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First multi-reactive polysaccharide-based transurf to produce potentially biocompatible dextran-covered nanocapsules

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ABSTRACT

A multi-reactive polysaccharide-based transurf (acting both as macro-Chain Transfer Agent and stabilizer) was used to confine RAFT polymerization of methyl methacrylate (MMA) at the oil/water (o/w) miniemulsion interface. Dithiobenzoate groups and hydrophobic aliphatic side chains were introduced onto dextran, conferring it both transfer agent properties and ability to stabilize direct miniemulsion of MMA in the presence of a biocompatible oil, used as co-stabilizer. Because of their amphiphilic character, transurfs were initially adsorbed at the (o/w) interface and their reactive sites mediated RAFT polymerization *via* the R-group approach. PMMA-grafted dextran glycopolymers were consequently produced at the o/w interface, thus leading to dextran coverage/PMMA shell/oily core nanocapsules (NCs) as evidenced by Cryo-TEM analyses. The influence of dextran-based transurf chemistry and oil amount on MMA RAFT polymerization control was investigated. Positive preliminary results on NCs cytotoxicity suggest the potential of these objects for biomedical applications.

KEYWORDS

Polysaccharide
Nanocapsules
Transurf
Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization
Miniemulsion
Poly(methyl methacrylate)

1. Introduction

Polymer nanocapsules (NCs) have attracted greater attention in recent years because of their unique properties and potential applications in the biomedical field, for instance as drug delivery systems (Mora-Huertas, Fessi, & Elaissari, 2010; Vrignaud, Benoit, & Saulnier, 2011; Poltorak, Durand, Léonard, Six, & Nouvel, 2015, Six, & Ferji, 2019). Miniemulsion polymerization is a promising technique that allows drug encapsulation and NCs production in one-pot (Steinmacher et al., 2010). To produce oily-core NCs by this technique, nanodroplets with size ranging from 50 to 500 nm are initially obtained by dispersion of a monomer/oil mixture in an aqueous phase. Nanodroplets are protected against coalescence by the presence of stabilizer and preserved from Ostwald ripening by the addition of an osmotic pressure agent (co-stabilizer), which may be the oil constituting the inner liquid core in the final NCs (Steinmacher et al., 2010; Landfester & Mailänder, 2013; Forero Ramirez et al., 2018). In such a case, oily core and polymeric shell will be formed during polymerization by means of induced-phase separation whereby polymer migrates to the droplet/water interface due to poor solubility in the oil. However, a well-defined core/shell structure can be difficult to obtain because of the strict thermodynamic and kinetic parameters involved in polymer/oil separation. Moreover, stabilizers are usually physically adsorbed at NCs surface and can be easily removed or replaced by surface-active molecules present in physiological media, which may thus affect the colloidal stability or enhance unwanted immune responses.

To overcome all the above limitations, some authors reported the combination of mono-reactive amphiphilic copolymers as miniemulsion stabilizers with Reversible-Deactivation Radical Polymerizations (RDRP) such as Atom Transfer Radical Polymerization (ATRP) or Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization to confine polymerization at the oil/water interface. While keeping their role as stabilizers, these copolymers acted either as mono-reactive inisurfs (initiators and stabilizers) (Li,

67 Matyjaszewski, Albrecht, & Möller, 2009; Li, Yoon, & Matyjaszewski, 2010; Tian et al.,
68 2015) or transurfs (transfer agents and stabilizers) (Luo & Gu, 2007; Lu, Luo, & Li, 2010; Yu,
69 Zhang, Zhan, & Chen, 2012). This strategy was shown to offer several advantages. Firstly,
70 polymer chains grow directly at, or in the proximity of the interface thus allowing formation
71 of polymeric shell at the droplet periphery. Secondly, the stabilizer provides a permanent
72 hydrophilic covalently-linked coverage in the final NCs. Thirdly, the use of ATRP or RAFT
73 polymerization offers the possibility of precise control of macromolecular parameters of the
74 polymeric shell. With similar objectives, our team recently reported the first multi-reactive
75 dextran-based inisurf used in ATRP polymerization of butyl acrylate (Wu et al., 2015) and of
76 methyl methacrylate (MMA) (Forero Ramirez et al., 2018). In such cases, grafted dextran-
77 based copolymers were synthesized leading to nanoparticles (NPs) with covalently-anchored
78 hydrophilic dextran coverage. This coverage is expected to enhance NPs biocompatibility as
79 well as to ensure their colloidal stability and stealthiness, while offering the opportunity to
80 further functionalize their surface. Nevertheless, this ATRP process requires additional
81 purification steps to remove remaining copper catalysts after polymerization. We herein
82 hypothesize that switching ATRP to RAFT polymerization (Scheme 1) will overcome this
83 issue and will lead to the production of more suitable NCs for biomedical applications. This
84 new approach required the synthesis of a novel dextran derivative (transurf) carrying
85 hydrophobic and chain transfer agent (CTA) groups and acting as stabilizer and macro-CTA
86 during the miniemulsion process. To the best of our knowledge, it is the first time that RAFT
87 polymerization has been performed in a miniemulsion using a multi-reactive macro-CTA and
88 the above described dextran-based transurf is the second example thereof. In fact, one mono-
89 reactive dextran-based macro-CTA was previously reported for vinyl acetate polymerization
90 in emulsion, but the transurf was only formed during the polymerization process (Bernard,
91 Save, Arathoon, & Charleux, 2008). Moreover, among all the works dealing with the use of

92 transurfs in miniemulsion polymerization, the production of oily-core NCs is scarcely related,
93 and the preparation of PMMA-shell NCs has never been approached, which contributes to the
94 originality of the present work.

95 Herein, we hypothesize that using a dextran-based multi-reactive transurf can help to produce
96 oily-core/PMMA shell NCs by RAFT interfacial miniemulsion polymerization Transurfs were
97 first designed with a precise control of hydrophobic and CTA groups numbers to provide
98 convenient stabilizing and transfer agent properties. After model studies on MMA
99 polymerization, RAFT polymerization of MMA in miniemulsion was carried out using such
100 dextran-based transurfs: polymerization kinetics, molar mass control and CTA groups'
101 efficiency were studied. Detailed characterization of NCs in terms of size distribution, amount
102 of dextran at the surface, morphology and cytotoxicity were performed to prove the formation
103 of oily-core NCs, usable as platform for drug delivery.

104

105

106 **Scheme 1.** Synthesis of dextran-covered oily-core NCs by MMA RAFT polymerization in
107 miniemulsion

108 **2. Materials and methods**

109 *2.1 Materials*

110 Dextran T40 ($\bar{M}_n$= 26 000 g/mol, Đ = 1.3; values determined by size exclusion
111 chromatography coupled to a multi-angle laser light scattering detector (SEC-MALLS) in
112 DMSO/$NaNO_3$ (0,1 M)) was purchased from Aldrich. Produced from *Leuconostoc*
113 *mesenteroides* B 512-F, it was having very low branching (< 5% as given by the furnisher).
114 Consequently, it could be considered as linear. MMA (99% Aldrich) was vacuum distilled
115 from CaH_2. 2,2-Azobis(isobutyronitrile) (AIBN, 99%, Aldrich) was purified by
116 recrystallization from methanol. All those were used without further purification: N-(3-
117 dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 99%, ABCR), 4-
118 (dimethylamino)pyridine (DMAP, 99%, Aldrich), carbonyldiimidazole (CDI, 99%, Aldrich),
119 2-cyanoprop-2-yl dithiobenzoate (CPDB, >97%, Aldrich), 4-cyanopentanoic acid
120 dithiobenzoate (CPADB, >97%, Aldrich). Miglyol®810 (M810, d = 0.94 g/cm^3, viscosity = 28
121 mPa.s at 20°C, water content = 0.02 wt%) was a gift from CREMER Oleo GmbH & Co.
122 Division. M810 belonged to triacylglycerols (also called triglycerides) and was made from a
123 mixture of saturated fatty acids (mainly caprylic acid -69.3 wt%- and capric acid -30.1 wt%),
124 giving it a log P value of 10.2 as evaluated by group contribution methods (Ramirez, Babin,
125 Durand, Six, & Nouvel, 2015).

126 *2.2 Transurf synthesis*

127 The transurf was synthesized in two steps. In the first step, the attachment of azide end-
128 functionalized side chains onto dextran was performed in DMSO by reaction of 6-
129 azidohexanoic acid activated with carbonyldiimidazole as previously described leading to
130 DexN_3-$\boldsymbol{\tau}$ derivatives (Laville et al., 2013). In the second step, introduction of dithiobenzoate
131 CTA groups was performed using classic EDC/DMAP catalyzed esterification and DexN_3-
132 $\boldsymbol{\tau}$CTA$\boldsymbol{\gamma}$ transurfs were obtained (see Supporting Information). $\boldsymbol{\tau}$ and $\boldsymbol{\gamma}$ correspond to the

number of N_3-end alkyl chains (N_3) and dithiobenzoate CTA groups per 100 glucopyranosic units, respectively, (i. e. Substitution Degrees) and were estimated by ^{1}H NMR in DMSO-d_6 using equations 1 and 2 (Figure 1). A_{N_3}corresponds to the area of 6 methylene protons of alkyl chains (1.2 to 1.7 ppm), A_R is the area of the 5 aromatic protons of dithiobenzoate groups (7.4 to 8.1 ppm) and A_{UG} (4.3 to 5.2 ppm) corresponds globally to 4 protons per glucopyranosic unit, whatever the modification yield. The chemical shift of protons "f" was equal to 3.3 ppm, according the ^{1}H NMR spectrum of the 6-azidohexanoic acid in DMSO-d_6 (not shown).

$$\tau = \frac{A_{N_3}/6}{A_{UG}/4} \times 100 \qquad (1)$$

$$\gamma = \frac{A_R/5}{A_{UG}/4} \times 100 \qquad (2)$$

Figure 1. ^{1}H NMR spectra (DMSO-d_6) of a) $DexN_{3\text{-}20}$, b) $DexN_{3\text{-}20}CTA_{3.7}$ and c) $DexN_{3\text{-}20}$-g-$1.8PMMA_{7800}$.

146 *2.3 RAFT polymerization of MMA in miniemulsion*

147 Typical experimental conditions were as follows: [DexN$_3$-τCTAγ]$_0$ = 10 g/L, DexN$_3$-
148 τCTAγ /organic phase (MMA + M810) was varied from 13 to 36 wt% and M810/MMA from
149 10 to 50 vol.%. [CTA groups]$_0$/[AIBN]$_0$ molar ratio was fixed to 3. For example, in case of
150 DexN$_{3\text{-}20}$CTA$_{3.7}$ transurf and M810/MMA = 10 vol.% (Run 1, Table 1), DexN$_{3\text{-}}$
151 $_{20}$CTA$_{3.7}$/organic phase (MMA + M810) was 13.2 wt%. 150 mg of DexN$_{3\text{-}20}$CTA$_{3.7}$ were
152 dissolved in 15 mL of MilliQ water. The organic phase was composed of 1.1 mL (10.3 mmol)
153 of MMA, 110 µL of M810 and 1.3 mg (7.9x10^{-3} mmol) of AIBN. The aqueous phase was
154 poured into this organic phase and the mixture was sonicated in an ice bath during 2 min
155 (51% amplitude (power 46 W), pulsed mode) using a Vibracell 600W (Sonics & Materials,
156 Dantbury, CT). Droplets size of the resulting miniemulsion was measured immediately after
157 sonication. The miniemulsion was then transferred to a Schlenk tube covered with a 3-way
158 stopcock and purged with a little nitrogen flow during 45 min at 10 °C. The reactor was then
159 immersed in a thermostatted oil bath at 80 °C to start polymerization. To follow reaction
160 kinetics and evolution of molar masses and particles size, the resulting suspensions were
161 divided in three parts. The first one was kept apart for size measurements. The second one
162 was used to determine monomer conversion (x) by a gravimetric method after vacuum drying
163 for 24 hours. NCs were washed twice with THF then dried to obtain the grafted
164 glycopolymers named DexN$_{3\text{-}}\tau$-g-$nPMMA_{\bar{M}_n}$, where n is the number of PMMA grafts
165 (having an average molecular weight equal to $\bar{M}_n$) per 100 glucopyranosic units, respectively.
166 In order to study PMMA grafts, the dextran backbone of such glycopolymers was completely
167 degraded under basic conditions as previously reported (Dupayage, Save, Dellacherie,
168 Nouvel, & Six, 2008). The uncoupled PMMA grafts were analyzed by ^{1}H NMR in DMSO-d_6
169 to verify the absence of dextran backbone. Their molar masses were then characterized by
170 SEC-MALLS in THF. The remaining suspension was centrifuged. Recovered NCs were

171 washed twice and dispersed in deionized water for biological tests, whereas the floating was
172 analyzed by anthrone titration (Wu et al., 2015) to estimate the quantity of $DexN_3$-τCTAγ
173 remaining in aqueous solution. By difference with the initially introduced amount, one can
174 estimate the quantity of dextran derivative adsorbed at the NC surface.

175 *2.4 Characterization*

176 ^{1}H NMR and 2D DOSY ^{1}H NMR spectra were recorded on a Bruker Avance 300 apparatus
177 (30,013 MHz, 25 ◦C) in DMSO-d_6. 1024 Scans were recorded.

178 Droplet and NCs sizes were measured by using a Laser Diffraction Particle Size Analyzer
179 (Mastersizer 2000 Malvern Instruments). Washed NCs suspension (8 wt% solid content) was
180 diluted by a factor of 200 and NCs morphology was observed by cryo-Transmission Electron
181 Microscopy (cryo-TEM) as reported (Forero Ramirez et al., 2018).

182

183 SEC-MALLS analyses of PMMA chains were performed in THF at 40ºC. Refractive index
184 increment (dn/dc) of 0.087 mL.g^{-1} was used (See Supporting Information).

185 NCs cytocompatibility was evaluated on rat vascular smooth muscle cell line (A-10, ATCC_
186 CRL-1476™) (See Supporting Information).

187

188 **3. Results and discussion**

189 *3.1 $DexN_3$-τCTAγ transurf*

190 To the best of our knowledge, relatively few examples of polysaccharides bearing RAFT
191 functionality have been described in the literature and only 4 examples were based on dextran.
192 One xanthate-end functionalized dextran RAFT agent was prepared by click chemistry
193 (Bernard et al., 2008) and three multi-reactive dextran-based macro-CTA were obtained by

194 esterification (Duong et al., 2012; Ferji, Venturini, Cleymand, Chassenieux, & Six, 2018,
195 Karmakar et al., 2018). Nevertheless none of them was also used as stabilizer in miniemulsion
196 polymerization.

197 Herein, the first multi-reactive dextran-based transurf for a RAFT miniemulsion
198 polymerization was synthesized and called DexN_3-τCTAγ, where τ and γ correspond to the
199 number of N_3-end alkyl chains (N_3) and of dithiobenzoate CTA groups per 100
200 glucopyranosic units, respectively (Scheme 2a). On the one hand, N_3-end alkyl chains have
201 been chosen as hydrophobic groups due to their efficiency to hydrophobize dextran, leading to
202 amphiphilic DexN_3-τ dextran derivatives exhibiting surfactant properties (see Supporting
203 Information). These derivatives have been successfully used to produce dextran-covered NPs
204 via emulsion/evaporation (Laville et al., 2013; Poltorak et al., 2015) or nanoprecipitation
205 (Laville et al., 2013) processes. In addition, azide functionality of such DexN_3-τ is expected to
206 be suitable for potential post-modification of final NPs surface by click chemistry.

207

208

209

Scheme 2. a) Synthesis of DexN$_3$-τCTAγ transurfs. b) RAFT polymerization of MMA from DexN$_3$-τCTAγ *via* the R-group approach.

On the other hand, a dithiobenzoate group bearing cyano functionality was chosen as the reactive side group. Indeed, molecular CTAs having similar structure (i.e. CPDB and CPADB) showed remarkable efficiency and high degree of control over MMA RAFT polymerizations in homogeneous (Biasutti, Davis, Lucien, & Heuts, 2005; Johnston-Hall, Stenzel, Davis, Barner-Kowollik, & Monteiro, 2007) and heterogeneous media (Yang, Luo, Liu, & Li, 2009; Zhou, Ni, & Yu, 2007). CTA groups were introduced (through R-group) by reacting previous DexN$_3$-τ with CPADB in the presence of EDC and a catalytic amount of DMAP, resulting in DexN$_3$-τCTAγ transurfs (Scheme 2a). No degradation of the dextran backbone was evidenced as attested by SEC-MALLS analysis (Table S1). The integrity of grafted CTA groups was as well confirmed by ^{1}H NMR spectroscopy as characteristic protons

224 of both Z and R moieties clearly appeared (Figure 1b), thus ensuring its reactivity as RAFT
225 agent. Modification degree of CTA groups (γ) could be tuned from 2 to 8% according to the
226 experimental conditions. Those results were in quite good agreement with those previously
227 obtained from native dextran (Duong et al., 2012).

228 To be used as transurfs, the designed $DexN_3$-$\tau CTA\gamma$ were expected to exhibit both surfactant
229 and transfer agent properties. To check their surface activity, steady state surface tension
230 measurements were performed with different series of $DexN_3$-τ and $DexN_3$-$\tau CTA\gamma$ (Figure
231 S1). $DexN_3$-τ ($9 < \tau < 27$) and $DexN_{3\text{-}20}CTA\gamma$ transurfs (τ=20 and γ=3.7 or 7.5) were able to
232 adsorb at the air/water interface and to reduce the surface tension of aqueous solutions. In the
233 case of transurfs, the contribution of CTA groups to the decrease of surface tension seems less
234 than that of N_3-end alkyl groups, at least in the range of CTA modification degree tested here,
235 i. e. below 8%. In fact, similar curves have been observed in case of $DexN_{3\text{-}24}$ (τ=24), $DexN_{3\text{-}}$
236 $_{20}CTA_{3.7}$ (global modification degree of $\tau + \gamma \sim 24$) and $DexN_{3\text{-}20}CTA_{7.5}$($\tau + \gamma \sim 27$), whereas
237 a bigger decrease in surface tension was observed for $DexN_{3\text{-}27}$ (τ=27). Even if CTA groups
238 have global hydrophobic character (log P~3.4 as calculated by group contribution methods,
239 Ramirez., Babin, Durand, Six, & Nouvel, 2015), these results could be ascribed to the
240 presence of thiocarbonylthio and cyano functions in CTA groups in the middle of the chain,
241 that make them less prone to segregate towards oil phase, with respect to N_3-end alkyl chains.
242 Nevertheless, we can expect that the hydrophobic character of those CTA groups (logP~3.4)
243 is high enough to allow direct contact of dithiobenzoate groups with the oil phase (log P
244 ranging between 1.8 and 2.4 for initial droplet phase with M810/MMA ratio ranging from 10
245 to 50 vol.%), thus enabling their role as transurfs.

246 *3.2 Miniemulsion polymerization using dextran-based transurf*

247 After checking both the stability of the MMA miniemulsion containing M810 (a
248 pharmaceutically acceptable triglyceride oil (Hippalgaonkar, Majumdar, & Kansara,
249 2010))/DexN_3-τ as co-stabilizer/stabilizer system under polymerization process, and the
250 control of the MMA RAFT polymerization in miniemulsion using model conditions
251 (molecular CTA + no-reactive DexN_3-τ stabilizer) (see Supporting Information), optimized
252 conditions were applied to DexN_3-τCTAγ transurfs. Our main challenge was to evaluate the
253 potential of the transurf to act both as oil/water interface stabilizer and macro-CTA to
254 generate DexN_3-τ-g-$nPMMA_{\bar{M}_n}$ at the organic interface, in order to produce dextran-covered
255 NCs with PMMA shell and M810 oily core. Thus, we first verified both the availability of
256 CTA groups at the interface and their effective participation in RAFT polymerization.
257 Secondly, we evaluated the effects of M810 content and transurf structure on the
258 polymerization kinetics and control.

259 Table 1 summarizes all experimental recipes used in the current study, as well as the average
260 size of initial nanodroplets and final NCs. In any case, latexes were very stable during
261 polymerization procedure. Distribution size of the final NCs were roughly similar to the one
262 of the initial nanodroplets, in agreement with preliminary stability assays performed with
263 Dex$N_{3\text{-}20}$ (see Supporting Information). Depending on experimental conditions, and especially
264 on M810 volume ratio, final solid content varied from 8 to 13 wt% in water.

Table 1. Miniemulsion polymerization of MMA at 80°C using DexN$_3$-τCTAγ transurf or DexN$_{3\text{-}20}$ stabilizer with 1.1 mL of MMA. [DexN$_3$-τCTAγ(or DexN$_3$-τ)] = 10 g/L and [CTA]$_0$/[AIBN]$_0$ = 3.

Run	(DexN$_3$-τCTAγ or DexN$_3$-τ)/ organic phase (wt%)	[AIBN]$_o$	M810/MMA (vol.%)	Stabilizer or Transurf	DexN$_3$-τCTAγ adsorbed (%) [a]	[MMA]$_o$/[CTA]$_o$ (or X) [b]	Nano-objects diameter (nm) [c]		Grafts number (n)[d]	Eff_{CTA} (%)[e]	F_{Homo} [f]
							After degassing	Final			
0	13.2	6.7	10	DexN$_{3\text{-}20}$	-	-	113 (1.30)	110 (1.10)	-	-	-
1	13.2	6.7	10	DexN$_{3\text{-}20}$CTA$_{3.7}$	94	393	102 (1.00)	102 (0.83)	1.8±0.3	48	20
2	11.6	5.7	25	DexN$_{3\text{-}20}$CTA$_{3.7}$	94	393	112 (1.49)	109 (1.30)	1.7±0.3	45	25
3	9.7	3.5	50	DexN$_{3\text{-}20}$CTA$_{3.7}$	92	402	117 (1.37)	119 (1.14)	1.8±0.3	48	25
4	11.6	11.9	25	DexN$_{3\text{-}20}$CTA$_{7.5}$	94	204	105 (1.31)	110 (1.30)	3.5±0.3	47	17

[a]Relative molar amount in NCs. Expressed as function of the initially introduced DexN$_3$-τCTAγ.

[b]Initial molar ratio in the organic phase, estimated with the real amount of DexN$_3$-τCTAγ at the interface evaluated in (a), and supposing that all the CTA groups were accessible for polymerization.

[c]Average nano-object diameter. Values under brackets correspond to Span (width of the distribution) calculated using $Span = (D(0.9) - D(0.1))/\,D(0.5)$. D(0.1), D(0.5) and D(0.9) were the particle diameters at 10%, 50% and 90% of the particle volume size distribution.

[d]Average number of PMMA grafts per 100 glucopyranosic units estimated from ^{1}H NMR spectrum (Figure 1c) using $n = [(4A_{A'B'}/5A_{UG}) \times \bar{M}_{n(SEC)DEX}/162)]/\bar{X}_n$. $A_{A'B'}$ and A_{UG} are the areas under peaks (A'B') and (OH^1, OH^2, OH^3 and H$^\alpha$), respectively. $\bar{M}_{n(SEC)DEX}$ is the $\bar{M}_n$ of native dextran, 162 the molecular weight of one glucopyranosic unit and $\bar{X}_n$ is the average polymerization degree of PMMA grafts.

[e]Average CTA groups efficiency for transurfs estimated using $Eff_{CTA} = n/\gamma \times 100$, where n and γ are the number of grafts and CTA groups per 100 glucopyranosic units, respectively.

[f]Weight fraction of PMMA homopolymers (non-attached to dextran) with respect to the total synthesized PMMA using $F_{Homo} = \left[1 - \left[(m_{CTA}/M_{UG-CTA}) \times n \times \bar{M}_{n(SEC)grafts}\right]/(m_{MMA}x)\right] \times 100$, with m_{CTA} the mass amount of DexN$_3$-τCTAγ transurf at NCs surface, M_{UG-CTA} the molecular weight of one glucopyranosic unit of DexN$_3$-τCTAγ, n the average number of PMMA grafts per 100 glucopyranosic units evaluated in (d), $\bar{M}_{n(SEC)grafts}$ the $\bar{M}_n$ of PMMA grafts, m_{MMA} the initial mass amount of monomer and x the monomer conversion. F_{Homo} was evaluated at two different conversions and average values are given.

281 *3.2.1 Relative amount of transurfs at nanodroplets/NCs surface.*

282 In the early stage of RAFT polymerization, $DexN_3$-τCTAγ transurfs were located at the
283 monomer/water interface because of their surfactant properties (Figure S1) and mediated
284 RAFT polymerization from their multiple reactive sites. As CTA groups were attached to
285 dextran molecules via their R moieties, re-initiating radicals ($R^{\bullet}$) were situated on the dextran
286 backbone after the first addition-fragmentation step (Scheme 2b). Therefore, PMMA chains
287 grew directly from the polysaccharide, via the R-group approach, to produce $DexN_3$-τ-g-
288 $nPMMA_{\bar{M}_n}$ at the oil/water interface that will constitute the NCs surface/polymeric shell at the
289 end.

290 For Runs 1-4 (Table 1), $[MMA]_o/[CTA]_o$ molar ratio (X) was estimated by taking into
291 account the total amount of monomer in the system and the quantity of $DexN_3$-τCTAγ
292 adsorbed at nanodroplets surface. This last quantity was considered in first approximation to
293 be similar to the one at the surface of the final NCs, and was indirectly determined after NCs
294 washing by dosage of the $DexN_3$-τCTAγ remaining in aqueous supernatant. In all cases, more
295 than 90% of initial transurf was found to be located at the interface (Table 1). In contrast,
296 similar analysis of PMMA latexes obtained with the model system (see Supporting
297 Information) showed that only 60% of $DexN_{3\text{-}20}$ was present in final NCs. This result could be
298 surprising as polymerization conditions and specially [$DexN_3$-τ]/[organic phase] weight ratio
299 were chosen to avoid the presence of stabilizer into the aqueous phase, so as to prevent
300 micellar or homogeneous nucleation. Nevertheless, in the model system, $DexN_{3\text{-}20}$ desorption
301 was more prone to occur under polymerization procedure or during particle washings as the
302 stabilizer was only physically adsorbed onto NCs surface, whereas covalent linkage between
303 dextran backbone and PMMA chains was expected to be formed in the case of transurfs, from
304 the first moments of the polymerization. Transurf derivatives should thus be immobilized at

305 NCs surface *via* their CTA groups, which would limit their desorption and explain their
306 quantitative presence in final NCs.

307 *3.2.2 Kinetics studies.*

308 Figure 2 shows the first-order kinetic plots for RAFT polymerization in miniemulsion of
309 MMA using $DexN_3$-τCTAγ transurfs. A linear relationship between $\ln([M]_o/[M]_t)$ and
310 polymerization time was observed until at least 70% conversion, whatever the transurf or the
311 M810 content. In addition, similar polymerization kinetics with reproducible delay period
312 were observed when increasing M810 amount from 10 vol.% to 50 vol.% compared to MMA,
313 while keeping $[MMA]_0/[CTA]_0$ constant (Runs 1-3). This result was quite surprising as these
314 experiments were carried out with the same transurf, $[CTA]_o/[AIBN]_o$ ratio (equal to 3), and
315 MMA volume.

316

317 **Figure 2.** $Ln([M]_o/[M]_t)$ *versus* time. RAFT polymerization of MMA in miniemulsion at
318 80°C using $DexN_3$-τCTAγ transurfs (or $DexN_{3-20}$ stabilizer). (*) Run 0, $DexN_{3-20}$, $[CTA]_o = 0$,
319 10 vol.% M810 (●) Run 1, $DexN_{3-20}CTA_{3.7}$, X = 393, 10 vol.% M810 (♦) Run 2, $DexN_{3-}$
320 $_{20}CTA_{3.7}$, X = 393, 25 vol.% M810 (▲) Run 3, $DexN_{3-20}CTA_{3.7}$, X = 402, 50 vol.% M810 (■)
321 Run 4, $DexN_{3-20}CTA_{7.5}$, X = 204, 25 vol.% M810 (see Table 1).

322

323 In such conditions, AIBN initial mass remained constant while organic phase global volume
324 increased with the amount of M810. Polymerization rate was thus expected to slow down
325 when increasing M810/MMA volume ratio as a result of the reduction of AIBN concentration
326 inside nanodroplets. Nevertheless, as observed in stability studies, the increase of M810
327 content from 10 to 50% vol.% (which corresponded to initial organic phase volumes of 1.1
328 mL and 1.65 mL, respectively) resulted only in a slight increment of initial nanodroplets
329 average size (from 102 to 117 nm) (see Supporting Information). As a consequence, the
330 number of nanodroplets considerably increased from one experiment to another, which led to
331 faster polymerization kinetics and, hence, offset the effect of AIBN concentration decrease,
332 resulting in similar kinetics.

333 As for the model system (see Supporting Information), both studied $DexN_3$-$\tau CTA\gamma$ transurfs
334 appeared to slow down the miniemulsion polymerization of MMA. Indeed, $DexN_{3\text{-}20}CTA_{3.7}$
335 ($[AIBN]_o$ = 5.7 mM, Run 2) and $DexN_{3\text{-}20}CTA_{7.5}$ ($[AIBN]_o$ = 11.9 mM, Run 4) showed much
336 slower kinetics when compared to a conventional radical polymerization system ($[AIBN]_o$ =
337 6.7 mM, Run 0). This slow down effect suggested both the availability of CTA groups at the
338 oil/water interface and their effective involvement in growth of PMMA chains. As expected,
339 $DexN_{3\text{-}20}CTA_{7.5}$ (Run 4) gave faster polymerization rate than $DexN_{3\text{-}20}CTA_{3.7}$ (Run 2). Since
340 $DexN_{3\text{-}20}CTA_{7.5}$ was bearing more CTA groups, AIBN concentration was almost doubled in
341 Run 4 with respect to Run 2 to keep constant the $[CTA]_o/[AIBN]_o$ ratio. Furthermore, no
342 delay period was observed with $DexN_{3\text{-}20}CTA_{7.5}$ once again probably because of the higher
343 initiator amount used in this experiment.

344 *3.2.3 $DexN_3$-$\boldsymbol{\tau}$-g-$\boldsymbol{nPMMA_{\bar{M}_n}}$ analysis.*

345 Attempting to confirm the covalent linkage between PMMA shell and dextran coverage of
346 NCs, $DexN_3$-τ-g-$nPMMA_{\bar{M}_n}$ glycopolymers produced at the oil/water interface were purified

347 from M810 and from free PMMA homopolymers (not attached to dextran), by several
348 washings of dried NCs with THF. These glycopolymers were also characterized with their
349 weight fraction of PMMA (F_{PMMA}) determined by equation 3, where M_{UG-CTA} is the
350 molecular weight of one glucopyranosic unit of $DexN_3$-τCTAγ.

351 $$F_{PMMA} = \frac{n\overline{M}_n}{n\overline{M}_n + 100M_{UG-CTA}} \qquad (3)$$

352 For copolymers produced in Runs 1 – 4, n was estimated from ^{1}H NMR spectra in DMSO-d_6
353 (Table 1). ^{1}H NMR spectrum of $DexN_{3\text{-}20}$-g-1.8$PMMA_{7800}$ elaborated from $DexN_{3\text{-}20}CTA_{3.7}$
354 (Run 3) is given in Figure 1c as example. Typical signals of dextran and PMMA parts are
355 clearly observed, especially methyl (B', 0.5-1.0 ppm), methylene (A', 1.5-2.0 ppm) and O-
356 CH_3 proton peaks (C', 3.6 ppm) attributed to the repeating unit of PMMA grafts, and
357 hydroxyl and anomeric proton peaks (4.1-5.4 ppm) from the dextran backbone. Unfortunately,
358 ester linkages between the two parts could not be proven by this method. $DexN_3$-τ-g-
359 $nPMMA_{\overline{M}_n}$ were therefore studied by 2D Diffusion-Ordered Spectroscopy (DOSY) ^{1}H NMR
360 (Figure 3). This technique allowed separation of the NMR signals of different species
361 according to their diffusion coefficient. It has previously been used to discriminate between a
362 grafted copolymer and the corresponding homopolymer mixture (Soliman, Colombeau,
363 Nouvel, Babin, & Six, 2016). On the one hand, the 2D DOSY ^{1}H NMR spectrum of $DexN_{3\text{-}20}$-
364 g-1.8$PMMA_{7800}$ was recorded in DMSO-d_6 (Figure 3a). As shown, a single self-diffusion
365 coefficient was observed for all the protons from both dextran and PMMA parts (D = 4.47×
366 10^{-10} m^2 s^{-1}), which suggested that PMMA grew effectively from CTA groups on dextran. On
367 the other hand, a mixture (same weight composition as for the copolymer $DexN_{3\text{-}20}$-g-
368 1.8$PMMA_{7800}$) of the precursor $DexN_{3\text{-}20}CTA_{3.7}$ transurf and a PMMA homopolymer having
369 roughly the same average molecular weight of PMMA grafts was also analyzed (Figure 3b).
370 In this last case, 2D DOSY spectrum provided two different diffusion coefficients (D = 3.16 ×

371 10^{-11} m^2 s^{-1}and D = 1.78 × 10^{-10} m^2 s^{-1}) for transurf and PMMA, respectively. The presence of
372 these two diffusion coefficients, compared to only one (at a different value) in case of $DexN_{3-}$
373 $_{20}$-g-1.8$PMMA_{7800}$, proved that PMMA grafts were really covalently linked to dextran
374 backbone, resulting in a $DexN_{3-}\tau$-g-$nPMMA_{\bar{M}_n}$ glycopolymer.

375

376 **Figure 3.** 2D DOSY ^{1}H NMR spectra of a) $DexN_{3-20}$-g-1.8$PMMA_{7800}$ (F_{PMMA}= 43%) obtained
377 from Run 3 (Table 1) at 12% of conversion and b) mixture of $PMMA_{7100}$ and $DexN_{3-20}CTA_{3.7}$
378 (43/57; w/w).

379

380 Finally, we wanted to characterize those $DexN_{3-}\tau$-g-$nPMMA_{\bar{M}_n}$ glycopolymers by SEC-
381 MALLS. Nevertheless, it was difficult to find an appropriate solvent able to well solubilize
382 both the hydrophilic polysaccharide and the hydrophobic PMMA parts. Despite solubility
383 problems resulting in the appearance of numerous aggregate peaks on the SEC trace,
384 chromatograms of purified $DexN_{3-}\tau$-g-$nPMMA_{\bar{M}_n}$ (i.e. after washing with THF- Figure S6)
385 clearly showed a shift of the molecular weight distribution toward higher molar masses in
386 comparison with the chromatogram of the precursor transurf, which further attested to the
387 copolymer formation. Nonetheless, accurate average molar masses were not accessible from
388 these SEC-data due to the above-mentioned solubility issues. Non-purified $DexN_{3-}\tau$-g-
389 $nPMMA_{\bar{M}_n}$ (i.e. before THF washings) were also investigated. In comparison with
390 chromatograms of purified glycopolymers, an additional smaller peak evolving with

391 conversion was detected at high elution time. This peak, disappearing after purification, was
392 found to correspond to PMMA homopolymers (non-attached to dextran) formed during
393 polymerization, as confirmed by ^{1}H NMR spectrum of the product extracted with THF. This
394 observation was not a surprise. The production of a small percentage of homopolymer is
395 inherent to the RAFT process since chains are initiated by AIBN decomposition. Thus, in
396 addition to PMMA chains re-initiated by CTA groups and growing from the dextran backbone
397 at the oil/water interface, there is also a small fraction of PMMA chains coming from AIBN
398 and propagating within the organic phase. Furthermore, irreversible transfer reactions could
399 also eventually lead to homopolymer formation. For all experiments, homopolymer fraction
400 (F_{Homo}) was evaluated to about 20 wt% of the total produced polymer amount (Table 1),
401 which remains quite reasonable and below the maximum amount evaluated for a
402 $[CTA]_0/[AIBN]_0$ ratio of 3. We can expect that this relatively low fraction would not have a
403 significant adverse impact on the desired core-shell morphology control.

404 *3.2.4 Polymerization control and CTA groups efficiency.*

405 In order to ascertain the degree of RAFT polymerization control, PMMA grafts were
406 deliberately uncoupled from dextran backbone, then characterized by SEC-MALLS. Total
407 PMMA recovery was achieved under basic conditions already proven to be harmless for
408 PMMA grafts (Dupayage et al., 2008; Forero Ramirez et al., 2018). SEC of uncoupled
409 PMMA chains exhibited monomodal distributions at low conversion and a clear shift toward
410 higher molar masses with conversion (Figure 4a). For conversions above 50%, a small
411 molecular weight shoulder was however observed suggesting a small proportion of
412 irreversible deactivation by bimolecular termination between two PMMA grafts, or more
413 probably between a PMMA graft and a PMMA homopolymer. When comparing
414 polymerizations carried out with the same transurf but different M810 contents, similar SEC
415 traces were observed at analogous conversions indicating that M810 amount has no influence

416 on RAFT polymerization control. Furthermore, molar mass distributions of PMMA
417 homopolymers (unlinked to dextran) overlaid pretty well with those of PMMA grafts obtained
418 at the same conversion (Figure S7). This showed that the exchange of thiocarbonylthio groups
419 between grafted and free PMMA chains was quite effective to allow the control of
420 macromolecular parameters of both types of chains. Overall these results indicated the
421 absence of noticeable transfer or termination reactions, except a small proportion of
422 termination by recombination of PMMA grafts (no shoulder was detected in case of
423 homopolymers). Bimolecular termination between a PMMA graft and a PMMA
424 homopolymer was more prone to occur at high conversion and with consequent amount of
425 M810 in the organic phase. Actually, sufficient amount of M810 in the organic phase was
426 expected to favor phase segregation between the oily core and the polymer shell (see 3.3), and
427 thus, the geographic proximity between free PMMA chains (growing into the organic phase)
428 and PMMA grafts (growing at the interface). Bimolecular termination between two PMMA
429 grafts was less probable to happen. Furthermore, such termination would have resulted in the
430 reticulation of the system, which was never observed in any case.

431

432 **Figure 4.** Evolution of a) SEC traces (Refractive Index detector) of PMMA grafts (Run 2) and
433 b) experimental $\overline{M}_n$ (full symbols), Dispersity Đ (open symbols) *versus* conversion.
434 Conversions are given in % on figure a). RAFT polymerization of MMA in miniemulsion at
435 80°C using $DexN_{3\text{-}20}CTA_{3.7}$ transurf and various amounts of M810 in the organic phase (●)

Run 1, 10 vol.% (♦) Run 2, 25 vol.% (▲) Run 3, 50 vol.%. See Table 1 for other conditions. Theoretical $\overline{M}_n$ (dotted lines) was evaluated using $\overline{M}_n = 100(x)(X)$ where x and 100 are the monomer conversion and the molecular weight of a PMMA unit, respectively. Curve for X = 393 is given.

The evolution of experimental $\overline{M}_n$ of PMMA grafts with conversion for Runs 1-3 is shown as a function of theoretical $\overline{M}_n$ in Figure 4b. In all cases, experimental $\overline{M}_n$ evolved linearly with conversion until 80% of conversion without appearance of noticeable transfer or termination reactions. In addition, Đ remained considerably below 1.2. All these results further attested of RAFT control during almost the whole polymerization. Experimental $\overline{M}_n$ were however much higher than the theoretical ones (calculated with the adjusted average value of X = $[MMA]_o/[CTA]_o$=393) indicating that not all CTA groups participated in the polymerization (Figure S8 for additional results of Run 4, X = 402). The comparison between the number of grafts (n) estimated from ^{1}H NMR spectra and the number of CTA groups along dextran backbone (γ) allowed us to evaluate an average efficiency of CTA groups (Eff_{CTA}) of about 48% (Table 1). Roughly half of the initial CTA groups of $DexN_3$-$\tau CTA\gamma$ transurfs located at the interface, effectively resulted in a PMMA grafted chain. Similar lower efficiencies have been already observed for other polysaccharides bearing reactive groups when used for controlling ATRP (Dupayage et al., 2008; Ferji et al., 2015) in homogeneous media. One possible cause seemed to be the presence of intramolecular termination reactions at the very beginning of the polymerization resulting in the loss of RAFT re-initiating radicals ($R^{\bullet}$). In our case, the reaction takes place in a heterogeneous environment. So, the transurf conformation at the oil/water interface is more likely the key factor limiting the availability of some CTA groups for polymerization if they are not facing to the organic phase, which was the main challenge of this work. Finally, CTA group efficiency appeared to be the same for both $DexN_{3\text{-}20}CTA_{3.7}$ and $DexN_{3\text{-}20}CTA_{7.5}$, and remained unchanged whatever the M810/MMA volume ratio in agreement with our previous results for miniemulsion ATRP of

463 MMA using dextran-based inisurfs (Forero Ramirez et al., 2018). A steric hindrance effect in
464 the vicinity of CTA groups restricting the access to C=S function does not seem to be present
465 in the range of studied CTA modification degrees.

466
467 *3.3 Nano-objects morphology*

468 Indirect confirmation of an oily-core/polymeric shell morphology of final objects was
469 provided by MDSC analysis. The obtained thermograms (Figure S9) showed that NCs made
470 with 25-50 vol.% of M810 (Runs 2, 3 and 4) were effectively two-phase systems having: i) a
471 plasticized amorphous phase of PMMA/M810 (11 / 89 w / w, T_g = 98 °C) and ii) a pure M810
472 liquid phase exhibiting one melting transition (T_m = - 4°C). On the contrary, nano-objects
473 formulated with 10 vol.% of M810 (Run 1) displayed only a single glass transition event
474 indicating a total miscibility of two components. These results were in quite agreement with
475 our previous studies on PMMA/M810 blends miscibility as M810 was shown to plasticize
476 PMMA until a weight fraction of 11 wt% (Forero Ramirez et al., 2018).

477 In order to prove NCs morphology in a direct way, final nano-objects were analyzed by
478 Cryogenic-Transmission Electron Microscopy (cryo-TEM). Electron micrograph of NCs
479 obtained with 25 vol.% of M810 in the organic phase (Run 2, Table 1) at 93% of conversion
480 is shown in Figure 5. Cryo-TEM image showed the sphericity of synthesized objects as well
481 as the average diameter determined by Laser granulometry, which varies from around 200 nm
482 to 50 nm. A few particles are even smaller in that case the inner oily core is not clearly seen.
483 The lack of sufficient contrast between plasticized PMMA and pure M810 phases render the
484 visualization of the oily-core/polymeric shell structure under low dose conditions not simple.
485 The core shell structure is best seen on small objects. On the larger objects the PMMA layer is
486 just slightly darker than the oily core. The shell thickness is around 25 nm on all objects of
487 run 2, Table 1. Internal liquid oil cavity was more visible for large objects after exposure to

488 the microscope electron beam since the polymeric shell was less sensitive to irradiation
489 damage (Figure S10). These observations provided direct evidence of core-shell structure
490 formation. Improved studies will be however needed to further characterize NCs morphology.

491

492 **Figure 5.** Assembly of Cryo-TEM micrographs of final NCs obtained from Run 2. The black
493 arrows point towards the shell of the particle. The scale bar is the same for each 3 images.

494

495 *3.4 Nano-objects cytocompatibility*

496 Formulated NCs are constituted of an oily liquid biocompatible M810 core, an inner shell of
497 PMMA (frequently used in implantable medical devices (Frazer, Byron, Osborne, & West,
498 2005)) and an outer shell of dextran (well-known biodegradable and bioeliminable
499 polysaccharide (Posocco et al., 2015)). In order to check the potential of these NCs for
500 intravenous drug administration, preliminary cytocompatibility studies on vascular smooth
501 muscle cells were performed *in vitro*. NCs prepared with the model system (see Supporting
502 Information), called MOD, and with transurfs (called CTA-PMMA) were both analyzed.
503 Metabolic activity of vascular cells was measured by monitoring the conversion of MTT to
504 formazan. The reduction of MTT is catalyzed by mitochondrial dehydrogenase enzymes and

505 is therefore a measure for cell viability. After 24 hours, results expressed as percentages did
506 not show any variation whatever the concentration or NCs type, when compared to the control
507 condition (no NC) (Figure 6). The MOD and CTA-PMMA NCs appeared to be
508 cytocompatible with vascular smooth muscle cells.

509

510

511 **Figure 6.** Cytocompatibility of MOD (prepared with a $[MMA]_o/[CPDB]_o$ molar ratio of 700
512 and 25 vol.% of M810 compared to MMA, see Supporting Information) or CTA-PMMA NCs
513 (Run 2, Table 1) towards rat vascular smooth muscle cells (A-10) after 24 h incubation at
514 37°C. 100% of metabolic activity (MTT assay) represents control cells incubated with PBS.
515 Results expressed as mean ± standard error of the mean, n = 3.

516

517 **4. Conclusions**

518 As stated, we designed dextran-based transurfs able to produce oily-core/PMMA shell NCs by
519 RAFT interfacial miniemulsion polymerization. The two major challenges of this work were
520 to introduce CTA functionalities onto dextran in order to synthesize a multi-reactive transurf
521 (acting both as macro-CTA and stabilizer) and to check its ability to confine RAFT
522 miniemulsion polymerization of MMA at the oil/water interface. About a half of the CTA
523 groups along dextran backbone were found to effectively initiate a PMMA grafted chain.

524 About 80 wt% of the total produced PMMA was effectively attached to dextran backbone. In
525 any case, polymerization was controlled until high conversion as concluded from the analysis
526 of uncoupled PMMA grafts deliberately removed from $DexN_3$-τ-g-$nPMMA_{\overline{M}_n}$. Phase
527 segregation between PMMA shell and M810 core was evidenced.

528 Overall these results highlight the value of our novel dextran-based transurf to produce
529 dextran-covered oily-core NCs having different oil contents. Such NCs can be potentially
530 used in drug delivery applications as indicated by preliminary *in vitro* cytotoxicity tests.
531 Complementary biological tests, deep characterization of covalently linked dextran coverage,
532 as well as post-functionalization of final NCs *via* the azide functions will be reported soon.
533 The possibility of extending this method to RAFT copolymerization of MMA with a pH-
534 responsive monomer will be also studied in the near future.

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540

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