Living Architecture

Micro performances of bio fabrication

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Abstract. *This ongoing research study explores novel modes of design and fabrication by combining digital tools and technologies with living biological systems within controlled environments in order to induce specific biological functions and material production processes. The main objective is to design and implement a biological fabrication technique, using bacteria, to produce physical components for architecture and product design.*
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This ongoing research study explores novel modes of design and fabrication by combining digital tools and technologies with living biological systems within controlled environments in order to induce specific biological functions and material processes. The main objective is to design and implement a biological fabrication technique, using bacteria, to produce physical components for architecture and product design.

The study focuses on the study of the Acetobacter Xylinus. This bacteria metabolizes glucose synthesizing cellulose. As opposed to plant cellulose, which through industrial treatments used to make paper, cardboard and other materials (Brown,1976) (Brown,1985), bacterial cellulose is chemically pure. While cellulose is the most abundant biopolymer on the planet and its economic importance is enormous, as it is based on a model of industrial mass processing of plant material, it is responsible for an explosive consumption of natural resources. Cotton crops and wood timber are major sources of cellulose and drive enormous industries, which are often the single most important industries driving a country's economy. The environmental impact of such global industry is massive, and this has triggered extensive research on alternative sources of cellulose. For decades it has been know that marine microbes and other microorganisms can also biosynthesize cellulose, and research has focused on understanding the growth processes involved, the resulting

Table 1
Reference: National Center for Biotechnology Information (NCBI, USA).

Acetobacter Xylinus	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alpha Proteobacteria
Order	Rhodospirillales
Family	Acetobacteraceae
Genus	Acetobacter
Species	Acetobacter Xylinus

cellulose structures and their material properties in addition to the genetic encoding responsible for the production of cellulose in such organisms. Most of the research has targeted biomedical applications, although some research has been done in applying bacterial cellulose to plant cellulose to enhance its material properties due to the high purity of bacterial cellulose.

A. Xylinus is a very prolific producer of cellulose. One single cell is typically able to synthesize up to 108 molecules of glucose per hour into cellulose. Since one single liquid droplet can contain as many as a million cells, the productive capacity of A. Xilinus is significant.

The goal of this research is to investigate how the design of environmental conditions and the control of its critical parameters can induce the fabrication of material structures and their performance. Moreover, the long-term objective of this investigation is to be able to genetically modify the bacteria in order to insert new genetic instructions that enable it to trigger or stop cellulose production. A second objective would be to transform the bacteria in order to include the biological mechanism to produce the enzyme cellulase, which through hydrolysis degrades cellulose back to glucose molecules. This would enable control of both an additive material process and a subtractive material process, both of which could be programmed or controlled via chemical or physical promoters. This study tried to develop a design methodology in order to implement a biological fabrication process that directs criticism toward contemporary manufacturing paradigm, which was inherited from the industrial revolution. That paradigm is one of extraction and exploitation of anisotropic and heterogeneous resources and materials that are processed in order to become homogenous and standardized. Remove and sterilize living matter in order to make it inert, sanitized before it can be consumed safely at the cost of making it hard to metabolize again when it is returned back to the environment. The implications of this paradigm have been thoroughly discussed in contemporary critical discourse, from its impact on ecological balances, to the economical and social costs of these methods of production and growth, to the political implications in terms of national and international dependency on scarce material sources.

From a design perspective, the paradigm of matter that acquires form though the creative act of design, the homogeneity of the material is key, both conceptually and physically. Form is what differentiates; matter is continuous and homogeneous, often flat and in squared proportions or in stable and pure liquid form.

This investigation proposes a new paradigm of symbiotic co-adaptation where anisotropy and irregularity are embraced and where the role of design is not to give form but to inform the environment in which active material performances take place. It proposes a rather reciprocal and dialogical approach with the inner and outer environments of the system that contrasts with the one directional linearity of the current industrial paradigm.

MATERIALS AND METHODS

Bacteria strain

Gram negative cellulose-producing bacteria Acetobacter Xylinum. We used bacterial strain ATCC number 10245 (http://www.atcc.org/). The original strain was received from Prof. David Kaplan from TERC (Tissue Engineering Resource Center), from the Department of Biomedical Engineering at Tufts University.

Growth medium

In our experiments we used Schramm–Hestrin (SH) medium containing 2.0% D-glucose, 0.5% yeast extract, 0.5% peptone, 0.51% di-sodium hydrogenphosphate heptahydrate, 0.115% citric acid (Hestrin & Schramm, 1954). Some attempts were made to optimize the medium as discussed in the next chapter.

Optical density (OD)

We used OD measurements to estimate and compare bacterial growth at the initial overnight cul-

tures. Samples of 1ml were measured and compared to pure HS media used as a blank. Average values of 0.1 read at 600 nm indicated overnight bacterial growth.

Static culture growth

In static mode of growth the bacteria is added to a measured volume of HS medium and placed in the incubator/ heated by applying heating pad to achieve optimal temperature for bacteria growth (+27°C according to literature).

Agitated culture growth

In agitated mode of growth, the culture is fixed on a vibrating platform and constantly shaken. This to create a continuous oxygen access to the bacteria in the liquid medium and accelerate growth. The agitated cultures were placed in incubator (+30°C).

Molding in vivo- experimentation using the whole, living organism)

Molds (*in vivo – "within the living" (from Latin*) with varying surface textures and texture resolutions were designed, modeled in Rhinocerus and 3D printed in Objet Convex.

The mold was fixed in a 100ml cylindrical glass container. 50ml HS medium was added to the containers. The cellulose membrane was pre-grown on a Petri dish and introduced to the containers. After the membrane was stabilized and the growth stopped, we took the mold with the membrane out and left it to dry in room temperature overnight. Then demolding was performed using scalpel to gently peel the membrane of the mold.

Molding in vitro

In the in vivo molding experiment we used a static culture growth in an aquarium tank of twenty-five gallon (*in vitro – "within the glass" (from Latin)– in controlled environment, using isolated components of living/dead organism*). Six liter of HS medium were added to the tank and seven days old pre-grown small cellulose membranes from six Petri plates were introduced. The thermostat-controlled heating pad kept temperature to the optimum of +27°C and time-lapsed web camera was programmed to three times a day shots. The constant volume of medium was kept by adding fresh medium every 3-4 days. When the membrane achieved its maximum dimensions of 120*220*8 mm and stabilized, it was removed from the tank, rinsed with tap water and placed on the CNC-milled wooden mold. Petroleum Jelly was applied on the mold for easier de-molding. It was left to dry for four days in room temperature.

Lyophilization

We used lyophilization to evaporate the water and yet preserve the spatial configuration of cellulose fibers. The samples were removed from the medium still on the 3D printed mold and gradually frozen: first at (-20°C) for overnight, then at (-80°C). The frozen samples were transferred to Labconco lyophilizer for several days and kept frozen at (-20°C) before de-molding attempt.

PARAMETRIC CONDITIONS

The fabrication process of material structures and their performance are directly affected by the environmental conditions. The central aspect of designing with living systems is carefully planning and controlling the external environmental conditions in order to induce the behavior of the organisms internal conditions. This is crucial both in the initial set up and over the growth time. Below we list some of the main conditions affecting material production processes in our experiments:

Nutrients optimization

The main input for the material production process is sugar (glucose) in the medium and oxygen. We are currently working on replacing the sugar in HS medium with sugar-rich waste from food industries. This will enable us to create a sustainable design process when the waste from one industry production is used as basis resource for another, but also because it would drop costs down allowing us to scale up the process towards construction material standards.

Oxygen supply

The bacteria needs both oxygen and nutrients for material production. In static culture, the cellulose membrane will be produced in the interface between the air and the liquid (medium containing the nutrients). By designing the mode of oxygen supply both in the initial set up and over time, we can control the spatial organization of the material and it's material properties.

Temperature, pH

The temperature and pH affect the rate of material production. The optimal conditions based on the literature are pH=6.0 and temperature of +27°C. Nonetheless, it has also been proven that different strains of bacteria are productive at different environmental conditions, aspect that is being investigated in order to fine tune the optimal pH and temperature conditions to grow/reproduce the bacterial colony, then to induce or stop material production, effectively orchestrating when and how cellulose structures are to be produced.

Timeline

As growth of material structure is a gradual process, the conditions of the material production can be controlled/ orchestrated over time. For example, by adding medium in measured time periods, the layering of cellulose structure with loose connections between the layers can be achieved, thus creating a panel of cellulose with varying material properties.

OBSERVATION 1: INHERIT VERSUS EMERGENT MATERIAL PROPERTIES

Methods: agitated culture growth

Observation

For this stage of initial growth we used 25 falcon tubes, each containing 3ml of HS medium inoculated with Xylinus. After an overnight growth in agitated culture in 30°C incubator, we observed variety of formations in the tubes (Fig.1). Some of the cellulose formations had a loose cloudy structure, other tubes presented dense granulated structures or even a combination of both. Same variation in shape was later observed in larger volume growth.

Discussion

Although the conditions of the 25 tubes were exactly the same, the variation probably resulted from spontaneous mutation during bacterial growth.

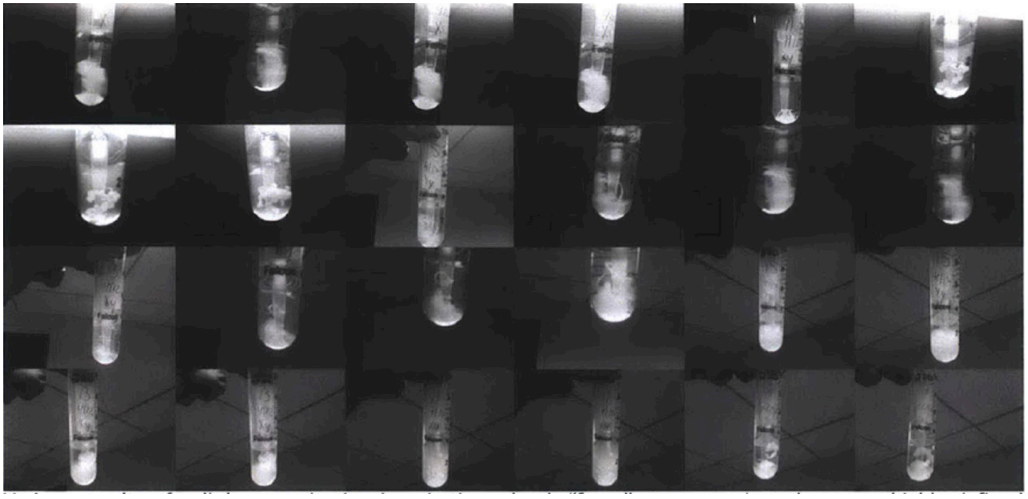

Figure 1
Spontaneous variation in the initial growth.

While working with living matter, there is a constant dialog between the designer and the artifice, involving decision-making, adaptation and alternation at each stage. For example, out of 25 different formations we can choose those with material properties that suit best our design intentions for further growth.

OBSERVATION 2: RESPONSIVE DESIGN SYSTEM, REGROWTH

Methods: static culture growth

Observation

10ml of HS medium were added on 10 Petri dishes and placed in 30°C incubator for static growth (Fig.2a). The next day the formation of cellulose membrane was evident, but also the evaporation of medium due to large surface/volume ratio. More medium was added to the plates and the samples were returned to the incubator. After 7 days the samples were taken out. Due to the continuous evaporation and temperature the pellicles were almost completely dry, some of them even became dry and brittle and ended up cracking up (Fig.2b,d). They were then removed from the incubator, were inoculated with new medium and returned to the 30°C incubator. The next day we observed renewed growth in the samples, and new cellulose growing over and between the cracked edges of the previously dry membrane (Fig.2e).

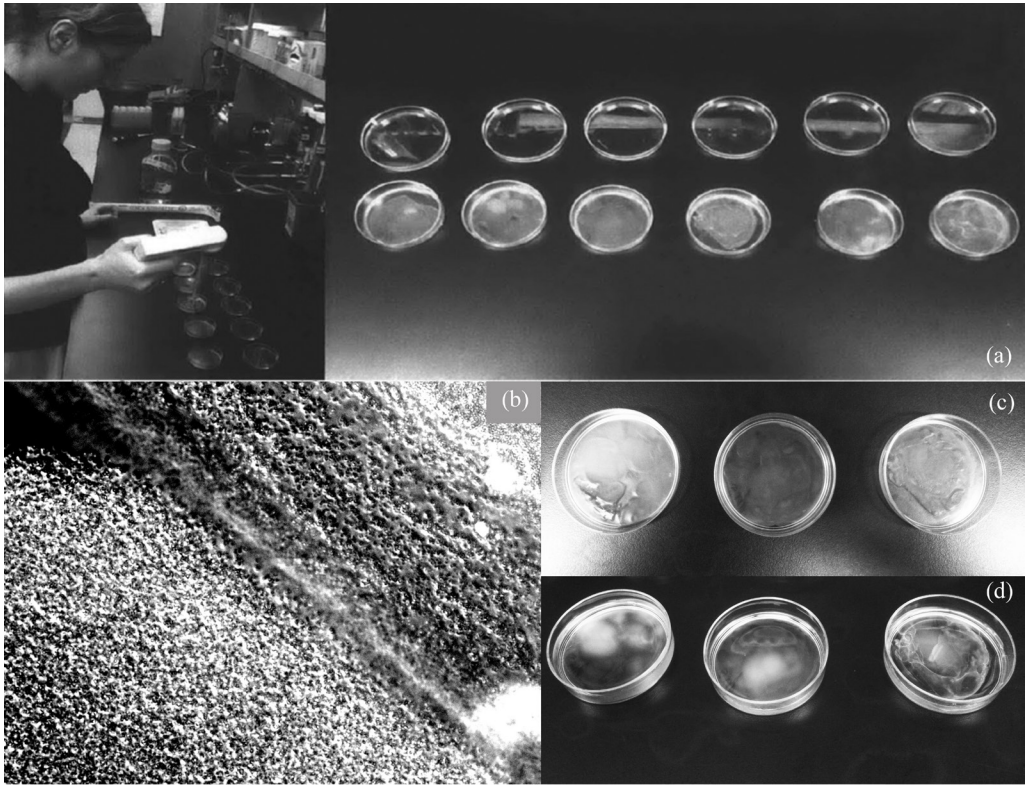

Figure 2
Regrowth: self-healing of the cellulose membrane.

Discussion

We observed cellulose production by the bacteria embedded in the cellulose membrane. observation under microscope showed that while almost all bacteria seemed to be dead or at least static, a small fraction still managed to survive (Fig.2e). Once the bacteria was restored to an optimal environment and medium was reapplied, they resumed their function and returned to cellulose production, completing or healing the previous structure. This experiment proved that bacteria may survive in a semi-inert state or lethargic state in adverse conditions, and that the growth and material production may be reactivated after conditions are restored. This gives an idea of the reactivation of growth and self-healing capabilities of such cellulose structures, which might be able to repair themselves after going through high stress and even fracture.

OBSERVATION 3: MOLDED GROWTH AS STRUCTURE

Methods: static culture growth, molding in vivo, lyophilization

Observation

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. We designed and 3d printed molds with various surface morphologies and texture resolutions (Fig. 3a, b). The molds were fixed in 100ml containers; medium and initial membrane 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 (Fig.3c,d). The membrane followed formation with good precision in a water-swallowed state. When dried, it lost the thickness significantly (Fig. 3e). The dried thin cellulose membrane was hard to remove from the molds, and while it was dry and brittle yet still fairly elastic, it preserved the features of the mold only partially after removal, where the mechanical procedure of removal was the main cause to deform and break the delicate and thin structures. In order to preserve the fibers configuration of the water-swallowed membrane, we then used a method of lyophilization (Fig. 3f).

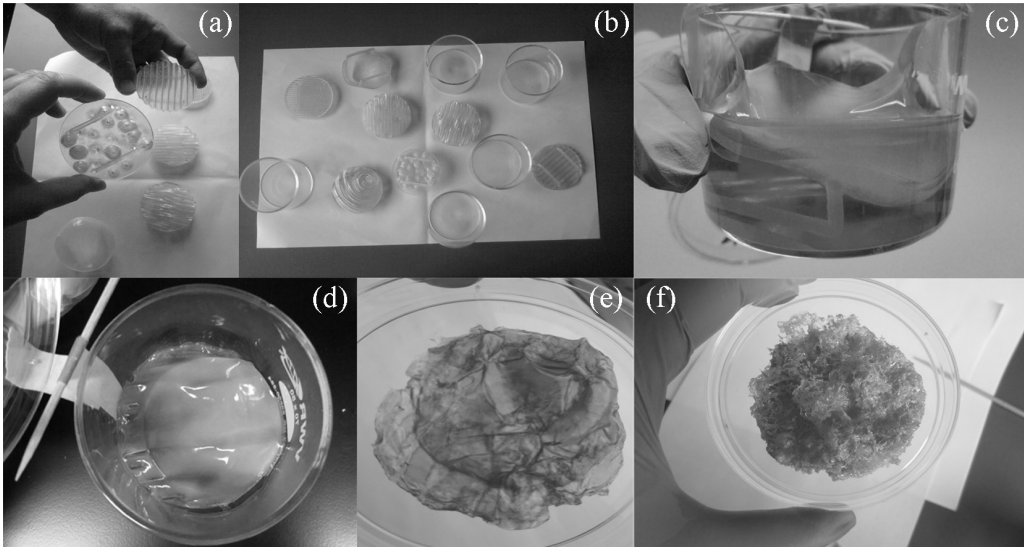

Figure 3
Molding in-vivo.

Discussion

We observed successful formation of the membrane on the mold and we achieved the desired shape in the water-swallowed state. In the post-growth stage though, we didn't manage to maintain the shape due to the significant thickness loss. The lyophilization resulted in spongeous structure with non uniform density. This is a promising path for further investigation, but so far limited by the capacity of the lyophilizer equipment being used, which only allows small volumes to be processed. Further evaluation of material properties of this resulting material is needed to find ways to stabilize and study the structure achieved in *in vivo* state.

OBSERVATION 5: MOLDED POST-GROWTH STRUCTURE

Methods: static culture growth, molding in vitro

Observation

For the larger –scale material production, seven days grown membranes were introduced into six-liter HS medium volume in a twenty-five-galloon tank. The heating pad was applied to keep temperature to (+27°C) and web camera was installed and programed to follow the growth process (a). After a few days of rapid growth, the membrane was stabilized and achieved average thickness of 8mm. The stabilized membrane was taken out of the medium, washed with tap water and placed on CNC-milled wooden mold for several days to dry. In this experiment, the wooden mold was treated with Vaseline used as a demolding agent in order to prevent adherence of the bacterial cellulose film to the plant cellulose structure of the mold. The resulting structure was successfully demolded and retained its shape in a dried state (c).

Discussion

Larger scale in vitro molding experiment was more successful then the in vivo in maintaining the structure achieved. Even the finest texture of the mold was visible on the resulting shape.

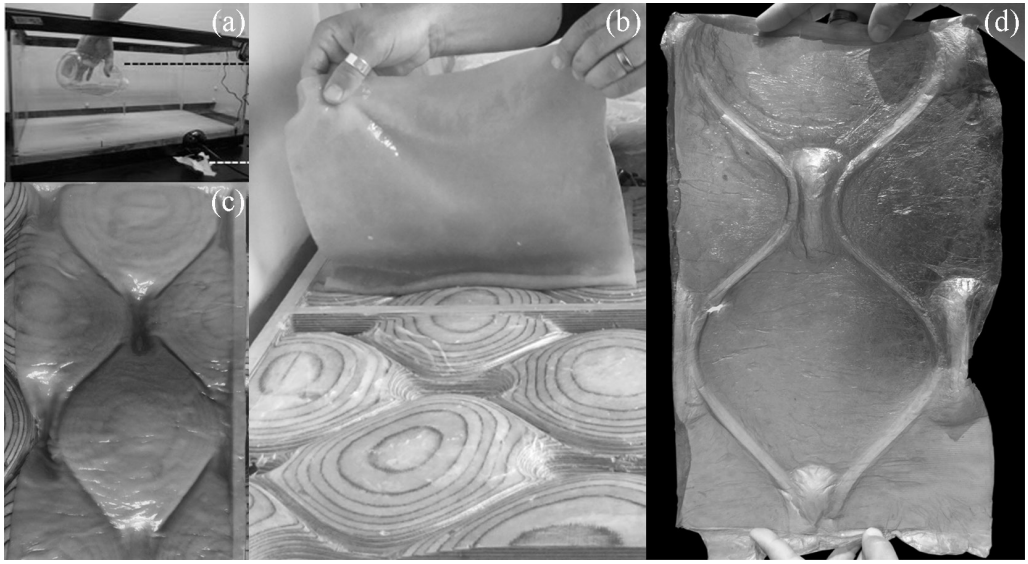

Figure 4
Molding in-vitro.

OBSERVATION 6: BIOLOGICAL 3D PRINTER

Methods: static culture growth

Observation

In the large-scale in vitro experiment described above, we observed the following material production mode: once pre-grown is introduced, it enters the stage of rapid growth. The nutrients and oxygen availability are the main limiting factors in this process and at certain thickness the cellulose is stabilized and no growth is observed. New layer can be initiated at this point by adding fresh medium on top of the old one. When fresh medium was added on top of the stabilized membrane, new membrane was initiated on the new surface level, while sending loose connecting fibrous formations to the old layer below (Figure 5).

Discussion

This layering technique gave us the idea of biological 3D printer – if the surface of the liquid was constantly moved by adding fresh medium, constant material production might be achieved. The layering mode can enable sequential build – up of an object. If we learn enough on how to control the configuration of each layer, we can build up (grow) a material formation (an object) into any desired shape. Conceptually, we can suggest two possible ways to control the configuration of each layer:

Genetically - using genetic engineering methods it is possible to control the production of cellulose. It is possible to engineer Xylinum cells to produce enzyme cellulaze that degrades the cellulose. By spatially distributing cellulose-producing and cellulose-degrading bacteria in each layer, it is possible to build up any desired shape.

Physiologically - it is also possible to combine genetic control with the physiological one. For example, by attaching UV light-sensitive promoter to the cellulose-producing complex it is possible to turn the production of cellulose on and off. The bacteria in the areas that receive UV light will produce cellulose, and those in the dark areas won't.

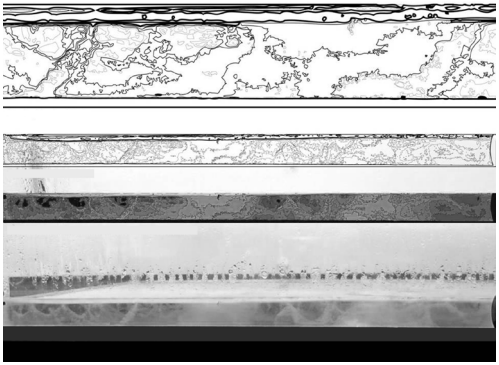

Figure 5
Schematic description of varying material properties in layered material formation.

Both of these ways to control cellulose production spatially will require extensive research and experimentation in collaboration between designers/architects and synthetic biologists.

Discussion

Although the results obtained so far and presented here are still preliminary and partial, we believe they offer important and relevant insight on critical aspects of this thesis.

We have obtained satisfactory results regarding production of bacterial cellulose pellicles and cloud-structures. The large-scale culture resulted in the growth of a twelve by twenty two inches surface pellicle, and with about eight millimetres (one

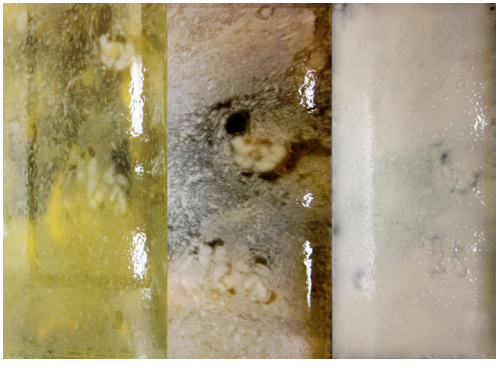

Figure 6
One layer development over time.

third of an inch approximately) of average thickness. The resulting membrane showed significant strength compared to the pellicles observed in Petri dishes, given its large size, we were able to extract it from the tank culture with no signs of stretching or any elastic deformations. It was placed on a tray for transportation, and was later placed over a CNC milled wooden mold prepared with Vaseline to allow for an easy de-molding process.

From the analysis of both series of experiments, the large-scale pellicle growing and the small scale mold growing, certain preliminary conclusions can be derived. First of all, the growth rate of bacteria and its consequent production of bacterial cellulose is directly affected by environmental parameters and its variation in time. Our parametric modelling of these environmental parameters -temperature, nutrient-medium (HS), and the variation of such parameters have resulted in variation of the cellulose structure produced. We were able to replicate successfully a layering effect -separate layers of cellulose being produced by the same culture, by affecting the nutrient-medium parameter: adding medium after one layer of cellulose pellicle had been formed. We also proved that these effects are transitory and that after they have been grown separate, the increased production of cellulose could grow structures between these and create one cohesive cellulose structure. We also proved that bacteria are actively producing cellulose even if entrapped in the cellulose film itself.

While we have yet to test different extreme environmental parameters for the cultures, we now know that the bacteria can resist and survive under unfavourable temperature conditions -dry media, complete evaporation and high temperature- and still be able to be reactivated after several days, and to resume cellulose production after a few hours and the proper medium and environmental conditions. This realization allows us to speculate about the capacity of cellulose structures to become self-healing structures. It would be possible to think of structures that under fracture or failure due to stress, might recover by locally reactivating the bacteria entrapped in the cellulose structures in those areas and opportunistically recreating optimal culture environments, to therefore grow new cellulose structures that would patch up those fractures. But it would also be possible to speculate about structures that could grow differentially in time responding to varying programmatic or functional requirements or to changing environmental conditions in large period of time.

The challenge of designing and operating with design principles and parametric logics at a chemical and physical level in order to produce three-dimensional material structures involved first of all a reframing of the goals and expectations, but also of the methods and techniques applied. Since there is no direct influence on the formal aspects of the produced, since the production process is first of all differed in time and secondly because it is the result of a complex balance of controlled but also of some uncontrolled variables -aspects of the biomechanics of the cellulose material fabrication by the bacteria for example- the actual design process happens in a sort of delayed feedback loop, where input operations affect certain conditions which in time change due to these effects and return new modified conditions with which to operate. The design process becomes a mutual self-adaptive set of relations where designer and artifice engage: the designer adapts and reacts based on the new artifice, the artifice adapts and reacts to the new operations and processes unleashed by the designer. Both designer and artifice can be understood as environmental conditions for the other one.

The ecological implications of developing such a manufacturing system are comparable to the scale of the impact of the cellulose industry and its ecological footprint. To imagine the fabrication of cellulose panels and products harvesting bacteria instead of harvesting forests is in itself quite a radical possibility with potentially large scale impact. But also relevant from the perspective of the environmental impact it could have if waste glucose-rich sources were to be utilized. Waste residues from

crops like corn, rice, and wheat could be used as glucose sources and therefore offer large-scale and low-cost alternative resources for the production of bacterial cellulose. And of course there is the fact that cellulose products could always be degraded back to glucose sources, using enzymatic cellulase protocols, especially since this particular type of cellulose is of a high degree of purity in comparison to that obtained from plants.

But leaving the appealing and sound ecological benefits of such a project, it also opens up interesting lines of exploration in terms of design manufacturing techniques. The layering effect observed and produced, could be analogue to similar layer-by-layer process of current 3D printing technologies, where in this case, the fabrication machine would be a biological one instead of an electro-mechanical one, and the input materials would be fed to and metabolized by the system instead of being just simply a raw input processed and deployed. Specially since the "fabrication mechanisms" are integral part of the fabricated material itself, being able to resume production and fabrication at a later time, modifying not just the state and configuration but its generative and productive capability with it.

These are but initial results and further investigation should be carried out, where a future goal aims at understanding, and then controlling and eventually programming the genetic sequence in A. Xyllinus in order to promote or deactivate selectively the cellulose production. Alternatively, we plan to attempt to genetically modify the bacteria to add a sequence that would enable the transformed bacteria in order to allow it to selectively produce the cellulase enzyme, which metabolizes the cellulose degrading it back to glucose. The capacity of controlling both production of cellulose and of cellulase is regarded from this research perspective as being able to control both additive and subtractive mechanisms in a material production system. It offers enormous opportunities to design a manufacturing system that would combine material techniques, material deposition and material subtraction, in order to provide a more accurate and versatile manufacturing system. Literature shows that oxygen supply and pH control are also key parameters in regulating the bacteria growth cycle and the cellulose production, which would, if regulated, allow a higher degree of control both on the ratio of growth and of cellulose production but also on the structural properties of the cellulose structure produced. Further investigations will attempt to control the formation of cellulose pellicle and cloud structures by the dynamic control of these environmental parameters. It is an objective of this research to provide methods and techniques to design and operate the anisotropic distribution of material properties on the cellulose structures produces, as much as to drive the fabrication of these three-dimensional structures. This responsive performative behaviour programmed in the system and induced by the environment enable a homeostatic, or even further transistatic, momentum where both artifice and environment dialogue, the environment by inducing transformations in the artifice but being transformed by the material production of the system, the artifice by responding to the dynamic conditions adapts to the changes and changes the physical relations between both by adding or subtracting material. Furthermore, bacterial cellulose structures are currently used in tissue engineering as scaffold structures where tissue cells can be allocated and grown. In a similar fashion we propose cocultures where cellulose structures would be grown as scaffolds initially then partially resorbed by another growing culture with different material properties. An ecosystem composed of symbiotic agents negotiating material configurations and adapting to changing local and global conditions. There is no doubt that the potential impact of developing such design techniques and material nano-technology can be of unprecedented relevance, both in terms of influencing a paradigm shift within the manufacturing industry and in terms of its ecological implications. The trans-disciplinary nature of this research case, spanning from biology, computer science, engineering and architecture is but a reflection on the scope of such an enterprise, designing a bacterial

nano-cellulose biofabrication technique for large scale anisotropic three-dimensional structures, or the possibility of growing your own chair, a house, maybe a city.

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