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► To cite this version:

Nadège Leray, Malika Talantikite, Ana Villares, Bernard Cathala. Xyloglucan-cellulose nanocrystalchitosan double network hydrogels for soft actuators. Carbohydrate Polymers, 2022, 293, pp.119753. 10.1016/j.carbpol.2022.119753 . hal-04193849

HAL Id: hal-04193849 https://hal.inrae.fr/hal-04193849

Submitted on 1 Sep 2023

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Xyloglucan-cellulose nanocrystal-chitosan double network hydrogels for soft actuators

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7 ABSTRACT

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8 Hydrogels are materials consisting in a three-dimensional hydrophilic polymer network swollen 9 by a large amount of water. An efficient strategy to elaborate hydrogels consists in establishing 10 double polymer networks in order to achieve high strengthening effect associated with other 11 properties such as transparency or tailored swelling capacities. In this work, we prepared 12 cellulose nanocrystals (CNC)-based hydrogels with double network architecture. The first 13 network, formed by CNC and xyloglucan (XG), takes advantage of entropic adsorption of XG on 14 the CNC surface while the second network relies on electrostatic interactions between cationic 15 Chitosan (Chi) and anionic CNC. Hydrogels with different compositions were successfully 16 prepared. Their rheological properties, stability and swelling capacities in acidic and alkaline 17 solutions were evaluated. Internal organizations of hydrogels were investigated by fluorescence 18 microscopy after polymer labelling and polarized optical microscopy (POM). Finally, hydrogels 19 demonstrated excellent mechanical properties and tuneable swelling capacities that can be 20 leveraged for the implementation of bilayer actuators. Therefore, we further prepared films 21 composed of two hydrogels layers, each one containing a different XG/CNC ratio. Bilavered

- films bended in water due to the asymmetric swelling of layers and the extent of bending can be
- 23 modulated by the XG/CNC ratio.
- 24
- 25 **Keywords**: xyloglucan, cellulose nanocrystals, chitosan, hydrogel, swelling, actuator.

26 **1. Introduction**

27 Hydrogels have attracted much attention due to their ability to hold large amounts of water. They 28 have been studied for decades as they found applications in various fields including medicine, 29 pharmaceutics, cosmetics, domestic uses and personal care (Patel & Thareja, 2022; T. Zhu et al., 30 2019). Hydrogels are composed of three-dimensional polymer networks that exhibit outstanding 31 features such as high water absorption, transparency and good mechanical properties among 32 many others. During last two decades, research has focused on natural hydrogels to gradually 33 replace synthetic hydrogels. Biopolymers have demonstrated a great potential, particularly, 34 polysaccharides are excellent candidates thanks to the presence of a high number of hydrophilic 35 groups (hydroxyl groups and others). Different polysaccharides have demonstrated gel-like 36 behavior, such as alginate, carrageenan, chitosan, cellulose or starch (Chang & Zhang, 2011; 37 Coviello, Matricardi, Marianecci, & Alhaique, 2007; Patel & Thareja, 2022; T. Zhu et al., 2019). 38 Nanocelluloses have also arisen as outstanding components of hydrogels (Du et al., 2019). 39 Indeed, cellulose nanocrystals (CNC), which are obtained by acid hydrolysis of the cellulose 40 fiber, provide excellent mechanical properties thanks to their rigid nature and rod-like shape 41 (Eichhorn, 2011; Moreau, Villares, Capron, & Cathala, 2016). The specific association of 42 cellulose with certain hemicelluloses, such as xyloglucan (XG), allows controlling the swelling 43 behavior (Villares, Bizot, Moreau, Rolland-Sabate, & Cathala, 2017), and results in gel-like 44 dispersions (Talantikite, Beury, Moreau, & Cathala, 2019; Talantikite, Gourlay, Le Gall, & 45 Cathala, 2019; Talantikite et al., 2021). The XG/CNC hydrogels are stabilized by hydrogen 46 bonds and dispersive forces, which allows the breakdown and re-formation of the gel under 47 shearing. However, hydrogen bonds do not confer enough stability to the network and the 48 XG/CNC hydrogels are mechanically weak and loose its shape under shear forces (Talantikite,

Gourlay, et al., 2019). One strategy to obtain more mechanically efficient networks is the formation of semipermanent junction points by interanchored long chains. In this field, the formation of interpenetrating networks significantly improves the mechanical properties, and the hydrogel stability (K. J. De France, Cranston, & Hoare, 2020; Kevin J. De France, Hoare, & Cranston, 2017). The physical interlocking of two networks, known as the double or interpenetrating network, creates junction points that stabilize the hydrogel and favor water uptake.

56 In this work, we took advantage of the specific interactions between polysaccharides and 57 nanocellulose to prepare all biobased hydrogels. Hydrogels were produced by a sequential two-58 step process, firstly creating the XG/CNC network, and then cross-linking a second network of 59 chitosan (Chi) within the first. The specific interaction between XG and CNC provides the 60 physical properties such as water uptake and mechanical resistance, and the electrostatic 61 interaction between the positive charge of Chi and anionic CNC facilitated the interaction 62 between the two networks. We obtained highly swollen and transparent hydrogels, showing good 63 mechanical resistance. By changing the XG/CNC ratio, we succeeded in tuning the hydrogel 64 properties in terms of water uptake and mechanical properties with a simple, efficient and green 65 process. The formation of anisotropic structures, such as successive layers showing different 66 swelling behavior and/or mechanical properties may trigger shape changes and motion, which is 67 the mechanism of actuation (McCracken, Donovan, & White, 2020; Shang, Le, Zhang, Chen, & 68 Theato, 2019). Soft actuators from biopolymers have attracted much attention thanks to their 69 environmentally friendly properties and the possibility to tune their response by non-covalent 70 interactions (Wu et al., 2013; Zhao et al., 2021; Q. Zhu et al., 2020). In this field, 71 polysaccharides have demonstrated to be excellent candidates because of their tunable water

swelling arising from their structural and chemical versatility (Chemin, Beaumal, Cathala, & Villares, 2020; W. Chen, Sun, Biehl, & Zhang, 2022). Therefore, in this study we focused on the impact of hydrogel composition (i.e. XG/CNC ratio) on their mechanical properties and water uptake. The different behavior of the XG/CNC assemblies permitted to fabricate bilayer actuators driven by the asymmetric swelling of layers when the films are immersed in water.

77

78 2. Materials and methods

79 2.1. Materials

80 Cellulose nanocrystals (CNC) were purchased from CelluForce (Canada). CNC were obtained by 81 acid hydrolysis of wood pulp and according to product specification, purity = 100% surface charge density = $0.023 \text{ mmol g}^{-1}$ (by conductivity), crystalline fraction = 0.88 (by XRD), 82 crystallite diameter = 2.3-4.5 nm (by AFM), crystallite length = 44-108 nm (by AFM), pH 83 (dispersion in water) = 6–7. Xyloglucan (XG), $M_w = 800$ kDa, from tamarin seed gum, was 84 85 purchased from DSP Gokyo Food and Chemical (Japan). Chitosan (Chi) medium molecular 86 weight, Brookfield viscosity 200-800 cP, was purchased from Sigma Aldrich. Rhodamine B 87 isothiocyanate (RBITC) was purchased from Sigma Aldrich. Acetic acid and dimethylsulfoxyde 88 (DMSO) were purchased from Merck.

89 2.2 CNC, XG and Chitosan solutions/dispersions preparation

90 CNC dispersion was prepared in Milli-Q water at a final concentration of 2 % w/w. Dispersion
91 was vigorously stirred during 24 h and finally sonicated. XG solution was prepared in Milli-Q
92 water at final concentration of 2 % w/w, and vigorously stirred during 24 h. Chitosan solution at
93 0.1 % w/w was prepared in acetic acid 1%, at room temperature and vigorously stirred for 24 h.

94 2.3. Fluorescence labelling of chitosan

95 RBITC-labelled chitosan was synthesized by the reaction between isothiocyanate group of 96 RBITC and amino group of chitosan. Chitosan (1 g) was dissolved at 1% in 0.1 M acetic acid. 97 RBITC (7.5 mg) dissolved in 20 mL DMSO was add to chitosan solution. The mixture was 98 stirred for 3 h, in dark, at room temperature. Labelled-chitosan was precipitated by addition of 99 NaOH. Labelled-chitosan was centrifuged and washed with ultra-pure water until no free RBITC 100 was detected in supernatant. Chitosan-RBITC was solubilized in acetic acid 1% and dialyzed 101 against ultra-pure water during 3 days in dark.

102 2.3. Films and hydrogel bilayers fabrication

103 5 mL of CNC and XG were mixed at different XG/CNC ratios (R1, R0.5 and R0.2) with same 104 final polysaccharide concentration (1.2 % w/w) and vortexed to obtain a homogeneous gel 105 solution. CNC and XG solution concentrations for the different ratio were : R=0.2 [CNC] = 1 % 106 w/w; [XG] = 0.2 % w/w; R=0.5 [CNC] = 0.8 % w/w; [XG] = 0.4 % w/w; R=1 [CNC]= 0.6 % 107 w/w; [XG] = 0.6 % w/w. Gels were casted into Petri dishes (diameter 3 cm), and 2.5 mL chitosan 108 at 0.1 % w/w was added above the gel. After floated gel in chitosan during one night, chitosan 109 supernatant was removed, and the gel was dried at room temperature for 24 h. Film thickness 110 was measured by a micrometer. The three different bilayer films by the successive deposition of 111 XG/CNC/Chi hydrogels at the selected XG/CNC ratios: R0.5/R0.2, R1/R0.2 and R1/R0.5. The 112 first hydrogel layer was casted into a Petri dish and the second one was immediately and 113 carefully put on the top of the first one. Then chitosan was added to cross-link the bilayered 114 films.

115 2.4. Transmittance

116 UV-visible spectra were obtained from films placed directly in a SPECORD S 600 spectrometer
117 (Analytik Jena GmbH). All spectra were recorded using WinASPECT software set to absorbance
118 mode after 10 accumulations and scanning from 182 to 1019 nm.

119 2.5. Rheology

Rheological measurements were performed using a stress-controlled rheometer AR-2000 (TA Instruments) equipped with a plane geometry (20 mm diameter). The storage (G') and loss (G'') moduli were measured at different frequencies range (100 to 0.01 Hz). The imposed stress was chosen within the linear response regime. Temperature was controlled using the Peltier and set at 20 °C for all measurements. Samples were covered with paraffin oil to prevent sample evaporation. Samples were shaped to fit the geometry. Three samples were used for each measurement; they displayed reproducible behaviour and values.

127 2.6. Water uptake of films

The obtained hydrogels were placed in water, or in acid or alkaline pH solutions, to swell until equilibrium at room temperature. The swelling degree was calculated by weighing the samples before and after immersing in acetate (50 mM, pH 3.5) and carbonate buffer (50 mM, pH 10.5). The mass swelling percentage was calculated using the following expression:

132
$$S(\%) = \frac{m_t - m_0}{m_0} 100$$
 (1)

133 Where m_0 and m_t are the weights of the dry gel at time 0 and the swollen gel at time t, 134 respectively.

For the actuation tests, films were immersed in water, and the bending curvature was determined by recording movies during the water uptake, and periodically extracting pictures. Films were fixed with a binder clip leaving a free length of approximately 30 mm.

138 2.7. Polarized optical microscopy (POM)

Polarized optical microscopy (POM) was performed using an upright Olympus System
Microscope Model BX51, with crossed polarizers and a 530 nm retardation plate (U-TP530).
Digital images were taken of samples between the microscopy slide and the cover glass using a
Sony XCD-SX90CR charge-coupled device camera.

143 2.8. Confocal microscopy

Films containing rhodamine-labelled Chitosan were imaged by confocal microscopy (Leica TCS-SP2). Confocal images of the microcapsules were acquired with LSM-510 UV–Vis equipped with 63×1.2 water immersion objectives (Zeiss, Germany). Excitation/emission wavelengths were 530/566 nm.

148 **3. Results and Discussion**

149 *3.1. Preparation of XG/CNC/Chi hydrogels and evaluation of their optical properties.*

150 XG adsorption on cellulose surface has been reported for decades and has been more recently 151 identified as an entropy driven process (Tobias Benselfelt et al., 2016; Kishani, Benselfelt, 152 Wagberg, & Wohlert, 2021). According to the XG/CNC ratio, the surface crowding can be 153 tailored to form a variable proportion of loops and tails as demonstrated by enzymatic hydrolysis 154 of XG/CNC complexes displaying various mass ratios (Dammak et al., 2015). Indeed, the trains 155 are defined as the part of the polymer that are adsorbed in contact with surface, while the loops 156 and the tails correspond to the parts of the polymers that are not directly attached to the surface 157 (Tobias Benselfelt et al., 2016), being the tails the end parts of the polymer chains. Thus the 158 loops and tails are accessible to enzymes while the trains are not. At low XG/CNC ratio, the 159 surface availability is high and leads to XG adsorption in an extended conformation while at 160 higher XG/CNC ratio, loops and tails are more frequent due to the surface crowding. These 161 results can be explained by the fast adsorption kinetics of XG on the cellulose surface and the

162 slow rate of the desorption that leads to almost irreversible adsorption (Park & Cosgrove, 2015; 163 Villares, Moreau, Dammak, Capron, & Cathala, 2015). In this work, the rationale was the base of 164 the choice of the XG/CNC ratio used. Indeed, the limit of the CNC surface saturation by XG was 165 found to be in the range of 250 µg of XG per mg of CNC, i.e. 0.25 w/w ratio (Dammak et al., 166 2015). We therefore studied three different XG/CNC ratios for the hydrogel fabrication. The 167 lowest one (XG/CNC 0.2 w/w; R0.2) corresponded to a XG concentration slightly lower than the 168 saturation limit so that CNC surface will be not fully crowded and XG may adopt an extended 169 conformation. Moreover, since the amount of CNC surface is lower than XG available, XG 170 chains can interact more easily with two CNC leading to the cross-link of the nanorods. The two 171 other XG/CNC ratios, 0.5 w/w (R0.5) and 1.0 w/w (R1), were clearly higher than the saturation 172 limit and corresponded to the formation of loops and tails due to surface crowding. In this case, 173 XG chains are more accessible as demonstrated by the enzymatic degradation (Dammak et al., 174 2015).

175 For all the studied ratios, when the total biopolymer concentration (XG + CNC) is high enough 176 (> 1% w/w), gel formation occurs (Talantikite, Gourlay, et al., 2019; Talantikite et al., 2021). 177 Indeed, in solution, the adsorption of XG on stiff CNC increases the effective hydrodynamic 178 volume of XG/CNC complexes, compared to CNC alone, leading to gel formation through steric 179 repulsion and transient network formation between XG chains (de Freitas et al., 2015; 180 Talantikite, Gourlay, et al., 2019). Similar gelation process was observed for other CNC 181 adsorbing polymers (Hu, Cranston, Ng, & Pelton, 2014). Variation of XG/CNC ratio influenced 182 the gel strength due to the modification of CNC cross-linking and XG/XG chains interaction 183 extent (Talantikite, Gourlay, et al., 2019; Talantikite et al., 2021). Nevertheless, the interaction 184 strength between XG chains is weak leading to hydrogels with poor mechanical properties

185 (Talantikite, Gourlay, et al., 2019). A general strategy to achieve hydrogel strengthening consists 186 in the elaboration of double network architectures (Q. Chen, Chen, Zhu, & Zheng, 2015). This 187 approach has been successfully implemented for nanocellulose-based materials either by using 188 ionic or covalent cross-linking (T. Benselfelt, Engstrom, & Wagberg, 2018; K. J. De France et 189 al., 2020). Similarly, our strategy relies on electrostatic interactions and takes advantage of 190 interplay capacities of negative sulfate groups present on CNC with polycations. Among bio-191 based polycations, chitosan has been widely used for different materials preparation including 192 hydrogels and was also found to be able to cross-link cellulosic substrates such as paper or 193 nanocellulose (Lindstrom, Wagberg, & Larsson, 2005; Toivonen et al., 2015).

194 Fig. 1a shows the scheme of the XG/CNC/Chi hydrogels fabrication. Hydrogels were prepared 195 by first mixing XG and CNC at the appropriate ratio (R0.2, R0.5 and R1) at a constant total dry 196 weight (XG + CNC) of 1.2 % w/w. The mixture was thoroughly stirred to achieve homogeneity. 197 Indeed, Talantikite et al. (Talantikite, Gourlay, et al., 2019) demonstrated that XG/CNC gels can 198 be easily broken by shearing and then reformed instantaneously. These self-healing properties 199 rely on transient no-covalent interactions between XG chains (de Freitas et al., 2015). The gels 200 were thereafter molded in a Petri dish to achieve cross-linking. Chi solution (0.1 % w/w) was 201 added on the XG/CNC dispersion. Several chitosan concentrations were tested (data not shown) 202 and since concentration have been found to have no effect on swelling and mechanical properties 203 (data not shown), 0.1 %w/w concentration was chosen. After Chi addition, for all the XG/CNC 204 ratios, hydrogel shrinkage was visually observed, suggesting that water was removed. This 205 behavior has been previously observed in the fabrication of CNC/Chi multilayers monitored by 206 quartz crystal microbalance with dissipation (QCM-D) (Lombardo, Cathala, & Villares, 2021). 207 These studies revealed that the adsorption of Chi onto CNC yielded lower adsorbed hydrated 208 mass than in the case of XG, which pointed at a shrinking effect of Chi mediated by the 209 electrostatic interaction between CNC and Chi. The strong interaction could therefore expel 210 water molecules and counter-ions from the CNC surface by an entropy-driven process 211 (Lombardo & Thielemans, 2019), which stabilized the hydrogel and provided the self-supporting 212 properties.



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Fig. 1. (a) Schematic illustration of the preparation of XG/CNC/Chi hydrogels; (b) photography
showing the transparency of the hydrogels; and (c) transmittance values of hydrated hydrogels in
the range of 400-800 nm for the three XG/CNC/Chi hydrogels studied, R0.2, R0.5 and R1.

217

The optical properties were examined by transmittance measurements of dry and hydrated films. As-prepared films were highly transparent, smooth and flexible (**Fig.** *I*b). When hydrogels were rehydrated, transmittance increased in all cases, being more noticeable in the case of R0.2 XG/CNC/Chi hydrogels (Table 1). For the higher XG/CNC ratios (R0.5 and R1) the transmittance was 80% whereas for the lower XG/CNC ratio, the transmittance was around 40% (**Fig.** *I*c and Table *1*). This lower transmittance suggested the presence of light-scattering aggregates. This difference between R0.2 and the two other systems could be ascribed to the association between CNC mediated by XG chains when available CNC surfaces are larger than
the amount of XG available to cover all CNC surfaces. This might result in tight contact between
CNC forming large particles and therefore less transparency.

- 228
- 229 Table 1 : Characteristics of XG/CNC/Chi hydrogels in terms of water uptake ratio, transmittance,
- 230 mechanical properties and thickness

| Composition | Water uptake | | Transmittance (% at λ = 550 nm) | | Mechanical properties | | | |
|-------------|--------------|------|---|-----|-----------------------|-------|-----------|---------------|
| | ratio (g/g) | | | | | | Thickness | |
| XG/CNC | nH 3 | pН | Drv | Wet | G' | G" | Dry | Wet |
| ratio | L 2 | 10 | Diy | | (kPa) | (kPa) | (µm) | (µm) |
| R0.2 | 4.9 | 3.5 | 31 | 41 | 68.4 | 5.1 | 91±18 | 812 ± 93 |
| R0.5 | 10.4 | 6.1 | 80 | 87 | 5.1 | 0.3 | 64 ± 7 | 653 ± 78 |
| R1 | 19.7 | 14.7 | 80 | 84 | 1.8 | 0.1 | 31 ± 5 | 700 ± 140 |

231

232 *3.2. Mechanical properties*

The dynamic mechanical properties of XG/CNC/Chi hydrogels were investigated by measuring the frequency dependence of the storage (*G'*) and loss (*G''*) moduli (**Fig.2**, Table 1). For the three studied XG/CNC ratios, R0.2, R0.5 and R1, the storage modulus was always higher than the loss modulus (G'>G''), which indicated a gel and elastic-like behavior (Pääkkö et al., 2007). The values of both storage and loss moduli were particularly high in comparison with CNC/polymer hydrogels (Hynninen et al., 2018; Talantikite, Gourlay, et al., 2019). 239 Results showed clear differences on the rheological behavior depending on the XG/CNC ratio. 240 Thus, the values of both G' and G'' decreased when the XG/CNC ratio increased, reflecting a 241 significant increase of rigidity when the amount of CNC increased. Nevertheless, the decrease in 242 G' and G'' was not correlated to the CNC concentration, which revealed the role of the XG 243 conformation on the mechanical properties. These results clearly showed the transition to a 244 different regime above 0.25 w/w (Dammak et al., 2015). Thus, the low XG concentration (R0.2), 245 which adsorbs in an extended conformation on the CNC surface, provided rigidity and cross-246 linking between CNC to the XG/CNC/Chi hydrogels whereas the XG conformation as loops and 247 tails prevailing for the higher XG concentrations (R0.5 and R1) provided more flexibility. 248 Moreover, the low coverage of surface by XG might also favor interactions between CNC sulfate 249 charges and Chi charges leading to a reinforcing effect.



Fig.2.The storage modulus (G') and the loss modulus (G'') as a function of the frequency for the three XG/CNC/Chi hydrogels studied, R0.2, R0.5 and R1, at 20 °C.

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The swelling properties of the XG/CNC/Chi hydrogels were determined by the water uptake in two different buffers, i.e. acetate (pH 3.5) and carbonate (pH 10.5) (**Fig. 3**). Similarly to mechanical properties, the swelling behavior was significantly influenced by the XG/CNC ratio. Thus, as the XG/CNC ratio increased, the hydrogels adsorbed more water. In all cases, absorption is extremely fast and equilibrium was reached after few hours of immersions. All hydrogels displayed high stability in the two pH conditions since the gels retained their full integrity for weeks (**Fig. 3**).

262 Swelling involves the diffusion of water molecules into the gel matrix and the subsequent 263 polymer chain relaxation within the hydrogel. The capacity of the hydrogel for water adsorption 264 depends on the interactions between the biopolymers. Swelling can be explained in terms of 265 equilibrium swelling pressure that is constituted of the contribution from interaction of water 266 with the polymers forming the gels, the osmotic pressure due to charged groups of polymer 267 constituents and the pressure due to the resistance of the gel network (T. Benselfelt et al., 2018; 268 Flory, 1953). If the first two pressures are higher than the later, the gel will be dissolved whereas 269 if the pressure generated by the network resistance dominates, the swelling will be limited. 270 Therefore, the formation of tight entanglements results in low swelling whereas loose and 271 flexible networks show high water uptake. In the case of low XG/CNC ratio (R0.2), the 272 formation of tight contacts between cellulose and xyloglucan in association with potentially more 273 efficient interaction between Chi and CNC charges resulted in less swollen networks. As the 274 XG/CNC ratio increases (R0.5 and R1), the formation of XG loops and tails on the CNC surface, 275 as well as the present of some unbound XG chains for the highest ratio, facilitated water 276 adsorption. These results are in agreement with previous studies showing that the XG adsorption 277 onto CNC forming loops and tails resulted in higher water uptake, and the hydration of XG

chains increased significantly their accessibility and the thickness of CNC/XG/CNC multilayer
films (Villares et al., 2017). The thicknesses of hydrogels were measured before and after drying
(Table 1). For the three hydrogels studied, the thickness increased between 800-2000%, which
demonstrated the hydrogel character of the XG/CNC/Chi systems.
Finally, it is necessary to underline the difference of swelling observed in the acetate buffer and
the carbonate buffer. The swelling was more important at acidic pH due to the protonation of the

amine groups of the chitosan which reinforced the electrostatic repulsion between the chitosan





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Fig. 3. Variation of water uptake with time in acetate (pH 3.5) and carbonate buffers (pH 10.5)

for the three XG/CNC/Chi hydrogels studied, R0.2, R0.5 and R1.



291 CNC are known to self-assemble into chiral nematic liquid crystal phases with twisted helicoidal 292 organization. This self-assembled organization is characterized by a specific fingerprint pattern. 293 This pattern is present when the band spacing is large enough to be resolved in the optical 294 microscope (Revol, Bradford, Giasson, Marchessault, & Gray, 1992). CNC/XG/Chi hydrogels 295 have been observed under cross-polarized light and birefringence has been observed, but finger 296 print pattern cannot be identified (Fig. 4). Hydrogel microstructures displayed anisotropic zones 297 as demonstrated by the birefringence indicating certain level of CNC organization but not as 298 highly organized as in chiral nematic phases. It is likely that the anisotropic zones visualized by 299 POM are constituted of XG and CNC and not only by highly organized and phase separated 300 CNC. Hydrogels microstructures were also revealed by fluorescence microscopy (Fig. 4). For 301 this purpose, Chi was labelled with rhodamine B. XG/CNC hydrogels were then cross-linked 302 with rhodamine-labelled Chi and were then further imaged to reveal the domains accessible to 303 Chi.

304 Both POM and fluorescence images reported in Fig. 4 indicate that the microstructures of 305 hydrogels were strongly influenced by the XG/CNC ratio. For the lowest ratio (R 0.2), both 306 microscopy modalities clearly indicated the formation of small size domains. The POM images 307 showed different colored domains suggesting varying preferential orientations and/or changes in 308 material density from one domain to another. Also, different fluorescence intensities were clearly 309 detected, indicating that some areas appeared richer in labeled Chi than others that seemed less 310 accessible. This result was consistent with the formation of CNC-rich domains that appeared 311 aggregated (and thus less accessible) due to the lack of XG that defaults to the amount of CNC 312 surface area available. When the XG/CNC ratio increased, the microstructure of the hydrogel 313 became more homogeneous. In the case of R0.5 ratio, regions with different birefringence were

314 clearly visible while in the case of R1 ratio, large areas displayed the same birefringence pattern.

- 315 Similarly, for R1 ratio, fluorescent labelling became completely homogeneous. These results
- 316 suggested that CNC, XG and Chi were uniformly distributed at the highest XG/CNC ratio and no
- 317 phase separation occurred at least at the microscale.



318

Fig. 4. Polarized optical microscopy images (crossed polarizers, 530 nm red wave plate); confocal microscopy images of hydrogels fabricated with rhodamine-labelled Chi; and schematic representation of the arrangement of biopolymers in the hydrogels for the three XG/CNC/Chi systems studied, R0.2, R0.5 and R1. XG and Chis are represented by a green and yellow strands respectively, CNC are represented by purple rods surrounded by pink light rods representing the electrostatic double layer.

326 **Fig.** 4 depicts an illustration of the biopolymer arrangement within the hydrogel. When XG 327 concentration was low (R0.2), XG adsorbed in an extended conformation on the CNC surface, 328 that favored the close interaction between CNC, and therefore, the formation of compacted 329 networks/aggregates consistent with le lowest transparency observed (Fig. 1). Chi stabilized the 330 system, forming more rigid hydrogels yielding higher mechanical properties. The tight contact 331 between biopolymers made therefore difficult to accommodate many water molecules, and the 332 water uptake of XG/CNC/Chi R0.2 hydrogels was low compared to the other XG concentrations. 333 When the XG concentration increased (R0.5), there was a transition to a regime XG forming 334 loops and tails on the CNC surface, resulting in a more swollen and flexible network, where Chi 335 interpenetrated and formed junctions that reinforced and stabilized the hydrogel. Accordingly, 336 transparency increased since the CNC are better dispersed, and swelling increased also due to the 337 lowest resistance of the network (less XG cross linking) and simultaneously the mechanical 338 properties decreased. The higher XG concentration (R1) resulted in a highly swollen hydrogel 339 where the lower CNC concentration prevented the formation large CNC rich domains. XG 340 showed a uniform distribution within the hydrogel and increase the water affinity and swelling 341 capability while lowering the mechanical properties.

342

343 *3.5.* Towards the implementation of soft actuators

We took advantage of the different rheological and swelling properties to fabricate water swelling-driven actuators. We prepared three different bilayer films by the successive deposition of XG/CNC/Chi hydrogels at the selected XG/CNC ratios: R0.5/R0.2, R1/R0.2 and R1/R0.5. Water uptake resulted in a volume increase; therefore, the combination of two layers differing in their swelling behavior resulted in an asymmetric expansion of the film. Depending on the geometry of the film, different responses can be obtained, such as bending, twisting, or selfrolling. Square-shaped films (10×10 cm) were cut into rectangles of 5×35 mm. The film thickness was significantly influenced by the film composition, varying from 95 µm for the R1/R0.5 film, to122 µm for the R1/R0.2 film, and to 155 µm for the R0.5/R0.2. When the films were immersed in water, they changed their shape from straight (time 0) to bended, reaching a maximum curvature over 30 min. **Fig. 5** shows the photographs of the films when they were immersed in water at time 0 and after 30 min.



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Fig. 5 Photographs showing the bending of R0.5/R0.2, R1/R0.2 and R1/R0.5 films when they
 were immersed in water at time 0 and 30 min; and schematic illustration of the behavior of the
 films in water.

361 The films R0.5/R0.2 and R1/R0.2 showed a simple bending actuation in water, in a higher extent 362 in the case of the R0.5/R0.2 film. Differently, the R1/R0.5 display a more complex behavior

363 since, both bending and twisting were observed. (Fig 5) According to the water uptake values of 364 starting films (Table 1), the highest difference in swelling between the layers corresponded to the 365 R1/R0.2 film, so the highest bending was expected for this film. Nevertheless, the experimental 366 results indicated a different tendency, and the greater bending was observed for the R0.5/R0.2 367 film. The bending of bilayer films triggered by the asymmetric expansion of the layers depends 368 not only on the difference in water uptake within the layers, but also on their storage modulus, 369 and the film dimensions and geometry (Le Duigou et al., 2019; Rueggeberg & Burgert, 2015; 370 Wang, Tian, Ras, & Ikkala, 2015; Zhang et al., 2015). In the case of R1, the storage modulus is 371 the lowest measured and is almost 2 decades lower than those of R0.2 which suggest that the 372 difference of mechanical behavior would limit the bending and counteract the anisotropic 373 swelling action. In the case of the R1/R0.5 actuation, the difference in swelling and storage 374 modulus are not that high however, the twisting and bending of the R1/R0.5 film may be 375 justified in terms of the lower thickness of the film (95 μ m) compared to the others (122-155 376 µm). Thus, it is likely that the actuating properties of XG/CNC/Chi films is combination of 377 several parameters and can finely be controlled by the hydrogel composition and film 378 architecture. Further works will be necessary to model and predict the actuating behavior 379 opening the door to the implementation of new soft bio-based materials but also to biomimetic 380 tools to investigate biology related issues such plant cell wall shaping and growth.

381

382 4. Conclusions

In this work, we took advantage of the interactions between cellulose, xyloglucan and chitosan to fabricate bio-based hydrogels. The specificity of the cellulose/xyloglucan association and the subsequent formation of a double network by the addition of a charged biopolymer (chitosan) 386 provided the hydrogel stability. By tuning the XG/CNC ratio, and therefore the adsorption 387 regime of xyloglucan on the cellulose surface, the physical properties of the hydrogel, such as 388 transparency, storage and loss moduli, and water uptake, can be easily modified. The 389 combination of two different XG/CNC ratios in a bilayer film allowed the fabrication of 390 actuators driven by the asymmetric swelling between the layers. This work opens new routes in 391 the field of actuators, we fabricated totally bio-based hydrogels without chemical cross-linking 392 and whose properties and response to stimuli can be tuned by the interactions between the 393 biopolymers.

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395 Acknowledgements

Camille Jonchère and Catherine Garnier are acknowledged for their excellent help for rheology
measurements. This work is a contribution to the HOBIT program financed by the Pays de la
Loire region.

399

400 Declaration of competing interest

401 The authors declare no conflicts of interest.

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403 Supporting Information.

404 No supporting material

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- 409 Author Contributions
- 410 The manuscript was written through contributions of all authors. All authors have given approval
- 411 to the final version of the manuscript.
- 412
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