Rheological Properties of PLGA-PEG-PLGA Copolymers for Ophthalmic Injection

Bochu Wang, Yang Cao, Lichun Yang, Yazhou Wang

College of Bioengineering, Chongqing University, Chongqing 400030, People's Republic of China

Received 13 March 2011; accepted 2 September 2011
DOI 10.1002/app.35584
Published online 19 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Polymer rheological property is one of intrinsic properties for the design and preparation of intravitreal injection systems. Rheological behaviors of thermosensitive poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly (lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers were investigated in this article. The rigidity phase angle (δ), elastic modulus (G'), viscous modulus (G''), and complex viscosity (η*) were determined. The injectability of polymers was evaluated as well. The results indicated both temperature and concentration were key parameters influencing elasticity of polymers. Owing to low complex viscosity (below 1 Pa s), PLGA-PEG-PLGA polymers can be successfully injected at room temperature. When the temperature was raised to 37°C, the complex viscosity increased (over 4 Pa s). Thus, suitable rheological properties ($G' > G''$; tan $\delta < 1$) were obtained for injection administration. Elastic modulus (G'), viscous modulus (G''), and complex viscosity (η*) were diminished when polymer solutions were ejected through syringe needles (25 gauge, G). Ejection force (from 4.21 to 19.42 N) was required in the process of injecting administration through syringe needles (24, 25, and 26 G) for polymer solutions at 20, 25, and 30% (w/v) concentration. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 370–375, 2012

Key words: rheology; PLGA-PEG-PLGA copolymers; phase behavior; drug delivery system

INTRODUCTION

Although eye drops represent 90% of all ophthalmic dosage forms, there is a significant effort directed toward new drug delivery systems for ophthalmic administration.[1] *In situ* forming hydrogel, as a kind of promising ophthalmic drug delivery system, has been extensively studied during last two decades.[2–7] It can respond to small changes in phenomenon such as temperature, pH, and electrolyte composition *in vivo*, forming physically crosslinked hydrogels by sol-gel phase transition.[4,6,8–10]

The vitreous body consists of collagenous fibers, which are organized as reticular structures. Approximately 99% of the vitreous is water. This viscous liquid system with collagen, hyaluronan, and proteoglycans acts as a shock absorber, supporting the shape of the eye and positioning the retina.[11] The administration of drugs into the eye requires specific rheological properties that are different during preparation of the drugs and when they are injected into the vitreous body. When preparing the drugs a low viscosity is beneficial because they will easily disperse into the polymeric solution. However, once the polymer is inside the eye, it is desirable that the solution should have the rheological properties similar to those of the native vitreous body, thus avoiding wastage of the drugs through the retina. In addition, some elasticity is necessary to maintain the physical structure of the vitreous body, whereas a high rigidity should be avoided to ensure no damage occurs to the surrounding tissue.[12–14]

In addition, it is preferable to inject polymers through small-gauge needles with small incisions, and the rheological properties should be retained after injection. Previous work with preformed polymeric hydrogels has demonstrated the influence of injection process on the viscoelastic properties and resiliency after injection through a small-gauge needle.[15] When the human vitreous was removed using a 30 gauge (G) needle, it was less viscous and visibly more homogenous than when removed using a larger 16 G needle. However, injection with smaller inner diameters requires more ejection force. Thus, it is important to evaluate polymer injectability in drug administration.

Recent years have witnessed the increasing number of researches on the intravitreal drug injection with thermosensitive polymers.[2,4,16–21] Poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers present excellent features such as biocompatibility and degradability as well as thermosensitivity, making the copolymer as an ideal

Correspondence to: B. Wang (wangbc2000@126.com).
Contract grant sponsor: Chongqing University Postgraduates' Innovative Team Building Project; contract grant number: 201105A1001.

© 2011 Wiley Periodicals, Inc.

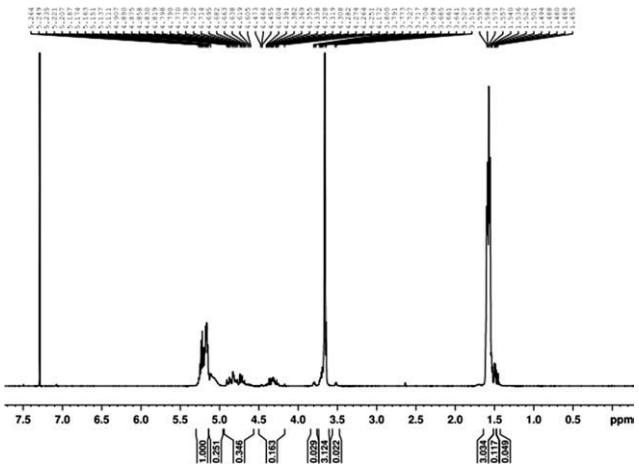

Figure 1 The ^{1}H-NMR spectra of PLGA-PEGP-PLGA copolymer.

candidate for injection *in vivo*.[22–27] Since most of the biological environment is hydrophilic in nature. Hydrophilic poly(ethylene glycol) (PEG) shell, which can suppress the adsorption of opsonin and clearance by the mononuclear phagocyte system, is supposed to improve the biocompatibility of the delivery carriers by alternating hydrophilicity degree of the surface, while the hydrophobic core of D,L-lactide (D,L-LA) and glycolide (GA) can solubilize hydrophobic drugs.[23]

When used as vitreoretinal drug delivery systems, PLGA-PEG-PLGA hydrogels should have proper rheological behaviors to fit the requirement of injection administration at different stages. In a former study, PLGA-PEG-PLGA copolymers were synthesized by a ring-opening method with D,L-LA and glycolide molar ratio of 6 : 1.[22] These copolymers are thermosensitive and will present different rheological behavior at ambient and body temperature. Considering the properties required for the preparation and delivery of the drugs, this article evaluates the rheological properties of the PLGA-PEG-PLGA copolymers to determine if they are suitable for use as intravitreal drug delivery systems. Meanwhile, the influence of the injection process upon polymer rheological properties was investigated by conduction of tests after injection and the determination of the ejection force necessary to inject the polymer.

EXPERIMENTAL

^{1}H nuclear magnetic resonance (NMR) and molecular weight evaluation

The structure and composition of PLGA-PEG-PLGA copolymers were determined by NMR. ^{1}H-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) using a NMR instrument (Bruker AVANCE-500, Switzerland) at 300 MHz. The chemical shift of tetramethylsilane was taken to be zero.[28,29] The average of molecular weight of tripolymers was calculated by end-group analysis, based on the ^{1}H-NMR spectra.[30,31]

Rheological behavior studies

Samples (1.5 mL) were taken and rheological behaviors were investigated by Bohlin rotational rheology instrument (Malverm, UK) at 1 Hz, with increasing temperatures by 1°C per minute from 5 to 60°C. Polymer solutions (20, 25, and 30%, w/v) were prepared and equilibrated at 8°C for 12 h. Syringes (1 mL, 25 G) were used to inject the solutions with given concentrations to the rotational rheology instrument.[16,32] The polymer solution was placed between parallel plates of 40 mm diameter and a gap of 0.5 mm. The sample plates were covered carefully to minimize solvent evaporation. The rigidity phase angle (δ), shear storage modulus or elastic modulus (G') and shear loss modulus or viscous modulus (G'') of copolymer solutions (20, 25, and 30%, w/v) were evaluated as a function of temperature. The experiments were repeated three times at each condition and the results presented are averages.

Injectability of copolymers

The injectabilities of hydrogels with different concentrations through needles were investigated by Instron universal testing machine. Polymer solutions (20, 25, and 30, w/v) were prepared and equilibrated at 8°C for 12 h. The solutions (1 mL) were ejected through needles with given inner diameters (24, 25, and 26 G) at room temperature.[33] Ejection force was measured by Instron universal testing machine at 100 mm/min. The experiments were repeated three times at each condition and the results presented are averages.

Statistical analysis

Values are presented as the mean ± standard deviation. Statistical comparisons were made using Student's *t*-test. A value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

^{1}H-NMR analysis and molecular weight evaluation

As shown in Figure 1, chemical structures of copolymers were analyzed by ^{1}H-NMR. The typical spectrum was very similar to the reported spectrum and all the signals were assigned on the spectrum.[22,28] The characteristic signals appearing at 5.187, 4.338, 1.571, 4.738, and 3.661 ppm are assigned to CH of D,L-LA (peak area = 1.000), CH of D,L-LA (peak area = 0.163),

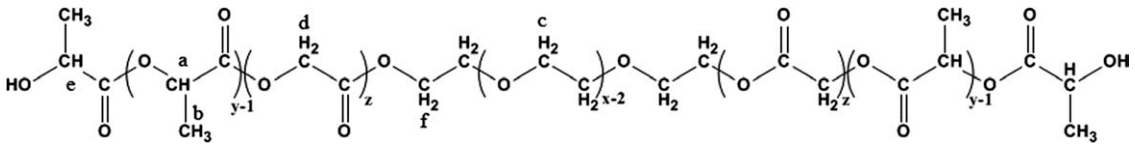

Figure 2 The molecular structure of PLGA-PEG-PLGA copolymer.

CH_3 of D,L-LA (peak area = 3.034), CH_2 of GA (peak area = 0.346), and CH_2 of PEG (peak area = 3.124). The complicated split in these peaks was due to the random copolymerization of glycolide and lactide.[28]

Average molecular weight of copolymers was calculated by end-group analysis.[30] Figure 2 showed the molecular structure of PLGA-PEG-PLGA copolymers. The peak areas of CH of D,L-LA, CH_2 of GA, CH_2 of PEG and CH_3 of D,L-LA were substitutes into eqs. (1), (2), and (3) and the average molecular weight obtained was 8337.

$$y - 1 = A_{CH_3 \text{ of D,L-LA}}/A_{CH \text{ of D,L-LA}} \quad (1)$$

$$2z/(y-1) = A_{CH_2 \text{ of GA}}/A_{CH \text{ of D,L-LA}} \quad (2)$$

$$4x/(y-1) = A_{CH_2 \text{ of PEG}}/A_{CH \text{ of D,L-LA}} \quad (3)$$

$$M_n = y \times 2 \times 72 + z \times 2 \times 58 + x \times 1500 = 8337.$$

Rheological behavior of copolymers

Rheological behavior of native copolymers

As shown in Figure 3, shear storage modulus (G', which gives the elastic nature of the gels) and shear loss modulus (G'', which gives the viscous nature) of copolymer solutions (20, 25, and 30%, w/v) were evaluated as a function of temperature. G' describes the elasticity of copolymer solutions under deformation (the loss modulus is neglected), whereas G'' demonstrates the viscous properties.[13] At temperature below room temperature (25°C), viscous behaviors is exhibited ($G'' > G'$), rigidity phase angle (δ) was near 90°, tan δ (G''/G')>1. Increasing temperature from 5 to 25°C, G' and G'' values remain constant. The polymer solution can freely flow, behaving as a Newtonian fluid with a low viscosity at this condition, suggesting the good injectability. When temperature increases over 25°C, G' and G'' curves raised sharply, especially at 30, 28, and 25°C for 20, 25, and 30% copolymer solutions, respectively. Usually, the abrupt increase of G' corresponds to the sol-gel transition.[32] When G' overpass G'', tan δ decreased and the elastic properties of copolymer solutions is enhanced, leading to low polymer flowability. When $G' = G''$ (tan δ = 1), the temperature is defined as sol-gel transition temperature. The sol-gel transition temperature for 20, 25, and 30% (w/v) PLGA-PEG-PLGA copolymer solutions were 34.5, 34.1, and 34.5°C in this study.

At temperatures below sol-gel transition temperature, some bridging micelles of PLGA-PEG-PLGA copolymers formed. They were not stable due to the low hydrophobicity of PLGA.[6] With increasing temperatures higher than sol-gel transition temperature, G' is larger than G'' and tan δ decreased continuously. A bridged micelle network was formed because of an increase in the hydrophobicity of the PLGA segment, leading to gelation.[6,32,34] Polymer solutions were transformed to translucent gelatins, suggesting the predominant state of elasticity. Increasing the temperature the PLGA ends groups reduce their hydrophobicity increasing their ability to move through water and promote micelles interconnection that are responsible for the gel

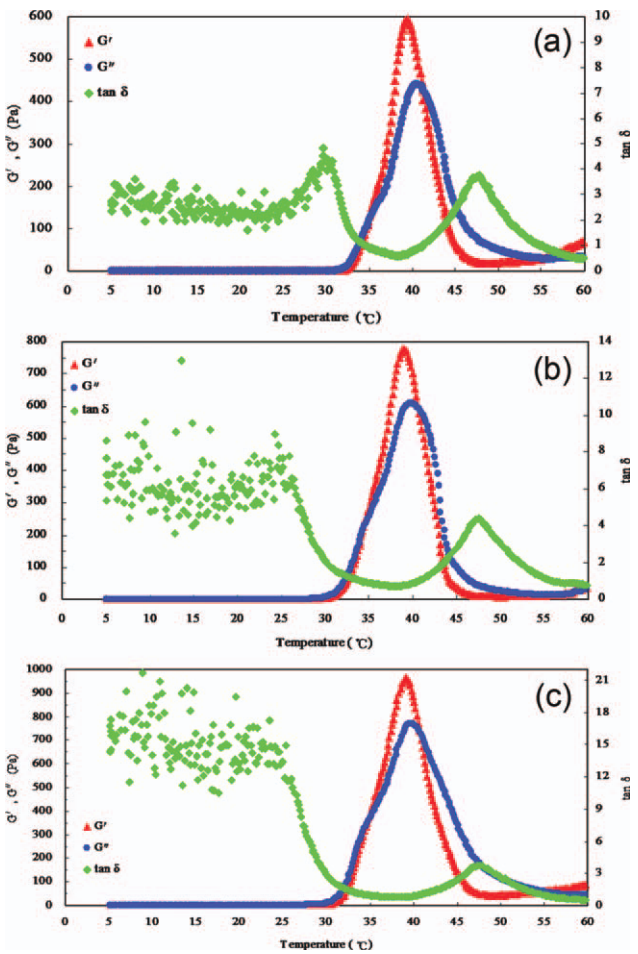

Figure 3 Rheological properties of PLGA-PEG-PLGA copolymers (a) 20% copolymers, (b) 25% copolymers, and (c) 30% copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I
Maximum G' and G'' Values Measured for PLGA-PEG-PLGA Copolymer Solutions by Rotational Rheology from 5–60°C and the Temperature at Which They Occurred

Samples[a]		20% (w/v)	25% (w/v)	30% (w/v)
G' max and corresponding temperature	Native solution (Pa; °C)	593.53 ± 3.23 39.4 ± 0.0	776.44 ± 7.32 38.9 ± 0.3	964.64 ± 10.39 39.2 ± 0.1
	Through 25 G needles (Pa; °C)	556.92 ± 4.19 39.4 ± 0.2	708.39 ± 8.93 39.1 ± 0.2	956.86 ± 11.73 39.0 ± 0.2
G''max and corresponding temperature	Native solution (Pa; °C)	440.19 ± 3.09 40.7 ± 0.3	608.70 ± 7.36 39.8 ± 0.2	772.31 ± 8.39 39.6 ± 0.1
	Through 25 G needles (Pa; °C)	428.99 ± 5.47 40.7 ± 0.1	517.02 ± 6.49 40.0 ± 0.0	748.20 ± 7.98 39.7 ± 0.2

[a] Maximum G' and G'' values of samples are significantly different ($P < 0.05$).

formation.[6] PLGA-PEG-PLGA copolymers achieve a maximum value for a specific temperature as shown in Table I. Then G' and G'' values decreased significantly ($G'' > G'$) and the polymer suspension came out. When both G' and G'' values were below 100 Pa and the temperature higher than the sol-gel transition, polymers were precipitated.

The results showed that both the viscous and elastic properties of copolymers depended on the solution concentrations (Fig. 3; Tables I and II). When compared to copolymer solutions with different concentration, it required different temperature to achieve G'max values.

Rheological behavior of copolymers after injection process

The rheological properties of PLGA-PEG-PLGA copolymers were studied after ejecting through 25 G needles as well. As shown in Figure 4, the curves were similar to the copolymers without ejection. G' and G'' values kept constant with increasing temperature before 25°C. PLGA-PEG-PLGA copolymer solutions exhibited as a Newtonian fluid. With increasing temperature, G' and G'' increased continually. The abrupt increase G' occurred at 32.3, 31.6, and 31.1°C for 20, 25, and 30% copolymer solutions and the sol-gel transition temperature were 34.6, 35.0, and 34.5°C, respectively.

In the injection administration, the applied shear causes a large deformation of the copolymer, and the degree of deformation under constant stress is related to the structure of the polymer chains.[15] The macromolecular chains overlap extensively creating a tunnel for each chain wherein the movement is restricted. The chain cannot move freely, because it cannot pass through or across other chains as this process would break other chains. Therefore, the crosslinkage and interaction of copolymers were broken to allow the chain movement.[15] PLGA-PEG-PLGA micelles and monomers in solutions increased while on the other hand, polymer aggregation was suppressed and diminished resulting in low elastic and viscous moduli. As shown in Tables I and II, G' and G'' values were both smaller after injection ($P < 0.05$).

Complex viscosities of PLGA-PEG-PLGA copolymers

Complex viscosity of PLGA-PEG-PLGA copolymers was also measured (Fig. 5). The complex viscosity describes the relationship between the dynamic viscosity and the out-of-phase viscosity, or imaginary part of the complex viscosity. The results showed that copolymer solutions exhibit a sol-like behavior at temperature below 25°C (room temperature) and at this condition, the complex viscosity (η^*) was independent of polymer concentration. However, when the temperature increased above 30°C, η^* increased sharply and the complex viscosity was significantly influenced by temperature as well as concentration ($P < 0.05$). The corresponding temperatures for abrupt complex viscosities increment were different for each polymer concentration here studied. η^* increased at a faster rate with increasing temperature. In addition, higher polymer concentration induced lower temperature phase transition and stronger gel rigidity. As shown in Figure 5, the

TABLE II
G' and G'' of PLGA-PEG-PLGA Copolymers at 37°C

Samples (Pa)		20% (w/v)	25% (w/v)	30% (w/v)
G'	Native solution	288.38 ± 1.76	525.71 ± 8.03	652.95 ± 4.98
	Through 25 G needles	286.20 ± 1.82	408.14 ± 7.42	651.95 ± 3.39
G''	Native solution	209.40 ± 0.78	397.49 ± 3.44	516.16 ± 5.33
	Through 25 G needles	200.17 ± 1.23	291.31 ± 4.01	499.43 ± 4.09

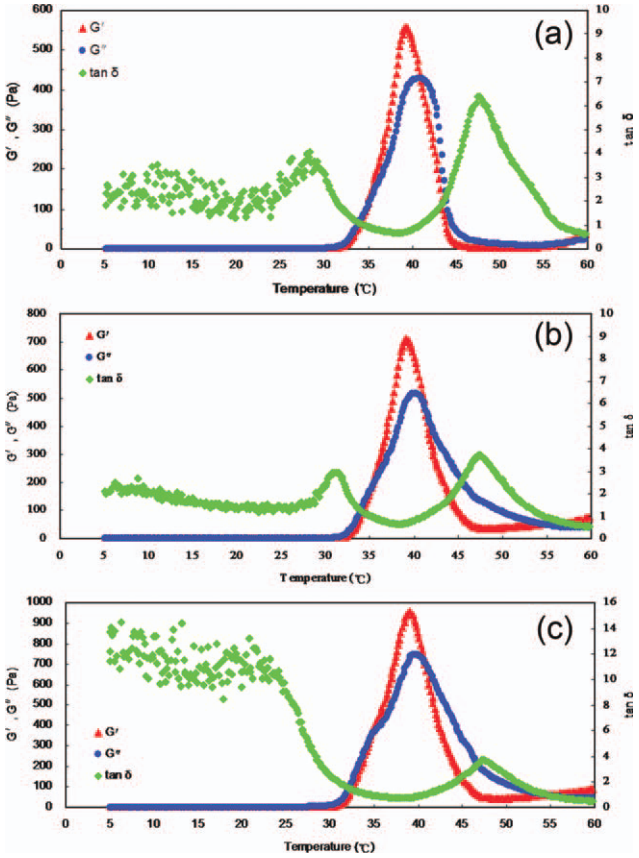

Figure 4 Rheological properties of PLGA-PEG-PLGA copolymers ejection through 25 G needles (a) 20% copolymers, (b) 25% copolymers, and (c) 30% copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

maximum complex viscosity values were, 114.58, 155.52, and 195.68 Pa s, for 20, 25, and 30% polymer concentrations, respectively. The complex viscosities of these three samples were 56.7, 106.00, and 132.47 Pa s at 37°C. As expected, polymer solution with 30% concentration had the highest complex viscosity. Our results show that after the samples passed through 25 G needles, the complex viscosities were all diminished ($P < 0.05$). Polymer solutions with 20, 25, and 30% had the maximum values of 110.00, 137.16, and 191.66 Pa s, respectively. At the temperature of 37°C, the corresponding complex viscosities were 55.58, 79.80, and 130.96 Pa s, respectively.

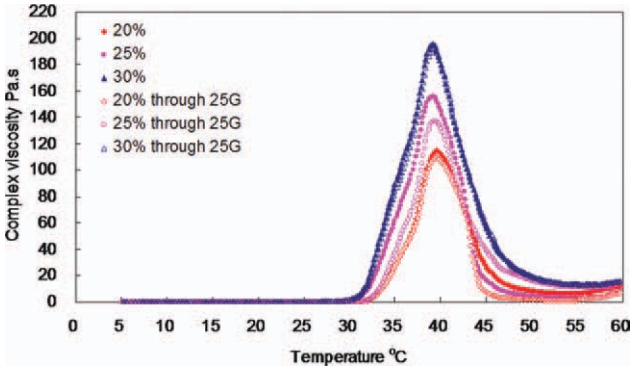

Figure 5 Complex viscosities of PLGA-PEG-PLGA copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Generally, solutions with complex viscosity <1 Pa s are able to go through needles smoothly at room temperature.[35] In this study, polymer solutions with 20, 25, and 30% concentrations had complex viscosities of 0.03, 0.06, and 0.18 Pa s, respectively, at 25°C. Therefore, all samples can be administrated by injection at room temperature. In addition, the viscosity of vitreous body *in vivo* is larger than 4 Pa s at a shear rate of 0.15/s and it can form a gel with $G' > G''$.[36] At temperature of 37°C, complex viscosities of polymers with three given concentrations were all larger than 4 Pa s and G' was larger than G'' (tan $\delta < 1$), indicating elasticity and viscosity that is similar to the vitreous body. These results confirmed that PLGA-PEG-PLGA copolymers were fit for vitreoretinal drug delivery.[13,15]

Injectability of copolymers

Injectability of hydrogel is an important parameter for intravitreal drug deliver systems, which are administrated by injection. To evaluate the influence of needles with different inner diameters on polymer solutions injectability, needles with inner diameter of 24, 25, and 26 G were chosen to measure the injectability of PLGA-PEG-PLGA copolymer in this study (Table III). Owing to low complex viscosity, polymer solutions with 20, 25, and 30% (w/v) can flow through all three needles smoothly at room temperature (25°C). As for the same needle, ejecting

TABLE III
Injectability of PLGA-PEG-PLGA Copolymers Through Needles at 25°C

	Samples[a]	Air	Water	20% (w/v)	25% (w/v)	30% (w/v)
24 G	Maximum force (N)	1.33 ± 0.00	1.67 ± 0.05	4.21 ± 0.22	8.46 ± 0.38	9.17 ± 0.42
	Time (s)	8.67 ± 0.03	8.59 ± 0.14	8.60 ± 0.41	8.42 ± 0.23	8.34 ± 0.42
25 G	Maximum force (N)	1.46 ± 0.02	2.71 ± 0.00	7.29 ± 0.15	12.40 ± 0.75	16.42 ± 0.25
	Time (s)	8.44 ± 0.02	8.44 ± 0.00	8.44 ± 0.28	8.74 ± 0.04	8.76 ± 0.46
26 G	Maximum force (N)	1.50 ± 0.00	2.71 ± 0.07	8.33 ± 0.22	17.75 ± 0.50	19.42 ± 0.42
	Time (s)	8.87 ± 0.29	9.17 ± 0.38	9.12 ± 0.46	8.76 ± 0.06	8.74 ± 0.26

[a] All the values are significantly different ($P < 0.05$).

force increases when increasing polymer concentration, which may be related with micelles assembled in the different concentration solutions. High concentration can induce the formation of more micelles and shorter distance among chains of copolymers that are acting as crosslinkage chains. The interaction of polymer chains may be enhanced. Therefore, high shear stress was needed to break the balance of copolymer systems.

Larger inner diameters of needles lead to smaller ejection force for polymer solutions with the same concentration. The drag force, however, increased in case of needles with small inner diameters. For example, among all PLGA-PEG-PLGA copolymer samples, only 4.21 N (minimum) was taken to eject 20% polymer solution through 24 G needles, whereas, it took 19.42 N (maximum) to eject 30% polymers through 26 G needles. It is reported that application of a maximum ejection force of 12 N over 10 s can be considered as a suitable development criterion.[37,38] In this article, some samples (25 G : 25%, 30%, w/v; 26 G : 25%, 30%, w/v) took more than 12 N ejection force within 10 s. Due to high ejection speed of the experiments, the force was enlarged correspondingly.

CONCLUSIONS

Elastic modulus (G') and complex viscosity (η^*) of PLGA-PEG-PLGA polymers were found significantly affected by polymer concentrations and temperatures ($P < 0.05$). Ejection of the polymer through a 25 G needle caused reduction of G', G'', and η^* values probably because polymer structure was affected by the shear force. The study showed that the complex viscosities of 20, 25, and 30% (w/v) polymer solutions were all <1 Pa s, indicating the solutions can be injected through syringe needles smoothly at room temperature, while the complex viscosities were increased to over 4 Pa s ($G' > G''$; $\tan \delta < 1$) at 37°C. The hydrogels had the similar elasticity and viscosity properties as those of the native vitreous body *in vivo*. The study of injectability of copolymer hydrogels through 25 G needle suggested that the ejection force ranging from 4.21 to 19.42 N was required for PLGA-PEG-PLGA hydrogels injection administration.

References

1. Kaur, I. P.; Garg, A.; Singla, A. K.; Aggarwal, D. Int J Pharm 2004, 269, 1.
2. He, C.; Kim, S. W.; Lee, D. S. J Controlled Release 2008, 127, 189.
3. Jeong, B.; Bae, Y. H.; Kim, S. W. J Controlled Release 2000, 63, 155.
4. Nanjawade, B. K.; Manvi, F. V.; Manjappa, A. S. J Controlled Release 2007, 122, 119.
5. L, X.; C, Y.; Lloyd, A. W.; Mikhalovsky, S. V.; Sandeman, S. R.; Howel, C. A.; Liao, L. Contact Lens Anterior Eye 2008, 31, 57.
6. Nguyen, M. K.; Lee, D. L. Macromol Biosci 2010, 10, 563.
7. Tang, Y.; Singh, J. Int J Pharm 2009, 365, 34.
8. Hsiue, G. H.; Hsu, S. H.; Yang, C. C.; Lee, S. H. Biomaterials 2002, 23, 457.
9. Liu, C. B.; Gong, C. Y.; Huang, M. J.; Wang, J. W.; Pan, Y. F.; Zhang, Y. D.; Li, G. Z.; Gou, M. L.; Tu, M. J.; Wei, Y. Q.; Qian, Z. Y. J Biomed Mater Res Part B 2007, 84B, 165.
10. Singh, S.; Webster, D. C.; Singh, J. Int J Pharm 2007, 341, 68.
11. Colthurst, M. J.; Williams, R. L.; Hiscott, P. S.; Grierson, I. Biomaterials 2000, 21, 649.
12. Edsman, K.; Carlfors, J.;Petersson R. Eur J Pharm Sci 1998, 6, 105.
13. Maltese, A.; Borzacchiello, A.; Mayol, L.; Bucolo, C.; Maugeri, F.; Nicolais, L.; Ambrosio, L. Biomaterials 2006, 27, 5134.
14. Jeong, B.; Kim, S. W.; Bae, Y. H. Adv Drug Delivery Rev 2002, 54, 37.
15. Chirila, T. V.; Hong, Y.; Dalton, P. D.; Constable, I. J.; Refojo, M. F. Prog Polym Sci 1998, 23, 475.
16. Swindle-Reilly, K. E.; Shah, M.; Hamilton, P. D.; Eskin, T. A.; Kaushal, S.; Ravi, N. Invest Ophthalmol Vis Sci 2009, 50, 4840.
17. Ruel-Gariépy, E.; Leroux, J. Eur J Pharm Biopharm 2004, 58, 409.
18. Yasukawa, T.; Ogura, Y.; Tabata, Y.; Kimura, H.; Wiedemann, P.; Honda, Y. Prog Retin Eye Res 2004, 23, 253.
19. Barbu, E.; Verestiuc, L.; Nevell, T. G.; Tsibouklis, J. J Mater Chem 2006, 16, 3439.
20. Chung, H. J.; Lee, Y.; Park, T. G. J Controlled Release 2008, 127, 22.
21. Diebold, Y.; Calonge, M. Prog Retin Eye Res 2010, 29, 596.
22. Qiao, M.; Chen, D.; Ma, X.; Liu, Y. Int J Pharm 2005, 294, 103.
23. Song, Z.; Feng, R.; Sun, M.; Guo, C.; Gao, Y.; Li, L.; Zhai, G. J Colloid Interface Sci 2011, 354, 116.
24. Choi, S.; Kim, S. W. Pharm Res 2003, 12, 2008.
25. Kwon, Y. M.; Kim, S. W. Pharm Res 2004, 21, 339.
26. Qiao, M.; Chen, D.; Hao, T.; Zhao, X.; Hu, H.; Ma, X. Pharmazie 2008, 63, 27.
27. Gao, Y.; Ren, F. Z.; Ding, B. Y.; Sun, N. Y.; Liu, X.; Ding, X. Y.; Gao, S. J Drug Target 2011, 19, 516.
28. Chen, S.; Pieper, R.; Webster, D. C.; Singh, J. Int J Pharm 2005, 288, 207.
29. Yu, L.; Chang, G. T.; Zhang, H.; Ding, J. D. Int J Pharm 2008, 348, 95.
30. Jeong, B.; Bae, Y. H.; Kim, S. W. Colloids Surf B 1999, 16, 185.
31. Lee, J. W.; Hua, F.; Lee, D. S. J Controlled Release 2001, 73, 315.
32. Yu, L.; Zhang, Z.; Ding, J. Biomacromolecules 2011, 12, 1290.
33. Gao, Z.; Shukla, A. J.; Johnson, J. R.; Crowley, W. R. Pharm Res 1995, 12, 857.
34. Lessard, D. G.; Ousalem, M.; Zhu, X. X.; Eisenberg, A.; Carreau, P. J. J Polym Sci Part B: Polym Phys 2003, 41, 1627.
35. Yang, Y.; Wang, J. C.; Zhang, X.; Lu, W. L.; Zhang, Q. J Controlled Release 2009, 135, 175.
36. Suri, S.; Banerjee, R. J Biomed Mater Res A 2006, 79, 650.
37. Martínez-Sancho, C.; Herrero-Vanrell, R.; Negro, S. J Controlled Release 2004, 99, 41.
38. Martínez-Sancho, C.; Herrero-Vanrell, R.; Negro, S. Int J Pharm 2006, 326, 100.