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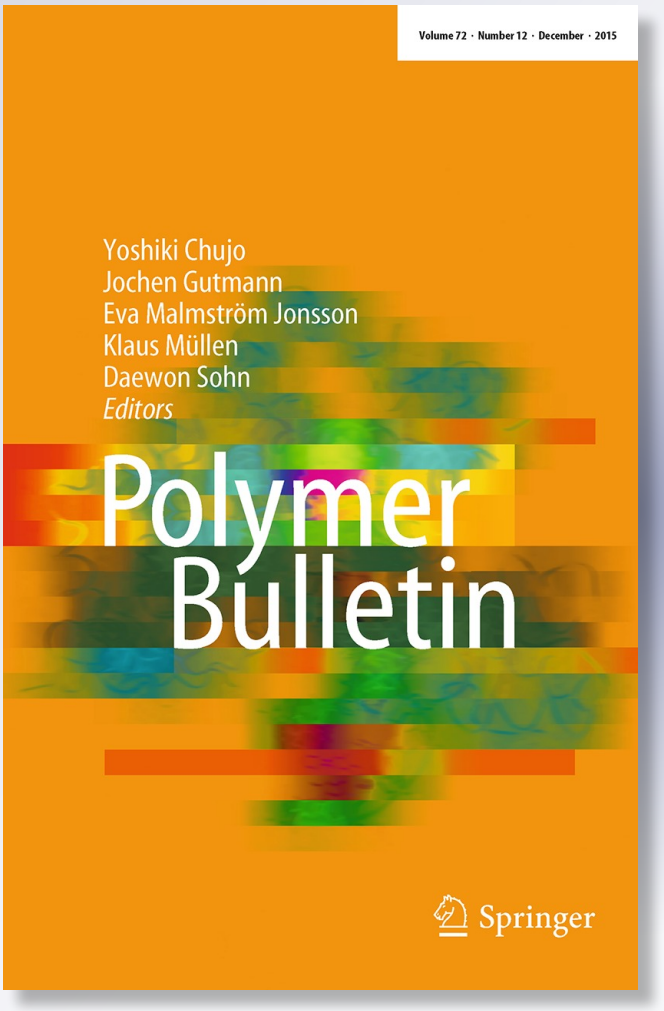
Polymer Bulletin

ISSN 0170-0839
Volume 72
Number 12

Polym. Bull. (2015) 72:3043-3062
DOI 10.1007/s00289-015-1452-y



Springer

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ORIGINAL PAPER

Gelatin films dendronized selectively on one side: enhancing antimicrobial properties and water repellence

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Received: 27 March 2015 / Revised: 29 May 2015 / Accepted: 2 July 2015 /
Published online: 16 July 2015

Abstract To develop a material with potential biomedical applications, novel gelatin films were prepared by cold-casting method using cerium(III) and genipin solution as cross-linking agents, and surface modified with dendritic molecules. The structure and properties of the synthesized gelatin films were investigated by ATR-FTIR, mechanical tests, swelling behavior and water vapor permeability (WVP). The results showed that cross-linking could improve the mechanical and microbiological properties and lower the hydrophilic property of gelatin films. According to ATR-FTIR analysis, it can be concluded that the dendronization took place on only one of the faces of the films. The results have shown that the experimental methodology performed allows one-surface modification, so a novel biomaterial was obtained in the form of a film with good properties and dendritic structure in one face (hydrophobic and hydrophilic faces), rendering a multivalent structure useful in biomedicine development.

Keywords Biomaterials · Gelatin films · Dendronization

Introduction

Biopolymers that include polysaccharides and animal protein-based biopolymers, such as gelatin, are some well-known examples of biorenewable resource-based environmental-friendly polymeric materials [1–3]. Rising environmental concerns

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have resulted in an increased interest in the use of waste materials such as protein from seafood industry. Around 30 % of squid waste and by-products are rich in collagen [4]. Collagen is a particularly important component that contributes significantly to meet toughness properties [5]. The extraction of collagen and its derivatives from marine sources has considerably increased in the last years, since it proves an appropriate alternative source to pig and bovine skin and bones, with promising functional properties [6]. Moreover, gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen. The widespread interest in gelatin is mainly attributed to its biodegradability. Food, pharmaceutical and photographic industries are the main users of gelatin and the other natural polymers [7], which has several other technical applications [4, 8]. The pharmaceutical applications of gelatin are based mainly on gel/film-forming properties.

Recently, an increasing number of new applications have been found for gelatin in products, such as emulsifiers, foaming agents, colloid stabilizers, hydrogels, packaging materials, wound dressing and micro-encapsulating agents [9–11]. Gelatin exhibits biocompatible, hydrophilic and biodegradable properties. Probably, gelatin readily dissolves in water and leads to thermal and mechanical instability, confining its possible applications as a biomaterial and this requires further development to achieve the targeted results and desired range of efficiency [12–16]. Previous studies showed that pure gelatin films are brittle, highly hydrophilic and easily attacked by bacteria [17, 18]. Noticeable improvement in the mechanical properties of gelatin films in the direction of deformation can be seen by inducing segmental orientation in gelatin films [19–22]. Chemical and physical cross-linking indicated improved, as has been previously reported, stability [22].

Particularly, genipin is a naturally occurring cross-linking agent, which seems to display promising characteristics [23]. Genipin-fixed tissue shows resistance against enzymatic degradation [24, 25]; in addition, genipin is not cytotoxic [26, 27]. Furthermore, it has been found that gelatin-derived bioadhesives display higher biocompatibility and less cytotoxicity when cross-linked with genipin rather than with other agents, such as formaldehyde and epoxy compounds [22].

Moreover, the antimicrobial properties of gelatin may be improved by the incorporation of cerium(III), an effective antibacterial agent against the whole spectrum of bacteria, such as *Staphylococcus aureus* and *Escherichia coli* [18]. However, there are few reports on gelatin/cerium(III) film. The amino acids in gelatin have active groups which can interact with cerium(III) [28]. Consequently, cerium nitrate is considered to have potential for the cross-linking of gelatin film by electrostatic forces between cerium(III) and the polar ionic groups in gelatin chains, which is helpful in improving thermal and mechanical properties as well as lowering hydrophilic property. At the same time, the film might have antibacterial activity.

Another interesting aspect of the modification process is the dendronization of natural and synthetic polymers using dendrons [29–33]. Although the dendritic fragments of these materials usually constitute only a minor part of the total volume, these components can significantly alter support properties, supplying entirely new features and functions to the hybrid material [34]. Superficially, dendronized polymers can display a higher loading capacity and allow the bulk incorporation of bioactive molecules of higher molecular weights and of different chemical structure,

while maintaining high internalization and transfection efficiency as compared to conventional dendrimers. The modification of natural polymers with dendritic structures is a novel and interesting path to synthesize highly functionalized and unconventional products that may be of interest in various applications, for example, as drug delivery systems, catalysts, disinfectants, and as components in cosmetics. A further property of this class of polymers is that the combination of specific dendrons with linear chains affords an opportunity to design a well-defined amphiphilic dendronized polymer system. Novel chitosan films dendronized dendron on one side were prepared by us [31]. This work highlights the superficial modification of chitosan films to yield different hydrophilic and hydrophobic properties on both sides of the system. The films obtained can be potentially applied as wound dressings are able to maintain a moist environment at the wound interface and act as a barrier to microorganisms, removing excess exudates. Stancu [35] reported the synthesis and characterization of dendronized gelatin hydrogel with amino-terminated PAMAM dendrimers. The dendritic molecules were chemically immobilized onto the surface of glutaraldehyde cross-linked thin gelatin scaffolds; the material showing biomedical and mostly hard tissue engineering application.

In this context, the chemical approach of the present work centered on developing dendronized nanostructured surfaces on cross-linked gelatin. Gelatin contains a high number of amine-ending side chains available for chemical reactions. However, the availability of amino groups on the surface of polymer scaffolds proves limited to be further functionalized with other biomolecules of interest.

In this paper, we develop a dendronized gelatin film with improved chemical, mechanical and microbiological properties on one side. The dendronization was carried out with a biocompatible hyperbranched molecule [36]; a cross-linking step was required to reinforce the chemical stability of the biosorbents in solution and their microbiological properties. Specifically, we aimed at increasing the efficiency of gelatin as biomaterial and to render an insoluble water material. This approach only addresses the synthesis, characterization and microbiological behavior against Gram (−) and Gram (+) of surface-dendronized gelatin scaffolds; the further applications are going to be developed in a future work.

Experimental section

Materials

The following chemicals were purchased and used: Type B gelatin (Aldrich); di-t-butyl-4-[2-(t-butoxycarbonyl)ethyl]-4-isocyanato-1,7-heptanedicarboxylate (Weiso-cyanate dendron, Frontier Scientific); genipin (Wako); dibutyltindilaurate (Aldrich); dimethylacetamide (DMA, Sintorgan); acetic acid glacial (Cicarelli); Acetic/acetate buffer (pH 3.5); phosphate buffer (pH 6.8); potassium bromide 99 % FTIR grade (Aldrich); dry calcium chloride; cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H2O$, Fluka); miliQ water; nitrogen; chloroform. Brain–heart infusion agar (BHI, Britania); *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC

25923 were used from the collection of microbiological cultures from the Center of Applied Chemistry (CEQUIMAP-UNC). Solvents were obtained from Sintorgan, purified by distillation, and dried.

Instruments and techniques

Fourier transform infrared spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer. Attenuated total reflectance Fourier transform infrared (ATR/FTIR) interferograms were acquired using the same spectrometer equipped with an Attenuated Total Reflectance accessory. A 45° ZnSe crystal was used to monitor samples. All spectra were obtained with 32 scans at a 4.0 cm^{-1} resolution in a range between 4000 and 650 cm^{-1}. Atomic force microscopy (AFM) was recorded using Innova (Bruker) equipment (Metrology Center, CEMETRO, Córdoba, Argentina). Mechanical properties were measured on Instron Universal Testing Machine 3342.

Mass variations in swelling behavior studies of the films were measured on a Mettler Toledo Newclassic MF balance (Model MS204S). The equilibrium swelling (E_{sw}) of the films was calculated at different pH. Each swelling experiment was performed twice and the average was taken as the E_{sw} value.

Methods

Preparation of films

Type B gelatin (0.2500 g) was dissolved in distilled water (about 3–4 mL) and homogenized at 40 °C for 2 h. First, 0.5 mL of genipin solution (0.5 % w/v) was added. After that, 0.0125 g $Ce(NO_3)_3.6H_2O$ was added according final product and was complete with water until 5 mL (5 % w/v). The ratios of genipin/gelatin and cerium(III)/gelatin studied were 1 and 5 % w/w, respectively. After stirring at 40 °C for 8 h, the mixture (5 mL) was cast on a polypropylene plate (5 cm in diameter), and gradually dried in air at room temperature. Thus, the cross-linked gelatin films were obtained and called GB-Ce, GB-gen and GB-Ce-gen. The GB film was obtained as control.

The films (about 100 μm thick) were carefully removed from the Petri dishes, and analyzed with different methodologies.

Dendronization of films

After reaction, gelatin films were dried under vacuum, washed and swelled in DMA. Dendronized equipment was designed to modify one selected face of gelatin films [31]. 25 mL of nitrogen flask equipped with a magnetic stirring bar was charged with a GB-Ce-gen film (0.1000 g) in 10 mL of DMA. Weisocyanate (0.150 g, 0.15 mmol) and dibutyltin dilaurate (0.02 mL) were added and stirred at 60 °C for 72 h. The film was washed with $CHCl_3$ to remove unreacted dendron, and the dendronized gelatin film was carefully removed from the flask and dried under vacuum. The film was characterized and the properties and morphology of dendronized network were analyzed.

Swelling studies

The water sorption capacity of the gelatin films was determined by their swelling in buffers of pH 3.5 and 6.8 at room temperature. A known weight (0.200 g) for each film was placed in the medium for 7 h, the appropriate dosage time. The swollen films were collected at different times, after having been superficially dried with tissue paper and weighed immediately on an analytical balance. The equilibrium swelling weight (E_{sw}) of the gelatin films in the medium was calculated using Eq. (1):

$$E_{sw}\,(\%) = \frac{W_s - W_o}{W_o} \times 100 \tag{1}$$

where W_S denotes the weight of the films at equilibrium swelling, and W_o is the initial weight of the films. Each swelling experiment conducted twice, and the mean value was informed as E_{sw}.

Water vapor permeability

Water vapor permeability (WVP) was determined in duplicate for all films according to the desiccant method described in the ASTM standard method (ASTM E96M-10). Each film of 3.14 cm^2 (without physical defects such as cracks, bubbles or pinholes) was sealed onto an aluminum permeation cup (2.0 cm in diameter and 2.5 cm in depth) containing dry $CaCl_2$ (0 % RH) with silicone vacuum grease and a ring to hold the film in place. The side in contact with the casting plate surface was exposed inside the test cups. Once the films were held, test cells were placed in a humidity chamber. The permeability cups with the films were weighted at intervals of 1 h until reaching 7 points. Linear regression was used to calculate the slope of a fitted straight line in a graph of variation of mass versus time. Water vapor transmission rate (WVTR, g m^2 day^{-1}) and WVP (g m^{-1} s^{-1} Pa^{-1}) were calculated from Eqs. (2) and (3), respectively:

$$\mathrm{WVTR} = \frac{F}{A} \tag{2}$$

$$\mathrm{WVP} = \frac{(\mathrm{WVTR} \times e)}{\left[S_p \times (\mathrm{RH}_1 - \mathrm{RH}_2)\right]} \tag{3}$$

where F is the slope of the graph of variation of mass versus time (kg s^{-1}), A is the test area (cup mouth area), e is the film thickness (m), S_p is the saturation pressure (Pa) at the test temperature, RH_1 is the relative humidity in the humidity chamber, and RH_2 is the relative humidity inside the cell test.

Mechanical properties

Tensile strength, elongation at break and Young's modulus were determined by performing mechanical measurements on films after drying, according to ASTM D882-02.

Atomic force microscopy

Atomic force microscopy (AFM) measures were recorded operating in tapping mode with commercial silicon nitride (Si_3N_4) tips of diameter 8 mm. A scan rate of 6 μm s^{-1} was used for scan size of 30–50 μm with 256 sample lines. Images were processed using NanoScope Analysis software. The root mean square (RMS) roughness values were obtained from AFM software simultaneously and the informed RMS was a mean of three different images from each sample. Three-dimensional images, height histograms and RMS values were used to analyze the surface morphology of GB-Ce-gen and GB-Ce-gen-W films.

Microbiological studies

The antimicrobial activity of the gelatin films, dendronized (GB-Ce-gen-W) and undendronized (GB-Ce-gen), was evaluated against a Gram-negative bacterium and a Gram-positive one. The strains *E. coli* and *S. aureus* were used, respectively. From stock cultures of both bacteria, stored at −80°C in broth brain heart with 20 % glycerol, working cultures were obtained from the corresponding culture stock to tubes with brain heart agar and incubating at 36 ± 1 °C for 36 h, to obtain fresh cultures during the exponential growth phase of both microorganisms.

Working inocula of both bacteria were prepared by their suspension in dilution buffer phosphate (pH 6.8–7.2), under aseptic conditions. From the mother suspension, a tenfold dilution was prepared to evaluate the antimicrobial activity of films against inocula with a lower microbial load to 600 colony-forming units per milliliters (CFU/mL). 5 cm^2 sections of the films were immersed in tubes containing 10 mL of microorganism suspensions; changes in microbial loads were evaluated every 2 h from the initial contact time (0) and for a period of 6 h (0, 2, 4 and 6). The bacterial population at each control point was determined in duplicate, using the pour plate technique in brain–heart infusion agar. Counts were performed after 48 h of incubation at 36 ± 1 °C.

To determine whether the microorganism suspension in phosphate buffer dilution was stable during the test period, the bacteria load of the same microorganism suspension used in each case without contact with the films was tested at equivalent time intervals compared to suspensions in contact with gelatin films, as a suspension control.

Statistical analysis

The data for each test were statistically analyzed. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between means. The Tukey's test was used for comparing mean values. Differences between means were considered significant when $p \leq 0.05$.

Results and discussion

Preparation of cross-linked gelatin films

Gelatin is a protein derived from the chemical degradation of collagen, with amino acid composition, molecular weight distribution and three-dimensional structures, depending on the method of production. Particularly, amino acid composition determined the isoelectric point (pI) of gelatin. Thus, two types of gelatin were identified: type A gelatin (GA) with a pI ranging between 8 and 9 and type B gelatin (GB) with a pI ranging between 4 and 5. Type B gelatin has more amino acid groups available to form bonds with different molecules [37, 38]. There are different ways of obtaining gelatin films, but it has been found that, in the solid state, the properties of gelatin depend on thermal history [39]. The conformational state of dehydrated gelatin films obtained by casting from gelatin aqueous solution differs when the solvent is evaporated at room, or lower (cold-cast film) temperatures, or at temperatures above 35 °C (hot-cast film). At low temperatures, a helical structure is formed, while at temperatures above 35 °C a loss of triple helix occurred during heating via breaking down hydrogen bonds between α-chains [40–45]. The films were then obtained by cold-casting methodology, for not being brittle and for not reaching the helix–coil transition temperature. Different cross-linked Type B gelatin films with cerium nitrate and/or genipin were yielded, as shown in Fig. 1. The effect of each cross-linking agent was evaluated and GB-gen and GB-Ce films were obtained. Both cross-linkers were then used together to yield GB-Ce-gen films.

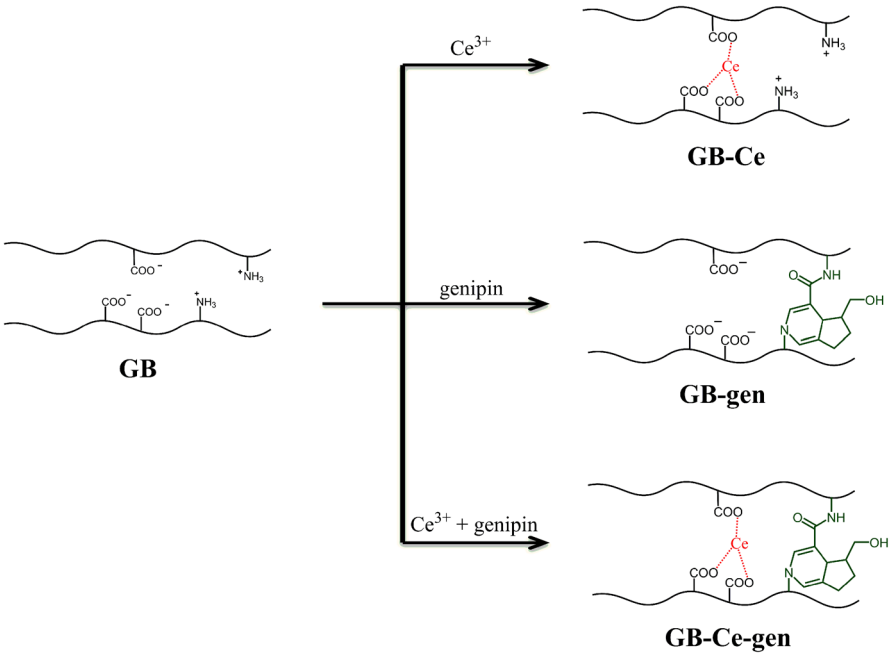

Fig. 1 Synthetic route of gelatin films

GB-Ce films

The electrostatic cross-linking between cerium(III) and gelatin could enhance the interaction between gelatin molecules and the stability of the film. Therefore, mechanical and thermal properties could be improved, in addition to microbiological properties. Figure 2 shows the ATR/FTIR of gelatin and GB-Ce films.

Gelatin is a polyamide, thus the infrared spectra of gelatin film in Fig. 2 exhibited pronounced amide and hydrogen bonding characteristic absorption bands at 1666 cm^{-1} (amide I), 1552 cm^{-1} (amide II), 1236 cm^{-1} (amide III), and 3300–3400 cm^{-1} (hydrogen bonding), respectively [46]. Amide I band corresponding to C=O stretching vibration coupled to contributions from C–N stretch, C–N deformation, and in-plane N–H bending modes. Amide II vibration was attributed to an out-of-phase combination of C–N stretch and in-plane N–H deformation modes of the peptide group. This band is generally considered to be sensitive to hydration of gelatin. The wide band between 3300 and 3400 cm^{-1} corresponding to N–H vibration of amine group overlapped with O–H stretching vibration of hydroxyl groups of water which form hydrogen bond [47–49].

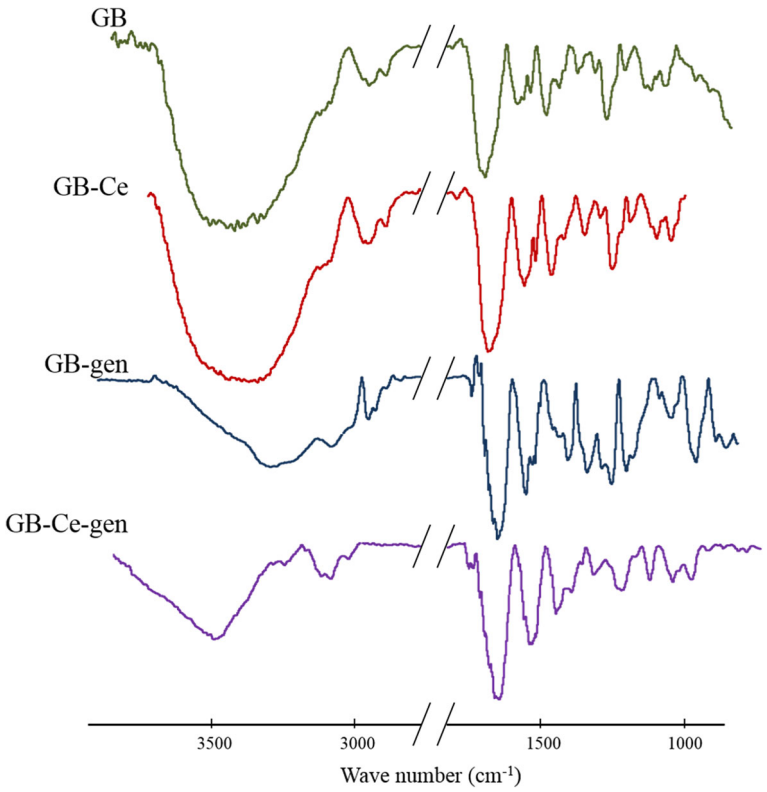

Fig. 2 ATR/FTIR spectra of gelatin and cross-linked gelatin films

After the reaction between gelatin and cerium(III), the change in signal of amide I was slight, indicating that there were no major changes in the secondary structure of gelatin. However, the spectra of GB-Ce show the band corresponding to the hydrogen bond (3357 cm^{-1}); amide band II (1546 cm^{-1}) is shown to shift to a wave number lower than that of the gelatin; and the band of hydrogen bond is found to be narrower. This shift pattern has been described by other authors in the literature [18]. They all suggest that the addition of cerium(III) decreases the formation of hydrogen bonds between water and gelatin molecules, which could reduce the hydrophilic character of gelatin. Such changes are possibly caused by the electrostatic interactions of cerium(III) with the polar groups present in gelatin, which partially interrupted the formation of intermolecular hydrogen bonding between gelatin and water molecules. The presence of cerium(III) enhances the degree of connection between gelatin chains, and reduces the amount of polar ionic groups reacting with water molecules. As a result, the hydrophilic behavior of the film might be altered.

GB-gen films

According to previous results reported by us [31] and other authors [22, 50–53], the mechanical properties of natural polymer could be improved by cross-linking with genipin. The films were then formed using genipin as covalent cross-linker. It reacts with gelatin chain as shown in Fig. 3 [54, 55]. All matrices showed an intense blue color, characteristic of cross-linked networks with genipin [56].

Figure 2 compares the spectra of GB and GB-gen films. After reaction with genipin to yield GB-gen, the signal characteristic of genipin (1681 cm^{-1}) disappears. The presence of a signal at 1647 cm^{-1} was attributed to the vibration of the C=O amide formed [54, 57]. By means of these results, covalent cross-linking between gelatin and genipin was evidenced.

To evaluate the mechanical performance of films synthesized, tensile strength, elongation at break and Young's modulus were calculated. Young's modulus shows the behavior of the solid component of the film. Table 1 displays the results found.

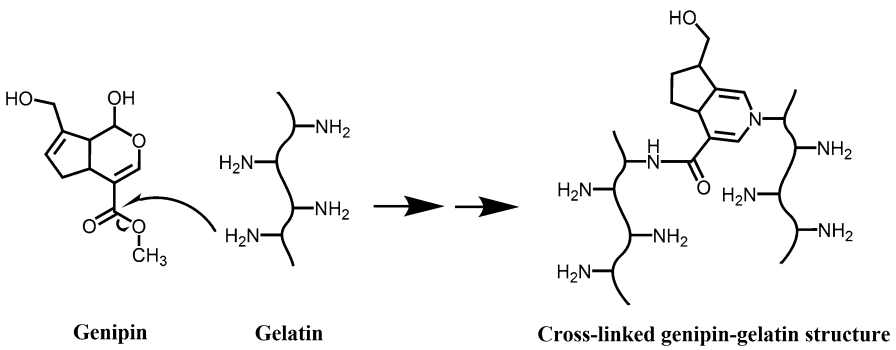

Fig. 3 Cross-linking reaction between gelatin and genipin

Table 1 Mechanical performance of films

Film	Tensile strength (MPa)	Elongation at break (%)	Young's modulus ($\times 10^3$ MPa)
GB	44 ± 6	1.7 ± 0.3	3.23 ± 0.03
GB-gen	60 ± 7	1.9 ± 0.4	3.83 ± 0.06

Mean ± SD ($n° = 3$)

As a result of cross-linking, GB-gen films showed an increase in Young's modulus and the tensile strength compared with gelatin films. These results show that the cross-linked gelatin films synthesized are materials more resistant than gelatin films, in agreement with bibliographic reports [22, 58].

GB-Ce-gen films

Since genipin is a covalent cross-linking and cerium(III) has antimicrobial properties, in addition to being an ionic cross-linking and to enhancing material performance, cross-linked films with genipin and cerium were prepared, as shown in Fig. 1. Following the descriptions of Fig. 2 above, the spectrum corresponding to GB-Ce-gen shows the shifts of the bands corresponding to the gelatin by cross-linking with cerium(III). In addition, the signal at 1647 cm^{-1} is observed, corresponding to vibration of C=O amide group formed between gelatin and genipin, coupled to amide III signal of gelatin.

Dendronization of GB-Ce-gen films

The multivalency provided by each dendron unit improves binding over comparable monovalent systems. The preparation of gelatin modified with dendrons on one face could provide a different hydrophilic/hydrophobic character. Hence, the experiment was carefully designed to modify one side of the cross-linked film, leaving the virgin gelatin on the opposite side. This can prove a proper balance in the film permeability to moisture and film functionality necessary for biomaterials, such as wound dressing, artificial skin, bone grafts and pharmaceutical applications [59].

A heterogeneous approach for the covalent binding of dendritic compounds affords a valuable opportunity to develop a high surface functionalization with regard to biofunctionalization.

The dendronized cross-linked gelatin films were successfully prepared by covalent union with weisocyanate dendron to yield GB-Ce-gen-W films (Fig. 4).

ATR/FTIR analysis of the sample shows that the expected product was obtained, as seen in Fig. 5. It is important to consider that the beam penetration is 2 μm and the thickness of the films is 100 μm, which states that dendronization has reached on only one surface of the film.

On this face, the characteristic peaks of the dendron were observed, as the *tert*-butyl methyl group at 849 and 718, 2946 cm^{-1} could be assigned to CH_3 stretching and the band at 1247 cm^{-1} corresponding to C–O–C of the ester group. The band at 1700 cm^{-1} can be assigned to the C=O stretching of the dendron or urethane or urea

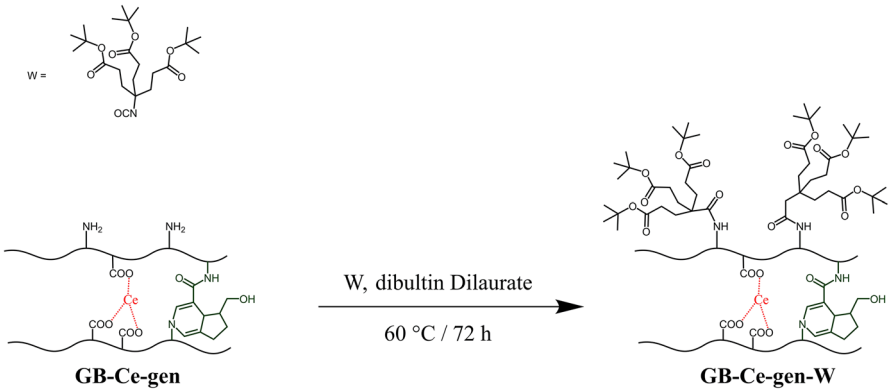

Fig. 4 Scheme of synthetic route of dendronized cross-linked gelatin films

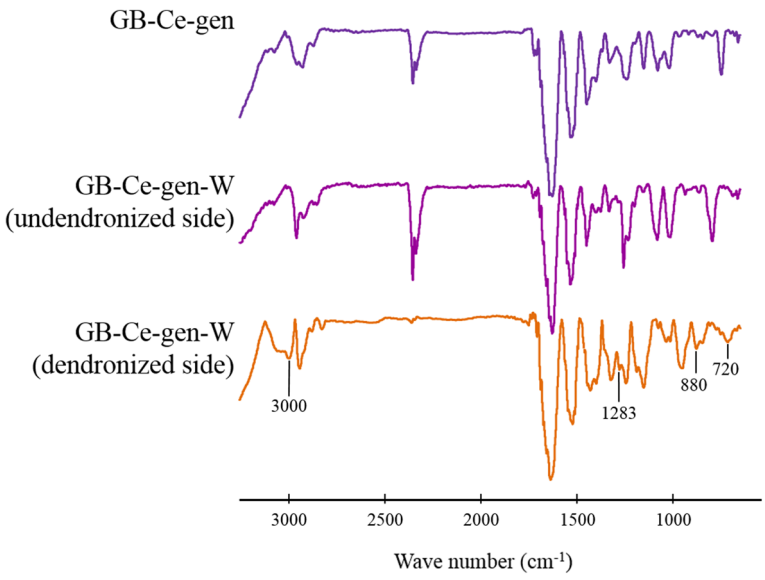

Fig. 5 ATR/FTIR spectra of modified film

bond formation, due to overlapping of vibrations. The characteristic bands of GB-Ce-gen showed no changes in the profiles of the spectra of the other side of the films.

AFM was used to characterize the surface topographic feature of cross-linking gelatin (GB-Ce-gen) and dendronized gelatin films (GB-Ce-gen-W). Undendronized gelatin presents quite homogeneous surfaces (Fig. 6a), while the dendronized face of GB-Ce-gen-W (Fig. 6b) reveals a predominantly hill–valley structure surface. The RMS of surface roughness, measured on scanning areas of 10×10 μm indicated values of $(1.9 \pm 0.5)^a$ and $(15 \pm 4)^b$ nm for GB-Ce-gen and GB-Ce-gen-

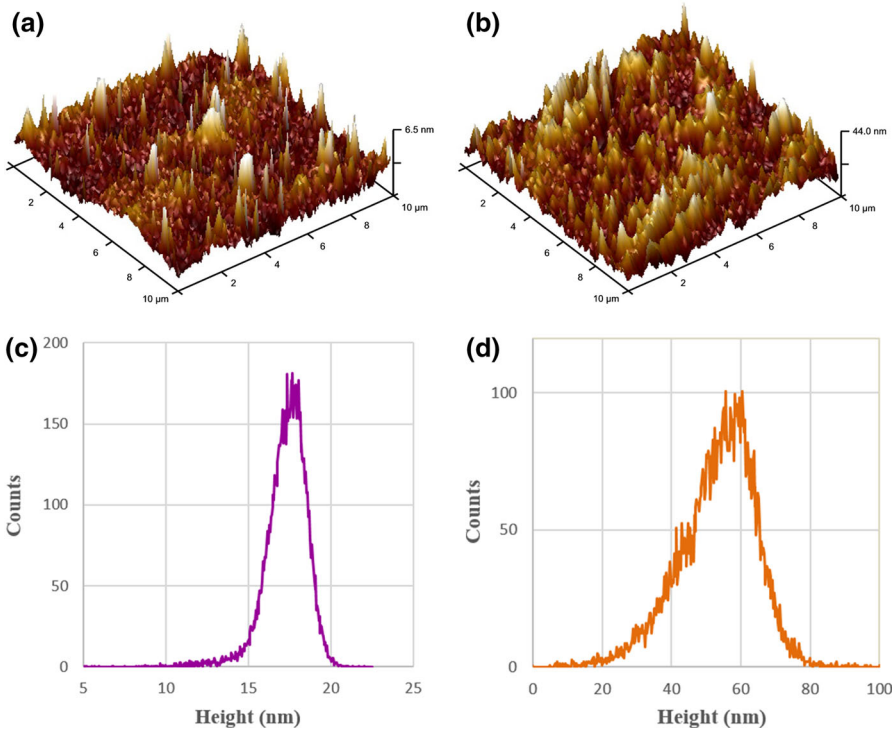

Fig. 6 AFM topographic image surface: three-dimensional images (3D) of **a** GB-Ce-gen and **b** GB-Ce-gen-W dendronized face. Height histograms of **c** GB-Ce-gen and **d** GB-Ce-gen-W dendronized face

W (dendronized face), respectively, where small letter indicates significative differences between RMS of each films.

The major population of heights is situated around 18 and 60 nm for undendronized gelatin and dendronized face gelatin films, respectively (Fig. 6c, d). Because of the dendronization of surface gelatin, the film was more roughened than unmodified cross-linking gelatin.

Swelling index

Gelatin is soluble in aqueous solution, and a few minutes of storage in physiological solution was sufficient to induce considerable film swelling (between 500 and 1000 % after 1 h) [22]. E_{sw} values were determined according to Eq. (1). Figure 7 shows that the degree of swelling markedly reduces with cross-linking and that swelling values depend on cross-linked agents. Considering the potential applications of the films as biomaterial and the isoelectric point of gelatin, their equilibrium swelling (E_{sw}) was studied at different pH: 3.5 and 6.8.

The swelling of the films was measured until reaching equilibrium and they were left in contact with each of the buffers for a week. After that period, the films were not degraded unlike gelatin (GB) and cross-linked gelatin with cerium(III) (GB-Ce).

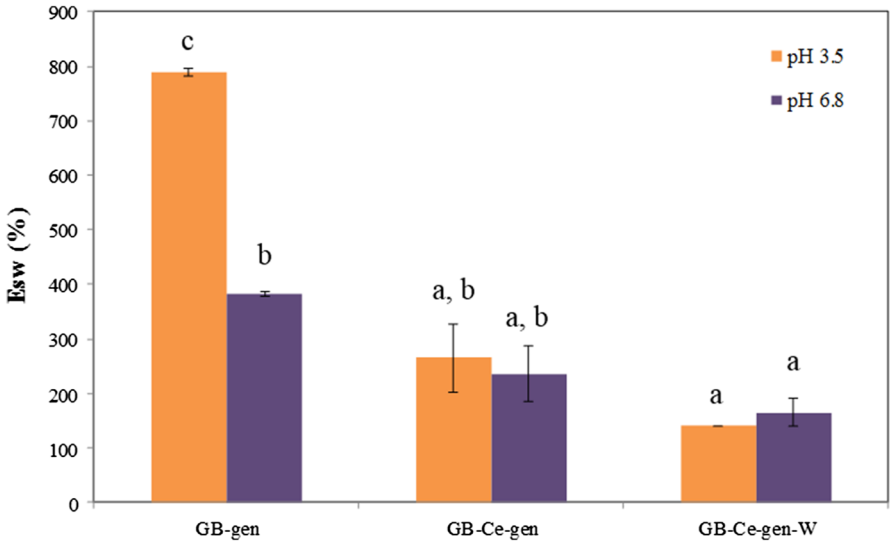

Fig. 7 Swelling index of cross-linked gelatin and dendronized cross-linked gelatin films at pH 3.5 pH 6.8; *small letters* represent a comparison between films by swelling values at different pH

This is important, considering their potential use of films as biomedical material [31].

GB-Ce-gen films showed a swelling lower than of the GB-gen. In addition to the formation of covalently cross-linked network between genipin and the amino groups of gelatin, carboxylate groups of gelatin are coordinated with cerium(III) and the ion is behaving as a cross-linking agent too [28]. This result agrees with the changes found in the FTIR spectra of cross-linked films with cerium(III). The addition of Ce^{3+} ions caused a decrease in the formation of hydrogen bonds between water and gelatin molecules, which could reduce the hydrophilic character of gelatin. Thus, a decrease in the swelling index is observed when increasing the degree of cross-linking of the network.

The swelling index of GB-gen also depends on pH values. This behavior may be explained by the isoelectric point values and the amino acid composition of gelatin. At pH values (pH 3.5) below the pI, the functional groups of gelatin are protonated (NH^{3+} and –COOH), while, at pH (pH 6.8) above the pI of type B gelatin, their functional groups are deprotonated (NH_2 and COO^-). The electrostatic repulsion between free ammonium or carboxylate groups governs the swelling value of GB-gen films. The behavior of GB-Ce-gen and GB-Ce-gen-W is similar to both pH, since most of the functional groups of gelatin are involved in chemical bonds or cross-linking.

Furthermore, the decrease of the hydrophilic character of the modified gelatin can be observed in dendronized films. The swelling indexes of these films are the lowest.

Water vapor permeability (WVP)

Generally, the main functional properties of different hydrophilic materials strongly depend on their water content and therefore on their surrounding humidity. Water vapor permeability results can be useful to understand possible mass transfer mechanisms and solute and polymer interactions in films. Permeability can be defined as the product of diffusivity and solubility only when Fick and Henry laws fully apply [60].

WVTR and WVP measures were performed and the results are shown in Table 2. For some films, water vapor strongly interacts with polymer structure, which results in diffusion and solubility coefficients dependent on driving force.

The dendronization process decreases WVP and WVTR (when the modified face is to air). It confirms that the non-polarity of the peripheral functional groups of the dendron gives a minor water absorption, a hydrophobic side and therefore, non-sticking properties. The WVP value for cross-linked films results from the formation of some densely cross-linked regions; it decreases the free volume in the films, thereby increasing the diffusion path of the water molecules. The presence of the dendron causes a slight decrease in the solubility and diffusion of water vapor through the films. The decrease in diffusivity with dendronization may be ascribed to the hindered motion of the polymer segments. The dendronized face also shows lower permeability than that of the unmodified face (Table 2), indicating that the hydrophobic nature of the dendrons decreases water absorption. This result reveals that the dendronized face could be potentially applied to the prevention of fluid accumulation by adsorption of the atmosphere.

The films obtained are considered semi-permeable materials which could be used in the treatment of different shallow wounds. This biomaterial could also be used to protect the skin from friction or continuous exposure to moisture, preventing breakdown which produce excessive amounts of exudates [31].

Microbiological studies

The values of antimicrobial activity are shown in Table 3. In *S. aureus* suspension control and exposed to dendronized and undendronized films, no significant differences were observed at different exposition times. However, considering the

Table 2 Water vapor transmission rate (WVTR) and water vapor permeability (WVP) values in films obtained

Film	WVTR ($\times 10^1$ g m^2 day^{-1})		WVP ($\times 10^{-12}$ g m^{-1} s^{-1} Pa^{-1})	
	Non-modified face to air	Modified face to air	Non-modified face to air	Modified face to air
GB-Ce	70.8 ± 1^{a}		2.22 ± 0.05^{a}	
GB-Ce-gen	$89.8 \pm 1^{b,c}$		2.9 ± 0.1^{c}	
GB-Ce-gen-W	92.6 ± 2^{c}	86.9 ± 1^{b}	$2.85 \pm 0.06^{b,c}$	2.67 ± 0.01^{b}

Mean ± SD ($n° = 2$)

Different lowercase in the same column indicate significant differences ($p < 0.05$)

Table 3 Antimicrobial behavior of dendronized and undendronized gelatin films against *S. aureus* and *E. coli*

Time (h)	*S. aureus*			*E.coli*		
	Log CFU/mL			Log CFU/mL		
	Control	GB-Ce-gen	GB-Ce-gen-W	Control	GB-Ce-gen	GB-Ce-gen-W
0	$2.5 \pm 0.1^{a,B}$	$2.3 \pm 0.1^{a,A,B}$	$2.33 \pm 0.01^{a,A}$	$2.65 \pm 0.04^{a,A}$	$2.4 \pm 0.1^{b,A}$	$2.65 \pm 0.09^{b,A}$
2	$2.5 \pm 0.1^{a,B}$	$2.2 \pm 0.1^{a,A}$	$2.30 \pm 0.02^{a,A}$	$2.62 \pm 0.07^{a,A}$	$2.2 \pm 0.1^{a,b,A}$	$2.28 \pm 0.09^{a,b,A}$
4	$2.37 \pm 0.04^{a,B}$	$2.26 \pm 0.03^{a,A,B}$	$2.20 \pm 0.04^{a,A}$	$2.7 \pm 0.1^{a,B}$	$2.0 \pm 0.2^{a,A}$	$2.20 \pm 0.04^{a,A,B}$
6	$2.40 \pm 0.04^{a,B}$	$2.26 \pm 0.01^{a,A}$	$2.1 \pm 0.1^{a,A}$	$2.70 \pm 0.04^{a,B}$	$1.8 \pm 0.3^{a,A}$	$1.9 \pm 0.1^{a,A,B}$

Mean $\pm$ SD ($n^{\circ} = 3$)

Different lowercase in the same column indicate significant differences ($p \leq 0.05$). Different uppercase indicate significant differences in the same row ($p \leq 0.05$)

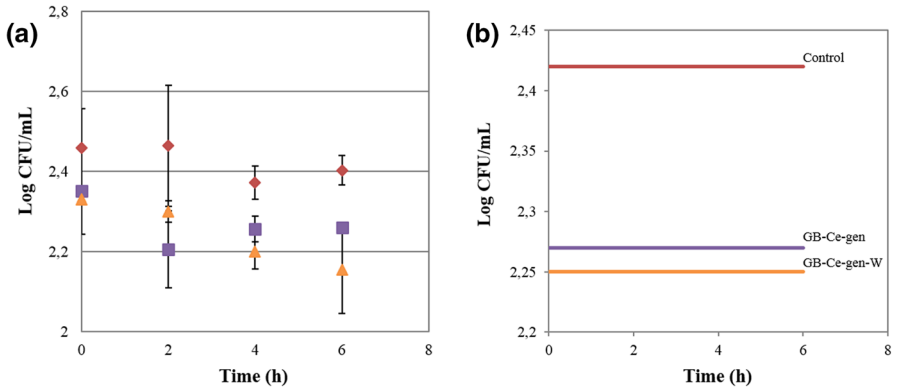

Fig. 8 **a** Log CFU/mL vs exposition time; (*filled rhombus*) *S. aureus* suspension control, (*filled square*) GB-Ce-gen and (*filled triangle*) GB-Ce-gen-W films. **b** Lineal correction of exposed films and *S. aureus* suspension control

counts obtained at different time intervals as repetitions of the same measure, it appears that the population densities in the inocula of the bacteria exposed to GB-Ce-gen and GB-Ce-gen-W films do not differ significantly from each other; they rather have a lower microbial burden with respect to *S. aureus* suspension control, reaching inhibition close to 30 % in the presence of films (Fig. 8).

These results show an inhibitory effect of both gelatin films on *S. aureus*. This inhibition occurs immediately upon contact of the bacterial population with the films, but not emphasized as time goes on interaction, indicating a sustained bacteriostatic effect after an initial partially bactericidal effect. In addition, the surface modification of cross-linking gelatin films with dendritic molecules does not change the antimicrobial activity, which is perhaps due to the presence of cerium(III).

The *E. coli* suspension control was stable throughout the test period. The counts obtained in the suspension of this bacterium in contact with films were lower than those in the control suspension, yet they showed significant differences after 4 h of interaction as shown in Fig. 9.

Unlike what was observed in *S. aureus* suspensions, contact with dendronized and undendronized gelatin films did not cause an immediate effect on the microbial load of a suspension of *E. coli*; inhibition, however, in terms of an effect partially bactericidal, was observed while contact time passed and accentuated gradually. Both films caused a decrease in the suspension of *E. coli* with respect to the suspension control reaching a value close to 80 % within 6 h of contact.

This results shown an antibacterial power of gelatin films modified with dendritic molecules and cerium(III). While the tests were performed only with *E. coli* and *S. aureus*, the results suggest that the antimicrobial activity of these films includes, to a different extent, bactericidal and bacteriostatic actions, and that both, or at least one, may depend largely on the chemical nature of the cell wall of the microorganisms inhibited.

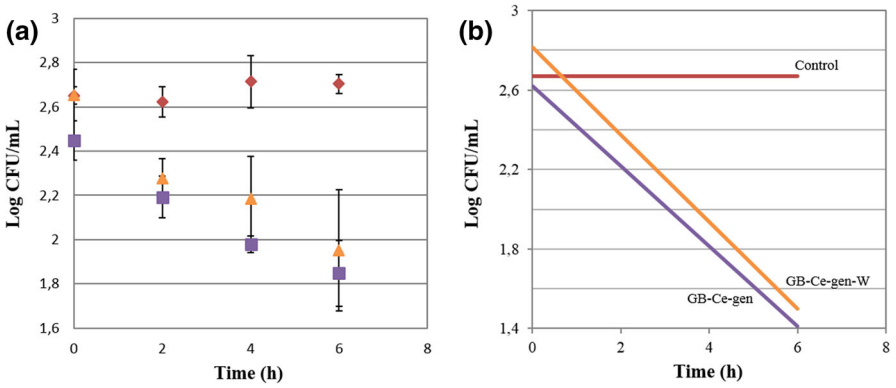

Fig. 9 **a** Log CFU/mL vs exposition time; (*filled rhombus*) *E. coli* suspension control, (*filled square*) GB-Ce-gen and (*filled triangle*) GB-Ce-gen-W films. **b** Lineal correction of films and *E. coli* suspension control

Conclusions

In this paper, we have synthesized and characterized stable cross-linked dendronized gelatin films on one side. These films were prepared by covalent binding between weisocyanate dendron and gelatin on cross-linking network. These materials were stable in aqueous medium at different pHs, overcoming the main limitation of gelatin as a biomedical material, its strong hygroscopic property. The dendron may form a shield and provide a multivalent surface. Dendronized hydrophobic face would represent a barrier to moisture; the hydrophilic/hydrophobic balance and permeability could then be controlled by cross-linking and dendronization.

GB-gen film was found to be more resistant up to high tensile stress, according to the mechanical properties, due to the formation of multiple covalent links between amine groups of gelatin and genipin.

This work implies an important contribution to the design of an attractive biomedical material based on natural polymer attachment to multivalent molecule. The highlight in this work centers on the fact that the material synthesized is shown to possess antimicrobial activity, in contrast to that reported in virgin gelatin, which is easily attacked by bacteria.

This research leads to surface-functionalized films; the superficial character of that dendronization is even more important than the chemistry involved, which is based on typical isocyanate–amine chemical affinity. The synthesis strategy presented in this study seems to open a new route of gelatin-based scaffolds that could be used for biomedicine, especially for applications in tissue engineering. Further studies will concern better control of dendrimer surface distribution as well as biofunctionalization with drugs, adhesion peptides and other molecules of interest for wound dressing.

Acknowledgments The authors gratefully acknowledge the financial assistance of FONCYT, CONICET, and SECYT of UNC. Schejtman Sergio D. thanks CONICET for the fellowship awarded.

Conflict of interest The authors declare no conflict of interest.

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