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Biomimetic thermo-responsive star diblock gelators†

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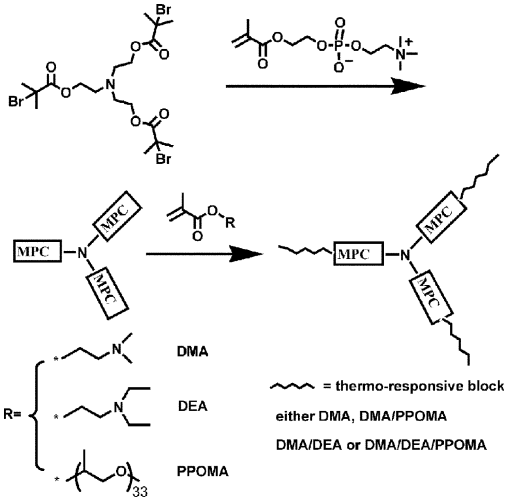
We report the synthesis of novel biomimetic gelators with star diblock copolymer architectures by sequential monomer addition *via* alcoholic ATRP at 20 °C; free-standing gels can be formed from 5% aqueous copolymer solutions at 37 °C.

Hydrogels[1–3] that can change from free-flowing liquids to free-standing gels on application of an external stimulus have recently attracted particular attention in the context of drug delivery[4–7] and tissue engineering.[8] These gelators normally comprise either amphiphilic block or graft copolymers that can self-assemble in water to form organized structures on the nanometer length scale. Gelation can be triggered by changes in solution temperature, solution pH, ionic strength, electric field, light or the presence of specific analytes.[6,9,10] Certain water-soluble polymers exhibit inverse temperature solubility behaviour between ambient temperature (20–25 °C) and physiological temperature (37 °C); in principle, such polymers can be used to produce injectable biomaterials that form biocompatible gels *in situ*. Indeed, there are a few reports concerning thermo-responsive polymeric gelators, but usually their biocompatibility is insufficient or else the minimum copolymer concentration required for gelation is too high.[11–13] Deming and co-workers have described the synthesis of efficient polypeptide-based gelators but unfortunately the *N*-carboxyanhydride monomers used in these studies are not commercially available.[14]

2-(Methacryloyloxy)ethyl phosphorylcholine (MPC) is a zwitterionic methacrylic monomer that usually confers clinically-proven biocompatibility to a wide range of copolymer coatings.[15] We recently reported the synthesis of novel biomimetic *pH-responsive* gelators based on ABA triblock copolymers, where the central B block comprised MPC and the outer A blocks were composed of 2-(diisopropylamino)ethyl methacrylate (DPA).[16] These ABA triblocks were readily synthesised using a commercially available bifunctional ATRP initiator. Herein we report the synthesis of new biomimetic *thermo-responsive* gelators based on star diblock copolymers, where the inner block is MPC-based and the outer blocks comprise a statistical terpolymer based on 2-(dimethylamino)ethyl methacrylate (DMA), 2-(diethylamino)ethyl methacrylate (DEA) and poly(propylene oxide) monomethacrylate (PPOMA), see Scheme 1 and Table 1. These new copolymers may have potential applications for drug delivery and/or tissue engineering.

Our initial attempts to prepare MPC-based thermo-responsive gels using DMA–MPC–DMA triblock copolymers prepared from bifunctional initiators were not very successful, since gelation only occurred at relatively high temperatures and free-standing gels could not be formed. Thus a new trifunctional initiator (TrisE) was synthesised by the direct esterification of triethanolamine with excess 2-bromoisobutyryl bromide in the presence of triethylamine in THF. Ma *et al.* reported that the ATRP of MPC in methanol had good living character when using monofunctional initiators, but were less well-controlled at higher target degrees of polymerisation (Dp's).[17] However, premature termination is more likely to occur when using multifunctional initiators, and high Dp's were considered essential for optimum gelator performance. For the homopolymerisation of MPC with this TrisE initiator, the mean target Dp for each arm was 125, making a total Dp of 375 for the three-arm star MPC homopolymer. Aqueous GPC using PEO as standards indicated relatively high polydispersities of around 1.48 for these homopolymers, but given their star architecture, the high conversions (>95%) and the relatively high target Dp, we considered these results to be encouraging. Moreover, self-blocking chain extension experiments (see ESI†) confirmed that when the target Dp was increased from 187 to 375, the molecular weight approximately doubled from 24 000 (M_w/M_n = 1.67) to 51 800 (M_w/M_n = 1.37), as expected. Thus the ATRP of MPC with the TrisE trifunctional initiator appears to be reasonably well controlled under these conditions.

DMA homopolymer exhibits inverse temperature solubility and its observed cloud point in neutral/alkaline solution (pH 8) varies from 32 to 46 °C, depending on its degree of polymerisation.[18] In principle, it is possible to prepare thermo-responsive gelators based



Scheme 1 Reaction scheme for the synthesis of the star diblock copolymers *via* atom transfer radical polymerization (ATRP) using the trifunctional ATRP initiator (TrisE).

Table 1 Formulation details for the ATRP syntheses of MPC-based star diblock copolymers (12.5 mmol MPC was used in all syntheses)

Expt no.	Target star diblock copolymer structure	DMA/DEA/PPOMA/ mmol			Conv. (%)
1	I–(MPC$_{125}$–DMA$_{100}$)$_3$	10.0	—	—	90
2	I–[MPC$_{125}$–(DMA$_{93}$/PPOMA$_7$)]$_3$	9.3	—	0.7	99
3	I–[MPC$_{125}$–(DMA$_{95}$/PPOMA$_5$)]$_3$	9.5	—	0.5	85
4	I–[MPC$_{125}$–(DMA$_{97}$/PPOMA$_3$)]$_3$	9.7	—	0.3	90
5	I–[MPC$_{125}$(DMA$_{50}$/DEA$_{50}$)]$_3$	5.0	5.0	—	92
6	I–[MPC$_{125}$–(DMA$_{50}$/DEA$_{50}$/ PPOMA$_3$)]$_3$	5.0	5.0	0.3	95

† Electronic supplementary information (ESI) available: Synthesis and characterisation data. See http://www.rsc.org/suppdata/cc/b4/b410150b/

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Table 2 Summary of the gelation behaviours of the various star diblock copolymers used in this study[a]

Expt. no.	Gelation behaviour	
	Aqueous solution	0.15 M PBS buffer
1	Weak gel at 20% and 80 °C	Not determined
2	20%, weak free-standing gel at 37 °C	15%, free-standing gel at 37 °C
3	8%, free-standing gel at 37 °C	7%, free-standing gel at 37 °C
4	6%, free-standing gel at 37 °C	5%, weak free-standing gel at 37 °C
5	7%, free-standing gel at 37 °C	6%, weak gel at 37 °C
6	6%, free-standing gel at 37 °C	5%, free-standing gel at 37 °C

[a] Compare with Table 1 for chemical composition data.

solely on MPC and DMA. Unfortunately, a three-arm MPC–DMA star diblock copolymer only formed a weak gel above 80 °C, even at copolymer concentrations exceeding 20% (see entry 1 in Table 2). Presumably the highly hydrophilic MPC chains raised the effective cloud point for the DMA blocks. Such copolymer gels are unlikely to be useful for biomedical applications.

Homopoly(propylene oxide) (PPO) of M_n = 2000 dissolves molecularly in dilute aqueous solution at low temperatures (< 5 °C) but at higher temperatures it becomes progressively more hydrophobic, usually exhibiting a cloud point at around 15–20 °C. A methacrylic PPO macromonomer (PPOMA) was synthesised by reacting monohydroxy-capped PPO with excess methacryloyl chloride (see ESI†). Statistical copolymerisation of this PPOMA macromonomer with DMA was expected to lower the cloud point of the outer blocks of the star diblock copolymers. Indeed, this new copolymer (see entry 4 in Table 2) formed free-standing gels at 37 °C. However, perhaps surprisingly, higher PPOMA contents (see entries 2 and 3 in Table 2) did not lead to lower critical gelation concentrations.

2-(Diethylamino)ethyl methacrylate (DEA) is similar to DMA in its chemical structure but is somewhat more hydrophobic. Thus replacing some of the DMA with DEA was expected to lower the critical gelation temperature of the copolymer. Indeed, free-standing gels were obtained at 37 °C (see entry 5 in Table 2) at a copolymer concentration of only 6%; this is a significant improvement compared to the MPC–DMA star diblock copolymer, which only formed weak gels at 80 °C even at 20% concentration. However, the best gelator performance was obtained from a star diblock copolymer in which the stimulus-responsive block comprised a statistical terpolymer of DMA, DEA and PPOMA (entry 6 in Table 2). A 5% aqueous solution of this copolymer was free-flowing in PBS at 20 °C, whereas at 37 °C a transparent free-standing gel was obtained. It is noteworthy that the pH of the PBS buffer increased from pH 7.4 to about pH 8 due to the strongly self-buffering nature of the tertiary amine methacrylate residues in the thermo-responsive blocks. Hence HCl was added to adjust the pH of the copolymer solution back to 7.4. Unfortunately these star diblock copolymers could not be analysed by aqueous GPC since a suitable eluent could not be identified. However, static light scattering studies indicated M_w values ranging from 232000 to 305000 (see ESI†).

The (I–[MPC_{125}–(DMA_{50}/DEA_{50}/$PPOMA_3$)]$_3$) star diblock copolymer was examined both as a free-flowing solution and also as a free-standing gel using variable-temperature ^{1}H NMR spectroscopy. Typical spectra are depicted in Fig. 1 for a 5% copolymer solution (entry 6 in Table 1) in D_2O at pH 7.4. At 2 °C, all of the NMR signals expected for the inner MPC block and the outer statistical terpolymer block are visible, indicating high degrees of solvation and mobility for both blocks at this temperature. In contrast, at 12 °C, the PPO signals at 0.75–0.80 ppm due to the pendent methyl group are both broadened and attenuated relative to the MPC signals. This indicates a significant reduction in solvation and mobility for the PPO blocks. At 37 °C physical gelation occurs, but the NMR signals due to the DMA and DEA residues remained visible (δ 1.85–2.40), although they are slightly broadened and attenuated. The lower temperature spectra can be reproduced on cooling the gel below its critical gelation temperature, confirming the physical, reversible nature of the gelation.

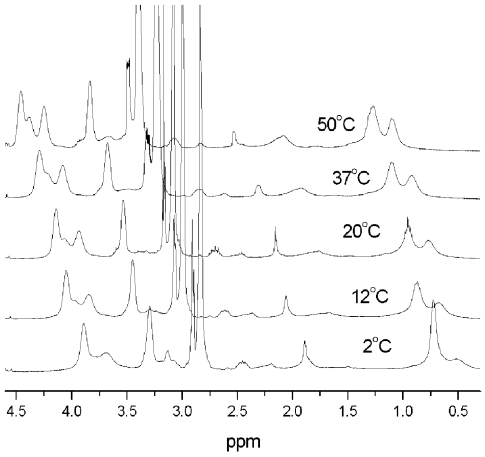

Fig. 1 Variable-temperature ^{1}H NMR spectra obtained for a 5% solution of the I–[MPC_{125}–(DMA_{50}/DEA_{50}/$PPOMA_3$)]$_3$ star diblock copolymer dissolved in D_2O (pH 7.4).

In summary, novel thermo-responsive gelators with star diblock copolymer architectures were synthesised by sequential monomer addition *via* alcoholic ATRP at 20 °C using a new trifunctional initiator to polymerize MPC, followed by the statistical copolymerisation of DMA, DEA and (optionally) PPOMA. A 5% aqueous solution of the most efficient copolymer gelator formed a free-standing gel at 37 °C. Cell viability studies of these new gels are now in progress.

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