Thermosensitive PNIPAM grafted alginate/chitosan PEC

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ABSTRACT

Smart biomaterial functionality such as controlled adhesion properties is crucial to limit strip-off injuries. Among functional polymers, poly-N(isopropylacrylamide) (PNIPAM) allows surface properties to be changed depending on the temperature, with a transition of its properties that occurs around 32 °C, called the lower critical solution temperature (LCST). This transition is expected to modify surface interactions. Alginate and chitosan are biocompatible polymers commonly combined as polyelectrolyte complex (PEC) and are suitable for wound dressing applications. As a complex system, however, it is not so trivial to achieve an efficient functionalization. Herein, we elaborated a procedure to functionalize the surface of alginate/chitosan PECs without altering their intrinsic properties. FTIR revealed that acidic treatment led to a partial decomplexation of the PECs. Therefore, while the N-Hydroxysuccinimide/N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (NHS/EDC) coupling usually requires an intermediate pH, we showed that a preliminary acidification seemed to increase the surface grafting efficiency. Water contact angle increased from 51° to 72°, showing that PNIPAM enhanced the surface hydrophobicity. The LCST transition modified the interaction forces between PNIPAM and model surfaces: it revealed an unexpected thermosensitive behaviour as hydrophobic transition favoured interactions with hydrophilic surfaces. It was presumably due to PNIPAM/PEC substrate interactions. Finally, the surface modification did not affect the release properties of the PEC biomaterial.

1. Introduction

Alginate and chitosan are well known polysaccharides that are widely used in the biomedical field [1,2]. Biocompatible, hemostatic and resorbable, these two polymers are commonly used as wound dressings [3 6]. It can, however, be required to control their adhesiveness toward tissues to limit injury during the application or to facilitate their manipulation. Adhesiveness toward tissues, i.e. bioadhesion, is controlled by the surface properties, which can be tailored by the grafting of a functional polymer, however. Poly N(isopropylacrylamide) (PNIPAM) is a smart thermosensitive polymer that is undergoing intense evaluation, as, for example, smart hybrid drug delivery vesicles or micelles [7,8]. This polymer changes its properties at its lower critical solution temperature (LCST), around 32 °C, changing its conformation. Below its LCST, interactions with water are favoured through its hydrophilic moieties, while above 32 °C, and thus at body temperature, the polymer externalizes its hydrophobic moieties to favour polymer/polymer interactions [9]. As a result, PNIPAM is able to give rise to smart surfaces with the desired thermocontrolled bioadhesion [10]. However, in order to facilitate surface characterization, PNIPAM grafted surfaces are most often elaborated from inorganic well controlled substrates such as gold [11,12], silica [13], and glass [14], although some polymer grafted surfaces such as polyethylene terephthalate (PET) [15], polycaprolactone (PCL) [16], or cellulose [17 19] are also reported.

A challenging task consists of the grafting of such smart polymers on more complex substrates such as an alginate/chitosan matrix surface. These two polymers are commonly combined as a so called complex polyelectrolyte (PEC) through the interaction of the anionic carboxylic groups of alginate and the protonated amines of chitosan. Lawrie et al. suggested that it is not so trivial to evidence the interactions of these polymers within the PECs [20]. However, to achieve an efficient surface functionalization, it is necessary to develop a strategy to ensure the availability of the reactive groups involved in the complex.

To the best of our knowledge, there are no studies reporting the grafting of PNIPAM in a brush structure on the surface of alginate/chitosan PEC films. The surface modification of such systems requires chemical modification to be performed in a solvent that does not allow the swelling of the PECs. As the nature of PECs involves interactions between the reactive moieties of alginate and chitosan, a low reactivity

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of the biomaterial toward the PNIPAM is expected. The study of the evolution of these polysaccharides is proposed in order to evaluate al ginate/chitosan interactions such as the elaboration of a method al lowing the enhancement of their reactivity for PNIPAM grafting. After their functionalization, we report the study of the surface properties, especially in terms of interactions with various surfaces through the measurement of their adhesive force below and above the LCST of PNIPAM.

2. Materials and methods

2.1. Materials

Sodium alginate, chitosan, calcium chloride, acetic acid, ethanol, phosphate buffer saline (PBS), poly (N Isopropylacrylamide) (PNIPAM, Mw = 5000 g mol^{-1}), hydrochloric acid, N Hydroxysuccinimide (NHS), sodium hydroxide, ammonia, N (3 Dimethylaminopropyl) N′ ethylcarbodiimide hydrochloride (EDC), and dimethylsulfoxide (DMSO), were provided by Sigma Aldrich. Eosin was furnished by DistriBS.

The alginate and chitosan were characterized as previously reported [21]. The G/M unit ratio of alginate was estimated using a water sup pressive NMR method to M/G = 2.7 [22]. Using the Mark Houwink equation, viscosimetry revealed the molecular weight to be 340 kDa, with α and κ parameters taken as 1.13 and 6.9 × 10^{-4} mL g^{-1} [23]. Using the Shigemasa method, the degree of acetylation (DA) of chitosan was estimated to be 23% [24] and Mw = 1 300 kDa (α = 0.93, κ = 1.81 × 10^{-1} mL g^{-1} [25]).

2.2. Biomaterials elaboration

2.2.1. Alginate/chitosan PEC film elaboration

The protocol for the elaboration of PEC films was derived from a previous study [21,26]. The sodium alginate was dissolved in water with a concentration of 1.5% w/v and mechanically stirred overnight (500 rpm). In parallel, the chitosan was dissolved in slightly acidic water (acetic acid, 0.34% v/v) with a polymer concentration of 1.5% w/v and mechanically stirred overnight (500 rpm). After both polymers were dissolved, a mix of 50 g of the alginate solution and 30 g of the chitosan solution was homogenised with an Ultra Turrax (11 000 rpm) for 10 min. After the PEC formation, the mixture was poured into a Petri dish and dried at 50 °C for 12 h. These films are referenced as **PEC**. If reticulated, the films were further treated with a $CaCl_2$ solution (20 mL, 1% w/v) for 1 h 30 min and then dried for another 12 h to obtain thin semitransparent PEC films, referenced as **PEC-$CaCl_2$**.

2.2.2. PNIPAM grafted PEC synthesis

Commercial PNIPAM (5000 g/mol) was grafted on the PEC by an NHS/EDC coupling. Briefly, the PNIPAM COOH (1.6 mmol/L) was activated for 3 h with NHS (17.5 mmol/L) and EDC (35.0 mmol/L) in DMSO (20 mL), after which the PECs (100 g) were added. The mixture was left to react for 12 h on a gyratory rocker. The films were then thoroughly washed two times in a DMSO bath then two times in an EtOH bath under slight agitation for 10 min to remove any unreacted species.

2.3. Characterizations

2.3.1. FT IR spectroscopic study: influence of an acidic treatment on PECs

In order to study the influence of an acidic pH on the alginate, chitosan and their blend, samples (20 × 20 mm) were treated in various media (Table 1). After the desired time, the samples were dried (50 °C) and ATR FTIR spectra were recorded on a Perkin Elmer Frontier spec trometer, with a resolution of 4 cm^{-1} and 32 accumulations.

For some samples, a second treatment in DMSO (acid free) was performed for 3 h. The drying procedure and ATR FTIR spectroscopy were performed similarly as described above.

Table 1
Medium used for treatments of PECs.

Solvent	Acid	pH
Water	HCl	2.5
DMSO	HCl	2.5

2.3.2. XPS analysis

X ray photoelectron spectroscopy (XPS) was performed on an ES CALAB 250, Thermo Electron. The excitation was monochromatic, Al Kα ray at 1486.6 eV. The diameter of analysis was 400 μm and a charge compensation was done through an electron beam at −2 eV. The signal of aliphatic carbons was used as reference and set to 285.0 eV.

Before analyses, samples were stored in glass containers, under vacuum to avoid pollution.

2.3.3. Water drops contact angle

The static contact angle measurements were performed on a Digidrop GBX analyzer. A drop (1 μL) was formed on a needle (diameter of about 800 μm) and deposited on the surface of the sample. A picture was recorded after 500 ms. The angle between the drop and the surface was measured on the picture and determined as the average of the angles measured to the left and right of the drop. Three measurements were realized on 3 different samples of each type.

2.3.4. SEM observations

Scanning electron microscopy (SEM) was performed on a FEG FEI Quanta 250 at 5 kV. Prior to observations, samples were covered with 10 nm platinum.

2.3.5. Adhesion study

Adhesive forces were recorded with a TA.XTplus texturometer, equipped with a 5 kg captor. Samples were fixed on a probe (diameter 10 mm). Model surfaces were immersed in thermoregulated water at 25 °C or 40 °C. Samples were put in contact with the surfaces for 5 min, with an application force of 500 g. The maximal forces needed for the separation were recorded and correspond to the adhesive force. The speed of the surface separation was set to 0.5 mm/s.

2.3.6. Drug release study

Release kinetics studies were done using a protocol close to that described in a previous study [21]. Briefly, samples (10 × 10 mm, m ≈ 15 mg) were loaded in a PBS buffer (pH 7.4, 0.01 M) containing 1.5 g/L of eosin, for 12 h. The amount of loaded eosin was evaluated by indirect quantification, i.e. by measuring the loss of eosin in the loading solutions. The release was then performed on a PBS eosin free solution (V = 5 mL) under gentle agitation on a gyratory rocker for 3 days. The volume was maintained constant by replacing aliquots by eosin free PBS solution. The cumulative percentage of the released eosin curve was plotted using the ratio % = $m_{released}$ × 100/$m_{incorporated}$. The eosin concentration n was evaluated by UV Vis spectroscopy using a SHIM ADZU UV 1800 spectrometer (517 nm).

3. Results and discussion

The grafting of a functional PNIPAM COOH on chitosan is possible using NHS/EDC to couple carboxyl groups to the primary amines of the chitosan. Usually, this type of coupling requires the amines to be in neutral form. However, the nature of PECs can hinder functional functions and limit their availability. In order to optimize the reactivity of the PECs, it is necessary to understand the interactions between the alginate and chitosan functions at the outer region of the materials. As the interactions between the two polymers are due to protic moieties,

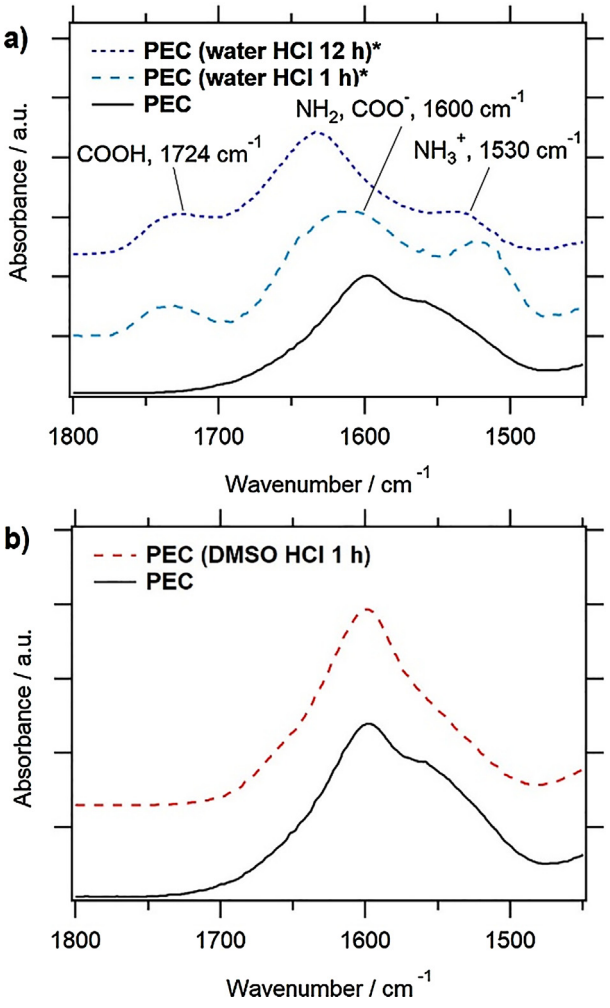

Fig. 1. FTIR spectroscopy of non-reticulated PECs treated with (a) HCl in water and (b) HCl in DMSO for 1 h and 12 h. * The sample was altered in aspect.

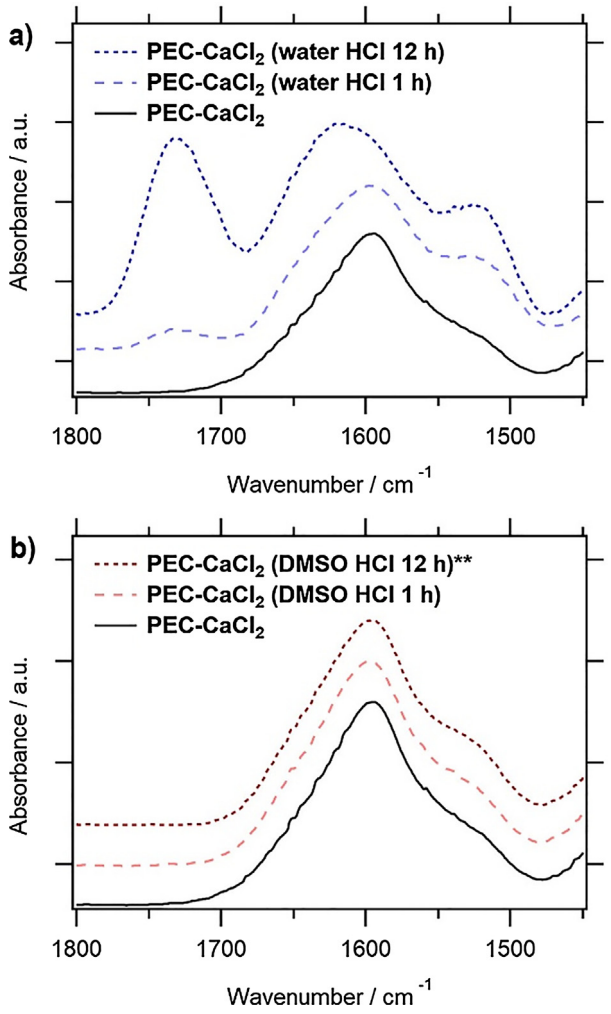

Fig. 2. FTIR spectroscopy of reticulated PECs treated with (a) HCl in water and (b) HCl in DMSO. *, ** the sample becomes sticky with a loss of mechanical strength or dissolved.

namely carboxylates for alginate and amines for chitosan, the evolution of the protonated state of these functions following acidic treatments was evaluated through ATR FTIR spectroscopy to provide information about their availability to elaborate a strategy for the subsequent functional grafting. The use of a non solvent such as DMSO would also limit the effect of the treatment at the surface of the PEC, thus it was studied and compared to water as the medium.

4. Spectroscopic studies of alginate/chitosan interactions

Firstly, attention was focused on non reticulated **PEC** (without $CaCl_2$, Fig. 1). The spectroscopic area of interest, between 1450 cm^{-1} and 1800 cm^{-1}, allowed the observation of the evolution of both the carboxyl (COOH, 1724 cm^{-1}; COO^-, 1600 cm^{-1}) and amine (NH_2, 1600 cm^{-1}; NH_3^+, 1530 cm^{-1}) moieties [20]. Acidic treatments (HCl) in water (Fig. 1a) revealed that, as expected, protonation of both car boxylates and amines occurred with time, as suggested by the increases at 1724 cm^{-1} and 1530 cm^{-1}, respectively. These results suggest that, before treatment, a non negligible part of the chitosan is in neutral form, which emphasizes that not all the chitosan was in interaction with the alginate. This partial complexation has already been reported in the literature [20]. The increase of the signal at 1641 cm^{-1} is attributed to water. We could also mention that after 12 h of treatment, the film was neither gellified nor dissolved, despite an alteration of their aspect.

Acidic treatment in DMSO showed no significant variation after one hour Fig. 1b). However, after 12 h, the PEC was gellified and the

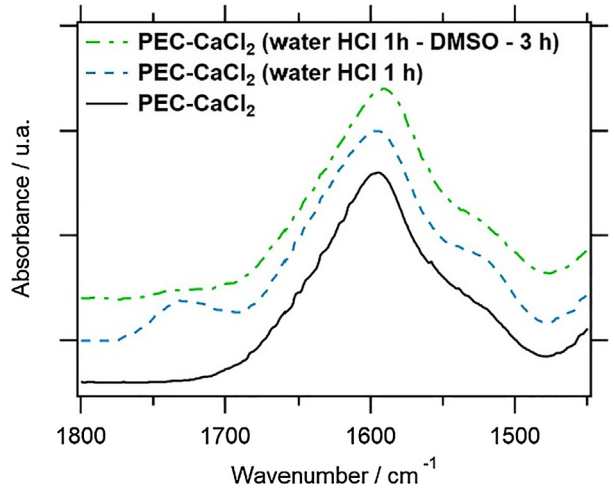

Fig. 3. FTIR spectroscopy of PEC-$CaCl_2$, PEC-$CaCl_2$ treated with HCl in water (1 h) and followed by an acid-free DMSO bath (3 h).

spectrum was not feasible. One could then suggest that carboxylic functions, poorly accessible as implied by the ionic interactions with chitosan amines and/or oriented toward the inner material as DMSO is a poor solvent, were protonated with a slow kinetic. Once the alginate

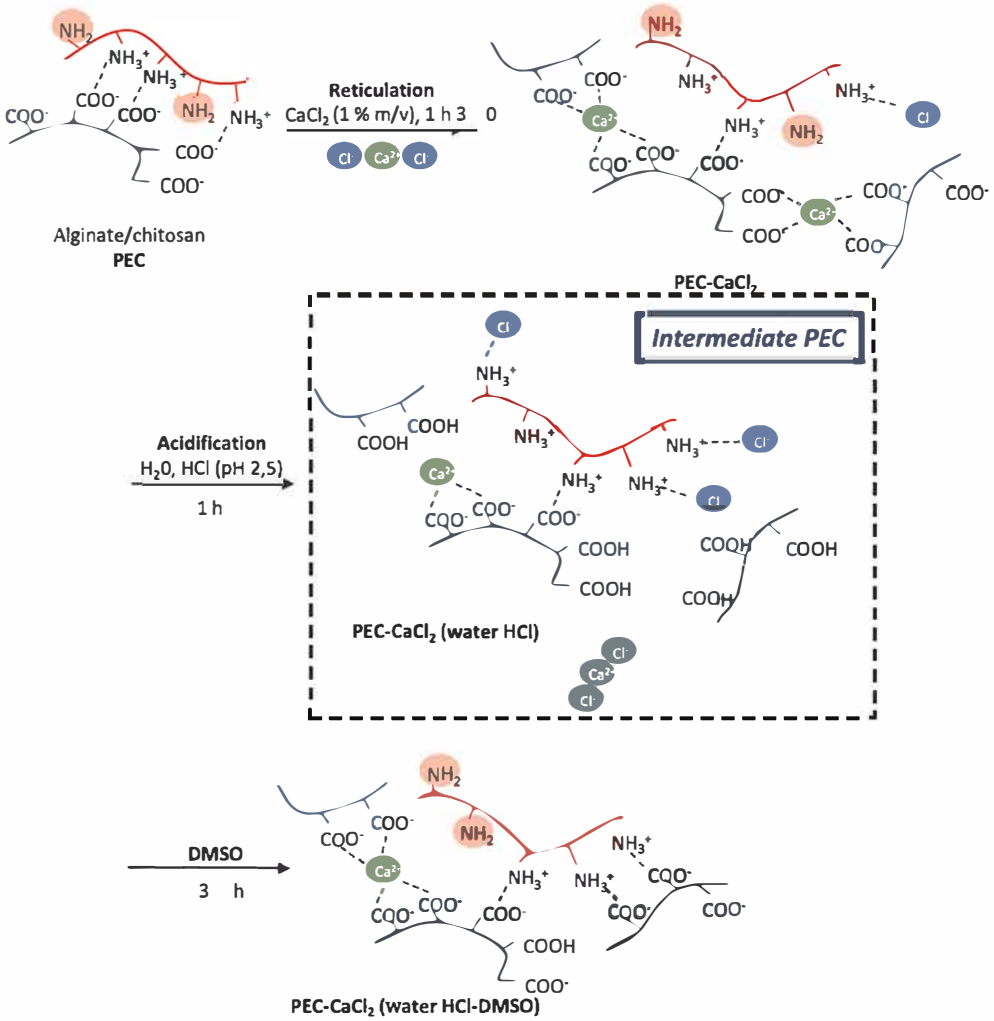

Fig. 4. Model presenting the behaviour of the PECs matrices through various treatments.

was protonated, the free chitosan was able to solubilize, partially, in the favourable acidic medium. This decomplexation of the PEC led then to the gelation of the material.

Interestingly, the chemical variations due to acidic treatments in water were much more important when the PECs were calcium reticulated (PEC-$CaCl_2$, Fig. 2a). Carboxylates seem more sensitive and, compared to those of non reticulated PECs, were significantly more protonated after 24 h of treatment. The presence of Ca^{2+} cations is thus responsible for a higher availability of COO^- functions. The calcium intercalation was probably done at the expense of alginate/chitosan interactions and the COO^-/Ca^{2+} is probably more labile. In addition, bonded to the calcium, carboxylates are more mobile and thus more available for protonation. The reticulation also allowed the material integrity to be maintained and no degradation was observed even after 12 h. The amine protonation was similar to that of non reticulated PECs, which emphasises the prevalence of the protonation of the carboxylates linked to calcium cations.

The acidic treatment in DMSO was not very effective on PEC-$CaCl_2$, and only a slight increase of the protonated amines around 1530 cm^{-1} could be observed. The stability of alginate carboxylates means that the poor solvation of the COO^- moieties did not favour their protonation, while favouring alginate/calcium interactions. However, if only the chitosan was slightly affected by the acidic organic environment, the destruction of the integrity of the material that was observed was presumably due to the formation of NH_3^+/Cl^- interactions instead of alginate/chitosan complex. Contrarily to the aqueous environment, the DMSO seemed to favour alginate/calcium interactions over protonation, whereas the alginate/chitosan complexes were still weak.

4.1. Towards the reactivity of PEC

From the above information, it was possible to propose a comprehension of the behaviour of the reactive functions of alginate and chitosan after acidic treatment, depending on the solvent. Acidic treatments in water led to the neutralisation of COO^- into COOH, which probably resulted in the decomplexation of the PECs. Therefore, one could expect a higher availability of the chitosan amines for reaction.

However, it should be considered that the surface modification of these PECs implies making the reaction without swelling of the matrices, i.e. working on a non solvent. DMSO is then a suitable solvent for the grafting, and the evolution of the reactive functions of the PECs within this solvent, after the acidic treatment in water, revealed that the functions return to their prior states, i.e. deprotonation of carboxylic acid (1724 cm^{-1}) and amines (1530 cm^{-1}, Fig. 3).

Owing to these results and in order to increase the PECs' reactivity, a model of the impact of the procedure was suggested and presented in Fig. 4. Briefly, during reticulation, calcium cations replace chitosan to interact with alginate. The obtained material is then more sensitive to acidic treatments, which give rise to a decomplexation of the material (Intermediate PEC). Consequently, the carboxyls and amines functions are possibly more available for reaction. However, the reactivity of the

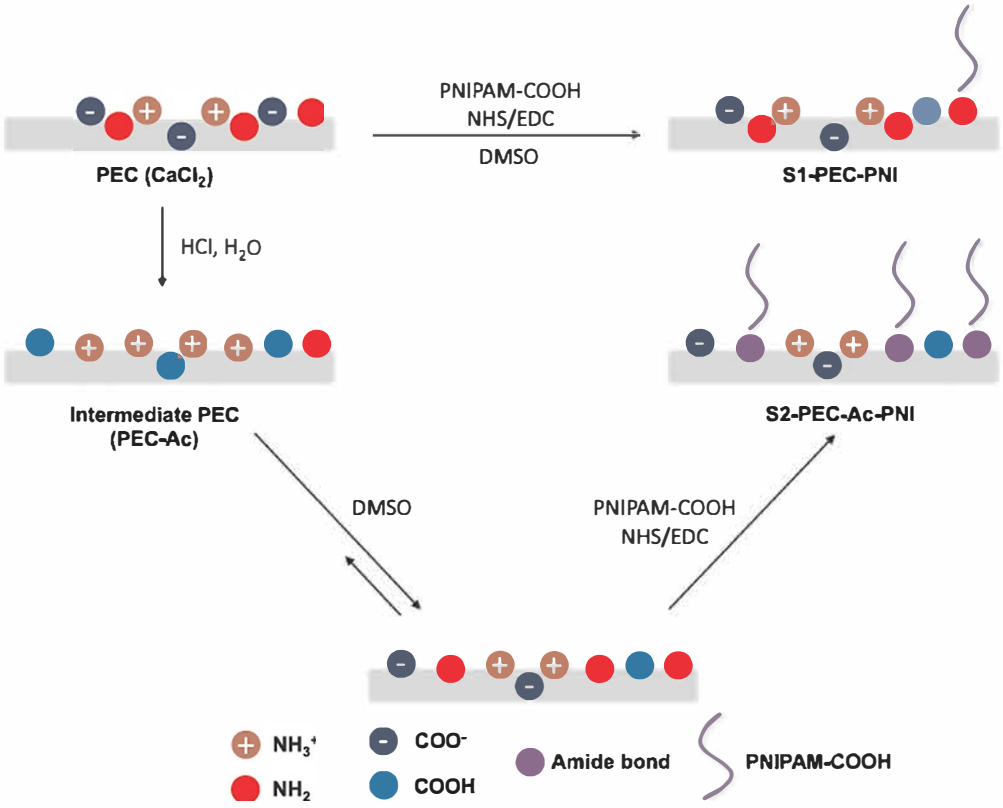

Fig. 5. Hypothesis of reaction following two different grafting procedures.

Table 2
XPS compositions of PEC and PNIPAM grafted following grafting protocols. EDC and NHS compositions are theoretical. If not marked, std inf. to 2%. *Std = 5%, **Std = 10%.

Sample eV	Acidification	CC, CH 285	CO, CN 286.6	COO, CON 288.2	C1s 285	N1s 400	O1s 533
PEC ($CaCl_2$)	–	37	45	18	61	3	35
EDC_{th}	–	25	63	13	73	28	–
NHS_{th}	–	50	–	50	50	13	38
PNIPAM-COOH	–	74	13	14	78	10	11
S1-PEC-PNI	No	29*	46*	24	59	1	40
S2-PEC-Ac-PNI	Yes	41**	46**	13	68	6	26*

ionic amines is known to be lower than those of the neutral form toward NHS/EDC coupling [27]. In DMSO, the recovery of the chemical initial states would lead to reactivating the functions, which supposedly increases their availability. This last point was further studied via grafting of the thermosensitive PNIPAM on the surface through NHS/EDC coupling.

4.2. PNIPAM grafting: effect of the acidic treatment on surface reactivity

As discussed above, an acidic treatment, prior to the surface grafting, could increase the PECs reactivity due to a higher reactivity of the intermediate PEC. Two different procedures were then followed in order to verify whether or not the acidic treatment made it possible to graft more efficiently the PNIPAM COOH on chitosan's amines via a NHS/EDC coupling (Fig. 5).

After reaction, the material surfaces were analyzed by XPS (Table 2). The analysis of a non modified PEC (PEC) shows a very low amount of nitrogen of 1.6% (N1s, 400.0 eV) and equal values for aliphatic carbons (CC, CH, 285.0 eV)) and CO, CN (286.6 eV), which each represents about 40% of all the carbons. A PNIPAM reference was analyzed (PNIPAM COOH). The measured percentages were close to the theoretical compositions, with a majority of carbons (78%), in particular aliphatic CC, CH (74%). In addition, the nitrogen content of 10% of the PNIPAM should involve an increase in the percentage of this atom in the composition of PNIPAM grafted surfaces. Nevertheless, the composition of NHS remains close to that of PNIPAM, apart from a higher COO, CON content (50%), and remnant coupling agents should be considered when analyzing the surfaces.

Two grafting protocols were performed, with and without prior acidification, for respectively S2-PEC-Ac-PNI and S1-PEC-PNI. The coupling must take place only between the carboxylic groups of PNIPAM COOH and the amines of chitosan. In the case of S1-PEC-PNI, the amount of aliphatic carbons decreased to 29% and CO, CN remained constant at 46% compared to PEC, along with an increase of COO, CON (24%). A very slight decrease of the nitrogen rate (1%) may also suggest the absence of PNIPAM, NHS or EDC on the surface.

The acidification of the PEC (S2-PEC-Ac-PNI) increased the level of nitrogen at the surface, which rises to 6%. In addition, an increase of the rate of CC, CH (41%) occurred, excluding the presence of EDC, in combination with a decrease in COO, CON (13%), excluding NHS. These signals, added to the high overall carbon percentage, suggest that the acidification made it possible to attach a larger amount of PNIPAM

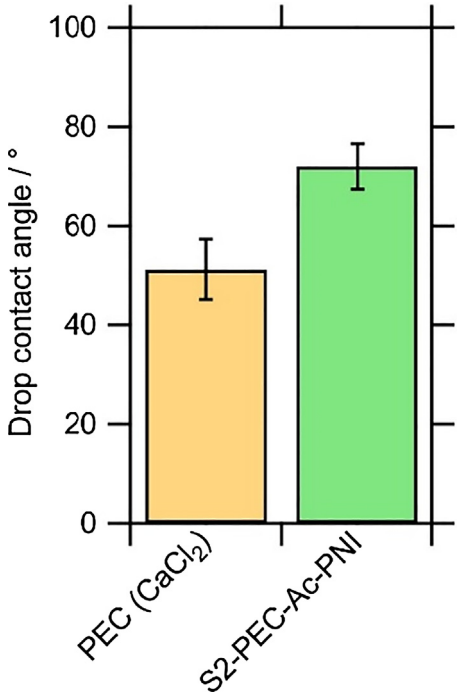

Fig. 6. Contact angle of water drops on surfaces before modification (PEC), after grafting of PNIPAM without (S2-PEC-Ac-PNI) or with the use of EDA. Recorded at RT.

COOH.

The acidification of PECs before the surface modification step al lowed a more effective PNIPAM grafting onto the surface, presumably due to the higher availability of chitosan's amines as suggested by the FTIR spectroscopy. However, the grafting involved amides formation, functions that were already present on PECs' chitosan. The grafted or deposited states of PNIPAM were then not able to be discriminated, due to the difficulty of harvesting such biomaterials surface. Nevertheless, to study the functionality of the surface elaborated through this pro cedure, **S2-PEC-Ac-PNI** was characterized in terms of its surface ther mosensitivity.

4.3. Characterization of PNIPAM grafted PEC surfaces

In order to study the surface state of PEC and PNIPAM grafted materials, the hydrophilicity of the prepared biomaterials was studied. Fig. 6 presents the contact angle of a water drop (static) measured at 25 °C. The materials were absorbent, so the measurements were made at the same time after the drop deposition (500 ms).

The **PEC** films exhibited a contact angle of 51 ± 6° and were therefore relatively hydrophilic. The surface modification, however, decreases the surface hydrophilicity, with measured angles around 72 ± 5° (**S2-PEC-Ac-PNI)**. PECs are indeed naturally rich in electric charges, with an anionic alginate and a cationic chitosan. The recovery of these polysaccharides by a grafted PNIPAM layer decreases the charge density and can explain the decrease of hydrophilicity. In the literature, the contact angles measured on the grafted surfaces of PNIPAM vary greatly, depending on the substrate, but the order of magnitude of the angles measured here seems consistent [28,29]. The thermosensitive effect could not be observed, because of the difficulty of achieving temperature controlled measurement of a small drop, as well as the high amplitude of measurements due to the roughness of the samples.

The adherence between these elaborated surfaces and some model surfaces was evaluated in order to observe the influence of the PNIPAM grafting below and above its LCST on the physical interactions. Model surfaces were chosen to provide an increasing hydrophilic gradient following Teflon < Ti < PMMA, and roughness was evaluated as the increasing order PMMA < Teflon ≤ Ti (Fig. 7).

The adherence between these surfaces and the biomaterials below (25 °C) and above (40 °C) the LCST of the PNIPAM are presented in Fig. 8. In the case of PMMA, which is moderately hydrophilic, we note that if unmodified the **PEC** showed no difference in adhesion between 25 °C and 40 °C (approx. 5 kPa), while the surface modification through PNIPAM COOH appeared to induce thermosensitivity with a higher affinity at higher temperature, in addition to an overall increase of the adhesive force whatever the temperature. A reverse trend was observed for the titanium surface (Fig. 8b), with a greater cold adhesion for PEC. This thermosensitivity was enhanced by the presence of PNIPAM (**S2-PEC-Ac-PNI**). The same phenomenon was observed for Teflon surfaces (Fig. 8c), with stronger adhesive force measured at 25 °C for **S2-PEC-Ac-PNI.**

The greatest adhesion thermosensitivity observed between **S2-PEC-Ac-PNI** and Ti, compared to other model surfaces, could be attributed to the higher roughness, and therefore contact interface, between the sample and the titanium surfaces. Considering unmodified **PEC**, it ap pears that an increase of the hydrophobicity of the model surface (PMMA < Ti < Teflon) correlates with a decrease in adhesion, whatever the temperature and regardless of the roughness observed through SEM. Considering the hydrophilic character of the latter, it therefore seems logical that the hydrophilic/hydrophilic type

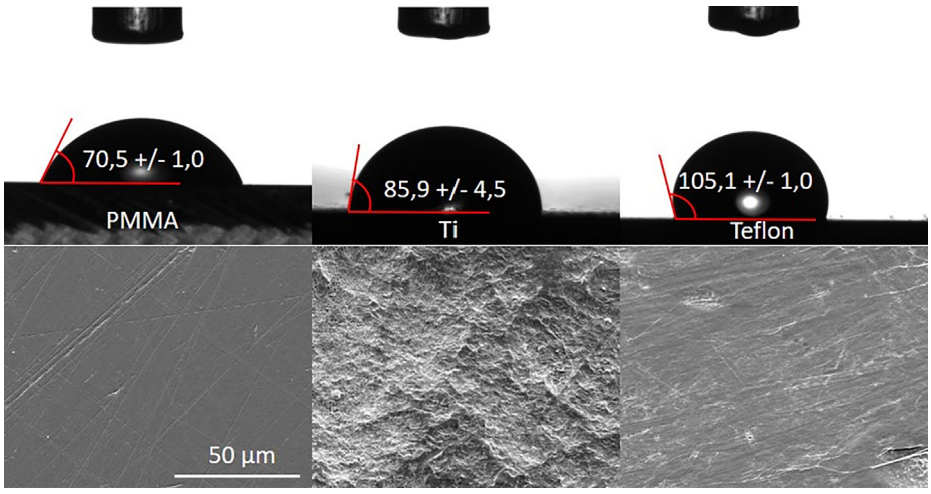

Fig. 7. Characterization of model surfaces: (top) water contact angles and (bottom) SEM images of the associated surfaces.

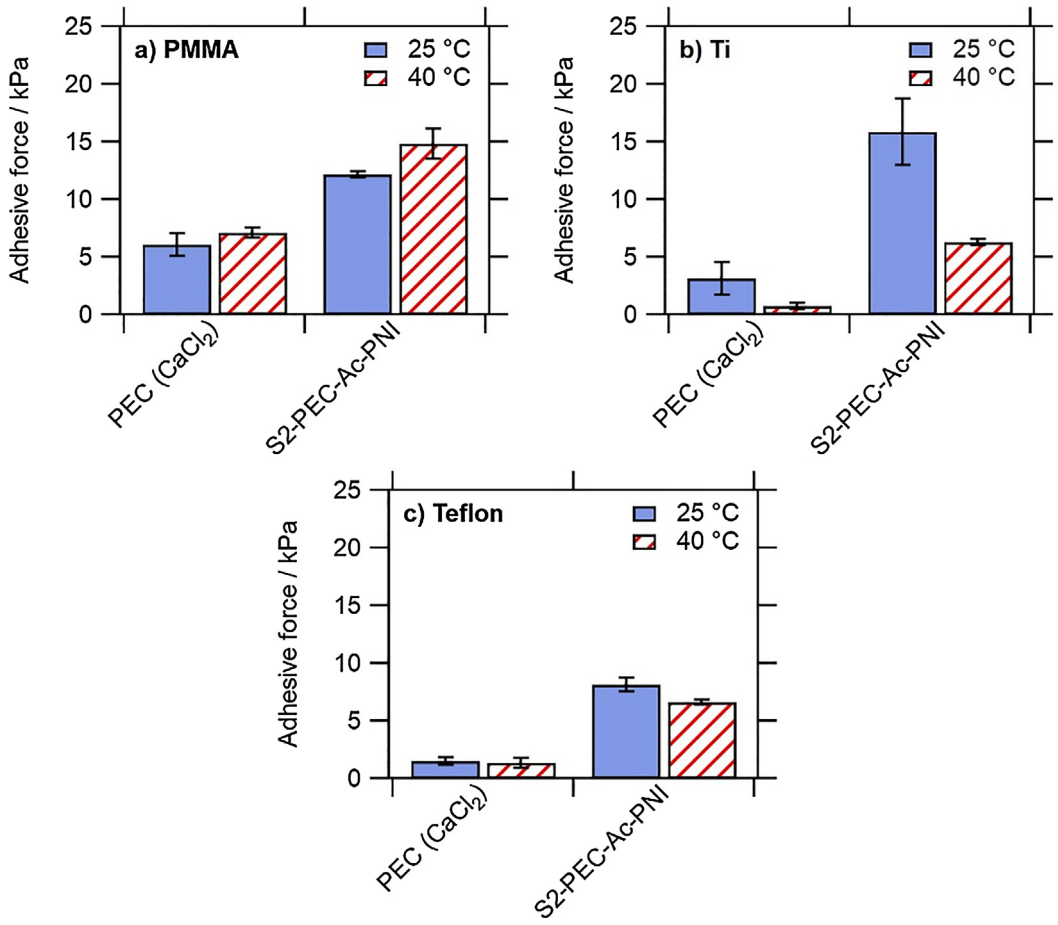

Fig. 8. Adhesive forces between the model surfaces and the PECs, at 25 °C and 40 °C, with or without surface modifications.

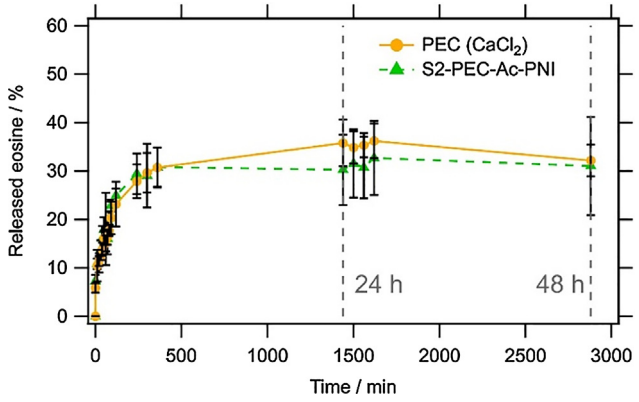

Fig. 9. Kinetics of eosin release by PEC and PNIPAM grafted PEC.

interactions are more favourable than hydrophilic/hydrophobic. In addition, for PNIPAM grafted PECs, the most hydrophilic surfaces (PMMA) tend to promote adhesion at 40 °C, while more hydrophobic surfaces (Ti, Teflon) promote cold interactions. PNIPAM is assumed to become hydrophobic above 32 °C, so this last observation seems to contradict the favourable character of hydrophilic/hydrophilic interactions (dipoles/dipoles, hydrogen bonds) or hydrophobic/hydrophobic (Van der Waals) interactions, and it therefore seemed that the supposed increase of the hydrophobicity of the surface above the LCST favors PEC's interactions with hydrophilic surfaces (PMMA). Without data on the contact angles of the biomaterial surfaces at 40 °C, it is difficult to affirm or deny the increase of surface hydrophobicity related to the passage of the LCST of PNIPAM. However, interactions phenomena can be more complex, with hydration of the external superficial layer of the grafted PNIPAM.

It is known that the transition of PNIPAM is a complex phenomenon involving polymer/water interactions at low temperature with polymer/polymer bond formation at the passage of the LCST [9]. This phenomenon is endothermic and requires the breakage of the hydrogen bonds that PNIPAM forms with water. It is also known that PNIPAM is, for example, able to form hydrophobic bonds with tetramethylated urea [30]. In this case, a decrease in the transition enthalpy was observed. The interaction with tetramethylated urea thus partially prevents the PNIPAM from interacting with water. In addition, the anions tend to destabilize, by polarization, the hydrogen bonds between polymer/water. Other authors believe that PNIPAM, water and anions form three types of competitive bonds, PNIPAM/water, anion/water and PNIPAM/anion [31]. Finally, it is known that the use of PNIPAM in the form of copolymer can modify its LCST by promoting, for example, intramolecular interactions [32].

From this literature the species present in the medium can influence the transition behaviour. One could then, for example, assume that below the LCST, the PNIPAM, through its amide functions, binds favourably to its alginate/chitosan PEC substrate, which is hydrophilic and able to form hydrogen bonds. The substrate would be in competition with water. The surface would then exhibit a more hydrophobic outer layer, as observed above by the contact angle. When passing through the LCST, the PNIPAM could then, as is conventionally the case, form intra and intermolecular interactions with itself. While externalizing its hydrophobic groups, it would then be in unfavourable interactions with both the aqueous medium and its PEC substrate. Some hydrophilic interactions, favourable with PMMA, hydrophobic, and favourable with Ti and Teflon, could then appear. In the literature, it

has already been assumed that, for short polymer chains, some grafted PNIPAM could interact with its substrate [33]. If this is only a hypothesis that cannot be demonstrated, the increase of the size of the PNIPAM chains would allow further study of the influence of PNIPAM/substrate interactions.

4.4. Influence of the grafting on release properties

A previous study reported the release of eosin from various PEC structures [21]. The release of this active hydrophilic molecule could be slowed by the surface of PECs becoming hydrophobic beyond the LCST of PNIPAM. The kinetics of release for 48 h is shown in Fig. 9. The amount of eosin released by **PEC** after 48 h was low, as already reported [21]. However, it appeared that surface modification did not influence the diffusion of eosin over time. The grafting of PNIPAM was limited to the chitosan's amines and the grafting density was thus relatively low. The small eosin molecule diffused between PNIPAM chains without being blocked or slowed down. This aspect is important since it is desirable that the surface modification brings advanced properties of thermosensitivity without altering the intrinsic properties of the PEC matrix.

5. Conclusion

In the present study, we report the evolution of alginate/chitosan PEC films in various environments to propose a protocol allowing an increase of the PECs' functions reactivity. It clearly appeared that the reticulation of PECs with $CaCl_2$ weakened the alginate carboxylates interactions with amino groups of chitosan, increasing their ability to welcome a proton in an aqueous acidic medium. The protonation of alginate led to the partial decomplexation of the PECs. Chemical modification showed that this effect allowed an increase in the surface affinity of chitosan's amine towards functional PNIPAM in a non solvent such as DMSO, while the grafting state of the PNIPAM was not clearly evidenced. An acidic activation may then be suitable to improve the chemical functionalization of such PECs.

PNIPAM grafted PECs' wettability showed a diminution of the surface hydrophilicity, probably due to the charge density decrease. However, it was not possible to perform such analyses above the LCST of PNIPAM. The missing data would thus be important as the adhesive force measurement revealed an unexpected behaviour: if thermosensitivity was effectively brought to PECs through the grafting of PNIPAM, interactions below the LCST were favoured with more hydrophobic surfaces, while above the LCST hydrophilic surfaces were more likely to adhere. The interactions of PNIPAM chains with their highly polar substrate could explain these observations, and further studies varying the PNIPAM chain length combined with strong surface analysis could provide a further understanding.

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