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Guillaume Conzatti, Shaan Chamary, Nathalie de Geyter, Sandrine Cavalie, Rino Morent, Audrey Tourrette

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G. Conzatti[a], S. Chamary[a], N. De Geyter[b], S. Cavalie[a], R. Morent[b], A. Tourrette[a,*]

[a] Université de Toulouse, CIRIMAT, UPS-INPT-CNRS, Faculté de Pharmacie, 118 Route de Narbonne, 31062 Toulouse cedex 4, France
[b] Research Unit Plasma Technology (RUPT), Department of Applied Physics, Faculty of Engineering & Architecture, Ghent University, Sint-Pietersnieuwstraat 41 B4, 9000 Ghent, Belgium

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ABSTRACT

Chitosan films were formulated and subsequently given thermosensitive properties by modifying the surfaces. The first step involved the incorporation of poly(ethylene glycol) (PEG) plasticizers at various ratios into the chitosan blends. Tensile tests showed that the molecular weight of the PEG impacted the mechanical properties, while the plasticizing effect was optimal for a PEG content of 20%. The addition of glycerol, in combination with PEG, increased the elongation at break without altering the tensile strength. In order to add thermosensitive properties to the chitosan films, poly(N-isopropylacrylamide) (PNIPAM) was graft polymerized on the surface via two methods: UV irradiation or plasma treatment. The surface modification was evaluated in terms of surface characteristics (XPS, SEM, contact angle) and bulk swelling abilities, with a specific focus on the thermosensitive nature due to the lower critical solution temperature (LCST) transition of the PNIPAM. The two grafting methods implied differences in terms of thermal responsiveness. Indeed, due to the presence of particles on their surfaces, UV grafted samples exhibited higher hydrophilicity and thermosensitivity, whereas their lower content of PNIPAM involved lower swelling thermal dependence. All of these results support the interest in PNIPAM grafted chitosan surfaces for the development of smart biomaterials.

1. Introduction

There is great interest in the development of smart biomaterials as results are widely encouraging. For example, wound dressings are common devices that can be used for absorption or drug delivery purposes. However, some considerations must be taken into account. For example, fabrication cost is a major issue, as expensive polymers are sometimes required to create efficient products. To overcome this issue, the use and compatibility of natural and inexpensive biopolymers have to be determined. Among several polymers, polysaccharides have already been intensively studied as potential biomaterials [1]. In particular, chitosan is already used in various biomedical applications, such as innovative drug carriers [2] or wound dressings [3]. This biopolymer, derived from the widely available chitin, is rather inexpensive as it can be obtained from a simple and well known process. Besides being biocompatible, polysaccharide can also possess various properties depending on its molecular weight and deacetylation degree (DA) and can even show antimicrobial activities.

Unfortunately, chitosan based films have the disadvantage of being brittle. Nevertheless, this problem can be resolved by the addition of a plasticizer. However, the choice of potential plasticizers is limited, as they should be compatible with a biomedical application. Polyethylene glycol (PEG) and glycerol have been shown to be good candidates for this purpose [4 6]. These two polymers act by different mechanisms. Glycerol is a small molecule that strongly interacts with acetamide groups of chitosan (internal plasticizer), avoiding the formation of H bonds between polymer chains. PEG, a larger molecule, is mobile in the blend and forms H bonds with acetamides (external plasticizer), increasing their mobility [5]. As these two plasticizers operate according to two different mechanisms, both of them can also be used as a chitosan plasticizer at the same time. In addition, it has also been shown that the molecular weight of PEG influences its ability to plasticize materials, both alone and in combination with glycerol [7].

At the same time, peeling off of the biomaterial used as a wound dressing is an important issue, as it can be painful for the patient. In order to overcome this problem, stimuli responsive surfaces can be used to tailor a controlled bioadhesion to the tissues, which would allow removing the biomaterial from the wound without further injuries. Stimuli responsive polymers exhibit a change in structure and conformation due an external stimulus, such as solvent composition, light,

* Corresponding author.
E-mail address: audrey.tourrette@univ-tlse3.fr (A. Tourrette).

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temperature, ionic strength or mechanical stress [8]. Poly(N iso propylacrylamide) (PNIPAM) is a thermosensitive polymer. This polymer has already been used to culture cells, and no toxicity reports have been found [9]. The PNIPAM has been of particular interest for several years as it shows a lower critical solution temperature (LCST), around 32 °C, which is between ambient and body temperatures [10]. This transition affects its hydrophilicity, being hydrophilic below this temperature and hydrophobic above it. This change in properties af fects its intermolecular interactions, especially with proteins involved in cellular adhesion (e.g. fibronectin). Thus, PNIPAM showed a thermo controlled bioadhesion, as used for the production of cell sheets [11]. This effect was particularly observed for cells, so a few studies recently also made the link with tissue adhesion, as it has been shown for retinal implants [12] or wound dressing [13,14]. Hence, PNIPAM grafted surfaces could be of particular interest to develop smart wound dres sings with thermo controlled skin adhesion.

The combination of chitosan based matrices with PNIPAM surfaces is an innovative strategy that allows for producing relatively in expensive biocompatible thermosensitive injectable hydrogels [15], water vapour permeable fabrics [16], flocculants [17] or multi functional thermo/pH dual responsive probes able to deliver drugs [18,19]. Various PNIPAM/chitosan grafting strategies are reported: “click” coupling [20], graft polymerization [21] using dispersion [16] or emulsion [22]. PNIPAM grafted on chitosan film surfaces are mostly used for cell sheet engineering [23,24] but are also suitable to develop smart thermosensitive wound dressing devices with easy peeling off properties. Only few studies report this type of system, which requires an efficient grafting of the thermosensitive polymer on the chitosan film without alteration of its integrity. Conventional graft polymerizations in liquid medium were reported [23] as well as NHS/EDC coupling [25]. UV and plasma induced graft polymerization are attractive be cause they are rapid and economical routes that, to the best of our knowledge, are not already reported for the grafting of PNIPAM on chitosan film surfaces, while it is the case for the PNIPAM grafting on poly(propylene) [26]. However, UV radiation induced graft poly merization has many additional advantages: (1) it causes no change in the inherent characteristics of the polymer, (2) the radiation used (gamma or UV rays) provides a sterilizing effect, (3) no potentially toxic initiators are needed and (4) no potentially hazardous chemical wastes are produced [27]. The technique has already been successfully used for the graft polymerization of PNIPAM on glass substrates, with the use of a cross linker [28]. Non thermal plasma induced graft polymerization is an equally suitable technique for surface modification, as it shares the same advantages as radiation induced graft polymerization. Usually two steps are needed: plasma surface activation (reactive chemical groups are incorporated on a surface by the interaction with plasma species) and bringing the activated surface into contact with monomers in order to induce polymerization (gaseous phase or liquid phase) [29].

In this work, the production of a robust chitosan material exhibiting a thermosensitive surface was explored through a novel, simple, eco nomic and two steps process. As a potential biomaterial and prior to surface modification, the plasticizer effects of PEG and glycerol, sepa rately and in combination, were studied in order to optimize chitosan’s mechanical properties. In addition, the influence of the PEG molecular weight (PEG 200 and PEG 400) on the plasticizing effect was evaluated. In a second step, the developed chitosan film surfaces were grafted with PNIPAM making use of both UV and plasma induced graft poly merization. Analysis of the grafted samples by various methods con firmed the presence or the absence of PNIPAM on the surface of the films. The behaviour of the films after grafting was also studied to see if the thermosensitivity of the polymer was impaired or modified by the performed grafting processes.

2. Materials and methods

2.1. Materials

Low molecular weight chitosan (M_w = 280 000 g/mol, degree of acetylation (DA) = 18%), PEG (200 and 400), glycerol (Gly), Acetic acid, Genipin, NIPAM, N,N′ methylenebisacrylamide (BIS), 2,2 di methoxy 2 phenylacetophenone (DMPAP), Ethanol (technical grade), Potassium persulfate (KPS) and NaOH were purchased from Sigma Aldrich.

2.2. Experimental methods

2.2.1. Preparation of chitosan physical gel

A chitosan solution was prepared by dissolving 3% (w/v) into an aqueous solution of acetic acid (1% (v/v)). The mixture was then me chanically stirred for about 12 h at 500 rpm. The mixture was sonicated in order to remove all the trapped bubbles and then poured into Petri dishes (40 g into 10 cm diameter Petri dishes). Mixtures were then dried at 50 °C for two days to obtain films. The films were removed from their moulds and the chitosan networks were neutralized with an aqueous solution of NaOH (1 M). Next, the films were rinsed with deionized water until the supernatant reached neutrality. Finally, the films were dried between two glass plates at room temperature (RT) for 48 h.

2.2.2. Incorporation of plasticizers

Two steps were added to the previous protocol in order to add the plasticizers. Once the chitosan was completely dissolved, the plastici zers were added (20 or 40% of the total polymer mass) to the mixture. The solution was then mechanically stirred at 500 rpm for 30 min. The plasticizers used were PEG and Gly, following the formulations listed in Table 1.

2.2.3. Surface functionalization

2.2.3.1. UV graft polymerization. Plasticized films (CS PEG400 GLY 20%, 1 g) were immersed into different solutions of reagents containing the monomer NIPAM (1.25 g, 111 mM) alone, or in combination with, a cross linker (BIS, 110 mg, 0.71 mM) and/or a radical initiator (DMPAP, 40 mg, 0.33 mM) in order to study their influence on the graft efficiency. Irradiation (λ = 365 nm) then took place for 40 min. The reactions were conducted at 70 °C for 2.5 h. The films were then thoroughly washed with an ethanol solution (80%) so that unreacted moieties could be eliminated. In a final step, the films were dried between glass plates at RT for 24 h.

2.2.3.2. Plasma graft polymerization. The plasma set up consisted of two circular copper plates with a diameter of 7 cm, both covered with a glass plate that acts as a dielectric barrier. The distance between the glass plates was 7 mm and the electrodes were placed within a cylindrical enclosure (inner diameter: 25 cm, height: 25 cm). The upper electrode was connected to an AC power source (frequency = 5 kHz), while the lower electrode was connected to earth through a capacitor of 10 nF. After placing the chitosan film on the lower glass plate, the chamber was evacuated by a rotary vane

Table 1
Formulations of the produced chitosan films.

Film code	PEG 200	PEG 400	Glycerol
CS	–	–	–
CS-PEG200-20%	20%	–	–
CS-PEG200-40%	40%	–	–
CS-PEG400-20%	–	20%	–
CS-PEG400-40%	–	40%	–
CS-PEG200-Gly20%	20%	–	20%
CS-PEG400-Gly20%	–	20%	20%

pump below a pressure of 2 kPa. Next, the plasma reactor chamber was filled with dry air (Air Liquide Alphagaz 1) to reach a pressure of 50 kPa. Afterwards, the chamber was again pumped down to 5 kPa while a constant air flow of 1 standard litre per minute (slm) was fed between the glass plates. By slightly pumping, the pressure in the discharge chamber was maintained at 5 kPa. After that, the AC power source was turned on and one side of the chitosan film was plasma treated for 20 s. In a next step, the chitosan film was flipped and the other side of the film was also plasma treated for 20 s using the same procedure as described before. A high voltage probe (Tektronix P6015A) was also connected to the upper electrode to measure the high voltage applied to the reactor, while at the same time the charge stored on the electrodes was obtained by measuring the voltage over the capacitor connected in series to the ground. Both waveforms were then recorded using an oscilloscope (Tektronix TDS210, 60 MHz) enabling the visualization of a Lissajous figure, which permitted determination of the plasma discharge power, which was found to be equal to 2.1 W in this work [30,31].

After plasma modification, the chitosan films (CS PEG400 GLY 20%, 1 g) were immersed into different solutions (H_2O, 200 mL) of reagents, containing the monomer (NIPAM, 1.25 g, 111 mM) alone, or in combination, with a cross linker (BIS, 110 mg, 0.71 mM) and/or a radical initiator (KPS, 90 mg, 0.33 mM). The reactions were conducted at 70 °C for 2.5 h. The films were then thoroughly washed with an ethanol solution (80%) to eliminate unreacted species. As a final step, the films were dried between glass plates at RT for 24 h.

2.3. Evaluation methods

2.3.1. Mechanical properties analysis

To characterize the mechanical properties of the chitosan films, tensile strength (TS) and elongation at break (EB) of the films were determined. For this purpose, small samples were cut out of the chitosan films and kept, prior to measurements, in a climate chamber at 25 °C and 60% relative humidity for five days to control the hydration state. In a next step, the samples were placed in a tensile tester (HSKT, France Scientifique) loaded with a 25 N load sensor. The samples were fixed between two crossheads covered with silicon gum to prevent slipping, after which the upper crosshead was driven upwards at a speed of 10 mm/s until breakage of the sample. During this process, the sample elongation was recorded against the applied force. The TS (MPa) of the sample was then calculated by dividing the maximum force (N) before breaking by the cross section area (mm^2) of the sample. In addition, the EB (%) of the films was determined as the ratio between the maximal extension of the sample before breaking and the initial length of the sample. To perform these calculations, each sample thickness was measured with a hand held micrometre and thickness measurements were conducted across each sample to determine an average thickness value.

2.3.2. X ray photoelectron spectroscopy (XPS)

The chemical composition of the chitosan films was obtained by XPS. This technique provided an insight in the chemical functionalities introduced on the chitosan surface by the different applied surface functionalization methods. The XPS measurements were performed on a K Alpha spectrometer (Thermo Scientific) employing a monochromatic Al K_α X ray source ($h\nu = 1486.6$ eV). Survey and high resolution scans were recorded at a take off angle of 90° relative to the sample surface with a pass energy of −2 eV on a sample size of 400 μm^2. The XPS scans were processed using Advantage software and from the peak area ratios, the elemental composition of the chitosan samples was determined.

2.3.3. Scanning electron microscopy (SEM)

The chitosan film coating morphologies were visualized using a scanning electron microscope fitted with a field emission gun (SEM FEG Quanta 250 FI) operating at 5 kV. Prior to observation, the samples were coated with 5 nm platinum. Observations were carried out at 2 distinct magnifications: 2000× and 20,000×.

2.3.4. Swelling tests

Swelling tests were carried out in order to assess the thermo sensitivity of the chitosan films. First, all samples were weighed and then were immersed in water at two different temperatures: 25 °C and 50 °C. After 4 h of water immersion, the samples were taken out, dried on absorbent paper and weighed again. Based on the weighing results, the swelling percentages of the chitosan films were determined through the following equation:

$$Swelling\ (\%) = ((W_t -- W_0))/W_0) \times 100$$

where W_t is the weight of the immersed sample and W_0 its initial weight.

2.3.5. Captive bubble

The hydrophilicity of the chitosan films was determined by means of the captive bubble method. For this purpose, the sample was first immersed in a thermo regulated water bath at 25 °C or 37 °C. Afterwards, an air bubble was slowly produced underneath the sample making use of a needle. In a final step, the contact angle between the air bubble and the surface was measured using a GBX DIGIDROP analyser.

3. Results and discussion

Mechanical properties are important factors that have to be taken into account when designing a biomaterial. For instance, a wound dressing should allow plastic deformation (high elongation at break, EB) to be compliant with the morphology and skin deformation. It should also be resistant enough (high tensile strength, TS) so that tearing of the film does not occur during storage, usage and peeling off. The major difficulty in plasticizing is that it results in an increase of the EB value combined with a decrease of TS while plasticizing. Although the first effect is favourable for wound dressing handling, the second one can lead to weak materials which cannot be used in several applications. Thus, an equilibrium has to be found between the two parameters, EB and TS.

3.1. Optimization of chitosan films

In order to improve the chitosan film mechanical properties, glycerol and PEG plasticizers were selected, as they have shown interesting properties on chitosan film plasticizing [6]. Two different molecular weights of PEG, namely PEG 200 and PEG 400, were studied alone and in combination with glycerol (see Table 1 and the resulting mechanical properties of the chitosan films analysed in Table 2). The introduction of PEG to rigid and brittle chitosan increased its elongation at break (EB). As the PEG content increased, it was also shown that the plasticizing effect diminished, both for CS PEG200 and CS PEG400. Indeed, the EB value increased respectively from 16.2% for CS to 40% and 36.9% for CS PEG200 20% and CS PEG400 20%, respectively, but then

Table 2
Elongation at break (EB) and tensile strength (TS) of chitosan films plasticized with PEG and glycerol at various concentrations.

Film designation	EB (%)	TS (MPa)
CS	16.2 ± 2.2	58.9 ± 2.9
CS-PEG200-20%	40.0 ± 4.7	35.7 ± 11.8
CS-PEG200-40%	24.9 ± 6.3	64.9 ± 11.3
CS-PEG400-20%	36.9 ± 0.5	49.1 ± 1.4
CS-PEG400-40%	26.4 ± 3.7	65.2 ± 2.4
CS-PEG200-Gly-20%	27.0 ± 4.2	44.1 ± 1.1
CS-PEG400-GLY-20%	59.6 ± 4.6	49.0 ± 4.1

decreased to 24.9% and 26.4% for CS PEG200 40% and CS PEG400 40%, respectively.

At the same time, the tensile strength (TS) was also lowered with the addition of 20% PEG, with a TS of approximately 35.7 MPa and 49.1 MPa for CS PEG200 20% and CS PEG400 20%, respectively, while pure chitosan exhibited a TS of 58.9 MPa. It is also seen from the results presented in Table 2 that a high incorporation of PEG (CS PEG 200 40% and CS PEG400 40%) resulted in a rigidification of the chitosan blends, as shown by an increase of the TS (64.9 MPa and 65.2 MPa, respectively). The plasticizing effect (increase of EB, decrease of TS) of the PEG consisted of breaking polymer/polymer interactions, thereby increasing the mobility of polymeric chains [5]. The efficiency of PEG as a plasticizer is then dependent on its ability to create hydrogen bonds between the OH groups and amine groups present on chitosan. Moreover, it was observed that at high PEG concentrations, an anti plasticizing effect arose. This can be attributed to the fact that preferential PEG/PEG interactions occurred instead of PEG being intercalated between acetamide or amine groups [6]. Consequently, PEG and chitosan had a lower mobility in this case and a more organized, rigid structure was obtained.

These results are, however, contradictory with those obtained by Suyatma et al. on highly deacetylated chitosan (DA = 8%, low content of acetamide groups) [32]. No difference was observed by Suyatma's group between a composition of 20% and 40% of PEG400. In the present study, it was observed that the higher content of acetamide groups on the chitosan film tended to increase the anti plasticizing effect, presumably because the PEG had better interactions with the amines than with the acetamide groups, perhaps due to a lower steric hindrance.

The effect of the molecular weight of PEG is mainly visible for low plasticizer contents (20%). Sample CS PEG200 20% (TS = 35.7 MPa) had a slightly lower tensile strength than CS PEG400 20% (49.1 MPa). It was assumed that the smaller size of PEG chains allowed better intercalation, and thus better interactions with the chitosan, resulting in a more efficient plasticizing effect. This size effect was less significant for high plasticizer contents (CS PEG200 40% and CS PEG400 40%), which can again be attributed to the poor contribution of PEG/chitosan interactions occurring at these compositions.

Addition of glycerol to the blend increased the EB value for CS PEG400 GLY 20% (EB = 59.6%) compared to CS PEG400 20% (EB = 36.9%), whereas an anti plasticizing effect was observed for CS PEG200 GLY 20% (EB = 27.0%) compared to CS PEG200 20% (EB = 40.0%). Glycerol is a small molecule which is known to act like an internal plasticizer by reducing the mobility of acetamide groups, and thus limiting H bonds between polymeric chains [5]. Its interactions with smaller PEG chains were, perhaps, able to form PEG/glycerol hydrogen bonds or susceptible to be modified in a way that PEG/chitosan interactions were weakened. In any case, by adding glycerol, CS PEG400 GLY 20% showed a high EB value combined with a high TS. The combination of glycerol and PEG 400 (CS PEG400 GLY 20%) with these proportions showed an optimal mechanical performance. It was therefore chosen for the further study of thermosensitive chitosan surfaces through UV and plasma surface functionalization taking in consideration that both glycerol and PEG plasticizers are known to be relatively stable under UV [33 36] or plasma exposure [37,38].

3.2. Functionalization of chitosan films surfaces

Chitosan surfaces, as such, do not present thermosensitive skin adhesion properties and should be functionalized in order to achieve these properties. In this work, thermosensitive PNIPAM was used to graft chitosan surfaces through UV and plasma treatments in an effort to engineer chitosan films with thermosensitive bioadhesive properties (Fig. 1) [10]. The functionalized chitosan films were subjected to several characterizations to evaluate the surface composition, surface morphology and thermal behaviour of the functionalized chitosan films.

3.2.1. XPS characterization

Chitosan surfaces were grafted with PNIPAM by two different techniques: UV and plasma. In addition to the monomer, the effect of the addition of a cross linker and/or a radical initiator was also investigated. In order to evaluate the presence of PNIPAM on the chitosan films, sample surfaces were analysed by XPS (Table 3), as the nitrogen percentage in the film could be an indicator of the presence of acrylamides on the surface. Sample UV5 exhibited the highest nitrogen content (3.0%) compared to the reference sample (1.6%) and the other UV treated films. For these latter samples, as a result of the absence of a cross linker (UV2 and UV3) and radical initiator (UV2 and UV4), nitrogen content remained below 2%. When NIPAM was used only in combination with the radical initiator, no PNIPAM was detected on the chitosan samples, which is most likely due to the fact that PNIPAM was not bound to the surface. In addition, when NIPAM was only combined with the cross linker BIS, no PNIPAM was detected on the chitosan samples, probably due to the non polymerization of NIPAM, which made its detection on the surface difficult. One could hypothesize that the introduction of BIS, which contains nitrogen atoms, could artificially increase the N content on the chitosan surface due to possible weak interactions on the surface. However, the addition of BIS in case of the sample UV4 did not increase the nitrogen content (1.0%) compared to the sample prepared under the same conditions, but without BIS (UV2, 1.6%). The results shown in Table 3 also clearly reveal that PNIPAM was only detected on the chitosan surface when both the initiator and the cross linker were present. The initiator was required to initiate the NIPAM polymerization and to create radicals on the chitosan surface [39], while the cross linker subsequently allowed the formed polymers to bind to the radicals present on the chitosan films [40 43].

In contrast with UV polymerization, which needs a radical initiator, air plasma was able to produce radicals on the chitosan surface by the decomposition of formed peroxides. Polymerization could also occur when dipping the samples in a NIPAM containing solution. As the plasma treatment of chitosan was performed in air, the oxygen content on all plasma treated samples (P1, P2, P3 and P4) was higher than on the pristine chitosan film. This oxygen increase can be attributed to the formation of oxygen groups on the chitosan surfaces, an effect that is typically obtained after air plasma treatment [44 46]. In addition, the nitrogen content on the plasma treated chitosan sample (P1) was also higher compared to the untreated chitosan film. This nitrogen incorporation was most likely due to plasma induced surface etching, which eliminated non chitosan compounds (impurities) from the sample surface, resulting in an increased nitrogen content. When the plasma treated chitosan film was exposed to a NIPAM solution (sample P2), no significant increase in nitrogen content was detected. A considerable decrease in oxygen content was observed, suggesting that some NIPAM or PNIPAM was grafted on the chitosan surface, resulting in the coverage of the underlying oxygen enriched chitosan film.

When the cross linker BIS with its linker properties was added to the NIPAM solution, a higher nitrogen content (P3, 7.5%) could be detected on the chitosan surface, combined with a significantly lower oxygen concentration. This would be the result of the grafting of the NIPAM or PNIPAM, through the action of the surface radicals induced by the decomposition of peroxides created during the plasma process. However, the lifetime of these surface peroxides and radicals is very short and the radical number can already be significantly reduced by the time the sample is transferred to the reaction mixture. To reinforce the initiation of the polymerization, KPS can be added to the NIPAM solution, as it is known to spontaneously create radicals in solution. The addition of this radical initiator was found to strongly increase the nitrogen content up to 11.7% (P4) on the chitosan samples combined with a strong decrease in oxygen content, which can be explained by a more efficient NIPAM polymerization process. The results obtained in

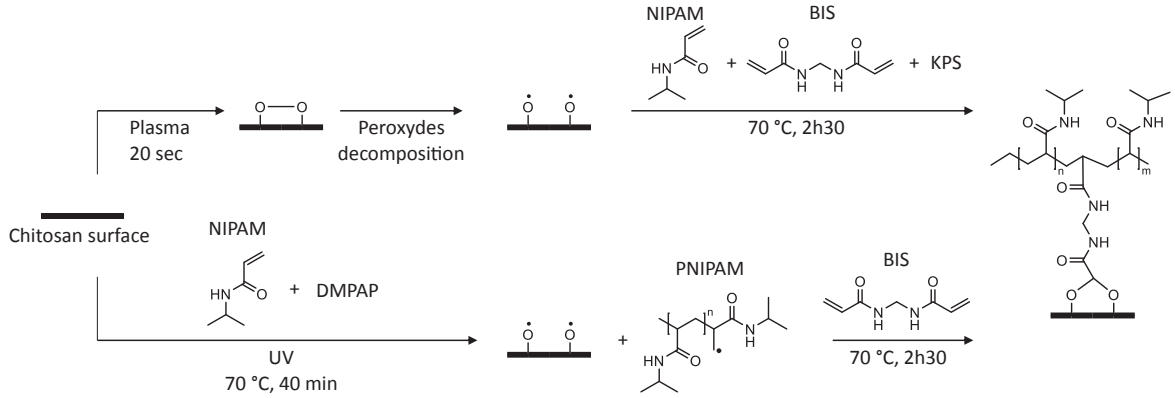

Fig. 1. UV and plasma grafting routes used to functionalize chitosan films.

Table 3
Atomic composition of different chitosan samples. Standard error associated with each measurement was < 5%.

	UV	Plasma	NIPAM	BIS	Radical Initiator	C 1 s (%) 285.0	N 1 s (%) 399.5	O 1 s (%) 532.3
CS-PEG400-Gly20%	–	–	–	–	–	82.5	1.6	14.4
UV1	X	–	–	–	–	78.1	2.2	17.1
UV2	X	–	X	–	–	81.8	1.6	13.4
UV3	X	–	X	–	DMPAP	81.1	1.0	16.1
UV4	X	–	X	X	–	81.6	1.0	14.1
UV5	X	–	X	X	DMPAP	72.9	3.0	21.5
P1	–	X	–	–	–	63.4	5.6	31.0
P2	–	X	X	–	–	69.4	5.8	23.5
P3	–	X	X	X	–	74.6	7.5	15.8
P4	–	X	X	X	KPS	73.7	11.7	13.9

this work thus show that plasma is a more efficient method for PNIPAM surface grafting than UV, perhaps due to a more efficient radical for mation on the surface of the chitosan films. However, due to the need of a radical initiator and the nature of the chemical bond, it was not possible to confirm whether the PNIPAM was bind through a covalent mechanism or not. In order to further study the presence, or not, of PNIPAM, surfaces were observed by SEM and sample thermo sensitivities were characterized by the swelling ability and surface hy drophilicities at two temperatures.

3.2.2. SEM imaging

The modified chitosan samples with the highest nitrogen content, namely P4 and UV5, were also observed by SEM and compared to a non modified chitosan reference (CS PEG400 GLY 20%, Fig. 2). The non modified chitosan surfaces were quite smooth as can be observed in Fig. 2(a) and (b). The UV treated samples (UV5, Fig. 2(c) and (d)) showed that in addition to the very thin PNIPAM coating, PNIPAM microaggregates were also homogeneously deposited all over the chit osan surface.

Based on these results, it can be assumed that the following process occurred: polymerization of the NIPAM in solution, leading not only to the surface coverage through the action of BIS (presumably very thin, but present as shown by the XPS results in Table 3), but also to the formation of PNIPAM aggregates on the chitosan surface. On the other hand, the plasma treated sample (P4, Fig. 2(e) and (f)) showed a homogeneous smooth surface, showing an efficient PNIPAM surface coverage as polymerization mainly occurred at the surface. These SEM observations revealed that the mechanisms of surface grafting were depended on the employed technique, being UV or plasma. The plasma induced grafting resulted into homogeneously covered chitosan sur faces, while the UV induced grafting resulted in multiple PNIPAM ag gregates on the chitosan surface.

3.2.3. Swelling properties

In order to study the thermosensitivity of chitosan films functiona lized with PNIPAM, swelling tests were conducted at two distinct temperatures: 25 °C and 50 °C (Fig. 3). These two temperatures were selected because the PNIPAM transition at 32 °C can be expected to influence the capacity of chitosan films to absorb water.

Swelling percentages obtained from CS PEG400 Gly 20% were used as a reference (Fig. 3, CS). It can be noted that the swelling percentage for non modified chitosan at 25 °C (80%) was lower than its swelling percentage at 50 °C (93%). The thermal energy input onto the matrix, which allowed its structure extension through thermal dilatation, can explain this trend. Differences in swelling percentage obtained at 25 °C and 50 °C were not significant for the UV treated samples (Fig. 3, UV2, UV3, UV4 and UV5). This can be explained by the fact that UV initiated surface polymerization was not effective enough, as measured by XPS. However, the adjunction of the BIS cross linker (UV4) involved a slight reduction of the swelling capacity of chitosan films (around 70% at both temperatures). Indeed, it is known that highly energetic radia tions, such as UV rays, tend to degrade chitosan and lead to the for mation of radicals, thus the chitosan's BIS cross linking could occur, resulting in a more rigid structure [47,48].

However, the use of a radical initiator (UV5), in addition to the BIS, led to a significant reduction of the swelling ability of the matrix, set around 65% at both temperatures, without significant thermal sensi tivity, despite the presence of PNIPAM measured by XPS. It seems that the amount of PNIPAM on the surface of UV5 was not sufficient to bring a thermal dependence of the swelling properties, and that an effective cross linking of the chitosan could occur in the same way as for UV4, namely due to the presence of radicals.

No statistical difference was observed for the P2 and P3 samples between the water uptakes at the two temperatures, with swelling percentages of approximately 95% for the latter. This suggests that the amount of PNIPAM on the P3 sample surface was not sufficient to counterbalance the thermal dilatation. As a result, it was difficult to

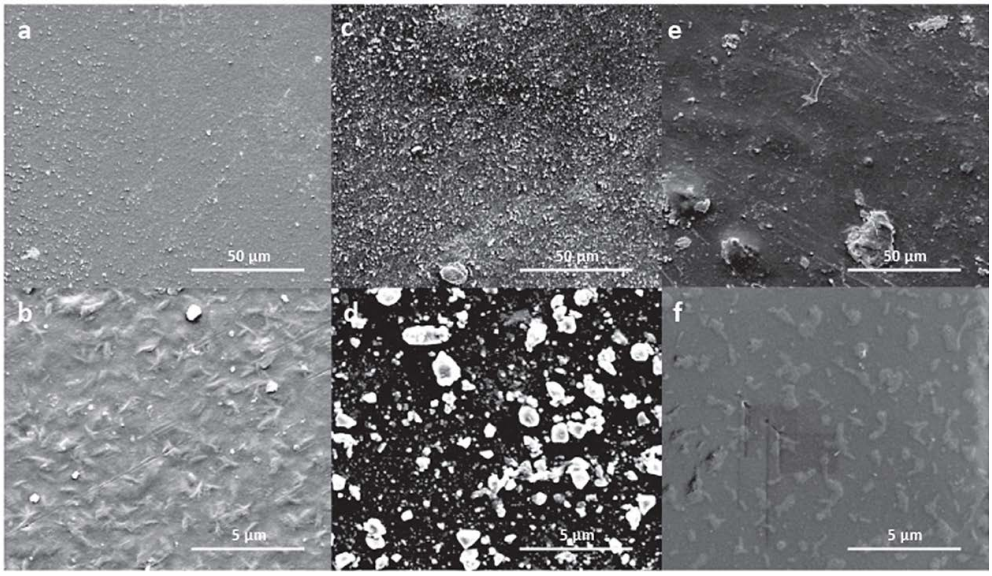

Fig. 2. SEM images of (a,b) CS-PEG400-GLY-20%, (c,d) UV5 and (e,f) P4.

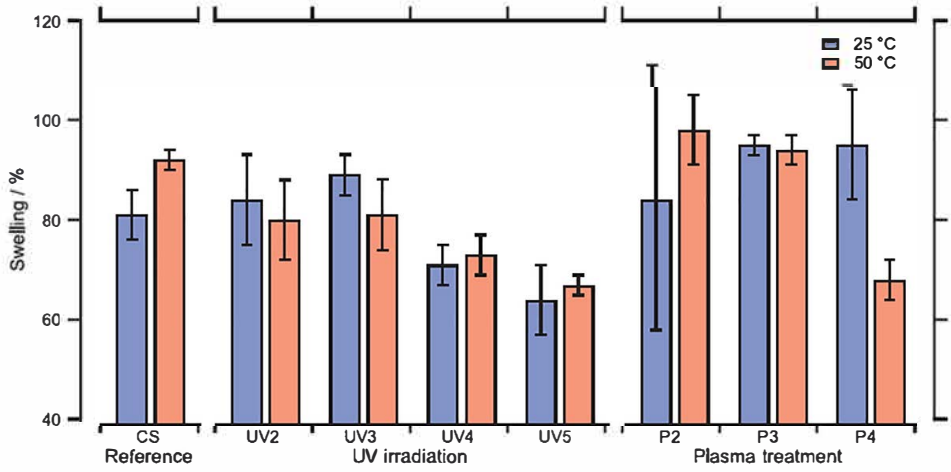

Fig. 3. Swelling properties in an aqueous medium for 4 h. Chitosan reference (CS) refers to CS-PEG400-Gly-20% without chemical modification.

confirm whether the nitrogen content measured by XPS was due to the monomer, which has no temperature sensitiveness, or the polymer, or a mixture of both. The sample P4 showed 96% of swelling at 25 °C, whereas it only exhibited a 69% of swelling at 50 °C. For this sample, the hydrophobicity of the PNIPAM grafted surface strongly decreased the ability of the material to absorb water above the PNIPAM LCST, leading to a thermosensitive effect. The plasma activated P4 sample additionally showed a thermosensitivity, whereas UV5 did not, in re lation to the higher amount of PNIPAM found on these sample surfaces owing to XPS analyses. Due to their water uptake abilities, all of these materials could be used for the treatment of moderately exudative wounds.

3.2.4. *Surface properties*

Fig. 4 shows the angle of a captive air bubble in aqueous media. With this technique, contrary to classic water drop contact angles, the higher the contact angle is, the higher the hydrophilicity. A slight sta tistical difference was observed for non modified chitosan, with an in crease from 102° to 112° (Fig. 4, CS, refers to CS PEG400 Gly 20%). Fig. 4 also shows that no statistical difference in bubble angles can be observed between the untreated chitosan film and the samples UV2, UV3 and UV4, which is consistent with the obtained XPS results showing no additional nitrogen content on these samples. At the same time, a significant increase of the angle, i.e. hydrophilicity, occurred for the samples UV4, UV5, P2, P3 and P4 at 25 °C. In case of the samples P2 and P3, this hydrophilicity increase was most likely due to the increase of the oxygen rates on these surfaces as measured by the XPS. Fig. 4 also shows that no statistical differences can be observed in the captive bubble angles at 25 °C and 37 °C for samples UV2, UV3, UV4, P2 and P3. This is consistent with the previously obtained XPS results showing no, or only a small increase, in nitrogen content on these chitosan films. For the two other samples UV5 and P4, however, the captive bubble angles at 37 °C are significantly lower than the ones at 25 °C, suggesting a hydrophobic transition between 25 °C and 37 °C, which is consistent with the presence of PNIPAM on these chitosan surfaces as observed from XPS. A higher surface thermosensitivity was observed for the sample UV5, which could be attributed to the presence of PNIPAM particles on the surface structure, resulting in a higher water/surface contact area.

Based on the above mentioned results, some general conclusions can be drawn:

(i) The presence of BIS (UV5) supported the grafting of NIPAM and/or PNIPAM, resulting in a strong increase of the surface hydro philicity.
(ii) The addition of a radical initiator (UV5), DMPAP, resulted in

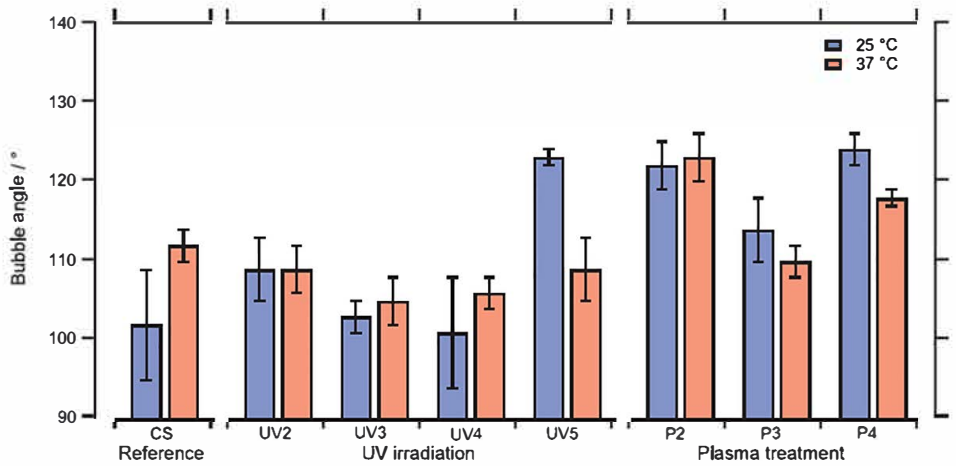

Fig. 4. Thermoregulated captive bubble angles of an air bubble in an aqueous media. Chitosan reference (CS) refers to CS-PEG400-Gly-20% without chemical modification.

thermosensitivity for the surface hydrophilicity. This confirmed the radical polymerization of NIPAM into PNIPAM, while no macroscopic thermosensitivity was observed on the swelling abil ities of the films. In addition, UV graft polymerization involved a significant decrease of these swellings, which would have a nega tive impact for wound dressing applications.

(iii) The plasma induced graft polymerization presumably allowed grafting of a small amount of NIPAM and/or PNIPAM on the sur face (P2).

(iv) The efficient grafting of PNIPAM was only achieved when BIS and a radical initiator were introduced through the use of KPS (P4), showing the low radical initiating efficiency of plasma when used alone.

(v) Compared to UV graft polymerization, plasma treatments obtained better swelling in addition to a surface sensitivity, making these materials attractive for the elaboration of themoregaluted skin bioadhesion innovative wound dressings.

4. Conclusions

Chitosan films were enhanced by strengthening their mechanical properties via plasticizing and subsequent functionalizing through UV and plasma induced polymerization to obtain thermosensitive proper ties. The combination of the two plasticizers, PEG and glycerol, is thus an interesting pathway. The optimal CS PEG400 GLY 20% was then functionalized by UV and plasma induced surface grafting. Both a cross linker and a radical initiator were needed to ensure an efficient graft polymerization. In such conditions, both UV and plasma treated samples exhibited thermosensitive properties: UV irradiated samples exhibited a better surface hydrophilicity and thermosensitivity, pre sumably due the presence of particles on its surfaces, whereas plasma grafting induced swelling thermosensitiveness confirming the larger amount of PNIPAM deposited through this procedure. Medium sup puration wounds would be an application target, but the adjunction of alginate could be an interesting insight to optimize the swelling ability of these biomaterials [49,50], and relationships between surface sen sitivity and bioadhesion toward, for example, skin still have to be in vestigated.

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