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Biodegradable thermoreversible gelling PLGA-g-PEG copolymers†

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The thermogelling aqueous solution of poly(DL-lactic acid-*co*-glycolic acid) grafted with poly(ethylene glycol)s is developed, and the elegant instrumental methods to determine sol–gel transition temperature and the method to control gel duration are reported.

Self-assembly of polymers by external stimuli has been studied extensively over the past decade.[1,2] *In situ* gelling systems have recently generated attention as promising biomaterials.[3–6] To apply *in situ* gelling systems for drug delivery and tissue engineering, the aqueous polymer solution should be free flowing at a certain temperature and form a gel at the physiological temperature. The resultant gel must have reasonable mechanical strength to persist as a drug-releasing depot over the designed lifetime.

The synthesis of thermogelling poly(ethylene glycol-*b*-(DL-lactic acid-*co*-glycolic acid)-*b*-ethylene glycol) (PEG-PLGA-PEG) triblock copolymers by Jeong *et al*. required two steps.[7,8] The ring-opening polymerization of DL-lactide and glycolide on methoxy poly(ethylene glycol), followed by the coupling of the PEG-PLGA diblock with hexamethylene diisocyanate (HMDI) requires strict anhydrous conditions. The resulting triblock copolymer contains two urethane linkages, which might affect the degradation profile of the polymer. However, there is a limitation of molecular weight for PEG-PLGA-PEG copolymers by a triblock topology. Because the sol–gel transition temperature strongly depends on the PEG length, the total molecular weight of PEG-PLGA-PEG triblock copolymers should be 4000–5000, if aqueous solutions are to show a sol–gel transition below 37 °C.[7]

In this paper, thermogelling aqueous solutions of biodegradable graft copolymers, poly(DL-lactic acid-*co*-glycolic acid) grafted with poly(ethylene glycol)s (PLGA-g-PEG) are reported and we will show that such a molecular weight limitation can be overcome by graft copolymer systems.

PLGA-g-PEG copolymers were synthesized by one-step ring opening polymerization of DL-lactide, glycolide, and epoxy terminated poly(ethylene glycol) (PEG; M = 600) using stannous octanoate, as a catalyst.[9] The final DL-lactic acid/glycolic acid/ethylene glycol mol ratio of 3.2/1/2.8 was determined by ^{1}H NMR spectroscopy. Therefore, the grafting frequency of PEG is 4.7 mol%. Gel permeation chromatography (GPC) using light scattering and refractive index detectors in series has been used to determine the absolute molecular weight of polymers.[10] GPC results found a unimodal distribution for our polymers. The number average molecular weight (M_n) and polydispersity (M_w/M_n) of the polymers determined by GPC using tetrahydrofuran (THF) as an eluting solvent were 9300 Daltons and 1.5, respectively. Therefore, the 4–5 PEGs are grafted onto the PLGA backbone.

At 23 °C, the viscosity of the aqueous solution (25 wt%) was *ca*. 0.3 P (g m^{-1} s^{-1}). This viscosity is suitable for injecting the solution through a 25-gauge syringe needle. With increasing temperature, the aqueous solution (25 wt%) of PLGA-g-PEG undergoes a sol–gel transition at 30 °C. In the practical application, the gel should keep its equilibrium-swollen state in an excess amount of water. Further increase in temperature above 50 °C of the PLGA-g-PEG aqueous solution results in a macroscopic phase separation between gel and water. The entire phase diagram will be reported elsewhere.[11] The gel state has been traditionally defined as a non-flowing semisolid in a test-tube inversion test. The method is controversial in its simplicity and lack of scientific rigidity.

To address this issue, the sol–gel transition of the graft copolymer aqueous solution was investigated using dynamic rheometry (Rheometric Scientific: SR 2000).[12] The polymer solution was placed between parallel plates of 25 mm diameter and a gap of 0.5 mm. The data were collected under a controlled stress (4.0 dyn cm^{-2}) and a frequency of 1.0 radian s^{-1}. The heating and cooling rate was 0.2 °C min^{-1}. According to dynamic rheometry, the sol–gel transition was identified in a more reproducible and quantitative manner than by the test-tube inversion method.

The storage moduli (G') of PLGA-g-PEG aqueous solutions varying from 22 to 29 wt% were measured at a heating rate of 0.2 °C min^{-1}. As the temperature increased, the storage modulus increased abruptly at the sol–gel transition temperature. The gels have a modulus of *ca*. 50 dyn cm^{-2} and are slightly affected by concentration in the range of 22–29 wt%. The sol–gel transitions occur at *ca*. 30 °C, suggesting easy formulation at room temperature.

To confirm the reversibility of the sol–gel transition, a 25 wt% PLGA-g-PEG aqueous solution was studied. The real part (η') of complex viscosity of the polymer solution, which is a measure of dissipated energy when cyclic deformation is applied to a material, is shown as a function of temperature in Fig. 1(a). During the first heating cycle (H1), η' increased 1000 times upon the sol–gel transition. The cooling curve (C1) shows that the gel phase persisted over the temperature range of 43–20 °C during the experiment. This fact results from the difficulty in molecular motion to occur in the gel phase; once the solution forms a gel, the gel resists rehydration. η' abruptly decreased at 15 °C due to the gel–sol transition during the cooling of the system. The second heating curve (H2) shows the sol–gel transition at practically the same temperature as the first heating curve (H1).

The storage modulus (G') of the PLGA-g-PEG aqueous solution (25 wt%), which is a measure of stored energy when a cyclic deformation is applied to a material, approaches zero in the sol state and is not shown in the heating curve [Fig. 1(b); H1]. G' sharply increased during the sol–gel transition at 32 °C as shown in the heating curves. The maximum value for G' was observed in the temperature range 35–39 °C, indicating a promising material for *in vivo* (37 °C) applications. During the cooling cycle (C1), the gel modulus increased over the temperature range 43–20 °C, exhibiting similar behavior to typical elastic materials, and dropped abruptly at 15 °C due to the gel–sol transition. During the first (H1) and second (H2) heating cycle, practically the same transition curve was measured for G', indicating reversible gelation. The decrease in G' at temperatures above 40–45 °C can be expected due to an increase in thermal motion. This trend was also observed in the ^{13}C NMR spectra (see ESI†).

† Electronic supplementary information (ESI) available: ^{1}H NMR spectrum of PLGA-g-PEG in $CDCl_3$. ^{13}C NMR (75 MHz) spectra of 25 wt% PLGA-g-PEG copolymer in D_2O as a function of T. See http://www.rsc.org/suppdata/cc/b1/b102819g/

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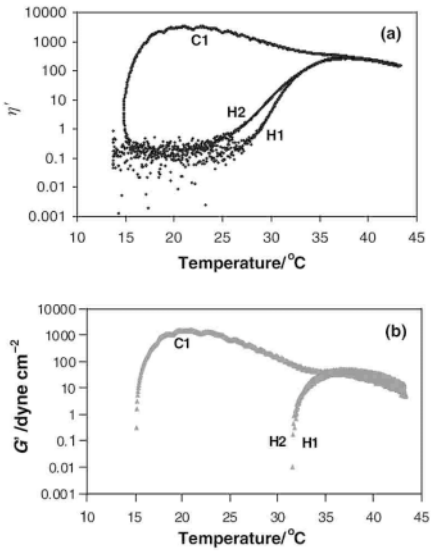

Fig. 1 Rheological study of PLGA-g-PEG copolymer aqueous solutions (25 wt%). The thermogram was obtained while heating and cooling with a rate of 0.2 °C min^{-1}. Temperature programming sequence was heating → cooling → heating. H1, C1 and H2 indicate the first heating cycle, cooling cycle and the second heating cycle, respectively. The real part (η') of complex viscosity, (a), and the storage modulus (G'), (b), of the copolymer solution were measured as a function of temperature.

The ^{13}C NMR spectra of a 25 wt% copolymer solution in D_2O were recorded with increasing temperatures. In the sol state (20 °C), the methyl peak of the hydrophobic PLGA (δ 18) is collapsed and broadened compared with PEG peak (δ 72) whereas that in $CDCl_3$ appears as a sharp peak, indicating a core–shell structure of this polymer in water. The core–shell structure of these amphiphilic copolymers was also confirmed by micelle formation in dilute aqueous solutions. The critical micelle concentration (CMC) determined by a dye solubilization method was 0.03 wt% at 20 °C.[13]

Just above the sol–gel transition temperature (33 °C) of an aqueous PLGA-g-PEG solution (25 wt%), the ^{13}C NMR peak shapes of both the hydrophobic PLGA methyl peak and the hydrophilic PEG peak are preserved except that the PEG peak is shifted down field about 0.3 ppm. With a further increase in temperature, the peak height of the PLGA methyl peak increases, and the PEG peak is split into two peaks, a sharp one at δ 72.4 and a broad one at δ 72.7. These behaviors are thought to be caused by an increase in molecular motion of the hydrophobic backbone and phase mixing between PEG and PLGA. Phase mixing between PEG and PLGA or PLLA has been previously reported.[14] A further increase in temperature resulted in macrophase separation between water and the polymer.

The reversibility of the sol–gel transition is also confirmed by deuterium NMR spectroscopy (Fig. 2). The peak at δ 4.8 at 20 °C (sol state) shifted to δ 4.6 at 33 °C (just above sol–gel transition), δ 4.58 at 37 °C (gel state), δ 4.56 at 40 °C, and δ 4.5 at 50 °C (syneresis). The change in chemical shift was most pronounced during the sol–gel transition (δ 0.2). When the system is cooled to 20 °C, the deuterium peak reappears at δ 4.8, indicating the reversibility of the transition. In a sol state, water moves more freely than in a gel state. During the sol–gel transition, PEG becomes more hydrophobic due to dehydration and the extent of hydrogen bonding between water molecules and polymers changes. Therefore, the time average environment around deuterium nuclei will be affected, leading to the changes in chemical shift of water during the sol–gel transition. This finding suggests that the deuterium NMR can be a good method for the determination of the sol–gel transition.

The sol–gel transition temperature could be controlled from 20 to 40 °C by changing PEG length and composition. When the PEG molecular weight of PLGA-g-PEG was increased from 600 to 1000 the sol–gel transition occurred at 40 °C, whereas the sol–gel transition occurred at 20 °C when the PEG composition was decreased by 20 mol%.[11]

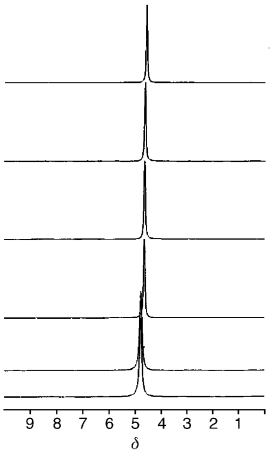

Fig. 2 Deuterium NMR showing reversibility of the sol–gel transition. The deuterium NMR spectra of PLGA-g-PEG in D_2O (25 wt%) were recorded at 20 (5th row from the top), 33 (4th row), 37 (3rd row), 40 (2nd row), 50 (1st row) °C, and after cooling to 20 °C (6th row). The sample was held at each temperature for 30 min.

To conclude, an aqueous PLGA-g-PEG system showing a reversible sol–gel transition at slightly elevated temperatures is developed and a dynamic mechanical test and deuterium NMR confirm the reversibility of this process. These tools can be suggested as standard methods to determine the sol–gel transition temperature than the more empirical test-tube inverting method reported most often.

The system developed in this study is very promising for injectable local delivery of pharmaceutical agents. *In vitro* study shows that the duration of gels could be controlled from one week to three months by mixing slow degrading PLGA-g-PEG and fast degrading PEG-g-PLGA in different ratios.[15] Both polymer aqueous solutions undergo a sol–gel transition around 30 °C. This is very important for applications in drug delivery and tissue engineering, which need the control of gel duration as a matrix or carrier.

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