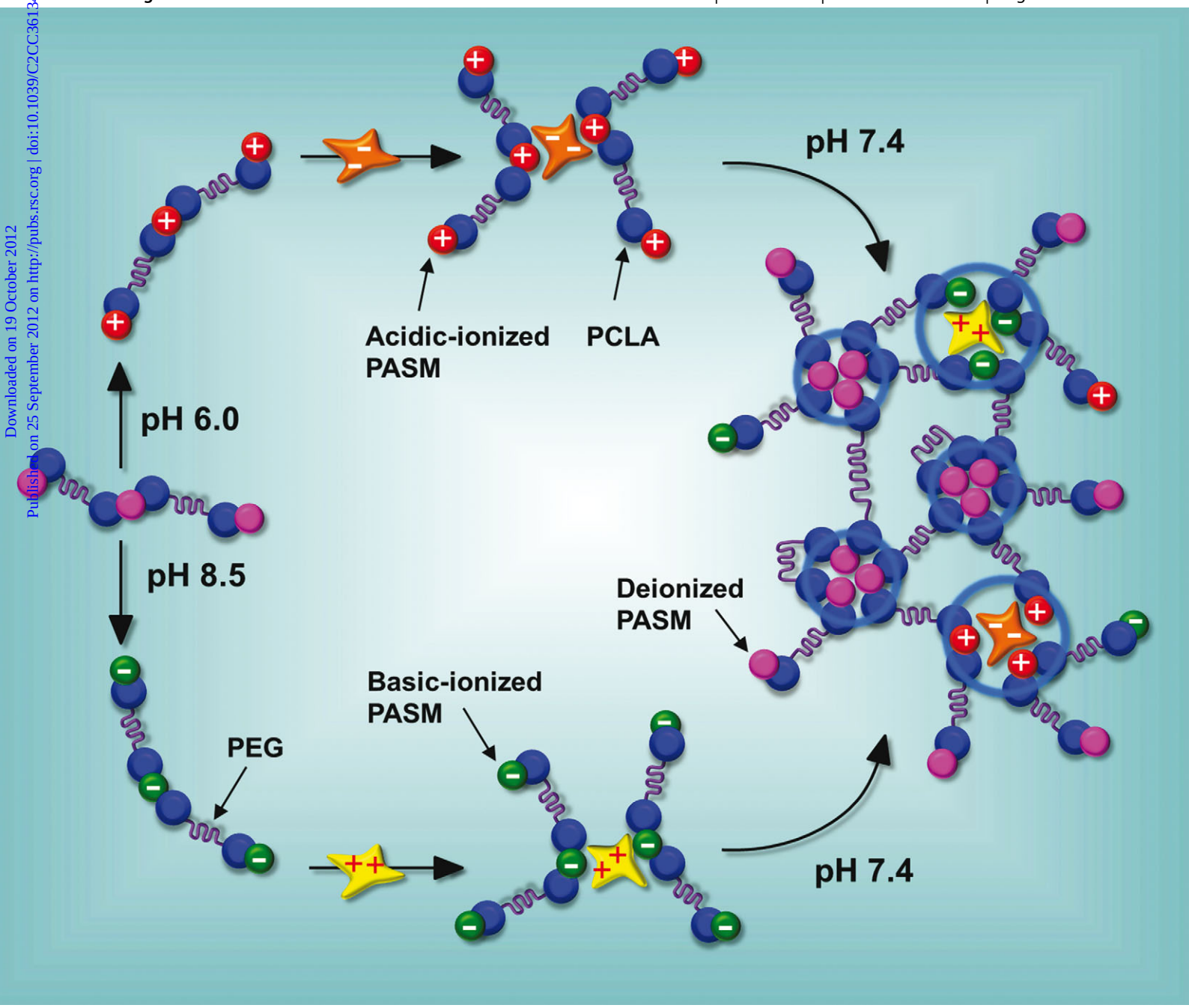
ChemComm

Chemical Communications

www.rsc.org/chemcomm

Volume 48 | Number 89 | 18 November 2012 | Pages 10921–11036



ISSN 1359-7345

RSC Publishing

COMMUNICATION

Doo Sung Lee *et al.*

Dually cationic and anionic pH/temperature-sensitive injectable hydrogels and potential application as a protein carrier

ChemComm

Dynamic Article Links
View Online

Cite this: *Chem. Commun.*, 2012, **48**, 10951–10953

www.rsc.org/chemcomm

COMMUNICATION

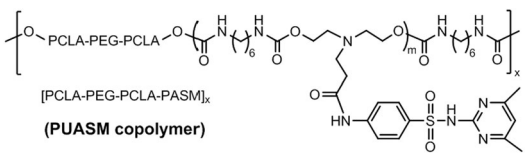
Dually cationic and anionic pH/temperature-sensitive injectable hydrogels and potential application as a protein carrier†

Cong Truc Huynh,‡ Minh Khanh Nguyen§ and Doo Sung Lee*

Received 23rd August 2012, Accepted 23rd September 2012
DOI: 10.1039/c2cc36134e

Novel copolymers containing both anionic and cationic pH-sensitive moieties were reported. These amphoteric copolymers exhibited special closed-loop reversible sol–gel–sol phase transitions in response to both pH and temperature.

pH/temperature-responsive therapeutic delivery provides a promising strategy for on demand release of a variety of bioactive factors. Injectable pH/temperature-responsive hydrogel systems capable of forming an ionic complex with oppositely charged bioactive factors have been widely explored for bioactive agent delivery vehicles.[1–3] Among them, anionic hydrogels may offer potential for complexation with cationic molecules (*e.g.*, bone morphogenetic protein (BMP), transforming growth factor beta (TGF-β), *etc.*),[4] whereas cationic hydrogels may interact with anionic macromolecules (*e.g.*, insulin, human growth hormone (hGH), *etc.*).[5–8] The ionic interaction may retain the therapeutics in the hydrogels. pH-responsive polymeric hydrogels based on poly(2-(diisopropylamino) ethyl methacrylate) (PDPAEMA), poly(2-(diethylamino) ethyl methacrylate) (PDEAEMA), poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA), poly(*N*-isopropylacrylamide-*co*-propylacrylic acid) (p(NIPAAm-*co*-PAAc)) copolymers, poly(NIPAAm-*co*-PAAc-*co*-butyl acrylate) (p(NIPAAm-*co*-PAAc-*co*-BA)), poly(amidoamine) (PAA), poly(amino urethane) (PAU) and poly(amino urea urethane) (PAUU) have been reported.[9,10] These materials display a non-biodegradability that may limit their practical applications. To overcome this shortcoming, different series of pH/temperature-responsive polymers based on oligosulfamethazine (OSM), poly(β-amino ester) (PAE) and poly(β-amino ester urethane) (PAEU) have been developed as biodegradable hydrogel systems.[6–8,11–13] Ionic biomolecules are retained within these pH/temperature-responsive hydrogels that will hydrolytically degrade to release the payloads. However, these hydrogels are either cationic or anionic, because the materials only bear basic (amine) or acidic (sulfonamide) groups.



Scheme 1 Chemical structure of PUASM copolymer.

Here, we introduce an innovative class of amphoteric copolymers, poly(urethane amino sulfamethazine)-based block copolymers (PUASM), capable of forming dually cationic and anionic hydrogels in response to pH and temperature. The copolymer aqueous solutions exhibited as a free flowing sol at both mildly acidic and basic pH, but a gel was induced at a pH range of 6.8–8.2 as temperature increased. The dually cationic and anionic characteristics, mechanical properties, cytotoxicity and potential application as a protein carrier of the amphoteric hydrogels were examined.

The PUASM block copolymers (Scheme 1) were synthesized by polyaddition of isocyanate groups of 1,6-diisocyanato hexamethylene (HDI) with hydroxyl groups of a synthesized dihydroxyl amino sulfamethazine monomer (DHASM) and of triblock poly(ε-caprolactone-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-lactide) (PCLA-PEG-PCLA) copolymers in the presence of dibutyltin dilaurate (DBTL) as a catalyst (Scheme S1 in ESI†).[12] The PCLA-PEG-PCLA triblock copolymers were synthesized and characterized as in the previous report.[11] The DHASM was prepared by Michael-addition reaction[12] between the secondary amine groups of diethanolamine (DEA) and the vinyl groups of acrylated sulfamethazine (SM-A), which was similarly synthesized to the previous procedure.[11] The synthesized PUASM block copolymers were characterized using ^{1}H NMR (Fig. S1 in ESI†) and gel permeation chromatography (GPC). The detailed characteristics of the synthesized PUASM copolymers are listed in Table S1 in ESI.†

To confirm the dual ionic properties of the synthesized amphoteric copolymer, zeta potentials of the synthesized amphoteric PUASM, cationic PAE-based (PAE-poly(ε-caprolactone)-PEG-poly(ε-caprolactone)-PAE, PAE-PCL-PEG-PCL-PAE:1250-1500-1650-1500-1250)[6] and anionic OSM-based (OSM-PCLA-PEG-PCLA-OSM:1140-1550-1750-1550-1140)[4] copolymer solutions were compared. As shown in Fig. 1, zeta potential of the cationic PAE-based copolymer solutions decreased with increasing pH.

Theranostic Macromolecules Research Center, Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon, Gyeonggi-do 440-746, South Korea. E-mail: dslee@skku.edu; Fax: +82 31 299 6857; Tel: +82 31 290 7282

† Electronic supplementary information (ESI) available: Detailed experimental procedures, characterization, sol–gel transition, degradation and cell cytotoxicity. See DOI: 10.1039/c2cc36134e

‡ Present address: Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia.

§ Present address: Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA.

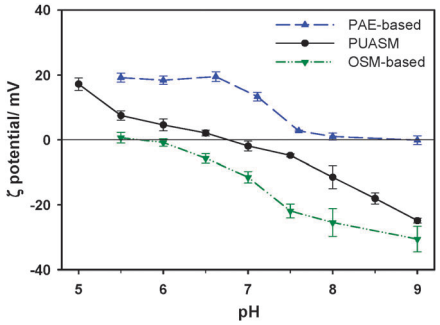


Fig. 1 Zeta potential of amphoteric PUASM (PUASM-2), cationic PAE-based (PAE-PCL-PEG-PCL-PAE) and anionic OSM-based (OSM-PCLA-PEG-PCLA-OSM) copolymers in water (2 mg mL^{-1}) at different pH.

It was +19.5 mV at pH 6.5, +2.7 mV at pH 7.5, and +1.0 mV at pH 8.0. In contrast, zeta potential of the anionic OSM-based copolymer solutions increased when lowering pH. Interestingly, as a combination, zeta potential of the amphoteric PUASM copolymer solutions was positive at acidic pH (+4.6 mV at pH 6.0), and became negative at basic pH (−11.4 mV at pH 8.0). This is a result of ionization of the tertiary amine at relatively acidic pH and sulfonamide groups at relatively basic pH, and their deionization at neutral pH.

The PUASM copolymers displayed special closed-loop reversible sol–gel–sol phase transition, determined using the tube inversion method,[13] when varying pH and temperature, as shown in Fig. 2. This behaviour results from the amphoteric properties of the copolymers combining anionic sulfonamide and cationic tertiary amine groups in the poly(amino sulfamethazine) (PASM) blocks. The closed-loop sol–gel phase transition has been reported in previous literature.[14–16] Thermosensitive copolymers of PEG and poly(ethyl-2-cyanoacrylate) (PEC) exhibited a closed-loop sol–gel–sol phase transition as a function of temperature and concentration.[14] Multiblock copolymers composed of a poloxamer (P88) and terephthalic anhydride showed a closed-loop sol–gel–sol phase transition at below physiological conditions relevant to the anionic properties of the copolymer.[15] Recently, the similar closed-loop sol–gel–sol phase transition was observed with Pluronic F127 end-capped by the polyamine hydrogel system.[16] However, the mechanism of their gel-to-sol transition at high pH was not

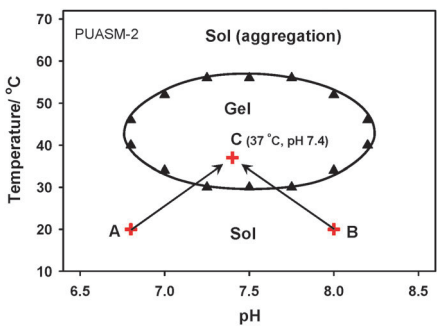


Fig. 2 Closed-loop sol–gel phase diagram of PUASM-2 hydrogel (25 wt%). The bioactive molecules and copolymer solution can be formulated and injected into the body at either slightly acidic pH (*e.g.*, A, pH 6.8) or slightly basic pH (*e.g.*, B, pH 8.0), and a gel forms rapidly (see Fig. S3 in ESI†) under physiological conditions (C, 37 °C, pH 7.4).

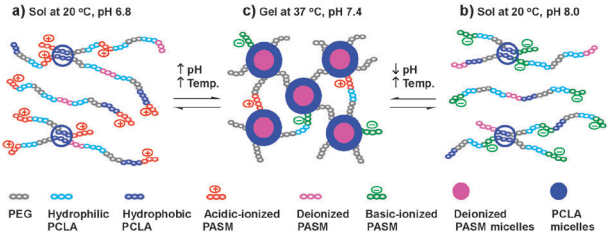


Fig. 3 Schematic showing reversible sol–gel–sol phase transition of PUASM hydrogels with changing temperature and pH. Ionization of basic portions containing amine groups in the PASM at slightly acidic pH (a) and of acidic portions composed of sulfonamide groups in the PASM at slightly basic pH (b) renders the polymer hydrophilic, resulting in a sol state in water. In contrast, relative deionization of both basic and acidic portions of the PASM and increases in hydrophobicity of the PCLA under physiological conditions led to formation and interconnection of nanostructured polymeric micelles, resulting in gelation (c). The positive and negative charges in c indicate that the PASM is not fully deionized.

completely clear. In this amphoteric PUASM hydrogel system, the combination of anionic and cationic pH-sensitive moieties accounted for the closed-loop sol–gel–sol phase transition mechanism that is depicted in Fig. 3. Specifically, the ionization of tertiary amine groups at relatively acidic pH (*e.g.*, pH 6.5) or sulfonamide groups at relatively basic pH (*e.g.*, pH 8.5) rendered the copolymers hydrophilic, resulting in sol states in the temperature range of 0–65 °C. At a pH range of 6.8–8.2, even though the PASM domains (including both cationic and anionic portions) became more hydrophobic as a result of an increase in degree of their deionization, the sol state still existed at low temperatures, due to the less hydrophobicity of the PCLA segments.[7,11] In this stage, the hydrophobic interactions among the PASM were not sufficient to induce gelation. In contrast, an increase in the hydrophobicity of the PCLA at higher temperatures led to formation of micelles bridged by PEG segments, and thus gelation occurred. The gel-to-sol transition appeared with further increasing temperature, which is relevant to the partial dehydration of PEG segments and the breakage of hydrophobic interactions.[11–13] The closed-loop gel regions of the PUASM hydrogels are adjusted by varying the molecular weight of the PEG block or copolymer concentration (Fig. S2 in ESI†).[11–13,17]

Variation in viscoelasticity of the PUASM copolymer aqueous solutions at different pH values as a function of temperature was examined using dynamic rheological analysis.[12] As shown in Fig. 4, while the copolymer solutions exhibited low viscosities

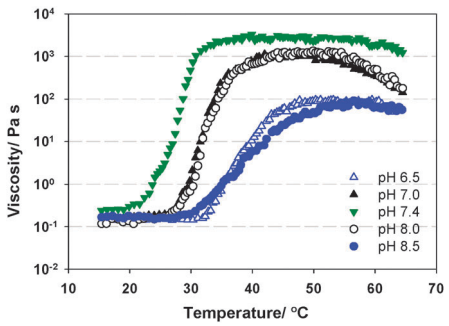


Fig. 4 Variation in viscosity of 25 wt% PUASM-2 copolymer solutions at different pH as a function of temperature.

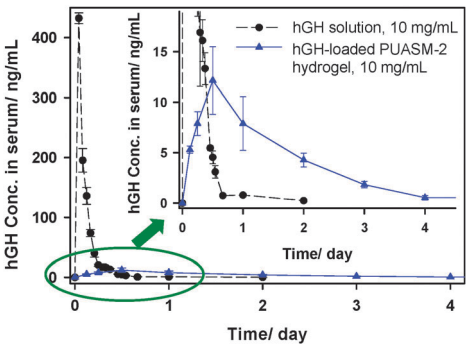


Fig. 5 Concentration of hGH in the plasma of SD rats after injecting 200 μL of the hGH solutions (10 mg mL^{-1}) and 200 μL of the hGH-loaded PUASM-2 (25 wt%) solutions (hGH 10 mg mL^{-1}) (±SD, $n = 5$).

(<1 Pa s) at low temperatures (<20 °C) in the pH range from 6.5 to 8.5, the viscosities significantly increased with increasing temperature to 65 °C. At pH 7.4, the viscosity markedly increased from 0.24 Pa s (a sol state) to 2381 Pa s (a gel state) as temperature was increased from 15 to 37 °C due to the enhancement in hydrophobicity of PCLA. At body temperature (37 °C), the viscosities of the copolymer solutions were 2.68, 450 and 2381 Pa s corresponding to pH values at 6.5 to 7.0 and 7.4. However, they decreased to 405 and to 1.51 Pa s with further increasing pH to 8.0 and 8.5, respectively. The low viscosities at both acidic (pH 6.5) and basic pH (pH 8.5) may facilitate homogeneous encapsulation of bioactive agents as well as subcutaneous injection of the copolymer solutions into the body (Fig. S3 in ESI† shows the photographs of the gels at five minutes and one week after injection of PUASM aqueous solutions at pH 6.8 and pH 8.0 into Sprague–Dawley (SD) rats).

The possibility of releasing anionic protein from the amphoteric PUASM hydrogel was examined using hGH as a model protein. An *in vivo* release profile of hGH from the PUASM hydrogels in SD rats is presented in Fig. 5. The hGH concentration in the serum of the SD rats with hGH-loaded hydrogels was maintained at higher regarded effective concentration ($\geq$1 ng mL^{-1})[18] for more than 3 days with a minimal initial burst. Meanwhile, the hGH solution group (as a negative control group) showed a significantly initial burst release profile. The controlled release of hGH from the complex hydrogel was governed by ionic complexation between the anionic hGH and cationic moieties in the PUASM copolymer. In addition, the PUASM hydrogel was confirmed to be a biodegradable (Fig. S4 in ESI†) and low-cytotoxic, according to the ISO/EN 10993 Part 5 Guidelines[19] (Fig. S5 in ESI†), hydrogel system.

In summary, dually anionic and cationic copolymers have been successfully synthesized. Aqueous copolymer solutions exhibited special closed-loop reversible sol–gel–sol phase transitions as a function of temperature and pH with controllable gel regions. The aqueous copolymer solutions were administered into SD rats at both acidic and basic pH. The biodegradability, low-cytotoxicity, injectability and potential application as a protein carrier of the hydrogel system suggest that the material may provide a promising versatile approach for bioactive molecules delivery.

This study was supported by The Basic Science Research Program through a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (2010-0027955) and the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean Government (MEST) (2011-0019391).

Notes and references

1 (*a*) C. T. Huynh, M. K. Nguyen and D. S. Lee, *Macromolecules*, 2011, **44**, 6629; (*b*) M. K. Nguyen and D. S. Lee, *Macromol. Biosci.*, 2010, **10**, 563; (*c*) L. Yu and J. Ding, *Chem. Soc. Rev.*, 2008, **37**, 1473.
2 (*a*) B. Jeong, Y. H. Bae, D. S. Lee and S. W. Kim, *Nature*, 1997, **388**, 860; (*b*) M. H. Park, M. K. Joo, B. G. Choi and B. Jeong, *Acc. Chem. Res.*, 2012, **45**, 424; (*c*) U. P. Shinde, M. K. Joo, H. J. Moon and B. Jeong, *J. Mater. Chem.*, 2012, **22**, 6072; (*d*) G. Chang, L. Yu, Z. Yang and J. Ding, *Polymer*, 2009, **50**, 6111.
3 (*a*) A. S. Hoffman, *Adv. Drug Delivery Rev.*, 2002, **53**, 3; (*b*) Z. M. O. Rzaev, S. Dinçer and E. Pişkin, *Prog. Polym. Sci.*, 2007, **32**, 534.
4 H. K. Kim, W. S. Shim, S. E. Kim, K.-H. Lee, E. Kang, J.-H. Kim, K. Kim, I. C. Kwon and D. S. Lee, *Tissue Eng., Part A*, 2009, **15**, 923.
5 M. K. Nguyen, C. T. Huynh, G. H. Gao, J. H. Kim, D. P. Huynh, S. Y. Chae, K. C. Lee and D. S. Lee, *Soft Matter*, 2011, **7**, 2994.
6 D. P. Huynh, M. K. Nguyen, B. S. Pi, M. S. Kim, S. Y. Chae, K. C. Lee, B. S. Kim, S. W. Kim and D. S. Lee, *Biomaterials*, 2008, **29**, 2527.
7 D. P. Huynh, G. J. Im, S. Y. Chae, K. C. Lee and D. S. Lee, *J. Controlled Release*, 2009, **137**, 20.
8 C. T. Huynh, S. W. Kang, Y. Li, B. S. Kim and D. S. Lee, *Soft Matter*, 2011, **7**, 8984.
9 (*a*) Y. Ma, Y. Tang, N. C. Billingham and S. P. Armes, *Biomacromolecules*, 2003, **4**, 864; (*b*) M. D. Determan, J. P. Cox and S. K. Mallapragada, *J. Biomed. Mater. Res., Part A*, 2007, **81**, 326; (*c*) F. Bossard, T. Aubry, G. Gotzamanis and C. Tsitsilianis, *Soft Matter*, 2006, **2**, 510; (*d*) J. C. Garbern, A. S. Hoffman and P. S. Stayton, *Biomacromolecules*, 2010, **11**, 1833; (*e*) J. C. Garbern, E. Minami, P. S. Stayton and C. E. Murry, *Biomaterials*, 2011, **32**, 2407.
10 (*a*) C. T. Huynh, Q. V. Nguyen, S. W. Kang and D. S. Lee, *Polymer*, 2012, **53**, 4069; (*b*) M. K. Nguyen, D. K. Park and D. S. Lee, *Biomacromolecules*, 2009, **10**, 728; (*c*) M. K. Nguyen and D. S. Lee, *Chem. Commun.*, 2010, **46**, 3583; (*d*) C. T. Huynh, M. K. Nguyen, D. P. Huynh, S. W. Kim and D. S. Lee, *Polymer*, 2010, **51**, 3843.
11 (*a*) W. S. Shim, J.-H. Kim, H. Park, K. Kim, I. C. Kwon and D. S. Lee, *Biomaterials*, 2006, **27**, 5178; (*b*) W. S. Shim, S. W. Kim and D. S. Lee, *Biomacromolecules*, 2006, **7**, 1935.
12 C. T. Huynh, M. K. Nguyen, J. H. Kim, S. W. Kang, B. S. Kim and D. S. Lee, *Soft Matter*, 2011, **7**, 4974.
13 (*a*) C. T. Huynh and D. S. Lee, *Colloid Polym. Sci.*, 2012, **290**, 1077; (*b*) C. T. Huynh, M. K. Nguyen, D. P. Huynh and D. S. Lee, *Colloid Polym. Sci.*, 2011, **289**, 301.
14 B. G. Choi, Y. S. Sohn and B. Jeong, *J. Phys. Chem. B*, 2007, **111**, 7715.
15 J. M. Suh, S. J. Bae and B. Jeong, *Adv. Mater.*, 2005, **17**, 118.
16 S. Y. Lee, Y. Lee, J. E. Kim, T. G. Park and C.-H. Ahn, *J. Mater. Chem.*, 2009, **19**, 8198.
17 (*a*) C.-Y. Gong, S. Shi, L. Wu, M.-L. Gou, Q.-Q. Yin, Q.-F. Guo, P.-W. Dong, F. Zhang, F. Luo, X. Zhao, Y.-Q. Wei and Z.-Y. Qian, *Acta Biomater.*, 2009, **5**, 3358; (*b*) M. S. Shim, H. T. Lee, W. S. Shim, I. Park, H. Lee, T. Chang, S. W. Kim and D. S. Lee, *J. Biomed. Mater. Res.*, 2002, **61**, 188.
18 M.-R. Park, C.-J. Chun, S.-W. Ahn, M.-H. Ki, C.-S. Cho and S.-C. Song, *Biomaterials*, 2010, **31**, 1349.
19 D. Park, W. Wu and Y. Wang, *Biomaterials*, 2011, **32**, 7.