JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Molecular and Translational Medicine

Research Article

AK12/AK19 PLGA-PEG-PLGA Triblock Polymer: Thermo-Gelation Properties and *in vivo* Biocompatibility

Cuifen Wang [1,2], Eric Blough [1,2], Ravikumar Arvapalli [1], Omolola Olajide [3], Henry Driscoll [3,4], Maali Milhem [3], Shahed Elhamdani [3], Nicole Winston [2], William E. Triest [4] , and Miaozong Wu [1,2,3,*]

[1]*Center for Diagnostic Nanosystems*

[2]*School of Pharmacy*

[3]*Department of Internal Medicine, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV*

[4]*Huntington Veterans Affairs Medical Center, Huntington, WV, USA*

**Corresponding author: Dr. Miaozong Wu, Ph.D. ,Center for Diagnostic Nanosystems, Marshall University, 1 John Marshall Drive , Huntington, WV 25755, Tel: 304-4296741 ext 2807; Fax: 304-6963766 ; E-mail: wum@marshall.edu*

Received: 03-18-2016

Accepted: 05-16-2016

Published: 06-07-2016

Abstract

This study was to determine a novel and applicable PLGA-PEG-PLGA triblock copolymer with ideal gelation at the physiological temperature. A maximum gelation at 37 °C was obtained by mixing two polymers at 1:3 ratio, PLGA1000-PEG1000-PLGA1000(named as AK12) and PLGA1500-PEG1500-PLGA1500 (AK19). The AK12/AK19 polymer rapidly initiated gelation when temperature was greater than 32°C and had a single gelation peak at ~37°C. Placebo-controlled animal study showed intraperitoneal administration of AK12/AK19 copolymer (20%w/v) did not affect body weight, food and water consumption during 21-day study. Similarly, blood biochemical analysis showed no difference of organ weight, indices of overall health, liver and kidney functions between the polymer and placebo groups. Taken together, these data suggest that the AK12/AK19 PLGA-PEG-PLGA copolymer exhibits desirable thermoregulable and biocompatible properties, which allow *in vitro* preparation at cool or room temperatures but ideal gelation after *in vivo* delivery, and may make it extremely attractive for drug delivery applications.

Keywords: PLGA-PEG-PLGA Copolymer; AK12; AK19; Thermo-gelation; Biocompatibility; Rheology; *in vivo* study

Introduction

With increased standards and demands for therapeutic efficacy and patient safety, more and more drugs are expected to be delivered specifically to their targeted tissue in a controlled manner [1, 2]. Controlled-release drug delivery technologies not only allow drugs to be released over a period of time with a more efficacious plasma drug profile, but also provide patient-specific benefits, such as less frequent dosing and reduced magnitude and frequency of adverse events [3, 4]. As such, several sustained release formulations, such as polymer conjugates, liposomes and biodegradable microspheres, have been of interest to researchers, clinicians, patients and pharmaceutical manufacturers.

Poly(lactic-co-glycolic acid) or PLGA is a Food and Drug Administration (FDA)-approved copolymer, that can be synthesized by cyclic polymerization of glycolic acid and lactic acid [5, 6]. Studies have shown that the thermosensitive gelation properties, crystallinity and degradation rates of PLGA polymers can be controlled by the ratio of monomers, lactide to glycolide [5-8]. The degradation of PLGA polymers occurs in the presence of water, primarily through non-enzymatic hydrolysis of ester bonds. Its end-products, lactide and glycolide,

are identical to those metabolites normally present in our bodies [9]. Because of its thermosensitive gelation, biodegradable and biocompatible properties, it is thought that PLGA polymers exhibit substantial potential for clinical application using multiple dosing routes including subcutaneous, submucosal, intraocular, intravitreal, intratracheal, and topical administration [10-17], and have been tested in many therapeutic fields, such as cancer, regenerative medicine, macular degeneration, tissue engineering, and nanoparticle formulation [14, 18-21]. To this end, the purpose of this study was to determine a novel and applicable copolymer with ideal gelation at the physiological temperature, and to evaluate its *in vivo* safety using placebo-controlled animal model. Our study shows that by mixing two PLGA-PEG-PLGA polymers AK12 and AK19 at 1:3 ratio, a desirable gelation at the physiological temperature can be obtained. Animal study further demonstrates great biocompatible properties of the AK12/AK19 polymer. These data suggest that the novel AK12/AK19 polymer is applicable for *in vitro* drug preparation at cool or room temperatures and *in vivo* drug delivery applications.

Materials and Methods

Materials

Polymer AK12 (PLGA-PEG-PLGA with an approximate molecular weight of 1000-1000-1000 Da, respectively; 75% lactide / glycolide (1:1) and 25% PEG), and AK19 (PLGA-PEG-PLGA with a molecular weight of ~1500-1500-1500 Da, respectively; 67% lactide / glycolide (1:1) and 33% PEG) were purchased from Akina Inc. (West Lafayette, IN). The PLGA-PEG-PLGA triblock copolymer was synthesized through self-assembly of PLGA and PEG (poly(D,L-lactide-co-glycolide), which can form the PLGA-PEG-PLGA copolymer micelles (poly(D,L-lactide-co-glycolide)-bpoly(ethylene glycol)-b-poly(D,L-lactide-co-glycolide)). The PEG was used to provide a hydrophilic block for the polymer. Sodium chloride solution (NaCl; 0.9%) was purchased from RICCA Chemical (Arlington, TX). VetScan® blood comprehensive diagnostic profile was purchased from Abaxis Inc. (Union City, CA).

Thermo-gelation Study

Each polymer (AK12 and AK19; 20% w/v) was completely dissolved in cold 0.9% NaCl by overnight shaking at 4 °C. The AK12/AK19 mixture was obtained by mixing the cold AK12 and AK19 polymer solutions at a 1:1 or 1:3 ratio, with a final concentration of 20% w/v. The rheology of four polymer solutions was determined using an AR550 rheometer (TA instruments; New Castle, DE) equipped with a 60mm 2-degree cone. The viscosity of each solution (1 minute peak hold, 5 second test intervals) was measured at 0.1 (sec^{-1}) and 5 °C. Rheology was performed by oscillating at constant 6.283 rad/s, 0.1% strain, in increments of 2.5 °C ranging from 5-50 °C with 3 minutes of temperature equilibration at each point [22]. The time required for gelation and the solution-gel transition at 37°C were tested by writing letters on a 37°C heated-plate, according to previous study [23].

Tube tip testing was used to visualize the gelation status and phase separation of AK12/AK19 polymer at room temperature and 37 °C incubation. Briefly, the AK12/AK19 mixed solution was aliquoted into two vials (1 mL each), and one vial was left at room temperature while the other was kept in a 37 °C incubator. The gelation state of solution was pictured after equilibrium [22]. The phase separation study was carried out by storing AK12/AK19 polymers (1:1 or 1:3 ratio) in a 37 °C incubator and allowing to equilibrate for two days [22].

Animal Study

Male Fischer CDF® rats (F344/DuCrl; Strain Code: 002) were purchased from Charles River Laboratories International (Wilmington, MA), and housed in an AAALAC approved vivarium under a 12 Hour: 12 Hour dark-light cycle and at 22 ± 2 °C. Rats were allowed to recover from shipment for at least one week and reach the body weight of 273.8 ± 2.9 g before study. Standard laboratory rodent chow LabDiet 5001 (LabDiet; St. Louis, MO) and water were provided *ad libitum*. Animals were randomly assigned to either placebo (0.9% NaCl) or AK12/AK19 polymer group (N = 4). The AK12/AK19 mixture (20% w/v) was prepared by mixing the cold AK12 and AK19 polymer solutions at 1:3 ratio in 0.9% w/v sterile saline solution, and intraperitoneally administrated to animal on day 0 (1 mL). Animals in the placebo group were given 1 mL of 0.9% sterile saline intraperitoneally. All animals were anesthetized by isoflurane inhalation during intraperitoneal injection. Body weight, food intake, and water consumption were recorded weekly. At the end of the 21-day study, animals were deeply anesthetized with isoflurane. The spleen, kidney, liver, heart and skeletal muscles (including soleus, plantaris and gastrocnemius) were dissected and weighted. Blood was collected via cardiac puncture. All animal procedures have been approved by the Marshall University's Institutional Animal Care and Use Committee (IACUC).

Blood Biochemistry

Blood total protein, albumin, alanine aminotransferase (ALT), bilirubin, globulin, creatinine, and urea nitrogen (BUN) were measured using a VetScan® blood chemistry analyzer.

Statistical Analysis

Data are presented as mean ± standard error. The data of body weight, food and water intake at different time points were analyzed using two way repeated measures ANOVA, while the Student's T-test was used to determine the statistical difference of other data between the placebo and polymer groups. A $P \leq 0.05$ was considered as statistically significant.

Results

Thermo-gelation Properties of Polymer AK12, AK19 and AK12/AK19 Mixtures

To determine the *in vitro* gelation properties of PLGA-PEG-PLGA triblock copolymers AK12 and AK19, the gelation of each polymer was determined at different temperature (5 - 50 °C) [22]. As shown in the Figure 1A, AK12 polymer had a gelation point with onset at ~15°C and end at ~40°C, and it had two peaks at ~20°C and ~30°C. The AK19 polymer had a gelation point with onset at ~37-40 °C (Figure 1B). Interestingly, when the AK12 and AK19 polymers were mixed together at the ratio of 1:3, the gelation initiated at ~27 °C but rapidly increased at ~32 °C, and there was only a single gelation peak appearing at ~37°C (Figure 1D).

However, when mixed at 1:1 ratio, the single gelation peak appeared at ~35°C (Figure 1C). The polymer formed gel instantly as tested on a 37°C heated plate (Figure 1E). Similar approach to test gelation has been reported on polymer PLGA1500-PEG1000-PLGA1500 [21]. The gel status of the AK12/AK19 mixture was clearly visualized during tube tip testing (Figure 1F), suggesting that 1:3 AK12/AK19 mixture was in solution status at room temperature, while in solid gel status at 37°C. Further phase separation study showed that equilibrating for 2 days at 37°C caused phase separation in the 1:1 AK12/AK19 mixture, but not in the 1:3 AK12/AK19 mixture (Figure 1G). Taken together, these data suggest that the thermo-gelation properties of AK12/AK19 mixture (1:3) is convenient for preparing polymer thermogel at cool or room temperature but achieving quick gelation at temperatures (~37°C) that are typically associated with *in vivo* applications.

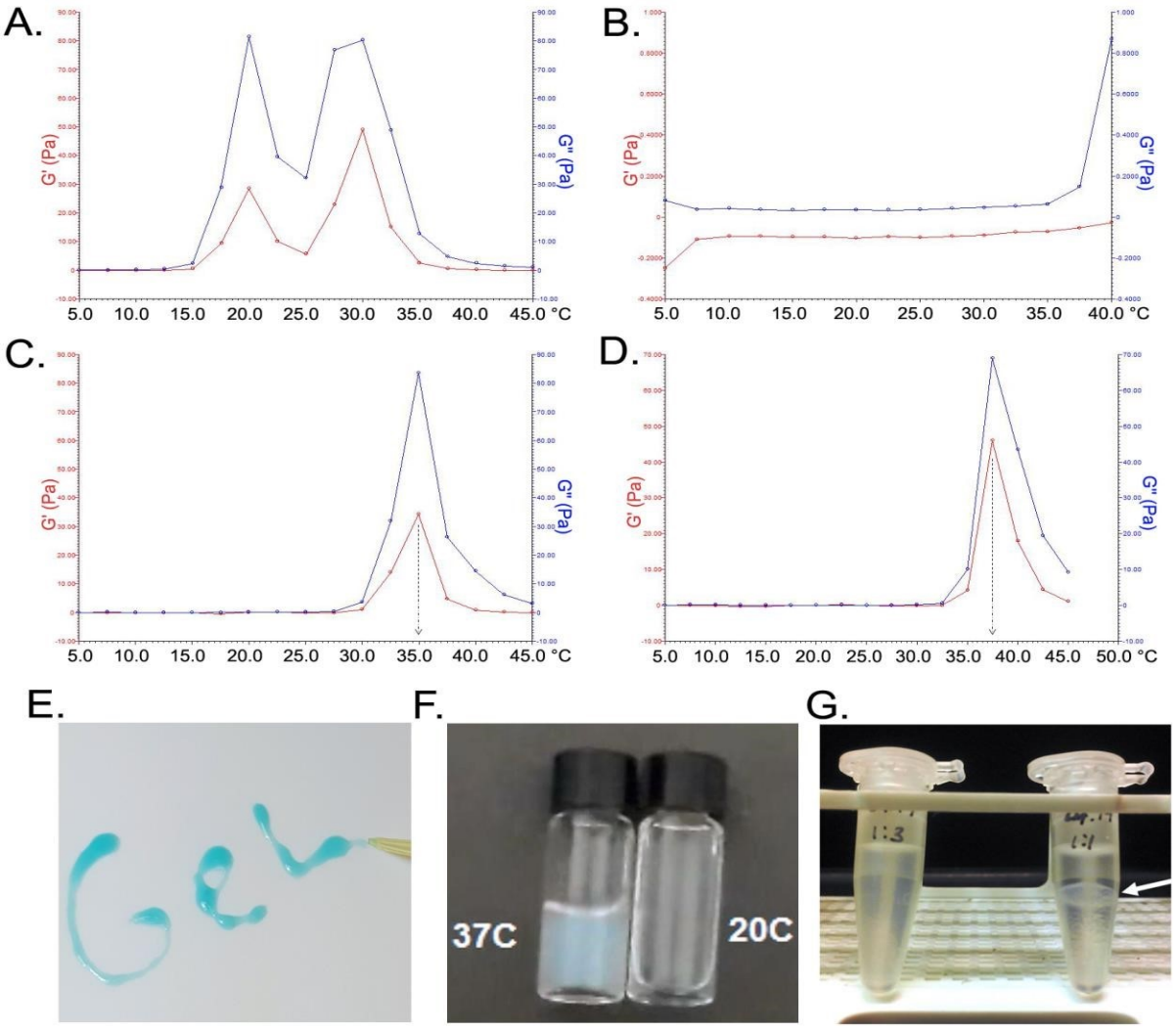

Figure 1. The thermo-gelation properties of polymer AK12, AK19 and AK12/AK19 mixture. The gelation process of polymer AK12 (panel A), AK19 (panel B) and AK12/AK19 mixture at a 1:1 (panel C) or 1:3 ratio (panel D) was determined using an AR550 rheometer, and expressed as storage modulus / elastic response (G', blue line) crossing over loss modulus / viscous behavior (G'', red line) at different temperature measured. Solution-gel transition was tested on a 37°C heated-plate to demonstrate instant gelation of 1:3 AK12/AK19 polymer at 37°C (panel E). Gelation state at different temperatures (20 and 37°C, respectively; panel F) and phase separation (panel G) were determined using tube tip testing. All polymer solutions had a final concentration of 20% w/v.

Effect of AK12/AK19 Polymer on Body Weight in Rats

To assess *in vivo* safety of AK12/AK19 polymer, male Fischer CDF® rats were intraperitoneally received with 1 mL of AK12/AK19 polymer (1:3 ratio; 20% w/v). No difference of body weight was observed between the placebo group and the rats receiving AK12/AK19 polymer throughout the experimental duration (P = 0.65; Figure 2).

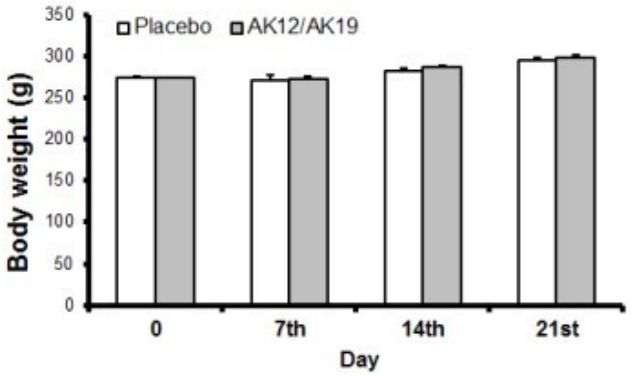

Figure 2. Body weight change. Placebo (0.9% NaCl) and AK12/AK19 polymer were intraperitoneally administrated on day 0, and body weight was measured on 0, 7th, 14th and 21st day of experiment.

Effect of AK12/AK19 Polymer on Food and Water Consumption in Rats

To investigate whether AK12/AK19 polymer can alter feeding behavior, the amount of food and water intakes was measured weekly, and the daily food intake and water consumption were calculated. Both daily food and water intakes were not different between the placebo and AK12/AK19 polymer groups (P = 0.88 and 0.58, respectively; Figure 3).

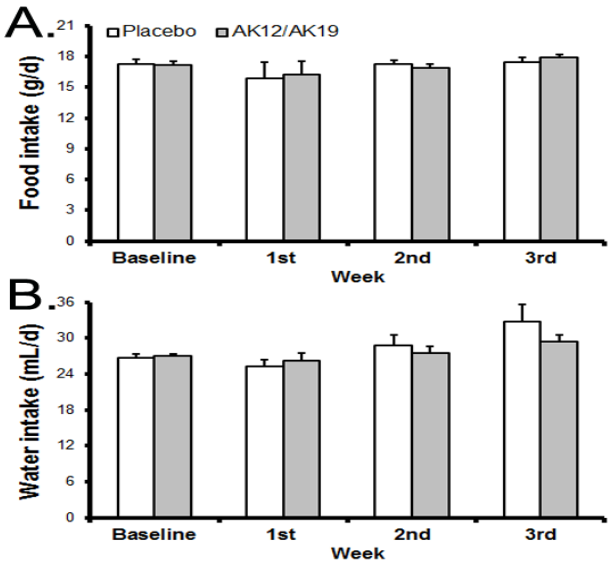

Figure 3. The amount of daily food and water intakes. Food intake (panel A) and water intake (panel B) were measured the week before study (as baseline) and throughout 21 days of study in rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer.

Effect of AK12/AK19 Polymer on Organ Weights in Rats

To evaluate whether AK12/AK19 polymer can affect the weight of internal organs, the spleen, kidney, liver, heart and skeletal muscles (including soleus, plantaris and gastrocnemius) were dissected and weighed after 21 days of study. As shown in Figure 4, the weight of these organs or the percentage of organ / body weight was not different between the placebo and AK12/AK19 polymer groups (P = 0.16, 0.71, 0.27, 0.74 and 0.96, respectively; Figure 4).

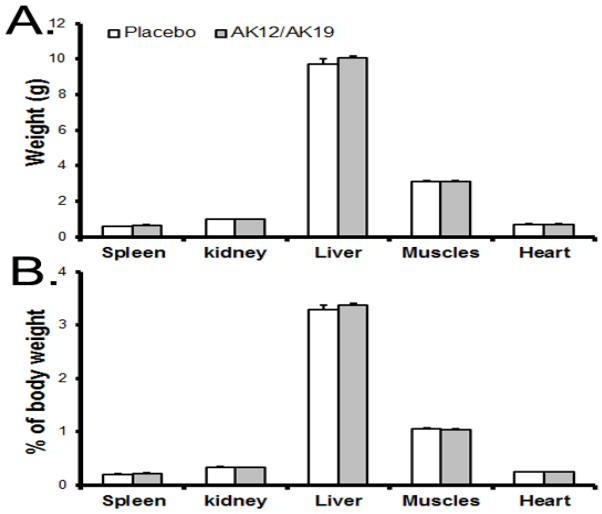

Figure 4. Organ weight. The wet weight (panel A) and organ/body weight (%; panel B) of spleen, kidney, liver, skeletal muscles (including soleus, plantaris and gastrocnemius) and heart in rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer.

Effect of AK12/AK19 Polymer on Blood Total Protein Content and Albumin to Globulin (A/G) Ratio

To evaluate the overall health of animals between placebo and AK12/AK19 polymer treatment, blood total protein content and the ratio of albumin to globulin (A/G) were measured. Both blood total protein content and A/G ratio were not different between the placebo and AK12/AK19 polymer groups (P = 0.30 and 0.77, respectively; Figure 5).

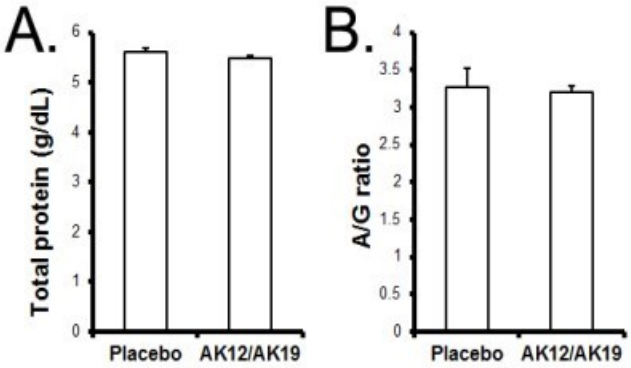

Figure 5. Blood total protein content (panel A) and albumin to globulin (A/G) ratio (panel B) in rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer.

Effect of AK12/AK19 Polymer on Liver Function in Rats

To determine whether AK12/AK19 polymer administration can change liver function, blood alanine aminotransferase (ALT) and total bilirubin content were measured. As shown in Figure 6, both ALT enzymatic activity and bilirubin content were not different between rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer (P = 0.77 and 0.35, respectively; Figure 6).

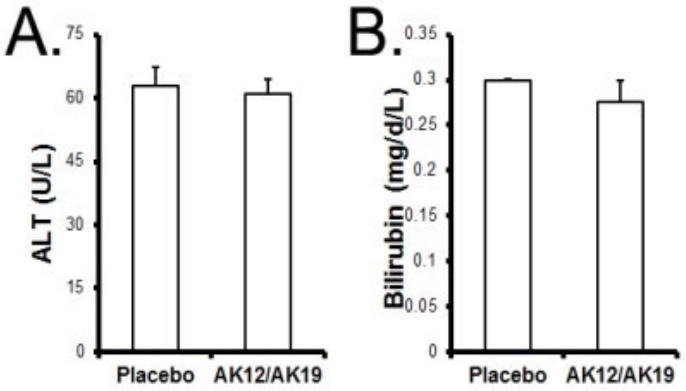

Figure 6. Liver function. Blood alanine aminotransferase activity (ALT; panel A) and total bilirubin content (panel B) were measured in rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer.

Effect of AK12/AK19 Polymer on Kidney Function in Rats

Blood creatinine and urea nitrogen (BUN) contents were measured to determine whether AK12/AK19 polymer administration can change kidney function. As shown in Figure 7, both creatinine and BUN contents were not different between the placebo and AK12/AK19 polymer groups (P = 0.75 and 0.36, respectively; Figure 7).

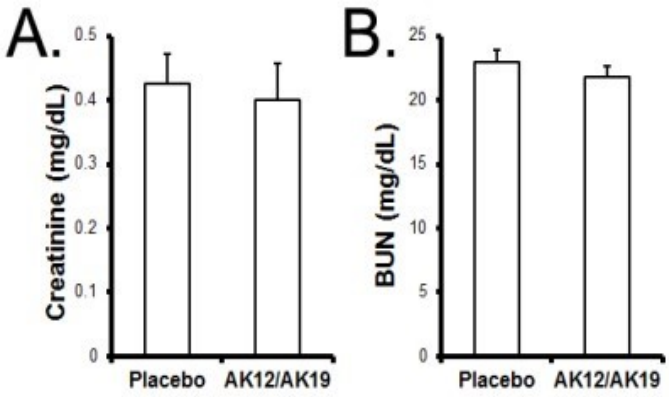

Figure 7. Evaluation of kidney function. Blood creatinine (panel A) and urea nitrogen (BUN, panel B) were measured in rats receiving placebo (0.9% NaCl) or AK12/AK19 polymer.

Discussion

Concerns over drug therapeutic effects and patient safety have significantly increased the demand for novel drug delivery technologies. Taking advantages of its biodegradability and biocompatibility [5-8, 12, 13, 17], here we identified an applicable PLGA polymer with ideal gelation at the physiological temperature by mixing two triblock copolymers, PLGA1000-PEG1000-PLGA1000 (named as AK12) and PLGA1500-PEG1500-PLGA1500 (named as AK19). We demonstrated that the thermo-gelation properties of AK12/AK19 mixture (1:3 ratio) allow for the preparation of a polymer thermogel at cool or room temperature, but undergo almost instant gelation at physiological temperature (37 °C). Additional *in vivo* experiments suggested that this polymer is highly biocompatible and has no adverse effects when compared to the placebo group.

The thermosensitive gelation of PLGA polymer is critical for its clinical application. Studies have shown that hydrophobic PLGA and hydrophilic PEG polymer blocks can aggregate and form micelles, and at a certain temperature they can gelatinize [6, 24]. As shown in Figure 1A and 1B, the AK12 polymer exhibits gelation beginning at ~15°C and ending at ~40°C, while the AK19 polymer has a gelation point with onset at ~37-40 °C. However, a 1:3 mixture of AK12 and AK19 led to a rapid gelation above ~32°C, and, more importantly, the formation of a single peak at ~37°C (Figure 1D). These rheological data suggest that mixing the AK12 and AK19 polymers can lead to the development of a polymer whose gelation occurs at a physiological temperature, which would enable the use of this polymer for *in vivo* applications possible. In support of this possibility, we further studied the phase separation of the mixed polymer and found that the gelation of AK12 and AK19 polymers (1:3 ratio) is remarkably stable at 37°C even after 2 days of *in vitro* incubation (Figure 1G). Consistent with these data, other research has shown that a similar polymer gel PLGA1500-PEG1000-PLGA1500 is stable *in vivo* for up to two weeks [21]. In addition, it should also be noted that the AK12 and AK19 polymer mixture (1:3 ratio) exhibits low-viscosity when held at a temperature under ~32°C (Figure 1D and 1F). This property is important, as it should allow drugs to be easily mixed with the polymer at cool or room temperatures but ensure in situ gel-forming and targeted tissue deposition after *in vivo* delivery [18, 24].

To investigate the *in vivo* safety profile of the AK12/AK19 polymer, a placebo-controlled animal study was performed. As shown in Figure 2 and 3, when compared to the placebo group, AK12/AK19 polymer administration (via i.p.) did not alter animal body weight as well as daily food intake and water consumption during the 21 day study. In support of body weight and feeding behavior data, organ wet weight and the percentage of organ/body weight of the spleen, kidney, liver, heart and skeletal muscles were also not different between the

placebo and experimental groups (Figure 4). Additional molecular analysis obtained from blood samples indicated that the polymer administration did not have a negative effect on blood total protein content and albumin to globulin ratio(indices of overall health [25]), liver function (blood alanine aminotransferase activity and total bilirubin content) [26] and kidney function (blood creatinine and urea nitrogen) [27]. These data, taken together, are consistent with previous reports, demonstrating the biocompatibility and *in vivo* safety of PLGA polymers [16, 28].

In conclusion, our study suggests that the new AK12/AK19 PLGA-PEG-PLGA triblock copolymer possesses ideal thermo-gelation characteristics for *in vivo* application and that it appears to be largely nontoxic in nature. Future investigation to examine the efficacy of using this polymer for drug delivery may be warranted.

Acknowledgments

This work was supported by NASA EPSCoR grant (# NNX13AN08A). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The thermo-gelation studies were conducted by Akina, Inc. All the authors and Akina, Inc. declare no competing financial interests.

The authors would like to acknowledge technical expertise and support from Mr. John Garner at Akina, Inc. We also acknowledge the support of the Huntington VA Medical Center and the Joan C. Edwards School of Medicine Training Program in Endocrinology for personnel, laboratory space and equipment.

References

1. Bertrand N, Leroux JC. The journey of a drug-carrier in the body: an anatomophysiological perspective. J Control Release. 2012, 161(2): 152-163.

2. Khodaverdi E, Tekie FS, Mohajeri SA, Ganji F, Zohuri G et al. Preparation and investigation of sustained drug delivery systems using an injectable, thermosensitive, in situ forming hydrogel composed of PLGA-PEG-PLGA. AAPS PharmSciTech. 2012, 13(2): 590-600.

3. Mutschler E, Knauf H. Current status of sustained release formulations in the treatment of hypertension. An overview. Clin Pharmacokinet. 1999, 37 (Suppl 1): 1-6.

4. Wang CH, Hwang YS, Chiang PR, Shen CR, Hong WH et al. Extended release of bevacizumab by thermosensitive biodegradable and biocompatible hydrogel. Biomacromolecules. 2012, 13(1): 40-48.

5. Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. J Biomater Sci Polym Ed. 2006, 17(3): 247-289.

6. Makadia, HK, Siegel SJ. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers (Basel). 2011, 3(3): 1377-1397.

7. Samadi N, Abbadessa A, Di Stefano A, van Nostrum CF, Vermonden T et al. The effect of lauryl capping group on protein release and degradation of poly(D,L-lactic-co-glycolic acid) particles. J Control Release. 2013, 172(2): 436-443.

8. Zhang Z, Ni J, Chen L, Yu L, Xu J et al. Biodegradable and thermoreversible PCLA-PEG-PCLA hydrogel as a barrier for prevention of post-operative adhesion. Biomaterials. 2011, 32(21): 4725-4736.

9. Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev. 1997, 28(1): 5-24.

10. Patel B, Gupta V, Ahsan F. PEG-PLGA based large porous particles for pulmonary delivery of a highly soluble drug, low molecular weight heparin. J Control Release. 2012, 162(2): 310-320.

11. Duvvuri S, Janoria KG, Pal D, Mitra AK. Controlled delivery of ganciclovir to the retina with drug-loaded Poly(d,L-lactide-co-glycolide) (PLGA) microspheres dispersed in PLGA-PEG-PLGA Gel: a novel intravitreal delivery system for the treatment of cytomegalovirus retinitis. J Ocul Pharmacol Ther. 2007, 23(3): 264-274.

12. Yang X, Shah SJ, Wang Z, Agrahari V, Pal D et al. Nanoparticle-based topical ophthalmic formulation for sustained release of stereoisomeric dipeptide prodrugs of ganciclovir. Drug Deliv. 2015, 1-11.

13. Yu L, Xu W, Shen W, Cao L, Liu Y et al. Poly(lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-co-glycolic acid) thermogel as a novel submucosal cushion for endoscopic submucosal dissection. Acta Biomater. 2014, 10(3): 1251-1258.

14. Hirani A, Grover A, Lee YW, Pathak Y, Sutariya V. Triamcinolone acetonide nanoparticles incorporated in thermoreversible gels for age-related macular degeneration. Pharm Dev Technol. 2016, 21(1): 61-67.

15. Cai X, Luan Y, Jiang Y, Song A, Shao W et al. Huperzine A-phospholipid complex-loaded biodegradable thermosensitive polymer gel for controlled drug release. Int J Pharm. 2012, 433(1-2): 102-111.

16. Yu L, Zhang Z, ZhangH, Ding J. Biodegradability and biocompatibility of thermoreversible hydrogels formed from mixing a sol and a precipitate of block copolymers in water. Biomacromolecules. 2010, 11(8): 2169-2178.

17. Pavot V, Berthet M, Rességuier J, Legaz S, Handké N et al. Poly(lactic acid) and poly(lactic-co-glycolic acid) particles as versatile carrier platforms for vaccine delivery. Nanomedicine (Lond). 2014, 9(17): 2703-2718.

18. Nagahama K, Kawano D, Oyama N, Takemoto A, Kumano T et al. Self-assembling polymer micelle/clay nanodisk/doxorubicin hybrid injectable gels for safe and efficient focal treatment of cancer. Biomacromolecules. 2015, 16(3): 880-889.

19. Qodratnama R, Serino LP, Cox HC, Qutachi O, White LJ. Formulations for modulation of protein release from large-size PLGA microparticles for tissue engineering. Mater Sci Eng C Mater Biol Appl. 2015, 47: 230-236.

20. Zou F, Jiang J, Lu F, Ma X, Xia X et al. Efficacy of intradiscal hepatocyte growth factor injection for the treatment of intervertebral disc degeneration. Mol Med Rep. 2013, 8(1): 118-122.

21. Ge J, Neofytou E, Cahill TJ 3rd, Beygui RE, Zare RN. Drug release from electric-field-responsive nanoparticles. ACS Nano. 2012, 6(1): 227-233.

22. Garner J, Skidmore S, WhitePaper: Thermogel Mixtures impact on Rheology. PolySciTech Division of AKINA INC. 2013, 1-6.

23. Garner J. Thermogels mixed Gelation morphology/speed. PolySciTech Division of AKINA INC., 2016: 1.

24. Gong C, Shi S, Dong P, Kan B, Gou M et al., Synthesis and characterization of PEG-PCL-PEG thermosensitive hydrogel. Int J Pharm. 2009, 365(1-2): 89-99.

25. Pagana K, Pagana T. Mosby's Manual of Diagnostic and Laboratory Tests (5th Edition). 2014.

26. Wu M, Desai DH, Kakarla SK, Katta A et al. Acetaminophen prevents aging-associated hyperglycemia in aged rats: effect of aging-associated hyperactivation of p38-MAPK and ERK1/2. Diabetes Metab Res Rev. 2009, 25(3): 279-286.

27. Wanga C, Blougha E, Arvapallia R, Daic X, Triest WE et al. Acetaminophen attenuates glomerulosclerosis in obese Zucker rats via reactive oxygen species/p38MAPK signaling pathways. Free Radic Biol Med. 2015, 81: 47-57.

28. Chen S, Singh J. Controlled release of growth hormone from thermosensitive triblock copolymer systems: *In vitro* and *in vivo* evaluation. Int J Pharm. 2008, 352(1-2): 58-65.