J Mater Sci: Mater Med (2008) 19:1803–1813
DOI 10.1007/s10856-007-3324-y

Hydrogel–elastomer composite biomaterials: 4. Experimental optimization of hydrogel–elastomer composite fibers for use as a wound dressing

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Received: 31 May 2007 / Accepted: 6 November 2007 / Published online: 6 December 2007

Abstract We report a novel 3-D cavity wound dressing based on a hydrogel–elastomer Interpenetrating Polymer Network (IPN) fabricated into an open-mesh architecture. IPN fibers used to form the dressing were produced by a wet spinning method and optimized in two steps. A factorial experiment was first conducted to identify key parameters that controlled fiber properties. We observed that gelatin wt% played a major role in determining fiber yield, swelling, strength and stability. Other contributing factors included coagulation solution composition, gelatin type, and pre- and post-UV irradiation time. The key factors were then further evaluated individually to achieve a condition that provided a combination of good swelling, mechanical properties and stability. The concentration of the gelatin/HydroThane^{TM} extrusion solution significantly affected fiber formation and properties, presumably due to the changes in solution viscosity. The effects of pre-UV irradiation were also ascribed to its impact on the solution viscosity and became negligible at higher concentrations when viscosity is mainly controlled by concentration. The composition of the coagulation bath influenced the fiber swelling and wet stress. These results, taken together with our previous studies, suggest that our biomaterial would provide a combination of mechanical and swelling properties suitable for wound dressing applications.

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1 Introduction

Polymer fibers of various diameters have been fabricated from single natural or synthetic polymers or their blends using a variety of processes. These fibers have been assembled into membranes or 3-D structures for tissue engineering, drug delivery, biochemical detection and protection, and molecular filtration applications [1–3]. Wet spinning and electrospinning are the two most widely used techniques to produce fibers from polymer solutions. Wet spinning typically involves extrusion of a polymer solution into a coagulation bath to make fibers with a diameter in the micrometer scale. Using this technique, the formation of chitosan fibers and 3-D mesh scaffolds for bone regeneration has been reported [4]. Electrospinning, a technique where a polymer solution is extruded by an electrostatically driven jet to produce fibers in a nano- and submicro-meter diameter range [5], has recently drawn much attention. Wet spinning has the advantage of producing single fibers that can be drawn, individually characterized, and woven into more complex structures. Electrospinning can generate finer fibers, but only in a non-woven form with short strands.

The performance of scaffolds and membranes fabricated from single-component fibers is constrained by the inherent properties of the constituent material. Composite fibers produced from natural and synthetic polymers can overcome this limitation by combining complementary and synergetic properties of different materials. Thus, chitosan–hyaluronic acid [6], human fibronectin–fibrinogen [7], polyacrylonitrile–chitosan derivatives [8], alginate–gelatin [9], chitosan–gelatin [10] fibers have been prepared using the wet spinning technique while gelatin–polycaprolactone [11] and polyurethane–collagen [12] fibers have been made by electrospinning.

Significant technological advances have been made in both material synthesis and fabrication process to create novel fibrous membranes or scaffolds. To our knowledge, the preparation of hydrogel–elastomer Interpenetrating Polymer Network (IPN) fibers that combine the advantages of absorbency and bioactive properties of hydrogel and mechanical integrity of elastomer has not been reported. Specifically, we were interested in developing a novel battlefield wound dressing based on a hydrogel–elastomer composite biomaterial with various architectures. To this end, we have completed serial studies on IPN films where gelatin formed the hydrogel component and HydroThane™, a commercial thermoplastic polyurethane, constituted the elastomer [13–15]. Gelatin and polyurethane are two classes of biomaterials with unique properties. The former is a biopolymer widely used in the biomedical [16], pharmaceutical [17] and food industries [18], and the latter is a synthetic polymer with excellent mechanical properties and biocompatibility for medical devices ranging from catheters to artificial hearts [19]. Both have been made into polymer fibers either alone [20, 21] or in combination with other polymers [9–12]. In addition, polyurethane fibers have been produced via an electrospinning process and deposited in the form of membranes for wound dressing applications [22].

We have recently investigated a process to produce fibers via extrusion of a solution comprising gelatin and HydroThane™ into a coagulation bath, followed by UV-irradiation. The resulting fibers may be further arranged into an open-mesh structure.

Many studies have been performed to understand the effects of material properties and fabrication process parameters on the performance of the blend fibers prepared from polymer solutions [9–12]. However, none of them described the influences of parameters in enough quantitative detail to optimize the production of IPN fibers. Factorial experimental design provides an efficient way to quantitatively analyze a large matrix of factors versus performance parameters for both main effects and factor interactions [23]. It can be used to effectively identify the most influential factors and provide direction to attain the optimum material and process conditions for the preparation of IPN fibers with desired properties.

In this study, we investigated the fabrication of gelatin–Hydrothane™ IPN fibers using a factorial experimental design involving 6 parameters at 2 levels each. More specifically, we intended to better understand the effects of solution properties and processing conditions on fiber physicochemical properties, and produce stable fibers with suitable physical and mechanical properties for applications in wound care. Key factors were identified and further investigated to optimize the fiber production. This study enabled the further development of the wound dressing for potential use on the battlefield.

2 Materials and methods

Gelatin type A with bloom numbers of 235 (type A LoMw) and 300 (type A HiMw), and gelatin type B with a bloom number of 250 were purchased from Great Lakes Gelatin (IL, USA), and were methacrylated using methacrylic anhydride (Sigma-Aldrich Co, ON, Canada), as previously described [13]. Sodium azide was obtained from Sigma-Aldrich Co. (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 purchased from Fisher Scientific (ON, Canada). Silicone oil standards were provided by 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 Preparation of gelatin–HydroThane™ IPN fibers

As shown in Fig. 1, the preparation of gelatin–HydroThane™ IPN fibers included: solution extrusion, definition of circular extrusion patterns and provision of fiber drawing effects using a robotic system with a spin table, precipitation, and UV irradiation. Specifically, a mixed solution of methacrylated gelatin, HydroThane™ and Irgacure 651 in dimethyl sulfoxide (DMSO) in a glass scintillation vial was pre-UV irradiated at 350 nm at an intensity of 9 mW cm^{-2} in a photochemical chamber reactor (RAYONET model RPR-200, Southern New England Company, CT, USA). The mixture was then loaded into a Pressure-lok glass syringe (Precision Sampling Corp., LA, USA) and extruded at 2.75 mL min^{-1} through a polyethylene tube (ID 0.76 mm) and a 21-gauge flat tip needle into a spinning acetone–water coagulation bath using an automated syringe pump (Harvard Apparatus, QC,

Fig. 1 Experimental set-up for fiber fabrication

Canada). The resulting fiber was then UV irradiated by a top-down UV exposure system (ABM Inc., CA, USA) at 3.8 mW cm^{-2} for 15 min, washed in 0.1% sodium azide, and freeze-dried. Gelatin and HydroThane™ fibers were prepared under experimental conditions very similar to those used in the preparation of IPN fibers. Thus, type B gelatin solution at 7.5 wt% was pre-UV irradiated for 3 min, extruded at 2.75 mL cm^{-1} into 95% acetone aqueous solution, spun at 10 rpm and post-UV irradiated for 15 min. HydroThane™ solution at 4 wt% was pre-UV irradiated for 6 min, extruded at 2.75 mL cm^{-1} into 91% acetone aqueous solution, spun at 10 rpm and post-UV irradiated for 15 min.

2.2 Characterization of gelatin–HydroThane™ IPN fibers

The fiber yield was calculated as the ratio between the mass of the fiber produced and the mass of polymers used.

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

Attenuated total reflectance (ATR) infrared spectra of each IPN fiber 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}.

2.2.2 Swelling study

The swelling was measured as the ratio between the mass of the fiber re-hydrated in a solution of 50% serum and 0.1% sodium azide at 37 °C for 2 and 4 days, and the initial dry mass of the fiber. The stability of the fiber was defined as the percentage of the fiber mass lost after immersion in the serum solution maintained at 37 °C.

2.2.3 Optical microscopy

Photos of the freeze-dried IPN fibers before and after rehydration 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).

2.2.4 Mechanical testing

Mechanical tests were conducted on freeze-dried fibers and on fibers immersed in the 37 °C serum-containing medium for 2 and 4 days. 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 of dry fibers and of fibers re-hydrated in the serum solution at 37 °C for 2 and 4 days 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 fiber.

2.2.5 Viscosity measurement

Methacrylated gelatin and HydroThane™ solutions were prepared at different concentrations in DMSO. The viscosity of each solution was measured at 25 °C using a viscometer equipped with a spindle (Brookfield Engineering Laboratories Inc., MA, USA). Silicone oil standards were used to calibrate the viscometer.

2.3 Experimental design and statistic analysis

We applied a 2^{6-2} factorial experiment design [24] to understand the effects of various factors on the fiber yield, the extent of swelling, mechanical properties and stability characteristics. The factors and their values in the design are summarized in Table 1. This type of design defines the minimum number of experimental combinations to obtain the maximum information. Data analysis was performed to determine the significance of the effects of each parameter with 95% confidence, as previously described [25].

Based on the factorial experiment, the experimental conditions for fiber production were further optimized by changing solution concentrations and compositions, gelatin type, and the concentration of the coagulation bath. The resulting fibers were then characterized as described above. Significant differences between two groups were evaluated using a two-tailed t test. When $p < 0.05$, the differences were considered to be statistically significant.

3 Results

3.1 General IPN characteristics

Figure 2 shows the FTIR spectra of the fibers made from extrusion of combined gelatin and HydroThane™ solutions into slightly different coagulation solvents. In addition, the polymer solutions were also extruded individually to confirm that they could coagulate and form a fiber in the solvent. The spectrum of the gelatin fiber shows

Table 1 Experimental variables used for the preparation of the fibers

Experimental condition #	Pre-UV irradiation time (min)	Gelatin wt%	Spin rate (rpm)	Post-UV irradiation time (min)	Gelatin type	Acetone vol%
1	5	40	10	15	A[a]	91
2	5	40	10	30	A	95
3	5	40	15	15	B	95
4	5	40	15	30	B	91
5	5	60	10	15	B	95
6	5	60	10	30	B	91
7	5	60	15	15	A	91
8	5	60	15	30	A	95
9	6	40	10	15	B	91
10	6	40	10	30	B	95
11	6	40	15	15	A	95
12	6	40	15	30	A	91
13	6	60	10	15	A	95
14	6	60	10	30	A	91
15	6	60	15	15	B	91
16	6	60	15	30	B	95

[a] Gelatin type A with a bloom number of 235

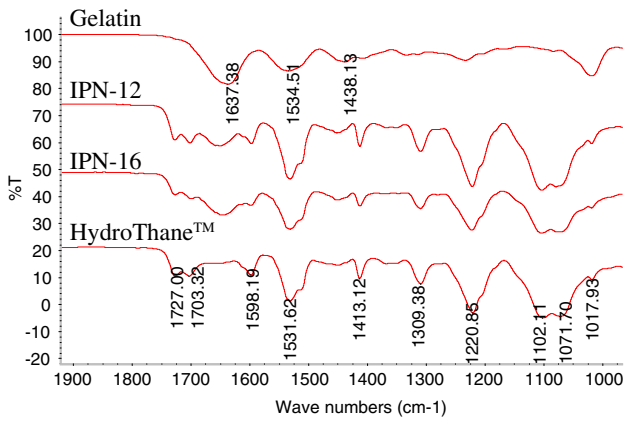


Fig. 2 FTIR spectra of gelatin, HydroThane™ and gelatin–HydroThane™ IPN fibers. The IPN fibers were prepared from experimental conditions #12 and #16 in Table 1. Spectra clearly show IPN to be a gelatin–HydroThane™ composite with tunable composition

a peak at 1,637 cm^{-1} likely due to the amide group of gelatin. The urethane group of the HydroThane™ fiber is responsible for the peaks observed at 1,727 and 1,703 cm^{-1}. These characteristic peaks were also identified in the spectra of the IPN fibers. Neither new nor significant shifts of characteristic absorption bands were noticed. Based on the relative intensity of the characteristic peaks, the data indicates that the fiber made in experimental condition #12 (Table 1) possessed a greater proportion of the gelatin component than the one made in experimental condition #16, a finding consistent with the amount of gelatin in their extrusion solutions (60 vs. 40 wt%).

Microphotographs of an IPN fiber in dry and hydrated state are presented in Fig. 3. Re-hydration increased the diameter of the IPN fiber from 200–300 μm to 600 μm. Furthermore, the re-hydrated fiber showed a brighter center core and a denser surface layer.

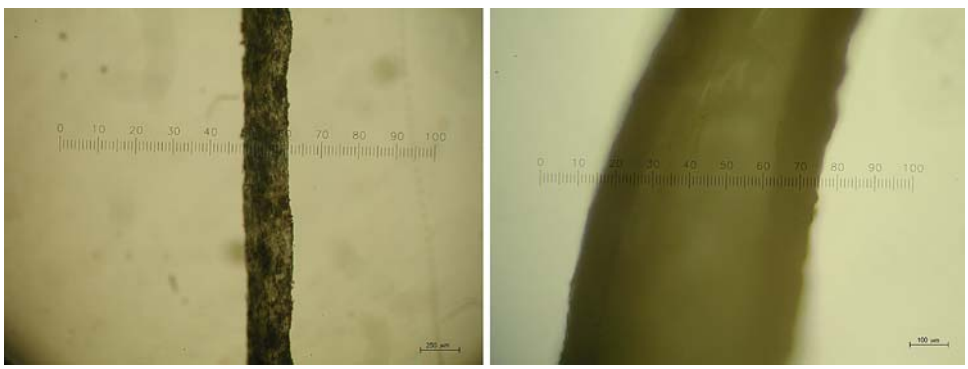


Fig. 3 Micrographs of an IPN fiber in dry (left) and hydrated states (right). The fiber was prepared from experimental condition #1 in Table 1. The scale bars represent 250 μm (left) and 100 μm (right), respectively

3.2 Factorial study

Statistical analysis of the factorial experimental data is summarized in Table 2. The chosen sign (+/−) denotes either positive or inverse relationship between each factor-performance parameter pair, while the magnitude suggests the strength of the factor's influence. The 95% confidence intervals are shown in parenthesis and indicate the statistical significance of the effect. The effects were colour-coded for better visualization. All responses were markedly influenced by gelatin percentage. For example, a greater gelatin wt% in the gelatin–HydroThane™ composition significantly reduced the yield, mechanical strength and stability of the fibers, but increased the swelling. It was the only significant factor affecting ultimate stress in the wet state. Overall, the factor that most strongly influenced the fiber yield and properties was the gelatin wt%, while the effects of other factors such as acetone vol% in the coagulation solution, type of gelatin, and pre- and post-UV irradiation times were less prominent. Although its effects showed some tendencies, the spinning speed of the coagulation bath that collected the fiber had no significant effect on any of the investigated properties, indicating a lack of drawing effects.

In addition to main factor effects, interaction effects were also studied. Table 3 summarizes the significant interactions found in the study. Particularly, the interaction between pre-UV irradiation time (factor 1) and gelatin composition (factor 2), and between gelatin type (factor 5) and acetone vol% (factor 6) warrant future investigations. The interaction effect results obtained should be interpreted with consideration of main effects confounding shown in Table 4.

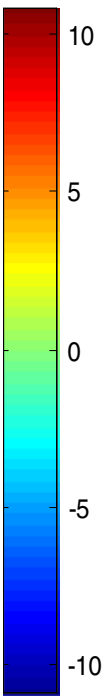
3.3 Final optimization

To further increase the quality of the IPN fibers, an experiment was designed to control the viscosity of the extrusion solution through altering either the concentrations of HydroThane™ and methacrylated gelatin or pre-irradiation duration or the molecular weight of gelatin (Table 5). Table 6 summarizes the properties of the fibers prepared using the various conditions detailed in Table 5. Figure 4 shows that increasing the polymer molecular weight and polymer concentration of different solutions increased their viscosity. The response is more sensitive to changes in concentration than to molecular weight differences.

The yield of the HydroThane™ fiber was well-above those of the gelatin and IPN fibers, which ranged from 40% to 60%. The HydroThane™ fiber also showed a greater

Table 2 Main effects of the experimental variables on various fiber properties

	Yield[a]	Dry ultimate stress[b]	Swelling ratio[b]	Wet ultimate stress[b]	Stability[b]
Pre-UV Irradiation time	+0.912 (4.018)	-2.785 (1.861)	-0.370 (0.743)	+0.059 (0.210)	+3.224 (2.183)
Gelatin wt%	-8.374 (4.070)	-7.375 (1.856)	+3.176 (0.752)	-1.374 (0.209)	-10.983 (2.212)
Spin rate	+0.156 (4.018)	+0.763 (1.871)	-0.651 (0.742)	+0.033 (0.215)	+1.722 (2.177)
Post-UV Irradiation time	-0.870 (4.085)	+1.892 (1.864)	-0.050 (0.739)	+0.032 (0.216)	+0.917 (2.168)
Gelatin type	+0.537 (4.053)	-1.295 (1.856)	+1.259 (0.741)	-0.120 (0.215)	+2.963 (2.152)
Acetone vol %	-8.416 (4.048)	-0.298 (1.863)	+0.316 (0.740)	+0.002 (0.212)	-2.395 (2.188)



[a] Experiments were conducted in duplicate; [b]Experiments were conducted in triplicate. Data in parenthesis represent 95% confidence intervals

Table 3 Significant interaction effects of the factors on the fiber yield and properties

Factors[a]	Parameters				
	Yield[b]	Dry ultimate stress[c]	Swelling ratio[c]	Wet ultimate stress[c]	Stability[c]
1 * 2		2.397 (1.875)			
1 * 4		−1.915 (1.870)			
3 * 5		2.397 (1.875)			
5 * 6		−1.915 (1.870)			
1 * 2 * 3			1.259 (0.741)		−2.963 (2.152)
1 * 3 * 5	−8.374 (4.070)	−7.375 (1.856)	3.176 (0.752)	−1.374 (0.206)	10.983 (2.212)
1 * 4 * 5	−8.416 (4.048)				2.395 (2.188)
1 * 4 * 6			1.259 (0.741)		−2.963 (2.152)
1 * 5 * 6		1.892 (1.864)			
2 * 3 * 4	−8.416 (4.704)				2.395 (2.188)
2 * 3 * 5		−2.785 (1.861)			−3.224 (2.183)
2 * 3 * 6		1.892 (1.864)			
3 * 4 * 6	−8.374 (4.070)	−7.375 (1.856)	3.176 (0.752)	−1.374 (0.206)	10.983 (2.212)
4 * 5 * 6		−2.785 (1.861)			−3.224 (2.183)

[a] Factors 1–6 represent pre-UV irradiation time, gelatin wt%, spin rate, post-UV irradiation time, gelatin type and acetone vol%, respectively

[b] Experiments were conducted in duplicate

[c] Experiments were conducted in triplicate. Data in parenthesis are 95% confidence intervals

Table 4 Confounding structure of the significant effects in the fiber 2^{6-2} fractional factorial design

Effects	Confounding effects	
1	2 * 3 * 5	4 * 5 * 6
2	1 * 3 * 5	3 * 4 * 6
4	1 * 5 * 6	2 * 3 * 6
5	1 * 2 * 3	1 * 4 * 6
6	1 * 4 * 5	2 * 3 * 4
1 * 2	3 * 5	
1 * 4	5 * 6	

wet strength and stability in the serum solution, but lowest swelling, while the gelatin fiber exhibited the highest dry strength and largest swelling, but lowest wet strength and stability. The stability was reflected by the percentage of gelatin loss during swelling, but no decrease in swelling was observed for up to 4 days despite the reduced gelatin content. The properties of the IPN fibers tended to fall in-between those of gelatin and HydroThane™ across all categories. The ultimate stress of the dry IPN fibers ranged from 6.44 to 18.09 MPa. In contrast, the ultimate stress of the re-hydrated fibers was about 10 times lower. The dry ultimate stress and swelling ratio increased with increasing gelatin concentration (fibers 3 vs. 4; Table 5). The swelling ratio tended to decrease ($p = 0.065$) as the HydroThane™ concentration was reduced from 5 to 4 wt% (fibers 4 vs. 5). Unlike the dry fibers, the wet fibers were more sensitive to

Table 5 Conditions used to prepare gelatin, HydroThane™ and gelatin–HydroThane™ IPN fibers

Fiber	Gelatin[a] concentration (wt%)	HydroThane™ concentration (wt%)	Gelatin: HydroThane™ by weight	Pre-UV irradiation (min)	Post-UV irradiation (min)	Acetone vol%
1	7.5	4	1:1	5	15	93
2	7.5	5		2		
3	10	5		0.5		
4	15	5		0.5		
5	15	4	1:1	0.5	15	93
6	15	4	1:1			95
7	15	4	7:3			93
8	7.5 (type A HiMw)	4	1:1	0.5	15	93
Gelatin	7.5	0		3	15	95
HydroThane™	0	4		6		91

[a] Type B gelatin, unless otherwise specified

Table 6 Effects of preparation conditions on fiber properties

Fiber	Parameters				
	Yield[a] (%)	Dry ultimate stress[b] (MPa)	Swelling ratio[b]	Wet ultimate stress[b] (MPa)	Stability[b] (%)
1	58.45	6.44	6.10	0.14	6.02
2	64.05	14.35	4.27	0.75	7.27
3	42.04	6.77	2.73	0.82	4.51
4	58.84	15.62	4.13	0.65	6.27
5	59.47	15.87	3.22	1.12	11.20
6	45.65	15.16	4.13	0.45	10.44
7	58.64	18.09	6.17	0.13	7.98
8	52.57 (0.06)[c]	7.91	4.29 (1.79)[c]	1.05 (0.59)[c]	1.80 (2.10)[c]
9	48.95[b]	3.33 (1.00)[c]	3.74 (0.21)[c]	0.44 (0.16)[c]	2.68 (0.50)[c]
Gelatin	45.26	25.33 (10.26)[c]	10.26 (1.58)[c]	0.026 (0.008)[c]	16.92 (8.37)[c]
HydroThane™	78.53	18.87 (3.37)[c]	1.33	12.86	0.02

Swelling, ultimate stress in the wet state, and stability were measured using fibers immersed for 2 days at 37 °C in a 50% fetal bovine serum solution supplemented with 0.1% sodium azide

[a] n = 1 unless otherwise specified

[b] Experiments were conducted in duplicate, unless otherwise specified

[c] Data in parenthesis represent standard deviation (n = 3)

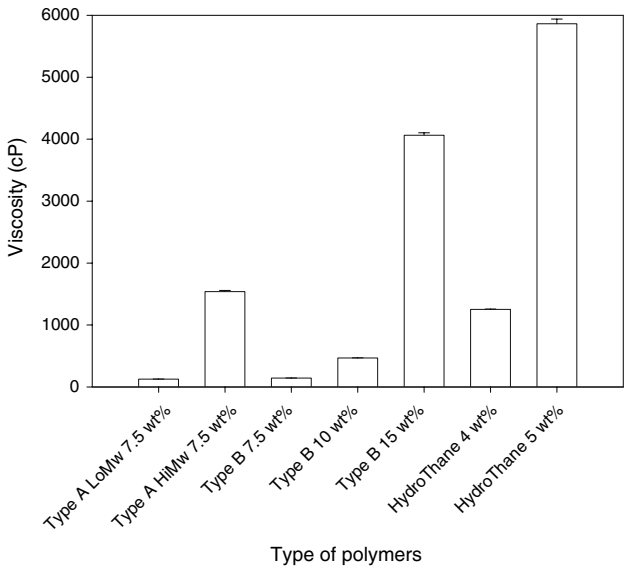


Fig. 4 Viscosity of different types of methacrylated gelatin and HydroThane™ solutions at varying concentrations. Data are expressed as mean ± standard deviation (n = 3)

changes in the polymer concentration. The increase in the amount of gelatin from 50 to 70 wt% led to a significant increase in the swelling ratio from 3.22 to 6.17, and a decrease in the wet strength from 1.12 to 0.13 MPa (fibers 5 vs. 7). The effects on swelling and wet strength are in agreement with the main effect of gelatin percentage observed in the factorial study, but no significant effects on the dry strength and stability were observed. However, it should be considered that the conditions in this experiment were different from those in the factorial one due to higher gelatin concentrations. Increasing acetone content from 93 to 95 vol% in the coagulation bath remarkably reduced the yield from 59.47% to 45.65% and wet stress from 1.12 to 0.45 MPa, but had no significant effects on other fiber properties (fibers 5 vs. 6). Simultaneously increasing the polymer concentration and decreasing pre-UV irradiation time did not significantly alter the fiber properties (fibers 1 vs. 2, 3, 4; Table 5), suggesting that the pre-UV irradiation time and gelatin concentration influenced the fiber properties similarly. Overall, changing the composition of the pre-IPN solution had the largest impact on mechanical properties and swelling, as observed in the factorial study, but less on the yield and stability. Gelatin concentration played a significant role in the dry strength and stability. The fiber properties remained comparable for the conditions that differed in the coagulation bath except for wet stress, which is very sensitive to bath formulation.

4 Discussion

Polymer fibers have been used as structural units for biomedical devices, including wound dressings [3]. It is well known that polymer properties and processing conditions control fiber formation and performance. Extrusion of polymer fibers possessing both high strength and absorbency is highly desirable but challenging because the processes and conditions required achieving such disparate attributes are very different [26]. We have succeeded in the synthesis of a novel IPN film that combines the two advantages by simultaneously cross-linking two dissimilar

polymers in a solution [13]. Based on this previous work, we extruded fibers from our formulation using a home-made wet spinning system, followed by UV cross-linking in the coagulation bath.

4.1 Experimental design

To better understand the process for IPN fiber production, we chose to study polymer and coagulation solution compositions, polymer types, and UV irradiation, as these factors have been reported to affect both fiber formation and properties [10, 27]. In addition, the spin rate was also examined considering the nature of experimental set-up. To obtain the maximum amount of information from the least number of experiments, we used a fractional factorial design, similar to that previously reported in a study on the influence of processing parameters on the structure and mechanical properties of as-spun polypropylene filaments [23]. Our experiment consisted of a resolution IV design with 16 duplicated runs. Preliminary trials were conducted to determine the appropriate levels for each factor so that fibers could be successfully produced under all experimental conditions to further characterize their properties. Solution viscosity is an important factor in the solution extrusion process, but choosing an appropriate value from a wide range is difficult and depends on application; viscosities between 20 and 5,000 poise have been used in various processes [28]. We investigated the viscosity of pre-IPN extrusion solutions to determine the most appropriate conditions for our experimental set-up. This was done by monitoring the curing process of a polymer solution containing 7.5 wt% methacrylated gelatin and 4 wt% HydroThane^{TM}. The glass vial containing the solution was removed from the irradiation chamber every minute to view the progress. The solution became slightly thicker after a minute of irradiation. Within 3 min, the solution still appeared thinner than honey, but began adhering slightly to the side of the glass when it flowed. After 5 min, the mixture was thicker than honey, with a viscosity likely in the range of 351–1,750 cP [29]. After 9 min, the solution had thickened to form a viscous gel. Based on these observations, it seems that lightly cross-linking for about 5–6 min is necessary to obtain a viscous and spinnable solution that can form fibers, but only within a narrow window of gelatin concentration, UV irradiation, and acetone percentage. A broader range may be achievable at higher polymer concentrations.

Swelling, mechanical properties and stability are the most important fiber physical properties for our wound dressing application. These parameters were thus selected to evaluate the performance of different fibers. The stability was reflected by the percentage of material loss during swelling, but no actual decrease in swelling was observed for up to 4 days.

4.2 Fiber morphology

The morphology of a fiber is controlled by its composition and coagulation conditions. Due to a significant difference between gelatin and polyurethane in their solubility parameters (35 $MPa^{1/2}$ [30] vs. 18.3–26.5 $MPa^{1/2}$ [31]), phase separation occurs during IPN formation [13]. In addition, different coagulation rates of the two polymers during wet spinning were confirmed by comparing the individual extrusion of gelatin and HydroThane^{TM} fibers (data not shown). This difference is expected to accelerate phase separation between the two polymers in the fiber. It has been reported that the difference in coagulation rate between poly(vinyl alcohol) and poly(acrylic acid) increased phase separation, resulting in larger phase domains in their fibers than in corresponding films [32]. The heterogeneity resulting from the phase separation is likely responsible for the rough surface of the fiber seen in Fig. 3. Furthermore, the coagulation rate is also a decisive factor in determining the internal morphology. A high rate of coagulation generally results in a porous morphology, while a slow rate yields a dense structure. Finally, the cross-sectional shape of solution-spun fibers is also determined by the coagulation rate. Indeed, a higher rate tends to result in a noncircular section [33]. The various demonstrated effects of coagulation rate on fiber formation warrant further studies to elucidate the coagulation effects on the morphology of our fibers.

4.3 Effects of fiber composition

FTIR results confirmed the positive correlation between the percentage of gelatin in the extrusion solution and in the fiber. This explains the effect of gelatin percentage in the extrusion solution on the swelling and strength of fiber. The most significant effects of gelatin wt% manifest in the fiber yield and stability. These effects can be ascribed to gelatin's greater solubility in the coagulation bath as well as in the solutions used for both washing and swelling compared to those of HydroThane^{TM}. As the hydrogel component in the fiber, gelatin contributed dominantly to swelling. On the other hand, the increased gelatin wt% compromised the mechanical properties of the fiber in both the dry and swollen states as HydroThane^{TM} provided most of the strength. These results are consistent with the literature showing increases in water absorbency and decreases in mechanical strength with higher content of hydrophilic components in blend fibers [34]. Type B gelatin resulted in

higher swelling than type A due to its ionization in the serum solution at neutral pH. This is consistent with our findings with type B IPN films [15]. Therefore, swelling is a function of both gelatin content and gelatin type, albeit to a lesser extent for the latter parameter.

The significant improvement in the wet strength of the IPN fibers over that of the gelatin fibers is ascribed to the HydroThane™ component, which is in agreement with our previous studies showing its contribution to the mechanical properties of IPN films [13]. Overall, the strength of the IPN fibers was lower than that of HydroThane™ itself due to the weak interactions between the two macromolecules and phase separation. Interestingly, the IPN fibers showed higher strength and stability, but lower swelling compared to the films. This may be attributed to the lower porosity of the fibers.

4.4 Effects of polymer concentration in extrusion solution

To understand the nature of the polymer concentration effects, the viscosities of methacrylated gelatin and HydroThane™ solutions at different concentrations were measured (Fig. 4). The results support that the effects of the polymer concentration were due to its influence on solution viscosity. The different degree of influences on fiber properties between HydroThane™ concentration and gelatin concentration may be a direct reflection of their abilities to control solution viscosity. In addition to changing coagulation kinetics and thus fiber properties, polymer viscosity also influences the mechanical process of extrusion by changing the flow. Low extrusion solution viscosity results in poor fiber spinnability due to the formation of droplets and dispersion in coagulation bath. On the other hand, too high viscosity increases pressure requirements and may form globular obstructions rather than continuous flow.

4.5 Effects of UV irradiation time

The pre-UV irradiation time is another factor whose effect may be interpreted in terms of its influence on viscosity. Irradiation increases the viscosity of the pre-IPN solution and improves extrusion properties, but the technique has its limitations as the uneven formation of gel eventually prevented extrusion flow. Altering polymer concentrations is the simplest method to control viscosity and was thus used to optimize fiber production. Therefore, when we increased the concentrations of gelatin and HydroThane™ to obtain the viscosity suitable for fiber production, the resulting fiber properties were further improved.

In contrast to pre-UV, post-UV irradiation only increased the dry stress, likely because nearly all the photoinitiators had already diffused out during the coagulation stage, thus leading to only limited cross-linking of the polymers. Therefore, use of higher photoinitiator concentrations may further increase the fiber strength. Alternately, enhancing the post drawing process might also strengthen our IPN fibers. Drawing has been known to increase fiber strength though alignment and interactions of polymers in the fiber [35]. Even though the spin table did not provide noticeable effects on any fiber properties, hand drawing may be a simple way to improve the fiber strength [36].

4.6 Factor interactions

Compared to the traditional optimization approach of examining factors individually, an important benefit of factorial optimization is its combinatorial approach, which allows for the simultaneous examination of a large number of factors and the interactions among them. Table 3 summarizes significant 2- and 3-factor interactions found in the study. Higher order interactions are assumed to be negligible. While the calculation of interaction effects is straight forward using established methods [25], the interpretation of the results requires more care due to confounding introduced through experimental design [37]. Because of this confounding, some interactions can not be estimated separately. However, three principles guide the interpretation of confounding factors and interactions without conducting additional experiments [38, 39]:

A. Hierarchical ordering principle

- Lower order effects are more likely to be important than higher order effects.
- Effects of the same order are equally likely to be important.

B. Effect sparsity principle

- The numbers of relatively important effects in a factorial experiment are small.

C. Effect heredity principle

- In order for an interaction to be significant, at least one of its parent factors should be significant.

By principle A, all three-factor interactions in Table 3 may be neglected due to confounding with main factor effects (Table 4). Of the two-factor interactions, 1 * 2 and 3 * 5, and 1 * 4 and 5 * 6 are confounded. 1 * 2 is more likely to be the real interaction than 3 * 5 by Principle C. Figure 5 shows that at higher gelatin wt%, pre-UV irradiation has a much more pronounced effect on dry strength. In addition,

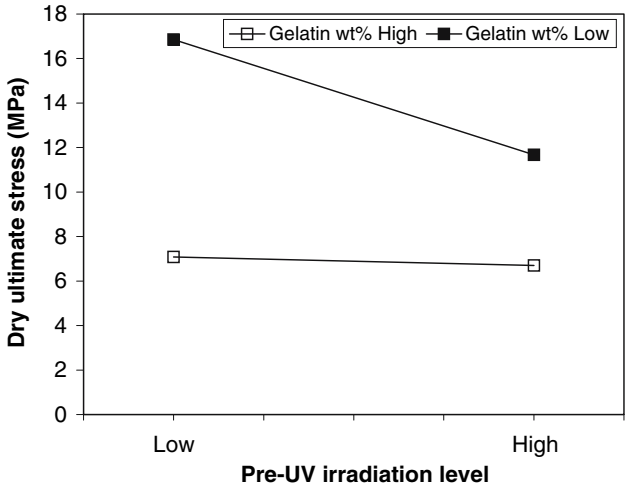


Fig. 5 Interaction effect between pre-UV irradiation time (factor 1) and gelatin wt% (factor 2). Pre-UV irradiation time is observed to have a larger effect at lower gelatin wt%

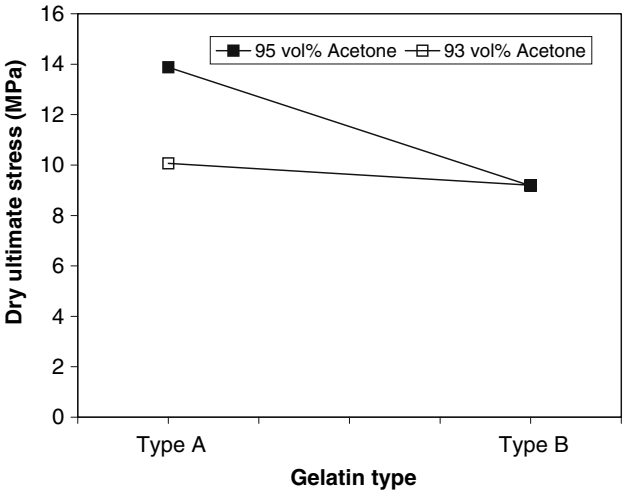


Fig. 6 Interaction effect between gelatin type (factor 5) and acetone vol% (factor 6). Gelatin type is observed to have a larger effect at higher acetone vol%

the general decrease of dry strength with increasing pre-UV irradiation time is also observed. The formation of gels in the extrusion solution during pre-UV irradiation can cause flow obstruction and influence the morphology of the extruded fiber. Too long pre-UV irradiation increases the likelihood of weak linkages in the fiber. Because gel formation is influenced more heavily by gelatin, flow disturbances may already occur at 5 min pre-UV irradiation when the gelatin wt% is high, and a longer duration has little influence (Fig. 5). In contrast, at low gelatin wt%, gel formation is postulated to progressively modify the fiber morphology as the pre-UV irradiation time is increased from 5 to 6 min, causing the observed decrease in the dry strength (Fig. 5).

The confounding of interactions 1 * 4 and 5 * 6 may be resolved using a priori knowledge that coagulation rate can influence the final fiber composition. Figure 6 shows that at higher acetone vol%, the effect of gelatin type on dry strength becomes more pronounced. This may imply that more gelatin precipitated and remained in the fibers at higher acetone vol%. Such explanation would lead one to conclude that 5 * 6 should have the most effect on swelling, which was not observed. This may be a consequence of not having enough statistical power in the experimental design. Further experiments should be carried out to study the interaction in more detail.

The capacity to study interactions in a factorial design unveiled new insights into the fiber formation process, which points way to new important experiments that are likely to produce positive findings. Ultimately, studying interactions gives us a deeper and more comprehensive understanding of the fiber formation process, and thus an optimization that would otherwise be unattainable.

Overall, our findings corroborate well with our own and other groups' previous results. Previous studies have shown the effects of gelatin type and solution viscosity on the morphology of IPN films [14, 15]. These parameters may similarly affect fiber formation and properties. Indeed, our measured main and interaction effects may be explained by differences in the morphologies of the fibers. It has been shown that solution concentration and composition, and coagulation bath affected fiber properties due to their effects on fiber morphologies [40, 41].

5 Conclusions

A number of parameters with profound effects on IPN fiber performance have been identified. Specifically, we found a strong influence of gelatin composition on the fiber properties. Meanwhile, polymer concentrations and pre-UV irradiation time affected the fiber production due to their effects on solution viscosity. A balance among these factors determined final properties of the fibers. Our systematic approach enabled us to optimize the process for fiber fabrication, and to make uniform continuous fibers with high swelling and relatively good strength for wound dressing applications.

Acknowledgements The authors are indebted to Ms. Michelle Mok and Mr. Doug Saunders for their expert technical assistance.

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