Molecular Weight Effect on Theta-Gel Formation in Poly(vinyl alcohol)–Poly(ethylene glycol) Mixtures

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ABSTRACT: Injectable hydrogel formulations that undergo *in situ* gelation at body temperature are promising for minimally invasive tissue repair. This work focuses on the investigation of injectable poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) mixtures. The injectable PVA–PEG aqueous solutions form a hydrogel as temperature is reduced to near body temperature, while filling a defect in the injection site. Gamma sterilization of these solutions compromises injectability presumably due to crosslinking of PVA. We hypothesized that by modifying the PEG molecular weight and its concentration, injectability of radiation sterilized PVA–PEG hydrogels can be optimized without compromising the mechanical properties of the resulting gel. The use of a bimodal mixture of higher and lower molecular weight PEG (600 and 200 g/mol) resulted in lower PVA/PEG solution viscosity, better injectability, and higher gel mechanical strength. The PVA/bimodal-PEG had a lower viscosity at 2733 ± 149 cP versus a viscosity of 5560 ± 278 cP for PVA/unimodal-PEG (400 g/mol). The gel formed with the bimodal PEG mixture had higher creep resistance (61% total creep strain under 0.5 MPa) than that formed with unimodal PEG (84%). These hydrogel formulations are promising candidates for minimally invasive tissue repair. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2890–2895, 2012

Key words: hydrogels; implant; polymer blends

INTRODUCTION

Injectable hydrogels may enable minimally invasive surgical procedures to decrease patient morbidity and are therefore promising for many regenerative and reconstructive applications, such as minimally invasive repair of early cartilage lesions,[1–3] tissue bulking for mitral regurgitation, urinary defects,[4] and gastroesophageal reflux disease,[5] vocal cord repair,[6] drug delivery,[7,8] and making scaffolds in cell-based tissue engineering systems.[9–11] Injectable hydrogels are free flowing aqueous solutions at ambient temperatures that solidify at body temperature to form a gel either via physical or chemical interactions. Chemically crosslinked hydrogels are usually formed by covalent interaction such as carbon–carbon and disulfide bond formation, or aldehyde-mediated crosslinking by temperature change or radiation exposure upon injection.[12–17] Physically crosslinked hydrogels are formed by introducing reversible physical interactions between polymer chains such as hydrogen bonding, ionic, or hydrophobic interactions.[18,19] As physically crosslinked hydrogels do not involve chemical reactions, the integrity and stability of the final gel *in vivo* could be compromised due to interactions with the physiological environment or exposure to mechanical stress in the body.[20,21]

Injectability at or near body temperature and high mechanical strength after gelation at injection site are key requirements for injectable hydrogel devices especially in applications where the injected hydrogel is subjected to static or dynamic loading. An injectable formulation should also be substantially resistant to dissolution during its injection and its subsequent *in vivo* lifetime. Rapid gelation could hinder dissolution and diffusion of the formulation components, while providing enough time to fully cure the gel and fill the intended defect. In some cases, the injectable solution is gamma sterilized, which could crosslink constituents of the solution and adversely affect its injectability.[22–24]

Ruberti and Braithwaite[25] described an injectable hydrogel that uses physical phase separation of a poly(vinyl alcohol) (PVA) solution by the use of a gelling agent that brings the solution near its theta point. In this method, the addition of a gelling agent, such as low-molecular weight poly(ethylene glycol) (PEG) into aqueous PVA solution, reduces the quality of the solvent with decreasing temperature, forcing

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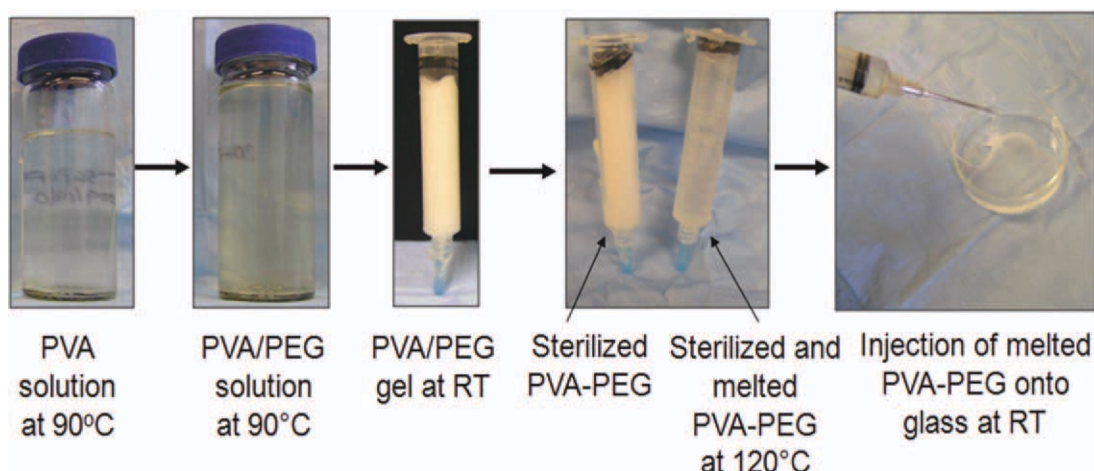

Figure 1 Preparation of injectable PVA–PEG hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the PVA to phase separate and crystallize, forming a physically crosslinked hydrogel network. These theta-gels are thermoreversible, in that heating to near 90°C dissociates the hydrogel and the gel remains injectable through a small gage needle within a temperature range of 90°C–55°C. However, when the gels are gamma sterilized, their solution viscosity increases and their injectability is compromised, likely because radiation crosslinks the PVA molecules. In a previous study, we showed that PEG at high concentrations could prevent the crosslinking of PVA during radiation sterilization.[26] We hypothesized that keeping the PVA concentration and molecular weight the same and increasing the concentration of the gelling agent, in this case PEG, would decrease the viscosity of the radiation sterilized solution, improving injectability. However, with higher molecular weight PEG, the viscosity of the formulation could increase and adversely impact injectability. Therefore, we propose using a low-molecular weight PEG as a weak gelling agent together with a high-molecular weight PEG, which would reduce viscosity without compromising gelation rate and final strength.

EXPERIMENTAL

Materials

PVA (molecular weight (MW) = 115,000 g/mol, Scientific Polymer Products, Ontario, NY) and PEG (MW = 200, 400, and 600 g/mol, Acros, NJ) were used to prepare the theta-solutions.

Hydrogel preparation

Several different PVA/PEG aqueous solutions were prepared. In all solutions, we started with a 17.5 wt % PVA solution in deionized (DI) water prepared by stirring at 90°C. One set of gels was prepared by adding PEG400 (MW = 400 g/mol) to the PVA solution at 90°C while stirring. The other was prepared by adding PEG600 (MW = 600 g/mol). PEG concentrations used were 17.5% and 39% (w/v), respectively. In another set, PEG200 was added to PVA–PEG400 and PVA–PEG600 mixtures at 17.5% (w/v). The solutions were conditioned in air at 90°C in an oven and poured into 10 cc syringes that were preheated to 90°C. The syringes were cooled to room temperature and packed in vacuum; subsequently, they were sterilized by gamma irradiation to a dose of 35–40 kGy (Fig. 1). PVA–PEG formulations that were prepared by either PEG400 or PEG600 were called "unimodal PVA–PEG systems." The PVA–PEG formulations where PEG200 was mixed with PEG400 or PEG600 were called "bimodal PVA–PEG systems."

Viscosity measurements

Viscosity measurements were done using a bubble-tube viscometer (Fig. 2). The gels were placed in an Erlenmeyer flask with a heat resistant cap and were heated in a convection oven set at 120°C until they melted completely, and a clear solution was obtained. Then, the solution was taken from the flask and poured into a bubble tube until the fill line. The tube was closed with a cork and packaged in vacuum-sealed packaging to prevent the tube from leaking at high temperatures. The samples were then placed in a water bath at 50°C. The tubes were inverted until the air bubble between the fill line and the cork traveled to the bottom of the tube. Then, the tube was reinverted, and the time that it took for the air bubble to travel from the bottom marker line to the top marker line was recorded.

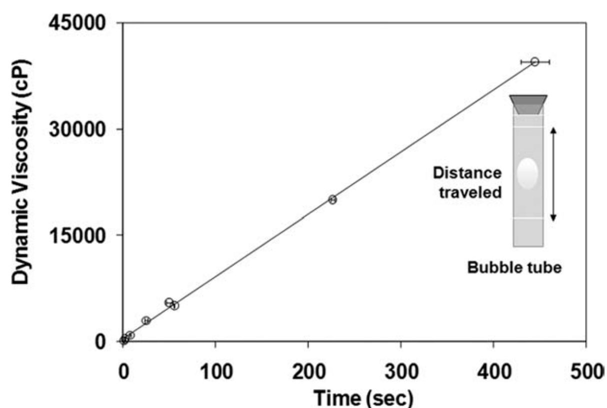

Figure 2 Viscosity measurements and calibration curve with oil standards.

Calibration of bubble travel time readings to viscosity was made by using four ISO 17025 viscosity reference standard oils at room temperature and 50°C (Fig. 2). These oils were N100, D5000, S8000, and N15000 (Koehler Instrument Co., Bohemia, NY).

In vivo injection simulation using a tissue mold

Bovine round eye muscle was bored (19 mm diameter) with a depth of 5 mm to fabricate "tissue molds." Molds were individually packed in vacuum and placed in a water bath to equilibrate at 40°C. The gamma sterilized hydrogels were melted in the syringe tube using a convection oven at 120°C, cooled to 55°C, and injected through a 16.5 gauge needle into the tissue cavity. The solutions were allowed to gel at temperature (40°C) for 24 h.

Equilibrium water content

The equilibrium water content (EWC) of gamma sterilized hydrogels was measured after gelation in a tissue mold for 24 h with subsequent equilibration in DI water at 40°C for 2 days. The samples were then cut into three pieces and placed in vacuum for 1 day at room temperature and then in a convection oven at 90°C for complete dehydration. The samples were periodically weighed during dehydration until no significant change (less than 2%) in weight was detected. The EWC was calculated by taking the ratio of the difference between the equilibrium hydrated and dehydrated weights and the equilibrium hydrated weight.

Creep test

Creep behavior of PVA gamma sterilized hydrogels was measured after gelation in tissue mold for 24 h followed by equilibrating in DI water at 40°C for 2 days. Hydrogels were cut into cylindrical disks with a 16-mm diameter trephine mounted on a drill press while submerged in DI at room temperature. The creep experiments were performed on an MTS 858 Mini Bionix servohydraulic machine with the test sample immersed in 40°C saline solution (Eden Prairie, MN). The test samples were placed between stainless steel compression plates, and the top plate was lowered until it made contact with the surface of the creep specimen. The displacement reading from the Linear Variable Differential Transformer (LVDT) on the Mechanical Testing and Simulation (MTS) was recorded as the height of the specimen. The compressive load was initially ramped at a rate of 50 N/min to a creep load of 100 N, resulting in an initial compressive stress of about 0.45 MPa. This load was maintained constant for 10 h. The load was then reduced at a rate of 50 N/min to a recovery load of 10 N. This load was also held constant for 10 h. Time, displacement, and load values were recorded once every 2 s during the loading and unloading cycles.

The data was plotted as compressive strain versus time to compare the creep behavior of different hydrogel formulations described above. The total creep strain (TCS) of the samples refers to the strain that was measured at the completion of 10 h of loading under 100 N. In addition to the TCS, we also reported the elastic strain (ES) at the completion of ramp up to 100 N load, the viscoelastic strain (VS) after 10 h of loading under 100 N, the elastic recovery (ER) on unloading from 100 N to 10 N, the viscoelastic recovery (VR) after 10 h of unloading under 10 N, and total recovery after 10 h of unloading.

RESULTS

Gamma sterilized unimodal 17.5% PVA–PEG theta hydrogels both with PEG400 and PEG600 at 17.5% (w/v) concentration and higher (up to 39%) resulted in gels that flowed under their own weight when melted at 120°C. The PVA–PEG hydrogels with higher PEG molecular weight (PEG600) showed lower viscosity and higher injectability than their lower molecular weight counterparts (Fig. 3). The unimodal PVA–PEG systems showed an increase in the viscosity with increasing PEG600 concentration and a decrease in the viscosity with increasing PEG400 concentration (Fig. 3). The gamma sterilized and melted unimodal PVA hydrogels with PEG400 did not form a gel and those with PEG600 formed a weak gel in the simulated tissue mold at 45°C [Fig. 4(a,b)].

Addition of PEG200 to create the bimodal systems with 17.5% PVA hydrogels containing either PEG400 or PEG600 lowered the melt viscosity of the mixtures after gamma sterilization (Fig. 5). The 17.5% PVA–PEG600 bimodal system with 17.5% PEG 200 showed lower viscosity (2733 ± 149 cP) than its

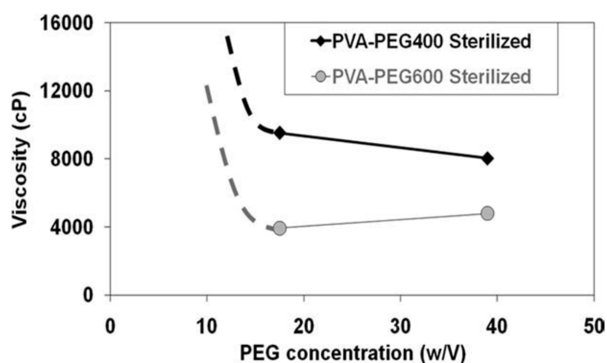

Figure 3 Effect of PEG concentration and molecular weight on the viscosity of melted PVA–PEG hydrogels.

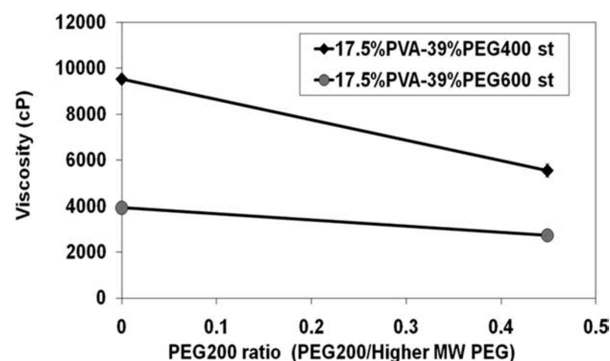

Figure 5 Effect of viscosity modifier (PEG200) on viscosity of melted PVA–PEG hydrogels.

PEG400 counterpart (5560 ± 278 cP) (Fig. 5). Gamma sterilized and melted bimodal PVA hydrogels with PEG400 and PEG600 gelled better when observed 24 h after injection into the simulated tissue mold at 40°C. The bimodal 17.5% PVA-39% PEG600-17.5% PEG200 formulation formed a more mature gel than its unimodal PEG400 counterpart [Fig. 6(a,b)].

Creep resistance of bimodal PVA–PEG600-PEG200 was higher than its unimodal counterpart. TCS values of gamma sterilized and melted 17.5 PVA-39% PEG600 and 17.5 PVA-39 PEG600-17.5 PEG200 were measured as 84% and 61%, respectively, 24 h after injection and gelation in the tissue mold (Fig. 7). The initial elastic response of the PVA–PEG gels to the 0.5 MPa uniaxial stress was lower with bimodal PVA–PEG600 than unimodal formulation. On unloading, both unimodal and bimodal PVA–PEG600 systems showed ER, which was higher with the bimodal system (Fig. 8). PVA–PEG600 unimodal system showed slightly higher EWC content than PVA–PEG600 bimodal system. The EWC of the 17.5%PVA-39%PEG600 and 17.5%PVA–PEG600-17.5%PEG200 was measured as 90% ± 1.3% and 87% ± 0.4%, respectively.

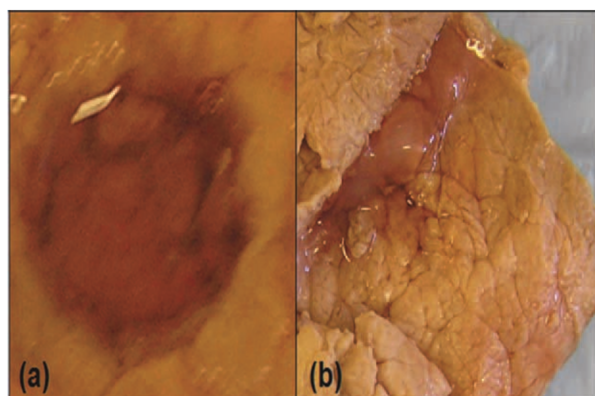

Figure 4 Gamma sterilized 17.5% PVA with (a) 39% PEG400 at 40°C and (b) 39% PEG600 at 40°C after 24 h gelation in tissue-mold [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

In an earlier study, we showed that the presence of gelling agent PEG prevented the physically crosslinked PVA-theta gel network from crosslinking when exposed to gamma irradiation.[26] In this study, our primary goal was to improve the *in vivo* simulated injectability and the mechanical strength of the theta-gel formulation after irradiation. We investigated the effect of molecular weight and concentration of gelling agent on injectability and mechanical strength. We also introduced a second gelling agent with a lower molecular weight than PEG400 and PEG 600 as a viscosity modifier by creating bimodal systems and compared properties of these systems with their unimodal counterparts.

We kept the PEG concentration at 39% due to the poor anti-crosslinking ability of PEG and the weak gelation behavior of the PVA–PEG solution on melting after gamma sterilization with PEG concentrations lower than 39%.[26] Above 39%, we were not able to prepare homogenous PVA/PEG solutions with

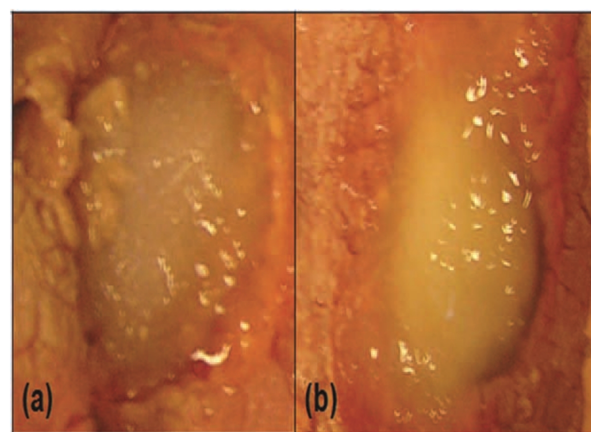

Figure 6 Gamma sterilized 17.5% PVA with (a) 39%PEG400/17.5%PEG200 at 40°C, and (b) 39%PEG600/17.5%PEG200 at 40°C after 24 h gelation in tissue-mold [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

either PEG400 or PEG600 due to the poor miscibility and fast phase separation. Increasing the concentration of PEG400 in gamma sterilized PVA–PEG markedly lowered the melt viscosity, corroborating our hypothesis (Fig. 3). Gels containing PEG600 showed a slight increase in the viscosity with increasing concentration, which may be explained by two competing effects: One is the reduction of crosslinking during irradiation in PVA by the increase in PEG concentration, which would result in decreased viscosity; and the other is the reduction in solvent quality of the PEG solution by the increase in PEG concentration. The resultant effect of these two factors could have caused lower viscosity for the gels with increased concentration of PEG600 (Fig. 3).

Increasing PEG molecular weight resulted in a mechanically stronger gel likely because of a corresponding reduction in solvent quality for PVA molecules with larger PEG molecules, corroborating our hypothesis [Fig. 4(a,b)]. Although sterilized PVA–PEG solutions using high concentration PEG had lower viscosity at injection, incipient phase separation was common in these samples likely due to the reduced solvent quality that came from having a larger number of PEG molecules. We achieved this by creating bimodal systems with the addition of PEG200 in PVA solutions that contained either PEG400 or PEG 600. In these bimodal PVA–PEG solutions, solvent quality at the injection temperatures would be improved without compromising gelation because PEG200 likely acted as a solvent for the higher molecular weight PEGs and reduced the solution viscosity. Our hypothesis tested positive, as these "bimodal" systems containing two different molecular weights of PEG resulted in lower viscosity at injection temperature than their unimodal counterparts (Fig. 6).

The gel formed *in situ* using the PEG600/PEG200 bimodal system resulted in a visibly stronger gel than its unimodal counterpart [Fig. 6(a,b)]. Creep strength measurements supported this observation: PVA–PEG600 system showed 84% TCS, while the PVA–PEG600-PEG200 bimodal system showed 61% (Fig. 7). Although the creep data analysis suggested that the unimodal system had a higher elastic component, the bimodal system showed higher ER (Fig. 8). Total recovery for the PVA–PEG600 bimodal system was also higher than unimodal PVA–PEG600. This result showed that using the bimodal system not only increased the ease of injectability but also increased the mechanical strength of radiation sterilized PVA–PEG injectable gels.

We showed that radiation sterilized PVA–PEG hydrogels could be injected in a simulated *in vivo* tissue environment, where they would form strong gels at body temperature. Furthermore, we presented a novel, injectable, PVA–PEG hydrogel with improved creep strength containing a bimodal PEG molecular weight composition. These hydrogel formulations are promising candidates for minimally invasive tissue repair.

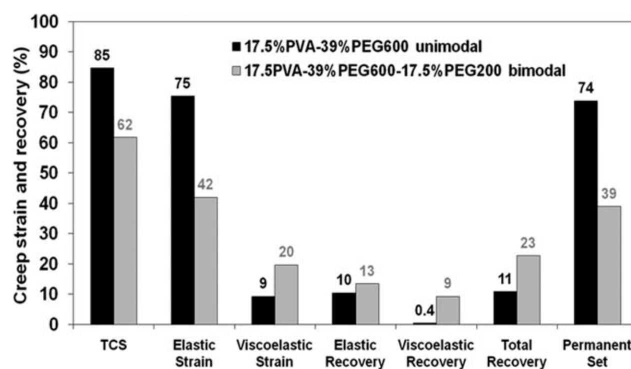

Figure 8 The creep strain and recovery of PVA–PEG600 and PVA–PEG600-PEG200 gels after 24 h gelation in tissue mold at 40°C.

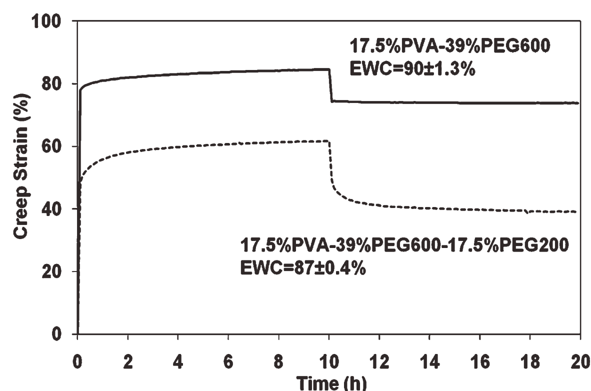

Figure 7 The creep and the relaxation responses of PVA–PEG600 and PVA–PEG600-PEG200 gels after 24 h gelation in tissue mold at 40°C.

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