MIT | 2019 | Art and Design Undergraduate Thesis

Creating Form

Biofabricated Cellulose

Priya Pillai
12-12-2018

Contents

Introduction

Biofabrication is a growing method of producing functional objects and structures that takes advantage of biological processes. This is often done by using or manipulating bacteria to produce a biochemical product. It has the potential to redefine manufacture- while it does take time, it is highly scalable, as nearly any quantity of bacteria can be grown once the original template is made. The result is often biodegradable and biocompatible, which can allow for more ecofriendly production. However, the tools to biofabricate are still in their infancy, and work needs to be done to allow for easy and mass-producible biofabrication.

In 2017, Katia Zolotovsky wrote her Ph. D. thesis in Design and Computation at MIT about inserting cellulose producing genes into the bacterium *G. xylinus* in order to grow controlled biomaterials. Cellulose is a polymer of glucose produced by plants that provides structure for the plants. By hacking bacteria to produce cellulose with conditional logic, a multipurpose and strong biomaterial can be created. Zolotovsky was able to successfully transform the bacteria with genes for controlling cellulose production and create 3D-printed compartments in which the bacteria could grow with differing media conditions. Each media condition gave the cellulose produced different properties. For example, Hesterin-Swann growth media produced standard cellulose, whereas Hesterin-Swann media with sucrose produced denser cellulose. Zolotovsky envisions expanding the current genetic circuit to account for more complex logic, such as incorporating green fluorescent protein into the cellulose, only if lactose is present in the growth media.

This research forms the basis of a system for producing controlled biomaterials and a new system of manufacturing. Biomaterials are environmentally sustainable, nontoxic,

and have a large range of physical properties; by manipulating them genetically and structurally, biomaterials have the potential to replace nonrenewable materials, like plastics. The range of possibilities involved in genetic manipulation is also much higher, and structure can be controlled and organized at the microscopic level.

The properties of the cellulose are not only characterized by the genetic circuit and the media, but also the physical structure in which the bacteria grows. Zolotovsky currently grows the bacteria in hexagonal polydimethylsiloxane (PDMS) compartments. PDMS, a type of silicone, is oxygen permeable, and the cellulose will accumulate wherever there is exposure to oxygen. This means that a hollow cellulose hexagonal prism is created, following the walls of the compartment and the media air boundary. This shape was chosen to allow for modularity and as a proof of concept.

The research proposed will work with Zolotovsky to consider how the shape of the compartments can affect the properties of the biomaterial, and will explore potential functions for the material produced by the shape. The starting shape this research will consider is a star shape module. This would change the surface area to volume ratio drastically, a property important in the biological context to determine the rate of transfer between membranes. It would also reduce the amount of unsupported cellulose, reducing the need for other methods to maintain structure. A final function this research will consider is forming a complex shape based on an input shape via aggregating compartments.

To generate and test the properties of each shape modification considered, scripts in Rhino using Grasshopper and Python will be developed to test different extents of each shape modification. For example, the script could edit the extent of curvature in each side of the previously mentioned star. Ideally, this research will produce a set of parameters that

can be modulated to form defined, variable properties in the cellulose. This research could allow manufacturers of biomaterials to use a set of standards to produce materials adaptable for a range of situations, in order to provide a basis for future researchers to design more complex properties in the cellulose.

In summary, this thesis studies design parameters for 3D printed molds for growing cellulose biomaterials to discover how cellulose's properties change with varying physical growth conditions in order to understand how manufacturers and researchers may be able to add more control to biological fabrication.

Literature Review

This thesis will focus on creating physical constraints to control the accumulation of cellulose generated by the bacteria *Glucoacetobacter xylinus*. It will combine different fields of research, including the use of cellulose as a material, the biological fabrication of cellulose and other biomaterials, and the geometric control of the three dimensional shape of the structure. This section will discuss work previously done in each of these areas.

Cellulose as a Material

Cellulose ($(C_6H_{10}O_5)_n$) is a polymer of glucose bonded with glycosidic bonds between the 1 and 4 carbons. This allows the polymer to form long, unbranched polymer chains. It has tight hydrogen bonding between each chain, which allows it to form into fibers called microfibrils and gives it strong structural properties. It is used extensively in nature-in wood, cotton, and every cell wall in plants. Cellulose is the most abundant carbohydrate polymer in the world (Esa).

Because of its natural availability, cellulose has been manipulated by scientists chemically to enhance its properties. Early experiments with plant cellulose produced some of the first artificial fabrics, like rayon, viscose, and acetate. (Hon) In 1983, Herrick et al. and Turbak et al. created a refined version of plant cellulose called microfibrillated cellulose (MFC) from wood. “Microfibrillated” refers to the clustering of microfibrils into fibers with larger diameters. Through acid hydrolysis, MFC can be split into “cellulose whiskers” that can then be crystallized into microcrystalline cellulose (MCC). MCC is used in pharmaceuticals to bind drugs in pills, but does not provide structural benefit[1]. (Siró and Plackett).

However, not all cellulose is created equal. Historically, applications of cellulose have relied on the purification of cellulose from plants. In 1988, A. J. Brown discovered a pellicle, a layer of material that bacteria grow on the top of their media, formed by *G. xylinus*[2] made of cellulose. This bacterial cellulose was chemically equivalent to plant cellulose. However, it is made up of bundled, longer, and more organized nanofibrils and is more crystalline. This allows it to be stronger and better able to retain water than plant cellulose (Siró and Plackett; Esa). This thesis will focus on using this version of the material, due to its mechanical properties and its potential to be manipulated with synthetic biology.

In the future, this work could be adapted to use even stronger versions of cellulose. Recently, cellulose nanofibers (another name for MFC) have been manipulated through the

[1] More recently, the acid hydrolysis process has been refined by using sulfuric acid to produced nanocrystalline cellulose (NCC). However, this is not used as frequently as MCC due to the high concentration of sulfuric acid necessary, as this leads to sulfate incorporation into the MCC making it less eco-friendly (Julkapli and Bagheri).

[2] *G. xylinus* is the modern reclassification of this bacteria-some (often older) literature refers to it as *Acetobacter xylinum* (Svensson).

use of flow-assisted assembly into highly organized macrofibers that are stronger than all known biomaterials, metals, alloys, and glass fibers (Mittal). If this methodology could be incorporated into the process of biologically growing cellulose into its final shape, it could, in theory, add additional 3-D structural strength to the material.

While cellulose's material characteristics are impressive, what may be its most useful trait is its biocompatibility. As a polymer of glucose and a dietary fiber, the mammalian body recognizes it, has no immune reaction to it, and can eventually biodegrade it. Cellulose does not serve the same structural purpose in animals that it does in plants, but in theory it could serve as cellular scaffolding temporarily. Therefore, cellulose has been proposed to be used in tissue engineering as a structural guide for cells (Svensson). This thesis work could be applied to this use, by giving researchers greater control over the physical structure of the scaffolding for engineered tissue.

Biofabrication of Cellulose

Cellulose is known to be a useful material, and by using biofabrication methods, it is possible to take advantage of a larger number of its properties. Biofabrication is defined as any method that uses bioactive sources to create biologically functional and organized materials. In tissue engineering, most biofabrication comes from an adaption of the techniques 3-D printing to biology (Moroni). However, these methods are limited by the necessity to manually create structures that are automatically generated with the body.

In contrast, Suzanne Lee's biocouture project does not create its structure through 3-D printing, but rather through sewing. Lee created "kombucha leather" from the cellulose pellicle called a scoby formed by bacteria in kombucha. The "leather" is then dried out and can be dyed for use as a fabric. Lee has sewn cellulose jackets, vests, dresses, and shoes

Figure 1: Biocouture by Suzanne Lee **(Deniz and Keskin Gundogdu)**

Figure 2: Malai, by Gombosova **(Tydlitátová, Lišková and Jůn)**

out of the material, illustrating its capability to become sustainable clothing. Unfortunately, the water absorption and temperature dependent strength of cellulose make it less functional as clothing, and the length of time it takes to produce make it unlikely that it will be adopted by the fast fashion industry. (Grushkin)

Zuzana Gombosova has solved some of these issues with her cellulose based fabric Malai. Malai is made from the pellicle of coconut water via *G. xylinus* bacteria. It is then treated with other plant fibers, oils, and starches to make it water resistant but still sustainable. Malai is still limited to a two dimensional form and by the number of dyes it can take on. (Tydlitátová, Lišková and Jůn). Modern Meadow's ZOA bioleather uses similar biofabrication methods on another material-collagen-which gives it more water resistance (Our Technology). However, all of these methods are limited to two dimensions still. If the chemical modifications applied to these two dimensional fabrics were applied to the 3D cellulose produced through the method proposed, objects with far greater durability would be able to be made

In Finland, the Designing Cellulose for the Future conference brought together designers and scientists interested in solving these problems. Researchers at the conference presented work on electrospinning cellulose thread to make yarns, foam forming to make three dimensional structure, and laminated forms of cellulose that require no glue to make. They have even created functional objects, like a load bearing bicycle and

a stool, via knit nanocellulose fibers (Kataja and Kääriäinen). While foam forming is a method of creating three dimensional structure out of cellulose, it is inherently weaker, as it contains air bubbles throughout the material (Rühs). Alternatively, Luiz Greca's team discovered a method of manipulating normal 3-D printing molds for cellulose to be superhydrophobic, which creates an oxygen interface and allows a pellicle to accumulate along the sides of the container (Greca).

Figure 3: Setup for Zolotovsky's growth process **(Zolotovsky)**

Katia Zolotovsky uses an easier method of providing oxygen to the bottom of the material-using the oxygen permeable material silicone as the scaffolding for the bacteria. Zolotovsky also takes the biofabrication method a step further by adding in genetic control of the bacteria. By adding genetic circuitry to the cells, Zolotovsky was able to conditionally grow cellulose in the presence of a signal from the media. The added biological control suggests a new method of controlling three dimensional shape intentionally (Zolotovsky). Combining this potential of biological control with physical controls could produce unique interplays that this thesis hopes to discover.

Control of 3D Shape

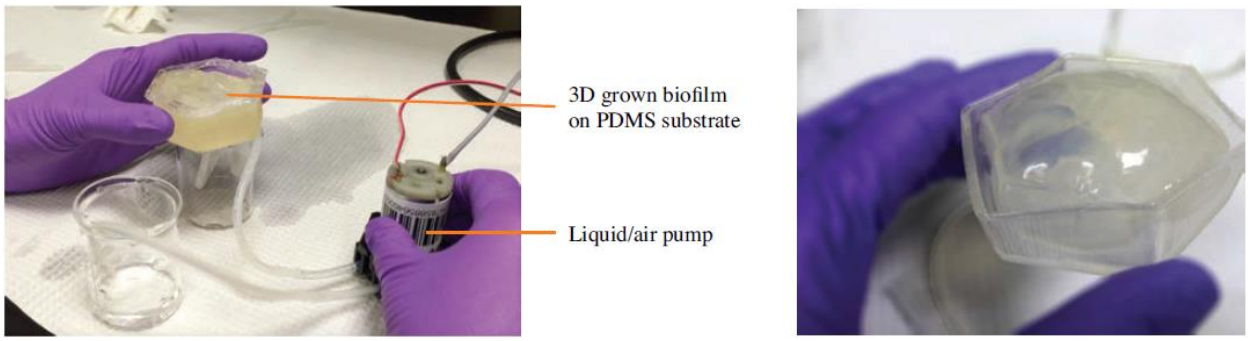

Figure 4: Zolotovsky's current compartments for growing cellulose biomaterial **(Zolotovsky)**

Zolotovky's structure of the current compartment is a hexagonal prism. It was chosen for its commonness in natural forms such as honeycombs and its ability to be tessellated to form larger forms. However, it lacks internal support for the top layer. While the cellulose is accumulating, this is not an issue; however, when the liquid media needs to be drained out, the top surface no longer has support. Zolotovsky solves this by pumping air into the hexagonal cellulose as the liquid is pumped out. This prevent the top layer from collapsing (Zolotovsky). After the cellulose has been drained, it is dried out, after which it is more able to retain its form. The shape could be advanced in one of two ways-creating support in or outside the cellulose to prevent deformation of the top layer or designing a shape for the cellulose to allow them to be aggregated in interesting ways. To determine methods of constructing supports, this section will compare and contrast current scaffolding techniques for non-biomaterials with the traits that would be necessary for biomaterials. To determine potential shapes to allow for more useful aggregates, it will consider biological patterns as inspiration for the final form, particularly examining Voronoi patterns and scutoids.

The mechanism behind creating supports for 3D printed material relies heavily on the type of model. The most common technique-fused deposition modeling (FDM)-involves extruding a liquid ink (usually ABS plastic) that freezes into a solid. It begins by making the bottom layer and then repeatedly adds layers of material on top. However, if there is an overhang of greater than 45 degrees or a bridge larger than 5 mm, ABS FDM 3D printers must add supports. To do this, two algorithms are used. The first is a method that builds thin walls in a zig zag fashion underneath the areas that need support straight to the base, called the accordion method. The second is a tree model, which can use less material, but is more computationally complex[3]. It uses a branching structure in order to provide support for the part. Both of these methods require manually cutting the support off and sanding the surface down, and so the surface of the model is usually scarred by the scaffolding, though the tree method has less contacts and is therefore cleaner. Alternatively, methods such as selective laser sintering and polyjet printing can enable support-free 3D printing by using easily removable material as support (Cain).

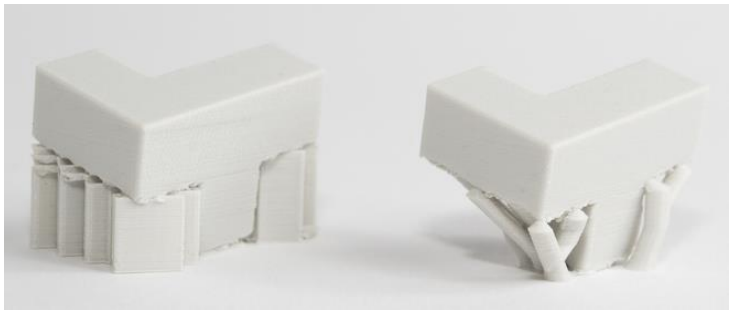

Figure 5: Accordion and tree supports **(Cain)**

To apply these methods to the biofabrication process would present a challenge, but they still offer potential insights. Walls underneath the top layer of cellulose would be almost impossible to remove due to the lack of an opening in the compartment. Therefore,

[3] The tree model is much more common in stereolithography (SLA), which builds models from the top down using light to harden the top surface of a liquid and progressively pulling the object up and out of the liquid.

the walls would need to be permanent structurally; in theory, they would need to be made from cellulose. To expose these areas of the media to oxygen, the silicone compartment could be modified to include thin walls within it that could provide exposure to air. Implementing a tree support structure would require a similar strategy. The method of using another material to support the structure already occurs with the presence of the liquid media; it is the removal that makes it require additional support. Alternatively, the shape could be designed such that it does not have large bridges or overhangs, which would enable the structure to support itself.

However, once the cellulose dries, it requires no support, so the current method is a functional, if perhaps arduous. Alternatively, the thesis will look at computationally generating shapes that can aggregate together to form larger structures. To do this, the research returns to its roots in biology for inspiration. Specifically, cells are able to pack together in limited space by using a Voronoi pattern. A Voronoi pattern is created by randomly selecting a set of points in a confined area, then dividing the area into parts based on whatever point is closest. These patterns can be generated in $O(n \log n)$ time using Fortune's algorithm (Fortune). Considering the cellulose compartments as "cells", these structures may be able to mimic their origins to aggregate together with Voronoi patterns.

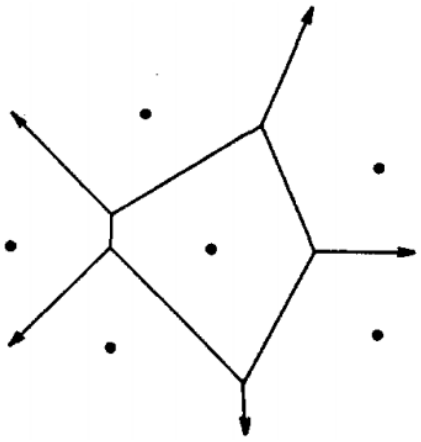

Figure 6: A Voronoi Diagram **(Fortune)**

Scientists have recently added further complexity to the Voronoi pattern by defining scutoids. A scutoid is similar to a prism, except the two faces have a different number of sides and a different size. This enables the shape to create the curvature common in epithelial cell linings. To form scutoids computationally, Gómez-Gálvez and his team calculated a Voronoi on two surfaces using seeds that were mapped between the two. They then connected the vertices of corresponding seed points to make the final scutoid shape (Gómez-Gálvez). The flexibility of this shape allows it to aggregate in a way that it can create arbitrary 3D forms. This could be a useful form for the cellulose compartments to take on, as they would be able to stick to each other using only water as a binding agent.

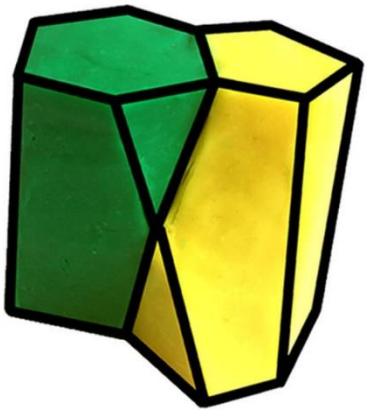

Figure 7: Two scutoids **(Gómez-Gálvez)**

Methodology

In order to understand the production of biomaterials, this thesis uses three processes to create cellulose biomaterial: the biological manipulation and growth of *G. xylinus*, the fabrication of the PDMS scaffolding in which to grow the bacteria, and the control of the material during and after the growth process. Each process that will be used to investigate the manufacture of cellulose is described in detail below.

The biological process starts with the bacteria *G. xylinus*. In order to manipulate the conditional function of the bacteria, a plasmid, or a circular piece of DNA that can be inserted into bacteria cells. The plasmid is constructed to have the gene for producing cellulose, along with other genetic controllers to add conditional logic to the production of

cellulose. *G. xylinus* is then transformed with the plasmid so that the genes on the plasmid are expressed in the bacteria. These bacteria are then cultured in a suspension of Hesterin-Swann growth media, sometimes with additional signals to turn genes on and off. Once the bacteria have reached an optimal concentration, the bacteria and growth media are transferred to the PDMS scaffolding.

The next step, the fabrication of the PDMS scaffolding, starts with a 3D model of the structure. An inverted mold of this model is then created and 3D printed in plastic. Liquid PDMS is then poured into the mold, and then the PDMS is given time to dry. Once the PDMS dries, the scaffolding is removed from the mold and the bacteria can be added in to grow.

The bacteria then grow and produce cellulose within the scaffolding. The cellulose only accumulates where it is exposed to oxygen. Since the scaffolding is oxygen-permeable, cellulose accumulates along the walls of the container as well as on the top of the growth media, creating an enclosed shape filled with growth media and bacteria. The scaffolding is designed to connect to tubing to pump out the liquid and pump in air to remove the bacteria while retaining the structural properties of the cellulose. The material is then dried out to produce the final material.

This thesis is focused on the second step of the fabrication of the scaffolding. At this point, the cellulose is a single hexagonal prism. As part of this investigation, Grasshopper and Python using RhinoScript will be used to produce a variety of shapes in which to grow the cellulose, varying parameters such as polygonal shape, thickness, and overall structure, in order to create material structures that have a variety of functional properties and applications for this material. This code will have explicit parameters that can be chosen by researchers to produce cellulose biomaterial with the properties to fit

specific tasks. This thesis will determine potential structures from existing literature on support, aggregation, and connection.

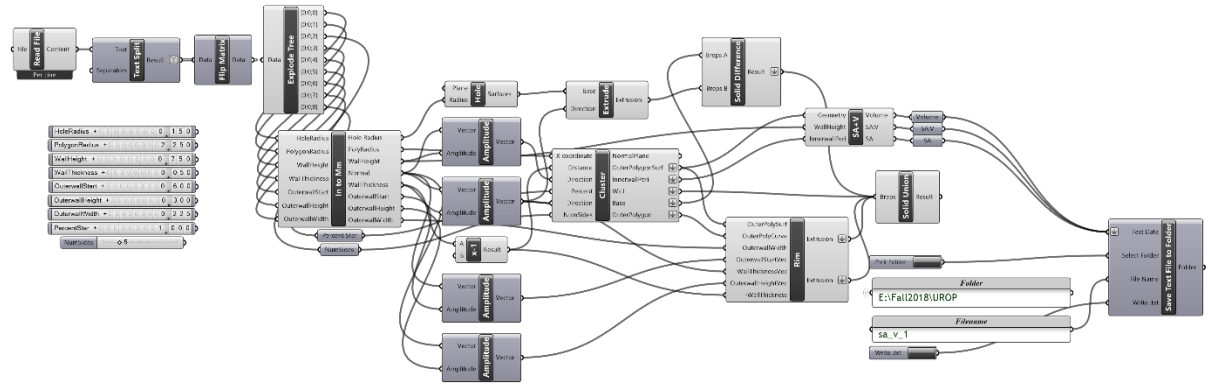

Figure 8: Grasshopper Script to create star module compartments

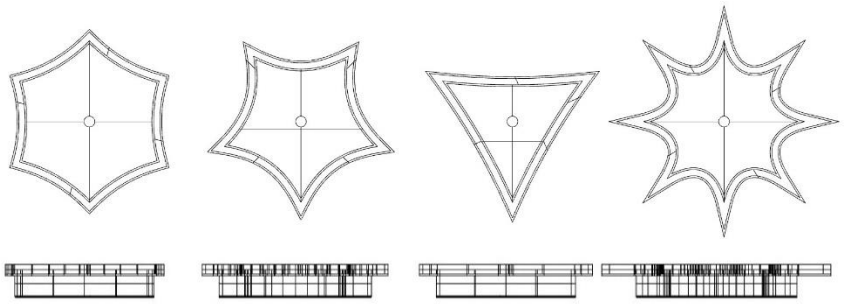

Figure 9: Some potential forms from the script, varying curvature and number of sides

A starting Grasshopper script has already been produced to generate star shaped forms. This script takes in as input the radius, height, thickness, number of sides, extent of curvature, as well as a number of other parameters. It then produces the shape, including a rim for mounting purposes and a hole at the bottom of the compartment for draining the media. Through this script, many iterations of the design can be realized and tested.

Timeline

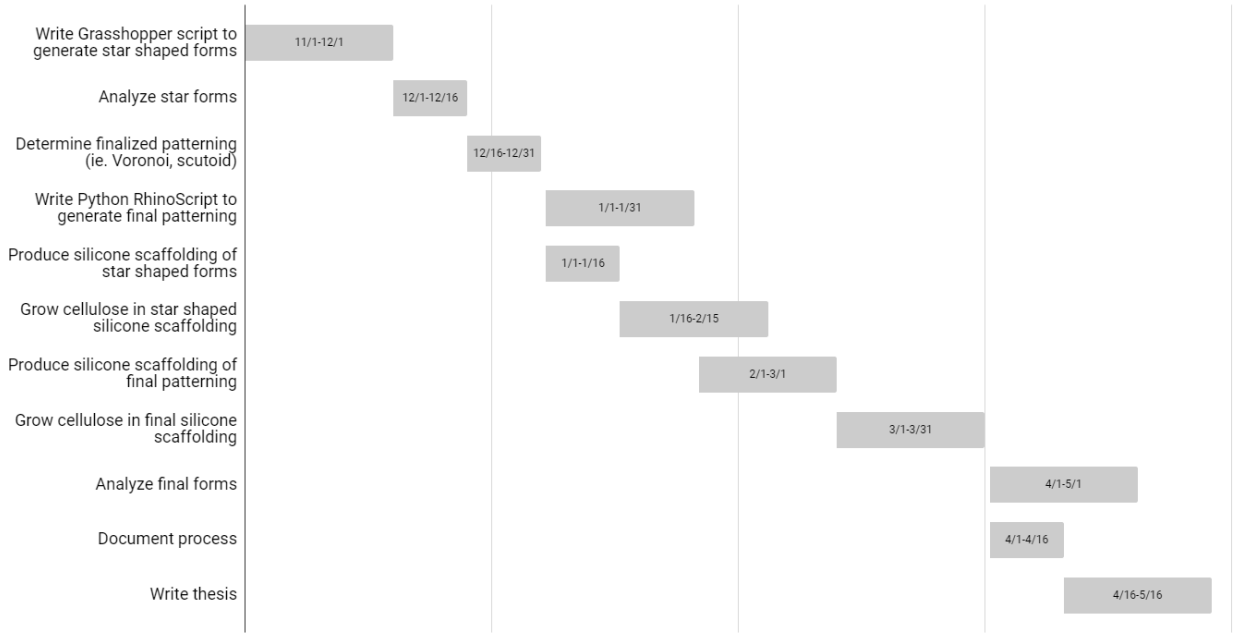

Figure 10: Gantt Chart of projected plan for thesis

Work on the thesis has already begun this semester with a Grasshopper script to produce star shaped compartments with varying curvatures. This shall function as a proof of concept. During January, the silicone compartments will be made and *G. xylinus* will be cultured in them, producing the first cellulose compartments. The final code to create a scutoid patterning will also be written in January. During spring semester, the silicone compartments for the scutoids will be produced and *G. xylinus* will grow in them. Throughout the process, some basic analysis of the strength and flexibility of the forms will be done. The final month and a half of the spring semester will be dedicated to documentation and writing the final thesis.

Bibliography

Cain, Perry. *Supports in 3D Printing: A technology overview*. n.d.

Deniz, Irem and Tugba Keskin Gundogdu. "Biomimetic Design for A Bioengineered World." *Interdisciplinary Expansions in Engineering and Design With the Power of Biomimicry*. Ed. Gulden Kokturk and Didem Akyol. 1. InTech, 2018. 57-75.

Esa, Faezah, et al. "Overview of Bacterial Cellulose Production and Application." *Agriculture and Agricultural Science Procedia* 2 (2014): 113–119.

Fortune, Steven. "A Sweepline Algorithm for Voronoi Diagrams." *Algorithmica* 2 (1987): 153-174.

Gómez-Gálvez, Pedro, et al. "Scutoids are a geometrical solution to three-dimensional packing of epithelia." *Nature Communications* 9 (2018). <https://www.nature.com/articles/s41467-018-05376-1>.

Greca, Luiz G, et al. "Biofabrication of multifunctional nanocellulosic 3D structures: a facile and customizable route." *Material Horizons* 5.3 (2018): 311-580. <https://pubs.rsc.org/en/content/articlepdf/2018/mh/c7mh01139c>.

Grushkin, Daniel. *Meet The Woman Who Wants To Grow Clothing In A Lab*. 17 February 2015. 1 November 2018. <https://www.popsci.com/meet-woman-who-wants-growing-clothing-lab>.

Hon, David NS. "Cellulose: a random walk along its historical path." *Cellulose* 1.1 (1994): 1-25.

Julkapli, Nurhidayatullaili Muhd and Samira Bagheri. "Progress on nanocrystalline cellulose biocomposites." *Reactive and Functional Polymers* (2017): 9-21. <https://www.sciencedirect.com/science/article/pii/S1381514816302401>.

Kataja, Kirsi and Pirjo Kääriäinen, "Designing Cellulose for the Future." *Design-Driven Value Chains in the World of Cellulose*. Helsinki, Finland: VTT Research, 2018. 1-139.

Mittal, Nitesh, et al. "Multiscale Control of Nanocellulose Assembly: Transferring Remarkable Nanoscale Fibril Mechanics to Macroscale Fibers." *ACS Nano* 12.7 (2018): 6378-6388. <https://pubs.acs.org/doi/10.1021/acsnano.8b01084>.

Moroni, Loreno, et al. "Biofabrication: A Guide to Technology and Terminology." *Trends in Biotechnology* (2018): 384-402. <https://www.cell.com/trends/biotechnology/fulltext/S0167-7799(17)30279-2?code=cell-site>.

Our Technology. 2018. 1 November 2018. <http://www.modernmeadow.com/our-technology/>.

Rühs, Patrick A, et al. "3D bacterial cellulose biofilms formed by foam templating." *Biofilms and Microbiomes* 4.21 (2018). <https://www.nature.com/articles/s41522-018-0064-3>.

Siró, István and David Plackett. "Microfibrillated cellulose and new nanocomposite materials: a review." *Cellulose* 17.3 (2010): 459–494.

Svensson, A., et al. "Bacterial cellulose as a potential scaffold for tissue engineering of cartilage." *Biomaterials* 26.4 (2005): 419-431.

Tydlitátová, Barbora, Tereza Lišková and Dominik Jůn. *Zuzana Gombošová Fed Coconut Water To Bacteria, And Developed The Unique Material Malai*. 25 April 2018. 1 November 2018. <https://www.materialtimes.com/en/our-focus/zuzana-gombosova-fed-coconut-water-to-bacteria-and-developed-the-unique-material-malai.html>.

Zolotovsky, Katia. *Guided growth : design and computation of biologically active materials*. PhD Thesis. Cambridge, Massachusetts: Massachusetts Institute of Technology, 2017. <http://dspace.mit.edu/handle/1721.1/113925>.