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Influence of drying methods on the physical properties of bacterial nanocellulose

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Abstract

Bacterial nanocellulose (BNC) is a promising material for the use in medical implants. BNC does not induce unwanted reactions in vivo, is long term stable and possesses unique mechanical properties. However, to make the most of these features, BNC must be carefully processed. Details of the cultivation and post-synthetic methods offer various ways to control the properties of BNC. The focus of this work is put on drying of the BNC. Different unconstrained drying methods (climate chamber at 23°C, oven at 100°C, freeze-drying) and constrained drying under exertion of uniaxial pressure at various temperatures have been investigated. The reduction of the high water content of native BNC (≈98%) causes a thickness reduction of the samples. For oven or climate chamber drying a thickness reduction of 98% is observed, while freeze-drying widely preserves the nano- or micro-structure of the fibrous material and leads to a thickness reduction of only ≈13%. During drying or pressing at high temperature (100°C), i.e. by evaporation of the water, intermolecular hydrogen bonds are formed and interconnect the individual fibres and strands. Consequently mechanical stiffening is observed in tensile tests at small strains. After drying, a densified cellulose nano-fibre network is observed by scanning electron microscopy. Due to the irreversibility of drying by evaporation, the water content and water retention capacity of BNC are not recovered by rehydration. Applying uniaxial pressure before drying further enhances the irreversible reinforcement of the fibre network, while this is not the case when pressing the samples after drying. The presented results show that the properties of BNC can be widely controlled by post-processing steps. Thus, taylor-made BNC can be produced for biomedical applications.

1. Introduction

Cellulose is the most abundant biopolymer on earth. Not only plants, but also microorganisms like fungi, algae and bacteria produce cellulose [1]. Bacterial nanocellulose (BNC) was first reported by Adrian J. Brown in 1886. He discovered BNC as a solid mass that was formed on the surface of a culture medium during vinegar fermentation [2]. Today, a variety of cellulose-producing bacteria is known, including Gluconacetobacter, Rhizobium, Agrobacterium, Rhodobacter and Sarcina [3–6]. Due to its exceptional mechanical stability and remarkable biocompatibility, BNC is used in different fields of application, like food and textile industry, cosmetics and biomedical engineering [7–15]. Especially in biomedical engineering, BNC is a promising alternative to plant cellulose, as it is of high purity and does not contain additional unwanted substances like hemicelluloses, lignin or pectin [16–18]. The fibre composite material consists of ≈ 99% water and presents as an ≈ 7 mm thick fleecy after week-long synthesis, depending on the bacterial strain that is used. De Oliveira Barud et al (2016) and Picheth et al (2017) reported that BNC in this native (as cultivated) state is a good choice for medical applications, like wound dressings and patches for variable lesions [8, 19]. The properties of BNC can be controlled in a wide range during its synthesis, as well as by post-synthetic processes. Thus taylor-made properties for various applications may be obtained [11, 20, 21]. Besides artificial skin for the treatment of
extensive burns, several other biomedical applications have been reported. Li et al (2017) demonstrated the potential of BNC as a biomaterial for artificial blood vessels for microsurgery. Nimeskern et al (2013) discussed scaffolds for tissue engineering of cartilage [22, 23]. When treating tumours or traumas, BNC can be used as a substitute for dura mater, as reported by Xu et al (2014) and Rosen et al (2011) [24, 25].

Further research activities aim at composites with other well established biomaterials, e.g. collagen (Zhijiang et al (2011)) to combine the advantageous properties of both materials [26]. In recent years, many products of start-up companies entered the market. Examples are DePuy Synthes, USA (Dura Repair (implant)), JeNaCell GmbH, Germany (wound dressing), and BOWIL, Poland (wound dressing) (Klemm et al (2018)) [27].

For minimally invasive cardiovascular applications, like e.g. heart valve prostheses, the use of very thin BNC layers is mandatory to keep the diameter of the catheter used for implantation as small as possible. Moreover, the possibility to store the implant in a dry state is desirable to ensure easy handling. To meet these demands, BNC can be chemically and physically modified. Simple air drying induces unwanted wrinkling and structural changes, e.g. reported by [28]. According to Klemm et al (2018) air-drying leads to the loss of the characteristic three-dimensional fibre network which in turn results in strong shrinkage, superficial hornification, fibre aggregation, and reduced porosity. For applications of BNC hydrogels in medical implants the problem of brittleness must be addressed [27]. A stepwise solvent exchange (e.g. ethanol, acetone, hexane), critical-point drying and freeze-drying are conceivable to resolve this issue. These methods are assumed to retain the original nanofibre structure by gently replacing water by air to form aerogels. Even though the resulting material is also completely dry, it is more compliant and not as brittle [27]. However, no significant thickness reduction of the BNC hydrogel is achieved by these structure-preserving methods.

According to Ul-Islam et al (2013), the details of the post-synthesis processing have a considerable influence on the structural and physico-mechanical properties of the resulting BNC. Drying of BNC under different conditions leads to strong variations of properties. Freeze-drying produces BNC with a spongy structure having plenty of pores, while air drying at different temperatures leads to compact and stiff BNC sheets [20]. As reported by Stanisławska et al (2020) the water content of the BNC strongly affects its mechanical properties. A detailed understanding of the processes occurring during dehydration and subsequent rehydration is essential to finally obtain the properties that are required for the use of BNC in cardiovascular implants [28].

In this work, the influence of post-synthetic processing on the physical properties of BNC is studied. The envisaged applications are, among others, vascular implants. Accordingly a high mechanical stability, a homogeneous structure, an adjustable thickness and some other properties must be controlled. A variety of drying methods (room- and high temperature drying, freeze-drying) as well as the application of uniaxial pressure at different temperatures are investigated to elucidate the range of control of the most important properties of BNC.

2. Materials and Methods

2.1. Materials

2.2. Bacterial Strain

For the biosynthesis of BNC the bacterial strain Gluconacetobacter hansenii (ATCC® 53582™) is used. The culture medium contains glucose (20g/l), peptone (5g/l), yeast extract (5g/l), dinatriumhydrogenphosphate (2.7g/l) and citric acid (1.5g/l) dissolved in 1l of distilled and pyrogen-free water (Carl Roth GmbH & Co. KG, B. Braun AG) [29]. For strain maintenance 25ml of the culture medium and 2ml of shredded cellulose fleece (bacterial suspension) containing the bacteria are blended in a tube (50ml, pyrogen-free, Sarstedt AG & Co. KG). The strain maintenance is repeated every seven days [30, 31].

2.3. Preparation of Sheets

For the preparation of the sheets stainless-steel boxes (300 x 125 x 60mm) are used. The boxes and a cover that allows oxygen to penetrate are both sterilised in an autoclave at 121°C for 20min. Subsequently the culture medium (480ml) and the bacterial suspension (40ml) are blended in the box. Under static conditions the synthesis takes place in an incubator (IPP260, Memmert GmbH & Co. KG) at 28°C and 90% relative humidity. After seven days of cultivation the cellulose fleece is rinsed in distilled water to remove remaining culture medium. For further processing the fleece is stored in distilled water at 4°C [30].

2.4. Post-Processing

The BNC sheets (300x125mm) are laser-cut into rectangles of 125 mm × 70 mm (CO2-Laser, Epilog Zing 24, Epilog Zing). Different drying methods are applied to reduce the thickness of the BNC. For freeze-drying, the sample is cooled to −45°C at normal pressure (1.013bar) in an airtight chamber. A freeze dryer Christ Epsilon
1–4 LSCplus (Martin Christ Gefriertrocknungsanlagen GmbH) is used. Subsequently, the pressure in the chamber is reduced to 0.07 bar, and ice sublimates. Over a period of 72h the temperature is slowly increased to room temperature (23°C).

Drying at room temperature (23°C) takes place in a climate chamber under controlled conditions. The relative humidity is 50% for the first 60h and reduced linearly to 10% during the last 12h. To avoid curling the cellulose sheets are covered with a grid and weights (300g, stainless steel) [27]. A drying process at high temperature (100°C) is performed in an oven (Memmert GmbH and Co. KG). The samples are treated like in the case of the drying in a climate chamber at room temperature. The drying at 100°C lasts 24h.

Another method for the initial water removal is the pressing of BNC sheets, either at room temperature or at elevated temperatures up to 100°C. The samples are placed between two heatable plates of a hydraulic press (LaboPress P150H, Vogt Labormaschinen GmbH). The desired temperature and uniaxial pressure are applied for 15min. To simulate the implantation case, a final rehydration step is performed. For this purpose the samples are rinsed for 24h in distilled water on a shaker in an incubator (37°C).

2.5. Methods

2.6. Thickness Measurement
The thickness of the cellulose samples is determined in the native, dried, pressed and rehydrated states. For its measurement, the gauge Absolute ID-C1012CXB (Mitutoyo Deutschland GmbH) is used. To ensure reproducible results, prior to readout a punch with a diameter of 20mm is placed on the sample for 2s exerting a force of 0.3N.

2.7. Water Retention Capacity
The water retention capacity (WRC) is also known as the swelling value and describes the amount of water retained in a material after centrifugation. According to DIN 53814:1974–10, the overall water content is the sum of swelling water in the fibres, capillary water between the fibres and adhesive water on the surface of the fibres [32]. Circular samples with a diameter of 8mm were laser-cut for the analysis. One sample of each set of samples was centrifuged for 20min (5920R, Eppendorf GmbH) in a standardised centrifugal vessel (DIN 53814:1974-10) at 1000g at a temperature of 20°C. After centrifugation, the wet weight (\( W_{w} \)) was determined (Mettler Toledo GmbH). The samples were then dried in an oven (Memmert GmbH & Co. KG) for 24h at 100°C and weighed again (\( W_{d} \)). The WRC is calculated by the following formula:

\[
\text{WRC} = \frac{W_{w} - W_{d}}{W_{d}} \cdot 100
\]

2.8. Water Content
The water content (WC) is measured after rehydration of the samples. The same sample geometry as for the WRC was used (discs with a diameter of 8mm). The samples were weighed in the moist state (\( W_{w} \)) and subsequently undergo a drying process (vacuum-drying) for 24h. The dry weight (\( W_{d} \)) is then measured and the water content calculated by

\[
\text{WC} = \frac{W_{w} - W_{d}}{W_{w}} \cdot 100
\]

2.9. Material Density
The density is determined from the dry mass (Mettler Toledo GmbH) of the samples and their volume in the native and rehydrated states. The samples have an initial area of 4cm². After processing, the area is determined from micrograph images. The thickness is measured with the gauge Absolute ID-C1012CXB (Mitutoyo Deutschland GmbH).

2.10. Morphological Analysis
The optical analysis of the surface and the bulk fibre network is carried out in a scanning electron microscope (EVO MA 15, Carl Zeiss Microscopy GmbH) using the detector for secondary electrons. The acceleration voltage is 10kV. To reduce disturbing effects of charge accumulation, the freeze-dried samples are sputter-coated with gold in a nitrogen atmosphere (Agar Sputter Coater, Plano GmbH).

2.11. Mechanical Properties
For mechanical characterisation uniaxial tensile tests are performed. In order to obtain reproducible and comparable data for all samples a small preload of 2g is exerted and the length of the sample determined. The
thus obtained value is subsequently used as the initial (unloaded) length of the samples. The specimen is stretched at a constant rate of 12 mm/min until failure occurs. The strain $\varepsilon$ is calculated from the actual length $\Delta l$ and the above mentioned initial length $l_0$. For the uniaxial tensile tests, a specimen geometry based on DIN EN ISO 527-2 (Type 1BA) is used. The samples have a total length of 50mm and a minimum width of 5mm. The free length between the fixing supports is 30mm. In order to better compare the mechanical properties of the samples in different processing states force-strain diagrams are analysed instead of stress-strain diagrams. The latter would be prone to misinterpretation as the thicknesses of the samples vary by orders of magnitude throughout the processing while the load supporting fibre network essentially remains the same. From the force-strain diagram the breaking force $F_{\text{max}}$ and the breaking strain $\epsilon_{\text{max}}$ are determined. The constant slope of the curve in the range before failure occurs characterises the stiffness of the sample and is designated as F-Modulus (FM). The stiffness of the sample at small strains below 5% is quantified by the initial modulus $F = \text{Modulus}_{5\%}$ ($FM_{5\%}$).

3. Results and Discussion

In the following sections data obtained on differently processed cellulose samples are presented. The analyzed parameters are water content, water retention capacity, density, thickness and mechanical properties. The microscopic morphology (SEM micrographs) is analysed after re-wetting the samples, i.e. in the rehydrated state. Additionally to separate drying and pressing of the samples, a combination of both processes is also investigated, for pressing and drying as well as for drying and subsequent pressing. Pressing first already removes most of the free interstitial water prior to drying, while pressing after drying causes a homogenisation of the
samples and leads to a uniform lateral thickness distribution. For pressing, a uniaxial force is applied at a temperature of 50°C. The force is chosen to obtain a resulting pressure of 10 N mm\(^{-2}\) independent of sample area. Drying is carried out in the climatic chamber, the oven, and by freeze-drying.

### 3.1. Material Thickness

The thickness of the samples in the native state after cultivation is in the range of 7.5–8.5 mm (figure 1(a)). After drying there is a reduction in thickness of \(\approx 98\%\) in case of oven and climate chamber drying. As expected, for structure-conserving freeze-drying the reduction of thickness is much smaller and amounts to only \(\approx 13\%\). The final rehydration step leads to slight swelling or shrinking of the samples, indicating that the formation of hydrogen bonds and fibre interconnection during drying is an irreversible process.

The initial thickness of about 8 mm can be reduced by about \(98\%\) by pressing only, i.e. without further drying. Only a slight dependence on pressure and temperature is found (figure 1(b)). As expected, homogeneous thickness distributions in the pressed state are obtained, also indicated by the small values of the standard deviations. However, the influence of pressing prior to drying is revealed after moistening in water. A higher swelling capacity, i.e. recovery of thickness is achieved at the lowest pressing temperature when compared to the high ones. After rehydration a more inhomogeneous lateral thickness distribution is observed, although less pronounced at the highest pressing temperature. Furthermore, there is only a relatively slight correlation of the swelling capacity to the applied pressure. In summary, pressing at a higher temperature leads to a higher reduction in thickness, corroborating the notion that irreversible inter-fibre bonding is enhanced by temperature.

For a look at the combination of pressing and drying an initial pressing step at 50°C and a pressure of 10 N mm\(^{-2}\) is chosen. The thickness is thus already reduced by \(\approx 99\%\) compared to the native state and lies below 0.1 mm, as can be seen in figure 1(c). Subsequently an additional drying process is carried out. As expected only small changes in thickness are observed. The subsequent rehydration results in an increase of thickness again, but the total swelling capacity is only about 20%–50% compared to the thickness after pressing-drying.

When drying before pressing, the initial thicknesses before the pressing are comparable for climate chamber and oven drying (figure 1(d)), while it is much larger for the freeze-dried samples, with their conserved highly porous structure. It is thus worthwhile to check whether additional pressing leads to different results for these different starting points. In fact it is observed that, when rehydrating the freeze-dried and subsequently pressed samples more than 50% of the thickness after freeze-drying and thus an only slightly lower thickness than in the native state is reached. In absolute values this means swelling from about 0.2 mm after pressing back to \(\approx 4.5\) mm, i.e. more than a factor of 20 or 2000%. For the samples dried in the climate chamber or the oven the swelling only amounts to a range of 10%–50%. It can be stated at this point that freeze-drying effectively prevents the formation of most inter-fibre connections. The nanostructure is preserved and thus no close contact between neighbouring fibres occurs, especially not in the last stages of drying when the hydrogen bonds are formed.

### 3.2. Water content and water retention capacity

After rehydration, the water content of the differently dried samples is always lower than in the never-dried state. Drying at 23°C in the climate chamber leads to a higher rehydrated water content than oven drying at 100°C (table 1). In agreement with the findings of the last paragraph it is safe to assume that the increased formation of inter-fibre hydrogen bonds is facilitated at higher temperatures during drying. Consequently the ability of the samples to absorb water decreases with increasing temperature [33]. Again the freeze-dried samples show a clearly different behaviour. Their initial water content in the native state is almost completely recovered after rehydration. At first sight this difference may be attributed to the pores that remain in the samples after freeze-drying, allowing a reversible uptake of water similar to never-dried samples.

| Drying process | WC [%] | WRC [%] |
|----------------|--------|---------|
| Non-dried      | 98.29 ± 0.16 | 1032.50 ± 88.29 |
| CC (23°C)      | 71.56 ± 4.51 | 133.50 ± 9.65 |
| O (100°C)      | 53.93 ± 2.09 | 92.65 ± 7.10 |
| Freeze-dried   | 96.57 ± 0.49 | 762.66 ± 43.90 |
As expected, the same is true for the water retention capacity. Samples dried at 23°C in the climate chamber, as well as those dried at 100°C in the oven exhibit a water retention capacity that is one order of magnitude smaller than for native samples. The freeze-dried samples have a WRC that nearly reaches the value of the native samples. Portela et al (2019) and Wang et al (2012) have reported that the WRC directly relates to the available pore volume and surface area. A more compact BNC structure with a dense fibre arrangement and only few pores left does not allow high WRC values. In general the water retention capacity of BNC ranges from 60 to 700 times of its dry weight, depending on synthesis conditions [33]. The WC and the WRC have also been investigated for constrained drying under uniaxial pressure at elevated temperatures. Both properties decrease with increasing pressure (table 2), since the fibre arrangement is more dense and less space is available to store interstitial water. The effect is further enhanced at higher pressing temperatures.

The results of uniaxial pressing combined with drying are shown in table 3. It can be stated that the water content of samples dried in the climate chamber is almost not affected by additional uniaxial pressing at 50°C and 10 N mm⁻², regardless of whether it happens before or after drying.

For the oven-dried samples it is interesting to note that pressing before drying leads to a significantly higher WC after rehydration. Pressing after drying has practically no influence on the WC, as is expected. The mechanism that causes the higher rehydrated WC after pressing before drying remains unclear at this moment, especially as the water retention capacity is not affected. The freeze-drying is again the exception to the rule. Pressing after freeze-drying preserves WC and WRC. Pressing before freeze-drying, however, leads to irreversible changes of the microstructure, inter-fibre bonds are formed and the resulting values of the WC and WRC in the rehydrated state are similar to those obtained for the other drying methods.

### 3.3. Microstructure

The microstructure of samples after rehydration has been investigated by scanning electron microscopy. In the native, never-dried state (figure 2(a)) a highly porous structure is visible. The same is true for the freeze-dried samples in (figure 2(d)). After drying in the climate chamber at 23°C areas of compressed fibres dominate the micrograph in figure 2(b). The densification, however, is not complete. Loosely packed strands of fibres are

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**Table 2.** Water content and water retention capacity of differently pressed BNC samples (n = 12) (No additional drying).

| Temperature °C | Pressing force (N mm⁻²) | WC [±] (%) | WRC [±] (%) |
|---------------|--------------------------|------------|------------|
| 23°C          | 5                        | 89.20 ± 3.86 | 362.66 ± 82.90 |
|               | 10                       | 87.87 ± 4.58 | 300.34 ± 40.87 |
|               | 20                       | 79.95 ± 5.26 | 246.13 ± 45.73 |
| 50°C          | 5                        | 87.30 ± 1.29 | 374.15 ± 44.55 |
|               | 10                       | 84.38 ± 3.76 | 291.67 ± 57.31 |
|               | 20                       | 78.62 ± 4.34 | 265.09 ± 22.02 |
| 100°C         | 5                        | 77.56 ± 1.94 | 223.21 ± 20.46 |
|               | 10                       | 69.45 ± 1.83 | 159.81 ± 12.12 |
|               | 20                       | 69.00 ± 3.98 | 154.75 ± 7.91 |

**Table 3.** Water content and water retention capacity after rehydration of pressed and dried, as well as dried and pressed BNC samples (n = 12).

| Drying process          | WC [±] (%) | WRC [±] (%) |
|-------------------------|------------|------------|
| Pressing before drying  |            |            |
| CC (23°C)               | 75.41 ± 5.64 | 139.37 ± 10.87 |
| O (100°C)               | 72.26 ± 5.59 | 89.23 ± 3.67   |
| Freeze-dried            | 80.53 ± 3.12 | 120.07 ± 12.05 |
| Pressing after drying   |            |            |
| CC (23°C)               | 69.88 ± 2.15 | 145.68 ± 5.73  |
| O (100°C)               | 55.64 ± 2.24 | 94.05 ± 2.79   |
| Freeze-dried            | 97.96 ± 0.32 | 678.94 ± 91.11  |
present everywhere between the dense areas. The latter are less frequently observed for the samples dried in the oven at 100°C, resulting in an almost uniform and dense structure (figure 2(c)) [34]. This is agreement with the findings of Fink et al (1997), who reported that drying at elevated temperatures leads to a collapse of the fine fibre network [35]. A densely packed microstructure is also observed for the pressed samples after rehydration. For room temperature pressing, the pores in the loose fibre network after pressing and rehydration are clearly visible (figure 2(e)). Pressing at 100°C, however, changes the microstructure completely and results in a densely packed structure without individually distinguishable fibres or strands of fibres (figure 2(f)). These observations strongly corroborate the hypotheses that have been developed in the preceding paragraphs of this section.

3.4. Density of Samples
To complete the investigated set of parameters, the physical density of the samples has been measured. The results are presented in table 4.
In good agreement with the other findings, the densities are very similar for the native and the freeze-dried samples. The density of the two other kinds of dried sample is higher by a factor of more than 25. This high density is caused by the irreversible inter-fibre connections that do not allow the re-formation of pores during rehydration. For the pressed-only samples, however, a portion of the pores is recovered during rehydration.

Figure 3. Comparison of the mechanical properties of differently dried or pressed BNC samples.

### Table 4. Mass, volume and density of differently dried and pressed BNC samples (n = 5) in the native state and after processing and rehydration.

| Process     | Mass [mg]  | Volume [cm³] | Density [mg/cm³] |
|-------------|------------|--------------|-----------------|
| Native      | 43.1 ± 1.1 | 2.95 ± 0.16  | 14.7 ± 1.1      |
| Drying      |            |              |                 |
| CC (23°C)   | 40.3 ± 1.4 | 0.07 ± 0.01  | 543.2 ± 31.7    |
| O (100°C)   | 41.8 ± 1.1 | 0.06 ± 0.01  | 692.6 ± 43.9    |
| Freeze-dried| 41.7 ± 1.0 | 1.98 ± 0.10  | 21.1 ± 1.4      |
| Pressing    |            |              |                 |
| 23°C, 5MPa  | 41.7 ± 1.2 | 0.39 ± 0.07  | 110.1 ± 17.9    |
| 23°C, 10MPa | 41.2 ± 1.8 | 0.30 ± 0.03  | 140.6 ± 15.0    |
| 23°C, 20MPa | 41.4 ± 1.6 | 0.25 ± 0.04  | 169.0 ± 22.3    |
| 50°C, 5MPa  | 41.5 ± 1.5 | 0.28 ± 0.04  | 150.6 ± 16.6    |
| 100°C, 5MPa | 41.7 ± 2.0 | 0.18 ± 0.03  | 241.8 ± 32.7    |
swelling occurs and consequently the density is much lower. As expected, higher pressure and temperature result in a higher density.

3.5. Mechanical Properties

In this subsection the mechanical properties of differently processed BNC will be correlated with the other investigated parameters, primarily with the microstructure. All tensile tests have been performed after rehydration, except for the native samples. As shown in figure 3(a), the force-strain curves of both native (d) and freeze-dried samples (c) exhibit very small initial slopes at small strains. For the samples dried in the climate chamber, however, the initial slope is about 70 times higher for strains below 5%. The oven dried samples have an even higher initial modulus (FM5%, table 5). Note that the values of the force at break are in general very similar for all samples, indicating that the load-bearing fibre network is not degraded by post-processing. It is thus justified to state that the tensile strength of a BNC fibre network can be enhanced by orders of magnitude by interconnecting the fibres with hydrogen bonds [36, 37]. As the individual fibres and strands are randomly oriented in the native state, they must be aligned after drying, at least partly. The fibre network is then somehow folded to allow a uniaxial compression by orders of magnitude without breaking the fibres. This notion is supported by the experimental observation that is shown in figure 3(b). When performing a tensile test on dried and rehydrated BNC a pronounced increase of the thickness perpendicular to the loading direction occurs. This

![Figure 4. Combination of drying and pressing: Mechanical properties of samples pressed before or after drying.](image)

| Drying process | Strain at break [%] | Force at break [N] | F-Modulus [N] | FM5% [N] |
|----------------|---------------------|--------------------|---------------|---------|
| Non-dried      | 56.38 ± 4.96        | 45.86 ± 3.62       | 143.00 ± 37.33 | 4.31 ± 3.59 |
| Climate Chamber (23°C) | 38.42 ± 2.72      | 55.11 ± 6.16       | 151.30 ± 12.34 | 280.52 ± 16.84 |
| Oven (100°C)   | 32.86 ± 1.70        | 57.35 ± 5.15       | 158.03 ± 8.45  | 424.17 ± 24.99 |
| Freeze-dried   | 42.06 ± 3.88        | 48.00 ± 3.82       | 177.61 ± 15.62 | 13.85 ± 9.49 |

| Drying process | Strain at break [%] | Force at break [N] | F-Modulus [N] | FM5% [N] |
|----------------|---------------------|--------------------|---------------|---------|
| Pressing before drying | Climate Chamber (25°C) | 29.15 ± 3.89     | 45.00 ± 3.52    | 91.11 ± 2.59    | 492.71 ± 77.65 |
| Oven (100°C)   | 21.00 ± 3.63        | 48.60 ± 5.32       | 146.70 ± 15.38 | 657.79 ± 37.02 |
| Freeze-dried   | 36.19 ± 4.30        | 58.80 ± 4.74       | 135.06 ± 12.81 | 421.15 ± 62.84 |
| Pressing after drying | Climate Chamber (25°C) | 42.53 ± 4.74     | 52.57 ± 5.50    | 135.06 ± 12.81 | 261.13 ± 17.30 |
| Oven (100°C)   | 32.30 ± 2.32        | 57.51 ± 5.62       | 152.29 ± 6.00  | 440.41 ± 25.80 |
| Freeze-dried   | 40.50 ± 2.77        | 45.55 ± 3.48       | 171.77 ± 12.00 | 14.35 ± 8.57 |

Table 5. Mechanical properties of BNC samples after different drying processes (n = 10).

Table 6. Mechanical properties of BNC samples after different drying processes, pressing and re-wetting (n = 10).
effect amounts to about 700% and can be attributed to the mentioned folding of the network. Stretching the network in the plane of the sheet induces the unfolding of those fibres that were originally oriented out of plane. Verma et al (2014) reported a similar behaviour for paper. Details of their model to explain the underlying mechanisms can be found in [38].

The strain at failure is lower for the dried samples. This can be attributed to the fact that starting at strains of 5% partial failure occurs. This failure may be either due to the breakup of fibre interconnections or due to failure of the fibres or strands themselves. Taking into account that the slope before failure is slightly smaller for the dried samples than for the native and freeze-dried samples it is justified to assume that both mechanisms contribute to the failure. However, as the slope before failure is only slightly smaller, only few fibres seem to be broken and the contribution of broken interconnections must be dominant. This assumption is also supported by the observation that the force at failure is even slightly higher for the dried samples. A detailed explanation, however, requires further investigation beyond the scope of the present work. The fibres and strands in the native state, but also the freeze dried samples are not interconnected by hydrogen bonds and can more easily slide past each other along the direction of strain, thus resulting in a more elastic behaviour.

The pressed samples also exhibit stiffening at small strains, as can be seen in figures 3(c) and (d). The effect is more pronounced at higher temperature, while the pressure only plays a minor role.

Pressing before drying leads to an increase of the initial slope of the force-strain curve for all types of post-processing, even freeze-dried samples (figure 4(a)). For the latter the effect is less pronounced than for the other types of samples as can be seen in table 6. Pressing after drying does not lead to stiffening of freeze-dried samples (figure 4(b)). Almost no change of the curves occurs for the climate chamber and the oven dried samples. The first dehydration is crucial for the formation of the inter-fibre hydrogen bonds while subsequent pressing steps have practically no effect [39].

4. Conclusion

From the observation of the effects of various drying protocols for BNC after cultivation it can be concluded that the way how the water is removed from the fibre network dominates the development of the final material properties of BNC. Removing the water by draining the samples under pressure or by evaporating the water at elevated temperatures is always accompanied by a stiffening of the samples. The observed strong increase of the initial slope of the stress-strain curves below 5% is caused by the formation of hydrogen bonds that interconnect individual fibres in the fibre network. Most of these bonds, however, break at strains between 5% and 15% depending on the details of the drying method. The formation of the hydrogen bonds can be avoided by employing freeze-drying to remove the water from the BNC. Only in this case a markable stiffening can be avoided.

By applying uniaxial pressure to the samples, their thickness can be strongly reduced. Whether the thickness can be recovered by rehydration crucially depends on the initial way of dehydration. Pressing after freeze-drying is the only combination that allows the water to enter the cellulose network in a reversible fashion.

Combining the mentioned drying methods with controlled 3D-growth of BNC, e.g. as tubes, novel fields of application for this interesting biomaterial will emerge, especially when cardiovascular implants are concerned. First results in this respect are very promising and will be presented in an upcoming publication.

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