Growth Based Fabrication Techniques for Bacterial Cellulose

Three-Dimensional Grown Membranes and Scaffolding Design for Biological Polymers

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1 Bacterial cellulose differential growing. Physical model of the membrane and representation of the funicular system.

ABSTRACT

Self-assembling manufacturing for natural polymers is still in its infancy, despite the urgent need for alternatives to fuel-based products. Non-fuel based products, specifically bio-polymers, possess exceptional mechanical properties and biodegradability. Bacterial cellulose has proven to be a remarkably versatile bio-polymer, gaining attention in a wide variety of applied scientific applications such as electronics, biomedical devices, and tissue-engineering. In order to introduce bacterial cellulose as a building material, it is important to develop bio-fabrication methodologies linked to material-informed computational modeling and material science. This paper emphasizes the development of three-dimensionally grown bacterial cellulose (BC) membranes for large-scale applications, and introduces new manufacturing technologies that combine the fields of bio-materials science, digital fabrication, and material-informed computational modeling. This paper demonstrates a novel method for bacterial cellulose bio-synthesis as well as in-situ self-assembly fabrication and scaffolding techniques that are able to control three-dimensional shapes and material behavior of BC. Furthermore, it clarifies the factors affecting the bio-synthetic pathway of bacterial cellulose—such as bacteria, environmental conditions, nutrients, and growth medium—by altering the mechanical properties, tensile strength, and thickness of bacterial cellulose. The transformation of the bio-synthesis of bacterial cellulose into BC-based bio-composite leads to the creation of new materials with additional functionality and properties. Potential applications range from small architectural components to large structures, thus linking formation and materialization, and achieving a material with specified ranges and gradient conditions, such as hydrophobic or hydrophilic capacity, graded mechanical properties over time, material responsiveness, and biodegradability.

INTRODUCTION

Contemporary digital fabrication tools are able to produce geometrically complex objects and structures, yet most of the constructs are not generally sustainable nor energy efficient (Oxman et al. 2012). In contrast, organic self-assembling processes produce little to no waste, using small amounts of energy to produce multi-functional and adaptable systems (Vincent 2012). Despite the recognized capabilities of natural processes to generate complex structures of organic and inorganic multi-functional composites (shells, corals, teeth, wood, silk, horn, collagen, and muscle fibers), the use of bio-materials for large scale architectural and engineering applications is still underdeveloped (Benyus 1997; Vincent 2012). Their structural and functional diversity, especially that of bio-polymers, underlines their capacity to replace existing synthetic polymers and to provide new methods of bio-fabrication as well as new applications and structures.

Cellulose, one of these bio-polymers, is one of the most abundant biodegradable materials in nature, and has been the topic of wide investigations in macromolecular chemistry (Mohite et al. 2014). The high water content of bio-synthetic cellulose (99%) and its mechanical properties make it a versatile material that can be manufactured in various sizes and shapes. Bacterial cellulose has many unique properties, including high purity, high water retention, and a hydrophilic nature, tensile strength, thermal stability, and biodegradability.

Because of these unique properties, it is an attractive candidate for a wide range of applications, including within architecture and engineering (i.e.: water retaining structures, architectural components, etc.), but due to the lack of suitable fabrication methods and digital design tools, cellulose is still disregarded as a building material. Although recent developments within biochemistry and microelectronic engineering have improved knowledge of biological materials, it is still not possible to produce bacterial cellulose on an industrial scale and control the three-dimensional (3D) outcomes through standard manufacturing and digital techniques (Fernandez et al. 2013). Current approaches towards virtual and physical prototyping with non-fuel-based materials also lack the capacity to model and fabricate with continuously varying material properties (Oxman 2011). In order to introduce cellulose as a building material, it is therefore important to develop bio-fabrication methodologies linked to materially informed computational modeling.

In nature, morphogenesis is a biological process describing the formation of a shape of an organism, inseparably linking formation and materialization (Menges 2007). By contrast, architecture is characterized by prioritizing form-generation over inherent material logic. The integration of morphogenesis within the field of architecture suggests a bottom-up, material-driven design with specified ranges and gradient conditions (Soldevila 2015). This paper emphasizes the development of new manufacturing technologies for the production of 3D-grown bacterial cellulose (BC) membranes.

In this paper, this claim is substantiated and a solution is offered, making the following contributions:

1. *Investigation of bio-inspired fabrication methodologies and virtual and physical prototyping. The factors affecting cellulose bio-synthesis are demonstrated—mainly growth medium, environmental conditions, and the formation of derivatives (§1.1 and §2.1). The design of the culture medium is a key influence for the growth of microorganisms, and therefore in stimulating the formation of three-dimensional membranes.*

2. *The transformation of the bio-synthesis of bacterial cellulose into BC based bio-composite is shown, leading to the creation of new materials with additional functionalities and properties. Furthermore, we clarify the factors that affect the bio-synthetic pathway of bacterial cellulose, such as bacteria, nutrients, and medium culture properties. Particular focus is given to the creation of a natural polymer which could grow to any thickness, shape, and robust structure (§1.2 and §2.3, 2.4).*

3. *In-situ self-assembly fabrication techniques and scaffolding techniques for bacteria cellulose are presented and show the creation of a bio-composite via the fermentation of bacteria strain A. Xylinum along the surface of natural fibers (§1.3 and § 2.2, 2.5).*

1.1 Bio-Inspired Fabrication Methodologies and Virtual and Physical Prototyping

Through the combination of non-fuel-based materials with

2 Consolidation through drying. Bacterial cellulose three-dimensional morphology, with variable thickness.

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3 Bio-synthesis of bacterial cellulose: strikers for preparation of *Acetobacter xylinum* culture.

4 Bio-synthesis of bacterial cellulose: Microfluidics system to provide continuous nutrient to the culture.

5 Bio-synthesis of bacterial cellulose: bacterial cellulose growing after 5 days.

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advances in biological sciences, genetics, and bio-engineering of bacteria, a new set of possible bio-fabrication technologies can be developed.

Current design practice is mostly characterized by the domination of shape over matter, consequently prioritizing virtual shape-defining parameters over physical material and fabrication constraints, leading to a geometric-centric design phase (Menges 2007; Oxman 2011). Nevertheless, some recent developments in direct digital manufacturing enable a shift towards a material-centric design practice, such as water-based fabrication techniques (Oxman 2011). Additive manufacture (AM) technologies for rapid prototyping employ virtual, computer-aided designed models, and translate them into thin horizontal successive cross-sections to define three-dimensional physical objects (Sachs et al. 1993).

AM technologies have become an efficient and common means to deliver geometrically precise functional prototypes in relatively short periods of time (Oxman 2012). At the same time, there is a need to expand manufacturing processes towards bio-fabrication-based approaches, borrowing techniques from biological science and tissue engineering. In contrast to AM technologies, which relate to a specific controlled output, bio-fabrication techniques in a water medium aim to create dynamic feedback or reciprocity within a specific context. This process certainly escapes the pitfalls of bio-mimicry in favor of bio-synthesis, as a substance forms through interaction with a living organism. As a result, this will proliferate methods to fabricate synthetic composites by generating novel morphogenetic mechanisms linked to bacteria and different states of matter. This process is clearly described within §2.3, focusing on the different levels of oxygen within the water medium. As a result, 3D growth of cellulose can be directly manipulated.

1.2 Bio-Nanocomposite and Bacteria Engineering

Engineering the bio-synthesis of bacterial cellulose (BC) into BC-based nanocomposites leads to better mechanical and thermal properties, or additional functionalities that are useful in many applications and fields. They could be categorized, for instance, as high-strength materials, plant-mimicking materials, electrically-conductive materials, catalytic materials, antimicrobial materials, thermo-responsive materials, and many others. More explicitly, the process describes a structure with growth-induced material properties reacting to external stimuli and resulting in hierarchically structured forms (Soldevila 2015). Bacterial cellulose production depends heavily on several factors, including culture medium and environmental conditions. The culture medium (Figure 3) contains a carbon source, nitrogen source, and other nutrients required for the bacteria to grow. In normal static and aerobic conditions, the bacteria will form a pellicle (flake) (Figure 5) at the surface of the culture medium. This pellicle will grow in thickness slightly, but the thickness is limited by the supply of oxygen and nutrients to the bacteria. Recent studies found it possible to optimize the thickness and strength of BC by creating continuous systems of sub-ministration of nutrients (Figure 4). This creates a potential condition where the natural polymer could grow to any thickness and shape to form a structure (Gateholm et al. 2012). The variation of culture medium components can alter the metabolism of the bacteria (Figures 13 and 14, and §2.3) and consequently the mechanical properties and bio-synthesis pathways. Such 3D bacterial cellulose-based structures and membranes can be prepared with a target topological condition, thickness, and strength. In this approach, the distribution of material properties is informed by structural and environmental performance criteria contributing to the internal physical makeup of the membrane. It thus requires a set of virtual and physical prototyping tools and methods to support variable fabrication approaches and modeling (Oxman 2011).

It is precisely this complex and dynamic exchange between organism, environment, and functionality that makes synthetic life valuable for architecture.

1.3 Self-Assembly Fabrication Techniques

Self-assembly fabrication of 3D structures and membranes can be described as a multi-step fermentation process in the presence of natural fibers or other polymers. This can furthermore lead to the formation of BC-based hybrids or nanocomposites (Qiu and Netravali 2014). Ultimately, advances in self-assembly fabrication techniques will lead to different manufacturing methods, implying a condition of growing and morphogenesis. Taking as reference the biosynthetic process of bacterial cellulose, these methods are able to control three-dimensional shapes and material behavior. Self–assembly fabrication can be achieved by providing a scaffold to support and guide the process of growing to define precise target geometry (Figures 6–9 and §2.2).

Cellulose is a polymer which forms the cell wall of eukaryotic plants and algae and can also be found as the major constituent of the cell wall of fungi. Despite this, a few bacteria can also secrete cellulose. A notable example is *Acetobacter xylinum*, which is known to secrete cellulose as part of its metabolism of glucose and other carbohydrates. The bacteria strains of *A. Xylinum* have been found to grow preferentially on the surface of natural fibers (Figures 17 and 18) or certain polymer molecules, rather than freely in the medium. Therefore, starch, soy resin, or polyvinyl alcohol (Figures 6 and 7) provide ideal substrates for the bacteria to grow on and can therefore lead to the formation of BC-based hybrids or bio-composites. Bio-composites are composite materials reinforced via natural plant-based fibers or certain polymers (Qiu and Netravali, 2014). Biosynthesizing and engineering the original BC into-BC based composites could potentially find several applications in the fields of architecture, engineering, and product design, opening up a substantial change in how products and buildings are form-found, designed, and fabricated (Oxman 2011). A variety of methods, digital and analog, have been utilized for achieving this purpose. Polyvinyl


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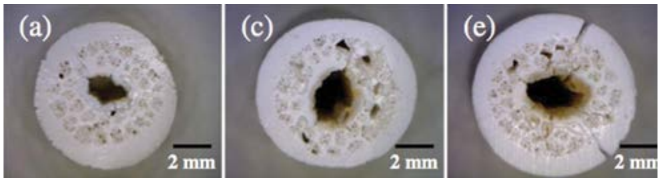
6 BC-induced growth over permanent scaffold. PLA scaffolding and PVA immersion.

7 BC-induced growth over permanent scaffold. Immersion of scaffold into culture medium.

8 BC-induced growth over permanent scaffold. Image of the membrane during the drying process.

9 BC-induced growth over permanent scaffold. Detail of the resulting membrane.



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alcohol hardening (Figures 6–9 and §2.2) and sisal fiber deposition (Figures 17 and 18 and §2.5) can be used as excellent in-situ bio-fabrication techniques.

A key point for potential future development is the augmentation of the mechanical properties of the polymer, including its strength and stiffness. In all cases, consolidation is usually achieved by drying, sintering, or solidification techniques. BC in particular has the capacity to be calcified (Figure 10). This solidification method is currently used in biomedical applications for tissue and bone engineering using hydroxyapatite, chitosan, alginate, and agarose. In this case, it may be possible to generate a material with properties similar to those of bones if we introduce an agent in its mineral phase into the bacteria culture medium during the formation of BC.

METHODS

This process is intended to show novel BC fermentation methods and scaffolding techniques for controlling shape, thickness, and material-driven computing experiments.

2.1 Preparation of *Acetobacter xylinum* (AXy) Culture (Figures 3–5)

Preparation of the AXy culture (Figure 3): The bacteria was grown in broth and subsequently streaked on agar with an incubation period of 24 hours. A single colony of AXy was inoculated in a flask containing mature coconut water, which was sterilized at 121 C° for 15 minutes. The flask was left at 25 C° or room temperature for 2–3 days.

Preparation of the culture medium (Figure 4): 5% sugar and 0.5% of ammonium sulphate were added to mature coconut water, and the mixture was brought to boil for 5 minutes. 1% acetic acid (99.85%) was added and mixed. A micro-fluid system (Figure 5) was applied to provide a continuous flow of nutrients in order to favor an unlimited growth of BC (Gateholm et al. 2012).

2.2 In Situ Self-Assembling (Figures 6–9, 11, 12)

We prepared different scaffolding methods to induce BC growth over a predetermined shape. Two strategies of scaffolding were introduced: permanent and bio-degradable. Figures 6–9 and 12 show the creation of shells and membranes using BC as a binding agent. The scaffold remains embedded within the structure. Bio-degradability (dissolving after the bio-synthesis process) of a scaffold is shown in figure 11, using a sodium-alginate scaffold.

The culture medium was prepared in a glass beaker (2 liters) and 10% of AXy culture was added. The flask was left at 25 C° for 7 days without disturbance. Oxygen was supplied during the photosynthetic period to direct the growth on the scaffolding.

2.3 Adhesion Growing (Figures 13 and 14)

This methodology showed a potential to grow three-dimensional morphology with a target geometry. Static and anaerobic conditions caused the inversion of the metabolism of the bacteria, producing a growing which was no longer superficial, but instead adhered to the morphology of the containing element.

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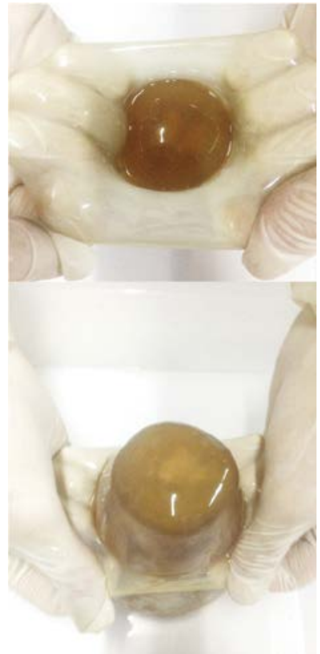

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10 Images of cellulose-based scaffolds a) pure cellulose and composite scaffolds of c) cellulose agarose, and e) chitosan–alginate.

11 Sodium alginate medium led to the creation of result of a bio-film soluble scaffolding.

12 BC-induced growth over permanent PLA scaffold led to the creation of a growth shell structure.

13 Three-dimensional growth morphology under static conditions: resulting geometry.

14 Three-dimensional growth morphology under static conditions,removal operations from the containing boundary.

The culture medium was prepared in a latex flask (1.5 liter) and 10% of AXy culture was added. The flask was left at 25 C° for 10 days without disturbance.

2.4 Bacterial Cellulose with Differential Growing Patterns (Figures 1, 15, 16)

We prepared BC membranes with different growing patterns and different thicknesses, and used a particle-spring system as a "tension-only" hypothetical funicular model to simulate the behavior of the membrane (Figure 16).

The culture medium was prepared in a glass beaker (2 liter) and 10% of AXy culture was added. The flask was left at 25 C° for 4 days without shaking and nutrients were continuously added. The flask was then left at 25 C° for 5 days. Drying method: freezing (4 hours) and drying (4 days at 10 C°) .

2.5 Scaffolding Technique Using Sisal Fiber (Figures 17, 18)

We prepared a scaffolding technique with BC-modified sisal fibers (produced in an incubating shaker), using 60 cm long sterilized sisal fibers. These were added to the culture media and sterilized. AXy was inoculated into the culture media and BC-modified sisal fibers were extracted after 3 days culture. Preliminary studies were done by simulating the material behavior according to the pattern deposition of the fibers.

RESULTS

This approach demonstrated the first steps in the design of a complementary technology able to expand the potential of

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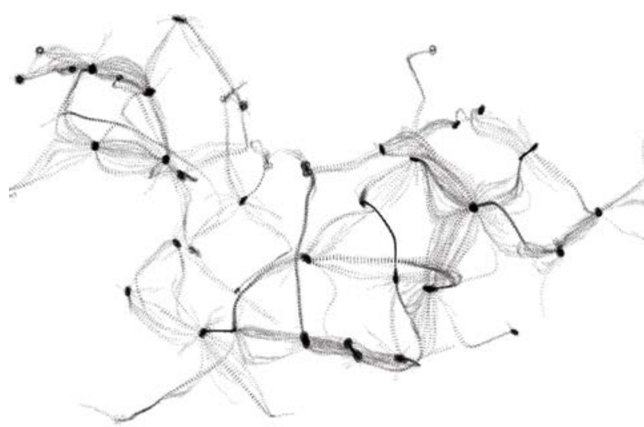

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15 BC differential growing. Physical model of the membrane and representation of the funicular system.

16 Particle spring simulation, "tension only" funicular system of the membrane.

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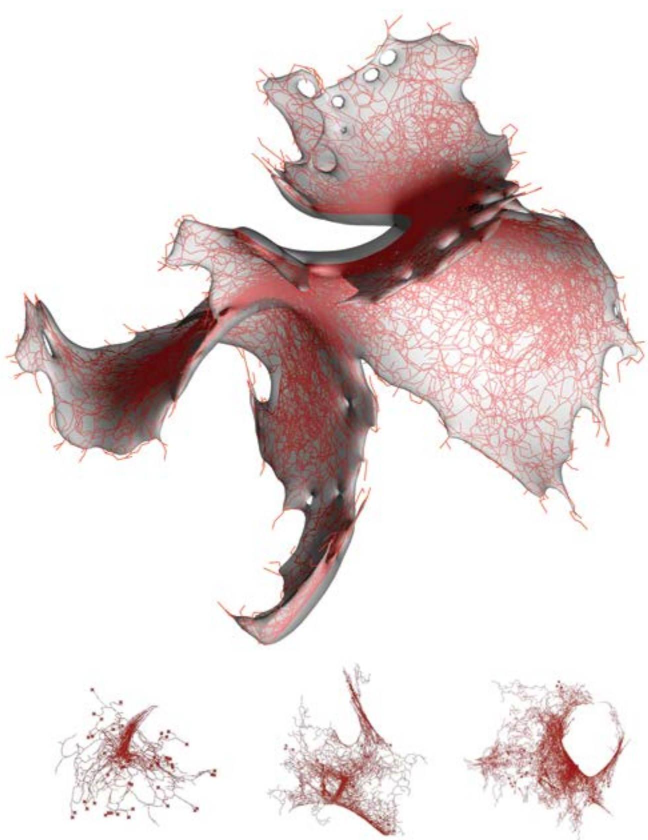

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17 Result of BC bio-composite using sisal fibers. Bacterial cellulose as binding material has been observed to grow preferably around natural fibers.

18 Recursive system for sisal fiber deposition and scaffolding creation. The aggregation of the fibers relative to a hypothetical growing ratio of BC.

prototyping processes for bio-synthesis. Specifically, experiments 2.1 and 2.4 showed that BC is moldable in cultivation. By meticulous control of the addition of fermentation media through the use of a microfluidic system, BC can grow to a potentially unlimited thickness and shape. Moreover, other experiments confirmed the hypothesis that BC grows preferentially on natural fibres such as sisal. It has been noted that the creation of BC-based bio-composites increase the mechanical properties of the bio-polymer, offering the potential to create structural and graded properties. Finally it has been successfully proven that it is possible to grow BC around a predefined shape; the addition of oxygen during the fermentation drove the cellulose to follow and adhere to specific surface conditions. Despite the results, all the experiments are still far away from a real application on a large scale and the accuracy of material-driven computing methodologies, relative to prototypes as grown, needs to be further explored.

CONCLUSIONS

This paper outlined novel bio-fabrication and scaffolding techniques for the control of 3D membranes and morphologies of bacterial cellulose, positioning the results in the field of bio-materials science, digital fabrication, and material-informed computational modeling. Future developments of the present research include:

- Developing a range of structural BC bio-composites using hydroxyapatite, chitosan, or lining as agents to calcify the 3D membranes.
- Exploring the conditions for target-geometry-based growing. Define the role of the scaffold compared to the growing pattern of BC.
- Investigating BC as a matrix for growth and cultivation of photosynthetic microorganisms such as algae and cyanobacteria in order to generate a material able to perform photosynthesis and react to photo stimuli.

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REFERENCES

Benyus, Janine M., 1997. *Biomimicry: Innovation Inspired by Nature*. New York: Quill.

Vincent, Julian. 2012. *Structural Biomaterials*. Princeton, NJ: Princeton University Press.

Fernandez, Javier G. and Donald E. Ingber. 2013. "Bioinspired Chitinous Material Solutions for Environmental Sustainability and Medicine." *Advanced Functional Materials* 23 (36): 4454–4466.

Mohite, Bhavna V. and Satish V. Patil. 2014. "A Novel Bio Material: Bacterial Cellulose and its New Era Applications" *Biotechnology and Applied Biochemistry* 61 (2): 101–110.

Oxman, Neri, Elizabeth Tsai, and Michal Firstenberg. 2012. "Digital Anisotropy: A Variable Elasticity Rapid Prototyping Platform." *Virtual and Physical Prototyping* 7 (4): 261–274.

Oxman, Neri. 2011. "Variable Property Rapid Prototyping." *Virtual and Physical Prototyping* 6 (1): 3–31.

Qiu, Kaiyan and Anil N. Netravali. 2014. "Fabrication and Applications of Bacterial Cellulose Based Nanocomposites" *Polymer Reviews* 54 (4): 598–626.

Sachs, Emanuel, Michael Cima, James Cornie, David Brancazio, Jim Bredt, Alain Curodeau, Tailin Fan, Satbir Khanuja, Alan Lauder, John Lee, and Steve Michaels. 1993. "Three-Dimensional Printing: The Physics and Implications of Additive Manufacturing." *CIRP Annals: Manufacturing Technology* 42 (1): 257–260

Menges, Achim. 2007. "Computational Morphogenesis: Integral Form Generation and Materialization Processes." In *Proceedings of the 3rd International ASCAAD Conference on Embodying Virtual Architecture*, edited by Ahmad Okeil, Aghlab Al-Attili, and Zaki Mallasi. Alexandria, Egypt: ASCAAD. 725–744.

Gateholm, Paul, Henrik Backdahl, Theodore Jon Tzavaras, Rafael V. Dovalos, and Michael B. Sano. 2012. Three Dimensional Bioprinting of Biosynthetic Cellulose (BC) Implants and Scaffolds for Tissue Engineering. US Patent 0190078A, filed September 28, 2010, and issued July 26, 2012.

Soldevila, Laia Mogas. 2015. "Water-based Digital Design and Fabrication" MS Thesis, Massachusetts Institute of Technology.

IMAGE CREDITS

Figures 1–9, 11–18: Derme, Mitterberger, Di Tanna, 2015
Figure 10: Jang et al., 2012

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