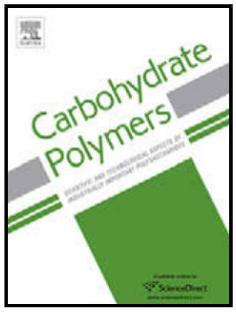

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Influence of Aloe vera on water absorption and enzymatic in vitro degradation of alginate hydrogel films

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Abstract

This study investigates the influence of *Aloe vera* on water absorption and the *in vitro* degradation rate of *Aloe vera*-Ca-alginate hydrogel films, for wound healing and drug delivery applications. The influence of *Aloe vera* content (5%, 15% and 25%, v/v) on water absorption was evaluated by the incubation of the films into a 0.1M HCl solution (pH 1.0), acetate buffer (pH 5.5) and simulated body fluid solution (pH 7.4) during 24 hours. Results show that the water absorption is significantly higher for films containing high *Aloe vera* contents (15% and 25%), while no significant differences are observed between the alginate neat film and the film with 5% of *Aloe vera*. The *in vitro* enzymatic degradation tests indicate that an increase in the *Aloe vera* content significantly enhances the degradation rate of the films. Control films, incubated in a simulated body fluid solution without enzymes, are resistant to the hydrolytic degradation, exhibiting reduced weight loss and maintaining its structural integrity. Results also show that the water absorption and the *in vitro* degradation rate of the films can be tailored by changing the *Aloe vera* content.

Keywords: Alginate; *Aloe vera*; Wound healing; Water absorption; *In vitro* degradation.

1. Introduction

Skin is the largest organ of the human body, protecting the internal organs from the external environment and preventing body dehydration (Groeber, Holeiter, Hampel, Hinderer, & Schenke-Layland, 2011; Pereira, Barrias, Granja, & Bártolo, 2013a). Skin can be damaged as a result of burn injuries, chronic wounds, excision of skin, tumours and other dermatological conditions. To repair and regenerate the damaged tissue, a dynamic and continuous cascade of events occurs. This is a complex process involving the interaction of cellular components, growth factors and cytokines within four sequential and overlapping phases: (i) hemostasis, (ii) inflammation, (iii) proliferation and (iv) tissue remodelling or maturation (Boateng, Matthews, Stevens, & Eccleston, 2008; Guo & DiPietro, 2010; MacKay & Miller, 2003; Wild, Rahbarnia, Kellner, Sobotka, & Eberlein, 2010). A great variety of wound-care products are used for the treatment of skin lesions, including autografts and allografts, creams and solutions, wound dressings and tissue-engineered skin substitutes (Boateng et al., 2008; Pereira, Mendes, Bártolo, 2013b; Wild et al., 2010). Among these products, wound dressings are widely used due to both its ability to cover and protect the damaged tissue, promoting a moist environment to stimulate the healing process and good relationship between clinical efficacy and manufacturing cost (Boateng et al., 2008; Huang & Fu, 2010; Jones, Grey, & Harding, 2006; Pereira et al., 2013c).

The dressings can be classified as traditional or modern dressings, according to the ability to provide a wound moist environment (Boateng et al*.,* 2008; Jones et al., 2006). Traditional dressings like bandages, gauzes or cotton wool, absorb large amounts of exudate, drying the wound bed and avoiding a moist wound environment, which can lead to cell death and inhibit the healing process (Jones et al., 2006; Wild et al., 2010; Skórkowska-Telichowska, Czemplik, Kulma, & Szopa, 2013). Due to the high absorption rate, these dressings may also adhere to the wound bed, making its removal difficult and causing pain (Boateng et al., 2008; Jones et al., 2006). Conversely, modern dressings are able to create and maintain a warm moist environment into the wound, providing the optimal conditions for an improved healing process (Boateng et al., 2008; Jones et al., 2006). Modern dressings can be obtained from either natural (Pereira et al., 2013c; Wang, Zhu, Xue, & Wu, 2012) or synthetic polymers (Elsner & Zilberman,

2010; Zahedi et al., 2012), or through a combination of both (Liu et al., 2010; Singh & Pal, 2011), being available as thin films, foams or gels (Boateng et al., 2008).

Alginate is an anionic polysaccharide widely used in wound healing applications, due to its biocompatibility, biodegradability, excellent film forming properties and easy formation of hydrogels (Bouhadir et al., 2001; d'Ayala, Malinconico, & Laurienzo, 2008; Lee & Mooney, 2012; Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011). Alginate hydrogels are commonly prepared through the ionic cross-linking method, in which ionic cross-linking agents like calcium ions are added to an aqueous alginate solution, resulting in the formation of a cross-linked 3D network (Goh, Heng, & Chan, 2012; Lee & Mooney, 2012). Calcium alginate hydrogels are attractive materials for the treatment of different kinds of wounds, due to the (i) ability to absorb the wound exudate, maintaining a moist environment; (ii) the haemostatic properties of calcium ions released into the wound bed; and (iii) the ability to act as a matrix for aggregation of platelets and erythrocytes (Boateng et al., 2008; Clark, 2012; d'Ayala et al., 2008; Lee & Mooney, 2012). These gels partly dissolve when in contact with the wound fluid, as a result of the ion exchange between the sodium ions present within the exudate and the calcium ions of the hydrogel. This process leads to the formation of a soluble hydrophilic gel that protects the wound and stimulates the granulation and epithelialization (Goh et al., 2012; Jones et al., 2006; Krahwinkel & Boothe, 2006; Lee & Mooney, 2012; Skórkowska-Telichowska et al., 2013). The biodegradation of alginate gels in a wound is very useful as the resulting fragments can be easily rinsed with saline solution without pain, avoiding destroying the granulation tissue (Boateng et al., 2008; Krahwinkel & Boothe, 2006). However, the biodegradation of hydrogels from the wound depends on the chemical composition of the alginate (Clark, 2012; Jones et al., 2006), which in turn is determined by some parameters, such as the marine source, algae specie, geographic location and season (d'Ayala et al., 2008; Lee & Mooney, 2012). Alginates rich in M units result in gel fragments that are easily removed from the wound through irrigation with saline solution, while alginates rich in G units present high integrity and should be removed in one piece (Jones et al., 2006). Although calcium alginate hydrogels are biodegradable in the wound bed, the dissolution process leads to a slow and poor controlled degradation kinetics *in vivo* (Bouhadir et al., 2001; Lee & Mooney, 2012). The biodegradation of hydrogels influences its performance during the application, namely the cell interaction (Boontheekul, Hill, Kong, &

Mooney, 2007), tissue formation (Alsberg et al., 2003) or the delivery of biomolecules (Bencherif et al., 2009), so the control over the degradation rate of hydrogel dressings is fundamental to improve the healing process.

Aloe vera, also referred as *Aloe barbadensis* Miller, is a plant native from South Africa widely used in folk medicine and of great interest for several biomedical, pharmaceutical and cosmetic applications (Hamman, 2008; Pellizzoni, Ruzickova, Kalhotka, & Lucini, 2012). Different products can be obtained during the processing of the *Aloe vera* leaves, like a bitter yellow juice called *Aloe vera* latex or *Aloe* juice and a clear mucilaginous *Aloe vera* gel (Hamman, 2008; Wynn, 2005). The gel, extracted from the parenchymal tissue of the plant, is composed of two phases: a water phase (99-99.5%), and a solid one (0.56-0.66%) containing several potentially active constituents, including soluble sugars, non-starch polysaccharides, lignin, lipids, enzymes, salicylic acids, proteins and minerals (Boudreau & Beland, 2006; Hamman, 2008; Wynn, 2005). There is a great deal of interest in the use of *Aloe vera* gel for the treatment of skin disorders, due to its therapeutic properties like anti-inflammatory, antibacterial, antiseptic, and its ability to promote the wound healing (Boudreau & Beland, 2006; Choi & Chung, 2003; Habeeb et al., 2007; Pellizzoni et al., 2012; Wynn, 2005). Chithra et al. (1998a, b) showed that *Aloe vera* gel significantly improves the synthesis of collagen and the degree of collagen cross-linking, after topical and systemic administration in wounds created in a diabetic rat model. Recently, Atiba et al. (2011) stated that the oral administration of *Aloe vera* significantly stimulates the proliferation of fibroblasts, the collagen deposition and the blood vessel formation (angiogenesis) in radiation-exposed rats. These healing properties are attributed to the biological activity of the polysaccharides and glycoproteins present in the *Aloe vera* gel, as well to the synergy established between the compounds (Hamman, 2008; Pellizzoni et al., 2012).

Recently, we developed novel *Aloe vera*-Ca-alginate hydrogel films, combining the occlusive and haemostatic properties of calcium alginate hydrogels with the therapeutic properties of *Aloe vera* (Pereira et al., 2011, 2013b, c). In this system, alginate hydrogel acts as a vehicle for the incorporation and release of *Aloe vera* compounds directly into the wound bed during the swelling, in order to improve the healing process and the tissue regeneration. The topical release of *Aloe vera* aims to circumvent the inadequate perfusion of the wound, which is a

limiting factor for the efficiency of systemic treatments, increasing the risk of infection (Boateng et al., 2008).

The purpose of this work is to investigate the influence of the presence of *Aloe vera* on both the water absorption and the *in vitro* degradation behaviour of *Aloe vera*-Ca-alginate hydrogel films. It is expected that the incorporation of *Aloe vera* into the hydrogel films can improve the control over the water absorption and degradation rate, which are important properties to wound dressing applications.

2. Materials and methods

2.1. Materials

The sodium alginate (54.09 ± 1% of M units (Pereira et al., 2013c)) was purchased from BDH Prolabo (VWR International, UK). The *Aloe vera* (ACTIValoe®, *Aloe vera* Gel Qmatrix 200X Flakes) was kindly offered by Aloecorp (Broomfield, U.S.A.) and the glicerol was obtained from Scharlau (Spain). The alginate lyase from *Flavobacterium sp.* (≥10,000 units/g) was purchased from Sigma Chemical Co. (St. Louis, USA). The reagents from analytical grade used in the preparation of the SBF solution were as follows: NaCl, $NaHCO_3$, KCl, $K_2HPO.3H_2O$, $MgCl_2.6H_2O$, HCl, $CaCl_2$ and $(CH_2OH)_3CNH_2$. The sodium acetate dihydrate and the acid acetic from analytical grade were used to prepare the acetate buffer.

2.2. Preparation of hydrogel films

Alginate films containing *Aloe vera* at different percentages were prepared through an experimental protocol previously reported (Pereira et al., 2013c). Briefly, an aqueous solution of sodium alginate (1.5%, w/v), containing glicerol at 15% (w/w, based on the mass of the alginate), was mixed with an aqueous solution of *Aloe vera* (1.0%, w/v), in order to obtain final alginate/*Aloe vera* proportions (v/v) of 100:0 (film AG) 95:5 (film AGA5), 85:15 (film AGA15) and 75:25 (film AGA25). Afterwards, 25 mL of each mixture was casted into petri dishes (ø=9.5cm) and left to dry in room at 25ºC, under 50% of humidity. After drying, the films were immersed into a calcium chloride aqueous solution (5.0%, w/v) for 5 minutes to allow the cross-linking reaction. The films were then washed with distilled water and dried at room temperature, before use.

2.3. Equilibrium water absorption and pH sensitivity

The equilibrium water absorption was determined through the immersion of pre-weighted cross-linked film samples with 20 mm of diameter and thickness in a range of 54.4-66.0μm, into 20 mL of solution during 24 hours. After this period, films were collected from the medium, the excess of water was removed with a filter paper, and the hydrated weight measured. In this test, solutions with different values of pH were used: (i) 0.1M HCl solution at pH 1.0 simulating the gastric fluid, (ii) acetate buffer at pH 5.5 (10mM) simulating the pH of the skin, and (iii) SBF solution at pH 7.4 (50mM of trishydroxylaminomethane and 45mM hydrochloric acid), simulating the body fluid. The water absorption equilibrium of the films was determined according to the Eq. (1):

$$\text{Water absorption } (\%) = \left(\frac{W_h - W_i}{W_i} \right) \times 100 \qquad (1)$$

where W_h represents the hydrated weight of the cross-linked film, and W_i corresponds to the dry weight of the film. Four samples were used to replicate each condition.

2.4. Contact angle measurements

Contact angle studies were performed using the OCA 20 (Data Physics®) equipment and distilled water as probe liquid (10 μl of volume drop and 2 μl/s of velocity). The tests were performed at room conditions, with a minimum of nine test measurements considered for each condition.

2.5. *In vitro* degradation studies

The enzymatic degradation behavior of the *Aloe vera*-Ca-alginate films was investigated by the immersion of film samples (50 mm x 15 mm) into a falcon tube containing 10 mL of SBF solution (pH 7.4, 37ºC) with the enzyme alginate lyase (10 U/g alginate). The films were dried in an oven at 37ºC until constant mass (W_i) before the test. At pre-determined periods, the samples were removed from the degradation medium, washed with distilled water, and the excess of water at the surface withdrawn by a filter paper. After this procedure, the hydrated weight (W_h) of the films was immediately evaluated to determine the water absorption. Then, the films were transferred to an oven and dried at 37ºC until constant mass (W_f) to determine its

weight loss. The films were also immersed into an SBF solution in the same conditions, without the enzyme (hydrolytic degradation), as a control. In both cases, enzymatic and non-enzymatic degradation, the medium was replaced weekly with a fresh medium. Nine samples were tested for each condition.

2.5.1. Film characterization

After each period of degradation, the films were characterized regarding its weight loss, water absorption, dimensional changes, chemical, morphological and mechanical properties.

During the degradation process, the water absorption of the films was determined according to Eq. (1), while the degradation was evaluated by determining the weight loss according to Eq. (2):

$$\text{Weight loss } (\%) = \left(\frac{W_i - W_f}{W_i} \right) \times 100 \qquad (2)$$

where W_f and W_i correspond to the dry weight of the film, after and before the degradation process respectively.

The thickness of the dry films was evaluated using a micrometer (Model 102-301, Mitutoyo) with 0.001 mm of accuracy. At least nine test measurements were considered for each condition.

The chemical characterization of the samples was performed using the Fourier Transform Infra-Red (FTIR) analysis, to evaluate and compare changes in the absorption bands as a result of the degradation process. For comparison, the spectrum of the enzyme was also recorded. The analysis was performed using an Attenuated Total Reflectance cell (ATR) on the spectrophotometer FT/IR-4200 type A (JASCO, USA), in a range from 4000 to 500cm^{-1} with 4 cm^{-1} of resolution and 64 scans. Each test was repeated three times for each condition.

The surface morphology of the films, before and after enzymatic degradation, was examined by scanning electron microscopy (SEM), using a Hitachi 2700 scanning electron microscope at an acceleration voltage of 20 kV. The samples were sputtered with gold before the analysis.

The mechanical properties of the dry films were evaluated by tensile tests, using an universal testing machine TCD-1000 (Chatillon^{TM}). The tests were carried out at room

temperature, using a gauge length of 20 mm and a crosshead speed of 15 mm/min. Results were expressed as a maximum force at rupture and an elongation ratio, being the last one determined by the Eq. (3):

$$Elongation\ ratio\ (\%) = L/L_0 \times 100 \quad (3)$$

where, L represents the final length of the sample at rupture, and L_0 corresponds to the initial length of the sample prior to the test. A minimum of three samples were used for each condition.

2.6. Statistical analysis

Results were expressed by the means of the measurements with the error bars representing the standard deviation. The statistical analysis of the data was performed using the one-way analysis of variance (ANOVA), while the comparisons between two means were made through the Tukey's test. The statistical significance was considered for a $p<0.05$. For each condition, a minimum of three specimens were considered.

3. Results and discussion

Results show that the films are easy to handle, presenting high transparency (Fig. 1a) and good adaptability to the human skin of a volunteer (Fig. 1b). These properties are fundamental for wound dressing applications, once they allow an easy application and removal of the film, and the visual inspection of the healing process with no need to remove the film. These films can be applied to the wound in both dry and hydrated state.

Dry films are particularly indicated for the treatment of superficial wounds with moderate to heavy exudate, due to its ability to absorb the exudate, avoiding maceration (Krahwinkel & Boothe, 2006; Wild et al., 2010). These films are also very useful for use in bleeding wounds, due to the haemostatic properties of calcium ions released into the wound bed. During its application, the films gradually dissolve as a result of the ion exchange process, leading to the formation of a hydrophilic gel that maintains a moist environment, reducing the bacterial infection and stimulating the cell migration and epithelialization (Lee & Mooney, 2012; Skórkowska-Telichowska et al., 2013). The hydrated films are used in an hydrogel form, being suitable for the treatment of dry and painful wounds (Wild et al., 2010). In this case, films are

firstly hydrated through the immersion into appropriated solutions (e.g. saline solution), and subsequently applied to the wound site. When placed on the wound, the films release the absorbed water, creating a moist environment that rehydrates the dry wound bed and promotes the debridement and cleaning of the wound (Jones et al., 2006; Krahwinkel & Boothe, 2006). In both, dry and hydrated state, the *Aloe vera* compounds can be released directly into the wound bed, increasing their therapeutic effectiveness.

The films were developed for application in the different phases of the wound healing process (Fig. 1c) and their properties address the specific needs for each phase. More specifically, (i) the haemostatic properties of the calcium ions present in the film are relevant for the haemostatic phase (Clark, 2012; Lee & Mooney, 2012); (ii) the antiseptic, anti-inflammatory and antibacterial activities of *Aloe vera* gel can be useful in the inflammatory phase, preventing or treating wound infections (Hamman, 2008; Pellizzoni et al., 2012); and (iii) the ability of *Aloe vera* gel to stimulate the fibroblast proliferation and collagen synthesis can be very important for the proliferation and tissue remodeling/maturation phases (Atiba et al., 2011; Boateng et al., 2008). Whenever the therapeutic properties of *Aloe vera* do not allow an adequate treatment of the wound infection, an alginate film containing a synthetic drug (e.g. gentamicin, nitrofurazone) can be used to prevent the wound to enter into a chronic state and fail to heal (Guo & DiPietro, 2010). Once the inflammatory response and infection are controlled, an alginate hybrid film, composed of a synthetic drug and *Aloe vera*, can be applied to promote a gradual transition for the next phase of the healing process. During this stage, the proportion between the drug and the *Aloe vera* within the film should be gradually adjusted, leading to the increase in *Aloe vera* content.

“Insert Fig. 1”

3.1. Equilibrium water absorption and pH sensitivity

The water absorption capacity is an important property of materials for wound dressing applications, once it determines the ability of the dressing to remove the exudate in excess from the wound, maintaining a moist environment. In addition, it is widely recognized that the water uptake affects the degradation kinetics of polymers and the release kinetics of biomolecules (Davidovich-Pinhas & Bianco-Peled, 2010; Göpferich, 1996). Fig. 2 shows the influence of both the *Aloe vera* content and the pH of the solution on the water absorption capacity of the films.

The increase in the *Aloe vera* content significantly increases the water absorption capacity of the films for all mediums. Films with 5% of *Aloe vera* and without *Aloe vera* showed no significant change in the water absorption, after 24 hours of immersion. On the other hand, films with 15% and 25% of *Aloe vera* exhibit a significant increase in the water absorption capacity. Fig. 2 also indicates that an increase in the pH of the medium produces a significant increase in the film's water absorption. The immersion of the films in a 0.1M HCl solution do not result in significant changes on the water absorption, because the pH is lower than the pK_a of the ionizable groups of the alginate, leading to a low water uptake. However, when immersed in acetate buffer (pH 5.5) and SBF solution (pH 7.4), films exhibit a significant increase on the water absorption capacity, due to the ionization of the carboxylic groups of the alginate. Independently of its composition, films immersed into an SBF solution have an higher water absorption. Results indicate that *Aloe vera* improves the capacity of the films to absorb and retain water, which can be due to the changes on the hydrophilic properties on the film's surface.

"Insert Fig. 2"

3.2. Contact angle studies

Contact angle tests were performed to evaluate the influence of *Aloe vera* on the hydrophilic properties of the hydrogel film's surfaces. The alginate neat film (Film AG) presents a hydrophilic surface with a water contact angle of 42.2 ± 1.9º, as indicated in Fig. 3. The incorporation of *Aloe vera* at 25% (Film AGA25) significantly decreases the contact angle of the film surface to 30.3 ± 4.7º, which can be due to the *Aloe vera* gel hydrophilicity (Saibuatong & Phisalaphong, 2010). Results suggest that *Aloe vera* improves the hydrophilicity of the films, which is in accordance with the water absorption tests.

"Insert Fig. 3"

3.3. *In vitro* degradation studies

The *in vitro* degradation tests were performed to evaluate the influence of *Aloe vera* on the *in vitro* degradation behaviour of the films. The experiments were carried out in accelerated condition through the incubation of the films into an SBF solution containing the enzyme poly(M) lyase [(1-4)-*β*-D-mannuronan lyase], which was used to improve the *in vitro* degradation rate of

the films. As a control, the films were also incubated in SBF solution without the enzyme (hydrolytic degradation).

3.3.1. Weight loss

The degradation of the films was evaluated by measuring the dry weight loss as a function of the degradation time. The weight loss or degradation rate of the films in SBF solution with alginate lyase depends on the *Aloe vera* content, as shown in Fig. 4a. The film with 25% of *Aloe vera* presents the fastest degradation rate, with completely dissolution into the medium at day 1 of degradation. On the contrary, the alginate neat film exhibits the slowest degradation rate, being completely dissolved in the solution after 12 days of degradation. For the films with 5% and 15% of *Aloe vera*, the degradation was observed at day 10 and day 3 of degradation, respectively.

The weight loss profiles of the control films, immersed into an SBF solution without the enzyme, indicate that the films are resistant to the hydrolytic degradation and maintain its structural integrity. After 7 days of degradation, the films exhibit a weight loss in a range from 6.6 ± 0.6% to 7.6 ± 0.7%, as indicated in Fig. 4b. As the films exhibited a very slow degradation rate, they were only characterized at day 7 of degradation. The reduced weight loss determined at this period can be attributed to the leaching of the plasticizer agent (glicerol) used in the processing of the films and/or release of *Aloe vera*, once no significant alterations, in both chemical and mechanical properties, were detected. Hydrolytic degradation tests performed during 24 weeks show that the films maintain its structural integrity, exhibiting weight loss comprised between 19.4% and 27.4% (AG: 19.4 ± 0.3%; AGA5: 19.7 ± 0.4%; AGA15: 22.4 ± 0.7%; AGA25: 27.4 ± 1.0%), indicates that *Aloe vera* enhances the degradation rate of the developed films for both enzymatic and non-enzymatic degradation.

3.3.2. Water absorption

The water absorption of the films during the degradation process was determined to monitor the changes on this property, as a function of the film composition and degradation medium. The water absorption of the films, in the presence of the enzyme, reflects the changes in their structure. In Fig. 4b, it is possible to observe an increase on the water uptake, reaching a

maximum at the second day of the degradation process. This is followed by a decrease on the absorbed water along the time, as a result of the diminishing in the film's dimensions with a consequent reduction of the available area to absorb and retain water. From Fig. 4d, it is possible to verify that the films AG and AGA5, degraded by the alginate lyase, present a significant decrease in their thickness until day 2 (reduction of 25.1% and 38.4%, respectively), followed by a slow and gradual reduction until complete dissolution, which agrees with the results obtained for the weight loss and water absorption tests. In the case of the films AGA15 and AGA25, it was not possible to determine the thickness variation, due to the dissolution of the films. Similarly to the thickness change, these films also exhibit a reduction in their length and width during the degradation time. In the case of the control films, the reduction on the thickness after 7 days of degradation was in a range of 7.4 % to 23.7 % (AG: 7.4 ± 3.0%, AGA5: 10.5 ± 4.0%, AGA15: 17.8 ± 1.7%, AGA25: 23.7 ± 1.8%), indicating that *Aloe vera* improves the degradation rate of the films in both presence and absence of the enzyme.

Water contact angle analysis indicates that the hydrophilicity of film's surface is enhanced by the introduction of high percentages of *Aloe vera*, which in turn increased the water uptake. It is assumed that the films with high content of *Aloe vera* are more susceptible to the hydrolytic degradation, due to the further penetration of water within the hydrogel network, which results in the cleavage of degradable linkages and film degradation. The increase on the film degradation can lead the formation of pores in the film structure, facilitating the penetration of water and enzymes, which increases the degradation rate. The films degraded in presence of the enzyme were characterized by a significant reduction on their dimensions along the degradation time, maintaining their original geometric shape, which is characteristic of the surface erosion mechanism (Chen, Zhou, & Li, 2011; Göpferich, 1996).

"Insert Fig. 4"

3.3.3. Chemical characterization

FTIR-ATR spectroscopy technique was used to analyze the changes occurred in the chemical composition of the films, during the degradation process. Fig. 5a-d shows the FITR spectra of the films AG and AGA5 at different periods of degradation. The FTIR spectra of the films AGA15 and AGA25 was not recorded, due to their dissolution. The relevant absorption bands were identified at around 3200 cm^{-1} (OH group), 1600 cm^{-1} and 1400 cm^{-1} corresponding

to the asymmetric and symmetric stretching vibration of COOH group, respectively, 1295 cm^{-1} (C-O group) and 1030 cm^{-1} attributed to the -C-O-C- in glycosidic bonds (Jejurikar et al., 2012; Pereira et al., 2013c). As previously reported, the incorporation of *Aloe vera* into the alginate films did not cause significant alterations in the chemical composition investigated through FTIR, due to their similar chemical constituents (Pereira et al., 2013c).

From Figs. 5a and 5b, it is possible to observe that the characteristic absorption bands of the films, in the presence of the enzyme, exhibit a gradual decrease along the degradation time, due to the degradation process with consequent film erosion and dissolution. At 1030 cm-1, a significant decrease in the peak intensity is observed, indicating the cleavage of the glycosidic bond (–C-O-C–) of alginate by the enzyme, through a β-(1,4)-elimination mechanism, as illustrated in Fig. 5e. Comparing the FTIR spectra's of the films AG and AGA5, it was found that the peaks of the film AGA5 present lower intensity, in particular at day 5 and day 8 of degradation, due to the higher degradation rate (Fig. 4a), and consequently lower alginate content. Contrary, the control films AG and AGA5 do not exhibit significant variations in the characteristic absorption bands after 7 days of degradation (Figs. 5c and 5d), as a result of the very slow degradation rate. Similar results were observed for the films AGA15 and AGA25 (Appendix Fig. A.1). The FTIR spectrum of the enzyme in powder form was also recorded for comparison with the spectra's of the films submitted to the enzymatic degradation. Results indicate that the enzyme was not present on the surface of the films (Appendix Fig. A.2).

"Insert Fig. 5"

3.3.4. Morphological characterization

Fig. 6 presents the surface morphology of the films AG and AGA5 before and after enzymatic degradation. Non-degraded films exhibit a smooth and homogeneous surface, as a result of the excellent film forming properties of alginate (Dong, Wang, & Du, 2006). The films incubated in the presence of the enzyme alginate lyase have a rougher surface, with the presence of large pores and holes in both top and bottom surfaces, as shown in Figs. 6b and 6d. Relatively to the films incubated in an SBF solution, no significant changes were observed on the surface morphology, indicating that the polymer degradation occurs due to the enzymatic hydrolysis.

"Insert Fig. 6"

3.3.5. Mechanical characterization

The mechanical properties of dry films were evaluated through tensile tests at different degradation times. It possible to observe that the films AG and AGA5, in the presence of the enzyme, present a great decrease in the maximum force at break at day 2 of degradation (AG: -48.3 %, AGA5: -62.5 %), followed by a gradual decrease along the degradation time, until dissolution (Fig. 7a). The same tendency was observed for the elongation ratio of the films degraded by the enzyme, i.e., the elongation ratio decreases in two well-defined stages as the degradation time increases (Fig. 7c). These results are in accordance with the weight loss profile, in which two well-defined stages were identified: the first one occurs until day 2 of degradation, while the second one proceeds from day 2 until the complete dissolution of the film. The mechanical properties of the control films AG and AGA5 do not present significant alterations in both maximum force at break and elongation ratio, as indicated in Figs. 7b and 7d. After 7 days of degradation, the maximum force at break of the films AG and AGA5 decreased 2.9 % and 8.6 %, respectively, while in the elongation ratio no significant changes were observed. Similar results were obtained for the films AGA15 and AGA25 (Appendix Fig. A.3). Results show that the weight loss determined after 7 days of degradation do not have significant influence on the mechanical properties of the films.

"Insert Fig. 7"

The *in vitro* degradation work indicates that the *Aloe vera* content has a great influence on the degradation rate of the *Aloe vera*-Ca-alginate films, for either enzymatic or non-enzymatic degradation. The films, in the presence of the enzyme, present changes in their physicochemical properties as a result of the enzymatic digestion. Conversely, the films in an SBF solution, without the enzyme, present a reduced weight loss without significant changes in its physicochemical properties. Results suggest that changing the *Aloe vera* content within the hydrogel films can be a promising approach to tailor their degradation rate and water absorption properties.

4. Conclusions

Aloe vera-Ca-alginate films exhibit high transparency, good handling and flexibility, which are fundamental properties for wound healing applications. The contact angle analysis shows an

increase in the hydrophilicity of the film's surface as a function of the *Aloe vera* incorporation. When immersed in solutions with different values of pH, it was observed that the capacity of the films to absorb water is dependent of both *Aloe vera* content and solution pH. The water absorption capacity of the films can be adjusted by changing the *Aloe vera* content, which can be useful for drug delivery applications, allowing to control the release kinetics of the biomolecules. The *in vitro* degradation works demonstrate that the films are susceptible to enzymatic degradation, as can be confirmed by monitoring the changes on the physicochemical properties (weight loss, dimensional changes, chemical and mechanical properties) during the degradation. In contrast, films immersed into an SBF solution, without the enzyme (hydrolytic degradation), show high stability and structural integrity, with no significant changes in their physicochemical properties.

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Figure captions

Fig. 1. Transparency of the hydrated film composed of alginate and *Aloe vera* at 25% (a) and its conformability to the human finger of a volunteer (b). Application of the Aloe vera-Ca-alginate films in the different phases of the wound healing process (c).

Fig. 2. Influence of *Aloe vera* content on the equilibrium water absorption of the films immersed in a 0.1M HCl solution pH 1.0 ( ), acetate buffer pH 5.5 ( ) and simulated body fluid pH 7.4 ( ). *The difference in water absorption is statistically significant (p<0.05).

Fig. 3. (a) Water contact angle of the alginate neat film (Film AG) and (b) alginate film containing 25% of *Aloe vera* (Film AGA25).

Fig. 4. Weight loss and water absorption of the films degraded in the presence (a,b) and absence (c) of the enzyme alginate lyase. Dry thickness of the films during enzymatic degradation (d). *After this period, the films are dissolved in the solution, which impeded the determination of water absorption and dry thickness. #The difference in water absorption and weight loss was statistically significant (p<0.05).

Fig. 5. FTIR spectra of the films AG and AGA5 at different periods of degradation in presence (a, b) and absence (c, d) of the enzyme alginate lyase. Degradation mechanism of alginate by the enzyme alginate lyase (e), adapted from Chen & Columbia (2011).

Fig. 6. SEM microphotographs of the *Aloe vera*-Ca-alginate films surface before and after degradation: film AG before (a) and after 10 days (b) and film AGA5 before (c) and after 8 days (d).

Fig. 7. Mechanical properties of the films AG and AGA5 at different periods of degradation in presence (a, c) and absence (b, d) of the enzyme alginate lyase. *At this time, the dimensions of the films did not allow performing the tensile test.

Captions to Appendix A. Supplementary data

Fig. A.1. FTIR spectra of the films AGA 15 and AGA25, before and after, 7 days of degradation in presence of alginate lyase.

Fig. A.2. Comparison between the FTIR spectra of the enzyme alginate lyase, film AG before and after 5 days of degradation in SBF supplemented with the enzyme, and film AG after 7 days of degradation in SBF without the enzyme.

Fig. A.3. Mechanical properties of the films AGA15 and AGA25, before and after, 7 days of degradation in SBF without the enzyme.

Fig. 1

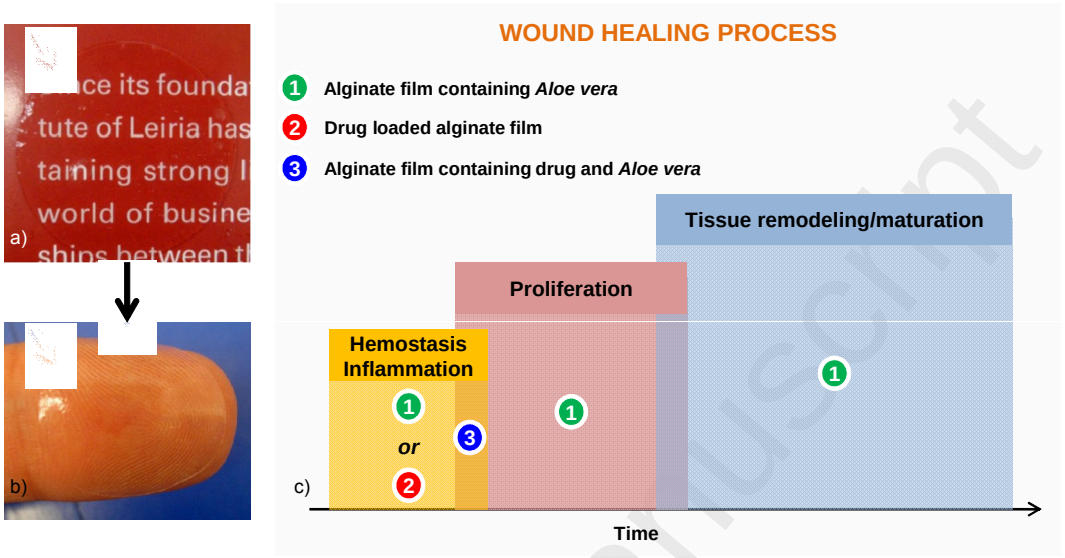


Fig. 2

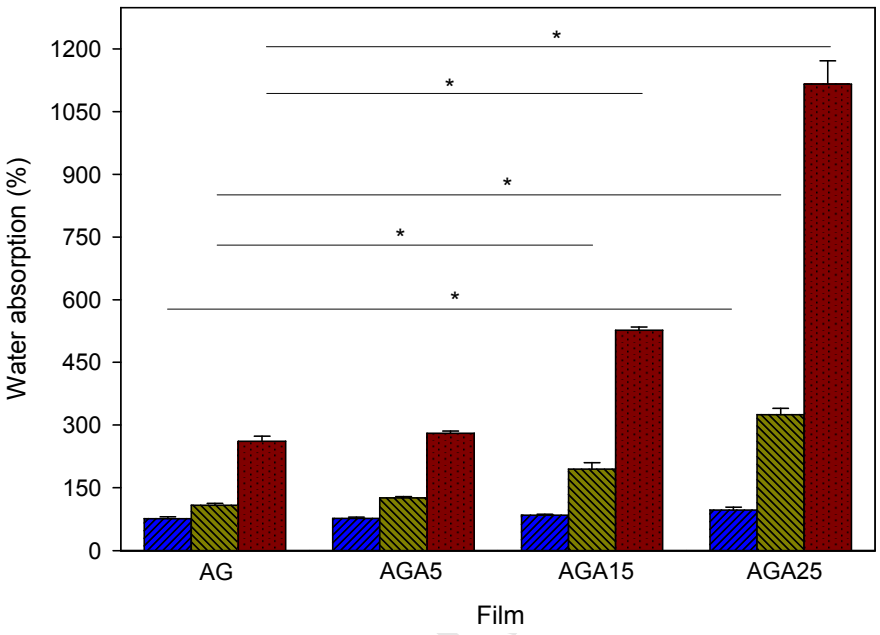


Fig. 3

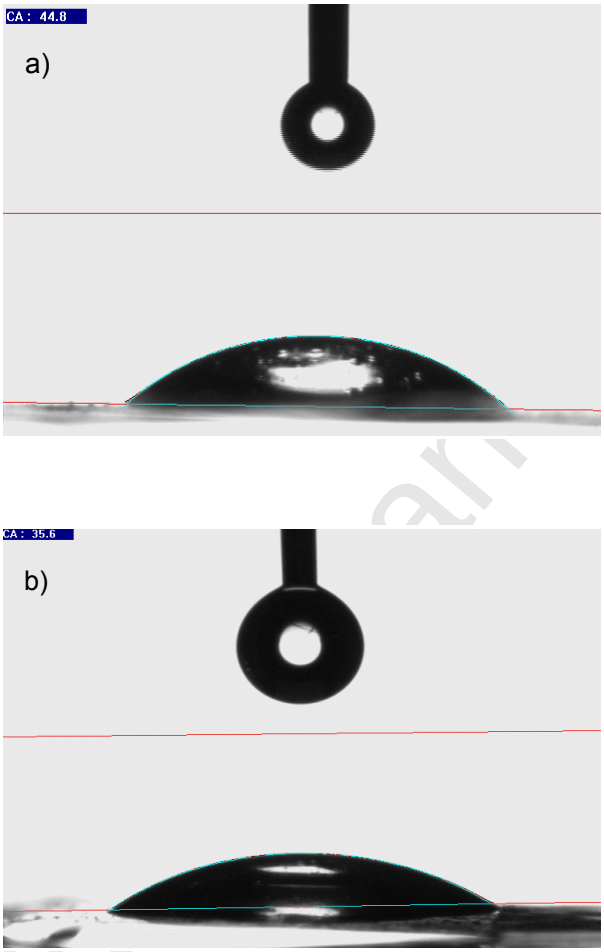
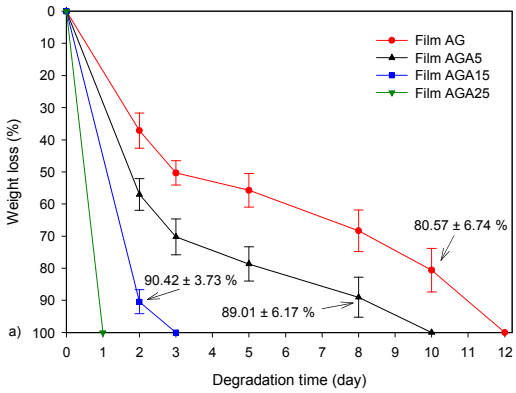
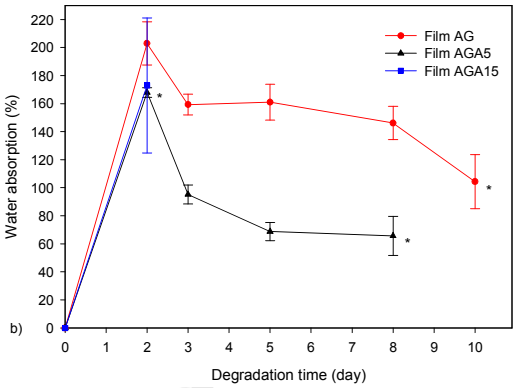
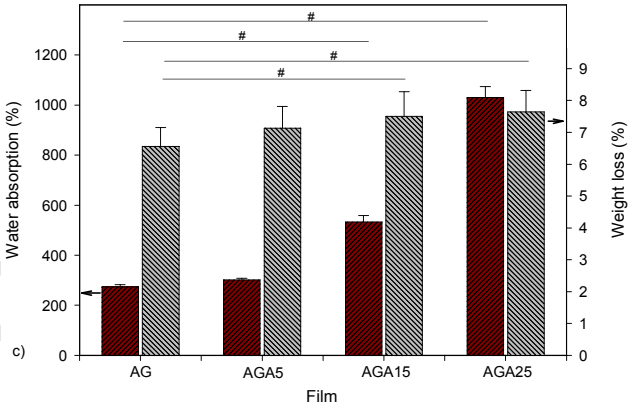


Fig. 4







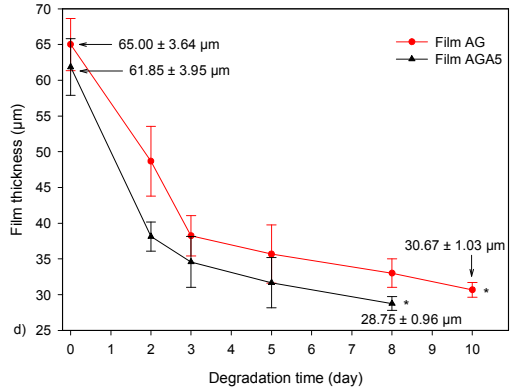
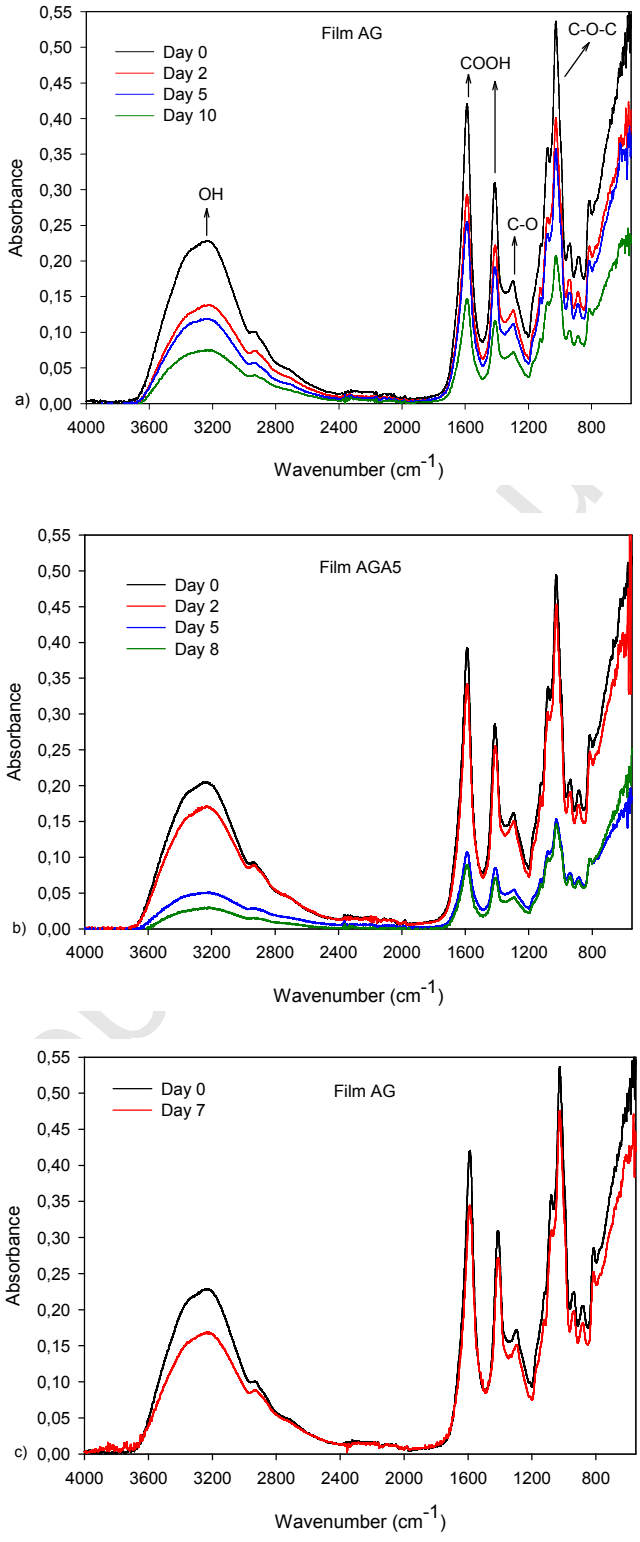
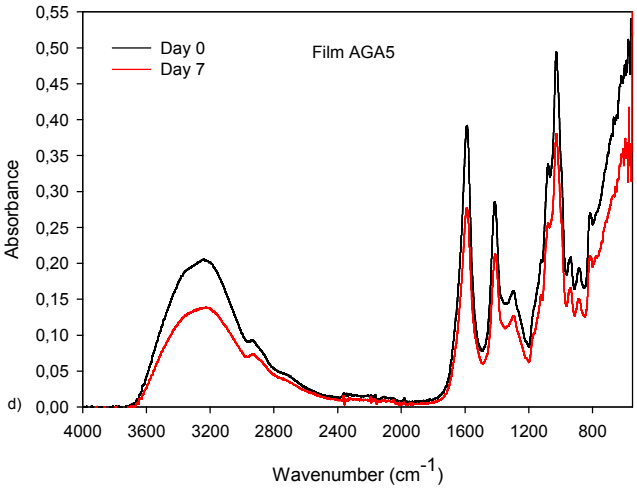
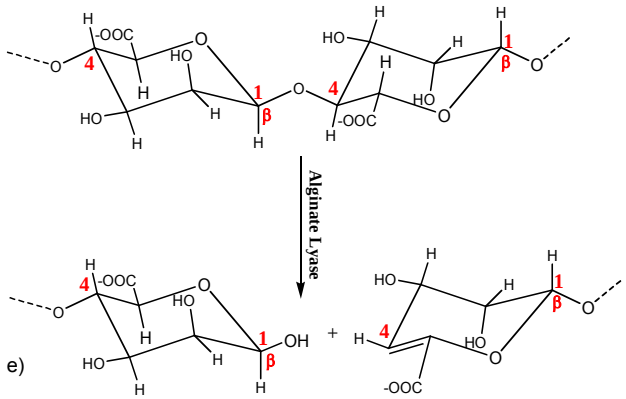


Fig. 5







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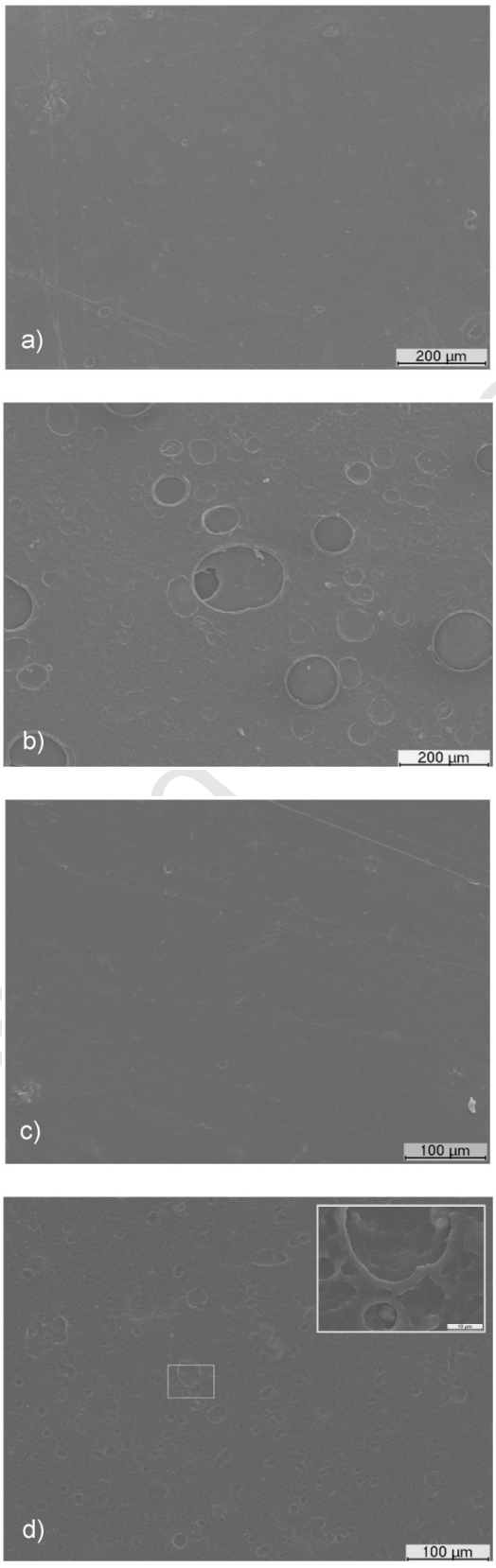
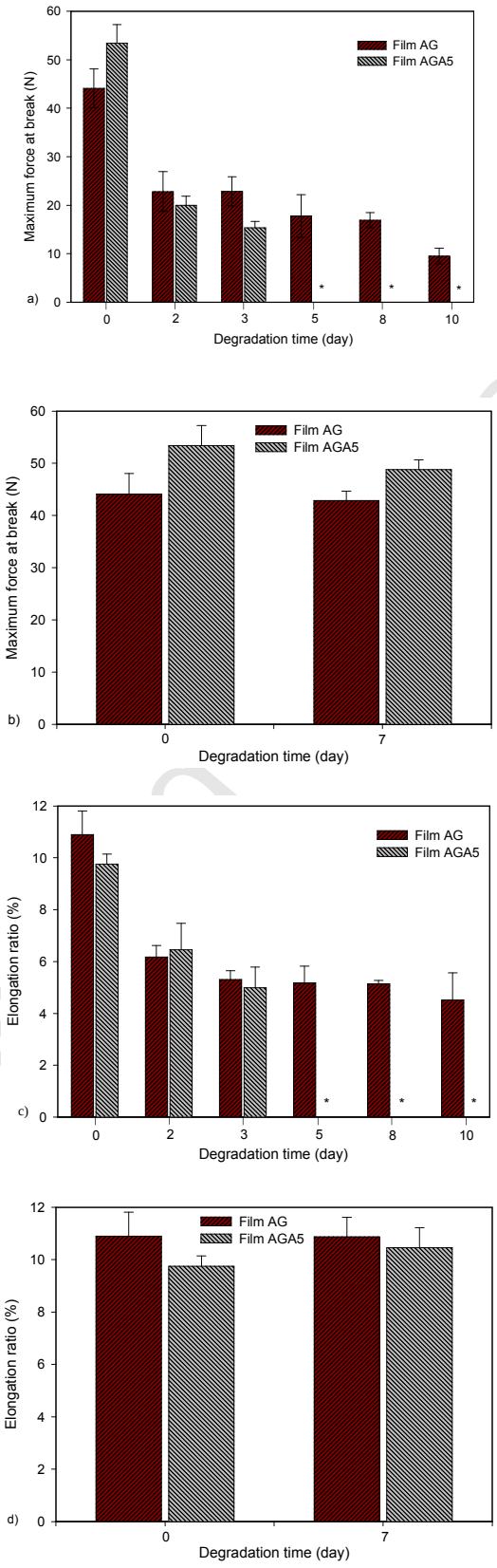


Fig. 7



Highlights

- Hydrogel films composed of alginate and *Aloe vera* gel were developed for wound healing applications
- Films with different formulations can be prepared for the different phases of the wound healing process
- *Aloe vera* gel significantly improves the water absorption and the *in vitro* degradation properties of alginate hydrogel films
- The properties of the hydrogel films can be tailored by changing the *Aloe vera* content