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Effects of Different Drying Processes on the Material Properties of Bacterial Cellulose Membranes

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Summary: Membranes of bacterial cellulose produced by *Gluconacetobacter xylinus* show a high water and gas permeability that can be altered by different drying techniques. It could be shown that freeze-drying reduces the swellability of the polymer membranes by a factor of 5 while evaporation drying causes a reduction by a factor of 50. The strong decrease of swellability for an evaporation dried membrane could be correlated with a reduction of the absolute number of polymer strands that form the network structure of the membrane, determined with oscillatory shear rheological experiments. The removal of network meshes by a complete aggregation of polymer strands could be confirmed by IR-spectroscopy with an increased degree of intramolecular hydrogen bonding of cellulose strands. In contrast to this, the freeze-drying process shows a slight increase of the number of network meshes due to partial aggregation of free polymer strands. Freeze-dried membranes show a gas permeability two orders of magnitude higher then evaporation dried membranes. The absolute permeability strongly depends on the bacterial strain used for the polymer membrane synthesis and varies by up to 1.5 orders of magnitude for the same drying process. The Young's modulus of the polymer membranes varies with the bacterial strain used, but does not show the same trends as the permeability. Finally, a comparison of the characterized properties shows that only one of the tested strains shows the capability to synthesize membranes that meets the requirements for an application as a wet wound dressing.

Keywords: bacterial cellulose; drying process; membrane; rheology

Introduction

Hydrophilic polymeric membranes have in general a high swellability, high permeability for water vapour and gases, a good fluid transport across the membrane, as well as a high selectivity for the transport of voluminous and apolar substances. These properties in combination with an adequate mechanical strength make them highly suitable for the treatment of wounds as a coverage material. As already mapped out by Winter in 1962[1] and recently rephrased by Turner,[2,3] modern wound dressings require a moist climate with a sufficient fluid transport away from the wound and a sufficient gas transport to the wound across the membrane to assure an aerobic climate, while at the same time providing a barrier function against infections and a sufficient thermal isolation. The exact tailoring of the polymers and their three-dimensional (3D) structure to achieve the properties of a moist wound dressing is of utmost importance, since synergistic effects of the transport phenomena may lead to undesired substance accumulation.[4]

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To date there are already several moist wound dressings commercially available, most of them based on a synthetic polyurethane carrier matrix in combination with an embedded hydrogel (agar, gelatine or carboxymethyl cellulose) or as a sole gel based on concentrated carboxymethyl cellulose gum, Ca-alginates or collagen matrices.[5] However, especially for the treatment of problematic wounds like burns and chronic wounds the commercially available wet wound dressings are insufficient. Chronic wounds, that caused 2 million lost working days per year in 2000 in Germany alone (according to the compulsory health insurance funds, Germany 2001), as well as burns require an atraumatic wound dressing that can easily be removed since these wounds are especially sensitive to a retraumatization and have a high degree of wound pain.[6]

A wet wound dressing membrane for the treatment of problematic wounds is bacterial cellulose. The ability of the bacterium *Gluconacetobacter xylinus* to produce a highly pure cellulose was discovered in 1886 by Brown.[7] The polymer chains extruded by the bacterium are woven into larger fibrils and meshed by the micro- and macro-brownian motion of the bacterium. The ability to form homogeneous membrane sheets under certain synthesis conditions[8,9] lead to various applications of bacterial cellulose membranes. Bacterial cellulose membranes are now used as electronic paper display,[10] are under investigation for a pervaporative separation of aqueous organic mixtures[11,12], have shown to be an excellent carrier material for metal catalysts,[13] and have a tremendous success as artificial blood vessels[14,15] or as acoustic membranes in loud speakers.[16]

Bacterial cellulose membranes as a wound dressing are already commercially available in a dry state as Biofill™ (Allvet Quimica Industrial Ltd, Curitiba, PR, Brazil) and in a wet state as X-Cell™ (Xylos Corporation, Langhome, PA, USA). The advantage of bacterial cellulose as a wet membrane in comparison to available polyurethane based membranes is their high water permeability and an increased wound healing process.[6,17,18]

Regular cellulose has a long tradition as a wound covering material, originating from its good wound compatibility. Microbiologically produced high-purity cellulose in form of bacterial cellulose has the additional advantage that their high swellability prevents them from adhering to the wound ground. Bacterial cellulose has already been tested as a wound dressing for burns.[19] In contrast to chitosan as a material for wet wound dressings,[20] bacterial cellulose does not have antimicrobial properties; however, due to its high swellability, the bacterial cellulose membrane can easily be imbued by antibacterial solutions of silver salts, antibiotics or disinfectants.

While Sokolnicki *et al.*[21] performed investigations on fluid permeability through bacterial cellulose membranes, so far there are no investigations on gas permeability and the causal membrane parameters for bacterial cellulose. On the other hand, an understanding of how this material property changes with the swelling state an the drying process and how it can be properly adjusted is highly desirable, since the amount of wound exudate can vary from large quantities for burns to relatively dry conditions in chronic wounds. To achieve the optimal wet wound climate while avoiding anaerobe states, the wound dressing and its fluid and gas transport capabilities need to be tailored to the specific conditions. This requires an effective understanding of the membrane structure to enable the adjustment of the desired permeability, barrier function and thermal isolation of a wound with at the same time a mechanically stable membrane. The capability of bacterial cellulose in a wet and never dried state right after synthesis to absorb or provide fluid can already be adjusted by a partial, mechanical dewatering as described empirically in Ref. [22]. Furthermore, for an easy application in the treatment of strongly exudating burns a completely dried and therefore strongly

absorbing membrane is highly desirable. However, to date there have been no investigations on the structural changes of bacterial cellulose membranes during a drying process and the resulting physical and mechanical properties.

We therefore present in this paper investigations on the alteration of bacterial cellulose membranes by processes as swelling and drying and also as a result of the use of different bacterial strains for the synthesis of the membrane. We demonstrate how the observed morphological changes in the network parameters, pore sizes and surface structure result in improved properties as gas and fluid permeability, elasticity and ductility.

The paper is structured as follows; the first part of the discussion deals with alteration methods of bacterial cellulose membranes by different drying processes and discusses in detail the achievable structural bandwidth. The second part compares the properties of the membranes produced from different bacterial strains regarding their permeability and strength and application properties as moist wound dressings.

Experimental Part

Bacterial Cellulose Membrane Preparation

Strains of *Gluconacetobacter xylinus* for the production of bacterial cellulose were supplied by DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) (Table 1).

Table 1.
Parameters of the Bacterial Strains *Gluconacetobacter Xylinus* and the Produced Membranes after Seven Days of Culturing.

Membrane	Strain	Thickness	Dry Weight	M_w
		mm	mg	Kg/mol
1	ATCC 14851	0.245	23.3	
2	ATCC 10245	1.132	95.2	1420
3	ATCC 11142	1.569	100.2	1450
4	ATCC 23768	0.910	68.5	

Twenty milligram of the freeze-dried bacteria were inoculated in 10 ml sterile Hestrin-Schramm solution[23] containing 2wt% glucose and 1% ethanol added to promote cellulose production[24] and kept for 20 minutes at 20 °C. One millilitre of the culture broth was then inoculated into 50 ml of the medium and incubated at 30 °C for 7 days in static culture. After 7 days a white membrane had formed at the liquid/air interface that was kept to a constant circular area of 225 mm^2. The cellulose membrane was then removed from the beaker and thoroughly washed with a 0.1 M sodium hydroxide solution and subsequently with deionised water. This wet membrane is referred to here after as the ''never dried'' state. Depending on the used strain the wet membranes obtained different thicknesses after the 7 day culturing time, values are given in Table 1. To remove the water, the membrane is either dried at 60 °C for 6 h (''normal dried'' state) or shock frozen with liquid nitrogen and subsequently freeze-dried (''freeze-dried'' state). Dry weights of the membranes are given in Table 1. Comparison of the ratio of wet membrane volume to dry weight shows that the cellulose density in the wet membrane is nearly independent of the used strain.

Viscometry

The molar mass M_w of the synthesized celluloses was determined from intrinsic viscosities in a solution of 9wt% lithium chloride in N,N-dimethyl acetamide via the Mark-Houwink-Sakurada relationship $[\eta]/(g/ml) = 1.28 \times 10^{-4}(M_w/(g/mol))^{1.19}$[25] and is given in Table 1. For dissolution in the solvent, the cellulose was swollen for 1 day in H_2O, subsequently 1 day in ethanol, 1 day in acetone and 1 day in dry N,N-dimethyl acetamide. The cellulose was then dried under vacuum and subsequently dissolved under slow stirring for 5 days at RT in 9wt% lithium chloride in N,N-dimethyl acetamide. Intrinsic viscosities were determined using a micro-Ubbelohde viscometer with a No. IIc

capillary (Ø = 0.95mm) (Schott-Geräte GmbH, Mainz, Germany).

Infrared Spectroscopy

The spectra were recorded on a Nicolet Impact 410 (Thermo Electron Corporation, Waltham MA, USA). To remove water from the cellulose membranes in the never dried state while retaining the friable structure, a successive replacement of solvents (methanol, acetone, hexane) was performed.[26]

Scanning Electron Microscopy (SEM)

Scanning electron microscopy pictures of the samples were obtained with a Philips SEM 515 (Philips Electronics N.V., Eindhoven, Netherlands). A cryofixation of the wet and swollen membranes was achieved by shock-frosting the samples in liquid nitrogen and the subsequent removal of the solvent by freeze-drying at −70 °C under high vacuum. The samples were sputtered with a 10nm gold layer to assure a thorough coverage of the highly porous samples.

Rheological Oscillatory Measurements

The rheology of the membranes in dynamic shear flow for the determination of the storage modulus G' and loss modulus G'' was investigated using a TA Instruments Rheometric Series ARES rheometer (TA Instruments, Newcastle, DE, USA) with plate and plate fixtures (Ø = 50 mm). All moduli were measured in the experimentally determined linear–viscoelastic limit of deformations of 0.5–1.9%. The plateau modulus G'_p, was determined from the frequency independent regime of the storage modulus G'. Since the growth process of the membranes starts in an insular way, the membrane is not in all parts completely regular. To assure a continuous contact of the not completely even surface of the membrane to the surfaces of the fixtures, a normal force of 3 N (never dried membrane), 5 N (freeze-dried membrane) and 20 N (evaporation dried membrane) was applied. The normal forces used lie in the applicable regime of normal forces that do not influence the measured moduli, determined by series of frequency sweeps at increasing normal forces as described in Ref. [27]

Rheological Stress - Strain Measurements

The stress – strain behaviour of the membranes was tested on a Z010 material tester (Zwick/Roell, Ulm, Germany). The test samples were cut from the membranes to a length of 50 mm and a width of 4.1 mm. The strain along the length of the sample was increased with a velocity of 0.1 mm/s.

Gas Permeation

The gas permeability of the membranes was tested with a custom built pressure-gradient apparatus (GKSS, Geesthacht, Germany), that allows the determination of the gas flux at discrete pressure gradients through the membrane. The pressure gradient was stepwise increased with time until steady flux conditions were reached. The apparatus allowed for the testing of permeabilities for different gases (H_2, O_2, CO_2 and N_2) in a single run.

Results and Discussion

Not all strains of *Gluconacetobacter xylinus* show the formation of a membrane, although all strains produce cellulose. In the following we compare the membranes of four different strains that were determined in preliminary experiments to actually show a membrane formation.

The mechanical strength and permeability of bacterial cellulose membranes were already reported to be sensitive to the treatment after the actual synthesis. Seifert et al. found that the swellability of a bacterial cellulose membrane depends on the subsequent drying process and that different swelling ratios are observed for reswollen membranes.[28] They also suggested a modification of the drying membrane by the addition of linear polymers. As a possible way to retain the sensible structure of the primary wet, Madihally et al. suggested a freeze-drying process for membranes[29] and we will follow this

approach to obtain cellulose membranes in addition to a normal drying process via simple evaporation of the solvent.

Influence of the Drying Process on Network Structures

A change of the network structure of the bacterial cellulose by the different drying processes is already visible with SEM images. As shown in Figure 1 the freeze-dried membrane shows more filigree and thinner fibres than the evaporation dried membrane. However, to compare of the network structure, in particular the mesh width and number of crosslinking points, simple visual observation cannot provide sufficient information.

A convenient way to directly access the network structure of a membrane is via non-intrusive oscillatory shear experiments. A determination of the network parameters of the membrane is possible via the frequency independent plateau region of the storage modulus G′ from a small amplitude oscillatory shear experiment ('plateau modulus') as shown in Figure 2.

In this frequency range the induced energy of an applied sinoidal shear deformation of the membrane is stored elastically by the polymer strand between two entanglement or network points, acting as entropic springs. Therefore the number density ν of these structural elements can directly be calculated from the measured plateau modulus[30] by the simple relation:

$$G'_p = \nu k_B T \tag{1}$$

where k_B is the Boltzmann constant. The plateau value of the storage modulus G'_p can then be related to the absolute number n of structural elements, once the volume V is known:

$$G'_p = \frac{n}{V} k_B T \tag{2}$$

A simple swelling of the network due to solvent does not change the absolute number n of polymer strands, however, the volume V and therefore the number density ν does. The definition of the swelling ratio Q as the volume ratio of two different states I and II

$$Q_{I\rightarrow II} = \frac{V_{II}}{V_I} \tag{3}$$

shows that the reciprocal ratio of the plateau moduli is directly correlated to the swelling ratio and the ratio of absolute numbers of strands:

$$\frac{G'_{p,I}}{G'_{p,II}} = Q_{I\rightarrow II} \frac{n_I}{n_{II}}. \tag{4}$$

If there is no structural change, the ratio of absolute numbers of polymer strands n_I/n_{II} is unity and the ratio of moduli reduces to the swelling ratio $Q_{I\rightarrow II}$.

It should be noted that, even for a constant number of strands, the netpoint size can increase and this will lead to shorter polymer strands. This will not affect the number density of netpoints, since the number density does not incorporate the length scale of the structural units. An increasing netpoint size is possible, due to

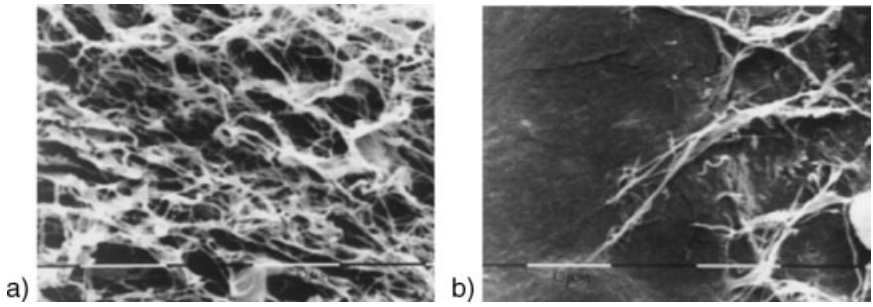

Figure 1.
SEM images of a) freeze-dried and b) evaporation dried bacterial cellulose membranes. The images are taken from the surface of the membranes. The length of the scale bar is 10 μm.

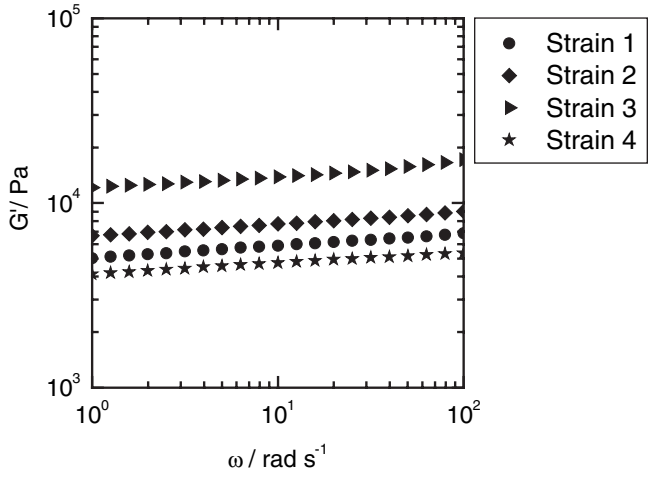

Figure 2.
Storage modulus G′ as a function of the applied frequency ω in an oscillatory shear experiment for never dried bacterial cellulose membranes from different strains with an applied normal force of 3 N.

the latent tendency in a polysaccharide-based network to expand the crosslinking via additional hydrogen bonds at the netpoints. However, in most cases for a self aggregating system the structural change should lead to either completely collapsing network meshes (therefore a ratio of $n_I/n_{II} < 1$) or to the formation of additional interconnections of polymer strands and therefore a ratio of $n_I/n_{II} > 1$.

For the bacterial cellulose membrane it is possible to compare structural changes for the initial wet state of the never dried (subscript nd), the freeze-dried (subscript fd) and the normally dried by evaporation (subscript ed) state. A general comparison of the swellability is shown in Figure 3 with swelling ratios Q referring to an initial volume of the dry membrane (obviously defined for the never dried membrane in a recursive way). The different strains show comparable swelling ratios with largest values for the never dried membrane around 80, for freeze-dried membranes at 25 and lowest values for the normally dried membranes of 4.

Comparing now the structural change from the never dried membrane induced by

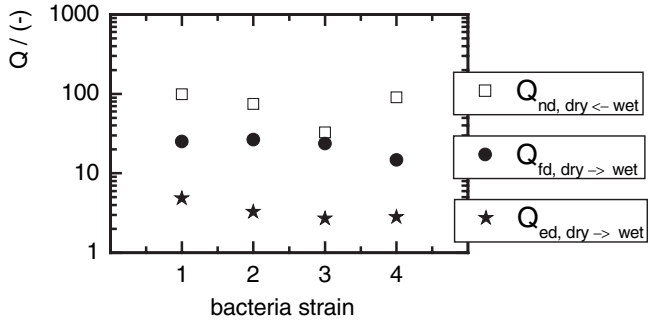

Figure 3.
Swelling of bacterial cellulose membranes from 4 different strains in a never dried (nd) state, evaporation dried (ed) state and freeze-dried (fd) state.

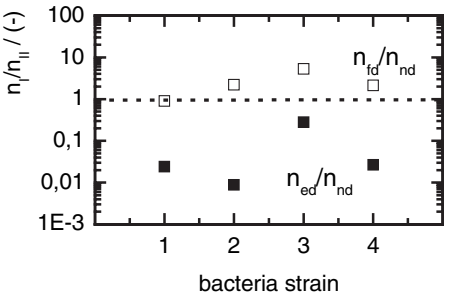

Figure 4.
The ratio of absolute numbers of polymer strands n_I/n_{II} for 4 different strains and two different drying processes: from the wet, never dried state (nd) to an evaporation dried membrane (ed) (closed symbols) or to a freeze-dried membrane (fd) (open symbols).

the different drying processes via the ratios

$$\frac{n_{fd}}{n_{nd}} = \frac{G'_{p,fd,wet}}{G'_{p,nd,wet}} \frac{Q_{fd,dry\rightarrow wet}}{Q_{nd,dry\leftarrow wet}} \quad (5)$$

for the freeze-drying process and

$$\frac{n_{ed}}{n_{nd}} = \frac{G'_{p,ed,wet}}{G'_{p,nd,wet}} \frac{Q_{ed,dry\rightarrow wet}}{Q_{nd,dry\leftarrow wet}} \quad (6)$$

for the normal evaporation drying process, Figure 4 shows that the freeze-dried membranes show values slightly above the critical value of 1. This indicates that during the freeze-drying process due to partial aggregation of free polymer strands the actual number of strands has increased. However, the original pore structure of the never dried membrane is mainly obtained.

In contrast to this, the membranes dried by normal evaporation show values up to 2 decades below the critical value of 1, indicating that during the slow evaporation drying process polymer strands completely aggregate and are removed from contributing to the elasticity of the membrane. This is also reflected in the lower swelling ratio of the evaporation dried membrane in Figure 3.

This interpretation of the changed ratios of structural elastic units is supported by the IR-spectra of the membranes shown in Figure 5. To retain the friable structure of the cellulose in the never dried state, a successive replacement of solvents (methanol, acetone, hexane) has been performed.[26]

As suggested by Putiev et al., the degree of supramolecular order in aggregated cellulose strands can be correlated with the ratio of absorption rates of the bands at 890 cm^{-1} and 1371 cm^{-1}.[31,32] As shown in Table 2, already for the freeze-dried membrane this ratio shows a slight increase

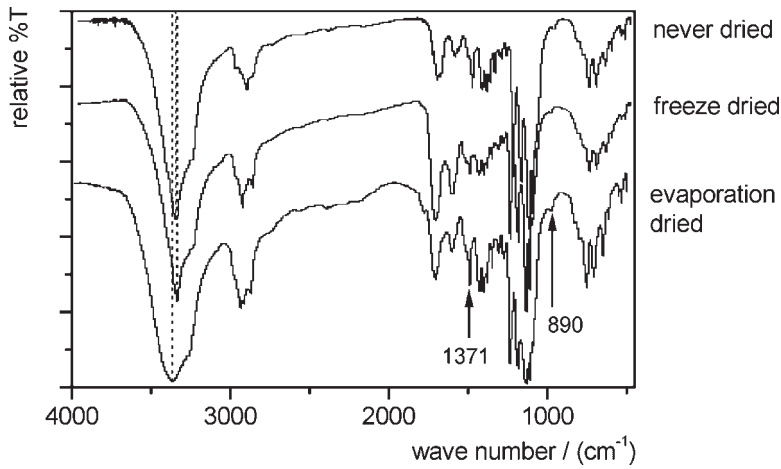

Figure 5.
FT-IR spectra of the never dried, evaporation dried and freeze-dried membrane. The spectrum of the never dried membrane was obtained in an apolar solvent and in the absence of water via a successive replacement of solvents.

Table 2.
Comparison of Critical Parameters of IR-Spectra for Different Drying Processes of Bacterial Cellulose Membranes.

Desiccation condition	Ratio of absorption bands' intensity I_{890}/I_{1371}	ν_{OH} - maximum cm^{-1}	OH-groups attitude of absorption right and left half-width	ν_{H2O} cm^{-1}
Solvent replacement	0.151	3347.4	1.35	1653.4
Evaporation dried at 21 °C (room temperature)	0.250	3346.7	1.05	1653.0
Freeze-dried	0.177	3346.2	1.77	1649.4

in accordance with the observed slight increase of the ratio of absolute numbers of polymer strands denoted to a partial aggregation of free polymer strands.

For the evaporation dried membrane, Table 2 shows a strong increase of aggregated celluloses strands in accordance with a complete collapse of elastic structural units in the membrane. In addition to this, the IR-spectrum for the evaporation dried membrane gives an increased ratio of right to left half-width of the OH-absorption band at ~3347 cm^{-1} compared to the never dried and freeze-dried membranes, along with a shift of the maximum to higher wave numbers, indicating an increased degree of intramolecular hydrogen bonding.[31,32]

Permeability of Bacterial Cellulose Membranes

The structural changes induced by the different drying processes reflect in the gas permeability L of the dried membranes. In this case the permeability is defined as the gas volume V per time t that permeates through an area A of membrane for a pressure gradient Δp across the membrane:

$$L = \frac{V}{tA\Delta p} \tag{7}$$

Since the gas permeability L depends on the thickness d of a membrane, for a better comparison Figure 6 shows the reduced permeability that depends only on the membrane structure.

$$L_{red} = Ld, \tag{8}$$

As can be seen in Figure 6 for the reduced permeability, the freeze-dried membranes have on average permeability two orders of magnitude higher than the evaporation dried membranes. Even taking into account that the evaporation dried membranes with an average thickness of ~60 μm are a factor 5 thinner than the freeze-dried membranes, the freeze-dried membranes still have a factor 20 higher permeability due to their nearly completely retained pore structure. However, the freeze-drying process is less reproducible with respect to the obtained permeability; the evaporation drying process gives in this respect relatively constant values for different drying runs. Therefore Figure 6 also allows for a comparison of the permeability of the membranes produced by different bacterial strains. Strains number 3 and 4 show in this respect a much less permeable evaporation dried membrane than the others.

Mechanical Strength

The importance of the bacterial strain used to synthesize a cellulose membrane does

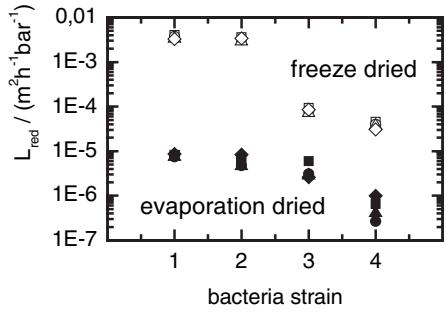

Figure 6.
Comparison of the reduced gas permeability L_{red} for freeze-dried (open symbols) and evaporation dried (closed symbols) bacterial cellulose membranes from four different strains (squares: N_2; circles: O_2; triangles: CO_2; diamonds: H_2O).

not only show in the permeabilities, but also reflects in the mechanical strength of the membrane. The capability to sustain a minimum stress without rupturing, while at the same not to exceed a limiting rigidity defines a narrow window of applicability of the membrane in particular with regard to a usage as a wound dressing. A stress-strain test that monitors the stress evolution in a continuously, uniaxially strained membrane up to the rupture level allows for a comparison to critical criteria that generally describe the applicability of membranes to a semi-soft surface as for example the human skin.

First of all the membranes have to meet a minimum fracturing stress level before rupturing. This limiting value, shown as a dotted line in Figure 7, depends also on the strain, as empirical tests have shown that it is easier to avoid stress peaks during application at larger deformations.[27] As it can be seen in Figure 7 for the evaporation dried membrane in the stress-strain test, the membranes show large differences in this sustainable stress. While two strains produce membranes that actually reach the order of required minimum stresses for applicability, the two other membranes rupture at much lower stress and strain levels.

The second key criterion for the applicability of a membrane is the Young's modulus E, observable as the initial slope of the curves and a measure for the stiffness:

$$E = \frac{\sigma}{\varepsilon} \tag{9}$$

Here σ is the stress and ε the strain or deformation. Membranes with Young's moduli above a critical level are simply not flexible enough to allow for a sufficient bending or stretching during hands-on application. Therefore, Figure 7 also gives an empirical critical Young's modulus of $E = 755$ MPa, introduced by Clasen et al.,[27] above which the rigidity of the membrane prohibits the practical applicability as a wound dressing.

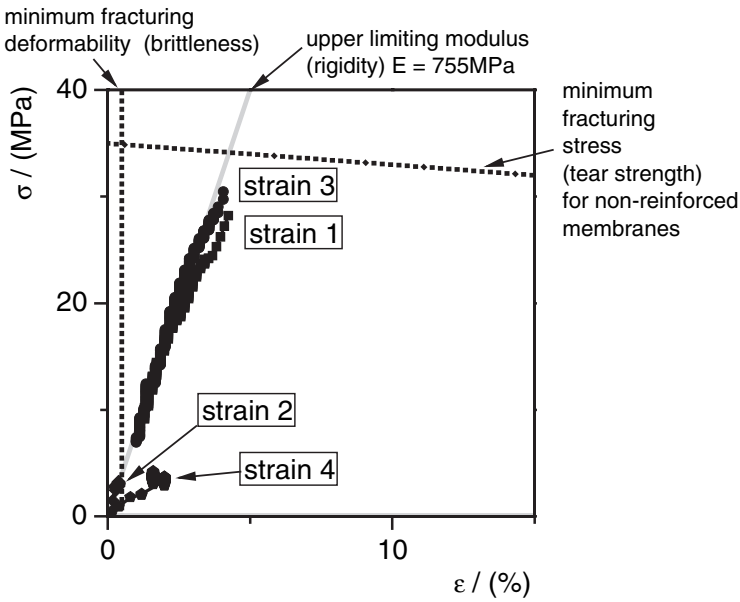

Figure 7.
Stress σ as a function of the applied strain ε in a uniaxial continuous deformation for normally dried bacterial cellulose membranes from four different strains. Values for critical levels of deformability, modulus and fracturing stresses for the applicability of membranes as wound dressings are taken from Ref. [27].

The investigated bacterial cellulose membranes are relatively stiff, showing Young's moduli that are close to this empirical upper rigidity limit for membranes of $E = 755$ MPa. Very rigid membranes show brittleness with rupturing conditions below a minimum fracturing deformability, however, this level is reached or surpassed by all investigated membranes.

A comparison of the mechanical strength of membranes produced by different strains shows marked differences with regard to the maximum stress that the membranes can sustain. In this context only membranes from strains 1 and 3 reach the minimum stress level indicated in Figure 7 and are applicable as wet wound dressings.

It is important to note that the mechanical strength and the permeability do not show the same trend for the different bacterial strains. Strain number 1 is in this respect the only suitable strain to produce membranes for the application as wet wound dressings as it shows the best permeability and a sufficient mechanical stability. However, it should be kept in mind that bacterial cellulose membranes can be synthesized to larger thicknesses by longer incubation times. Therefore even though the structure of a membrane by a certain strain might be less stable, the overall strength can be increased to an applicable level, if the permeability is high enough to assure a sufficient fluid and gas transfer even for the thicker membranes.

Conclusions

The properties of bacterial cellulose membranes for the application as a wet wound dressing strongly depend on the bacterial strain and on the drying procedure of the membrane. It could be shown that freeze-drying as well as evaporation drying reduces the swellability of a bacterial cellulose membrane significantly. While the swellability for a freeze-dried membrane is reduced on average by a factor of 5, the evaporation drying process causes a reduction by a factor of 50. This strong decrease of swellability for an evaporation dried membrane could be correlated with a reduction of the absolute number of polymer strands that form the network structure of the membrane. The removal of network meshes by a complete aggregation of polymer strands could be confirmed by IR-spectroscopy with an increased degree of intramolecular hydrogen bonding of cellulose strands.

In contrast to this, the freeze-drying process shows a slight increase of the number of network meshes due to partial aggregation of free polymer strands.

Measurements of the gas permeability show values that are on average two decades larger for freeze-dried membranes than for evaporation dried membranes. However, the absolute permeability strongly depend on the bacterial strain used for the polymer membrane synthesis and may vary up to 1.5 decades for the same drying process. Also the mechanical stability of the membranes varies with the utilized bacterial strain, but does not show the same trends as the permeability. Therefore only one of the tested strains (ATCC 14851) shows the capability to synthesize membranes that meet the requirements for an application as a wet wound dressing. This demonstrates the possibility to improve the membran's properties by a systematic selection and testing of *Gluconacetobacter xylinus* strains.

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