Journal of Applied Polymer Science

Preparation and Characterization of a Nanofiber Mat Consisting of Tetra-PEG Prepolymers

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ABSTRACT: Tetra-PEG gel, which has been known as a mechanically tough and biocompatible gel, was processed into a nanofiber mat by electro-spinning (ES) and 2-step treatment process using w/o type emulsion consisted of the Tetra-PEG prepolymer 1-octanol/water solution. The 2-step treatment was carried out in order to increase the cross-linking points to the as-spun nanofibers. From this study, we succeeded in insolubilizing poly(ethylene glycol) (PEG) nanofiber, and it was found that the Tetra-PEG gel nanofiber mat showed high tensile property even at swollen state. The elastic modulus at equilibrium swollen state was 4.5 kPa. In addition, we compared the differences of the structure and tensile property between the Tetra-PEG nanofiber mat and porous Tetra-PEG gel prepared by freeze-dry method. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41353.

KEYWORDS: electrospinning; fibers; gels

Received 21 May 2014; accepted 31 July 2014
DOI: 10.1002/app.41353

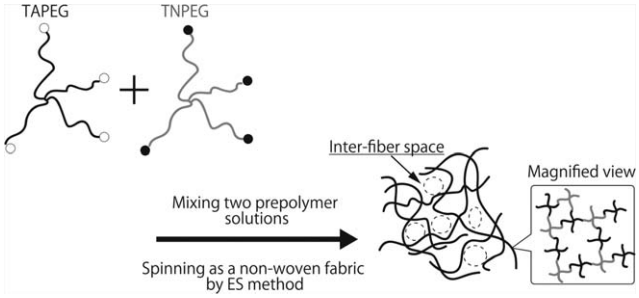
INTRODUCTION

Nanofibers have attracted increasing attentions especially in the fields of separation engineering,[1] energy engineering,[2] and biomedical applications.[3] By processing polymer into nanofiber, we can enlarge the surface area, and by depositing the nanofibers as a mat, many inter-fiber spaces can be obtained. There are several methods for processing nanofibers:[3,4] for example, self-assembly method, phase separation method, and electro-spinning (ES) method. Especially, the ES is a well-established method capable of producing ultra-fine fibers by electrically charging a suspended droplet of polymer melt or solution. Until today, not only usual polymer, but also nano-composite polymer,[5] ceramics,[6] and polymer gel have been processed by ES, showing the versatility of the method.

There are a few examples of the gel nanofiber prepared by ES, such as pH-sensitive nanofibrous gel material using poly(acrylic acid),[7–9] high performance ultrafiltration membranes based on PVA scaffold coated with PVA hydrogel.[10] Because a gel is usually prepared as a film by casting into mold at solution state, it scarcely has air spaces or voids. In this article, we call this conventional gel "cast-gel". On the other hand, by processing gels into nanofiber mat, we can obtain gels with many inter-fiber spaces and make the hydrophilic polymer not to dissolve in water, resulting in the many applications to scaffold, biofilter, and so on. The main problems for preparing gel nanofiber by ES are the difficulties of spinning. Generally, in ES method, a polymer at solution state is set in a syringe with <1 mm inside diameter needle, and pushed out by a syringe pump under high voltage, resulting in producing nanofibers. On the other hand, because a gel is in quasi-solid state, it cannot be spun from the needle of ES apparatus. Therefore, in order to prepare the gel nanofiber, it is necessary to introduce cross-linking points after spinning the polymer solution.

Tetra-PEG gel is the biocompatible gel, which consists of two types of 4-armed PEG prepolymer; tetra-amine-terminated PEG (TAPEG) and tetra-NHS-glutarate terminated PEG (TNPEG).[11] Here, NHS means *N*-hydroxysuccinimide. Due to its extremely homogeneous network structure, the compressive strength of Tetra-PEG gel is very high ($\approx$ 27 MPa), comparable to the

Additional Supporting Information may be found in the online version of this article.



Scheme 1. Schematic representation of hierarchical structure of Tetra-PEG gel nanofiber mat developed in this study.

native articular cartilage.[12] In spite of the recent vigorous studies on structure and mechanical properties of Tetra-PEG gel,[13] applicative studies have not been reported yet. The biocompatibility and high mechanical properties of Tetra-PEG gel should provide excellent materials for medical use.

Then, in this article, we report the preparation method of new type gel nanofiber made of Tetra-PEG prepolymers, and show the structure and physical properties of Tetra-PEG gel nanofiber both in dried and swollen states. By this study, we could fabricate the nanofiber mat of Tetra-PEG gel, where the crosslinking points could not be formed by the previous methods.[7–9] Scheme 1 shows the schematic structure of the Tetra-PEG gel nanofiber mat prepared in this study. The gel nanofiber was made from the two prepolymers via ES method, resulting in the non-woven fabric mat with many inter-fiber spaces, and each gel nanofiber was formed with biocompatible network. Thus obtained Tetra-PEG gel nanofiber mat is expected to be used as an anti-adhesive material for biomedical application, biofilter, and so on.

EXPERIMENTAL

Materials

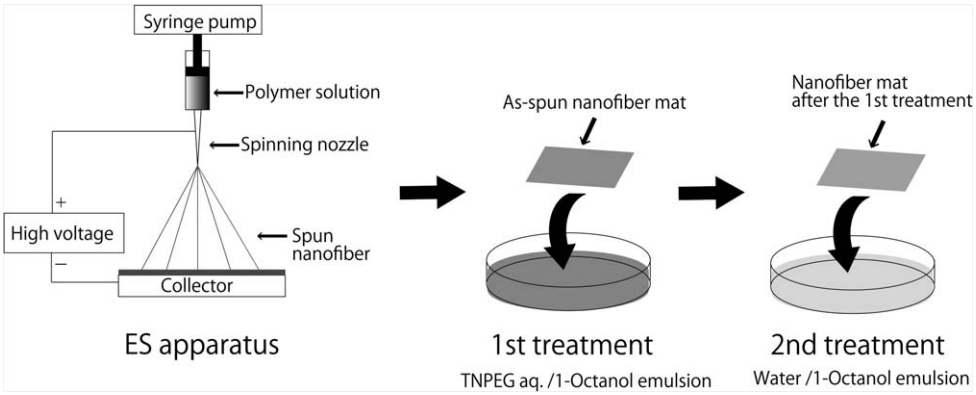
Tetra-amine-terminated PEG (TAPEG) and tetra-NHS-glutarate-terminated PEG (TNPEG) were prepared from tetrahydroxyl-terminated PEG (THPEG) having equal arm lengths. Here NHS represents *N*-hydroxysuccinimide. The details of TAPEG and TNPEG preparation are reported elsewhere.[11] The molecular weights (M_w) of the prepolymers were both 40 kg/mol. Linear 500 kg/mol PEG and 1-Octanol was purchased from Wako Pure Chemical Industries (Osaka, Japan), and Nacalai tesque (Kyoto, Japan), respectively, and used as received.

Preparation Method of the Tetra-PEG Gel Nanofiber Mat

The Tetra-PEG gel nanofiber mat was prepared as follows: at first, TAPEG (140 mg) was dissolved in 50 m*M* phosphate buffer (0.7 g, pH 7.4) and mixed with 9 wt % 500 kg/mol linear PEG solution (0.3 g), which enables easy spinning of the solution. Then, a small amount of TNPEG (25 mg) dissolved in 50 m*M* citric-phosphate buffer (0.5 g, pH 5.8) was added to the above TAPEG/linear PEG solution. The reason why the content of TNPEG was much lower than TAPEG was not to cause gelation of the spinning solution, but to connect the prepolymers moderately. In addition, because TNPEG can easily undergo hydrolysis with time,[14] the excess component of the spinning solution must be TAPEG. After stirred for 1 h at room temperature, the solution was spun by ES apparatus (Scheme 2, left). The viscosity of the solution measured by a vibratory viscometer (VM-10A-M; Sekonic Corporation, Japan) was 306 mPa·s at 24°C. The important points for the spinning solution were (i) to avoid gelation by mixing excess amount of TAPEG, and (ii) to add linear PEG with M_w ranging from 400 to 500 kg/mol. As for the factor (1), we summarized the states (sol/gel) at the various compositions in Supporting Information, Table SI. As for the factor (2), the adding linear PEG must have to proper M_w: in the case of 8000 kg/mol linear PEG, the viscosity of the solution was too high to spin. On the other hand, in the case of 100 kg/mol linear PEG, it was difficult to spin continuously.

The ES was carried out at 20 kV with 0.75 mL/h flow rate, and the nozzle-collector distance was 23 cm. This spinning condition was an optimized one: If the flow rate was high or the nozzle-collector distance was short, the solvent of the spinning solution did not completely evaporated before reaching the corrector plate. The gauge size and the inside diameter of the needle for the spinning nozzle was 21 gauge, and 0.51 mm, respectively. The ground plate was a copper plate covered with aluminium foil. The obtained as-spun fiber mat was completely dried state, and it could be easily taken off from the ground plate.

Here, note that the as-spun fiber can easily dissolve in water, because the spun solution contains excess amount of TAPEG comparing with TNPEG, and so the cross-linking reaction is



Scheme 2. Preparation method of Tetra-PEG gel nanofiber mat.

still insufficient. Then, in order to increase the cross-linking points, we carried out two-step treatment as follows: in the 1st treatment process, the as-spun fiber mat was soaked in the w/o type emulsion, which consists of TNPEG aqueous solution and 1-octanol (Scheme 2, middle). 1-Octanol was suitable for a dispersion medium because it was a poor solvent for PEG. After soaked for 1 h, the fiber mat was immersed in water/1-octanol emulsion as the 2nd treatment process (Scheme 2, right). The role of the 1st treatment is to increase the cross-linking points to the fiber, and that of the 2nd treatment is to wash the unreacted polymer. Here, we varied the TNPEG content used in the 1st treatment from TN/TA = 1 to 3: TN/TA was defined as the ratio of TNPEG weight used in the 1st treatment against TAPEG weight contained in the as-spun nanofiber mat. The water content used in the 1st treatment was fixed as 1.6 wt %, which was low enough not to dissolve the as-spun nanofibers. The water content of the 2nd treatment emulsion was 3.2 wt %.

Preparation of the Porous Tetra-PEG Gel (FD-Gel)

The porous Tetra-PEG gel (FD-gel) was prepared as follows: TAPEG and TNPEG (both M_w s were 40 kg/mol) were dissolved in phosphate buffer (50 m*M*, pH 7.4) and citric-phosphate buffer (50 m*M*, pH5.8), respectively. The concentration of the both solutions was 100 mg/mL. Equal amount of these two solutions were mixed and poured into the mold (30 × 20 × 1 mm). The obtained cast-gel was freeze-dried by a freeze-drier (FDU-1200; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for 3 h at around −46°C, under 20 Pa.

Scanning Electron Microscopy (SEM) Observation and Diameter Measurement

The SEM observations were carried out by using Keyence scanning electron microscope (VE-9800; Keyence Co., Osaka, Japan). The fiber samples were gold-sputter coated with an ion coater (SC-701; Sanyu Electron Co., Tokyo, Japan). The average and the standard deviation of the fiber diameters were determined from 100 measurements using Adobe Photoshop CS3 extended program.

Pore Size Measurement

The pore size was measured by a permporometer (Capillary flow porometer, CFP-1200-AEXLTC; Porous Materials, Inc.). The pore size was evaluated using the following equation[15]

$$D = \frac{4\gamma\cos\theta}{P}, \quad (1)$$

where D was the diameter of the pore, γ was the known surface tension of the test solution which penetrated into the pores, θ was the contact angle of the test solution, and P was the air pressure which could push out the test solution from the pore. The dried sample was immersed in a test solution (Galwick, Porous Materials, Inc.) with known surface tension (15.9 dyn/cm).

Porosity Measurement

The porosity was evaluated by the average weight and volume of six specimens as shown in the following equation:

$$\text{porosity} = 1 - \frac{w_s}{v_s \rho_{\text{PEG}}} \quad (2)$$

where w_s and v_s are the weight and the volume of the specimen, and ρ_{PEG} is the density of dried Tetra-PEG cast-gel (1.176 g/cm^3).[16]

Tensile Measurement

The tensile measurements were performed by a mechanical testing apparatus (Tensilon UTM-III; Toyo Baldwin Co., Tokyo, Japan) at room temperature. The rectangular shaped samples were used and the tensile speed was 10 mm/min. The initial length was 10 mm.

RESULTS AND DISCUSSION

Characterization of the Tetra-PEG Gel Nanofiber

Figure 1(a) shows the scanning electron microscopy (SEM) image for the as-spun fiber mat. The average diameter of the as-spun fiber was 0.31 ± 0.06 μm. Figure 1(b) shows the fiber mat after the 1st treatment. As can be seen, spine-like structure was formed on the fibers. This structure should be attributed to the precipitated TNPEG, because it developed as increasing TNPEG content added in the treatment emulsion (Figure 2). However, this spine-like structure disappeared after the 2nd treatment [Figure 1(c)], indicating that the un-reacted TNPEG was washed out by the 2nd treatment. For the sample prepared at TN/TA = 3, the diameter after the 2nd treatment was 0.39 ± 0.09 μm, and the average pore size measured with the permporometer was 413 nm. Here, the pore size means the size of the inter-fiber space. The porosity was 0.69 ± 0.12.

Then, in order to check whether the cross-linking points were formed in the fiber or not, we soaked the finally obtained fiber mat in water for 1 h. Figure 1(d) shows the image of the fiber mat after swollen and then dried. As you can see, the nanofiber retained its fibrous form even after the swelling process. Considering that the conventional PEG nanofiber easily dissolves in water, it was concluded that the cross-linking points were surely introduced inside the Tetra-PEG gel nanofiber, though the formed network structure should not be a homogeneous one like previously reported Tetra-PEG gel films.[17] However, the swelling and drying processes caused contraction and wrinkles on the nanofiber [Figure 1(d)]. This is the similar phenomenon to the macroscopic contraction of the nanofiber mat, as will be shown later [Figure 6(a)].

Comparison with the Porous Tetra-PEG Gel

Secondly, we show the structural difference between Tetra-PEG gel nanofiber [Figure 1(c)] and the other porous Tetra-PEG gels (FD-gel) prepared by frozen-dried method (Figure 3). The FD-gel was prepared by freeze-drying the Tetra-PEG cast-gel. Figure 3 shows the SEM images of (a) the surface and (b) the cross-section of the FD-gel. The figure (c) shows the magnified view of the figure (b). The cross-section was obtained by breaking the frozen sample. Comparing with the Tetra-PEG gel nanofiber mat [Figure 1(c)], the FD-gel seemed to have larger pores than the Tetra-PEG gel nanofiber had. However, as shown in the magnified view [Figure 3(c)], the pores of the FD-gel were not continuous, but each pore existed independently. In addition, we could not measure the pore size of the FD-gel sample by the permporometer. This is because the permporometer evaluates the pore size by assuming the pores pass through a sample. Therefore, we concluded that the FD-gel did not have any through-pores, while the Tetra-PEG gel nanofiber mat had many small through-pores. The porosity of FD-gel was

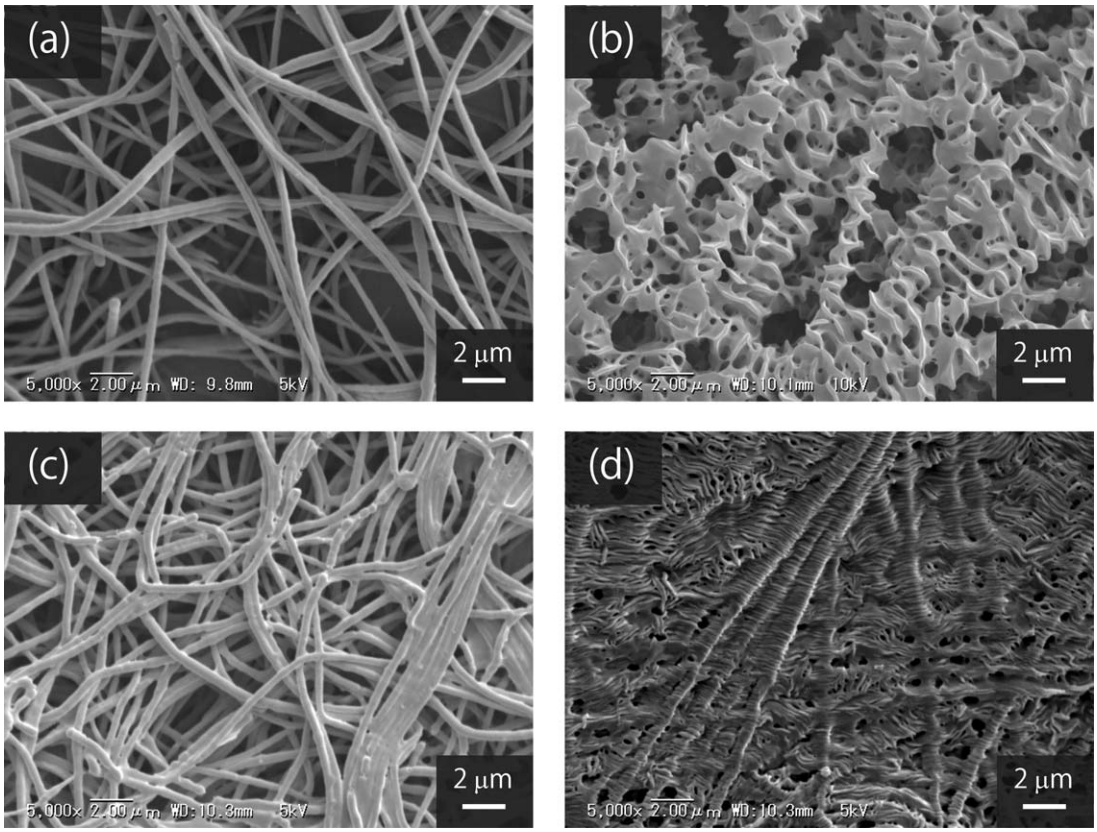


Figure 1. SEM images of the Tetra-PEG gel nanofiber. Image (a) as-spun, (b) after the 1st treatment, and (c) after the 2nd treatment. After these two-step treatments, the fiber mat was swollen in water and dried for SEM observation under tension in order not to shrink during drying process [image (d)].

0.44 ± 0.16, which was lower than that of the nanofiber mat ("Characterization of the Tetra-PEG gel nanofiber").

Tensile Properties at Dried State

Figure 4 shows (a) the stress–strain (s–s) curves of the Tetra-PEG gel nanofiber mat at dried state as a function of TN/TA, and (b) the variation of the Young's moduli (E) evaluated from the initial slopes of the s-s curves. From these figures, it was found that the tensile property can be controlled by TN/TA ratio, indicating that the TNPEG used in the post-treatment did not perfectly react with the TAPEG contained in the as-spun nanofiber. According to the literature,[18] the Young's modulus and breaking stress of the conventional 900 kg/mol PEG nanofiber was respectively about 4 MPa and 0.09 MPa, which was much lower than the case of Tetra-PEG gel nanofiber (the average values were 10.5 MPa and 2.1 MPa, respectively, at TN/TA = 3). The high tensile strength of the Tetra-PEG gel nanofiber should be attributed to the network structure inside the nanofiber.

Tensile Properties at Swollen State

Then, we also measured the tensile properties at swollen state. Figure 5(a) shows the s–s curves of the nanofiber mat as a function of the polymer weight fraction (PWF). From the initial slopes of the s–s curves in Figure 5(a), we evaluated the E values and showed them in Figure 5(b). In Figure 5(b), the E values of the FD-gel and Tetra-PEG gel prepared by usual casting method were also plotted as a comparison.[19] The s–s curves of the FD-gels were shown in Supporting Information, Figure S1. All of the molecular weights of Tetra-PEG prepolymers used in

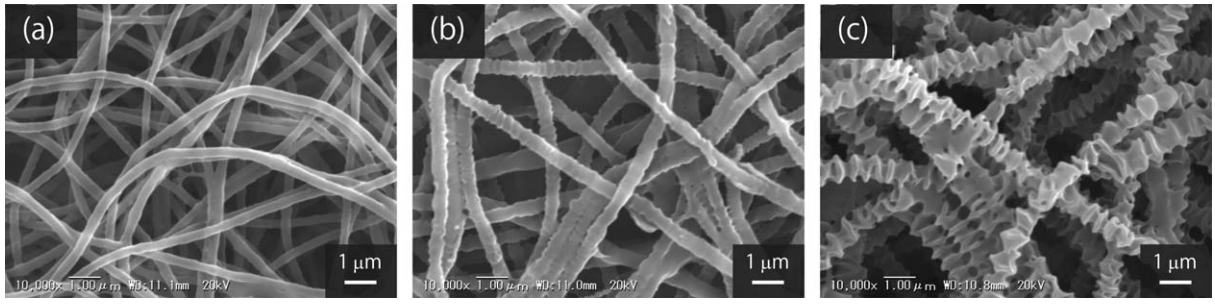


Figure 2. The SEM photographs after the 1st treatment samples with different TN/TA ratios. (a) TN/TA = 2, (b) TN/TA = 2.5, and (c) TN/TA = 3.3.

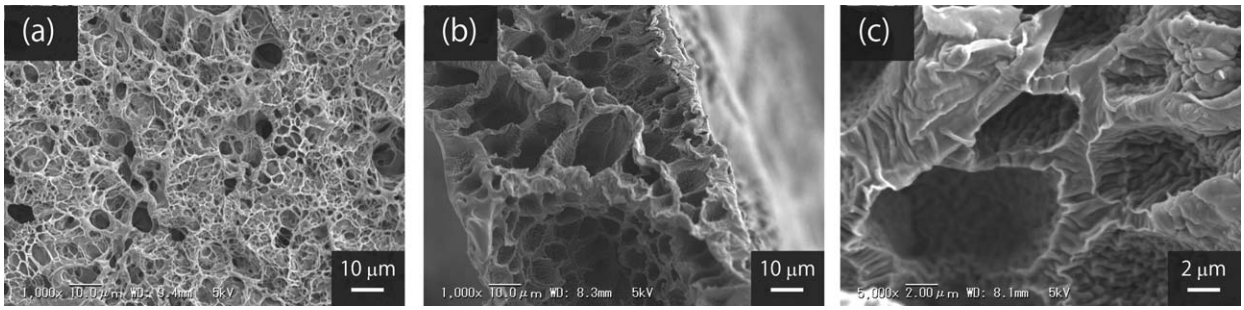


Figure 3. The SEM photograph of the porous Tetra-PEG gel (FD-gel) prepared by freeze-drying the usual Tetra-PEG cast-gel. Image (a) surface, (b) cross-section, and (c) magnified view of the cross section.

the nanofiber mat, the FD-gel, and the cast-gel were the same (40 kg/mol). The nanofiber mat samples were prepared at TN/TA = 3. Among $0.6 < PWF < 1$, the E values of the nanofiber mat decreased steeply with decreasing PWF, but in the range of the $PWF < 0.6$, the slope became gradual, compared to the case of the cast-gel. The E value of the nanofiber mat at equilibrium swollen state was 4.5 kPa. Interestingly, at PWF $\approx$ 0.08, the E values of the nanofiber mat were the same order as those of the corresponding cast-gel,[19] although they should have many inter-fiber spaces and network defects comparing with the cast-gel. In addition, the equilibrium swelling ratio by weight was 12.5 ± 0.3 (PWF = 0.08), which was much smaller value than that of the Tetra-PEG cast-gel.[17] Here, the swelling ratio was defined as the ratio of the weight after swelling against the weight at dried state. The low swelling ratio may be attributed to the inter-fiber spaces: because of the existence of the many inter-fiber spaces, the gel nanofiber mat could not retain water comparing with the cast-gel did. In addition, although the nanofiber mat absorbed up to about 12.5 times its own weight in water, it immediately shrunk to ca. 49% area, contrary to the case of the cast-gel (Figure 6). Considering that the weight increased after the swelling, each nanofiber should absorb water and shrink at the same time, resulting in the contraction of the overall mat. Similar shrinking behaviour was also reported by Mather et al.[20] This shrinkage may be due to the inter-fiber spaces and the entropic effect, which caused the relaxation and the contraction of the fibers during swelling. We conjectured that the shrinkage might also induce the entanglement between the nanofibers: Such entanglement might cover the loss of the E values come from the inter-fiber spaces and network defects, resulting in the same order E values as corresponding cast-gel.

The FD-gels elongated much longer than the corresponding nanofiber mats (Supporting Information, Figure S1), and the E values were higher [Figure 5(b)]. These results should be attributed to the structural difference between the nanofiber mat and the FD-gel, as shown in Figure 1(c) and Figure 3, respectively. In the case of the FD-gel, the wall forming the gel had continuous structure and the thickness of the wall was about 2 μm, while the nanofiber mat consists of the nanofibers with submicron order diameter, and had many inter-fiber spaces. In addition, at $PWF > 0.3$, the E values of the FD-gel were on the extension line of those of the cast-gel, but they became higher than those of the cast-gel at $PWF < 0.3$. Considering that the FD-gel had many large independent pores (Figure 3), this result can be explained as follows: At $PWF > 0.3$, that is, at near dried state, the water should not be in the pore region, but in the Tetra-PEG network region. This situation is similar to that of the cast-gel, therefore, the E results of the FD-gel at near dried state should be on an extension line of the results for the cast-gel. On the other hand, at $PWF < 0.3$ (at near equilibrium swollen state), the water should also be in the pore regions, and the

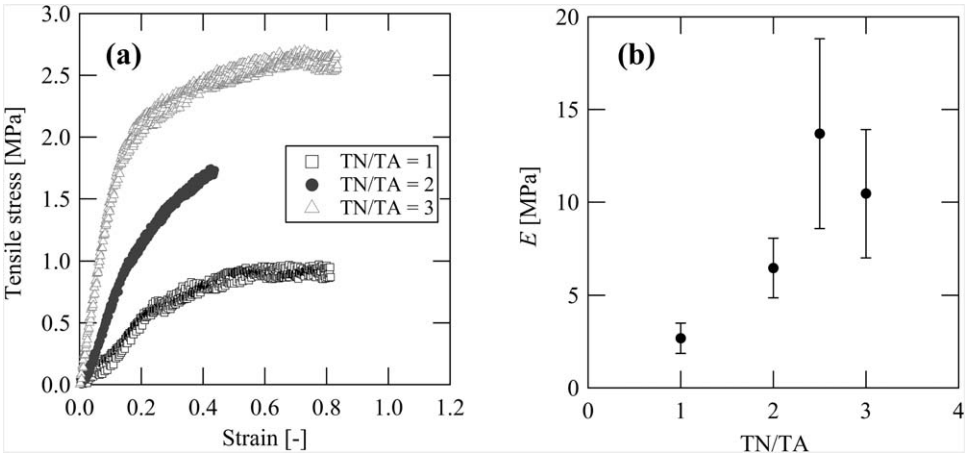


Figure 4. (a) Tensile stress–strain curves of the Tetra-PEG gel nanofiber mat at dried state, and (b) the variation of the Young's moduli (E) as a function of TN/TA. The shown s–s curves were the typical results.

Tetra-PEG network region should be fully swollen. Because the water inside the pore should not have an influence on the *E* value, the variation of the *E* values near equilibrium swollen state did not largely decrease as decreasing PWF.

CONCLUSIONS

We successfully prepared the novel type gel nanofiber consisting of 4-armed poly(ethylene glycol) (Tetra-PEG) network. The gel nanofiber was prepared by electro-spinning the polymer solution, which consisted of the two types of Tetra-PEG prepolymers and linear PEG with high molecular weight. Then cross-linking points were added to the as-spun nanofiber through 2-step treatment processes using w/o type emulsion. The obtained nanofiber mat did not dissolve in water, suggesting that the cross-linking points were introduced inside the nanofiber. Secondly, we showed the structural difference between the Tetra-PEG gel nanofiber mat and the other porous Tetra-PEG gel (FD-gel) prepared by freeze-drying method. The FD-gel did not have any through-pores, while the Tetra-PEG gel nanofiber mat had many small through-pores. The tensile properties of the nanofiber mat, such as the Young's moduli were also evaluated, and it was found that they could be controlled by the TN/TA ratio, which was defined as the ratio of TNPEG weight used in the 1st treatment against TAPEG weight contained in the precursor nanofiber mat. In addition, the swelling ratio of the nanofiber mat was smaller than that of cast-gel, and the nanofiber mat shrunk immediately when immersed in water. This shrinkage may be due to the inter-fiber spaces and the entropic effect, which caused the relaxation and the contraction of the fibers during swelling.

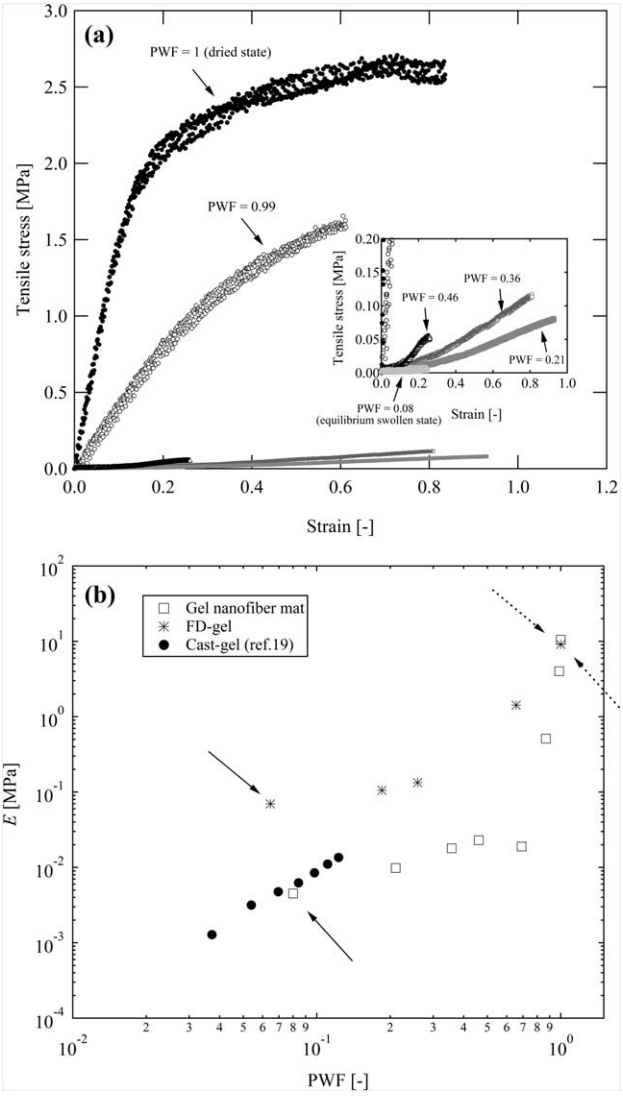


Figure 5. (a) Tensile stress–strain curves of the swollen Tetra-PEG gel nanofiber mat with different polymer weight fraction (PWF) prepared at TN/TA =3. (b) The Young's moduli for the gel nanofiber mat, FD-gel, and cast-gel as a function of PWF. The data of the cast-gel in part (b) was reproduced from Ref. 19 and changed to the function of PWF. As PWF decreases, the water content inside the gels increases. The broken and solid arrows indicate the results at dried and equilibrium swollen states, respectively, both for the nanofiber mat and the FD-gel.

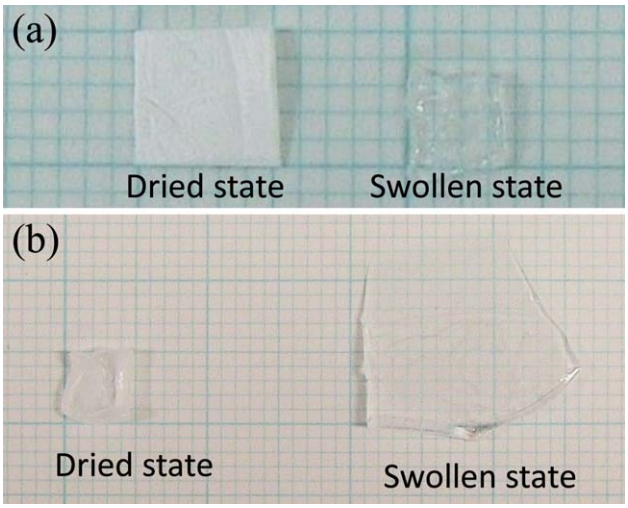


Figure 6. Comparison of the dried and equilibrium swollen state for (a) Tetra-PEG nanofber mat and (b) the corresponding cast-gel. One tick indicates 1 mm. In the case of the conventional cast-gel [image (b)], the area enlarged after equilibrium swelling state. However, the nanofiber mat [image (a)] shrunk to about 49% area after equilibrium swelling state. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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