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Novel Alginate/Aloe Vera Hydrogel Blends as Wound Dressings for the Treatment of Several Types of Wounds

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Currently, there is a significant increase in the use of natural materials for biomedical applications, mainly due to their biocompatibility, biodegradability and similar properties to the constituents of natural tissues. In this work, hydrogel films composed of alginate and Aloe vera gel were prepared and characterized for application in the treatment of several types of wounds (e.g. exuding, painful and dry). The films were prepared through a two-step procedure encompassing the film formation by a solvent-casting method and an additional crosslinking step to improve their properties. The physical, morphological and water absorption properties were characterized by introducing several variations in the Aloe vera content. The *in vitro* degradation behaviour of the films was investigated in the presence of the enzyme alginate lyase for 2 weeks. Results showed that Aloe vera improves the water absorption and the *in vitro* degradation rate of the films. In conclusion, this study indicates that the alginate/Aloe vera hydrogel films can be potentially explored as wound dressing for the treatment of different wound types.

1. Introduction

The repair or regeneration of tissue defects caused by trauma, disease or genetic disorders represents a great challenge in regenerative medicine (Melchels et al., 2012; Rigogliuso et al., 2012). Skin is the largest organ of the human body, acting as a protective barrier between the internal body and the external atmosphere (Yildirimer et al., 2012; Groeber et al., 2011). The direct exposure of skin to the external environment can result in different types of damage (e.g. burns, wounds or ulcers) with loss of variable volumes of extracellular matrix in either epidermal or dermal layer. According to the depth (epidermal, superficial partial-thickness, deep partial-thickness and full-thickness wound) and type (burn, ulcer, acute and chronic wound) of the injury, different strategies can be used to promote the skin regeneration, including auto/allografts, tissue-engineered skin substitutes and wound dressings (Groeber et al., 2011; Shevchenko et al., 2010; Boateng et al., 2008; MacNeil, 2007). The clinical golden standard for the treatment of skin lesions consists in the removal of split-thickness grafts from healthy sites of the body to treat injured areas. However, the use of autografts and allografts is characterized by several limitations like patient morbidity, risk of immunogenic rejection, transmission of diseases and ethical issues (Groeber et al., 2011; MacNeil 2007). To address these drawbacks and solve the problem of donor graft shortage, both cellular and acellular tissue-engineered skin substitutes are a clinically viable solution. When applied to the lesion site, these products provide protection from contamination and dehydration, enabling the regeneration of different skin layers (Shevchenko et al., 2010). Although the clinical effectiveness of tissue-engineered skin substitutes, it is necessary reduce their fabrication cost and improve their efficiency on the regeneration of full-thickness wounds and skin appendages (Groeber et al., 2011; Boateng et al., 2008). The application of either traditional or modern dressings represents an effective strategy for the treatment of different types of wounds due to their excellent relationship between clinical efficacy and manufacturing cost. Among these products, alginate dressings are widely used by the clinicians as a result of their ability to create and maintain a wound moist environment that improves the healing process (Boateng et al., 2008; Jones et al., 2006). Furthermore, alginate dressings are also biocompatible, biodegradable and exhibit an easy gelation (Pereira et al., 2013; Lee and Mooney, 2012). Although these important properties, alginate dressings by itself do not present therapeutic activity, which can limit their use in some

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types of wounds like infected wounds. To address this need, we are investigating the potential of blending alginate with Aloe vera gel in order to produce hydrogel films with therapeutic activity for the treatment of different types of wounds (Pereira et al., 2013, 2011). Aloe vera is a photosynthetic plant of great interest for several biomedical and pharmaceutical preparations due to its therapeutic properties. The gel extracted from the parenchymal tissue of the plant is composed of several potentially active constituents (soluble sugars, polysaccharides, salicylic acids, proteins, minerals, etc.) and thereby has been used as topical agent for the treatment of different skin disorders (Boudreau and Beland, 2006). Several therapeutic properties have been assigned to the Aloe vera gel including antibacterial, anti-septic, anti-inflammatory and ability to stimulate the proliferation of fibroblasts and the synthesis of collagen (Pellizzoni et al., 2012; Atiba et al., 2011; Chithra et al., 1998a, 1998b). Our main goal is to combine the occlusive and haemostatic properties of calcium alginate hydrogels with the therapeutic properties of Aloe vera. We hypothesized that the Aloe vera compounds incorporated within the alginate films can be released into the wound bed during the swelling, improving the healing process. In this work, Aloe vera-Ca-alginate hydrogel films were prepared and their potential for application as wound dressing material investigated. The films were characterized regarding the thickness, morphology, water absorption and enzymatic *in vitro* degradation behavior.

2. Experimental procedure

2.1 Preparation of crosslinked hydrogel films

The hydrogel films were prepared through a two step procedure as previously reported (Pereira et al., 2013). Briefly, solutions of sodium alginate at 1.5% (BDH Prolabo, VWR International, UK) containing glicerol at 15% (w/w) (Scharlau, Spain) and Aloe vera at 1.0% (Aloecorp, Broomfield, U.S.A.) were mixed to obtain the 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). After drying at controlled conditions, the films were immersed into a 5% (w/v) calcium chloride solution to allow the crosslinking and subsequently washed with distilled water.

2.2 Film thickness, surface morphology and swelling behaviour

The thickness was measured using a manual micrometer (Model 102-301, Mitutoyo) at nine different positions. The morphology of film samples previously sputtered with gold was examined by scanning electron microscope (SEM) using the Quanta 600F system (FEI Company) operating at 15kV. The swelling behaviour of the crosslinked films was investigated by the immersion in 20 mL of simulated body fluid (SBF, pH 7.4) at 37ºC. At different periods of time, the samples were removed from the solution, the excess of water at surface withdraw with a filter paper and their wet weight immediately determined. The water absorption was calculated according to the Equation 1:

$$\text{Water absorption} = \left[\left(W_w - W_i\right)/W_i\right]\times 100 \quad (1)$$

where, W_w and W_i represents the wet and dry weight of the films, respectively. Four samples were used for each condition.

2.3 *In vitro* enzymatic degradation studies

Accelerated *in vitro* enzymatic degradation tests were performed through the incubation of film samples (15 mm x 50 mm) in 10mL of SBF solution at 37ºC, supplemented with the enzyme alginate lyase (10 U/g alginate). Before the test, the samples were dried in an oven at 37ºC until constant mass. At pre-determined periods, the samples were collected from the degradation medium, washed with distilled water and the excess of water removed using a filter paper. After this procedure, the wet weight of the samples was immediately accessed in order to determine the water absorption (Equation 1). Afterwards, the films were transferred to an oven and dried at 37ºC until constant mass to evaluate the loss of weight. As control, the films were immersed in SBF solution in the same conditions but without the enzyme. The weight loss was determined using the Equation 2:

$$\text{Weight loss} = \left[\left(W_i - W_f\right)/W_i\right]\times 100 \quad (2)$$

where, W_i and W_f correspond to the weight of the film before and after degradation. Nine samples were used for each condition.

2.4 Statistical analysis

The statistical analysis of the results was done through the one-way analysis of variance (ANOVA), while the comparisons between two means were done using the Tukey's test. The statistical significance was considered for a $p<0.05$. For each condition a minimum of three samples were considered.

3. Results and Discussion

In this work, hydrogel films composed of alginate and Aloe vera gel were prepared using a simple and fast methodology for use in the treatment of different types of wounds.

3.1 Film thickness and surface morphology

The influence of both composition and crosslinking on the film thickness is shown in Figure 1. The non-crosslinked films exhibited thicknesses in a range 66.14-69.00 μm, while the crosslinked films in dry state presented thicknesses comprised between 59.7 μm and 75.3 μm. The wet thickness of the films, determined after crosslinking in an aqueous calcium chloride solution, was in a range 139.0 μm to 211.0 μm. Results showed that the dry films with and without crosslinking exhibit similar thickness, independently of their composition. Contrary, the wet thickness of the films is significantly affected by the composition. In this case, the increase in the Aloe vera content leads to a significant increase on the thickness.

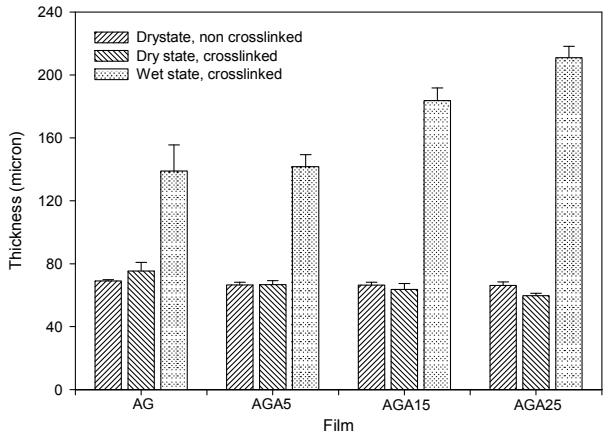

Figure 1: Influence of composition and crosslinking on the film thickness

As it is seen in Figure 2, the hydrogel films exhibited high transparency in both dry and wet state, which is of great interest for the proposed applications. The transparency is an important property for materials aimed to be used as wound dressings, once it allows the visual inspection of the lesion without need to remove the dressing. Regarding the surface morphology of the films, the SEM analysis revealed a smooth and homogeneous surface without significant defects (Figure 2c), which can be attributed to the excellent film-forming properties of alginate (Dong et al., 2006).

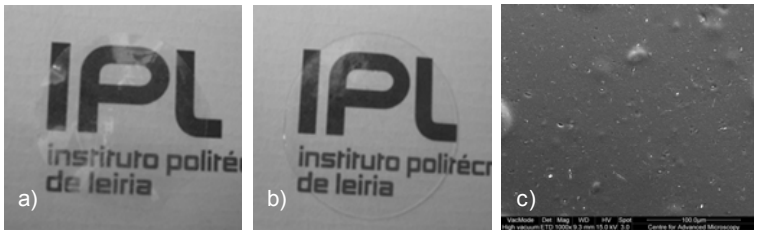

Figure 2: Macroscopic images showing the transparency of the crosslinked alginate film containing 25% of Aloe vera in dry (a) and wet state (b). SEM image of the surface of the crosslinked alginate film (c)

3.2 Swelling behaviour

As the films were developed for application as wound dressings, the evaluation of their capacity to absorb aqueous solutions is fundamental. This property determines the ability of the films to absorb exudate from

the wound, avoiding maceration and maintaining a moist environment. The effect of Aloe vera content on the water absorption capacity of the hydrogel films immersed in SBF solution for 24 h is shown in Figure 3. Results indicated that the increase on the Aloe vera content significantly increased both the film's water absorption and the necessary time to reach the equilibrium. From the results, it is possible to observe that the neat alginate film (film AG) and the alginate film containing 5% of Aloe vera (film AGA5) presented a quickly absorption of water along the first 30 min after immersion, reaching the equilibrium in approximated 120 min. The water absorption profiles of the films AG and AGA5 were very similar, which suggest that 5% of Aloe vera does not have a significant influence on the water absorption. Contrary, the incorporation of 15% (film AGA15) and 25% (film AGA25) of Aloe vera within the films significantly affected the water absorption capacity. The film with higher Aloe vera content exhibited a quickly absorption of water after immersion, which was followed by a progressive and slower absorption of water, not reaching the equilibrium during the 24 h of the test. These results clearly showed that the water absorption capacity of the films, and consequently, their ability to remove exudate from the wound can be tailored by changing the Aloe vera content.

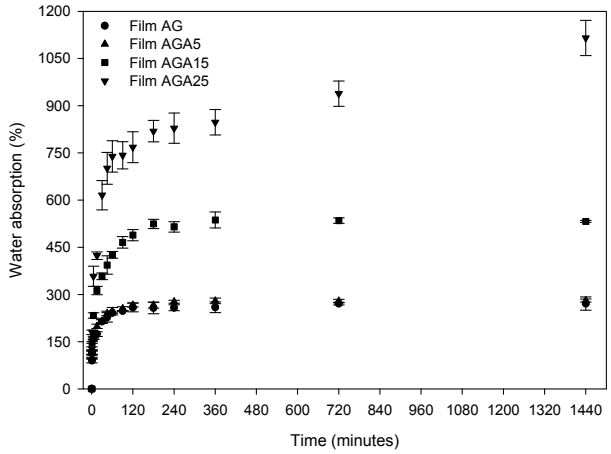

Figure 3: Influence of Aloe vera content on the water absorption of the films during immersion in simulated body fluid at physiologic temperature

3.3 *In vitro* enzymatic degradation studies

The accelerated *in vitro* enzymatic degradation tests were performed to evaluate the influence of Aloe vera content on the degradation behaviour of the films. The films were incubated in SBF solution supplemented with the enzyme alginate lyase, which degrades the alginate through a mechanism that involves the cleavage of the glycosidic bonds β-1,4 (Kim et al., 2011).

As seen in Figure 4a, the degradation rate of the films in SFB solution supplemented with the enzyme was dependent on the Aloe vera content. The films AG and AGA5 presented the lower degradation rate, being completely dissolved in the medium 12 and 10 d after immersion, respectively. Contrary, the films AGA15 and AGA25, exhibited the fastest degradation rate, being completely dissolved in the medium only after 3 and 2 d of degradation, respectively. The enzymatic degradation tests clearly showed that the increase on the Aloe vera content leads to a significant increase on the *in vitro* degradation behaviour of the films. As control, the films were immersed in SBF solution without the enzyme for determine the non-enzymatic weight loss. From the Figure 4b, it is possible verify that the films exhibited weight loss values in a range 6.6±0.6% to 7.6±0.7% after 7 d of degradation, which indicates the stability and the resistance of the films to the hydrolytic degradation. As can be seen in Figure 4b, the films with high percentage of Aloe vera presented high loss of weight, indicating that Aloe vera improved the degradation rate, similarly to what happened in the enzymatic degradation.

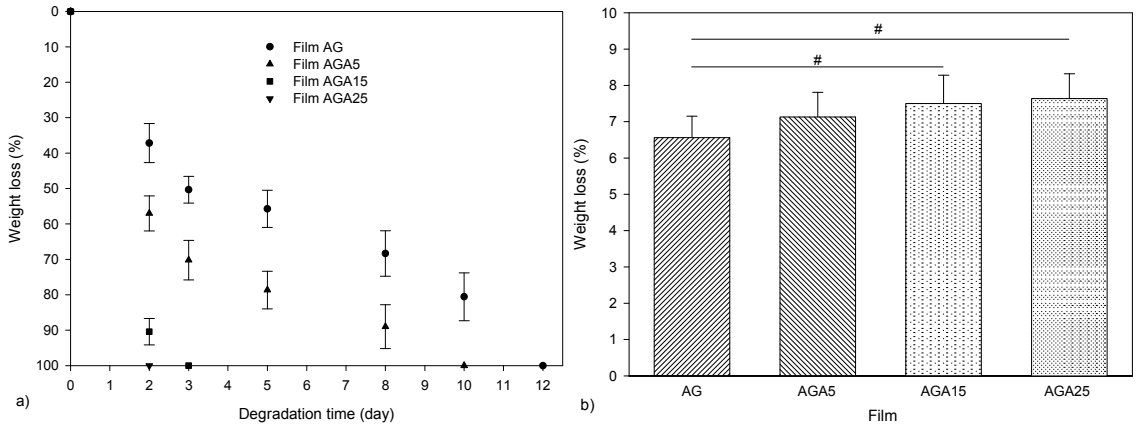

Figure 4: Weight loss profiles of the films degraded in presence of the enzyme alginate lyase for 12 d (a). Weight loss values of the developed films after 7 d of immersion in SBF solution without the enzyme (b). #Statistically significant when compared with film AG (p < 0.05)

From Figure 5, it is possible observe that the Aloe vera content has a great influence on water uptake of the films submitted to the hydrolytic degradation, as previously observed in the water absorption test. After 7 days of degradation, the films with high Aloe vera content exhibited high water absorption values, which can explain the differences in the degradation rate. In the case of enzymatic degradation (data not show), this behaviour was not verified because the fast dissolution of the films resulted on the decrease of the available area to absorb and retain water. The data of the *in vitro* degradation tests clearly showed that the Aloe vera improves the degradation rate of the films. Based on both water absorption and weight loss results, it is hypothesized that the films containing high Aloe vera content are more susceptible to the hydrolytic degradation due to the high absorption of water within the hydrogel network, which leads the cleavage of degradable linkages and raises the film degradation.

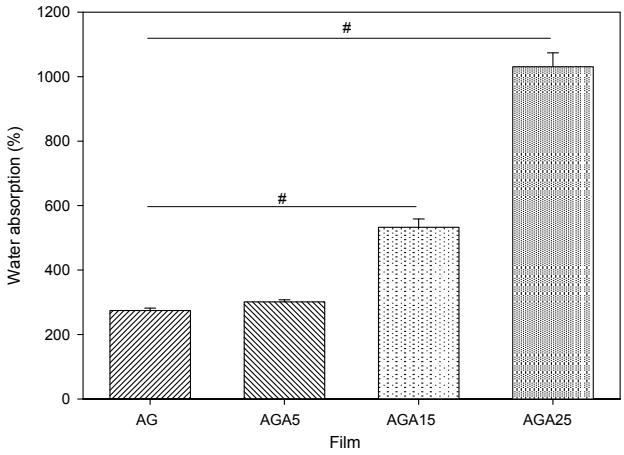

Figure 5: Water absorption of the films after 7 d of degradation in SBF solution. #Statistically significant when compared with film AG (p < 0.05)

4. Conclusions

In this study, a series of alginate/Aloe vera hydrogel films were fabricated using a simple a fast methodology. The films were prepared with different Aloe vera contents, exhibiting high transparency in either dry or wet state. The water absorption properties of the films are dependent on the Aloe vera content, i.e., the increase on Aloe vera content significantly improved the water absorption. Enzymatic *in vitro* degradation studies clearly showed that Aloe vera increased the degradation rate of films, probably due to the high water absorption. Together, these results indicated that the water absorption and the *in*

vitro degradation properties of the films can be tailored by the introduction of different contents of Aloe vera.

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