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Bioprinting bacterial cellulose

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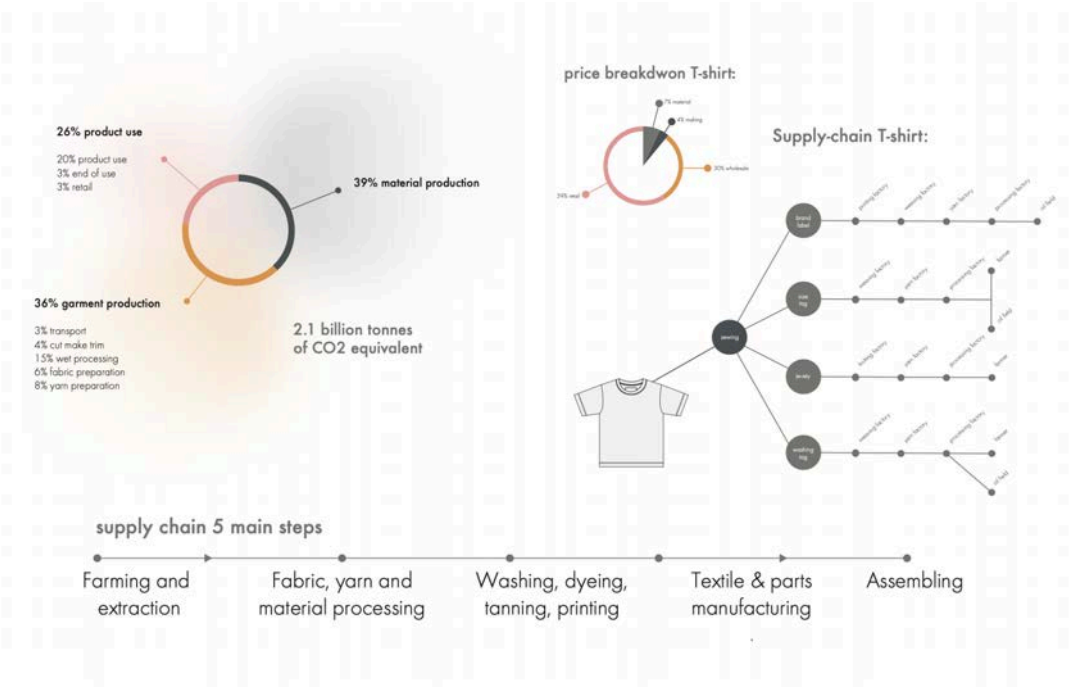
01. abstract

A research project that investigates how bioprinting bacterial cellulose can be used to tackle the root of the problem with today's supply chain in fashion, the assembly type of manufacturing. What if we instead could create distributed networks of designers and consumers growing garments straight from raw material to finished product?

02. introduction

02.01 The problem:

Inequality is built into all the different aspects of the industry, and the exploitation of natural resources and people is constitutive. Initiatives are being taken, especially in material extraction and recycling, to reduce resource wastage. However, this takes place within a structure (supply chain) that, by its nature, is energy-intensive and resource-wasting. Implementing improvements is important, but do not get to the bottom of the structural problems. According to the IPCC, the UN's Intergovernmental Panel on Climate Change, (Wigan, 2020) 38 percent of the fashion industry's CO2 emissions come from the extraction of raw materials, 36% from fabric and clothing production, and a further 23% from products' End-use.



Pictogram showing the breakdown of the supply chain in fashion.

This project aims to find alternatives to fashion's outdated production system. In a narrow sense, the proposal focuses on finding alternatives to the wasteful way that fabrics and garments are produced, but it is not limited to this. The idea of locally produced materials, as opposed to extracted from nature and transported to production sites and consumers, opens entirely altered ways of perceiving fashion. What happens when we start from the other end of the production chain, with consumers and designers controlling design, materials, and distribution? Thinking away from the current set-up to allow space for consumers and independent designers to find new ways of relating to fashion.

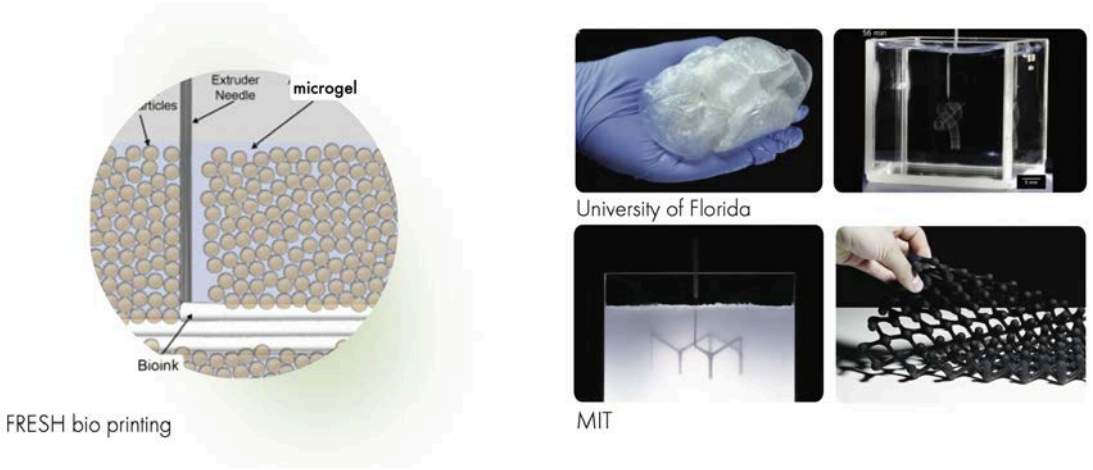
02.02 The solution

Additive manufacturing has for the past 20 years revolutionised many industries however it has proved challenging for fashion, mainly because of the material (filament) traditionally used for 3D printing. In this study, we will investigate the possibility to utilise recent innovations in the field of bioprinting and the possibility to create a distributed supply chain where small-scale "factories" can produce garments from "seed" to finished garments.

02.02.01 bioprinting

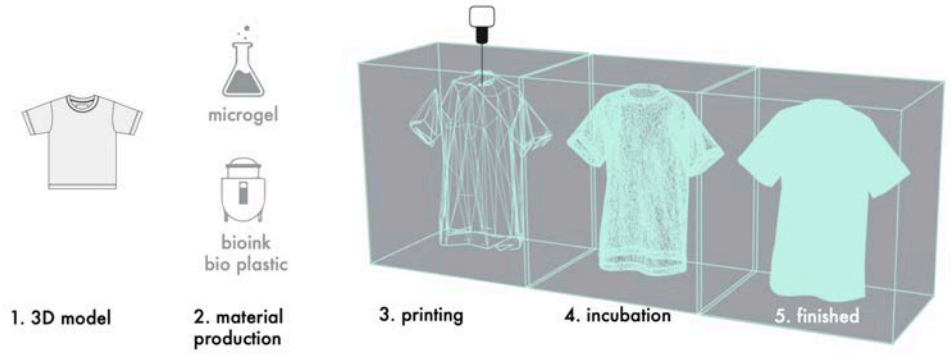
The study takes particular interest in the "FRESH" protocol in bioprinting, which stands for "Freeform Reversible Embedding of Suspended Hydrogels."

The FRESH method is a 3D printing technique that enables the printing of complex, high-resolution, and free-standing structures using hydrogels. It involves embedding a hydrogel structure in a support bath, which provides mechanical support during printing. Once the printing is complete, the support bath can be dissolved, leaving behind the printed hydrogel structure.



Top University of Florida, Bottom: (merdami, 2022) Rapid liquid printing (MIT, n.d.)

The FRESH protocol is particularly useful for bioprinting because it allows the printing of complex and intricate structures. It has primarily been developed for tissue engineering and can produce intricate structures such as blood vessels, which are difficult to produce with traditional 3D printing methods. But it could also be one technique to develop 3D garments without stitching.



02.02.02. Bio-ink

Hydrogel is a type of polymer that can absorb and retain large amounts of water, they are commonly used as bioprinting inks due to its ability to support the growth and viability of living cells. They can be made from a range of different polymers (alginate, gelatin, chitosan, etc) and be crosslinked with the following methods:

1) thermal Collagen, Gelatin, Poloxamer
2) photo-activated Pegda, GelMA, HAMA
3) chemical Alginate, Fibrinogen, Silk

02.02.03. Support baths

Are generally made from a jammed microgel which is a material that acts like a solid when still but transforms into a liquid when put under stress. Hand sanitizer is a good example of that. The reason why the air bubbles inside the bottle don't rise to the surface is that under low levels of applied stress, jammed microgel behaves like elastic solids trapping the bubbles inside the liquid, however, when we pump out the liquid through the nozzle it flows like a "normal" liquid. (Angelini, 2021)

Support baths have been made from a range of different microgels, but the most commonly used is gelatin.

02.02.04. materials

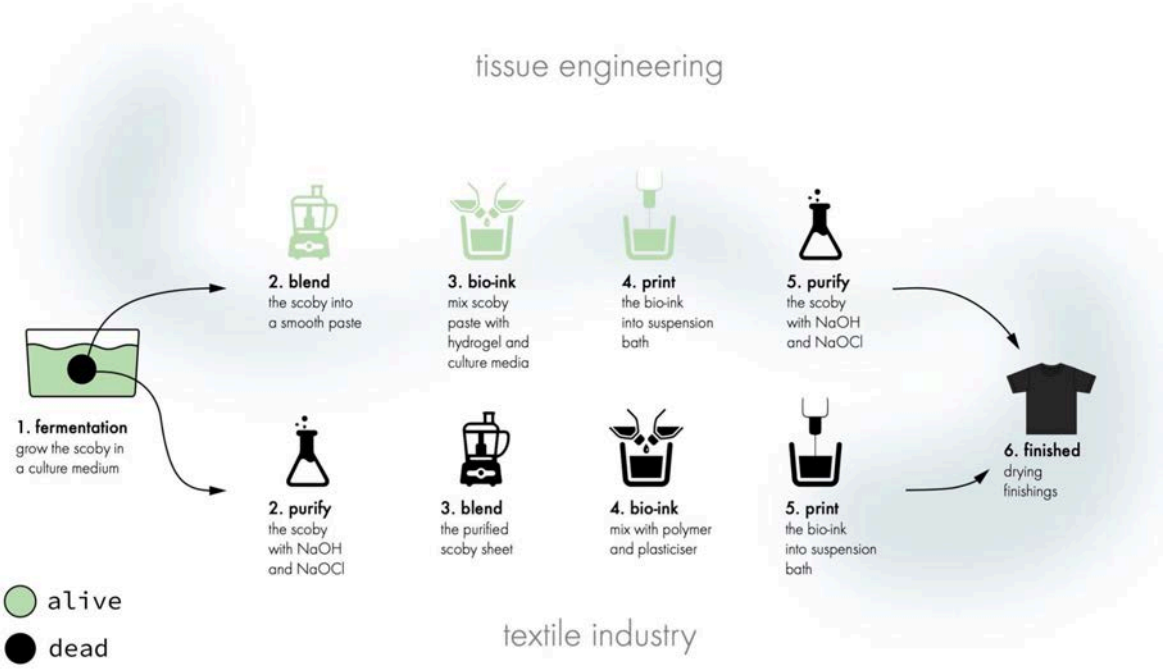
Bacterial cellulose (BC) is a highly pure form of cellulose produced by certain types of bacteria. It is known for its high tensile strength and high modulus, which makes it a good candidate for use in medical implants, textiles, and other applications that require strong and stiff materials.

BC was chosen for this project because of the relatively fast growth and positive yield-to-land ratio making it ideal to grow in a micro-factory set-up.

There are two different approaches to working with BC. Which way would be the best for the given application would form the main question to answer for this project:

1) work with it as a living-ink meaning printing it out while it is still a living bacterial culture. This is a technique that has been proposed within the field of tissue engineering however not yet for fashion applications

2) As a "dead" purified bio-ink a tested technique in the fashion field. Few companies like Scobytex grow BC membranes that after purification can be used as a leather alternative, however, me knowingly have never been used as a bioprinting ink.



03. methodology

To be able to analyze my experiment in a simple but reliable way without much of the expensive equipment often used in labs I decided on a simple DIY protocol from Clara Davis thesis (Davis, 2022)

03.01. protocol for living BC-ink

1) Yield in g/L- will give an idea of how different hydrogel recipes are affecting the growth speed of BC
2) Water loss in %— will give an indication of how different hydrogel recipes will affect the quality and density of the BC.

" the calculation of the yield in g/L and the water loss in %. The first provides information on the quantity of cellulose produced, the second gives an idea of the quality. The production

yield of BNC can be defined as the productivity and capacity of bacteria to secrete cellulose. Concerning the loss of water, the hypothesis is that the more the membrane loses liquid, the greater the rearrangement of internal structure in the polymer matrix.
Moreover, if less water is lost, the denser the inter- and intramolecular hydrogen bonds of cellulose, and the higher the degree of crystallinity. Therefore, a BNC membrane with a high-water loss percentage will theoretically have a lower tensile strength." (C. Davis)

Yield:

$$BNC\ yield\ (g/L) = \frac{BNC\ W_d\ (g)}{culture\ medium\ V_i\ (L)} \qquad (1)$$

*The production yield of the samples was obtained gravimetrically by using the dry weight (**W**) **d** in (g) of the collected membrane and the initial volume of the culture medium (**V**) in (L). BNC yield **i** was calculated according to the following equation:*

BNC is a highly porous fibrillar network that is synthesised as a gel-like membrane in a liquid medium. In its never-dried state (see Figure 1-22), the BNC matrix can hold a tremendous amount of water because most molecular hydrogen-bonds are not yet formed. Calculating the initial water loss from the BNC provides information on the density and quality of the fibrillar structure. Water loss from BNC can be determined using the following formula:

density and tensile strength:

$$Water\ loss\ (\%) = \frac{BNC\ W_w\ (g) - BNC\ W_d\ (g)}{BNC\ W_d\ (g)} * 100 \qquad (2)$$

*Where **BNC W** is the wet weight of BNC (never-dried state) and **BNC W** is the dry weight of BNC **wd** after first drying.*

03.02 for purified BC-ink

The weight was determined using a digital scale Mettler, model AE240, with an accuracy of 0.0001g. The dimensions were taken with a millimeter ruler and the thickness with a gauge

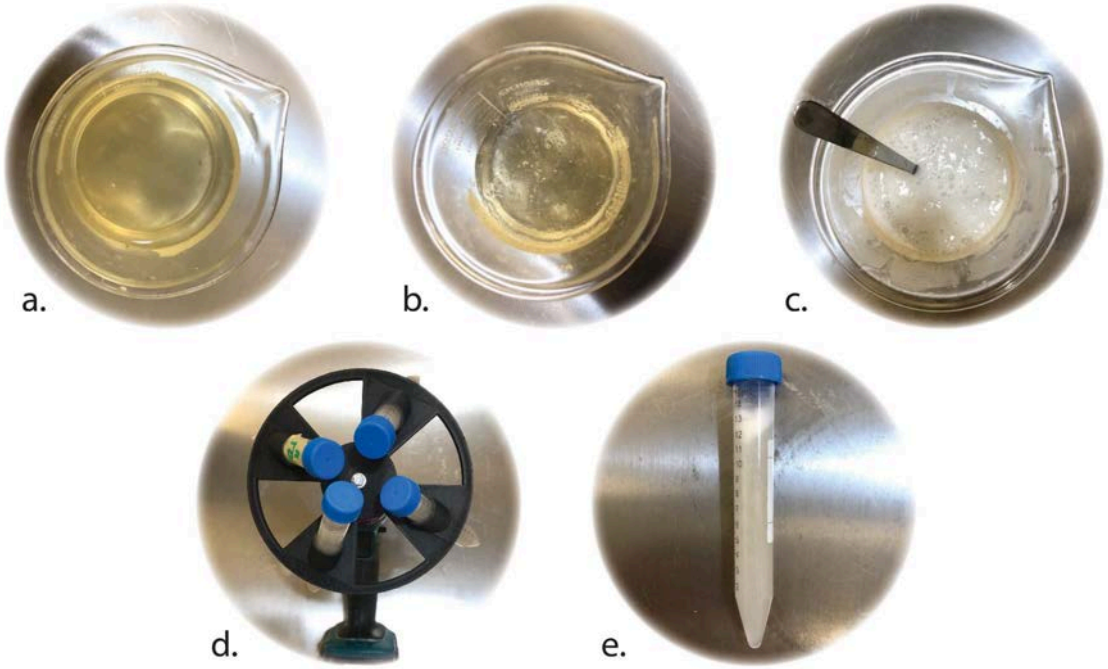
micrometer SHAHE with a precision of 0.01mm. With these measurements, different data could be approximated and calculated such as the density d and the grammage G (surface density) of the samples. The density and grammage were calculated using the following equations:

$$d = \frac{m}{v} \quad \text{eq. (1)} \quad \text{with} \quad v = l * w * t \quad \text{eq. (2)} \quad , \text{ and} \quad G = \frac{m}{A} \quad \text{eq. (3)}$$

Where m the masse (g), v the volume (mm3), l the length (mm), w the width (mm), t the thickness (mm), and A the surface area (m2) of the sample.

04. preparation of bio-ink and support bath

04.01. protocol for support bath



ingredients Support-bath:

* 4% Gelatin
* 0.16% Calcium chloride
* 500 ml water

tools:

* magnetic stirrer
* glass jars
* centrifuge
* fridge
* blender

How-to:

* Dissolve the gelatin with 250 ml water at 55° C on a magnetic stir plate
* Dissolve the calcium chloride in the same water at 34° C on a magnetic stir plate
* Let the solution rest for 24 hours at 20 ° C (fig a)
* Mix in 250 ml calcium Chloride solution with the gelatin slurry (fig b)
* Place the solution for 1 hour in a fridge at 6 ° C (to avoid overheating)
* Blend the solution for 1 min using "pulse" mode (fig c)
* Move the solution into 15 ml centrifuge tubes
* Centrifuge the slurry 3 times (for 1-2 min) (fig d)
* for the first and second centrifuges extract the supernatant and resuspended the gelatin with 0.16% (fig e)
* After the last centrifuge, the gelatin was not resuspended but dislodged and placed in a petri dish for printing
* Cool the gelatin again in a fridge at 6° C

04.02. protocol for bio-ink

* 2% v/w Sodium Alginate
* 2% v/w Glycerin
* purified SCOBY paste
* 0.5% v/w Mica powder
* magnetic stirrer

* glass jars
* polymers, plasticizers, and colourants combined
* dissolved and vortexed at 40o C for a minimum of 30 min

05. BC fermentation

05.01. methodology & tools

The Kombucha fermentation was made inside a growth box with 3 seedling heat mats to regulate temperature. The temperature was held between 30-35 °C PH value 2-4.
For the fermentation bath, regular plastic boxes were used.

How-to:

1) Boil the water for 30 min to sterilize before letting it cool down to 30 C°
2) Ad sucrose, nitrogen source as well as a small piece of SCOBY mother
3) Add the ingredients to the vessel and tie a cotton fabric cover as a lid, this will protect the fermentation bath from flies and mosquitos while still allowing it to breathe.
4) Do not move or shake the fermentation bath before harvesting.
5) Monitor the temperature and PH values during the process.

Note: Be sure to properly sterilize all equipment before inoculation; wipe the growth box, working table, and fermentation vessel with alcohol. Smaller objects like fabric lids and tools can be sterilized in a pressure cooker.

05.02. recipes

Recipe K-A.

· 3L Water
· 30 g Green tea
· 300 g Sugar
· 150 ml Natural vinegar

Recipe K-B

· 3L Water
· 30 g Green tea
· 300 g Sugar

Recipe K-C.

· 1L Water
· 1L Beer (Argus Suave)
· 100 g White sugar
· 100 g Brown sugar

Recipe K-D

· 1L Water
· 1L Beer (Argus Suave)
· 100 g White sugar

· 100 g Brown sugar
· 110 ml reciepe K-C

Recipe K-E

· 45 gram tea
· 300 gram sugar
· 3 litre water

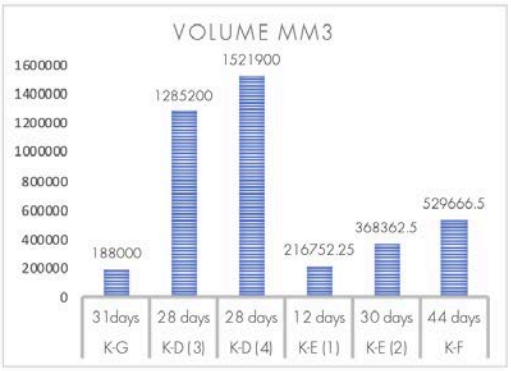
Reciepe K-F

· 15 gram coffe
· 540 gram sugar
· 3 litre water

05.03. Reflection of the BC fermentation process:

Though optimal fermentation conditions were not this research's main point, some observations could be made. K-A and K-B failed because of contamination. The beer recipe K-D showed impressive growth with more than double the speed of growth. This is likely because of the existing yeast culture in beer that seemed to kickstart the fermentation process. However, the quality of the cellulose production was poor, the sheets were "puffy" and fragile, easily falling apart during handling.

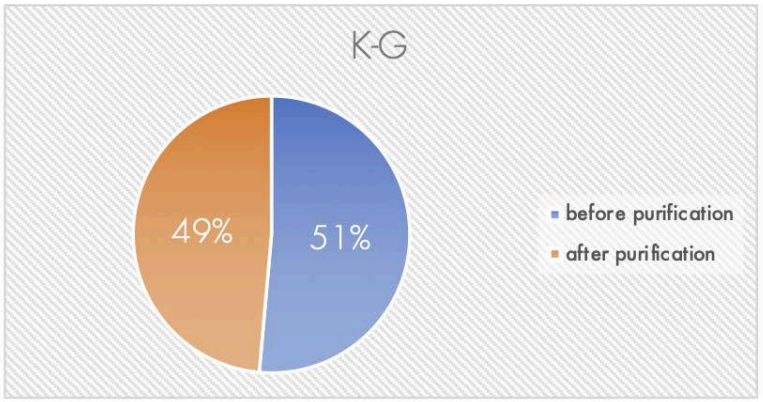
name	days	volume mm3
K-G	31days	188000
K-D (3)	28 days	1285200
K-D (4)	28 days	1521900
K-E (1)	12 days	216752.3
K-E (2)	30 days	368362.5
K-F	44 days	529666.5



No comparison of purified yield was made but my guess would be that the actual cellulose production from the beer fermentation would not be more than other bacterial cellulose

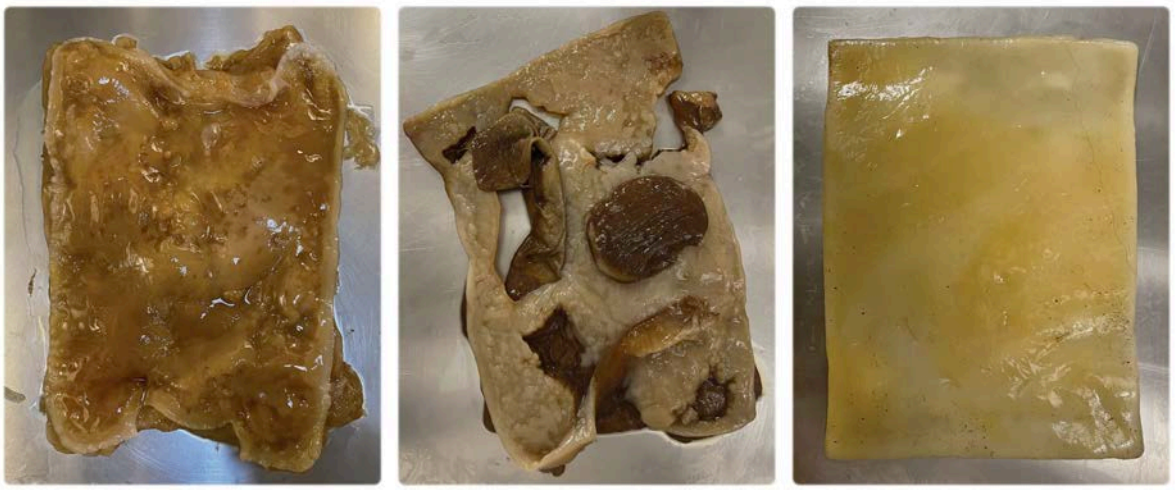
recipes.

Purification experiment with K-G indicates only 2% loss in volume from the process.



	volume mm3	
	before purification	after purification
K-G	188000	177600

K-E was the clear winner producing even and strong pellicles with a far superior yield (compared with K-F and K-G). K-E was harvested after 12 days (K-E(1)) and the bath was left growing (K-E(2)). One explanation for the high yield could be that the removal of top layers allowed new layers to free oxygen supply and thereby increased cellulose production.

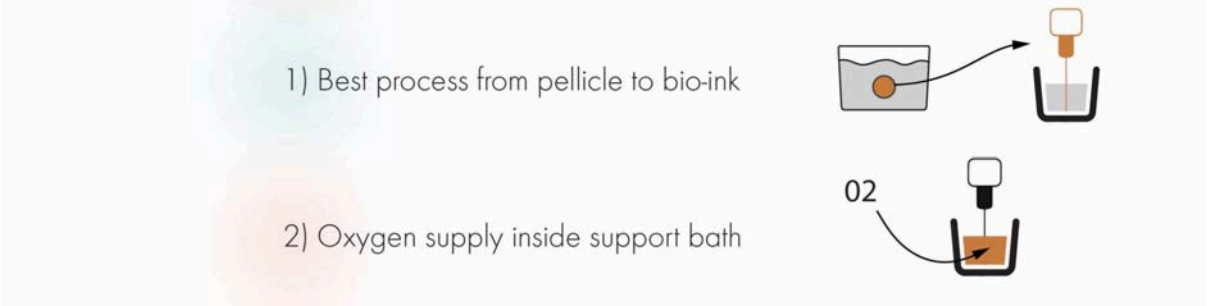


Left: K-D middle: Reciepe K-F right: K-E

06. living/purified BC-ink

05.01. living BC-ink

I identified 2 different main problems to overcome in trying to grow BC as a "living-ink"

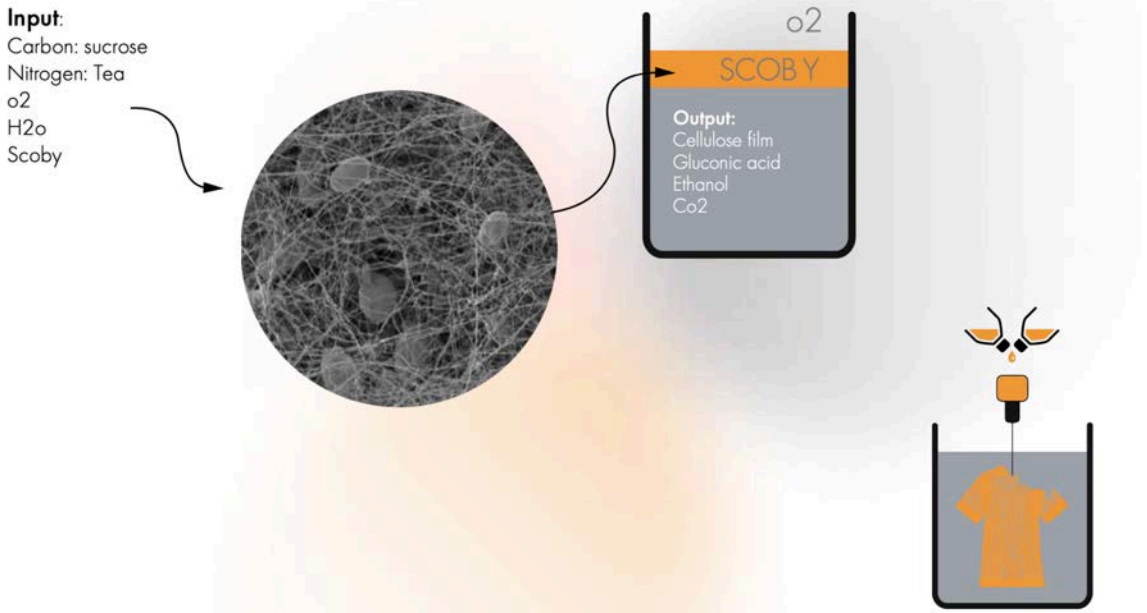


06.01.01 Problem 1: Pellicle to bio-ink

The bacteria and yeast in the SCOBY secrete a sticky, slimy substance called extracellular polymeric substances (EPS), which helps them adhere to surfaces and form a biofilm. This biofilm is a complex and highly organized structure composed of layers of microorganisms, EPS, and other organic and inorganic materials growing on the surface of the culture media where it is in contact with air.

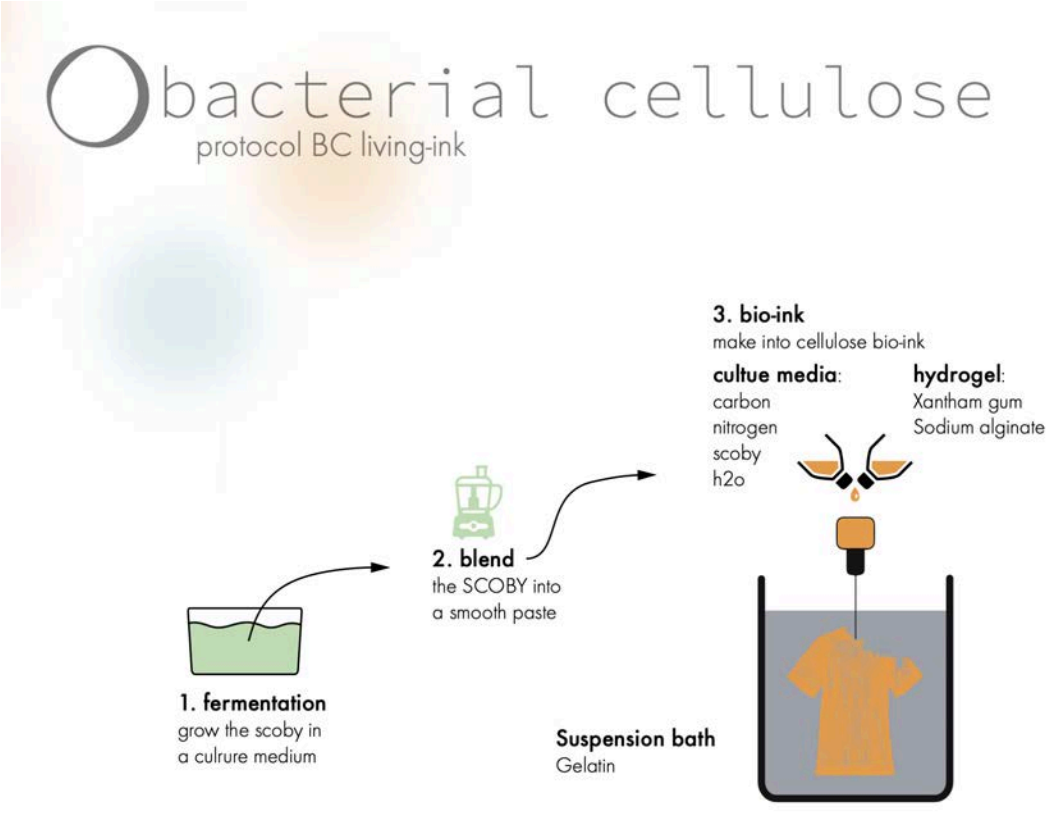
The EPS produced by the SCOBY serves several important functions in the formation of the biofilm. It helps to anchor the microorganisms to the surface of the tea, provides a protective barrier against environmental stresses such as changes in temperature and pH, and facilitates communication and cooperation between different species of bacteria and yeast within the biofilm.

Overall, the formation of a SCOBY biofilm is a complex and dynamic process that involves symbiotic interactions between multiple species of microorganisms and their environment.

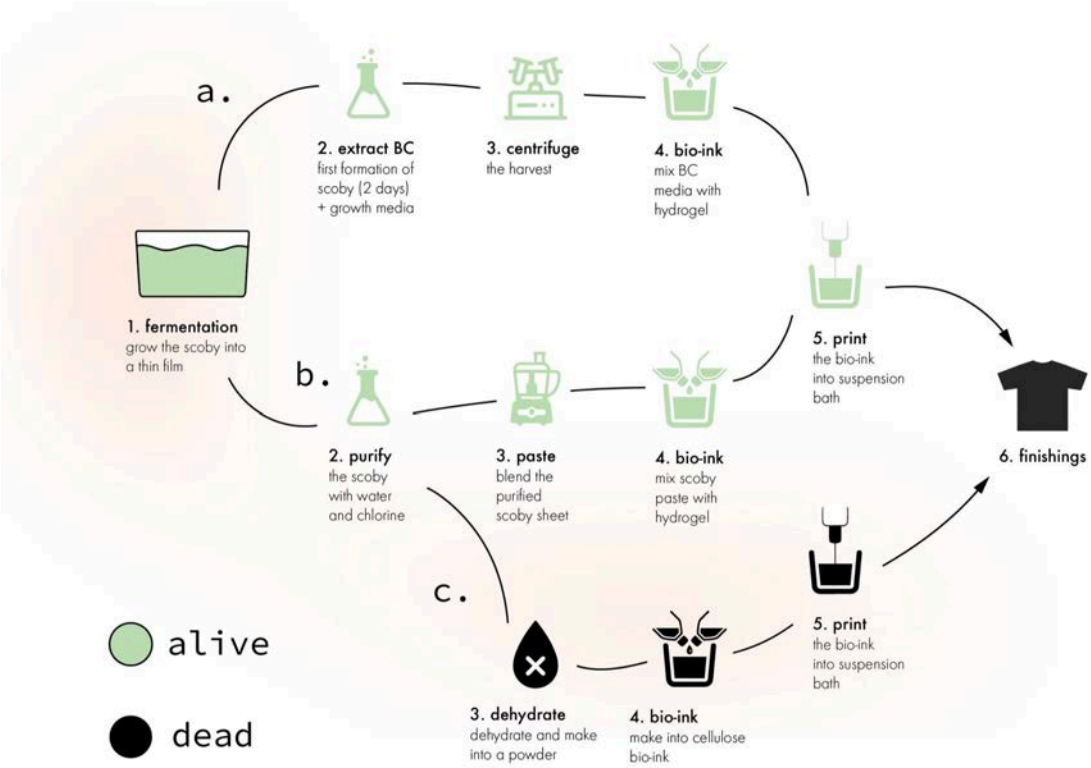


If we wanna print living BC we need to find a way of guiding the cells to grow in a completely new way that it would not naturally do.

This raises a few different challenges:



When fermenting the BC culture it will form a SCOBY, but what is the best way to extract the BC into a culture smooth enough that it is suitable for printing?



3 different roadmaps outlined:

06.01.02. problem 2: Oxygen supply

One of the bigger problems around printing living cells into a support bath is the oxygen supply that all living organisms need. This is a study outlining a gas exchange that supplies BC with enough supply that it can form a network (Binelli, 2022)

However, it is worth noting that the BC is not growing as well in the gel as it would with an unlimited supply of air

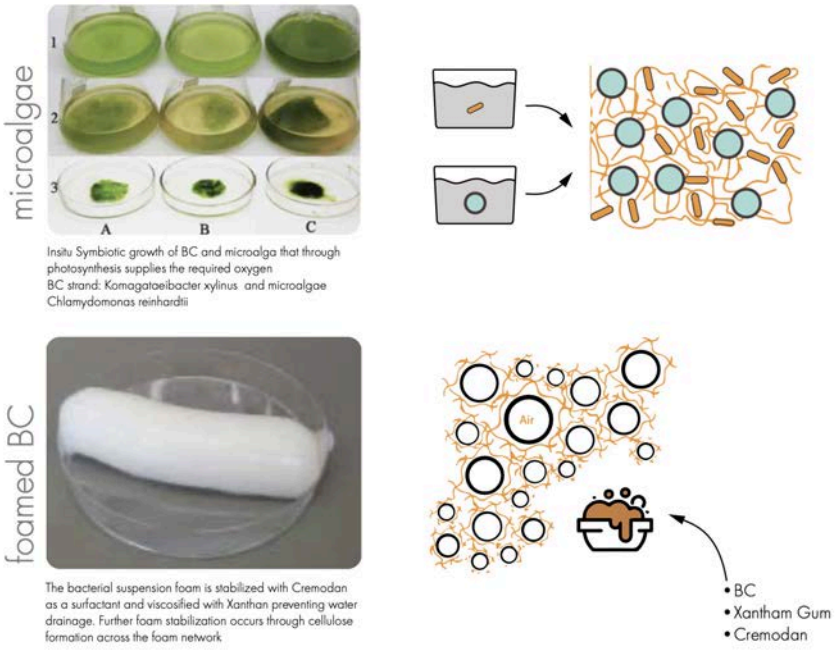
"Microscopic images reveal that bacteria embedded in the granular gel lead to a more heterogeneous cellulose network with a lower fiber density compared to biofilms exposed to

air. Indeed, the density of cellulose fibers in ink decreases from 5.23 to 1.65 vol%". (Binelli, 2022)

06.01.03 Experimentation 1

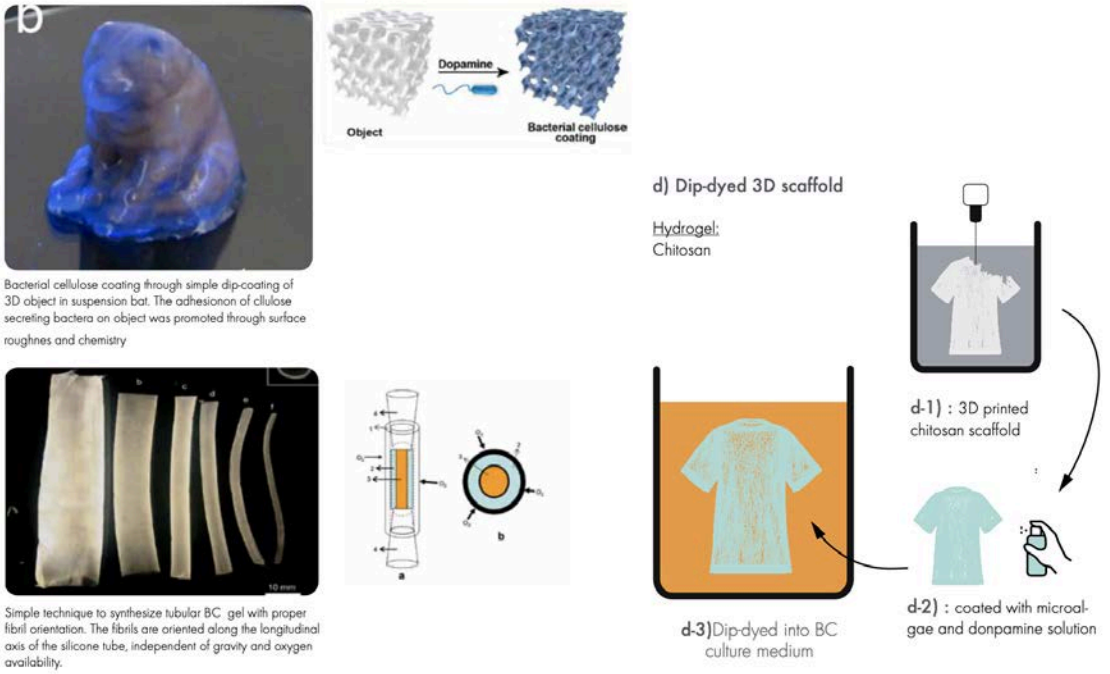
During initial experiments, foaming attempts have been made with household soup however they don't seem to be able to sustain its volume over a longer time, instead attempts using an ice cream stabilizer for the foaming (Ruhs, 2022) allowed the creation of a more stable foam with smaller bubbles.

Based on this study 4 new road maps were developed for future experiments:

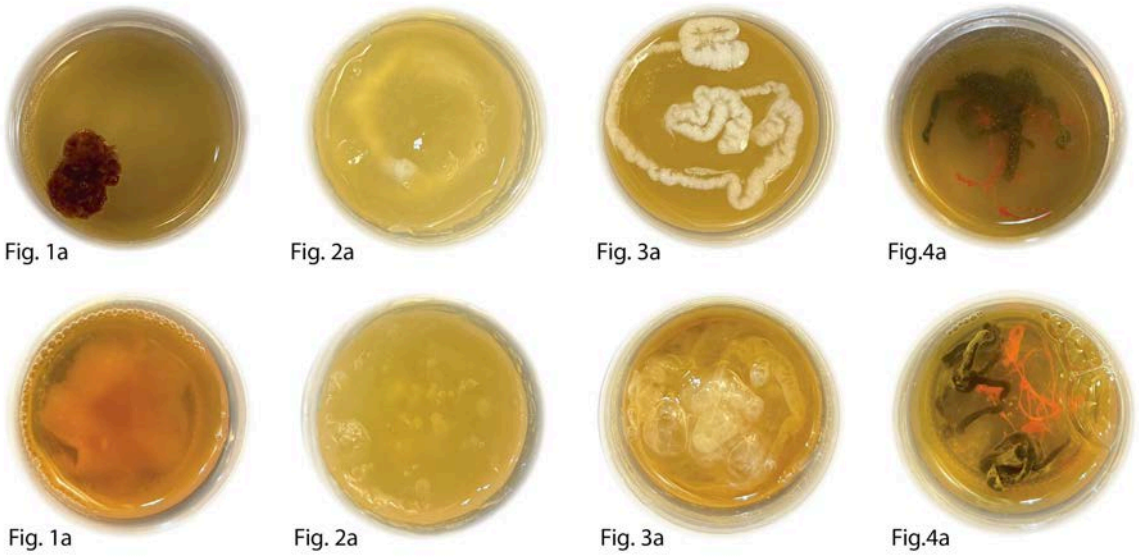


Top: microalgae: (Huang, 2018) Bottom: (Ruhs, 2022)

roadmaps:



Top: (Ruhs, n.d.) bottom: (Putra, 2022)



From left to right: Fig.1a&b S-70 dried SCOBY Xanthan, Fig.2 a&b S-67 foamed BC ink+ alginate support bath, Fig.3a&b S-66 foamed BC ink+ gelatin support bath, Fig.4a&b Alginate and Chitosan scaffold in cultural media

Conclusion: Samples were prepared according to the Excel sheet below and left in a sealed plastic container for 1 week at 30°C.
The container for Samples 66, 67, 70 had significantly expanded indicating CO2 production. Sample S-70 and S-66 had clear visual signs of growth while 67 seemed to have dissolved into the support bath leaving few air bubbles in the support-bath. The chitosan scaffold showed little growth.

The microalgae experiments were not possible to conduct due to slow growth of the algae however the foaming using an ice cream stabilizer significantly increased the stability of the foam. It could hold its volume during the 2 weeks test period without significant loss. However, the cell growth was limited.

	date:	name	polymer	ml	liquid code	scoby %w/v	treat. scoby	treatment		printing
roadmap: A	03-Jan	sample 30	1% alginate	20	K-C					petridish
	03-Jan	sample 31	2% alginate	20	K-C					petridish
	03-Jan	sample 32	0.5% xantham gum	20	K-C					petridish
	03-Jan	sample 33	1% xantham gum	20	K-C					petridish
	03-Jan	sample 34	2% alginate	20	K-C			foamed		petridish
	03-Jan	sample 35	2% alginate	20	K-C			1% glycerin		petridish
		sample 36	1% alginate	20	K-C			foamed		susp. Bath
	03-Jan	sample 37	2% alginate	20	K-C			foamed		susp. Bath
	03-Jan	sample 38	0.5% xantham gum	20	K-C					susp. Bath
	03-Jan	sample 39	1% xantham gum	20	K-C			foamed		susp. Bath
		sample 41	2% alginate	20	K-C			1% glycerin		susp. Bath
roadmap: B	03-Jan	sample 43	1% xantham gum	20	K-C	10%	blender			petri dish
	03-Jan	sample 44	1% xantham gum	20	K-C	30%	blender			petri dish
	03-Jan	sample 45	1% xantham gum	20	K-C	60%	blender			petri dish
	03-Jan	sample 46	0.5% xantham gum	20	K-C	60%	blender	1 glycerin		petri dish
	03-Jan	sample 47	1% xantham gum	20	K-C	60%	blender	foam		petri dish
	03-Jan	sample 48	1% xantham gum	20	K-C	60%	blender	foam		susp. Bath
	03-Jan	sample 49	1% xantham gum	20	K-C	30%	blender	amed no dish liquid		petri dish
	03-Jan	sample 50	1% xantham gum	20	K-C	30%	blender	amed no dish liquid		susp. Bath
		sample 51	1% xantham gum	20	K-C	30%	cellulase			petri dish
		sample 52	1% xantham gum	20	K-C	60%	cellulase			petri dish
		sample 53	1% xantham gum	20	K-C	30%	cellulase	foamed		susp. Bath
		sample 54	1% xantham gum	20	K-C	60%	cellulase	foamed		susp. Bath
scaffold	15-Jan	sample 68	1% xantham gum	30 ml				1% coco glucoside		
	16-Jan	sample 69	alginate+ chitosan					2 samples		susp. Bath
	16-Jan	sample 70	bioink d (dry?)					xantham		susp. Bath
	16-Jan	sample 71	bioink d (dry?)					alginate		susp. Bath

overview of the 2nd round of samples launched

06.01.04. Experimentation 2

Several variations of earlier samples were relaunched using different SCOBY concentrations and ratios of polymer. Every sample also used fresh culture media recipes using instant coffee or green tea as a nitrogen source and sucrose from the supermarket. In some cases, a yeast extract was used to jumpstart the fermentation process. The recipes were printed

1) In the support bath either in 1.5 % Xanthan gum or 4% w/v gelatin.
2) In a Petri dish with a covered lid
All samples were incubated for at least 2 weeks at 30°C

Fig. 1

Fig. 2

Fig. 3

97 was printed using a hand syringe (fig.1) and after being printed covered with culture media (fig2.) Fig 3. Most growth seemed to be rather around the print in the liquid culture media. The result was one of the strongest pellicles we managed to achieve.

Fig. 1

Fig. 2

left S91 (printed in S.92) right S95

The jars swallowed up during the incubation period and clear signs of air bubbles indicated Co2 production, the print also seemed to gain in strength indicating cellulose production. However, the samples were too small to be able to analyze them further.

Fig. 1

Fig. 2

Fig. 3

Fig1. 105b Fig2. 103c Fig3. 102 c

Many of the Petri dish samples didn't show any proof of growth and were falling apart when treated, however, samples were quite strong and showed some cellulose production.

Fig. 1

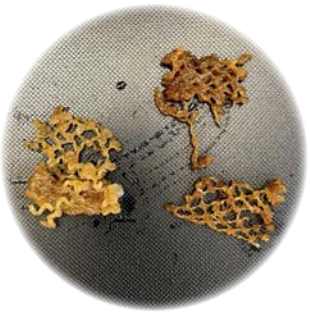


Fig. 2

100a coffee/alginate scaffold Fig1 freshly printed fig2 after two weeks of incubation

The scaffold experiments did not show any growth

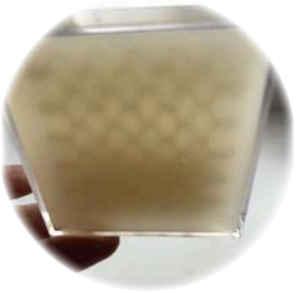


Fig. 1

Fig. 2

Co2 production in XG suspension bath

06.01.05. Reflection of the BC living-ink protocol:

Around 100 tests were conducted trying to print bacterial cellulose harvested from a DIY kombucha fermentation process. We outlined some of the difficulties with oxygen supply for this process and suggested a few research studies tackling this problem. The experiments were conducted both as prints in suspension baths as well as in Petri dishes.

The reference research studies that were used as inspiration for this research were conducted with isolated BC strands and using expensive specialized culture media. In this study, we used simple DIY kombucha recipes that sometimes made the translation of the experiments difficult. We outlined a few different recipes using different culture media recipes, polymers, and SCOBY concentrations. Because of the lack of advanced lab

equipment, the results were often hard to analyze however some conclusions could be made.

The 3ed round of samples that contained a higher SCOBY concentration and lower polymer concentration seemed to have better growth. Also, adding fresh culture media indicated a longer growth time compared to earlier experiments. However, none of the samples showed a dense enough growth that they would be suitable to make garments out of. When purified after incubation time even the dense samples fell into pieces.

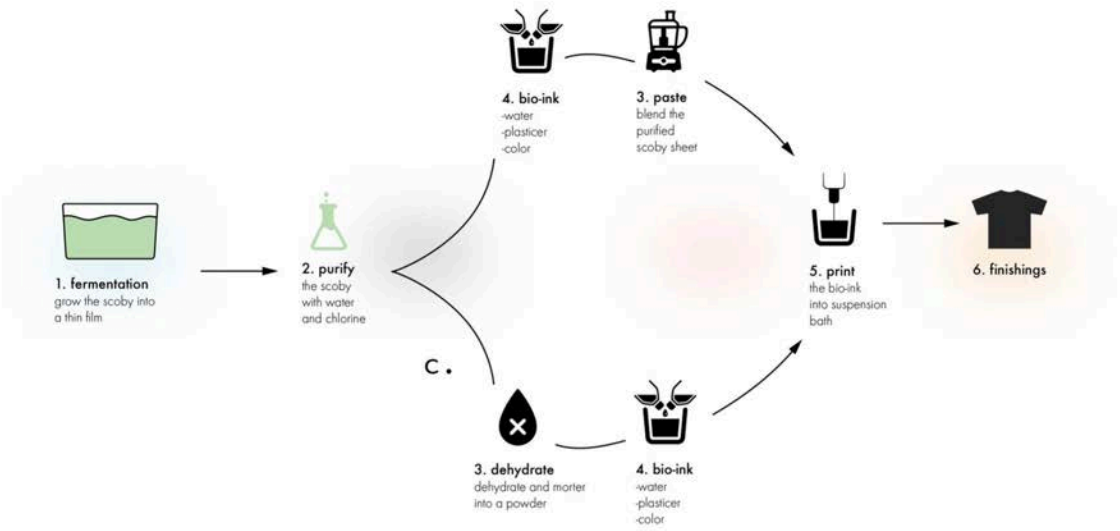
It is possible that further experimentation would give better results however it is clear that this process shows a lot of problems and very few advantages compared to the purified roadmap. The most important hurdle is that the process is complex and requires a sterile lab environment that would make scaling the process for a fashion manufacturing process highly challenging.

06.02. purified BC-ink

When purifying the SCOBY we basically remove residues from the fermentation process like sugar and yeast so that we are left with pure nano cellulose fibre. There are several studies online describing slightly different procedures but most of them are using a combination of aqueous sodium hydroxide, (available in chemist stores) and sodium hypochlorite Which is the main component in standard household bleach.

After the purification process, there are two ways that we can turn it into printing ink; either we can simply blend it into a paste or we can dehydrate it and turn it into a nano cellulose powder. During my experiments, I did not see any clear difference in the sense of strength and mechanical properties between the two methods however for the lab experiments I mainly used the fresh paste since it is a quicker process. However, the nano cellulose powder method has two advantages, one is storage and secondly maybe most importantly since we know exactly how much liquid we add to the powder we can formulate much more

precise recipes compared to the paste method.



possible roadmaps

06.02.01 Purification of kombucha pellicle:

Fig. 1 Fig. 2 Fig. 3

Fig. 1 Fig. 2 Fig. 3

Fig 1 Kombucha pellicle was covered with 1.0 M aqueous sodium hydroxide solution thermostated at 90 °C for 1.0 h (recipe for 1.0 M here) . Then removed from the sodium hydroxide solution and washed with deionized water six times, pat dried with Kleenex tissues.
Fig 2 The pellicle portion was then placed in a second 1.0 M aqueous sodium hydroxide bath thermostated at 90 °C, allowed to stand for 1.0 h washed with deionized water, and dried.
Fig 3 Next the pellicle portion D was covered with aqueous sodium hypochlorite (Bleach 3.5%), and allowed to stand at room temperature (23 °C) for 2.0 h. Then removed from the sodium hypochlorite bath and washed with deionized water (6 times), pat dried with Kleenex tissues.
Fig 4 Finally, the pellicle portion D was transferred to a Petri dish and air-dried for 20 h before being mortared down into a fine powder
Fig 5 The powder was dehydrated with 3 ml of sterilised water and 1 drop of glycerin Fig 6 air-dried for another 24 hours Purification process following this study

Conclusion: the purification process was overall successful however the final bioplastic material was very weak and brittle, and more fabrication research was needed.

Fig1 dried SCOBY Fig 2&3 BC powder

06.02.02. Dried powder:

Process The purified SCOBY was placed in a dehydrator for 20 hours at 20°C. Once the pellicles were dried they were grinded in a coffee grounder for 2 min. Then strained to divide the bigger pieces from the finer powder using a strainer.

06.02.03. blended SCOBY :

Fig1.

Fig2.

55.

56.

57.

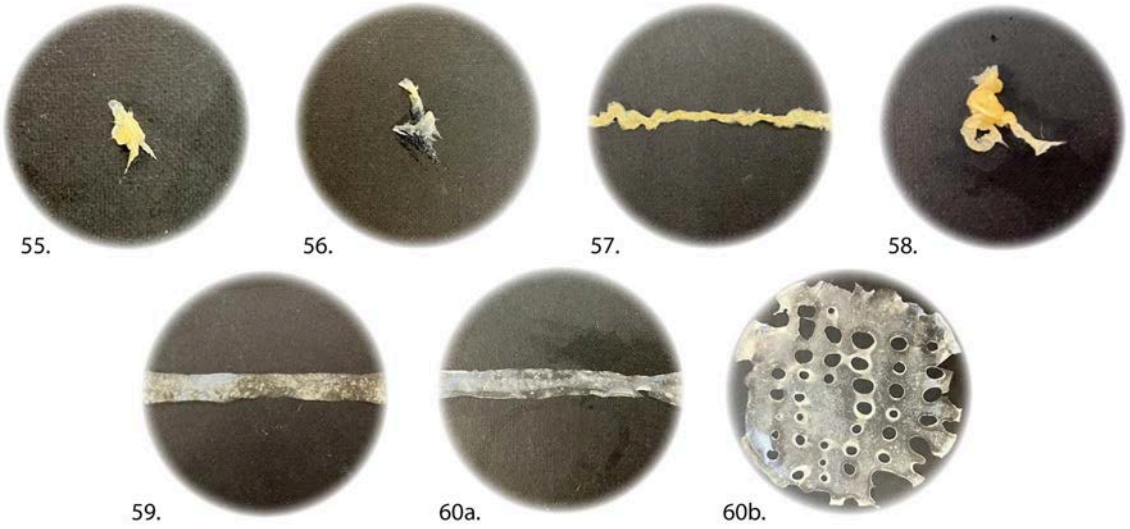
58.

59.

60a.

60b.

Process Scoby was purified according to the above-described protocol after that blended with a household blender for 2 min. Once it reached a slurry consistency it was mixed according to the above recipes and extruded (1ml) into individual Petri dishes and left to air dry for 48 hours.



Conclusion: Samples 55, 56, and 58 still had not dried, for 55 and 56 it could be explained by the high water percentage but not for 58. An alternative explanation might be the presence of glycerol in all three samples. Shrinkage: all the samples were shrinking during drying, naturally Sample 57 had the least amount of shrinkage due to the relatively low water percentage.

Color: All samples got a more yellowish tone after drying

Tensile strength: no test was conducted however all the samples appear to be too weak for the target application.

fresh BC: S-101 BC powder: S-109

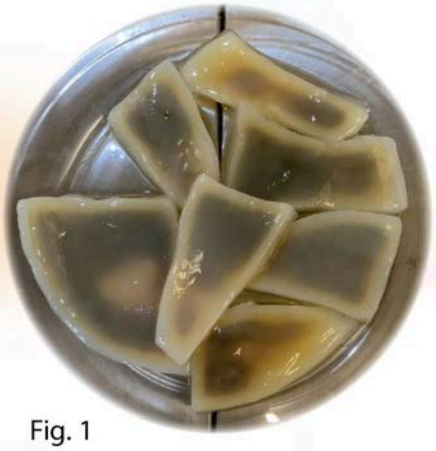


Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig1 pellicle during the last Sodium hypochlorite wash Fig 2&3 first and second sodium hydroxide wash Fig4. sodium hypochlorite wash

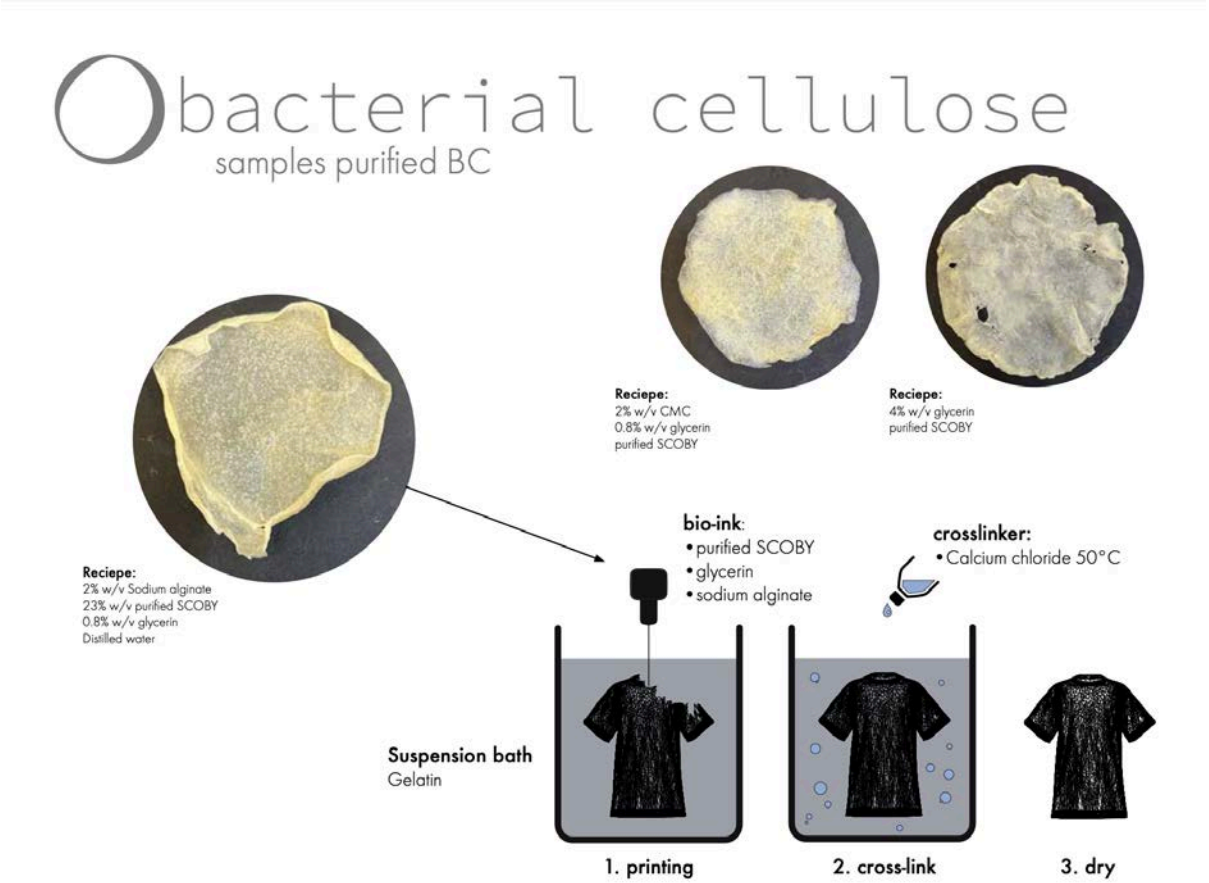
One of the problems that I encountered during the purification process was that the SCOBY pellicles didn't purify evenly Fig1, they tend to bleach quickly on the surface but required a long time to penetrate all the cellulose layers. Logically this means that the outer layers of the pellicle were put under more chemical stress than necessary. To avoid that I experimented with blending the SCOBY into a paste before purifying it. This reduced the last sodium hypochlorite step dramatically from 1+ hour to 5-7 min.

It also made the deionized water wash much more effective since we didn't have the problem of residues of bleach stuck inside the pellicle. Resulting in dried samples without crystallisation residues.

06.02.04. purified BC-ink recipes 1 :

SCOBY was purified according to the previous recipe, blended, and mixed with polymers and plasticizer on a magnetic stirrer at 60°C for five min.
Then spread out on a Petri dish and left to dry at room temperature with a fan circulating the air.

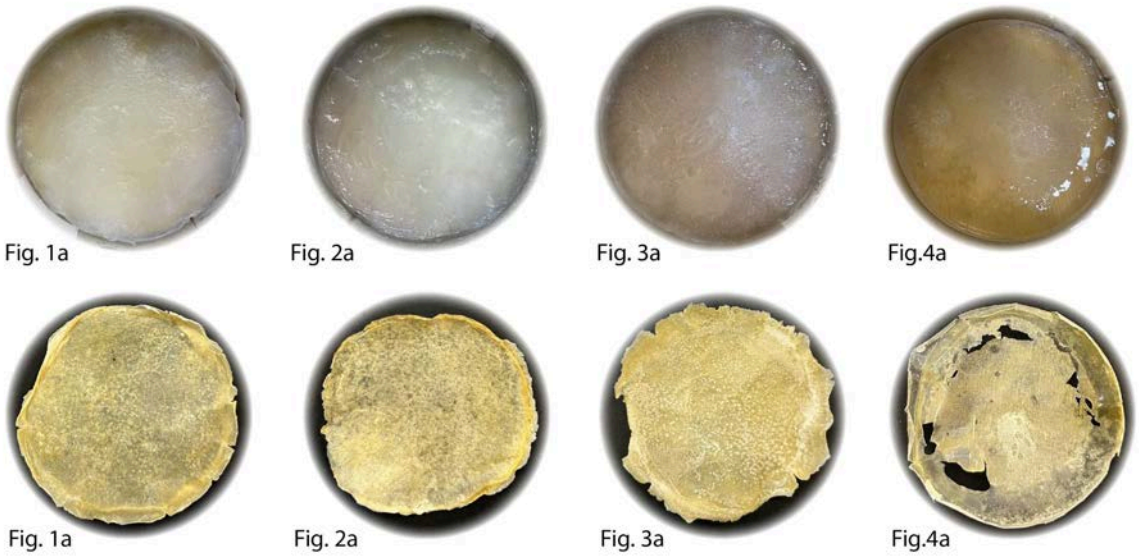
The two 100% SCOBY samples with 4% and 6% percent glycerin never fully dry (even after one week) and were very weak, easily being able to pull apart. The two other samples with sodium alginate or CMC powder as a binding polymer were much stronger, more flexible, and satisfactory.



Conclusion: Both the Sodium alginate and CMC recipes were satisfactory however I choose to proceed with the Sodium alginate recipe because of the possibility to cure the print by crosslinking with Calcium chloride which would simplify the printing process significantly.

05.02.05. purified BC-ink recipes 2 :

SCOBY was purified according to the previous recipe, blended and mixed with polymers and plasticizer on a magnetic stirrer at 60°C for five min.
Then spread out on a wooden board (to speed up drying) and sprayed with calcium chloride before and after moulding. The round mould shapes were held in place by a cookie mould.



From left to right: Fig.1 a&b S-87 100% SCOBY 1% Sodium alginate, Fig.2a&b S-89 60% SCOBY 2% Sodium Alginate, Fig.3a&b S-88 100% SCOBY 2% Sodium Alginate, Fig.4a&b S-86 20% SCOBY 2% Sodium alginate. All samples contained 1% w/v Glycerin

The samples were left to dry at room temperature with a fan circulating the air.
The density and grammage of the dried samples were calculated according to the below method.

Conclusion: Sample 86, 87, and 89 were overall satisfactory, they seemed to be strong and flexible sample 88 was slightly brittle. All the samples showed some crystallisation on the surface this would most likely be because of some residue of hypochlorite from washing the SCOBY.

The samples might also benefit from adding a bit more glycerin to increase flexibility.
The shrinkage was as expected significantly less with the 100% SCOBY samples and the extra percent Sodium alginate for sample 86 seemed to also help with "only" 15 times the loss in volume.


Fig. 1

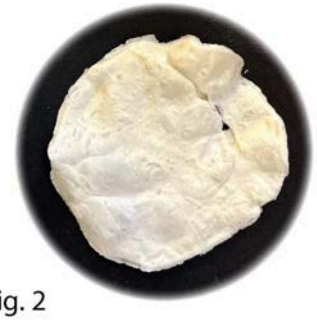


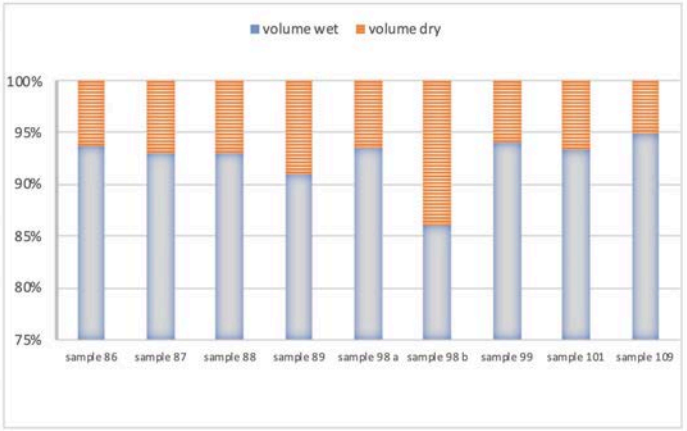
Fig. 2


Fig. 3

From left to right: Fig.1 s-98a Fig 2 s-98b (with 2% surfactant) s-99 All samples was made from 100% purified SCOBY blended and mixed with 2% Sodium alginate and 2% w/v Glycerin

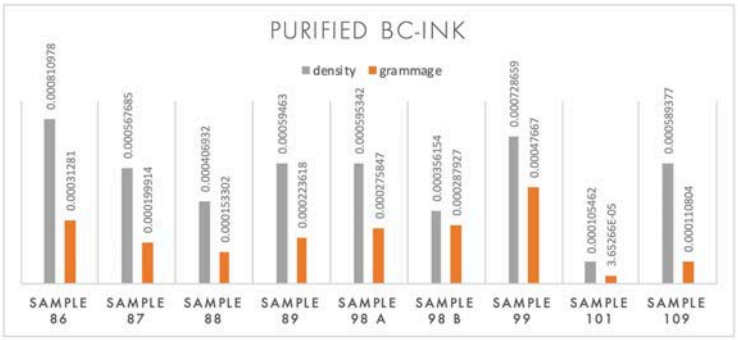
The samples were left to dry at room temperature with a fan circulating the air.

Conclusion: Sample s-99a was formed using an ice cream stabiliser (surfactant) that gave the sample a softer touch and significantly less shrinkage most likely this is because when the water evaporates the air bubbles helps to stabilise the volume and the porous nature of the final sample makes it soft.



	volume wet (g)	volume dry (g)
sample 86	40,183.79	2,675.78
sample 87	38917.04303	2,924.16
sample 88	39022.0114	2,973.47
sample 89	25721.70529	2,539.39
sample 98 a	30603.56358	2,150.02
sample 98 b	45728.15455	7,412.53
sample 99	28003.22928	1,756.65
sample 101	25458.49262	1801.590163
sample 109	28446.73726	1527.037004

Density and grammage seemed to be higher with the higher percentage of SCOBY in the sample.



	density g/mm3	grammage g/mm2
sample 86	0.00081098	0.0003128
sample 87	0.00056769	0.0001999
sample 88	0.00040693	0.0001533
sample 89	0.00059463	0.0002236
sample 98 a	0.00059534	0.0002758
sample 98 b	0.00035615	0.0002879
sample 99	0.00072866	0.0004767
sample 101	0.00010546	3.653E-05
sample 109	0.00058938	0.0001108

06.02.06. Reflection of the BC purification protocol:

We have experimented with several modifications of purification methods as well as studied how different polymers and plasticizers are affecting the nano cellulose fibre.

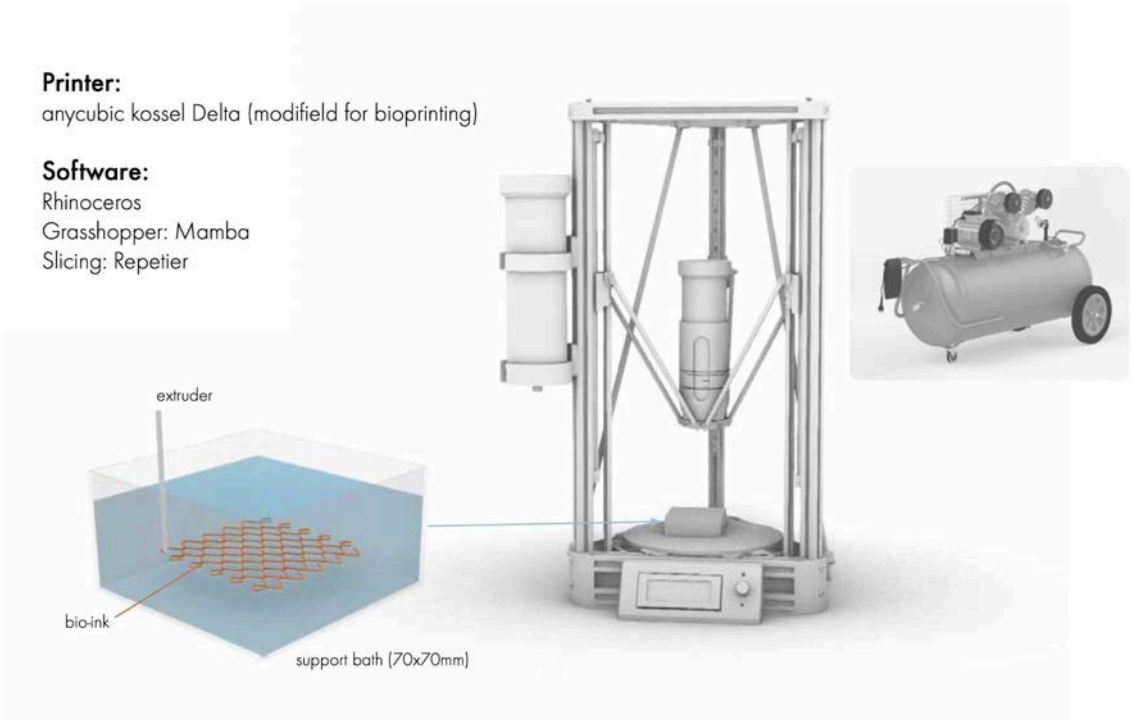
For the purification process, we have discovered that by blending the SCOBY before purifying it we can get a much faster bleaching process that would suggest a gentler treatment of the fibre.

We have experimented with different drying processes and concluded that air drying on an absorbing wood surface is the most efficient as that gives a beautiful slightly papery touch, a strong membrane that should be strong and flexible enough to make garments out of.

We have studied how different percentages of plasticizers are affecting the fibre and concluded that over 6% w/v inhibits the drying process and concluded that 2% w/v is the optimal concentration for our requirements. We have also studied additions of different polymers like cellulose powder and sodium alginate and concluded that it doesn't have a significant effect on the final material however we have decided to use 2% w/v Sodium alginate blend because it allows us to cure the print (crosslinking with calcium chloride) inside the suspension bath.

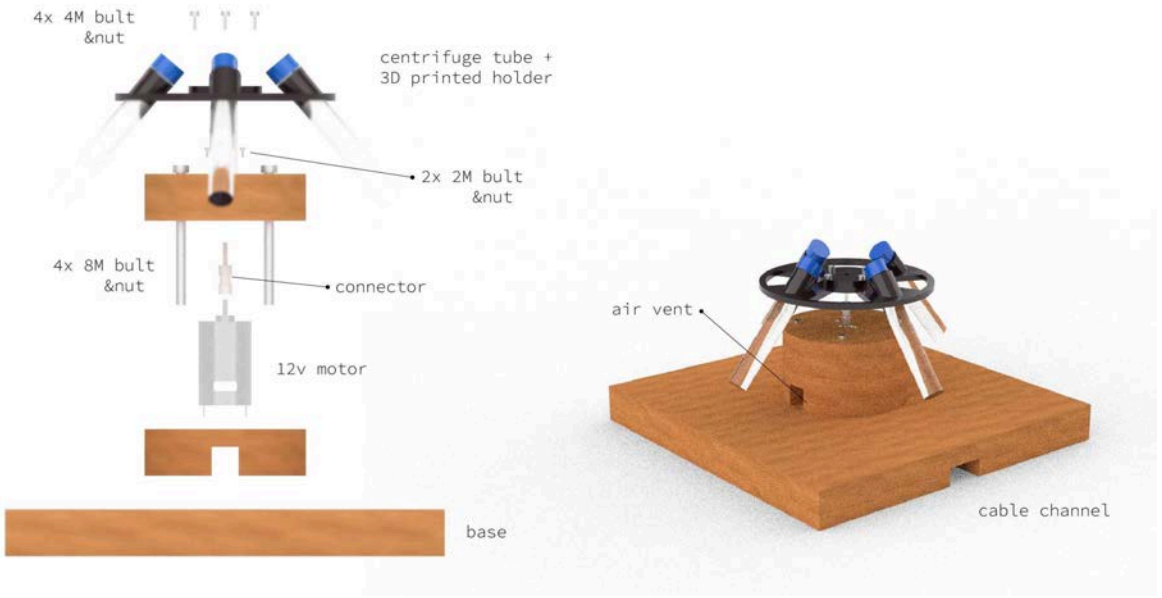
07. fabrication

07.01. methodology and DIY- tools



For the printing, I used a modified Delta desktop printer that we had in the lab.
All the artworks were developed in Rhino.
G-code was generated through the grasshopper plugin Mamba This plugin is designed by eduardo chamorro (fablab Barcelona) for both generic 3D printer FDM(fused deposition modelling) & paste printers (ceramic-biomaterials with pressure or motor control), up to large-scale Robotic Fabricators using FFF technologies and running from Gcode or for simple path generator on them.

Slicing was done with Repetier, an open-source slicing software. The support bath was prepared in transparent 7x7x4cm containers.
Bio-inks were colored with Mica powder to differentiate from the support bath.
When printing in a support bath finding the right balance between the viscosity of bio-ink and support bath, print settings such as speed, pressure and the geometry of the artwork is crucial and takes some trial and error to perfect. It is recommended to start with simple geometries and light straight lines before moving to more complex artworks.



Initially, for my experiments, I used a 3D printed centrifuge holder attached to an electric drill but realized that it was too slow to efficiently vortex the gelatin slurry (especially over 5% v/w) also risking overheating the motor of the drill. Therefore, a new DIY centrifuge motor was developed.

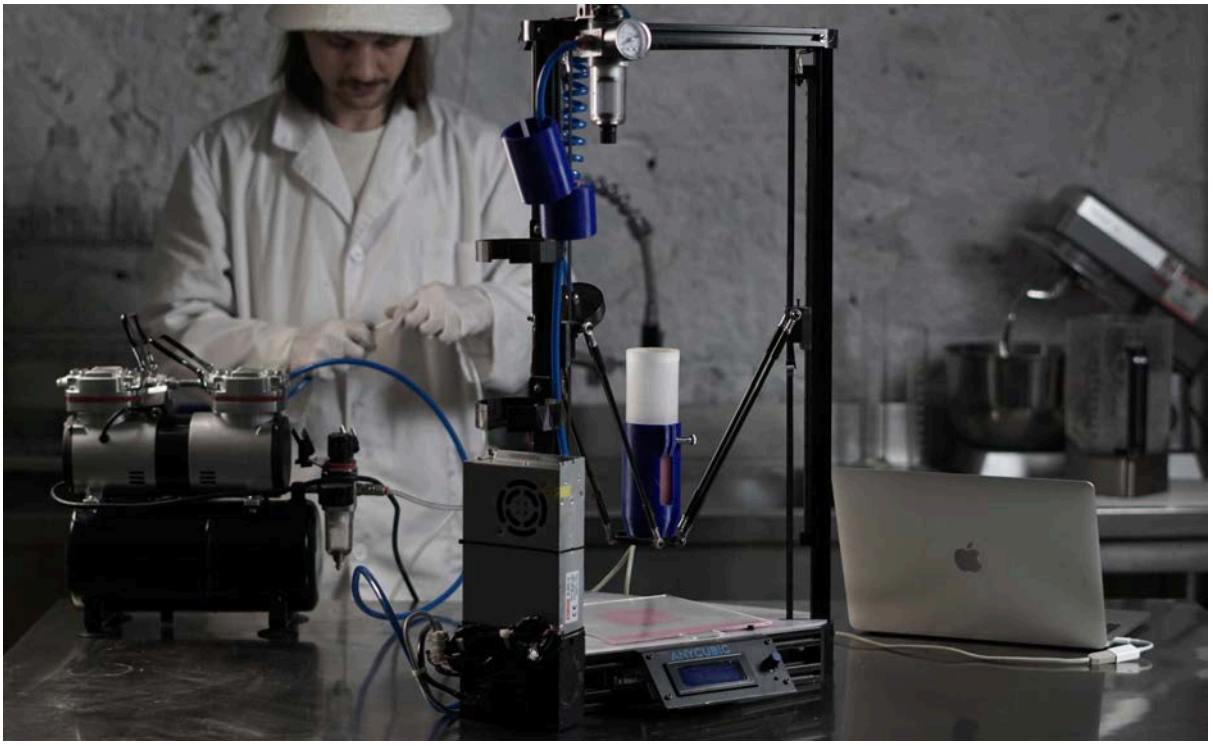
The machine is using a 12v rotation motor attached to wood construction that I cut out in the lab with our CNC machine.
The electric motor is simply connected to a high-voltage power supply, for future development, it would be good to install a separate potentiometer and a power adapter so that I could plug the machine straight into the wall.

07.02. Workflow:

soft/ hardware:
Step1: make artwork in Rhino.
Step2: set z-0 in Repetier
Step3: Generate G-code in Mamba
Step4: Export G-code to Repetier
Step5: connect the printer to the computer and compressor.
Step6: test air pressure (adjust accordingly)
Step7: print

Step8: crosslink print (cure)

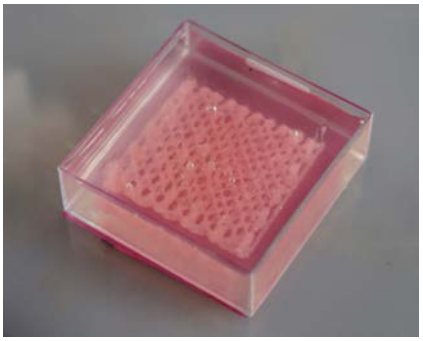
Step8: dry



printer set-up

material prep:

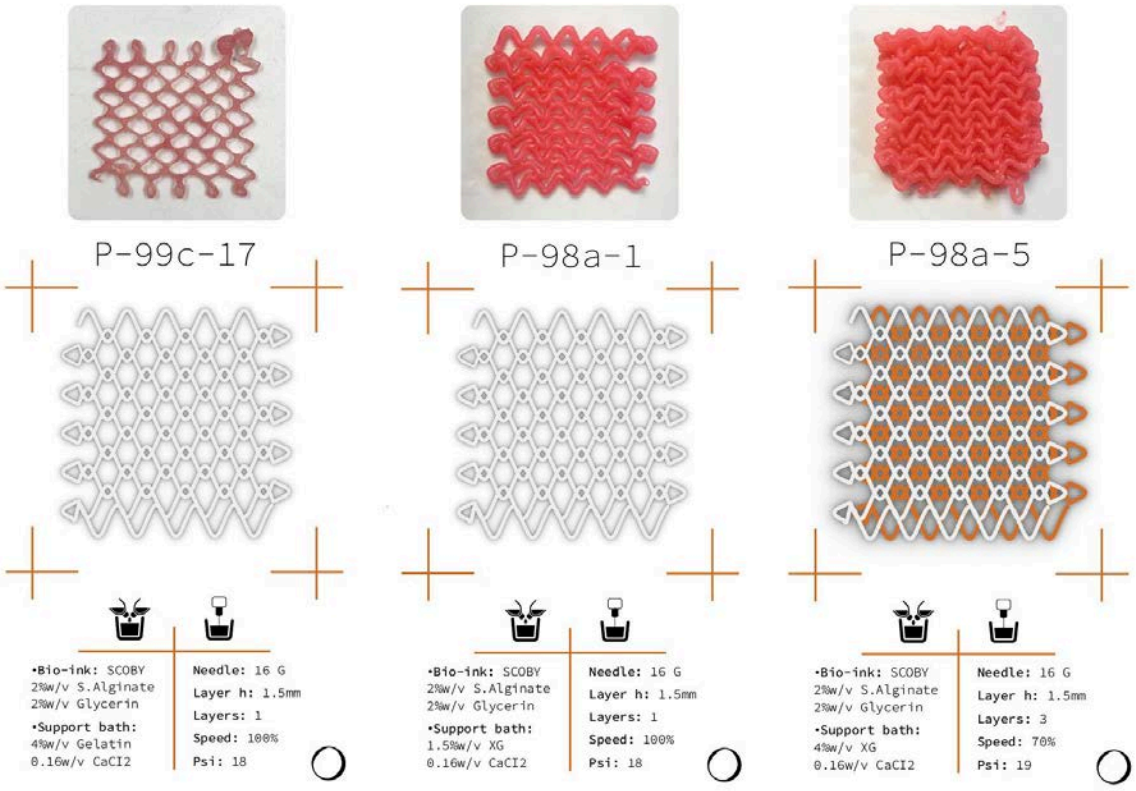
* 2% v/w Sodium Alginate
* 2% v/w Glycerin
* purified SCOBY paste
* 0.5% v/w Mica powder



short video describing the process

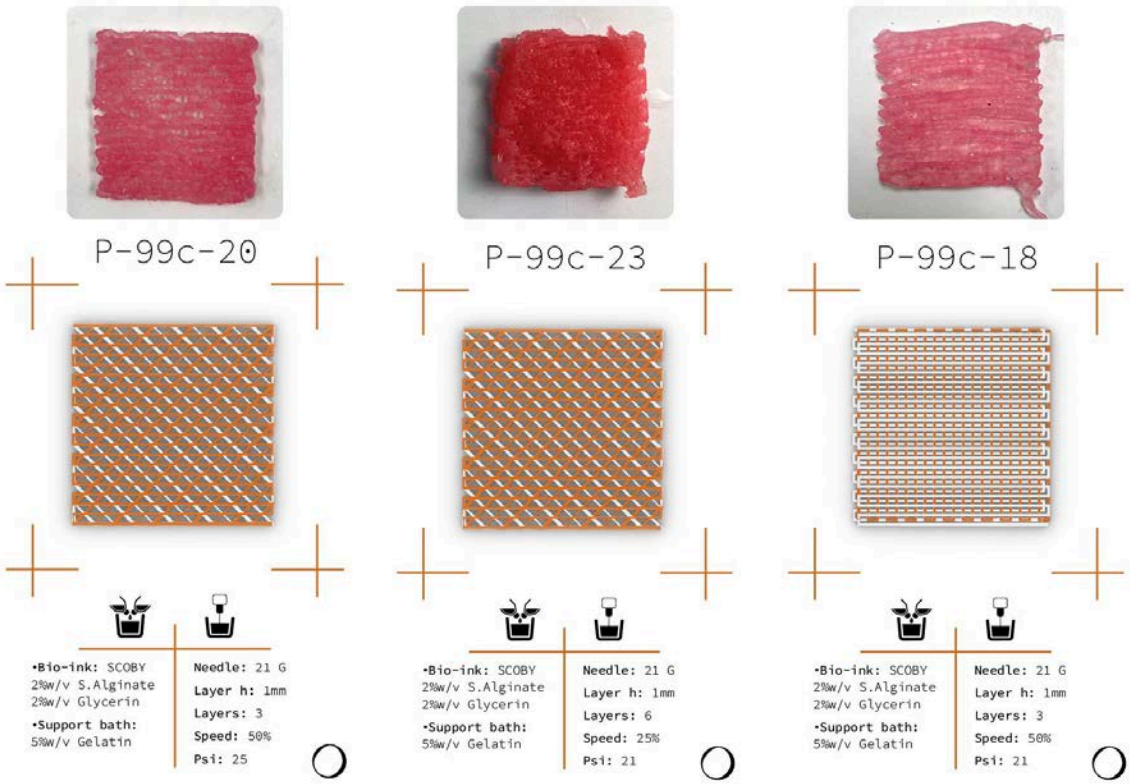
07.03. printing:

During my printing experiments, I mainly focused on variations of two different artworks one knit and one woven inspired geometry. One of the bigger differences between them was that for the knit-inspired artwork, I could use the original support bath recipe with 0.16% calcium chloride this made the removal of the print much easier since the print was slowly curing while printing, the problem with this solution is that it is not possible to cross "old" print lines since the needle would "drag" previous lines. It also made timing challenging since the overlapping points had to glue together before the print had cured something that would become more and more challenging the bigger the artwork I would experiment with.
For the woven artwork, I, therefore, excluded calcium chloride from the artwork.



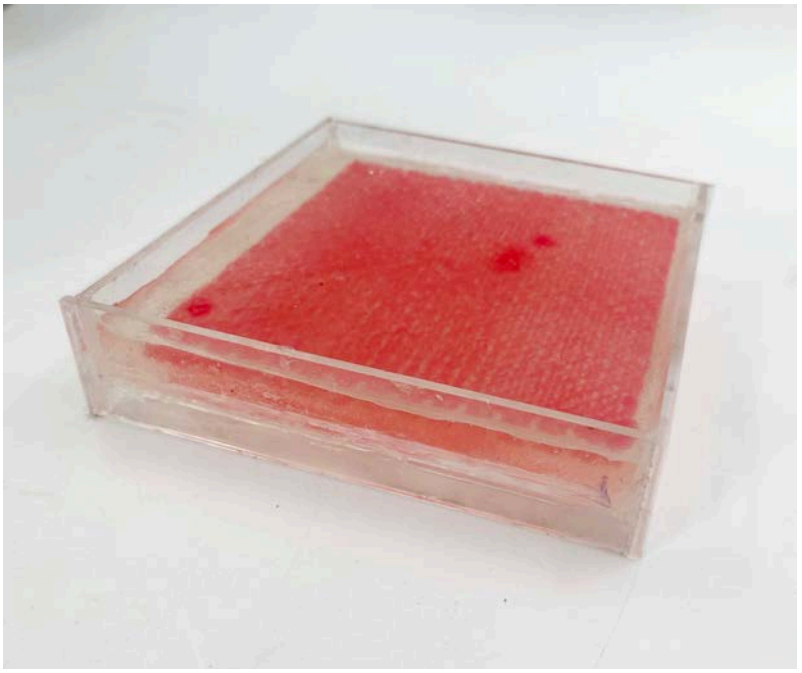
variations of "knit" artwork

The challenge with this artwork was to print fast enough that the contact points were "sticking" with each other keeping in mind that the faster the printing speed the bigger "cave" the needle leaves behind causing inaccuracy in the print.



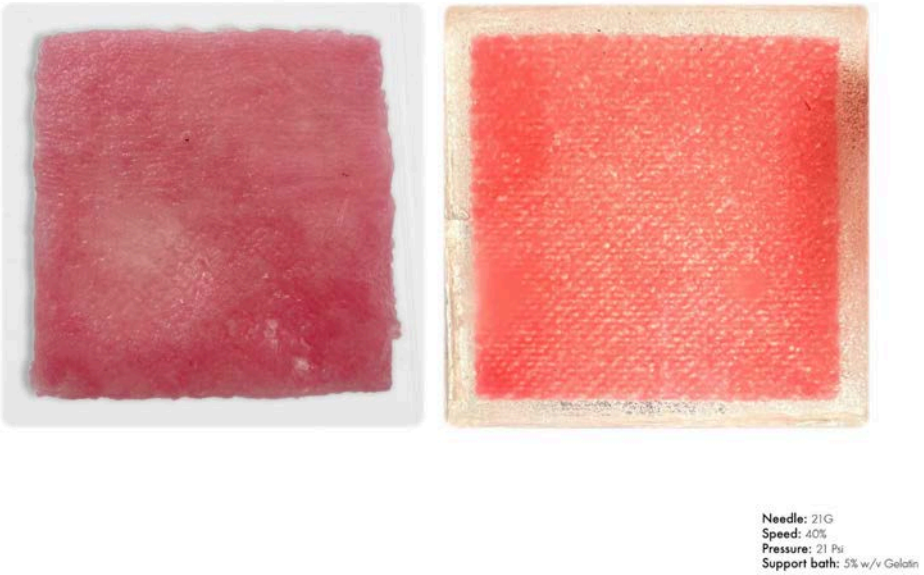
variations of "woven" artwork

Excluding the calcium chloride from the suspension bath opened up a lot more freedom in the artwork however releasing the print without destroying the geometry was challenging. The best method seemed to be to put the support bath into a water bath slowly heating up the gelatin just under melting point and then carefully pouring hot Calcium chloride from the top. Leave the print in calcium chloride solution for a minimum of 30 min.



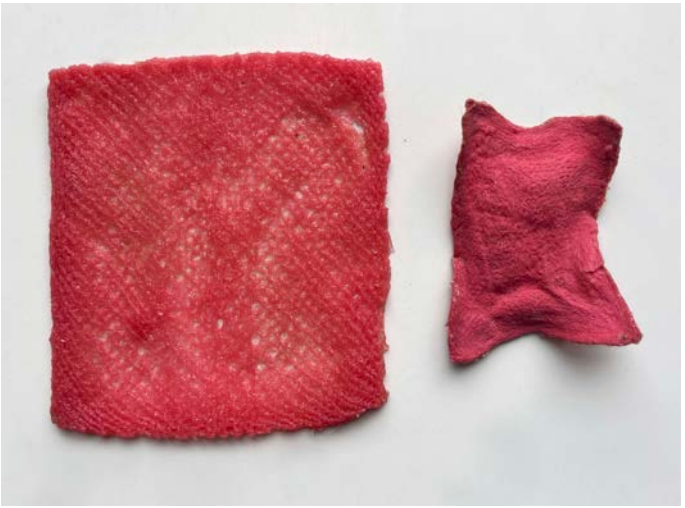
Experiments with scaling up the container from 70 x 70 to 160 x 160 mm were also made. Using the woven artwork.

the *first tryout after the print is cured (still wet)*



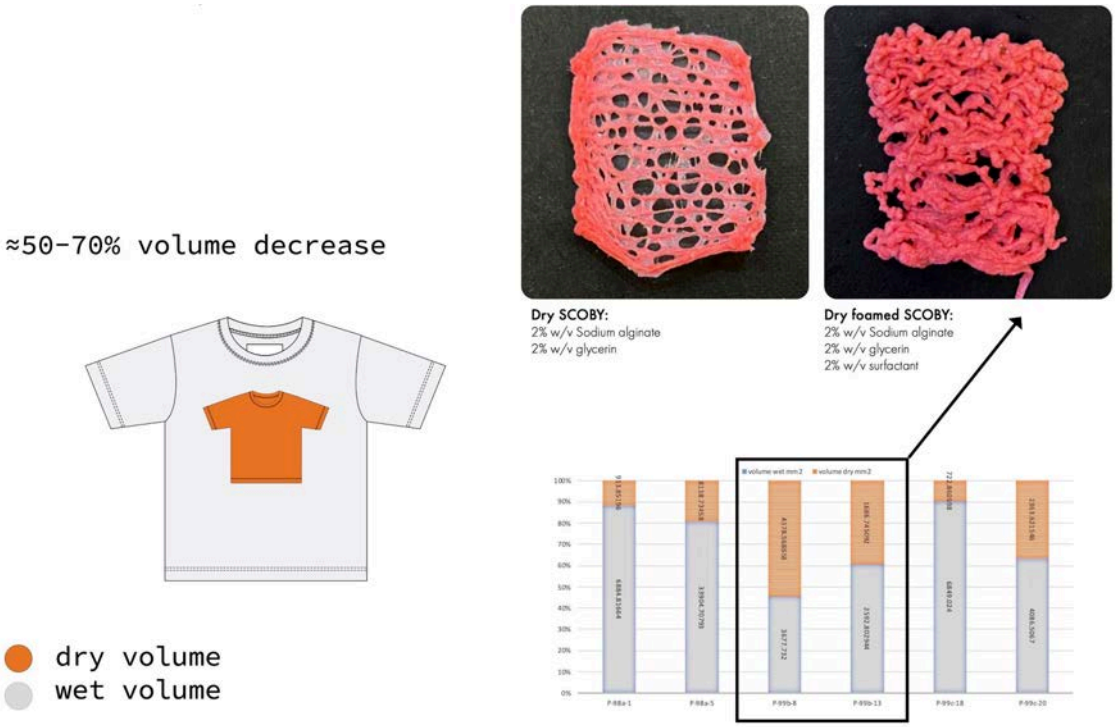
the second tryout left cured right: still in the support bath

Even though I followed the same methodology when curing the print the first tryout was more successful. The comparison image of before and after curing of the second tryout clearly shows the details lost. Further, prove the difficulties of replicating the protocol on a bigger scale and accuracy.

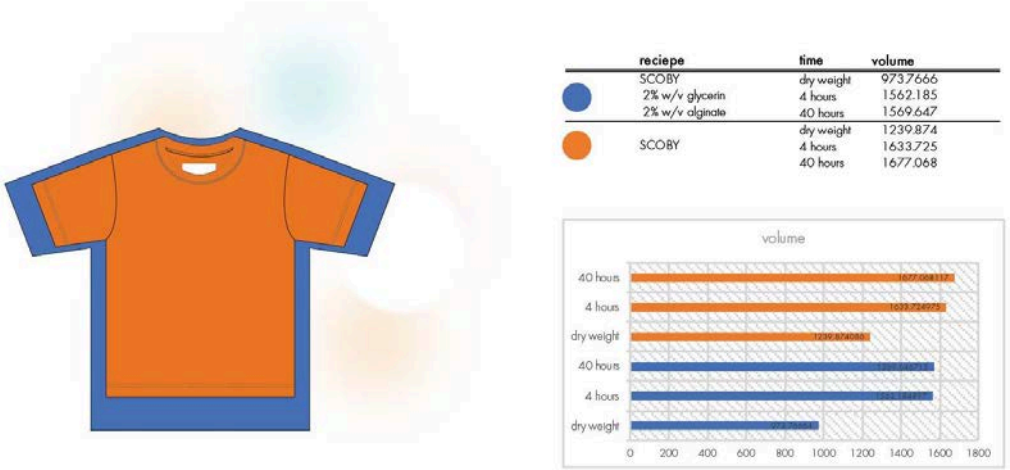


first and second samples after drying

Interestingly the two samples showed a significant difference in shrinkage. Both samples were dried at room temperature with the only difference being that the first sample was dipped in a 50/50 glycerin bath for 2 hours before drying which made it significantly softer with an almost oily surface. It is not clear if this also affected the shrinkage. An alternative explanation for the shrinkage difference could be that the first sample crosslinked better with the calcium chloride resulting in a stronger wet structure that inhibited drastic volume loss.



Even previous samples showed significant shrinkage, sometimes up to 70%. I have experimented by mitigating it by foaming the ink which decreased the volume loss (see P99b-13 and P99b-8) however the foaming also made the printing more difficult.



An absorption test with dried samples was also conducted. The test was performed with two samples, one pure BC sample and another one with 2% v/w alginate and 2% v/w glycerin dipped into distilled water for 4 and 40 hours. For both samples, the experiment showed

some volume increase after 2 hours but then the expansion plateaued. Moreover, the experiment indicated that the alginate and glycerin sample absorbed less water than the pure BC sample.

07.04. Reflection of the BC bioprinting protocol:

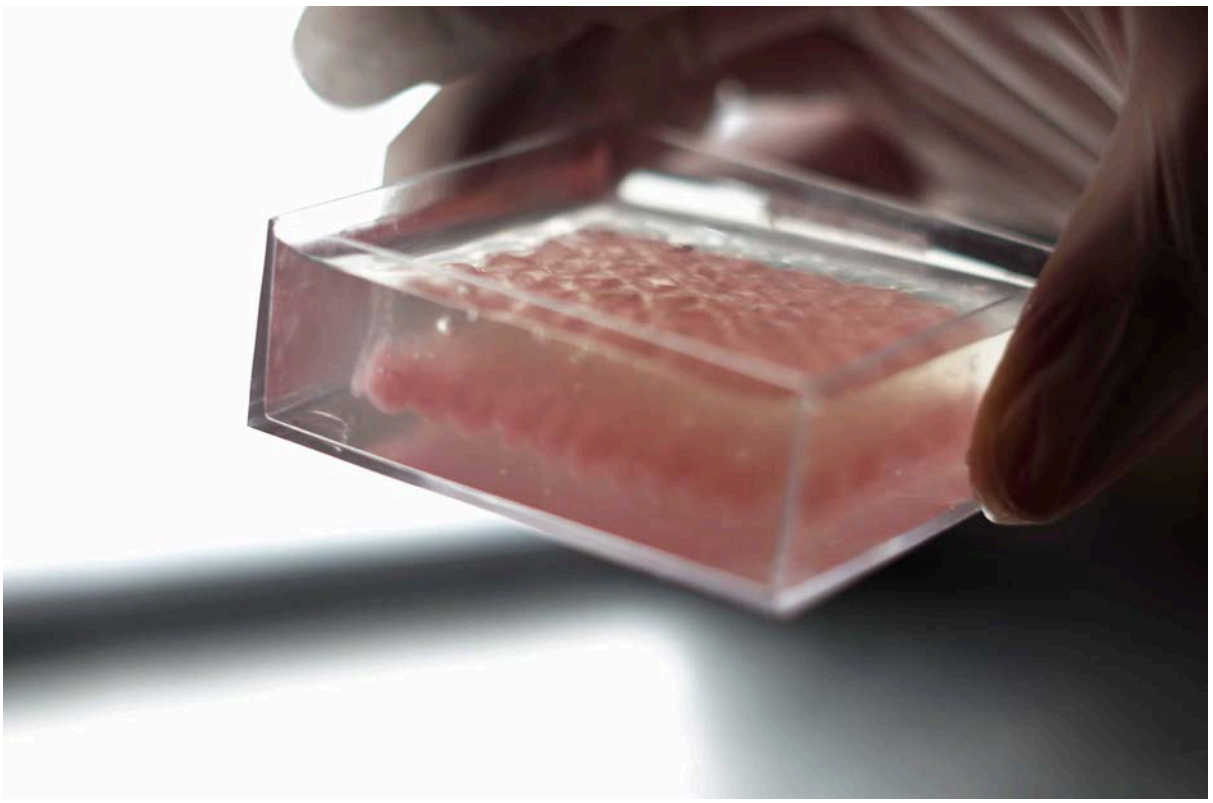
We have developed a process for bioprinting bacterial cellulose using the FRESH protocol. We have experimented using different artwork mainly focusing on modifications of two different geometries. We have seen how we could create open lace-like structures as well as more dense solid materials using two different suspension baths; gelatin and xanthan gum-based.

We have shown how the FRESH protocol can create complex 3D structures mimicking traditional fabrics, but we have also encountered problems, especially in the drying and post-processing phases. Here further research is necessary. One suggested solution could be to freeze-dry the prints, it is a process that should dramatically reduce the shrinkage but of course, also be quite an energy-heavy process.

Another option could be to use the shrinkage as a feature, a method to create small-scaled detailed artworks that otherwise would not be physically possible with a 3D printer. Of course, this would require that we print oversized garments that then shrink down into the finished size. Printing the garments folded could be a solution to this problem see nervous system Another problem with this idea is that a 3D garment would dry differently in different parts dependent on gravitation direction possibly a rotational drying process could be a solution rotational casting We have also seen how the material after drying lose most of its absorption quality which suggest that it would be washable and partly water repellent. That should be a good material property for garments.

Another problem we have encountered is curing the print in an even and replicable way without using 0.16% v/w Ca2I infusion. Further research into different curing processes such as photocrosslinking would be interesting for further development of the project.

08. Conclusion of the study



During a 3-month period we investigated the possibility of bioprinting bacterial cellulose and how this process could be used to tackle the root of the problem with today's supply chain in fashion, the assembly type of manufacturing.

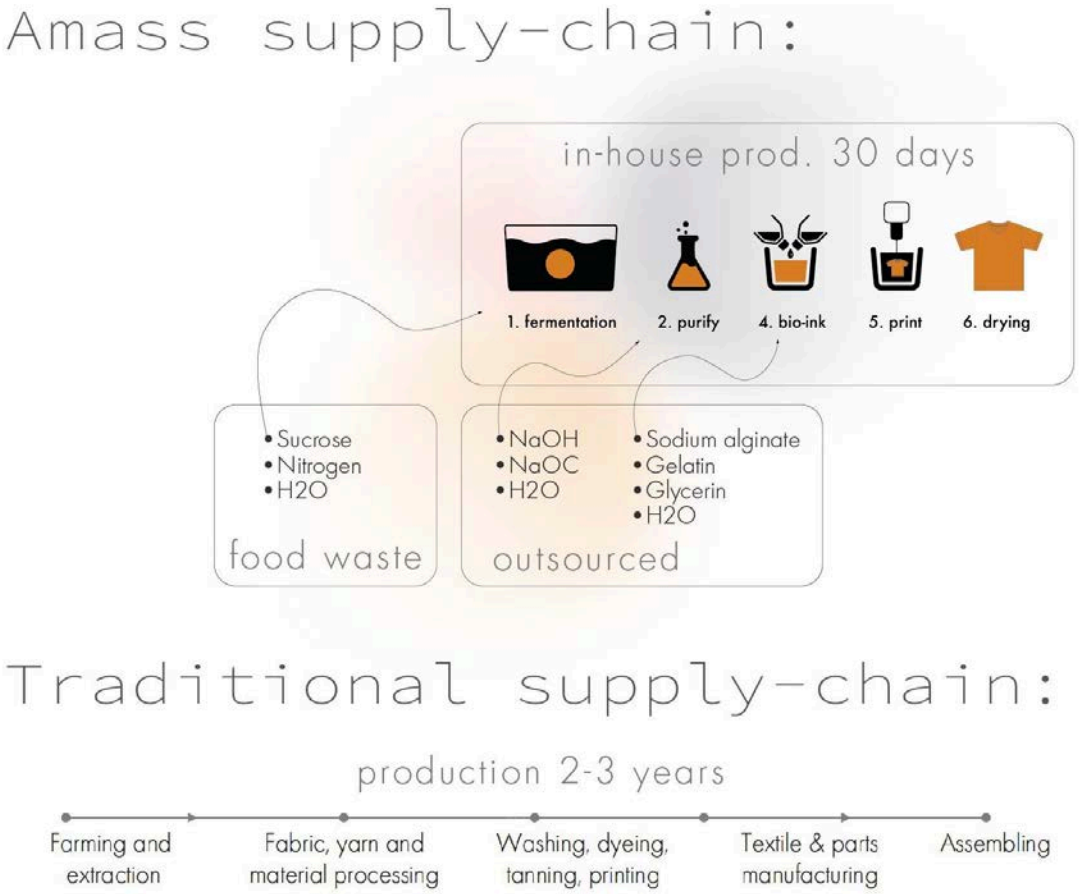
We have investigated two main roadmaps on how to work with the bacterial cellulose; as living cells and purified "dead" material even though we haven't been fully successful with the living-ink we still learned enough that we are able to conclude that the purified roadmap is the most practical and efficient method for our application.

Furthermore, we tested different culture media for growing BC and concluded that green tea and sucrose showed the best yield. We have experimented with low-cost DIY methods and recipes for bioprinting BC in a gelatin support bath. This has included a simplified purification process and ink/ support bath recipes using simple tools to realise. We have also presented how we can use a low-cost desktop printer for the printing process and provided instructions for building a DIY centrifuge machine necessary for the process.

So, what promises does this method hold?

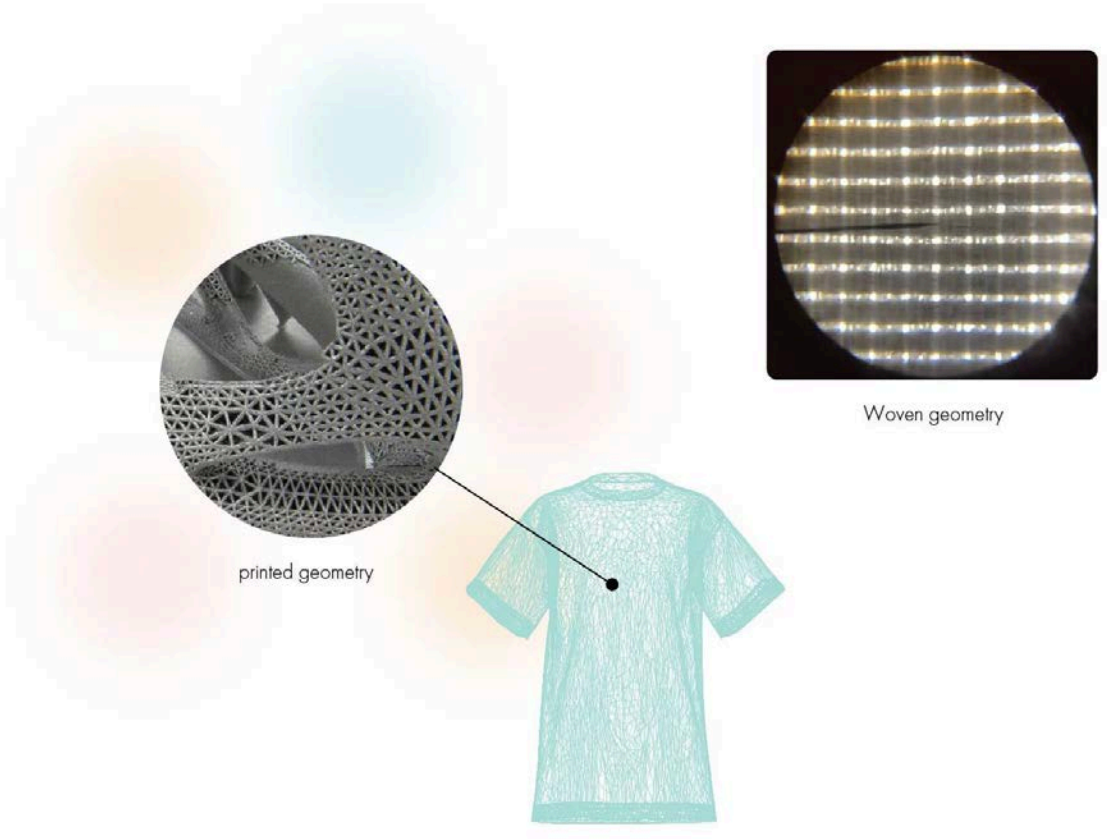
introduction video for the project

The traditional supply chain in fashion from seed to finished product is a slow process (2-3 years) that requires an intricate and opaque web of suppliers that can only be profitable in an unsustainable and unfair world. This project suggests a new method that drastically reduces production time and almost completely excludes outsourced suppliers.



It is not only the simplified production model that is promising with this project. From a design perspective, the ability to design the garment from microscopic structures to finished shapes opens endless possibilities of different combinations of colours, materials, and

geometries in the same garments, a process more like knitwear than cut and sew, a promising method to integrate mass customization in a cost-effective a sustainable way.



What is the main challenge for this project?

design perspective We still need to investigate how we further could improve and diversify the hand feel of the material by improving geometry and post-treatments. Furthermore, more research into the curing/ drying process is required (discussed in Chapter 05).
Business angel The hardware cost is of course a challenge, cheapest robotic arms start from 25.000 and more types of equipment like dryers, centrifuges, etc would also be required. Without having studied economics, it seems that it should be possible to mitigate the high hardware cost with low material and labour costs.
To grow the BC film I have used raw materials from the supermarket, this is of course not an ideal method for scaling the production however there is a lot of available research on growing BC film from food waste, so it is easy to envision collaborations with local restaurants and supermarkets. Another environmental concern is, of course, the chemicals required in the purification process and electricity used for the machine even though they most likely

have a neglectable environmental impact compared to traditional garment manufacturing it is still worth analysing further.

Besides all the above-mentioned challenges, the biggest task would be (as always) people and their habits. For designers how to convince them to completely change their design process? And for customers will they be willing to wear something constructed in such a completely new way?

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