Guided Growth:

Design and Computation of Biologically Active Materials

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In this dissertation, I propose ways for designers and architects to engage with the material units of biological computation and fabrication, namely the living cells. Microscopic cells, the basic units of life, are bits of material with embedded computation, fabrication, and regulation mechanisms. Their genetic code in the DNA performs complex computations, allowing cells to grow, interact with their environment through exchange of matter and energy, and even produce structural materials such as cellulose. These materials are renewable, self-assembling, self-healing, and biologically active. Our rapidly growing ability to re-program living cells to produce materials they would not produce in their natural state presents future possibilities as far-reaching as growing houses from seeds. This dissertation explores synthetic biology as a computational method to program cells so as to grow biologically active materials for architecture, with the possibility of designing them for new functions, including air filtering and purification, self-repair, and photosynthesis. To realize the potential for biologically active materials, I propose a Guided Growth design process, using the cellulose-producing bacterium *Gluconacetobacter xylinus* as an example. The Guided Growth design process employs three scales of resolution: nano (engineered living cells), meso (biomaterials that inhabit cells), and macro (bio-computational interface):

· *Nano-scale*, the nanometer scale of DNA design: using tools of synthetic biology, I program the behavior of bacterial cells to respond to changes in their environment and pattern their function and properties.

· *Meso-scale*, the micro- to centimeter scale of guided material self-assembly: using tools of materials science, I develop workflows to grow, shape, harvest, and process living-non-living composite biofilms, while keeping the bacteria cells alive and biologically active.

· *Macro-scale,* the centimeter and up scale of bio-computational interface: using tools of digital fabrication, I design and fabricate a bio-computational interface that through computationally regulated flow of nutrients, added substances, and air allows the designer to interact with the process of growth.

In the Guided Growth design process, I collaborate with synthetic biologists and computational designers to integrate the rigor of scientific research and the openness of material-based explorations. This multi-scale collaborative process can be further generalized to other material systems where programmed living cells act as matter-organizing agents. My experimental methodology proposes new ways of computational making in architecture, a new class of biologically active materials, and a new application domain for synthetic biology.

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CHAPTER ONE

INTRODUCTION

Throughout history, architecture has embodied technological and cultural values through innovations in materials and tools. The invention of concrete enabled Romans to manifest their imperial greatness and build on an unprecedented scale, erecting soaring arches and domes over huge spans. The introduction of steel and reinforced concrete during the Industrial Revolution in the early 20th century made high-rise buildings and skyscrapers possible, fostering urbanization and industrialization. In the Information Age, digital tools and fabrication methods have allowed freedom from geometrical constraints and led to the sophistication of architectural shape and data-based fabrication methods.

Now we are entering a new era of biotechnology, a technology that is based on biology, that harnesses cells and the biochemical processes inside them to develop processes and products that help improve the health of both humans and the planet. I believe we can use this new technology to invent new products and uses for design and architecture. In architecture, beginning in the twentieth century, the construction of higher and higher and more technologically sophisticated buildings along with the transportation of materials around the globe, has resulted in pollution and overuse of natural resources. According to the U.S. Green Building Council, 40% of the world's raw materials are used in the construction of buildings, and buildings are responsible for 38% of all CO_2 emissions. At the end of the 20th century, ecology, energy conservation and a call for sustainable development led to the search for materials and methods of construction that can sustain harmonious relationships with the environment. The question has become, "Can architecture be developed in a way that is in symbiosis with nature and perhaps also provide things that were not there before?" (McDonough, 2016).

Biotechnology has great potential to solve the environmental problems created by previous technological revolutions if we can find a way to apply materials of biology to the domain of architecture. Born at MIT, synthetic biology has become an opportunity for designers and engineers to design new synthetic functions from biological functions that exist in nature. Synthetic biologists reprogram DNA, establish libraries of biological parts, and develop tools that allow non-biologists to use biology in their designs.

This research explores synthetic biology as a computational method to program cells and to grow bio-active and adaptive building components for architecture, with the possibility of designing them for new functions, such as air filtering and purification, self-repair, and photosynthesis.

Biological materials support life on earth and flow through cycles of growth, decay and rebirth, thereby recapturing nutrients to create new life (McDonough, 2016). Simply put, waste equals food—the

"waste" of one system becomes food for another. Biological materials can biodegrade safely and restore the soil after use. Today even the most advanced building or factory in the world is still a kind of steamship, polluting, contaminating, and depleting the surrounding environment, and relying on scarce amounts of natural light and fresh air. People are essentially working in the dark, and they are often breathing unhealthful air. This research proposes first steps towards a vision of a building as a kind of tree. It would be photosynthetic and biologically active, accruing solar energy, cycling nutrients, releasing oxygen, fixing nitrogen, purifying air, and changing with the seasons.

Recently, two developments in the area of materials science and architecture call for the application of synthetic biology. First, biomaterials that are grown instead of fabricated have been used in architecture. For example, architects have used *mycelium*, a mushroom grown on wood chips, to produce structurally sound material (Stamets 2005) and have used bacteria to aggregate minerals into hard blocks (Dosier 2014). Growing materials on site helps save resources and energy. However, after the growth process, these materials no longer preserve the biological advantages of the organisms that created them, such as the ability to heal in response to damage.

Second, in materials science, a new generation of energy-transforming materials has been developed. Examples include silk fibers mass produced by bacteria, microbial fuel cells, and synthetic leaves that convert sun energy and fix carbon dioxide from the air. In these examples, biological organisms are not merely used for the fabrication of inanimate materials, but for active materials that are continuously responsive, sensing their environment and designed to transform it. I believe these materials could change building construction, making it a sustainable and active participant in the ecological cycle rather than one that harms the ecology. This dissertation proposes a new area of synthetic biology in architectural construction.

Traditionally, architectural construction uses dry structural materials such as concrete, metals, glass, plastics, wood, asphalt, and bricks. These materials are often produced in a non-sustainable way. Their transportation pollutes the environment, and the destruction and renovation of buildings result in large amounts of waste disposed of either in landfills or incinerators, polluting the air and water. Work with biomaterials for architectural construction already is tackling this important environmental problem by suggesting construction methods with biological materials that are renewable, degradable, and natural participants in the ecological cycle of materials. Using biomaterials in architecture will help reduce pollution and save natural resources.

This dissertation looks further into the future and asks an important question: Can we not only make construction less harmful to the environment, but actually design materials that will improve the environment? My vision in this research is to design engineered living cells as active components that are capable of biological functions such as self-repair, air filtering, metabolizing harmful chemicals into

harmless ones, binding carbon dioxide from the air, and perhaps even performing photosynthesis.

In this dissertation, I developed an experimental methodology that takes the first steps toward this vision. To develop new materials that embed biological responsiveness, architects need to learn to design using new mediums of materials in liquid or hydrogel phase. Whether this means creating breathable walls, or designing windows that perform photosynthesis and neutralize harmful chemicals in the environment, biologically active materials will contain living biological cells. To sustain the biological function of these cells, some general conditions need to exist within the material system:

- These materials will exist with high water content to allow signals to travel and to sustain life.
- A circulation of elements and liquids will exist to let materials in and out.
- Biological sensors will be engineered to respond to the environment in certain ways, to interface with other materials, and to change over time.
- A feedback system will exist within the material, providing a way to process information and adapt behavior.
- The material system will have a structural component (a way to self-support and bear load) and functional component (a way to sense, process information, and adapt behavior).

My vision in this research is to combine the benefits of biomaterials (renewable, degradable, healthful) with the benefits of synthetic biological function (selective air-filtering, tunable properties, and possibly even photosynthesis). Therefore, this dissertation explores this new class of synthetic biological materials for design and architecture. It focuses on converting a particular biological material – bacterial cellulose – into a programmable material system that includes both living cells with sensing and processing capabilities, and structurally-sound membrane material, relevant for architectural application. Bacterial cellulose has long been a research target because, unlike plant cellulose, it is produced in its pure form, free from other chemical compounds. This makes the biomaterial mechanically both strong and flexible, extremely malleable, and renewable.

To realize my vision in part, I propose a new design process called Guided Growth. The Guided Growth design process I propose here employs three scales of resolution: nano-scale (engineered living cells), meso-scale (biomaterials that inhabit cells), and macro-scale (bio-computational interface). In Guided Growth, I collaborate with synthetic biologists to program the behavior of bacterial cells to respond to changes in their environment and pattern their function and properties. I then design and modulate the growth environment as scaffolding on which to grow, shape, harvest, and process composite biofilms, while keeping the bacteria cells alive and responsive. Collaborating with another designer, I build a computationally controlled bio-pneumatic envelope. Through the computationally controlled flow of liquids and air, this envelope facilitates the growth, patterning, pneumatic actuation, and post processing of the composite cellulose membranes into three-dimensional components. The bio-pneumatic

envelope creates a novel bio-computational interface with which a designer can interact with the process of growth and guide the self-assembly of material architecture and its function.

Through concepts, illustrated methods, and hands-on experiments, this dissertation shows ways to program materials to sense their environment, process information, and adapt their structure, properties and biological function. This work develops materials, tools, methods, and workflows for introducing bio-active materials in architecture. Working with soft, biological materials, and designing and maintaining their living function, Guided Growth opens new possibilities for future designers.

I believe the contribution of this project goes beyond the specific domain of architectural application. This study unites three disciplines -- synthetic biology, materials science, and architectural design -- and makes contributions to all three. For synthetic biology, this project bridges the invisible scale of DNA design with the design of materials, patterns and 3D shapes in the tangible scale of human experience.

For materials science, the proposed methodology of applying synthetic gene networks for biofilm patterning and functionalization has the potential to generate a new class of materials. These biologically active materials not only combine shape with materiality for properties amplification, but also can be selectively functionalized for programmable behavior and tailored for applications that require biological sensing and responsiveness, and maybe even self-repair and self-assembly.

For architectural design this research asks new questions about the possibilities of design in interaction with biological growth and material formation. Moreover, the kind of materials I am using (liquids and semi-liquids, or gels) and the way I am using them (reprogramming them on the DNA level, guiding their growth process, and designing their biological function) can change the culture of architecture from one that uses materials to one that creates them. Through dissemination of this project and through teaching, I hope to introduce a new generation of designers and scientists to the notion of approaching biological growth as a creative design process.

The Roadmap

This dissertation is comprised of four chapters, as follows.

Following this introduction in **Chapter 1**, in **Chapter 2: Design and Computation with Biology** I provide the background literature for my approach of design and computation with living cells and biologically active materials. First, I introduce the idea of computation with living cells as a design strategy in a section I name: How Life Got Designed (title adapted from (Roosth 2017). The goal of the literature review that follows is three-fold. The first goal is to discuss the design strategies that are being developed through the new cross-disciplinary field of synthetic biology. I review the history and the main concepts of this novel technology and discuss its potential for patterning synthetic biologically active structural materials, namely synthetic morphogenesis. The second goal, on the level of materials, is to

discuss the unique features of structural biological materials that grow instead of being fabricated. I review the multi-scale hierarchical organization of biological materials that result from the growth process. The third, on the architectural scale, is to discuss the relevance of living, biologically active materials and existing precedents of their use in architecture, design, and fashion. I divide this chapter into three sections according to the scale of resolution each section deals with – Nano-scale for 2.2, Meso-scale for 2.3, and Macro-scale for 2.4.

Following my discussion of background literature and motivations, I present the methodology and the experiments of the Guided Growth design approach in **Chapter 3: Guided Growth of Bacterial Cellulose Biofilms**. First, I present the Guided Growth methodology and discuss how each scale of resolution complements one other in one multi-scale design process. Next, I focus on one material system: the case of bacterial cellulose and discuss its unique characteristics in Section 3.2. The rest of the chapter is divided into sections that focus on the Nano-, Meso-, and Macro- scales of resolution.

· *Nano-scale*, the nanometer scale of DNA design: using tools of synthetic biology, I program the behavior of bacterial cells to respond to changes in their environment and pattern their function and properties.

· *Meso-scale*, the micro- to centimeter scale of guided material self-assembly: using tools of materials science, I develop workflows to grow, shape, harvest, and process living-non-living composite biofilms, while keeping the bacteria cells alive and biologically active.

· *Macro-scale,* the centimeter and up scale of bio-computational interface: using tools of digital fabrication, I design and fabricate a bio-computational interface that through computationally regulated flow of nutrients, added substances, and air allows the designer to interact with the process of growth.

The experimental chapter includes for each section: a relevant literature review, an illustration of the methods used in the laboratory, presents results, and discusses future work. In this chapter I show integration of the design strategies of genetic design and regulation, with the materials shaping and patterning, with the design of digitally controlled flow in a bioreactor. Using the Gluconacetobacter *xylinus* bacterium and a biofilm of cellulose it produces, I grow active and adaptive hybrid materials and three-dimensional components. Biofilm here is a structural layer of cellulose produced by G.*xylinus* bacteria cells grown on a surface of nutrient rich liquid into which bacteria cells have been embedded. The Guided Growth methodology of combining genetic engineering with environmental regulation and scaffold design can be further generalized to other material systems where bacteria act as a matter-organizing agent.

In the last chapter, **Chapter 4: Discussion**, I discuss the integration of methods and disciplinary cultures in my work, outline the main contributions of this work, and propose future developments of these experiments.

CHAPTER 2:

DESIGN AND COMPUTATION WITH BIOLOGY

In this chapter I review the background literature and projects for my approach of design and computation with living cells and biologically active materials. First, I introduce the idea of computation with living cells as a design strategy in a section I name: How Life Got Designed (title adapted from (Roosth 2017). The goal of the literature review that follows is three-fold. The first goal is to discuss the design strategies that are being developed through the new cross-disciplinary field of synthetic biology. I review the history and the main concepts of this novel technology and discuss its potential for patterning synthetic biologically active structural materials, namely synthetic morphogenesis. The second goal, on the level of materials, is to discuss the unique features of structural biological materials that grow instead of being fabricated. I review the multi-scale hierarchical organization of biological materials that result from the growth process. The third, on the architectural scale, is to discuss the relevance of living, biologically active materials and existing precedents of their use in architecture, design, and fashion. I divide this chapter into three sections according to the scale of resolution each section deals with – Nano-scale for 2.2, Meso-scale for 2.3, and Macro-scale for 2.4.

2.1 How Life Got Designed

This section tells a story about form, material, and computation, and how working with the materials of biology can completely reinvent relations between the three. I tell this story through the historical lens of two realms of computation that were the focus of research and development during the twentieth century: genetic computation and digital computation. In the section below I discuss the sequence of discoveries, inventions, and theories about both types of computation and their mutual influence on each other. I focus on the relationships between shape, material, and information organization and processing (computation), as the main aspects in both biological and digital computing.

I claim that with the beginning of synthetic biology at the turn of twenty first century, the two trajectories began to converge, giving a new opportunity to designers to engage with genetic computation and actual living matter. Synthetic biologists are developing programmable cells that can be integrated into the domain of architectural design. These programmable cells have the potential to introduce a new kind of computation to architectural design: a computation that is embedded in the DNA of bits of material, allowing the material to interact with its environment through biochemical processes. I call this new kind of computation *genetic computation in design*.

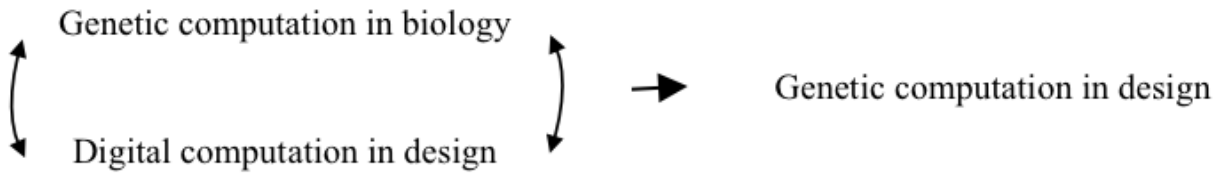


Figure 2.1.1 Diagram of section organization. Mutual influence between discoveries and innovation in genetic computation in biology and the birth and development of digital computation during the last century may evolve into a hybrid end of genetic computation in design: programmable cells integrated in architectural design and related fields of fashion, industrial, and product design.

Figure 2.1.1 and Figure 2.1.2 show an organizing visual for this section, a timeline with the main historical milestones in the development of genetic computation in biology and digital computation in design, moving toward a hybrid end: genetic computation in design. The upper side of the timeline shows how genetic computation in biology developed throughout the twentieth century. It shows a progression from early ideas about design with biological materials in the beginning of the century, then moves to a sequence of discoveries recognizing the computational aspects of cell biology. Toward the end of the twentieth century came the realization that if cells perform computationally, we can program them, giving birth to synthetic biology.

The parallel track at the bottom of the figure traces the evolution of digital computation and the introduction of biological concepts in design and architecture. The events highlighted on the two tracks inform each other: discoveries in biology led to revelations in digital computing and vice versa. In biology, I show how discoveries of computational aspects of biological growth led to opportunities to design with it. In architectural design, I trace the transition from drawing upon biological form to drawing on its material substrate. I conclude with framing my own work proposing integration of programmable cells in materials for application in design and architecture.

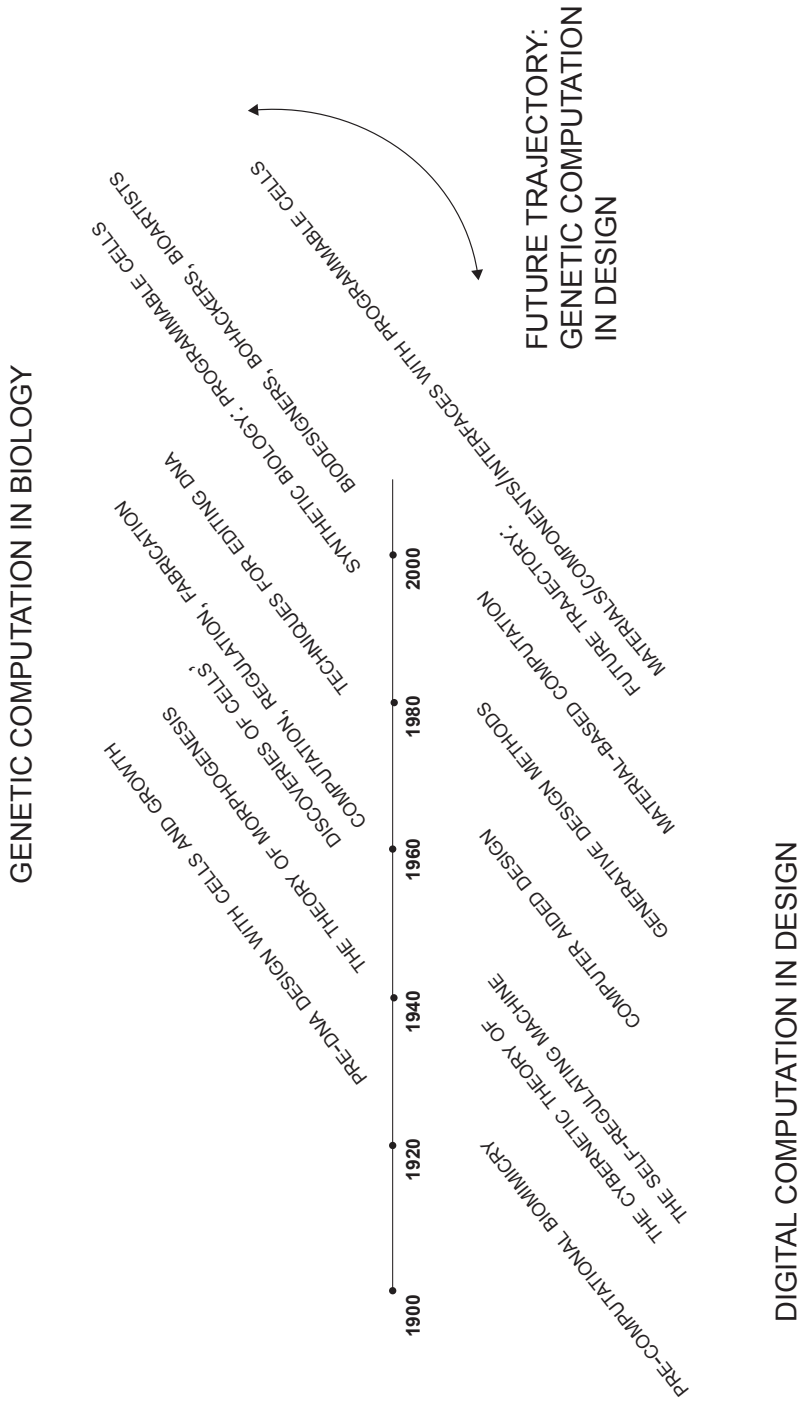


Figure 2.1.2 Genetic computation timeline. An organizing visual for this section: a timeline with main historical milestones contributing to the development of genetic computation in biology and digital computation in design towards a hybrid end: genetic computation in design.

Pre-computational biomimicry.

Engineers, architects and designers have long gained inspiration from forms in nature, a strategy referred to as *biomimicry*. There are many examples from the nineteenth century of how architects and designers mimicked nature's form, both for its aesthetic value and its robust engineering. One example of the aesthetic inspiration provided by natural forms is the entrance gate to the World Exposition pavilion in Paris in 1900, whose design was inspired by Ernst Haeckel's drawings of tiny mineral skeletons, or *radiolaria* (Ball 2001). Another example is Gustave Eiffel's tower supports, whose elegant curves, supporting immense weight, were inspired by bone structure (Ball 2001).

Biomimicry today brings innovation in materials and architectural solutions that are very forward-looking, including solar cells, smart sensors, advanced robotics and aerospace materials. In addition to its creative and innovative potential, biomimetics (Benyus, 2002) has been identified as a promising discipline for the environmentally responsive innovations that will be needed in the future, such as climate-responsive building envelopes (Gamage and Hyde 2012; Mazzoleni 2013; Weston 2012; Zari 2010; Badarnah and Kadri 2015). Several biomimetic design strategies have been developed in the last decade (Weston 2012; Vincent 2009; Helms, Vattam, and Goel 2009). For example, Ilaria Mazzoleni in her book "Architecture Follows Nature" investigates animals' skin functions: communication, thermoregulation, water balance, and protections. She then proposes concepts for new architectural "skins" based on material organization principals derived from the animal world (Mazzoleni 2013).

However, one of the biggest obstacles to taking full advantage of what nature has to offer is that the living world has an awesomely elaborate means of construction, which is difficult if not impossible to mimic with human-made fabrication techniques. As British science writer Phillip Ball pointed out in his paper, "There is no assembly plant so delicate, versatile and adaptive as the cell" (Ball 2001). Indeed, to begin to approach the complexity and efficiency of nature's own constructions, we need new materials and new methods. In the next section I trace the history of how a new methodology has emerged, one that merges nature's machinery with synthetic constructs on the molecular scale.

Pre-DNA design with cells and growth.

Biology has always been about understanding what life is and how it operates. Nonetheless, at the end of nineteenth century biology became more experimental; new approaches began to be formulated that would change the way living matter was treated (Roosth 2017).

The shift in understanding living matter began when the theory of spontaneous generation -- the belief that life could arise from non-living matter upon the action of some 'vital force' -- was finally disproved. In 1862, French biologist Louis Pasteur conducted experiments with bacterial growth in broth that proved that living things only come from other living things (Berche 2012; Porter 1961). In addition,

around the mid-nineteen century**,** cell theory -- the idea that all living matter is composed of cells -- emerged and became widely accepted among scientists (Harris 2000).

In the years that followed, biologists studied cells under the microscope, extending their understanding of life and its production machinery beyond the cellular level. The more they learned about the amazing mechanism of life in the cell, the more their desire grew to experiment with it, blurring the boundaries between 'nature' and 'artifice'.

The early twentieth century can be seen as a beginning of the era of design and computation with biology. The first visionaries to create new designs with living matter were Jacques Loeb and Stephane Leduc. Jacques Loeb, a German physiologist who was working at the center for marine biology in Woods Hole, Massachusetts, showed that scientists could manipulate living cells in a laboratory to create, as he called it, "the beginning forms of life." In his experiments, Loeb induced an unfertilized sea-urchin egg to divide and develop by exposing it to certain salts (Pauly 1987). In an interview in 1902, Loeb shared his broader vision: "I wanted to take life in my hands and play with it. I wanted to handle it in my laboratory as I would any other chemical reaction—to start it, stop it, vary it, study it under every condition, to direct it at my will!" (Ball 2001).

At the same time Leduc, a French biologist, coined the term *la biologie synthétique* -- synthetic biology (Leduc and Butcher 1911). In his book *The Mechanism of Life,* he announced in 1911 the beginning of the synthetic era in biology, proposing that all science passes through three stages of development: observational and descriptive; analytical; and synthetic. Leduc wrote: "Up to the present time, biology has made use only of the first two methods, the descriptive and the analytical. Now, at last, the science has become synthetic" (Keller, 2009). In the same book, Leduc presented his experiments of synthesis of biological organisms under the laws of inorganic chemistry: by introducing various metal salts into solutions, Leduc created "artificial organisms" which both looked and behaved like living creatures. To me, the significance of Leduc's experiments lies in the fact that in both his writing and experiments, he created a vision of design with the actual machinery of biology. This was vision that could only be realized a century later, when the technology of synthetic biology was developed.

The theory of morphogenesis.

Following these early attempts to join biology to design, new theories were born that not only explained biological growth but also provided fertile ground for the generative computational methods that architecture would adopt later on. It is in *morphogenesis*, a theory first formulated by that genetic computation in biology and digital computation in design coincide.

The theory of morphogenesis was first introduced by the biologist and mathematician D'Arcy Wentworth Thompson. In his book *Growth and Form*, which became famous across disciplines, Thompson theorized shape generation in nature as material organization under physical forces, or

morphogenesis (Thompson 1945). He explained that the key to biological growth and form generation lies in understanding of the forces operating between material substructures during the process of growth. Thompson called for mathematics to take biomimicry to the next step, revealing the forces underlying morphogenesis.

In Chapter 1 of his book he gives an example of the amoeba, a primitive, single cell organism, explaining how it forms, moves, and grows in a steady-state equilibrium with physical forces acting on it. The creeping motion of the amoeba is a result of contradicting forces of molecular cohesion and friction, and its shape is determined by surface tension forces. The amoeba grows by drawing in water, salts, and food materials, and chemically transforming them. "Matter as such produces nothing, changes nothing, does nothing…[matter can act] only as seats of energy and as centers of forces," Thompson wrote.

Architects later on picked up Thompson's ideas about form generation and transformations to develop digital algorithms for shapes and pattern generation; however, in his book Thompson's idea about form and growth are intimately linked to a material's chemical and physical properties. He sees the generation of shapes and patterns in biology as a consequence of physical and chemical forces and the diffusion processes. In his book he further theorizes the *Principle of Similitude*, showing how the forces that act on matter vary in hierarchical manner with its dimensions and scale.

Thompson proposes three levels of resolution at which to examine biological design. The first level is the scale of resolution of the molecules and chemical substances in a single cell. Surface tension is most influential in the generation of a cell's shape: minimal surfaces are created and cells are shaped as drops.

The second level is the scale of resolution of cell aggregates and tissue growth. Here diffusion processes define tissue tessellation patterns. To demonstrate, Thompson refers to diffusion experiments by Leduc: patterns formed in a solution of gelatin on a glass plate when it was infused with drops of weak potassium cyanide solution.

The third level is the scale of resolution of structural elements produced by living tissues, or exoskeletons. Thompson gives an example of Radiolaria, or tiny mineral skeletons, as drawn by biologist Ernest Haekel in 1904 (**Figure 2.1.3**). In Radiolaria, the formation process of the skeletal element is influenced by the chemical nature of crystalline inorganic matter (calcium or silica) and the conformation of the laid material with the form assumed by cells/tissues/organs, or molding. The pattern evolves by material accumulating first on the edges and corners, and develops radially from the center.

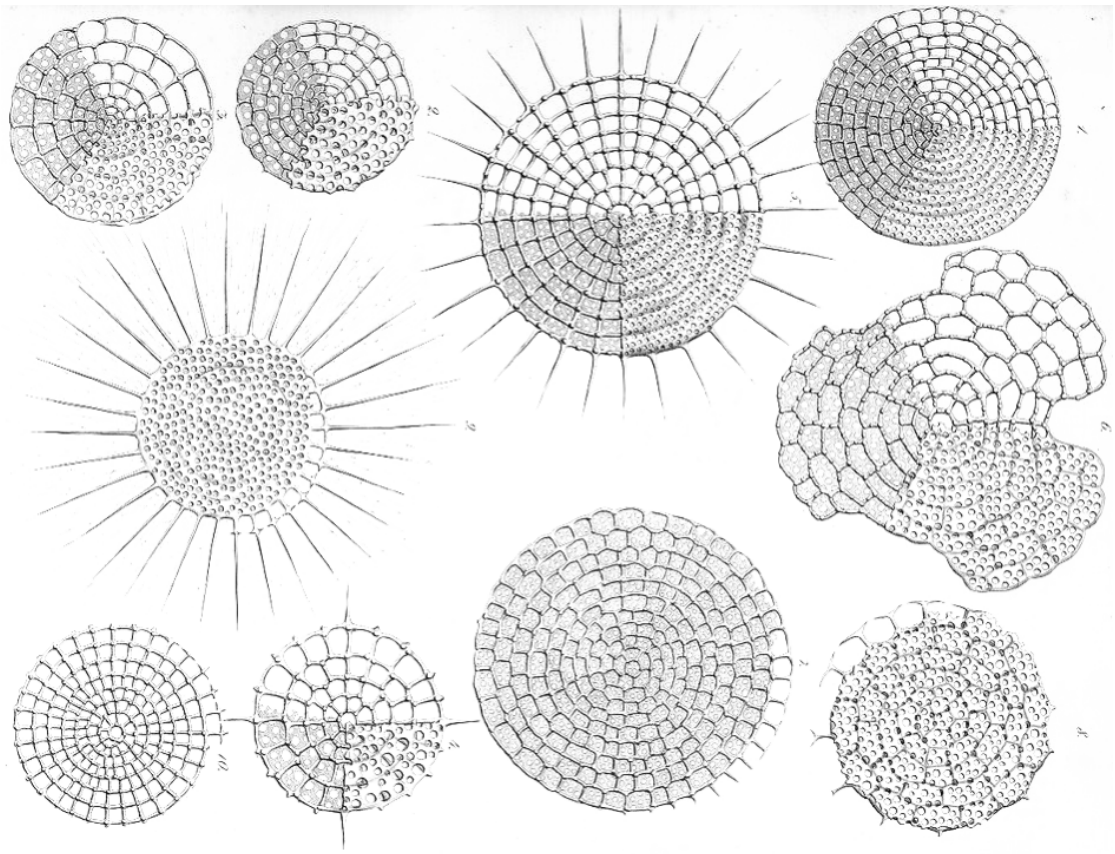


Figure 2.1.3 Drawings of Radiolaria, tiny mineral skeletons, by biologist Ernest Haeckel in 1904.

In Section 2.3 of this chapter, I go into further detail and show examples of how material organizes itself during the growth process. The three levels of resolution that Thompson recognizes resonate with the three levels of resolution I define in my design work: the nano-scale of DNA design, the meso-scale cells integrated in materials, and the macro scale of scaffolding (see Chapter 3).

Thompson's ideas about biological growth and pattern formation were soon translated into mathematics, where the materiality and physicality of his insights were lost to abstraction. In 1952, seven years after Thompson's famous book was published, Alan Turing, a mathematician and a pioneer of computer science, published "The Chemical Basis of Morphogenesis," a paper in which he proposed his mathematical model for a reaction-diffusion theory of morphogenesis (Turing 1952). Turing proposed a mathematical model for the reaction-diffusion process as a disruption to a stable system that causes it to evolve into a new pattern or structure. Turing modeled pattern formation in nature as a combination of two processes: a chemical reaction between elements (morphogens) and their diffusion through tissues.

It is interesting to note that Turing engaged with the problem of morphogenesis after inventing the Logic Computing Machine in 1936, a machine that laid the foundation for the development of the modern digital computer (Longo 2009). The two directions of Turing's work: the inquiry about

continuous processes of biological growth and the development with discrete states of digital computing continued to evolve in parallel throughout the second half of the twentieth century, till their integration in the work of synthetic biologists at the turn of millennia.

Next, I would like to introduce the physical thread of computation in biology, then return to digital computation and discuss how digital simulations of morphogenesis were adopted by architects as form-generation strategies.

Discoveries of cells' computation, regulation, and fabrication.

In living organisms, the organization of information (computation) is intimately linked with the processes of molecular production (fabrication) and its regulation. In what follows, I describe the trio of computation, fabrication, and regulation in biology as physical processes involving transformation of matter and energy.

Computation. The first part is DNA computation within cells. The Austrian physicist Erwin Schrödinger was the first to propose the idea of genetic programming (Schrödinger 1992). In his book, he proposed the idea of a molecular "code-script" that determined the entire pattern of biological growth and functioning in the mature state. He described groups of atoms encoding a simple unit; a combination of these units would provide endlessly complex combinations that encode instructions for the development plans of all the living organisms and also provide the means to put the plan into operation.

In 1953, Schrodinger's predictions were realized in James Watson and Francis Crick's discovery of the double helix structure of the DNA molecule (James D. Watson 1969; J. D. Watson and Crick 1953). Simple units, four atomic groups or bases named with letters A, T, C, G assemble into a gigantic molecule of DNA. The order in which the bases appear on the DNA molecule encode the instructions for molecular units to assemble into larger biological molecules -- proteins -- that build up and perform all function in living cells, tissue, and organisms.

Of particular importance to our current ability to design DNA is another discovery of a small circular form of DNA present in many bacteria cells. These cells were first discovered in 1952 by the American molecular biologist Joshua Lederberg (Lederberg 1998). *Plasmids* can replicate within the bacteria host, but can also be transmitted from one bacterium cell to another. Because of their ability to carry DNA sequences, replicate, and move between cells, plasmids are widely used to replicate the newly designed DNA sequences within the host organisms or transmit them between different hosts.

Because bacteria are the simplest living organisms, each composed of one cell, and because they are the subject of this research, I use bacteria to demonstrate genetic coding in **Figure 2.1.4** below. Reading the figure from top to bottom: a bacterium cell contains two types of molecules that store genetic information: the large and complex chromosomal DNA and the small and simple round molecules of plasmid DNA. Both chromosomal and plasmid DNA have the same double helical structure, and the

sequence of the four bases will later determine the amino acid sequence in the protein.

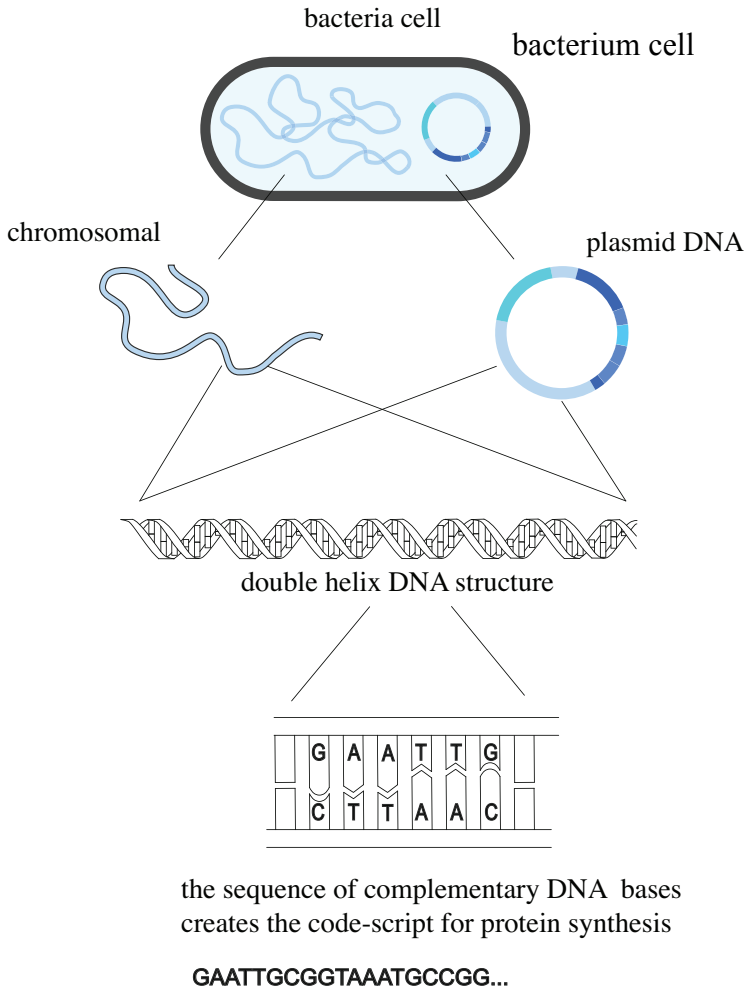
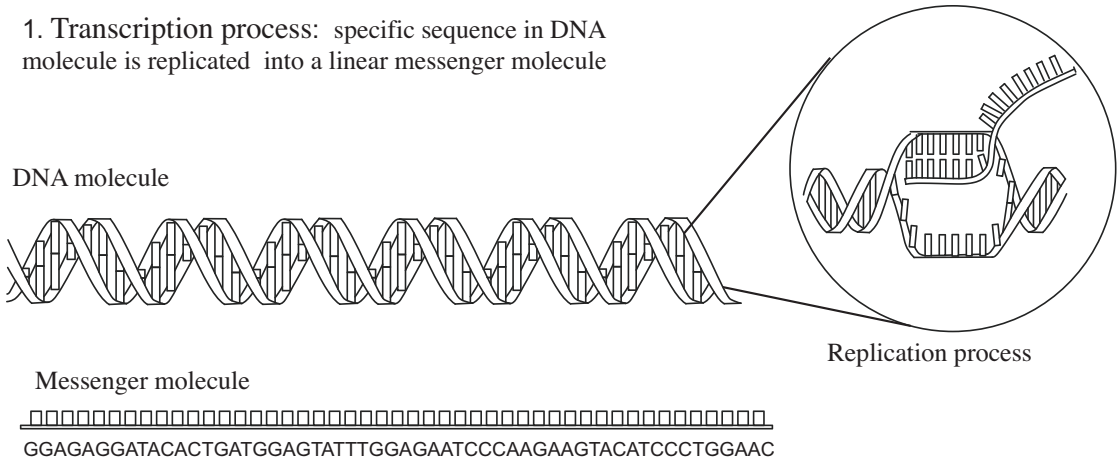


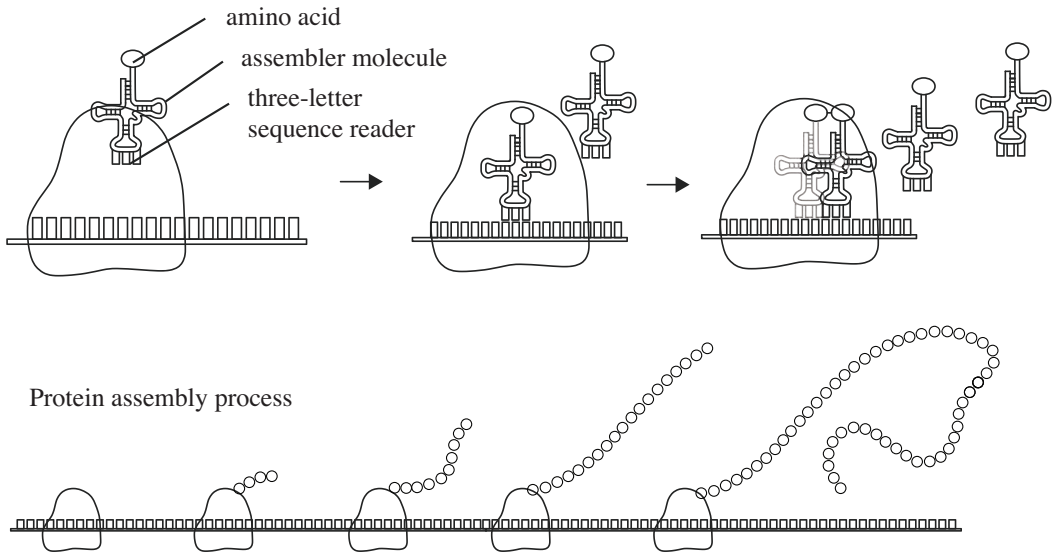
Figure 2.1.4: DNA structure diagram. Living cells as material units with embedded computation: DNA is a code script storing all the information of the cell.

Fabrication. The second part of the design and computation with living cells is the fabrication machinery of cells, complementing the computation encoded in the DNA. The two processes of reading these instructions (*transcription*) and protein assembly (*translation*) allow the fabrication of the building blocks that build the cell and also perform all the cell's biological functions. **Figure 2.1.5** below shows a schematic description of the cell fabrication process, reading the DNA instructions, or *transcription*, and protein assembly, or *translation*.

1. Transcription process: specific sequence in DNA molecule is replicated into a linear messenger molecule



2. Translation process: the messenger molecule is used as a template for assembly of protein building blocks (amino acids).



The assembled sequence of amino acids folds into a functional protein molecule

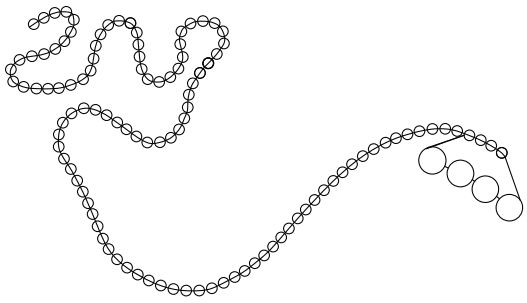


Figure 2.1.5: From DNA computation to protein fabrication: transcription and translation.

In general, chromosomal DNA is a huge molecule containing all of the cell's information. A small specific sequence is replicated to a special messenger molecule, which is then used as a template for protein assembly. Each of the three letters in the messenger molecule encodes one amino acid, a protein building block, out of a repertoire of twenty amino acids. In turn, a specific length and composition of amino acids defines the three-dimensional folding of a protein, and therefore its specific biological function in the cell or outside it. Once the linear sequence of a protein is assembled, it folds into a final protein. This protein then either serves as a building block in the cell's structure (structural protein) or participates in other biochemical processes within the cell as a regulating agent (functional). The regulatory mechanisms in cell are briefly touched upon below.

Regulation. The third part of the design and computation with living cells is the regulation mechanism. The first mechanism of genetic regulation was described by biologists François Jacob and Jacques Monod (Jacob and Monod 1961).

All cells of one organism contain exactly the same set of instructions stored in the DNA. Jacob and Monod discovered that while many genes are always 'on,' other genes are activated only when the cell needs their products. The regulation happens through feedback loops when products of certain genes activate/inhibit processes of transcription and translation from other genes in response to signals from the environment (see *Fabrication* above). They studied the example of *Lac operon*, a set of genes that synthesizes proteins that digest a certain sugar, lactose. Jacob and Monod showed how the cell activates production from Lac operon genes only when lactose is available. This type of regulatory relations between genes is called a *gene network* (see synthetic gene networks below).

As can be seen from the three mechanisms of DNA computation and molecular fabrication and regulation in biology, the three processes are in constant influx with each other and are intimately linked. In other words, molecules are synthesized and assembled from a set of pre-defined DNA instructions, but in response to signals from environment using feedback regulation loops and genetic networks.

Throughout the second half of the twentieth century, the development of molecular biology was influenced by parallel ideas developed in computer science. According to the American scientist and a writer Evelyn Fox Keller, a constant exchange of concepts occurred between developing computation and molecular biology: the linguistic terms *program*, *script* and *code* were borrowed from computation (Keller 2009). In his 1974 book *The Logic of Life; a History of Heredity* Jacob combined Turing's computational vision of a thinking machine with the cybernetic vision of a machine as purposive, goal-directed, and self-regulating (Wiener 1965; Jacob 1974; Turing 1950). In turn, the organization of biological organisms has continuously inspired the development of computation (see examples of Artificial Life and other generative design methods below).

From this point, scientists began to develop techniques to use chemistry to modify the instructions

for life. This was the business of genetic engineering, which took off in the 1970s when scientists figured out how to use natural enzymes to edit and paste portions of "recombinant" DNA (Watson 2007). The language of this new science is that of the engineer and designer, not the natural philosopher discovering how nature works. Indeed, genetic engineering of organisms has been used for production in medicine, food, materials, and many other areas. I will come back to design with computation of the actual mechanisms of computation, regulation, and fabrication in biology after I discuss below how these concepts were reflected in the digital computation, specifically in the domain of digital computation in architectural design.

The cybernetic theory of the self-regulated machine.

The field of cybernetics defined common ground between the development of digital computation and the computation in biology. This common ground was "the rules of control and communication in the animal and the machine" as defined by the mathematician and philosopher Norbert Wiener (Wiener 1965). In other words, cybernetics dealt with universal rules of regulation in complex systems without regard to their specific materialization, blurring the distinction between man-made and nature-made machines (Ashby 1961).

The Chilean biologist Humberto Maturana claimed that any unity can be defined in terms of static or dynamic relations between its components or processes (Maturana 1980). He defined such unities as '*Autopoetic machines'* by their dynamic relations, or processes, of component production. If processes stop, the relations of production will vanish; therefore the relations of productions must be continuously regenerated by the components which they produce. Maturana describes it this way:

"*Autopoetic machine is a machine organized (or defined as unity) as a network of processes of production (transformation and destruction) of components that produces the components which: (i) through their interactions and transformations continuously regenerate and realize the network of processes (relations) that produces them; and (ii) constitute it (the machine) as a concrete unity in the space in which they (the components) exist by specifying the topological domain of its realization as such a network." (*Maturana 1980)

In architecture, cybernetic theories were reflected in the design of indeterminate, responsive and flexible built environments that adopt to the changing needs of the user. One example of such a project is the Fun Palace proposed by an architect Cedric Price in 1961 (Mathews 2006). This is how the Fun Palace responsive plan is described:

"*With an open ground-level deck and with multiple ramps, moving walkways, moving walls, floors, and ceilings, hanging auditoriums, and an overall moving gantry crane, the physical volumes of the spaces could be changed as different usages were adopted. The kit of parts for these operations included charged*

static vapour barriers, optical barriers, warm air curtains, a fog dispersal plant, and horizontal and vertical lightweight blinds (Mathews S., 2005)."

Although the Fun Palace was never built, cybernetic ideas of "architecture as living, evolving thing" (Frazer 1995) continued to mature.

Computer-aided design; morphogenesis and generative design methods.

With the development of the digital computer in the second half of the twentieth century, early ideas of computation in biology evolved into generative design approaches in architecture and other fields. The theories proposed by Thompson, Turing and others (see above) were reflected in the field of digital morphogenesis. In architecture, digital morphogenesis is a type of generative computational method in which complex shape development is driven by computational algorithms and adapts them to a known environment (Hensel, Menges, and Weinstock 2004; Steadman 2008)

Until recently, application of morphogenesis to architectural design led to focus on form and organization, disconnected from material considerations. However, during the last decade or so, as computational simulation methods advanced, there has been a turn toward the integration of material properties and behavior (Oxman et al. 2014; Menges 2012; Hensel et al. 2010). As a result, fabrication techniques and material systems are being developed to integrate sustainable, responsive, renewable biological materials into architectural construction. See Section 2.4 for examples.

In addition to form-generation, generative design methods help the designer or architect explore alternative solutions, using computational algorithms as variant-producing engines and generating unexpected solutions for a given design problem. (Negroponte 1975). One such generative algorithm is Cellular Automata (CA) first introduced by a mathematician John Von Neuman (Von Neumann 1951). In CA, biological phenomena are simulated "bottom-up" through local rules of interaction, in an abstract space represented as a lattice of cells. The state of each cell evolves by reading the state of the neighbors to which it is connected through pre-specified and uniform rules. By changing the initial conditions or the rules of interactions, the outcome changes (Langton 1997). Mutation and recombination as strategies for increasing diversity and survivability in organisms are translated into operations of exchange in programming code (Forbes 2005). Examples of application of CA to architectural design include high density architecture and more abstract stacking method explorations (Coates et al. 1996; Bays 1987).

A larger field of Artificial Life (AL), named by the computer scientist Christopher Langton in 1986, aimed to simulate organization of biological organisms in a digital medium. In his 1997 book, *Artificial Life: an Overview,* Langton defined life as a property of the organization of matter free of its material implementation (Langton 1997). AL researchers wanted to reproduce living processes in digital media and even create new life forms, imagining "life-as-it-could-be." Although the main consequence of the AL enterprise was to develop computers that were more like living organisms, metaphoric

assimilation of computers and organisms was guiding research toward the literal realization of a hybrid end -- namely, synthetic biology (Keller 2009).

The enterprise of AL, conjoined by the ideas of cybernetics and the techniques of molecular biology, gave birth to this new interdisciplinary field. Synthetic biology, offers biology as a new technology for re-inventing production, manufacturing, and design using living matter. In the next section I will discuss this new field of synthetic biology, and its limitations and opportunities.

From techniques for editing DNA to synthetic biology

The discovery of *Lac operon* by Jacob and Monod in 1961 gave insight into the existence of regulatory circuits that define the response of a cell to its environment. Consequently, basic research in molecular biology during the twentieth century revealed that cellular networks were organized as a hierarchy of functional modules, similar to many engineered systems (Jacob and Monod 1961). Gradually, a new vision developed: by tuning or rearranging the functional modules of biological regulatory networks, it would be possible to create new biological devices.

In 1972, cloning was made possible by the discovery of enzymes, called *restriction enzymes*, that could cut the DNA sequence at specific locations. Segments of interest can be recombined into DNA vector molecules such as plasmids, which naturally replicate inside host bacteria. In this way, large quantities of purified recombinant DNA molecules can be produced in bacterial cultures. Polymerase Chain Reaction, or PCR, a technique for making numerous copies of recombinant (designed) DNA, was developed in 1983. Next, in the mid 90s, a technique for automated DNA sequencing was developed, enabling biologists to 'read' and verify the recombinant DNA sequences fast and inexpensively.

The techniques for cutting and combining, amplifying and reading DNA, together with advanced computational modeling techniques, provided the techniques with which to realize this vision. By the end of the 1990s, a small group of engineers, physicists and computer scientists recognized the opportunity and began to migrate into molecular biology to try their hand at the bench. Synthetic biology arose from this vision of using the ever-expanding list of molecular 'parts' to engineer synthetic regulatory networks and make new, synthetic biological devices. Life has become designable. Even the minimal unit of operation of life, the living cell, is challenged by synthetic biologists when they build synthetic programmable cells *de novo*, from scratch.

Biodesigners, biohackers, bioartists.

The impact of synthetic biology goes far beyond the boundaries of academic and industrial laboratories. In her book *Synthetic: How Life Got Made*, published this year, science anthropologist Sophia Roosth describes the cultural phenomena of deskilled biological making: "I observe how amateurs and hobbyists use the same genetic parts developed by synthetic biologists to engineer living systems outside

professional laboratories: in kitchens, garages, and community hobby workshops"(Roosth 2017). Roosth makes a connection between this movement and the slow making and crafting of the nineteenth-century Arts and Crafts movement. She also emphasizes the aspect of *hacking*, described by the 'playful, clever, anonymous, and *funny* engineering' that originated from MIT pranks, and later became associated with computer hacking. Roosth writes that the biohacking movement identifies itself with the 1970s-era electronics hobbyists such as The Homebrew Computer Club (HCC), which counted Apple founder Steve Jobs among its members. To me, the significance of the movement is that I see it as an opportunity for designers and architects to engage with biologically engineered living systems both in architectural design studios and in practice.

Material-based computation; programmable synthetic cells in architecture.

Since its birth in 2000, synthetic biology has sparked the imagination of architects and other designers. Most of the work so far involves speculations and theorizations of how living cell computations could be integrated into designs. For example, a special edition of an architectural design book entitled *Protocell* collected projects that imagine integrations of programmable synthetic cells – *protocells* -- in architectural designs (*Protocell Architecture: Architectural Design* 2011).

Among other projects, sustainable innovator Rachel Armstrong imagines how protocells could be engineered to produce limestone-like substances and reinforce the wooden piles that support the city of Venice. Another project featured in the book is an architectural proposal for the 2010 Venice Biennale by a Canadian interdisciplinary team led by architect Philip Beesley. The proposal was for an interactive, responsive system that integrates lightweight kinetic scaffolding with the technology of biochemistry and synthetic biology – namely, protocell technology. Beesley explained that through slow biochemical processes, this active filtration system would gather toxins from the environment and convert them into hard substances like limestone and carbonates (Beesley and Armstrong 2011; Beesley 2009).

Another important recent publication dealing with integrating synthetic biology with design and architecture is the book *Synthetic Aesthetics: Investigating Synthetic Biology's Design on Nature*, which describes a project run by the University of Edinburgh and Stanford University that brought together synthetic biologists, designers, artists and social scientists to explore collaborations between synthetic biology, art and design (Ginsberg et al. 2014). The book raises important questions about the possibility of integrating synthetic biology with design and architecture. What does it mean to design with living things? How do designers and architects fit into the process of making machines with biology? Architects work with structural engineers, product designers work with mechanical engineers – what kind of new relationships could develop with synthetic biologists? (Ginsberg et al. 2014). The answers to these questions are still evolving, and I hope that my research will contribute to the discussion.

The most important aspect synthetic biology is that synthetic biologists treat life and its computational processes as inseparable from biological matter. Section 2.2 below focuses on the design with synthetic biology in details.

In this dissertation, I aim to provide some tools for the realization of the integration of computational processes within biological matter. My vision is to develop a methodology to design materials, components, and interfaces using programmed cells. Chapter 3 is dedicated to my experimental work toward the realization of this vision.

2.2 Nano-scale: Synthetic Morphogenesis

In this section, on the level of living cells, I discuss the design strategies that are being developed through the new cross-disciplinary field of synthetic biology. The molecular mechanisms on the nano-scale ultimately dictate the fate of cells, their organization, material production, and organization into larger structures. The molecular processes occurring within the cells define the properties of all the larger structures and materials they build (Harris 2000). This hierarchical principle of organization lies at the center of my interest in biologically active materials: large-scale material properties and function emerge from, and are dependent on, small-scale behavior of living cells and their response to their environment (Davies 2013).

Synthetic Gene Circuits

The conventional process can be thought of through an analogy to computer programming. Synthetic gene networks are built to carry out functions, similar to software applications, within a living cell or in a liquid solution, which is considered the "operating system" (Pardee et al. 2014). Synthetic gene networks allow to rationally design synthetic, biological mechanisms to carry out specific functions.

Concept 1: Parts.

This notion of genetic circuitry is the conceptual platform on which synthetic biology is being built. In the BioBricks scheme (Knight 2003) created by researchers at the Massachusetts Institute of Technology (MIT), genes may be combined and compiled into a Registry of Standard Biological Parts: an open-access catalogue of biological gene circuits that you can peruse just as you would a RadioShack catalog of electronic components to find the devices you need to realize your design. The aim is that with sufficient attention to standardization, these biological parts will work as "plug and play," without needing a lot of refinement and tuning for each application. Synthetic biologists develop biological devices such as synthetic gene networks that count (Friedland et al. 2009), synthetic gene networks that smell (Farzadfard and Lu 2017).

As discussed above, a sequence can be written in a form of script-code of the four letters A, T, C, G with a fairly long length (2000 characters per page, 3000 pages, and human genome is 1000 times longer) even for the simplest one-cell organisms (**Figure 2.2.1**).

```
ATGGGTGATGTTGAGAAAGGCAAGAAGATTTTTATTATGAAGTGTTCCCAGTGCCACACC
GTTGAAAAGGGAGGCAAGCACAAGACTGGGCCAAATCTCCATGGTCTCTTTGGGCGGAAG
ACAGGTCAGGCCCCTGGATACTCTTACACAGCCGCCAATAAGAACAAAGGCATCATCTGG
GGAGAGGATACACTGATGGAGTATTTGGAGAATCCCAAGAAGTACATCCCTGGAACAAAA
ATGATCTTTGTCGGCATTAAGAAGAAGGAAGAAAGGGCAGACTTAATAGCTTATCTCAAA
```

Figure 2.2.1: DNA base sequence, encodded as a sequence of four letters: A,G,C,T.

Synthetic biologists 'read' the DNA sequence in terms of its functional parts and interpret it in a schematic way (**Figure 2.2.2**).

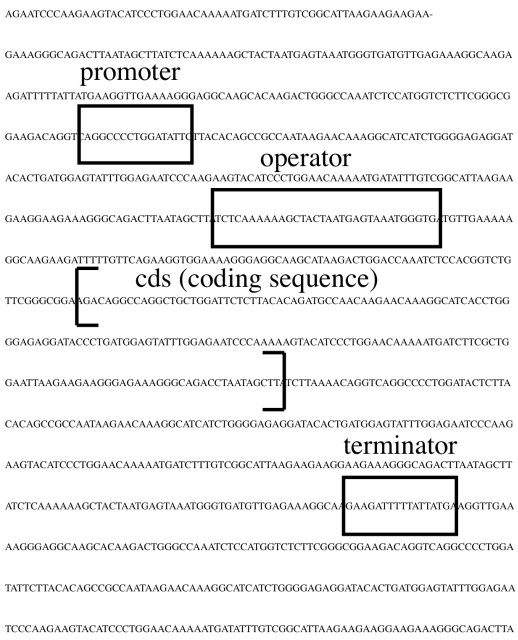
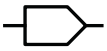


Figure 2.2.2: Functional DNA parts. An example of functional parts in a DNA sequence: promoter, operator, coding sequence (cds), and terminator.

Synthetic biologists decompose DNA sequences that appear in a particular order in a specific organism to then assemble them into a new synthetic gene circuits. Examples of basic circuit parts for design include *promoters*, *operators*, *coding sequences*, and *terminators*. To support the culture of synthetic biology in sharing parts and processes across the scientific community, a Synthetic Biology Open Language (SBOL) was developed (Galdzicki et al. 2014) (**Table 2.2.1**). Sharing functional biological parts enables modular

designs of synthetic gene circuits that are made accessible to designers who are not biologists through open access registries of the Registry of Standard Biological Parts (http://parts.igem.org/)

Table 2.2.1: Synthetic Biology Open Language (SBOL) for Standard Biological Parts.

Symbol	Part	Definition
	Promoter	A portion of a sequence recognized by cell machinery to start transcription
	Operator	A sequence of DNA to which a repressor binds to regulate gene expression
	Coding sequence (CDS)	A portion of a sequence that codes for a protein
	Terminator	A portion of a sequence recognized by cell machinery to start transcription

Concept 2: Rules and Models.

In addition to dissecting a DNA sequence into parts, synthetic biologists understand how these parts work together, which is defined by the rules that cells follow to make a sequence of parts functional. For this, synthetic biologists use rules (such as Boolean logic rules "gene on" or gene "off") and mathematical models to predict how genetic parts works in the cell.

Figure 2.2.3 below shows an example of a biological gene circuit and how the events of genetic activation unfold in three steps. First, gene A will be transcribed and then translated into protein A by cell machinery. Second, the protein A will bind to the DNA sequence at a specific site, depicted as a little square, or operator. Protein A is a sensory protein with activating functions. It is capable of sensing a particular chemical signal (signal x) that interacts with this protein and then activates a different promoter, the promoter for a gene B. Cell machinery will then bind to promoter B and transcribe protein B. This small schematic structure shows how a regulatory circuit gives instructions to the cell: start here automatically, make a protein, bind that protein that can intercept the signal, and then transcribe another protein. This shows a set of rules that are encoded in this DNA sequence.

In short, a computational rule by which this regulatory gene circuit operates can be described as follows: If signal x is present in the environment, make protein B. As with all genetic circuits, it links a state of cell and its production to the state of the environment outside the cell.

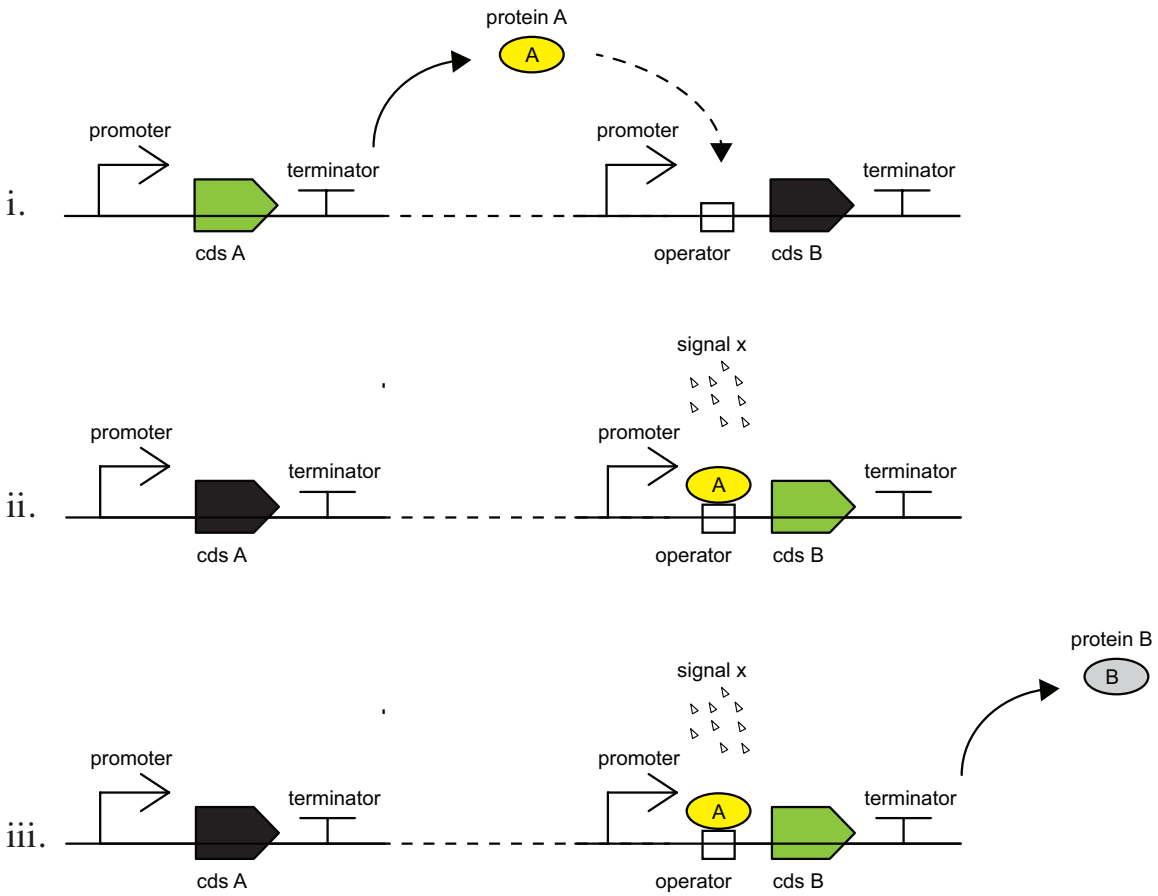


Figure 2.2.3: Schematic description of a regulatory gene network.

Figure 2.2.4 below gives general schematic description for the basic synthetic gene networks – constitutive, repressible, inducible, and a more complex toggle switch one.

Constitutive – in a constitutive network, the gene is always 'on'. Product A will be constantly produced, and its concentration will increase over time.

Repressible - in a repressible network, signal x inhibits the synthesis of product A from gene A, and product A will not be produced when signal x is present.

Inducible - in an inducible network, signal x activates the synthesis of product A from gene A, and product A will only be produced when signal x is present.

Bi-state toggle switch – this network encodes a more complex behavior. Product A inhibits synthesis of product B from gene B and is induced by signal x. Product B inhibits synthesis of product A from gene A and is induced by signal y. This system has two states: one is when product A is low and product B is high, and the other when product B is low and product A is high.

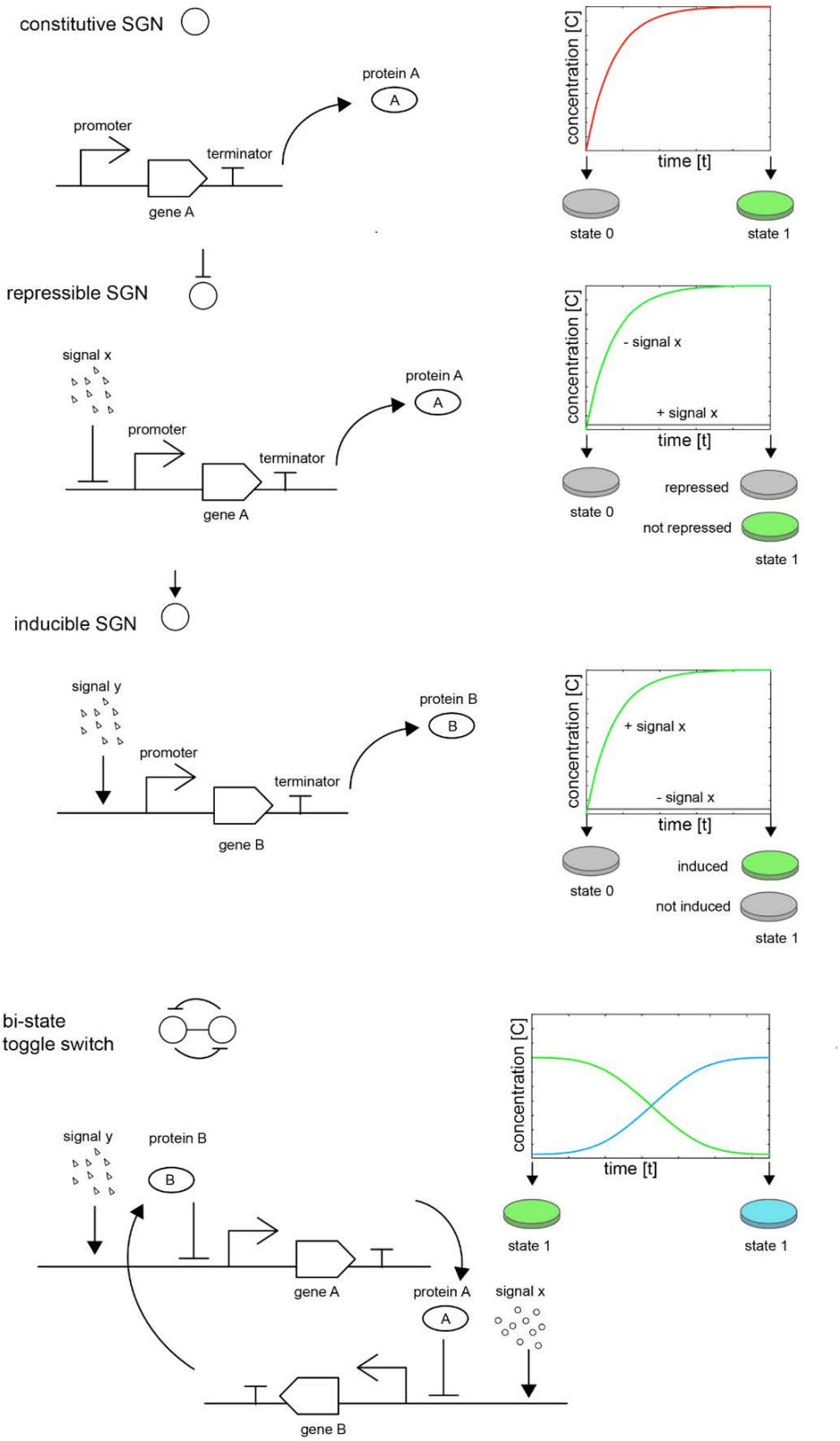


Figure 2.2.4: Synthetic gene networks (SGN) architectures: constitutive, repressible, inducible, and toggle switch.

Concept 3: Accessibility.

In addition to creating biological parts and devices, synthetic biologists make the design of biology accessible to non-expert designers by creating open libraries of parts, simplifying methods of work, and developing computational tools for simulation and modeling of biological devices. Beyond the production of molecules, synthetic biology is open for new applications and designs. To spread technology through standardization, a large enough community of users should adopt the new standards. To create such a community and to foster innovation with living organisms, iGEM, the International Genetically Engineered Machine, was created in 2004. iGEM is an international competition in synthetic biology for undergraduate students. For the last decade, this group has driven innovation and development in the field. The idea of iGEM is simple: a team of undergraduate students from various backgrounds is given a kit of biological parts with which it works throughout the summer to design new biological machines. Teams are encouraged to use BioBricks for their designs and also create and submit new BioBricks to the registry (Shetty, Endy, and Knight 2008; Knight 2003; Weiss and Knight 2000). In the fall, all teams gather in Cambridge to present their projects.

As each year more and more teams from all over the worlds participate in the iGEM and more and more BioBricks are submitted to the library, there are also more questions that arise both within and outside the field. Is synthetic biology simply a way of pumping more out of biology from what we already have? Are the new manufacturing methods as sustainable as they claim to be? Do we really need more and better fuel, plastic, and so on? Is this the time, perhaps, to challenge the new technology and the new possibilities it presents?

The geneticist, molecular engineer, and chemist George Church describes how Tom Knight, the originator of the standardization idea as applied to biology, became frustrated with the unpredictability of living organism behavior when he first began working with the stuff? of biology: “There was nothing routine or standardized in experimenting with them (organisms). It drove me crazy, as it would any self-respecting engineer.” Knight said, “The path forward was clearly to standardize the part definition and the assembly process.”(Church and Regis 2014). Drew Endy agreed: “I hate emergent properties. I like simplicity. I don’t want the plane I take tomorrow to have some emergent properties. I like to build stuff and biology is the best technology to build stuff: trees, people, computing devices, food, chemicals, you name it.” (Ginsberg et al. 2014)

Whether this vision of robust function and predictability yields expected results remains a question. “Evidently, putting biobricks together and getting them to work successfully would be slightly more challenging than anyone had thought,” Church admits. “Perhaps engineers need to learn to dream differently.” (Church and Regis 2014). Eugene Thacker is even more critical: “We are the victims of biotech Imagineering’ … There is blatant disparity between hyper-optimism and a lack of concrete

results." (Thacker 2003)

I propose that dreaming differently can come from the superposition of synthetic biology culture with another culture, the culture of design and architecture. With the development of synthetic biology, living matter is becoming more accessible for these other disciplines. As a result, designers and architects are becoming engaged with life as raw material for design (Ginsberg et al. 2014; Roosth 2017).

Recently some developments have increased the accessibility of synthetic biology to designers. Similar to iGEM, but coming from an art and design perspective, the Biodesign Challenge offers art and design students the opportunity to envision future applications of biotechnology. Students from top design schools around the country spend the semester envisioning how cells, microbes, and other living things can remake the products and processes of our made world. The Biodesign Challenge themes include architecture, water, food, materials, energy, medicine, and others areas where biological design could make a dramatic difference.

In addition to institutionalized initiatives, many community labs have emerged in major US cities that promote accessibility of biotechnology and synthetic biology to non-experts. To support this movement, and promote education for synthetic biology, a piece of desktop synthetic biology equipment is being developed (**Figure 2.2.5**).

Figure 2.2.5: Desktop synthetic biology equipment (retrieved from http://www.biofabricate.co/biofabricate-2016/).

2.3 Meso-scale: Design Principles for Structural Biological Materials

In this section, I discuss the unique features of materials with a biological origin, or biomaterials. I review the design strategies for multi-scale organization of biomaterials in nature in relation to their biological function, adaptivity, and their living component, namely their living cells. This section asks an important question – if we were to design living cells in the materials to make them bio-active, what would some of the characteristics of these materials be?

To answer this question, I take a look at the defining features of structural biological materials in nature. Most natural (or biological) materials are complex composites whose mechanical properties are often outstanding, considering the weak constituents from which they are assembled (Ortiz & Boyce, 2008; Meyers, Chen, Lin, & Seki, 2008). These are materials that are 'hard', with superior mechanical properties, generated by 'soft' living tissues. Some examples of these 'hard' biological materials are:

- Calcium phosphate: teeth, bone, antlers.
- Calcium carbonate (aragonite): mollusk shells, some reptile eggs; (calcite): bird eggs, crustaceans, mollusks.
- Amorphous silica: spicules in sponges, diatoms.
- Iron oxide (Magnetite-Fe3O4): teeth in chitons (a weird looking marine worm), bacteria.
- Collagen: organic component of bone and dentine, tendons, muscle, blood vessels.
- Chitin: arthropod and insect exoskeletons.
- Cellulose: plant cell walls, some bacteria biofilms
- Keratin: bird beaks, horn, hair.
- Elastin: skin, lungs, artery walls.

Of the above, iron oxide, carbon phosphate, calcium carbonate, silica, and iron oxide are minerals. Chitin and cellulose are polysaccharides. Collagen, keratin and elastin are proteins.

As a subject for this dissertation, I chose cellulose biofilm produced by bacteria. The reasons for this choice are summarized in the section 3.2 Bacterial Cellulose, below. In this material system living bacteria cells are directly integrated in the biofilm as it grows, and are relatively easy to genetically engineer so as to explore the relation between cell function and material properties. In this background section I would like to focus on a more general issue of the unique properties of structural biological materials and the processes of their formation. In Chapter 3, I propose ways to use DNA design and synthetic gene networks to pattern and build up these hierarchical materials.

The defining features of these materials are that they are self-assembling, multi-functional, self-healing, and hierarchical. Below I discuss these features in more detail.

Self-assembling

Shell formation is a good example of the biological mechanism of adaptive self-assembly, where the construction of inorganic materials occurs through organic processes. In addition, shells are a good example because they occupy an iconic status within design and have been used as an emblem of scale and proportion. Moreover, shells have provided the material basis of many types of building materials.

An abalone constructs its shell by first forming soft tissues through morphogenesis, a process by which the organism's cells organize into tissue patterns (**Figure 2.3.1**). The organism does not build its shell directly. Instead, the tissues act like a scaffold that makes it possible for the abalone to alter the chemical composition of its outer surface, inducing the calcium in its environment to combine with carbon and crystallize through the process of *biomineralization*. Furthermore, by altering the chemical environment through the addition of extra cellular substances, the shell shifts between different crystalline structures, from an inner layer of flat, plate-like crystals (sometimes referred to as mother of pearl) to the outer layer, which is made up of vertical crystals that create an exceptionally strong surface (Cartwright and Checa 2007). By controlling this process, the abalone assembles a single material with properties of a composite material and creates a structure with significant strength, all with very little expenditure of energy.

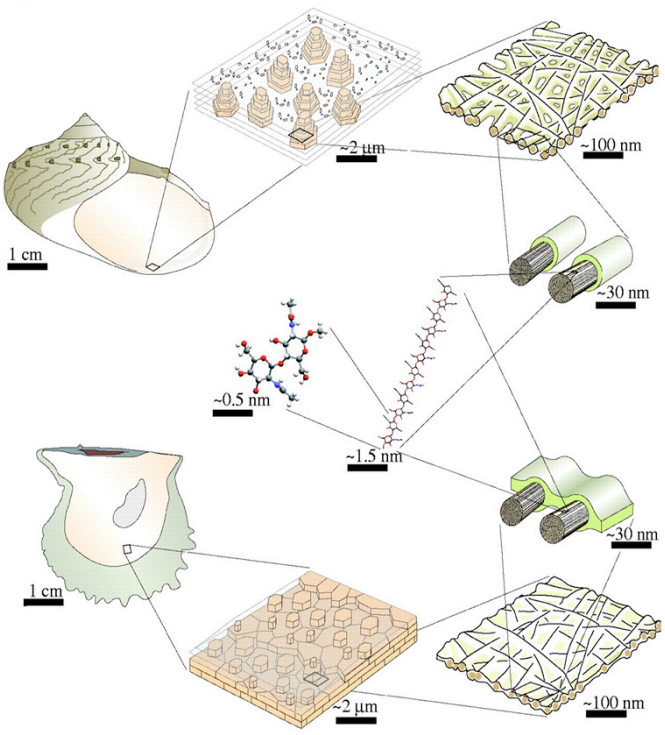


Figure 2.3.1: Guided biomineralization in the abalone shell. Adopted from (Cartwright and Checa 2007).

Multifunctional

Most biological materials are multifunctional (Marc A. Meyers et al. 2011), they accumulate functions:

(a) Bone: structural support for body plus blood cell formation.

(b) Chitin-based exoskeleton in arthropods: attachment for muscles, environmental protection, water barrier.

(c) Sea spicules: light transmission plus structural support.

(d) Tree trunks and roots: structural support and anchoring plus nutrient transport.

(e) Mammalian skin: temperature regulation plus environmental protection.

(f) Insect antennas: mechanically strong and self-repair. They also detect chemical and thermal information from the environment. They can change their shape and orientation.

Self-healing

Another defining characteristic of biological systems, in contrast with current synthetic systems, is their self-healing ability. This is nearly universal in nature. Most structures can repair themselves after undergoing trauma or injury. For example, bone, skin, and muscle undergo constant cycles of breaking down and regenerating, to prevent accumulation of defects due to aging and fatigue (Brochu, Craig, and Reichert 2011). A new field of materials science is developing biomimetic self-healing polymers (Wu, Meure, and Solomon 2008). In general, these materials have a three stages: first, actuation; second, transportation of materials to damaged areas; and third, the chemical repair process. However, in biomimetic polymers the material doesn't go back to its original mechanical properties and to its original strength, since flaws are introduced during the repair process. Biologically active materials have the ability to restore to their pristine condition.

Hierarchical

In bio-materials, shape and microstructure emerge together during the growth process. Growth implies that "form" and "microstructure" are created in the same process. The subject of functional adaptation was pioneered by D'Arcy Thomson, relating the "form" (or shape) of biological objects to their function (see Section 2.1). The shape of a branch is created by the assembly of molecules into cells, and of cells to wood with a specific shape. Hence, at every size level, the branch is both form and material – the structure becomes hierarchical (Fratzl and Weinkamer 2007b).

For example, bone is built from two types of material: on the surface there is a compact tubular arrangement with blood vessels passing through (osteons) and in the middle, looser spongy material (**Figure 2.3.2** upper row). On the next level, osteons have a layered structure, with each layer consisting

of fibers arranged in geometrical patterns. On the next level, each fiber is comprised of several mineralized collagen sub-fibers with a triple helical geometry.

Bamboo has a radial density gradient that increase its flexural rigidity with hollow tube cross-sections (**Figure 2.3.2** bottom row). This honeycomb arrangement is composed of cellulose fibers embedded in a lignin–hemicellulose matrix shaped into hollow prismatic cells of varying wall thickness. Each fiber is comprised of a bundle of sub-fibers of cellulose.

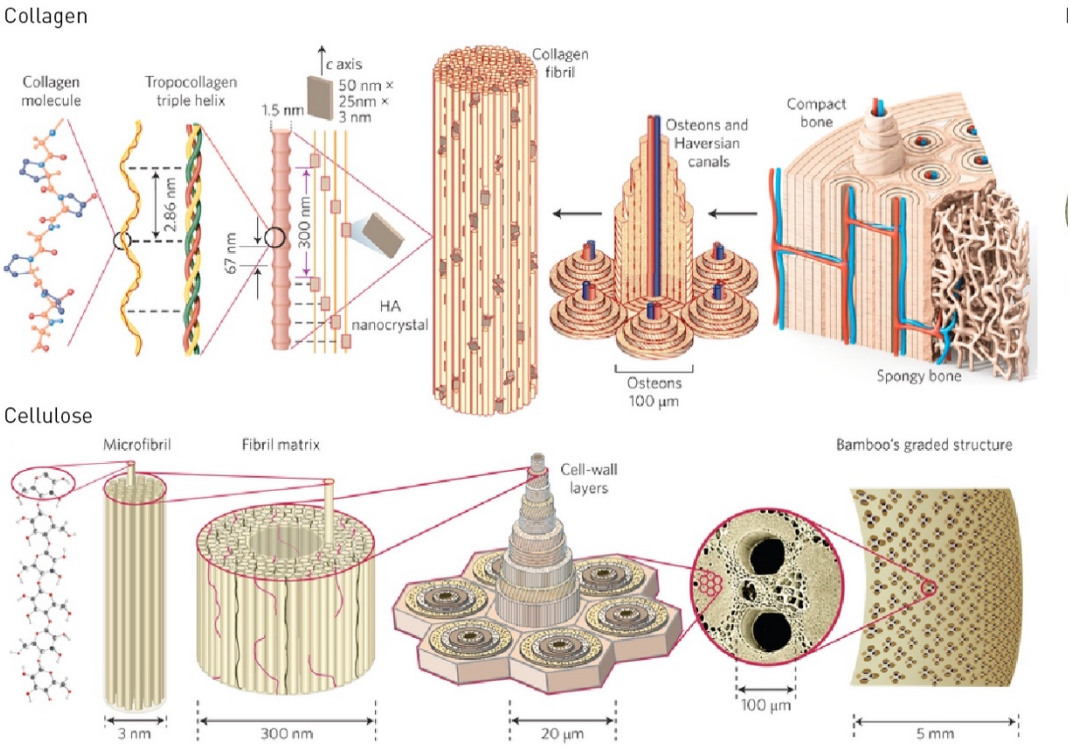


Figure 2.3.2: Hierarchical organization of structural biological materials. Adopted from Wegst, Bai, Saiz, Tomsia, and Ritchie, 2015.

One of the biggest obstacles to taking full advantage of what nature has to offer is the complexity of its construction. There is no assembly as elaborate, versatile, and adaptive as that of the living cell. Despite advances in digital fabrication in general, and additive manufacturing in particular, the principles that give biological materials their superior properties are extremely difficult, if not impossible, to replicate with man-made fabrication methods.

Biological materials have traits that are impossible to replicate with synthetic materials. Efforts in materials science to mimic these properties are often "biologically inspired," but they lack the

sophistication and ability to adapt that characterize biological materials. Biological materials are composed of some combination of cells, biopolymers (e.g., cellulose, collagen, and keratin), and minerals (hydroxyapatite, calcium carbonate, and silica). Living systems assemble these components using a process of guided, feedback-controlled self-assembly (Davies 2013) at the molecular, cellular, and macro scale. As shown in the examples above, these multiple scales of feedback mean that biomaterial structure is universally hierarchical (Fratzl and Weinkamer 2007a).

As the principles of materials' organization in nature become better understood, opportunities arise to engineer, program, grow, and maintain biological systems with complex structures. The field of synthetic biology is uniquely positioned to enable these efforts. The key to this synergy is the conception of synthetic biology as an engineering discipline (Cartwright and Checa 2007) (Arpino et al. 2013) that combines traditional engineering concepts such as reusable parts, modularity, and abstraction, with novel rules specifically suited to engineering biology (Slusarczyk et al. 2012). These concepts have served to propel the field's rapid development. In the last decade, synthetic biology has progressed from simple bacteria systems (Elowitz and Leibler 2000; Gardner et al. 2000) to sophisticated synthetic gene networks in almost every branch of life, including plants (Schaumberg et al. 2015) and mammalian systems both *in vitro* (Xie et al. 2011) and *in vivo* (Ye et al. 2011).

As the tools of the discipline become more powerful, they are beginning to enable the construction of systems that have dynamic behavior and nontrivial emergent properties similar to those of natural morphogenetic processes. Synthetic gene circuits can detect a cell's type (Miki et al. 2015), metabolic state (Callura et al. 2012), (bio-) chemical signals (Weber et al. 2007), and light (Muller et al. 2014; Schmidl et al. 2014). They can use these inputs, combinatorially or sequentially, to alter the cell's shape (Yeh et al. 2007), motility (Park et al. 2014), differentiation program (Wamaitha et al. 2015; Guye et al. 2016), or even kill the cell outright (Xie et al. 2011). Synthetic intercellular signaling allows cell populations to make decisions and coordinate behaviors both locally (Sprinzak et al. 2010; Matsuda et al. 2012) and globally (Tabor et al. 2009; Prindle et al. 2011; Chen et al. 2015b). These diverse sensors, actuators, and communication channels could implement complex morphogenic systems through a combination of top-down approaches in which cells are patterned by external signals, and bottom-up programs in which collective properties emerge through cells' local decision-making. The key insight is that the assembly of biomaterials, like most morphogenesis, is guided by interlocking molecular feedback loops at multiple length scales (Davies, 2013).

In the next section I propose methods of synthetic biology, in particular the field of synthetic morphogenesis, as a way to program materials that are not only hierarchical and have superior mechanical properties, but are also programmable, regulated, and bio-active.

2.4 Macro-scale: Biologically Active Materials for Architecture

This section answers the question of why architects should bother to design with living materials. In other words, what are the benefits of introducing biological materials to architectural construction? The goal of this section is to show the reader the relevance of bio-active materials to architecture through a sequence of projects in the areas of architecture and closely related fashion and product design. These projects demonstrate emerging efforts to search for material-based approaches that have positive impacts on the environment.

The design of biologically active materials requires development on the nano and micro-scales, both very far from the traditional disciplinary domain of architecture. By briefly reviewing the history of the relation between biology and architecture, and by reviewing projects in architecture, design, and fashion from the last decade, I hope to demonstrate the shift in architectural thinking that supports my multi-scale design approach. Design with biology in architecture is shifting from borrowing ideas and solutions from nature to using actual living mechanisms. This dissertation develops methods and processes to realize this vision. In the following sections I focus on biological materials and propose processes of synthetic morphogenesis as the next step for material innovation for architecture.

I will first review projects from the last decade that represent the current material-based approach in design with biology. I divide the projects into three groups. The first group of projects – Projects 1, 2, 3, and 4 – deal with *bio-materials*, where living, responsive organisms are used in fabrication methods. After the fabrication is completed, the material is rendered inert, and the bio-responsiveness is lost. The second group of projects showcases *bio-responsive* materials. This group – Projects 5, 6, and 7 - is different from the first in that properties of materials are integrated as a function of the architectural element itself. This group demonstrates design processes that take into consideration material behavior and integrates responsiveness of the final architectural element.

The third group of projects – Projects 8, 9, and 10 - deals with *biologically active materials* and takes full advantage of living organisms' biological functions. Here, the material system incorporates the living organism as part of its functionality. The living agents within the material system are sensitive to biological signals, and interact with or are actuated by them, with the potential of acting upon their environment and transforming it through chemical, biological, mechanical changes (Y. Lu et al. 2016). The difference here between biologically active materials and bio-materials or bio-responsive materials is that with biologically active materials a signal not only causes a change in the material itself, but also triggers/activates a biochemical process that transforms the environment. In such a process, materials are taken from the environment, produced and released into the environment, and energy is consumed. There is thus a tremendous potential here not only to reduce the carbon footprint and decrease the overuse of

natural resources and harmful waste, but also to create a positive impact on the environment because of the biochemical processes of living cells. However, today there is a gap between the vision of using biologically active materials for architecture and the actual methods and processes for realizing such work. The one, full scale architectural example that exists to date is the algae façade system of the BIQ house (Project 8), which demonstrates the possibility of using a living biomass (algae) and its biochemical processes to power a building (Holopainen et al. 2016). Projects 9 and 10 exemplify developments at the intersection of synthetic biology and architecture that allow programming living cells to perform functions, such as photosynthesis and chemical recognition, which have the potential for healthful energy generation for the environment.

Group One: Projects 1-4 (Bio-Materials)

Here, I define bio-materials as all materials of biological origin that either are, or used to be, a part of living organisms or were produced by them (Fernandez 2006). This definition differs from the common scientific definition of biomaterials as any substance that has been engineered to interact with biological systems for a medical purpose. In this section, I show projects that demonstrate the use of bio-materials in architecture and fashion and the use of living organisms in the fabrication process. All of these materials are benign, pose no threat to human health or the environment, can be locally sourced, and are renewable (Fernandez 2006). Projects 1 and 2 show grown materials that are fully developed technologies certified for use in architectural construction and available on the market (**Figure 2.4.1**). The third and fourth projects show the creative use of a biological process (silk fiber spinning by silk worms; bacteria grows sheets of cellulose) as a fabrication method. The benefits of using living organisms in fabrication of bio-materials are threefold: using locally sourced materials (such as the aggregates in Projects 1 and 2) avoids transportation of materials which lessens pollution; fabrication processes such as the kiln firing in traditional masonry avoid high-energy coasts, and material returns safely to the ecological cycle of natural materials at the end of the process.

Figure 2.4.1: Group One: Projects 1-4 (Bio-materials): Using living organisms as a fabrication method.

Project 1: Grow Bricks by BioMason, 2012 (Dosier 2014)

Organism: Calcite-precipitating bacteria from a genus Bacillus.

Process: Bacteria produces enzyme that facilitates calcium carbonate deposition and fuses aggregates such as sand or pebbles at room temperature.

Fabrication: A bacteria culture is mixed with aggregates such as sand or pebbles; the wet bio-cement mix is then poured into brick molds. Hydraulic press and vibration are applied for 3-4 days at room temp (incubation), enabling calcium carbonate deposition to occur. Several bricks from each batch are then tested in compression to meet industrial standards.

Responsiveness: During the fabrication process; changes in growth process of bacteria and incubation conditions affect the mechanical performance of the bricks.

Additional science: This project uses the biochemical process of common soil bacteria to fuse aggregates (De Muynck, De Belie, and Verstraete 2010; Hills et al. 2016). The grown brick method is developed as an alternative to traditional masonry methods, saving energy in two ways. One is that the biochemical process occurs at room temperature and avoids burning fossil fuels in kilns; the other is that calcination, a chemical process triggered by heating the limestone to split it into calcium oxide and carbon dioxide (CO_2), eliminates emissions. Beyond neutralizing carbon emissions, there is an opportunity to use the same biological process to create bio-functional, self-healing materials. One example is a self-healing concrete that is being developed by researchers at Delft University (Wiktor and Jonkers 2011).

Project 2: Hy-Fi Pavilion by The Living, 2014 (Benjamin 2017)

Organism: Fungus

Process: Fungus grows mycelium, a network of fungal threads that fuses together agricultural waste into solid bricks.

Fabrication: Agricultural waste is purchased from local farmers and mixed with fungi; it is then placed in molds. The mycelium grows for several days before a drying mixture is introduced to kill fungi and spores. The final product is used like a traditional brick.

Responsiveness: During fabrication process; simulation of pavilion structural performance provided feedback for fabrication of blocks. Changes in growth process of the mycelium allows control over the strength, flexibility, and even water-resistance of the bricks.

Variable properties: a range of different material properties can be produced by regulating mycelium growth conditions, from dense sheets to lightweight foams.

Additional science: Over the last decade, a wide variety of products have been developed. For example, Ecovative produces a range of materials such as engineered wood boards and insulation foams, using fungal mycelium growth (Consulting 2017), Mycoworks produces flexible leather-like materials ("MycoWorks: Redefining Leather" 2017), and a NY-based designer makes curved lamp shades ("Lighting" 2016)**.** Another project proposes 3D printing straw substrate for mushroom growth (Eric Klarenbeek, 2013). Beyond their use in making structural bio-material**,** fungi's bio functions could be used to remediate environmental problems. Fungi have a highly evolved external digestive system that can decompose plastic, soak up toxic heavy metals including lead, arsenic and mercury with no apparent side effects, and even neutralize radioactive waste (Stamets 2005).

Project 3: The Silk Pavilion by the Mediated Matter Group at MIT, 2013 (Duro-Royo and Oxman 2015)

Organism: Silk worm

Process: Silk worms are purchased/grown and then released on a frame to spin their silk on the frame as they move from bottom to top searching for a corner to build their cocoons.

Fabrication: A metal dome is assembled that acts as a frame: a thread network is built on the frame using computationally controlled robotic arm. Silk worms are then released on the bottom of the frame; as they move from bottom to top, they spin the silk on the network of threads.

Responsiveness: During fabrication process the worms respond to the geometry of the surface they move upon, searching for a corner to make a cocoon. Post fabrication material is inert.

Variable properties: Variable density and properties of silk fiber network will result from behavior of silk worms and their movement on the frame.

Additional science: The mechanism of silk production and spinning has been transformed to host a variety of organisms, such as yeast, mammalian cells, and goat.

Silk fibers are known for their excellent mechanical properties comparable to Kevlar fibers. The properties of fibers are the result of the hierarchical organization and unique spinning mechanism of the silk worm that are difficult to replicate synthetically. Researchers develop soluble silk fibers for medical applications and beyond (Kaplan et al. 2009; Omenetto and Kaplan 2010; Vepari and Kaplan 2007). Recently, a method for 3D printing aqueous silk is being developed (Mogas-Soldevila and Oxman 2015).

Project 4: Biocouture by Suzanne Lee, 2010 (Lee, du Preez, and Thornton-Jones 2005)
Organism: A kombucha mix of microorganisms including cellulose-producing bacteria Gluconacetobacter *xylinus*.
Process: A kombucha scoby (an initial microorganism culture within a cellulose biofilm) is introduced into a growth medium (a mix of sugar and green tea); the culture grows to form a cellulose sheet on the surface of the liquid; the cellulose sheet is then harvested, washed and dried. The resulting material is used to sew clothes.
Fabrication: The material is biologically grown instead of fabricated.
Responsiveness: The responsiveness of the cellulose material to its growth environment will be discussed in details in the experimental chapter, Chapter 3.
Variable properties: The properties of cellulose are determined by the biological process of growth and vary based on the chemical (such as growth media composition and acidity) and physical (such as temperature and turbulence).
Additional science: Please see the Chapter Three for my work with this material system.

Group Two: Projects 5-7 (Bio-Responsive Materials)

Bio-responsive materials can change their properties or shapes in response to signals in the environment. Signals in the environment to which materials respond include pH, toxins, mechanical cues such as pressure, temperature (polymers undergo phase change in response to pressure), sunlight, and so on. Materials respond to humidity change either by opening for ventilation (Projects 5 and 6) or by evaporating water (Project 7) (**Figure 2.4.2)**.

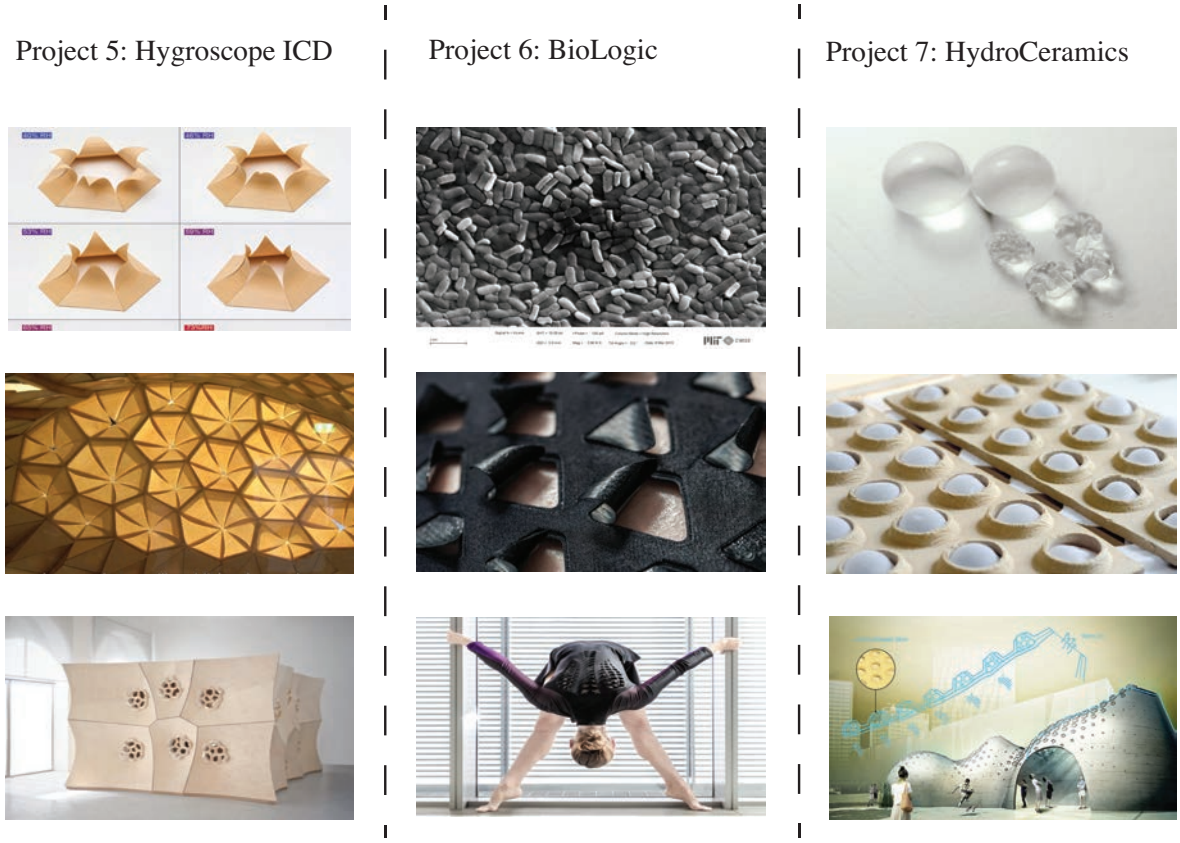


Figure 2.4.2: Group Two: Projects 5-7 (Responsive Bio-materials): Bio-materials that change their properties or shape in response to signals in the environment.

Project 5: HygroScope: Meteorosensitive Morphology by Achim Menges and Steffen Reichert at the Institute for Computational Design (ICD), 2012. (Menges and Reichert 2012)

Bio-responsive material: Thin wooden plates, a combination of maple veneer and synthetic composites.

Local responsiveness: Based on the level of humidity in the environment, water molecules penetrate the wood tissue and change the distance between microfibers in the wood cell tissue, resulting in both a change in strength and a significant decrease in overall dimension. The design of elements, their dimensions, shapes, and fiber orientations allow the use of the hygroscopic (humidity-absorbing) behavior as a passive actuation mechanism that does not require any energy or mechanical elements.

Global responsiveness: The surface or façade of a pavilion responds to a change in humidity in the environment. The flower-like surface elements open and close in response to humidity changes.

Variable properties: Fiber directionality, length-width-thickness ratio, geometry of the elements, and humidity during the fabrication process are digitally computed and fabricated.

Use of computation: For this project the computational design research and the related development of the generative code is as important as the material system research. The way machine computation is used to generate the system is directly related to the way material computation is employed to enable the

system's responsiveness. The code simulates the material behavior and correlates its parameters: shapes, dimensions, fiber orientations, and humidity during fabrication are tuned to the desired response to humidity levels of the final element.

Additional info: This project exemplifies the climate responsive architectural systems that do not require any sensory equipment, motor functions, or even energy. Here, the responsive capacity is ingrained in the material's hygroscopic behavior and anisotropic characteristics. A similar approach is taken in the collaborative project between ICD and the Self-Assembly Lab at MIT, where computation is used to program the responsive behavior of material post-fabrication, through material structure, composition and direction-dependent 3D printed layering (Correa et al. 2015).

Project 6: BioLogic by Tangible Media Group at MIT Media Lab, 2015 (Yao et al. 2015)

Bio-responsive material: *Bacillus Subtilis* endospores, a dormant life form of bacteria.

Process: A liquid cell culture is applied to a thin (0.2mm) silicone substrate; water is vaporized to obtain the composite membrane.

Local responsiveness: Bacteria cells absorb water from the environment and change dimensions. While bound to a substrate, humidity causes the entire surface to bend, acting as a passive actuation mechanism.

Global responsiveness: The surface (or a garment) responds to a change in humidity in the environment. The synthetic bio-skin reacts to body heat and sweat, causing flaps around heat zones to open, enabling sweat to evaporate and cool the body.

Variable properties: The properties of the substrate, such as its elasticity and thickness, the concentration and application area of the cell.

Use of computation: Cell are deposited via custom-built 3D printer. The team uses computation to translate the relation between the shape and material parameters and the responsive behavior into surface design: 1D linear transformation, 2D surface expansion and contraction, 2.5D texture change, and 3D folding.

Additional info: This project exemplifies hygromorphic behavior of cells for responsive surface actuation. Although this project uses bacteria cells, there is no use of their biological function and biochemical processing; only the mechanical properties of the cells are used, their water-absorbing and shape-changing passive behaviors.

Project 7: Hydroceramics by Institute of Advanced Architecture of Catalonia, 2014 (Decker 2016)

Material: Hydrogel (not a bio-reponsive material, but could incorporate living cells)

Process: Hydrogel elements are nested on a stretchable fabric and clay substrate.

Local responsiveness: When temperatures rise, water evaporates from the hydrogel and cools the

environment. Porous clay absorbs water to create larger cooling surface area.

Global responsiveness: The composite system creates a passive cooling mechanism for an architectural façade.

Use of computation: Computational set up was used to test performance of different materials.

Additional info: This project exemplifies a passive cooling mechanism that relies on material properties of hydrogel. Although no bio-material was used in this project, the use of hydrogel for architectural applications is important to the scope of this research. Because of their high hydroscopic properties, some hydrogels can hold water up to 500 times their weight, and create aquatic environment that can support viability and biological function of engineered living cells.

See Project 10 below for a potential use of hydrogel materials to support the biological function of synthetic living cells.

Group Three: Projects 8-10 (Bio-Active Materials)

The third group of projects, *Bio-Active Materials*, is the most relevant to the scope of this research, because I design this type of material. Bio-active materials are sensitive to biological signals and interact with or are actuated by them; they have the potential to act upon their environment and transform it through chemical, biological, and mechanical changes (Y. Lu et al. 2016). The difference between bio-responsive materials and biologically active materials is that in the latter, the signal not only causes a change in a material itself, but also triggers/activates a biochemical process that transforms the environment. In such a process, materials are taken from the environment, produced and released to the environment, and energy is consumed (**Figure 2.4.3**).

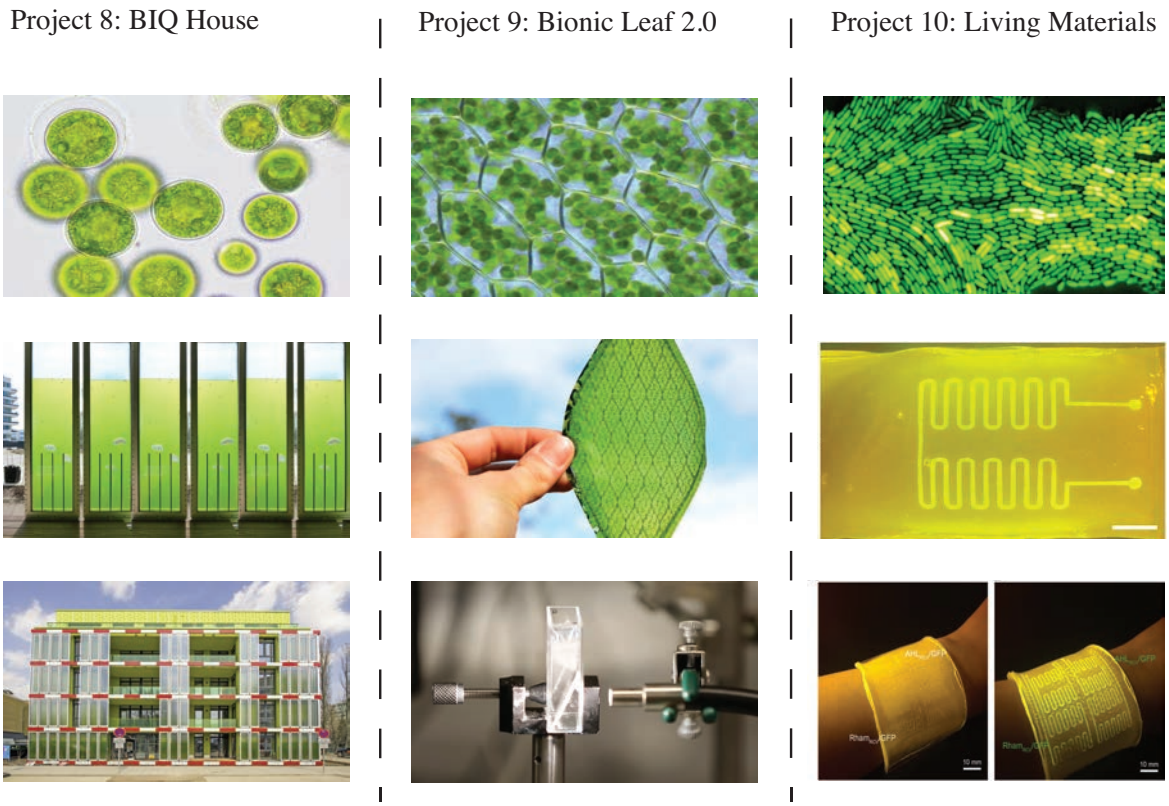


Figure 2.4.3: Group Three: Projects 8-10 (Biologically Active Materials): Act upon and transform their environment in response to signals.

Project 8: Solar Leaf Bioreactor Façade at the BIQ House by Strategic Science Consult of Germany (SSC), Colt International and Arup, 2013 (Wurm and Pauli 2016)

Bio-active material: Living algae

Process: Flat bioreactors are installed on a building façade, each with a 24-liter capacity, using insulation and antireflective glass to minimize heat loss. Compressed air is introduced to the bottom of each bioreactor at intervals; the gas generates an upstream water flow and turbulence to stimulate the algae to grow and take in CO_2 and light, transporting the biomass and heat generated by the façade by a closed loop system to the building's energy management center. The biomass is then harvested through floatation (chemical treatment is used to separate the biomass from water by floating it to the surface) and the excess heat is used to supply hot water or heat the building, or stored for later use.

Local responsiveness: Because microalgae absorb daylight, bioreactors can also be used as dynamic shading devices. The cell density inside the bioreactors depends on available light and the harvesting regime. When there is more daylight available, more algae grows – providing more shading for the building.

Use of bioreactors: One hundred and twenty-nine bioreactors measuring 2.5m x 0.7m and made from structural glass are installed on building facades. SolarLeaf integrates all servicing pipes for the inflow

and outflow of the culture medium and the air into the frames of its elements. It provides around one third of the total heat demand of the 15 residential units in the BIQ house.

Additional info: This is the first façade system in the world to cultivate micro-algae to generate heat and biomass as renewable energy sources. The bio-active façade aims to create synergies by linking different systems for building services, energy and heat distribution, diverse water systems, and combustion processes. The key to a successful implementation of photobioreactors on a wider scale will be cooperation between stakeholders and designers. It is a technology that benefits from strong interdisciplinary collaboration, combining skills in environmental design, façades, materials, simulations, services, structural engineering and control systems.

Project 9: Bionic Leaf (Yuan, Ye, and Li 2014)

Water splitting–biosynthetic system with CO2 reduction efficiencies exceeding photosynthesis

Bio-active material: Bacteria *Ralstonia eutropha***;** Hybrid inorganic-living system; the bacteria consume H_2 and synthesize biomass and fuels.

Process: Water is split chemically into oxygen and hydrogen; bacteria uses the hydrogen to synthesize biomass and fuels.

Additional science: Photosynthesis fixes CO_2 from the air by using sunlight. This project demonstrates the potential of hybrid synthetic-living materials for building construction, as it is scalable and even more efficient than photosynthesis in nature, and can be used to fix CO_2 and reduce greenhouse effect. In natural systems, energy conversion processes limit the overall efficiency of photosynthesis (Blankenship et al. 2011). Most plants do not exceed 1%, and microalgae grown in bioreactors do not exceed 3%; however, efficiencies of 4% for plants and 5 to 7% for microalgae in bubble bioreactors may be achieved in the rapid (short-term) growth phase (Blankenship et al. 2011). Artificial photosynthetic solar-to-fuel cycles may occur at higher efficiencies (Luo et al. 2014), This hybrid living-nonliving system has a CO_2 reduction energy efficiency of ~50% when producing bacterial biomass and liquid fuel alcohols, producing 180 grams of CO_2 per kilowatthour of electricity. Coupling this hybrid device to existing photovoltaic systems would yield a CO_2 reduction energy efficiency of ~10%, exceeding that of natural photosynthetic systems.

Project 10: Living Material by Zhao Lab and Lu Lab at MIT (Liu et al. 2017)

Bio-active material: E. Coli bacteria genetically engineered to sense toxins in the environment and omit fluorescent light.

Process: Programming bacteria; fabricating hydrogel sheet with internal channels using 3-D printing and micro-molding techniques; fusing the hydrogel to a layer of elastomer, or rubber, that is porous enough to let in oxygen; injecting bacteria cells into the hydrogel's channels

Variable properties: The thickness of the hydrogel layer to be used, the distance between channels, how to pattern the channels, and how much bacteria to use.

Additional science: This project responds to the challenge of maintaining those living cells within the material to keep them viable and functional. Cells require humidity, nutrients, and oxygen. The tough, highly stretchable, biocompatible material hydrogel is made from a mix of polymer and water and contains up to 95 percent water, providing an environment suitable for sustaining living cells. The material also resists cracking even when repeatedly stretched and pulled — a property that could help contain cells within the material (Yuk et al. 2016).

Discussion

My Guided Growth approach to designing bio-active materials for architecture integrates many aspects of the projects reviewed above. The focus of my work is the same bacterial cellulose biofilm as in Project 4, where it is grown into sheets for sewing clothes. I guide the growth of biomaterial with a digitally fabricated scaffold similar to the Silk Warm Pavilion in Project 3. Unlike Projects 1 and 2, where bacteria is used to 'glue' the locally sourced aggregates, in the bacteria system I work with, the structural component, cellulose biofilm, is produced by bacteria cells directly.

Although the main aspect of biological function and responsiveness that I discuss in my thesis is a result of the engineered biochemical processes within the biofilm, I also explore some aspects of 'passive responsiveness' that occur because of the hydroscopic, or water absorbing capacity of the cellulose hydrogel (see Section 4.3 below). The approach I am proposing is similar to the one used in Project 8. However, in Project 8, the algae are used in their native state and require high maintenance in order to be applied to architecture (glass construction, cleaning, overgrowth). My research envisions the ability to design synthetic materials systems that will be engineered for specific functions as construction materials, such as the living hydrogel system in Project 9 or the Bionic Leaf in Project 10.

The next section focuses on design strategies to intimately integrate the structural, shape and material-forming aspects of biological materials – their morphogenic aspects – with their biochemical activity.

CHAPTER THREE:

GUIDED GROWTH OF BACTERIAL CELLULOSE BIOFILMS

A note on collaborations.

In this note I would like to acknowledge my collaborators, who helped me perform the experimental and design work on this project. The synthetic biology work was conducted in the Weiss Lab for Synthetic biology at the MIT Center for Synthetic Biology under the supervision of Professor Ron Weiss. The experiments were conducted with Trinh Nguyen, who worked on this project under my mentorship as part of the undergraduate research opportunities program (UROP). The work on the in-situ and post-growth molding with a former MIT PhD student in the Design and Computation group in the Department of Architecture Sergio Araya, for the last seven years a Professor at the Universidad Adolfo Ibanez, Chile. The work on the meso- and macro- scales experiments was done in collaboration with a former MIT Master's student in the Design and Computation group in the Department of Architecture Merav Gazit, who also developed the pneumatic actuation and the custom-made electronic components for this project. To acknowledge these collaborations, I use 'we' when discussing the experiments.

3.1. Guided Growth Design Process

This research explores synthetic biology as a computational method to program cells to grow biologically active and adaptive building components for architecture, with the possibility of designing them for new functions, including air filtering and purification, self-repair, and photosynthesis. My vision is to create hybrid materials composed of engineered living cells and non-living scaffolds that support the cells' long-term viability and continuous exchange of matter and energy with their environment. To realize the potential for self-assembling, self-healing, adaptive, and biologically active materials, I propose a Guided Growth design process using the bacterium Gluconacetobacter *xylinus* and the re-programming of its living functions. In Guided Growth, using methods of synthetic biology, I combine methods of genetic computation with environmental regulation and scaffold design. This methodology can be further generalized to other material systems where bacteria act as a matter-organizing agent. The experimental work is conducted on three levels of resolution that complement each other in one continuous Guided Growth design process as described on **Figure 3.1.1** below:

Nano-scale: The nanometer scale of DNA design. Using synthetic gene networks, we regulate and guide the natural process of biofilm formation by cellulose-producing bacteria Gluconacetobacter *xylinus* to produce self-assembling biologically active biofilms with tunable structure, properties, and

function.

Meso-scale: The micro- to centimeter scale of shaping and patterning materials through regulated material self-assembly. We regulate the physical and chemical parameters of the growth environment to tune the composition and properties of biofilms as they grow.

Macro-scale: The centimeter and up scale of regulating the growth environment. We design and fabricate a macro-fluidic pneumatic scaffolding that allows a computationally-regulated flow of nutrients, added substances, and air for biological growth and material processing.

As shown schematically in **Figure 3.1.1,** design strategies of the next scale of resolution are embedded in the previous one.

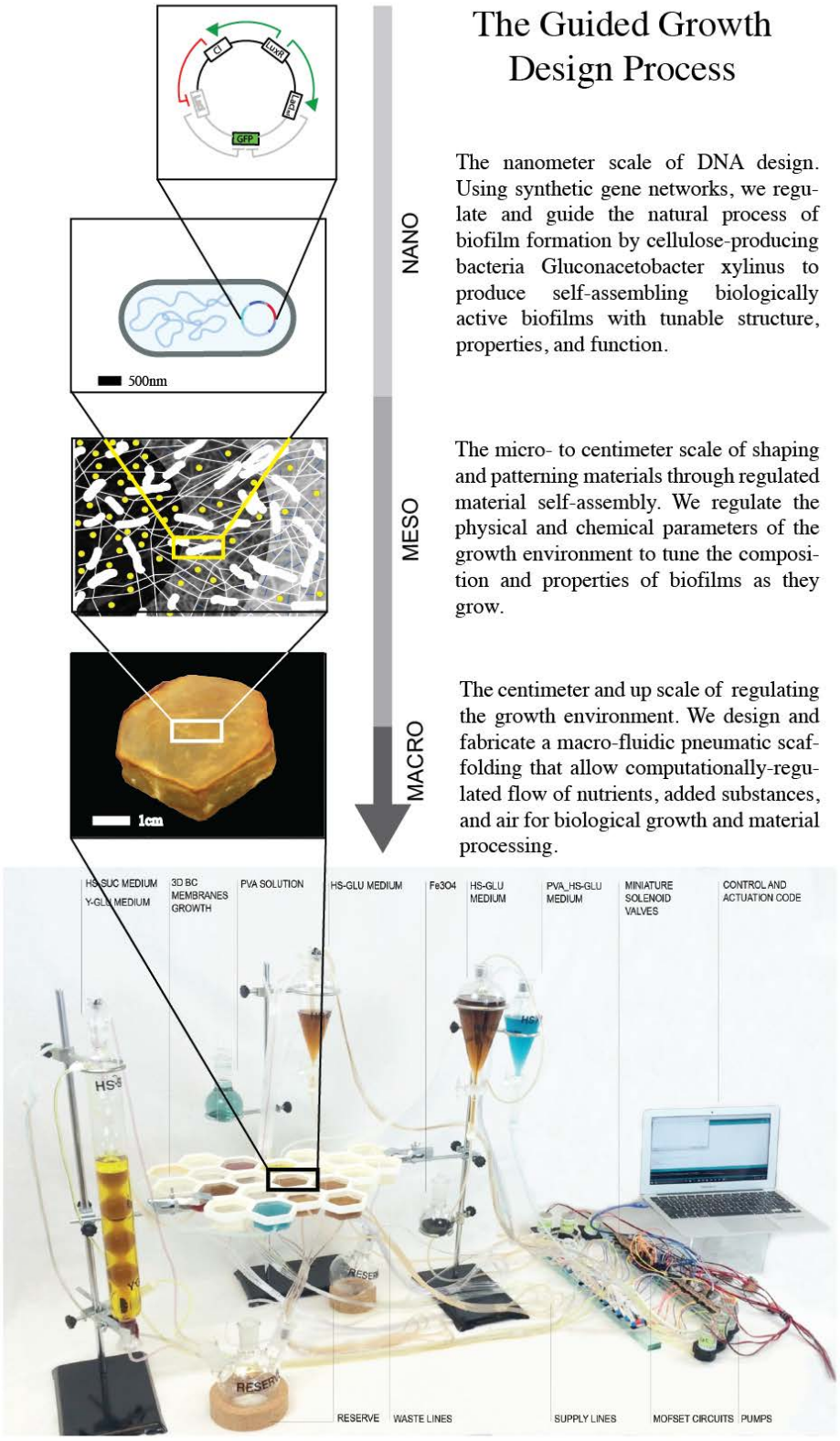


Figure 3.1.1: Schematic description of the Guided Growth design process.

3.2 Bacterial Cellulose

Structural biological materials are produced by living cells based on instructions encoded in DNA and therefore have hierarchical structure. In Section 2.3 I discussed the properties of these multi-scale hierarchical structural materials – including, for example, barks, exoskeletons of marine animals, shells, bones, and biofilms. The focus of my research is one such system – biofilm assembled by the bacteria Gluconacetobacter xylinus (G.xylinus). As one of its basic functions, G.xylinus cells swim around in a sugar-rich liquid and assemble sugar molecules into long chains of cellulose (**Figure 3.2.1** left) through a multi-step metabolic process occurring inside the cell (**Figure 3.2.1** right). These chains then self-assemble in a hierarchical fashion into structural cellulose biofilm (**Figure 3.2.2**).

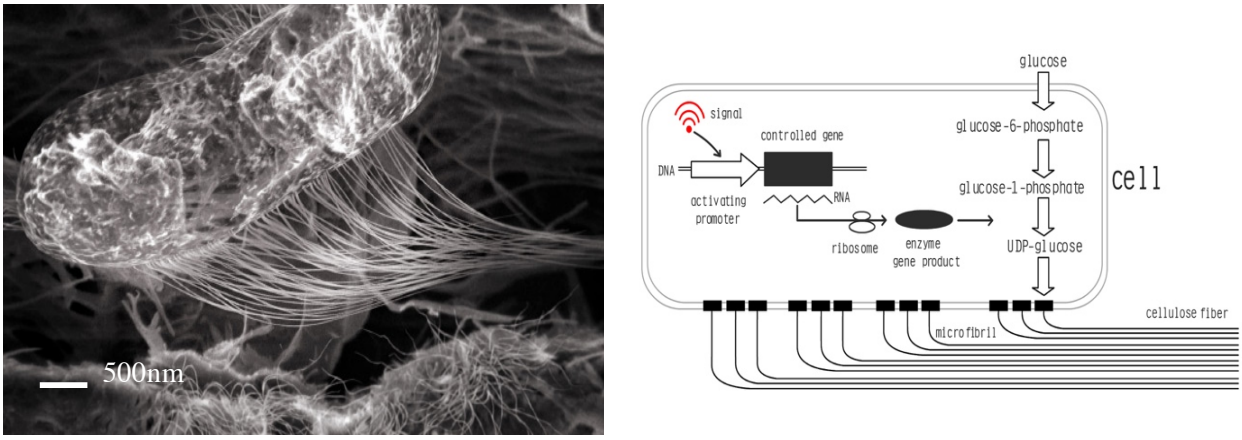


Figure 3.2.1: Bacteria Gluconacetobacter *xylinus* assembly of glucose into cellulose fibers.

Five main reasons make this material system a great candidate for the design of biologically active materials:

- G.xylinus bacteria cells stay embedded in the three-dimensional network of cellulose fibers, and can remain biologically active (**Figure 3.2.2** upper left)
- G.xylinus bacteria cells are the simplest one-cell organisms and are relatively easy to engineer and introduce new functions (See Section 3.3.1 for more detailed discussion)
- The high water content of bacterial cellulose can support long-term viability of the biologically active cells embedded in it (Qin, Panilaitis, and Kaplan 2014)
- Unlike the plant cellulose, bacterial cellulose is produced in its pure form and has excellent mechanical properties discussed below
- Cellulose biofilm grows rapidly into sheets as large as the surface area of a vessel they grow in – one of the reasons many fashion and product designers show interest in this material system (See Biocouture project in Section 2.4)

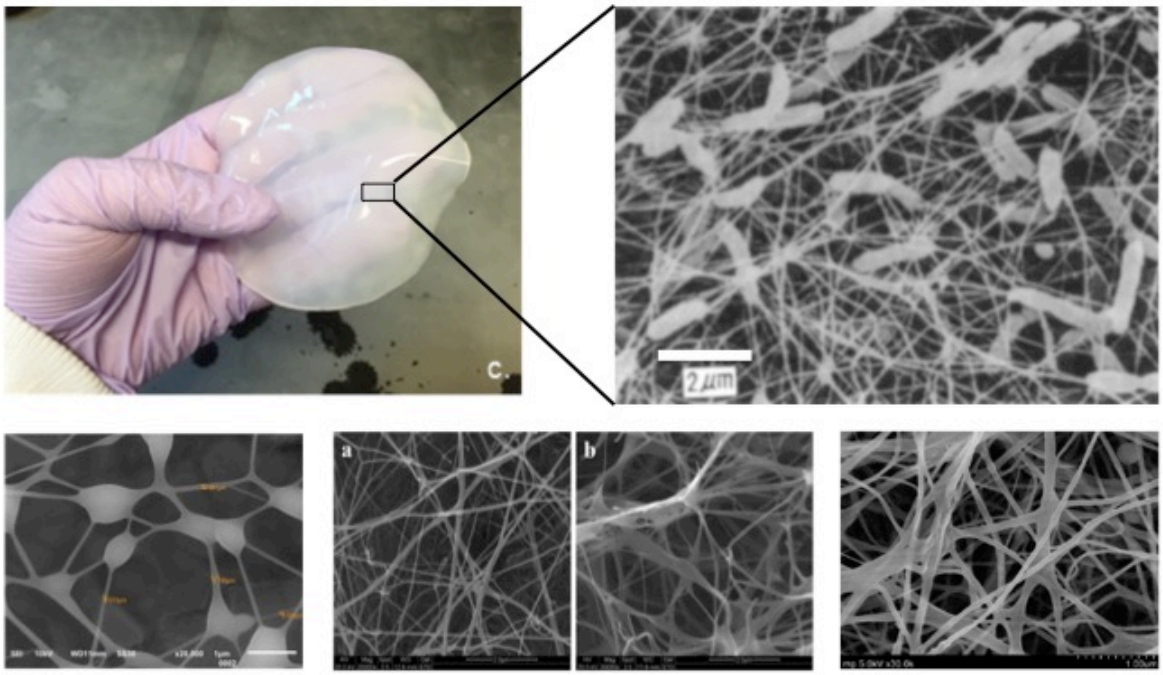


Figure 3.2.2: Material architecture of bacterial cellulose biofilm.

Cellulose is the main structural component of wood and is produced in its pure form by G. *xylinus*. Cellulose produced by bacteria, or *bacterial cellulose (BC)*, has recently received extensive attention from researchers due to its unique properties, such as high water capacity, high crystallinity, ultrafine fiber networks with a diameter of 20–100 nm, high purity, and high tensile strength (Yamanaka et al. 1989; Svensson et al. 2005; Bäckdahl et al. 2006). The Young's modulus of a BC sheet is about 20 GPa (Brown 1985; Johnson and Neogi 1989). Meanwhile, the modulus of a single BC fiber estimated by Raman spectroscopy techniques is 130 GPa which is comparable to Kevlar and steel (Lin et al. 2013; Brown 1985).

Due to its unique properties, BC holds great potential for a range of applications, such as textiles (Yamanaka et al. 1989) biomedical applications (e.g. drug delivery, tissue engineering scaffolds) (Svensson et al. 2005; Bäckdahl et al. 2006) and sustainable building components (Long and Rolison 2007). The mechanism by which bacteria are producing the cellulose fiber is well documented (Brown 1985). Cellulosic fibrils are released into the growth environment through cell pores and aggregated into cellulose fibers. The structure of bacterial cellulose has been studied by standard materials characterization methods and is summarized in **Figure 3.2.3** (Iguchi, Yamanaka, and Budhiono 2000; Czaja et al. 2007; Lin et al. 2013; Johnson and Neogi 1989). The hydrogel accumulated on the air-medium interface (**Figure 3.2.3a**) is comprised of a random assembly of fibrils, <130 nm wide, with bacteria still embedded in it (**Figure 3.2.3b**); the fibrils are composed of finer microfibrils, 2-4 nm in diameter (**Figure 3.2.3c**).

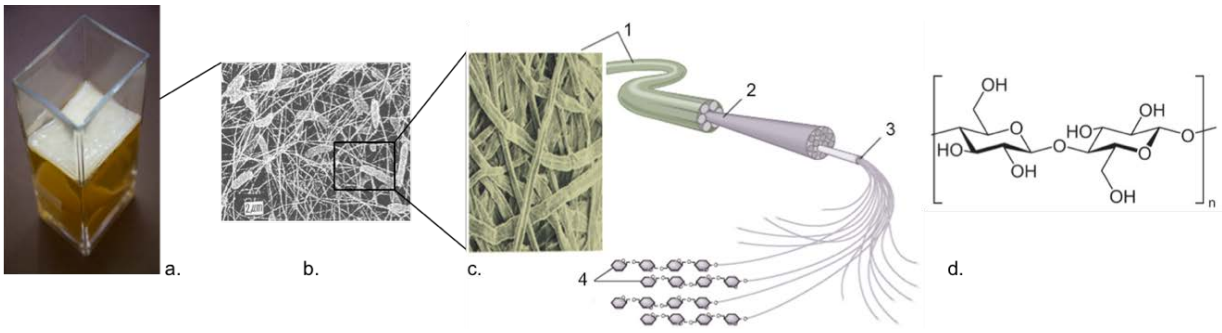


Figure 3.2.3: The hierarchical structure of bacterial cellulose produced by Gram-negative bacteria *Gluconacetobacter xylinus*. a. Bacterial cellulose hydrogel (white) is formed on an air-liquid interface [image by authors]. b. Scanning electron micrograph of the surface of freeze-dried bacterial cellulose hydrogel (Iguchi, Yamanaka, and Budhiono 2000) c. Schematic of the hierarchical structure of cellulose fiber [image from (Brown 1985). d. chemical composition of bacterial cellulose (Watanabe and Yamanaka 1995a).

Bacteria cell communication within biofilm in combination with superior mechanical properties of cellulose and its versatility, make this system an ideal candidate for self-organizing structural materials for building construction applications.

Molecular chemistry and structure of bacterial cellulose

Celluloses found in nature is called native cellulose. It has crystalline structure of polymorph, meaning that cellulose molecular crystals organize in different forms within the material. Native cellulose, or Cellulose I, crystallizes in two phases, I_α and I_β, both co-exist with amorphous cellulose in different cellulose types (Atalla and Vanderhart 1984; Šturcová et al. 2004). Both cellulose I_α and I_β are metastable and can only be synthesized by living organisms. Bacterial and algae cellulose mostly exists in I_α form, while in plant cellulose I_β is dominant (Qian et al. 2005). There exist several additional forms of synthetic and regenerated celluloses, cellulose II, III, and IV that differ in their molecular structure from native cellulose. Both crystalline cellulose I_α and I_β are formed by stacking planar cellulose sheets, but in different ways (Qian et al. 2005). The cellulose sheets are in turn composed of linear cellulose chains bounded by hydrogen-bonding interactions. The differences in the atomic structures and hydrogen-bonding networks of has been well characterized by synchrotron X-ray and neutrons and neutron diffraction analysis (Nishiyama, Langan, and Chanzy 2002). Crystallinity study and comparison between agitated and static cultures of G.xylinus strains were conducted (Johnson and Neogi 1989).

Table 3.1.1 below provides a brief literature review on molecular chemistry and structure. The table is divided into two sections by the material's state. First is a hydrogel or a polymer network suspended in water - this is the natural state of bacterial cellulose as it grows (Atalla and Vanderhart 1984; Watanabe et al. 1998; Huang et al. 2010). The second is an aerogel, obtained from cellulose hydrogel by freeze drying or critical point drying – an open porous foam in which all the water is replaced with air, and a polymer

network is preserved (Liebner et al. 2013; Mauda et al. 2006). BC hydrogel density as low as 300 kg m^{-3} compared to 1500 kg m^{-3}[v] in plant cellulose (Gibson, 2013).

Table 3.1.1. Literature review of molecular chemistry and structure of bacterial cellulose

	Property	Method	Notes	Materials	Source
BC hydrogel	Vanderhart's model suggests that Acetobacter cellulose is 60 to 70 percent I_α, whereas plant cellulose, such as cotton is approximately 60 to 70 percent I_β	NMR analysis	Crystallinity study of celluloses from different sources	G.xylinus, Static culture	Vanderhart and Atalla, 1984
	• Transition temperature (T_g) =48.9±1.2 • Crystallinity index (CrI, %)=70.5±7.5 • FTIR: shoulder peak at 3240 cm^{-1} is attributed to hydrogen bonds in cellulose I_α	Rheological analysis X-ray diffraction and degree of crystallinity FT-IR analysis SEM micrographs	BC network structure study	G.xylinus, Static culture	Huang et al. 2010
	Studied the effect of agitation during growth on molecular structure and crystallinity of BC hydrogel: in agitated BC decreased degree of crystallinity, crystallites of smaller size, less of cellulose I_α than static	X-ray diffractometry, NMR analysis, Gel permeation chromatography	See Sugiyama et al., 1991 for structural analysis of native bacterial cellulose	G.xylinus Static/agitated culture	Watanabe et al. 1998
BC aerogel	• Cellulose weight fraction in cellulose aerogel 1w%; • Poisson ratio, between 0.1 to 0.3 for silica aerogels, is apprx. 0 for BC in all directions; • Microstructure of interconnected micro-, meso-, and macropores. Smaller macropores of ~100nm in diameter	Small-angle X-ray scattering (SAXS); Scanning electron micrographs SEM; Thermoporosimetry with o-xylene, nitrogen sorption	Aerogels prepared through freeze-drying preserve fiber network; includes discussion of methods to study porosity	G.xylinus, HS media, static culture	Liebner et al. 2013
	• Ultra lightweight, low density foams ~6 mg cm^{-3} (compared to 16 mg cm^{-3} for polystyrene foams) • Open porous aerogel composed of 20-60nm thick fibrils, with porosity of 99%	Aerogels obtained by super critical fluid of ethanol at 6.38 MPa and 243°C	Foams density compared to 7-63 mg cm^{-3} for foams from plant cellulose (Gibson, 2013) ;	G.xylinus, HS media, static culture	Maeda et al. 2006

*BC = bacterial cellulose

3.3. Nano-scale: Regulating Genes of Cellulose-producing Bacteria

This section proposes synthetic biology as a computational method to program cells to grow biologically active and adaptive materials for architecture.

Figure 3.3.1 demonstrates the schematic workflow on the nano-scale: engineering living cells to make new bio active materials.

We use tools of synthetic biology, namely synthetic gene networks, as computational devices to introduce new functions into cells and link their regulation to signals in environment.

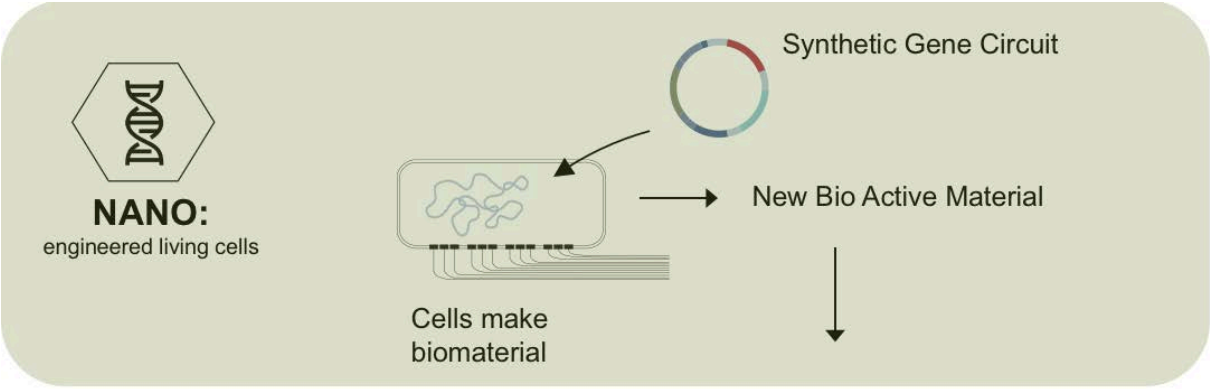


Figure 3.3.1 The diagram of nano-scale engineering of living cells to make new bio active materials.

More specifically, in the Guided Growth experiments presented in this section, we develop new design strategies to guide the self-assembly of cellulose biofilms, its structure and properties, and integration of new biological functions into them. For this, we implement state-of-the-art cell-to-cell communication devices in the cellulose-producing bacteria *G.xylinus*. We work in three directions:

1. Increasing the complexity of synthetic gene networks step-by-step to achieve cell-to-cell communication and signal propagation within the structural biofilm.
2. Introducing variation in structure, properties, and biological functionalities of the cellulose biofilm.
3. Combining signaling devices from aim 1 with the functional actuators from aim 2 to achieve a responsive biofilm patterning of properties and structure based on signals from the environment

3.3.1 Background

Combining communication devices with the functional patterning of biomaterials has recently been defined as an area of synthetic biology called "synthetic morphogenesis." Synthetic morphogenesis relates the molecular mechanisms within cells and the properties of the structures these cells form. One basic principle is the hierarchical organization of all material structures in biology in which higher-level properties emerge from, and are dependent on, lower-level properties of system components (Davies 2013).

I reviewed the hierarchical organization of biological materials or morphogenesis in Section 2.2 above, and in this section, I discuss the design strategies to guide the natural morphogenesis towards new designs, namely synthetic morphogenesis. These processes occur on the molecular and the sub-cell (smaller-than-cell) scale of resolution and can be grouped into a small number of recurring motifs, or morphogenic modules, such as spatial orientation of cells by a gradient of morphogen, a chemical signal.

Synthetic biologists develop useful morphogenic devices. For example, synthetic cell-to-cell signaling allows cell populations to make decisions and coordinate behaviors both locally and globally (Teague, Guye, and Weiss 2016; A. Y. Chen et al. 2014a). These various sensors, actuators, and communication channels could implement complex morphogenic systems through a combination of top-down approaches in which cells are patterned by external signals, and bottom-up programs in which collective properties emerge through cells' local decision-making (Teague, Guye, and Weiss 2016).

In this dissertation, I propose a domain of application for these synthetic morphogenic gene networks in designing bio-active materials for architecture. An existing closely related area of research is the application of these networks to the design of biomaterials. Initial proof-of-concept studies on the ability to genetically engineer the organism have shown that synthetic plasmids can be inserted into *Gluconacetobacter xylinus* (Coucheron 1991). However, the use of genetic engineering tools on *Gluconacetobacter xylinus* to control material properties of cellulose structures has not been attempted.

Synthetic biology is a new, bottom-up engineering discipline, where biological parts (such as transcription factors, gene promoters, or proteins) are constructed from DNA, and then are combined into gene networks with predefined behavior such as signal processing and communication within their living biological hosts (Gardner, Cantor, and Collins 2000). For example, digital processing of external inputs within bacterial cells is possible using memory (T. K. Lu, Khalil, and Collins 2009), multi-input logic (Rinaudo et al. 2007) or specialized circuits such as oscillators (Elowitz and Leibler 2000) and synthetic multicellular distributed computing systems (Subhayu Basu et al. 2005; S. Basu et al. 2004).

I propose to harness cellulose-producing bacterial host *G.xylinus* with novel biological functions by harnessing these networks for sensing of inputs (i.e. small molecules, specific light wavelengths, temperature), signal processing (boolean logic operations), and resulting actuation mechanisms (change

of gene expression to produce light, color, or material). These modules have already been employed to produce systems on bacterial layers for 'photography' that transduce light signals to directed spatial pigment formation to capture images (Levskaya et al. 2005) or 'bandpass filters' that transduce chemical signals to colored concentric ring formation (S. Basu et al. 2004). A change in actuation mechanisms from colorimetric readouts to peptides or proteins that bind inorganic materials would enable spatial genetic control of bio-mineralization or bio-templating (A. Y. Chen et al. 2014b).

I propose, tuning material formation to create materials with spatial variation in composition and properties. Using synthetic biological methods, we can engineer synthetic gene networks with extracellular regulation that will allow the control of material properties of cellulose structures by spatial location, timing, and intensity. Recently, genetic circuits were introduced into another material-producing system, *Escherichia coli,* that enabled user-controllable regulation of amyloid fibril assembly that could then be interfaced with nanoparticles for the generation of nanowires (A. Y. Chen et al. 2014a). In *G.xylinus*, genome sequencing and plasmid transformation that increased the yield of cellulose production has been demonstrated. However, while these efforts brought material formation under genetic regulation, neither of these approaches modifies the actual morphology of the biomaterial.

By creating novel genetic networks for engineering bacterial material formation and quantitatively understanding how these networks impact material properties, we can create engineering frameworks that work across length of scales to efficiently leverage intracellular genetic control of material formation/functionalization alongside more traditional extracellular manipulation of material properties. In turn, with experience, we can develop the ability to create materials that may capture other biological phenomena such as self-repair, self-assembly, or environmental responsiveness.

3.3.2 Methods and Results

The goal of this project is to engineer regulation of cellulose production in *G.xylinus* by environmental signals. We start from the native state of bacteria and a set of functions which, it is designed by nature to perform. This includes swimming around, metabolizing sugar in the environment and synthesizing and spinning cellulose fiber which then self-organize into structural biofilms of pure cellulose with previously characterized materials properties. The first step is converting this native biological cellulose-producing system into a designable material system and develop engineering platform that will work in these bacteria.

Figure 3.3.2 summarizes the experimental workflow we developed for the genetic engineering G.xylinus for functionalization and patterning of the biofilm it produces.

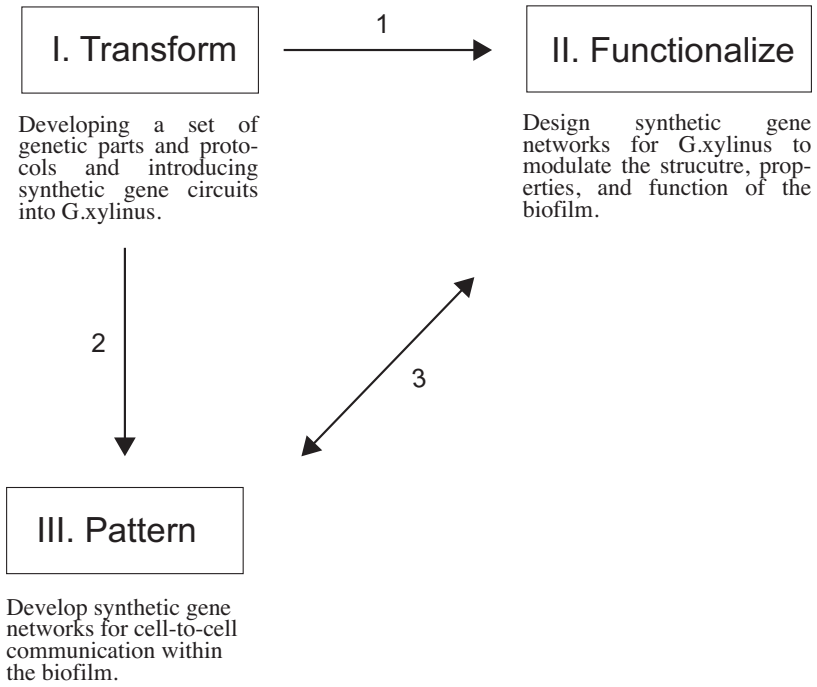


Figure 3.3.2: Workflow diagram of genetically engineering G.xylinus for functionalization and patterning of the biofilm it produces.

This Results section presents experimental results for the first aim (I. Transform) and detailed proposals for future experimental work for the second (II. Functionalize) and third (III. Pattern) aims. I will continue to work on this research project as a postdoctoral researcher at the Weiss Lab for Synthetic Biology.

I. Transform.

Our goal was developing a set of genetic parts and protocols for engineering *G.xylinus*. Since **no** platform for genetic engineering of cellulose-producing bacteria G.xylinus previously existed in the Weiss lab, we had to build this platform from the scratch. We developed the workflow summarized in a diagram below, under which you will find a short description of each step with a link to a methods section describing the materials and procedures we used in detail.

Workflow for engineering G.xylinus.

We developed the workflow described on **Figure 3.3.3** to design, introduce, and verify synthetic gene networks into the cellulose-producing bacteria *G.xylinus*.

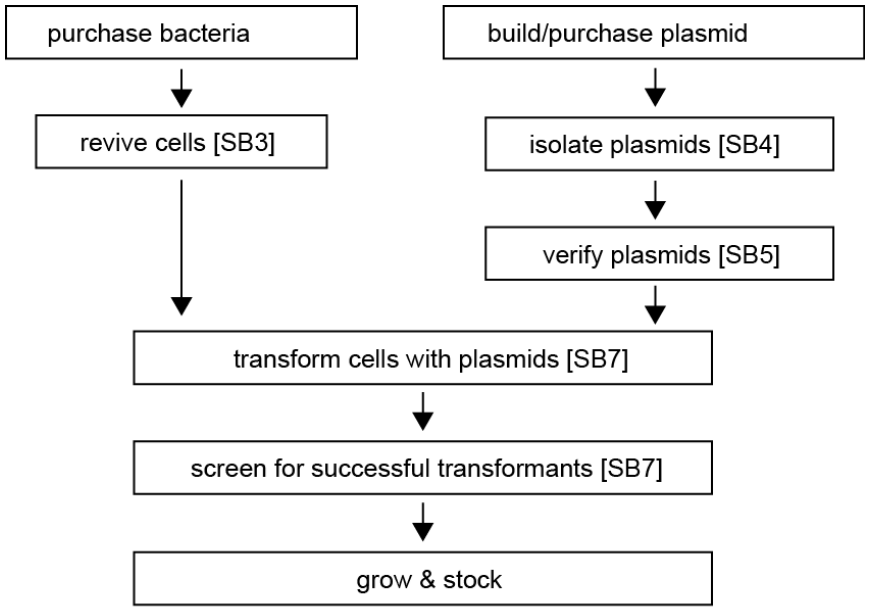


Figure 3.3.3 - General workflow for introducing new genetic parts into *G.xylinus*

1. ***Purchase bacteria*** - in addition to cellulose-producing bacteria *G.xylinus* we used *E.coli*, a most commonly used bacteria for genetic engineering. We used *E.coli* to test our designs and to assist with the transformation of *G.xylinus* through conjugation. All the bacteria strains we used in our experiments are summarized in **Table 3.3.1** below.

2. ***Revive cells*** - bacteria purchased online arrived as a freeze-dried culture in a glass capsule. To revive the cells from their dormant state, we mixed the dry culture with a growth medium and placed it in an incubator to grow [SB3] in Appendix.

3. ***Build/purchase plasmid*** - we started with purchasing and testing several backbone plasmids that were reported in the literature to work in G.xylinus. Once we developed a successful transformation protocol, we transformed the bacteria with plasmids of increasing complexity (see below **Table 3.3.2**).

4. ***Isolate plasmid*** - plasmids purchased online arrive inside bacteria cells as a cell culture. We used miniprep, a technique for DNA extraction and purification [SB4] in Appendix. The methods consist of transferring cells containing plasmids between different solutions. During this process, the cells break and release the plasmids, and a centrifuge is used to get rid of all other cells parts, leaving purified plasmids in the solution.

5. ***Verify plasmid*** - after the miniprep [SB5], we apply techniques of gel electrophoresis and DNA sequencing [SB6] to verify that we got the right plasmids.

6. ***Transform cells with plasmids*** - plasmids and cells are combine and undergo a transformation procedure together to insert plasmids into cells. We tried two different methods - electrophoresis and conjugation - for transformations, while only the conjugation was successful. During conjugation, we use an additional bacteria E.Coli that has a natural ability to inject plasmids into other bacteria cells. We use a three step conjugation process - we first transform our plasmids into E.Coli using electroporation, and then grow the two strains together for E.Coli to inject plasmids into G.Xylinus, and then get rid of E.Coli. For detailed protocols, please see [SB7] in Appendix.

7. ***Screen for successful transformants*** - plasmids contain antibiotic resistance gene that helps grow only the cells that got the plasmid. Since G.xylinus has Chloramphinicol antibiotic resistance in its natural state, we only used plasmids that encode resistance to other antibiotics.

8. ***Grow and stock*** - we grow transformed bacteria, prepare glycerol stocks and store in -80 degrees Celsius for future use.

Using the workflow we developed, our main achievements were as follows:

Accomplished to date:

- Developed protocols for transformation of G.xylinus via conjugation
- Transformed plasmid with antibiotic resistance
- Established a library of basic parts that work in G.xylinus
- Demonstrated cellulose production rate and water content of genetically modified biofilms

In progress:

- Transforming G.xylinus with constitutive fluorescent proteins
- Transforming G.xylinus with secretion sequence to secrete FPs from cells
- Fusing FPs with cellulose binding domains (CBD)
- Building synthetic gene network with inducible promoter, fusion protein with CBD, and secretion sequence
- Transforming with above SGN
- Demonstrating tunable change in material structure and properties based on signal
- Quantification of cellulose production. Water content. Visualization.

For the synthetic biology experiments, we used the bacterial strains and the DNA parts that we purchased online. **Tables 3.3.1** and **3.3.2** summarize the bacteria strains and the plasmids we used in this research.

Table 3.3.1. A list of bacteria strains used in this research.

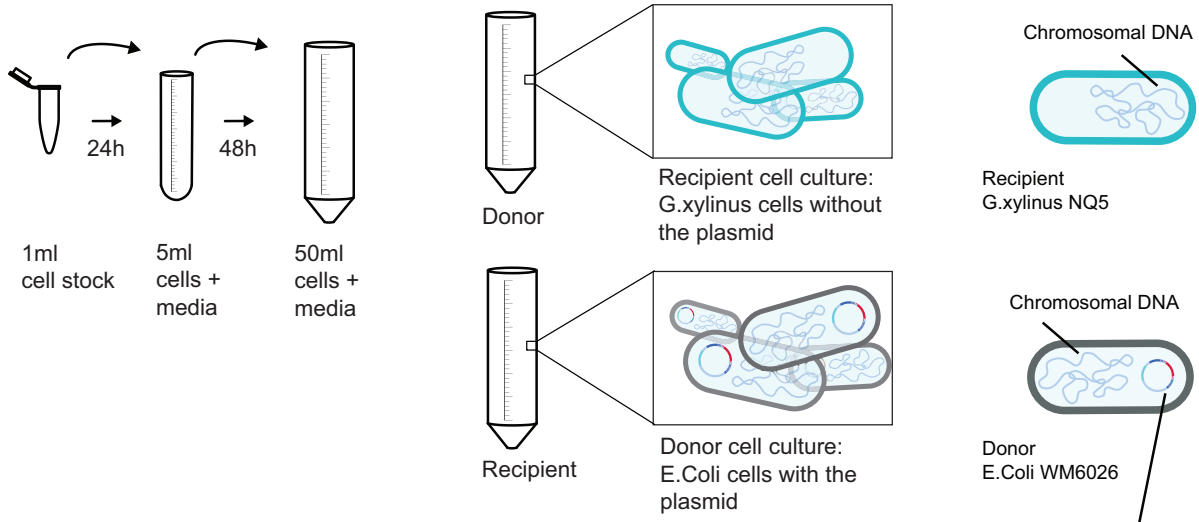
Bacteria Strains	Description	Reference/ Sources
Gluconacetobacter hensenii NQ5	The most rapid producer of cellulose, previously classified as Gluconacetobacter xylinus NQ5. I use the old name in this thesis.	ATCC
Gluconacetobacter xylinus NCIB 8031	The most extensively studied species, formerly known as Acetobacter xylinum and since reclassified as Komagataeibacter xylinus.	ATCC
Escherichia coli WM6026	Defected in DAP gene (DAP auxotroph). Defected in RP4 gene incorporated in the chromosome (can't be transported via conjugation)	Lina Gonzelez from Chris Voigt Lab

Table 3.3.2. A list of plasmids used in this research.

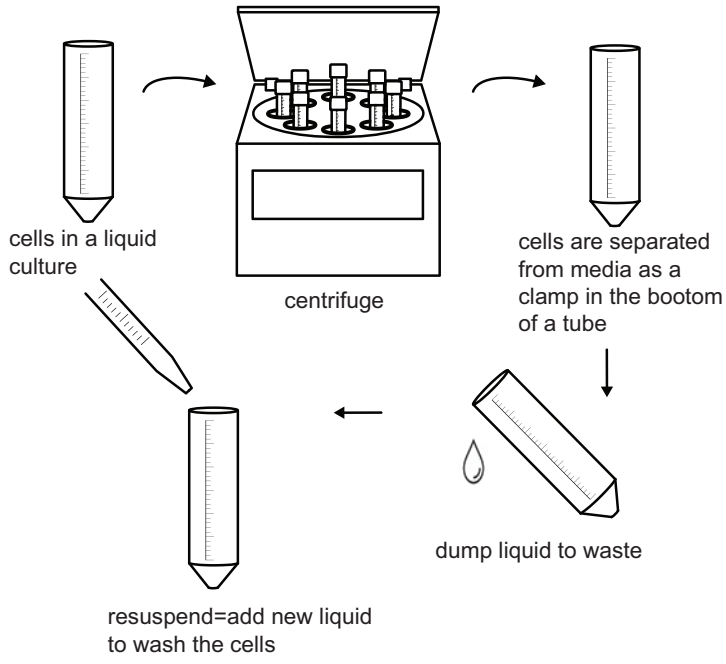
Plasmids	Antibiotics	References/ Sources	Expressed protein
pSEVA331Bb	Chloramphenicol	Addgene	_
pSEVA351	Chloramphenicol	SEVA	_
pSEVA251	Kanamycin	SEVA	_
pSEVA551	Tetracyclin	SEVA	_
pSEVA227Y	Neomycin	SEVA	yellow fluorescent protein (YFP)
pSEVA237Y	Neomycin	SEVA	yellow fluorescent protein (YFP)
pSEVA227R	Neomycin	SEVA	red fluorescent protein (mCherry)
pSEVA237R	Neomycin	SEVA	red fluorescent protein (mCherry)

For the detailed description of the methods we developed and used please see the Appendix: Basic Methods of Synthetic Biology for Non-Biologists. **Figure 3.3.4** below provides a detailed step-by-step graphic description of the conjugation process we developed.

1. Grow cells to 50ml of liquid culture



2. Wash cells to prepare for conjugation



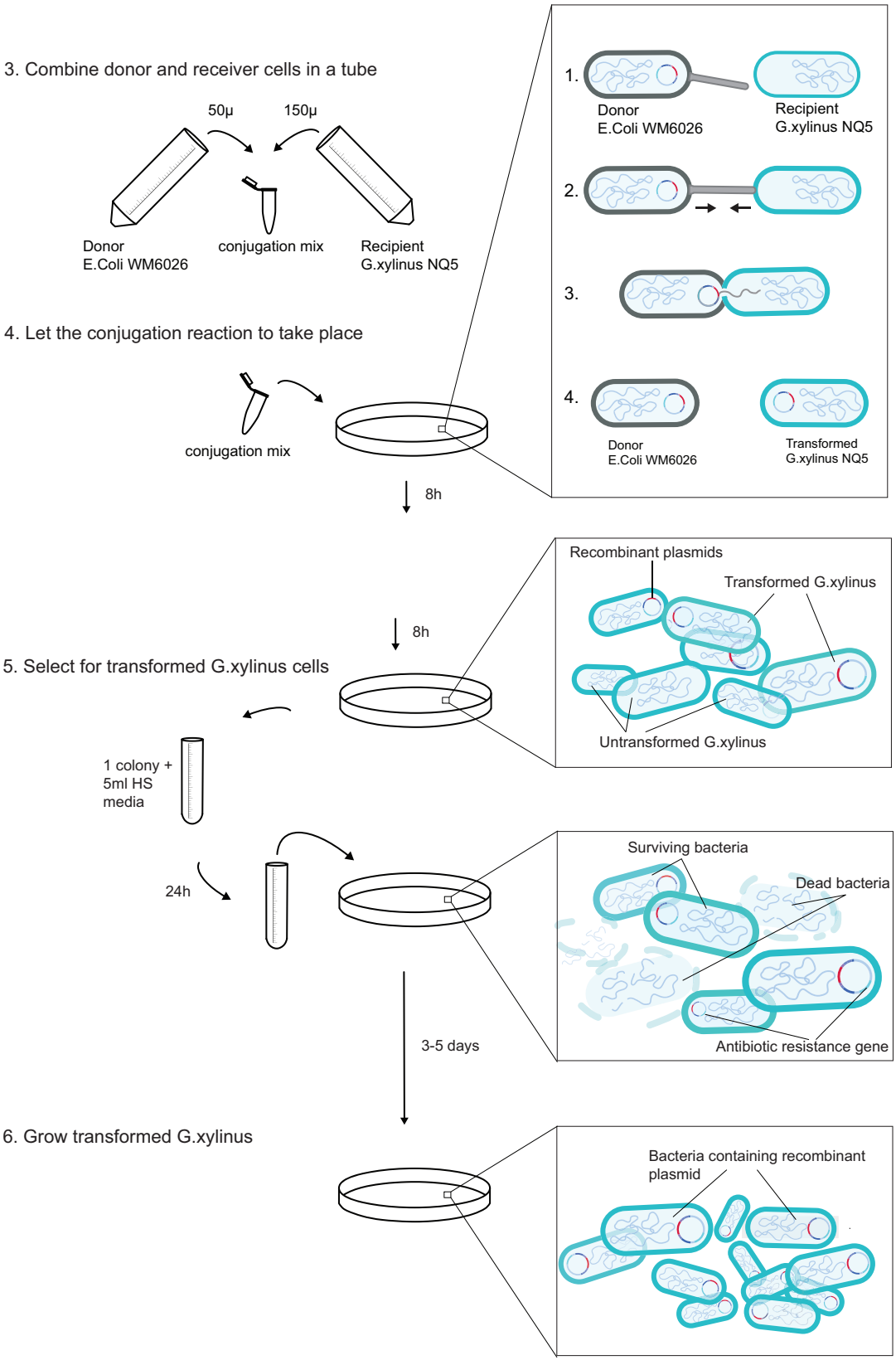


Figure 3.3.4: Schematic description of introducing synthetic gene networks into G.xylinus via the conjugation process with the donor bacteria strain E.coli WM6026

Figure 3.3.5 below shows an interactive map of plasmid available online at the SEVA collection (part pSEVA351). The top part of the figure shows the graphic description of the circular DNA molecule. This plasmid consists of 5120 base pairs, and it carries the gene CamR, providing its host with resistance to an antibiotic. The bottom part of the figure shows the actual DNA sequence of a part of plasmid, including the sequence of the gene CamR. Once we developed the technique for introducing the backbone plasmid into *G.xylinus*, we can start 'plug and play,' introducing new genes and network on this same plasmid and designing it to carry new functions into bacteria. The various labels on the outer side of the plasmid indicate cutting sites for the restriction enzyme, allowing cutting and pasting of new genes into the plasmid. See the next section for a detailed proposal.

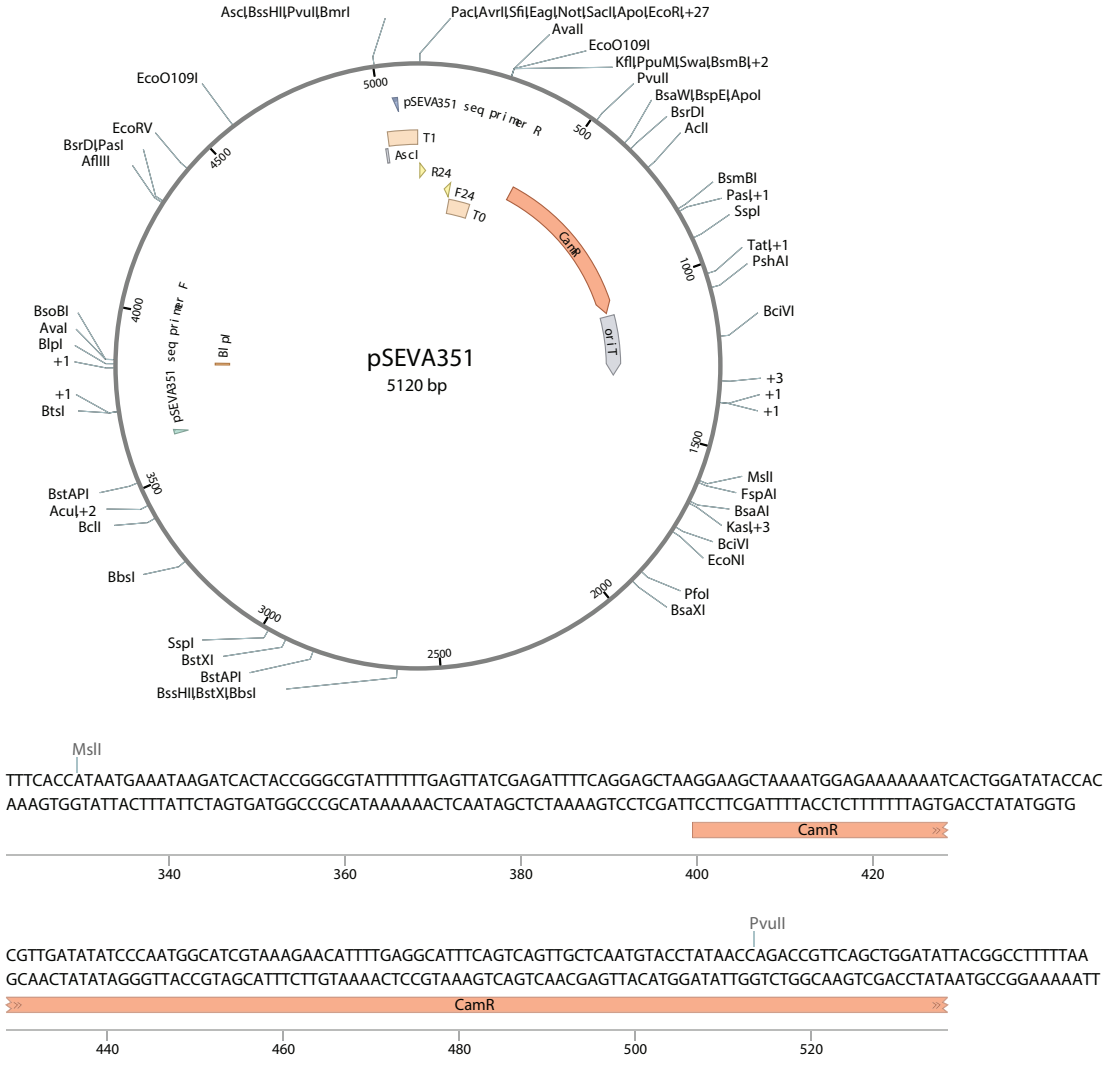


Figure 3.3.5. An interactive map of a backbone plasmid from SEVA collection (screenshot from www.benchling.com)

Table 3.3.3 Summarizes the results of the conjugation experiment we conducted introducing two different backbone plasmids into G.xylinus (pSEVA251 and pSEVA551) and the control samples to verify that the growth we obtain is actually due to the introduction of the new plasmid.

Table 3.3.3. A table of samples for the conjugation experiment

	Samples	Additions	Growth Results
1.	Control w/o cells	(+DAP)	No
2.	Control w/ cells not pulsed	(+DAP)	Yes
3.	Control w/ pulsed cells	(+ DAP)	Yes
4.	Control w/ pulsed cells	(- DAP)	No
5.	pSEVA251	(Kam + DAP)	Yes
6.	pSEVA551	(Tet + DAP)	No

We obtained the transformation of the plasmid pSEVA 251; **Figure 3.3.6** shows successful growth of the G.xylinus colonies with the newly introduced plasmid. The selection of transformed colonies was performed by using their newly acquired resistance to Kanamycin. We grew colonies on the solid medium containing Kanamycin, so only the cells containing the plasmid could grow (**Figure 3.3.6**).

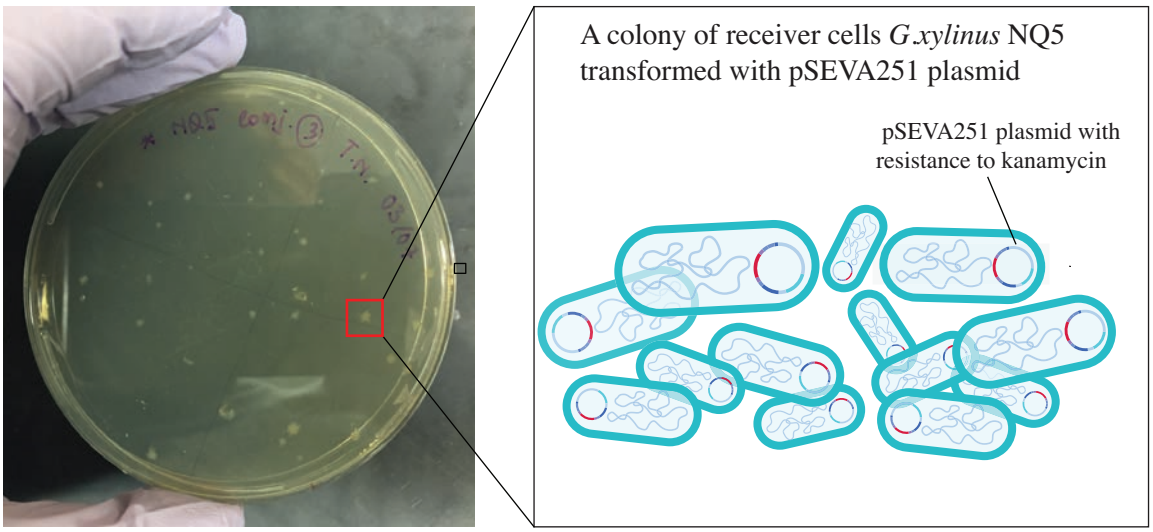


Figure 3.3.6. Conjugation results. left: A picture of transformed bacteria colonies grown on a plate with kanamycin antibiotic. right: Schematic description of a transformed colony of cells containing the pSEVA251 plasmid they received via conjugation from the E.Coli WM6026 cells.

Once we successfully introduced a new plasmid into G.xylinus, we experimented to verify that the cellulose production capabilities of G.xylinus were not interrupted. The method we used is [SB 8] in the Appendix. **Table 3.3.4** shows that cellulose production in the transformed sample (NQ5+251) is similar to the cellulose production in the native control sample (NQ5 Control), and in the sample mixed with Kanamycin to eliminate the possibility of the native bacteria growth, the cellulose production is even higher (**Figure 3.3.7**).

Table 3.3.4. A table of post-conjugation cellulose production results

	5 days	16 days	
NQ5 Control	3mm	10mm	1 layer
NQ5+251	3mm	10mm	3 layers
NQ5 +251+Kam	4mm	20mm	4 layers

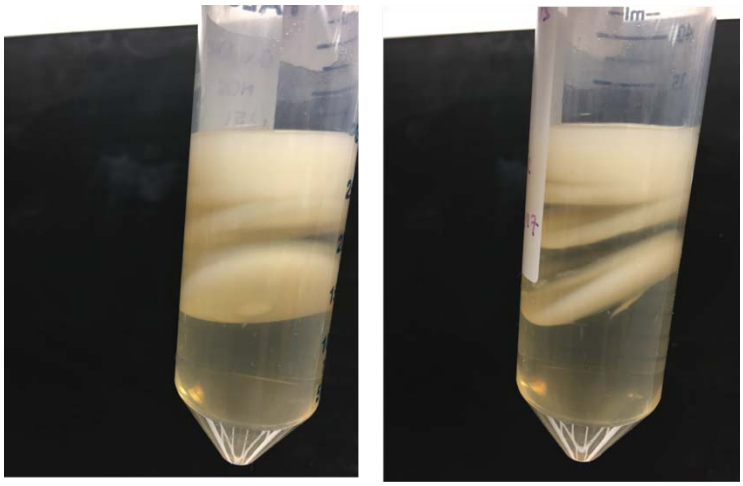


Figure 3.3.7. Cellulose production in static culture of *G.xylinus* transformed with pSEVA251 plasmid.

Proposal: Programmed Pattern Formation by Cell-to-Cell Computation

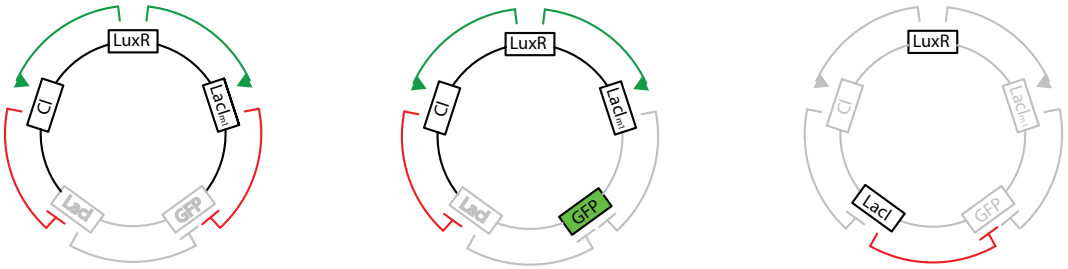
Once we have shown we can transform the cellulose-producing bacteria with synthetic gene parts without interrupting its cellulose production; we can say that we have a platform for genetically engineering this material system. Now we can design and compute with this system. If we want to design by principles of material organization in nature, we need to master two design principles: differentiation (programming similar cells to exhibit different behavior) and pattern formation (coordinated cell behavior).

The design process starts with generating a high-level idea of a behavior we want to program. Synthetic biology methods foster modularity and standardization, so each part we introduce to *G.xylinus* can potentially be used in many different designs. Also, designing with the cellulose-producing material system, we can use network architectures and patterning devices that were developed for other cell types and biomaterials. Below I propose a general design framework and then a more detailed breakdown of modules and parts of bacterial cellulose.

We want to create a design space for the cellulose material system and be able to pattern properties and biological functions onto the cellulose biofilm. Once we put the material production under the genetic regulation with synthetic gene network, there is a large space of possible designs we can 'plug-and-play.' Below I show a proposal of designing a responsive bio-active material able to detect center and edge and produce gradient of material properties. I will then show how the high level design can be broken down to model and engineering of its specific parts, and show how the same parts/modules/network that we develop for one design can be used for other designs. This proposal will be realized as I continue working in the next two years as a postdoctoral research in the Weiss Laboratory for Synthetic Biology at MIT.

The patterning gene network has already been developed for the common bacterial model organism, E.Coli (Subhayu Basu et al. 2005). My innovation in this proposal is to implement the pattern-forming cell-to-cell communication device for biomaterial functional patterning. The design below shows bull-eye pattern of cellulose biofilm, demonstrating a band of higher density by localize activation of crossl-inking proteins production (**Figure 3.3.8**).

Bull-eye Pattern through Cell-to-Cell Communication and Crosslinking of Cellulose Biofilm



Inner band -
High concentrations of AHL - no crosslinker production

Middle band -
Concentrations of AHL in the middle range -
production of crosslinkers - high density of cellulose

Outer band -
Low concentrations of AHL - no crosslinker production

gradient of signal molecules from the sender cells creates allows differentiation of cellular response, and

crosslinking between cellulose fibers increase he density of cellulsoe fiber network

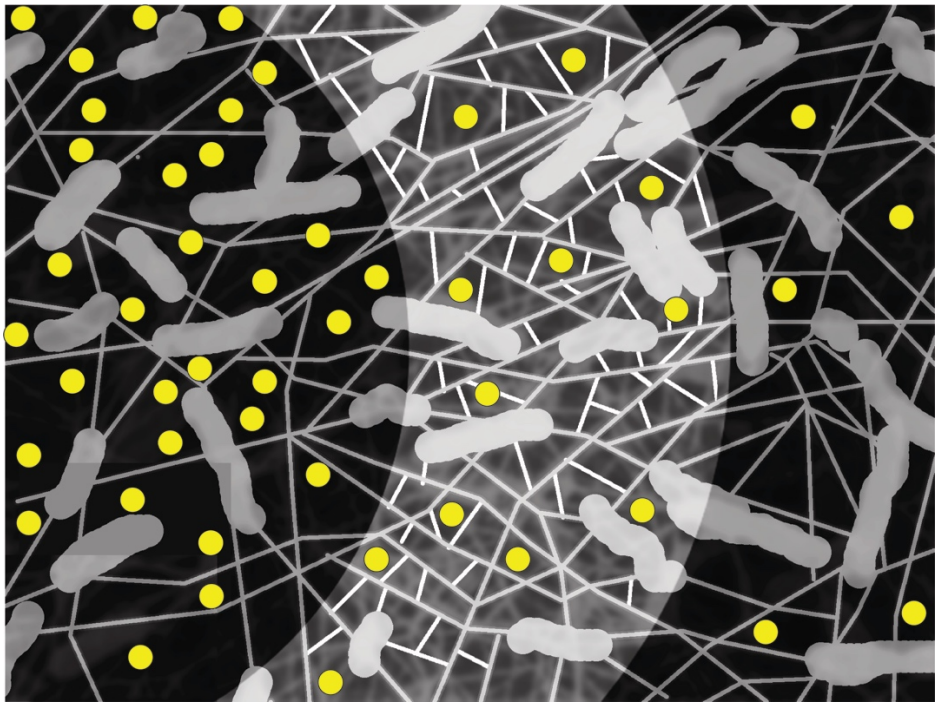


Figure 3.3.8. Schematic description of a density gradient pattern within the hybrid cellulose biofilm.

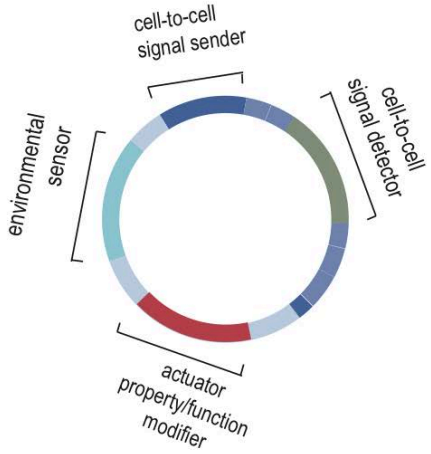
Gradient of density: bacteria cells produce a *fusion* protein that creates connections between cellulose fibers within the biofilm and increases its density (Habibi 2012). The higher the concentration of the

crosslinker, the higher the density of the biofilm. Please see the section 'parts' below for a discussion on fusion proteins and crosslinking.

To generate this pattern, we need to program three types of computational constructs (or plasmids):

a) the sender plasmid that are placed in the middle and are responsible for sending the signal and creating the spatial orientation gradient;
b) the high-detect plasmid that produces fusion proteins with high sensitivity to the signal concentration;
c) the low-detect plasmid that produces fusion proteins with low sensitivity to the signal concentration;

The cells containing these plasmids will follow the following computational rules:



Sender cells:

If no ES:
Do nothing

If ES:
Emit cell-to-cell signaling

Receiver cells:

if [C] < x
Do nothing

If x < [C] < y
Actuate modification

If [C] > y
Do nothing

*[C] = concentration of cell-to-cell signal

Figure 3.3.9. Computation rules for the sender and receiver cells to generate bull-eye pattern.

A more detailed explanation of the biological regulatory behavior that allows cells to perform computation. As a first step, the pattern formation is initiated by placing a colony of 'sender's cells on the undifferentiated lawn of 'receiver's cells. Senders initiate cell-to-cell communication by producing LuxI enzyme, which activates production of AHL, a small chemical that can exit cells, diffuse freely into the environment, and enter neighboring cells. Over time, a chemical gradient of AHL is formed from high concentrations close to the origin and reduced concentration with the distance (**Figure 3.3.10**). The band

of higher density will form due to the non-uniform response of the receiver cells to different concentrations of AHL.

Only cells at a certain distance from the origin will be activated for production. In the 'inner band' close to senders, where the concentrations of AHL are higher than a certain threshold, receiver cells will produce high levels of proteins Cl and $LacI_m$ which causes repression of production. In the outer band, where the receivers are far from senders, both Cl and $LacI_m$ are produced at a basic low level, which allows activation of LacI that represses the GFP, so again no GFP is produced. Only at a certain pre-defined range of AHL concentrations, the levels of Cl and $LacI_m$ are such, that there is not enough $LacI_m$ to repress GFP, but there is enough Cl to repress LacI that in turn doesn't repress GFP, so GFP is produced only in this middle band. In short, the mid-range expression is possible due to a pre-programmed and experimentally calibrated difference in efficiency between Cl and $LacI_m$.

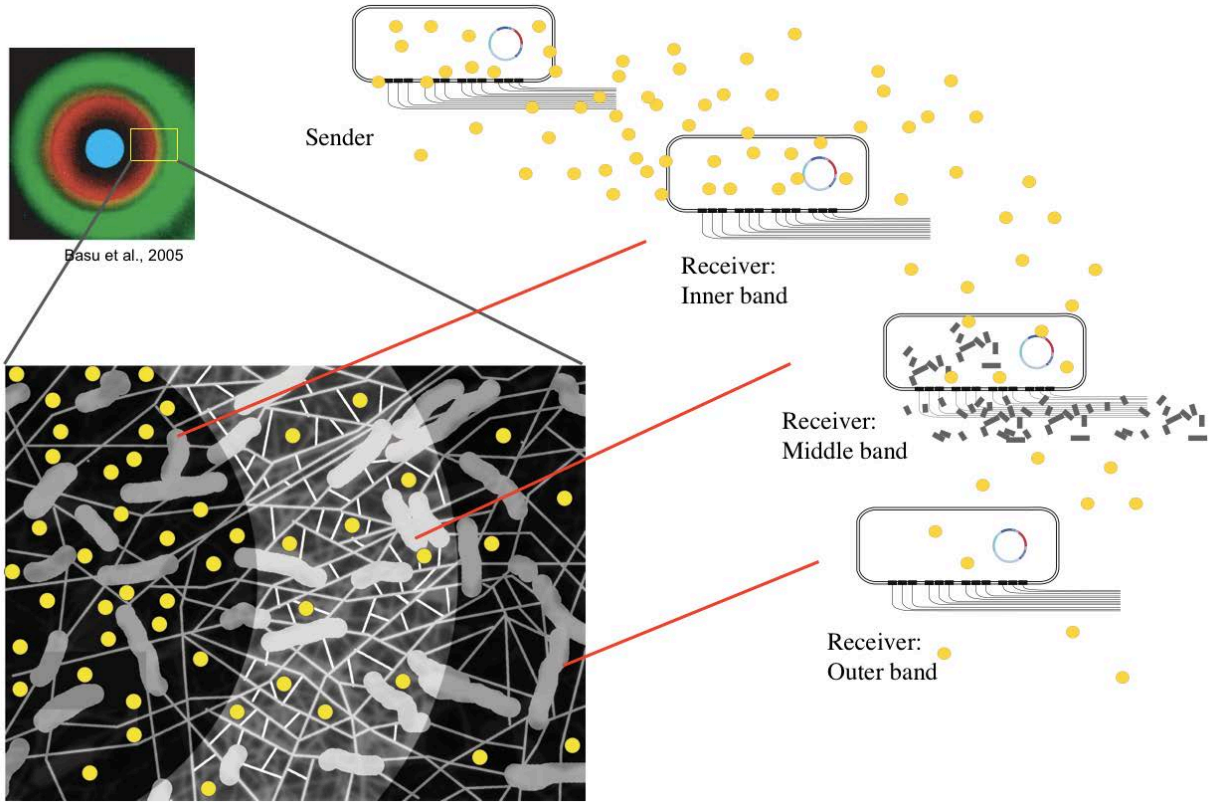


Figure 3.3.10. Schematic description of the middle band activation by signal concentration range. Receiver cells in the middle band, at a certain distance from the sender colony produce cross-linking protein, resulting in denser area.

The specific range and the intensity and type of the middle band response can be user specified and engineered. We can design, computationally predict, and experimentally tune the following parameters to design materials with pre-programmed responsive properties:

- Location - the distance and width of the band in which the gene will be expressed, or the range of signal concentrations to which receiver's cells respond.
- Type of response - please see below a discussion about fusion proteins and functional variation for the bacterial cellulose system.
- Intensity and duration of response – these parameters need to be experimentally tuned by choosing the right type of promoter, or directed mutation, such in the LacIm1 part in the bull-eye patterning system.

As always in work with living biological systems, the behavior depends on the complex context of the organism. Transferring the patterning device to *G.xylinus* will result in technical and experimental challenges, and the design process will involve calibrating parts and building a computational predictive model.

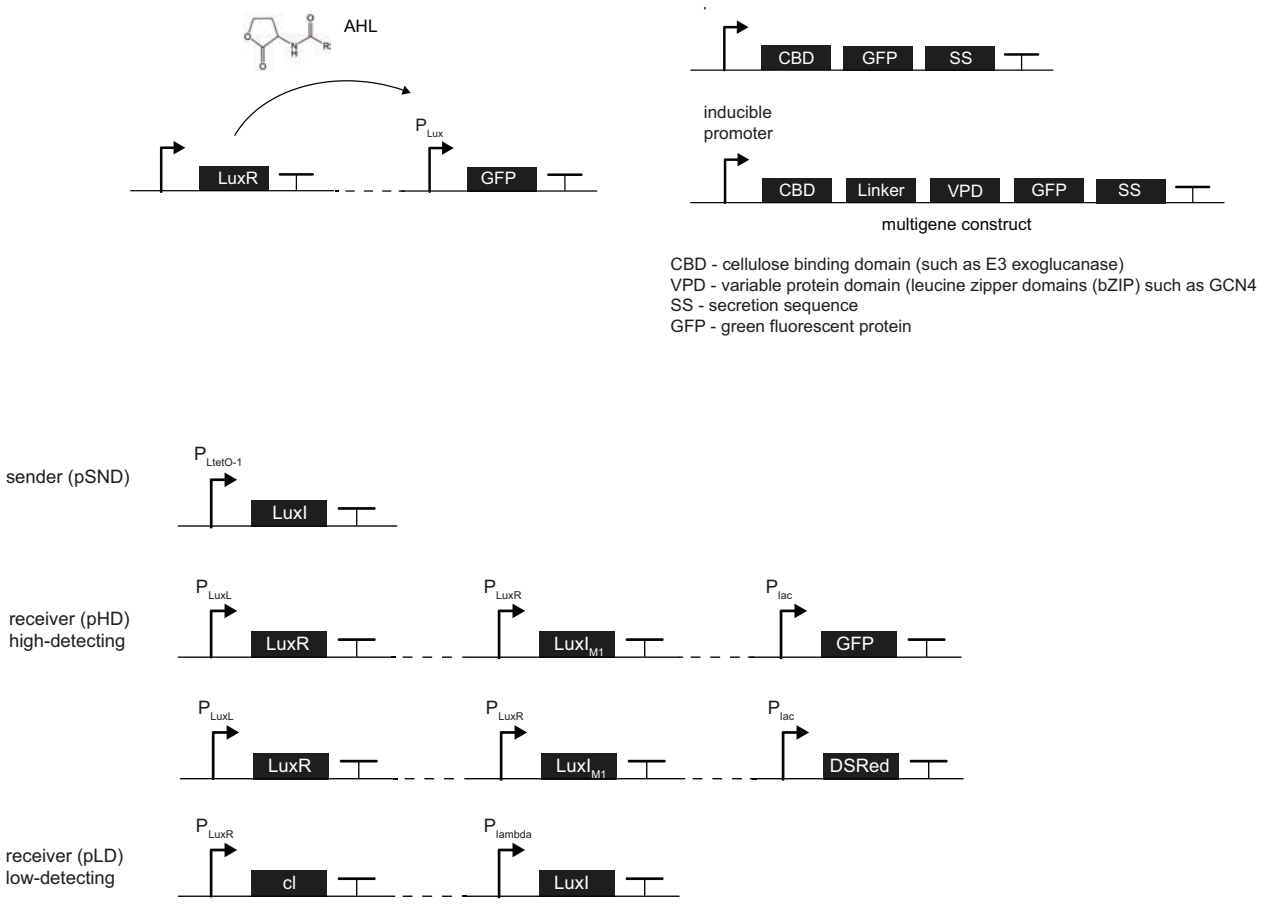


Figure 3.3.11. Schematic description of DNA parts to be built to realize the bull-eye pattern design of density.

Functionalization.

As a next design step, we will create networks specific to G.xylinus and its cellulose production mechanism. We will create networks that modify cellulose properties and functionalize, meaning addition of a biological function that does not exist in the native material system.

To do so, we will cast the networks from the library to produce fusion proteins rather than express colored fluorescent proteins upon UV exposure/ chemical gradient. Proteins are complex large molecules made from long chains of amino acid and folded into unique 3D shape. The sequence and the 3D shape of a protein define functional domains, which are the areas that will bind other molecules and perform specific functions. Fusion proteins combine several functional domains. Our goal will be using a protein that has one constant domain that binds to cellulose, and one variable domain, that will change its function based on our design needs. These functions include:

- Crosslinking
- Metal
- Biomineralization

Our fusion proteins will include one cellulose-binding domain and another functional domain that will vary in function. To achieve cross linking under the regulation of synthetic gene networks, we will design a fusion protein with the cellulose-binding domain and a cross-linking domain. One of the cross linking systems to be tested in G.xylinum is the E3 exoglucanase cellulose-binding domain and basic leucine zipper domains (bZIP). These zipper domains (such as GCN4, FOS, and JUN) enable homodimerization between the bZIP domains and thus cross-linking between cellulose strands to modify bacterial cellulose properties. By choosing from the available toggle variants and the magnitude of input applied, the degree of cross-linking can thus be tuned.

First, we will expand our library of coding sequences to include E3 exoglucanase – bZIP fusion proteins. These coding sequences can be ordered from commercial DNA synthesis providers, and thus we can readily investigate multiple bZIP domains for use in cross-linking. Once the library is filled with potential cross-linking parts, we will utilize one-pot Golden Gate assembly (see Methods) to build a set of toggle networks spanning a range of input-output switching dynamics and production levels of cellulose-crosslinking protein. Then, we will conduct screening of constructs with and without UV light exposure and subsequently determine material properties from stress-strain measurements and the degree of cross-linking as measured by western blotting or immunofluorescence.

Once we design and calibrate cross linking fusion protein for G.xylinus and its cellulose production, we can expand this system to other designs. We can design fusion proteins comprising the cellulose-binding domain and various small proteins or peptides. Examples include beta-lactamase enzyme, collagen, silica-binding sequences, or metal-binding hexahistidine tags, to functionalize bacterial cellulose fibers during production for use in catalysis (enzymes), improved biocompatibility (collagen) or mineralization (silica and metal binding peptides). As with the cross-linking fusion protein, the degree of functionalization will be tunable, and networks will be regulated by UV light and small chemicals.

The methods are in the appendix in the form of tutorials. Since designing with biologically active materials is a new area for architects, I decided to present the main methods used here as a detailed tutorial, so the reader will get a sense how to repeat it if she would want to engage in work with DNA design. All methods of synthetic biology are ways to manipulate tiny amounts of liquids, and extracting, amplifying, cutting and pasting, reading DNA sequences. These manipulations of DNA are done through chemical solutions, temperature cycles, and additions of biologically active molecules, or enzymes. Below is a one sentence description of each of the methods, for the full tutorial including materials, quantities, and procedures, see Appendix A.

3.2.5 Discussion and Future Work

Building and implementing synthetic gene networks in new biological species is not an easy task. So far, genetic engineering achievements presented above include introducing a plasmid with antibiotic resistance genetic the cellulose-producing bacteria, selectively growing the transformed bacteria.

Designing and guiding living cells to do new functions can face unpredictable challenges due to a complex biological context of living organism. Also, building complex gene networks in a hierarchical manner such as proposed in this section can be a challenging engineering task - changing the behavior of one gene or gene network alters the behavior or structure of every context in which that gene or network is used. Therefore, synthetic gene networks that were designed for one organism, such as the bull-eye patterning networked for *E.coli* discussed above, might not work in *G.xylinus*.

If complex circuits fail in *G. xylinus* due to contextual effects arising from the bacterial host and the circuits we engineer, we will instead focus on an *E. coli* system engineered with both a plasmid containing our synthetic gene circuits. This approach of integrating another bacteria species in the biofilm produced by G.xylinus has been shown to work. Researchers in Tufts designed a "living membrane" system based on recombinant Escherichia coli bacterial strains entrapped in cellulosic membranes produced by Gluconacetobacter xylinus (Qin, Panilaitis, and Kaplan 2014). The advantage of this approach is that there are many ready-to-use genetic devices for sensing, communication, and patterning that have been designed for the *E.coli* bacteria.

Co-culturing can provide new exciting opportunities to introduce biological functions into G.xylinus biofilm. For example, integrating cyanobacteria in the biofilm can produce photosynthetic materials (Whitton and Potts 2007).

3.4 Meso-scale: Regulating Growth of Cellulose Biofilms

3.4.1 Background

My goal in the experimental part of this research is to create methods and processes for designing hybrid living/non-living biofilms. The combination of synthetic gene networks discussed in the previous section, with the strategies for growing, shaping, in-situ composites will allow tuning both biological function, material structure and properties, in space and time. In addition, design workflows presented in this section, mediate between two traditionally separated scales of resolution: the scale of molecular assembly (or Nano-scale in this dissertation) and the scale of architectural fabrication (or Macro-scale in this dissertation).

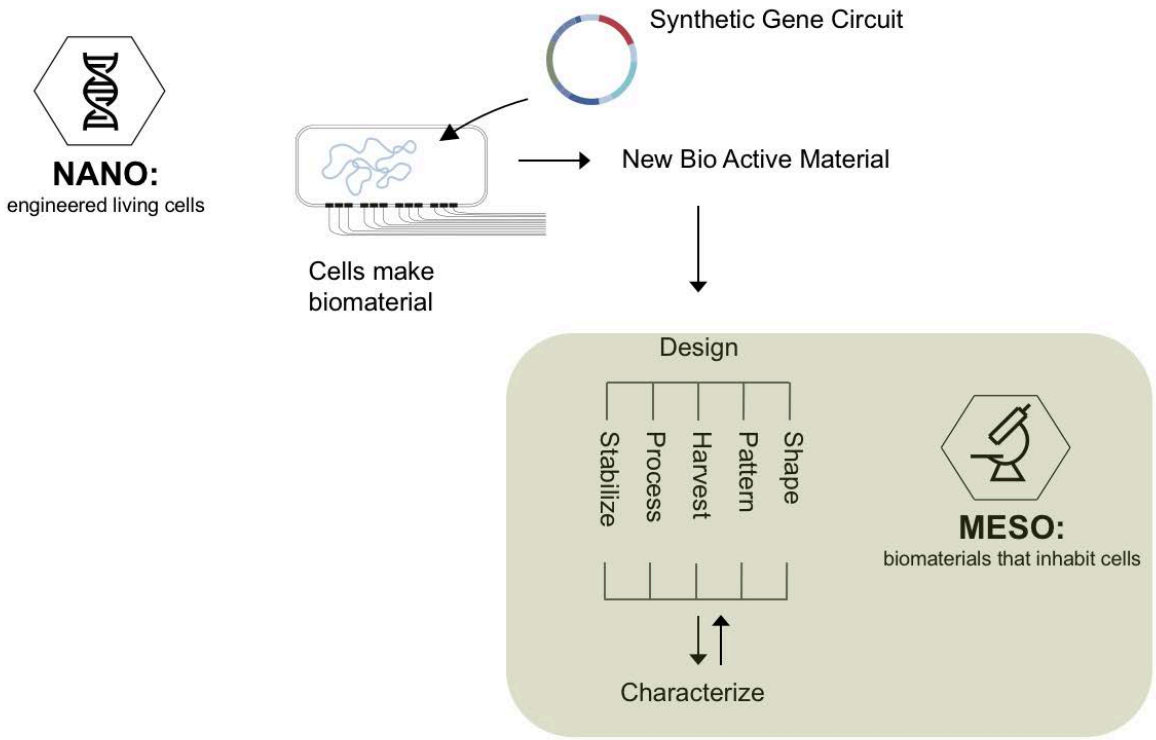


Figure 3.4.0 A diagram of the meso-scale design workflow - biomaterials that inhabit cells.

Unlike the dry structural materials that are commonly used for architectural applications, I am proposing here to use materials that exist in three different phases:

1) The liquid phase, as a cell culture is just initiated.
2) The hydrogel phase, as water-swollen cellulose biofilm is formed on the interface between air and liquid.
3) The solid phase, as the cellulose biofilm is harvested and post-processed (air-dried, oven-dried, or freeze-dried).

Out of these three, only the liquid and the hydrogel phases can support life and biological function of synthetic living cells. If we were to design engineered living materials for architectural applications, we need to develop methods and processes of work for materials with high water contents.

I utilize materials science methods to bridge the gap between work on engineering cells and large scale architectural application. Previous research has demonstrated how modifications of the bacteria growth environment can induce changes in the properties of bacterial cellulose (Watanabe and Yamanaka 1995b; Johnson and Neogi 1989). For example, varying oxygen pressure and agitation caused changes in the density and toughness of the fiber network of bacterial cellulose (Bodin et al. 2007; Watanabe and Yamanaka 1995a; Hult et al. 2003; Johnson and Neogi 1989). Post-processing procedures, such as washing in various solutions and critical point drying has been shown to change material properties by improving the contact between cellulose fibers post-growth (Nishi et al. 1990). Also, taking advantage of high moldability of bacterial cellulose, many recent studies have proposed that shaping the air-medium interface allows to shape the bacterial cellulose as it grows. Oxygen-permeable substrates were fabricated from PDMS in simple tube shapes to create bacterial cellulose structures with oriented fibers (Yoshino, Asakura, and Toda 1996; Putra et al. 2007, 2008; Bodin et al. 2007).

In this section, I present new design strategies that combine shaping, molding, pneumatic actuation, and post-processing to fabricate three-dimensional components from material cellulose with tunable material structure and mechanical properties. These components have a closed shape (spherical or hexagonal , but could be any other shape), which allows them to be filled with water to support biological functions of engineered living cells (see Section 3.3.4).

In the fabrication of the workflow we integrate additive manufacturing technologies, such as 3D printing, that provide powerful methods to create precisely designed structures, molds, and composites with 30μm feature resolution (Singh 2009; Melchels, Feijen, and Grijpma 2010; Yan and Gu 1996). We use PDMS substrates fabricated with the use of 3D printed molds, to grow and shape the biofilms. The ability to grow macroscopic three-dimensional structures from bacterial cellulose will open up avenues for new range of larger scale sustainable applications for architecture.

3.4.2 Methods and Results

Using the methods from materials science, we developed the workflows for bacterial cellulose below. These include culturing cells; harvesting sheets of various sizes of bacterial cellulose; vary growth conditions and making in-growth composites to tune materials structure and properties; in-growth molding and shaping material to achieve three-dimensional components; inflating post-growth to stabilize 3D shapes; post-processing to terminate growth or solidify the material with dormant cells; and structure and properties characterization of the resulting material architectures. Below I summarize these design

strategies. This section will not include a separate Methods section, but rather the methods we developed are integrated in the results portion of this section. Each new material design strategy below includes some steps from the previous design strategy before it, so I list all the steps and capitalize the new one. The color of each step indicates the phase of the material: liquid (green), hydrogel (blue), and solid (gray). This is important since only the liquid and the hydrogel state support biological function of the cells. The letters next to each step indicate the state of cells in the material: active living (l) meaning they are fully functioning; dormant living (d) means they are not fully functioning, but can be revived by adding fresh growth medium; sterile (s) meaning no living cells remain in the material.

Stock (d) ➔ AGITATED (l)

Bacterial cellulose is a unique material system in which invisible microscopic bacteria cells produce, when provided the right growth conditions; they produce macroscopic sheets (or biofilms) of cellulose with superior mechanical properties. Using this natural growth process for larger scale applications, such as building construction here, requires development of a fabrication process "bottom-up" which I present here.

Bacteria cells were purchased as a freeze-dried cell culture, in which cells exist in their inactive, dormant state (See [SB3] in Appendix A for reviving cells stock). Once stock is prepared (**Figure 3.4.1**), cells can be used to gradually scale up the production to the desired size of cellulose sheet. First stage in the process is a two-day agitated culture.

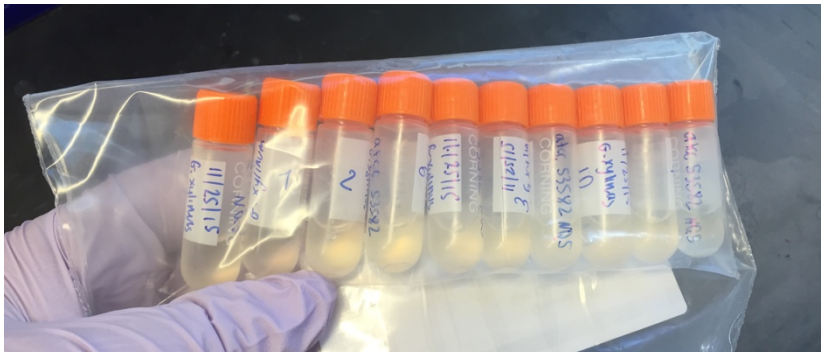


Figure 3.4.1: Freeze dried stock of *G.xylinus* cells.

The macroscopic material sample of bacterial cellulose was produced by cultivation of Gluconacetobacter xylinus. The microorganism used was G. xylinus (American Type Culture Collection (ATCC) 53582). Hestrin-Schramm's medium was used as a culture medium, the constituents were as follows: 2.0% D-glucose, 0.5% yeast extract, 0.5% peptone, 0.51% di-sodium hydrogenphosphate heptahydrate, 0.115% citric acid.

First step in the growth is amplification of cells; it is a short term (24-48hours) culturing to produce enough cells that, once transferred to a larger volume of medium, will start rapidly producing the

cellulose. For this stage we use 15ml falcon tubes, each containing 5ml of HS medium inoculated with *G. xylinus*. Cells are grown in agitation, a constant shaking, which allows oxygen to mix well with the cells. Unlike other bacterial cultures, where cell growth will result in turbidity, in *G.xylinus* culture the cells are trapped or encapsulated in the loose cellulose formations, and the rest of the liquid remains clear. We observed a variety of formations in the tubes, from loose cloudy structure to dense granulated structures or a combination of both (**Figure 3.4.2**). Once there are enough cells, next step is transferring them to a larger volume for cellulose growth.

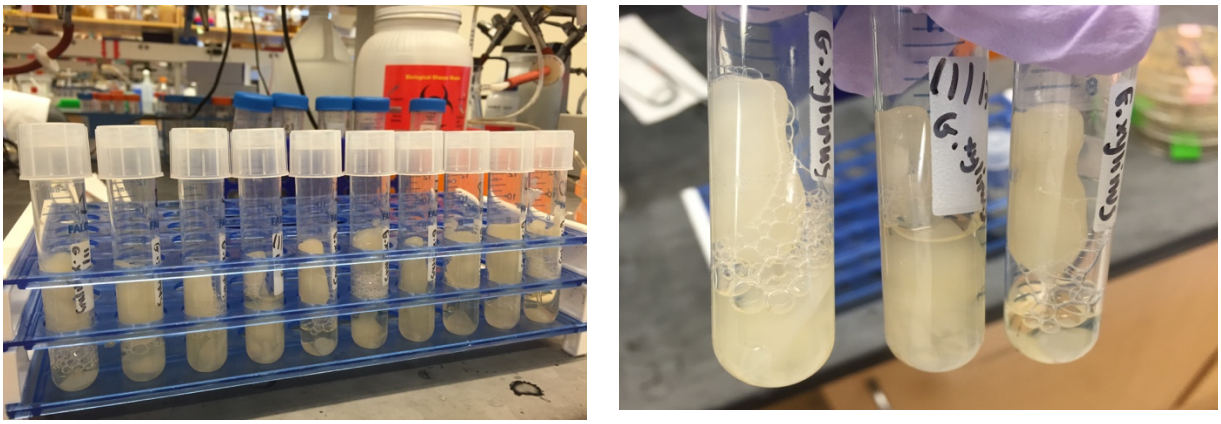


Figure 3.4.2: Overnight agitated culture

Stock (d) ➔ agitated (l) ➔ STATIC (l)

The static culture is achieved by transferring the cells from 1-2 days of agitated culture from cell stock to a larger volume. As cells swim around the liquid and metabolize glucose into cellulose fibers, a white gelatinous substance accumulates on the interface between the medium and the air. The initial rate of cellulose production is approximately 10mm per seven days, and the final thickness will be determined by the availability of nutrients and air. The surface area is not a limiting factor in cellulose production, cellulose sheets can grow as large as a few meters in dimensions.

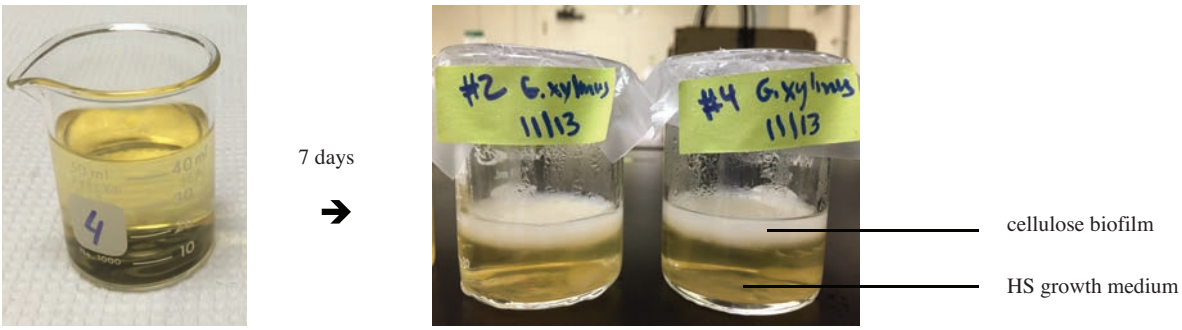


Figure 3.4.3: Static culture of bacterial cellulose.

Stock (d) ➔ agitated (l) ➔ static + COMPOSITE (l)

During the growth molding process, we discovered that the BC membranes fail to maintain their 3D shape after de-molding. After removing the liquid growth medium, they lose significant thickness when transforming from hydrogel to dry membranes, and tend to be very brittle in their dry state. We developed a novel workflow to overcome the aforementioned difficulties. We designed the workflow to include composition of the 3D BC membranes with PVA to achieve greater toughness and increased tensile strength, performing pneumatic actuation in a view to maintain desired 3D shape, and freeze drying to preserve 3D fiber network and porosity of the material.

BC-PVA Composite

Dried BC membranes tend to be very brittle, and lose significant thickness when transforming from hydrogel to dry membranes. That can be a major setback when aiming to create BC based materials that are applicable to design and architecture. In order to achieve greater toughness and increase elongation at break; we fabricated a composite between the BC membrane and Poly(vinyl alcohol) – PVA. Poly(vinyl alcohol) is a water-soluble synthetic homopolymer (Leitão, Silva, Dourado, & Gama, 2013).
PVA is used in textiles, as a coating material, and in other applications. PVA fibers also perform as reinforcement in concrete. The combination of PVA and BC has been previously proposed mainly for biomedical applications (Leitão, Silva, Dourado, & Gama, 2013). BC-PVA composites consider to be a good match due to PVA's water solubility, biocompatibility and good mechanical properties (Figueiredo, Vilela, Neto, Silvestre, & Freire, 2014).

The preparation of the BC-PVA composites was made by solvent exchange and produced as follows: BC membranes, grown for 7 days, with a thickness of ~3mm were washed from the growth medium with distilled water and purified by immersing in NaOH. The samples were then immersed in PVA solution with a concentration of 6% or 10%(Gea et al. 2010)7 for 18H in ~35c, followed by 14H in ~90c. The samples were then frozen for 17H in -20c and thawed for 6H in room temp. After thawing, the samples were reheated in ~65c to remove excess PVA. They were then frozen again at -20c followed by -80c in preparation for freeze-drying. The samples were then freeze-dried for 6 days (**Figure 3.4.4**).
7 PVA solution was prepared by mixing PVA and distilled water for 30 min in 80c.

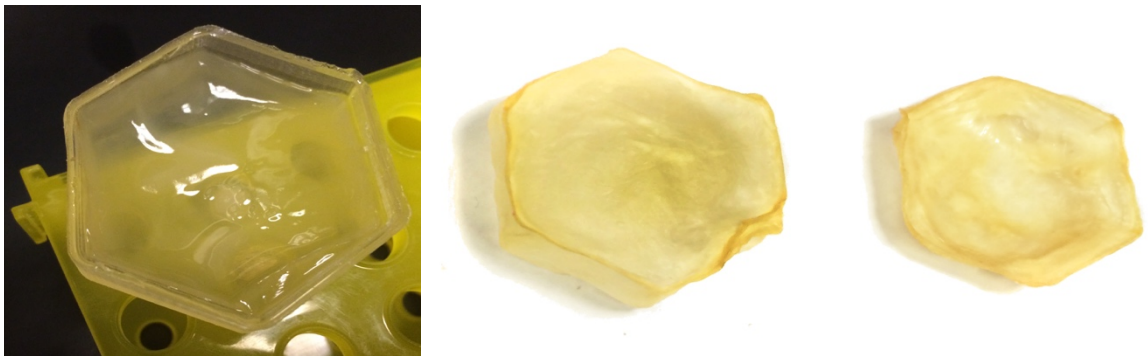


Figure 3.4.4: A composite of bacterial cellulose and Polyvinyl alcohol (with M.Gazit).

BC-Magnetite

Fe3O4 is the formula of the chemical compound iron oxide (also called Magnetite). Magnetite is a ferrimagnetic mineral and it is the most magnetic of all natural minerals on earth. It is drawn to magnets and can become a magnet itself if being magnetized (Wasilewski & Kletetschka, 1999). Composing BC and Magnetite was previously proposed and reported (Zhang et al., 2011). The main objective of fabricating composites is the magnetic properties achieved by the addition of the iron oxide nanoparticles (Figueiredo, Vilela, Neto, Silvestre, & Freire, 2014). For this research we fabricated the BC-Fe**3**O4 composites to enhance the cellulose membrane with magnetic features, and to explore the possibility of producing in-situ composites. BC-Fe**3**O4 composites can have compelling applications in design and architecture due to their flexibility, high tensile strength, their ability to perform as magnetic connectors between different parts and components, and more. The composite was prepared by adding magnetite particles into the culture medium. During growth, the BC nano-fibril network uses as a matrix for an in-situ synthesis of the magnetite particles. Due to the unique section of the PDMS vessels, the magnetite particles were added to the growth medium only in desired areas, resulting in enclosed 3D BC membranes with magnetic features at the edges and the bottom part (**Figure 3.4.5**). Compelling future work in that context includes performing tests to evaluate whether the magnetic particles got linked to the bacteria (and not merely to the cellulose nano-fibrils). In that case, the location and the orientation of nano-fibrils might be guided by guiding the motion in a specific direction using magnets and stirring.

Figure 3.4.5: A composite of bacterial cellulose and Magnetite (with M.Gazit)..

Stock (d) ➔ agitated (l) ➔ static ± composite (l) ➔ POST-PROCESSING (d/s)

The hierarchical structure of bacterial cellulose is a result of the growth process and self-assembly of cellulose fibers. In the static culture, cellulose biofilm is formed as a water-swollen three dimensional network of fibers. Typically, cellulose biofilm will consist of 99% water. As the biofilm is harvested and removed from the growth medium, the structure of the material is determined by the post-processing. We experimented with two post-processing technique: air/oven drying and freeze-drying. For tensile testing described in the section below, we washed in samples in distilled water and purified by boiling in 0.1M NaOH, 60^0C, for 30min, then washed again with water (**Figure 3.4.6**). The material samples were then air dried for 48 hours. As a result, the three dimensional structure of the biofilm collapses, and the thickness is lost to up to 1% of the initial volume.

Figure 3.4.6: Post processing of cellulose biofilms (with M.Gazit).

Stock (d) ➔ agitated (l) ➔ static ± composite (l) ➔ FREEZE-DRYING (s?)

To preserve the three-dimensional fiber network of the biofilm, and also its thickness, we post-processed the material using the freeze-drying technique. The freeze drying technique allows making aerogel by replacing all the water in the material with air. Freeze drying process includes two stage: first rapidly freezing the material and then subjecting it to a high vacuum that removes ice by sublimation.

The preparation of samples after harvesting them included wither one of the following: to sterilize the culture - washing in distilled water and purified by boiling in 0.1M NaOH, 60^0C, for 30min, then washed

again with water; or freezing without washing, such as with the samples on molds or in composites. After this stage, all samples were gradually frozen: first at -20^{0}C and then in -80^{0}C in preparation for freeze-drying. The samples were then placed in the Labcono flask and attached for six days to the Labcono benchtop freeze-dryer (volume 4.5L, temperature -50^{0}C, 115V) (**Figure 3.4.7).**

Table 3.4.1 A summary of freeze-drying experiment results

	Before freeze-drying 12/14/16	After freeze-drying 12/18/16
Diameter	45mm	45mm
Thickness	8.5mm	7.5mm
Weight	17.50gr	0.32gr
% Dry weight	1.8% of wet weight	

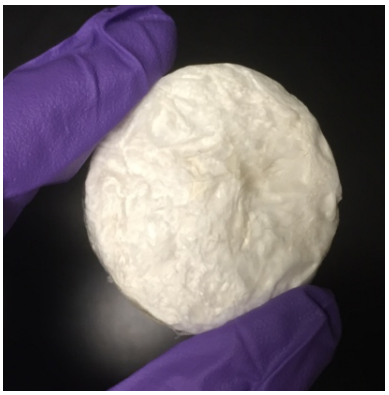
Water content:

We calculate water content by the following formula:

Water content (%) = $(m_{wet} - m_{dry})/m_{wet} \times 100$

Water content (%) of cellulose biofilms = 98%

The water content of cellulose biofilms is 98%, and only 2% of the freeze-dried airogel is cellulose fibers. This makes the cellulose an extremely light aerogel. The lightweight, high porosity, and the irregularity of the cellulose three-dimensional fiber network suggest that cellulose aerogel may have excellent thermo and acoustic properties relevant for architectural applications.

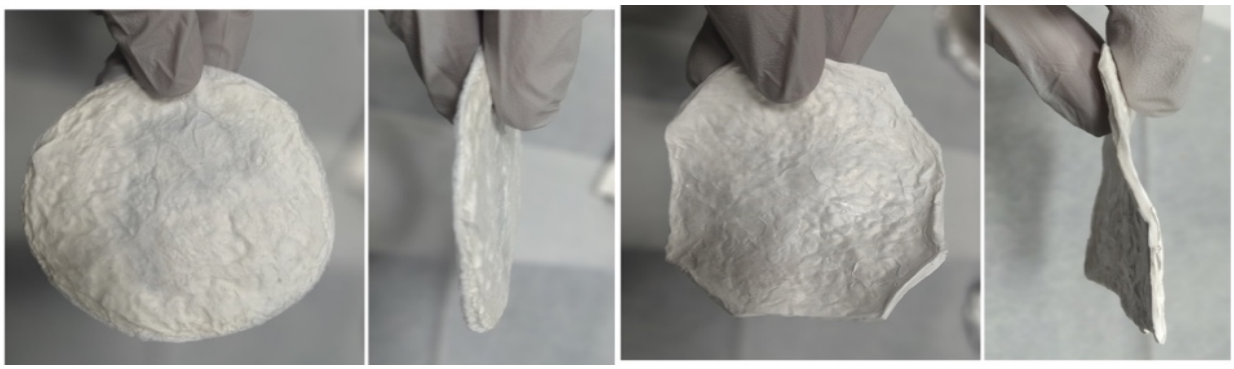


Figure 3.4.7: Freeze drying cellulose biofilms to produce aerogels and maintain three-dimensional fiber network structure (with M.Gazit)..

Stock (d) ➔ agitated (l) ➔ static ± composite (l) ➔ air-drying (s) ➔TESTING (s)

A 1x1cm fragment of a two-week culture membrane of G. xylinus was used in a 20x20 cm container with 200 ml of Hestrin-Schramm's medium (typical formulation with 20 g/L of glucose) and incubated as static culture for 7 days at 30oC. After that BC hydrogel was removed from growth culture, washed in distilled water and purified by boiling in 0.1M NaOH, 60^0C, for 30min, then washed again with water (**Figure 3.4.6** left). To dry the material, the bacterial cellulose was sealed in plastic and placed in a stove 70^0C for 48 hours.

The dried material was laser cut using 120Watt Epilog machine, with parameters of speed 40, power 3, into dogbone geometries (ASTM-638-V) for tensile testing (**Figure 3.4.8**). The dimensions of samples were 9.51mm*3.18mm*0.14mm and the weight of the sample 0.02 g. The samples were loaded in uniaxial testing until failure on a Zwick mechanical tester (Zwick Z010, Zwick Roelle, Germany) under displacement controlled loading at a strain rate of 0.5 mm/min using a 2.5 kN load cell (**Figure 3.4.8 right**). A representative stress-strain curve for tested samples was plotted and results shown in Figure X. The sample showed a small region of linear elastic loading until 2.5% elongation with E = 13.5 MPa. The sample continued to extend in the plastic regime with strain hardening until failure at 34% elongation and ultimate tensile strength 7.4 MPa. Please see results below.

Figure 3.4.8: Lasercutting bacterial cellulose samples for tensile testing (with M.Gazit)..

Stock (d) ➔ agitated (l) ➔ static + IN-GROWTH MOLDING (l)

In this experiment, the attempt was to control the three-dimensional structure of the cellulose membrane by changing the physical set up of the growth. 3d printed molds with various surface morphologies and texture resolutions were designed and 3d printed (**Figure 3.4.9**). The molds were fixed in 100ml containers; medium and starter culture were added. We observed that the cellulose membrane attached itself to the mold instead of following the surface of the liquid as it usually does in static culture (**Figure 3.4.9**). The membrane followed formation with good precision in a water-swallowed state. When dried, it lost the thickness significantly (**Figure 3.4.9**).

Figure 3.4.9: Molding bacterial cellulose biofilm as it grows on 3D printed molds and introducing texture (with S.Araya).

Stock (d) ➔ agitated (l) ➔ static ➔ air-dry + POST-GROWTH MOLDING (d/s)

For the larger –scale material production, seven days grown membranes were introduced into six-liter HS medium volume in a twenty-five-gallon tank. The heating pad was applied to keep the temperature to (+27°C), and web camera was installed and programmed to follow the growth process (**Figure 3.4.10** upper left). After 20 days of growth, the membrane was stabilized and achieved an average thickness of 8mm. The stabilized membrane was taken out of the medium, washed with tap water and placed on a CNC-milled wooden mold for several days to dry. Even the finest texture of the mold was visible on the resulting shape.

Figure 3.4.10: Molding bacterial cellulose biofilm post- grows on CNC-milled mold (with S.Araya).

Stock (d) ➔ agitated (l) ➔ static + SHAPING (l)

We successfully fabricated 3D complex shapes from bacterial cellulose for the first time and designed a workflow that allows creating arbitrary shaped surfaces from bacterial cellulose shapes.

BC grows only at the interface between medium and air. PDMS has a unique property of being liquid-proof and at the same time oxygen-permeable. Therefore, culturing BC cells in 3D PDMS vessels filled with growth medium enables the replication of the shape of the oxygen-medium interface created by the PDMS substrate, hence allowing shaping BC as it grows **(Figure 3.4.12, 13,14).** The growth vessels were designed to fit into the 3D printed framework of the macro-fluidic device **(Figure 3.5.2).**

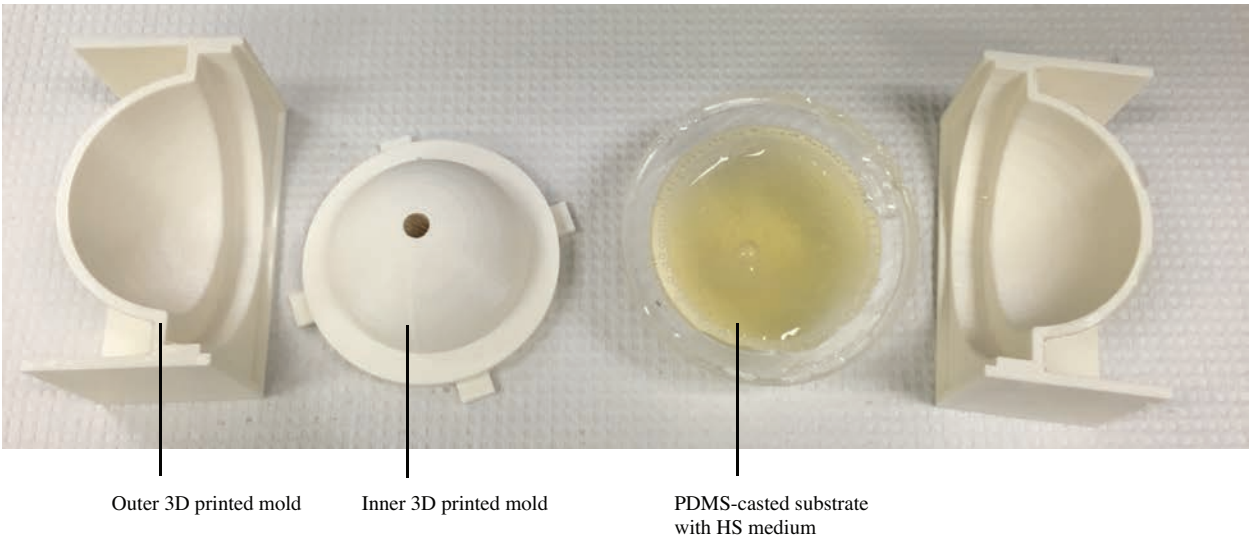


Figure 3.4.11: Inverse-molding of silicone growth vessels for cellulose growth and shaping (with M.Gazit).

Invert molds design and 3D printing and PDMS molds casting

3D Printing: Molds for PDMS casting are fabricated using multi-material additive manufacturing with Objet Connex 500 (Stratasys Ltd., EdenPrairie, MN). VeroWhite, a rigid ABS-like material, and TangoPlus are utilized. The Young's moduli of TangoPlus and VeroWhite have been experimentally determined to be 0.636 +- 0.02MPa and 2.06 +- 0.09 GPa, respectively, and the tensile and shear strength have been determined to be 1.86 +- 0.94MPa and 1.46 +- 0.11MPa, respectively.

Hexagon PDMS growth vessels: In order to enable proper BC growth inside the PDMS vessels, we maximized the air-medium interface and designed the vessels as thin as possible – up to 1.5mm thickness. We cast PDMS into 3D printed molds made of rubber-like materials for easy de-molding **(Figure 3.4.12).**

Preparation of PDMS mixture for casting: Silpot 184 and Silpot 184 catalyst were purchased from Dow Coning Inc. The elastomer and curing agent were mixed in 10:1 ratio. The mixture was then placed in a vacuum chamber for 10 minutes in order to avoid air bubbles. After de-gassing, the mixture was poured into the 3D printed molds and baked for 20min at 125^0C in an oven until cured.

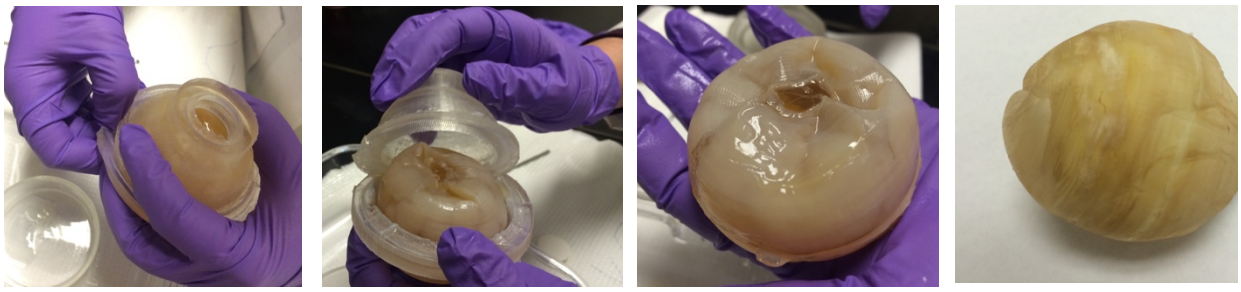


Figure 3.4.12: Removing cellulose biofilm from the mold. Cellulose sphere still holds the liquid (third from left) (with M.Gazit).

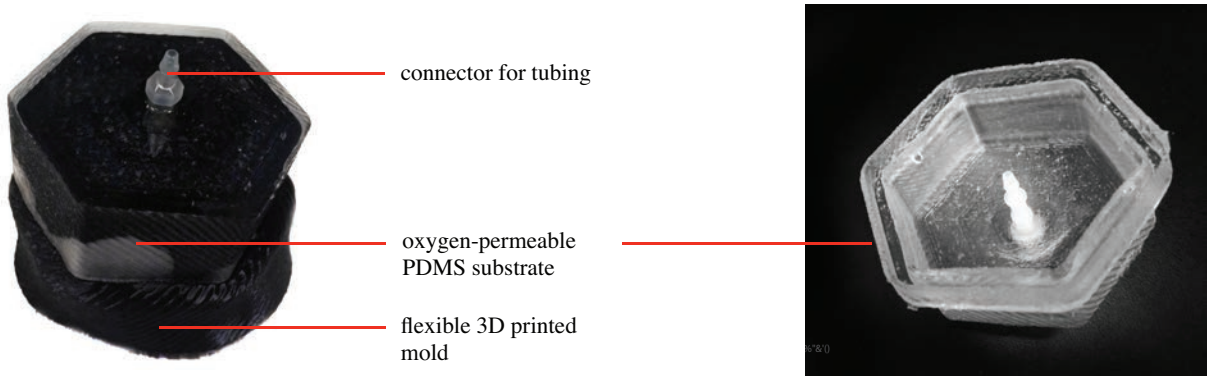


Figure 3.4.13: Flexible 3D printed mold (left) and the resulting silicone vessel with embedded inlet and outlet (right) (with M.Gazit).

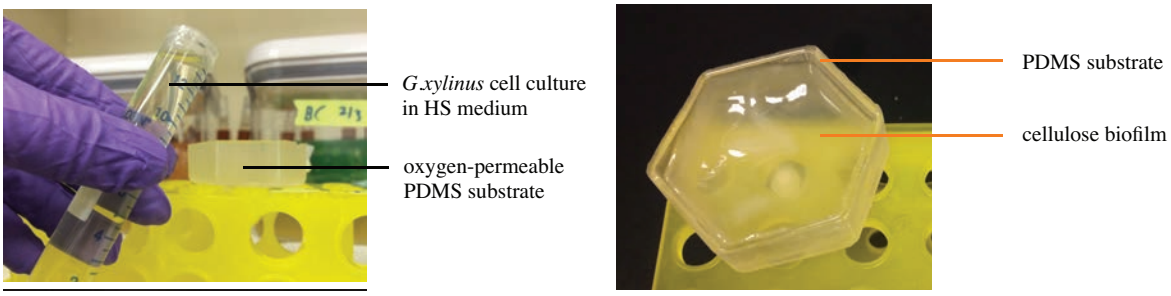


Figure 3.4.14: Growing three-dimensional cellulose biofilm in the growth vessel. Introducing overnight cells culture into the growth vessel (left) and biofilm growth (right) (with M.Gazit).

Stock (d) ➔ agitated (l) ➔ static + shaping (l) ➔ PNEUMATIC ACTUATION (l)

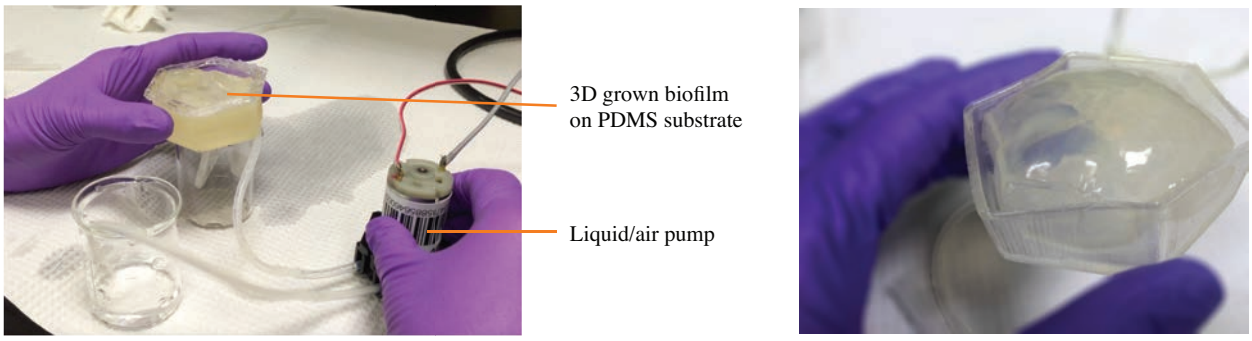


Figure 3.4.15: Pneumatic actuation of the three-dimensional biofilm (part one). Draining liquids and pumping air using air-liquid pump (left) and the resulting three closed hexagonal air-filled cellulose biofilm ready to be removed from the growth vessel (right) (with M.Gazit).

We designed a process of draining liquids from inside the grown 3d cellulose biofilm, and replacing water with air using dual liquid-air pump (Figure 3.4.15 above). This process allows the preservation of the closed biofilm shape and produce 3d cellulose components that can exist both in hydrogel (**Figure 3.4.16**) and dry state (**Figure 3.4.17**)

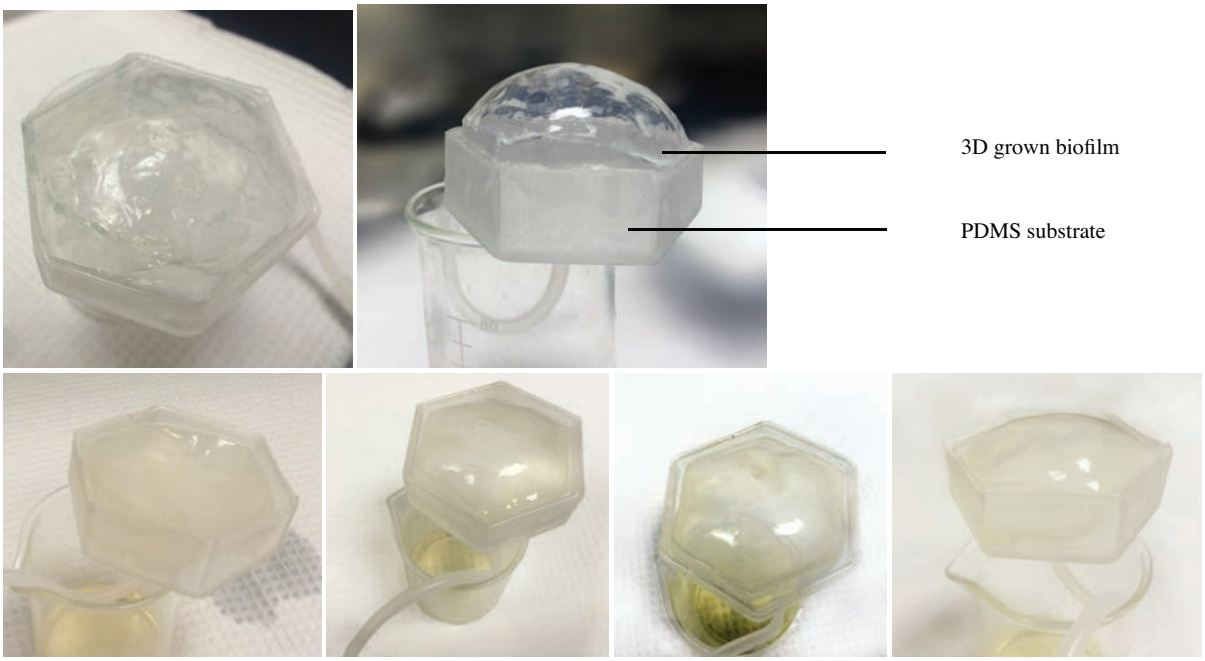


Figure 3.4.16: Pneumatic actuation process of the three-dimensional biofilm (part two) (with M.Gazit).

The hexagonal geometry of the cellulose components allows their aggregation to produce larger assemblies for architectural applications **(Figure 3.4.17).**

Figure 3.4.17: An aggregation of freeze-dried cellulose components (with M.Gazit).

3.4.3 Discussion and Future Work

I develop hybrid materials that combine structural properties of cellulose, the main component of wood traditionally used in building construction, with the living capabilities of a bacterial biofilm. In this section I experimentally demonstrated how to regulate the environment and guide the growth of cellulose biofilms. I also developed ways to tune the biofilm's shape, structure and properties by molding, shaping, post-processing, and introducing composites (such as PVA, and Magnetite) in-growth.

The next step would be integrating DNA computation described in the previous Section 3.1 with the chemical and physical parameters of guiding the growth of biofilm presented here. Combining these two levels will allow us to design and guide the bacteria to autonomously produce dynamic materials whose structure and composition change over time and adapt to its environment. This vision will require systematic studies and tune the parameters of growth to the resulting structure and properties of the cellulose material. This will require mathematical and computational predictive modeling.

The proposal for using genetic computation for patterning cellulose biofilms discussed in Section 3.2.4 above would be the first step toward this vision.

3.5 Macro-scale: Macro-Fluidic Pneumatic Interface

On the scale of centimeters and up, relevant for architecture, we design computationally controlled macro-fluidic pneumatic interface. Through the computationally controlled flow of liquids and air, this envelope facilitates the growth, patterning, pneumatic actuation, and post processing of the composite cellulose membranes into three-dimensional components. The macro-fluidic pneumatic interface is a novel bio-computational interface with which a designer can interact with the process of growth and guide the self-assembly of material architecture and its function. We built custom made microcontrollers and designed computational scripts to control physicochemical conditions in each growth vessel and allow post-growth pneumatic actuation of bacterial cellulose in each vessel. Designing the flow of matter, and energy in the interface. I then design and modulate the growth environment as scaffolding for patterning, shaping, harvesting, and processing composite biofilms (cellulose membranes), while keeping the bacteria cells alive and responsive. These methods of combining genetic engineering with environmental regulation and scaffold design can be further generalized to other material systems where bacteria acts as a matter-organizing agent, sensors and actuators of pre-programmed biological functions. **Figure 3.5.0** shows a diagram of the full nano-meso-macro design cycle of Guided Growth.

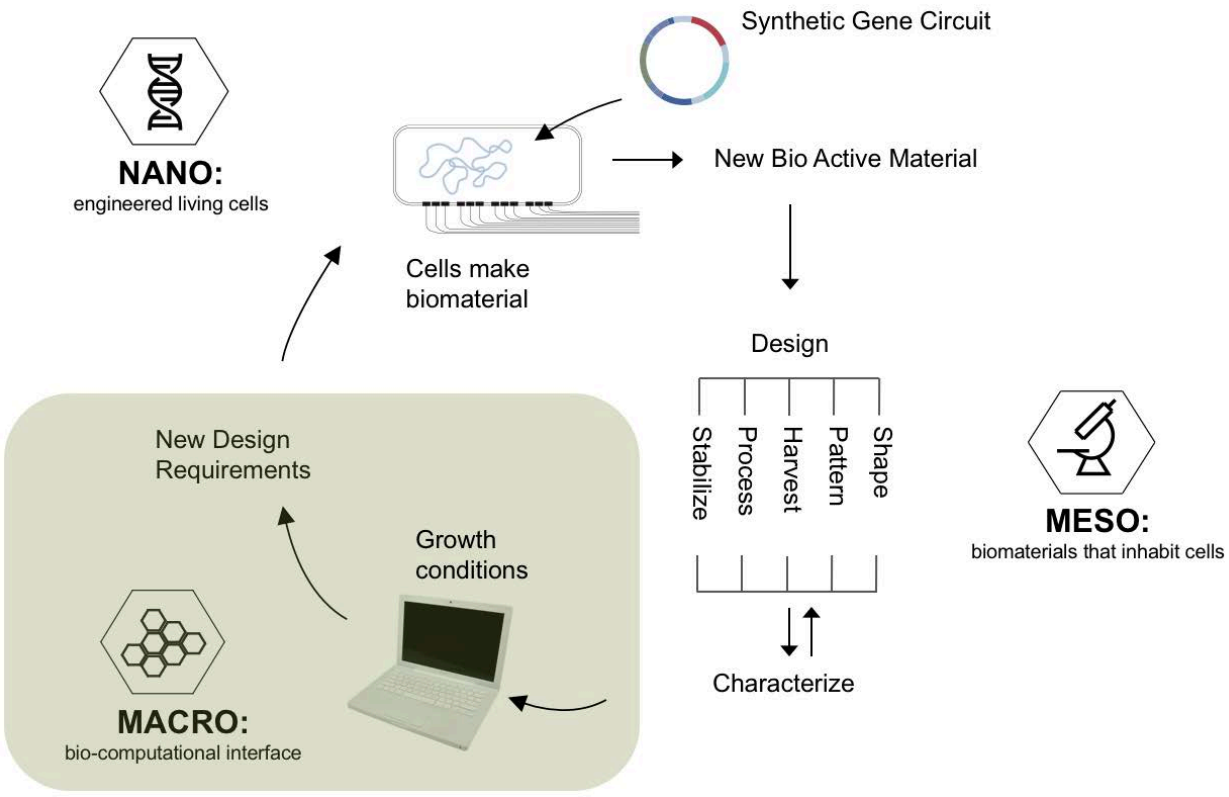


Figure 3.5.0: A diagram of the full nano-meso-macro design cycle of Guided Growth.

3.5.1 Background

This section begins to deal with question of how we can scale up biological processes and design strategies described in the previous sections.

In architecture, severalideas are being proposed for integrating living cells with non-living scaffolding or structure. Project Protocell imagines bacteria introduced into the pillar structure in the Venice canal (Hanczyc and Ikegami 2009; Armstrong 2011; *Protocell Architecture: Architectural Design* 2011). Once bacteria get exposed to light and water due to a crack, they start producing reinforcing materials that repair the pillars. In Delft, similar technology is already being developed (Wiktor and Jonkers 2011). Researchers add dormant bacteria to the concrete mix, when the concrete cracks, and water enters the gaps, it comes into contact with the bacteria and the food source, setting the healing process off. The bacteria then feed on the calcium lactate, joining the calcium with carbonate to form limestone, thereby, fixing the crack. In the Biota Architectural Studio at Bartlett School of Design, researchers develop porous hygroscopic materials that can act as scaffolds for living cells and materials systems due to their structural integrity on one hand, and high water content on the other (Cruz and Beckett 2016).

The proposed solutions, although not developed as an actual materials systems with integrated living components, offer seamless integration of both the living and non-living component in one material system. Such is the biofilm of cellulose. Cells are embedded in the biofilm, and in the previous section I showed design strategies to create three-dimensional structural components from bacterial cellulose with the possibility to maintain living function: shaping, making composites to tune properties, making aerogels, etc. (see Section 3.4). Once techniques of growth and patterning using synthetic gene networks are developed, there will be no need in additional scaffolding – material components will grow from cells.

Meanwhile, the primary challenge and limitation seem to be that living cells need a continuous supply of nutrients and oxygen to grow. A homeostasis, relatively steady living conditions, needs to be kept in order to maintain the function of biologically active materials. The area of tissue engineering for biomedical applications face a similar challenge: tissues need the infrastructure of channels to feed nutrients in and waste out and maintain the homeostasis for the functioning of living cells. Innovations in this area include surprising solutions such as 'ghost heart'(Maher 2013) … or an apple (Modulevsky et al. 2014). In the 'ghost heart', scientists would remove the donor's cells from the tissue, use the remaining porous tissue as a scaffold, repopulate it with the patient's stem cells and then transplant the organ back into the patient. In apple, once all the cells are removed, the material that provides structure — the scaffolding — for new cells population is apple cellulose. However, it is very hard to regulate the flow of nutrients, liquids, and air through such porous substrates.

To regulate the flow and create an optimal condition for growth and functions of living cells, bioreactors are used. In the laboratory settings or an industrial-scale production of biological processes, a bioreactor is set up to support living function. Bioreactor is a vessel or an apparatus in which a biological reaction or process is carried out, and favorable conditions for living cells are maintained.
Recently, development of bioreactors have been miniaturized and democratized. Microfluidic devices control and manipulate fluids to support cells growth. Recently, Metafluidics was developed by the MIT community. Metafluidics is an open-source, community-driven repository that hosts digital design files, assembly specifications, and open source software to enable users to build, configure, and operate a microfluidic device. This device and controller are applied to build genetic circuits using standard DNA assembly methods including ligation, Gateway, Gibson, and Golden Gate and is intended to enable a broad community to engage with design with synthetic biology.

In this project, we construct macro-fluidic bio-pneumatic scaffolding to regulate the process of biofilm growth and support the function of living cells.

3.5.2 Methods & Results

We aim to add control of environmental conditions to the genetic regulation of bacterial cellulose structure and properties. In this part of the research, we systematically investigate the role of physicochemical conditions. We designed and fabricated a macro-fluidic pneumatic device presented in **Figure 3.5.1**. We build custom made microcontrollers and design python script to:

a) control physicochemical conditions in each growth vessel
b) allow post-growth pneumatic actuation of BC in each vessel

The macro-fluidic pneumatic device consists of 21 hexagon growth vessels, 4 types of growth medium for variation in in-situ growth conditions, PVA solution and Fe3O4 powder for post-growth bio-composites, reserve containers for renewable waste, 10 miniature solenoid valves, 6 liquid and air pumps, custom made MOSFET circuits, and Arduino microcontroller connected to a laptop in order to actuate, monitor and receive feedback from the system (Gazit 2016). Each cell has an inlet and outlet, and the entire system is routed by silicone tubes (**Figure 3.5.2).**

The macro-fluidic pneumatic device is computationally controlled, enabling a tunable flow of air and liquids in and out of each growth vessel in the system. This facilitates the growth of hybrid cellulose-based materials with different properties in each cell. Variation in material properties is achieved by regulating physicochemical growth conditions and post-growth processing. The system allows the adjustment of medium composition, in-situ substance concentration, and solvent exchange in order to create bio-composites, and post-growth pneumatic actuation (**see Section 3.4.2**). Also, computation and

fabrication techniques such as monitoring pneumatic and fluid actuation by code, molding and casting, 3D printing, and the development and fabrication of custom electronic circuits were also employed.

Figure 3.5.1: A diagram of the macro-fluidic pneumatic interface and its basic parts (with M.Gazit).

Figure 3.5.2: Perspective views of the macro-fluidic pneumatic interface (with M.Gazit).

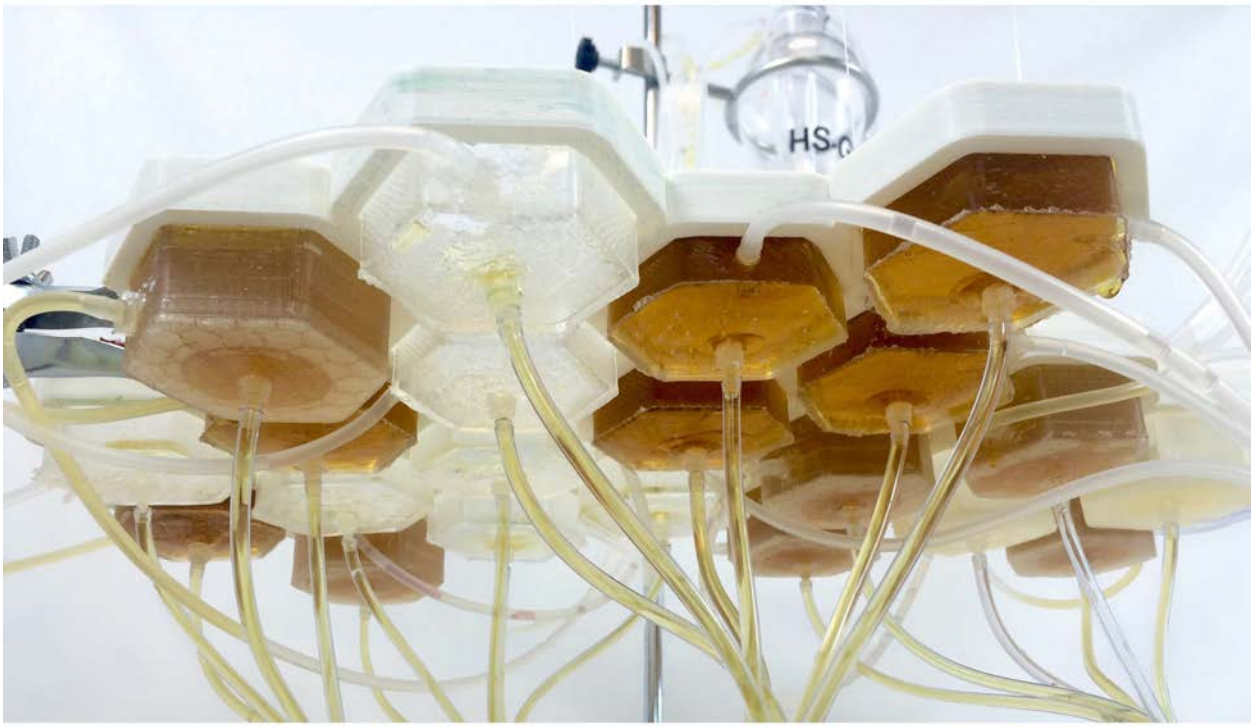


Figure 3.5.3. A zoom in view on the growth vessels with inlet and outlet supply lines (with M.Gazit).

Designed air and fluids actuation and controls for the macro-fluidic device.

In order to computationally control a measured flow of air and liquids in and out of each growth envelope in the device, we designed the following system:

Two miniature solenoid valves (X-Valve by Parker) were connected to each pair of growth vessels. One solenoid monitors incoming flows and the other monitors waste flows. The solenoids are also connected to pumps which either injected or vacuumed fluids in and out of the cells and the different growth medium and other substances in the system. The solenoids are connected to a MOSFET circuit, which were designed and fabricated by Merav Gazit. The MOSFET circuits are connected to an Arduino microcontroller, which enables communication and feedback with the programming environment. A script that actuates and monitors flows in different conditions and compositions was written in Arduino IDE. The custom-made electronics control system for macro-fluidic device: solenoid valves, pumps, MOSFET circuits, and Arduino microcontroller (**Figure 3.5.4**)

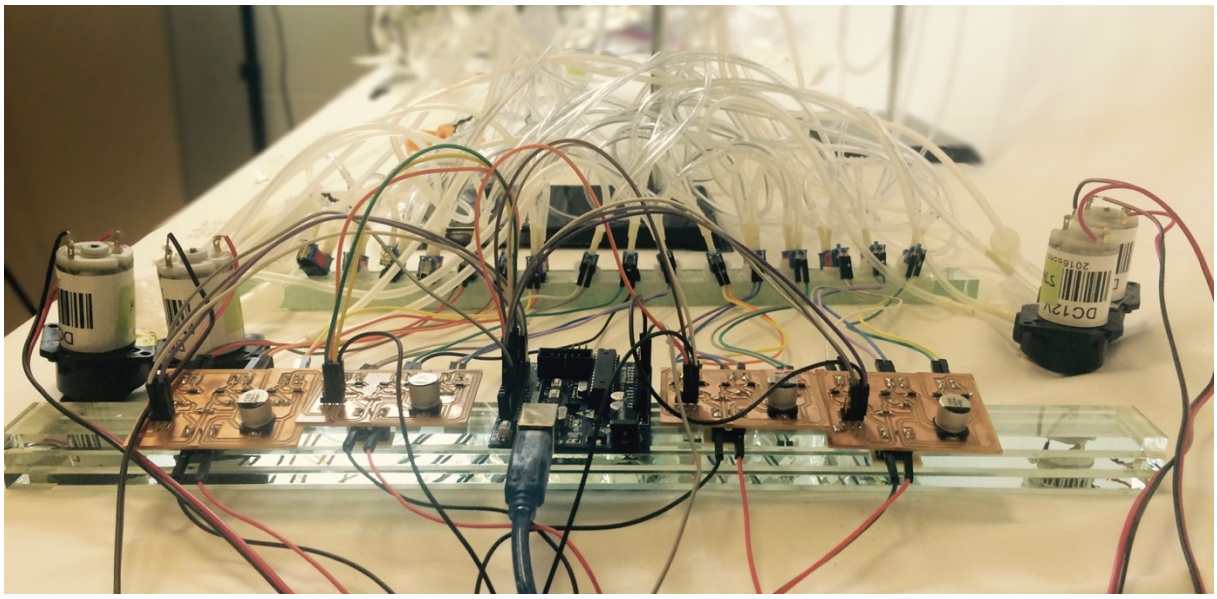


Figure 3.5.4: The custom-made electronics control system for macro-fluidic device: solenoid valves, pumps, MOSFET circuits, and Arduino microcontroller (with M.Gazit).

A matrix of environmental parameters

Using the macro-fluidic device we built, we conducted experiments to investigate the role of environmental conditions on the structure and properties of bacterial cellulose. We fabricated 21 hexagon growth vessels, 15 of them were made from PDMS, and 6 were made of 3D printed PLA. Using the Macro-Fluidic Device, we investigate a matrix of parameters and their effect on BC structure and properties (**Figure 3.4.8** in Section 3.4). Each hexagon cell in the system has an inlet connector through which to insert growth medium, composite solution and air, and an outlet connector to remove liquids into the reserve container. Each pair of cells contains a unique composition of the parameters described in **Table 3.5.1**, in order to grow BC membranes with varied structure and properties. The table summarizes the modifications to the growth environment, and references the source of the protocol. For more detailed protocols of growth modifications, please see section 3.4 above.

Table 3.5.1 A summary of the growth modifications in macro-fluidic interface.

Name	Modification of growth conditions	Protocol
Control	A static culture of G.xylinus in Hesterin-Swann growth medium containing glucose as a carbon source	
Interval_Control_0	A static culture of G.xylinus in Hesterin-Swann growth medium containing glucose as a carbon source	(Mikkelsen et al. 2009)
Interval_1 - 3	A flow of medium in and waste out the growth vessels is regulated through custom script over time (see … for details)	-
HS_SUC_Growth Medium	A modified growth medium containing sucrose as a carbon source, which has been shown to increase cellulose production	(Ruka, Simon, and Dean 2012)
Y_GLU_Growth Medium	A modified growth medium, which has been shown to improve mechanical properties of BC	(Yamanaka et al. 1989)
BC - Fe_3O_4 Composite	Flexible magnetic membranes prepared by adding magnetic nanoparticles into growth medium that are incorporated into BC membrane as it grows	(Zhang et al. 2011)
BC - 6% PVA Composite	Bio-nanocomposites of BC and PVA (Cellulose-poly(vinyl alcohol)) were prepared by treating BC with PVA solution post-growth. PVA acts as plastisizer, interrupting hydrogen bonding between cellulose fibrils, and resulting in more ductile membranes (see below for mechanical data)	(Gea et al. 2010)
BC - 10% PVA Composite		
***in-situ* BC - PVA Composite**	Bio-nanocomposites of BC and PVA (Cellulose-poly(vinyl alcohol)) were prepared by adding PVA solution to the HS growth medium	(Gea et al. 2010)

In **Figure 3.5.5** below maps growth modifications to the macro-fluidic device layout. Each cluster of growth vessels comprises a unique composition of parameters to grow BC membranes with varied structure and properties. To visually distinguish different growth environment, we introduced food coloring into the medium (**Figure 3.5.6**).

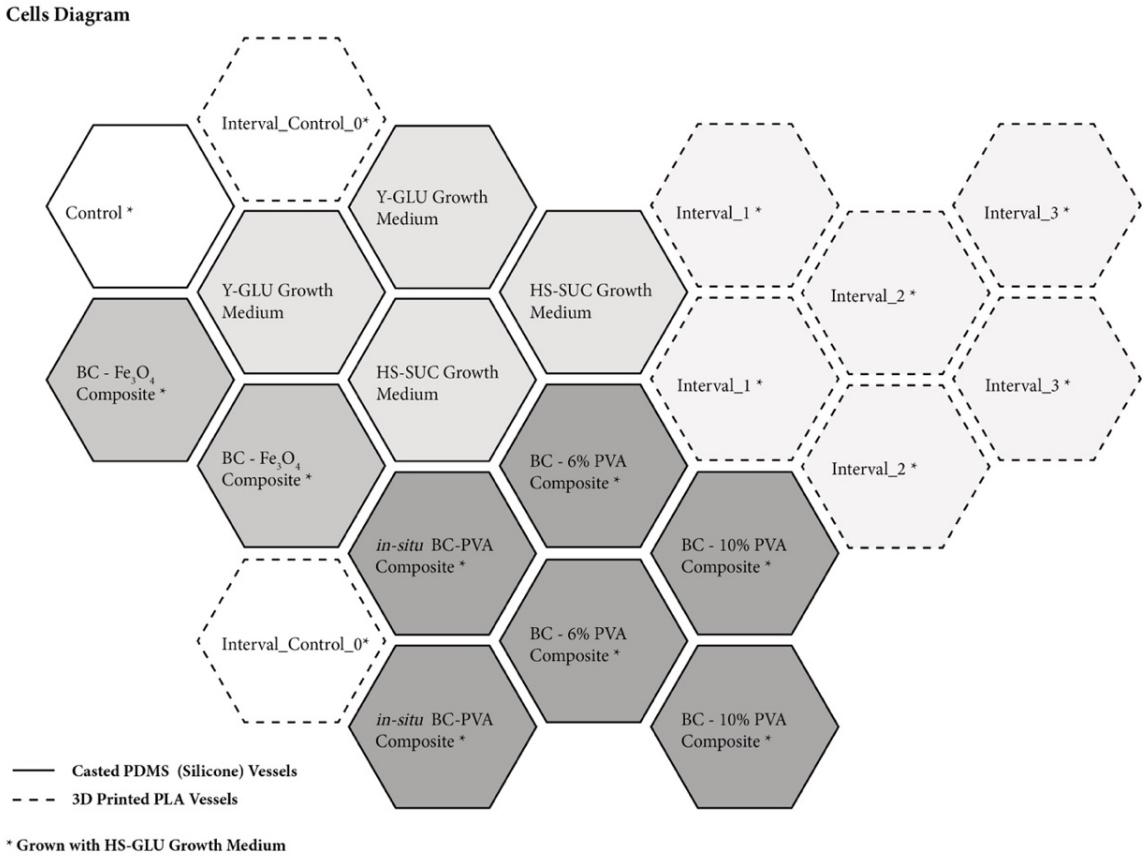


Figure 3.5.5. Mapping growth modifications to the macro-fluidic device layout. Each cluster of growth vessels comprises a unique composition of parameters to grow BC membranes with varied structure and properties (Diagram by M.Gazit).

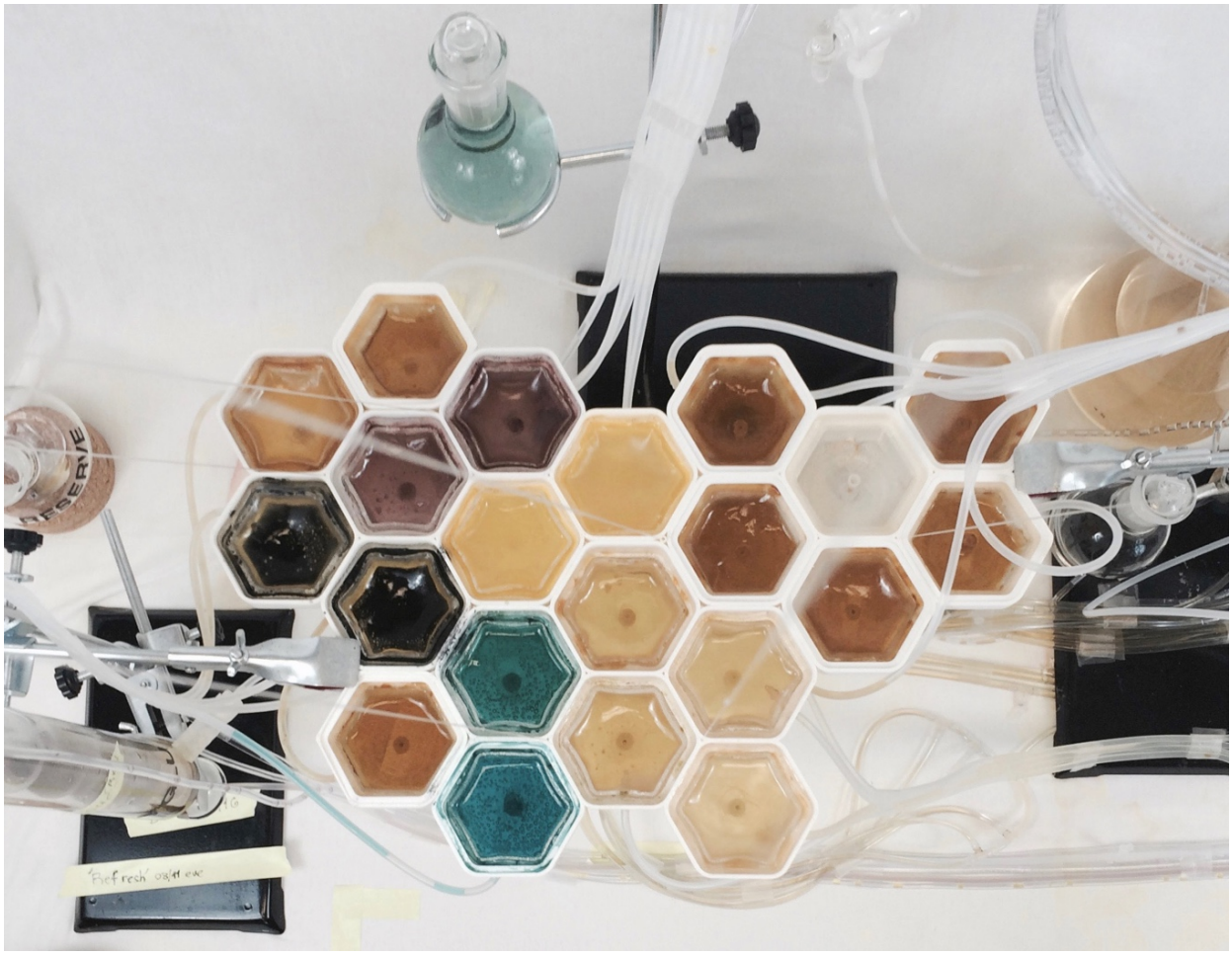


Figure 3.5.6. A plan view of the growth vessels and their different clusters (with M.Gazit).

Using the macro-fluidic device we demonstrated a matrix of environmental parameters. The effect of modifications in growth parameters on bacterial cellulose structure and properties was also investigated. The macroscopic material samples of bacterial cellulose were produced by cultivation of *Gluconacetobacter xylinus* in static culture. The microorganism used was *G. xylinus* (American Type Culture Collection (ATCC) 53582). Hestrin-Schramm's medium was used as a culture medium, the constituents were as follows: 2.0% D-glucose, 0.5% yeast extract, 0.5% peptone, 0.51% di-sodium hydrogenphosphate heptahydrate, 0.115% citric acid. A 1x1cm fragment of a two-week culture membrane of *G. xylinus* was used in a 20x20 cm container with 200 ml of Hestrin-Schramm's medium (typical formulation with 20 g/L of glucose) and incubated as a static culture for 7 days at 30ºC.

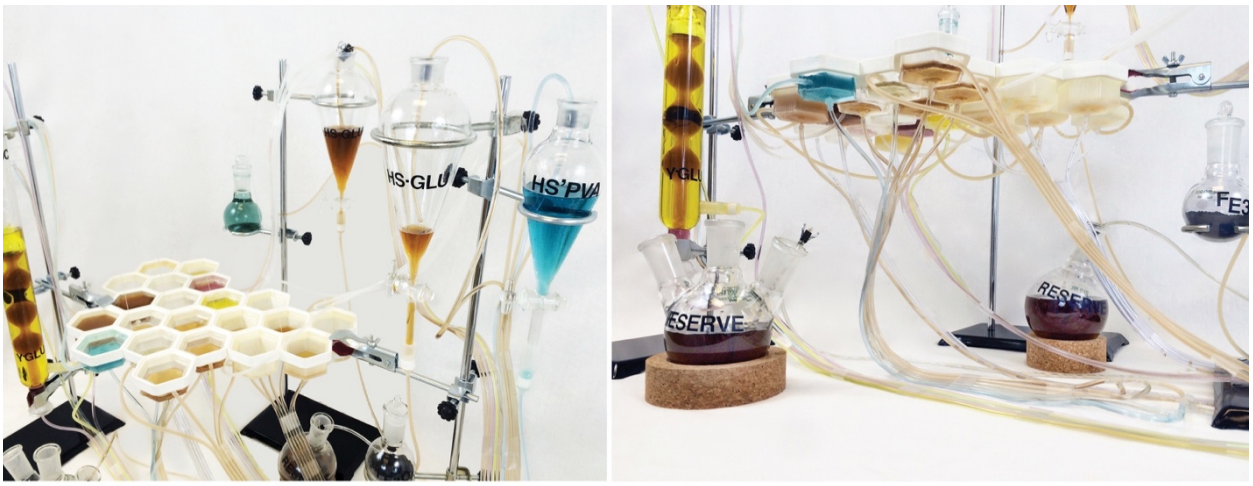


Figure 3.5.7. Growth vessels of varied composition (color-coded using food coloring) in the macro-fluidic device(with M.Gazit).

After 7 days of growth, BC hydrogel was removed from growth culture, washed in distilled water and purified by boiling in 0.1M NaOH, 60^0C, for 30min, then washed again with water. To dry the material, the bacterial cellulose was either air-dried (AD) for 48 hours or freeze dried (FD) (**Table 2**). The dried material was laser cut using 120Watt Epilog machine, with parameters of speed 40, power 3, into dogbone geometries (ASTM-638-V) for tensile testing **(Figure 3.4.8)**. The samples were loaded in uniaxial testing until failure on a Zwick mechanical tester (Zwick Z010, Zwick Roelle, Germany) under displacement controlled loading at a strain rate of 0.5 mm/min using a 2.5 kN load cell.

Table 3.5.2. A Summary of samples description

Sample	**Sample description**	**Protocol**
BC sheet with cells	BC sheets were grown in a static culture from G.xylinus and HS medium for 7 days. BC sheets were harvested, washed with distilled water twice and air-dried until no change of weight was recorded (see section 3.4.2 for full procedure).	(Iguchi, Yamanaka, and Budhiono 2000)
BC foam	BC sheets were grown as above, then treated with alkaline solution (NaOH) to digest bacteria cells and medium residue, see section 3.4.2 for full procedure. The samples were then gradually frozen to -80^0C, and then freeze dried for 6 days in Labcono benchtop freeze-dryer.	(Liebner et al. 2013)
BC-PVA 6% composite foam	Cellulose-poly(vinyl alcohol), or PVA, nanocomposites. BC sheets were grown as above and then immersed in PVA solution with concentration of 6%, see section 3.4.2 for full procedure. After that foams were prepared by freeze drying for 6 days.	(Gea et al. 2010)
BC-PVA 10% composite foam	Cellulose-poly(vinyl alcohol), or PVA, nanocomposites. BC sheets were grown as above and then immersed in PVA solution with concentration of 10%, see section 3.4.2 for full procedure. After that foams were prepared by freeze drying for 6 days.	(Gea et al. 2010)

Table 3.5.3. A summary of mechanical testing results.

Sample	Density (g/cm^3)	Elongation at break (%)	Ultimate tensile strength (MPa)	Elastic modulus (MPa)	Toughness (MJ/m^3)
BC sheet with cells	-	6.77 +/- 0.52	48 +/- 4	728 +/- 21	1.52 +/- 0.48
BC foam	0.11+/- 0.05	5.27 +/- 1.37	24 +/- 10	674 +/- 312	0.42 +/- 0.21
BC-PVA 6% composite foam	0.26 +/- 0.02	4.6 +/- 0.59	13 +/- 2.5	289 +/- 41	0.37+/-0.15
BC-PVA 10% composite foam	0.69 +/- 0.28	43 +/- 9.2	26 +/- 0.5	60 +/- 7	11.62+/-3.74

Elastic modulus (GPa) - We calculate the elastic modulus as the slope of its stress–strain curve in the linear elastic deformation region. We measured an order of magnitude higher elastic modulus (16.5GPa) in the air-dried bacterial cellulose. The elastic modulus of sample 3 is similar to that reported in the literature, of 7-day grown BC air-dried at 20^0C that had an elastic modulus of 17GPa (Iguchi, Yamanaka, and Budhiono 2000).

Elongation at break (%) – We calculate the elongation at break as the percentage (%) of the changed length from the initial length the breakage point of the test specimen. It expresses the capability of material to resist changes of shape without crack formation. The elongation at break is the highest by order of magnitude in BC-PVA 10% composite, standing at almost 100% compared to below 10% in other samples.

Toughness (MJ/m^3) - The toughness was calculated by calculating the area under the stress-strain curve. A custom-made script summarized values of d(toughness) function, which calculated trapezoid sub-areas between each set of two points on the curve. The highest toughness was 25.8 MJ/m^3, in order of magnitude higher than non-composite samples, ranging 0.2-2 MJ/m^3.

Ultimate tensile strength (UTS) (MPa) – We calculate the ultimate tensile strength as the stress value at the highest point of the stress–strain curve, this value is independent of the size of the test specimen. We observed that BC samples broke very sharply, without plastic deformation, in a brittle failure. However, the BC-PVA 10% composites were much more ductile and experienced plastic deformation

and necking before fracture. The highest UTS of 227 MPa was measured in the air-dried bacterial cellulose (sample 3), and it was order of magnitude higher than the UTS of other samples ranging 15-45 MPa. The UTS of sample 3 is similar to that reported in the literature, of 7-day grown BC air-dried at 20^0C that had UTS of 256MPa. Representative stress-strain curves for tested samples were plotted and results shown in summarized below.

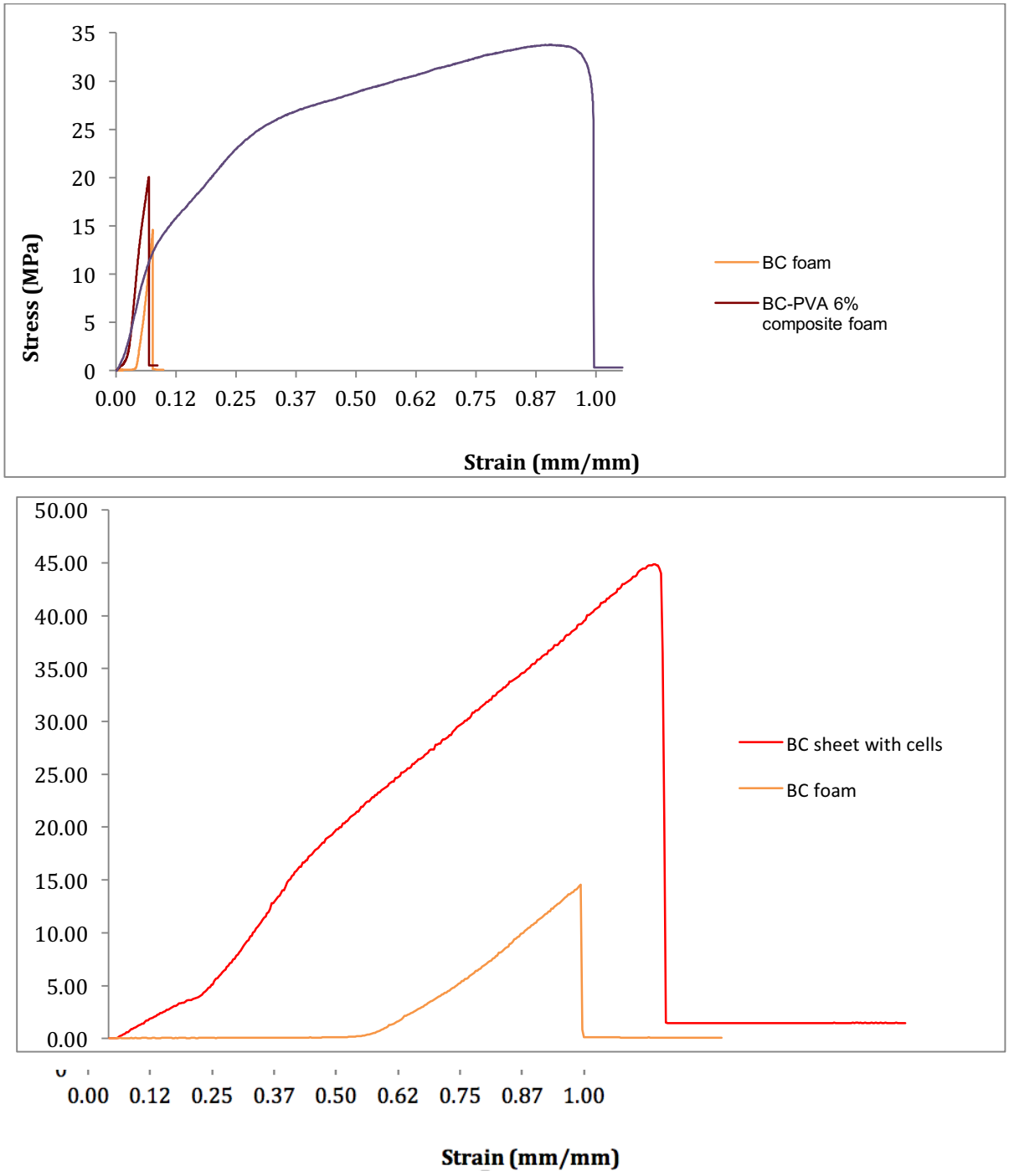


Figure 3.5.8 Stress-strain curves of cellulose biofilms with growth modifications

The samples of both bacterial cellulose sheet and foam display behavior of brittle material (**Figure 3.5.8**). After initial horizontal portion that results from wrinkles on the material, the curve shows linear relation between stress applied and the resulting elongation (strain) of the material. This phase is the elastic phase, where the deformation of the material is reversible. If tension was released in this phase, the sample would return to its initial state. For the brittle material such as native cellulose, the failure of the sample occurs in the elastic phase. The failure is sudden and the sample breaks at once (we could hear the crack sound occurring as the sample broke). It breaks very sharply, without plastic deformation, in what is called a brittle failure. The 6% BC-PVA composite was somewhat weaker, withstanding stress of 13MPa, compared to 24MPa of the control sample (**Table 3.5.3**). This may be explained by the interruption to the hydrogen bonding between cellulose nanofibers by the added PVA (Gea et al. 2010).

However, the BC-PVA 10% composite demonstrates completely different deformation mechanism. This composite exhibits ductile stress-strain behavior. The initial liner region of elastic deformation (**Figure 3.5.8**), is followed by a region of plastic deformation. This plastic deformation shows the ability of the composite to deform under stress without fracture, or failure. Plastic deformation is irreversible, meaning that if the stress is removed, the material won't return to its initial shape. Due to the ability to deform plastically, the BC-PVA 10% composite shows order of magnitudes higher toughness – 11.6 MJ/m^3 compared to 0.42 MJ/m^3 (**Table 3.5.2**). This demonstrates the versatility of bacterial cellulose properties and the ability to modify properties of BC by post-growth treatment, in this case PVA 10% solution. As future work, it is possible to regulate BC properties in-situ, by adding PVA solution to the medium during growth.

3.5.3 Discussion

Working with the tools of synthetic biology, I aim to reduce the scaffolding needs, and to grow materials bottom-up, exploring the relations between living and non-living components of a self-supporting material system that is engineered to grow. The relation between the biological growth and its scaffolding remains an open and challenging question. In this research, we use the macro-fluidic interface to regulate and interact with the growth process. However, the scaffolding itself introduces additional materials and fabrication methods. The silicone molds, tubing, 3D printed frames, and electronic components are all unwanted 'technical' material we are trying to eliminate its use. In future developments, once we master the tools of genetic engineering as applied to this system, we will reduce the need to use scaffolding. Instead, we will utilize the genetic engineering platform to grow emerging self-supporting structural patterns and assemble them into architectural enclosures.

CHAPTER 4

CONCLUSION

This research promotes the integration of biological material systems into architectural design. Through elucidating concepts, methods, and hands-on experiments, this dissertation demonstrates ways to program materials to sense their environment, process information, and adapt their structures, properties and biological functions. Working with biological materials to design and maintain their living functions, Guided Growth opens new possibilities for future designers.

Architects have long been inspired by biological systems, both in form and in materials, and have used them to inform their design processes (see Section 2.1). This dissertation proposes a strategically different approach for innovation with biological systems. I see the future of biological integration not as an attempt to emulate nature, but to integrate biological systems —their matter, computations, and metabolisms —into materials for architectural, fashion, and product design. In the Guided Growth approach, we do not simply learn from biology and apply this knowledge to design artificial systems, but synthetically regulate biological materials to become agents for interaction with and alteration of the environment. I believe this work proposes a new model of interaction between the architectural object and its environment.

In the theoretical chapter of this dissertation, I show how discoveries of genetic computation in biology and the generative design methods in digital computation developed in parallel, mutually informing each other. In particular, I trace the concept of morphogenesis and its implications for architectural design. I recognize the shift that began in the beginning of this century in the use of computation as a tool for form generation and representation, to a new approach of material-based computation. Following the historical literature review, I demonstrate biological growth as physical and chemical processes defined by the computation within cells and the tools of synthetic biology to design these computations.

In the experimental chapter of this dissertation, I demonstrate ways to design microorganisms, such as the cellulose-producing bacteria *G.xylinus*, to guide the formation of shapes, composites, and patterns. Design in continuous interaction with the living system, like that which I demonstrate in the Guided Growth experiments, offers a shift toward new modes of computation normally associated with electronics and electronic circuitry. This new design involves augmenting living organisms and using their existing unique capabilities for communication, material patterning, and energy transformation. I also demonstrate how digital fabrication and computation can be integrated with biological growth processes to continuously guide and regulate living functions in space and time.

In Guided Growth I tune material structure and properties. Bacterial cellulose is a very versatile material. I show how it is possible to use cellulose to make super lightweight and tough membranes and foams. Moreover, I engineer cellulose-producing cells to absorb particles into fiber structure as a way to make completely new materials, such as magnetic films or hard mineralized shells. The applications currently suggested for cellulose and its composites include medical applications, textiles, and high performance acoustic materials. However, by combining genetic regulation of this material with the physico-chemical environment that supports biological growth, we can preserve the biological 'magic' of these materials.

As an example of a possible application, recent research proposed aerogel-filled sandwich panels for architectural applications. These panels reduce a building's energy consumption due to the excellent insulation properties of aerogels (K. Chen et al. 2014). The aerogel is embedded in sandwich scaffolding, which provides mechanical support. By integrating genetic engineering and design strategies as described in Chapter 3, we could pattern panels from bacterial cellulose to have both a structural frame (by bio-mineralization processes as described in the proposal in Section 3.3.2) and a lightweight aerogel filling (for thermal and acoustic insulation). Moreover, by keeping some areas in a water-saturated hydrogel state, we could make self-healing panels and even integrate new functions, such as air purification and light emission.

4.1 Specific Contributions

Through the Guided Growth experiments, I demonstrate synthetic gene regulation of structural biological material (cellulose), and show ways to integrate shaping, the patterning of properties, and functionalization into the structural cellulose membranes. I list below the main contributions of the Guided Growth experiments:

On the nano-scale of computation with living cells:

- Transformation – developed a method to insert synthetic DNA into the cellulose-producing bacteria and demonstrated that it does not interrupt cellulose production.
- Developed design strategies to pattern and add functions to the cellulose biofilm – a proposal for constructing cell-to-cell communication devices, patterning of properties, and integration of new biological functions of cellulose biofilms.
- Protocols and workflows – documented and stored the protocols, materials, and methods for genetic design of cellulose-producing bacteria on www.benchling.com, an online shareable research platform.
- Parts – built a collection of parts for 'plug and play' design of the cellulose-producing bacteria.

On the meso-scale of guiding the growth of biomaterials into shapes and patterns, and tuning their properties:

- Developed design strategies for guiding the growth of cellulose biofilm for the following:
    - Composites – in-growth composites for tough and magnetic biofilms.
    - Post-processing – cellulose membranes or lightweight cellulose foams.
    - Pneumatic actuation — draining the growth medium out and pumping air in to maintain the three-dimensional shapes of biofilms.
    - Testing and characterizing – performed tensile tests, and identified water content and cellulose production as ways to characterize material structure and properties.
    - Molding – both in-growth and post-growth molding techniques.
    - Shaping – the growth interface between liquid and air was designed and shaped to guide the growth process of biofilms and produce stable three-dimensional components from bacterial cellulose.

On the macro-scale of regulating the flow of air and liquids:

- A digital computational interface – allows designers to interact and regulate the growth process. The interface provides access to the invisible computations of living cells by regulating the growth environment.
- Components — bioreactor components were designed and fabricated to facilitate the flow of nutrients, liquids, and air, and allow guidance of the growth of bacterial cellulose biofilms and interface them with regulating substances (such as composites, chemicals, and environmental signals).
- Flow system – a system of incoming and outgoing tubing was developed to channel the materials, liquids and air in and out of the bioreactor components.
- Computational control – through computational control of the flow and actuation of a sequence of pumps and solenoids, an interface was developed for a designer to interact with the growth process.
- Integration of material design strategies into macrofluidics – using the macrofluidic system, a three-dimensional cellulose biofilms was fabricated, and demonstrated varying material structure and properties by regulating the physical and chemical parameters of the growth environment.

4.2 Future Work

Biologically active materials will combine the structural properties of traditional building materials with functions of living systems, including the ability to rapidly grow, self-repair, and adapt to the environment. Some specific challenges, and future steps to overcome these challenges, include the following:

- **Viability:** The long-term viability of cells in a new, designed environment, is an important challenge. The use of a hydrogel (bacterial cellulose) with extremely high wettability (as high as 98% water content) will allow us to maintain living cells. It has been previously demonstrated that cells can maintain their living functions within the biofilm post-growth (Qin, Panilaitis, and Kaplan 2014). In our prior experiments bacteria cells recovered their cellulose-producing function upon reintroducing a growth medium after two weeks of being embedded in the dry cellulose membrane (Sergio Araya, Katia Zolotovsky, and Manuel Gidekel 2012). The computationally controlled scaffolding allows to introduce cycles of wetting and drying the biofilms over time.
- **Scaffolding:** The relation between the biological growth and its scaffolding remains an open and challenging question. In this research, we use the macro-fluidic interface to regulate and interact with the growth process. However, the scaffolding itself introduces additional materials and fabrication methods. The silicone molds, tubing, 3D printed frames and electronic components are all unwanted 'technical' materials that I am trying to eliminate the use of. In future developments, once I master the tools of genetic engineering as applied to this system, I will reduce the need to use scaffolding. Instead, I will utilize the genetic engineering platform to grow emerging self-supporting structural patterns and assemble them into architectural enclosures.
- **Biological Context:** As always in the case of genetic design, nothing starts from a blank canvas. The design is in a context of biological organism, and needs to be compatible with its normal functioning. In the case of cellulose-producing bacteria, I am adding synthetic regulation over the natural mechanisms of movement, consumption of raw materials from the environment, and weaving cellulose fibers from them. Using a synthetic gene network I can tune and modulate these natural self-assembly processes.

4.3 Integration of Methods and Cultures

Through elucidating workflows, illustrated methods, and hands-on experiments, this dissertation promotes a multi-scale design process and develops new modes of collaborative innovation with a continuum of methods from nano-to-macro scales of resolution. My experimental work spans three levels of resolution and proposes ways of making a new class of biologically active materials in architecture,

and a new application domain for synthetic biology. I outline the integration of methods of work with biological materials within the domain of architecture in more detail below:

For materials science, the proposed methodology of applying synthetic gene networks for biofilm patterning and functionalization has the potential to generate a new class of materials. These biologically active materials not only combine shape with materiality for properties amplification, but also can be selectively functionalized for programmable behavior and tailored for applications that require biological sensing and responsiveness, and perhaps even self-repair and self-assembly.

For architectural design this research asks new questions about the possibilities of design in interaction with biological growth and material formation. Moreover, the kind of materials I am using (liquids and semi-liquids, or gels) and the way I am using them (reprogramming them on the DNA level, guiding their growth process, and designing their biological function) can change the culture of architecture from one that uses materials to one that creates them

One continuum:

- Introduced a new dimension to the traditional duality of shape and material of architectural objects; synthetic living systems evolve and change over time and are subjects for regulation and continuous design.
- Introduced new biological functions to the traditionally defined functionality of architectural materials (structural support, thermal and acoustic regulation, protection from the environment). The new functionalities will potentially allow the materials to first sense changes in the external and internal environment and then perform computations to respond to them. These new
- biological functions will potentially include filtering and metabolizing harmful elements, fixing carbon dioxide, self-healing, and perhaps even photosynthesis.
- Demonstrated design strategies for working with biological materials that exist in liquid and hydrogel state.
- Demonstrated workflows that include design and computation across multiple scales.
- Integrated the creativity and imagination of design processes with the rigor of science and engineering processes, by engineering a synthetic biological system that can be used creatively for new spatial designs (example below).

In biology, design is an outcome of two components. The first component is pre-defined: the DNA of cells contains all the information for their potential behavior, development, and growth. The second is emergent: the way in which this information in the DNA will be materialized is context-dependent. The cell is an active and responsive element – it senses the environment, and based on this information will express its DNA differently. Cells also communicate between themselves and create patterns of growth

relative to each other. This is how each cell in our body evolves differently and in coordination with its neighbors and with the tissue in which it is embedded.

I have two examples of this design logic in my work. In the patterning proposal in Section 3.3.2, I engineer cells to respond to certain concentrations of signal by producing a property modifier, a certain protein that will bind to cellulose fibers and increase the density of the material. This way, the change in property is both spatial (defined by a certain distance from the sender colony) and time-dependent: as the signal propagates through the membrane, its concentration will increase, the property-modifier will be produced at a certain rate, and the change in the material structure will unfold over time.

I use rigorous methods of synthetic biology to engineer bacterial DNA so it will behave in a predictable manner: it will respond to a certain signal concentration by producing a property modifier. However, in the design process that I envision, the designer can now use this engineered system in a creative way: by introducing signal-producing colonies in different locations and over time, the designer can generate new patterns.

The macro-fluidic interface is the second example of how design emerges from the combination of engineered behavior on the nano-scale and the design of the growth environment on macro-scale. Here, I create an interface to externalize the invisible processes of growth. The growth environment in each vessel is regulated by the flow of materials and air in and out of the growth vessel. The interface between liquid and air forms the material in a three-dimensional shape. The designer can write a script to control the flow of materials into a growth vessel, and this scenario will define the growth process and the outcome.

The grow-regulate-sense-actuate processes in the living material are dynamic, responding to different environmental conditions, to each other, and to the designer's changes in situ. When engaged in this design method, designers may perceive (embed) new patterns and imagine new ways of materializing them. This creative perception and imagination at the macro-scale on the designer's part can inspire previously unconsidered steps. Designers can co-design with the living material by dynamically changing the environment – hence, guide the behavior -- and creating gradient. Newly perceived shapes in the patterns can generate design requirements for engineering of a completely new behavior or bacteria.
In conclusion, designing material forms through the manipulation of molecular and microscopic scale structures offers a radically different way of thinking about the design process and possible outcomes. It also raises questions about the role of a designer in this new field, where material formation is guided by the computations encoded in DNA, and synthetic is the new natural.

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APPENDIX A:

BASIC METHODS OF SYNTHETIC BIOLOGY

[SB1] MAKING LIQUID GROWTH MEDIUM

For this project, we used Hestrin-Schramm growth medium.

Materials for 1L HS media:

- 1L of DI H_2O (distilled water)
- 20g Glucose
- 5g Peptone
- 5g Yeast
- 2.7g Na_2HPO_4 (5.4g $Na_2HPO_4.7H_2O$
- 1.5g Citric acid
- Weights + measuring plates and spoons
- 1L glass bottles
- Autoclave

Procedure:

- Use weights to weight ingredients
- In the first bottle, mix half volume water with glucose
- In the second bottle, mix half volume water with the rest of dry ingredients
- Autoclave both bottles at 121^0C for 20min
- Store glucose solution and HS separately
- Mix half:half to use as a complete HS medium
- Store in room temperature

[SB2] MAKING SOLID GROWTH MEDIUM

Materials:

- One sleeve of Petri dishes (or 'plates')
- 250ml of HS media (no glucose)
- 250ml of 40% Glucose solution
- 7.5gr BactoAgar
- 1000X antibiotic solution

Procedure:

- Add agar to HS media
- Mix 250mL HS-agar with 250mL glucose for total of 500mL of HS-agar solution
- Add antibiotic. Unless otherwise note, the stock antibiotic solution is 1000X
- Pour ~25mL HS-agar for each agar plate
- Store plates in refrigerator

[SB3] REVIVING FREEZE-DRIED BACTERIA CULTURE

Materials:

- Freeze-dried bacteria culture purchased from www.atcc.com.
- Growth medium (see SB2, SB3)
- 70% Ethanol for cleaning
- Bunsen flame or sharp object
- Sterile forceps
- Pasteur pipettes
- 15ml culture tube

Procedure:

- Clean the glass ampoule from outside with 70% Ethanol.
- Crack the glass vessel open by pouring cold water on it and then holding above bunsen burner (alternatively, use sharp object to break the glass vessel).
- Using sterile forceps, carefully remove the cotton plug.
- Using a single tube of the recommended media (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette.
- Use this to rehydrate the entire pellet.
- Transfer the entire suspension back into the broth tube and mix well.
- The last few drops of this suspension may also be transferred to an agar slant.
- Incubate cultures under the appropriate conditions (see [SB1])

See similar protocol with picture guide here: https://www.phe-culturecollections.org.uk/media/103570/m201_how-to-open-nctc-glass-ampoules-with-photos_small.pdf

[SB4] EXTRACTING PLASMIDS WITH MINIPREP

Modification to the standard Qiagen Miniprep protocol: For the last step, diH2O was used to elute the DNA from the spin column to prevent salt in the DNA solution, which will reduce efficacy of electroporation for transforming the DNA into E. coli in later step.

Materials:

- Qiagen Miniprep Kit
- Cell Culture
- 1.6 ml Microcentrifuge tubes (2 per a miniprep)
- TE (1:10)

Procedure:

- Resuspend pelleted bacterial cells in 250 μl Buffer P1 and transfer to a microcentrifuge tube.
- Add 250 μl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.
- Add 350 μl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.
- Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
- Apply 800 μl of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting.
- Centrifuge for 30–60 s. Discard the flow-through.
- Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.
- Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60s.
- Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.
- Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 μl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.

[SB5] DIAGNOSTIC RESTRICTION DIGEST

Materials:

- 200uL pCR strip tubes (one per reaction).
- Restriction enzyme (1uL per reaction).
- 10X restriction enzyme buffer (1uL per reaction).
- Minicentrifuge
- Vortex

Procedure:

- Choose restrictions enzymes and relevant working buffers.
- Retrieve the minipreped plasmids and the appropriate 10X buffer concentrate from the freezer. Thaw on the benchtop.
- Label PCR tubes.
- Vortex the minipreps and the 10X buffer concentrate briefly, then centrifuge in the microcentrifuge.
- For each miniprep, set up a PCR tube containing the following in order (total volume of 10uL per reaction):
    - 5uL di H2O..
    - 1uL enzyme buffer.
    - 3uL miniprep DNA.
    - 1uL enzyme.
- Flick the tubes to mix, then pulse down in the strip tube microcentrifuge.
- Incubate at the appropriate temperature for at least 1 hour and no more than 16 hours.
- Stop the reaction by adding 1uL of 6X NEB purple gel loading dye to each reaction (or heat stop depending on enzymes used).
- Flick the tubes to mix, then pulse down.
- Proceed to gel electrophoresis.

[SB6] DNA SEQUENCING

DNA samples are sent for DNA sequencing at Genwitz, www.genewiz.com

Materials:

- DNA stock
- Nuclease-free water
- Primer (5nM stock)
- 2 microcentrifuge tubes per plasmid

Procedure:

- Identify primer' sequences and order from Intergrated DNA Technologies (IDT) www.idtdna.com if needed.
- Dilute sequencing primers to 5uM (pmol/uL) using water. Need total of 5uL for each sequencing reaction.

- Using the recorded concentration, dilute the DNA template to the correct concentration. For each plasmid, at least 20uL is needed.
- Mix 10uL of DNA template and 5uL in each microcentrifuge tube. Each DNA template requires one sample for forward primer and one sample for reverse primer.
- Submit the samples to Genewiz www.genewiz.com.

[SB7] TRANSFORMATION BY CONJUGATION

Materials:

- E. coli WM6026 with transforming plasmids
- G. xylinus
- HS + glucose media
- 40% glucose solution
- DAP enriched LB media
- HS + glucose agar plates
- HS + glucose agar with relevant antibiotics

Procedure:

- Grow G.xylinus cells in (50mL) HS+glucose media for 2-3 days (until OD600 = 0.8-1.0)
- Grow E. coli WM6026 in (50mL) LB + DAP + antibiotics media overnight
- Transfer both bacterial cultures into 50mL Falcon tubes
- For G. xylinus, vortex the culture vigorously to release the cells from the cellulose bed. Let the cellulose settle. Then, collect the supernatant into another Falcon tube
- Centrifuge the G. xylinus for 10min at max speed
- Re-suspend the G. xylinus pellet using 5mL of HS+glucose
- For WM6026, wash (x2) with DAP enriched LB to remove all the antibiotics from the overnight culture
- Re-suspend WM6026 in 5mL DAP enriched LB (NO antibiotics)
- Add 150uL of G. xylinus and 50uL of n into a 1.5mL tube
- Plate the mixture of cells in HS+glucose agar with NO antibiotics. Let the mating reaction takes place for 8hr.
- Pick the colonies, and grown the transformant in 5mL HS+glucose (NO antibiotics) overnight
- Vortex the overnight culture to release the cells from cellulose matrix
- Plate the cells on HS+glucose+antibiotics agar plates. Incubate at 30^{o}C, 230 rpm for 3-5 days until colonies appear

[SB8] CELLULOSE PRODUCTION QUANTIFICATION

Materials:

- 250 mL conical flasks/50 mL Corning tubes
- HS medium
- Plastic weighing boats/baking paper
- (High sensitivity) scale
- 65 °C heat-box

Procedure:

- Add 50 mL of HS medium (or other medium of choice) to 250 mL conical flask. Alternatively, can use 10-20 mL HS in 50 mL Corning tubes if you have a high-sensitivity scale.
- Grow G.*xylinus* in HS medium for 7 days standing, at 30 °C. Don't seal the flasks hermetically in order to allow diffusion of oxygen (seal using foam buns). When using Corning tubes, leave caps loose or cover tubes with Breathe-Easy membrane to allow increase aeration.
- After 7 days of growth, wash the cellulose twice with distilled water.
- Add 50 mL of 0.1M NaOH to cellulose, incubate at 65 °C for 4 hours.
- Wash the cellulose twice using distilled water.
- Cut plastic weighing boats or baking paper to identical weight (measuring with a high-sensitivity scale if possible).
- Place the formed cellulose pellicle on baking paper or plastic weighing boats and air-dry the pellicle at 65 degrees for 24 hours. Increase drying time if some pellicles are not completely dry and crisp. Dry the pellicle together with the paper or weighing boat, as the pellicle will invariably stick to the surface, and removal of it results in loss of cellulose.
- Weigh the pellicle+paper/weighing boat using a high-sensitivity scale. Subtract the weight of the paper or weighing boat to determine the weight of cellulose.

[SB9] WATER CONTENT QUANTIFICATION

- Water content (%) = $(\underline{m}_{wet} - \underline{m}_{dry})/\underline{m}_{wet} \times 100$
- $\underline{m}_{wet}$ - weigh of the wet biofilm
- $\underline{m}_{dry}$ - weigh of the dry biofilm