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Hydrogel-elastomer composite biomaterials: 3. Effects of gelatin molecular weight and type on the preparation and physical properties of interpenetrating polymer networks

Henry T. Peng · Lucie Martineau · Pang N. Shek

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Abstract To optimize the preparation of a gelatin-HydroThane™ Interpenetrating Polymer Network (IPN) and obtain optimum physical properties for its use as a wound dressing, we studied IPN films prepared with two types of gelatin having different molecular weights. The effects of the gelatin molecular weight and type on the IPN film's structure, morphology, swelling and mechanical properties were determined. While FTIR did not reveal any noticeable differences between the IPNs prepared using different gelatin, light microscopy showed a lesser phase separation of the film prepared with a high-molecular-weight type A gelatin. Furthermore, these films displayed slightly less swelling, higher strength and lower strain, compared to the IPNs prepared with either low-molecular-weight type A or type B gelatin. The IPN prepared with type B gelatin showed higher swelling in serum-containing medium than those prepared with type A gelatin, because of its ionic charges under the condition. Increases in viscosity were observed with increasing molecular weight, type A being more viscous than type B gelatin despite having a lower bloom number. The viscosity of the high-molecular-weight gelatin was in the same magnitude as that of HydroThane™, which might lead to less phase separation. A better understanding of the effects of alterations in the gelatin molecular weight and type on the formation and properties of the gelatin-HydroThane™ IPN should facilitate the development of promising composite biomaterials for wound dressing applications.

H. T. Peng (✉) · P. N. Shek
Defence Research and Development Canada – Toronto,
1133 Sheppard Avenue West, P.O. Box 2000, Toronto, ON,
Canada M3M 3B9
e-mail: henry.peng@drdc-rddc.gc.ca

L. Martineau
Defence Research and Development Canada – Valcartier, 2459
Pie-XI Blvd North, Quebec, QC, Canada G3J 1X5

Introduction

Interpenetrating polymer networks (IPNs) are a special class of composite materials. They have been extensively studied because of their simplicity in combining the properties of a wide range of materials, ranging from plastics and elastomers to hydrogels that are desirable for different biomedical applications [1–3]. Moreover, the formation of IPNs tends to enhance the desirable properties of each constituent polymer, resulting in what is commonly known as positive synergism effects [4].

An IPN is generally prepared through the formation of two polymer networks either simultaneously or subsequently, with at least one cross-linked network formed in the presence of other components [1]. It is well known that the phase structure and morphology of an IPN determine its physical properties. Therefore, it is important to control phase-separated structures in a scale ranging from nanometer to micrometer. IPNs have been produced by using a variety of methods to obtain different morphologies, in order to understand their structure-property relationships [1, 5]. Most of the studies were directed at understanding the impact of alterations in compositions and preparation chemistry on IPN structures, morphologies and properties [5, 6]. Controlling IPN morphology through changes in irradiation intensity [5], reaction temperatures [6] and chemical structures [7] were also investigated at a constant composition. There were a few studies that examined the effects of secondary structures of constituent polymers, in

particular molecular weights and side groups, on miscibility and properties of IPNs composed of polyurethanes and derivatives of polysaccharides [8, 9].

We have previously described a gelatin-HydroThane™ IPN prepared by simultaneously cross-linking each constituent polymer in solution to combine the unique properties of a hydrogel (gelatin) and a thermoplastic polyurethane elastomer (HydroThane™; Cardiotech International Inc.) [10, 11]. Specifically, gelatin was modified by methacrylation, followed by photo cross-linking in solution with HydroThane™. We confirmed that both polymers were simultaneously photo cross-linked to form a full IPN. In these previous studies, we prepared the IPNs under different conditions and characterized their swelling and mechanical properties. One of our goals was to optimize the IPN preparation to obtain good reproducibility and best performance for our wound dressing applications.

Gelatin is a biopolymer widely used in biomedical [12], pharmaceutical [13] and food industries [14]. Depending on the processing method, two types of gelatin can be produced: type A and B, with different bloom numbers. Both types are derived from collagen by either acid or alkaline treatment, resulting in different isoelectric points, namely, 7–9 for type A and 4–5 for type B [13]. The bloom number is a standard industrial measure used to indicate the mechanical strength of physical gelatin gels. It decreases with the temperature used to process collagen [15]. Investigators have evaluated some effects of gelatin molecular weight and type on the properties of itself and its biomaterials. For example, Tabata et al. [16] have demonstrated different release profiles of basic fibroblast growth factor from hydrogels made from type A and B gelatin. Both bloom number and type were found to strongly affect the mechanical properties and atomistic structures of gelatin [15]. In addition, the triple-helix content of gelatin increased with its bloom number, leading to an increase in the stress and Young's modulus of either native or cross-linked gelatin films [17].

A number of IPNs containing type A or B gelatin have been reported [18, 19]. However, these composites comprised mainly hydrophilic polymers, and the effects of gelatin molecular weight and type were not elucidated. It is generally acknowledged that the formation of an IPN is strongly affected by the rheological properties and chemical compositions of constituent polymers. As the viscosity and chemical structure were influenced by the gelatin molecular weight and type [13, 20], we hypothesized that these two factors would influence the formation of an IPN by the gelatin with other polymers and also the properties of the resultant IPNs. Specifically, we expected that the restricted molecular mobility of the highly entangled gelatin chains would tend to produce an IPN with less phase separation, thus improving the overall IPN properties. Furthermore, since the chemical structure is dependent on the type of gelatin used [13], we compared the IPNs prepared from the two types of gelatin. To our knowledge, no studies have been reported to examine the effects of gelatin molecular weight and type on IPN formation and properties.

In the present study, we assessed the effects of altering the gelatin molecular weight and type on the structure, morphology, swelling and mechanical properties of the gelatin-HydroThane™ IPN. The study enabled us to control the production of our IPN biomaterials with good reproducibility and optimal performance.

Materials and methods

Gelatin type A with bloom numbers of 235 and 300, and gelatin type B with a bloom number of 250 were purchased from Great Lake Gelatin (IL, USA). Methacrylic anhydride (94% purity) and sodium azide were obtained from Sigma-Aldrich (ON, Canada). Poly(sodium styrenesulfonate) standards with monodispersity less than 1.2 and with molecular weights over the range of 210–350,000 g/mol, were purchased from Sigma-Aldrich (ON, Canada). HydroThane™ (AR25-80A) 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 Sigma-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).

Methacrylation of gelatin

About 10 g of gelatin were dissolved in 100 mL phosphate buffered saline (pH 7.4) and stirred at 50 °C. A volume of 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 constant weight was reached. The methacrylation was characterized using Thermo Mattson Infrared-100 with 128 scans at a resolution of 4 cm^{-1} [FTIR wave number (cm^{-1}): 1,720 (C=O of methacrylate)].

Gel permeation chromatography (GPC)

Methacrylated gelatin solutions were prepared at 1 mg/mL in 0.1% sodium azide aqueous solution and were analyzed using a GPC system composed of: PL aquagel-OH 30 and 40 GPC columns (Polymer Laboratories Inc., MA, USA) connected in series; a Waters 2690 separation module; and a Waters 996 UV detector (Waters Ltd., MA, USA). The manufacturer's Millennium software was used for data acquisition. The running conditions and sample preparation procedures were developed based on a published method [21]. The mobile phase (1.8% sodium dodecyl sulphate in Milli-Q water) was filtered through 0.45-μm filters (Waters Ltd., MA, USA) prior to its use. Each sample (20 μL) was run for 20 min at a flow rate of 1 mL min^{-1}. The temperatures of both sample and column compartments were set at 37 °C. UV detection were carried out at 220 nm.

Using a calibration curve constructed from the poly(-sodium styrenesulfonate) standards, we calculated the corresponding molecular weight of methacrylated gelatin [21]. Using the GPC instrument software, the chromatogram generated was accordingly subdivided into two areas representing high- ($HiMw_{gelatin}$) and low-molecular-weight fractions of gelatin ($LoMw_{gelatin}$). The ratio between the two areas was designated as $HiMw_{gelatin}/LoMw_{gelatin}$ and calculated to indicate the molecular-weight distribution [22].

Preparation of gelatin-HydroThane^{TM} IPN films

Three types of IPN films were prepared by combining equal amounts of polymers in their respective solutions of 4 wt% HydroThane^{TM} and 7.5 wt% methacrylated gelatin, the latter being prepared using one of the two bloom numbers or gelatin types. 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^{TM} in DMSO in a glass scintillation vial. Then 91-μL 10 wt% Irgacure 651 in DMSO was added. The mixture was 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 referred to herein as washed films. The washed films were then freeze-dried until constant weight was reached; these films are referred to herein as freeze-dried films.

Characterization of gelatin-HydroThane^{TM} IPN films

Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy

Attenuated total reflectance (ATR) infrared spectra of each IPN film were obtained with a Thermo Nicolet IR 100 system using a Zn–Germanium ATR accessory (Thermo Electron Corporation, PA, USA). Each sample was placed against the ATR element and the spectra were collected in the range 800–4,000 cm^{-1} using 64 scans at a resolution of 4 cm^{-1}.

Morphology analysis

Washed and freeze-dried IPN films were sectioned near the middle of the samples using 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^{TM} component in the washed films and preferentially stained it in the freeze-dried films, while HE preferentially stained the Hydrothane^{TM} component in both types of films. The two stains allowed the distribution of each component to be easily identified.

Images were taken with a Nikon CoolPix880 digital camera (Nikon Corporation, ON, Canada) through the eyepiece of an Olympus BH-2 optical microscope (Olympus, ON, Canada) at 100 × magnification. About 10 images were taken at the middle section of each sample. All the images were transferred to a computer, and the relative areas of gelatin, HydroThane^{TM} and pores were calculated using a combination of the HE and rhodamine-stained samples, with the aid of an image-processing program [23]. If the image had two components, one component was rendered black and the other white. For images with three components, we selected which components to differentiate. By performing this process twice, we could take the difference to calculate all individual components.

Swelling study

The freeze-dried IPN films were rehydrated at 37 °C in a solution of 50% fetal bovine serum, supplemented with 0.1% sodium azide solution to prevent bacterial growth. 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 dry mass of the film.

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 strips of 2 × 1 × 0.2 cm. The force and elongation at break point were measured using a Zwick materials testing machine (TC-FR005TN.A50, Zwick USA, GA, USA) at a test speed of 5 cm 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.

Viscosity measurement

Vials of methacrylated gelatin solutions were prepared at 7.5 wt% in DMSO to quantify the effects of the gelatin molecular weight and type on viscosity, and to understand their subsequent effects on IPN morphology and properties. The viscosity of each solution was measured at room temperature using a viscometer equipped with a spindle (Brookfield Engineering Laboratories Inc., MA, USA). Silicone oil standards were used to calibrate the viscometer.

Statistical analysis

Data are expressed as means ± standard deviation, unless otherwise specified. Significant differences between two groups were evaluated using a 2-tailed student *t* test with a level of significance of $p < 0.05$.

Results

GPC

Figure 1 illustrates typical gel permeation chromatograms of the methacrylated gelatin solutions used to prepare our IPN films. Two peaks were identified, corresponding to the

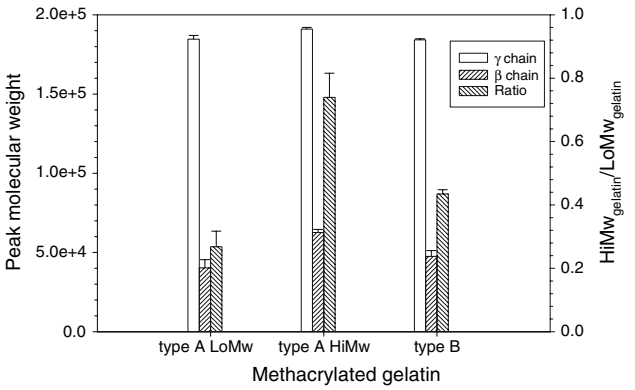

Fig. 2 Molecular weights and molecular-weight distributions of type A and B gelatin with different bloom numbers. $HiMw_{gelatin}$, high-molecular-weight fraction of gelatin. $LoMw_{gelatin}$, low-molecular-weight fraction of gelatin

γ and β chains in the macromolecule [24] and representing the high- (i.e. $HiMw_{gelatin}$) and low-molecular-weight fractions of gelatin (i.e. $LoMw_{gelatin}$), respectively [24].

Figure 2 shows the peak molecular weights of each fraction of gelatin as well as the weight ratio between the $HiMw_{gelatin}$ and $LoMw_{gelatin}$. As expected, type A gelatin, with a larger bloom number of 300, possessed higher molecular weights of the γ and β chains, and a much larger ratio between the two fractions, findings in agreement with the reports that gelatin with a higher bloom number has a higher molecular weight [25] and contains a larger amount of the $HiMw_{gelatin}$ [26]. The peak molecular weights and $HiMw_{gelatin}/LoMw_{gelatin}$ ratio of type B gelatin with a bloom number of 250 were comparable to those of type A gelatin with a bloom number of 235, with no significant difference in their molecular weights.

FTIR

Figure 3 shows the FTIR spectra of the freeze-dried IPN films, prepared with either type B gelatin or type A gelatin

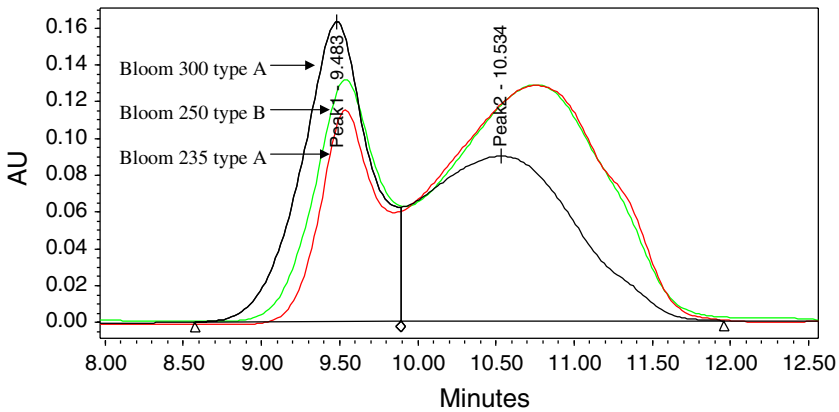

Fig. 1 Typical gel permeation chromatograms of methacrylated gelatin prepared using either type A (bloom 235 or 300) or type B gelatin (bloom 250). AU UV absorbance at 220 nm.

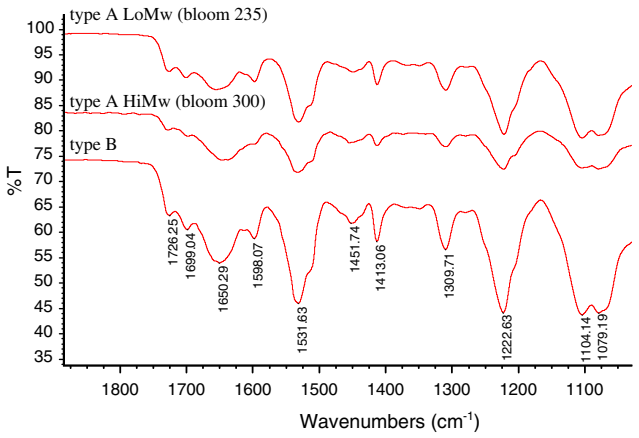

Fig. 3 FTIR spectra of freeze-dried gelatin-HydroThane™ IPN films prepared using either type A or B gelatin with different bloom numbers. LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

of different bloom numbers. The bands corresponding to each component were identified. For the HydroThane™ component, characteristic peaks of an ether group were observed in the region of 1,070–1,110 cm^{-1} [27], along with its characteristic urethane group showing two split peaks in the region of 1,700–1,730 cm^{-1} of C=O [10]. For the gelatin component, its characteristic amide showed one peak at approximately 1,650 cm^{-1} due to C=O and one peak at approximately 1,530 cm^{-1} due to N–H [10]. There were no noticeable differences between the three IPN spectra.

Morphology analysis

We conducted both qualitative and quantitative studies of the morphologies of the IPN films. The IPNs prepared with bloom 300 type A gelatin were designated as HiMw IPN; those prepared with bloom 235 type A gelatin and type B gelatin were designated as LoMw and Type B IPN, respectively.

Figure 4A shows the images of washed IPN films, prepared by using gelatin with different bloom numbers. The bright and dark regions of the HE-stained washed films correspond to the gelatin- and HydroThane™-rich domains, respectively. The unstained regions correspond to the pores. All IPN films showed phase separation with distinct gelatin- and HydroThane™-rich domains and pores. For the LoMw and type B IPNs, the hydrogel domain was interspaced into the HydroThane™ network.

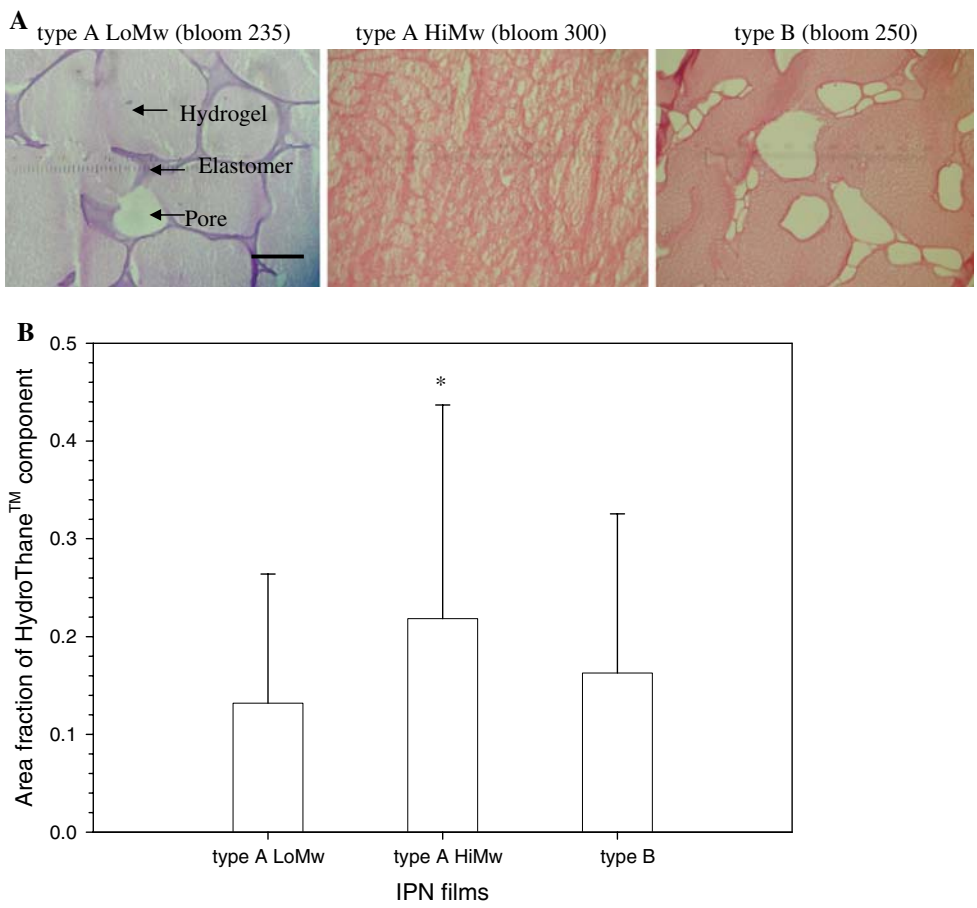

Fig. 4 Typical light microscopy photographs of washed IPN films (panel **A**) and area fractions of their HydroThane™ component (panel **B**). Each film was prepared with methacrylated gelatin at 7.5 wt% and HydroThane™ at 4 wt% in DMSO. The methacrylated gelatin was prepared using either type A or B gelatin with different bloom numbers. Films were sliced near the middle of the sample and stained with hematoxylin and eosin after the washing process. Scale bar is 120 μm. Data represent means ± standard deviation ($n = 10$). *Different from type A LoMw and type B ($p < 0.05$). LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

In contrast, the HiMw IPN had strikingly low phase-separation, with structures much smaller than those of the LoMw and type B (Fig. 4A). Accordingly, the HiMw film showed smaller gelatin domains and a relatively denser and more compact network structure of HydroThane™. In addition, the two-phase structure of the HiMw film was much different from that of the other two samples, showing a bicontinuous morphology in contrast with the dispersed, continuous morphology of the LoMw and type B IPN films. The interface between the two phases in the HiMw film became indistinct implying an improvement in interpenetration. The HiMw film also possessed a larger area fraction of the HydroThane™ component compared to that of the LoMw and type B films. More specifically, Fig. 4B shows about 14%, 20% and 16% of the HydroThane™ component by volume in the type A LoMw, HiMw and type B washed films, respectively. Lastly, the type B film possessed more pores than the LoMw film.

The HE-stained freeze-dried HiMw and LoMw IPN films were highly porous and showed a heterogeneous morphology of gelatin networks interspaced into HydroThane™ networks (Fig. 5A). The pores were irregular in shape and non-directional. The LoMw IPN film showed looser gelatin networks and larger pores compared to the HiMw IPN film. Accordingly, the architecture of the HiMw IPN film was relatively denser.

Figure 5B quantitatively depicts the percent areas of different domains in the LoMw and HiMw films after the freeze-drying process. The LoMw IPN film possessed a smaller overall area fraction of the HydroThane™-rich domain than the HiMw IPN film (16% vs. 34%, $p < 0.05$), but larger area fractions of gelatin (34% vs. 20%, $p < 0.05$) and pores (48% vs. 40%, $p < 0.05$). While the HydroThane™ area fractions of the LoMw IPNs were unaltered by the freeze-drying process (Figs. 4B vs. 5B), they were increased ($p < 0.05$) for the HiMw film (20% vs. 34%, $p < 0.05$). The presence of the pores in the freeze-dried films changed the balance of the two polymer domains, especially in the HiMw IPN film. In addition, the gelatin area fractions were significantly reduced in the freeze-dried IPNs compared to those of the washed IPNs (i.e. 20–34% vs. 80%).

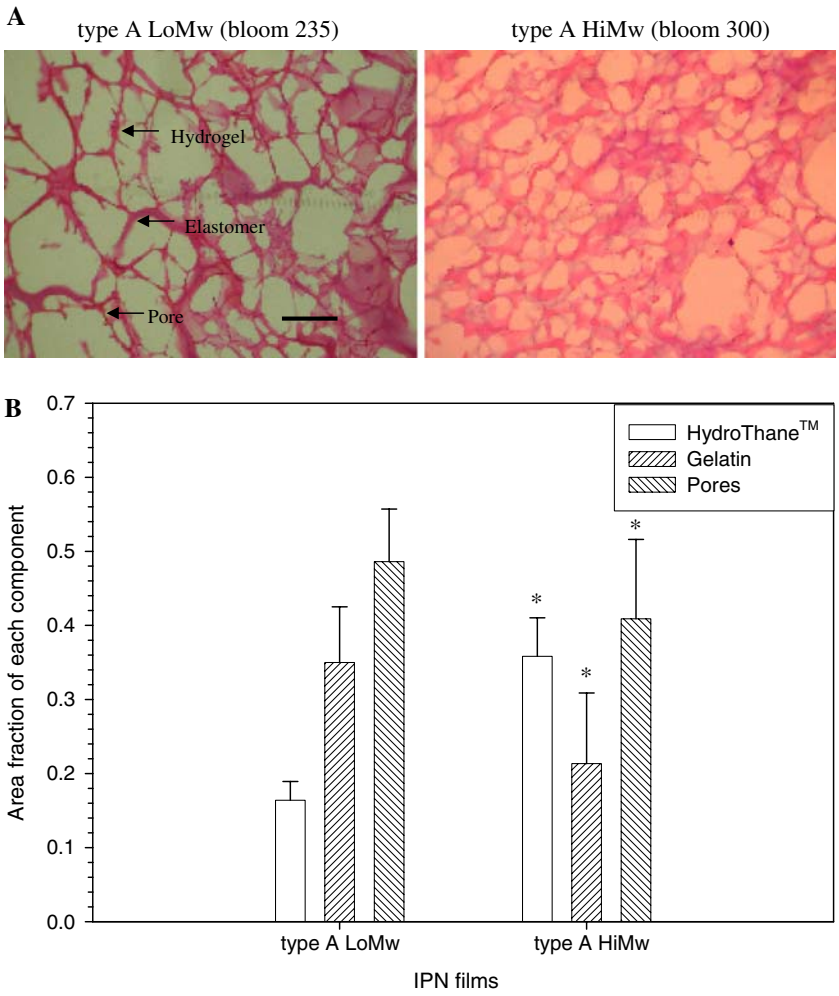

Fig. 5 Typical light microscopy photographs of freeze-dried IPN films (panel **A**) and area fractions of their individual components (panel **B**). Each film was prepared with methacrylated gelatin at 7.5 wt% and HydroThane™ at 4 wt% in DMSO. The methacrylated gelatin was prepared using type A gelatin with different bloom numbers. Freeze-dried films were sliced near the middle of each sample and then stained with hematoxylin and eosin. Scale bar is 120 μm. Data represent means ± standard deviation ($n = 10$). *Different from LoMw ($p < 0.05$). LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

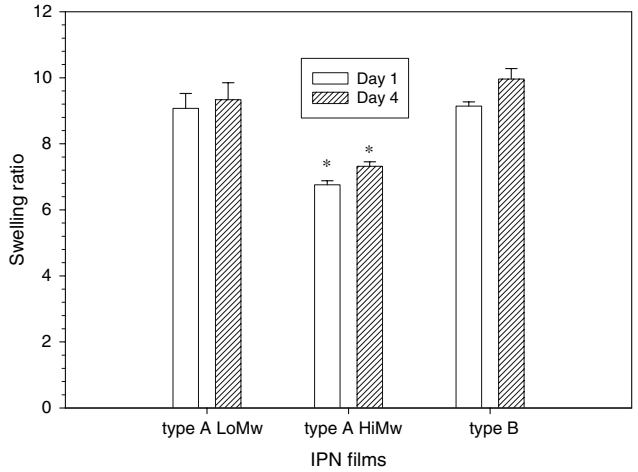

Fig. 6 Absorbency of freeze-dried IPN films after immersion at 37 °C in a 50% fetal bovine serum supplemented with 0.1% sodium azide solution. Data are expressed as means ± standard deviation ($n = 3$). * Difference from LoMw ($p < 0.05$). LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

Swelling studies

Swelling profiles of the freeze-dried IPN films were monitored over 4 days in a serum-containing medium supplemented with 0.1% sodium azide. Fig. 6 shows the difference in swelling stability between the different types of IPNs. The hydration values of the IPN films ranged from 7 to 10. Minimal changes in swelling were observed throughout the study, regardless of the IPN type. For example, the LoMw IPN film increased its hydration by less than 3% from day 1 to day 4. However, the absorbency of the type A LoMw gelatin-HydroThane™ IPN film was 22–25% greater than that of the HiMw IPN film but comparable to that of the type B IPN, the swelling ratio averaging 9.6 ± 0.5 throughout the 4-d study period.

Mechanical properties

Ultimate stress and strain of all IPN films were measured after their rehydration in the serum-containing medium at 37 °C for 4 days (Fig. 7). The stress values for the HiMw and type B IPN films were comparable but greater than that of the LoMw IPN film (0.14 vs. 0.11 MPa, $p < 0.05$). In contrast, the strain values for the LoMw and type B IPN films were comparable but greater than that of the HiMw IPN film (4.3 vs. 2.6, $p < 0.05$).

Viscosity measurement

Figure 8 compares the viscosity of methacrylated gelatin solutions prepared using different types of gelatin to that of HydroThane™. The viscosity of the type A HiMw methacrylated gelatin solution was much higher than that of both the type A LoMw and type B solutions (1538.0 ± 19.1 vs. 240.4 ± 1.3 and 189.4 ± 0.1 cP, $p < 0.05$), but in the same magnitude as that of the HydroThane™ solution. Interestingly, the viscosity of the type A LoMw solution was slightly higher than that of the type B solution even though the former contained a larger amount of the low-molecular-weight fraction (Fig. 2).

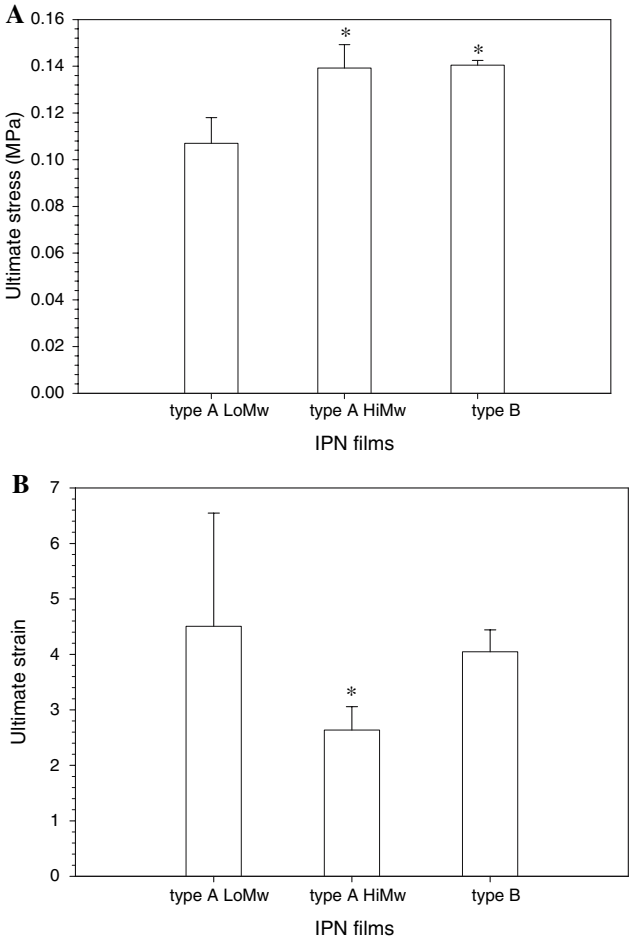

Fig. 7 Effects of the molecular weight and type of gelatin on the ultimate stress (panel **A**) and strain (panel **B**) of freeze-dried films following a 4-d immersion at 37 °C in a 50% fetal bovine serum supplemented with 0.1% sodium azide solution. Films were prepared using either type A (bloom 235 or 300) or type B gelatin (bloom 250). Data represent means ± standard deviation ($n = 3$). *Difference from the LoMw ($p < 0.05$). LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

Discussion

Although gelatin has been widely used for biomedical applications including multi-component wound dressings [28], no studies have been carried out to understand the

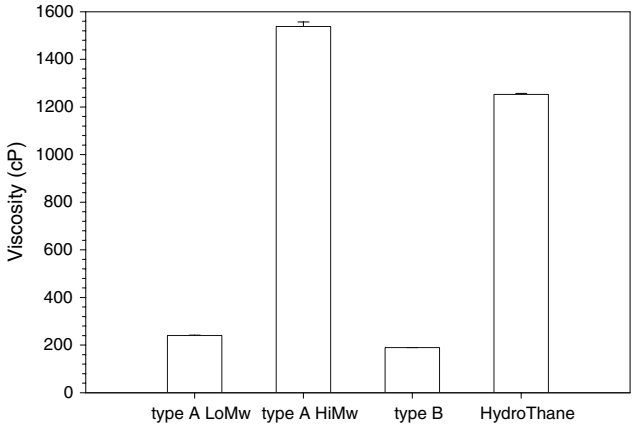

Fig. 8 Viscosities at room temperature of the methacrylated gelatin and HydroThane™ solutions used in the preparation of the different IPN films. Methacrylated gelatin solutions were prepared at 7.5 wt% in DMSO using either type A gelatin (bloom 230 or 300) or type B gelatin (bloom 250). The HydroThane™ solution was prepared at 4 wt% in DMSO. Data represent means ± standard deviation (n = 3). LoMw, low-molecular-weight, bloom 235; HiMw, high-molecular-weight, bloom 300

effects of gelatin molecular weight and type on the preparation and properties of an IPN.

In the present study, we used commercially available gelatins, the latter being heterogeneous mixtures of polypeptides. Only a bloom number (a measure of stiffness of a gelatin hydrogel) was provided by the manufacturer. Therefore, it was necessary to estimate the molecular weight and molecular-weight distribution as these parameters are considered to have a strong impact on the properties of gelatin such as gelation, processability and emulsion stability [29, 30]. For example, using higher proportions of low-molecular-weight gelatin was reported to reduce both viscosity and gelation temperature while increasing the gelation time [22]. The methacrylation process itself may affect the molecular weight and molecular-weight distribution of gelatin, subsequently affecting the properties of its composite biomaterial. Therefore, it is important to determine the molecular weight and molecular-weight distribution of methacrylated gelatin.

We could identify only two main fractions in our GPC chromatograms using the same method as that reported by Dupont [21]. The very low-molecular-weight fractions were likely removed in the membrane-dialysis step following gelatin methacrylation. On the other hand, the molecular weight that we measured for our methacrylated gelatin is similar to the values reported for gelatin with comparable bloom numbers [31, 32], implying limited effects of the methacrylation process. The broad molecular mass distribution was also confirmed, although the samples were not completely separated, especially in the range of high-molecular-weight masses [32].

A common way to characterize the molecular-weight distribution of a polymer is to use the ratio between weight-average molecular weight and number-average molecular weight, known as polydispersity [33]. Our instrument software was not designed for GPC analysis and could not calculate the average molecular weights of polymers. Therefore, the ratio between the areas under peaks 1 and 2 was calculated to indicate the molecular-weight distribution. The increase in the amount of the $HiMw_{gelatin}$ fraction with the gelatin bloom number, regardless of gelatin types, is consistent with the literature [26, 31]. The ratio also seems to correlate better with the bloom number than with the molecular weight. Furthermore, our GPC analysis confirmed that the methacrylated gelatin used for the preparation of the IPNs contained mixed fractions, with a wide range of molecular weights. Our observation is consistent with the reported molecular weight heterogeneity of gelatin [26]. The bloom number depends on both the molecular weight and molecular-weight distribution of gelatin, perhaps more related with the latter. Gelatin with different bloom numbers may contain different amounts of each fraction, and the higher-bloom-number gelatin contains a larger amount of the $HiMw_{gelatin}$ fraction [26].

While our FTIR analysis confirmed the methacrylation of gelatin, there were no effects of alterations in either the gelatin type or molecular weight (data not shown). Therefore, the extent of chemical cross-linking through free-radical polymerization of the methacrylate groups may be similar between all gelatins. However, the alkaline treatment of collagen to produce type B gelatin hydrolyses the amide groups of asparagine and glutamine into carboxyl groups. In contrast, the acidic treatment has little effect on the amide groups [13]. This deamidation may result in the difference in the formation of helix structure and thus, physical cross-linking of each type of gelatin [34].

The peaks observed at 1,700 cm^{-1} and 1,730 cm^{-1} in the FTIR spectra of our biomaterial are due to hydrogen-bonded and free C=O, respectively [12]. The ratio between the hydrogen-bonded and free C=O of HydroThane™ in our IPN was reduced compared to that of HydroThane™ itself, due to the interruption of the hydrogen-bonding formation of HydroThane™ resulting from the presence of gelatin [12]. The relative intensity of the two bands was used to indicate the extent of interpenetration between the constituent polymers in an IPN [9]. The absence of a difference in the relative intensity and of a shift of the bands of the functional groups from each component suggests limited chain interactions, at a molecular level between gelatin and HydroThane™ in all IPNs. Our FTIR results also suggest that there are no significant differences in the cross-linking reactions of each polymer, during the formation of the three types of IPNs.

The phase morphology of an IPN is a complex function of many variables including miscibility of components, composition, cross-linking density and reaction kinetics [1, 35]. We have previously confirmed that the biopolymer (gelatin) and polyurethane (HydroThane^{TM}) used in the preparation of our IPN were incompatible, as indicated by their very different glass transition temperatures and solubility parameters [12]. In our system, phase separation involved demixing of the pre-IPN solution and polymer coalescence during cross-linking process. In such a system, the viscoelastic effect may play an important role in phase separation behavior [36], which could be associated with gelatin molecular weight and its polydispersity.

Our data show that gelatin and HydroThane^{TM} could form IPNs with a morphology spectrum from dispersed-continuous to co-continuous structures with interpenetration occurring at the interface. Similar structures have also been observed in other polymer systems induced by photochemical reactions [5]. The differences in morphological features among the IPNs might be related to variations in the viscosities of the methacrylated gelatin solutions. The reduced phase separation in the HiMw IPN could be attributed to the increase in viscosity of the methacrylated gelatin solution, which reduced demixing of the pre-IPN solution and restricted phase separation [37]. Accordingly, the increase in viscosity prevented the gelatin component from coalescence separation into individual domains, leading to a continuous phase of gelatin and improved interpenetration, as indicated by dark interface between the two networks.

The gelatin molecular weight may also affect phase separation in the IPN through its influence on the cross-linking density of gelatin and chain entanglement/interpenetration with HydroThane^{TM} [38]. As there were no significant differences in the degree of methacrylation of gelatin with different molecular weights, the effect of the molecular weight on the chemical cross-linking of gelatin via free radical polymerization of the methacrylate groups are likely marginal. This is consistent with Mühlebach et al.'s study [39] showing very small differences in the solid content of 2-vinyl-4,4-dimethylazlactone-modified poly(vinyl alcohol) gels despite variations in the molecular weight of the polymer ranging from 31,000 to 67,000. However, the cross-link density of the gelatin network might be increased as a result of augmented physical cross-links, due to an enhanced formation of helix structure with increasing gelatin molecular weight [22]. The increased cross-link would prevent phase separation and domain growth, leading to a decrease in the domain size and an increase in the number of domains.

The chain entanglement between gelatin and HydroThane^{TM} might be enhanced through increases in molecular weight of gelatin and result in two continuous phase morphology, as demonstrated by Chiang et al. [38] for an IPN based on poly(urethane-epoxy) and allyl novolac resin. This is also in accordance with the lack of a phase separation in an IPN, consisting of poly(carbonate urethane) and poly(methyl methacrylate) with increases in the molecular weight of the latter polymer, as a result of reduced molecular mobility [40]. The mechanical strength was improved by the formation of the IPN with the high-molecular-weight poly(methyl methacrylate), but not the low-molecular-weight one. In contrast, studies on the effects of alterations in the molecular weight of polysaccharide derivatives on the miscibility and properties of their IPNs formed with polyurethane showed better miscibility with decreasing molecular weight [8]. This apparent discrepancy may be related to different mechanisms of IPN formation as well as the fact that the gelatin formed a cross-linked network in our IPNs as opposed to linear polymers of varying molecule weights dispersed into another network (i.e. cross-linked matrix; 8–9).

On the other hand, the increase in viscosity of the methacrylated gelatin solution might have affected the formation of the HydroThane^{TM} phase due to lower polymer and free radical diffusion coefficients in our pre-IPN solution. Indeed, Zhang et al. [41] have shown that increasing the molecular weight of polylactide from 2,096 to 63,000 reduced the cross-linking reaction of poly(ethylene glycol) dimethacrylate during formation of their IPN, resulting in a lesser phase separation in the IPN formed with the polymer with a lower molecular weight. However, this could be partially due to different reaction conditions (e.g. solution vs. solid). The decrease in curing rates of vinyl monomers with the increasing viscosity of a pre-IPN solution has also been reported [42]. The different morphology of a simultaneous IPN could be also attributed to the formation sequence of two networks [7]. In general, it is preferable for IPN formation with the cross-linking reactions in each network proceeding at comparable rates [43]. An increase in gelatin molecular weight might reduce the cross-linking rate of HydroThane^{TM} in such a way that the cross-linking rates of these two polymers became comparable with each other, leading to a less phase separation and smaller domain size.

In addition to the domain size, phase inversion took place in the IPNs prepared with the HiMw methacrylated gelatin solution due to an increase in viscosity. The dispersed-continuous (island–sea) morphology seen in the LoMw IPN was likely to appear, because phase separation occurred via nucleation and growth, while the bicontinuous morphology in the HiMw IPN was formed via spinodal decomposition [44]. The bicontinuous structure with a vague interface was likely developed from the closed network-like structure, seen in the LoMw IPN, by shrinking the network-like phase as a result of reduced or incomplete phase separation.

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 correlated with a smaller fraction of gelatin area in the HiMw IPN compared to the LoMw and type B due to less gelatin swelling as a result of the increased physical cross-link and chain entanglement previously discussed. In contrast, the freeze-dried IPNs showed porous structures, likely formed by the removal of water in the gelatin phase [45] and influenced by the water content [46]. 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 those of the washed IPNs were likely due to a significant increase in the number of pores. The greater porosity in the freeze-dried LoMw IPN film may be explained by the initially higher water content in the gelatin network after washing, as indicated by a larger area fraction of gelatin in Fig. 4A (corresponding to a lower area fraction of HydroThane™ in Fig. 4B).

Swelling of the freeze-dried IPNs was measured at different time intervals. Swelling ratios could be related to a number of factors including the network structure, cross-linking density and ionization of each network, especially the gelatin network as it provided a dominant swelling capability [47]. Normally, increased cross-links would increase mechanical strength, but compromise the extent of swelling [48]. Phase separation, poor interpenetration and the presence of large interfaces makes it easier for solvents to penetrate via the interfaces, resulting in higher swelling. On the other hand, the compatible IPN systems decrease the ability of a solvent to swell the network, due to the constrained molecular chains and lower free volume [49]. Because of the hydrophobic nature of HydroThane™ relative to gelatin, the swelling would become mainly dependent on gelatin network and porosity. The higher swelling of the LoMw film suggests less cross-links of the gelatin network, due to limited interpenetration between the two polymers, as observed in the morphology. In addition, the type B IPN showed the highest swelling due to the positive charges of type B gelatin in the neutral serum-containing medium. These results are consistent with studies reporting a decreased swelling of gelatin networks with increasing molecular weight/bloom number, as a result of increased cross-links [22] as well as of IPNs with greater miscibility [49].

The different morphologies seen in the IPNs may be also responsible for the differences in their mechanical properties. Specifically, the improvement of phase mixing and the chain entanglement of the network components led to a stronger interfacial adhesion between the two polymer phases and the increased strength. On the other hand, a reduction/interruption of HydroThane™ network by the gelatin component in the IPNs could have a negative effect on their mechanical properties. The HydroThane™ component, through forming a continuous phase, would contribute most to the mechanical properties and improve IPN strength and elasticity. This observation was consistent with reports showing stronger IPNs possessed better network connectivity, despite less compatibility between two polymer phases [49] and larger domain sizes [50].

The observed mechanical properties may not only depend on morphology, but also on the extent of cross-linking within the HydroThane™ network [51]. As previously discussed, the increased viscosity of the HiMw methacrylated gelatin solution could lead to a lower cross-link degree of the HydroThane™ network, compromising the positive effects on the mechanical strength and strain. A very high molecular weight gelatin solution is too viscous to mix well with a HydroThane™ solution, therefore retarding the formation of a HydroThane™ network. It has been reported that the first polymer network formed is expected to exert a dominant control over its morphology and mechanical properties [7].

In an attempt to understand the nature of the molecular-weight effects, the viscosities of methacrylated gelatin solutions were measured. The method has been used to characterize the properties of gelatin solutions [52]. It is noteworthy that the viscosity of type B gelatin is slightly lower than that of LoMw type A gelatin, despite the latter having a larger amount of $LoMw_{gelatin}$ fraction. This implies that chemical structures, in addition to the molecular weight, can affect the resultant viscosity. The increase in viscosity may be also related to the chain organization of gelatin in solution, e.g. the formation of a helix structure. The increase in viscosity was closely related to the bicontinuous phase morphology, consistent with the report revealing the formation of bicontinuous structures, as a function of the volume fraction and viscosity of each phase in solution [53]. It is expected that a highly viscous solution would have a different impact on IPN formation.

Conclusions

Several gelatin-HydroThane™ IPNs were prepared using methacrylated type A and B gelatin with different bloom numbers, the latter being associated with different molecular weights and molecular-weight distribution. Our results show that the biopolymer and polyurethane selected could form IPNs, exhibiting two-phase morphologies from dispersed-continuous to co-continuous structures with interpenetration occurring at the interface, although no difference was observed in the FTIR characterization. The different morphologies might be associated with an

increase in viscosity, as a result of increases in the molecular weight and the amount of high-molecular-weight fractions, via a viscoelastic phase separation mode. Our data revealed that optimal molecular weight, molecular-weight distribution, and the selection of gelatin type would reduce phase separation and improve both the swelling and mechanical properties of the IPN. The molecular-weight effects may be attributable to both the physical structuring of gelatin at a molecular level and the increase in solution viscosity of constituent polymers. Our study clearly indicates that the physical properties of an IPN strongly depend on its morphology and can be tailored through the polymer molecular weight and type, thus providing another approach to the development of IPN materials.

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