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Bioartificial Polymeric Materials Based on Collagen and Poly(N-isopropylacrylamide)

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Films of collagen (CLG) and poly(N-isopropylacrylamide), PNIPAAm, were prepared by casting from water solutions. These bioartificial polymeric materials were studied to examine the influence of PNIPAAm content and glutaraldehyde vapor cross-linking on the thermal and biological stability of CLG. Mixtures, ranging from 20-80 wt% CLG composition, were cross-linked through exposure to glutaraldehyde vapors. Thermal and morphological properties of the films, cross-linked or not, were investigated by differential scanning calorimetry, thermogravimetry, and scanning electron microscopy, with the aim of evaluating miscibility, thermal stability, and interactions among the constituents. The experimental results indicated that the homopolymers are not thermodynamically compatible. However, there is good evidence that effective interactions, probably due to hydrogen bond formation, takes place between the constituents. These interactions are more evident on the samples that were not cross-linked. DSC studies revealed that PNIPAAm exerts a thermal stabilizing effect on uncross-linked CLG, while the cross-linking with glutaraldehyde affects only the biological polymer, preventing the interactions with PNIPAAm. SEM micrographs of the uncross-linked mixtures showed that the morphology, in all compositions studied, remains similar to the pure collagen. In the corresponding cross-linked samples, a more compact aggregation is observed although no appreciably changes can be seen.

Keywords: *collagen, poly(N-isopropylacrylamide), bioartificial polymeric materials*

1. Introduction

"Bioartificial polymeric materials" is a term to designate a new class of materials based on blending synthetic and natural polymers for biomedical applications[1,2]. Originally, these new materials were conceived to overcome the poor biological performance of synthetic polymers and also to enhance the mechanical characteristics of biopolymers, in order to be employed as biomaterials or as low-environmental impact materials[1-7]. In this context, our group has investigated several systems involving both biological (primarily fibrin, hyaluronic acid, and collagen) and synthetic components (polyurethanes, poly(vinyl alcohol), and poly(acrylic acid))[8-13].

Reconstituted collagen (CLG) has been used in a wide variety of biomedical applications[14]. However, the lack of mechanical properties of CLG, due to the chemical treatment used to isolate it, as well as the high biodegradation rate after implantation are factors that have limited its application[15].

To solve the biodegradation problem, we have aimed to cross-link the collagen in order to reduce its degradation rate and to avoid the rapid dissolution of the material when it comes in contact with biological fluids. Previously, collagen-based materials have been cross-linked by chemical treatment with gaseous glutaraldehyde[11,12] and by a dehydro-thermal procedure[8,9,13]. The thermal cross-linking method has been a good alternative to the chemical method as there are no release of cytotoxic residuals that could affect the biocompatibility[9].

Poly(N-isopropylacrylamide), PNIPAAm, is a thermo-responsive polymer, i.e. a polymer that dissolves in water at room temperature, but undergoes a phase separation when heated to approximately 32 °C, exhibiting a lower critical solution temperature (LCST)[16]. Many studies have been published utilizing this thermo-sensitive behavior of PNIPAAm in fields such as drug delivery systems[17,18] and thermo-sensitive membranes[19-21]. PNIPAAm has been extensively studied under the hydrogel state as a polymeric matrix for use in biotechnology and bioengineering[22], and has been found to be cell compatible[23]. However, PNIPAAm, in a gel or linear form, shows low mechanical strength, which limits its practical application. To solve this problem, many studies have focused on the preparation of blends and copolymers and on the formation of interpenetrating polymer networks. Following this principle, we have previously reported[24,25] the preparation of blends of PNIPAAm with poly(vinyl alcohol) (PVAl), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), and poly(ethylene-*co*-vinyl alcohol) (EVAL).

Collagen and PNIPAAm are well known for their interesting biological properties, however the interactions between these polymers in blends has not been studied previously. PNIPAAm contains a proton accepting amide group, while collagen contains a carbonyl moiety and an N–H group (amide bonds) and hydroxyl groups as side groups, suggesting some possible interactions between these two macromolecules. Blends can be either miscible or immiscible, in a thermodynamic sense (i.e., miscible polymers blends behave as a single-phase system down to the segmental level of dispersion, and are usually associated with $\Delta Hm < 0$). The term "compatible blend" is an utilitarian term indicating a material to be commercially attractive, usually homogeneous to the eye with enhanced physical properties.

The aim of this work was to study the interactions between collagen and PNIPAAm in the solid state (thin films) and draw conclusions regarding the miscibility of these components. The interactions

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between these macromolecules were examined from a physicochemical point of view. Thermal behavior and morphological aspects of the CLG/PNIPAAm blends were considered in the solid state, in the form of thin films, cross-linked or not with gaseous glutaraldehyde. Differential scanning calorimetry (DSC), thermogravimetry (TG) and scanning electron microscopy (SEM) were used as effective methods of evaluating stability, miscibility, and compatibility among the components, respectively.

2. Experimental Section

2.1. Materials

Soluble collagen, CLG, type III from calf skin, was supplied by Sigma (St. Louis, MO, USA) and used as received.

PNIPAAm was synthesized through a free radical mechanism, under a nitrogenous atmosphere, according to the method described by Freitas[26]. The monomer (NIPAAm; Aldrich, Milwaukee, WI, USA) and the initiators (ammonium persulfate and sodium metabisulfite; Reagen, Rio de Janeiro, RJ, Brazil) were used at analytical grade without further purification.

Glutaraldehyde, GTA, 25% aqueous solution (Sigma, St. Louis, MO, USA), was used as received.

2.2. CLG/PNIPAAm films preparation

A 1% (w.v^{-1}) CLG solution was prepared in acetic acid 0.5 M at 0 °C with mild stirring. A PNIPAAm solution (1% w.v^{-1}) was prepared in water at room temperature with continuous stirring. Different amounts of the two solutions were mixed together, while stirring for 30 minutes at room temperature, in order to obtain various CLG/PNIPAAm weight ratios. Films were cast on Petri plates by water evaporation at room temperature and were then stored in a desiccator.

2.3. Films cross-linking

CLG/PNIPAAm films were cross-linked with gaseous glutaraldehyde. Films of the blends were fixed on the upper side of a desiccator which contained, in the lower part, 5 mL of an 8% GTA water solution. The container was placed in an oven at 37 °C for 18 hours in the dark.

2.4. Methods

The comparative studies of the thermal and morphological behaviors of the cross-linked and uncross-linked films were accomplished by differential scanning calorimetry, DSC, thermogravimetry (TG), and scanning electron microscopy, SEM.

2.4.1 Differential scanning calorimetry

DSC curves were obtained using a Perkin Elmer DSC 7 equipment. Samples at about 5 mg and nitrogen flow were used.

Dried samples. Aluminum pans were used with the following thermal cycles: from ambient to 160 °C, back to the ambient temperature and then to 300 °C; all at 10 °C/min. The first cycle was used to dry the samples. The results given are from the second heating cycle.

Non-dried samples. Samples were heated from ambient to 130 °C with a scan rate of 5 °C/min in sealed stainless-steel pans.

2.4.2. Thermogravimetry

TG was carried out under nitrogen flow (30 mL/min) using a Thermogravimetric Analyzer Perkin-Elmer TGA 6. Samples of about 4 mg were heated from 30 to 800 °C at a heating rate of 20 °C/min.

2.4.3. Scanning electron microscopy

SEM micrographs were carried out on a JEOL JSM-5600 (at 12 kV) using samples which were ripped at ambient temperature and sputter coated with gold.

3. Results and Discussion

The thermodynamic properties of collagen, PNIPAAm, and their blends were studied using differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) techniques.

As reported previously[27], the amorphous homopolymer PNIPAAm used in these studies was synthesized in a linear form and showed, by light scattering analysis, weight average molecular weights in the range of 10^5. Thermogravimetric results suggest that PNIPAAm is stable until 350 °C, losing 74% of its mass, in a single stage, from 350 to 450 °C. By DSC analysis, PNIPAAm presented a glass transition temperature of 135 °C and a degradation temperature of 433 °C [27].

3.1. Thermal analysis - DSC

The thermal and mechanical properties of CLG are strongly dependent on the water content in the starting material[11]. To avoid the influence of water, two different experimental conditions were used in the DSC studies: in the first, using aluminum pans, the samples were dried during the first heating cycle. In the second condition, stainless-steel pans were filled with the non-dried material and then sealed to suppress vaporization and water loss.

3.1.1. Uncross-linked films

Non-dried samples. Results of DSC analysis carried out on uncross-linked and non-dried samples are reported in Figure 1 and Table 1.

Pure CLG showed an endothermic peak at 104 °C related to its denaturation process. The denaturation temperature (Td) increased in the samples up to 50 wt. (%) of PNIPAAm and then remained almost constant. The enthalpy of denaturation (ΔHd), on the other hand, did not change with the presence of a synthetic polymer.

These results indicate that PNIPAAm exerts a stabilizing effect on the uncross-linked CLG, overcoming the effect of bulk water that normally lowers its denaturation temperature[28].

Dried samples. The dried samples showed a substantially similar trend (Figure 2). CLG is thermally stabilized by the presence of PNIPAAm, as verified by the shifting of the denaturation peak towards higher temperatures, with no appreciable changes on the enthalpy of denaturation. As expected, the absence of water led to an increase in the Td of the dried collagen compared to the non-dried one.

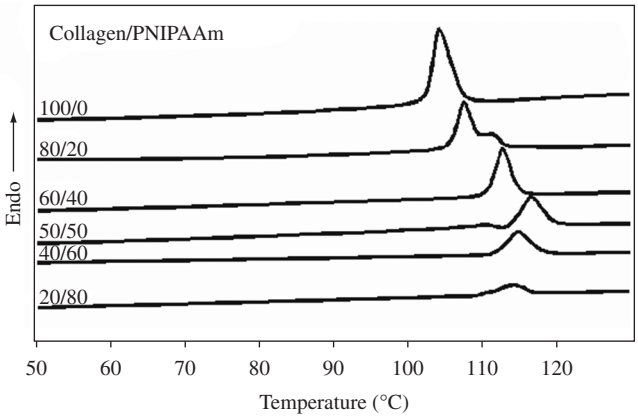


Figure 1. DSC curves of uncross-linked and non-dried samples. Stainless steel pans; heating rate of 5 °C/min.

Table 1. DSC results (temperature and enthalpy of CLG denaturation process) for non-dried CLG/PNIPAAm blends (uncross-linked and cross-linked samples).

CLG (%)	Uncross-linked			Cross-linked		
	Tg PNIPAAm (°C)	Td CLG (°C)	ΔHd CLG (J.g^{-1})	Tg PNIPAAm (°C)	Td CLG (°C)	ΔHd CLG (J.g^{-1})
100	-	104	35	-	112	37.5
80	122	108	33	135	118	34.8
60	111	113	33	138	111	38
50	123	117	33	140	114	36
40	127	115	33	140	109	34.4
20	130	114	34	140	114	27
0	137	-	-	137	-	-

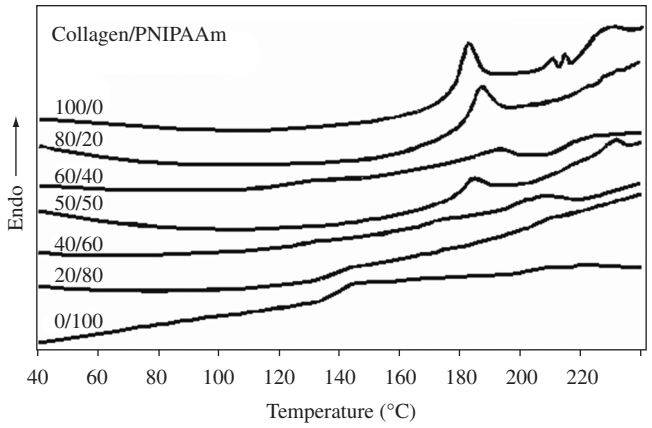


Figure 2. DSC curves of uncross-linked and dried samples. Aluminum pans; heating rate of 10 °C/min.

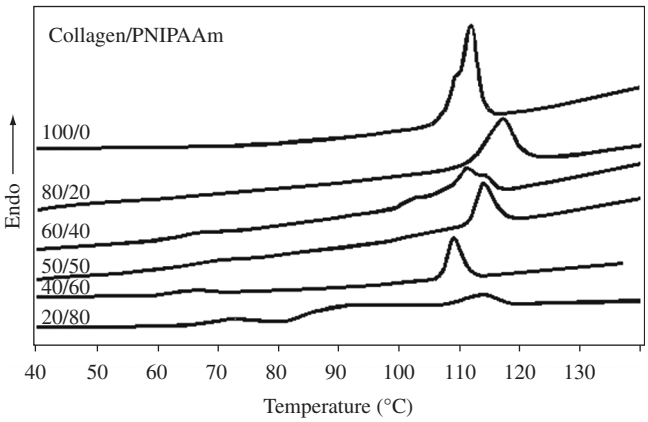


Figure 3. DSC curves of cross-linked and non-dried samples. Stainless steel pans; heating rate of 5 °C/min.

The glass transition temperature (Tg) of PNIPAAm, in contrast to the Td CLG behavior, shifted to lower temperatures with increases in the CLG content (from 137 to 122 °C for 0/100 and 80/20 CLG/PNIPAAm blends, respectively). Although it is difficult to observe the Tg at low concentrations of PNIPAAm, the decrease in the Tg values could be explained by the interactions of CLG and PNIPAAm. Collagen, which is a hydrogen donor, should form hydrogen bonds with the carbonyl group from PNIPAAm. The synthetic polymer contains a proton-accepting carbonyl moiety, while collagen presents hydroxyl and amino groups as side groups. Therefore, a hydrogen-bonding interaction may take place between these two chemical moieties in a blend of collagen and PNIPAAm. The formation of hydrogen bonds between the two different macromolecules competes with the interactions between molecules of PNIPAAm, resulting in a decreasing of Tg PNIPAAm values.

The calorimetric results obtained for uncross-linked CLG/PNIPAAm blends, either dried or not, indicate that there are strong interactions between the synthetic and biological polymers. These interactions, probably due to the hydrogen bonding, appear to exert a thermal stabilizing effect on collagen and promote the shift of Tg to lower temperatures.

3.1.2. Cross-linked films

The cross-linking of the CLG/PNIPAAm mixtures was carried out as in the case of pure CLG where it was verified that the best results have been obtained by exposing the CLG films to the GTA vapors for 18 hours[12].

Non-dried samples. In Figure 3 and Table 1 the typical DSC curves of non-dried and cross-linked samples and the values of temperature and enthalpy of denaturation, as a function of CLG concentration are presented, respectively. As reported in previous works[11,12], the cross-linking method affects the biological polymer, which can be verified by the enhancement of its denaturation temperature and the related enthalpy.

By comparing the values on Table 1, we can observe that the influence of PNIPAAm on CLG denaturation seems smaller in the case of cross-linked samples. The cross-linking prevents an efficient interaction between the two components.

Dried samples. The denaturation of CLG is prevented by the stabilization due to the cross-linking promoted by GTA. This phenomenon can be verified comparing Figures 2 and 4. The Td of pure CLG increases from 183 °C in the absence of cross-linking to 206 °C with GTA cross-linking. The same behavior is observed at all concentrations. As observed for the non-dried samples, the thermal stabilizing effect on CLG, related to the cross-linking, overcoming that one due to the interactions with PNIPAAm. Although it is difficult to observe the Tg in the films at low concentrations of PNIPAAm in Figure 4, it is possible to observe insignificant variations on the Tg of PNIPAAm in relation to the uncross-linked blends (Figure 2). The cross-linking of CLG macromolecules most likely diminishes the number of possible interactions with PNIPAAm through hydrogen bonding as observed in uncross-linked blends.

3.2. Thermal analysis - TG

Figure 5 illustrates typical TG curves of uncross-linked films. The cross-linked samples show a substantially similar trend. The desiccation treatment did not completely remove the water from the films, as it appears from the presence in all samples of the band at about 70 °C associated with the evolution of freely bound water. Pure

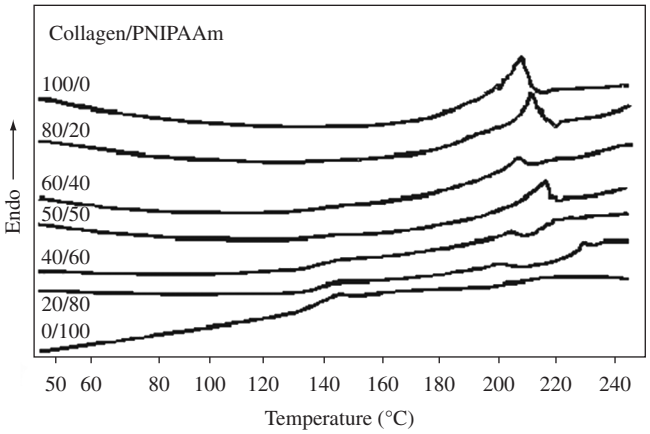


Figure 4. DSC curves of cross-linked and dried samples. Aluminum pans; heating rate of 10 °C/min.

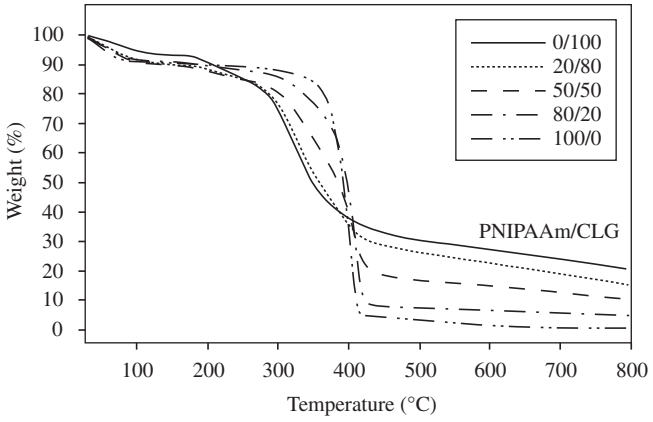
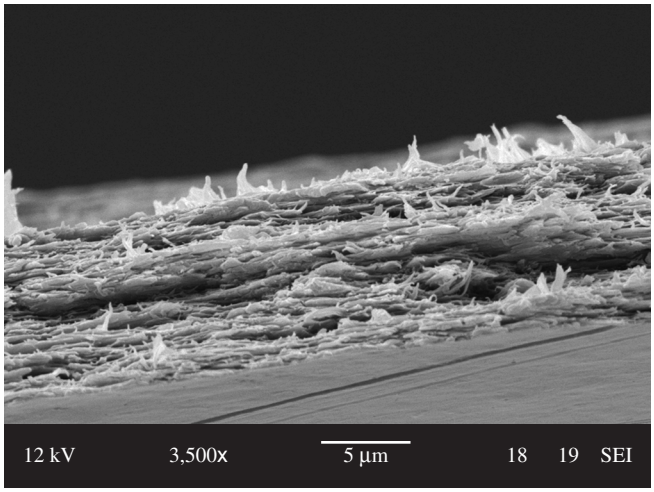
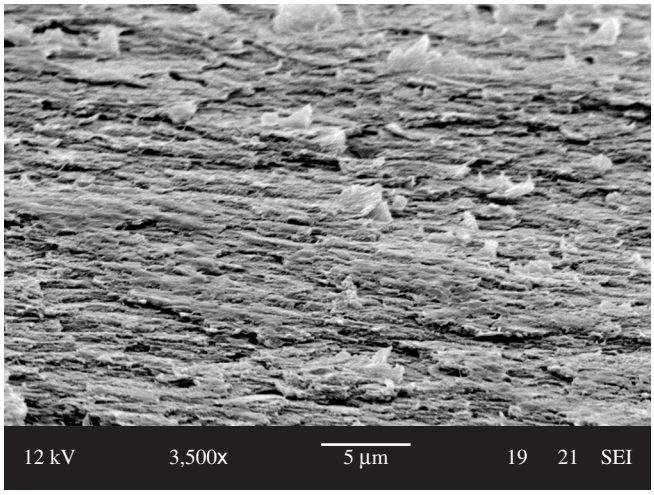


Figure 5. TG curves of uncross-linked films.



(a)



(b)

Figure 6. SEM micrographies of fractured section of uncross-linked samples: a) pure CLG; and b) CLG/PNIPAAm 40/60.

collagen is characterized by an additional two losses of weight. The first one, with maximum at about 200 °C, is related to the loss of structural water (strongly bound to the collagen); the second event with a maximum at 320 °C is related to decomposition of collagen molecule. The TG curve of PNIPAAm, beyond the transition due to the loss of freely bound water, shows another thermal event in the temperature around 400 °C, which is attributed to the loss of weight by the degradation of the synthetic polymer.

The curves of the blends show all the phenomena present in the pure components. We can observe that CLG is thermally more stable in the blends than the pure CLG, since the degradation temperature is shifted to higher temperatures. These temperatures are dependent on the blends concentration and we can explain this behavior by the thermal stabilizing effect due to interactions among the polymers primarily through hydrogen bonds. For the cross-linked blends, the thermal stabilizing effect on CLG is due to the cross-linking, as observed by the DSC results (Figures 3 and 4).

3.3. Morphological studies

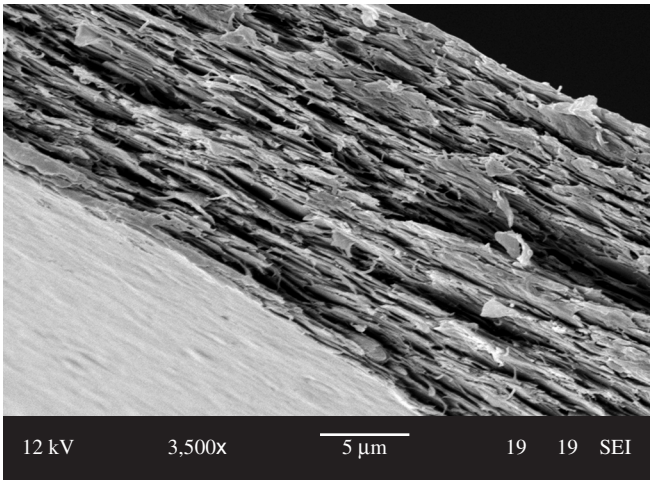
The electron microscope images of uncross-linked mixtures show that the appearance of the fractured sections remains quite similar to that of pure collagen, as can be observed from Figure 6. The longitudinal arrangement, due to the fibrous structure of CLG, is evident in all samples including those in high PNIPAAm concentrations (Figure 6b).

In the whole range of the composition studied, it was impossible to observe the presence of different phases, suggesting that strong interactions between the constituents are present, in agreement with the DSC data.

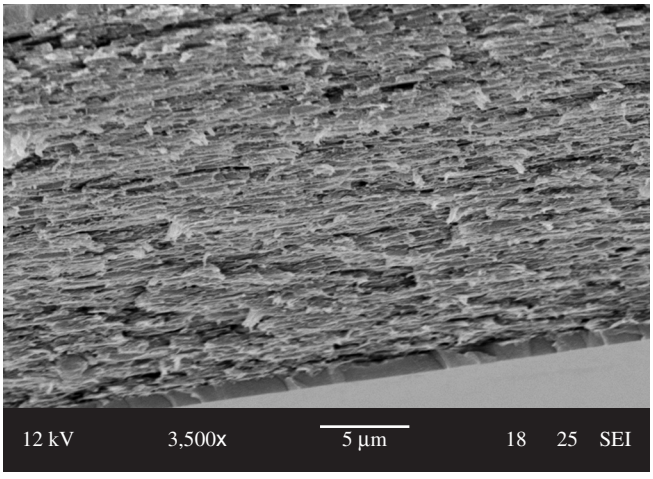
In comparison with the uncross-linked samples, films cross-linked with GTA seem to result in a more compact aggregation, which does not considerably change the original structure (Figure 7). It is still possible to observe the typical longitudinal disposition of the untreated collagen, although this kind of morphology is less evident after cross-linking. In all concentrations studied, SEM did not distinguish the presence of separated phases, using the indicated magnification grade.

4. Conclusions

The experimental results obtained in this work on mixtures of CLG/PNIPAAm, either cross-linked or uncross-linked with glutaraldehyde, indicate that the components are thermodynamically incompatible. However, there is good evidence that effective interactions, probably due to hydrogen bond formation, take place between the constituents. These interactions are more evident on the samples that



(a)



(b)

Figure 7. SEM micrographies of fractured section of cross-linked samples: a) pure CLG; and b) CLG/PNIPAAm 40/60.

were not cross-linked. In this respect, the most remarkable results are the PNIPAAm stabilizing effect on the denaturation temperature of CLG and the shift to higher temperatures of the PNIPAAm and CLG thermal degradation temperatures. The cross-linking with glutaraldehyde also promotes a thermal stabilizing effect on the biological polymer. The CLG denaturation temperature is enhanced after exposing the films to glutaraldehyde vapors, as a result of the cross-linking. On the other hand, cross-linking prevents the effective interactions among CLG and PNIPAAm, which were observed on the uncross-linked samples. In all concentrations studied, on both cross-linked or uncross-linked samples, SEM micrographies showed that the morphology of the blends remains quite similar to that of pure collagen, indicating the longitudinal arrangement due to the fibrous structure of collagen.

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