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Cécile Nouvel

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# 1 First multi-reactive polysaccharide-based transurf to produce potentially

# 2 biocompatible dextran-covered nanocapsules

4	Laura Marcela Forero Ramirez <sup>a</sup> , Jérôme Babin <sup>a</sup> , Ariane Boudier <sup>b</sup> , Caroline Gaucher <sup>b</sup> , Marc
5	Schmutz <sup>c</sup> , Mériem Er-Rafik <sup>c =</sup> , Alain Durand <sup>a</sup> , Jean-Luc Six <sup>a</sup> , <u>Cécile Nouvel</u> <sup>a</sup> *±
6	
7	<sup>a</sup> Université de Lorraine, CNRS, LCPM, F-54000 Nancy, France
8	<sup>b</sup> Université de Lorraine, Cithefor EA 3452, Nancy F-54001, France
9 10	<sup>c</sup> Université de Strasbourg-CNRS- Institut Charles Sadron UPR22, Strasbourg, France.
11	± Current address Université de Lorraine, CNRS, LRGP, F-54000 Nancy, France
12	Correspondence to: Cécile Nouvel (E-mail: cecile.nouvel@univ-lorraine.fr)
13 14	= Current address DTU Nanolab, Technical University of Denmark, 2800 Kgs, Lyngby Denmark
15	
16	Fax : 33(0) 3 83 32 29 75; Tel : 33(0) 3 72 74 38 32
17	
18	Additional Supporting Information will be found in the online version of this article.
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#### 21 ABSTRACT

A multi-reactive polysaccharide-based transurf (acting both as macro-Chain Transfer Agent 22 23 and stabilizer) was used to confine RAFT polymerization of methyl methacrylate (MMA) at 24 the oil/water (o/w) miniemulsion interface. Dithiobenzoate groups and hydrophobic aliphatic 25 side chains were introduced onto dextran, conferring it both transfer agent properties and 26 ability to stabilize direct miniemulsion of MMA in the presence of a biocompatible oil, used 27 as co-stabilizer. Because of their amphiphilic character, transurfs were initially adsorbed at the 28 (o/w) interface and their reactive sites mediated RAFT polymerization via the R-group 29 approach. PMMA-grafted dextran glycopolymers were consequently produced at the o/w 30 interface, thus leading to dextran coverage/PMMA shell/oily core nanocapsules (NCs) as 31 evidenced by Cryo-TEM analyses. The influence of dextran-based transurf chemistry and oil 32 amount on MMA RAFT polymerization control was investigated. Positive preliminary results 33 on NCs cytotoxicity suggest the potential of these objects for biomedical applications.

#### 34 **KEYWORDS**

- 35 Polysaccharide
- 36 Nanocapsules
- 37 Transurf
- 38 Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization
- 39 Miniemulsion
- 40 Poly(methyl methacrylate)
- 41

42

## 1. Introduction

43 Polymer nanocapsules (NCs) have attracted greater attention in recent years because of their 44 unique properties and potential applications in the biomedical field, for instance as drug delivery systems (Mora-Huertas, Fessi, & Elaissari, 2010; Vrignaud, Benoit, & Saulnier, 45 46 2011; Poltorak, Durand, Léonard, Six, & Nouvel, 2015, Six, & Ferji, 2019). Miniemulsion 47 polymerization is a promising technique that allows drug encapsulation and NCs production 48 in one-pot (Steinmacher et al., 2010). To produce oily-core NCs by this technique, 49 nanodroplets with size ranging from 50 to 500 nm are initially obtained by dispersion of a 50 monomer/oil mixture in an aqueous phase. Nanodroplets are protected against coalescence by 51 the presence of stabilizer and preserved from Ostwald ripening by the addition of an osmotic 52 pressure agent (co-stabilizer), which may be the oil constituting the inner liquid core in the 53 final NCs (Steinmacher et al., 2010; Landfester & Mailänder, 2013; Forero Ramirez et al., 54 2018). In such a case, oily core and polymeric shell will be formed during polymerization by 55 means of induced-phase separation whereby polymer migrates to the droplet/water interface 56 due to poor solubility in the oil. However, a well-defined core/shell structure can be difficult 57 to obtain because of the strict thermodynamic and kinetic parameters involved in polymer/oil 58 separation. Moreover, stabilizers are usually physically adsorbed at NCs surface and can be 59 easily removed or replaced by surface-active molecules present in physiological media, which 60 may thus affect the colloidal stability or enhance unwanted immune responses.

To overcome all the above limitations, some authors reported the combination of monoreactive 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., 2015) or transurfs (transfer agents and stabilizers) (Luo & Gu, 2007; Lu, Luo, & Li, 2010; Yu, 68 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 during the miniemulsion process. To the best of our knowledge, it is the first time that RAFT 86 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 transurfs in miniemulsion polymerization, the production of oily-core NCs is scarcely related,
and the preparation of PMMA-shell NCs has never been approached, which contributes to the
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



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

#### 108

#### 2. Materials and methods

#### 109 2.1 Materials

110 Dextran T40 ( $\overline{M}_n$  = 26 000 g/mol,  $\overline{D}$  = 1.3; values determined by size exclusion 111 chromatography coupled to a multi-angle laser light scattering detector (SEC-MALLS) in 112 DMSO/NaNO<sub>3</sub> (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<sub>2</sub>. 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-(dimethylamino)pyridine (DMAP, 99%, Aldrich), carbonyldiimidazole (CDI, 99%, Aldrich), 118 119 2-cyanoprop-2-yl dithiobenzoate (CPDB, >97%, Aldrich), 4-cyanopentanoic acid dithiobenzoate (CPADB, >97%, Aldrich). Miglyol<sup>®</sup>810 (M810, d = 0.94 g/cm<sup>3</sup>, viscosity = 28 120 121 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<sub>3</sub>- $\tau$  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<sub>3</sub>-132  $\tau$ CTA $\gamma$  transurfs were obtained (see Supporting Information).  $\tau$  and  $\gamma$  correspond to the number of N<sub>3</sub>-end alkyl chains (N<sub>3</sub>) and dithiobenzoate CTA groups per 100 glucopyranosic units, respectively, (i. e. Substitution Degrees) and were estimated by <sup>1</sup>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 <sup>1</sup>H NMR spectrum of the 6-azidohexanoic acid in DMSO- $d_6$  (not shown).

140 
$$\tau = \frac{A_{N_3}/6}{A_{UG}/4} \times 100$$
(1)

141 
$$\gamma = \frac{A_R/5}{A_{UG}/4} \times 100$$
(2)



144 **Figure 1.** <sup>1</sup>H NMR spectra (DMSO- $d_6$ ) of a) DexN<sub>3-20</sub>, b) DexN<sub>3-20</sub>CTA<sub>3.7</sub> and c) DexN<sub>3-20</sub>-g-

145 1.8*PMMA*<sub>7800</sub>.

147 Typical experimental conditions were as follows:  $[DexN_3-\tau CTA\gamma]_0 = 10$  g/L, DexN<sub>3</sub>-148  $\tau$ CTA $\gamma$  /organic phase (MMA + M810) was varied from 13 to 36 wt% and M810/MMA from 149 10 to 50 vol.%. [CTA groups]<sub>0</sub>/[AIBN]<sub>0</sub> molar ratio was fixed to 3. For example, in case of 150  $DexN_{3-20}CTA_{3.7}$  transurf and M810/MMA = 10 vol.% (Run 1, Table 1),  $DexN_{3-20}CTA_{3.7}$ 151 20CTA3.7/organic phase (MMA + M810) was 13.2 wt%. 150 mg of DexN3-20CTA3.7 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<sup>-3</sup> 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<sub>3-</sub> $\tau$ -g-nPMMA<sub> $M_n$ </sub>, where n is the number of PMMA grafts 165 (having an average molecular weight equal to  $\overline{M}_n$ ) 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, Nouvel, & Six, 2008). The uncoupled PMMA grafts were analyzed by <sup>1</sup>H NMR in DMSO- $d_6$ 168 169 to verify the absence of dextran backbone. Their molar masses were then characterized by SEC-MALLS in THF. The remaining suspension was centrifuged. Recovered NCs were 170

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  $DexN_3-\tau CTA\gamma$ 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* 

<sup>1</sup>H NMR and 2D DOSY <sup>1</sup>H NMR spectra were recorded on a Bruker Avance 300 apparatus

177 (30,013 MHz, 25 °C) in DMSO-*d*<sub>6</sub>. 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 40°C. Refractive index
 184 increment (dn/dc) of 0.087 mL.g<sup>-1</sup> was used (See Supporting Information).

185 NCs cytocompatibility was evaluated on rat vascular smooth muscle cell line (A-10, ATCC\_
186 CRL-1476<sup>TM</sup>) (See Supporting Information).

187

#### 188 **3. Results and discussion**

189  $3.1 \text{ DexN}_3$ - $\tau CTA\gamma$  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<sub>3</sub>- $\tau$ CTA $\gamma$ , where  $\tau$  and  $\gamma$  correspond to the 199 number of N<sub>3</sub>-end alkyl chains (N<sub>3</sub>) and of dithiobenzoate CTA groups per 100 200 glucopyranosic units, respectively (Scheme 2a). On the one hand, N<sub>3</sub>-end alkyl chains have 201 been chosen as hydrophobic groups due to their efficiency to hydrophobize dextran, leading to 202 amphiphilic DexN<sub>3</sub>- $\tau$  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<sub>3</sub>- $\tau$  is expected to 206 be suitable for potential post-modification of final NPs surface by click chemistry.

207

208



210

211 **Scheme 2.** a) Synthesis of  $DexN_3-\tau CTA\gamma$  transurfs. b) RAFT polymerization of MMA from 212  $DexN_3-\tau CTA\gamma$  via the R-group approach.

213

214 On the other hand, a dithiobenzoate group bearing cyano functionality was chosen as the 215 reactive side group. Indeed, molecular CTAs having similar structure (i.e. CPDB and 216 CPADB) showed remarkable efficiency and high degree of control over MMA RAFT polymerizations in homogeneous (Biasutti, Davis, Lucien, & Heuts, 2005; Johnston-Hall, 217 218 Stenzel, Davis, Barner-Kowollik, & Monteiro, 2007) and heterogeneous media (Yang, Luo, 219 Liu, & Li, 2009; Zhou, Ni, & Yu, 2007). CTA groups were introduced (through R-group) by reacting previous  $DexN_3-\tau$  with CPADB in the presence of EDC and a catalytic amount of 220 221 DMAP, resulting in DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs (Scheme 2a). No degradation of the dextran backbone was evidenced as attested by SEC-MALLS analysis (Table S1). The integrity of 222 grafted CTA groups was as well confirmed by <sup>1</sup>H NMR spectroscopy as characteristic protons 223

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

228 To be used as transurfs, the designed DexN<sub>3</sub>- $\tau$ 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  $DexN_3-\tau$  and  $DexN_3-\tau CTA\gamma$  (Figure 231 S1). DexN<sub>3</sub>- $\tau$  (9 < $\tau$  <27) and DexN<sub>3</sub>-<sub>20</sub>CTA $\gamma$  transurfs ( $\tau$ =20 and  $\gamma$ =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<sub>3</sub>-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  $DexN_{3-24}$  ( $\tau=24$ ),  $DexN_{3-24}$  $_{20}$ CTA<sub>3.7</sub> (global modification degree of  $\tau + \gamma \sim 24$ ) and DexN<sub>3-20</sub>CTA<sub>7.5</sub>( $\tau + \gamma \sim 27$ ), whereas 236 237 a bigger decrease in surface tension was observed for DexN<sub>3-27</sub> ( $\tau$ =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<sub>3</sub>-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 pharmaceutically acceptable triglyceride oil (Hippalgaonkar, Majumdar, & Kansara, 248 249 2010))/DexN<sub>3</sub>- $\tau$  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<sub>3</sub>- $\tau$  stabilizer) (see Supporting Information), optimized conditions were applied to  $DexN_3$ - $\tau CTA\gamma$  transurfs. Our main challenge was to evaluate the 252 253 potential of the transurf to act both as oil/water interface stabilizer and macro-CTA to generate DexN<sub>3-</sub> $\tau$ -g-*nPMMA*<sub> $M_n$ </sub> at the organic interface, in order to produce dextran-covered 254 255 NCs with PMMA shell and M810 oily core. Thus, we first verified both the availability of CTA groups at the interface and their effective participation in RAFT polymerization. 256 257 Secondly, we evaluated the effects of M810 content and transurf structure on the 258 polymerization kinetics and control.

Table 1 summarizes all experimental recipes used in the current study, as well as the average size of initial nanodroplets and final NCs. In any case, latexes were very stable during polymerization procedure. Distribution size of the final NCs were roughly similar to the one of the initial nanodroplets, in agreement with preliminary stability assays performed with DexN<sub>3-20</sub> (see Supporting Information). Depending on experimental conditions, and especially 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<sub>3</sub>- $\tau$ CTA $\gamma$  transurf or DexN<sub>3</sub>- $_{20}$  stabilizer with 1.1 mL of MMA. [DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurf or DexN<sub>3</sub>- $_{20}$  stabilizer with 1.1 mL of MMA. 265  $\tau CTA\gamma$  (or DexN<sub>3</sub>- $\tau$ )] = 10 g/L and [CTA]<sub>0</sub>/[AIBN]<sub>0</sub> = 3. 266

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

267 <sup>a</sup>Relative molar amount in NCs. Expressed as function of the initially introduced DexN<sub>3</sub>- $\tau$ CTAy.

268 <sup>b</sup>Initial molar ratio in the organic phase, estimated with the real amount of DexN<sub>3</sub>-τCTAy at the interface evaluated in (a), and supposing that all the CTA groups were accessible 269 for polymerization.

270 <sup>c</sup>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) 271 were the particle diameters at 10%, 50% and 90% of the particle volume size distribution.

272 273 274 <sup>d</sup>Average number of PMMA grafts per 100 glucopyranosic units estimated from <sup>1</sup>H NMR spectrum (Figure 1c) using  $n = [(4A_{A'B'}/5A_{UG}) \times \overline{M}_{n(SEC)DEX}/162)]/\overline{X}_n$ .  $A_{A'B'}$  and  $A_{UG}$ are the areas under peaks (A'B') and (OH<sup>1</sup>, OH<sup>2</sup>, OH<sup>3</sup> and H<sup> $\alpha$ </sup>), respectively.  $\overline{M}_{n(SEC)DFX}$  is the  $\overline{M}_n$  of native dextran, 162 the molecular weight of one glucopyranosic unit and  $\overline{X}_n$  is the average polymerization degree of PMMA grafts.

275 276 <sup>e</sup>Average CTA groups efficiency for transurfs estimated using Eff<sub>CTA</sub> =  $n/\gamma \times 100$ , where n and  $\gamma$  are the number of grafts and CTA groups per 100 glucopyranosic units, respectively.

277 278 279

<sup>f</sup>Weight fraction of PMMA homopolymers (non-attached to dextran) with respect to the total synthesized PMMA using  $F_{Homo} = \left[1 - \left[(m_{CTA}/M_{UG-CTA}) \times n \times \overline{M}_{n(SEC)grafts}\right]/(m_{MMA}x)\right] \times 100$ , with  $m_{CTA}$  the mass amount of DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurf at NCs surface,  $M_{UG-CTA}$  the molecular weight of one glucopyranosic unit of DexN<sub>3</sub>- $\tau$ CTA $\gamma$ , n the average number of PMMA grafts per 100 glucopyranosic units evaluated in (d),  $\overline{M}_{n(SEC)grafts}$  the  $\overline{M}_n$  of PMMA grafts,  $m_{MMA}$  the initial mass amount of monomer and x the monomer

280 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,  $DexN_3$ - $\tau CTA\gamma$  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 dextran molecules via their R moieties, re-initiating radicals (R<sup>•</sup>) were situated on the dextran 285 286 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  $DexN_{3-}\tau$ -g-288  $nPMMA_{\overline{M}_n}$  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]<sub>o</sub>/[CTA]<sub>o</sub> molar ratio (X) was estimated by taking into 291 account the total amount of monomer in the system and the quantity of  $DexN_3-\tau CTA\gamma$ 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 washing by dosage of the DexN<sub>3</sub>- $\tau$ CTA $\gamma$  remaining in aqueous supernatant. In all cases, more 294 295 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<sub>3-20</sub> was present in final NCs. This result could be 298 surprising as polymerization conditions and specially  $[DexN_3-\tau]/[organic phase]$  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<sub>3-20</sub> 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<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs. A linear relationship between ln([M]<sub>0</sub>/[M]<sub>t</sub>) 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]<sub>0</sub>/[CTA]<sub>0</sub> constant (Runs 1-3). This result was quite surprising as these 314 experiments were carried out with the same transurf, [CTA]<sub>0</sub>/[AIBN]<sub>0</sub> ratio (equal to 3), and 315 MMA volume.



#### 316

321 Run 4,  $DexN_{3-20}CTA_{7.5}$ , X = 204, 25 vol.% M810 (see Table 1).

<sup>317</sup> Figure 2.  $Ln([M]_o/[M]_t)$  versus time. RAFT polymerization of MMA in miniemulsion at

<sup>318 80°</sup>C using DexN<sub>3</sub>-τCTAγ transurfs (or DexN<sub>3-20</sub> stabilizer). (\*) Run 0, DexN<sub>3-20</sub>, [CTA]<sub>0</sub> = 0, 319 10 vol.% M810 (•) Run 1, DexN<sub>3-20</sub>CTA<sub>3.7</sub>, X = 393, 10 vol.% M810 (•) Run 2, DexN<sub>3-20</sub>

 $<sup>320 \</sup>quad _{20}CTA_{3.7}, X = 393, 25 \text{ vol.}\% \text{ M810} (\blacktriangle) \text{ Run } 3, \text{DexN}_{3-20}CTA_{3.7}, X = 402, 50 \text{ vol.}\% \text{ M810} (\blacksquare)$ 

323 In such conditions, AIBN initial mass remained constant while organic phase global volume increased with the amount of M810. Polymerization rate was thus expected to slow down 324 325 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<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs 334 appeared to slow down the miniemulsion polymerization of MMA. Indeed, DexN<sub>3-20</sub>CTA<sub>3.7</sub> 335  $([AIBN]_{0} = 5.7 \text{ mM}, \text{Run } 2)$  and  $\text{DexN}_{3-20}\text{CTA}_{7.5}$   $([AIBN]_{0} = 11.9 \text{ mM}, \text{Run } 4)$  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<sub>3-20</sub>CTA<sub>7.5</sub> (Run 4) gave faster polymerization rate than DexN<sub>3-20</sub>CTA<sub>3.7</sub> (Run 2). Since 340 DexN<sub>3-20</sub>CTA<sub>7.5</sub> was bearing more CTA groups, AIBN concentration was almost doubled in 341 Run 4 with respect to Run 2 to keep constant the [CTA]<sub>o</sub>/[AIBN]<sub>o</sub> ratio. Furthermore, no 342 delay period was observed with DexN<sub>3-20</sub>CTA<sub>7.5</sub> once again probably because of the higher 343 initiator amount used in this experiment.

344 3.2.3  $DexN_3$ - $\tau$ -g- $nPMMA_{\overline{M}_n}$  analysis.

345 Attempting to confirm the covalent linkage between PMMA shell and dextran coverage of 346 NCs,  $DexN_3.\tau$ -g- $nPMMA_{\overline{M}_n}$  glycopolymers produced at the oil/water interface were purified from M810 and from free PMMA homopolymers (not attached to dextran), by several washings of dried NCs with THF. These glycopolymers were also characterized with their weight fraction of PMMA ( $F_{PMMA}$ ) determined by equation 3, where  $M_{UG-CTA}$  is the molecular weight of one glucopyranosic unit of DexN<sub>3</sub>- $\tau$ CTA $\gamma$ .

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

352 For copolymers produced in Runs 1 – 4, n was estimated from <sup>1</sup>H NMR spectra in DMSO- $d_6$ 353 (Table 1). <sup>1</sup>H NMR spectrum of DexN<sub>3-20</sub>-g-1.8PMMA<sub>7800</sub> elaborated from DexN<sub>3-20</sub>CTA<sub>3.7</sub> 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<sub>3</sub> 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<sub>3- $\tau$ -g-</sub>  $nPMMA_{\overline{M}_n}$  were therefore studied by 2D Diffusion-Ordered Spectroscopy (DOSY) <sup>1</sup>H NMR 359 360 (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, Nouvel, Babin, & Six, 2016). On the one hand, the 2D DOSY <sup>1</sup>H NMR spectrum of DexN<sub>3-20</sub>-363 364 g-1.8PMMA<sub>7800</sub> was recorded in DMSO-d<sub>6</sub> (Figure 3a). As shown, a single self-diffusion 365 coefficient was observed for all the protons from both dextran and PMMA parts ( $D = 4.47 \times$  $10^{-10} \text{ m}^2 \text{ s}^{-1}$ ), which suggested that PMMA grew effectively from CTA groups on dextran. On 366 367 the other hand, a mixture (same weight composition as for the copolymer DexN<sub>3-20</sub>-g-368 1.8PMMA7800) of the precursor DexN3-20CTA3.7 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 =  $3.16 \times$ 

371  $10^{-11} \text{ m}^2 \text{ s}^{-1}$  and  $\text{D} = 1.78 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ) 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<sub>3</sub>. 373  $_{20}$ -g-1.8PMMA<sub>7800</sub>, proved that PMMA grafts were really covalently linked to dextran 374 backbone, resulting in a DexN<sub>3</sub>- $\tau$ -g- $nPMMA_{\overline{M}n}$  glycopolymer.



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

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375

Finally, we wanted to characterize those  $DexN_{3-}\tau$ -g- $nPMMA_{\overline{M}_{n}}$  glycopolymers by SEC-380 381 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<sub>3</sub>- $\tau$ -g- $nPMMA_{\overline{M}_n}$  (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 $nPMMA_{\overline{M}_n}$  (i.e. before THF washings) were also investigated. In comparison with 389 390 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 <sup>1</sup>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]<sub>0</sub>/[AIBN]<sub>0</sub> 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 absence of noticeable transfer or termination reactions, except a small proportion of 421 422 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 M810 in the organic phase. Actually, sufficient amount of M810 in the organic phase was 425 426 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.



Figure 4. Evolution of a) SEC traces (Refractive Index detector) of PMMA grafts (Run 2) and b) experimental  $\overline{M}_n$  (full symbols), Dispersity D (open symbols) *versus* conversion. Conversions are given in % on figure a). RAFT polymerization of MMA in miniemulsion at 80°C using DexN<sub>3-20</sub>CTA<sub>3.7</sub> transurf and various amounts of M810 in the organic phase (•)

436 Run 1, 10 vol.% (•) Run 2, 25 vol.% (•) Run 3, 50 vol.%. See Table 1 for other conditions. 437 Theoretical  $\overline{M}_n$  (dotted lines) was evaluated using  $\overline{M}_n = 100(x)(X)$  where x and 100 are the 438 monomer conversion and the molecular weight of a PMMA unit, respectively. Curve for X = 439 393 is given.

440

441 The evolution of experimental  $\overline{M}_n$  of PMMA grafts with conversion for Runs 1-3 is shown as 442 a function of theoretical  $\overline{M}_n$  in Figure 4b. In all cases, experimental  $\overline{M}_n$  evolved linearly with 443 conversion until 80% of conversion without appearance of noticeable transfer or termination 444 reactions. In addition, D 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 445 446 higher than the theoretical ones (calculated with the adjusted average value of X =447 [MMA]<sub>0</sub>/[CTA]<sub>0</sub>=393) indicating that not all CTA groups participated in the polymerization 448 (Figure S8 for additional results of Run 4, X = 402). The comparison between the number of 449 grafts (n) estimated from <sup>1</sup>H NMR spectra and the number of CTA groups along dextran 450 backbone ( $\gamma$ ) allowed us to evaluate an average efficiency of CTA groups (Eff<sub>CTA</sub>) of about 451 48% (Table 1). Roughly half of the initial CTA groups of DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs located at 452 the interface, effectively resulted in a PMMA grafted chain. Similar lower efficiencies have 453 been already observed for other polysaccharides bearing reactive groups when used for 454 controlling ATRP (Dupayage et al., 2008; Ferji et al., 2015) in homogeneous media. One 455 possible cause seemed to be the presence of intramolecular termination reactions at the very 456 beginning of the polymerization resulting in the loss of RAFT re-initiating radicals (R<sup>•</sup>). In 457 our case, the reaction takes place in a heterogeneous environment. So, the transurf 458 conformation at the oil/water interface is more likely the key factor limiting the availability of 459 some CTA groups for polymerization if they are not facing to the organic phase, which was 460 the main challenge of this work. Finally, CTA group efficiency appeared to be the same for 461 both DexN<sub>3-20</sub>CTA<sub>3.7</sub> and DexN<sub>3-20</sub>CTA<sub>7.5</sub>, and remained unchanged whatever the M810/MMA volume ratio in agreement with our previous results for miniemulsion ATRP of 462

MMA using dextran-based inisurfs (Forero Ramirez et al., 2018). A steric hindrance effect in
the vicinity of CTA groups restricting the access to C=S function does not seem to be present
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 liquid phase exhibiting one melting transition ( $T_m = -4^{\circ}C$ ). On the contrary, nano-objects 472 473 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 black493 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 is therefore a measure for cell viability. After 24 hours, results expressed as percentages did not show any variation whatever the concentration or NCs type, when compared to the control condition (no NC) (Figure 6). The MOD and CTA-PMMA NCs appeared to be cytocompatible with vascular smooth muscle cells.

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510

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

516

#### 517 **4.** Conclusions

As stated, we designed dextran-based transurfs able to produce oily-core/PMMA shell NCs by RAFT interfacial miniemulsion polymerization. The two major challenges of this work were to introduce CTA functionalities onto dextran in order to synthesize a multi-reactive transurf (acting both as macro-CTA and stabilizer) and to check its ability to confine RAFT miniemulsion polymerization of MMA at the oil/water interface. About a half of the CTA groups along dextran backbone were found to effectively initiate a PMMA grafted chain. About 80 wt% of the total produced PMMA was effectively attached to dextran backbone. In any case, polymerization was controlled until high conversion as concluded from the analysis of uncoupled PMMA grafts deliberately removed from  $DexN_{3-}\tau-g-nPMMA_{\overline{M}_n}$ . Phase 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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