J Mater Sci: Mater Med (2008) 19:2397–2405
DOI 10.1007/s10856-007-3275-3

Chitosan composite films. Biomedical applications

Galo Cárdenas · Paola Anaya · Carlos von Plessing · Carlos Rojas · Jackeline Sepúlveda

Received: 11 May 2006 / Accepted: 7 September 2007 / Published online: 29 December 2007

Abstract Chitosan acetate films have been prepared using chitosans from shrimps (*Pleuroncodes monodon*) of low and high molecular weight (LMv = 68,000 g/mol and HMv = 232,000 g/mol) and deacetylation degree of 80 and 100%, respectively. The chitosan films were obtained by addition of several additives to acetic acid chitosan solutions, such as: glycerol, oleic acid and linoleic acid in different proportions. The pH of the solutions before casting ranged from 5.0 to 6.0. The composite film thickness are reported. The films have been analyzed by FTIR showing characteristic bands corresponding to the additives. The scanning electron microscopy (SEM) studies reveals the different morphology of the composite films. The films exhibit different physical properties depending upon the additives and/or mixture of them. The addition of glycerol to composite improves the elasticity of the films. The swelling in glucose and saline solutions for several films was evaluated, being higher in the glucose solution. The bactericide test against *Staphylococcus aureus, Pseudomona aeruginosa and Acinetobacter baumanii* in plates with either blood and or agar tripticase showed that the molecular weight influences on the bactericidal properties of the chitosan composite films and over its effect against gram positive and gram negative bacteria. Medical applications of the composite films were done in patients with burns, ulcers and injuries, the films containing glycerol showed good adhesion in comparison with those without it. The composite films tested were mainly three (1) chitosan acetate with glycerol, (2) chitosan acetate with oleic acid and (3) chitosan acetate with glycerol and oleic acid. Excellent results in the skin recovery were obtained after 7–10 days. Since the chitosan is biodegradable by the body enzymes it does not need to be removed and increases the gradual grows of the damage tissues.

G. Cárdenas (✉) · P. Anaya
Departamento de Polímeros, Facultad de Ciencias Químicas, Universidad de Concepción, Concepcion, Chile
e-mail: galocardenas@udec.cl

C. von Plessing
Facultad de Farmacia, Universidad de Concepción, Concepcion, Chile

C. Rojas
Hospital Naval de Talcahuano "Almirante Adriazola", Talcahuano, Chile

J. Sepúlveda
Facultad de Ciencias Biológicas, Universidad de Concepcion, Concepcion, Chile

1 Introduction

There are enough evidences to ensure that chitin and chitosan can be used in animals and humans without any risk. In fact, several benefits can be obtained when human body is damaged by surgery scarf, injuries, burns and ulcers.

The chitosan has shown an speed effect in the injuries recovery process. Additionally, the chitosan is an attractive product for the specific treatment of burns, due to the easy film formation, resistant, biocompatible, water absorbent and naturally degraded by body enzymes. Other advantage of this treatment is that allows optimum oxygen permeability for all the tissues and avoids the loss of body liquids [1]. This is very important to prevent the oxygen flow to damage tissues.

The massive fluid loss and the infection are two main problems in the treatment of individuals who has suffered

skin loss. In the case of victims with deep burns extending more than 50% of the body, current treatment recommends the closure of wounds with autografts, cadaver skin or pig skin following the excision of dead tissue [2].

All the efforts in the acute phase of burns are dedicated to cover the exposed surface. Heterografts, obtained from animals, especially pigs, are available commercially and are widely used to achieve shorter wound closure. Normally, they are removed after the third and ninth day application. A number of natural and synthetic polymer membranes have been reported in the treatment of burns and their use has not presented infection [3].

The problem of burn treatment for a wound caused by an injury from explosives and chemicals is even more severe than other burn wounds. In severe burns, the complete thickness of the skin has been destroyed. One treatment is to remove a thin film of healthy skin from the patients own body and graft it onto the burned area. In order to protect a skin from infections and dehydration in the period between hospitalization and grafting, temporary closure of a wound by the use of material other than the patients own skin has become very common during the past years [4–6].

Usually the autologous graft is the best and definitive solution to repair the damaged tissues specially in burn injuries. The technique consist in the skin removal with a dermatome from the health area to avoid the graft loss. The period between the injury and graft placed depends on several factors. Often surgeons have to use temporary cover materials to avoid the infections and other systemic disorders [7].

Among the wound dressing, bilayer artificial skin or wound dressing composed of a dense top layer (skin) and a lower sheet of sponge layer (sub layer) may be excellent. The skin layer (silicone) can present bacterial penetration and dehydration of the wound surface while the sub layer (collagen sponge) is designed to achieve high absorption for fluid damage of the wound and infiltration by fibroblasts for tissue regeneration [8, 9].

The burn injury, is an ideal substrate for the bacterial growing and provides a great entrance for the microbial invasion. The colonization by microorganisms (presence of bacteria in the necrotic tissue) of the open injury, mainly originated from an endogenic source with frequency is established during the first week. The infection is promoted by the loss of the epithelial barrier, the malnutrition induced by the hypermetabolic answer and the generalized immune suppression, due to the liberation of immune active agents from the injury.

The burn produces a reduction in the production of interleukina-2, T cell, cytosis of cells NK and reduction of the help index (T cells suppressor). It is probed that plasma infusion from a burn patient to a non burned or in experimental animals, can transmit some of these immune suppressors, presumably by the transfer of one suppressor of activated T lymphocyte. The incident time to get pathogen bacteria infection is during the first 10 days (*Staphylococcus aureus, Pseudomona aeruginosa and Acinetobacter baumanii*) [10]. The burn scar (is natural part of the healing process, due to the skin repair wounds) vascularized, is quickly colonized after the 5th day post burn, even with the use of antimicrobial agents.

Since chitosan is degraded by body enzymes, it does not need to be eliminated from the injury because the gradual grows of damage tissue and avoid a surgical operation to remove it. For this reason we decide to use it in several injuries and with some additives to improve their antimicrobial and mechanical properties.

2 Experimental

2.1 Chitosan

Two chitosans of low and medium molecular weight of different deacetylation degree were studied, they were prepared in our facilities. The chitosan was obtained in our pilot plant in a 0.5 m^3 stainless steel reactor from chitin obtained from shrimps (*Pleuroncodes monodon*) by normal procedures previously described by Cardenas [11, 12].

The molecular weight was determined by viscosity using the constant reported by Brugnerotto [13]. For the lowest Mv, the viscosity molecular weight was calculated by the Mark–Houwink equation; $[\eta] = KM^a$, where $K = 7.6 \times 10^{-2}$, and $a = 0.76$, respectively. Two different chitosan were used, one of Mv = 232,620 g/mol and deacetylation degree = 100% and other Mv = 68,340 g/mol and deacetylation degree = 80%.

The deacetylation degree was determined by potentiometric titration. The acid–base titration of the group $NH_3^+Cl^-$ from chitosan was carried in a known excess of 0.1 N NaOH as a title.

A pH meter WTW pH 531, with a combined glass electrode WTW Sen Tix 41, pH 0–14/0–80 °C is used and the change in pH due to neutralization with the advance of reaction is followed.

2.2 Chitosan films

Chitosan solutions at 1% (w/v) in 1% acetic acid were prepared and from them the other solutions containing the mixture with glycerol (0.5%), oleic acid (0.05%) and linoleic acid (0.075%) were obtained under magnetic stirring for 1 h. were obtained. The films were obtained by casting on glass plates, previously washed and with a relative humidity of 55% at room temperature, the pH was adjusted

Table 1 Chitosan film composition

Sample	Molecular weight of chitosan	Initial volume (mL)	Additive1	Additive2	Final pH
1001	68000	15	None	None	5.5
1002	68000	20	None	None	5.5
1003	68000	15	None	None	6
1004	68000	20	None	None	6
1005	68000	15	Glycerol	None	5.5
1006	68000	20	Glycerol	None	5.5
1007	68000	15	Glycerol	None	6
1008	68000	20	Glycerol	None	6
1009	68000	15	None	Linoleic acid	5.5
1010	68000	20	None	Linoleic acid	5.5
1011	68000	15	Glycerol	Linoleic acid	5.5
1012	68000	20	Glycerol	Linoleic acid	5.5
1013	68000	15	None	Oleic acid	6
1014	68000	20	None	Oleic acid	6
1015	68000	15	Glycerol	Oleic acid	6
1016	68000	20	Glycerol	Oleic acid	6
1017	230000	15	None	None	6
1018	230000	20	None	None	6
1019	230000	15	Glycerol	None	6
1020	230000	20	Glycerol	None	6
1021	230000	15	None	Linoleic acid	5.5
1022	230000	20	None	Linoleic acid	5.5
1023	230000	15	Glycerol	Linoleic acid	5.5
1024	230000	20	Glycerol	Linoleic acid	5.5
1025	230000	15	None	Oleic acid	6
1026	230000	20	None	Oleic acid	6
1027	230000	15	Glycerol	Oleic acid	6
1028	230000	20	Glycerol	Oleic acid	6

at 5.5 and 6.0 respectively (Table 1). After that the films were rinsed with HPLC grade water and dried. The films were irradiated on both sides with UV light of 20 W for 3 min in a clean area in order to sterilize the surface and then storage in sterilized sealed aluminum bags provided by Fisher Scientific [14–16].

The thickness of the films was measured with an Electronic Digital Micrometer from Vetto & Co., USA with a resolution of 0.001 mm. The thickness values showed in Table 2 are the media of five repetition.

2.3 Solubility test

Since the dried films are stored in sealed sterilized bags, previous medical application they are hydrated for that reason it was necessary to study the solubility of the films in different media like saline and glucose serum. Film pieces of 2 × 2 cm were immersed in 30 mL of saline (0.9% w/v) or/and glucose (5% v/v) solutions in order to test their solubility and swelling. They were left in the solution for 24 h, dried under vacuum and finally weigh.

2.4 FT-IR studies

Infrared spectra were measured by using a FT-IR Nicolet Magna 5PC spectrophotometer coupled to a PC with OMNIC software analysis. The films were placed in the holder directly in the IR laser beam. Spectra were recorded at a resolution of 4 cm^{-1} and 64 scans were accumulated.

2.5 Scanning electronic microscopy (SEM)

The samples under study were covered with a gold layer during 3 min to obtain 150 Å thickness using an Edwards S 150 Sputter Coater. The samples were analyzed in a scanning electron microscope ETEC AUSTOSCAN Model U-1.

Table 2 Chitosan film thickness

Sample	Thickness (mm)	Standard deviation	Sample	Thickness (mm)	Standard deviation
1001	0.024	0.0029	1015	0.035	0.0020
1002	0.032	0.0023	1016	0.045	0.0030
1003	0.024	0.0065	1017	0.021	0.0035
1004	0.031	0.0019	1018	0.040	0.0012
1005	0.038	0.0034	1019	0.015	0.0011
1006	0.043	0.0035	1020	0.021	0.0015
1007	0.023	0.0043	1021	0.015	0.0035
1008	0.046	0.0033	1022	0.027	0.0014
1009	0.018	0.0023	1023	0.014	0.0020
1010	0.044	0.0029	1024	0.024	0.0012
1011	0.035	0.0025	1025	0.021	0.0019
1012	0.045	0.0030	1026	0.039	0.0025
1013	0.015	0.0010	1027	0.018	0.0032
1014	0.021	0.0015	1028	0.034	0.0041

2.6 Bacterial test

This test was carried out by a culture on surface with bacteria. A volume of bacteria from a culture of 24 h that had 109 bacteria/mL was taken and added to the culture media with agar tripticase in order to have approximately 105 bacteria/mL.

Once the plates have the culture media and the bacteria (*S. aureus, P. aeruginosa* and *A. baumanii*) 10 μL of chitosan and chitosan composite solutions (2%) are added to the plates.

The plates were incubated for 24 and 48 h at 37 °C in order to evaluate the growth of the bacteria.

2.7 Medical applications of the films

The sterile films stored in aluminum sealed bags were opened and immersed in saline serum previous application to the patient in order to hydrate the film. The film was extended over the injury avoiding air occlusion. No further treatment was carried out.

Different kind of injuries were treated with chitosan films, some of the cases are describe below:

Case 1: A male patient, 21 years old. A tattoo from his left arm was removed. The injury caused by the tattoo removal was covered for a skin graft, chitosan acetate with glycerol films were used on the tattoo removal and the donor site.

Case 2: A male patient, 23 years old. An expose fracture of the patient need a skin graft, the injury cased by the fracture and the skin donor area were covered by the chitosan acetate with oleic acid films.

Case 3: A male patient, 43 years old. The diagnosis indicated that 1.5% of the body surface with AB/B type burn was damaged, on the same way, chitosan acetate with oleic acid films were used in this case.

3 Results and discussion

3.1 Solubility test

The solubility test showed a high swelling of the films in glucose serum which after a while become partially soluble. In Table 3, it is possible to see the increased of swelling depending on the additive and the changes in weight and size.

It is possible to observe a similar behavior in all the films depending on the solution used for the study, for instance, in all the cases the films increased their weight dramatically with the glucose serum, and all of them lost weight with the saline serum; on the other hand, the change in weight with the saline: glucose serum mixture is not very important because their weight increase but not more than 40% of the initial weight.

In the case of saline serum, the weight of the films decreased in all the cases, around 35%, being the low decrease for the sample 1023, chitosan of high molecular weight with glycerol and linoleic acid.

In the case of glucose serum, the weight of the films increased too much, for instance, the film 1005 reached 208% more than its initial weight and the less increase was 33% more than the initial weight for the sample 1023, from these results it is possible to affirm that the molecular weight of the chitosan influences on the swelling.

Table 3 Swelling of chitosan composite films

Sample	Clinical solution	Initial weight	Final weight	Change of weight	Size changes after 24 h
1005	Saline serum	0.0142	0.0090	−36.6%	Increase of 0.2 cm each side
1005	Glucose serum	0.0132	0.0407	208.3%	Increase of 1 cm each side
1005	Saline and glucose serum 1:1	0.012	0.0149	24.2%	Increase of 0.4 cm each side
1009	Saline serum	0.0138	–	–	No measurement
1009	Glucose serum	0.0164	–	–	No measurement
1009	Saline and glucose serum 1:1	0.0233	–	–	No measurement
1011	Saline serum	0.0127	0.0073	−42.5%	Increase of 0.3 cm each side
1011	Glucose serum	0.0201	0.0497	147.3%	Increase of 1.3 cm each side
1011	Saline and glucose serum 1:1	0.0272	0.0369	35.7%	Increase of 0.5 cm each side
1012	Saline serum	0.013	0.0084	−35.4%	Increase of 0.2 cm each side
1012	Glucose serum	0.016	0.0295	84.4%	Increase of 0.8 cm each side
1012	Saline and glucose serum 1:1	0.0147	0.0148	0.7%	Increase of 0.3 cm each side
1013	Saline serum	0.026	–	–	No measurement
1013	Glucose serum	0.0235	–	–	No measurement
1013	Saline and glucose serum 1:1	0.0178	–	–	No measurement
1015	Saline serum	0.0114	0.0068	−40.4%	Increase of 0.1 cm each side
1015	Glucose serum	0.0117	0.0199	70.1%	Increase of 0.5 cm each side
1015	Saline and glucose serum 1:1	0.0132	0.0139	5.3%	Increase of 0.2 cm each side
1023	Saline serum	0.0129	0.0092	−28.7%	Increase of 0.2 cm each side
1023	Glucose serum	0.0118	0.0157	33.1%	No measurement
1023	Saline and glucose serum 1:1	0.0083	0.0158	90.4%	Increase of 0.3 cm each side
1025	Saline serum	0.0184	–	–	No measurement
1025	Glucose serum	0.0204	–	–	Increase of 3 cm each side
1025	Saline and glucose serum 1:1	0.0206	–	–	No measurement

It is interesting to observe that the films without plasticizer were difficult to handle and they were rolled forming a sort of tube as soon as they were added to the serum and it was impossible to measure the swelling and the change of size.

3.2 FT-IR

The Table 4 summarizes the most relevant absorption bands of the additives incorporated in the films.

As we can see the amide bands remind without further changes in most of the composite films. Only the film 1005 exhibit the amide band at 1632 cm^{-1} due to their lower interaction.

In all the films containing either oleic or linoleic acid or mixtures between them we can observe the $\nu C = C$ around 1650 cm^{-1} which disappears when we have the film with the three additives.

The films FT-IR shows the presence of additives incorporated in them (see Fig. 1).

3.3 SEM

The morphology of the films was analyzed by SEM in order to see the porosity of the films. This is quite important since the oxygen permeation through a wound

Table 4 Comparison of bands in FT-IR [17]

Sample	νN–H (cm^{-1})	νO–H (cm^{-1})	$\nu C–H_{CH3}$ (cm^{-1})	$\nu C–H_{CH2}$ (cm^{-1})	Amide I (cm^{-1})	νC–O ($cm^{-1)}$	$\nu C = C$ (cm^{-1})
1001	3452	3366, 3313	2915	2870	1562	1085	–
1005	3650	3431	2927	2877	1632	1050	–
1013	3509	3321, 3261, 3196	2960	2857	1584	1079	1641
1015	3523	3373, 3275, 3115	2945	2888	1576	1089, 1032	1645
1009	3490	3415, 3277,3 109	2923	2878	1575	1078, 1034	1640
1011	3517	3368, 3270, 3148	2931	2880	1576	1095, 1041	1650

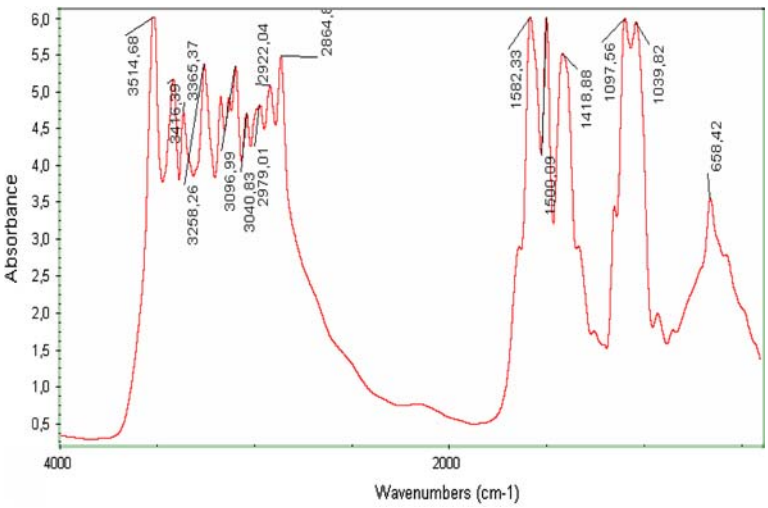

Fig. 1 FT-IR spectrum of film 1015

dressing is important because a high CO_2 pressure reduces the pH value and slows the heating rate. Besides, a low oxygen concentration makes possible the proliferation of anaerobic bacteria. The addition of additives to pure chitosan films makes better the mechanical handling.

The film 1003, chitosan (pH = 6), exhibits a surface with certain porosity, some residues from salt crystals due to the neutralization are observed (see Fig. 2a).

The composite film 1005, chitosan with glycerol (pH = 5.5), shows a more regular surface with some crinkles forming some layers due to the presence of the plasticizer (see Fig. 2b).

The composite film 1013, chitosan with oleic acid (pH = 6), exhibit a regular surface with lower porosity and few crystals coming from the neutralization (see Fig. 2c).

The composite films 1009, chitosan with linoleic acid (pH = 5.5), shows the best surface with no crinkles, pores or salts residue. This is probably due to different polarity compared with oleic acid (see Fig. 2d).

3.4 Bactericidal test

Infection organisms preferentially target wounds beneath dressing materials, leading to serious infections that

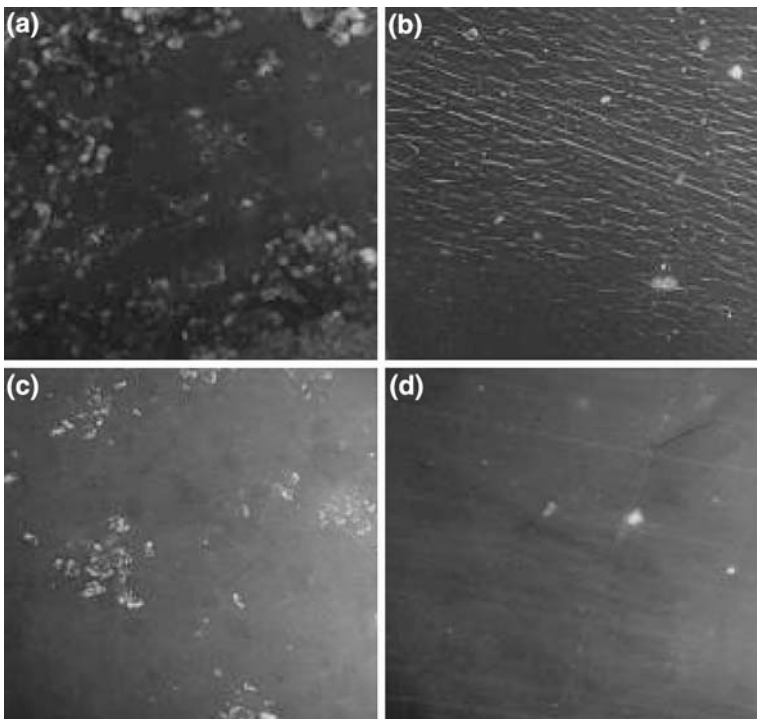

Fig. 2 SEM of composite films 500× of magnification (**a**) Film 1003, (**b**) Film 1005, (**c**) Film 1013 and (**d**) Film1009

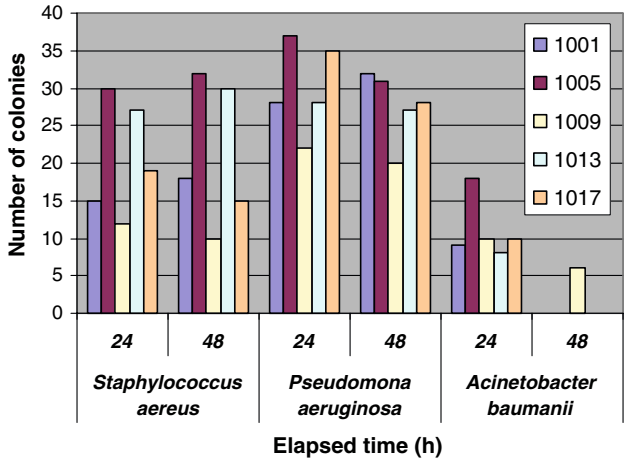

Fig. 3 Antimicrobial activity of chitosan composite films

frequently required removal of the wound dressing and excision of cutaneous wound [18–23].

On Fig. 3 we can see a comparison between the chitosan composite solutions of low (1001) and high (1017) molecular weight, the figures show the number of colony that have grown after 24 and 48 h. The x-axis shows the elapsed time since the beginning of the culture and the y-axis shows the number of the colony that were found, each colony should be formed from a cell of microorganisms. The chitosan activity over gram negative bacteria is higher than the activity over gram positive bacteria.

3.5 Medical applications

In order to show the chitosan acetate and chitosan acetate composite films properties as wound healing, two set of films were prepared and use in medical applications. Some medical reports about the applications are presented here.

3.5.1 Case 1 (chitosan acetate with glycerol film)

A male patient of 21 years old. A tattoo from the left arm was removed. The cruent area from the thigh, skin donator

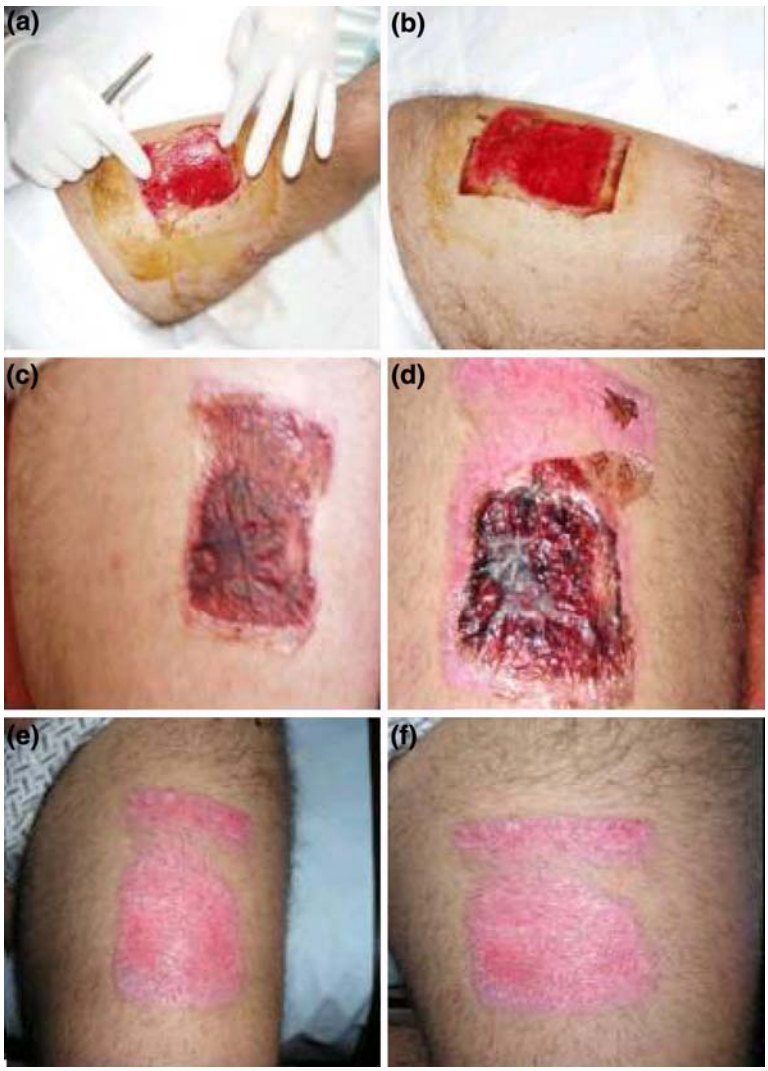

Fig. 4 Films application on a tattoo removal. (**a**) The film set up, (**b**) The film after the application, (**c**) The third day evolution, (**d**) The sixth day evolution, (**e**) and (**f**) Fourteenth day of evolution

for skin graft to cover the defect originated to remove the tattoo on another extremity were covered with a composite film.

The Fig. 4(a) shows film set up previous compressive hemostasis, (b) shows the film after application.

The Fig. 4(c) shows the third day evolution. An absence of liquid collections and the presence of clots under the film are observed. On 4(d) it is possible to see the sixth day evolution, there is partial epithelialization and some partial film falls off is observed.

The Fig. 4(e) Shows a great degree of epithelialization. Figure 4(f) the injury after 14th day evolution.

3.5.2 *Case 2 (chitosan + oleic acid)*

A male patient of 23 years old. Also is a case of covering the skin donor area in the muscle. The objective was to remove the skin for skin graft damage produced by an expose fracture of the patient.

The Fig. 5(a). The film was placed with sterilized instrument avoiding the presence of bubbles under the film. Figure 5(b) shows the initial retraction of the film in their periphery after 3 day. At the same time permanent epithelialization with some clots are observed. Figure 5(c) shows a positive centrifuge epithelialization and the films on the boundaries is detached. On 5(d), the epithelialization after 12 days it is showed.

3.5.3 *Case 3 (chitosan + oleic acid)*

A male patient 43 years old. A burn with 1/3 of the superior extremity is damaged. The diagnosis indicates 1.5% of the body surface with AB/B type burn is damaged. A scarf is present on the surface.

The Fig. 6 (a) and (b) shows a 6th day of evolution, showing epithelialization almost complete and the film is gradually removed. Local hyperemia and absence of bleeding areas is observed. On 6 (c) and (d), it is possible to see the evolution after 15th days. Complete epithelialization, good appearance of the scarf with original functionality.

4 Conclusions

Several types of chitosan composites films were obtained. Different amounts were tested in order to obtain the best films being chitosan 1% concentration the most appropriate.

The FTIR allows to identify the presence of the additives in the films, the presence of the additives modifies the physical characteristics of the films.

The films exhibit a good effect in the control of the most relevant intra hospital bacteria that infest the burn injuries. The bacterial activity shows a relative dependence of the chitosan molecular weight.

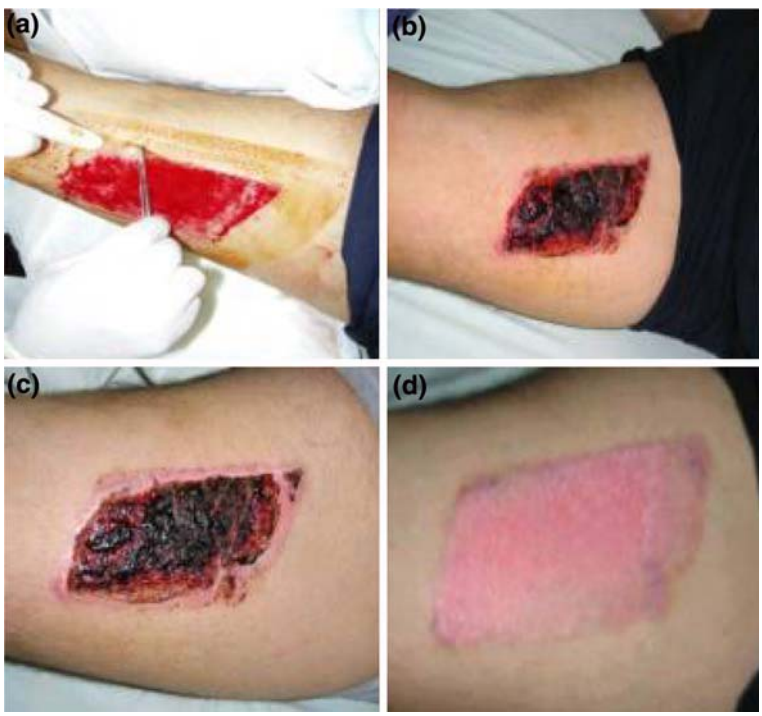

Fig. 5 Films application on a expose fracture. (**a**) The film placed on the skin donor place, (**b**) Third day evolution (**c**) The ephithelization (**d**) After 12 days

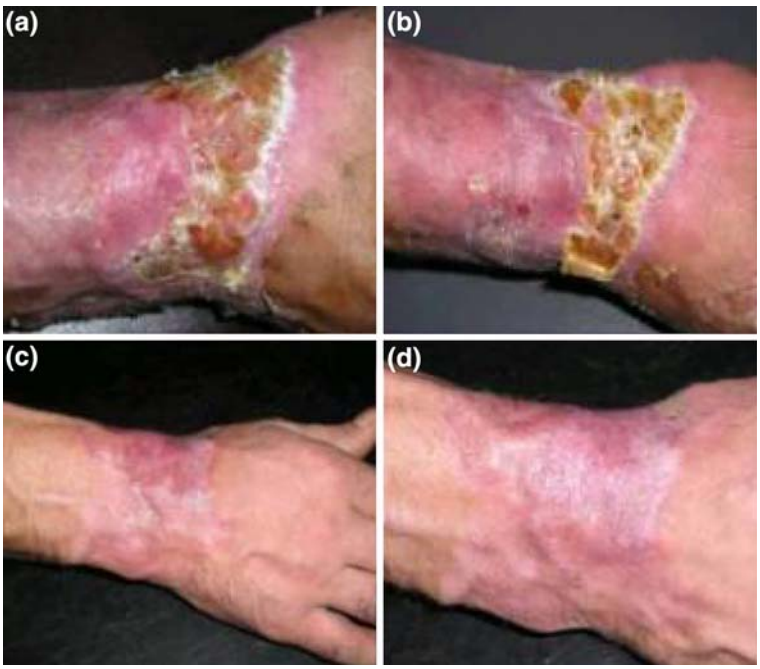

Fig. 6 Films application on a A/AB burn. (**a**) and (**b**) Sixth day evolution, (**c**) and (**d**) Fifteenth day evolution

The SEM shows the different characteristics of the surface and porosity of the film depending on the additive. The porosity of the films is important for oxygen permeability which was achieved with this composites.

The complete epithelialization was observed in the patients treated with the composite films.

Ackowledgements The authors would like to thank the financial supports from Innova Bío-Bío (Grant 03-B1-212-L1). P.A. would like to thank the scholarship from CIPA.

References

1. S. M. HUDSON, *J. Mater. Sci.* **C34**(3) (1994) 375
2. I. V. YANNES, J. F. BURKE, D. P. ORGILL and E. M. SKRABUT, *Science* 215 (1982) 174
3. I. V. YANNES and J. F. BURKE, *J. Biomed. Mater. Res.* **14** (1980) 65
4. K. MASTSUDA, S. SUSUKI, N. ISSHIKI, K. YOSHIOKA, R. WADA, S. H. ION and Y. ISADA, *Biomaterials* **14** (1993) 1030
5. L. J. HINRICHS, E. J. LOMNEN, C. R. H. WILDERRUR and J. FEIJEN, *J. Appl. Biomater*. **3** (1992) 287
6. Y. W. CHO, Y. N. CHO, S. H. CHUNG, G. YOO and S. W. KO *Biomaterials* **20** (1999) 2139
7. Y. OHSHIMA, K. NISHINO, Y. YONEKURA, S. KISHIMOTO and S. WAKABAYASHI, *Eur. J. Plast.Surg*. **10**(2) (1987) 66
8. H. UENO, H. YAMADA, I. TANAKA, N. KABA, M. MATSURA, M. OKUMARA, T. KADOSAWA and T. FUJINAGA, *Biomaterials* **20** (1999) 1407
9. K. KOJIMA, Y. OKAMAOTO, K. MIYATAKE, Y. KITAMURA and S. MINAMI, *Carbohydr. Polym.* **37** (1998) 109
10. Y. USAMI, Y. OKAMAOTO, S. MINAM, A MATSUSHASHI, N.H. KUMASAWA, S. TANIOKA and Y. J. SHIGEMASA, Vet. Med. Sci. **56** (1994) 761
11. G. CÁRDENAS, O. PARRA and E. TABOADA, *Int. J. Biol. Macromol*. **28** (2001) 167
12. E. TABOADA, Ph.D Thesis, Estudio de la Retención de Metales Pesados desde Residuos industriales usando Quitosana y Derivados. Universidad de Concepción (2003)
13. J. BRUGNEROTTO, J. DESBRIÉRES, L. HEUX, K. MAZEAU and M. RINAUDO, *Macromol. Symp*. **168** (2001) 1–20
14. L. CRUZAT, A. GARCIA, G. CÁRDENAS, J. BECERRA and S. P. MIRANDA, *Biological Res.* **3–4** (2003) 58
15. L. CRUZAT, Pharmacy thesis, Síntesis y Caracterización de Películas de Quitosano, U. de Concepción, 2003, Chile
16. (i) S. P. MIRANDA, G. CÁRDENAS, O. GARNICA and V. LARA-SAGAHON, *J. Chil. Chem. Soc*. **49** (2004) 173. (ii) G. CARDENAS and S. P. MIRANDA, *J. Chil. Chem. Soc*. **49** (2004) 291
17. R. SILVERSTEIN, C. BASSLER and T. MORRILL, in "Spectrometric Identification of Organic Compounds" 5th Ed. (Wiley, 1990)
18. Y. KUROYANAGI, Y. KGANEI and N. SHIOYA, *Jpn. J. Burn Inj.* **13** (1987) 206
19. C. L. FOX, B. W. RAPPOLE and W. STANFORD, *Surg. Gynecol. Obstet.* **128** (1969) 1021
20. D. G. SAYMAN, P. NATTHAN and I. A. HOLDER, *Appl. Microbiol.* **25** (1973) 921
21. C. EDWARD, B. S. ROBB and P. WATHAN, *J. Trauma* **21** (1981) 889
22. R. MUZZARELLI, R. TARSI, O. FILIPPINI, E. GIOVANETTI, G. BIAGNINI and P.E. VARALDO, *Antimicrob. Agents Chemother.* **34** (1990) 2019
23. R. A. MUZZARELLI, A. ISOLATI and A. FERRERO, Editors, in "Chitosan Membranes. Ionic Exchange and Membranes" Vol 1 (Gordon and Breach, 1974), p. 193