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Hydrogel–elastomer composite biomaterials: 1. Preparation of interpenetrating polymer networks and in vitro characterization of swelling stability and mechanical properties

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Abstract We prepared interpenetrating polymer networks (IPNs) composed of a gelatin hydrogel and a HydroThane^{TM} elastomer to combine the advantages of both polymers into one biomaterial. Fourier transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) confirmed the co-existence of the two polymers in the IPNs. Optical light microscopy confirmed hydrogel domains were interspaced into an elastomer network. Hydration and stability studies in aqueous solution showed that, although the IPN biomaterials exhibited stable swelling for more than 30 days, approximately 10% and 50% loss of the hydrogel component were confirmed at room temperature and 37 °C, respectively, using gel permeation chromatography (GPC). The swelling study in the serum-containing medium indicated the biomaterials maintained their swelling stability for different periods, depending on the extent of gelatin methacrylation, photoinitiator concentration and incubation temperature. Lastly, the biomaterials exhibited higher failure stress and lower failure strain in a dry state than in a swollen state, and showed limited changes in both stress and strain at room temperature and at 37 °C, in contrast with a decrease at 50 °C. No significant effects of gelatin methacrylation on mechanical properties were noticed. The preparation and characterization methods were well established and formed the basis of further developing the biomaterials.

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1 Introduction

Hydrogels have been widely used as biomaterials, including as wound dressing biomaterials, due to their structural similarities to the extracellular matrix of many tissues. In particular, hydrogels exhibit high absorbency for body fluids, possess soft and rubbery consistency, and have low interfacial tension [1, 2]. However, one drawback with most hydrogel biomaterials is their poor mechanical strength. Elastomers, most commonly polyurethanes and polysiloxanes, are another type of promising biomaterials. Their biocompatibility and mechanical properties suit a variety of biomedical applications, including wound dressings [3, 4]. However, they are normally hydrophobic and cannot absorb a large amount of body fluid for the treatment of highly exudating wounds. Therefore, it is highly desirable to combine the unique properties of hydrogels and elastomers into one biomaterial.

A number of approaches may be taken to combine the advantages of each type of biomaterial: coating an elastomer with a hydrogel layer; forming a composite containing each component (e.g., by dispersing hydrogel particles in a silicone matrix [5]); and forming interpenetrating polymer networks (IPNs). Among these approaches, only the formation of IPNs has led to enhanced properties of each constituent polymer, known as positive synergism effects [6]. Therefore, we investigated this approach to combining hydrogel and elastomer.

An IPN is defined as a combination of two crosslinked polymers, at least one of them synthesized or crosslinked in the immediate presence of the other [7]. IPNs have been extensively investigated to combine

properties of a variety of materials. For example, IPNs have been prepared to combine the mechanical property of polyurethane with the blood compatibility of a phospholipid polymer [8]. IPN-based biomaterials have been studied for drug delivery [9], tissue prosthesis and repair [8, 10] as well as wound dressings [11]. Recently, some IPNs comprising hydrophilic polymers and hydrophobic elastomers, typically biopolymers and polyurethanes, have been reported [12]. However, these IPNs are semi-IPNs prepared by crosslinking polymerization of polyurethane prepolymers in the presence of the biopolymers. As a result, the biopolymers were not crosslinked and thus could not perform as hydrogels.

To develop a novel hydrogel–elastomer composite biomaterial (HECB) for its potential use as a wound dressing for battlefield wound care, we developed an IPN to combine the unique properties of a hydrogel (gelatin) and an elastomer (HydroThane^{TM}) [13]. Gelatin is a biopolymer derived from collagen and is widely used for biomedical [14], pharmaceutical [15] and food industries [16]. Methacrylated gelatin can be crosslinked through polymerization to form a hydrogel component [17]. Gelatin has also been reported to be hemostatic [18]. Although gelatin-based IPNs have been prepared, these composites comprised mainly hydrophilic polymers [10, 19], and their mechanical strength is therefore expected to be weak. Incorporation of an elastomer component in the gelatin IPN would provide good mechanical strength and elasticity. To our knowledge, a hydrogel–elastomer IPN has never been reported.

There are a number of analytical methods for characterizing IPN and for demonstrating the successful combination of different polymer components. Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and optical light microscopy are mostly used to characterize IPN formation, polymer miscibility and phase morphology [7]. For example, DSC is a powerful tool to determine the glass transitions of parent polymers in an IPN to indicate polymer–polymer immiscibility.

Swelling, degradation and/or dissolution of a biomaterial are important design parameters affecting significantly its desired functions [20]. Depending on the application, the biomaterial may be required to show different degree of swelling and to be degradable or soluble at different rates or even non-degradable [21]. Stable swelling and good absorbency are required for a wound dressing to treat highly exudating wounds. Mechanical properties are also important for a biomaterial to function properly [22]. For example, elasticity may be required for a wound dressing to conform to irregular surfaces, while mechanical properties close to that of bone are required for a bone-repair biomaterial.

In this first paper on a series of studies of the hydrogel–elastomer composite biomaterials, we describe the synthesis of a gelatin-HydroThane^{TM} IPN composite and characterize its features by FTIR spectroscopy, DSC and optical light microscopy. We used swelling measurement to evaluate the IPN's hydration capacity and stability in aqueous solution and serum-containing medium. In addition, gel permeation chromatography (GPC) was used to characterize its stability in an aqueous solution. The mechanical properties of the IPNs were determined in dry state and during the swelling measurement. Our successful preparation and characterization of the HECB strongly implicate the merit of its further development as a novel biomaterial for wound management and other biomedical applications.

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), sodium dodecyl sulphate and sodium azide were obtained from Aldrich (ON, Canada). HydroThane^{TM} (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). Dialysis membranes with a molecular weight cut-off of 12000–14000 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 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 and then dialyzed against distilled water at 37 °C for 1 week before freeze-drying until constant weight was reached. The methacrylation was characterized using two methods: ^{1}HQS 400-1 Nuclear Magnetic Resonance (NMR) with an internal maleic acid standard in deuterated DMSO, and Thermo Mattson IR-100 with 128 scans at a resolution of 4 cm^{-1} [^{1}H-NMR (DMSO-d_6, chemical shifts, δ, in ppm): 5.3–5.6 (=CH_2); FTIR wave number (cm^{-1}): 1720

(C = O)]. The methacrylation degree is described as the percentage of methacrylate group over total ε-amino groups in gelatin and calculated using the equation:

$$\text{Wt\% } (=CH_2)/14 \Big/ 4.7/146.2 + 1.2/162.2$$

In this formula, wt% (=CH_2) is the weight percentage of =CH_2 group in methacrylated gelatin calculated from the relative intensity of =CH_2 proton and maleic acid proton, and 14 is the molecular weight of =CH_2. The numbers 146.2 and 162.2 represent the molecular weights of lysine and hydroxylysine with 4.7 and 1.2 wt% in gelatin, respectively, as specified by the manufacturer of the gelatin. The guanidino and hydroxyl groups were not included as they are not usually reactive with anhydrides in aqueous gelatin solution [23].

2.2 Preparation and analysis of gelatin-HydroThane™ IPN films

To prepare the IPN, we first mixed methacrylated gelatin (either 9% or 18% methacrylation) and HydroThane™ solutions in DMSO at different concentrations and compositions. The mixtures were then vortexed vigorously with 10 wt% Irgacure 651 solution in DMSO in a glass scintillation vial. The resulting solutions were flushed with nitrogen gas for 5 min. A photochemical chamber reactor (RAYONET model RPR-200, Southern New England Company, CT, USA) was used to provide a 15-min UV irradiation at 350 nm at an intensity of 9 mW cm^{-2}. After the irradiation, the mixed solutions remained as liquid or turned into gel-like and solid films. The resulting films were then washed in distilled water for up to 20 days and their swelling was measured as a ratio between mass in a swollen state and initial mass of polymers used to prepare the films. Based on our previous study of the effects of polymer concentration and composition on the IPN formation [24], the IPN films were prepared at 7.5 wt% methacrylated gelatin and 4 wt% HydroThane™ solution, containing an equal amount of each polymer, for further studies. More specifically, a 0.67 g 7.5 wt% methacrylated gelatin in DMSO was mixed with 1.25 g 4 wt% HydroThane™ in DMSO in a glass vial. Then 91-μL 10 wt% Irgacure 651 in DMSO was added. Irgacure 651 was also added at 5 and 20 wt% to study its concentration effect on the IPN swelling. The mixture was vigorously vortexed, purged with nitrogen for 5 min and UV irradiated for 15 min as described above. The resulting films were washed in 0.1% sodium azide aqueous solution at ambient temperature for a week to remove DMSO and then freeze-dried until constant weight was reached. Each film had a diameter of 2.5 cm and thickness of 0.2 cm.

2.2.1 *Attenuated total reflectance (ATR) Fourier transform infrared (FTIR)*

Attenuated total reflectance (ATR) infrared spectra of each (gelatin, HydroThane™ and IPN) film were obtained with a Thermo Nicolet IR 100 system using a Zn-Germanium ATR accessory (Thermo Electron Corporation, Shippack, PA, USA). Each side of a sample was placed against the ATR element and the spectra were collected in the range 800–4000 cm^{-1} using 64 scans at a resolution of 4 cm^{-1}.

2.2.2 *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 –70 to 250 °C to determine the glass transition and melting temperatures of the IPN as well as its elastomer and hydrogel components. The crosslinked gelatin and IPN films were prepared using gelatin with 9% methacrylation. 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.

2.2.3 *Morphology analysis*

IPN films were prepared using gelatin with 9% methacrylation, washed, and freeze-dried as described previously. Washed and freeze-dried IPN films were sectioned with a Microm HM560 cryostat (MICROM International GmbH, Walldorf, Germany) and then stained using Haematoxylin and Eosin. 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. The relative area of each component was calculated using image analysis software downloaded from a web site [25].

2.3 Stability study

Swelling measurement has been widely used as a simple method to characterize water absorption and stability of biomaterials [19]. We therefore conducted

stability studies by rehydrating each film in two different media. In the first study, freeze-dried IPN films (total of 24 films) were prepared using gelatin with 18% methacrylation and were rehydrated in 25 mL 0.1% sodium azide aqueous solution at room temperature and 37 °C. At specific time intervals, each film was blotted dry, weighed and then re-immersed in the medium. The swelling ratio of each film was calculated using the following equation: Wt/Wo, where Wt and Wo are the weight at time t and at time 0 (initial dry weight), respectively.

In addition, a 1-mL aliquot of the aqueous medium was collected and replaced with 1 mL of a fresh 0.1% sodium azide aqueous solution. This aliquot was analyzed using a GPC system composed of: PL aquagel-OH 30 and 40 GPC columns (Polymer Laboratories Inc., Amherst, MA, USA) connected in series; a Waters 2690 separation module; and a Waters 996 UV detector (Waters Ltd., Milford, 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 [26]. The mobile phase (1.8% sodium dodecyl sulphate in Milli-Q water) was filtered through 0.45-μm filters (Waters Ltd., Milford, 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 was carried out at 220 nm. The amount of residuals in solution was calculated at each time interval from the entire area under the chromatogram using a standard curve. The standard curve was created by analyzing a series of gelatin standard solutions (0.05, 0.2, 0.5 and 1 mg mL^{-1}) under the same running conditions. The loss of hydrogel component was calculated as the percentage of cumulative amount of residuals in the solution over initial amount of gelatin component in the IPN film. Gelatin and HydroThane™ films (2 films for each type) were prepared using the same methodology as for the preparation of IPN films, and used as controls.

In the second study, freeze-dried IPN films were prepared using 9% and 18% methacrylated gelatin (total of 146 films) and rehydrated in a solution of 50% fetal bovine serum and 0.1% sodium azide in distilled water at room temperature, 37 °C and 50 °C. The swelling ratio of each film was determined by the same method as described above.

2.4 Mechanical testing of films

The mechanical tests were conducted on both freeze-dried films and those immersed in the serum-containing medium. The films were cut into strips 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 failure 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. The Young's modulus was then calculated as the ratio between the stress and the strain.

2.5 Statistical analysis

Experiments were conducted in triplicate, unless otherwise specified, and data were expressed as mean ± standard deviation. Significant differences between two groups were evaluated using a two-tailed student t test. When $p < 0.05$, the differences were considered to be statistically significant.

3 Results

3.1 Methacrylation of gelatin

The FTIR spectrum of gelatin methacrylated using 0.5 mL of methacrylic anhydride, showed no absorption corresponding to the methacrylate group. In contrast, the spectrum of gelatin methacrylated using 1 mL of methacrylic anhydride, showed absorption at 1720 and 722 cm^{-1} due to the presence of the methacrylate group, confirming methacrylation. The methacrylation using both conditions was confirmed by H-NMR, showing the appearance of new peaks at about 5.6 ppm, ascribed to the methacrylate group. The NMR quantification with an internal maleic acid standard indicated the methacrylation degree was increased from 9% to 18% as the amount of methacrylic anhydride was increased from 0.5 to 1 mL (data not shown).

3.2 IPN formation and analysis

3.2.1 FTIR analysis

Figure 1 shows the FTIR spectra of the IPN and its hydrogel and elastomer components. The bands corresponding to each component were identified. For the HydroThane™ elastomer, its characteristic urethane group showed two split peaks in the region of 1700–1730 cm^{-1} of C=O and one peak at about 1530 cm^{-1} of N-H. For the gelatin hydrogel, its characteristic amide

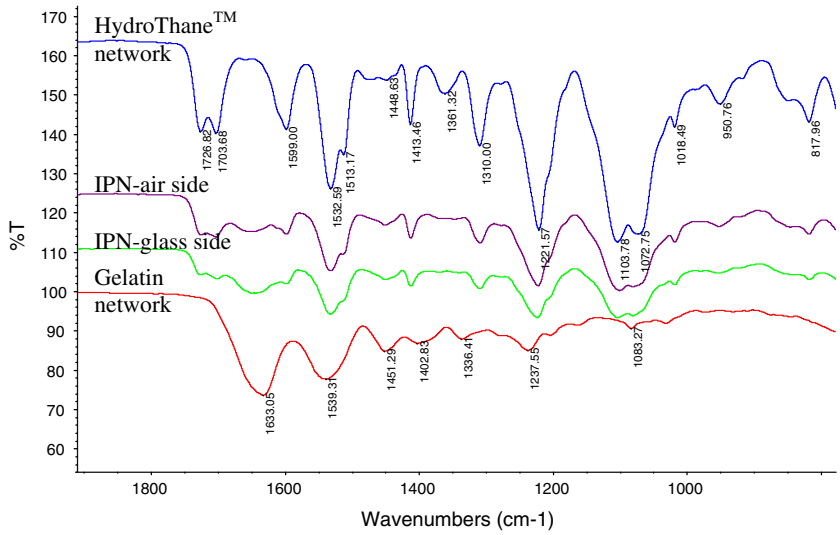

Fig. 1 FTIR spectra of crosslinked gelatin, HydroThane™ networks and gelatin-HydroThane™ IPN. The crosslinked gelatin network and IPN were prepared using gelatin with 9% methacrylation

showed one peak at 1633 cm^{-1} due to C=O and one peak at 1539 cm^{-1} due to N-H. In the IPN spectrum, no new characteristic absorption bands and no significant shifts of these bands were noticed. Finally, Fig. 1 shows the IPN depicted relatively higher intensity of the urethane peaks on the side interfacing air during its preparation than on the side interfacing glass, implying more elastomer components due to higher surface tension on the airside.

3.2.2 DSC analysis

Figure 2 shows the phase transition temperatures of individual gelatin and HydroThane™ networks as well as those of the gelatin-HydroThane™ IPN. The glass transition temperatures of individual gelatin (T_g ~ 155°C) and HydroThane™ (T_g ~ –14°C) networks were far away from each other. The IPN displayed two glass transition temperatures T_{g2} and T_{g1} at 170 and –31°C, respectively, corresponding to the gelatin and HydroThane™ components. On the other hand, the gelatin component in the IPN exhibited an increased melting transition temperature and a decreased enthalpy compared to the methacrylated gelatin control.

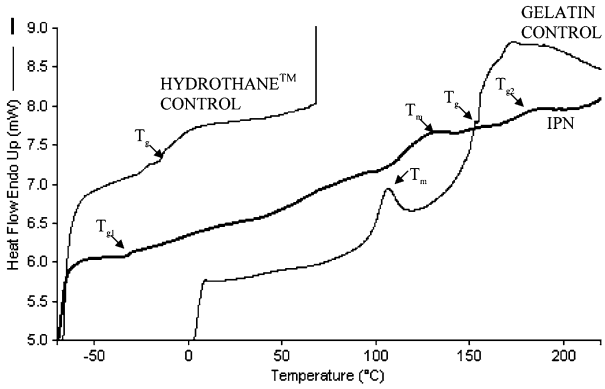

Fig. 2 Typical DSC curves of crosslinked gelatin, HydroThane™ networks and gelatin-HydroThane™ IPN. The glass transition temperature, T_g is indicated by the corresponding arrow for the HydroThane™ control and the gelatin control. In the case of IPN, there are two glass transition temperatures, T_{g1} and T_{g2}. The glass transition temperatures were determined by the inflection points of each curve. T_m represents the melting temperature of gelatin

3.2.3 Morphology analysis

Figure 3 shows the images of three sections located at the top, middle and bottom of the IPN film after the washing and freeze-drying processes. The bright and dark regions correspond to the hydrogel and elastomer component, respectively. The unstained regions correspond to pores.

The film washed in the aqueous solution had a heterogeneous IPN morphology of a hydrogel domain interspaced into an elastomer network. The hydrogel component was in a swollen state and occupied most of the area (more than 80% as calculated using the image analysis software), while the elastomer component was in a collapsed state and occupied less than 20% of the space with few pores. By contrast, the hydrogel component in the freeze-dried film collapsed into a fine network interspaced into the elastomer network, leaving about 60% pores behind.

In addition, there was a small but significant (4%, $p < 0.05$) decrease in the total area of the elastomer component of the washed film with a concomitant increase in the total area of the hydrogel component from the side interfacing air to the side interfacing glass

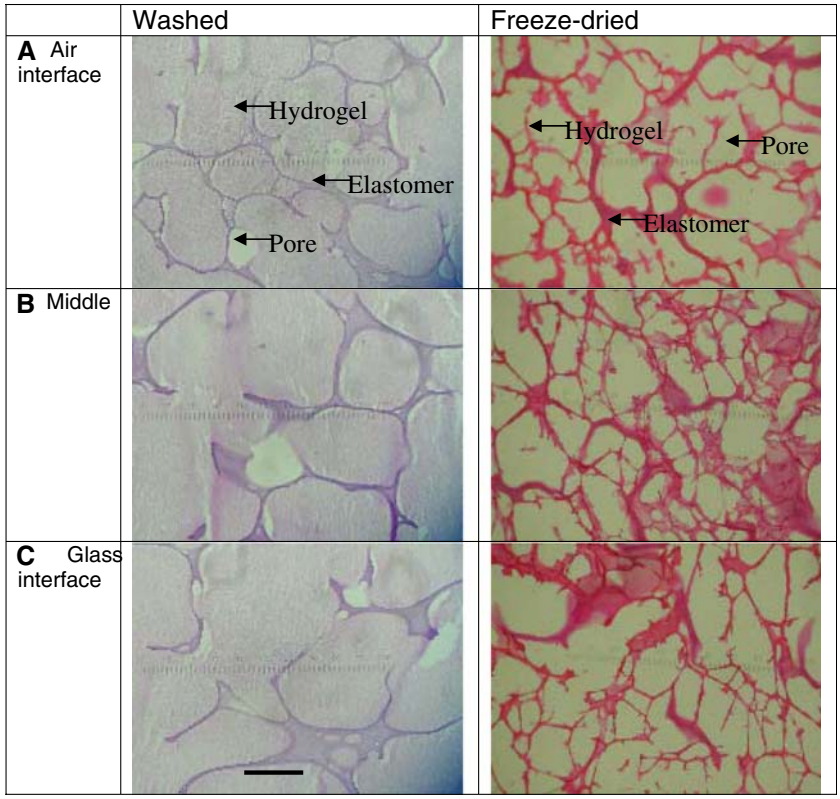

Fig. 3 Typical light microscopy photos of IPN films sliced at the top, middle and bottom, and stained with hematoxylin and eosin. Scale bar is 120 μm

during IPN formation (panels A to C in Fig. 3). In contrast, this parameter was not significantly altered for the freeze-dried film.

3.3 Stability of the composite biomaterials in aqueous solution

3.3.1 Swelling stability of the IPN films in aqueous solution

Figure 4 shows the rehydration properties of the IPN films and the photo-crosslinked gelatin films at room temperature and 37 °C. The freeze-dried IPN films could rehydrate above a swelling ratio of 10 within a day and maintain the hydration level over the 34-day immersion period in the aqueous medium, irrespective of the medium temperature. Both types of films showed slightly higher hydration at 37 °C than at room temperature; however, the difference was not always significant within the investigation period. In contrast, the photo-crosslinked gelatin films rehydrated to a significantly higher extent ($p < 0.05$) than the IPN films at both temperatures and retained the water-absorbing capacity for up to 31 days. Furthermore, the extent of rehydration of the gelatin films was significantly greater ($p < 0.05$) at 37 °C than at room temperature throughout the study, except for day 31 when an insignificant decline in swelling appeared at 37 °C.

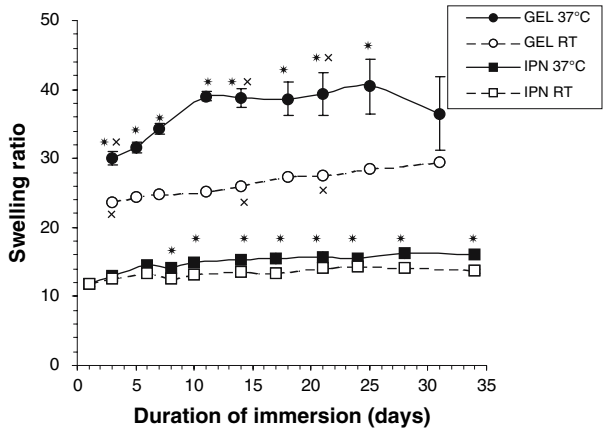

Fig. 4 Rehydration characteristics of freeze-dried gelatin-HydroThane™ IPN and crosslinked gelatin (GEL) films immersed in a 0.1% sodium azide aqueous solution at room temperature (RT) and at 37°C for up to 34 days. Values represent mean ± standard deviation ($n = 3$)

3.3.2 GPC study of stability of the IPN films in aqueous solution

Although the films remained swollen for more than 30 days, significant gelatin loss took place during the period, as confirmed by GPC characterization. Figure 5 shows typical gel permeation chromatograms of the aqueous solution in which the photo-crosslinked

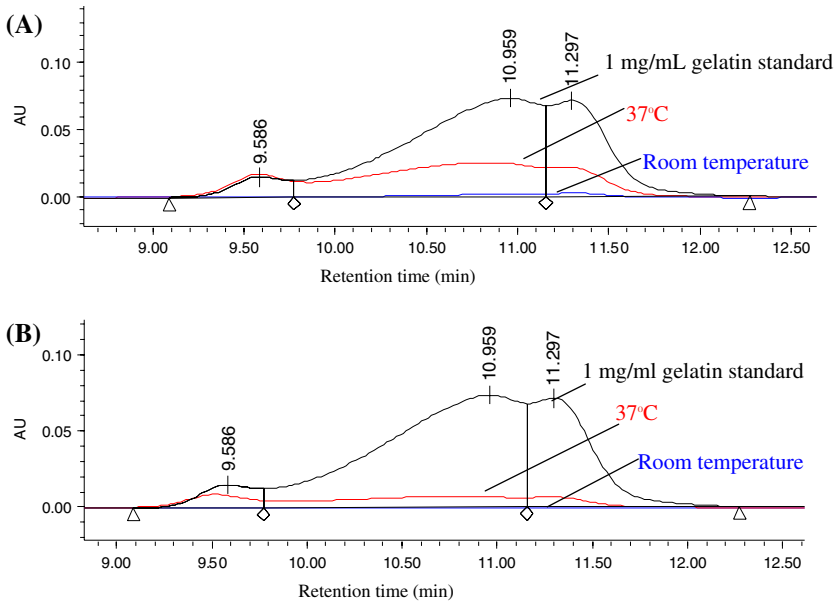

Fig. 5 Typical gel permeation chromatograms of photo-crosslinked gelatin (**A**) or IPN films (**B**) immersed in 0.1% sodium azide aqueous solution for 5 and 6 days, respectively. ^{AU} UV absorbance at 220 nm

gelatin and IPN films were immersed at room temperature and 37 °C for up to 6 days. Lower amounts of gelatin residuals were detected at room temperature than at 37 °C for both IPN and gelatin films over the immersion period. Furthermore, HydroThane™ was very stable, as suggested by the lack of residuals in the solution in which photo-crosslinked HydroThane™ films were incubated for 34 days. In fact, less than 1% of the HydroThane™ was recovered from the incubation medium over a 2-month immersion period in 0.1% sodium azide solution at 37 °C (data not shown).

Figure 6 shows a time-dependent release of gelatin residuals from both types of films, the gelatin loss at 37 °C being significantly greater ($p < 0.05$) than that measured at room temperature at all time intervals.

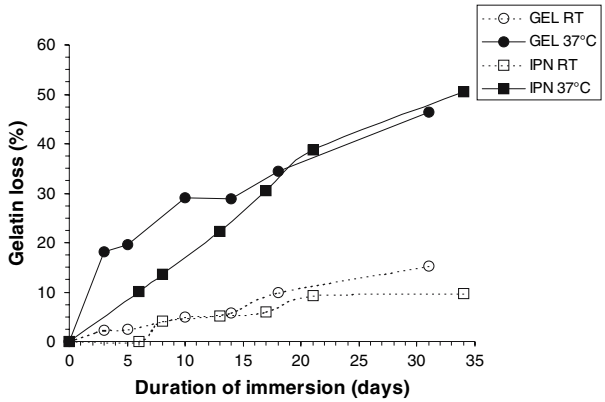

Fig. 6 Stability through gelatin loss of the freeze-dried cross-linked gelatin (GEL) and gelatin-HydroThane™ IPN films, immersed in 0.1% sodium azide solution at room temperature (RT) and 37°C. Data are expressed as means ($n = 2$)

Moreover, the gelatin loss from the photo-crosslinked gelatin-HydroThane™ IPN films remained lower than that of the photo-crosslinked gelatin films for at least the first 14 days of immersion at 37 °C, likely due to its entanglement with HydroThane™ in the IPN.

3.4 Swelling stability in serum-containing medium

3.4.1 Effect of gelatin methacrylation

Figure 7A shows that the IPN films rehydrated mostly within the first 15 min and then exhibited a slower increase in swelling that plateaued after 2–3 days. The films prepared using gelatin with 9% methacrylation started to show a decrease in swelling after a 5-day immersion at 37 °C. In contrast, the films prepared using gelatin with 18% methacrylation retained their hydration level for about 15 days. In addition, the IPN films swelled significantly more ($p < 0.05$) and tended to maintain their hydration twice as long in the aqueous solution than in the serum-containing medium (30 days vs. 15 days for the IPN films prepared using gelatin with 18% methacrylation; Figs. 4 vs. 7).

3.4.2 Effect of photoinitiator concentration

Figure 7B depicts the expected effect of the photoinitiator concentration on the swelling stability. It appears that the IPN films prepared using 5 and 10 wt% Irgacure 651 solutions showed a decline in swelling after a 5-day immersion in the serum solution at 37 °C, while the IPN film prepared at a higher Irgacure concentration had minimal changes in swelling throughout the study.

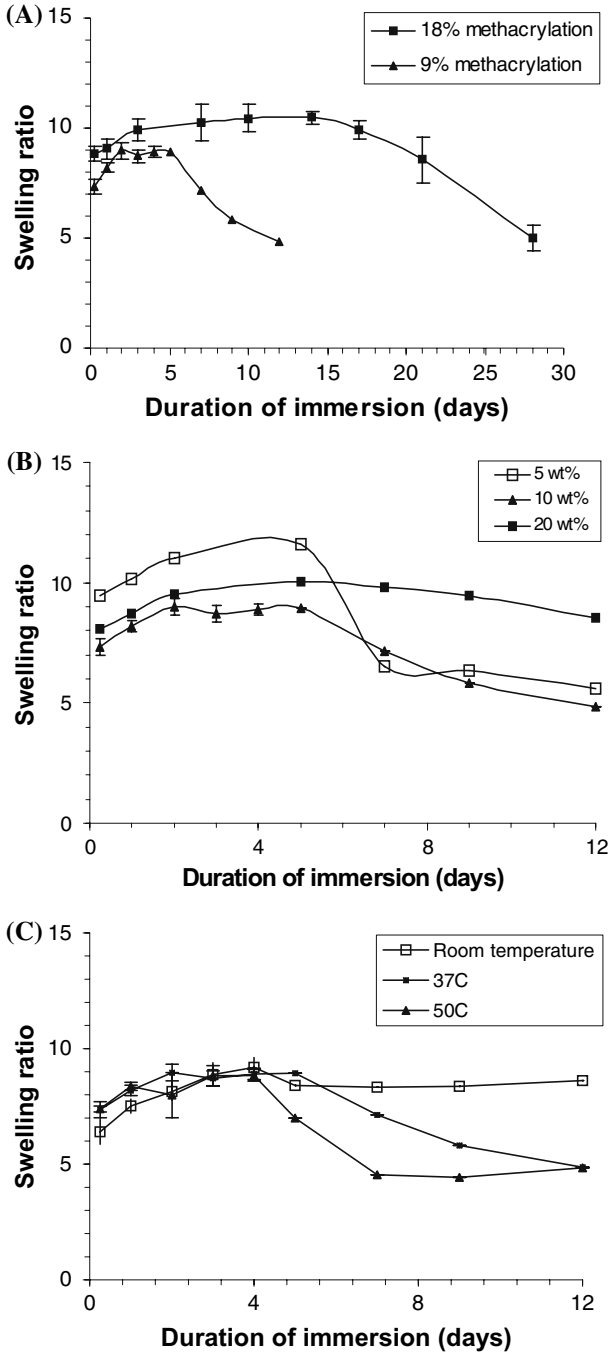

Fig. 7 Effects of the methacrylation degree of gelatin (**A**), photoinitiator concentration (**B**) and incubation temperature (**C**) on rehydration and stability of the freeze-dried IPN films in a solution of 50% fetal bovine serum and 0.1% sodium azide. Data with error bars represent means ± standard deviation ($n = 3$), otherwise $n = 1$. The films were prepared using gelatin with 9% methacrylation

3.4.3 Effect of incubation temperature

Figure 7C shows the medium temperature had a strong effect on the swelling stability. Although the IPN films exhibited comparable swelling degree for up to 4 days, irrespective of the temperature of the serum, there was a tendency towards a temperature-dependent reduction in the absorbency of the biomaterial after a 4-day immersion. At room temperature, the IPN films could maintain the hydration level over the 12-day investigation period. In contrast, they tended to decrease in swelling after 6 days of immersion at 37 °C and 4 days of immersion at 50 °C, respectively.

3.5 Mechanical testing in serum-containing medium

Figures 8A and B show the stress–strain curves for the IPN and its controls (i.e. photo-crosslinked gelatin and HydroThane™ films) in dry and wet states, respectively. Overall, the IPN showed a mechanical behavior in between those of gelatin and HydroThane™. The IPN exhibited increases in both elasticity in the dry state and stress in the wet state compared to the gelatin film. The latter showed the mechanical behavior of plastics in the dry state and a converse stress–strain behavior in the wet state. The HydroThane™ film displayed similar mechanical behaviors in the dry and

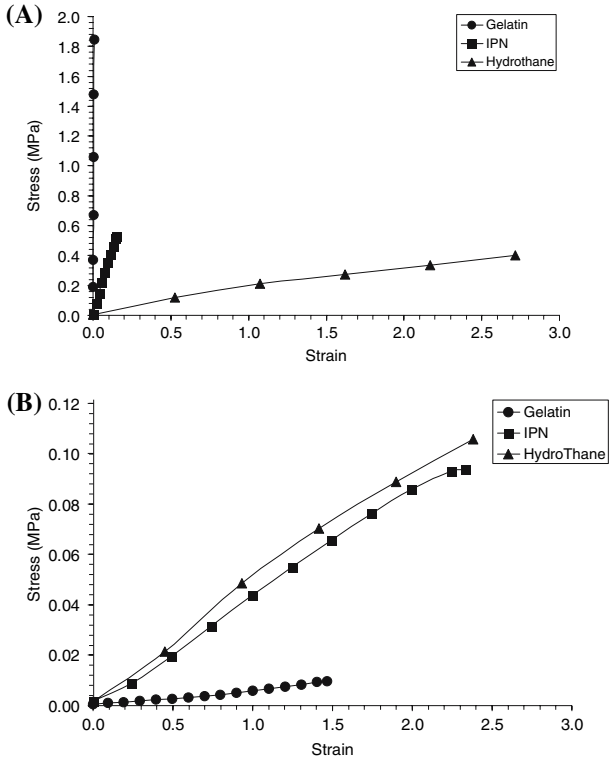

Fig. 8 Typical stress–strain curves for crosslinked gelatin, HydroThane™ and gelatin-HydroThane™ IPN films in the dry state (**A**) and immersed in the serum solution at 37 °C for 4 days (**B**). The films were prepared using gelatin with 9% methacrylation

Table 1 Mechanical properties of the freeze-dried gelatin-HydroThane™ IPN films before and after immersion in the serum solution

Methacrylation degree of gelatin	Medium temperature	Duration of immersion (days)	Failure stress (MPa)	Failure strain	Young's modulus (MPa)
9%	Room temperature	Dry	0.456 ± 0.097	0.145 ± 0.008	3.122 ± 0.503
		4	0.146 ± 0.040*	3.618 ± 0.996*	0.043 ± 0.004*
		12	0.108 (n = 1)	NA	NA
	37 °C	2	0.124 ± 0.021*	3.822 ± 0.496*	0.033 ± 0.001*
		4	0.110 ± 0.023*	3.177 ± 0.538*	0.035 ± 0.005*
		12	0.107 (n = 1)	NA	NA
	50 °C	4	0.120 ± 0.021*	3.717 ± 0.655*	0.033 ± 0.003*
		12	0.088 (n = 1)	NA	NA
18%	Room temperature	4	0.099 (n = 2)	3.248 (n = 2)	0.040 (n = 2)
		7	0.106 ± 0.008	1.946 ± 0.202	0.055 ± 0.006
	37 °C	2	0.089 ± 0.020	3.015 ± 1.177	0.031 ± 0.007
		4	0.107 ± 0.011	4.507 ± 2.040	0.027 ± 0.011
		7	0.089 ± 0.004	2.241 ± 0.360	0.040 ± 0.007
	50 °C	2	0.134 ± 0.019	5.848 ± 2.754	0.025 ± 0.008
		4	0.125 ± 0.025	5.992 ± 4.373	0.0275 ± 0.014

Data are expressed as means ± standard deviation (n = 3, unless specified)

* $p < 0.05$ vs. respective IPN films before immersion in the serum solution

wet states. The mechanical testing results are summarized in Table 1. First, the IPN showed much higher rigidity in the dry state than in the wet state (i.e. a Young's modulus of 3.1 vs. 0.043 MPa). Second, the IPN in the wet state exhibited about the same strength and elasticity at room temperature as at 37 °C and 50 °C, despite the physical gelation of the hydrogel component at room temperature. No significant decrease in mechanical stress with immersion time was observed at room temperature and 37 °C, although a decrease in swelling of the IPN prepared from gelatin with 9% methacrylation was observed at 37 °C, as shown in Fig. 7A. On the other hand, there was a decrease in the stress after 12-day immersion at 50 °C for the IPN prepared from gelatin with 9% methacrylation. It is noteworthy that the IPN exhibited a high elasticity comparable to that of the HydroThane™ control, and was much stronger than the corresponding hydrogel. However, it showed a much lower failure stress than the tensile stress of 39.5 MPa provided by the manufacturer for a solution-casted HydroThane™ film immersed in distilled water at 23 °C for 24 h. Lastly, no significant effects of the extent of gelatin methacrylation on mechanical properties were observed, as they were mainly controlled by the HydroThane™ component.

4 Discussion

We prepared a novel IPN through simultaneously crosslinking polymers in the presence of one another in a solution. Due to a significant difference between gelatin and polyurethane in their solubility parameters (35 $MPa^{1/2}$ [27] vs. 18.3–26.5 $MPa^{1/2}$ [28]), it is very challenging to find a common solvent for both to form a homogeneous mixture. We examined a number of commercial polyurethanes in various solvents for gelatin and identified HydroThane™, a thermoplastic polyurethane, soluble in DMSO likely due to the hydrophilic nature of its polytetramethylene glycol units [29]. The functionalized polyurethane has potentials for a number of biomedical applications [29] and is UV-curable using photoinitiators [30]. On the other hand, gelatin was methacrylated, which may decrease its solubility parameter and thus increase its miscibility with HydroThane™ in DMSO. Moreover, methacrylated gelatin could be crosslinked through free radical polymerization of the methacrylate groups on gelatin to form the hydrogel component.

The methacrylation was conducted using a reported method [17] and characterized qualitatively by FTIR and quantitatively by H-NMR. The extent of methacrylation we obtained was lower than that reported by Schacht et al. [17], but the increase in methacrylation obtained by using a larger amount of methacrylic anhydride was consistent with their results. The extent of methacrylation is an important consideration, as it would affect the IPN properties.

It is well known the polymer composition and solution concentration play important roles in IPN formation and its properties [7]. Therefore, we prepared the HECB films under different polymer compositions and solution concentrations and characterized their swelling and mechanical properties, two most relevant properties for our applications, to

determine an optimum condition. Having established the condition to prepare the HECB, we used FTIR, DSC and light microscopy to confirm the physico-chemical structure and molecular interactions in the composite biomaterial.

The peaks we observed at 1700 cm^{-1} and 1730 cm^{-1} are due to hydrogen-bonded and free C=O, respectively [31]. The ratio between the hydrogen-bonded and free C=O of HydroThane™ in our IPN was reduced compared to that of the HydroThane™ itself. This finding may be due to the interruption of the hydrogen-bonding formation of HydroThane™ resulting from the presence of gelatin. Alternatively, the absence of a shift of the bands of the functional groups from each component suggests weak molecular interactions between the two polymers. Although crosslinking reactions of each polymer were confirmed (as both polymers became insoluble in DMSO after the completion of the process) and the photo-crosslinking likely proceeded with free radical polymerization of methacrylate groups on gelatin and unsaturated groups on HydroThane™, our results suggest that no chemical reactions took place between the two component polymers during the formation of the IPN.

The large difference in the glass transition temperatures between the two components in the IPN implies limited chemical reactions and physical mixing at the molecular level [32]. While the two polymers appeared sufficiently mixed in the DMSO solution, phase separation took place during the course of the crosslinking reaction due to the immiscibility of the constituent polymers, as indicated by the difference in their glass transition temperatures [33]. The increase in the phase transition temperatures and decrease in the melting enthalpy of the gelatin component may be accredited to reduced mobility of the polymer segments, as a result of physical restriction from the HydroThane™ component in the IPN [34]. Theoretically, the blend of two immiscible polymers would not achieve a homogenous phase. Depending on the competitions between the crosslinking reaction and the phase separation process, various morphologies may be obtained. We are currently investigating the effects of preparation conditions on IPN morphologies.

Our process for preparing an IPN yielded a composite material with a larger domain of each component and porous structure compared to a simultaneous IPN, in which at least one network was formed from polymerization of a monomer followed by a wash in a solvent compatible for both components [32]. The porous structure was created by the removal of entrapped water in the gelatin phase following lyophilization and would lead to a rapid and considerable fluid absorption during rehydration of our material in both aqueous and serum-containing solutions.

Our finding of lower swelling ratios for the IPN material compared to those of gelatin films, taken together with the presence of the HydroThane™ network into which the gelatin domains were interspaced, suggests that the hydrophobic HydroThane™ (relative to gelatin) component was physically restricting the expansion of gelatin. This finding, taken together with our observation that many commercially available polyurethane-based wound dressings absorb approximately 8–10 times their dry weight in wound exudates (unpublished data), would suggest a modest advantage of this processing method for the use of our material as a wound dressing for combat casualties. The difference in swelling at room temperature and 37 °C of both IPN and gelatin films is due to a sol–gel phase transition of gelatin between the two temperatures [35].

Gel permeation chromatography (GPC) has been used to study the stability of different types of biomaterials, including hydrogels [36] and elastomers [37]. It provides information for both degradation rate and mechanism [38]. In addition, dissolution and degradation effects on biomaterial stability can be distinguished by molecular weight analysis of solutes in solution, where a biomaterial is immersed. Moreover, since swelling and degradation may occur immediately upon the immersion of a biomaterial in a medium, swelling changes cannot be correlated directly with the biomaterial stability [39]. Therefore, we used gel permeation chromatography (GPC) to further assess the stability of the composite biomaterial immersed in an aqueous solution.

The patterns of the chromatograms obtained for both gelatin and IPN films immersed at 37 °C were similar to that of the gelatin standard, indicating that gelatin was released from these films in the incubation medium. Since the films were washed for at least 1 week, to remove DMSO and any uncrosslinked gelatin before being freeze-dried, the residuals released into the solution during incubation were likely due to hydrolysis of the gelatin component. The degradation of native and crosslinked gelatin has been previously reported [28]. In the absence of any microorganisms and enzymes, gelatin could be hydrolyzed through cleavage of peptide bonds, pentosidine and pyridinoline crosslinks [40]. The temperature-dependent stability of our IPN was likely due to higher solubility and faster hydrolysis at 37 °C than at room temperature [28, 40].

The gelatin loss may compromise its functions as a hydrogel component for sustained drug release and water absorbency, possibly affecting its biological

response. While almost 50% of gelatin was lost after immersion of our IPN in an aqueous solution for 30 days, there were no significant changes in hydration. This finding may be explained by the fact that the loss of gelatin in the IPN material led to the formation of pores that were then filled with the aqueous solution, thereby leading to the maintenance of the absorbency of the photo-crosslinked films. Alternately, it is also possible that the loss of gelatin from the photo crosslinked films was responsible for loosening up their network structure, resulting in a greater hydration of the material [36]. Nevertheless, this finding is likely to be of little importance to our intended application, since the IPN is expected to remain in a wound site for up to 4 days only.

Although assessing the stability of hydrogel-based biomaterials in aqueous solutions provides a basic understanding of the extent of hydrolysis the material will undergo, it is known that both the swelling and stability of a biomaterial are also affected by solution composition, pH, temperature, and the presence of biocatalysts and enzymes [41]. While technical difficulties prevented us from determining the amount of gelatin lost of our gelatin-HydroThane^{TM} IPN film in a serum-containing solution, we assessed its ability to rehydrate in this medium to better understand the performance of the composite biomaterials under conditions of mimicking those of highly exudating wounds.

Considering that the photo-crosslinking of gelatin in our IPN was based on the free radical polymerization of the methacrylate group on gelatin, the extent of gelatin methacrylation also affected the IPN formation and properties, as suggested by the higher swelling stability of films prepared using gelatin with a higher degree of methacrylation.

Since the photo-crosslinking reaction involved in the preparation of our gelatin-HydroThane^{TM} IPN films was initiated by the UV-decomposition of the photo-initiator Irgacure 651, one could speculate that its relative concentration in the pre-IPN solution could also affect the IPN formation and its properties: the higher the photoinitiator concentration, the higher the initiation radical yield, which may, in turn, lead to a higher crosslinking and swelling stability.

The decrease in swelling stability with increasing temperature is due to increased gelatin hydrolysis and solubility. This is consistent with our GPC results, showing a decrease in stability with the medium temperature.

An ideal wound dressing must possess a relatively good strength and elasticity to provide high conformability to different wound contours and ease of dressing removal. We therefore attempted to improve the mechanical properties of the gelatin hydrogel, typically increasing the elasticity in the dry state and ultimate stress in the wet state, by including the HydroThane^{TM} elastomer. Our results show that due to the formation of a continuous HydroThane^{TM} phase in the IPN, the mechanical properties of the gelatin hydrogel were significantly improved. The stress and strain of our IPN were, however, lower than the corresponding properties of HydroThane^{TM} elastomer. The absence of the mechanical synergy was likely due to limited interactions between the two polymeric components. On the other hand, the mechanical properties of HydroThane^{TM} could be compromised due to changes in macromolecular structure and organization as a result of formation of the IPN film [42]. The IPN film appeared more elastic in the wet state than in the dry state due to accordingly increased strain of the gelatin network. Furthermore, the gelatin component in the IPN showed high rigidity in the dry state. One could speculate that increasing interactions between the two polymers at the molecular level would further enhance both the strength and the strain of our IPN material.

As polyurethanes may be biodegradable depending on their chemical and physical structures [43] and the degradation would compromise the mechanical properties of the IPN, we examined our IPN's mechanical properties as a function of immersion time in the serum solution at different temperatures. No significant differences were observed within the investigated periods and temperatures. The higher mechanical stability compared to the swelling stability may be because the stress and strain were mainly provided through the more stable HydroThane^{TM} component, while swelling resulted mainly from the gelatin component, which degraded and dissolved out.

5 Conclusions

Our investigation demonstrates that it is possible to create a hydrogel–elastomer composite biomaterial through the simultaneous formation of an interpenetrating network of two physically incompatible polymers. The composite biomaterial retained, to a certain extent, the unique properties of each component in terms of fluid absorbency and good mechanical properties despite limited interactions between the two constituent polymers at the molecular level. Although the composite biomaterial showed gelatin loss, the

total gelatin loss was relatively small over the period of application for our intended use, and did not reduce the overall absorbency of the IPN material. Furthermore, the biomaterial maintained its swelling stability for more than 30 days in the aqueous solution. While the biomaterial showed lower swelling stability in the serum-containing medium than in the aqueous solution, it could still maintain its swelling level in the serum for at least 4 days even at 50 °C, and the swelling stability was further increased by increasing the degree of gelatin methacrylation and photo-initiator concentration. The stability in both aqueous and serum solutions was decreased with increasing incubation temperature. The composite biomaterial showed good mechanical properties arising from the HydroThane™ component. There was neither a measurable degradation of the elastomer nor an impact on the mechanical properties of the IPN films. Fewer changes in mechanical properties were noticed compared to changes in swelling. Our study clearly indicates that there is an alternative, effective way to combine the advantages of different biomaterials and suggests the merit for further developing this novel composite biomaterial for use as a relatively stable battlefield wound dressing.

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