

1 **Bacterial Cellulose Films: Influence of bacterial strain and** 2 **drying route on film properties**

3 Muling Zeng, Anna. Laromaine*, Anna Roig

4 Institut Ciència de Materials de Barcelona, Campus UAB, 08193 Bellaterra, Spain.

5 E-mail: alaromaine@icmab.es

6
7 Keywords: bacterial cellulose, films, transparent, bacterial strain, water adsorbent

8 9 **Abstract**

10 Bacterial cellulose is increasingly used as reinforcing material or scaffolds for smart
11 electronics or biomedical applications due to its multifaceted advantages like natural
12 abundance, eco-friendliness, cost-effectiveness, biocompatibility and easy chemical
13 modification. Structural and functional properties of bacterial cellulose depend on the
14 microstructure of the material, which in turn is influenced by the cellulose's origin. This paper
15 reports the production of bacterial cellulose thin films from two bacterial strains,
16 *Gluconacetobacter Xylinus* and *Gluconacetobacter Europaeus*, and three methods of drying
17 the thin films; at room temperature, freeze drying and supercritical drying. We have
18 undertaken for the first time a comparative study of how several material's properties such as
19 porosity, transparency, water absorption capacity and mechanical properties are or not
20 affected, and thus can be tuned to some extent, by selecting the bacterial strain or the drying
21 method. For instance, using supercritical drying, we obtained mechanically robust and
22 extremely light films with up to 96 % of porosity, and with a water absorption capacity up
23 110 times their dried weight. Finally, we suggest the appropriate choice of strains and drying
24 methods for different applications, for instance to obtain cellulose composites with high
25 efficiency in the loading of the components.

26 **Introduction**

27 The interest in cellulose and cellulose composites materials have recently expand due to their
28 sustainable and environmentally friendly sources (vegetable or bacterial), their functional and
29 structural properties and applications.(Hu et al. 2014) Its facile derivatization and
30 formulations yields organic-inorganic cellulose composites materials which usually as films
31 find application in oil and water absorbents,(Sai et al. 2013; Nata et al. 2011; Ul-Islam et al.
32 2012b; Jin et al. 2011) organic light emitting diodes, (Ummartyotin et al. 2012) flexible and
33 transparent electronics(Wicklein and Salazar-Alvarez 2013) to name a few.(Klemm et al.
34 2011)

35 Vegetal cellulose is extracted from plants and wood and bacterial cellulose (BC) is produced
36 by microbial fermentation. Although with higher production cost than vegetal cellulose, BC
37 offers a pure biopolymer that exhibits a higher degree of polymerization and better
38 crystallinity than plant's cellulose.(Ross et al. 1991) They exhibit high water content, high
39 elasticity and mechanical stability. Importantly, BC does not contain lignin, hemicelluloses
40 and pectin, non-degradable molecules, associated to the toxicity of celluloses.(Ul-Islam et al.
41 2012a; Czaja et al. 2007) Therefore, BC composites not only find application in electronics or
42 optics but also in the food industry(Shi et al. 2014) (calorie-free dessert)(Kalia et al. 2011)
43 and in medical fields (tissue engineering,(Svensson et al. 2005; Andrade et al. 2013; Saska et
44 al. 2012) and wound healing patches(Ul-Islam et al. 2012a; Fu et al. 2013)).

45 The blending of components to form cellulose composites although it is simple, offers a poor
46 control of the mixture's homogeneity decreasing the performance of cellulose composites. In
47 order to fabricate homogeneous cellulose composites with high loading of components,
48 researchers take advantage of the high adsorption capability and structural properties of the
49 cellulose fibers. The cellulose origin and processing treatment strongly influence the final
50 characteristics of the composites.(Pinto et al. 2012)

51 In order to improve the BC films adsorption capabilities and porosity, we dried the harvested
52 BC thin films using three different methods, room temperature (RD), freeze drying (FD) and
53 using CO₂ supercritical (SCD) without any further mechanical treatments to the cellulose
54 fibers. We obtained a plethora of materials with different adsorption capabilities and
55 improved structural properties with few fabrication steps. The selected physical processes
56 evaporate the solvent content within the film, while minimizing hornification processes of the
57 cellulose fibers. When drying at room temperature (RD), capillary pressures of the water
58 meniscus exert a compressive force in the pores of the films that may induce the modification
59 of the structure, density and porosity of BC films. Freeze drying (FD) process dries the film
60 through sublimation of solid water after the BC films have been plunge-frozen in liquid
61 nitrogen. Supercritical drying (SCD) involves the exchange of the water solvent within the
62 BC film by ethanol, a final exchange with liquid CO₂ in the reactor and the final evacuation of
63 the CO₂ in supercritical phase. Evacuation of solvents in supercritical conditions is commonly
64 used to produce aerogels which is a term given to open pore materials presenting low density
65 and large specific surface area. The most known aerogels are silica,(Murillo-Cremaes et al.
66 2010; Moner-Girona et al. 2003; Martin et al. 2008; Budunoglu et al. 2011) carbon,(Wu et al.
67 2013; Mecklenburg et al. 2012) organic-inorganic aerogels(Hendel et al. 2013; Ennajih et al.
68 2012) and more recently thick pieces of BC aerogels have also been reported (Hendel et al.
69 2013; Liebner et al. 2010; Cai et al. 2008; Russler et al. 2012).

70 Additionally, we compared the drying methods along with the BC films produced from two
71 bacterial strains, *Gluconacetobacter Xylinus* (GX) and *Gluconacetobacter Europaeus* (GE), to
72 evaluate the impact of the bacterial origin. *Gluconacetobacter Xylinus* is the most extended
73 bacteria used to produce cellulose, some reports justifies its commercial interest due to the
74 fast cellulose production speed. *Gluconacetobacter Europaeus* (formerly *Acetobacter*
75 *Europaeus*) is one of the most prominent acid acetobacter bacteria species isolated from

76 industrial submerged vinegar fermenters with high resistance to acetic acid and it is much less
77 studied for cellulose production than the GX counterpart.(Andrés Barrao et al. 2011)

78 We compared the microstructure, water holding capacity, transparency and mechanical
79 properties of BC films of less than 100 μm in thickness, and produced by two bacterial strains.

80

81 **Experimental Section**

82 Bacterial strains *Gluconacetobacter Xylinum* (GX) (ATCC 11142)(Yamada et al. 1997) and
83 *Gluconacetobacter Europeaus* (GE) (MF03)(Yamada et al. 1997) were purchased from CECT
84 (Spain).

85 Glucose, peptone, yeast extract and agar were purchased from Conda Lab, and the NaOH,
86 $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and citric acid monohydrate were bought from Sigma Aldrich and used as
87 received.

88

89 **Synthesis of BC**

90 GX and GE were grown on a solid agar and a single colony was expanded in liquid media for
91 3 days. Then, 8 ml solution was transferred to an Erlenmeyer of 1L with 200 mL of liquid
92 media. A thin film of bacterial cellulose grew on top of the liquid media over 5 days for GX
93 and 10 days for GE. Culture media for GX consisted of 20 g/L glucose, 5 g/L peptone, 5 g/L
94 yeast extract, 1.15 g/L citric acid monohydrate and 6.8 g/L $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. Culture media
95 for GE was composed of glucose 50 g/L and yeast extract 10 g/L.(Liebner et al. 2010; Kim et
96 al. 2010; S. Hestrin 1954)

97 BC films harvested from the air/liquid interface were immersed in ethanol. Subsequently they
98 were transferred to deionized (DI) water and boiled for 40 min, four times more with 0.1 M
99 NaOH at 90 °C for 20 min and finally, neutralized with DI water for 24 h.

100

101

102 **Drying methodologies**

103 Never dried Bacterial cellulose (BC) films were cut into a rectangular shapes of 1 x 2 cm.
104 Before drying, samples were placed over a sheet of chromatography paper and kept between
105 two glass slides during the drying process. We created an accordion-like setup for each drying
106 methodology, up to 10 BC films were placed in the same setup. BC fibers were not
107 mechanically modified and the BC film maintains the shape from the bacterial culture. Figure
108 S1 presents schematics and pictures of the drying setup.

109

110 **Room temperature drying method (RD)**

111 BC films placed in the accordion setup were kept at room temperature for 4 days until the BC
112 films were completely dried.

113 **Freeze-drying method (FD)**

114 We plunge-freezed the samples in the accordion setup with liquid nitrogen for 5 min in a 50
115 mL falcon tube. After, they were placed in the freeze-drier for 12 h. Freeze-drying of the
116 samples was performed with a LYOQUEST-85 freeze drier (Telstar) at -80 ° C, below 0.005
117 mbar for 12 h.

118 **Supercritical CO₂ drying method (SCD)**

119 BC films were subjected to a solvent exchange step, which was performed by gently shaking
120 them in absolute ethanol. After 6 and 12 h, the gels were transferred two times to a fresh
121 ethanol bath without shaking. After another 6 h in ethanol, the resulting alcogels were dried.
122 Supercritical process was performed on SCF 300 ml Plant in MATGAS, Spain. Alkogels BC
123 films were placed in the accordion setup and loaded into a 300 ml autoclave vessel filled with
124 ethanol. The autoclave was pressurized to 100 bar at room temperature. We exchanged the
125 ethanol with liquid CO₂ flowing at 1 kg/h for 1.5 h. We heated the reactor at 45 °C; with a
126 CO₂ flow of 1 kg/h for 1 h (these are the SCD conditions, 45 °C, 100 bar). After the drying
127 period, we slowly depressurized the autoclave and removed the dry aerogels.

128

129 **Characterization of BC films**

130 **Fourier Transform Infra-Red Spectroscopy with Attenuated Total Reflectance (FTIR-** 131 **ATR)**

132 We folded 5 times the BC films and placed them onto the Universal diamond ATR top-plate
133 (Perkin Elmer). IR spectra were acquired using a PerkinElmer FT-IR Spectrum One with U-
134 ATR at 4 cm^{-1} resolution, between 4000 to 500 cm^{-1} using 4 scans and a pressure force
135 between 80 % and 90 %.

136 **X-Ray Diffraction (XRD)**

137 XRD patterns of the BC films were recorded using an X-Ray Diffractometer (Siemens, Model
138 D-5000), using a Cu anode with $\lambda_{K\alpha 1}=1.540560\text{ \AA}$ and $\lambda_{K\alpha 2}=1.544390\text{ \AA}$ in the 2θ range of 3
139 -60° using a step of $0.02^\circ/\text{min}$. All samples were fixed flat on a silicon wafer to perform the
140 measurement.

141 We calculated the crystallinity of BC films using the height of peaks (CrI)(Segal et al. 1959;
142 Wang et al. 2008) computed them using Spectrum Viewer Basic 2.6. The height of the peak at
143 (200) represents the crystalline part $I_{(200)}$ and the minimum height between (200) and (110)
144 peaks the amorphous part, $I_{(am)}$. We also used the same equation 1 but instead of the height of
145 the peaks, we used the area, and we obtained the same results. (Table S1)

146 Equation 1
$$\text{CrI} = (I_{(200)} - I_{(am)}) / I_{(200)}$$

147 The crystal size using the (200) peak was computed using Scherrer's equation:(Mansikkamaki
148 et al. 2007; Zhang et al. 2001)

149 Equation 2
$$L(hkl) = k\lambda / (B\cos\Theta)$$

150 Where L is the crystallite domain, λ is the X-ray wavelength, Θ is diffraction angle in radians
151 and B is the full width at half maximum of peak ($B^2 = B_{\text{total}}^2 - B_{\text{instrument}}^2$), B_{total} is the integral
152 breadth in radians of diffraction angle and $B_{\text{instrument}}$ is the instrumental integral breadth in
153 radians and k (Scherrer's constant) is a dimensionless shape factor. The shape factor has a

154 typical value of about 0.9, but varies with the actual shape of the crystal, $k = 0.94$ was chosen
155 in our case. (Das et al. 2010; Dietrich et al. 1987)

156

157 **Scanning Electron Microscopy (SEM)**

158 All BC film samples were gold coated for 1 min at 20 mA, approximately with 1 nm of gold
159 with a K550 Sputter Coater (Coating Attachment Emitech. Ashford, UK). Samples were
160 placed on a SEM aluminum substrate over a carbon tape adhesive. Images of Scanning
161 Electron Microscopy (QUANTA FEI 200 FEG-ESEM) were taken at high vacuum
162 conditions, an acceleration voltage of 10-30 kV, an electron beam spot of 2.5, a pressure of 2
163 to 8×10^{-4} Pa and a distance of 2 – 6 mm. SEM images of BCE-RD samples were acquired at
164 the low vacuum conditions, an acceleration voltage of 10 kV, an electron beam spot of 2.5, a
165 pressure of 40 Pa and a distance of 5 mm to improve the image acquisition.

166

167 Thickness of the films were computed using an optical microscope in differential interference
168 contrast mode (n=10) (Olympus IX-71, program of Stream Essential 1.7).

169

170 **Thermal Gravimetric Analysis (TGA)**

171 Thermal gravimetric analysis of the BC films was performed with a TGA-DSC/DTA analyzer
172 (NETZSCH STA 449 F1 Jupiter, ICMAB) with a heating rate 20 °C/min from room
173 temperature to 800 °C in air.

174

175 **Water Absorption Capacity (WAC)**

176 BC films grown for 10 days were dried and weighted. We immersed them in DI water for 1 h,
177 2 h and 2 days. Excess water was removed at each time and measured the weight again. The
178 water absorption capacity was defined by:

179 Equation 3

$$W = (W_{\text{wet}} - W_o) / W_o$$

180 W_{wet} (weight of wet BC films), W_o (initial weight of the dried BC films).

181

182 **Optical transmittance**

183 The optical transmittance of the films was measured using an ultraviolet-visible (Uv-vis)
184 spectroscopy (Shimadzu UV/VIS UV-2102 spectrometer) in the 250 nm to 800 nm range.

185 Samples were carefully cut into the rectangle shapes as one side of the cuvette; they were
186 placed in the front side of the Uv-vis spectroscopy cuvette in order to take the spectrum.

187

188 **Mechanical properties**

189 Penetration depth at maximum load (0.4mN), Hardness, Young's modulus and Elastic
190 parameter (EP%) of BC films were obtained with a nanoindenter (Nanoindenter XP system)
191 with a Berkovich diamond indentation tip. All samples were cut into 0.5 x 1 cm and were
192 fixed flat on the holder using double-sided tape, without any further preparation. Indentation
193 curves were obtained at a loading and unloading rate of 0.08 mN/s and maximum load of 0.4
194 mN. BC films were measured 5-10 times in regions separated within 5 μm each measurement
195 in order to evaluate and average the whole surface. Two different BC films samples were
196 evaluated for each type of material.

197 EP parameter was computed with:

198 Equation 4: EP % = (penetration depth at max loading - penetration depth after unloading)/
199 (penetration at max loading) %.

200 **Evaluation of mechanical properties and directionality of fibers**

201 BC-SCD films (1 \times 2 cm) were cut into 2 smaller pieces (1 \times 1 cm). One was fixed flat on the
202 holder by double-sided tape; another piece was turned 90 $^\circ$ and then fixed on the same holder.
203 Pieces were tested with the same setup conditions by Nanoindenter XP system.

204

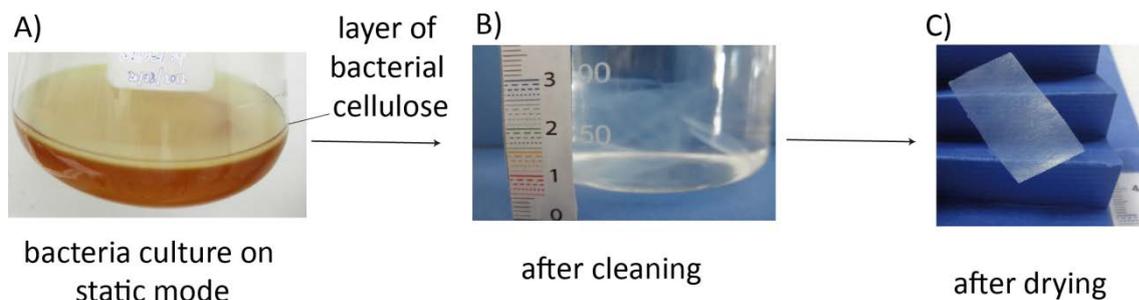
205

206

207 **Results and discussion**

208 **Production of thin BC films**

209 Two bacterial gram-negative strains, *Gluconacetobacter Xylinum* (GX) and
210 *Gluconacetobacter Europeaus* (GE) produced thin films of bacterial cellulose (BC). First,
211 harvesting times of the two strains were adjusted to obtain similar film thicknesses. 10 days of
212 culture for GE and 5 days for GX yielded BCE and BCX films of comparable thicknesses at
213 the top of the culture liquid media of an erlenmeyer at 25°C, Figure 1A. Prior to films drying,
214 BC layers were cleaned to remove any bacterial detritus following a previously reported alkali
215 procedure.(Liebner et al. 2010; George et al. 2008) After the alkali treatment, BC films
216 increased their transparency, Figure 1B.



217

218 **Figure 1. (A) Picture of a BC layer on top of the bacteria culture liquid media. (B)**
219 **Cleaned BC films obtained after the NaOH treatment. (C) Picture show a final BC film**
220 **dried and the accordion setup were BC films are held during the drying process.**

221

222 Films were then dried using three methods: a) solvent evaporation at room temperature (RD),
223 b) freeze drying (FD) and c) using supercritical CO₂ (SCD). In all cases, wet films were
224 placed in a home-made holder. A paper accordion construct held BC films in between two
225 glass slides to ensure regular shapes and prevent bending during drying, Figure S1. This
226 design afforded flat and thin films of less than 100 µm in thickness, Figure 1C, with few steps
227 and different properties.

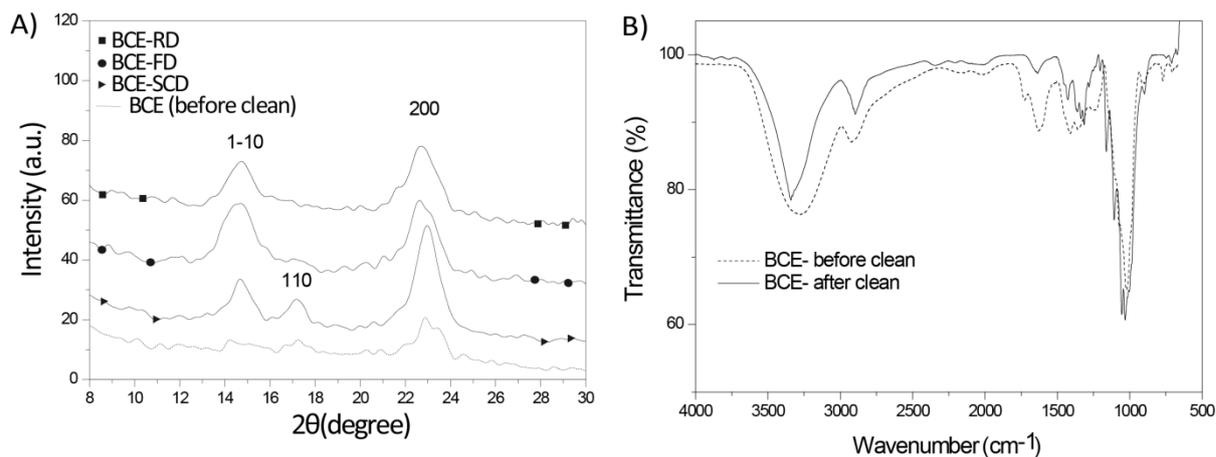
228

229 **Characterization**

230 BC films are a semi crystalline material and produce broad diffraction peaks. Figure 2A
231 shows the XRD patterns of BCE films treated with different drying methods. Native cellulose
232 is present in two different crystalline cellulose I modifications (I_α and I_β), which can be found
233 alongside each other at $2\Theta = 14.24$ and 17.36 corresponding to $\langle 1-10 \rangle$, $\langle 110 \rangle$ respectively,
234 the I_α/I_β ratio depends on the origin and the processing of the cellulose. (Klemm et al. 2005;
235 Atalla and Vanderhart 1984) After the alkali treatment, all BC films showed two main peaks
236 at $2\Theta = 14.6$ and 22.6 arising from crystalline planes $\langle 1-10 \rangle$ and $\langle 200 \rangle$, respectively, and
237 BCE-SCD also showed a small peak at $\sim 17.1^\circ$ (2Θ) arising from the $\langle 110 \rangle$ plane.
238 Relative crystallinity (CrI) of BCE-SCD, BCE-FD and BCE-RD films was calculated using
239 the intensity of the $\langle 200 \rangle$ peak considering equation 1 and yielding 82.9 %, 75.6 % and
240 72.5 %, respectively. In addition to the highest crystallinity observed for the BCE-SCD films,
241 those also present a slightly smaller crystalline domain (15 nm) as compared to FD (16 nm)
242 and RD (17 nm). Since all BC films derive from the same initial sample, any variations in the
243 crystallinity and crystal size are caused by the drying methods. The higher temperature of the
244 drying with SCD, 45°C , could afford this small increase of crystallinity. We obtained the same
245 patterns for BCX films, indicating that the crystallinity of the cellulose fibers are independent
246 of the bacterial strain.

247

248 The IR spectrum of the BC films are characterized by a strong and broad band around ν (CH)
249 (OH): $3342, 3347 \text{ cm}^{-1}$ corresponding to the overlap of the stretching vibrations of C-H and O-
250 H respectively and a strong peak at ν (CH_2 and CH) around 2895 cm^{-1} . Cleanliness of the
251 materials can be monitored by the decrease of the amide I (ν (strong) $_{\text{C=O}}$: 1642 cm^{-1}) peak,
252 Figure 2B.(Klemm et al. 2001)



253

254 **Figure 2. (A) X-ray diffraction pattern for BCE- RD, BCE- FD, BCE- SCD and non**
 255 **cleaned BCE film. (B) FTIR spectra for a non-cleaned BCE film and a BCE film after**
 256 **cleaning.**

257 Thermogravimetric analysis of BC films showed the typical single step thermal degradation
 258 profile with a decomposition temperature around 350 °C, Figure S2. The residual mass of BC
 259 films is less than 4 % indicating BC films were free of impurities. These values agree to
 260 previously TGA analysis reported for BC materials after alkali treatment. (George and
 261 Ramana 2005)

262

263 The thickness and size of wet and dry films were measured using an optical microscope in
 264 differential interference contrast microscopy mode. Table 1 summarizes the film thicknesses
 265 of the dried films (n=10). For both strains, films dried in SCD conditions are thicker than
 266 films obtained by FD and RD. The elimination of the solvent meniscus during supercritical
 267 solvent evacuation, avoids the effects of the capillary forces, as described previously for other
 268 aerogel materials. The initial thickness of the wet BC films were around 200 μm and after
 269 drying the computed thickness of all BC films decreased, ranging from 67 μm to 24 μm .

270

271 Density values (ρ) were determined from weighting the materials and measuring the BC films'
 272 volume. Films produced by GE are extremely light materials with densities around 0.08 - 0.05

273 g/cm³ showing only a slight dependency of the density with the drying method but strong
274 influence of the bacterial source. Densities of the BCX films are in the 0.16 - 0.6 g/cm³ range
275 and they strongly depend on the drying method used. BCX films dried by SCD are lighter
276 than FD and RD ($\rho_{\text{BCX-RD}} > \rho_{\text{BCX-FD}} > \rho_{\text{BCX-SCD}}$), although they are still two times denser than
277 any BCE film ($\rho_{\text{BCX}} > \rho_{\text{BCE}}$), see **Table 1**.

278 From the density values one can evaluate the total porosity of the films from the equation:
279 Total porosity (ϕ) = $1 - (\rho_{\text{BC film}} / \rho_{\text{cellulose}})$; where we have assumed a $\rho_{\text{cellulose}} = 1.5 \text{ g/cm}^3$
280 (Mwaikambo and Ansell 2001; Sehaqui et al. 2011; Sun 2005) for both strains. Following the same trend as for the
281 density, we observed that BCE films have a porosity larger than 94 % for the three drying
282 methods. On the other hand, BCX films are less porous and their porosity strongly depends on
283 the drying method used. We believe that the slow formation of the cellulose fibrils in GE
284 influences the microstructure of the film, forming fibrils more resistant to compact and finally
285 affording a more spongy structure.(Mwaikambo and Ansell 2001; Sehaqui et al. 2011; Sun
286 2005).The porosity, density are strongly affected by the bacterial strain and the drying
287 methods.

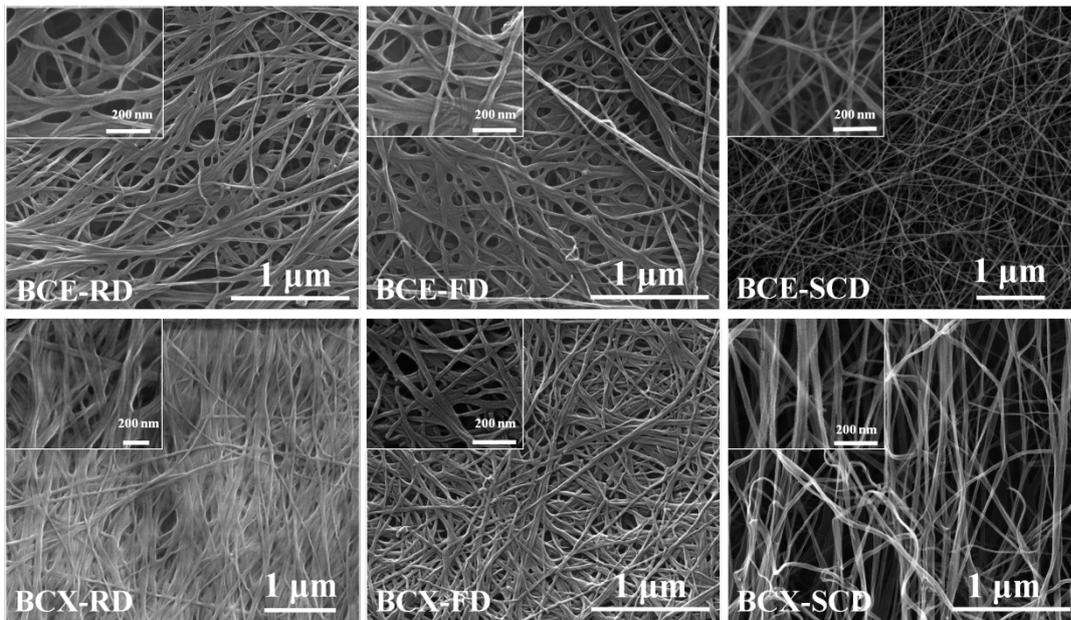
288

289 **Microstructure of the BC film**

290 BC films present an open porous network of cellulose fibers confirmed by scanning electron
291 microscopy (SEM), Figure 3. Similarly to previous reports,(Siro and Plackett 2010; Schutz et
292 al. 2012) BC films show a hierarchical structure with pores of different sizes from macro to
293 micro. For both strains, SCD drying method offer the most open structure and a higher
294 presence of individual fibrils than FD and RD films. BET measurements of the porosity of the
295 thin films provided partial results of the porosity of the thin BC films skewed towards the
296 mesoscale.

297 Individual cellulose fibrils measured by SEM are approximately $18 \pm 2 \text{ nm}$ in thickness
298 independently of the strain and the drying method used. Thus, the strain used or the drying

299 methods do not seem to influence the fibril size which is somehow surprising considering the
 300 difference in the cellulose growth rate of the two strains. In contrast, a detailed observation of
 301 the images showed that the drying method impact the fiber entangling. Analyzing in detail
 302 several SEM images, we computed that for both strains, $85 \pm 2 \%$ of the fibers form bundles
 303 in the films dried at RD and FD whereas we detect just a $38 \pm 2 \%$ of the fibers forming
 304 bundles in SCD films. Moreover, BCX-SCD is the material with the most differentiated
 305 microstructure since we distinguish higher number of individual fibers, less entanglement and
 306 some degree of directionality of the fibers in comparison to the rest of the films. Some
 307 detailed studies to elucidate if fiber directionality induces anisotropic mechanical behavior
 308 will be analyzed.



309
 310
 311 **Figure 3. SEM images of the BC films obtained after different drying conditions. Inserts**
 312 **show higher magnification images of the films.**

Drying method		RD		FD		SCD	
		BCE	BCX	BCE	BCX	BCE	BCX
Structural	Film Thickness (μm)	24 ± 2	37 ± 2	34 ± 2	40 ± 4	58 ± 3	67 ± 2
	Density (g/mL)	0.08 ± 0.01	0.59 ± 0.03	0.06 ± 0.01	0.32 ± 0.02	0.05 ± 0.01	0.16 ± 0.01
	Total Porosity (%)	94 ± 2	60 ± 2	95 ± 2	79 ± 2	96 ± 2	89 ± 2
	Fibers diameter	17 ± 2	19 ± 3	20 ± 4	19 ± 3	16 ± 2	20 ± 4

	(nm)							
	WAC	1 st cycle	39.5	37.3	15.8	15.8	109.4	66.6
		after 3 cycles	15.3	14.2	92.3	50.2	19.8	19.4
	Decrease (%)	61	62	42	68	82	71	
Mechanical	Penetration depth at 0.4mN load (μm)		3.9 \pm 0.4	1.1 \pm 0.1	4.1 \pm 0.4	1.4 \pm 0.3	3.2 \pm 0.5	4.3 \pm 1.5
	Young modulus ("E")(MPa)		198 \pm 50	659 \pm 90	204 \pm 50	601 \pm 200	238 \pm 20	198 \pm 30
	Hardness ("H")(MPa)		34 \pm 20	39 \pm 9	20 \pm 8	26 \pm 10	20 \pm 5	19 \pm 20
	EP (%)		59 \pm 7	39 \pm 3	53 \pm 4	33 \pm 3	52 \pm 5	48 \pm 10

314

315 **Table 1. Summary of the structural and mechanical values obtained for the BC films.**

316

317

318 **Water absorption capacity (WAC)**

319 Cellulose materials are good absorbents, this property identifies them as excellent candidates

320 for instance for organic pollutants, absorbents and catalysts (improve efficiency of enzyme

321 loading).(Wu et al. 2013; Shezad et al. 2010) This property is also exploited in order to create

322 cellulose composites with high yield of the different components, for instance in

323 antibactericidal BC patches that need a homogeneous and high loading of Ag

324 nanoparticles.(Ul-Islam et al. 2012a; Ul-Islam et al. 2011) We measured the water absorption

325 capacity and evaluated how the different drying methods and therefore, the microstructure

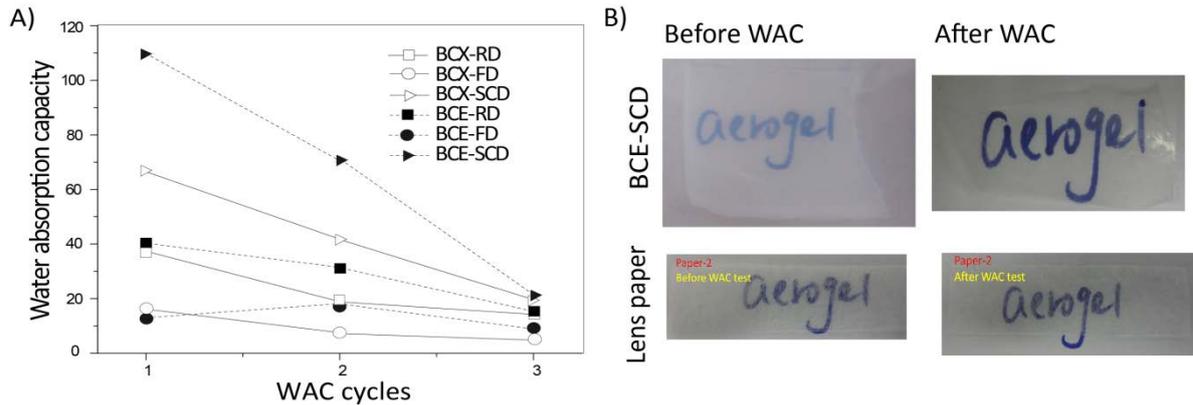
326 obtained influence the water absorption capacity (WAC). We dried and weighted BC films,

327 upon immersion in distilled water at room temperature for two days they were weighted again.

328 WAC quantifies the percentage of water absorbed in each film. We repeated two times the

329 water absorption cycle drying them at room temperature. The weight of the dry films at the

330 start of each WAC cycles did not change.



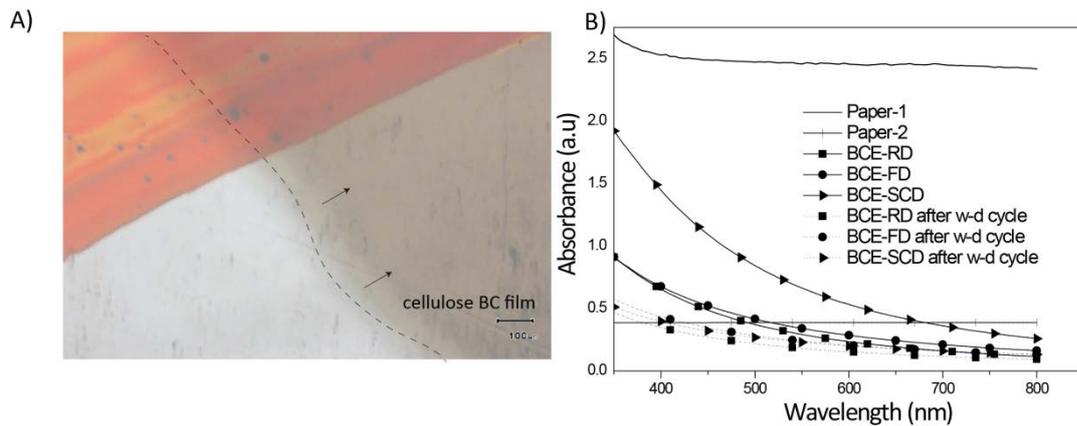
331
 332 **Figure 4. (A) Percentage of the water absorbed in the BC films after three wetting-**
 333 **drying cycles. (B) Images of the BCE-SCD films and lens paper before and after wetting.**

334
 335 WAC shows a strong influence of the drying method used. Supercritically dried films present
 336 the highest water absorbance capability of all the films, obtaining water absorption capacities
 337 of up to 110 times its own weight for BCE-SCD films, Figure 4A. FD films showed lower
 338 water absorption than RD for both strains. SCD drying method emerges as an ideal
 339 methodology to improve WAC in native BC films without additional mechanical processes or
 340 additives.

341 The ability of the films to recover the WAC capacity after drying at room temperature was
 342 evaluated performing some wetting-drying cycles. After three wetting-drying cycles, the
 343 WAC values converged for all the drying methods and the BC films still held up to 20 times
 344 their weight of water after 3 cycles, Figure 4A. We hypothesized, since in the WAC cycles we
 345 dry the films at room temperature, we compact the tridimensional structure of the film in each
 346 cycle until they all become similar. The extraordinary WAC achieved with supercritical
 347 drying methods indicates that the structure achieved could afford a methodology to create
 348 cellulose composites with high concentration of different compounds in an homogeneous
 349 fashion by absorption, such higher and more reproducible absorption of enzymes to improve
 350 their response.

351 **Transparency**

352 Evaluation of the water absorption capacity also revealed that the films increase their
353 transparency upon wetting. Their applications in electronics could also be expanded in water-
354 based applications, for instance in cell-based membranes, in skin-patches with flexible
355 electronics since they could help to monitor wound
356 healing, (<http://www.ricoh.com/about/company/technology/tech/033.html>) in sensors devices
357 where we could envisage while the liquid is transported without the need of external power,
358 we could image its reactivity from the change in transparency. Pictures depicting the
359 qualitative measurement of the transparency for lens paper, 35 μm , and the BCE-SCD film
360 before and after WAC cycle are shown in Figure 4B and Figure 5A. (Films are placed in
361 contact with the paper below). BCE-SCD film became more transparent than the lens paper
362 after a WAC cycle, likely related to the collapse of pores in the size range with larger visible
363 wavelengths scattering effect. Pictures of all the films are in Supplementary information
364 Figure S3. Quantification of the transparency using Uv-vis Spectroscopy was performed for
365 BC films. (Figure 5B for BCE and Figure S4 for BCX). BC films do not absorb above 500 nm
366 before and after WAC showing a higher optical transmittance compared to regular
367 chromatography paper of 200 μm (Paper 1) and lens paper of 35 μm (Paper 2). Initially BCE
368 films are more transparent than BCX films that could indicate the existence of different light
369 scattering effects in the films and they could reveal different surface roughness. (Yano et al.
370 2005) After the different WAC cycles, the optical transmittance improved for all the films.
371 High transparency of BC films at visible wavelengths and the biological origin of the
372 cellulose could appoint this material as a suitable matrix to biological applications where the
373 optical visualization through the material is necessary as for instance in cell culture surfaces.



374 **Figure 5. (A) Optical image of the BCE-SCD film immersed in a petri dish with water.**
 375

376 **The red line on the Petri dish surface is clearly distinguishable through the BC film. (B)**

377 **Uv-vis spectra of BC films before and after WAC and commercial papers.**

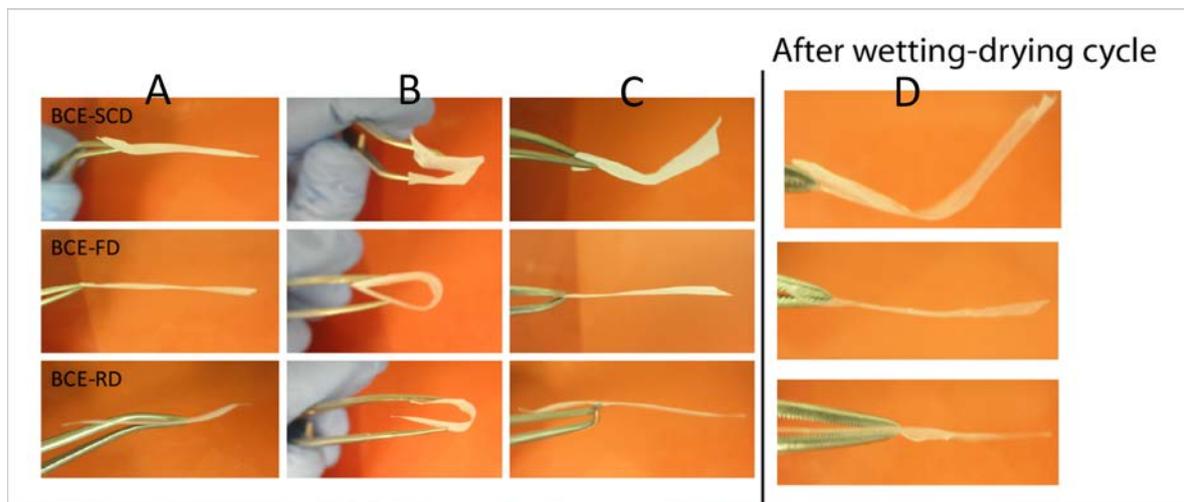
378

379 **Mechanical properties**

380 Mechanical properties are a key factor for BC films to be used as reinforcing materials,
 381 sensors or scaffolds in many applications.(Yano et al. 2005) In particular, for cell studies, the
 382 possibility of tuning the mechanical properties of the scaffold for each cell type is very
 383 attractive. Biological tissues are soft and elastic and they show a broad range of Young
 384 modulus from $1.5 \times 10^4 - 3 \times 10^4$ MPa found in bones to $10^{-4} - 10^{-3}$ MPa in softer tissues such
 385 as brain tissue. (Moore et al. 2010) Numerous researchers have revealed that cells have the
 386 ability to probe and respond to the rigidity of the substrates they are grown upon.(Pelham and
 387 Wang 1997; Rowlands et al. 2008; Engler et al. 2004; Guo et al. 2006) The cellular tensegrity
 388 model attempts to suggest that the homeostatic balance of forces governs the reciprocal
 389 relationship between cells and the physical properties of their microenvironment.(Cameron et
 390 al. 2011)

391 Firstly, we evaluated qualitatively the elastic behavior of the films by bending them with a
 392 tweezers. Figure 6 shows a series of images of the bending test performed. We clearly see that
 393 BCE-RD and BCE-FD films fully recovered and a flat sheet was recorded again. SCD films

394 did not recover fully and some creased was imaged after bending, although it did not break.
395 After wetting-drying cycles, the BC films do not break, indicating that the films keep their
396 elasticity even after a WAC cycle. Films produced by GX followed the same trend, Figure S5,
397 therefore the bacterial strain do not modify their film's elasticity. Unlike silica aerogels that
398 are brittle and fragile materials, BC aerogels have higher degree of elasticity.(Yano et al.
399 2005)



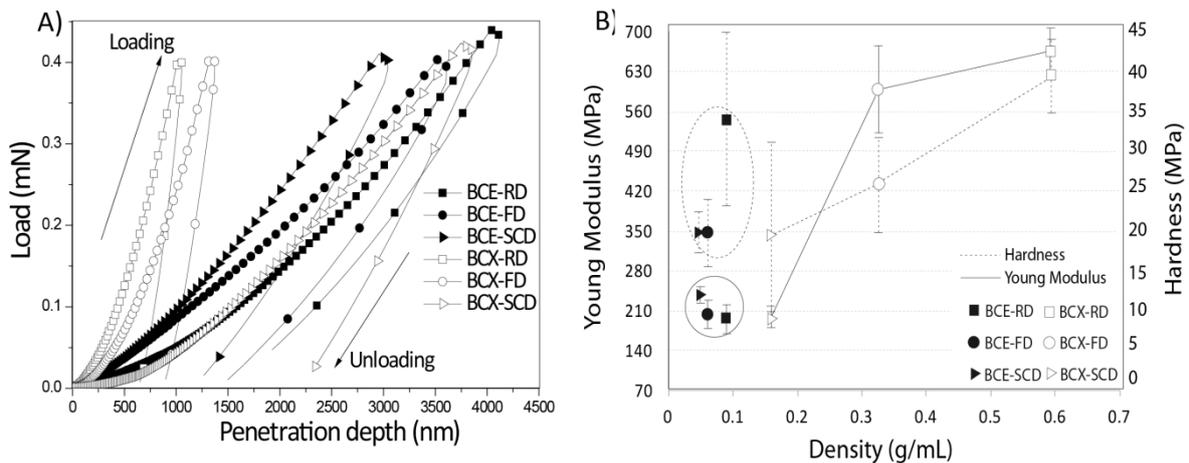
400
401 **Figure 6 Pictures of the bending test performed: (A) BCE films before the bending test**
402 **from the different drying methods, (B) process of the bending test, (C) the resulting**
403 **shapes of the BC films after the test and (D) the shape of the films obtained on a BCE**
404 **film that was wetted-dried (WAC cycle).**

405 To measure the mechanical behavior of the BC films we have used nanoindentation. For the
406 indentation cycles we used a constant maximum load of 0.4 mN to ensure that the penetration
407 depth of the tip into the films was smaller than their thickness. Smaller loads from 0.05 – 0.1
408 mN did not give us reproducible results. Moreover, we evaluated the effect of the adhesive
409 tape underneath the sample (presence and not presence) and the results did not change,
410 therefore we conclude the adhesive under the sample did not affect the measurements.
411 Figure 7 and Table 1 gather the load-displacement curves and the values obtained for the
412 penetration depth at maximum load, "*Young Modulus, E*", "*Hardness, H*" and "*elastic*
413 *parameter, EP*" for two samples (n=14). The load-displacement curves obtained are

414 representative of a material with elastoplastic properties. BC films show similar load-
 415 displacement curves except for BCX-RD and BCX-FD that show lower penetration depths
 416 and steeper unloading curves.

417 The analysis of the load-displacement curves shows that the BC films have creep and some
 418 literature review this property as a very desirable for scaffolds that interact with
 419 cells.(Cameron et al. 2011) Although we have use the slope of the curve to calculate the
 420 "Young Modulus", ("E") we have to notice that this estimation is not completely correct as the
 421 material has a strong elastoplastic behavior, as it happens in soft tissues. The analysis of "E"
 422 shows that GE affords less stiff films than GX and they are independent of the drying method,
 423 while BCX films are sensitive to the drying method obtaining stiffer BC films if we dry them
 424 by RD or FD.

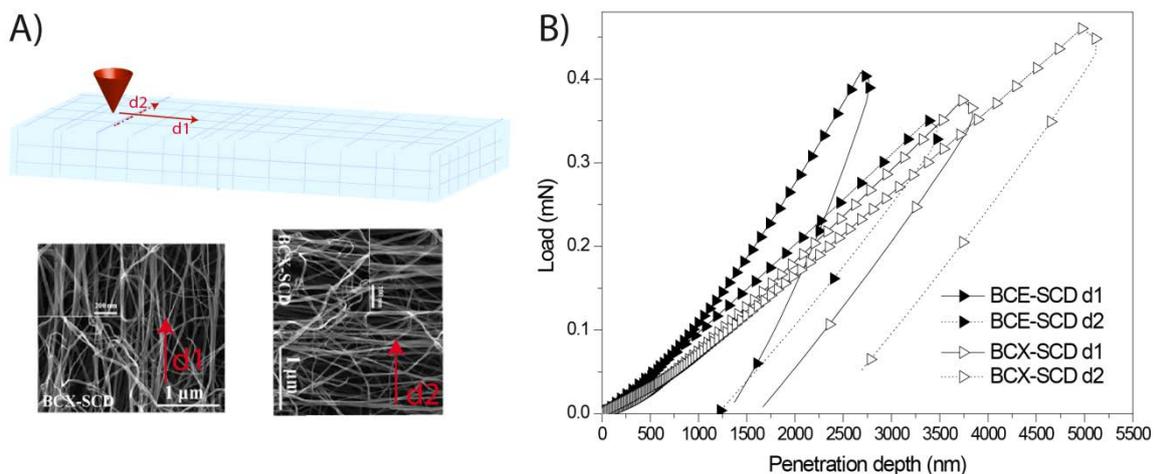
425 The "E" values obtained are around 200 MPa except for BCX-RD and BCX-FD that we
 426 observed E of 600 MPa, suggesting that their stiffness could be comparable to tissues as bone
 427 ($1.5 \times 10^4 - 3 \times 10^4$ MPa) and of soft arteries (0.1 – 1 MPa).(Moore et al. 2010)



428

429 **Figure 7. (A) Representative loading-unloading curve for each BC film obtained using**
 430 **the nanoindenter under a load of 0.4 mN. (B) Mean values of "Young Modulus", ("E")**
 431 **and Hardness ("H") obtained for each BC film as a function of their density (n=14). We**
 432 **circled the "E" and "H" for BCE for clarity.**

433 The averaged measured *Hardness* values ("*H*") of BC samples are around 20 – 34 MPa,
 434 which indicates the resistance of BC films to the nanoindenter, BC-RD films shows higher
 435 "*H*" values than the others BC films. The elastic component of the BC films was analyzed
 436 using the *elastic parameter* ("*EP*").(Moner-Girona et al. 1999) BC films presenting large
 437 "*Young modulus*" (BCX-RD and BCX-FD) also exhibit a low elastic character. On the other
 438 side, "*E*" and penetration depth at 0.4 mN values for BCE films are similar to the values found
 439 for BCX-SCD film even though they present a much smaller density (0.5- 0.8 g/cm³ for BCE
 440 and 0.16 g/cm³ for BCX-SCD) possibly pointing to a different mechanical strength of the
 441 cellulose microfibrils for each strain. The evaluation of the mechanical parameters with the
 442 density shows that the combination of BCX and the drying method used allow us to obtain
 443 broader range of mechanical properties for BCX films than for the BCE films.



444
 445 **Figure 8. A) Schematic representation of the mechanical measurements performed over**
 446 **two different directions. B) Load- displacement curves for BC films dried on SCD**
 447 **conditions for the two bacterial strains.**

448

	BCE-d1	BCE-d2	BCX-d1	BCX-d2
"Young modulus, E" MPa	233±50	300±50	150±40	242±100
Penetration depth at 0.4 mN (µm)	3.1±0.5	3.5±0.9	4.9±0.8	4.4±0.7

449

450 **Table 2. Values of "Young Modulus" (MPa) and penetration depth at 0.4mN (μm) from**
451 **BC films dried in SCD conditions measured in two directions of the BC films.**

452 BC- SCD films show in SEM images some anisotropy, possible directionality of the fibers
453 therefore we evaluated " E " and penetration depth if we placed the sample in two opposite
454 directions. The load-displacement curves show different profiles as a function of the direction
455 of the measurement, Figure 8. The evaluation of " E " of BC-SCD show some anisotropy in the
456 measurements although the penetration depth remains unchanged. We found for BCX-SCD
457 and BCE-SCD a difference in " E " as a function of the direction where we performed the
458 nanoindentation measurements. These results confirm the SEM images that we observed
459 some directionality of the fibers in BCX-SCD; we also found some directionality in BCE-
460 SCD even though we could not detect it in SEM, Table 2. We believe these are interesting
461 results that recommend a further and deeper analysis of the mechanical properties of the BC-
462 SCD films that will be continued with extensive loads, time and different humidity conditions,
463 since BC-SCD films also offer high absorption capacity.

464

465 **Conclusions**

466 The use of two strains and three drying methods allowed us to obtain different BC thin films
467 with different structural properties that can improve their application in cellulose composites
468 materials and others. Room temperature drying, freeze-drying, and supercritical drying
469 methods modify the porosity, mechanical properties and water absorption capability of the BC
470 films expanding their range of the properties.

471 Even after 10 days of culturing, BCE films are more transparent and lighter independently of
472 the drying methods used in comparison to BCX films, harvested for 5 days. BCE-SCD shows
473 the highest water absorbance capacity that allow us to suggest BCE films as suitable
474 candidates for applications such as absorbents, removal of contaminants or wound healing
475 dressings.(Jin et al. 2011) Even though BCE films production cost would be higher, BCE-

476 SCD film could provide cellulose composites with high loading efficiencies and
477 homogeneous mixtures. Additionally the use of SCD drying improves the cristallinity of the
478 cellulose fibers.

479 BC films from GX source offer a more versatile platform since we can control the porosity,
480 mechanical properties and water absorption capacity with only one-step, selecting the
481 appropriate drying method. Additionally, GX strain offers the advantage of faster production
482 of BCX films. We hypothesize that these films could be good candidates to interface with
483 tissues since their mechanical properties could be tailored to mimic the final tissue
484 replacement and their mechanical properties are not hampered in aqueous media.

485

486 **Supporting Information**

487 Supporting Information is available online or from the author.

488

489 **Acknowledgements**

490 The research leading to these results has received funding from the People Program (Marie
491 Curie Actions) of the European Union's Seventh Framework Program (FP7/2007-2013) under
492 REA grant agreement n° 303630 and cofounded by the European Social Fund. Authors
493 acknowledge the funding from Spanish Ministry of Economy MAT 2012-35324, COST
494 Action MP1202 and Ramon y Cajal grant RYC-2010-06082 (AL), Chinese Scholarship
495 Council fellowship (MZ). The group of Dr. Alex Peralvarez for their help in the bacterial
496 culture, Dr. Josep PuigMartí and the group of Dr. David Amabilino for the use of the optical
497 microscope, Prof. Elies Molins and Toni Pons for the use and advice in the use of the freeze
498 drier and Dr. Roberto L. Guzman de Villoria for his advices in the mechanical measurements.

499

500

501 **Notes and references**

502 Institut Ciència de Materials de Barcelona, Campus UAB, 08193 Bellaterra, Spain.

503 E-mail: alaromaine@icmab.es

- 504 Andrade F, Alexandre N, Amorim I, Gartner F, Mauricio A, Luis AL, Gama M (2013)
505 Studies on the biocompatibility of bacterial cellulose. *J Bioact Compatible Polym* 28
506 (1):97-112
- 507 Andrés Barrao C, Falquet L, Calderon Copete S, Descombes P, Perez R, Ortega Pérez R,
508 Barja F (2011) Genome sequences of the high-acetic acid-resistant bacteria
509 *Gluconacetobacter europaeus* LMG 18890T and *G. europaeus* LMG 18494 (reference
510 strains), *G. europaeus* 5P3, and *Gluconacetobacter oboediens* 174Bp2 (isolated from
511 vinegar). *J Bacteriol* 193 (10):2670-2671
- 512 Atalla RH, Vanderhart DL (1984) Native Cellulose: A Composite of Two Distinct Crystalline
513 Forms. *Science* 223 (4633):283-285. doi:10.1126/science.223.4633.283
- 514 Budunoglu H, Yildirim A, Guler MO, Bayindir M (2011) Highly Transparent, Flexible, and
515 Thermally Stable Superhydrophobic ORMOSIL Aerogel Thin Films. *ACS Appl Mater*
516 *& Interf* 3 (2):539-545. doi:10.1021/am101116b
- 517 Cai J, Kimura S, Wada M, Kuga S, Zhang L (2008) Cellulose aerogels from aqueous alkali
518 hydroxide-urea solution. *ChemSusChem* 1 (1-2):149-154
- 519 Cameron AR, Frith JE, Cooper-White JJ (2011) The influence of substrate creep on
520 mesenchymal stem cell behaviour and phenotype. *Biomaterials* 32 (26):5979-5993.
521 doi:<http://dx.doi.org/10.1016/j.biomaterials.2011.04.003>
- 522 Czaja WK, Young DJ, Kawecki M, Brown RM (2007) The future prospects of microbial
523 cellulose in biomedical applications. *Biomacromolecules* 8 (1):1-12.
524 doi:10.1021/bm060620d
- 525 Das K, Ray D, Bandyopadhyay NR, Sengupta S (2010) Study of the Properties of
526 Microcrystalline Cellulose Particles from Different Renewable Resources by XRD,
527 FTIR, Nanoindentation, TGA and SEM. *J Polym Environ* 18 (3):355-363
- 528 Dietrich A, Goring DAI, Revol JF (1987) Effect of mercerization on the crystallite size and
529 crystallinity index in cellulose from different sources. *Can J Chem* 65 (8):1724-1725
- 530 Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D (2004) Substrate
531 Compliance versus Ligand Density in Cell on Gel Responses. *Biophys J* 86 (1):617-
532 628. doi:[http://dx.doi.org/10.1016/S0006-3495\(04\)74140-5](http://dx.doi.org/10.1016/S0006-3495(04)74140-5)
- 533 Ennajih H, Bouhfid R, Essassi E, Bousmina M, El Kadib A (2012) Chitosan–montmorillonite
534 bio-based aerogel hybrid microspheres. *Microporous Mesoporous Mater* 152:208-213
- 535 Fu L, Zhang J, Yang G (2013) Present status and applications of bacterial cellulose-based
536 materials for skin tissue repair. *Carbohydr Polym* 92 (2):1432-1442.
537 doi:<http://dx.doi.org/10.1016/j.carbpol.2012.10.071>
- 538 George J, Ramana KV (2005). *Int J Biol Mole* 37:189
- 539 George J, Sajeevkumar VA, Kumar R, Ramana KV, Sabapathy SN, Bawa AS (2008)
540 Enhancement of thermal stability associated with the chemical treatment of bacterial
541 (*Gluconacetobacter xylinus*) cellulose. *J Appl Polym Sci* 108 (3):1845-1851.
542 doi:10.1002/app.27802
- 543 Guo W-h, Frey MT, Burnham NA, Wang Y-l (2006) Substrate Rigidity Regulates the
544 Formation and Maintenance of Tissues. *Biophys J* 90 (6):2213-2220
- 545 Hendel T, Lesnyak V, Kuhn L, Herrmann AK, Bigall NC, Borchardt L, Kaskel S, Gaponik N,
546 Eychmuller A (2013) Mixed Aerogels from Au and CdTe Nanoparticles. *Adv Funct*
547 *Mater* 23 (15):1903-1911. doi:10.1002/adfm.201201674
548 <http://www.ricoh.com/about/company/technology/tech/033.html>.

549 Hu W, Chen S, Yang J, Li Z, Wang H (2014) Functionalized bacterial cellulose derivatives
550 and nanocomposites. *Carbohydr Polym* 101:1043-1060.
551 doi:10.1016/j.carbpol.2013.09.102

552 Jin H, Kettunen M, Laiho A, Pynnonen H, Paltakari J, Marmur A, Ikkala O, Ras RHA (2011)
553 Superhydrophobic and Superoleophobic Nanocellulose Aerogel Membranes as
554 Bioinspired Cargo Carriers on Water and Oil. *Langmuir* 27 (5):1930-1934.
555 doi:10.1021/la103877r

556 Kalia S, Kaith BS, Kaur I (2011) *Cellulose Fibers: Bio- and Nano-Polymer Composites*.
557 Springer, Berlin

558 Kim S, Kim HJ, Jeon NL (2010) Biological applications of microfluidic gradient devices.
559 *Integrative Biol* 2 (11-12):584-603. doi:10.1039/c0ib00055h

560 Klemm D, Heublein B, Fink HP, Bohn A (2005) Cellulose:Fascinating Biopolymer and
561 Sustainable Raw Material. *Angew Chem* 44:3358-3393

562 Klemm D, Kramer F, Moritz S, Lindström T, Ankerfors M, Gray D, Dorris A (2011)
563 Nanocelluloses: A New Family of Nature-Based Materials. *Angew Chem Int Ed* 50
564 (24):5438-5466. doi:10.1002/anie.201001273

565 Klemm D, Schumann D, Udhardt U, Marsch S (2001) Bacterial synthesized cellulose -
566 artificial blood vessels for microsurgery. *Prog Polym Sci* 26:1561-1603

567 Liebner F, Haimer E, Wendland M, Neouze MA, Schlufte K, Miethe P, Heinze T, Potthast A,
568 Rosenau T (2010) Aerogels from Unaltered Bacterial Cellulose: Application of
569 scCO₂ Drying for the Preparation of Shaped, Ultra-Lightweight Cellulosic Aerogels.
570 *Macromol Biosci* 10 (4):349-352. doi:10.1002/mabi.200900371

571 Mansikkamaki P, Lahtinen M, Rissanen K (2007) The conversion from cellulose I to cellulose
572 II in NaOH mercerization performed in alcohol–water systems: An X-ray powder
573 diffraction study. *Carbohydr Polym* 68 (1):35-43. doi:10.1016/j.carbpol.2006.07.010

574 Martin L, Osso JO, Ricart S, Roig A, Garcia O, Sastre R (2008) Organo-modified silica
575 aerogels and implications for material hydrophobicity and mechanical properties. *J*
576 *Mater Chem* 18 (2):207-213. doi:10.1039/b712553d

577 Mecklenburg M, Schuchardt A, Mishra YK, Kaps S, Adelung R, Lotnyk A, Kienle L, Schulte
578 K (2012) Aerographite: Ultra Lightweight, Flexible Nanowall, Carbon Microtube
579 Material with Outstanding Mechanical Performance. *Adv Mater* 24 (26):3486-3490.
580 doi:10.1002/adma.201200491

581 Moner-Girona M, Roig A, Benito M, Molins E (2003) Aerogel thin film synthesis by a
582 supercritical fluid-assisted sol-gel route in a single processing unit. *J Mater Chem* 13
583 (9):2066-2068. doi:10.1039/b307057c

584 Moner-Girona M, Roig A, Molins E, Martinez E, Esteve J (1999) Micromechanical properties
585 of silica aerogels. *Appl Phys Lett* 75 (5):653-655. doi:10.1063/1.124471

586 Moore SW, Roca-Cusachs P, Sheetz MP (2010) Stretchy Proteins on Stretchy Substrates: The
587 Important Elements of Integrin-Mediated Rigidity Sensing. *Dev Cell* 19 (2):194-206.
588 doi:<http://dx.doi.org/10.1016/j.devcel.2010.07.018>

589 Murillo-Cremaes N, López-Periágo AM, Saurina J, Roig A, Domingo C (2010) A clean and
590 effective supercritical carbon dioxide method for the host–guest synthesis and
591 encapsulation of photoactive molecules in nanoporous matrices. *Green Chemistry* 12
592 (12):2196. doi:10.1039/c004762g

593 Mwaikambo LY, Ansell MP (2001) The determination of porosity and cellulose content of
594 plant fibers by density methods. *J Mater Sci Lett* 20 (23):2095-2096.
595 doi:10.1023/a:1013703809964

596 Nata IF, Sureshkumar M, Lee C-K (2011) One-pot preparation of amine-rich
597 magnetite/bacterial cellulose nanocomposite and its application for arsenate removal.
598 *RSC Advances* 1 (4):625-631. doi:10.1039/c1ra00153a

599 Pelham RJ, Wang Y-l (1997) Cell locomotion and focal adhesions are regulated by
600 substrate flexibility. *Proceedings of the National Academy of Sciences* 94 (25):13661-
601 13665

602 Pinto RJB, Neves MrC, Neto CP, Trindade T (2012) Composites of Cellulose and Metal
603 Nanoparticles, Nanocomposites - New Trends and Developments. *Nanocomposites -*
604 *New Trends and Developments*. InTech, September 27, 2012 under CC BY 3.0 license.
605 doi:10.5772/50553

606 Ross P, Mayer R, Benziman M (1991) Cellulose biosynthesis and function in bacteria.
607 *Microbiol Rev* 55 (1):35-58

608 Rowlands AS, George PA, Cooper-White JJ (2008) Directing osteogenic and myogenic
609 differentiation of MSCs: interplay of stiffness and adhesive ligand presentation.
610 *American Journal of Physiology - Cell Physiology* 295 (4):C1037-C1044.
611 doi:10.1152/ajpcell.67.2008

612 Russler A, Wieland M, Bacher M, Henniges U, Mieth P, Liebner F, Potthast A, Rosenau T
613 (2012) AKD-Modification of bacterial cellulose aerogels in supercritical CO₂.
614 *Cellulose* 19 (4):1337-1349

615 S. Hestrin MS (1954) Synthesis of Cellulose by *Acetobacter xylinum*. *Biochem J* 58:345-353

616 Sai H, Xing L, Xiang J, Cui L, Jiao J, Zhao C, Li Z, Li F (2013) Flexible aerogels based on an
617 interpenetrating network of bacterial cellulose and silica by a non-supercritical drying
618 process. *Journal of Materials Chemistry A* 1 (27):7963-7970. doi:10.1039/c3ta11198a

619 Saska S, Teixeira LN, de Oliveira PT, Gaspar AMM, Ribeiro SJL, Messaddeq Y, Marchetto
620 R (2012) Bacterial cellulose-collagen nanocomposite for bone tissue engineering. *J*
621 *Mater Chem* 22 (41):22102-22112. doi:10.1039/c2jm33762b

622 Schutz C, Sort J, Bacsik Z, Oliynyk V, Pellicer E, Fall A, Wagberg L, Berglund L, Bergstrom
623 L, Salazar-Alvarez G (2012) Hard and Transparent Films Formed by Nanocellulose-
624 TiO₂ Nanoparticle Hybrids. *Plos One* 7 (10). doi:10.1371/journal.pone.0045828

625 Segal L, Creely JJ, Martin AE, Conrad CM (1959) An Empirical Method for Estimating the
626 Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Textile*
627 *Research Journal* 29 (10):786-794. doi:10.1177/004051755902901003

628 Sehaqui H, Zhou Q, Ikkala O, Berglund LA (2011) Strong and Tough Cellulose Nanopaper
629 with High Specific Surface Area and Porosity. *Biomacromolecules* 12 (10):3638-3644.
630 doi:10.1021/bm2008907

631 Shezad O, Khan S, Khan T, Park JK (2010) Physicochemical and mechanical characterization
632 of bacterial cellulose produced with an excellent productivity in static conditions using
633 a simple fed-batch cultivation strategy. *Carbohydr Polym* 82 (1):173-180.
634 doi:<http://dx.doi.org/10.1016/j.carbpol.2010.04.052>

635 Shi ZJ, Zhang Y, Phillips GO, Yang G (2014) Utilization of bacterial cellulose in food. *Food*
636 *Hydrocolloids* 35:539-545. doi:10.1016/j.foodhyd.2013.07.012

637 Siro I, Plackett D (2010) Microfibrillated cellulose and new nanocomposite materials: a
638 review. *Cellulose* 17 (3):459-494. doi:10.1007/s10570-010-9405-y

639 Sun CC (2005) True density of microcrystalline cellulose. *J Pharm Sci* 94 (10):2132-2134.
640 doi:10.1002/jps.20459

641 Svensson A, Nicklasson E, Harrah T, Panilaitis B, Kaplan DL, Brittberg M, Gatenholm P
642 (2005) Bacterial cellulose as a potential scaffold for tissue engineering of cartilage.
643 *Biomaterials* 26 (4):419-431. doi:<http://dx.doi.org/10.1016/j.biomaterials.2004.02.049>

644 Ul-Islam M, Khan T, Park JK (2012a) Nanoreinforced bacterial cellulose–montmorillonite
645 composites for biomedical applications. *Carbohydr Polym* 89 (4):1189-1197.
646 doi:<http://dx.doi.org/10.1016/j.carbpol.2012.03.093>

647 Ul-Islam M, Khan T, Park JK (2012b) Water holding and release properties of bacterial
648 cellulose obtained by in situ and ex situ modification. *Carbohydr Polym* 88 (2):596-
649 603. doi:10.1016/j.carbpol.2012.01.006

650 Ul-Islam M, Shah N, Ha JH, Park JK (2011) Effect of chitosan penetration on physico-
651 chemical and mechanical properties of bacterial cellulose. *Korean J Chem Eng* 28
652 (8):1736-1743. doi:10.1007/s11814-011-0042-4

653 Ummartyotin S, Juntaro J, Sain M, Manuspiya H (2012) Development of transparent bacterial
654 cellulose nanocomposite film as substrate for flexible organic light emitting diode
655 (OLED) display. *Ind Crop Prod* 35 (1):92-97. doi:10.1016/j.indcrop.2011.06.025

656 Wang Y, Zhao Y, Deng Y (2008) Effect of enzymatic treatment on cotton fiber dissolution in
657 NaOH/urea solution at cold temperature. *Carbohydr Polym* 72 (1):178-184

658 Wicklein B, Salazar-Alvarez G (2013) Functional hybrids based on biogenic nanofibrils and
659 inorganic nanomaterials. *Journal of Materials Chemistry A* 1 (18):5469-5478.
660 doi:10.1039/c3ta01690k

661 Wu Z-Y, Li C, Liang H-W, Chen J-F, Yu S-H (2013) Ultralight, Flexible, and Fire-Resistant
662 Carbon Nanofiber Aerogels from Bacterial Cellulose. *Angew Chem Int Ed* 52
663 (10):2925-2929. doi:10.1002/anie.201209676

664 Yamada Y, Hoshino K, Ishikawa T (1997) The phylogeny of acetic acid bacteria based on the
665 partial sequences of 16S ribosomal RNA: the elevation of the subgenus
666 *Gluconoacetobacter* to the generic level. *Biosci, Biotechnol, Biochem* 61 (8):1244-
667 1251

668 Yano H, Sugiyama J, Nakagaito AN, Nogi M, Matsuura T, Hikita M, Handa K (2005)
669 Optically transparent composites reinforced with networks of bacterial nanofibers.
670 *Adv Mater* 17 (2):153-+. doi:10.1002/adma.200400597

671 Zhang L, Ruan D, Zhou J (2001) Structure and Properties of Regenerated Cellulose Films
672 Prepared from Cotton Linters in NaOH/Urea Aqueous Solution. *Industrial &*
673 *Engineering Chemistry Research* 40 (25):5923-5928
674
675

676 **Figures and Tables**

677 **Figure 1. A) Picture of a BC layer on top of the bacteria culture liquid media. B)**
678 **Cleaned BC films obtained after the NaOH treatment. C) Picture show a final BC film**
679 **dried and the accordion setup were BC films are held during the drying process.**

680

681 **Figure 2. A) X-ray diffraction pattern for 1) BCE- room temperature, 2) freeze drying 3)**
682 **supercritical drying and 4) non cleaned BCE film. B) FTIR spectra for a non-cleaned**
683 **BCE film and a BCE film after cleaning.**

684

685 **Figure 3. SEM images of the BC films obtained after different drying conditions. Inserts**
686 **show higher magnification images of the films.**

687

688 **Figure 4. A) Percentage of the water absorbed in the BC films after three wetting-drying**
689 **cycles. B) Images of the BCE-SCD films and lens paper before and after wetting.**

690

691 **Figure 5. A) Optical image of the BCE-SCD film immersed in a petri dish with water.**
692 **The red line on the Petri dish surface is clearly distinguishable. B) Uv-vis spectra of the**
693 **papers before and after WAC in comparison to commercial papers.**

694

695 **Figure 6. Pictures of the bending test performed: A) BCE films before the bending test**
696 **from the different drying methods, B) process of the bending test, C) the resulting**
697 **shapes of the BC films after the test and D) the shape of the films obtained on a BCE**
698 **film that was wetted-dried (WAC cycle).**

699

700 **Figure 7. A) Representative loading-unloading curve for each BC film obtained using**
701 **the nanoindenter under a load of 0.4 mN. B) Mean values of Young Modulus (E) and**

702 **Hardness (H) obtained for each BC film as a function of their density. (14 measurements**
703 **for each sample).**

704

705 **Figure 8. A) Schematic representation of the mechanical measurements performed over**
706 **two different directions. B) Load- displacement curves for BC films dried on SCD**
707 **conditions for the two bacterial strains.**

708

709 **Table 1. Summary of the structural and mechanical values obtained for the BC films.**

710

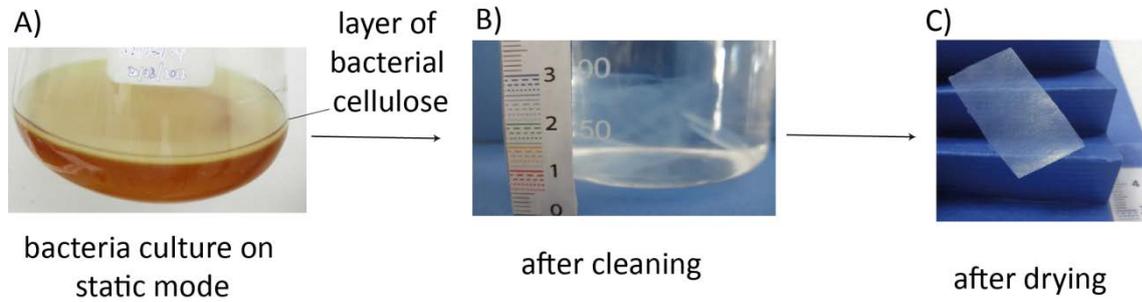
711 **Table 2 Values of "*Young Modulus*" (MPa) and penetration depth at 0.4mN (μm) from**
712 **BC films dried in SCD conditions measured in two directions of the BC films.**

713

714

715 **Figure 1. A) Picture of a BC layer on top of the bacteria culture liquid media. B)**
716 **Cleaned BC films obtained after the NaOH treatment. C) Picture show a final BC film**
717 **dried and the accordion setup were BC films are held during the drying process.**

718

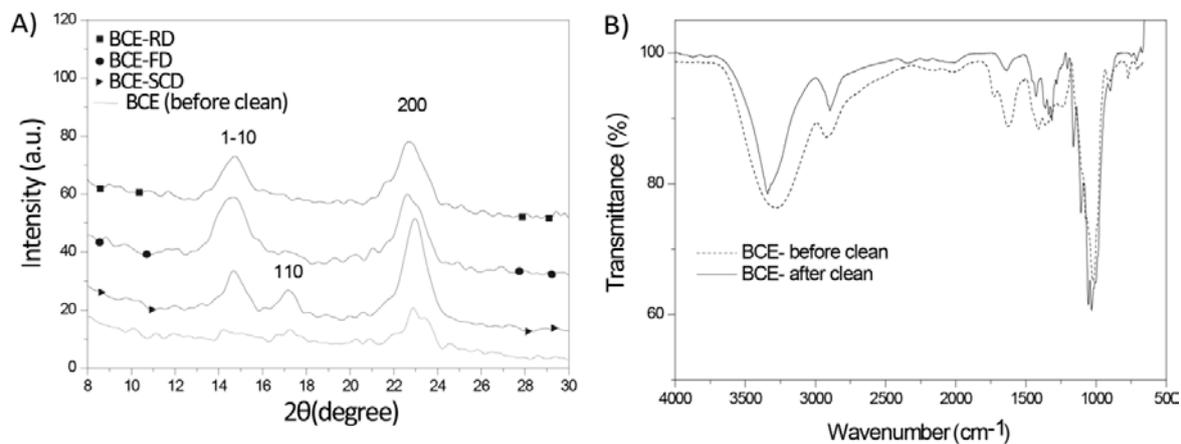


719

720

721

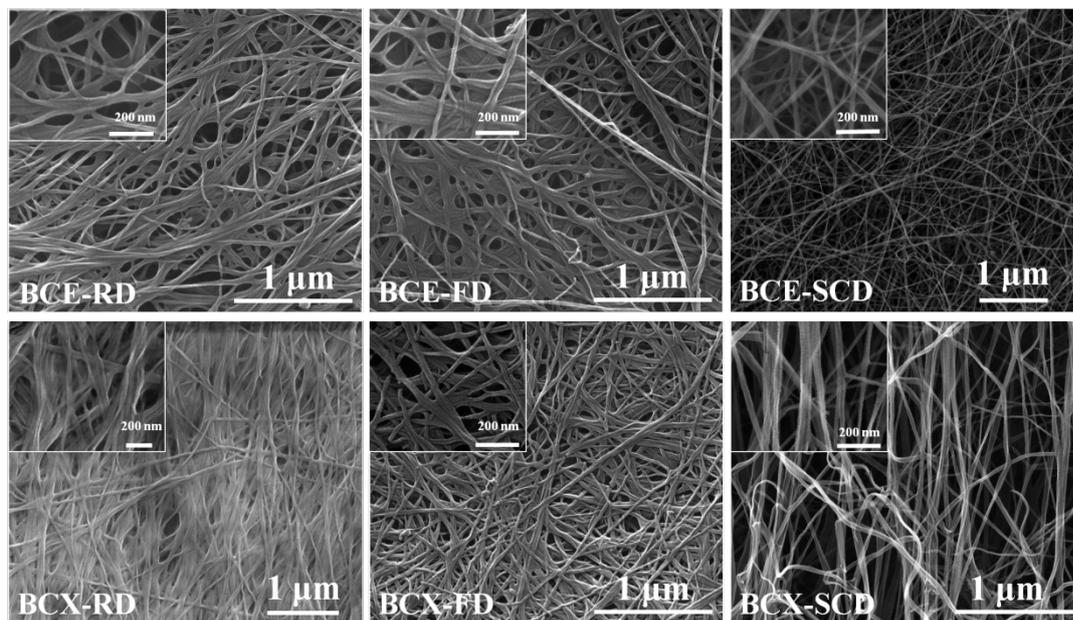
722 **Figure 2. A) X-ray diffraction pattern for 1) BCE- room temperature, 2) freeze drying 3)**
723 **supercritical drying and 4) non cleaned BCE film. B) FTIR spectra for a non-cleaned**
724 **BCE film and a BCE film after cleaning.**



725

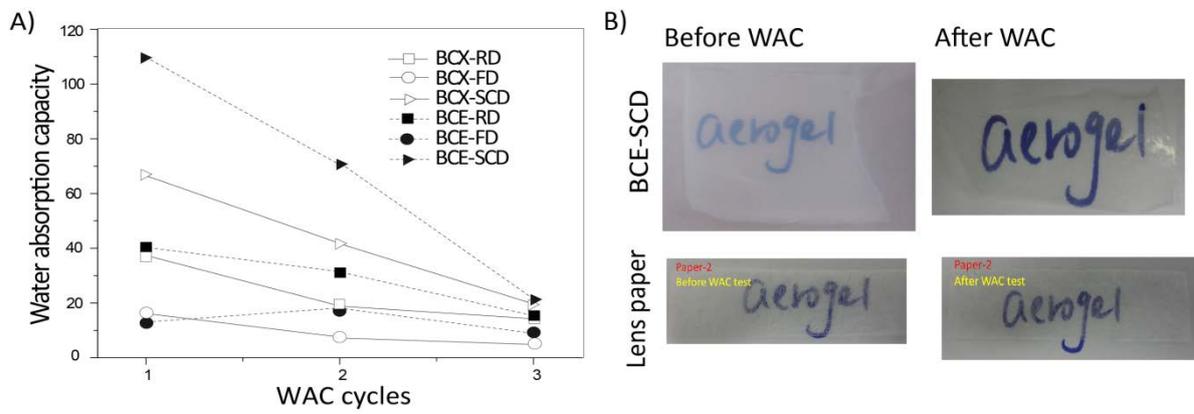
726

727 **Figure 3. SEM images of the BC films obtained after different drying conditions. Inserts**
728 **show higher magnification images of the films.**



729
730

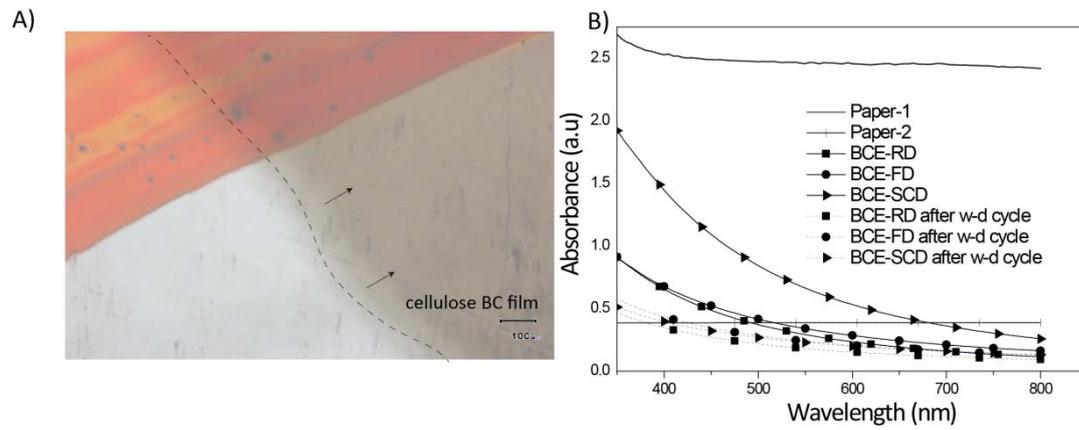
731 **Figure 4. A) Percentage of the water absorbed in the BC films after three wetting-drying**
 732 **cycles. B) Images of the BCE-SCD films and lens paper before and after wetting.**



733

734

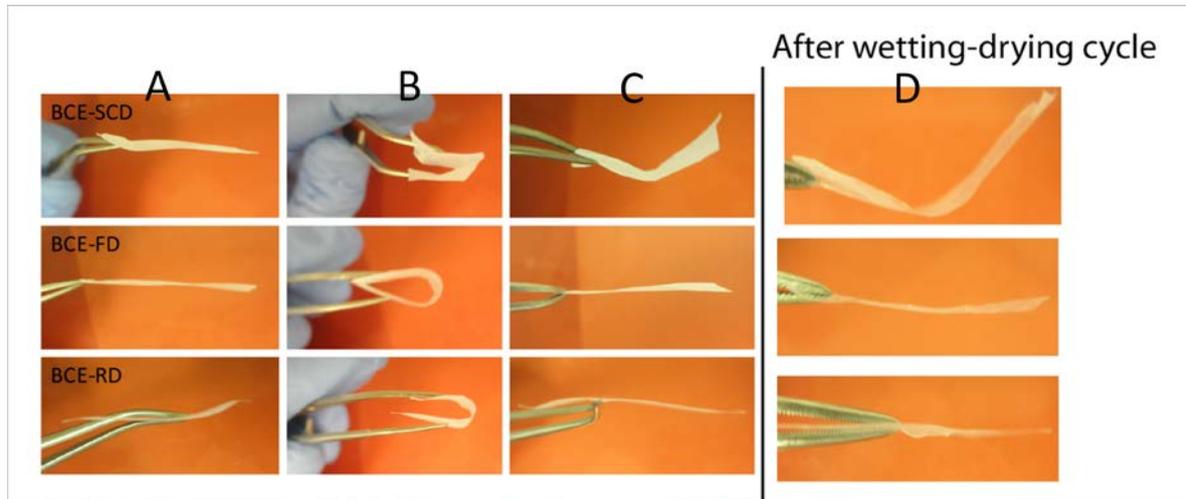
735 **Figure 5. A) Optical image of the BCE-SCD film immersed in a petri dish with water.**
736 **The red line on the Petri dish surface is clearly distinguishable. B) Uv-vis spectra of the**
737 **papers before and after WAC in comparison to commercial papers.**



738

739

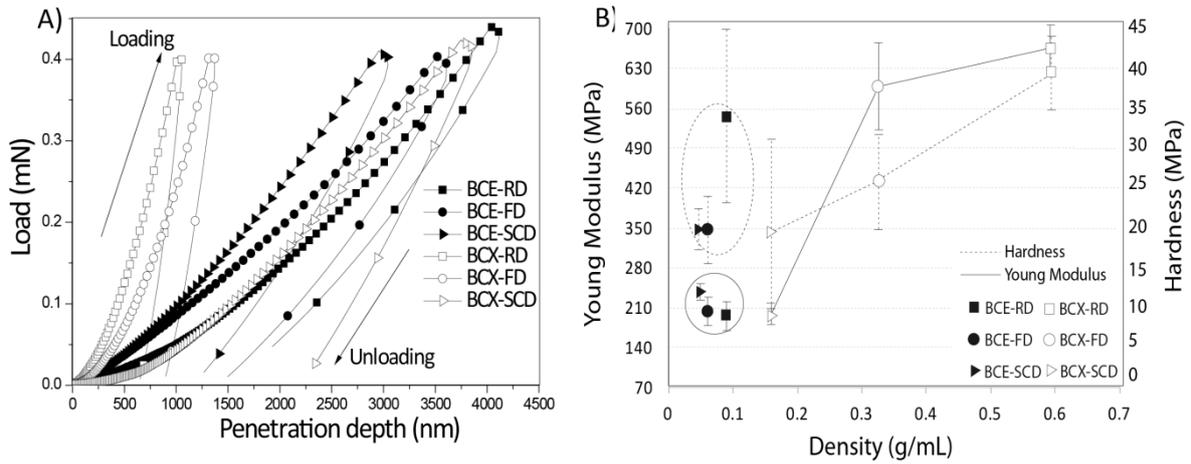
740 **Figure 6 shows pictures of the bending test performed: A) BCE films before the bending**
741 **test from the different drying methods, B) process of the bending test, C) the resulting**
742 **shapes of the BC films after the test and D) the shape of the films obtained on a BCE**
743 **film that was wetted-dried (WAC cycle).**



744

745

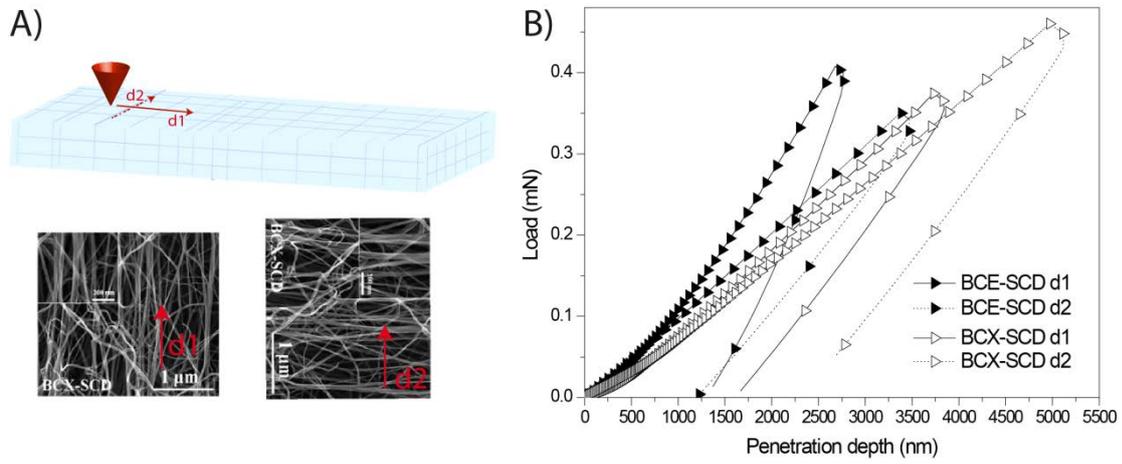
746 **Figure 7. A) Representative loading-unloading curve for each BC film obtained using**
 747 **the nanoindenter under a load of 0.4 mN. B) Mean values of "Young Modulus", ("H")**
 748 **and Hardness ("H") obtained for each BC film as a function of their density (n=14). We**
 749 **circled the "E" and "H" for BCE for clarity.**



750

751

752 **Figure 8. A) Schematic representation of the mechanical measurements performed over**
753 **two different directions. B) Load- displacement curves for BC films dried on SCD**
754 **conditions for the two bacterial strains.**



755

756

757

758

759

760

761 **Table 1. Summary of the structural and mechanical values obtained for the BC films.**

762

Drying method		RD		FD		SCD		
Strain		BCE	BCX	BCE	BCX	BCE	BCX	
Structural	Film Thickness (μm)	24 \pm 2	37 \pm 2	34 \pm 2	40 \pm 4	58 \pm 3	67 \pm 2	
	Density (g/mL)	0.08 \pm 0.01	0.59 \pm 0.03	0.06 \pm 0.01	0.32 \pm 0.02	0.05 \pm 0.01	0.16 \pm 0.01	
	Total Porosity (%)	94 \pm 2	60 \pm 2	95 \pm 2	79 \pm 2	96 \pm 2	89 \pm 2	
	Fibers diameter (nm)	17 \pm 2	19 \pm 3	20 \pm 4	19 \pm 3	16 \pm 2	20 \pm 4	
	WAC	1 st cycle	39.5	37.3	15.8	15.8	109.4	66.6
		after 3 cycles	15.3	14.2	92.3	50.2	19.8	19.4
		Decrease (%)	61	62	42	68	82	71
Mechanical	Penetration depth at 0.4mN load (μm)	3.9 \pm 0.4	1.1 \pm 0.1	4.1 \pm 0.4	1.4 \pm 0.3	3.2 \pm 0.5	4.3 \pm 1.5	
	Young modulus ("E")(MPa)	198 \pm 46	659 \pm 85	204 \pm 46	601 \pm 155	238 \pm 25	198 \pm 34	
	Hardness ("H")(MPa)	34 \pm 22	39 \pm 9	20 \pm 8	26 \pm 12	20 \pm 5	19 \pm 21	
	EP (%)	59 \pm 7	39 \pm 3	53 \pm 4	33 \pm 3	52 \pm 5	48 \pm 13	

763

764

765 **Table 2 Values of "Young Modulus" (MPa) and penetration depth at 0.4mN (μm) from**
766 **BC films dried in SCD conditions measured in two directions of the BC films.**

	BCE-d1	BCE-d2	BCX-d1	BCX-d2
<i>"Young modulus, E"</i> <i>MPa</i>	233 \pm 50	300 \pm 50	150 \pm 40	242 \pm 100
Penetration depth at 0.4 mN (μm)	3.1 \pm 0.5	3.5 \pm 0.9	4.9 \pm 0.8	4.4 \pm 0.7

767

768

769

Supplementary Material

[Click here to download Supplementary Material: Supporting Information Cellulose.pdf](#)