BME 4908 SENIOR DESIGN PROJECT
EXECUTIVE SUMMARY

Manufacturing Process of a SCOBY Bio-Thread

BIOMEDICAL ENGINEERING EXPO

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Project Team 7

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1. Recognition of Need/Opportunity

Among the population about 6.5 million people experience Chronic Wounds. A chronic wound is one that does not progress through the healing process properly. These wounds have usually been open for more than a month, have lengthy healing times and lead to serious infections. Currently, bacterial cellulose seems to be a potential material that can aid the healing process. After the completion of this project, there will be a biocompatible, biodegradable solution primarily composed of Cellulose that will aid in the wound healing process of Chronic Wounds. This solution will contain the ideal wound dressing conditions of applicability, flexibility, strength, biocompatibility and stability. This thread will also be one of the first successful manufactured processes of bacterial cellulose

Cellulose in particular Bacterial Cellulose is considered a prominent material that can help revolutionize the wound care industry. It has previously demonstrated chemical and mechanical properties that can be useful within a wound dressing and overall aid the healing process. Those properties include high tensile strength, flexibility, water absorption, gas and liquid permeability and biocompatibility. These properties have allowed for cellulose to cause epithelial regeneration when applied to a wound. The nanostructure of cellulose is a 3D network structure that is already similar to the extracellular matrix allowing for high levels of biocompatibility. Bacterial Cellulose enhances exudate absorption. Removing the necrotic tissue will stop the wound from spreading and eventually head more towards healing. These properties give bacterial cellulose a high potential to be implemented into the wound care industry.

Essentially our project will be creating a four step process that will develop a bioactive thread composed of cellulose. A thread like this will be used as a wound dressing and is capable of decreasing the healing time for chronic wounds.

2. Problem Formulation

a. Project Objectives:

Our objective was to develop a cellulose-based SCOBY (Symbiotic Culture of Yeast and Bacteria) Bioactive-Thread towards wound dressing applications. To produce the thread a process and a mold will need to be designed. Within an allotted time of four months the group designed a four-step process along with the construction of a silicone mold basin for the growth of the bio-thread. The process and mold together allowed for the completion of the market requirements and design inputs. The process design is necessary since the SCOBY is produced through the fermentation of Kombucha tea. The following variables: material dimensions, fermentation time, drying time and temperature were all optimized to allow for the proper production of the SCOBY. The designed process can be applied to mass production and the physical molds can be altered to fit the desired dimensions of thread. This was accomplished within a budget of $500 USD by April of 2022.

b. Design Specifications:

The following design requirements were taken into consideration will designing our thread and process.

Requirements:

1. The Bio-Thread will contain bioactive compounds
2. An uninterrupted Bio-Thread with dimensions that will facilitate large scale manufacturing

3. The Bio-Thread must be string and flexible for future use in wound dressings
4. The SCOBY must not be acidic

Design Inputs:

1. The final product will have cellulose.
2. The Bio-thread must present repeatability with a length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm to allow for future large scale production
3. The Bio-thread must endure a tensile stress of 32 MPa and fit around a spool with a diameter of 1.0 cm
4. The pH of the SCOBY must not be below 4.7

c. Constraints and other considerations

Constraints occurred within manufacturing of the thread and the environment that the thread was produced in. The length of the thread is determined by the dimensions of the silicone tube mold that it is grown in. A larger thread will require a mold and proper storage. The original workspace was unable to fit the 6.25 foot tubes. Since this design proved to be the most feasible arrangements were made to meet the space requirement.

Since SCOBY is an organic material every from of equipment that comes in contact with it must be properly cleaned between batches to prevent contamination and the presence of mold. All equipment was cleaned with an antibacterial soap and water and then rinsed with isopropyl alcohol to support a clean environment.

The Kombucha tea is originally fermented within room temperature. Research determined that the temperature range between 22°C-28°C was considered room temperature. The facility's thermostat was set to 26°C. A thermometer was placed next to the tube molds to monitor the temperature. If the SCOBY were to increase above 28°C there will be a greater risk for mold developing.

3. Solution Formulation

There were multiple opportunities to present creative solutions to the problem. The first design concept involved placing the silicone tubes in a snake like formation the base of a plastic bin. The second design consisted of wrapping the silicone tubes around 5-gallon buckets. The finalized concept involved the tubes being secured to a wooden base.

Pros and cons of each design were thought of to make the final decision. For example, since the SCOBY takes the shape of its container, different geometric shapes were utilized in designing the structure to grow the thread. In Design Concept 1, the tubes are arranged in a snake formation, to allow for a smaller space of occupancy and controlled environment in a bin. For Design Concept 2, the tubes are wrapped around a large cylinder, modeled by a bucket. This also allows it to take up a small amount of space, but does not constrain the SCOBY to grow in sharp angles, as in Design Concept 1. Lastly, Design Concept 3 focused less on minimizing its size and more on simplicity of growth. Later in the process the sponsor provided more space to be utilized by the team, which lessened the constraint on the design. This concept allowed the SCOBY to grow uniformly in an extended tube.

4. Engineering Analysis and Decision-Making

SolidWorks was utilized to depict the design for the three proposed design concepts. By creating these three designs in SolidWorks it allowed for the team to see each design and manipulate it slightly to determine its effectiveness.

A heat transfer simulation was conducted with COMSOL to determine that the Kombucha tea solution did not vary in temperature. The following equation used to perform the analysis involves the net inward heat flux, as shown below:

$$-\ n\,x\,q = \ \varepsilon\sigma(T^4_{amb} - T^4)$$

In this equation, ε represents the surface emissivity, which is selected from the material specified. This value ranges from 0 to 1, where 0 means that no radiation is emitted from the surface and 1 means that the surface is a perfect emitter, or a blackbody. The σ variable represents the predefined Stefan-Boltzmann constant, which is $5.6704*10^{-8}$ W/m^2*K. Lastly, T_{amb} is the ambient temperature in Kelvin, which is manually inputted by the user.

This simulation was performed on the project to determine how long it would take the tea within the tubes to return to room temperature. The temperature of the tea (T) was inputted as 30 °C, and the ambient temperature (T_{amb}) was 25.5 °C. The results demonstrated that it would take approximately 8 minutes for the temperature to return to the modified room temperature. The results can be shown within Figure 6.4 and 6.5 of the written report.

To prepare the NaOH for the purification stage, calculations were determined to achieve the proper concentration of 1 M NaOH solution from NaOH pellets. The predetermined amount of NaOH was added to the water to obtain the solution.

5. Detailed Design

The final design consisted of a 6ft by 1.5 ft 1in thick plywood base with 3 silicone tubes secured with clamps. The silicone tubes were 6.25 ft long with an inner diameter of 0.5 in. The three tubes were placed 4.5in apart along the width of the base. Along the length of the silicone tubes clamps were secured with screws every 12in. An image of the completed structural design can be referenced within Figure 7.61 and 7.62 of the written report.

The protocol for producing the thread involved a 4 phase system divided into brewing, fermentation, purification and spooling. The following materials were used to produce the SCOBY, black tea, spring water, white sugar and previously brewed unpasteurized unflavored Kombucha. These materials within the brewing stage are mixed together to create the solution. A 100ml of this solution was poured within the tubes to ferment for the respective time. After the threads have fermented, remaining waste solution was drained. Within the purification stage the threads were treated with around 20ml of 1 M NaOH and then rinsed with distilled water to remove any impurities. These threads were then left to dry. After the thread was dried it was twisted on to itself with the usage of a Dremel with a hook extension. Once the thread has been twisted it can then be wrapped around a spool and is read for use. Figure 7.7 demonstrates all 4 phases and steps.

Each tube had its own fermentation variables. The first tube will be fermented for 2 weeks within a closed system, the middle tube fermented for 3 weeks within a closed system and the last tube fermented for 2 weeks within an open system. These different variables were used to determine the most viable thread.

6. Testing

The following forms of verification tests were performed; a test for bioactive compounds, test for thread dimensions, test for strength and flexibility and a test for pH.

To test for bioactive compounds, more specifically cellulose, the SCOBY Bio-thread was placed at the base of an open container and using a measuring cylinder, 50 mL of Schulze's reagent was measured. Using a syringe, Schultze's reagent was applied along the length of the Bio-thread. All color changes present and any other observations which may be present were recorded and if the Bio-thread changed to a purple color, cellulose was present, and the verification test was passed.

To test for an uninterrupted Bio-thread with a continuous length of 152.4 cm and a diameter of less than 2 +/- 0.2 mm to allow for future large-scale production, the SCOBY Bio-thread was first placed along a long, flat surface and using a tape measure, the total length of the Bio-thread was measured. Keeping the Bio-thread in the same position, the width was measured two dimensionally across every 2.5 centimeters of the Bio-thread using calipers and all results were tabulated. The standard deviation of the width across every 2.5 centimeters of the Bio-thread was measured and compared to the standard deviation across the entire length. The standard error of the mean was determined by dividing the standard deviation by the square root of the sample size. Once the Bio-thread had a continuous, uninterrupted length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm, the verification test was passed.

To test for strength and flexibility, a 40 cm specimen of the SCOBY Bio-thread was spooled on a spool with 1.0 cm diameter. Next, an 11.5 cm specimen of the SCOBY Bio-thread was loaded into a tensile stress test machine. The bio-thread was pulled at a constant rate of extension specified in the standard and the test was stopped once the bio-thread fractured. Once the biothread endures a tensile stress of 32 MPa and is successfully spooled on a spool with 1.0 cm diameter without fracturing, the verification test is passed.

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7. Evaluation of Verification Testing

To evaluate the final results, test protocols were designed to confirm all design inputs. The final SCOBY Bio-thread was required to contain cellulose, have a continuous length of 152.4 +/- 15.42 cm with a diameter of 2 +/- 0.2 mm, endure a tensile stress of 32 MPa, be spooled on a 1.0 cm diameter spool, and have a pH of greater than 4.7. The PASCO Capstone hardware and software were used to perform the tensile stress verification test, an electronic caliper was used to measure the width of the Bio-thread along its entire length, a solid pH meter was used to determine the pH of the Bio-thread along its entire length and Microsoft Excel was used to determine any averages, standard deviations and standard errors of the means.

8. Relevance to the BME Curriculum.

Specific courses within the curriculum that pertained to the completion of our project included BME 4100 Biomaterials, BME 4050L & 4052 BME Lab 1&2, BME 3632 BME Transport, BME 2740 BME Modeling & Simulations and BME 4800C Design of Biomedical Systems. The lab courses and Biomaterials allowed for us to determine what mechanical characteristics were needed for the success of our thread as well as how to properly test for those characteristics. BME Transport and BME Modeling & Simulations allowed for the possibility to conduct the heat transfer simulation to determine the capability of our fermentation system. Lastly, Design of Biomedical Systems gave the team the knowledge to determine a design from a list of requirements as well as organization of team dynamics and project organization.

BSBME Program Learning Outcomes

1. Ability to apply knowledge of mathematics (including differential equations and statistics), physical and life sciences, and engineering to carry out analysis and design to solve problems at the interface of engineering and biology;
2. Ability to design and conduct experiments, as well as to measure, analyze and interpret data from living systems;
3. Ability to design a system, component, or process to meet desired needs, including systems that involve the interaction between living and non-living materials, within realistic constraints such as economic, environmental, social, political, ethical, health and safety, manufacturability, and sustainability;
4. Ability to identify, formulate, and adapt engineering solutions to unmet biological needs,
5. Ability to use the techniques, skills, and modern engineering tools necessary for engineering practice, including the ability to model and analyze biological systems as engineering systems;
6. Ability to function on multi-disciplinary teams;
7. Ability to communicate effectively;

Courses Outcomes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
EGN 1100 Intro to Engineering						**X**	**X**	**X**
BME 2740 BME Modeling & Simulation	**X**				**X**		**X**	
EEL 3003 Electrical Engineering I	**X**	**X**	**X**		**X**	**X**	**X**	**X**
EGM 3503 Applied Mechanics	**X**			**X**	**X**			
BME 3032 BME Transport	**X**			**X**	**X**	**X**	**X**	
BME 3700 EABS I	**X**			**X**	**X**		**X**	
BME 3701 EABS II	**X**			**X**	**X**		**X**	
BME 3710 BME Data Evaluation Principles	**X**	**X**			**X**			**X**
BME 4011 Clinical Rotations		**X**						**X**
BME 4050L Lab I	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
BME 4051L Lab II	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
BME 4090 Design Project Organization	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
BME 4100 Biomaterials Science	**X**	**X**	**X**	**X**	**X**		**X**	**X**
ELR 4202C Medical Instrumentation Design			**X**	**X**	**X**			
BME 4332 Cell and Tissue Engineering	**X**	**X**	**X**	**X**	**X**		**X**	**X**
BME 4908 Senior Design Project	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
BME 4800 Design Biomedical Systems Dev	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

8. Awareness of the characteristics of responsible professional engineering practice, including ethical conduct, consideration of the impact of engineering solutions on society in a global and contemporary context, and the value of life-long learning.

Relationship between the BSBME Courses and Program Learning Outcomes