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Hydrogel-elastomer composite biomaterials: 2. Effects of aging methacrylated gelatin solutions on the preparation and physical properties of interpenetrating polymer networks

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Abstract This study was conducted to understand the effects of aging methacrylated gelatin solutions on the properties of gelatin-HydroThane™ Interpenetrating Polymer Network (IPN) films. The latter were prepared from methacrylated gelatin solutions that were either freshly made or stored at different concentrations and temperatures for various periods. The morphology, swelling stability and mechanical properties of the IPNs were then accordingly characterized. The IPNs prepared with aged solutions showed a reduced phase separation; changed from a network-like structure to a continuous phase structure; and demonstrated higher swelling stabilities and higher elasticity under optimal aging conditions, compared to the IPN prepared with a fresh methacrylated gelatin solution. An increase in viscosity and a change in phase transition of aged methacrylated gelatin solutions were also observed, presumably due to the physical structuring of methacrylated gelatin chains (e.g., by the formation of a helix structure), thus altering the resulting IPN characteristics. A better understanding of the effects of aging methacrylated gelatin solution on the formation and properties of gelatin-HydroThane™ IPNs should enable us to further develop our composite biomaterials for different dressing applications.

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1 Introduction

Interpenetrating polymer networks (IPNs) are a special class of composite materials in which at least one crosslinked network is formed in the presence of other components [1]. They have been extensively studied because of their potential for combining properties of a wide range of materials ranging from plastics and elastomers to hydrogels suitable for biomedical applications [2, 3]. Moreover, the formation of IPNs has led to improving the properties of each constituent polymer, known as positive synergism effects [4].

Since it is generally known that the phase structure (morphology) of an IPN determines its physical properties, it is important to control phase-separated structures in the scale ranging from nanometer to micrometer. IPNs have been produced by a variety of methods to obtain different morphologies and to establish their structure-property relationships [1, 5]. Most of the studies were directed toward the effects of IPN compositions on their morphologies and properties [5, 6]. The roles of irradiation intensity [5], reaction temperatures [6, 7] and chemical structures [8] on IPN morphology have also been investigated. Moreover, a few studies have identified the effects of secondary structures of constituent polymers, in particular molecular weights, on the miscibility and properties of IPNs composed of polyurethanes and derivatives of polysaccharides [9, 10]. However, little or no studies were conducted to determine the effects of other intrinsic properties of a constituent polymer (e.g., conformations) on IPN formation.

Aging of polymers is a phenomenon observed either in their solid states or in solutions [11, 12]. It can lead

to changes in physicochemical properties of a polymer. For example, gelatin continued to rearrange its physical structure for weeks after the initial gelation process [12]. We have previously reported on a gelatin-HydroThane™ IPN prepared by simultaneously crosslinking each constituent polymer in solution [H.T. Peng et al. in press]. Specifically, gelatin was modified by methacrylation and then photo crosslinked in solution with a thermoplastic polyurethane, HydroThane™. We also confirmed that both polymers were indeed photo crosslinked to form a simultaneous full IPN. In our previous study, we prepared the IPNs under different chemical conditions and characterized their swelling and mechanical properties [H.T. Peng et al. in press]. One of our goals was to optimize the IPN preparation to achieve good reproducibility and properties for potential wound dressing applications. Our initial work with methacrylated gelatin solutions, stored for a prolonged period, has led us to study the influence of solution aging on IPN formation and properties, thus allowing us to further optimize our IPN system. It was hypothesized that the aging of a methacrylated gelatin solution would alter the morphology of an IPN prepared with the aged solution. Since there is a strong dependence of gelatin solution properties on its concentrations and storage temperatures [13], we also studied the aging effects at different solution concentrations and temperatures. To our knowledge, there are no reports of aging effects of polymer solutions on IPN formation and properties.

In this study, we assessed the effects of aging methacrylated gelatin solutions on the morphology, swelling stability and mechanical properties of a gelatin-HydroThane™ IPN. More specifically, we were able to modulate and correlate the structure-property relationship associated with the IPN morphology, and their swelling and mechanical properties, allowing us to produce our IPN biomaterials with good reproducibility and optimal performance. Based on the available results, we also explored possible mechanisms underlying the aging effects of the methacrylated gelatin solution.

2 Materials and methods

Gelatin Type A (Lot 39107-22409) with a bloom number of 235 was purchased from Great Lakes Gelatin (IL, USA). Methacrylic anhydride (94% purity) and sodium azide were obtained from Aldrich (ON, Canada). HydroThane™ (AR25-80A; Lot CTB-H26A-1113) was provided by Cardiotech International Inc. (MA, USA). The photoinitiator, 2, 2-dimethoxy-2-phenylacetophenone (Irgacure 651) was obtained from Ciba Specialty Chemicals (ON, Canada). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific (ON, Canada). Haematoxylin, eosin and rhodamine B were purchased from Aldrich (ON, Canada). Silicone oil standards were obtained from Brookfield Engineering Laboratories Inc. (MA, USA). Dialysis membranes with a molecular weight cut-off of 12,000–14,000 were obtained from Fisher Scientific (ON, Canada). Sterile fetal bovine serum was purchased from Cansera International Inc. (ON, Canada).

2.1 Methacrylation of gelatin

Ten g of gelatin were dissolved in 100 mL of phosphate buffered saline (PBS, pH 7.4) and stirred at 50°C. A volume of 0.5 or 1 mL of methacrylic anhydride was added. The reaction mixture was stirred for 60 min at approximately 50°C, dialyzed against distilled water at 37°C for 1 week, and freeze-dried until a constant weight was reached.

2.2 Preparation of gelatin-HydroThane™ IPN films

The IPN films were prepared using freshly made 4 wt% HydroThane™ and 7.5 wt% methacrylated gelatin solutions (1:1 by polymer weight). More specifically, a 0.67-g aliquot of 7.5 wt% methacrylated gelatin in DMSO was mixed with 1.25 g of 4 wt% HydroThane™ in DMSO in a glass scintillation vial, followed by the addition of 91-μL of a 10 wt% Irgacure 651 in DMSO. The mixture was then vigorously vortexed, purged with nitrogen for 5 min and UV-irradiated at 350 nm at an intensity of 9 mW cm^{-2} for 15 min in a photochemical chamber reactor (RAYONET model RPR-200, Southern New England Company, CT, USA). The resulting films were washed in a 0.1% sodium azide aqueous solution at ambient temperature for a week to remove DMSO; these films are designated as washed films. The washed films were then freeze-dried until a constant weight was reached; these films are designated as freeze-dried films. To study the aging effects, methacrylated gelatin solutions were prepared in DMSO at 7.5 and 18 wt%, and stored in sealed glass vials at either room temperature or 50°C for different time intervals. The solutions were then used directly (if aged at 7.5 wt%) or diluted to 7.5 wt% (if aged at 18 wt%) to prepare IPNs under the same conditions as previously described.

2.3 Characterization of gelatin-HydroThane™ IPN films

2.3.1 Morphology analysis

Washed and freeze-dried IPN films were sectioned with a Microm HM560 cryostat (MICROM International GmbH, Walldorf, Germany) and then stained, using either Haematoxylin and Eosin (HE) or rhodamine. Rhodamine selectively stained the Hydrothane™ component in the washed films and preferentially stained it in the freeze-dried films, while the HE preferentially stained the Hydrothane™ component in both types of films. The two types of staining allowed the distribution of each component to be easily identified.

Images were taken with a Nikon CoolPix880 digital camera (Nikon Corporation, Mississauga, ON, Canada) through the eyepiece of an Olympus BH-2 optical microscope (Olympus, Brampton, ON, Canada) at 100 × magnification. Three sections were taken for each film: the top section interfacing with air, the middle section, and the bottom section interfacing with glass during material preparation, to determine changes in morphology over the Z-direction. Ten images were taken at different locations of each sample section. All the images were transferred to a computer and the relative areas of gelatin, HydroThane™ and pores were calculated, using a combination of the HE and rhodamine-stained samples, with the aid of an image processing program [14]. If the image had two components, one component was rendered black and the other white. For images with three components, specific components were selected for further differentiation. By performing this process twice, we could take the difference to calculate all individual components.

2.3.2 Stability study

Swelling measurement has been widely used as a simple method to characterize water absorption and stability of biomaterials [15]. We therefore conducted stability studies by measuring the swelling of each washed film in the 0.1% sodium azide aqueous solution at room temperature. At specific time intervals, each film was blotted dry, weighed and then re-immersed in the medium. The swelling ratio of each film was measured as a ratio between mass in a swollen state and initial mass of polymers used to prepare the film.

In addition, freeze-dried IPN films were rehydrated in a solution of 50% fetal bovine serum and 0.1% sodium azide in distilled water at 37°C. The swelling of each film was measured by the same method as previously described and calculated as a ratio between mass in a swollen state and initial dry mass of the film.

2.3.3 Mechanical testing of films

Mechanical tests were conducted on the freeze-dried films immersed in the serum-containing medium at 37°C for 4 days. The films were cut into pieces of 2 cm × 1 cm × 0.2 cm. The force and elongation at break point were measured using a Zwick materials testing machine (TC-FR005TN.A50, Zwick USA, Kennesaw, GA, USA) at a test speed of 50 mm min^{-1}. The ultimate stress and strain parameters were calculated, respectively, as the force at the break divided by the cross-section area, and as the elongation at the break divided by the initial length of the IPN film.

2.4 Viscosity measurement

Vials of methacrylated gelatin solutions were prepared at 7.5 and 18 wt% in DMSO, and stored at room temperature and 50°C for up to 36 days to quantify the effects of aging, temperature and concentration on viscosity, and also to understand their subsequent effects on IPN morphology and properties. At specific time intervals, the viscosity of each solution was measured at room temperature and 7.5 wt%, using a viscometer equipped with a spindle (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). Silicone oil standards were used to calibrate the viscometer.

2.5 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed using a Perkin Elmer Pyris 6 system (PerkinElmer Canada Ltd., Woodbridge, ON, Canada), over a temperature range of 20 to 150°C to determine any thermal transitions of the methacrylated gelatin solution, aged at 7.5 wt% and 50°C for different time intervals. The temperature range is above the freezing point (18°C) and below the boiling point of DMSO (189°C), to avoid the interference by the thermal transitions of the solvent. Each sample was weighed and sealed in a 50-μL aluminum pan and heated at 10°C min^{-1} under nitrogen. The reference was an empty pan. The system was calibrated using indium with a melting temperature of 156.6°C and enthalpy of 28.71 J g^{-1} as a standard.

2.6 Statistical analysis

Data are expressed as means ± standard deviation, unless otherwise specified. Significant differences

between two groups were evaluated using a two-tailed student t test. When $P < 0.05$, the differences were deemed statistically significant.

3 Results

3.1 Morphology analysis

We conducted both qualitative and quantitative analyses of the morphologies of IPN films prepared with either a fresh or aged solution of methacrylated gelatin. The IPN prepared with a fresh solution was designated as non-aged; those prepared with aged solutions were designated as aged. In addition, aging concentration, temperature and incubation period were specified to designate IPNs, prepared with different aged solutions. For example, the IPN prepared with a solution aged at 7.5 wt% and room temperature for 3 weeks was designated as aged–7.5 wt%-RT-3 weeks.

Figure 1 depicts a series of images of washed and freeze-dried films sliced near the top, middle and bottom of the sample. The bright and dark regions of the HE-stained washed films correspond to the gelatin- and HydroThane^{TM}-rich domains, respectively (Fig. 1). The unstained regions correspond to the pores. Both non-aged and aged films showed extensive phase separation with distinct gelatin- and HydroThane^{TM}-rich domains and few pores. The hydrogel domain was interspaced into the HydroThane^{TM} network. There was an apparent increase in the domain size of gelatin and a decrease in the area size of HydroThane^{TM} domain from the top section to the bottom section for both types of films. Compared to the non-aged film, the aged film showed smaller gelatin domains and thus a relatively denser network structure of HydroThane^{TM}. Accordingly, the aged film possessed a larger area fraction of HydroThane^{TM} component compared to the non-aged.

The HE-stained freeze-dried films were highly porous and showed a heterogeneous gelatin network interspaced into the HydroThane^{TM} network from the top section to the bottom section of both non-aged and aged films (Fig. 1). The pores were irregular in shape and non-directional. The non-aged films showed looser gelatin networks and larger pores compared to the aged films. Accordingly, the architecture of the aged film was relatively denser.

Figure 2 further illustrates the different morphologies between the non-aged and aged films that were stained with rhodamine after the washing process. It also shows smaller gelatin domains for the aged films

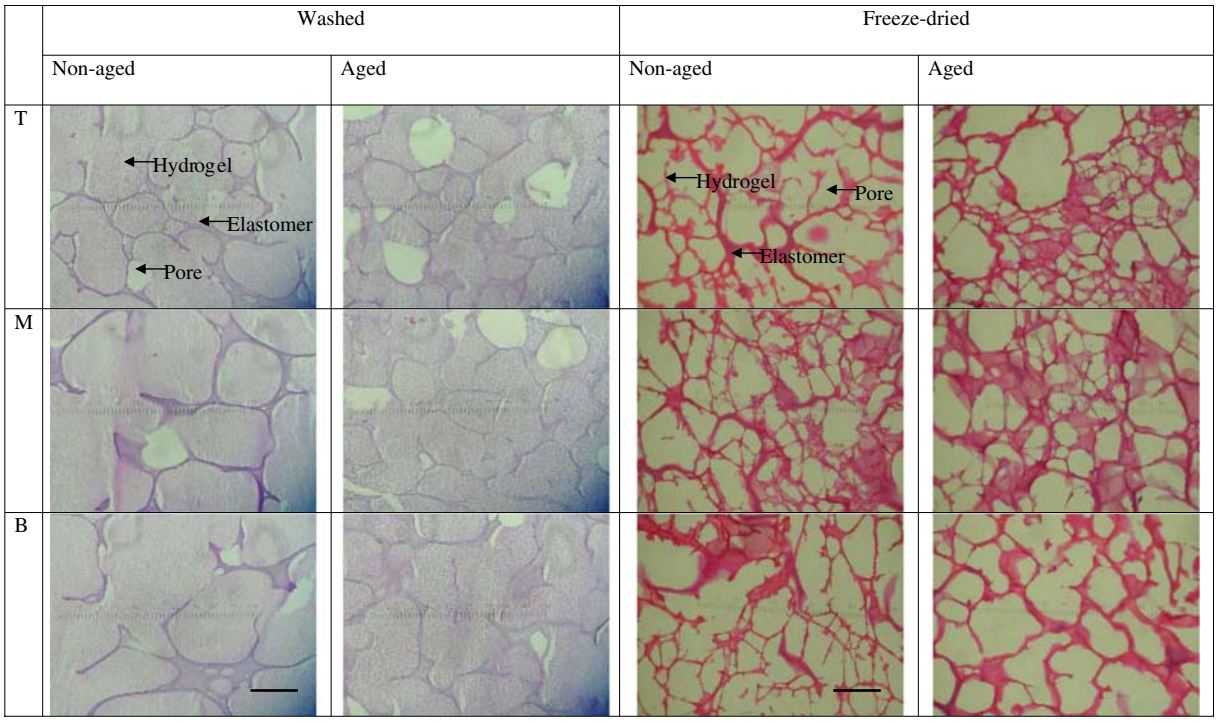

Fig. 1 Typical light microscopy photographs of IPN films prepared with either a fresh (i.e. non-aged) solution of methacrylated gelatin at 7.5 wt% or a solution aged at 7.5 wt% and room temperature for 3 weeks (i.e. aged). Films were sliced and stained with hematoxylin and eosin at the top (T), middle (M) and bottom (B), after the washing and freeze-drying processes. Scale bar is 120 μm

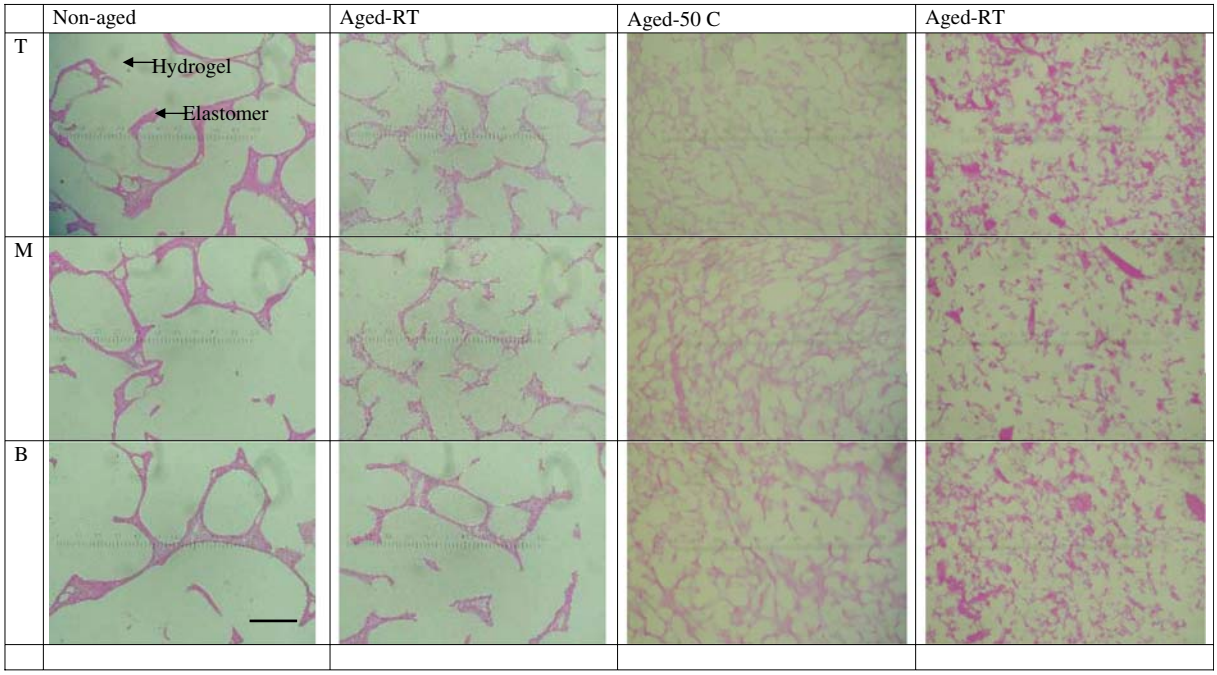

Fig. 2 Typical light microscopy photographs of non-aged and aged washed IPN films sliced and stained with rhodamine at the top (T), middle (M) and bottom (B). Films were prepared with methacrylated gelatin solutions at 7.5 wt% (first three columns) or with the solution aged at 18 wt% and diluted to 7.5 wt% (last column). Scale bar is 120 μm

compared to the non-aged films. In addition, the two-phase structures among the films prepared with the solutions aged under different conditions were definitely different from each other. The films prepared with solutions aged at a higher concentration (18 wt%) or temperature (50°C) exhibited less phase separation and a different morphology with no pores. Another noteworthy observation was the changes from a network-like morphology to a morphology of HydroThane™ dispersed in a continuous gelatin matrix to a bicontinuous morphology, as aging concentrations and temperatures were increased from 7.5 wt% to 18 wt% and from 20°C (room temperature) to 50°C, respectively. The interface between the two phases became merged, implying an improvement in interpenetration.

Figure 3 quantitatively depicts the percent areas of different domains in both types of films. Figure 3A shows different area fractions between the non-aged and aged IPNs after the washing and freeze-drying processes. The non-aged washed IPN possessed a smaller overall area fraction of the HydroThane™-rich domain than the aged film (14% vs. 17%, $P < 0.05$), while gelatin almost made up the remainder. The presence of pores in the freeze-dried films changed the balance of the two polymer domains. Overall, Hydrothane™ domain constituted about 14% of the total area, gelatin about 26% and pores the remaining 60% in the non-aged IPN. The corresponding values in the aged freeze-dried IPN were approximately 26%, 26% and 48%. Gelatin area fractions were therefore dramatically reduced in the freeze-dried IPNs (both non-aged and aged) compared to approximately 80% in the washed IPNs. On the other hand, compared to the washed IPNs, HydroThane™ area fractions in the freeze-dried IPNs remained almost the same in the non-aged, but were increased ($P < 0.05$) in the aged.

Figure 3B shows a non-uniform distribution of HydroThane™-rich domains throughout the whole thickness of washed IPN films, with about 5% and 6% decrease between the top and middle sections of the non-aged and aged films, respectively, the corresponding values between the top and bottom sections being 4% and 8%. Larger area fractions ($P < 0.05$) were observed in the middle section of the aged compared to that of the non-aged IPN.

3.2 Swelling studies

Swelling profiles of the washed and freeze-dried IPN films were monitored in a 0.1% sodium azide solution and in a serum-containing medium, respectively. Figure 4 shows the difference in swelling stability between the non-aged and aged IPNs. The absorbency of the non-aged IPN film was initially 35% greater than that of the aged gelatin-HydroThane™ IPN films, the former film remaining more absorbent for at least

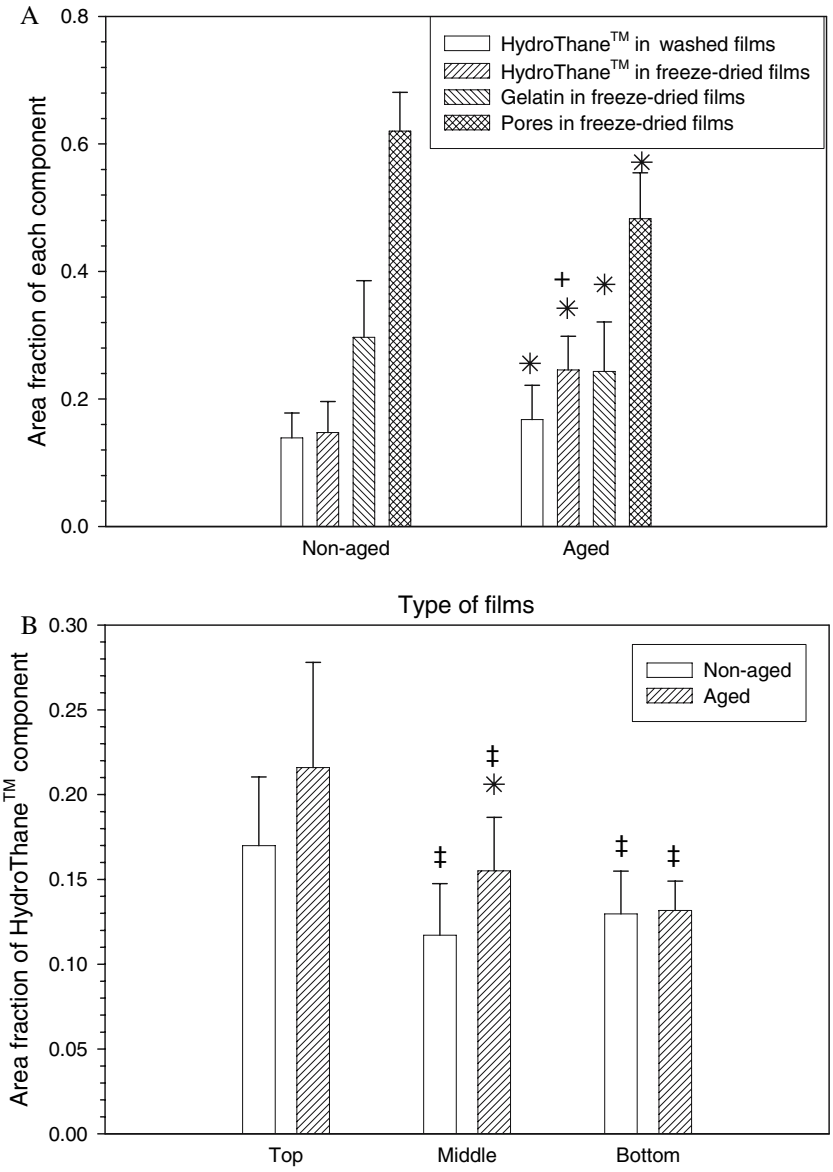

Fig. 3 Area fractions of individual components in IPN films prepared under different conditions. Films were prepared with either a fresh (non-aged) or an aged methacrylated gelatin solution, the latter solution being prepared at 7.5 wt% and stored for three weeks at room temperature prior to its use. Data represent means ± standard deviation (panel A: $n = 30$; panel B: $n = 10$). ✳ Different from non-aged films ($P < 0.05$). + Different from washed films. ‡ Different from the top section ($P < 0.05$)

14 days (Fig. 4A). The hydration values of the aged IPN films ranged from 12 to 16 for at least 60 days. Furthermore, the hydration of the IPN film prepared with fresh (i.e., non-aged) methacrylated gelatin steadily declined over the duration of the study, being reduced by 2.4-fold after 48 days.

The absorbency of freeze-dried gelatin-HydroThane™ IPN films prepared with the diluted methacrylated gelatin solution aged for 3 weeks was approximately 20% greater after 4 days than that of the control (i.e., non-aged films, Fig. 4B). The non-aged films swelled within the first 2 days, maintaining a swelling ratio of about 9 over the next 3 days, before starting to show a reduction in swelling. In contrast, the aged film swelled continuously for 8 days before a reduction in swelling was observed. The rate of decline in swelling for the non-aged film was also higher than that of the aged film, the overall reduction being twice greater for the non-aged than the aged film.

3.3 Mechanical properties

Ultimate stress and strain of both non-aged and aged films were measured after their rehydration in the serum-containing medium at 37°C for 4 days. The stress value for the non-aged film was 0.11 MPa. Interestingly, the stress values for the films prepared from the solutions aged at 7.5 wt% and room temperature increased from 0.092 MPa to 0.137 MPa, as the aging period was increased from 14 to 42 days, thereafter decreasing to 0.094 MPa over the next 14 days of immersion. Although there was no statistically

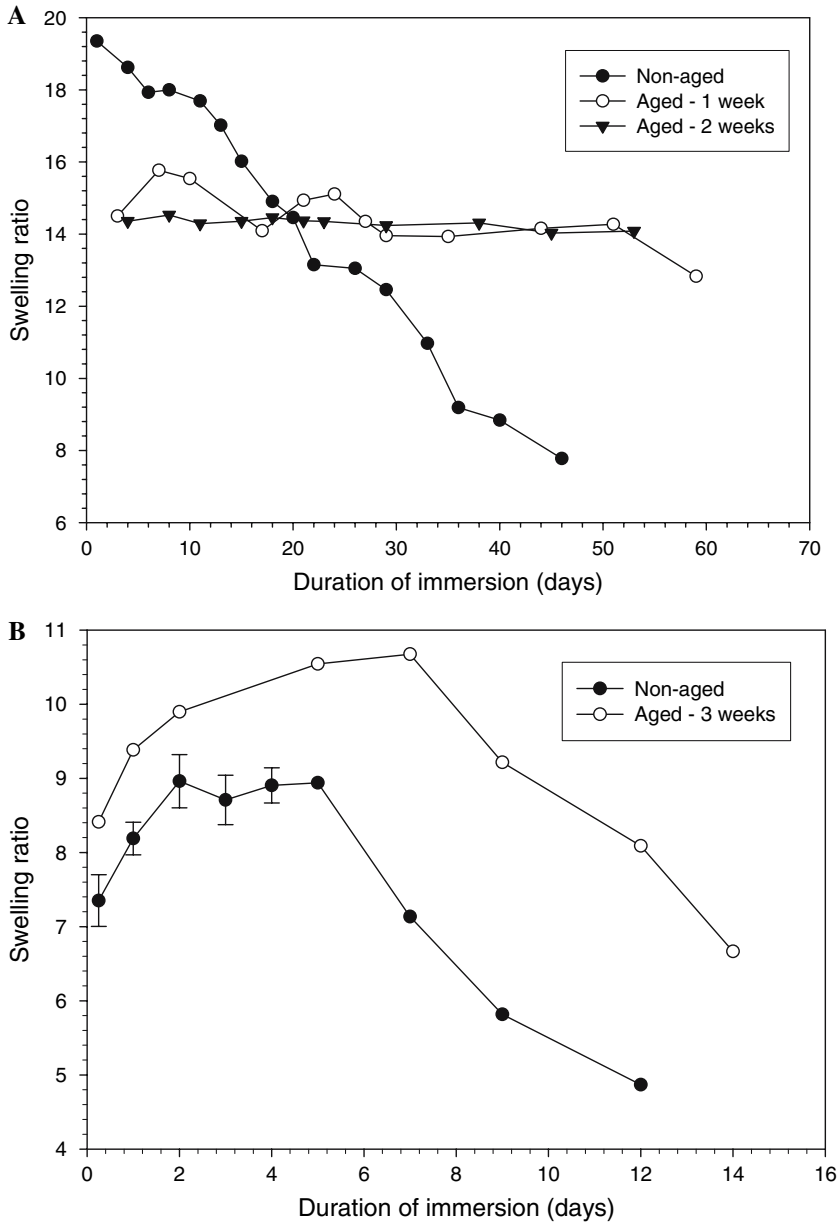

Fig. 4 Swelling stability of IPN films washed and immersed in a 0.1% sodium azide solution at room temperature (panel A) or freeze-dried and rehydrated in a solution of 50% fetal bovine serum and 0.1% sodium azide at 37°C (panel B). Films were prepared with either a fresh (non-aged) or an aged diluted methacrylated gelatin solution, the latter solution being prepared at 18 wt%, stored for up to three weeks at room temperature, and diluted to the same concentration as the fresh solution (7.5 wt%) prior to its use. Data represent means ± standard deviation ($n = 3$), otherwise $n = 1$

significant increase in ultimate stress of the aged films compared to that of the non-aged, a 56% increase in ultimate strain was observed for the aged, 42-day film and the increase in the ultimate strain was maintained for the next 14 days (Fig. 5).

3.4 Viscosity measurement

Figure 6 shows the changes in the viscosity of methacrylated gelatin solutions as a function of the aging period. The viscosity of the solution aged at 7.5 wt% and room temperature remained constant for up to 36 days. On the other hand, an abrupt increase in viscosity of the solutions aged at 7.5 wt% (50°C) and 18 wt% (room temperature) was observed, as the aging period was extended beyond 7 days. Moreover, the solution stored at 50°C showed a more rapid increase in viscosity, the latter averaging twice that of the solution kept at room temperature after 21 days.

3.5 DSC characterization

Figure 7 depicts the DSC thermograms of methacrylated gelatin solutions aged at 7.5 wt% and 50°C for different time intervals. An endothermic peak, a typical characteristic of first-order phase transitions (e.g., melting transitions, polymer conformation transitions) [16], was observed for the solution aged for

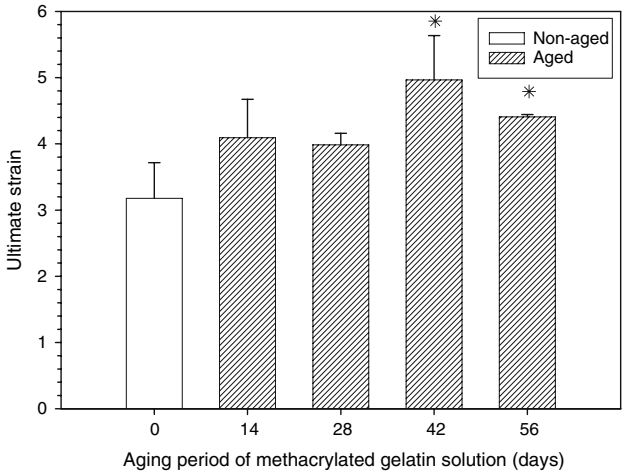

Fig. 5 Aging effects on the ultimate strain of freeze-dried films immersed in a 50% fetal bovine serum and 0.1% sodium azide solution maintained at 37°C for 4 days. Films were prepared with either a fresh (non-aged) or an aged 7.5 wt% methacrylated gelatin solution, the latter solution being stored for up to 56 days at room temperature. Data represent means ± standard deviation ($n = 3$). ✳ Different from non-aged films ($P < 0.05$)

13 days. This peak was well correlated with a dramatic increase in the viscosity of the solution aged under the same conditions.

4 Discussion

The phase morphology of an IPN is a complex function of several variables, including miscibility of components, composition, crosslinking density and reaction kinetics [1, 17]. These variables have been manipulated to control IPN preparations and their properties. To

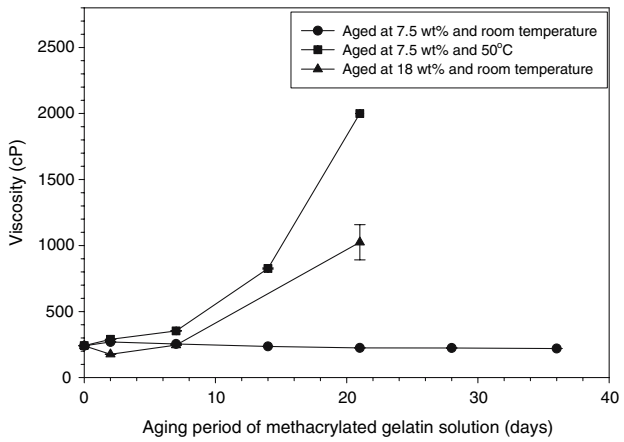

Fig. 6 Aging effects on the viscosity of methacrylated gelatin solutions. Methacrylated gelatin solutions were prepared at 7.5 and 18 wt% in DMSO and kept either at room temperature or 50°C for up to 36 days. Data represent means ± standard deviation ($n = 3$)

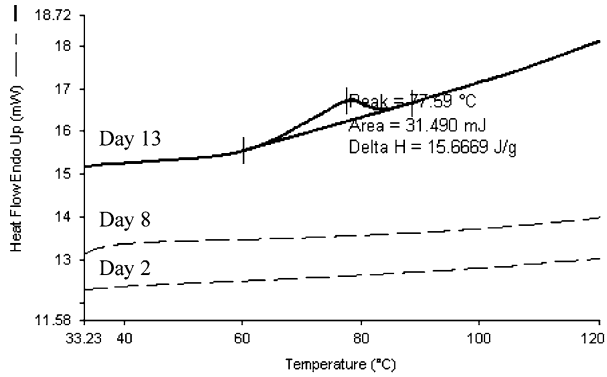

Fig. 7 Typical DSC curves of methacrylated gelatin solutions aged at 7.5% and 50°C for different time periods

our knowledge, there are no reports on the effects of aging polymer solutions on the preparation and properties of an IPN. In this study, we found a significant effect of aging methacrylated gelatin solutions on our IPN's morphologies, swelling and mechanical properties, and ascribed the aging effects to an increase in viscosity of the aged solutions. Furthermore, the physical structuring of methacrylated gelatin chains was proposed as the mechanism underlying the aging effects. Our finding of an aging effect on IPN morphologies and physical properties should provide a new approach for developing and preparing new IPN materials.

We have previously shown that gelatin (a biopolymer) and HydroThane™ (a polyurethane) are incompatible. The incompatibility led to the phase separation of the two polymers and a heterogeneous IPN morphology with distinct domains [H.T. Peng et al. submitted]. In such a system, phase separation involved demixing of the pre-IPN solution and polymer coalescence during the crosslinking process, with the viscoelastic effect likely playing an important role in the phase separation behaviour of the IPN [18, 19]. Our current results demonstrate that gelatin and HydroThane™ can form IPNs with a broad morphology spectrum from dispersed-continuous to co-continuous structures with interpenetration occurring at the interface. More specifically, the dispersed-continuous (island-sea) morphology seen in the aged-18 wt%-RT-3 weeks IPN was likely a result of phase separation occurring via nucleation and growth, while the shred network-like bicontinuous morphology in the aged-7.5 wt%-50°C-3 weeks IPN was formed via spinodal decomposition [20]. The shred network-like bicontinuous structure with vague interface observed in the non-aged and aged-7.5 wt%-RT-3 weeks IPNs was likely developed from the closed network-like structure, the latter shrinking as a result of reduced or

incomplete phase separation. The differences in morphological features among the IPNs prepared with solutions of aged methacrylated gelatin under various conditions might be related to an increase in their viscosities, which reduced demixing of the pre-IPN solution and restricted phase separation [7, 21]. Thus, the increase in viscosity might have prevented the gelatin component from coalescence separation into individual domains in the IPNs, prepared with the solutions aged at 18 wt% or 50°C. As a result, the IPNs possessed a continuous gelatin phase and improved interpenetration between the two networks.

Another factor affecting the morphology of our IPN was likely the formation of pores. Indeed, there were only a few pores in the washed films; the larger area of the HydroThane™ component represented a smaller fraction of gelatin area in the aged IPN. In contrast, the freeze-dried IPNs showed porous structures, likely formed by the removal of water in the gelatin phase [22] and influenced by the water content [23]. Furthermore, the freeze-drying process caused a collapse of the gelatin network into polymeric strands disentangled from the HydroThane™ network, the latter remaining essentially unchanged. The changes in the area fractions of the freeze-dried IPNs compared to the washed IPNs were likely due to a significant increase in the number of pores. The greater porosity in the non-aged, freeze-dried film may be explained by initially higher water content in the gelatin network after washing. The higher water content, in turn, could result in a larger fraction of gelatin area in the non-aged film compared to that of the aged IPN. Finally, the variation in morphology along the Z-direction of both washed and freeze-dried films might be related to the use of a glass vial for preparing the IPN films. Indeed, the non-uniform morphological features across the IPN films were likely due to the affinity of the HydroThane™ and gelatin components for air and the glass surface, respectively. The distribution of the two components is suggestive of different biocompatibility and mass transfer properties at the two interfaces, consistent with reported substrate effects on IPN formation [24].

In an attempt to understand the nature of the aging effects, some exploratory experiments were conducted in which methacrylated gelatin solutions were aged under different conditions and their viscosities and thermal behaviour were measured. Both methods have been used to characterize gelatin solution properties [25]. The abrupt increase in viscosity was closely related to the appearance of the thermal transition for the solution aged at 7.5 wt% and 50°C for 21 days. It is expected that the combined concentration and temperature effects (e.g. 18 wt% and 50°C) on aging process would act synergistically to yield a highly viscous solution.

It is noteworthy that the phase transition was not a result of the polymerization of the gelatin-associated methacrylate groups during the analysis, as evidenced by the manifestation of an endothermic change rather than an exothermic change, normally observed in a polymerization process [26]. Therefore, the observed viscosity increase and thermal transition may well be mediated by a chain organization of methacrylated gelatin in the solution, e.g., by the formation of a helix structure. Although it is known that helix structures can be formed in aqueous gelatin solution and the formation increases with a prolonged storage time [12], this phenomenon has not been observed in DMSO [27].

The proposed mechanism underlying the aging effects is consistent with the report revealing the formation of ordering structures (i.e., physical crosslinks) over time in polyvinyl alcohol in solution [28]. These structures consisted of higher molecular weight fractions not visible when freshly prepared, contributing to a higher viscosity because of an increase in physical crosslinking. In addition to the physical crosslinks, chemical changes of methacrylated gelatin during aging cannot be ruled out. It has been indicated that chemical crosslinking occurred during the storage of solid collagens [29], from which gelatin was derived. The precise nature of the structural changes of methacrylated gelatin in the solution during aging needs to be further studied.

The in vitro stability of both washed and freeze-dried IPNs appeared to be correlated to their structures and to the stability of each constituent polymer. Phase separation, poor interpenetration and the presence of large interfaces between different polymer components allow a better penetration of the solvent via the interfaces, resulting in higher swelling. On the other hand, compatible IPN systems become resistant to the penetration of the solvent to swell the network due to the constrained molecular chains and lower free volume [30]. Due to the hydrophobic nature of HydroThane™ relative to gelatin, the swelling would become mainly dependent on the configuration of the gelatin network and its porosity. Previous studies have confirmed that the HydroThane™ component was stable in the IPN immersed in an aqueous solution for over one month, and the decline in swelling was suggested to be due to the hydrolysis of the gelatin network via at least two mechanisms: chain scission and dissolution [H.T. Peng et al. in press]. The higher swelling and decreased stability of the non-aged washed film suggest a reduction in the number of

physical crosslinks in the gelatin network, as a result of limited interpenetration between the two polymers. For the freeze-dried film, despite the fact that the aged, 3-week film showed a higher swelling, the stability appeared to be more related to chain distribution and entanglement than to the water content. This is in contrast to the washed films, which showed a higher swelling, but lower stability, perhaps due to the formation of porous structure after the freeze-drying process. These results are in agreement with the report demonstrating that IPNs with better miscibility exhibits less degradation [31].

The different morphologies of the non-aged and aged IPNs might be responsible for the observed differences in their mechanical properties. Specifically, the improvement of phase mixing and the chain entanglement of the network components led to stronger interfacial adhesion between the two polymer phases and an increase in strength and strain. The HydroThane^{TM} component, through the formation of a continuous phase, would contribute most to the mechanical properties of the IPN, also improving its strength and elasticity. Alternatively, the interruption of HydroThane^{TM} network in the IPNs, prepared with the methacrylated gelatin solution aged at 18 wt%, might negatively impact on their mechanical properties. Nevertheless, our findings are consistent with reports showing that stronger IPNs correlate with a better network connectivity, despite larger domain sizes [32] and less compatibility between two polymer phases [30]. It appears that the mechanical property of our IPN biomaterial not only depends on its morphology, but also on the extent of crosslinking within the HydroThane^{TM} network [33]. The increased viscosity of the aged solution of methacrylated gelatin could weaken the crosslinked HydroThane^{TM} network, probably due to a lower diffusion coefficient of the polymer in our pre-IPN solution [34].

5 Conclusions

Gelatin-HydroThane^{TM} IPNs were prepared from a fresh (i.e., non-aged) methacrylated gelatin solution and from solutions aged under different concentrations and temperatures. Our results showed that gelatin and HydroThane^{TM} can form IPNs exhibiting a two-phase structure with a broad morphology spectrum, from dispersed-continuous to co-continuous structures with interpenetration occurring at the interface. It appears that the different morphologies are related to an increase in viscosity of the methacrylated gelatin solution, as a result of aging via a viscoelastic phase separation mode. Our results also revealed that aging methacrylated gelatin solutions reduce phase separation, thereby improving the biomaterial's swelling stability and mechanical properties. The aging effects may be attributed to the physical structuring of the methacrylated gelatin at the molecular level. In summary, the results of this study support the notion that the physical properties of an IPN strongly depend on its morphology, which can be manipulated by incorporating an IPN constituent solution (e.g., a methacrylated gelatin solution) aged under various conditions. Thus, our study results substantiate the availability of an alternative approach for the development of IPN materials.

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