

Mechanical Properties of Bacterial Cellulose Implants

Master of Science Thesis in Biomedical Engineering

GIUSEPPE SCIONTI

Department of Chemical and Biological Engineering
Division of Biopolymer Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden, 2010
Report n.366

THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Mechanical Properties of Bacterial Cellulose Implants

GIUSEPPE SCIONTI



Department of Chemical and Biological Engineering

Division of Biopolymer Technology

CHALMERS UNIVERSITY OF TECHNOLOGY

Göteborg, Sweden, 2010

Report n.366

Mechanical Properties of Bacterial Cellulose Implants

Giuseppe Scionti

© Giuseppe Scionti, 2010.

Technical report n.366

Department of Chemical and Biological Engineering

Biopolymer Technology

Chalmers University of Technology

SE-412 96 Göteborg

Sweden

Telephone + 46 (0)31-772 1000

Conducted by:

Giuseppe Scionti

Examiner and supervisor:

Paul Gatenholm

Approved date:

2010-06-08

Paul Gatenholm

Mechanical Properties of Bacterial Cellulose Implants

Master of Science Thesis in Biomedical Engineering

GIUSEPPE SCIONTI

ABSTRACT

Cellulose is a biopolymer that has long been used as a biomaterial and its different sources, as Bacterial Cellulose (BC), have shown to possess impressive mechanical properties, which can lead to the development of new biomedical implants. Bacterial Cellulose is a polysaccharide secreted by bacteria as *Gluconacetobacter xylinus*: BC is composed of highly hydrated nano-fibrils and it is characterized by high mechanical strength, high water content, high crystallinity and an ultra-fine highly pure nano-fibril network structure. The reasons for the great mechanical properties of Bacterial Cellulose have to be searched in the nano- and micro- level morphology of the material. The nano-scale morphology shows the presence of nano-fibrils; however, it is more likely the structure of the entire network at a micro-level that can explain the extremely good mechanical properties of Bacterial Cellulose implants. Thanks to its biocompatibility, BC can be used in a wide range of applications, such as blood vessels, skin and meniscus replacements etc. BC is a highly effective wound-dressing material, as skin of patients with burns heals faster when covered with membranes of bacterial cellulose, than if conventional wound-dressings are applied. Another medical application of bacterial cellulose is in vascular surgery, where tubular pieces of BC serve as bypass grafts. BC has Young's modulus in compression similar to the meniscus one, and shows even better mechanical properties than the native collagen material. The low price for the production of BC and the possibility to manufacture BC implants in different shape and size give to the Bacterial Cellulose a great potential among the biomaterials of the future.

Although the BC implants are having great success as biomaterials, it is still necessary to reach a deep understanding of the reasons behind such good mechanical properties of the material. The reasons have to be found through an analysis of the morphology of the BC at a nano- and micro-level. The present work has been based on the hypothesis that it is the network of fibrils at a micro-level which provides the mechanical performance of the material. The purpose of this work has been to study the effect of the BC networks on the mechanical properties of the implants. By changing the water content of the Bacterial Cellulose network, it was possible to produce BC materials with mechanical properties more similar to the native soft tissue that have to be replaced. The objective is to reach a prediction of the mechanical performances of the material, knowing the components, the morphology of the network and the amount of water content.

Several mechanical tests were performed, with a focus on tensile testing, to evaluate the mechanical properties of the Bacterial Cellulose samples: strength, Young's modulus, strain of BC pellicles were determined and analyzed. Due to the lack of Standards for this kind of mechanical tests on hydrogel materials, it was necessary to develop a new Standard Operating Procedure for all the experiments performed.

Index

ABSTRACT.....	4
THEORY	7
Cellulose	7
Bacterial Cellulose	8
Synthesis.....	8
Properties.....	9
Biomaterial: Biomedical applications.....	10
Mechanics	10
Tensile stress, elongation, Young’s modulus	10
MATERIALS AND METHODS.....	12
Preparation of the BC pellicles	12
Mechanical Characterization.....	12
Experimental Challenges	13
Tests on wet BC pellicles (99,12% water).....	18
Tests on partially dried BC pellicles.....	19
Scanning Electron Microscopy	21
RESULTS	22
Longitudinal Tensile tests.....	22
Clamping the specimens	22
Effect of hydration	22
Tests on ‘wet’ BC pellicles (0,88% cellulose content)	23
Tests on partially dried BC pellicles.....	25
Scanning Electron Microscopy	30
DISCUSSION.....	31
Cellulose content percentage	31
Properties of the representative curves: stress, strain, modulus	31

Effect of BC network on mechanical properties	32
Optimization.....	33
Future work.....	33
CONCLUSIONS.....	34
ACKNOWLEDGEMENTS.....	34
REFERENCES.....	35

THEORY

Cellulose

Cellulose is a biopolymer present in nature as structural component of the cell walls of plants and algae, and it is the most abundant organic compound on Earth. This linear polymer, with molecular formula $(C_6H_{10}O_5)_n$, is composed of repeating $\beta(1\rightarrow4)$ linked D-glucose units, as shown in Figure 1.

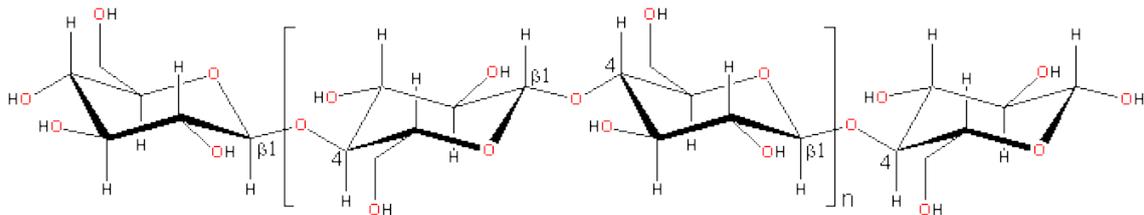


Figure 1: Representation of the structural unit of the cellulose polymer [18].

The $\beta(1\rightarrow4)$ bonds give a linear alignment to the molecule, allowing the formation of two intramolecular hydrogen bonds within every glucose residue: one bond links the O(6) to the O(2)H of the next residue, and the other bond links O(3)H to O(5). The inter-molecular hydrogen bonding pattern links the different chains of cellulose, from O(3) to O(6)H [1].

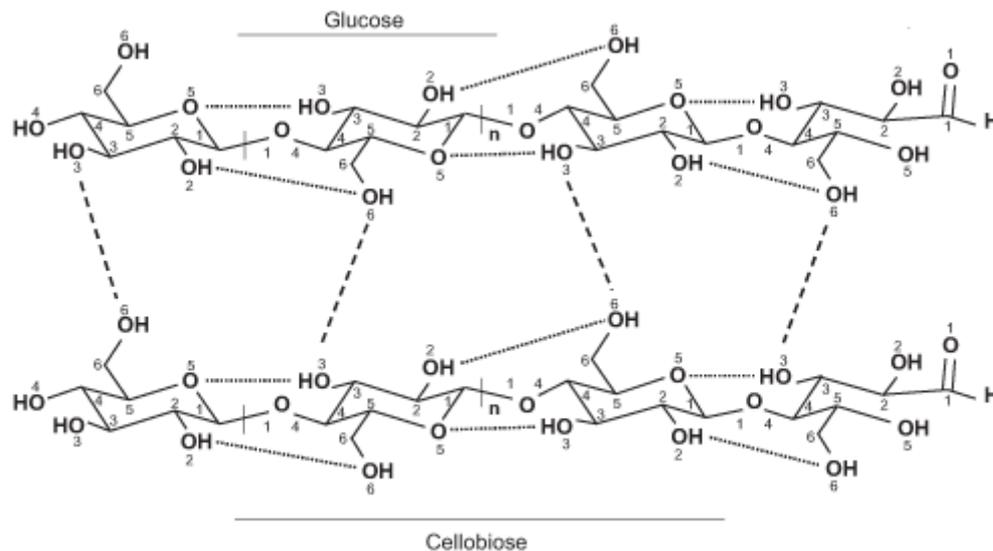


Figure 2: Representation of the inter- and intra-chain hydrogen bonding network [19]. Dashed lines show inter-chain hydrogen bonding, while dotted lines show intra-chain hydrogen bonding.

The cellulose can have different crystal structures: the native cellulose shows the structure called Cellulose I, characterized by a parallel disposition of the cellulose strands, which can be converted, through alkaline treatment, to the structure Cellulose II, which presents anti-

parallel disposition of the strands. In the case of Cellulose I, the dominant binding forces are van der Waals, while in Cellulose II crystal there are two hydrogen bondings between the layers.

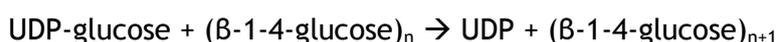
In nature, Cellulose I structure is found in allomorphic forms I α or I β , depending on the arrangement of the chains between each other: cellulose belonging to plant cell walls shows a higher percentage of structure I β , compared with cellulose from algae and bacteria, that shows higher percentage of structure I α , which seems to be a less stable displacement.

Bacterial Cellulose

As mentioned above, cellulose is not only produced by plants, but it is also synthesized by some fungi and bacteria. *Gluconacetobacter xylinus*, belonging to the family Acetobacteraceae, is a non-pathogenic, Gram-negative, rod-shaped, aerobic bacteria able to excrete cellulose extracellularly into long non-aggregated nano-fibrils [2,3,4,5]. In nature, *Gluconacetobacter xylinus* forms biofilms of cellulose on fruits and flowers surfaces: the reasons why the bacteria produces cellulose have been discussed among several biologists, and it seems that the film serves to the bacteria to keep its position near the nutrients, to protect themselves against enemies and UV-radiation, and to float on oxygen-reach surfaces [6].

Synthesis

The cellulose excreted by *Gluconacetobacter xylinus* has identical chemical structure as the Cellulose I found in plants, as observed through X-ray diffraction in the last century [6]. The synthesis of bacterial cellulose (BC) occurs at the cytoplasmatic membrane level, catalyzed by the “cellulose synthase” enzyme. *Gluconacetobacter xylinus* is able to convert several carbon sources to cellulose, but the synthesis mechanism always starts with the precursor UDP-glucose and ends forming a chain [2]. The mechanism which results in the production of a BC fibril is shown below:



Outside the bacterial cell surface, van der Waals forces assemble the cellulose chains to form sub-elementary fibrils, which then are linked together by hydrogen bonds to form micro-fibrils. Finally the micro-fibrils are assembled together into a ribbon, as shown in the figure below.

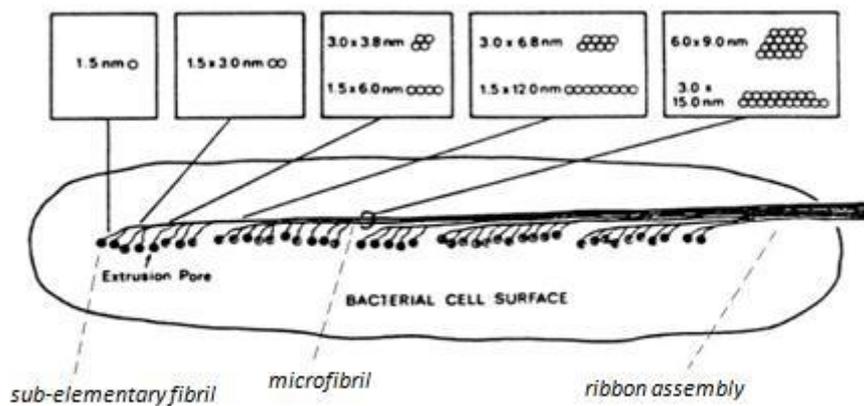


Figure 3: Ribbon formation process in *Gluconacetobacter xylinus*. Adapted from Ross et al. [20]

Properties

Gluconacetobacter xylinus produces “extracellular cellulose as a pure, ultra-fine random fiber network, possessing high crystallinity, high water absorption capacity and mechanical strength” [5,34,40]. Even though bacterial cellulose has the same chemical composition of the cellulose from the plants, the BC shows exceptional physical and mechanical properties, resulting from its special fibrillar network structure at a micro-level [7,30,31].

The great liquid-absorption ability is a result of the broad space between the different fibers, creating a large surface area, while the high mechanical strength is due to the inter-fibrils hydrogen bonds, which give stability to the structure [2,4].

In drying process of bacterial cellulose, the nano-fibers arrange parallel to each other and form layered sheets. These give the dried cellulose sheets high stability and strength, as there is the formation of more hydrogen bonds among the fibers [8,9].

The first mechanical tests on Bacterial Cellulose were conducted by Yamanaka et al. in 1989 [9] and the dried material was tested in sheet form, with Young’s modulus up to 15 GPa. Later on values of modulus of around 30/40 GPa were reported by Nishi et al. in 1990 [10] and Tajima et al. in 1995, treating the material with alkaline and oxidative solutions. For what concern the direct measurement of BC fibrils, Hsieh et al. [5] estimated the modulus of a single fibril to be of 114 GPa.

Biomaterial: Biomedical applications

Due to its attractive physical and mechanical properties, bacterial cellulose has been utilized in many applications: it had a great success in food industry in the Philippines as component of the dessert Nata de Coco, in audio-components as speaker diaphragms to achieve the best sound transduction, in paper industry, and in health-care as wound-dressing material [1].

Regarding the biomedical applications of bacterial cellulose, it is not only an interesting material for skin healing solutions, but recent studies have proposed BC as biomaterial for artificial blood vessels and meniscus replacement [3,11,12,13]. Moreover, “bacterial cellulose has gained interest in recent years due to the ability to use it both as a composite material, and also as a tissue engineering substrate” [14], as it possesses not only great water holding ability and mechanical properties, but also excellent biocompatibility and biodegradability [15,16,32,35,36,37,38,39].

Although bacterial cellulose has a great potential to become an important biomaterial in the future, at the moment commercialization is limited: for example the companies “Cellulose Solutions” and “Xylos Corporation” have BC products for wound dressing on the market, respectively with the brand names Dermafill™ and XCell®. Some of the reasons why the market of BC did not grow yet are the necessity of an optimization in the fermentation process, and a complete analysis on the physico-mechanical properties of the material [2,33].



Figure 4: Examples of biomedical applications of BC are meniscus replacements (pig meniscus on the left, BC meniscus on the right), artificial blood vessels and wound-dressing for skin healing. [12,21,22]

Mechanics

Tensile stress, elongation, Young’s modulus

Tensile tests provide important information regarding the mechanical properties of a material, and are commonly used in for analyses of new materials for engineering applications, as they are a very effective methods to compare the properties of different materials. The measurement of the tensile strength is often of fundamental importance: it “may be measured

in terms of either the stress necessary to cause appreciable plastic deformation or the maximum stress that the material can withstand" [17].

In the present work, after geometrical measurement of the samples, the values of stress and strain were calculated starting from the values of tensile force, measured directly by the tensile testing machine as a function of the increase in gage length. It has been chosen to focus our study on the stress-strain profiles and not on the load-elongation curves, to obtain results independent of sample dimension.

The stress, strain and Young's modulus were calculated as follows:

Engineering stress (σ) = (F/A_0) , where A_0 is the initial cross-sectional area inside the gage sector, and F is the force in Newton registered by the machine.

Engineering Strain (ϵ) = DL/L_0 , where L_0 is the extension of the gage-length section at the starting point, and DL is the difference between the present length (L) and L_0 .

The modulus of elasticity (Young's Modulus) is a basic property of the material, measuring the stiffness of the materials: it is defined as the stress-strain ratio in the linear region of the stress-strain curve, which represents the region of elastic response of the material [17].

MATERIALS AND METHODS

Preparation of the BC pellicles

The Bacterial Cellulose pellicles were prepared with 1ml of pre-culture bacteria (bacterial strain BPR2001) and 75ml of culture medium. The pellicles were then left for 4 days in a 30 degree incubator, grown in 10cm petri dishes. A purification method with NaOH was used: the pellicles were left in a 0.1 M NaOH solution over night, then they were moved to a new NaOH solution and put in a 60°C shaking water bath over night, and finally the pellicles were put in a distilled water solution and put back in water bath. Finally the cleaned BC pellicles were stored in a new distilled water solution.

Mechanical Characterization

The mechanical properties of the BC pellicles were analyzed through uniaxial tensile tests, using a testing machine Instron 5565A, equipped with a biobath system containing distilled water, and a temperature-controlling system. The specimens were cut in a rectangular shape using a paper cutting machine, producing samples with dimensions of 1cm X 6cm individual sample thickness. A Multitoyo digital thickness indicator was used to measure the thickness of each specimen. Two samples were cut per pellicle, and three pellicles were used in each experimental session, resulting in a total of 6 characteristic curves for every type of sample. Among the curves belonging to the same kind of sample, one was selected as the most representative curve: the selection was made through a research of the curve with the values of stress-at-break, strain-at-break and Young's Modulus more similar to the respective average values of the same parameters.

Using the Bluehill software, connected to the Instron machine, it was sufficient to insert the dimensions of the samples before the beginning of the experiments, and the software calculated automatically the values of stress and strain during the test. The Young's modulus was calculated using the same software, selecting manually the section of the stress-strain curves where it was required to measure it: the modulus was not measured at the same value of strain, but it was calculated in the first linear part of the curve.

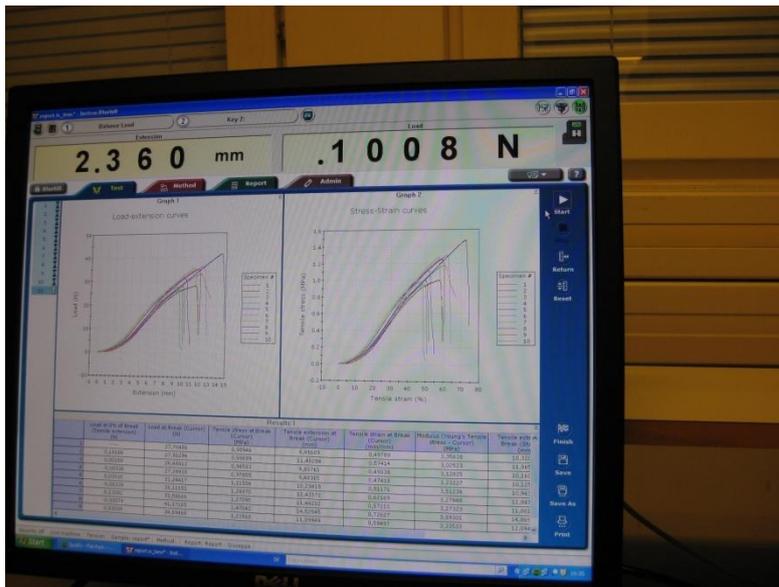


Figure 5: A view of the Bluehill Software, connected to the Instron testing machine, and used in all the tensile tests

Experimental Challenges

Mechanical testing on hydrogels involves several practical challenges for what concern the clamping of the specimen, the effect of the hydration, the cross sectional area measurement and the strain quantification.

Clamping the Specimens

Clamping hydrogel samples for a correct tensile testing presents many difficulties, as it is necessary to ensure a firm grip between the sample and the clamps: there is no definite standard regarding how to clamp hydrogels for tensile tests, so it is necessary to develop a Standard Operating Procedure depending on the particular kind of samples going to be analyzed.

The specimens tested in this work are pellicles made of Bacterial Cellulose. For what concern the samples shape, cutting the pellicles in rectangular shaped samples was found to be the more efficient way to obtain many samples from the same pellicles. Moreover, testing the rectangular specimens was proved to induce good results in the experiments, as the tested samples often presented fractures in the middle of the free-length region between the clamps. On the contrary, it was difficult to cut adequately samples with a dog-bone shape: when tensile tests on dog-bone shaped samples were conducted, the experiments were always characterized by premature fractures of the specimen, due to defects in cutting. Cutting the

pellicles in rectangular shaped samples makes also easier the calculation of the extension to failure, as it can be easily measured from the grip-to-grip separation.

After studying from literature the best solutions for clamping polymer hydrogels [24], tendons [23] or other and soft biological tissues [24], it has been proved experimentally that the best interface was characterized by the presence of a fine sandpaper adherent onto smooth jaw faces. Moreover, to avoid the break of the specimen at the jaw faces, it was added a silicon film below the sandpaper layer and a little piece of silicon film was adhered at the edge of every clamp.



Figure 6: The structure of the clamps used for the tensile test of wet BC pellicles (over 90% of water content percentage).

Effect of hydration

It was chosen to have two different hydration systems, as happened for the two clamping designs developed, to analyze properly the wet BC samples (water content percentage of 99,12%) and the partial dried samples (water content percentage below 99,12%). The main challenge is to create a testing environment able to keep the BC samples to the same hydration condition during all the time of the test: this means that the water content of the specimen has to be constant.

The Instron BioBath and submersible grips (parts of the BioPuls™ solutions) were used to test the wet BC samples [25,26,27]. Moreover, to keep physiological conditions throughout the mechanical evaluation of the BC pellicles, it was necessary the use of a temperature controlled bath at 37°C. Using a Biobath, it was possible to maintain the cellulose content percentage of the wet pellicles to the same value (0,88%).

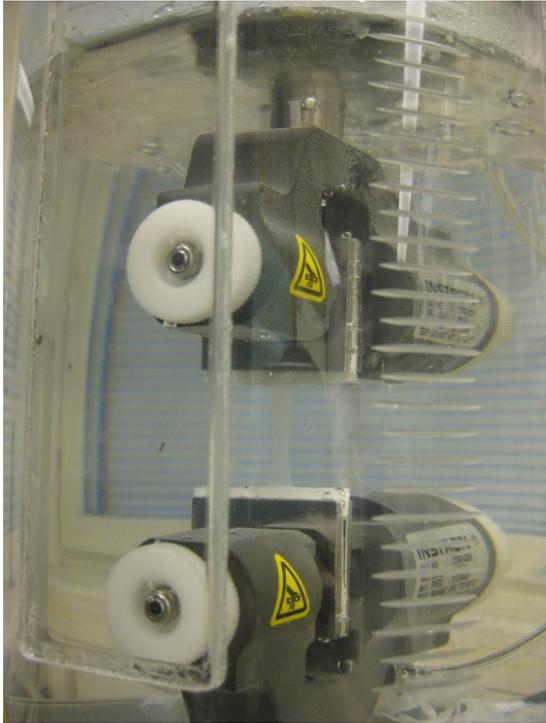


Figure 7: View of the biobath during a tensile test on wet BC pellicle.

For what concern the partial dried BC pellicles, it was decided not to test them in the Biobath: during the project it was proved that the partial dried pellicles tend to re-absorb water when re-immersed in aqueous solution after the partial drying, varying their cellulose content percentage; to avoid that, these pellicles were tested in air using normal rubber jaw faces, as already reported. In fact, the BC pellicles left in air undergo a slow drying, and the time between the sample preparation and the end of the experiment was considered enough short to avoid any sensible drying of the specimen.

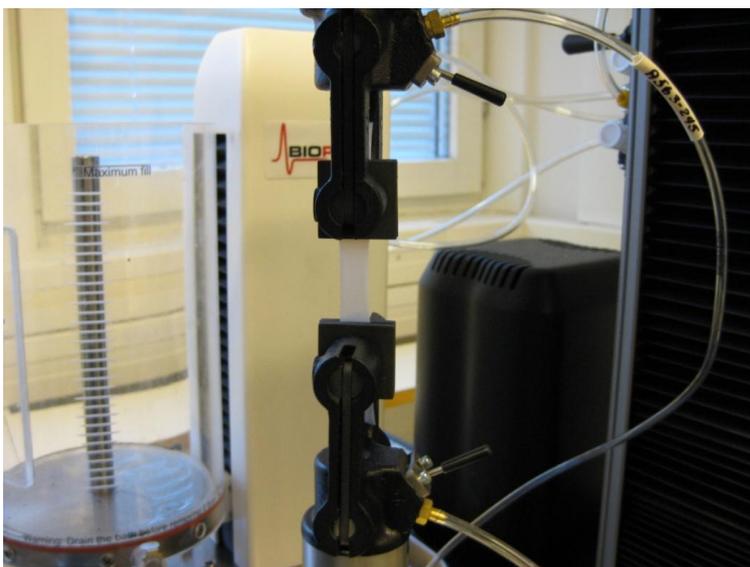


Figure 8: The partial dried pellicles were tested in air, with rubber clamps.

Cross sectional area measurement

To calculate the mechanical properties of the BC pellicles, as the tensile stress, it is critical to measure accurately the cross-sectional area of the samples. In literature many techniques for cross sectional area have been used, as ultrasonography [28], gravimetric methodologies and area micrometers [23].

In this work the cross sectional area of the samples was calculated with the use of a caliper and a Multitoyo digital thickness indicator.

The width of the samples was calculated using a caliper: this measurement was not the most critical because cutting adequately the rectangular specimens (with a paper-cutting machine) permitted to obtain samples with uniform width.

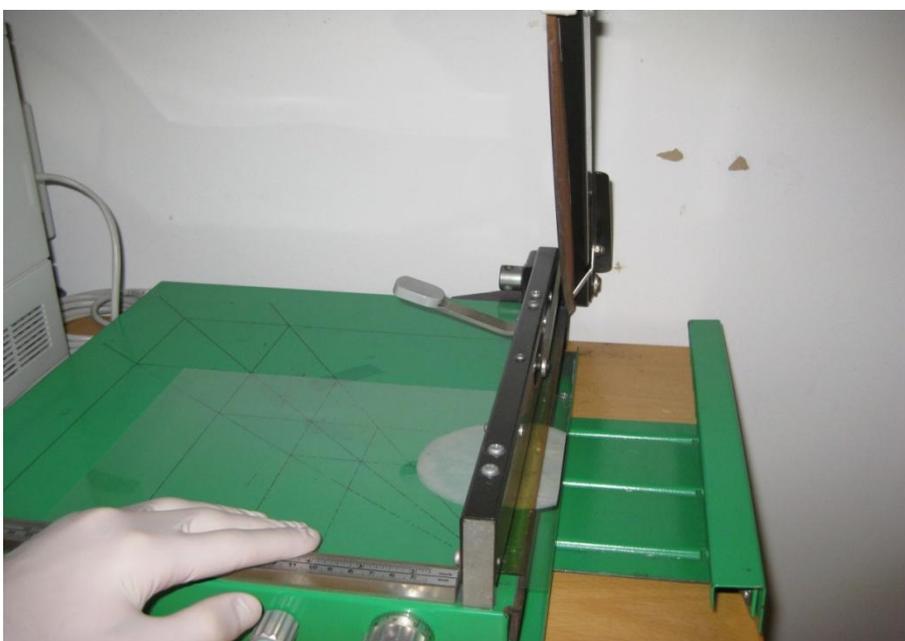


Figure 9: Cutting the BC pellicle into rectangular pieces, to prepare the specimen for the mechanical tests.

Measuring correctly the dimension of a piece of hydrogel with a caliper is difficult, but during the cutting procedure the pellicles were placed on a thin transparent plastic film, so the width of the sample could be calculated measuring the width of the plastic film, which is a easier material to measure with a caliper.

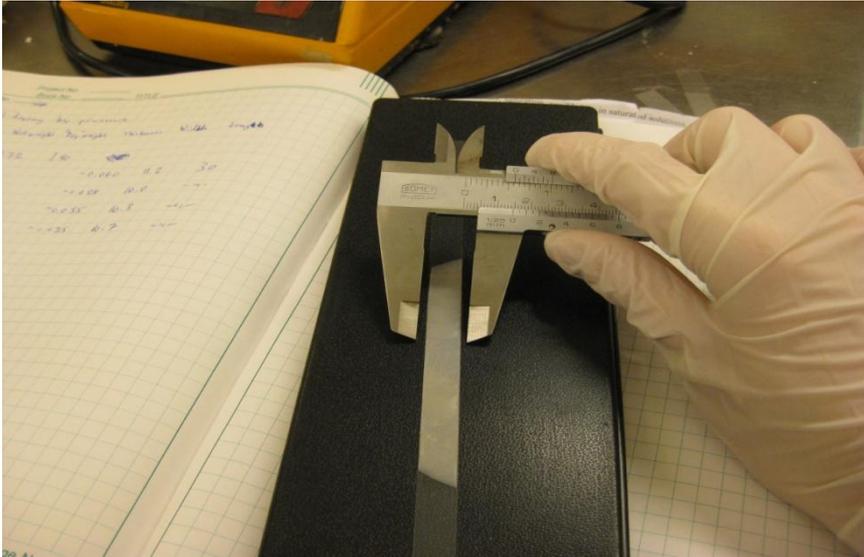


Figure 10: Measurement of the width of a BC pellicle sample with a caliper. The specimen is placed on a plastic film, which makes the measure of the width easier to calculate.

The thickness of the samples was measured through a digital thickness indicator on a stand set. The BC pellicles used for the mechanical tests, produced at the BBV Lab of Chalmers, were not characterized by a uniform thickness, as it varied along the pellicle: this required the thickness of the sample to be measured as an average of several local measurements, with a digital thickness indicator. The local measurements were limited to the median part of the specimen, where the sample was not clamped by the jaws.



Figure 11: Measurement of the thickness of a BC pellicle sample with a Mitutoyo digital thickness indicator: the plastic film below the sample makes the calculation of the thickness more precise, as its presence avoids that the tip of the instrument could squeeze the hydrogel and affect the measurement.

Strain measurement

Testing rectangular-shaped specimens permits the calculation of the strain from the grip-to-grip displacement, which is not possible using dog-bone shaped specimens.

It could be also possible to calculate local strains through the use of a video-extensometer and dog-bone shaped specimens, whose shape leads to a better defined point of fracture in the specimen. One of the practical challenge for the use of video extensometer on BC pellicles would be the research of an adequate method to draw markers on the hydrogel, which can be even more problematic when testing the hydrogel in the Biobath.

Tests on wet BC pellicles (99,12% water)

Operating procedure

The following steps were followed to make the experiments on wet BC pellicles:

1. Two samples were cut from three BC pellicles using a paper-cutting machine, with dimensions of 1cm X 6cm X individual sample thickness.
2. The thickness of each specimen was measured with a Multitoyo digital thickness indicator.
3. The extremities of the rectangular samples were partially dried with filter paper, for a better clamping of the specimen.
4. The samples were installed carefully between the clamps, avoiding any stretching or twisting.
5. The gauge length between the couples of clamps was fixed to 2 cm.
6. The pressure going to close the clamps was regulated manually to reach a gentle closure: it is necessary to avoid both the slipping of the sample (low pressure) and the breaking of the sample near the clamps (high pressure).
7. The jaw faces were closed and the Biobath moved up.
8. The BC samples were stretched at a constant speed of 5 mm/min, until the failure of the sample occurred. The force required to extend uniformly the samples was measured as a function of time.

Equipment

- Filter paper
- Paper-cutting machine

- Tensile testing machine: Instron 5565 A
- 100 Newton load cell
- Biopuls™ system [26]
- Distilled water in the Biobath
- Temperature of water: 37 °C
- Surfalloy jaw faces covered with layers of silicon and sandpaper



Figure 12: Picture showing the surfalloy jaw faces covered by silicon and sandpaper layers.

Tests on partially dried BC pellicles

Operating procedure

The following steps were followed to make the experiments on partially-dried/dried BC pellicles:

1. The weight of the wet pellicle going to be dried was measured.
2. The value of weight that has to be reached after the drying process was calculated; for example, if it is desired to obtain a pellicle with 20% of cellulose content, this is the weight that the pellicle should reach:

$$\text{Final weight} = [(\text{wet weight} * 0.88) / (\text{cellulose content } \%)] =$$

$$= [(\text{wet weight} * 0.88) / 20]$$
3. Partial drying the sample: press the sample with the hands, placing filter paper on both sides of the pellicle. The samples were dried to different extents of cellulose content percentages: 10%, 20%, 30%, 35% and 40%.
 Complete drying the sample: leave the sample in air, into a humidity controlled room, until the weight reaches the equilibrium. In these dry samples the cellulose content percentage was found to be 92%.

4. For every level of cellulose content percentage, two samples were cut from three BC pellicles, using a paper-cutting machine, with dimensions of 1cm X 6cm X individual sample thickness.
5. The thickness of each specimen was measured with a Multitoyo digital thickness indicator.
6. The samples were installed carefully between the clamps, avoiding any stretching or twisting.
7. The gauge length between the couples of clamps was fixed to 2 cm.
8. The pressure going to close the clamps was regulated manually to reach a gentle closure.
9. The partially dried BC samples were stretched at a constant speed of 5 mm/min and the dried samples at 1 mm/min, until the failure of the sample occurred.



Figure 13: Weighting the wet pellicle before the drying process (step 1).



Figure 14: Partial drying of the sample, pressing the pellicle with the hands. Filter paper is placed on both sides of the pellicle, and normal tissues are used on the top to soak up more quantities of water.

Equipment

- Filter paper
- Paper-cutting machine
- Tensile testing machine: Instron 5565 A
- 5000 Newton load cell
- Rubber jaw faces

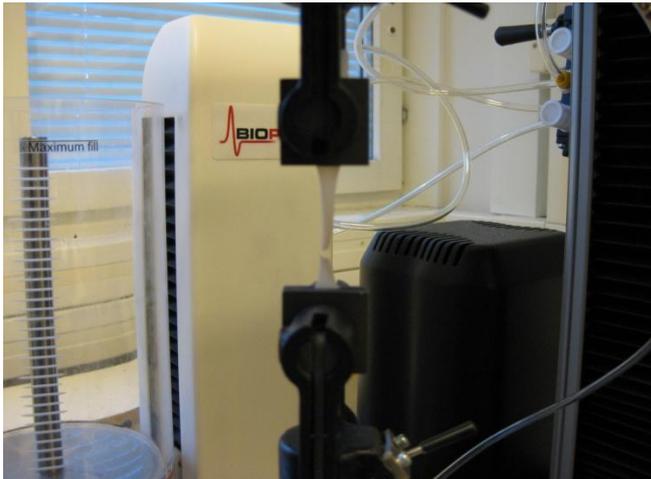


Figure 15: Failure of the sample after tensile test with rubber jaw-faces.

Scanning Electron Microscopy

SEM was used to study the morphology of the outer, and cross sectional surfaces of the pellicles. The material was frozen in liquid nitrogen before freeze-drying (with a Jouan LP3 freeze dryer machine) for 24 h at -52.

Small BC samples were cut-out and coated with gold particles attached to the sample holders with silver glue, and then magnified and photographed with the SEM machine Zeiss DSM 940.

RESULTS

Longitudinal Tensile tests

Clamping the specimens

It is fundamental to avoid the slipping of the samples away from the clamps, as well as avoiding that the grip of the clamps could damage the specimen. “A firm grip is a function of an optimum matching of the clamping device and the specimen” [23]. In this work several designs of clamping devices have been tested: rubber jaw faces, surfalloy jaw faces, serrated jaw faces, covered with sandpaper and covered with a silicon film.

Much time and work has been dedicated to design a Bacterial Cellulose “friendly” clamping system. As this work involved mechanical testing on specimens with different water content percentage, separated clamping systems have been used. The investigation to find the optimal gripping system ended with the selection of two different designs: testing the BC samples with water content percentage of 99,12% (wet samples) involved the development of an advanced clamping system, presenting an interface with sandpaper and silicon film, while the samples with water content percentage below 99,12% (partial dried samples) were tested using normal rubber jaw faces. In both the cases, to ensure the specimen not be damaged during the tests, and to achieve a firm grip without slipping of the sample, it was necessary to have a sample length of at least 2cm to be inserted into each clamp.

Among all the kinds of samples tested in this work, the specimens more difficult to clamp were the ones presenting the highest water content. Using normal surfalloy jaw faces, the wet pellicles were easily slipping away when closing the clamps and during the tensile test. But if trying to close the pneumatic clamps with higher pressure, the jaw faces always damaged the samples near the edge of the clamps, resulting in premature failure of the specimen, and this caused lower values of strain at break. As the thickness of the specimen varied greatly between the different samples, it was also necessary to adjust manually the pressure going to the pneumatic clamps, to provide the adequate gripping force.

Effect of hydration

The choice of the adequate clamping system for the tests on BC pellicles was not the only experimental challenge solved during this project. In fact, as the longitudinal tests on BC pellicles presented in this work include tests on samples with different water content percentages, this great variation required the development of separate hydration-control systems.

Tests on 'wet' BC pellicles (0,88% cellulose content)

1st generation of clamps: results using surfalloy jaw-faces

Figure 16 shows the effect of the clamping system preparation on the stress-strain curves: these 1st generation tests reported a failure of the specimens mainly located near the clamps.

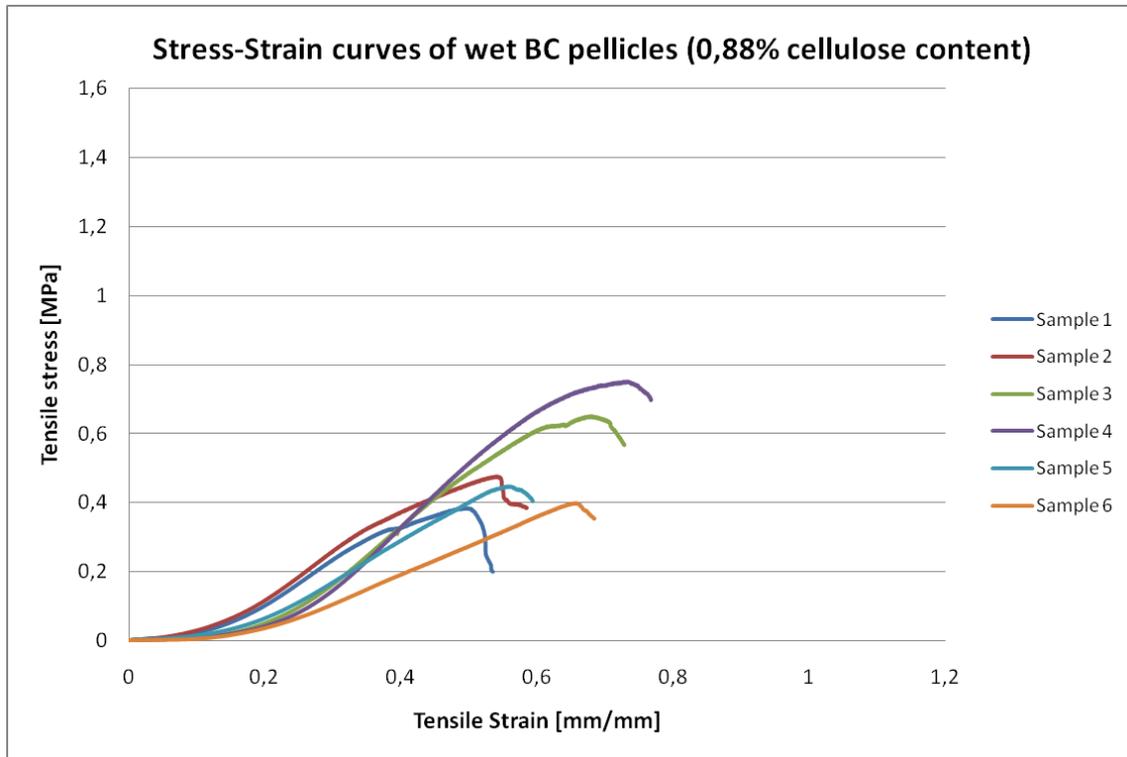


Figure 16: Stress-strain curves for mechanical evaluation of wet BC pellicles, using normal surfalloy jaw faces.

Table 1 is a summary of the results from the tensile tests on wet pellicles, before the development of the BC “friendly” clamping system.

	LOAD AT BREAK (N)	TENSILE STRESS AT BREAK (MPa)	TENSILE EXTENSION AT BREAK (mm)	TENSILE STRAIN AT BREAK (mm/mm)	MODULUS (MPa)
Mean	21,94098	0,51671	9,60706	0,60928	1,46508
Standard Deviation	10,51016	0,1497	1,05722	0,08929	0,38079

Table 1: mean values of load, tensile stress, extension, tensile strain (all values calculated at break) and Young’s modulus (calculated in the first linear part of the curve). In the lower part of the table you can also see the standard deviation related to each mean value.

2nd generation of clamps: results after the development of the BC “friendly” clamping system

Figure 17 shows the stress-strain data obtained from testing wet BC pellicles, when the sample preparation and the gripping method were developed, and the failure of the specimens occurred mainly in the middle of the samples.

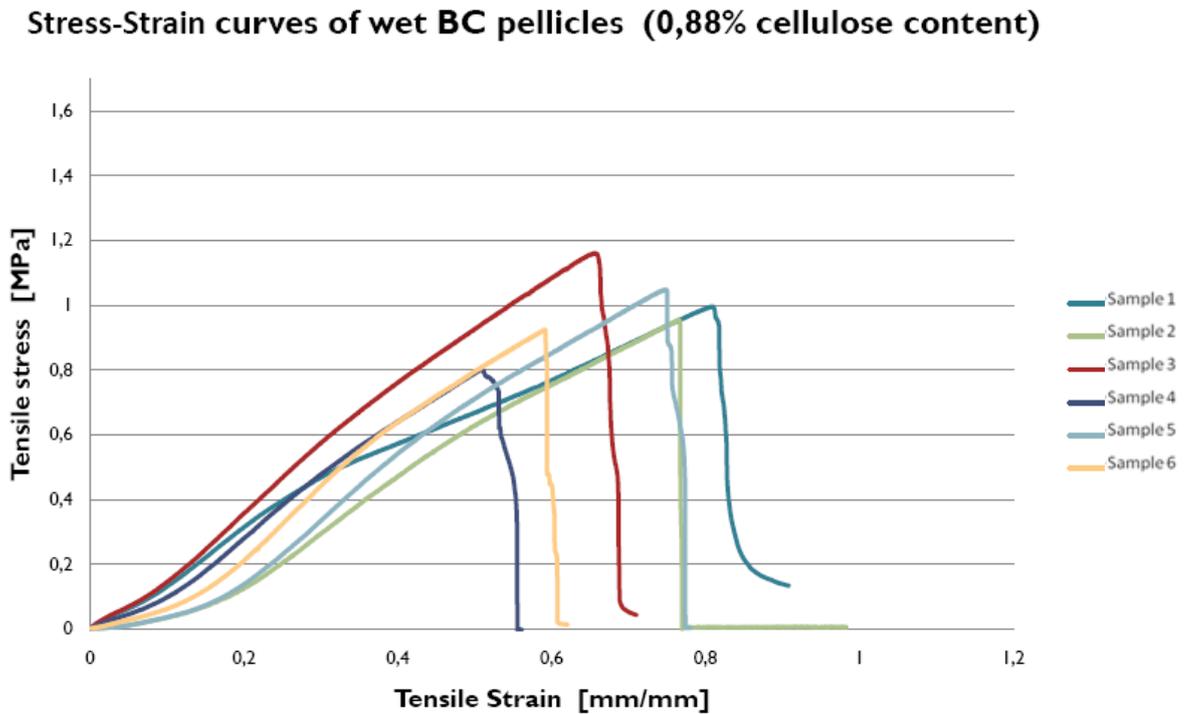


Figure 17: Stress-strain curves for mechanical evaluation of wet BC pellicles, after the development of the sandpaper and silicon clamping system.

Table 2 is a summary of the results from the tensile tests on wet pellicles, after the development of the clamping system. Comparing the values from tables 1 and 2 it is easy to see that the mean values of the stress at break and the Young’s modulus are much higher after the development of the BC “friendly” clamping system, with an average increase of respectively 70% and 44%. It is interesting to see that the values of tensile strain at break did not change to a big extent, with an average increase of only 4%.

	LOAD AT BREAK (N)	TENSILE STRESS AT BREAK (MPa)	TENSILE EXTENSION AT BREAK (mm)	TENSILE STRAIN AT BREAK (mm/mm)	MODULUS (MPa)
Mean	32,57055	0,8766	11,53878	0,63665	2,1101
Standard Deviation	9,96203	0,155	1,244	0,11178	0,27478

Table 2: average values of load, tensile stress, extension, tensile strain (all values calculated at break) and Young's modulus (calculated in the first linear part of the curve). In the lower part of the table you can also see the standard deviation related to each mean value.

Tests on partially dried BC pellicles

Figure 18 shows the stress-strain graphs resulted from testing BC pellicles with different cellulose content percentages. As from figure 18 it is difficult to see clearly the shape of the curve for 'wet' pellicles, figure 19 provides a zoom on that part of the graph.

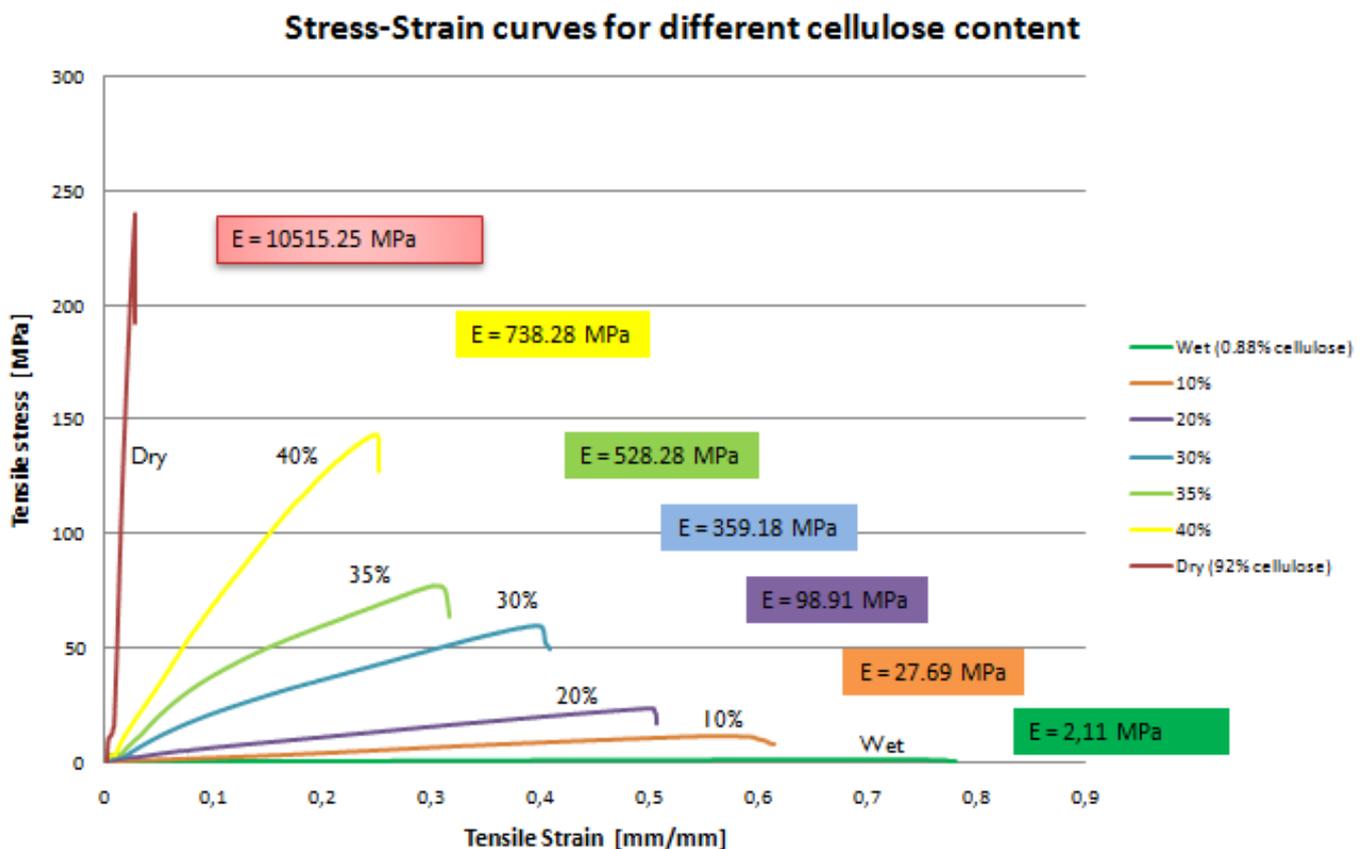


Figure 18: Stress-strain curves for mechanical evaluation of BC pellicles with cellulose content percentage equal to 0.88%,10%, 20%, 30%, 35%, 40%, and 92%.

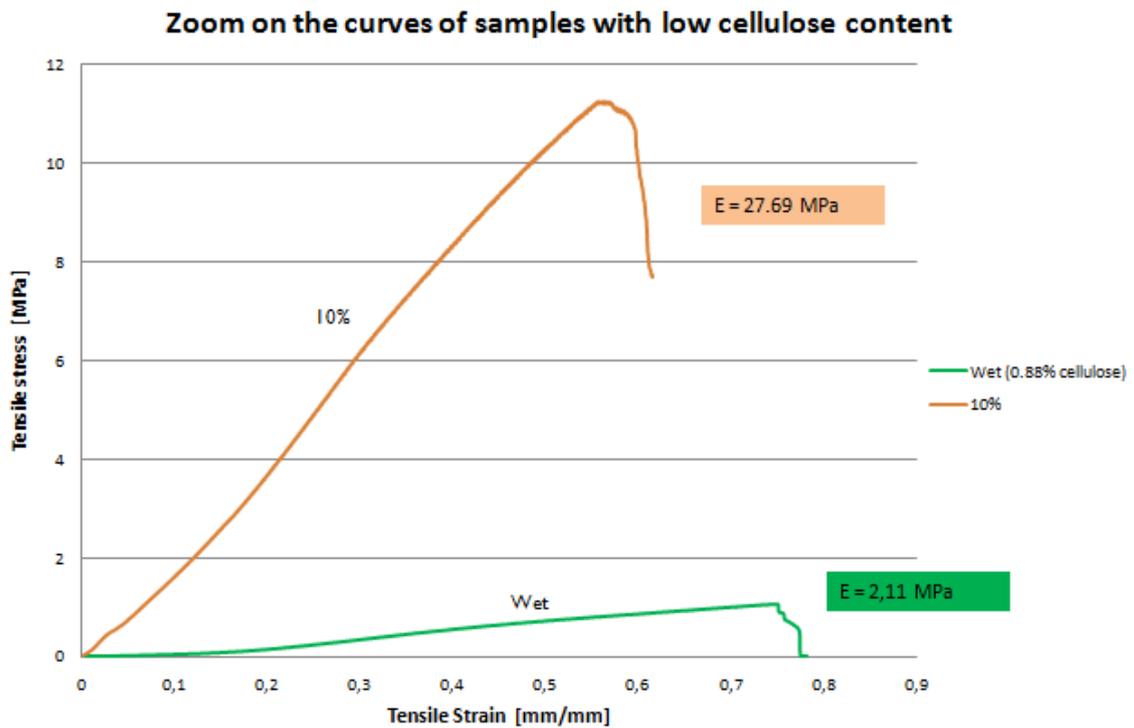


Figure 19: Zoom on the stress-strain curves of BC samples with cellulose content equal to 0.88% and 10%.

Table 3 shows the average values of load, stress, extension and strain (all values calculated at the break of the samples) and Young's modulus (calculated in the first linear part of the curves), referring to the curves presented in figure 18 and 19.

	LOAD AT BREAK (N)	TENSILE STRESS AT BREAK (MPa) (MPa)	TENSILE EXTENSION AT BREAK (mm)	TENSILE STRAIN AT BREAK (mm/mm)	MODULUS (MPa)
Wet (0,88%)	32,57	0,88	11,54	0,637	2,11
10%	56,14	11,23	8,37	0,558	27,69
20%	32,53	23,24	7,52	0,501	98,91
30%	47,23	59,64	5,97	0,398	359,18
35%	46,58	76,86	4,61	0,307	528,28
40%	91,54	142,14	4,39	0,293	738,28
Dry (92%)	56,91	239,63	0,822	0,0272	10515,25

Table 3: Summary of the results from the tensile tests on BC pellicles, with different water content percentage.

The histograms in figures 20A and 20B show the values of the tensile Stress at break measured from the tensile tests on BC pellicles with different cellulose content percentages.

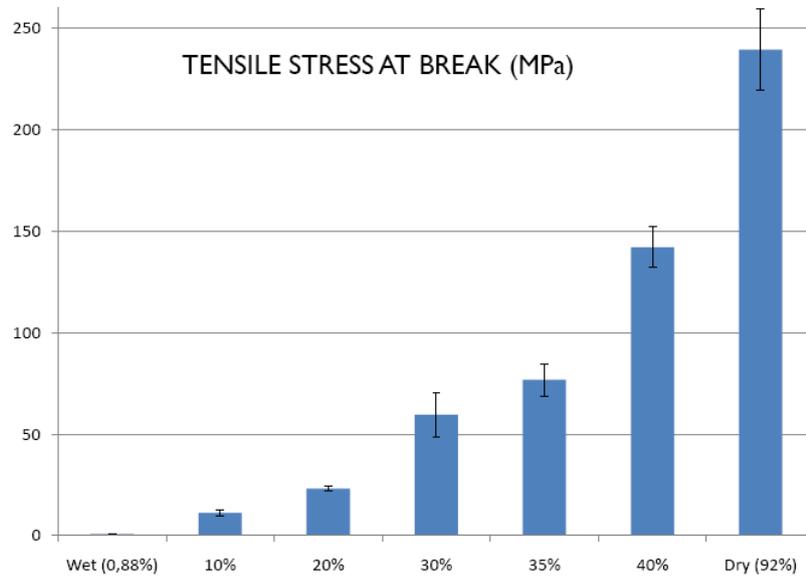


Figure 20A: Tensile stress at break (in MPa) and error bars, for BC pellicles with cellulose content percentage equal to 0.88%,10%, 20%, 30%, 35%, 40%, and 92%.

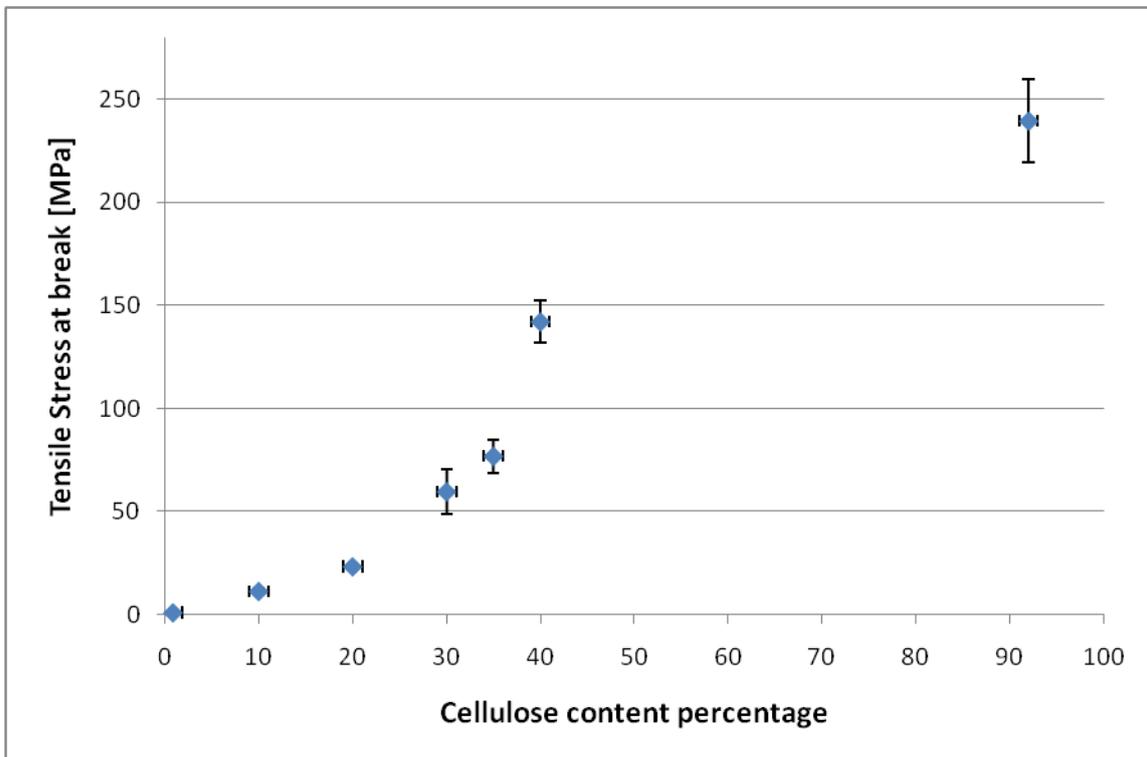


Figure 21B: Tensile stress at break (in MPa) for BC pellicles with varying cellulose content percentage.

The histograms in figure 21A and 21B show the values of the tensile Strain at break measured from the tensile tests on BC pellicles with different cellulose content percentages.

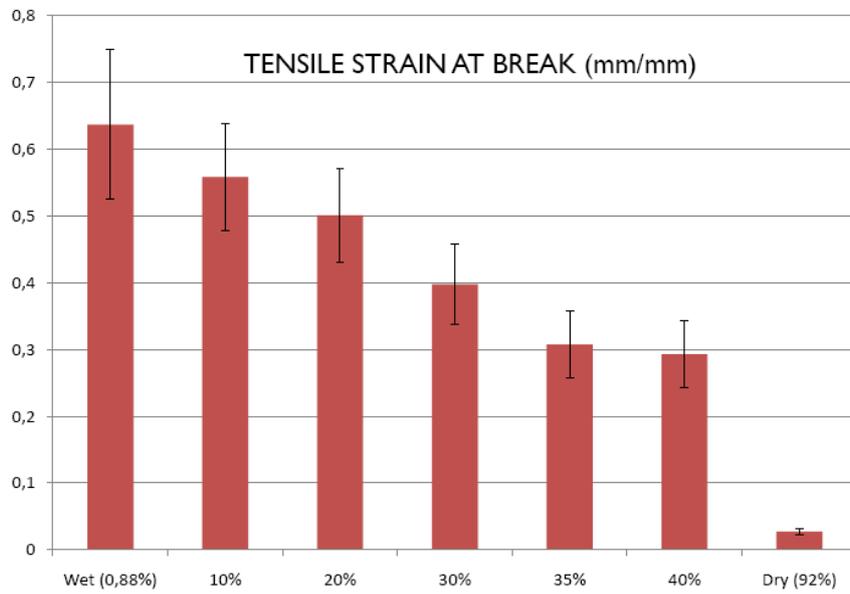


Figure 22A: Tensile strain at break (in mm/mm) and error bars, for BC pellicles with cellulose content percentage equal to 0.88%,10%, 20%, 30%, 35%, 40%, and 92%.

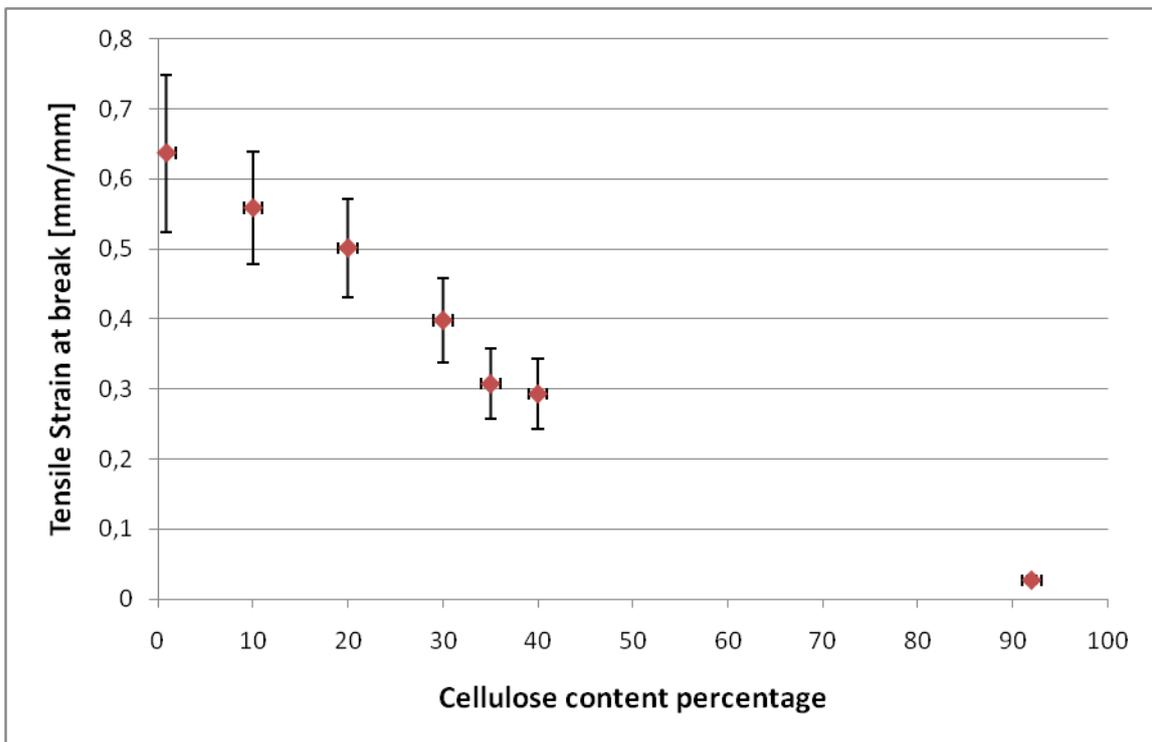


Figure 21B: Tensile strain at break (in mm/mm) for BC pellicles with varying cellulose content percentage.

The histograms in figure 22A and 22B show the values of the tensile Strain at break measured from the tensile tests on BC pellicles with different cellulose content percentages. The modulus was calculated in the first linear part of the stress-strain curves.

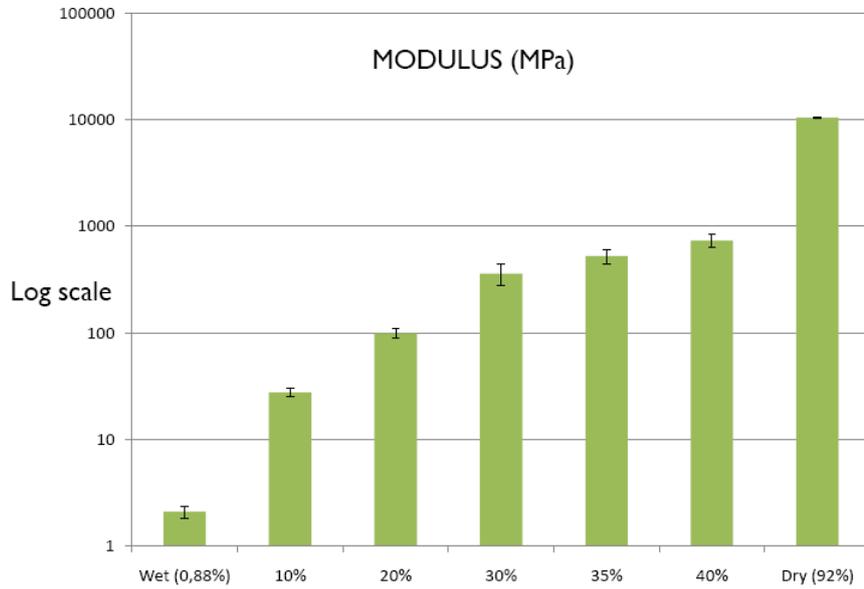


Figure 23A: Young's modulus (in MPa) and error bars, for BC pellicles with cellulose content percentage equal to 0.88%,10%, 20%, 30%, 35%, 40%, and 92%. Note that the scale of the y axis of the graph is logarithmic.

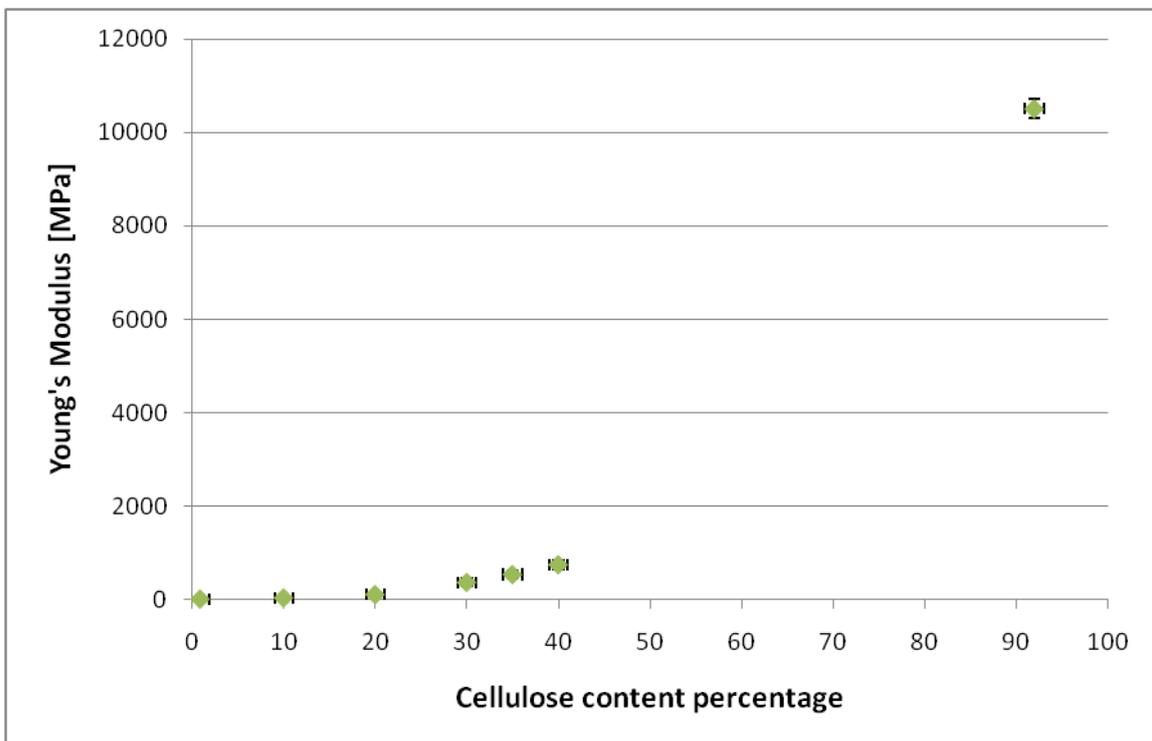
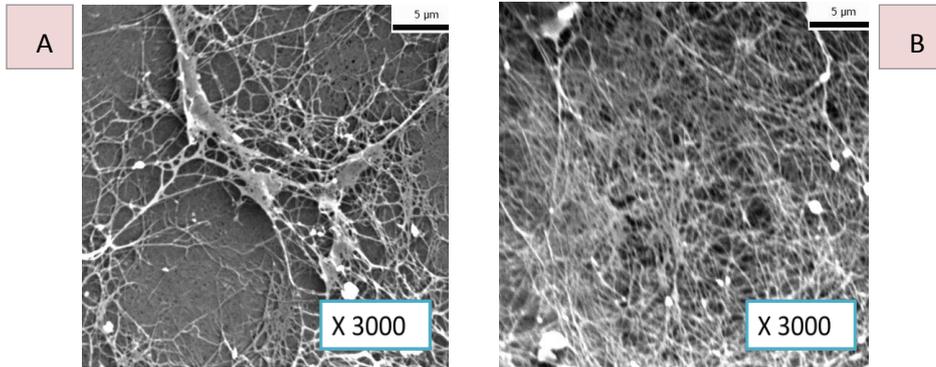


Figure 22B: Young's Modulus (in MPa) for BC pellicles with varying cellulose content percentage.

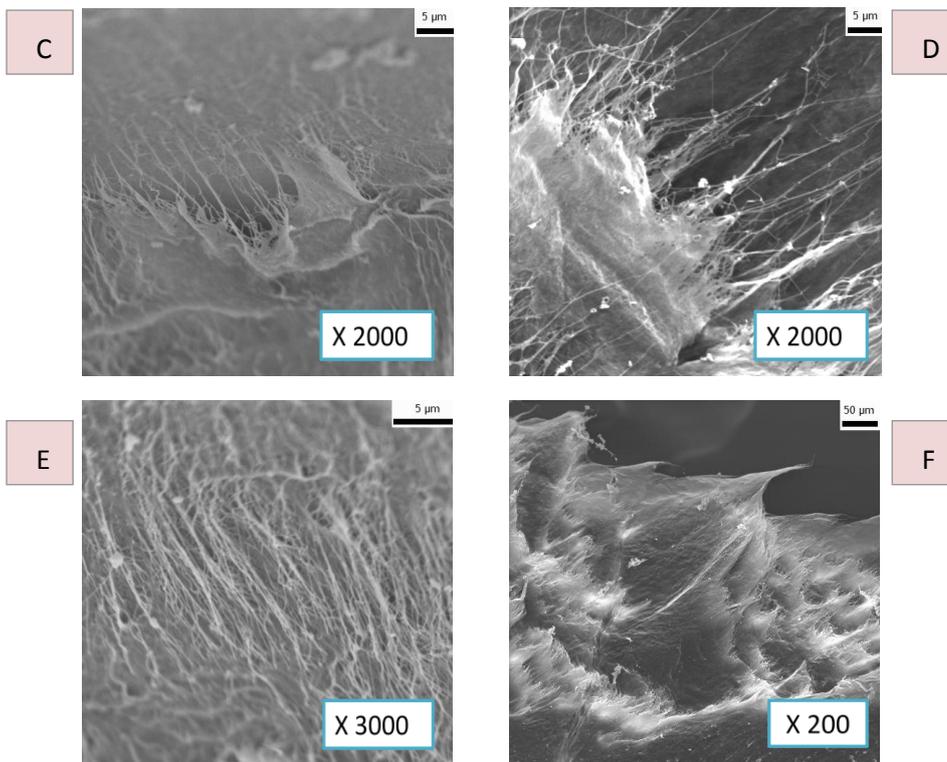
Scanning Electron Microscopy

The results from SEM, reported in figure 23, show the micro-architecture of the material at different magnification levels. Samples which were not tested (figure 23A, 23B) show a random alignment of the nano-fibers, while samples analyzed after the tensile test (figure 23C, 23D, 23E, 23F) show a definite orientation of the fibrils.



A: Wet BC pellicle (0,88% cellulose)

B: BC pellicle with 10% cellulose content.



C,D,E,F: SEM images taken at different points of the fracture region of a BC pellicle (10% cellulose content) after a tensile test. The magnification level changes between the four images.

Figure 24: SEM micrographs showing the micro-architecture of BC microfibers. The first two pictures refer to pellicles not tested, while the last four images refer to fractured surfaces of bacterial cellulose samples, after the tensile test.

DISCUSSION

Cellulose content percentage

Properties of the representative curves: stress, strain, modulus

Figure 17 shows the stress-strain curves of wet BC pellicles (0,88% cellulose content percentage) under uniaxial tensile test. The results show high variability among the different samples: this is due to the fact that the BC samples tested were not exactly the same, mainly because the fermentation process caused differences in the values of the thickness both between and within pellicles, as the thickness was not uniform along the single pellicles. Obviously this variability in thickness affected the calculation of the stress and caused big differences in the results, summarized in table 2. A part for the problem regarding the thickness of the samples, this heterogeneity “should be expected for a biological sample such as a randomly deposited network of cellulose micro-fibrils”. [5]

As reported in table 2, the stress-strain curves regarding the wet pellicles showed mean values of stress at break of about 0,88 MPa and strain at break of 64%. The modulus was calculated in the first linear section of the graph, showing a mean value of 2,11 MPa.

As a comparison with past studies, the values of stress and strain are consistent with the results obtained by Backdahl et al. (2006) and McKenna et al. (2009). The value of the modulus is in agreement to the previous studies with uniaxial mechanical tests, reporting values between 1-10 Mpa. [11,5] The differences in the results could depend from the use of different strain rates for the tests, or the use of dumb-bell shaped instead of rectangular shaped samples, or the use of pellicles with different water content percentage, or also the use of a different bacterial strain.

Figure 18 shows the stress-strain curves representing the tests on BC pellicles with different values of cellulose content. As clearly visible in the graph, the modulus of the curves increases when the cellulose content increases. In the same way the stress at break becomes higher and the strain at break becomes lower: when the water content becomes lower in the samples, the material act more as a fragile material. Table 3 contains a summary of the values of stress, strain and modulus of all the representative curves for different water content percentage. The value of the modulus varies in a huge interval between 2 Mpa (wet pellicles) and 10 GPa (dry pellicles).

As reported in the theory part, this variation finds an answer at a micro-level of the BC network, where the ramifications and entanglements of the ribbons exist; the high tensile strength is due to the hydrogen bonds between the fibrils. Values of moduli in the range of 15 GPa were reported by Yamanaka et al. in 1989 [9].

From Figure 18 and 19 it is interesting to analyze the shape of the curves, which is “characteristic of a viscoelastic material that does not exhibit linear stress/strain behavior at low deformations. The absence of a linear response at low deformations suggests there is some rearrangement of the network whilst undergoing deformation.” [5]

Effect of BC network on mechanical properties

The microscopy analysis conducted with SEM permitted to obtain the micro-graphs shown in Figure 23. Figure 23A and 23B represent the micro-structure of BC pellicles respectively with 99,12% and 90% of water content percentage, not tensile tested. The architecture shows a random orientation of the fibers.

On the other hand, Figure from 23C to 23F show the surface of the fractured region of a BC sample, after tensile test. During a test, the stress is applied and distributed between all the fibrils of the sample, and the sample breaks where the cross-linking among the fibrils is weaker. These figures show a definite orientation of the fibrils: previous studies with X-ray scattering have proposed a realignment model of BC nano-fibrils under uniaxial tensile strength, which consists of a fast first orientation of the fibrils, then there is a period of slow re-orientation, and when the sample breaks the fibrils undergo relaxation. [67]

It is interesting to remember that, for biomaterials in tubular forms, the uniaxial mechanical properties are not of primary importance. The potential of Bacterial Cellulose as a biomaterial becomes even better, if we think that this material gives the best performance under biaxial deformation. A previous study by Chanliaud et al. [29] has reported extraordinary values of modulus and stress-at-break for BC pellicles undergoing biaxial deformation. In this case there is no linear pulling of the fibrils, because biaxial tension does not permit realignment of the fibers. This amazing property of this material is intuitive, if we think that cellulose exists in nature as constituent of the cell wall of the plants, and its function is to resist biaxial stress of the cells against the wall.

Optimization

There are several aspects of this work that could be optimized in the future, to obtain better results:

- Better clamping system.
- Better fermentation conditions, to obtain BC pellicles with more uniform thickness.
- Dumb-bell shape of the samples to avoid that the sample could break near the clamps.
- Slicing the samples with microtome.
- Use of video-extensometer to measure of the local strain.
- Higher resolution for the SEM images, to analyze the structure of the samples at a nano-level.

Future work

To analyze completely the mechanical properties of the material it will be necessary to run tensile (uni-axial and bi-axial), compression (uniaxial and biaxial), shear on BC pellicles, but also perform stress relaxation studies. The speed of the tests can be changed, to characterize completely the response of the material. The tests on the wet samples could be run in a physiological solution that mimics better the environment inside the human body, but also at different temperatures. As already studied by many authors, the material could be chemically treated, or composites made of bacterial cellulose could be produced, to achieve even higher mechanical properties.

It will be also important to make mechanical tests on the implants made of Bacterial Cellulose, like skin, artificial meniscus and tubes for replacing blood vessels. When thinking about biomedical applications, engineering of the fermentation process could affect the properties of the material. It would be of interest to run all these experiments on different kinds of Bacterial Cellulose samples, varying the bacterial strain and the fermentation methods.

CONCLUSIONS

A mechanical evaluation of the properties of Bacterial Cellulose pellicles was performed: it was found that Young's Modulus, Tensile Strength and Tensile Strain vary to a great extent, when changing the cellulose content of the sample. This property of the material makes it very interesting for new possible biomedical applications.

Through SEM analysis of the network of BC pellicles at a micro-level, it is possible to recognize the realignment of the fibers. All the extraordinary mechanical properties of the material depend on its nano-structure, thus a complete analysis of the mechanical properties related to morphological studies, focused on the nano-level, will give to the scientific community all the knowledge about the material which is needed to turn Bacterial Cellulose into an amazing biomaterial.

The growth of the commercial applications of around Bacterial Cellulose is all depending on discovery and utilization of all its unique properties, as the potential of this material is great.

ACKNOWLEDGEMENTS

- Professor Paul Gatenholm
- Nikolaj Vest, Magdalena Zaborowska, Aase Bodin at BBV-lab, Chalmers
- Guillermo Toriz, Anders Höije, Anders Mårtensson at Polymer technology, Chalmers
- Kristoffer Drotz at Arterion AB

REFERENCES

1. SJOESTROM, Wood Chemistry: Fundamentals and Applications, 2nd edition, 1993.
2. AASE BODIN, Biomedical applications of bacterial cellulose: Fermentation, Morphology and Surface Properties, Chalmers University of Technology, Goteborg, Sweden 2007.
3. HENRIK BÄCKDAHL, Engineering the shape of bacterial cellulose and its use as blood vessel replacement, Chalmers University of Technology, Goteborg, Sweden 2008.
4. KRISTOFFER DROTZ, Production Optimization and Biomechanics of Biosynthetic Blood Vessels made of Bacterial Cellulose, Thesis for the Degree of Master of Science, Diploma Thesis n°350, Chalmers University of Technology, Goteborg, Sweden 2008.
5. BRIGID A. MCKENNA, DEIRDRE MIKKELSEN, J. BERNHARD WEHR, MICHAEL J. GIDLEY, NEAL W. MENZIES, Mechanical and structural properties of native and alkali-treated bacterial cellulose produced by *Gluconacetobacter xylinus* strain ATCC 53524, *Cellulose* (2009) 16:1047–1055.
6. M. IGUCHI, S. YAMANAKA AND A. BUDHIONO, Bacterial cellulose—a masterpiece of nature's arts, *Journal of Materials Science*, Volume 35, Number 2 / January, 2000, 261-270.
7. CZAJA WK, YOUNG DJ, KAWECKI M, BROWN RM JR, The future prospects of microbial cellulose in biomedical applications, *Biomacromolecules*, 2007 Jan;8(1):1-12.
8. JONAS R., FARAH L.F, Production and application of microbial cellulose, *Polymer Degradation and Stability*, Volume 59, Number 1, January 1998 , pp. 101-106(6).
9. YAMANAKA S, WATANABE K, KITAMURA N, IGUCHI M, MITSUHASHI S, NISHI Y, URYU M, The structure and mechanical properties of sheets prepared from bacterial cellulose, *J Mater Sci*, 1989, 24:3141–3145.
10. NISHI Y, URYU M, YAMANAKA S, WATANABE K, KITAMURA N, IGUCHI M, MITSUHASHI S, The structure and mechanical-properties of sheets prepared from bacterial cellulose. 2. Improvement of the mechanical-properties of sheets and their applicability to diaphragms of electroacoustic transducers, *J Mater Sci* 25:2997–3001, 1990.
11. HENRIK BÄCKDAHL, GISELA HELENIUS, AASE BODIN, ULF NANNMARK, BENGT R. JOHANSSON, BO RISBERG AND PAUL GATENHOLM, Mechanical properties of bacterial cellulose and interactions with smooth muscle cells, *Biomaterials*, Volume 27, Issue 9, March 2006, Pages 2141-2149.

12. AASE BODIN, SEBASTIAN CONCARO, MATS BRITTEBERG, PAUL GATENHOLM, Bacterial cellulose as a potential meniscus implant, *Journal of Tissue Engineering and Regenerative Medicine*, Volume 1 Issue 5, Nov 2007, Pages 406 – 408.
13. KLEMM D., SCHUMANN D., UDHARDT U., MARSCH S, Bacterial synthesized cellulose - artificial blood vessels for microsurgery, *Progress in Polymer Science*, Volume 26, Number 9, November 2001 , pp. 1561-1603(43).
14. Y.-C. HSIEH, H. YANO, M. NOGI, S. J. EICHHORN, An estimation of the Young's modulus of bacterial cellulose filaments, *Cellulose* (2008) 15:507–513.
15. GISELA HELENIUS, HENRIK BÄCKDAHL, AASE BODIN, ULF NANNMARK, PAUL GATENHOLM, BO RISBERG, In vivo biocompatibility of bacterial cellulose, *Journal of Biomedical Materials Research Part A*, Volume 76A Issue 2, Nov 2005, Pages 431 – 438.
16. JIA YUAN-YUAN, TANG WEI-HUA, LI FEI, JIA SHI-RU, Performance Improvement for Biomedical Material---Bacterial Cellulose, 2007, Tianjin University of Science and Technology, Tianjin, P.R. China.
17. DAVIS, J.R., *Tensile Testing* (2nd edition), 2004, Cleveland (OH): ASM International.
18. MARTIN CHAPLIN, *Water structure and science*, August 2009, <http://www1.lsbu.ac.uk/water/hycmc.html>, visited 03/06/2010.
19. Cellulose bonding network, <http://www.scielo.br/img/revistas/bjpp/v19n1/a01fig01.gif>, visited 03/06/2010.
20. ROSS, MAYER, BENZIMAN, Cellulose Biosynthesis and Function in Bacteria. *Microbiological Reviews*, 1991, 55 (1), 35-58.
21. BBV scientific teams - Chalmers University of Technology, <http://www.chalmers.se/chem/EN/divisions/biopolymer-technology/tissue-engineering/bbv-scientific-teams>, visited 03/06/2010.
22. Barbara J. Aung, Does A New Cellulose Dressing Have Potential In Chronic Wounds?, 2004, <http://www.podiatrytoday.com/article/2311>, visited 03/06/2010.
23. *Structural Biological Materials - Design and Structure-Property Relationships*, Edited by: Manuel Elices, Volume 4, Pages 3-361 (2000).
24. Instron: Polymers and hydrogels testing grip solution, http://www.instron.se/wa/solutions/Polymer_Hydrogels_Testing_Grip_Solution.aspx, visited 03/06/2010.

25. Instron BioPuls bath and submersible grips,
<http://www.asminternational.tv/mvlnstronBiopulsBathAndSubmersibleGrips.htm>,
visited 03/06/2010.
26. Instron BioPuls Submersible Pneumatic Grips and Temperature-Controlled Bath,
<http://www.instron.se/wa/library/StreamFile.aspx?doc=1156>, visited 03/06/2010.
27. Accessories for Biomedical Applications,
<http://www.instron.com/wa/library/StreamFile.aspx?doc=1807>, visited 03/06/2010.
28. MASAHIKO NOGUCHI, TOSHIYA KITAURA, KAZUYA IKOMA, AND YOSHIKI KUSAKA, A
method of in-vitro measurement of the cross-sectional area of soft tissues, using
ultrasonography, *Journal of Orthopaedic Science* (2002) 7:247–251
29. CHANLIAUD E, BURROWS KM, JERONIMIDIS G, GIDLEY MJ, Mechanical properties of
primary plant cell wall analogues, *Planta*, 2002, 215:989–996.
30. R. MALCOLM BROWN JR., Cellulose structure and biosynthesis: What is in store for the
21st century?, *Journal of Polymer Science Part A: Polymer Chemistry*, Volume 42 Issue
3, February 2004, Pages 487 – 495.
31. STEPHAN REILING, JÜRGEN BRICKMANN, Theoretical investigations on the structure
and physical properties of cellulose, *Macromolecular Theory and Simulations*, Volume
4 Issue 4, July 1995, Pages 725 – 743.
32. MIYAMOTO T, TAKAHASHI S, ITO H, INAGAKI H, NOISHIKI Y, Tissue biocompatibility of
cellulose and its derivatives, *J Biomed Mater Res.* 1989 Jan;23(1):125-33.
33. E.J. VANDAMME, S. DE BAETS, A. VANBAELEN, K. JORIS AND P. DE WULF, Improved
production of bacterial cellulose and its application potential, *Polym Degrad Stab* 59
(1998), pp. 93–99.
34. STANISŁAW BIELECKI, Bacterial cellulose – biosynthesis, properties and applications,
Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences,
Technical University of Łódź, Poland.
35. LUCIA INDRARTI AND RIKE YUDIANTI, Effect of Water Soluble Polymer on Structure
and Mechanical Properties of Bacterial Cellulose Composites, *Journal of Applied
Sciences* , Volume 8, 2008, Page No.: 177-180.
36. KRZYSZTOF A, CZAJA W, WIKTOROWSKA-JEZIERSKA A, GONÇALVES-MIŚKIEWICZ
M, TURKIEWICZ M, BIELECKI S., Factors affecting the yield and properties of bacterial
cellulose, Institute of Technical Biochemistry, Technical University of Lodz,
Stefanowskiego 4/10, Lodz 90-924, Poland.

37. SHOICHIRO YANO , HIDEAKI MAEDA, MEGUMI NAKAJIMA, TOSHIKI HAGIWARA AND TAKASHI SAWAGUCHI, Preparation and mechanical properties of Bacterial Cellulose nanocomposites loaded with silica nanoparticles, *Cellulose*, Volume 15, Number 1 , February 2008, 111-120.
38. S. KESHK, Physical properties of bacterial cellulose sheets produced in presence of lignosulfonate, *Enzyme and Microbial Technology*, Volume 40, Issue 1, 6 December 2006, Pages 9-12.
39. ASTLEY OM, CHANLIAUD E, DONALD AM, GIDLEY MJ, Tensile deformation of bacterial cellulose composites, *Int J Biol Macromol* 2003, 32:28–35.
40. MARC ANDRE´ MEYERS, PO-YU CHEN, ALBERT YU-MIN LIN, YASUAKI SEKI, Biological materials: Structure and mechanical properties, *Progress in Materials Science* 53 (2008) 1–206.