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1 **Optimization of the methacrylation of**
2 **carboxymethylcellulose and use for the**
3 **design of hydrogels and cryogels with**
4 **controlled structure and properties**

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13

14 **ABSTRACT**

15 Carboxymethylcellulose (CMC) was functionalized using methacrylic anhydride (MA) to produce
16 photo-crosslinkable methacrylated carboxymethylcellulose (mCMC). Optimization of the synthesis
17 led to a wide range of mCMC samples with well-controlled degrees of methacrylation (DM) up to
18 $76 \pm 6 \%$, **surpassing the literature data**. Aqueous mCMC formulations with different DM and
19 concentrations of mCMC were UV-cured to obtain hydrogels, which were transformed into
20 cryogels after freeze-drying. An innovative ¹³C CP-MAS solid-state NMR methodology was used to
21 calculate the crosslinked methacrylate density in cryogels and thus in hydrogels. These values
22 (from 4.3×10^{-3} to 1.3×10^{-2} mmol/cm³) were correlated with both the DM and the mCMC
23 concentration in formulations. These parameters were used to control the material microstructure
24 and rheological properties. As a result, the swelling ratio of hydrated cryogels could be modulated
25 for applications requiring high or low water absorption. **Moreover, it was shown that the elastic**
26 **moduli G' of the hydrated cryogels were superior to those of the original hydrogels, over the entire**
27 **frequency range (0.1-10 Hz).**

28

29 Keywords: Carboxymethylcellulose, methacrylation, photo-crosslinking, hydrogel, cryogel

30

31 Introduction

32 Polysaccharides such as hyaluronic acid, dextran, or cellulose, have been used for many
33 years in biomedical applications due to their interesting features such as biocompatibility,
34 biodegradability or biological properties (mucoadhesion, antimicrobial properties, etc.)
35 (Rebelo, Fernandes, & Fangueiro 2017). These raw materials can eventually be chemically
36 modified to improve their mechanical properties, to confer biological properties (specific
37 cell targeting, biodegradability, etc.) (Luo et al. 2021) or defined shapes and porosities
38 (Muir & Burdick 2020). For example, the methacrylation of these natural polymers can be
39 used to form hydrogels by photo-crosslinking. The resulting materials are three-
40 dimensional polymer networks with entrapped water, with potential applications in bone
41 reconstruction (Poldervaart et al. 2017), tissue engineering (Chen et al. 2012), wound
42 healing (Baier Leach et al. 2003), drug delivery (Matricardi et al. 2008) or 3D cell culture
43 (García-Lizarriar et al. 2018). It is also possible to remove the entrapped water by freeze-
44 drying to obtain cryogels (Darpentigny et al. 2020; Hossen et al. 2018), which are more
45 stable and robust than hydrogels (Memic et al. 2020). These spongy materials can absorb
46 a large amount of solvent thanks to their high porosity (Pacelli et al. 2021), which is
47 interesting for the transport of nutrients or cells through scaffolds (Razavi, Qiao & Thakor
48 2019). Their robustness is also useful for bone tissue regeneration (Gu et al. 2020).

49 Although dextran and hyaluronic acid are the most studied polysaccharides for such
50 applications (Dovedytis, Liu & Bartlett 2020), carboxymethylcellulose (CMC) is also of
51 great interest for the elaboration of hydrogels by photo-crosslinking (Reeves et al. 2010).
52 This cellulose derivative is biocompatible, water-soluble, and displays carboxylate and
53 hydroxyl groups amenable to chemical functionalization (Rahman et al. 2021). One of the
54 most studied reaction for the grafting of photo-crosslinkable methacrylate groups onto
55 CMC consists of the condensation of the hydroxyl groups with methacrylic anhydride (MA)
56 to yield methacrylated carboxymethylcellulose (mCMC) (García-Lizarriar et al. 2018,
57 Hossen et al. 2018, Dutta, Samanta, & Dhara 2016). After photo-crosslinking, CMC
58 hydrogels have been considered for biomedical applications such as cell encapsulation
59 (Reza et Nicoll 2010), tissue engineering (Lin et al. 2016) or drug delivery (Dutta, Samanta,
60 et Dhara 2016). mCMC cryogels have also been studied for their water absorption capacity
61 (Hossen et al., 2018).

62 In order to benefit from tunable structures and properties (e.g. mechanical) of the CMC
63 hydrogels and cryogels, which will allow targeting one or the other biomedical application,
64 it is firstly of crucial importance to be able to control the degree of methacrylation (DM)
65 of the mCMC, as already highlighted for other polymers such as methacrylated dextran
66 (van Dijk-Wolthuis et al. 1997) and gelatin (Chen et al. 2012). However, in all previously
67 reported studies, the DM of the mCMC never exceeded 60 % (Melilli et al., 2020), which
68 limits the possible uses of such products. The influence of parameters such as the extent
69 of methacrylate conversion and polymer concentration, which can also play a major role
70 in the structural and mechanical properties of hydrogels and cryogels based on mCMC,
71 have also been poorly investigated yet.

72 Herein, we report successful efforts to control the degree of methacrylation of mCMC
73 samples and extend its range to values never achieved before. Beyond this optimization
74 of the DM, we also investigated for the first time to the best of our knowledge the
75 influence of the DM and concentration on the structural and physical properties of photo-
76 crosslinked mCMC materials, either in the form of hydrogels or cryogels, and studied their

77 swelling behavior. Useful information were also obtained from the evaluation by solid-
78 state NMR of the conversion ratio in photo-crosslinked mCMC samples (cryogels).

79

80 **Materials and Methods**

81 **Materials**

82 Sodium salt of CMC (NaCMC, reference BLANOSE 7LP EP, Ashland, France) with a weight-
83 average molar mass (M_w) of 90.5 kg/mol and a degree of substitution (DS) of 0.7, was used
84 as received. Methacrylic anhydride (MA) containing 2,000 ppm topanol A as inhibitor, 2-
85 hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959), anhydrous
86 sodium hydroxide (NaOH) pellets and deuterium oxide (D_2O) were purchased from Sigma
87 Aldrich (France).

88 **Methacrylation of carboxymethylcellulose**

89 The protocol was adapted from Melilli et al. (2020). NaCMC was solubilized at 2 wt% in
90 deionized water at room temperature under stirring. MA was then added dropwise to the
91 CMC solution. Different molar ratios between MA and CMC hydroxyl groups were used
92 (namely 1.24, 2.48, 3.72 and 4.96) to study the effect of this parameter on the degree of
93 methacrylation of the resulting mCMC samples. Homogenization during the reaction was
94 performed by magnetic stirring at 700 rpm or by mechanical stirring at 2,000 rpm with a
95 **glass shaft anchor stirrer**, to investigate the influence of the stirring method on the
96 reaction. The reaction was continued for 24 h at room temperature. The pH of the
97 medium was constantly adjusted to 8 with 10 M NaOH during the reaction. The resulting
98 mixture was purified for a week by dialysis against deionized water using a cellulose mixed
99 ester membrane (MWCO = 12-14 kg/mol; Roth, Germany). Finally, the mCMC product was
100 freeze-dried for five days with a CHRIST Beta 2-8 LS plus lyophilizer (Martin Christ
101 Gefriertrocknungsanlagen, Germany).

102 **Hydrogel and cryogel preparations**

103 The freeze-dried synthesised mCMC samples were dissolved at 2, 3 or 4 wt% in deionized
104 water at 50 °C during 12h under magnetic stirring. The photoinitiator Irgacure 2959 was
105 then added at room temperature with a constant molar ratio compared to the number of
106 moles of methacrylate (MA) in solution (**$nPI/nMA = 0.55$**). Irgacure 2959 was chosen
107 because it is water-soluble (0.5 wt% maximum), non-toxic, and absorbs at 365 nm (Tomal
108 & Ortyl 2020). The formulations were treated for 15 min in an ultrasonic bath at 50 °C and
109 then agitated with a multi-rotator PTR-35 (Grant, UK) at 6 rpm for 12 h at room
110 temperature. 650 μ L of each formulation was then placed in a PTFE circular mold of 15
111 mm diameter and 1 cm height and then insolated for 5 min under a UV-light source with
112 a wavelength of 365 nm and an irradiance of 82 mW/cm² (Promaker V6000, Prodways,
113 France). All resulting hydrogels had a thickness of 3.6 mm and a diameter of 15 mm. They
114 were placed in an aluminum basket, filled with liquid nitrogen and freeze-dried overnight
115 with the same lyophilizer to yield cryogels.

116 **Characterization techniques**

117 *Nuclear magnetic resonance (NMR)*

118 **¹H NMR spectroscopy**

119 ¹H NMR spectroscopy was used to quantify the degree of methacrylation (DM) of the
120 mCMC samples. 4.5 mg of mCMC was dissolved in 650 μL of D₂O at 50 °C for 12 h using a
121 ThermoMixer C (Eppendorf, Germany). All ¹H NMR spectra were recorded on a
122 Spectrospin 300 MHz spectrometer (Bruker, Germany) at 25 °C from 64 successive scans
123 with a relaxation time D1 of 3 s.

124 **¹³C Non-Quaternary Suppression (NQS) and Cross-Polarization Magic-Angle Spinning 125 (CP-MAS) solid state NMR spectroscopy**

126 ¹³C NQS and CP-MAS solid-state NMR experiments were carried out to identify and
127 quantify carbons before and after crosslinking of mCMC. ¹³C CP-MAS solid-state NMR
128 spectroscopy was also used to measure the crosslinking ratio of UV-cured cryogels. 80 mg
129 of crosslinked mCMC cryogel was crushed and placed in a 3 mm diameter rotor. All ¹³C
130 NQS and CP-MAS solid-state NMR spectra were recorded on a Neo 500 MHz spectrometer
131 (Bruker, Germany) operating at 125.8 MHz. The acquisition time was 28 ms and the sweep
132 width was 37 kHz and at least 2,048 successive scans were recorded at 25 °C.

133 *Fourier-transformed infrared (FTIR) spectroscopy*

134 FTIR by attenuated total reflectance (ATR) was performed with a MIRacle 10 instrument
135 (Shimadzu, Japan) to analyze the characteristic chemical bonds of CMC and mCMC
136 samples. Absorbance spectra were acquired within the 4,000 – 550 cm⁻¹ wavenumber
137 range (64 scans, 4 cm⁻¹ resolution).

138 *Scanning electron microscopy (SEM)*

139 SEM was used to investigate the cryogel morphology and measure the pore size
140 distribution. Samples were fractured horizontally and pasted on metallic stubs with
141 carbon cement, fractured and sputter-coated with Au/Pd. Secondary electron images
142 were recorded with a FEI Quanta 250 (Thermo Fisher Scientific, US) scanning electron
143 microscope equipped with a field emission gun operating at 2.5 kV. All pores were
144 observed on the cutted surface. The pore size was determined by image treatment with
145 ImageJ. Horizontal or vertical lines were drawn on each pore giving the average pore size.
146 This procedure was performed on 25 pores per image and 4 images per sample. Pores
147 whose walls were broken by the cutting method were not measured.

148 *Swelling ratio and hydrogel mesh size calculation*

149 Swelling tests were performed on cryogels. Measurements were made on 3 cryogels per
150 condition. The samples were immersed in deionized water at room temperature and the
151 cryogels transformed this way into hydrogels (or re-hydrated cryogels) were regularly
152 weighed. These steps were repeated until mass equilibrium was reached to determine
153 the swelling ratios (SR) according to Eq. (1).

154 $SR = w_t/w_0$ (1)

155

156 where w_0 is the cryogel weight and w_t is the weight at measurement time t . Swelling ratios
157 were averaged over measurements carried out on three different samples.

158 The hydrogel mesh size, ξ , was estimated from swelling tests, based on the previously
159 described Flory-Rehner theory used by Baier Leach et al. (2003) and Lin et al. (2016). First,
160 the mass ratio between the hydrogel and cryogel, Q_w , was calculated using Eq. (2):

161 $Q_w = w_h/w_0$ (2)

162

163 where w_h is the hydrogel weight and w_0 is the cryogel weight. The volumetric ratio, Q_v ,
164 between the hydrogel and the polymer volumes, was determined from Q_w using Eq. (3):

165 $Q_v = 1 + \left(\frac{\rho_{mCMC}}{\rho_{water}} (Q_w - 1) \right)$ (3)

166

167 where ρ_{mCMC} is the density of the considered dried mCMC measured by pycnometry, by
168 analyzing pellets of each mCMC using an AccuPyc II 1340 pycnometer (Micromeritics, US)
169 (Supplementary data - Table S1); ρ_{water} is the solvent density ($\rho_{water} = 1 \text{ g. cm}^{-3}$). The
170 average molecular weight between two crosslinking points (\overline{M}_c) was calculated using a
171 simplified version of the Flory-Rehner equation (4) demonstrated by Hoti et al. (2021):

172
$$\overline{M}_c = \frac{V_{water} \left[\left(\frac{w_H}{w_{hC}} \right)^{\frac{1}{3}} - \left(\frac{2}{f} \frac{w_H}{w_{hC}} \right) \right]}{- \left[\ln \left(1 - \frac{w_H}{w_{hC}} \right) + \frac{w_H}{w_{hC}} + \chi \left(\frac{w_H}{w_{hC}} \right)^2 \right]}$$
 (4)

173

174 where V_{water} is the molar volume of the solvent at room temperature ($V_{water} = 18$
175 mol.cm^{-3}), w_H is the hydrogel weight, w_{hC} is the weight of the hydrated cryogel at the
176 maximum of swelling, χ is the Flory polymer-solvent interaction parameter and f is the
177 functionality of the crosslinking, *i.e.* the maximum number of chemically linked polymer
178 chains at a crosslinking point ($f = 2.3$ for CMC samples with DS = 0.7). Considering that
179 CMC and mCMC have a similar structure, a value of 0.45 was used for χ , which
180 corresponds to the CMC-water interaction parameter determined by Xia et al. (2013). The
181 differences between CMC, mCMC and crosslinked mCMC were assumed to be negligible
182 based on the work of Baier Leach et al. (2003) on hyaluronic acid (HA), methacrylate HA
183 (mHA) and the crosslinked mHA.

184 The hydrogel mesh size, ξ was determined by Torres-Lugo and Peppas (1999) using Eq.
185 (5):

186 $\xi = Q_v^{\frac{1}{3}} \left(\frac{\sqrt{\overline{M}_c}}{\sqrt{M_w}} \right) \sqrt{\overline{r}_0^2}$ (5)

187 where M_w is the molar mass of the mCMC, calculated depending on the DM, and \overline{r}_0^2
188 is the root-mean-square distance between two crosslinking points that can be linked to the
189 molar mass M_n of the mCMC by Cleland (1970) using Eq. (6):

$$\sqrt{r_0^{-2}} = \sqrt{\frac{2C_n l^2}{M_r}} \sqrt{M_w} \quad (6)$$

191

192 where M_r is the molecular weight of the mCMC unit. C_n is the Flory characteristic ratio of
 193 CMC (11.5) determined by Picout & Ross-Murphy (2002) and l is the length of a virtual
 194 bond spanning a monosaccharide unit (0.545 nm) determined by Holtzer, Benoit & Doty
 195 (1954).

196 Finally, the hydrogel mesh size was calculated using Eq. (7):

$$\xi = \sqrt{\frac{2C_n l^2}{M_r}} \sqrt{M_c Q_v^{\frac{1}{3}}} \quad (7)$$

198 *Rheology measurements*

199 The rheological properties of each hydrogel were measured at least on three different
 200 samples per hydrogel composition using a Netzsch Kinexus Pro+ parallel plates rheometer
 201 (Germany). Frequency sweep measurements were performed at a constant temperature
 202 (25 °C) from 0.1 to 10 Hz. According to preliminary amplitude sweep measurements, all
 203 tests were carried out in the linear viscoelastic region at 1 % strain. The gap between the
 204 plate and the mobile plane was set to 0.4 mm. Rheology measurements were also
 205 performed on hydrated cryogels at equilibrium swelling using the same procedure.

206 **Results and discussion**

207 **Control and optimization of the methacrylation of CMC**

208 Photo-crosslinkable methacrylate groups were successfully grafted onto CMC using the
 209 reaction between methacrylic anhydride (MA) and the polymer hydroxyl groups (Fig. 1a).
 210 In the literature, the reaction was only performed at low temperature (0 or 4 °C) (Reza &
 211 Nicoll 2010, Qian et al. 2014, Dutta et al. 2016, Melilli et al. 2020). However, no influence
 212 of temperature was observed in our study when comparing reactions performed at 0 °C
 213 or at room temperature (Supporting Information Fig. S1). Therefore, the reactions
 214 described below were more conveniently performed at room temperature and were fully
 215 optimized by varying protocol parameters.

216 All mCMC samples were characterized by FTIR spectroscopy, and the appearance of a
 217 carbonyl band at 1,705 cm^{-1} on the spectra of methacrylated samples when compared to
 218 the CMC spectrum (Supporting Information Fig. S2) qualitatively proved the success of the
 219 methacrylation (Supporting Information Fig. S2). Then, the samples were characterized
 220 by liquid ^1H NMR and compared to raw CMC (Fig. 1b) to determine the degree of
 221 methacrylation. According to the literature, protons of the CMC unit (H_{CMC}) are located in
 222 the bulk between 2.8 and 4.7 ppm (Kono et al. 2016), and account for 8.4 protons
 223 considering a DS of 0.7. For mCMC samples, three additional peaks corresponding to the
 224 vinyl (H_a : 6.0-6.4 ppm, H_b : 5.65-6.0 ppm) and methyl (H_c : 1.7 - 2.2 ppm) protons of the
 225 methacrylate groups were also found after purification, indicating that methacrylation
 226 occurred under our experimental conditions (Van Hove, Wilson & Benoit 2013). **The**
 227 **deprotonation of hydroxyl groups is achieved by adding NaOH. The resulting hydroxide**
 228 **ion reacts with one of the carbons in the carbonyl group of MA, leading to the liberation**

229 of methacrylic acid. CMC methacrylation should first preferentially occur on the remaining
230 30 % of C6 primary hydroxyl groups, which are more reactive (Fedorova et Rogovin 1963).
231 Once all C6 hydroxyl groups are consumed, methacrylation will then occur on the C2 and
232 C3 secondary hydroxyl groups. The reaction between the carboxylate group of CMC and
233 MA is unlikely, as the carboxylate group is stabilized by mesomeric effects. Furthermore,
234 mCMC remains water-soluble, proving the conservation of carboxylates. It is however
235 difficult to verify these hypotheses by conventional characterizations. Nevertheless,
236 recent advances in dynamic nuclear polarization NMR (DNP NMR) may allow determining
237 the regioselectivity of the reaction as Berruyer et al. (2021) did with a substituted
238 methylcellulose.

239 The degree of methacrylation of mCMC samples was calculated from the respective
240 integrals of vinyl ($H_a + H_b$) and CMC (H_{CMC}) protons, according to Eq. (8).

$$241 \quad DM (\%) = \frac{H_a + H_b}{H_{CMC}} \times \frac{8.4}{2 \times 2.3} \times 100 \quad (8)$$

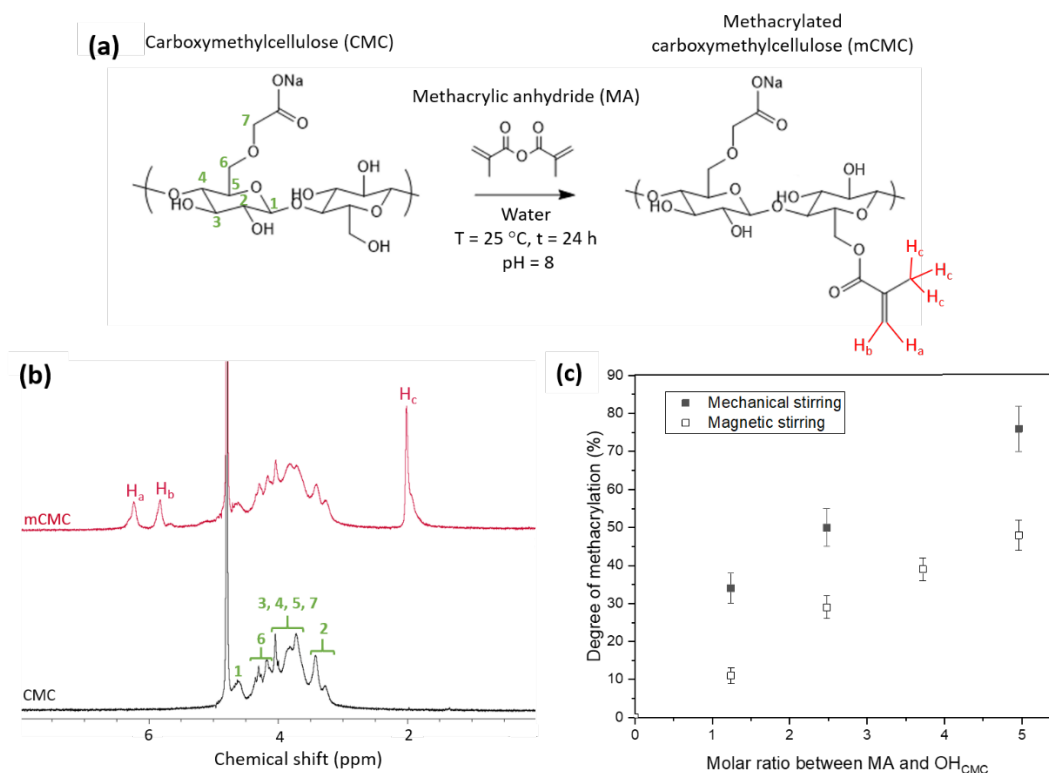
242 The influence on the final DM of both the molar ratio between MA and CMC hydroxyl
243 groups (MA/OH_{CMC}) and the agitation method was investigated. First, by increasing the
244 MA/OH_{CMC} ratio from 0 to 4.96, a continuous increase in the integrals of all methacrylate
245 peaks ($H_a - H_c$) was noticed (Fig. 1b), leading to a linear increase in the calculated DM (Fig.
246 1c; $R^2 > 0.94$). A maximum DM value of 76 ± 6 % was obtained for the mCMC prepared
247 using a MA/OH_{CMC} ratio of 4.96 under mechanical stirring at 2,000 rpm, indicating that
248 methacrylate groups successfully substituted 76 of the initial hydroxyl groups over 100
249 units. Such results therefore show a very easy and high degree of tunability of the DM by
250 simply playing with the MA/OH ratio.

251 As for reactions performed using magnetic stirring at 700 rpm, a linear increase with the
252 number of equivalents was observed. However, lower DM values were systematically
253 obtained for the same molar ratio, which was attributed to a lower diffusion of MA in the
254 solution. For example, for a MA/OH_{CMC} ratio of 4.96 under magnetic stirring, a DM of only
255 47 ± 4 % was obtained. The reaction was therefore found to be more efficient with
256 mechanical stirring at 2,000 rpm.

257 It is worth noticing that the maximum DM obtained in this work is 76 ± 6 %, which is 16
258 percentage points higher than the maximum DM reported in the literature ($DM = 60$ %)
259 by Melilli et al. (2020). According to the present results and since no plateau value was
260 reached, it is quite likely that even higher DM values could be obtained by using a higher
261 initial MA/OH_{CMC} ratio, but the influence of molar ratios larger than 4.96 was not studied
262 here.

263 This methacrylation could be considered for other cellulosic derivatives. Methylcellulose
264 has already been functionalized by MA and the development of the synthesis could be the
265 same as the one presented here (Stalling, Akintoye, et Nicoll 2009; Gold et al. 2015).
266 Similarly, hydroxypropyl cellulose has been functionalized by MA in N,N-
267 dicyclohexylcardodiimide with pyridine instead of NaOH (A. Qi et al. 2014). The synthesis
268 conditions described in this work could be adapted to this cellulose derivative. However,
269 these conditions could not be suitable for cellulose acetate which is not water-soluble.

270 The mCMC samples discussed below were all synthesized using mechanical stirring, with
271 initial MA/OH_{CMC} ratio of 1.24, 2.48 and 4.96 and had final DM values of 34 ± 4 % (sample
272 A), 50 ± 5 % (sample B) and 76 ± 6 % (sample C), respectively.



273

274 **Fig. 1 (a)** Scheme of the methacrylation of CMC with MA in water at 25 °C to yield mCMC. **(b)** ¹H
 275 NMR spectra for CMC in black and mCMC in red (2.48 eq, mechanical stirring, sample B). **(c)**
 276 Evolution of the degree of methacrylation with the molar ratio between MA and CMC hydroxyl
 277 groups for syntheses performed with mechanical and magnetic stirring. Error bars depend on the
 278 repetition of the synthesis (n = [1-3]) and the protons integration processing of the NMR spectra.

279 Hydrogels and cryogels characterization

280 The three mCMC samples (A-C) were then used to prepare eight photo-crosslinkable
 281 liquid formulations with mCMC concentrations between 2 and 4 wt% (Table 1). Due to its
 282 lower solubility in water, sample C (DM = 76 ± 6 %) was only used at 2 and 3 wt%. The
 283 influence of the UV insolation duration (30, 60 and 300 s) was then investigated by
 284 characterizing photo-crosslinked cryogel samples C3 using ¹³C CP-MAS solid-state NMR
 285 (Supporting Information Fig. S2). The methacrylate conversion ratio calculated for these
 286 cryogels were 45 %, 54 % and 62 %, respectively, and rapidly reached a plateau value
 287 around 60 % (Supporting Information Fig. S4). Based on these results, the photo-
 288 crosslinking reaction was considered completed after 5 min, and this time was selected
 289 for the subsequently-described experiments.

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295

296 **Table 1** Composition of the photo-crosslinkable mCMC formulations, and structural
 297 properties of the corresponding hydrogels and cryogels.

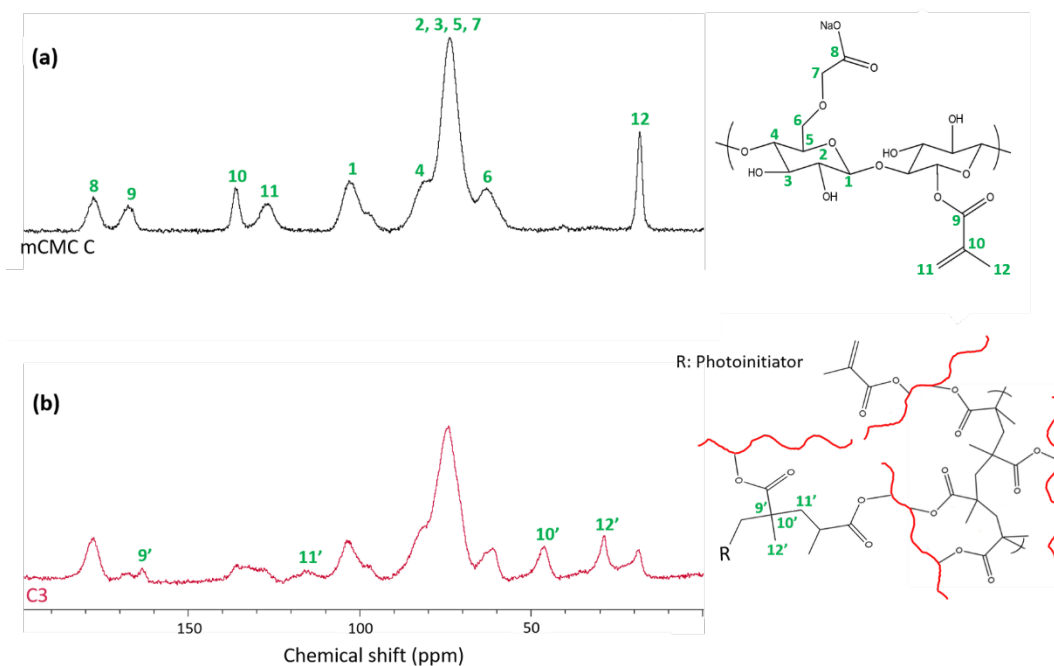
Formulation	mCMC			Cryogel	Cryogel	Hydrogel
	Sample	DM (%)	Concentration (wt%)	Conversion ratio of methacrylates (CR) (%)	Crosslinked methacrylate density (10^{-3} mmol/cm ³) ^a	Mesh size ξ (nm)
A2	A	34 ± 4	2	53 ± 2.0	4.3 ± 0.29	5.4 ± 1.6
A3			3	59 ± 2.0	6.4 ± 0.35	3.9 ± 1.2
A4			4	50 ± 2.0	8.1 ± 0.41	2.8 ± 0.96
B2	B	50 ± 5	2	58 ± 2.0	6.6 ± 0.39	4.2 ± 1.0
B3			3	54 ± 2.0	9.1 ± 0.50	2.8 ± 0.50
B4			4	53 ± 2.0	12 ± 0.65	0.79 ± 0.27
C2	C	76 ± 6	2	62 ± 2.0	9.9 ± 0.62	1.3 ± 0.38
C3			3	54 ± 2.0	13 ± 0.95	0.56 ± 0.14

298 ^a Crosslinked methacrylate density = $\frac{n_{meth}(mmol) \times CR}{100 \times V_{cryogel}(cm^3)}$

299 *Determination of the conversion ratio in photo-crosslinked mCMC*

300 The methacrylate conversion ratio (CR) of all 8 cryogels was investigated by NMR
 301 spectroscopy. First, the ¹³C CP-MAS spectra of the mCMC samples before crosslinking
 302 were used to attribute the signals of the different carbons, as already detailed (H. Qi et al.
 303 2009). The heterocyclic carbons (C₂₋₅) were found between 55 and 90 ppm, while the
 304 anomeric carbon was found at 104 ppm (Fig. 2a). The signals at 168 and 176 ppm were
 305 attributed to the carbonyl of the methacrylate (C₉) and carboxylate (C₈) groups,
 306 respectively. (Capitani et al. 2000) showed that the raw unmethacrylated CMC only has a
 307 carbonyl signal at 176 ppm, in good accordance with this attribution. Finally, the signals
 308 at 18, 123 and 133 ppm were respectively attributed to the methyl carbon (C₁₂) and to
 309 both vinyl carbons (C₁₀₋₁₁) of the methacrylate group (Fig. 2a). A few differences were
 310 observed in the ¹³C CP-MAS and NQS spectra of photo-crosslinked samples and attributed
 311 using sample C3 spectra (Fig. 2b and Supporting Information Fig. S3). During the UV
 312 insolation, the methacrylates of the C3 sample were progressively converted by vinyl
 313 polymerization, leading to a progressive decrease of carbons C₉₋₁₂ signal intensities.
 314 Simultaneously, the apparition of new carbon signals was observed and attributed to the
 315 crosslinking points formed by vinyl conversion: C_{9'} (163 ppm), C_{10'} (46 ppm), C_{11'} (115 ppm)
 316 and C_{12'} (28 ppm).

317



318

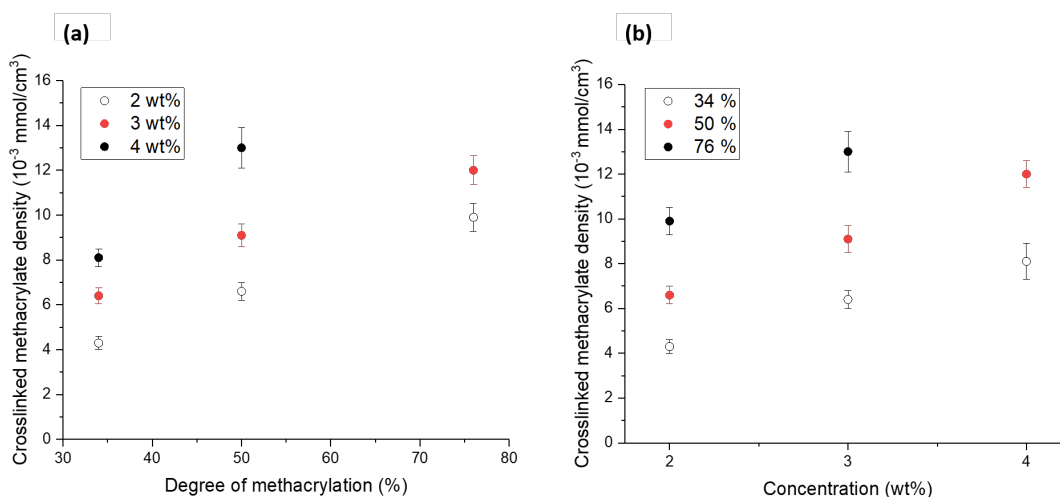
319 **Fig. 2** ^{13}C CPMAS solid-state NMR spectra of (a) C mCMC sample (DM = 76 %) and (b) C3 cryogel.

320 The conversion ratio of methacrylates in the cryogel samples was then calculated by
 321 integrating the area of the C12' carbon peak from the C12 of the crosslinked mCMC,
 322 normalized by the anomeric carbon 1, which remained constant during crosslinking. Two
 323 carbons of Irgacure 2959 were also integrated at the same chemical shift (24 ppm).
 324 However, considering a constant molar ratio between the photoinitiator and the
 325 methacrylate groups (0.55), the Irgacure contribution does not modify the calculation of
 326 CR. The CR was therefore calculated with Eq. (9):

$$327 \quad CR = \frac{A(C_{12'})}{A(C_{12}) + A(C_{12'})} \quad (9)$$

328 where $A(C_{12'})$ and $A(C_{12})$ correspond to the integrals of the C12' (28 ppm) and C12 (18 ppm)
 329 carbon signals of crosslinked mCMC, respectively.

330 Finally, the CR of methacrylates was calculated for all cryogels to determine its
 331 dependence with the initial degree of methacrylation (DM) and concentration of the
 332 mCMC in the formulations. Table 1 summarizes the extracted values that lie between $50 \pm 2 \%$
 333 $\pm 2 \%$ and $62 \pm 2 \%$. It is very interesting to note that the mCMC concentration negatively
 334 impacted the final CR while the DM had little influence (Table 1). Indeed, the CR did not
 335 change significantly with increasing DM for the same initial concentration, while, on the
 336 other hand, for the same DM, the higher the concentration, the lower the CR. Considering
 337 that the volume of all cryogels was constant and equal to 0.64 cm^3 , it was possible to
 338 calculate the density of crosslinked methacrylates in mmol/cm^3 from the DM and CR
 339 values (Table 1). Interestingly, the crosslinked methacrylate density increased linearly
 340 with the DM at a constant concentration ($R^2 > 0.99$; Fig. 3a), as well as with the
 341 concentration for a constant DM ($R^2 > 0.98$; Fig. 3b). The hydrogel crosslink density can
 342 thus be controlled over a significant range (3-fold increase between the lowest and
 343 highest densities) by modulating the initial DM of the considered mCMC, but also the
 344 mCMC concentration in the photo-crosslinkable formulation.



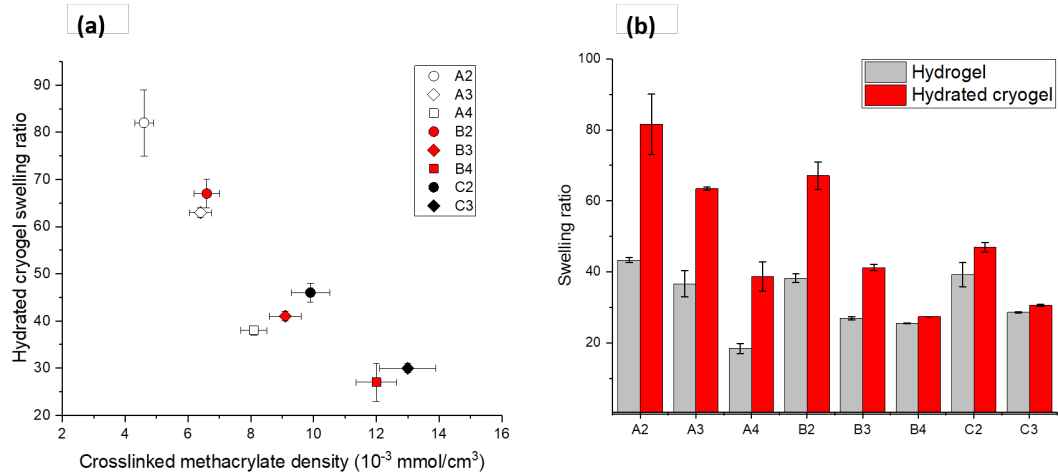
345

346 **Fig. 3** Density of crosslinked methacrylates according to (a) mCMC DM for a given concentration,
 347 and (b) mCMC concentration for a given DM.

348 *Relationship between swelling and hydrogel and cryogel structure*

349 Water swelling ratios (SR) reflecting the capacity of cryogels to absorb and retain water
 350 without breaking were measured. A swelling equilibrium was achieved in less than 10
 351 hours for all cryogels (Supporting Information Fig. S5). Interestingly, the final swelling ratio
 352 decreased with increasing concentration, with A2, A3 and A4 cryogels absorbing 81, 66
 353 and 45 times more water than their initial mass after 48 h at room temperature,
 354 respectively. Similarly, the swelling ratio decreased with increasing DM. For example,
 355 swelling ratios of 81, 62 and 37 were obtained for the cryogels A2 (DM = 34 %), B2 (DM =
 356 50 %) and C2 (DM = 76 %), respectively. The same trends were observed between the
 357 swelling ratio and the crosslinked methacrylate density (Fig. 4a). When the crosslinked
 358 methacrylate density in the cryogel increased, the network became denser and less
 359 stretchable, and thus absorbed less water. The hydrated cryogel swelling ratios were then
 360 compared to the water content of initial hydrogels (Fig. 4b). All cryogels absorbed more
 361 water than that contained in the hydrogels, as the swelling was conducted to its maximum
 362 capacity for the hydrated cryogels, whereas the equilibrium was probably not reached for
 363 the initial hydrogels. Interestingly, differences in the swelling ratio between the two
 364 materials were observed depending on the sample. For example, hydrated cryogels A2
 365 with the lowest crosslink density was twice as heavy as the initial hydrogel A2, whereas
 366 the most crosslinked cryogel B4 absorbed only 7 % more water than the initial hydrogel
 367 B4 had. This could be explained by the freezing, which causes the polymer chains to
 368 aggregate (Cassanelli, Norton, et Mills 2018). The swelling ratio at equilibrium strongly
 369 depends on the intrinsic capacity of the polymer chains to absorb water (Ganji,
 370 Vasheghani-Farahani, et Vasheghani-Farahani 2010). In the case of cryogels, the collapsed
 371 polymer chains were held together by hydrogen bonds, making it more difficult for water
 372 to hydrate them. This phenomenon became even more significant when mCMC
 373 concentration increased, which could involve enlargement of the size of the pore walls as
 374 observed by Cassanelli et al. (2018) with gellan gum cryogels. Finally, the water absorption
 375 capacity of cryogels also appeared to depend on the free methacrylate density
 376 (Supporting Information Fig. S6), since methacrylates are hydrophobic. The possibility to
 377 finely tune the swelling capacity of hydrated cryogels makes it possible to consider several
 378 types of medical applications. Indeed, it has been reported that the higher the water

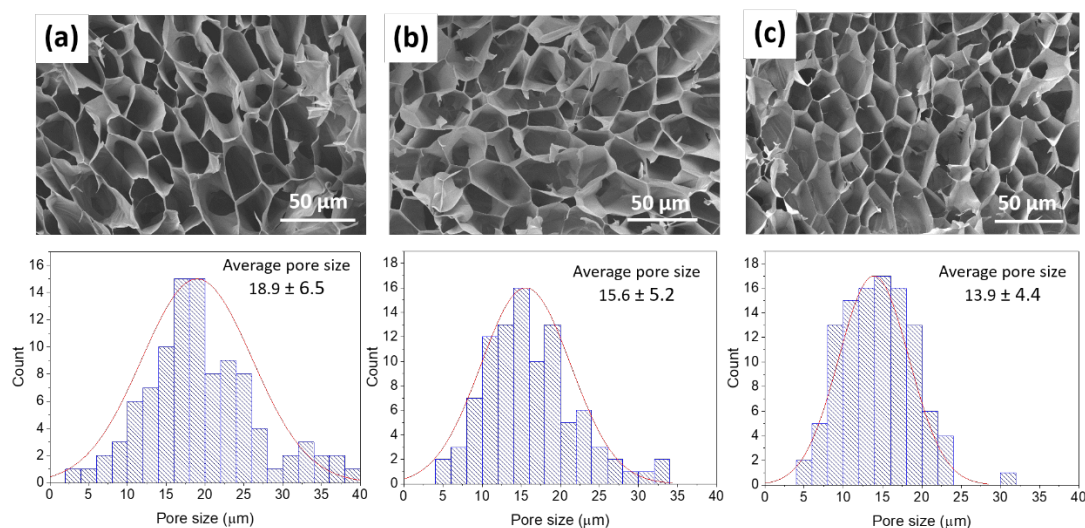
379 content of hydrogels, the more they provide a functional environment for cells. Such a
 380 property would be beneficial for applications such as drug delivery (Chang et al. 2010) or
 381 wound healing (Kanikireddy et al. 2020; Uzun 2018). Moreover, higher water content
 382 allows for better biodegradability (Capanema et al. 2018; Chang et al. 2010). However, for
 383 other applications such as bone repair, a lower swelling ratio is recommended (Bai et al.
 384 2018).



385

386 **Fig 4 (a)** Influence of the density of crosslinked methacrylates in cryogel samples on the swelling
 387 ratio of the hydrated cryogel after 48h. **(b)** Comparison of hydrogel **water content** and hydrated
 388 cryogel swelling ratio at equilibrium. Error bars are standard deviations for 3 different samples per
 389 formulation.

390 The density of crosslinked methacrylates also had an influence on the material structure.
 391 The hydrogel mesh size, representing the distance between two crosslinks, was evaluated
 392 using the Flory-Rehner theory for each formulation (Eq. 7). As expected, the mesh size
 393 decreased as the concentration and DM of mCMC increased (Table 1). The more
 394 methacrylate groups in the formulation, the more crosslinks and the smaller the mesh
 395 size. However, these results did not lead to significant variations in the cryogel pore sizes
 396 determined by SEM (Fig. 5, Supporting Information Fig. S7) as the most crosslinked sample
 397 (B4) had an average pore size of $13.9 \pm 5.3 \mu\text{m}$ and the less crosslinked sample (A2)
 398 displayed an average pore size of $18.9 \pm 6.5 \mu\text{m}$. The variations would rather be reflected
 399 in the thickness of the walls constituting the pores (Plieva et al. 2005). In fact, the pore
 400 size and direction in the cryogels happen to be predominantly controlled by the freeze-
 401 drying process, and not directly by the material crosslink density. The influence of the
 402 freeze-drying process is currently under investigation. Regarding the size of the pores,
 403 they are too small for applications such as bone repair, for which pores around $200 \mu\text{m}$
 404 are required to allow osteoblasts to penetrate the network (Abbasi et al. 2020; Sindhu,
 405 Prasanth, et Thakur 2014). To overcome this issue, it could be possible to shape grids by
 406 3D printing with an adequate porosity. However, the range of pore size we could reach
 407 would be able to allow neovascularization or fibroblast growth (Annabi et al. 2010).



408

409 **Fig. 5** SEM images displaying the cryogel morphologies and corresponding pore size distributions
 410 for **(a)** A2, **(b)** B2 and **(c)** C2. The average pore size was determined after around 25 measurements
 411 on 4 images per sample. Pores whose walls were broken by the cutting section method were not
 412 taken into account.

413

414 *Rheological behavior*

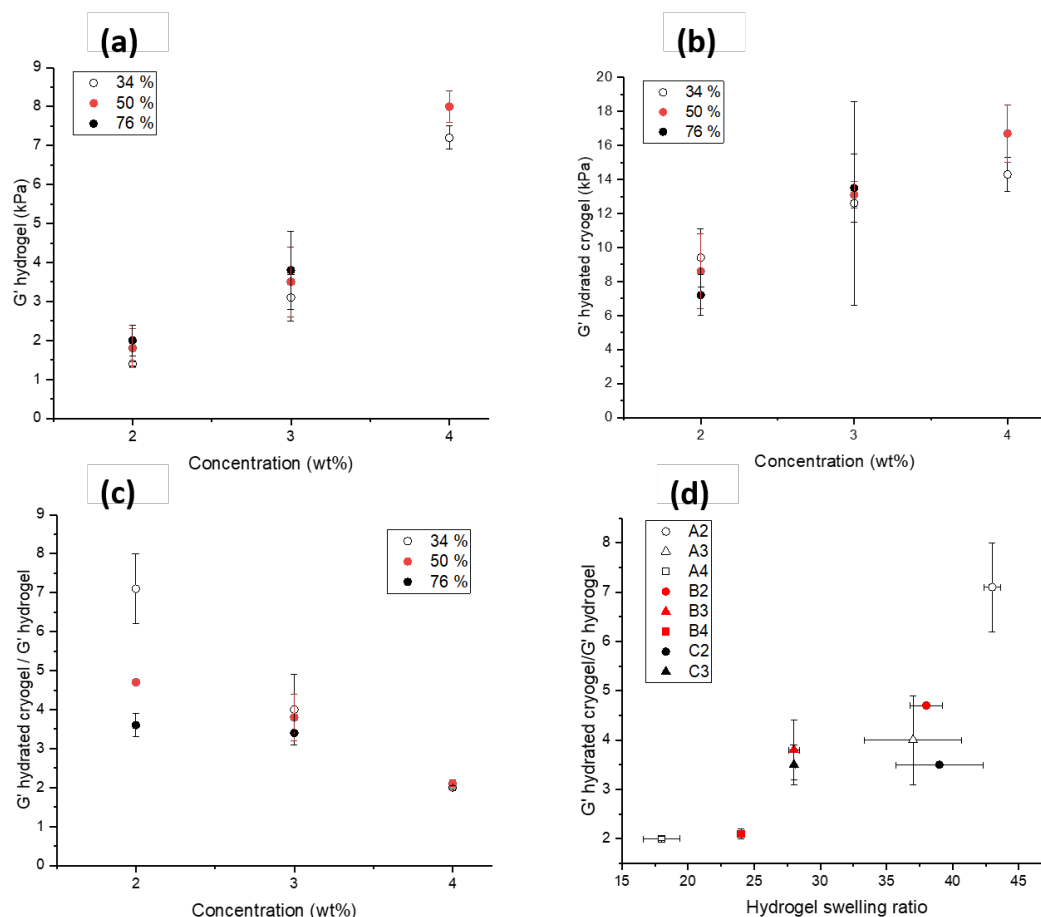
415 The impact of the crosslink density on the hydrogel mechanical properties was
 416 investigated by rheology. The evolution of the elastic modulus, G' , viscous modulus, G''
 417 and $\tan(\delta) = \frac{G''}{G'}$ were first compared for A2 and B4, the hydrogels bounding the range of
 418 rheological properties (Supporting Information Fig. S8). Over the entire frequency range
 419 (0.1 to 10 Hz) at 1 % strain and at 25 °C, G' is always superior to G'' , which evidenced the
 420 solid behavior of the gels. Moreover, the same behavior was observed for all the
 421 hydrogels, irrespective of the formulation. Finally, the elastic and viscous moduli of
 422 hydrogels and hydrated cryogels maintained the same order as a function of DM and
 423 mCMC concentration in the formulations (Supporting Information Fig. S9). Consequently,
 424 the comparison of the solid rheological properties of the materials was performed by
 425 considering G' at 1 Hz. The same study could be done with G'' for the viscous behavior.

426 For hydrogels, $G'(f)$ of the initial hydrogels increased with mCMC concentration (Fig. 6a).
 427 At 1 Hz, hydrogel $G'(1 \text{ Hz})$ values of 1.8 ± 0.50 , 3.5 ± 0.90 and 8.0 ± 0.40 kPa were measured
 428 for B2, B3 and B4, respectively. However, there was no significant trend observed with
 429 the DM. The same trend was observed with mCMC concentration for the hydrated
 430 cryogels (Fig. 6b). Hydrated cryogel $G'(1 \text{ Hz})$ values of 8.6 ± 2.2 , 13.1 ± 0.8 and 16.7 ± 1.7
 431 kPa were measured for B2, B3 and B4, respectively. At 1 Hz, the ratio of elastic moduli
 432 ($G'(\text{hydrated cryogel})/G'(\text{initial hydrogel})$) was superior to 1 for each formulation.
 433 Noticeably, even if they contain more water, the elastic moduli of the hydrated cryogels
 434 were significantly higher than those of the initial hydrogels. This ratio also decreased as
 435 the mCMC concentration increased. While at 2 wt% mCMC, the G' ratio increased with
 436 decreasing DM, no significant G' ratio evolution with DM was observed at 3 wt% and 4
 437 wt% (Fig. 6c).

438 Such a difference in the rheological properties could be due to the liquid nitrogen-freezing
 439 step before freeze-drying. As observed in the SEM images along the cross-section

440 (Supporting Information Fig. S10), cryogel pores were oriented along the freezing axis
441 (from the bottom to the top) after freeze-drying, whereas they could be randomly
442 oriented in the hydrogels. It is conceivable that this orientation implies a stronger network
443 organization in the cryogel than in the initial hydrogel. Indeed, cryogel swelling did not
444 affect the variation of the elastic modulus G' of hydrated cryogels as a function of DM and
445 mCMC concentration compared to hydrogels (Fig. 6a,b). The elastic moduli of both
446 materials increased with mCMC concentration and did not show significant variation with
447 DM. However, the less crosslinked methacrylate density in the formulation, the more
448 water the initial hydrogel contained (Fig. 4b). It is possible that upon freezing the pore
449 orientation and network organization is partly governed by the quantity of water in the
450 initial hydrogel. In other words, the formed crystals could be larger in the presence of
451 larger amounts of water. The microstructure could have deformed more easily and
452 become more robust upon freeze-drying when more water was entrapped in the initial
453 hydrogel. Moreover, the freezing process collapses the chains and can strengthen the
454 structure of the 3D network. Indeed, the G' ratio decreased with the hydrogel water
455 content (Fig. 6d). For B2, B3 and B4, G' ratios were 4.7 ± 0.10 , 3.8 ± 0.61 and 2.1 ± 0.10 for
456 a hydrogel water content of 38 ± 1.2 , 28 ± 0.43 and 24 ± 0.010 , respectively (Fig. 6d).

457 Finally, our results evidence that the elastic modulus G' of both initial hydrogels and
458 hydrated cryogels can be tuned by varying the mCMC concentration, rather than the DM.
459 Depending on the material targeted application, specific DM and concentration can be
460 selected to achieve suitable microstructure and mechanical properties. As an example,
461 hydrogels are generally mechanically brittle for bone repair applications (Bai et al. 2018;
462 Sindhu, Prasanth, et Thakur 2014). It is shown here that it is possible to increase the
463 viscoelasticity of hydrogels without increasing the amount of water in the hydrogel, as in
464 the case of the B4 sample. However, the obtained elastic moduli are too low for skeletal
465 tissues. On the other hand, they could be considered for use as extracellular matrix (ECM)
466 or for soft tissue repair, such as lungs or brain (Chaudhuri et al. 2020).



467

468 **Fig 6 (a)** Evolution of the elastic moduli G' of the initial hydrogels (at 1 Hz, 1% strain and 25 °C)
 469 versus the mCMC concentration in each formulation. **(b)** Evolution of the elastic moduli G' of the
 470 hydrated cryogels (at 1 Hz, 1% strain and 25 °C) versus the mCMC concentration in each
 471 formulation. **(c)** Ratios of the elastic moduli of hydrated cryogels over that of the corresponding
 472 initial hydrogels versus mCMC concentration. **(d)** Evolution of the ratios of the elastic moduli of
 473 hydrated cryogels over that of the corresponding hydrogels versus hydrogel swelling ratio. Error
 474 bars come from the standard deviation of three independent measurements.

475 4. Conclusion

476 In this study, the reaction between carboxymethylcellulose and methacrylic anhydride
 477 was first investigated to produce photo-crosslinkable methacrylated
 478 carboxymethylcellulose. By controlling the synthesis parameters, a wide range of mCMC
 479 samples with degrees of methacrylation ranging between 0 and 76 ± 6 % were obtained.
 480 Eight aqueous formulations with different DM and concentrations of mCMC were used to
 481 prepare UV-cured hydrogels, which were subsequently transformed into cryogels by
 482 freeze-drying. A new ^{13}C solid-state NMR method was used to measure the methacrylate
 483 conversion ratio (CR, from 50 to 62 %) and deduce the density of crosslinked
 484 methacrylates (from 4.3×10^{-3} to 1.3×10^{-2} mmol/cm³) of the final cryogels, which was
 485 found to be correlated with both DM and initial concentration of the mCMC formulation.
 486 Furthermore, correlations between the density of crosslinked methacrylate and the
 487 swelling ratio of cryogels on the one hand, and the microstructure on the other hand,
 488 were demonstrated. In addition, these results help to explain the evolution of the elastic
 489 moduli of hydrogels and hydrated cryogels, which depended mainly on the initial

490 concentration of mCMC in the formulation. Furthermore, the difference between the two
491 elastic moduli depended on the quantity of water in the initial hydrogel and the
492 crosslinked methacrylate density. In conclusion, we highlighted the possibility of fine-
493 tuning the microstructure (crosslinked methacrylate density; mesh size between 0.56 and
494 5.4 nm) as well as the cryogel swelling ratio (25-80) and rheological properties (2-8 kPa
495 for hydrogels, 4.5 - 17 kPa for swollen cryogels) of UV-cured mCMC hydrogels and
496 cryogels, by controlling the methacrylation conditions of a commercial CMC. According to
497 the selected properties, these crosslinked mCMC hydrogels and cryogels could serve
498 different purposes in biomedicine, such as 3D scaffolds for soft tissue regeneration, or cell
499 encapsulation. Additionally, photo-crosslinkable mCMC could be easily shaped by 3D
500 printing technics to obtain complex 3D-printed artefacts. Finally, **these materials could**
501 **also be considered in fields such as agriculture, food and textile where porous materials**
502 **are generally used to absorb solvents, dyes, water or liquids harmful to the analyzed**
503 **environment (Rahman et al. 2021).**

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519 free of charge in the supporting information.

520 **Declarations**

521 **Conflict of interest** The authors have no conflicts of interest to declare.

522 **Ethical approval** All authors have read and understood the "Ethical Responsibilities of Authors" in
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524 computer software.

525 **Consent for publication** All authors have fully read, agreed, and approved the final version of the
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References

- 528 Abbasi, Naghmeh, Stephen Hamlet, Robert M. Love, et Nam-Trung Nguyen. 2020.
529 « Porous Scaffolds for Bone Regeneration ». *Journal of Science: Advanced*
530 *Materials and Devices* 5 (1): 1-9. <https://doi.org/10.1016/j.jsamd.2020.01.007>.
- 531 Annabi, Nasim, Jason W. Nichol, Xia Zhong, Chengdong Ji, Sandeep Koshy, Ali
532 Khademhosseini, et Fariba Dehghani. 2010. « Controlling the Porosity and
533 Microarchitecture of Hydrogels for Tissue Engineering ». *Tissue Engineering Part*
534 *B: Reviews* 16 (4): 371-83. <https://doi.org/10.1089/ten.teb.2009.0639>.
- 535 Bai, Xin, Mingzhu Gao, Sahla Syed, Jerry Zhuang, Xiaoyang Xu, et Xue-Qing Zhang. 2018.
536 « Bioactive Hydrogels for Bone Regeneration ». *Bioactive Materials* 3 (4):
537 401-17. <https://doi.org/10.1016/j.bioactmat.2018.05.006>.
- 538 Baier Leach, Jennie, Kathryn A. Bivens, Charles W. Patrick Jr., et Christine E. Schmidt.
539 2003. « Photocrosslinked Hyaluronic Acid Hydrogels: Natural, Biodegradable
540 Tissue Engineering Scaffolds ». *Biotechnology and Bioengineering* 82 (5): 578-89.
541 <https://doi.org/10.1002/bit.10605>.
- 542 Berruyer, Pierrick, Martin Gericke, Pinelopi Moutzouri, Dörthe Jakobi, Michel Bardet,
543 Leif Karlson, Staffan Schantz, Thomas Heinze, et Lyndon Emsley. 2021.
544 « Advanced Characterization of Regioselectively Substituted Methylcellulose
545 Model Compounds by DNP Enhanced Solid-State NMR Spectroscopy ». *Carbohydrate Polymers* 262 (juin): 117944.
546 <https://doi.org/10.1016/j.carbpol.2021.117944>.
- 547 Capanema, Nádia S.V., Alexandra A.P. Mansur, Anderson C. de Jesus, Sandhra M.
548 Carvalho, Luiz C. de Oliveira, et Herman S. Mansur. 2018. « Superabsorbent
549 Crosslinked Carboxymethyl Cellulose-PEG Hydrogels for Potential Wound
550 Dressing Applications ». *International Journal of Biological Macromolecules* 106
551 (janvier): 1218-34. <https://doi.org/10.1016/j.ijbiomac.2017.08.124>.
- 552 Capitani, Donatella, Matteo Alessandro Del Nobile, Giuseppe Mensitieri, Alessandro
553 Sannino, et Anna Laura Segre. 2000. « ¹³ C Solid-State NMR Determination of
554 Cross-Linking Degree in Superabsorbing Cellulose-Based Networks ». *Macromolecules* 33 (2): 430-37. <https://doi.org/10.1021/ma9914117>.
- 555 Cassanelli, Mattia, Ian Norton, et Tom Mills. 2018. « Role of Gellan Gum Microstructure
556 in Freeze Drying and Rehydration Mechanisms ». *Food Hydrocolloids* 75 (février):
557 51-61. <https://doi.org/10.1016/j.foodhyd.2017.09.013>.
- 558 Chang, Chunyu, Bo Duan, Jie Cai, et Lina Zhang. 2010. « Superabsorbent Hydrogels Based
559 on Cellulose for Smart Swelling and Controllable Delivery ». *European Polymer*
560 *Journal* 46 (1): 92-100. <https://doi.org/10.1016/j.eurpolymj.2009.04.033>.
- 561 Chaudhuri, Ovijit, Justin Cooper-White, Paul A. Janmey, David J. Mooney, et Vivek B.
562 Shenoy. 2020. « Effects of Extracellular Matrix Viscoelasticity on Cellular
563 Behaviour ». *Nature* 584 (7822): 535-46. <https://doi.org/10.1038/s41586-020-2612-2>.
- 564 Chen, Ying-Chieh, Rwei-Zeng Lin, Hao Qi, Yunzhi Yang, Hojae Bae, Juan M. Melero-
565 Martin, et Ali Khademhosseini. 2012. « Functional Human Vascular Network
566 Generated in Photocrosslinkable Gelatin Methacrylate Hydrogels ». *Advanced*
567 *Functional Materials* 22 (10): 2027-39.
568 <https://doi.org/10.1002/adfm.201101662>.
- 569 Cleland, Robert L. 1970. « Ionic Polysaccharides. IV. Free-Rotation Dimensions for
570 Disaccharide Polymers. Comparison with Experiment for Hyaluronic Acid ». *Biopolymers* 9 (7): 811-24. <https://doi.org/10.1002/bip.1970.360090707>.
- 571 Darpentigny, Clémentine, Sonia Molina-Boisseau, Guillaume Nonglaton, Julien Bras, et
572 Bruno Jean. 2020. « Ice-Templated Freeze-Dried Cryogels from Tunicate
573 Cellulose Nanocrystals with High Specific Surface Area and Anisotropic
574
575
576
577

- 578 Morphological and Mechanical Properties ». *Cellulose* 27 (1): 233-47.
579 <https://doi.org/10.1007/s10570-019-02772-8>.
- 580 Dijk-Wolthuis, W. N. E. van, J. A. M. Hoogeboom, M. J. van Steenberg, S. K. Y. Tsang,
581 et W. E. Hennink. 1997. « Degradation and Release Behavior of Dextran-Based
582 Hydrogels ». *Macromolecules* 30 (16): 4639-45.
583 <https://doi.org/10.1021/ma9704018>.
- 584 Dovedytis, Matthew, Zhuo Jie Liu, et Samuel Bartlett. 2020. « Hyaluronic Acid and Its
585 Biomedical Applications: A Review ». *Engineered Regeneration* 1: 102-13.
586 <https://doi.org/10.1016/j.engreg.2020.10.001>.
- 587 Dutta, Sujana, Pousali Samanta, et Dibakar Dhara. 2016. « Temperature, PH and Redox
588 Responsive Cellulose Based Hydrogels for Protein Delivery ». *International
589 Journal of Biological Macromolecules* 87 (juin): 92-100.
590 <https://doi.org/10.1016/j.ijbiomac.2016.02.042>.
- 591 Fedorova, A.F., et Z.A. Rogovin. 1963. « A Study of the Relative Reactivity of the Hydroxyl
592 Groups of Cellulose in Esterification in an Acidic Medium ». *Polymer Science
593 U.S.S.R.* 4 (5): 1189-94. [https://doi.org/10.1016/0032-3950\(63\)90350-2](https://doi.org/10.1016/0032-3950(63)90350-2).
- 594 Fernández-Garibay, Xiomara, María A Ortega, Estefanía Cerro-Herreros, Jordi Comelles,
595 Elena Martínez, Rubén Artero, Juan M Fernández-Costa, et Javier Ramón-Azcón.
596 2021. « Bioengineered in Vitro 3D Model of Myotonic Dystrophy Type 1 Human
597 Skeletal Muscle ». *Biofabrication* 13 (3): 035035. <https://doi.org/10.1088/1758-5090/abf6ae>.
- 599 Ganji, Fariba, Samira Vasheghani-Farahani, et Ebrahim Vasheghani-Farahani. 2010.
600 « Theoretical Description of Hydrogel Swelling: A Review ». *Iranian Polymer
601 Journal* 5 (19): 375-98.
- 602 García-Lizarribar, Andrea, Xiomara Fernández-Garibay, Ferran Velasco-Mallorquí, Albert
603 G. Castaño, Josep Samitier, et Javier Ramon-Azcon. 2018. « Composite
604 Biomaterials as Long-Lasting Scaffolds for 3D Bioprinting of Highly Aligned
605 Muscle Tissue ». *Macromolecular Bioscience* 18 (10): 1800167.
606 <https://doi.org/10.1002/mabi.201800167>.
- 607 Gold, Gittel T., Devika M. Varma, Peter J. Taub, et Steven B. Nicoll. 2015. « Development
608 of Crosslinked Methylcellulose Hydrogels for Soft Tissue Augmentation Using an
609 Ammonium Persulfate-Ascorbic Acid Redox System ». *Carbohydrate Polymers*
610 134 (décembre): 497-507. <https://doi.org/10.1016/j.carbpol.2015.07.101>.
- 611 Gu, Lihua, Yifan Zhang, Liwen Zhang, Yiqian Huang, Dawei Zuo, Qing Cai, et Xiaoping
612 Yang. 2020. « Comparative Study of Gelatin Cryogels Reinforced with
613 Hydroxyapatites with Different Morphologies and Interfacial Bonding ». *Biomedical Materials* 15 (3): 035012. <https://doi.org/10.1088/1748-605X/ab7388>.
- 616 Holtzer, Alfred M., Henri Benoit, et Paul Doty. 1954. « The Molecular Configuration and
617 Hydrodynamic Behavior of Cellulose Trinitrate ». *The Journal of Physical
618 Chemistry* 58 (8): 624-34. <https://doi.org/10.1021/j150518a009>.
- 619 Hossen, Muhammad R., Nayereh Dadoo, David G. Holomakoff, Aimee Co, William M.
620 Gramlich, et Michael D. Mason. 2018. « Wet Stable and Mechanically Robust
621 Cellulose Nanofibrils (CNF) Based Hydrogel ». *Polymer* 151 (août): 231-41.
622 <https://doi.org/10.1016/j.polymer.2018.07.016>.
- 623 Hoti, Gjylje, Fabrizio Caldera, Claudio Cecone, Alberto Rubin Pedrazzo, Anastasia
624 Anceschi, Silvia Lucia Appleton, Yousef Khazaei Monfared, et Francesco Trotta.
625 2021. « Effect of the Cross-Linking Density on the Swelling and Rheological
626 Behavior of Ester-Bridged β -Cyclodextrin Nanosponges ». *Materials* 14 (3): 478.
627 <https://doi.org/10.3390/ma14030478>.
- 628 Kanikireddy, Vimala, Kokkarachedu Varaprasad, Tippabattini Jayaramudu,
629 Chandrasekaran Karthikeyan, et Rotimi Sadiku. 2020. « Carboxymethyl
630 Cellulose-Based Materials for Infection Control and Wound Healing: A Review ».

- 631 *International Journal of Biological Macromolecules* 164 (décembre): 963-75.
 632 <https://doi.org/10.1016/j.ijbiomac.2020.07.160>.
- 633 Kono, Hiroyuki, Kazuhiro Oshima, Hisaho Hashimoto, Yuuichi Shimizu, et Kenji Tajima.
 634 2016. « NMR Characterization of Sodium Carboxymethyl Cellulose: Substituent
 635 Distribution and Mole Fraction of Monomers in the Polymer Chains ». *Carbohydrate Polymers* 146 (août): 1-9.
 636 <https://doi.org/10.1016/j.carbpol.2016.03.021>.
- 637 Lin, Huizi A., Michelle S. Gupta, Devika M. Varma, M. Lane Gilchrist, et Steven B. Nicoll.
 638 2016. « Lower Crosslinking Density Enhances Functional Nucleus Pulposus-like
 639 Matrix Elaboration by Human Mesenchymal Stem Cells in
 640 Carboxymethylcellulose Hydrogels: Crosslinking Density Impacts HMSC
 641 Chondrogenesis ». *Journal of Biomedical Materials Research Part A* 104 (1):
 642 165-77. <https://doi.org/10.1002/jbm.a.35552>.
- 643 Luo, Moucheng, Xinyu Zhang, Jun Wu, et Jinmin Zhao. 2021. « Modifications of
 644 Polysaccharide-Based Biomaterials under Structure-Property Relationship for
 645 Biomedical Applications ». *Carbohydrate Polymers* 266 (août): 118097.
 646 <https://doi.org/10.1016/j.carbpol.2021.118097>.
- 647 Matricardi, Pietro, Marilena Pontoriero, Tommasina Coviello, Maria Antonietta Casadei,
 648 et Franco Alhaique. 2008. « In Situ Cross-Linkable Novel Alginate-Dextran
 649 Methacrylate IPN Hydrogels for Biomedical Applications: Mechanical
 650 Characterization and Drug Delivery Properties ». *Biomacromolecules* 9 (7):
 651 2014-20. <https://doi.org/10.1021/bm800252c>.
- 652 Melilli, Giuseppe, Irene Carmagnola, Chiara Tonda-Turo, Fabrizio Pirri, Gianluca Ciardelli,
 653 Marco Sangermano, Minna Hakkarainen, et Annalisa Chiappone. 2020. « DLP 3D
 654 Printing Meets Lignocellulosic Biopolymers: Carboxymethyl Cellulose Inks for 3D
 655 Biocompatible Hydrogels ». *Polymers* 12 (8): 1655.
 656 <https://doi.org/10.3390/polym12081655>.
- 657 Memic, Adnan, Mahboobeh Rezaeeyazdi, Pierre Villard, Zachary J. Rogers,
 658 Tuerdimaimaiti Abudula, Thibault Colombani, et Sidi A. Bencherif. 2020. « Effect
 659 of Polymer Concentration on Autoclaved Cryogel Properties ». *Macromolecular
 660 Materials and Engineering* 305 (5): 1900824.
 661 <https://doi.org/10.1002/mame.201900824>.
- 662 Meyvis, Tom, Stefaan De Smedt, Barbara Stubbe, Wim Hennink, et Joseph Demeester.
 663 s. d. « On the Release of Proteins from Degrading Dextran Methacrylate
 664 Hydrogels and the Correlation with the Rheologic Properties of the Hydrogels »,
 665 7.
- 666 Michelle T. Poldervaart, Birgit Goversen, Mylene de Ruijter, Anna Abbadessa, Ferry P. W.
 667 Melchels, F. Cumhur Öner, Wouter J. A. Dhert, Tina Vermonden, et Jacqueline
 668 Alblas. 2017. « 3D Bioprinting of Methacrylated Hyaluronic Acid (MeHA)
 669 Hydrogel with Intrinsic Osteogenicity ». Édité par Masaya Yamamoto. *PLOS ONE*
 670 12 (6): e0177628. <https://doi.org/10.1371/journal.pone.0177628>.
- 671 Muir, Victoria G., et Jason A. Burdick. 2020. « Chemically Modified Biopolymers for the
 672 Formation of Biomedical Hydrogels ». *Chemical Reviews*, décembre,
 673 [acs.chemrev.0c00923](https://doi.org/10.1021/acs.chemrev.0c00923). <https://doi.org/10.1021/acs.chemrev.0c00923>.
- 674 Pacelli, Settimio, Laura Di Muzio, Patrizia Paolicelli, Valeria Fortunati, Stefania Petralito,
 675 Jordan Trilli, et Maria Antonietta Casadei. 2021. « Dextran-Polyethylene Glycol
 676 Cryogels as Spongy Scaffolds for Drug Delivery ». *International Journal of
 677 Biological Macromolecules* 166 (janvier): 1292-1300.
 678 <https://doi.org/10.1016/j.ijbiomac.2020.10.273>.
- 679 Picout, David R, et Simon B Ross-Murphy. 2002. « On the Chain Flexibility of
 680 Arabinoxylans and Other B-(1 → 4) Polysaccharides ». *Carbohydrate Research*, 4.
- 681

- 682 Plieva, Fatima M, Malin Karlsson, Maria-Rosa Aguilar, David Gomez, Sergey Mikhailovsky,
683 et Igor Yu Galaev'. 2005. « Pore Structure in Supermacroporous Polyacrylamide
684 Based Cryogels », 7.
- 685 Qi, Aisha, Siew Pei Hoo, James Friend, Leslie Yeo, Zhilian Yue, et Peggy P. Y. Chan. 2014.
686 « Hydroxypropyl Cellulose Methacrylate as a Photo-Patternable and
687 Biodegradable Hybrid Paper Substrate for Cell Culture and Other
688 Bioapplications ». *Advanced Healthcare Materials* 3 (4): 543-54.
689 <https://doi.org/10.1002/adhm.201300155>.
- 690 Qi, Haisong, Tim Liebert, Frank Meister, et Thomas Heinze. 2009. « Homogenous
691 Carboxymethylation of Cellulose in the NaOH/Urea Aqueous Solution ». *Reactive
692 and Functional Polymers* 69 (10): 779-84.
693 <https://doi.org/10.1016/j.reactfunctpolym.2009.06.007>.
- 694 Qian, Hanqing, Xin Wang, Kangjun Yuan, Chen Xie, Wei Wu, Xiqun Jiang, et Lijiang Hu.
695 2014. « Delivery of Doxorubicin in Vitro and in Vivo Using Bio-Reductive
696 Cellulose Nanogels ». *Biomater. Sci.* 2 (2): 220-32.
697 <https://doi.org/10.1039/C3BM60176E>.
- 698 Rahman, Md. Saifur, Md. Saif Hasan, Ashis Sutradhar Nitai, Sunghyun Nam, Aneek
699 Krishna Karmakar, Md. Shameem Ahsan, Muhammad J. A. Shiddiky, et
700 Mohammad Boshir Ahmed. 2021. « Recent Developments of Carboxymethyl
701 Cellulose ». *Polymers* 13 (8): 1345. <https://doi.org/10.3390/polym13081345>.
- 702 Razavi, Mehdi, Yang Qiao, et Avnesh S. Thakor. 2019. « Three-dimensional Cryogels for
703 Biomedical Applications ». *Journal of Biomedical Materials Research Part A* 107
704 (12): 2736-55. <https://doi.org/10.1002/jbm.a.36777>.
- 705 Rebelo, Rita, Margarida Fernandes, et Raul Fangueiro. 2017. « Biopolymers in Medical
706 Implants: A Brief Review ». *Procedia Engineering* 200: 236-43.
707 <https://doi.org/10.1016/j.proeng.2017.07.034>.
- 708 Reeves, Robert, Andreia Ribeiro, Leonard Lombardo, Richard Boyer, et Jennie B. Leach.
709 2010. « Synthesis and Characterization of Carboxymethylcellulose-Methacrylate
710 Hydrogel Cell Scaffolds ». *Polymers* 2 (3): 252-64.
711 <https://doi.org/10.3390/polym2030252>.
- 712 Reza, Anna T., et Steven B. Nicoll. 2010. « Characterization of Novel Photocrosslinked
713 Carboxymethylcellulose Hydrogels for Encapsulation of Nucleus Pulposus Cells ». *Acta Biomaterialia* 6 (1): 179-86. <https://doi.org/10.1016/j.actbio.2009.06.004>.
- 714 Sindhu, Karthika Ammini, Raghavan Prasanth, et Vijay Kumar Thakur. 2014. « Medical
715 Applications of Cellulose and Its Derivatives: Present and Future ». In
716 *Nanocellulose Polymer Nanocomposites*, édité par Vijay Kumar Thakur, 437-77.
717 Hoboken, NJ, USA: John Wiley & Sons, Inc.
718 <https://doi.org/10.1002/9781118872246.ch16>.
- 719 Sk, Md Moniruzzaman, Prativa Das, Amit Panwar, et Lay Poh Tan. 2021. « Synthesis and
720 Characterization of Site Selective Photo-Crosslinkable Glycidyl Methacrylate
721 Functionalized Gelatin-Based 3D Hydrogel Scaffold for Liver Tissue
722 Engineering ». *Materials Science and Engineering: C* 123 (avril): 111694.
723 <https://doi.org/10.1016/j.msec.2020.111694>.
- 724 Stalling, Simone S., Sunday O. Akintoye, et Steven B. Nicoll. 2009. « Development of
725 Photocrosslinked Methylcellulose Hydrogels for Soft Tissue Reconstruction ». *Acta Biomaterialia* 5 (6): 1911-18. <https://doi.org/10.1016/j.actbio.2009.02.020>.
- 726 Tomal, Wiktoria, et Joanna Ortyl. 2020. « Water-Soluble Photoinitiators in Biomedical
727 Applications ». *Polymers* 12 (5): 1073. <https://doi.org/10.3390/polym12051073>.
- 728 Uzun, Muhammet. 2018. « A Review of Wound Management Materials ». *Journal of
729 Textile Engineering & Fashion Technology* 4 (1).
730 <https://doi.org/10.15406/jteft.2018.04.00121>.
- 731 Van Hove, Amy H., Brandon D. Wilson, et Danielle S. W. Benoit. 2013. « Microwave-
732 Assisted Functionalization of Poly(Ethylene Glycol) and On-Resin Peptides for
733
734

735 Use in Chain Polymerizations and Hydrogel Formation ». *Journal of Visualized*
736 *Experiments*, n° 80 (octobre): 50890. <https://doi.org/10.3791/50890>.
737 Xia, Zhiyong, Marcia Patchan, Jeffrey Maranchi, Jennifer Elisseeff, et Morgana Trexler.
738 2013. « Determination of Crosslinking Density of Hydrogels Prepared from
739 Microcrystalline Cellulose ». *Journal of Applied Polymer Science* 127 (6):
740 4537-41. <https://doi.org/10.1002/app.38052>.
741