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Cellulose nanofibrils and silver nanowires active coatings for the development of antibacterial packaging surfaces

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Abstract: An active ink composed of cellulose nanofibrils and silver nanowires was deposited on flexible and transparent polymer films using the bar coating process, achieving controlled thicknesses ranging from 200 nm up to 2 μ m. For 350 nm thick coating on polyethylene terephthalate films, high transparency (75.6% transmittance) and strong reduction of bacterial growth equal to 89.3% and 100% was noted respectively against Gram-negative *Escherichia Coli* and Gram-positive *Staphylococcus Aureus* bacteria using AATCC contact active standard test. Retained antibacterial activity was found with films produced by reverse gravure roll-to-roll process, showing the promising capability of this antibacterial solution to be deployed industrially. Finally, the same ink was also deposited on polylactic acid substrate to investigate barrier properties: for 350 nm thick coating, a reduction of 49% of oxygen transmission rate (dry conditions) and 47% reduction of water vapor transmission rate was noted, proving the enhanced barrier properties of the coatings.

Keywords: cellulose nanofibrils, silver nanowires, transparent coatings, antibacterial activity, barrier properties, up-scaling

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31 **1. Introduction**

32 In a high consumption society, production and global movement of food products is considerable
33 and increasing continuously (Food and Agriculture Organization of the United Nations, 2019). **Food**
34 **products are exposed to harsh conditions throughout all of the supply chain stages and packaging is today**
35 **expected to meet more and more requirements.** Recent innovation has focused on the development of
36 active packaging, which refers to the ability of a package to respond to an external stimulus (Yildirim et
37 al., 2018). Within this framework, active packaging aims at increasing the shelf-life of a product by
38 optimizing the condition inside the package, thus improving food quality and reducing food waste.

39 Antimicrobial food packaging has been studied extensively over the past 20 years and aspires to
40 reduce pathogenic contamination **against bacteria, fungus or viruses (Sofi et al., 2018) with a preferred**
41 **mode of action working by contact for consumer safety and environmental concern. (Kaur & Liu, 2016).**
42 Silver nanoparticles (Ag NPs) have recently attracted a lot of attention since they display strong
43 antibacterial activity and can help fighting against the increasing antibiotic resistance in pathogens (Rai et
44 al., 2012). It has been established that the antibacterial effect of Ag NPs is attributed to the nanoscale of
45 the particles itself along with the continuous release of silver ions (Morones et al., 2005). The morphology
46 of Ag NPs plays a major role on their antimicrobial activity: the smaller the nanoparticle (the higher
47 surface area) the more active they are against bacteria (Carlson et al., 2008). The shape of the nanoparticle
48 is also important and the antibacterial activity decreases on progression from pyramids, cubes, spheres, to
49 wire-like shape (Hong et al., 2016; Pal et al., 2007). **Different parameters also influence the antibacterial**
50 **activity of Ag NPs such as surface charge, the presence of residual synthesis surfactants or capping agent,**
51 **and Ag NPs crystallinity. (El Badawy et al., 2011; Kvítek et al., 2008; Smetana et al., 2008).**

52 One way to use Ag NPs in packaging applications is to incorporate them into a nanocomposite to limit
53 nanoparticle migration. **Ag NPs have been recognized as potentially hazardous for health as studies have**
54 **focused on evaluating their toxicity against in vivo and in vitro studies. There is still a lack of information**
55 **and knowledge concerning the global health hazard on human, but Ag NPs are suggested to be mainly**
56 **dangerous by oral and inhalation adsorption and silver accumulation in different organs leading to specific**
57 **malfunctions (Korani et al., 2015). The potent activity of Ag NPs is dependent on a lot of factors such as**
58 **size, surface chemistry, shape, etc. and more research is needed to have a full overview of the toxicity of**
59 **Ag NPs toward the human body. It then appears obvious that the leaching of Ag NPs in antibacterial**
60 **composite for food packaging application is an important parameter to address. Nanocellulose materials**
61 **are biobased, biodegradable and biocompatible nanomaterials that are defined as cellulose particles with a**
62 **nano-scale size for at least one of their dimensions. One of the established class of nanocellulose are**
63 **cellulose nanofibrils (CNF) which come from the mechanical fibrillation of pulp suspension (Herrick et**

64 al., 1983; Turbak et al., 1983). CNF have been of particular interest because they display interesting
65 properties for packaging applications: high oxygen barrier property, vast chemical functionalization
66 possibilities and good mechanical properties. CNF can be used either alone as a free standing film or as a
67 coating on classic plastic packaging or paper substrates for its reinforcement properties (Afra et al., 2016;
68 Fukuzumi et al., 2009; Rodionova et al., 2012) and also display good matrix properties that can be used
69 successfully to immobilize nanoparticles.

70 Combinations of Ag NPs and nanocellulose composites have been investigated extensively by the
71 scientific community (Xu et al., 2017) and different composite preparation techniques exist. **In the most
72 common in-situ synthesis methods, CNF serve as a template matrix for adsorption of silver salts, followed
73 by in-situ chemical reduction (Uddin et al., 2017; Yu et al., 2019). Covalent bonding is another option ,yet
74 to our knowledge**, the only reported example comes from Ramaraju, Imae, & Destaye (2015), who
75 reported the immobilization of Ag NPs stabilized with a dendrimer by covalently bonding the NH₂-
76 terminated dendrimer on TEMPO-oxidized CNF. Finally, the physical mixing approach is just mixing
77 separately produced components. **To our knowledge**, the only example of simple mixing of CNF materials
78 with Ag NPs for antibacterial applications has been reported by Martins et al. (2012) who prepared
79 CNF/Ag NPs composites based on a polyelectrostatic assembly for antibacterial coating on a paper
80 substrate. **Simple mixing is a straightforward easily up-scalable procedure, however immobilization of the
81 Ag NPs on cellulose materials is relatively poor (Ilić et al., 2009), leading to reduced life-time of the
82 system and possible non-desired release of Ag NPs.**

83 This work presents the development of antibacterial packaging based on a combination of silver
84 nanoparticles and nanocellulose materials. Indeed, based on the precedent literature review, it was then
85 hypothesized that combining silver nanowires with cellulose nanofibrils could achieve easy-to-process
86 systems that can be deposited on flexible substrate to achieve contact killing antibacterial mode of action
87 along with improved barrier properties. In this study, the technical challenges were firstly to
88 straightforwardly produce a transparent and antibacterial coating on plastic polymer sheets. Secondly the
89 objectives were to prepare similar active coatings on biobased polymer sheets and to evaluate the
90 enhancement of their barrier property. Finally, the challenge of preparing active surfaces with a roll-to-roll
91 process for up-scaling purposes was addressed.

92 To tackle the three core challenges, a hybrid ink composed of TEMPO-oxidised cellulose nanofibrils and
93 silver nanowires was firstly deposited on polyethylene terephthalate substrates using laboratory-scale bar
94 coating process. **Thickness and transparency of the coatings were investigated and they were tested against
95 bacterial contamination.** The same technique was used to deposit the active ink on bio-based polylactic
96 acid sheets and the barrier properties of the produced films were characterized. Finally, a new lower cost

97 active ink was specifically formulated by the authors to assess the up-scaling possibility by using a reverse
98 gravure roll-to-roll coating process.

99 **2. Materials and methods**

100 **2.1. Materials**

101 A polyethylene terephthalate (PET) sheet substrate (Melinex® ST726 – 175 µm) and polylactic acid
102 (PLA) substrate (Earthfirst® BCWC – 75 µm) was respectively purchased from Dupont (France) and
103 Sidaplast (Belgium). A hybrid ink system composed of silver nanowires (Ag NWs) and TEMPO oxidised-
104 cellulose nanofibrils (T-CNF) in water (PolyBioWire® 9830C) was supplied by Poly-Ink (France) **with an**
105 **approximate 1% wt total mass and 1:1 ratio given by the supplier. However to investigate a roll-to-roll**
106 **semi-industrial deposition process, a new specific formulation was prepared, using T-CNF suspension**
107 **(CNF-A13) from Betulium (Finland) with approximately length of 50-400 nm and diameter of 5-15 nm,**
108 **at 5 %wt (charge density of $1.6 \pm 0.1 \text{ mmol.g}^{-1}$, estimated aspect ratio of 22.5) and using 2.5% wt Ag NWs**
109 **aqueous suspension (NGAP NF Ag-3170) from NanoGap (USA) with estimated length 10-50 µm,**
110 **diameter 50-100 nm (and so with an estimated aspect ratio of 400).**

111 Escherichia Coli ATCC 8739 and Staphylococcus Aureus ATCC 6538 were purchased from
112 Microbiologics (USA). PCA (Plate Counting Agar) was purchased from BD Difco (USA) and contains
113 beef extract (3g.l^{-1}), peptone (5g.l^{-1}), agar (15g.l^{-1}). Standard nutrient broth 1 was purchased from Roth
114 (Germany) and contains beef extract (3g.l^{-1}), peptone (15g.l^{-1}), sodium chloride (6 g.l^{-1}) and glucose (1g.l^{-1}).
115 Sodium thiosulphate (>99%), L-histidine (>98.5%), potassium dihydrogen phosphate (>99%),
116 Tween®80 and calcium chloride (>96%) were also obtained from Roth (Germany). L α -phosphatidyl
117 choline (>99%) was purchased from Sigma Aldrich (France). All materials were used as received.

118 **2.2. Active layer production by bar coating process**

119 **Coated films were first prepared using the supplied T-CNF/Ag NWs ink and using the bar coating process**
120 **(KCC101, Erichsen, Germany). Prior to coating, the films were corona treated (Klwar Calvatron SG2,**
121 **Germany): 2 passes, 90° rotation between the pass. The coatings were then oven-dried and different**
122 **thickness were achieved using different threaded rod diameter (Supplementary data). Coatings, corona**
123 **treatment and drying step parameters were optimized through extensive research with visual observation**
124 **of possible defects, transparency and sheet resistance measurements. Optimized corona treatment settings**
125 **are the following: 2.9 m.min^{-1} speed and 330 mA intensity for PET substrate, 3.5 m.min^{-1} speed and 150**
126 **mA intensity for PLA substrate. Optimized coatings and drying parameters are the following: coating**
127 **speed of 5.4 m.min^{-1} for both PET and PLA coatings, oven-drying step at 120°C for 60s for PET and at**

128 room temperature overnight for PLA coatings. Each coating was performed at least three times to assess
129 consistency and reproducibility of the process.

130 **2.3. Structural and quality characterization**

131 Field-Emission Gun Scanning Electron Microscope (FEG-SEM) was used to investigate the hybrid
132 nanostructure after 2 nm-coating of Gold /Palladium, at 5.4 mm working distance and 3 kV accelerating
133 voltage. A cross-section through the coated samples was prepared using a LEICA UC6 (Germany)
134 ultramicrotome apparatus equipped with a diamond knife, at room temperature and with a cutting speed of
135 1 mm.s⁻¹. The thickness of the coatings was then estimated by image analysis using FIJI software
136 (Schindelin et al., 2012; Schneider et al., 2012). At least 6 measurements in three different positions in the
137 films were considered.

138 Transmittance (%) was measured on a UV-spectrophotometer (Shimadzu Manufacturing Inc., USA),
139 using the photometric mode. Six different measurements were conducted on each sample at 550 nm
140 wavelength. Sheet resistance (Ω /sq) of the coated layer was measured by a four-probe system (Jandel
141 Universal, USA). The measurement was repeated at six positions on the film and the average computed
142 along with associated standard deviation.

143 **2.4. Antibacterial characterization**

144 Prior to antibacterial testing, a leaching assay was performed by putting a 2 x 2 cm specimen of coating
145 (24 μ m estimated wet thickness) in 10 ml of deionized water (DI), under agitation (100 rotation per
146 minute) at 37°C. After 72 hours, the liquid media was recovered and full UV-vis spectra (300-700 nm
147 wavelength, 1 nm scan rate) were recorded on a UV-spectrophotometer (Shimadzu Manufacturing Inc.,
148 USA), and compared with Ag NWs suspensions at 0.005% wt.

149 All the glassware, consumables, tools and solutions used for the antibacterial characterization were
150 sterilized prior to use in autoclave for 20min at 120°C and 1.034 bar.

151 Antibacterial activity was assessed initially using an inhibition zone qualitative test following a modified
152 AFNOR EN 1104 standard (AFNOR standard, 2005). To exaggerate the antibacterial activity of the
153 samples, the test was carried out on multilayer coating (10 layers) prepared with the same parameters as
154 single layer coating (threaded rod with 1.27 diameter –100 μ m estimated wet thickness). Ag NWs only
155 coating was used as control and prior to deposition, the starting suspension was diluted to 0.5% wt in DI
156 and dispersed using an Ultra Turrax high shear disperser (30s, 10,000 rpm). The produced samples were
157 cut into 5 cm x 5 cm specimens and dry-sterilized for 24 hours at 60°C. 10 ml of pre-inoculated PCA at

158 10^5 CFU.ml⁻¹ (Colony Forming Unit) was poured in a petri dish and after cooling the samples were put on
159 top with the coated side facing the agar. The incubation period was 72 hours at 37°C and the inhibition
160 zone was assessed visually after incubation.

161 Quantitative tests were performed on single layer coatings with different thickness using a protocol
162 adapted from the AATCC TM100-1998 standard (AATCC standard, 1998). The samples were cut into 2
163 cm x 2 cm specimens and dry-sterilized for 24 hours at 60°C. 200 µl of bacterial suspension at 5.10^5
164 CFU.mL⁻¹ in 20% nutrient broth (5 g.l⁻¹ nutrient broth, 6.8 g.l⁻¹ sodium chloride) were deposited on the
165 samples specimen and incubated at 37°C for 24 hours. After incubation, the bacterial suspension was
166 recovered by washing with a prepared neutralizing solution (3 g.l⁻¹ of L- α -phosphatidyl choline, 5 g.l⁻¹ of
167 sodium thiosulphate, 1 g.l⁻¹ of L-histidine, 30 g.l⁻¹ of Tween 80, 10 ml of potassium dihydrogen phosphate
168 at 0.0425 g.l⁻¹, controlled pH of 7.2 ± 0.2) and the final concentration was determined by the plate
169 counting numbering method. The antibacterial activity (AA, %) of the samples was compared
170 quantitatively using the following equation (1):

$$171 \quad AA (\%) = \frac{BC_{reference} - BC_{sample}}{BC_{reference}} \times 100 \quad (1)$$

172 Where BC_{reference} (log CFU) is the remaining bacterial concentration on the PET substrate with no coating
173 and BC_{sample} (log CFU) is the remaining concentration on the tested sample.

174 **2.5. Barrier properties characterization**

175 The Oxygen Transmission Rate (OTR) of the coated samples was measured at 0, 50 and 80 % humidity
176 rate following the ASTM-F 1927-98 standards (ASTM standard, 2014) on a Systech Illinois Permeation
177 Analyser (USA) equipped with a coulometric detector. **The tests were conducted at 23°C and with a 6.15**
178 **cm⁻¹ exchange surface.** At least 3 different samples for each coating were tested and an average is
179 computed along with the associated standard deviation.

180 Water Vapour Transmission Rate (WVTR) was also measured on the same samples following a slightly
181 modified T 448 om-09 standard (TAPPI Standard, 2009). Around 30 g of anhydrous calcium dichloride
182 salt was put in a metal cup covered by the test sample and closed by a rubber gasket and screw-down cap.
183 The test was conducted at a regulated temperature (23°C) and humidity (50 %). The water-uptake of the
184 anhydrous salt through the tested sample was monitored by weighing at least twice a day for at least one
185 week. The WVTR value was calculated using the following equation (2):

$$186 \quad WVTR (g.m^{-2}.day^{-1}) = \frac{24 \times \Delta m}{\Delta t \times S} \quad (2)$$

187 Where Δt (h) is the time between two measurements, Δm (g) the corresponding weigh-uptake and S (m²)
188 is the exposed surface (6.15 cm²).

189 **2.6. Specific ink formulation and characterization for up-scaling test**

190 A specific, lower cost and in bigger volume formulation was prepared using T-CNF suspension
191 (Betulium) and Ag NWs suspension (NanoGap) redispersed together at 1% wt total mass with a mass ratio
192 of 1:1 using an Ultra Turrax high shear disperser (30s, 10,000 rpm).

193 T-CNF was imaged using a Philips CM 200/FEI (USA) Transmission Electron Microscope (TEM)
194 equipped with a TemCam F216 from TVIPS (Germany), at 200 kV acceleration voltage. A diluted
195 suspension of T-CNF was drop-casted on a copper grid with an amorphous carbon coating and uranyl
196 acetate (2%) dye was deposited on the drop-casted sample. AFM images were recorded on a Veeco
197 NanoScope-V apparatus (Canada). Ag NWs suspension was drop-casted on a Mica substrate and dried
198 overnight. A tapping mode with an OTESPA Bruker (USA) silicon cantilever was used covering a 3.3 μm
199 x 3.3 μm surface area.

200 The sedimentation evolution of the formulated ink was characterized by putting 10 ml of ink into a
201 cylindrical glass vial of 29 mm diameter section and measuring the sediment height after a fixed time. The
202 sediment height (S , %) was calculated using the following equation (3):

$$203 \quad S = \frac{H}{H_0} \times 100 \quad (3)$$

204 Where H (mm) is the measured sediment height and H_0 (mm) the total liquid height.

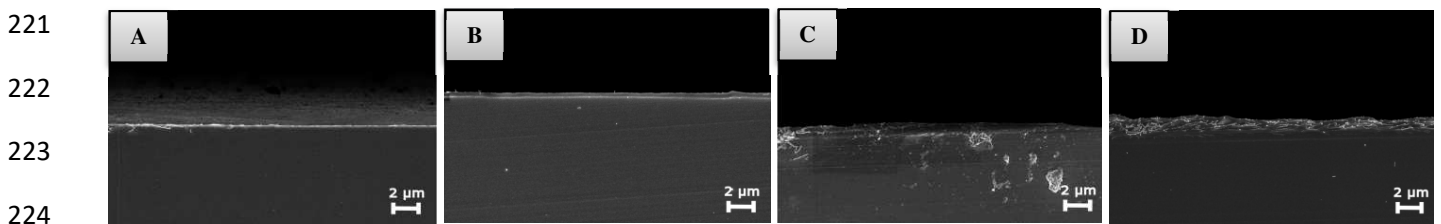
205 The rheological studies were performed on a rotational rheometer (MCR02, Anton Paar) at a set
206 temperature of 20°C with a cone-plate geometry (1.013° angle, 50 mm diameter and 55 μm truncate)
207 along with a set gap of 0.112 mm. Flow curves experiment were performed with increasing shear rate
208 ranging from 0.1 to 1000 s⁻¹.

209 Reverse gravure coating was conducted using a Mini-LaboTM apparatus from MIRWEC Film, Inc./ Yasui
210 Seiki company (USA). Coating was applied at a speed of 1 m.min⁻¹ and dried using infrared heaters. Two
211 different coating weights were produced using two different gravure rolls: roll 30 (estimated surface
212 volume of 150 cm³.m⁻², estimated transfer fraction of 0.33 and estimated approximate wet coat thickness
213 of 30-45 μm), and roll 120 (estimated surface volume of 34.7 cm³.m⁻², estimated transfer fraction of 0.28
214 and estimated approximate wet coat thickness of 5-11 μm).

215 **3. Results and discussion**

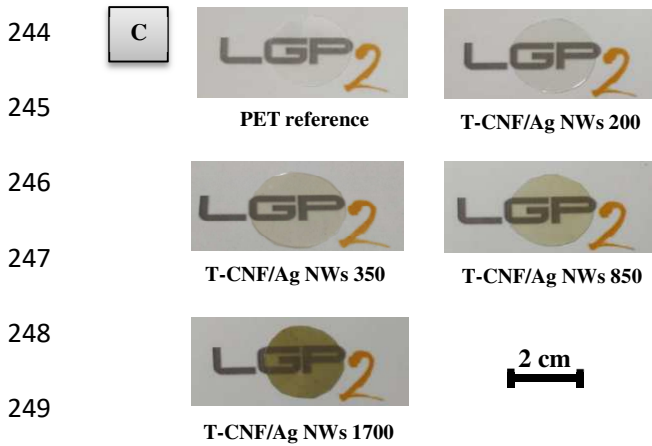
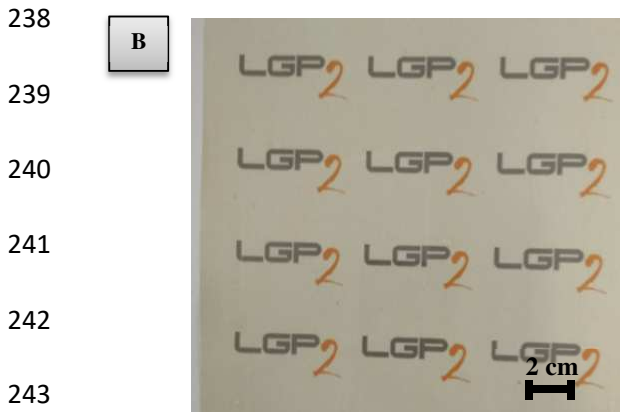
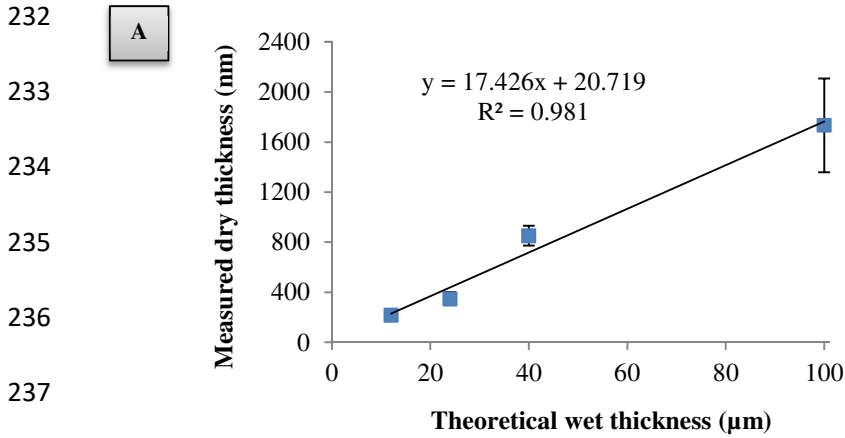
216 **3.1. Bar coating deposition on PET and antibacterial properties.**

217 The active ink was bar coated at 4 different thicknesses on PET sheets and the dry thickness was
218 determined from a cross section captured by FEG-SEM imaging (Figure 1). For the thicker coatings the
219 results demonstrated a higher standard deviation because the ultramicrotome cuts damaged the coated
220 layer.



225 **Figure 1: FEG-SEM cross section images of A) 12 μm wet estimated thickness coating B) 24 μm wet**
226 **estimated thickness coating C) 40 μm wet estimated thickness coating D) 100 μm wet estimate thickness**
227 **coating**

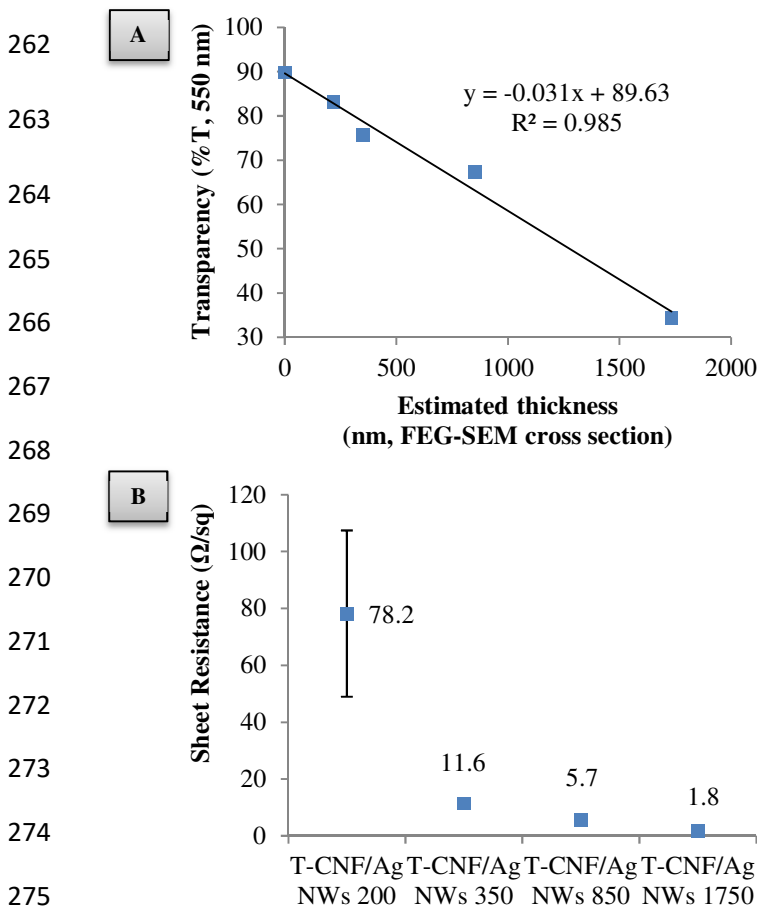
227 The measured average thicknesses are respectively 217 ± 27 , 349 ± 52 , 851 ± 79 and 1733 ± 375 nm. A
228 good linear correlation was found between wet and dry values (Figure 2A) proving a controlled thickness
229 deposition and accurate measurement. Consequently, in this study the samples will be designated T-
230 CNF/Ag NWs 200, T-CNF/Ag NWs 350, T-CNF/Ag NWs 850, T-CNF/Ag NWs 1750, referred according
231 to their dry thickness.



250 **Figure 2: Coating quality investigated by A) measured**
 251 **dried thickness (nm) vs theoretical wet thickness (µm), B)**
 252 **qualitative picture of 15 cm x 15 cm large area of T-**
 253 **CNF/Ag NWs 350 sample and B) qualitative picture of**
 254 **reduced area of all produced samples (2 cm diameter) and**
 255 **scale bar.**

253 From a macroscopic point of view, the coatings were observed to be uniform with no local uncoated
 254 patches (Figure 2B). For packaging applications, one of the key parameters for active coatings is to retain

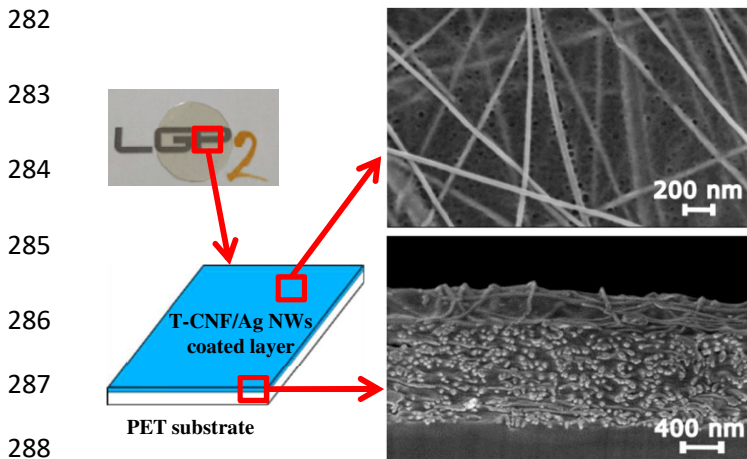
255 transparency for the purpose of aesthetics and product display. The coatings were found to be relatively
 256 transparent as the LGP2 logo is clearly visible under the different samples displayed in Figure 2C. The
 257 transmittance at 550 nm was measured at 83.3, 75.6 and 67.3% respectively for T-CNF/Ag NWs 200, T-
 258 CNF/Ag NWs 350 and T-CNF/Ag NWs 850 samples, when compared to the 89.8% reference uncoated
 259 PET. Coating quality may also be determined by measuring conductivity (Fahad et al., 2019) and except
 260 for T-CNF/Ag NWs 200, the produced coatings display high conductivity (low sheet resistance) with a
 261 very low standard deviation, confirming coating uniformity (Figure 3B).



276 **Figure 3: Coatings quality assessment using A)**
 277 **transparency measurement (% T, 550 nm) vs**
 278 **estimated dry thickness (nm) and B) sheet resistance**
 279 **measurements by 4-probe system (Ω /sq).**

278 The morphology of the coated layer was also investigated using electron microscopy imaging (Figure 4)
 279 and the coated layer was found to be highly organized in a dense network, yet not aggregated, with an
 280 orientation of the Ag NWs toward the direction of shearing during the bar coating process.

281

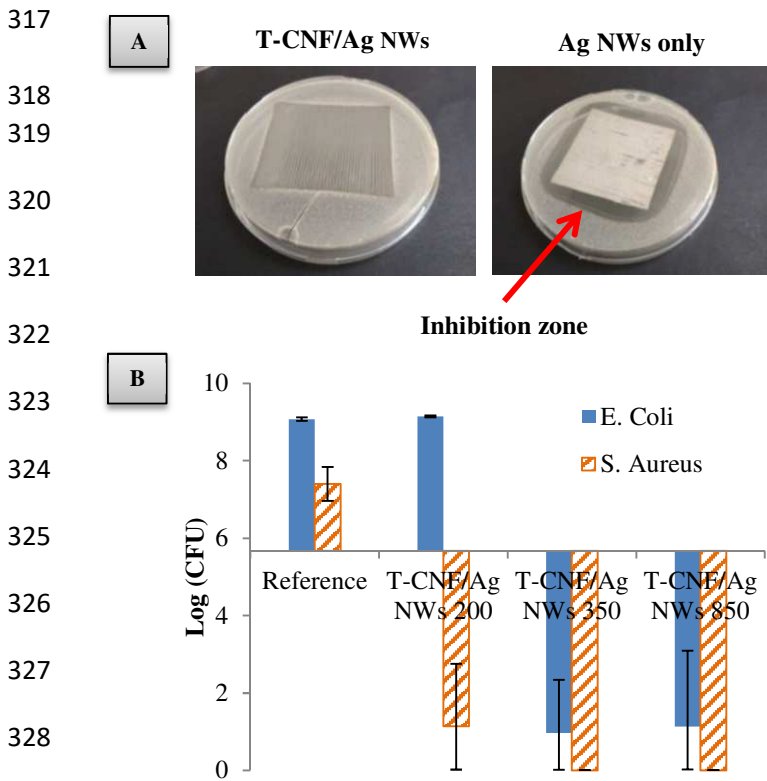


289 **Figure 4: Schematic view of the T-CNF/Ag NWs 850**
 290 **coated samples and FEG-SEM pictures of surface and**
 291 **cross-section**

291 A preliminary leaching study was performed prior to antibacterial characterization. The test showed that
 292 no peaks corresponding to Ag NWs could be seen after recording UV-vis spectra of the recovered liquid
 293 media after the leaching assay (Supporting Information). It can then be concluded that there is only limited
 294 leaching of Ag NWs measured by this technique. The antibacterial activity of the active coatings was then
 295 investigated with qualitative inhibition zone test (Figure 5A). For the sample coated only with Ag NWs, a
 296 broad inhibition zone is visible proving the antibacterial activity of Ag NWs. The antibacterial activity of
 297 Ag NWs was established to be mainly due to the release of silver ions because of their lower surface area
 298 and thus lower adhesion with bacteria cell walls compared to spherical or cubic silver nanoparticles (Hong
 299 et al., 2016; Visnapuu et al., 2013). It can then be assumed that the broad inhibition zone of the sample is
 300 due to the release of silver ions into the media. For the sample coated with the mixture of both T-CNF and
 301 Ag NWs no inhibition zone can be seen which suggests that physically entrapping the Ag NWs within the
 302 T-CNF matrix leads to a contact killing mode of action with no or minimal release of active material.

303 To quantitatively verify the activity of the sample the AATCC standard method was performed against
 304 both Gram-positive *Staphylococcus Aureus* and Gram-negative *Escherichia Coli* (Figure 5B). The T-
 305 CNF/Ag NWs 200 sample show a surprising activity: it displays no impact against *E. Coli* whereas for *S.*
 306 *Aureus*, a strong reduction of 6.3 log of colony forming unit compared to the uncoated reference sample
 307 was noted, which corresponds to a 86.5% activity. Indeed, the bacterial activity in the presence of silver
 308 nanoparticles is usually higher for *E. Coli* than *S. Aureus* (Chernousova & Epple, 2013). This is explained
 309 by the difference in the bacteria cell wall: Gram-positive bacteria display a more permeable layer made of
 310 peptidoglycan only whereas Gram-negative bacteria have a thinner yet more impermeable
 311 lipopolysaccharide layer coupled to a thin peptidoglycan layer (Slavin et al., 2017). The difference in

312 activity observed when compared with the literature could be explained by the very thin coating layer for
 313 T-CNF/Ag NWs 200 which is probably close to the antibacterial activity limit of the coating. The T-
 314 CNF/Ag NWs 350 and T-CNF/Ag NWs 850 coatings displayed more or less the same performance
 315 respectively 89.3% vs *E. Coli*/100% vs *S. Aureus* and 87.6% vs *E. Coli*/100% vs *S. Aureus*, showing that a
 316 350 nm thick coating is enough to achieve almost 100% antibacterial activity.

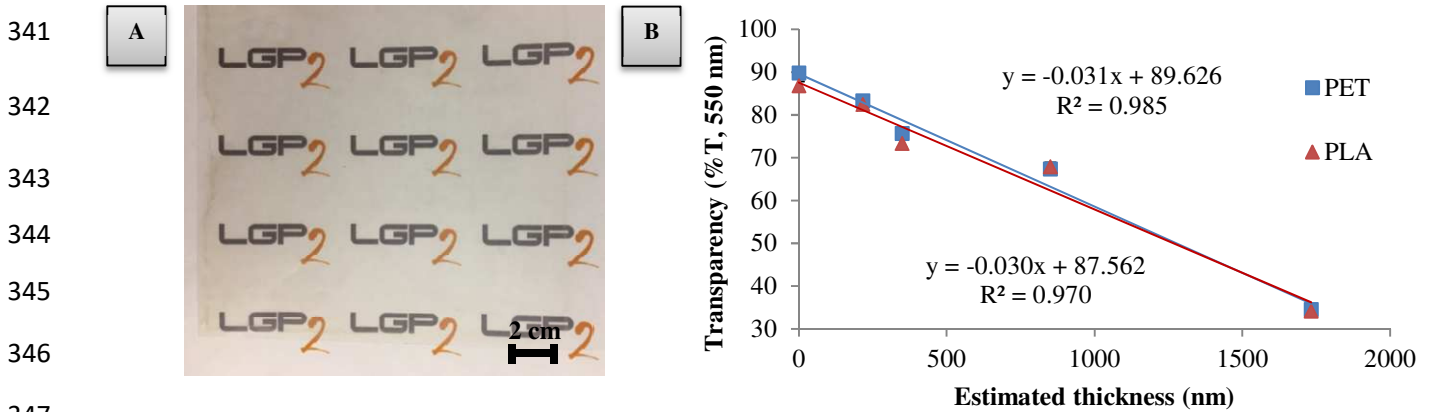


329
 330 **Figure 5: Antibacterial characterization of the coated samples by A) inhibition qualitative analysis for *E. Coli* bacteria strain B) qualitative analysis using AATCC standard method 100-1998 vs *E. Coli* and *S. Aureus* with results expressed in log of colony forming units (log CFU).**

333 **3.2. Bar coating on PLA and barrier properties.**

334 In order to explore a bio-based substrate, the same ink was also deposited on polylactic acid (PLA) films
 335 using the bar coating process. **The coatings were found to be homogeneous, with no uncoated patches and**
 336 **no visual defects (Figure 6A).** The different deposited thickness for the PLA coatings were estimated to be
 337 relatively close to the ones for the PET coatings. Indeed, the decrease of the transparency of the reference
 338 films due to the coating are more or less the same for both the PET and PLA coatings (Figure 6B). The

339 samples were then named with the same code, for instance T-CNF/Ag NWs 350 PLA corresponds to the
340 T-CNF/Ag NWs coating on PLA with an approximated thickness of 350 nm.



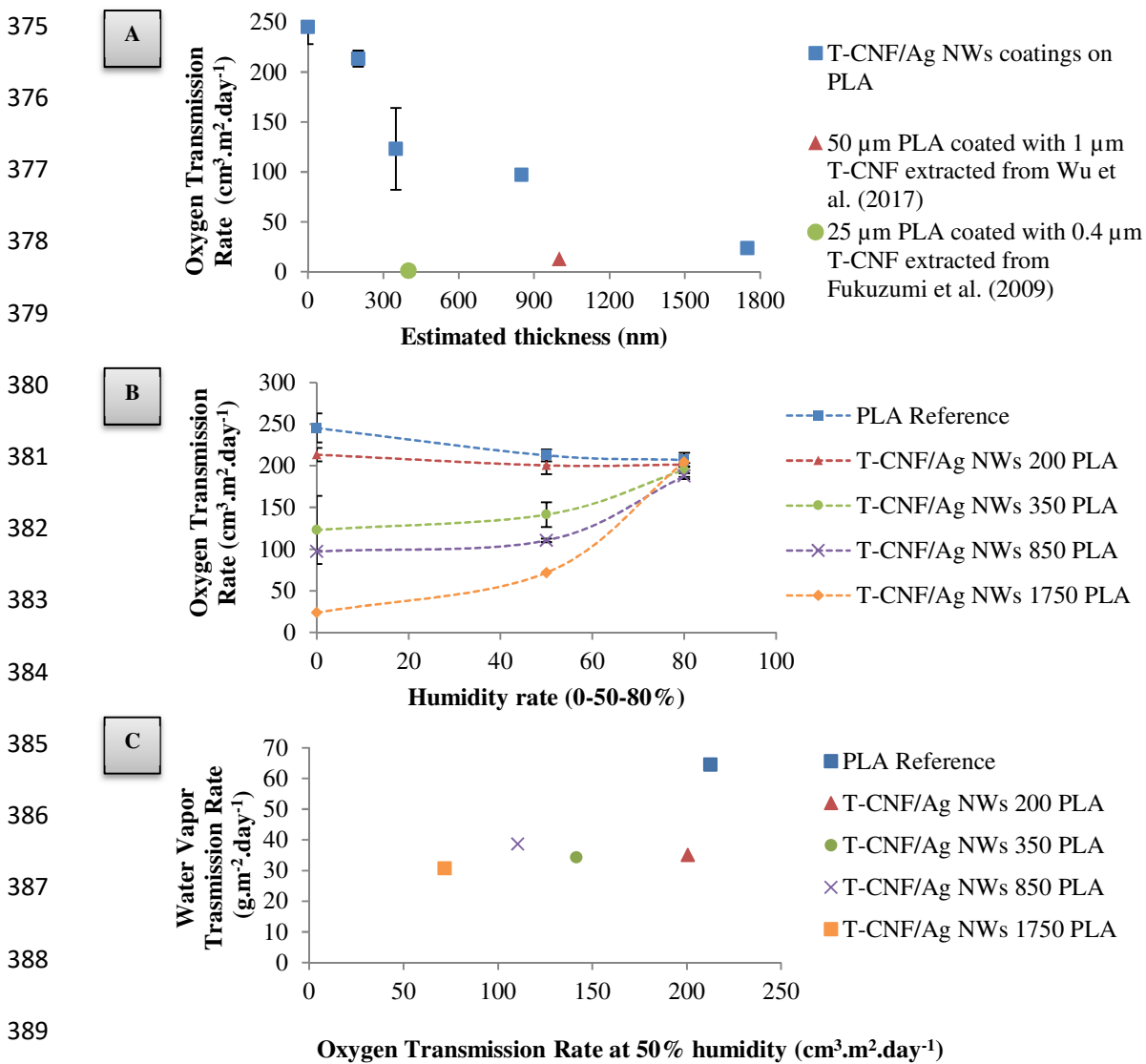
347 **Figure 6: T-CNF/Ag NWs coating on PLA quality assessment with A) picture of 15 cm x 15 cm large area of**
348 **T-CNF/Ag NWs 350 sample and B) transparency (% T, 550nm) measurements and comparison with coatings**
on PET.

349 **CNF are often used in packaging application for their interesting barrier properties (Ferrer et al., 2017)**
350 **and so the barrier properties of the coated PLA was investigated.** Under dry conditions, the oxygen
351 transmission rate (OTR) of the produced films was significantly decreased achieving more than 90%
352 reduction for an estimated 1750 nm thick coatings (Figure 7A) but the measured values are however
353 higher than those classically found in the literature for a T-CNF coating (Fukuzumi et al., 2009; Wu et al.,
354 2017) (Figure 7A). Surprisingly in this study coating only T-CNF material was not possible due to high
355 **dewetting** and so it was not possible to compare T-CNF/Ag NWs coatings to T-CNF coating only. A
356 stronger surface treatment may be required to achieve T-CNF only coatings. **CNF performance as a barrier**
357 **layer is generally explained first by the large specific surface area and dense network of hydrogen bonding**
358 **of the material which makes it difficult for any molecules to pass through (Ferrer et al., 2017). The CNF**
359 **network also presents high tortuosity due to the impermeable crystalline regions and strong entanglement**
360 **of the flexible fibres presenting a capability to seal any gaps within the network (Lagaron et al., 2004;**
361 **Syverud & Stenius, 2008; Belbekhouche et al., 2011).** Adding larger and rigid Ag NWs into the T-CNF
362 matrix probably led to a physical disruption of the sealed network and thus explaining the difference
363 between the measured properties and the ones described in the literature.

364 As expected from using CNF materials, the OTR measurement is humidity dependent (Figure 7B) and at
365 high humidity (80%) the OTR values of the different coatings are close to the uncoated PLA reference.
366 **The different coatings also showed a significant improvement of the WVTR values (Figure 7C) which is**
367 **relatively similar (around 50% reduction) for all of the samples. This is interesting as coating CNF on**
368 **polymer substrate usually leads to unchanged or slightly reduced water vapor permeability (Aulin et al.,**

369 2013; Vartiainen et al., 2017), due to the hydrophilic nature and low water resistance of such materials.
 370 (Nair et al., 2014).

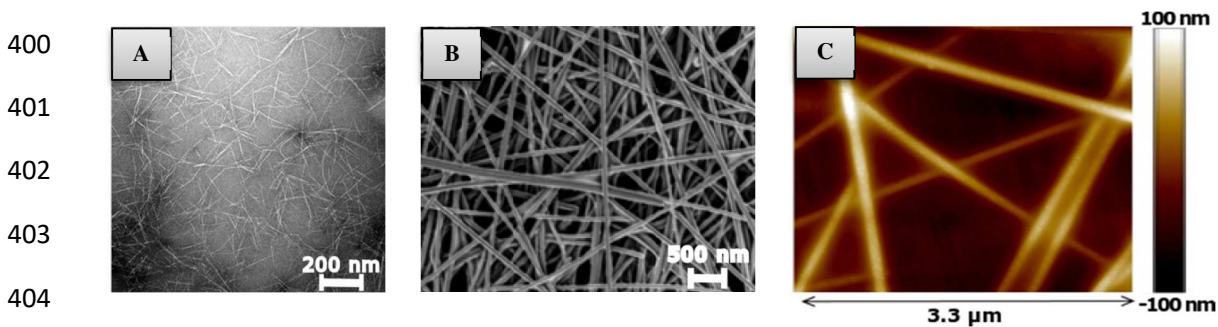
371 Coatings on biopolymer PLA sheets were successfully conducted and the coatings were found to be
 372 homogeneous with no uncoated patches and no visual defects. The deposited layers were found to be
 373 relatively similar to the coatings on PET and significant decrease for both the oxygen transmission rate
 374 and water vapour transmission rate was noted, proving the enhanced barrier properties.



390 **Figure 7: PLA coatings barrier properties characterizations with A) Oxygen Transmission Rate**
 391 **($\text{cm}^3 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) measurements in dry conditions vs estimated coating thickness (nm) and**
 392 **comparison with literature data B) Oxygen Transmission Rate ($\text{cm}^3 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) measurements at**
 393 **different humidity rate (0-50-80%) and C) Water Vapor Transmission Rate ($\text{g} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) vs**
 394 **Oxygen Transmission Rate ($\text{cm}^3 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) at 50% of humidity.**

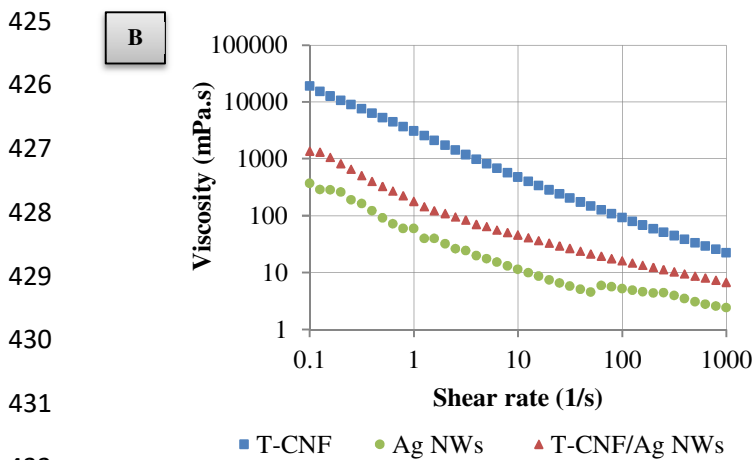
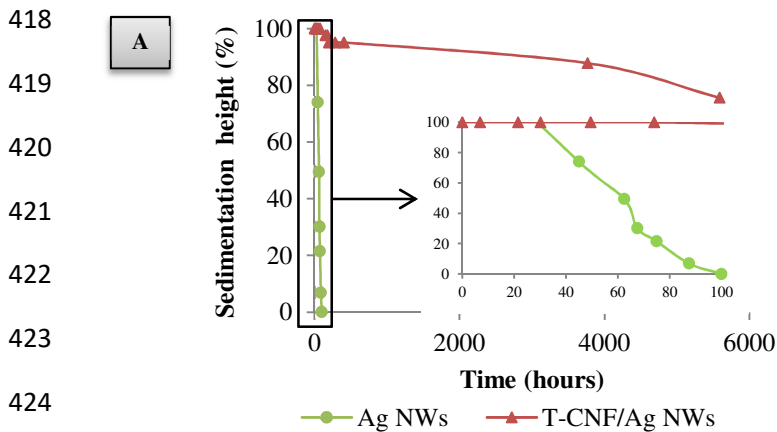
393 **3.3. Up-scaling**

394 As a more direct route and from a more economical point of view, a similar antibacterial ink was
395 specifically formulated by simple mixing and redispersion of the separately supplied components T-CNF
396 and Ag NWs in deionized water (see Materials and methods). The purchased T-CNF possess small
397 dimensions (approximately length 50-400 nm, diameter: 5-15 nm) and are rather rigid, as shown in the
398 TEM images in Figure 8A. The silver nanowires dimensions were approximately one order of magnitude
399 higher than the T-CNF: estimated length 10-50 μm , diameter 50-100 nm (Figure 8B and C).



405 **Figure 8: Raw materials microscopy imaging for A) TEM images of T-CNF B) SEM**
406 **images of silver nanowires C) AFM images of silver nanowires**

406 As it has already been described elsewhere (Hoeng et al., 2016), the T-CNF/Ag NWs ink is relatively
407 stable over time and exhibits a very low sedimentation rate, for example 75% sedimentation height after 8
408 months of testing, as compared to the Ag NWs suspension without T-CNF, that sedimented completely
409 after 4 days (Figure 9A). Flow curve rheology experiments were performed to assess the possibility to use
410 coating process for ink deposition and both materials and the final formulation display classic strong shear
411 thinning behaviour (Herrick et al., 1983; Hoeng et al., 2017) (Figure 9B). These behaviours can be
412 explained by the high aspect ratio of the nanoparticles in these colloidal suspensions and the induced
413 alignment of the particles at high shear rate, thus decreasing the viscosity. Ink viscosity at high shear rate
414 was found to be in line with was expected for the bar coating process. It was found that for the different
415 tested shear rate, viscosity of T-CNF suspension was one order of magnitude higher than Ag NWs. T-CNF
416 dictates the viscosity in the T-CNF/Ag NWs ink by acting as a rheological modifier which was already
417 shown in the literature for other aqueous CNF suspension coatings (Grüneberger et al., 2014).



433 **Figure 9: Ink formulation characterization compared to raw materials with A) sedimentation height measurement (%) and B) rheological flow curves. All formulations are at 1% wt total concentration.**

435 **3.3. Roll-to-roll process deposition**

436 The potential for this formulation to be applied to industrial-like roll-to-roll coating process was also

437 investigated using a reverse gravure coating process. **Reverse gravure coating is interesting for packaging**

438 **applications because of the wide range of inks and substrate that can be used and was also chosen because**

439 **of the versatility of the process and the possibility to deposit low coat weights with a uniform and**

440 **controlled thickness (Kipphan, 2001; Vak et al., 2016).** Two different coating thicknesses were

441 investigated and designated after their estimated wet thickness and previously established wet

442 thickness/dry thickness relation for the bar-coated samples. In other words, T-CNF/Ag NWs 850RR for

443 the coating using the roll 30 (wet coat thickness of 30-45 μm), and T-CNF/Ag NWs 200RR for the

444 coating using roll 120 (wet coat thickness of 5-11 μm). **The coatings were found to be visually of high**

445 **quality with no uncoated patches, no visual defects and good homogeneity (Figure 10A).** The antibacterial

446 properties of the T-CNF/Ag NWs 200RR is relatively low whereas T-CNF/Ag NWs 850RR display a

447 strong antimicrobial effect (Figure 10B). The T-CNF/Ag NWs 850RR sample showed a strong and
448 significant bactericidal effect, precisely 7.22 log reduction corresponding to 77.9% calculated antibacterial
449 activity against *E. Coli* and 100% against *S. Aureus*. On the other hand, the T-CNF/Ag NWs 200RR
450 sample showed no activity at all against *S. Aureus* whereas it showed a 7.57 log reduction corresponding
451 to 81.7% activity against *E. Coli*. The measured antibacterial activity for the roll-to-roll coated samples
452 using the up-scaled ink formulation were found to be in line with the results for the bar coated samples
453 and thus the proposed solution for antibacterial packaging could hence be easily adapted to an industrial
454 scale.

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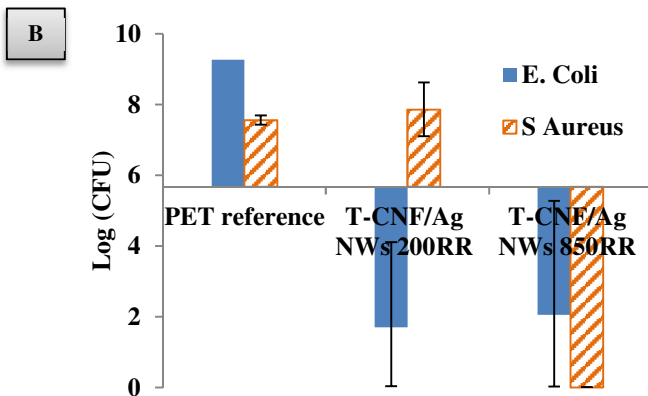
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Figure 10: Roll-to-roll coatings with A) visual aspect of the coatings and B) AATCC qualitative method for antibacterial activity assessment.

470 **4. Conclusion**

471 This work demonstrated the antibacterial activity of a hybrid system composed of TEMPO-oxidised
472 cellulose nanofibrils and silver nanowires, coated as thin layers on different flexible polymeric substrate.
473 The controlled thickness of the coatings on PET ranged from 200 to 1750 nm with a high relative
474 transparency superior to 65%. The antibacterial activity was suggested to work by contact active killing
475 mode of action and for the 350 nm thick coating, a significant AATCC standard antibacterial activity of
476 89.3% and 100% was noted respectively against Gram-negative *Escherichia Coli* and Gram-positive
477 *Staphylococcus Aureus* bacteria. Moreover, the same ink was also deposited on PLA sheets with similar
478 estimated thickness and quality. After coating, oxygen and water vapour permeability of the PLA
479 substrate was significantly reduced, respectively 49% of oxygen transmission rate (dry conditions) and
480 47% reduction of water vapor transmission rate for 350 nm thick coating. Finally, a similar ink with lower
481 cost materials was specifically formulated for up-scaling purposes and producing active surfaces using a
482 roll-to-roll process was proved using the reverse gravure process, showing a strong retained antibacterial
483 activity. Perspectives for a future work would be investigation of the migration of silver ions within the
484 cellulose nanofibrils matrix and the influence of matrix surface chemistry.

485 **Declaration of competing interest**

486 The authors declare that there is no conflict of interests with this manuscript.

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