implementation

Materials

The system was designed to be scalable and inexpensive. This initial prototype utilized consumer materials sourced from various scientific equipment vendors, however the materials were purchased in sizes that most closely matched the current materials used in the silk processing procedure. This scale consideration factored into the choice of heat source, boiling vessel, inlet pump, and outlet pump. To control the system, a microcontroller that had adequate processor speed and various output pins was researched and purchased. Future iterations of this prototype will feature a microcontroller that is capable of operating without an attached computer. A physical relay system was used to control the various consumer components. This four relay system allowed for the computer control of 120V AC power to be delivered to the different components of the system. These components were all necessary in the construction of the system, however the total cost of the system remained below $700.
Table 3: Bill of materials

<table>
<thead>
<tr>
<th>Component</th>
<th>Abstract Function</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food-Grade 300 Series Stainless Steel Round Batch Can 1.1 Gallon Capacity</td>
<td>Boiling Vessel</td>
<td>$30.80</td>
</tr>
<tr>
<td>General Purpose Hot Plate One 5-1/2” Burner, 1100 Watts, 120 VAC</td>
<td>Heat Source</td>
<td>$108.33</td>
</tr>
<tr>
<td>Extended-Life Plastic Pump for Water/Coolants 1/30 hp, 120V AC, 1/2 NPT Female Intake Connection</td>
<td>DI Water Pump In</td>
<td>$164.76</td>
</tr>
<tr>
<td>bayite BYT-7A014A DC 12V Solar Hot Water Circulation Pump with DC Power Supply Adapter Low Noise 3M Head 8LPM 2.1GPM</td>
<td>Waste Pump Out</td>
<td>$23.99</td>
</tr>
<tr>
<td>Straight-Blade Receptacle Economy, Three-Slot, Female, Grounded, NEMA 5-15</td>
<td>Power Distribution</td>
<td>$2.13*4</td>
</tr>
<tr>
<td>High-Purity Silicone Rubber Soft Tubing for Metering Pumps, 3/8” ID, 0.627” OD (5 feet)</td>
<td>Water and Waste Transportation</td>
<td>$6.45*2</td>
</tr>
<tr>
<td>Arduino Uno Microcontroller</td>
<td>Computer Control</td>
<td>$18.82</td>
</tr>
<tr>
<td>4 Mode Relay</td>
<td>Power Control of Components</td>
<td>$8.49</td>
</tr>
<tr>
<td>Miscellaneous Wiring</td>
<td>Power Connection</td>
<td>$24.29</td>
</tr>
<tr>
<td>20.00” X 16.00” X 6.00” CARBON STEEL ENCLOSURE</td>
<td>Electrical and Pump Housing</td>
<td>$247.25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>$648.15</td>
</tr>
</tbody>
</table>

Other Components Designed

Some new components were also designed as a part of this project to help expedite the silk processing procedure and ensure safe use of the machine. Some of these components were implemented in the system however some will require redesign before they can be fully implemented.

Dialysis Cassette Rack

A dialysis cassette rack was designed as a part of this project. The aim of this design was to ensure proper water flow over the dialysis cassette membranes and to expedite the dialysis process through constant flow dialysis. This rack was designed using CAD software to hold two dialysis cassettes. The 3D printed part consisted of a main water conduit (with the ability to attach to the water in pump), and two brackets in which dialysis cassettes could fit. The main water conduit at the top of the rack was able to interface with the water inlet of the boiling vessel. Deionized water would flow into the rack and through holes that faced both sides of the dialysis membrane on the cassette. This would more efficiently dialyze the solution within the cassettes, thus leading to a higher rate of dialysis. A more modular redesign of this system will be implemented in future prototypes of the system.
Boiling Vessel Braces

To prevent the boiling vessel from tipping, braces were designed and implemented in the prototype silk processor. This was partly developed to ensure safe use of the machine, and partly developed to ensure that components of the system did not fall off of the surface of the machine. The braces were made from aluminum and laser-cut acrylic. The braces did not contact the sides of the boiling vessel so that the heat would not affect the structure of the acrylic. The system was mounted to the surface of the hot-plate using bolts.

Construction

Construction was separated into mechanical, electrical, and programming phases. A large part of the construction involved boring holes in both the boiling vessel and in the project box. These holes were able to accommodate a $\frac{1}{2}$ inch NPT threaded pipe. Boring was completed using metal drill bits, a drill press, and a rotary cutting tool. Once boring was complete, wires and tubes were fed through the bored holes, and the proper hardware was attached to the components of the system. An electrical panel was created on the laser cutter to house the relay, Arduino, and outlets that controlled the various systems.

Wiring was completed by connecting the electrical outlets to the 120V AC power through a main shutoff switch. One pole of each outlet passed through the relay controller’s load cells to modulate the voltage seen by each outlet. Wiring for the computer controlled components of the system followed an online guide. The temperature probe was placed outside of the box, and its wires attached directly to the microcontroller.

After the wiring was organized, the components of the system were framed in the system. Water systems were separated from electrical systems, and all wires and tubes were pulled through the appropriate ports in each system. The deionized water intake was mounted to the deionized water holding tank, and the waste hose was placed in the sink adjacent to the system. After these systems were all in place, the system was programmed.

Programming of the system utilized specific inputs, outputs, and member functions. Simple modular sub-functions for the system such as “fill boiling vessel” were recycled in multiple steps of the process. Each step in the process was coded independently, and then each step was run in series. This produced the full activity of the silk processing system. PI control was implemented in the heating function to establish temperatures in the boiling vessel that were below 100°C. Control of the heating element was accomplished using PWM principles.
Results

Final Prototype v1.0

Figure 7: The Cocoon prototype version 1.0. (Right) inside wiring and tubing. (Left) the footprint of the machine without its external water tank is 45cm X 35cm.

Temperature Control

Figure 8: Signal averaging was implemented to reduce the noise seen by the temperature control loop. (Left) no averaging produces sharp spikes that would interfere with the PID control loop. (Middle) signal averaging over .01s interval produces a smoother temperature profile, and (right) signal averaging over .1s produces a profile that produces an appropriately responsive behavior in the heat source.
Endurance Testing

Figure 9: Oxidation of bottom of stainless steel vessel (left) after DI water immersion for 10 days. Sericin buildup on vessel walls after boiling 10g of raw silk at 100°C for 30 minutes (middle) and after 1 rinse for 20 minutes (right).

Design of Dialysis Cassettes

Figure 10: A dialysis cassette rack designed in Fusion360 (left) to hold multiple dialysis cassettes. This rack was 3D printed and designed to flow water over the membranes of the dialysis cassette to decrease dialysis time. Unfortunately this design could not accommodate the thickness of 12mL cassettes (right), thus it must be redesigned. Future designs of this cassette could include a modular feature in which racks can be connected.
Discussion

Results

The preliminary proof of function tests of the silk system were a success. The process was able to take raw silk cocoons, and with slight researcher oversight, the machine was able to complete each step of the silk processing protocol besides dissolution. Late development of the prototype reduced the amount of tests that were possible due to schedule constraints. This design serves as a functional initial prototype which can be improved upon substantially. The tests that were performed on the system indicated that the controls of the system and material choices of the system are adequate for initial prototypes, but may require redesigns later. These results serve as a concrete first step towards creating a commercial grade product that is able to solve the challenges outlined in the clinical needs of this project. This prototype was able to mostly accomplish the engineering challenges outlined in this project, however its design must change before it becomes a non-experimental design.

The temperature filtering results obtained in component experiments show how signal averaging can reduce the amount of noise in a measurement as seen in figure 8. By averaging the positive and negative deviations from each measurement, a smooth and accurate signal was able to be extracted from the raw data. This was an important part of the control algorithms that were developed for this system because sharp temperature changes could induce positive feedback loops in the control of the heating system. The smooth signal acquired by the .1 second interval average was a good choice to use in the feedback system, thus it should be implemented in future prototypes.

Durability tests of the vessel indicate that in future prototypes, it may be advantageous to the performance of the system to change what materials contribute to the construction. While this stainless steel vessel was inexpensive, corrosion could adulterate the fibroin product that is produced, and damage other components of the system. While corrosion is an important effect to consider in the construction of this machine, the surface oxidation that accumulated on the boiling vessel was superficial and easily cleaned off of the vat’s floor figure 9. To obviate corrosive effects, the vessel could be coated in a non-stick material such as PTFE. This could also additionally protect the boiling vessel from corrosion due to LiBr contact. Sericin buildup on the vat wall was also consistent with buildup on glass boiling vessels used in conventional silk processing.

The dialysis cassette rack unfortunately was not designed correctly to be used in this project. Tolerances within the 3D printed part were too high for the rack to accommodate a 12ml dialysis cassette as seen in figure 10. This meant that the rack could not be used in the initial prototype testing for this system. Redesigning this rack would allow for efficacy testing of the dialysis system, however continuous water flow through and dialysis floats were able to be used in the initial prototype testing. Dialysis rates of this system are unconfirmed, but initial estimates indicate that dialysis timing could be cut as much as three times with the continuous flow system implemented. Upon examining the silk processing protocol, researchers realized that water changes were centered on convenient times for researchers. Water changes often happened in the morning or at night in a non-continuous fashion. By increasing the flow over dialysis membranes
in this system, the dialysis step would be effectively expedited, thus increasing the rate of the whole protocol.

**Difficulties**

There were a wide range of difficulties in this project. Challenges ranged from design issues to access to workspaces. The design and optimization of systems before their construction delayed construction of the system and sacrificed time scheduled for testing of the systems. Part of this optimization was centered on increasing the time efficiency of components, however more time efficient components contained their own on-board computer systems which were not easily relay controllable. Over-optimizing design before initial construction served as a bottleneck for development of this project. Instead optimization should have been considered in the redesign of the system after initial construction of the prototype.

**Future Work**

**Weight Scale System**

An integrated weighing system would increase the automation of this system. Much of the silk processing protocol deals with specific masses and quantities of different reagents, so to more fully automate this process, it is important to keep the precision of the protocol through weight measurement. Challenges presented by this design are separation of systems. It is difficult to weigh the mass of something that is also going to be at a high temperature. A possible solution to this problem is utilizing related controls to dispense the proper amount of reagent to the system. This could take the shape of a hopper/screw assembly, or a hopper/ram assembly. Alternatively the entire hot plate assembly could be weighed with the added reagents. This would be difficult because the addition of reagents would only change the mass on the scale by a small percentage.

**Design Report Integration**

With the ultimate aim of making this system into a “smart” device, computer diagnostics should be collected and reported to an administrator of the lab so that analysis can be done on different aspects of the products of the silk machine. These diagnostics could prove valuable to researchers and to lab coordinators because they could better focus their time and energy into formulations of fibroin solution that have worked in the past, and stop wasting time on formulations that do not work.

**Redesign of Components**

**Dialysis Cassette Rack**

The dialysis cassette rack should be redesigned to accommodate the thickness of 12ml dialysis cassettes. Also creating a rack that is more modular would be a good addition to this design. Future designs of this rack should only accommodate one cassette, but they should be
able to connect to other cassette racks so that water can flow through the system of racks, thus flowing over all cassette racks in the system.

Bracing system

While the bracing system accomplished its goal of maintaining safe operation of the system, future designs of this component could include a built in agitator. This agitator could be driven by a planetary gearing system that could effectively mix the fibroin bundle while it is degumming and rinsing. This system could be mounted above the boiling vessel, and could be easily placed and removed. The bracing system will also need to be better attached to the structure of the electrical panel before this agitation system can be implemented.

Fill and Drain Feedback

The current prototype does not feature dynamic filling and draining protocols. Filling and draining are “hard” coded into the system utilizing the fill rate of each pump and the capacity of the boiling vessel. More adaptive and modular code would implement feedback mechanisms such as limit switches, level indicators, or float valves to control the level of the water in the tank and the pumping rate of the tank. This feedback is simple enough to be used in non-computer controlled plumbing products such as toilets (see figure 11).

Figure 11: A toilet float valve is an inexpensive and reliable solution to dynamically fill a reservoir

Temperature Probe

The temperature probe does not have adequate protection from boiling temperatures and corrosive chemicals. A heat tolerant plastic sheath should be used to encase the temperature probe so that it may be used in a more direct way with the system.
**User Interface**

The current user interface of this prototype is difficult to use. Its control flow diagram does not give users freedom within the system, and it is very linear. Redesigning this aspect of the system would allow for new users to intuitively learn how to use the system quickly. Also creating a computer independent control interface would allow for more flexible use of the system. Arduino hardware has the built in capability to be very modular with independent displays and control hardware. Future iterations of this system could include more powerful computers that are able to interface with full screen monitors capable of producing high quality graphics for the user to understand the prototype’s function better.

**Conclusion**

The success of this project is one of the first concrete steps towards creating a fully automated silk processing machine. The ultimate goals of this machine are to usher in a new era of silk research that could have substantial consequences in the biomedical engineering, mechanical engineering, environmental engineering, and chemical engineering disciplines. The inception and completion of this project represents the high level of interdisciplinary study within the systems engineering discipline because this project involved principles from every type of Tufts engineering major.

This project accomplished the specific aims set out in its initial proposal. The silk processing protocol was broken into its discrete pieces. Each of these pieces were abstracted based on their inputs and outputs. These inputs and outputs became the cornerstones of the design of the first prototype of the silk processing system. Each system was designed independently, and then design objectives were applied to increase the space and time efficiency of the system. After each system was fully designed, the constituent systems were put together in series to effectively create the entire linear system. Once this plan was carried out on paper, the designed systems were constructed using inexpensive materials. Each system was integrated into a unified prototype which was able to complete the silk processing procedure with a small amount human oversight. The testing of the system served as a proof of function for the system, however further calibration and testing of the prototype must occur before it is able to address the clinical needs of this machine.

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References


