Journal of Materials Chemistry

Dynamic Article Links

Cite this: J. Mater. Chem., 2012, 22, 22670

www.rsc.org/materials

PAPER

Chitosan films modified selectively on one side with dendritic molecules

Ana Agustina Aldana,[a] Ricardo Toselli,[b] Miriam C. Strumia[a] and Marisa Martinelli*[a]

Received 15th May 2012, Accepted 6th September 2012
DOI: 10.1039/c2jm33100d

Novel chitosan films dendronized on one side were prepared by reaction between a cross-linked biopolymer and weisocyanate dendron. According to ATR-FTIR analysis, it can be concluded that the dendronization took place on only one of the faces of the films. The highlight of this work is that the modification grants different hydrophilic and hydrophobic properties on both sides of the system. Interactions between the dendron-polymer network and the hydrophobic characteristics of the dendron modify the physical properties of the films. As the thermal stability of the films increases, the degree of swelling and WVP values decrease, and the elastic modulus increases, turning the network more rigid. The dendronized films were swollen at pH 3.4 and 6.8 which are the pHs of the different wound healing stages. The films remained stable for an extended period. The films obtained can be potentially applied as wound dressings able to maintain a moist environment at the wound interface and act as a barrier to microorganisms, removing excess exudates. The product is made from a readily available biomaterial that requires minimal processing, possesses antimicrobial properties and could promote wound healing.

1. Introduction

Dendrons have nano-dimensions and their immobilization on surfaces would lead to structural features within this dimensional range. Different kinds of dendrons can be used to modify several surfaces. Thus, tailored nanostructured surfaces can be obtained having good control over the morphology and other surface properties such as wettability, roughness, chemical reactivity and hardness. On account of their controllable geometry, size and functionality, dendrons are interesting for surface modification and for enlargement of active surfaces.

By having a high density of functional groups on their surfaces, dendrimers and dendrons are considered ideal candidates as spacers and linkers between substrate surfaces and biological macromolecules. There are interesting cases where the dendronization of the surface leads to new and important properties. For instance, a significantly positive dendritic effect was observed in the antibody binding capacity of immobilized bovine serum albumin (BSA) coupled to a dendronized support. In addition, our research group found a prominent example of a dendron as a mediator in the electrocatalysis of nicotinamide adenine dinucleotide oxidation onto carbon electrodes.[1] The electrochemical properties are directly related to the number of electroactive functional groups incorporated in the dendritic molecule, which can be controlled.[2]

Another interesting example has been well established by Haag and co-workers[3] who demonstrate that the presence of highly flexible and hydrophilic groups, combined with a highly branched architecture can lead to good resistance to protein adsorption.

Particularly, the treatment of wounds has evolved from ancient times. A wound dressing system should present several adequate properties for its intended final application as a barrier to microorganisms, allowing gaseous exchange, maintaining a moist environment at the wound interface, showing the capacity to absorb fluids exuded from the wounded area, and simultaneously controlling water loss.[4] It should also be non-toxic, non-allergenic, non-adherent and easily removed without trauma. It should be made from a readily available biomaterial that requires minimal processing, possesses antimicrobial properties and promotes wound healing.

In recent years, a large number of research groups have devoted efforts to producing a new and improved wound dressing by synthesizing and modifying biocompatible materials.[5–7] Biological-synthetic dressings are bilayered and consist of a high degree of polymer and biological materials.[8–11] The wound dressing has a skin top-layer supported with a sponge-like sublayer which can meet the requirements of higher gas permeation and protection of the wound from infection and dehydration. An asymmetric film as a wound dressing could be designed to be able to absorb the wound exudates—the hydrophilic layer—as well as to simultaneously control water loss—the hydrophobic layer.

[a]*Departamento de Química Orgánica-IMBIV, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre esq Medina Allende, Córdoba, Argentina. E-mail: aaldana@fcq.unc.edu.ar; mcs@fcq.unc.edu.ar; mmartinelli@fcq.unc.edu.ar; Fax: +54-0351-4333030; Tel: +54-0351-4333030*
[b]*Centro de Química Aplicada CEQUIMAP, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre esq Medina Allende, Córdoba, Argentina. E-mail: rtoselli@fcq.unc.edu.ar; Fax: +54-0351-4333030; Tel: +54-0351-4333030*

This journal is © The Royal Society of Chemistry 2012

In particular, efforts have been focused on the use of biologically derived materials such as chitin and its derivatives, which are capable of accelerating the healing processes at molecular, cellular and systemic levels. Chitin and its derivative, chitosan, are biocompatible, biodegradable, non-toxic, and have antimicrobial and hydrating agents. Due to these properties, they show good biocompatibility and positive effects on wound healing. Various forms of wound dressing materials based on chitosan derivatives are commercially available.[12] The ordered regeneration of wounded tissues requires the use of chitosan in the form of non-wovens, nanofibrils, composites, films, scaffolds and sponges. So far a number of research works have been published on chitosan as a wound dressing material.[13–16] Moreover, natural polymers are normally inexpensive, readily available from renewable sources, potentially biodegradable and capable of a multitude of possible chemical modifications.[17,18]

However, native chitosan usually suffers from poor water solubility and weak buffering capacity at physiological pH. To overcome these limitations, some chitosan derivatives, films, foams or combinations of both, including composite laminates of two or more materials, may be applied. The modification of chitosan by means of blending or bilayer structure with other polymers may be a convenient and effective method to improve the physical properties for practical application. For example, it has been reported that the hydrophilic property of chitosan could be modified *via* blending with PEG and PVA.[19] Chitosan was also blended with several polymers such as polyamides, poly(acrylic acid), gelatine, silk fibroin and cellulose to enhance mechanical properties.[20]

In this context, the chemical approach of the present work aimed at developing dendronized nanostructured surfaces on natural polymer supports that seem to be appropriate for specific biomedicine applications. The results of this research report on innovative nanomodified biomaterials.

Therefore, we present the application of a dendronization process on only one side of a chitosan film with the aim of conferring improved properties to its potential application as a wound dressing material. The presence of one dendronized surface polymeric film yielded a material with different hydrophilic/hydrophobic properties on each side and improved its mechanical properties. The dendronization was carried out with a biocompatible Newkome's type dendron with an isocyanate focal point and hydrophobic peripheral functional groups.[21] The present approach targets only the synthesis and characterization of surface-dendronized chitosan; the further applications are going to be achieved in a future work.

2. Experimental section

2.1. Materials

The following chemicals were purchased and used: chitosan, Ch (85% DA, LMW, Aldrich); polyvinyl pyrrolidone (P OR PVP, Todo Droga); genipin (Wako); di-*t*-butyl-4-[2-(*t*-butoxycarbonyl)ethyl]-4-isocyanato-1,7-heptanedicarboxylate (Weisocyanate) (dendron, Frontier Scientific); dibutyltindilaurate (Aldrich); dimethylacetamide (DMAc, Sintorgan); acetic acid glacial (Cicarelli); ninhydrin (Aldrich); buffers (glycine, chloride acid, potassium dibasic phosphate, sodium hydroxide); potassium bromide 99% FT-IR grade (Aldrich); *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923; brain-heart agar (Britania); plate count agar (PCA, Britania); solvents were obtained from Sintorgan, purified by distillation, and dried.

2.2. Instruments and techniques

Fourier Transform Infrared (FT-IR and DRI-FT) spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer. Attenuated Total Reflectance Fourier Transform Infrared (ATR/FT-IR) 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}.

Contact angle measurements performed on the samples were carried out using a Data Physics OCA20 instrument, fitted with an automatic image capture system. The contact angles were measured carefully from the modified side of the drop and subsequently averaged. The liquid used was distilled water. These experiments were performed at room temperature.

DSC measurements were taken using a V5.4A TA instrument on samples with mass of about 2 mg, at a heating rate of 10 °C min^{-1}, and a testing temperature ranging from 50 to 400 °C. TGA measurements were conducted using a 2950 TGA HR V5.4A TA instrument, on samples with masses of about 5 mg at heating rates of 10 °C min^{-1}; the samples were tested in nitrogen from 40 to 400 °C.

2.3. Methods

2.3.1. Preparation of films. Cross-linked chitosan films with different cross-linking (PVP and genipin) were prepared. The ratios of PVP/chitosan and genipin/chitosan studied were 18 and 0.1% w/w, respectively.

All the chitosan films were prepared by the following method: 1000 g of chitosan was dissolved in 1.5% acetic acid solution (100 mL) and homogenized at room temperature overnight. After that, PVP powder (0.1800 g) or genipin 0.5% w/v solution (160 μL) was added to the chitosan solution. Then, this solution (50 mL) was cast on a glass plate (10 cm in diameter), and gradually dried in air at room temperature. Thus, the PVP or genipin chitosan films were obtained and called ChP and ChG, respectively.

The films were carefully removed from the Petri dishes and analyzed by ATR-FTIR spectroscopy. The films were also characterized by thermo-gravimetric, contact angle and swelling studies.

2.3.2. Dendronization of films. A 25 mL nitrogen flask equipped with a magnetic stirring bar was charged with a ChP or ChG film (0.1000 g) in 10 mL dimethylacetamide. Weisocyanate (0.150 g, 0.15 mmol) and dibutyltin dilaurate (0.02 mL) were added and stirred at 60 °C for 4 days. A special flask was designed to hold the film in its base. The film was washed with $CHCl_3$ to remove unreacted dendron, and the dendronized chitosan films were carefully removed from the flask and dried under vacuum. The ChPW and ChGW films were characterized

by ATR-FTIR spectroscopy, thermo-gravimetric analysis, contact angle, swelling index and rheological studies.

2.3.3. Determination of amine groups. Ninhydrin-based monitoring systems are among the most widely used for the quantitative determination of the amino acid content of proteins. Ninhydrin reacts with primary amines to form a colored complex known as Ruhemann's purple.[22] This same reaction can also be used to measure the amount of free primary amino groups attached to an insoluble support.

The ninhydrin solution in ethanol (0.02 M) was freshly prepared on the day of the assays. For each assay, 1 mL of reagent was added to 0.002 g of the different chitosan-based films in a glass tube with 3 mL of water; the mixtures were heated in boiling water for 10 min to allow the reaction to proceed. The absorbance of each solution was measured on a UV spectrophotometer (Shimadzu AEU-210) at 570 nm.

The ninhydrin assay was carried out on the cross-linked films; the results were compared with the dendronized films. Native chitosan (degree of deacetylation 85%) was employed as blank and the degree of modification (DM) of samples was calculated following eqn (1):

$$\mathrm{DM} = \frac{\left[(\mathrm{NH_2})_{\mathrm{fresh}} - (\mathrm{NH_2})_{\mathrm{fixed}}\right]}{(\mathrm{NH_2})_{\mathrm{fresh}}} \times 100 \quad (1)$$

where "fresh" is the mole fraction of free NH_2 in the non-modified sample and "fixed" is the mole fraction of free NH_2 remaining in the modified samples. Three samples of each type of film were evaluated.

2.3.4. Swelling studies. The water sorption capacity of the cross-linked chitosan films was determined by swelling the films in buffers of pH 3.4 and 6.8 at room temperature. A known weight (200 mg) of each film was placed in the medium for 7 h, which is 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 percentage swelling (E_{sw}) of the chitosan cross-linked films in the medium was calculated using eqn (2):

$$E_{sw}(\%) = \frac{W_s - W_o}{W_o} \times 100 \quad (2)$$

where W_S denotes the weight of the cross-linked chitosan films at equilibrium swelling, and W_o is the initial weight of the cross-linked chitosan films. Each swelling experiment was repeated twice, and the average value was taken as the E_{sw} value.

2.3.5. Water vapor permeability (WVP). The 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, the test cells were placed in a humidity chamber. The permeability cups with the films were weighed at intervals of one hour during 7 h. Linear regression was used to calculate the slope of a fitted straight line in a graph of variation of mass *versus* time. The water vapor transmission rate (WVTR) (kg s^{-1} m^{-2}) and the WVP (kg m Pa^{-1} s^{-1} m^{-2}) were calculated from eqn (3) and (4) respectively:

$$\mathrm{WVTR} = \frac{F}{A} \quad (3)$$

$$\mathrm{WVP} = \frac{(\mathrm{WVTR} \times e)}{\left[S_p \times (\mathrm{RH_2} - \mathrm{RH_1})\right]} \quad (4)$$

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.

2.3.6. Rheological studies. Rheological properties were measured on an Antor Paar Physica MCR 301 rheometer equipped with a P-PTD 200/I accessory. A film fixture was chosen for performing extensional measurements on dendronized and non-dendronized films at a constant extensional rate of 0.001 s^{-1} and at room temperature. The tensile stress and extensional viscosity were measured under a constant extensional shear load.

2.3.7. Microbiological studies. The microbial growth inhibitory activity of the films was evaluated with Gram negative and Gram positive bacteria. Strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 respectively, belonging to the culture collection of the Centre of Applied Chemistry (CEQUIMAP – UNC), were used. Cultures of these bacteria were obtained on brain-heart agar. Then, they were suspended in phosphate buffer dilution (pH 6.8–7.2) to fit the absorbance at 620 nm in order to obtain an inoculate of approximately 1000 CFU mL^{-1}. The films were sterilized by exposure to UV light on both sides for 15 minutes and their antimicrobial activity was evaluated using two experimental models. In one of them, the films were deposited on the surface of each solidified plate of brain-heart agar previously inoculated by raking with 200 μL of the bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus*, respectively. In the other design, the films were deposited on the surface of each solidified plate of plate count agar (PCA). Then, a second layer of brain-heart agar, previously inoculated with the appropriate inoculums and kept at a temperature above the solidification point was poured. In both models, the resulting plates were incubated for 24 h at 37 °C. After the incubation period, the antimicrobial activity was evaluated by comparing the growth of colonies in the culture medium in contact with the film to an equivalent area of culture medium without film.

2.4. 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 Turkey test was used for comparing mean values. Differences between means were considered significant when $p \leq 0.05$.

 This journal is © The Royal Society of Chemistry 2012

3. Results and discussion

Chemical modifications of chitosan were proposed to improve the mechanical strength and chemical stability of the natural polymer in acidic media. They may increase its resistance to biochemical and microbiological degradation. A cross-linking step is required to reinforce the chemical stability of the biosorbents in acidic solution. We used PVP and genipin as the ionic and covalent cross-linker respectively, because they are biocompatible.[23]

3.1. Preparation of chitosan films

3.1.1. Chitosan–PVP cross-linked films. Chitosan was mixed with PVP by blending,[14,15] one of the most effective methods for providing new materials suitable for use in the biomedical field. Chitosan and PVP were mixed and the film was made by casting. PVP is physically entangled in the chitosan network. According to the ninhydrin test, the degree of cross-linking was 93.7%.

Fig. 1 shows the ATR/FT-IR spectra of chitosan and the cross-linked films. The spectrum of the film showed the PVP band at 1649 and 1400 cm^{-1} corresponding to the carboxyl group and C–N lactam group, respectively. The amine band of chitosan at 894 cm^{-1} decreased.

3.1.2. Chitosan–genipin cross-linked films. The film was prepared using a constant ratio of genipin to chitosan (0.1% w/w). Genipin is known to crosslink only chemically the amino groups of the chitosan chains.[24] The cross-linking degree was 75.0% according to the ninhydrin test.

Fig. 1 shows the ATR/FT-IR spectra of the cross-linked films. After the cross-linking reaction no signal corresponding to the ester group of genipin was observed, whereas the amide band at 1653 cm^{-1} appeared, thus suggesting that the carboxymethyl group of genipin had reacted with the amino groups of chitosan to form a secondary amide. At 1564 cm^{-1} the H–N–H clipping vibration of the amine group of chitosan overlaps with the band corresponding to the N–H vibration of the amide group.[25,26] The signal absorption of CN of the amide group appears at 1408 cm^{-1}.

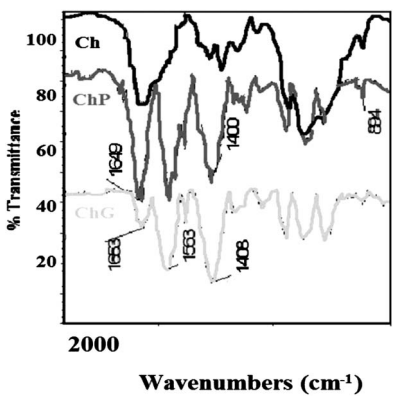

Fig. 1 ATR/FT-IR spectra of Ch (–), ChP (–) and ChG (–) films.

3.1.3. Dendronization of films. The experiment was carefully designed to dendronize one side of the cross-linked film, leaving the pristine chitosan on the opposite side, Fig. 2.

The dendronized chitosan films were successfully prepared by covalent union with weisocyanate dendron to yield ChPW and ChGW films. The degree of modification was 8.67×10^{-5} and 1.29×10^{-5} mol g^{-1} of film for ChPW and ChGW, respectively, and the thickness of the films was approximately 60–110 μm. Fig. 3 shows the synthetic route.

The chitosan–PVP films have the largest network of cross-linking degree and the highest degree of dendronization. Chitosan and PVP are poorly miscible and their miscibility is driven by hydrogen bond formation between the polymer chains. The PVP repeating unit contains a highly polar amide group which determines the hydrophilic and polar attracting properties, and apolar methylene and methine groups conferring hydrophobic properties on PVP.[27] In addition, PVP has an extraordinary adsorptive and complexing ability towards different types of small-molecular substances. In contrast, ChG films are covalently cross-linked networks, leaving amino groups less available for subsequent reactions. Thus, these latter networks present a major entanglement, preventing the linking of the dendron.

ATR/FT-IR analysis of the sample shows that the expected product was obtained, and that dendronization occurred only on one surface of the film (Fig. 4). On this face, the characteristic peaks of the dendron were observed, as the *tert*-butyl methyl group at 850 and 756 cm^{-1}, 2968 cm^{-1} assigned to the CH_3 stretching and the band at 1225 cm^{-1} corresponding to C–O–C of the ester group. The band at 1729 cm^{-1} can be assigned to the C═O stretching of the dendron or urethane or urea bond formation, because the vibrations overlap. The characteristic bands of ChP or ChG showed no changes in the profiles of the spectra of the other side of the films. Fig. 2 shows the comparative spectrum of ChP/ChPW and ChG/ChGW, non-modified and modified side.

3.2. Swelling index of chitosan films

The swelling behavior of the dendronized film changed compared to that of the unmodified cross-linked chitosan.

Before and after the dendronization process, the equilibrium swelling (E_{sw}) of the film was studied at different pH: 3.4 and 6.8, which simulates the pH of exuding wound and normal stages,[35] respectively. E_{sw} values were determined according to eqn (1), Fig. 5. The swelling was higher at minor pH due to the positive charges of the amino groups of chitosan and the electrostatic repulsion of their chains.

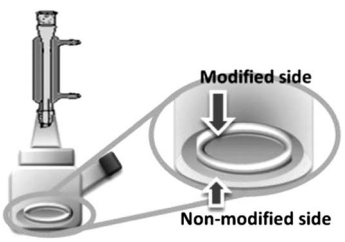

Fig. 2 Scheme showing the system of the synthesis.

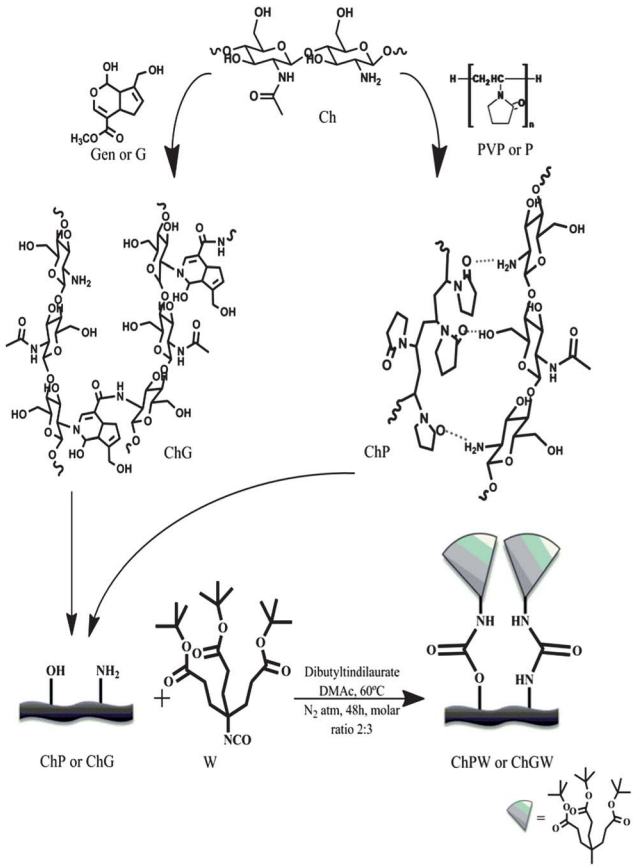

Fig. 3 Scheme of synthetic route of ChGW and ChPW.

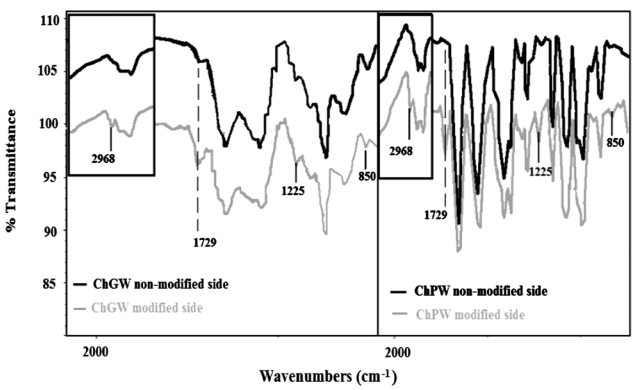

Fig. 4 ATR/FT-IR spectra of Ch and non-modified side and modified side of ChGW and ChPW films.

At pH 3.4, the ChP film showed a swelling index higher than that of the ChG film. This would indicate that the major hydrophilic nature of PVP is a key factor for this property, since the ChP films are networks more cross-linked than ChG films.[28] However, the ChPW and ChGW films showed a similar swelling index. The dendronization of the cross-linked chitosan caused a marked decrease in the swelling value of the films.

At pH 6.8, there were no significant differences in the swelling of non- and dendronized films. Probably, it derives from the fact that few free amine groups of chitosan are not charged, so the swelling does not change with a modification.

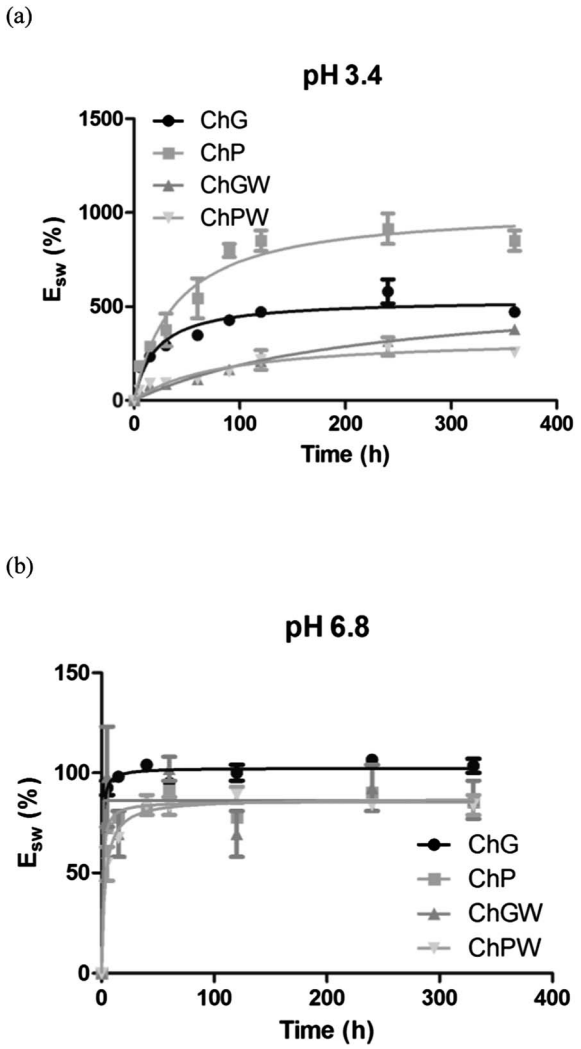

Fig. 5 Swelling index of obtained films at (a) pH 3.4 and (b) pH 6.8.

The swelling of the films was measured during 7 hours; they were left in contact with each of the buffers for a week. After that period, the films were not degraded. This is particularly important considering their potential use as a wound dressing material.

3.3. Thermal analysis of chitosan films

The thermal behavior of the films was analyzed by DSC and TGA, as shown in Table 1 and Fig. 6.

Table 1 Maximum peaks of the different films on TGA and DSC curves

	TGA	DSC	
Film	Peak of maximum degradation (°C)	Absorbed water (°C)	Degradation (°C)
ChG	268.8	169.9	285.8
ChGW	279.6	140.3 and 197.7	292.6
ChP	285.6	155.5	288.9
ChPW	290.1	143.5	296.3

This journal is © The Royal Society of Chemistry 2012

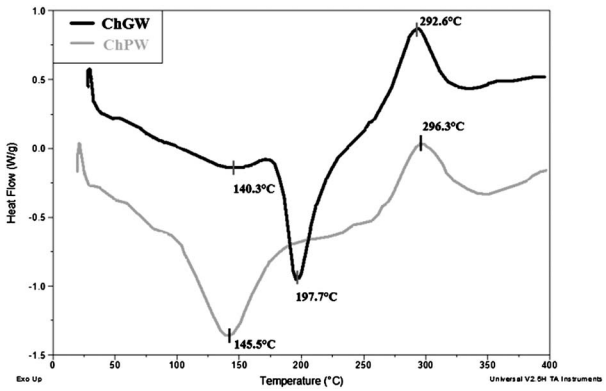

Fig. 6 DSC analysis of ChPW and ChGW films.

The onset temperature of the thermo-oxidative degradation of the films was also observed at around 280 °C, accompanied with a massive weight loss up to 100% at around 400 °C.[29] By comparing all decomposition temperatures, the thermal stability of the dendronized films proved higher than that of the cross-linked chitosan. The presence of the dendron creates new interactions between the polymer chains; functional groups have enough affinity to stabilize the polymeric network. A synergy is created between the cross-linking and covalent union of the dendron, increasing the thermal stability of the polymeric network. The superficial dendronized cross-linked films form an organized network by interactions between the chains at the molecular level.

The exothermic peak of adsorbed water appeared at 140 °C and 143 °C in ChPW and ChGW, respectively. In the DSC curve of ChGW, another exothermic peak was observed at 198 °C due to absorbed water in the cross-linked network. In addition, the DSC curves showed another endothermic peak around 290 °C ascribed to other polymer degradation, including saccharide rings, dehydration, depolymerization and decomposition, and deacetylated and acetylated chitosan units. These peaks have been reported in several other studies.[30] The increase in the thermal stability of the dendronized films would indicate that there was a distribution of crystal sizes in the composites as the dendrons got the crystalline order over one face of these films.[31]

3.4. Water vapor permeability (WVP)

The water vapor permeability of a wound dressing should prevent excessive dehydration and build-up of exudates. The water loss rates from injured skin would provide an adequate level of moisture without risking wound dehydration. If the wound is allowed to turn too dry, the healing process may also be delayed. However, the accumulation of excess fluid can cause maceration or infection. This means that, to provide optimum conditions, depending on the exudation level of the wound, films with appropriate permeability may be applied in each case.[32] The water vapor transpiration rate (WVTR) of chitosan films and blended chitosan-cellulose has been reported;[29] they are 1063 and 816–864 g m^{-2} per day, respectively. As shown in Table 1, the water loss data for the films of cross-linked chitosan are approximately 500 g m^{-2} per day.

Usually, water vapor permeation through a hydrophilic film is closely related to the solubility and diffusivity of water molecules in the polymer matrix.

The dendronization process decreases the WVTR and WVP. 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.

Both faces of chitosan-PVP films showed WVP values lower than those of chitosan–genipin films. This result demonstrates that the intrinsic nature of the cross-linkers has some ability to affect the WVP of the films as a result of the formation of different cross-linking structures. The WVP value for chitosan–genipin film 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 due to the hindered motion of the polymer segments. The dendronized face also shows lower permeability than that of the unmodified face (Table 1), 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 materials obtained are considered semi-permeable dressings which could be used in the management of shallow wounds, minor burns, donor sites, post-operative wounds, abrasions and minor lacerations. These dressings can also be used to protect the skin from friction or continuous exposure to moisture, preventing breakdown producing excessive amounts of exudates.[33]

3.5. Extensional rheological studies

Fig. 7 shows tensile stress *versus* tensile strain. A significant increase may be observed in the elastic modulus of the dendronized films. These results suggest that dendronized films are more rigid and resistant up to high tensile stress. The dendronized films break as soon as non-dendronized films. It could be related to the fact that the effect of the cross-linker is more important than the dendron effect.

3.6. Contact angle of chitosan films

Contact angle measurements provide a quantitative characterization of material surface energy. An increase in contact angle

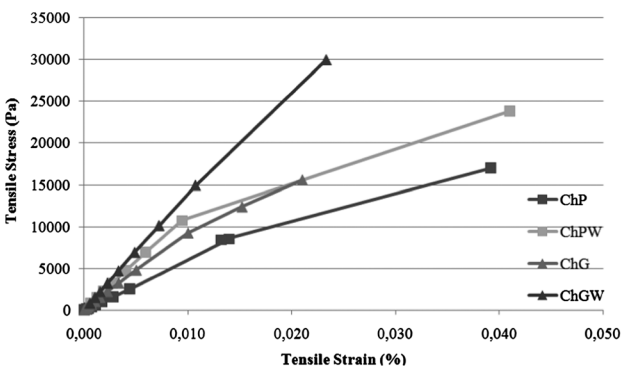

Fig. 7 Stress *vs.* strain curves of ChG, ChGW, ChP and ChPW films.

with water indicates an enhancement in the hydrophobic character of surface and a lower value for the polar component of the surface energy.

To compare the hydrophilic character of dendronized crosslinked films with that of the unmodified chitosan, the contact angle (θ) between the sample surfaces and water was measured in air using the sessile drop method. Water was carefully dropped on the films and the contact angles were quickly measured. The reported values (Table 2) suggest the average of three different measurements.

As seen in Table 2, the water contact angles of cross-linked films range from 80° to 112°. The contact angle of unmodified chitosan[33] is lower than that of the films, meaning that the incorporation of cross-linkings renders the surface less water-wettable. After dendronization, the films were found to be more hydrophobic, indicating that the amino and hydroxyl groups had engaged in chemical union with the Weisocyanate dendron. In addition, the dendron possesses hydrophobic *tert*-butyl groups on its periphery.

3.7. Microbiological studies

In the first design, the films showed a completely inhibited growth of *E. coli* and *S. aureus*. There was no inhibition halo of microbial growth around the films. This bacteriostatic effect of the polymer is due to the direct contact of the films with the agar surface and no diffusion from the films. However, this inhibition could be due to a certain physical impediment to develop visible colonies on the agar–film interface. To quantify the inhibition, the second experimental design was used. Table 3 displays the microbial inhibitions. In this design, the brain-heart agar layer was not sufficiently thin and colonies were able to grow on the agar that was not in contact with the film. Chitosan has been widely studied as an antimicrobial substance for bacteria and the possible mechanism of its antibacterial activity is attributed to amino groups.[34] A possible reason for the lower antibacterial activity of chitosan films cross-linked with genipin compared to the films prepared with PVP is that the covalent bond may obstruct the interaction between chitosan and bacterial cells. On the other hand, the dendronization of ChP increases the bacterial inhibition. The structural and chemical changes of the film may improve the interaction with bacterial cells.

The results obtained show that the films inhibit the normal *in vitro* development of the two bacteria tested in this assay, *S. aureus* and *E. coli*, despite the fact that the microbiological inhibition may be overestimated and underestimated in the first and second design, respectively.

Table 3 Percentage of bacterial inhibition of films obtained[a]

	Inhibition%	
Film	*S. aureus*	*E. coli*
ChG	46 ± 4^{a}	—
ChGW	23 ± 5^{b}	—
ChP	35 ± 2^{c}	36 ± 6^{a}
ChPW	68 ± 4^{d}	77 ± 2^{b}

[a] ANOVA analysis: small letters represent a comparison between films by row and capital letters between films by column.

4. Conclusions

In this paper we have synthesized and characterized novel chitosan films dendronized on one side only. These films were prepared by covalent binding between weisocyanate dendron and chitosan in a cross-linking network. A dendronization process allowed obtaining a chitosan film with one hydrophobic side with non-adherent properties and improved mechanical properties. The dendronized hydrophobic face presented low permeation to vapour water, while the hydrophilic face allowed good absorption of exuding liquid. There are transparent films which would also facilitate visual examination of the wound without disturbance. Moreover, they conserve the known advantages of chitosan, such as biocompatibility, biodegradability, antimicrobial and antifungal properties.

Both different crosslinked chitosans were prepared. Specifically, one was crosslinked with PVP and the other with genipin. The degree of dendronization was higher for the films crosslinked with PVP; however, the other showed better mechanical properties. Both ChGW and ChPW films hold promise for their potential application as wound dressings thus providing an alternative material for biomedical applications.

The synthesis strategy presented in this study seems to open a new route of chitosan-based materials that could be used for biomedicine, especially for application as wound dressings. Further studies will concern better control of the dendrimer surface distribution as well as biofunctionalization with drugs, adhesion peptides and other molecules of interest for wound dressing.

Table 2 Water vapor transmission rate (WVTR), water vapor permeability (WVP) and contact angle (CA) values of films obtained[a]

	WVTR ($\times 10^{1}$ g m^{-2} day^{-1})		WVP ($\times 10^{-12}$ g m^{-1} s^{-1} Pa^{-1})		
Film	Non-modified face to air	Modified face to air	Non-modified face to air	Modified face to air	CA (°)
ChG	$54 \pm 2^{a,A}$	$51 \pm 2^{a,b,A}$	$3.2 \pm 0.2^{a,A}$	$3.1 \pm 0.3^{a,A}$	84.6 ± 0.2^{a}
ChGW	$55 \pm 2^{a,A}$	$46 \pm 2^{b,B}$	$2.1 \pm 0.2^{b,A}$	$1.7 \pm 0.2^{b,c,A}$	89.9 ± 0.3^{b}
ChP	$56 \pm 3^{a,A}$	$53 \pm 3^{a,A}$	$2.1 \pm 0.2^{b,A}$	$2.0 \pm 0.2^{b,A}$	81.7 ± 0.2^{c}
ChPW	$58 \pm 2^{a,A}$	$46 \pm 2^{b,B}$	$1.8 \pm 0.2^{b,A}$	$1.4 \pm 0.1^{c,B}$	111.5 ± 0.4^{d}

[a] ANOVA analysis: small letters represent a comparation between films by column. Capital letters represent a comparation between column with same film.

This journal is © The Royal Society of Chemistry 2012

Acknowledgements

The authors gratefully acknowledge the financial assistance of FONCYT, CONICET, and SECYT of UNC. Aldana AA thanks CONICET for the fellowship awarded. We thank Prof. Alicia Susana Lorenti of Buenos Aires University for discussions.

Notes and references

1 J. I. Paez, M. C. Strumia, M. C. G. Passeggi Jr, J. Ferrón, A. M. Baruzzi and V. Brunetti, *Electrochim. Acta*, 2009, **54**, 4192.
2 F. E. Appoh and H. B. Kraatz, *J. Appl. Polym. Sci.*, 2009, **111**, 709.
3 M. Wyszogrodzka and R. Haag, *Langmuir*, 2009, **25**, 5703.
4 A. M. A. Dias, M. E. M. Braga, I. J. Seabra, P. Ferreira, M. H. Gil and H. C. De Sousa, *Int. J. Pharm.*, 2011, **408**, 9.
5 H. Shibata, N. Shioya and Y. Kuroyangi, *J. Biomater. Sci., Polym. Ed.*, 1997, **8**, 601.
6 J. P. Draye, B. Delaey, A. Van Den Voorde, A. Van Den Bulcke, B. De Reu and E. Schacht, *Biomaterials*, 1998, **19**, 1677.
7 K. Ulubayram, A. Nur Cakar, P. Korkusuz, C. Ertan and N. Hasirci, *Biomaterials*, 2001, **22**, 1345.
8 P. Bruin, M. F. Jonkman, H. J. Meijer and A. J. Pennings, *J. Biomed. Mater. Res.*, 1990, **24**, 217.
9 K. Matsuda, S. Suzuki, N. Isshiki, K. Yoshioka, T. Okada and Y. Ikada, *Biomaterials*, 1990, **11**, 351.
10 K. Matsuda, S. Suzuki, N. Isshiki, K. Yoshioka, R. Wada and S. H. Hyon, *et al.*, *Biomaterials*, 1992, **13**, 119.
11 S. Suzuki, K. Matsuda, N. Isshiki, Y. Tamada and Y. Ikada, *Biomaterials*, 1990, **11**, 356.
12 M. A. Brown, M. R. Daya and J. A. Worley, *J. Emerg. Med.*, 2009, **37**, 1.
13 H. Ueno, T. Mori and T. Fujinaga, *Adv. Drug Delivery Rev.*, 2001, **52**, 105.
14 M. N. V. Ravi Kumar, *React. Funct. Polym.*, 2000, **46**, 1.
15 I. Y. Kim, S. J. Seo, H. S. Moon, M. K. Yoo, I. Y. Park and B. C. Kim, *et al.*, *Biotechnol. Adv.*, 2008, **26**, 1.
16 R. A. A. Muzzarelli, *Carbohydr. Polym.*, 2009, **76**, 167.
17 A. D. Sezer, F. Hatipoglu, E. Cevher, Z. Ogurtan, A. L. Bas and J. Akbuga, *Pharm. Sci. Technol. Today*, 2007, **8**, 1.
18 S. L. Prabu, A. A. Shirwaikar, A. Shirwaikar, A. Kumar and A. Jacob, *Ars Pharm.*, 2008, **49**, 211.
19 M. Zhang, X. H. Li, Y. D. Gong, N. M. Zhao and X. F. Zhang, *Biomaterials*, 2002, **23**, 2641.
20 Y. B. Wu, S. H. Yu, F. L. Mi, C. W. Wu, S. S. shyu, C. K. Peng and Ac. Chao, *Carbohydr. Polym.*, 2004, **57**, 435.
21 A. A. Williams, E. W. Sugandhi, R. V. Macri, J. O. Falkinham and R. D. Gandour, *J. Antimicrob. Chemother.*, 2007, **59**, 451.
22 M. M. Leane, R. Nankervis, A. Smith and L. Illum, *Int. J. Pharm.*, 2004, **271**, 241.
23 A. Kamari, W. S. Wn Hgah and L. K. Liew, *J. Environ. Sci.*, 2009, **21**, 296.
24 S. Fujikawa, Y. Fukuy and K. Koga, *Biotechnol. Lett.*, 1987, **9**, 697.
25 F. L. Mi, H. W. Sung and S. S. Shyu, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 2804.
26 F. L. Mi, H. W. Sung and S. S. Shyu, *Carbohydr. Polym.*, 2002, **48**, 61.
27 K. Lewandowska, *Thermochim. Acta*, 2011, **517**, 90.
28 A. A. Aldana, A. Gonzalez, M. Strumia and M. Martinelli, *Mater. Chem. Phys.*, 2012, **134**, 317.
29 A. P. Martínez-Camachoa, M. O. Cortez-Rochaa, J. M. Ezquerra-Brauera, A. Z. Graciano-Verdugo, F. Rodriguez-Félixa and M. M. Castillo-Ortegac, *et al.*, *Carbohydr. Polym.*, 2010, **82**, 305.
30 C. L. Silva, J. C. Pereira, A. Ramalhoc, A. C. C. Pais and J. S. Sousa, *J. Membr. Sci.*, 2008, **320**, 268.
31 S. Balaji, R. Kumar, R. Sripriya, P. Kakkar, D. Vijaya Ramesh and P. Neela Kanta Reddy, *et al.*, *Mater. Sci. Eng., C*, 2012, **32**, 975.
32 A. M. A. Dias, M. E. M. Braga, I. J. Seabra, B. P. Ferreira, M. H. Gil and H. C. De Sousa, *Int. J. Pharm.*, 2011, **408**, 9.
33 D. Enescu, V. Hamciuc, R. Ardeleanu, M. Cristea, A. Ioanid and C. V. Harabagiu, *et al.*, *Carbohydr. Polym.*, 2009, **76**, 268.
34 D. Raafat and H. G. Sahi, *Microb. Biotechnol.*, 2009, **2**, 186.
35 G. Gethin, *Wound*, 2007, **3**, 52.