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Degradation behavior of biopolymer-based membranes for skin tissue regeneration

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Abstract

This research work investigates the long term *in vitro* degradation behavior of alginate-aloe vera composite membranes for skin regeneration. The membranes were prepared through a two-step procedure, which involves the synthesis of thin membranes and the crosslinking reaction. Degradation tests were performed through the immersion of the membranes into simulated body fluid solution at pH 7.4 and physiological temperature, during 6 months. Alginate-aloe vera membranes are resistant to the hydrolytic degradation and exhibit weight loss values in a range of 20.37-26.32%. Results show that an increase in the aloe vera content leads to a slight increase in the weight loss during the degradation process. Preliminary drug release studies, using nitrofurazone as a model drug, suggest the ability of the developed membranes to be used as a drug delivery system.

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Keywords: Degradation; Alginate; Aloe vera; Membrane; Skin.

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1. Introduction

The regeneration of skin remains a huge challenge for the clinicians, due to its multilayer structure and the presence of multiple cell types in a well-organized way. In order to promote the regeneration of healthy skin, several therapies (traditional and advanced) and biomaterials have been developed and used. Traditional therapies involve, for example, the use of medicinal plants, honey, larval therapies and gauzes (Jin et al. 2013; Sell et al. 2012; Turkmen et al. 2010). Despite the reduced costs of these therapies, they have been replaced by advanced ones, which include the use of wound dressings, tissue-engineered skin substitutes, cellular therapies and *in situ* biofabrication approaches (Pereira et al. 2013a; Wu et al. 2007). In advanced therapies, either natural or synthetic polymers play a pivotal role, enabling the development of either 2D or 3D matrices that mimic the function of the natural extracellular matrix (ECM) and support the repair and regeneration of the new tissue (Melchels et al. 2012).

Biopolymers represent a class of materials obtained from the nature that have been widely used in the field of tissue engineering and regenerative medicine, due to its useful properties such as the biocompatibility, biodegradability, hydrogel formation and compositional similarities with the ECM of the human tissues (Van Vlierberghe et al. 2011). Alginate is a natural polymer obtained from brown algae that contains β-D-mannuronic acid and α-L-guluronic acid units in its chemical composition (Pereira et al. 2011; Rezende et al. 2009). This material has the ability to easily form hydrogels in mild conditions without the use of toxic solvents, for example, through the interaction with divalent and trivalent cations (Pereira et al. 2013b). As a result of the high water content, elasticity, permeability and ability to create a moist environment in the wound bed, alginate-based hydrogels have been processed into different physical forms like films (Pereira et al. 2013c), scaffolds (Kim et al. 2011) and nanofibers (Shalumon et al. 2011) for skin applications.

In recent years, the use of medicinal plants as topical agents to induce the regeneration of different types of skin lesions has been increasing. Aloe vera is tropical plant composed of a water fraction and a solid fraction, containing potentially therapeutic constituents such as soluble sugars, lipids, proteins, polysaccharides, glycoproteins, vitamins and minerals (Boudreau and Beland, 2006). Due to its therapeutic properties, which include the anti-inflammatory, antibacterial and ability to improve the collagen content and the blood vessel formation during the healing process, aloe vera has been used as therapeutic agent to promote the skin repair (Pellizzoni et al. 2012; Atiba et al. 2011; Boudreau and Beland, 2006).

In this work, we investigate the *in vitro* degradation behavior of alginate-aloe vera membranes for skin tissue engineering applications. Preliminary drug release studies are also performed to evaluate the ability of the membranes to act as a drug delivery system.

2. Materials and methods

2.1. Materials

Sodium alginate (BDH Prolabo, VWR International, UK) was used with 54.09 ± 1% of M units, determined by ^{1}H NMR (Pereira et al. 2013b). The aloe vera (ACTIValoe®, Aloe vera Gel Qmatrix 200X Flakes) was kindly offered by Aloecorp (Broomfield, U.S.A.) and the glycerol was obtained from Scharlau (Spain). Calcium chloride and the drug nitrofurazone (NFZ) were obtained from Sigma Aldrich (Portugal). All other chemicals were reagent grade and used as received.

2.2. Preparation of biopolymer-based membranes

Membranes were prepared using a two-step experimental protocol previously developed (Pereira et al. 2013b). In the first step, aqueous solutions of aloe vera (1.0% w/v) and sodium alginate (1.5% w/v) containing 15% of glycerol were mixed at 600 rpm during 30 min, in order to obtain different alginate-aloe vera proportions, as shown in Table 1. Afterwards, the solutions (25 mL) were casted into the petri dishes (9.5 cm of diameter) and allowed to dry at 25ºC. In the second step, the dry membranes were immersed into a calcium chloride aqueous

solution (5.0%, w/v) for 5 minutes to allow the formation of hydrogel membranes. Then, the membranes were extensively washed with distilled water and dried at room temperature until constant weight.

To prepare drug-loaded membranes, nitrofurazone was added to the alginate solution at a concentration of 0.2 mg/mL, during preparation. The obtained membranes were designated as AG/NFZ, AGA5/NFZ, AGA15/NFZ and AGA25/NFZ.

Table 1. Composition of the composite membranes prepared using alginate, aloe vera and glycerol

Membrane abbreviation	Membrane composition (v/v)		
	Alginate (1.5% w/v)	Glycerol (%, w/w based on the alginate mass)	Aloe vera (1.0% w/v)
AG	100	15	0
AGA5	95	15	5
AGA15	85	15	15
AGA25	75	15	25

2.3. In vitro degradation behavior of membranes

The degradation behavior of the biopolymer-based membranes was investigated by the immersion of rectangular samples (50 mm x 15 mm) into a falcon tube containing 10 mL of simulated body fluid (SBF) solution at pH 7.4. The tubes were incubated at 37ºC during 24 weeks. Prior to test, the samples were dried in an oven at 37ºC until constant mass. The specimens were removed from the medium at defined time periods and the excess of water at the surface was withdrawn using a filter paper. Then, the samples were immediately weighted to determine the water uptake, and subsequently dried in an oven at 37ºC until constant mass to evaluate its weight loss. The medium was replaced weekly with fresh solution. Nine samples were used for each condition.

2.3.1. Thickness change, water uptake and weight loss

The thickness of the membranes in dry state was evaluated using a micrometer (Model 102-301, Mitutoyo) with 0.001 mm of accuracy before and after the degradation process.

The water uptake during the degradation process was determined by dividing the wet weight of the membrane after immersion by the initial dry weight before immersion, while the membrane's weight loss was calculated as follows:

$$Weight\ loss = \left(\frac{W_i - W_f}{W_i} \right) \times 100 \tag{1}$$

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

2.4. In vitro drug release studies

To investigate the properties of the membranes for drug delivery applications, drug-loaded membranes (30 mm x 30 mm) were incubated into acetate buffer (30 mL) at physiological temperature and 100 rpm (Siewert et al. 2003). At pre-determined periods of time, a sample of the medium was collected and the amount of the released drug was quantified using an UV spectrophotometer (Genesys 10UV) at 365 nm. Three replicates were tested for each condition.

3. Results and discussion

Alginate-based membranes were successfully prepared using aloe vera contents between 5% and 25%. The membranes exhibit high transparency in both dry and wet state, good homogeneity and a homogeneous surface.

3.1. In vitro degradation behavior

The degradation behaviour of biomaterials for tissue regeneration is a critical property, strongly influencing the regeneration rate of the new tissue (Alsberg et al. 2003). The degradation tests were carried out in SBF solution at 37ºC to investigate both the influence of the aloe vera content and the durability of the membranes in aqueous environment.

Fig. 1a shows the weight loss profiles of the alginate-aloe vera membranes along the degradation time. All membranes exhibit a quick loss of weight during the first week, which is attributed to the leaching of the plasticizer and/or the release of aloe vera compounds. After this stage, membranes present a slower and gradual loss of weight during the next weeks, reaching weight loss values comprised between 20.37 ± 0.94% and 26.32 ± 1.75%, after 24 weeks of incubation. From Fig. 1a, it is also possible to verify that the increase in aloe vera content leads to a slight increase in the weight loss. The influence of the aloe vera content in the degradation rate of membranes is explained by the differences in water absorption, as shown in Fig. 1b. The membranes with high aloe vera percentage present a significant increase in the water uptake during the degradation process due to the hydrophilic properties of aloe vera (Pereira et al. 2013b). Similarly to the weight loss profiles, the water absorption results indicate that the use of 5% of aloe vera does not have significant influence in the water uptake, while 15% and 25% of aloe vera strongly improve the water absorption. Based on these results, it is expected that the further penetration of water molecules within the membranes with high aloe vera content leads to an increase in the cleavage of degradable linkages, which in turn improves the weight loss (Fig. 1c).

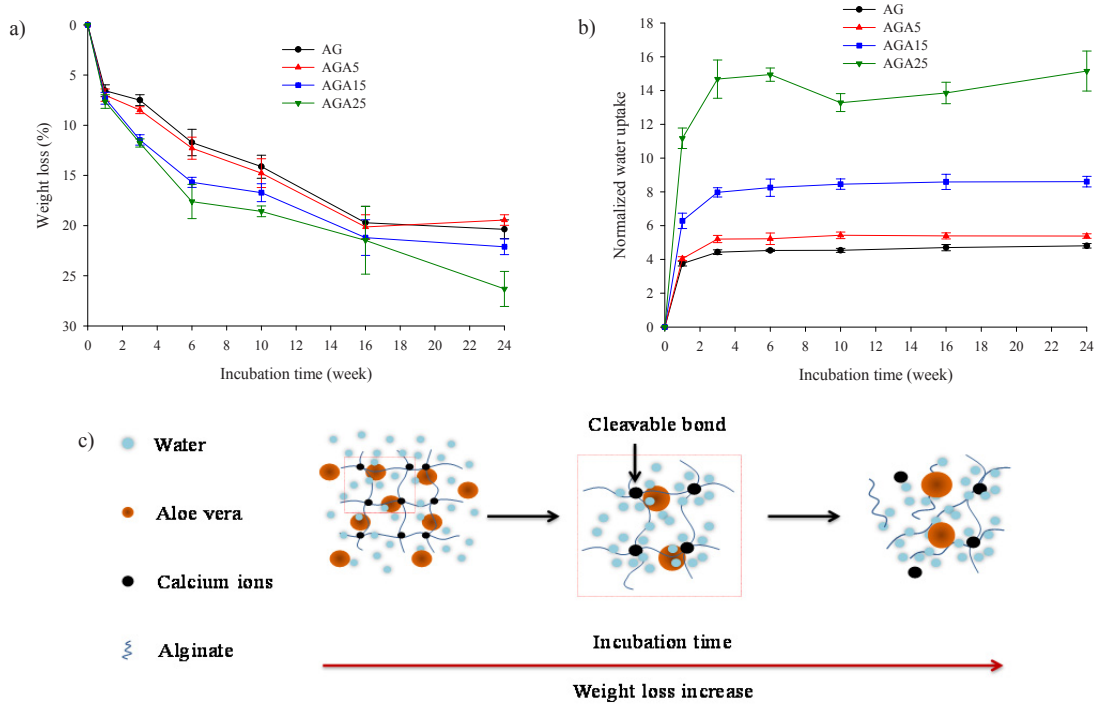

Fig. 1. (a) Weight loss and (b) water absorption profiles of the alginate-aloe vera membranes during the degradation process; (c) illustration of the degradation process with cleavage of degradable bonds and consequent release of calcium ions and aloe vera compounds in the lesion site.

Fig. 2a displays the changes in the wet thickness of the membranes during the degradation process. Results show that the membranes with high aloe vera contents suffer a great increase in the thickness values in the first weeks of degradation, which is in accordance with the water absorption data. Along the incubation time, the dry weight of the membranes was also monitored as an indicator of the degradation process. Dry membranes exhibit a great reduction in their thickness during the first 10 weeks of degradation, which is followed by a slow and gradual reduction during the following 14 weeks (Fig. 2b). After 24 weeks of degradation, the thickness of the membranes shows a reduction in a range of 33.8-38.5%.

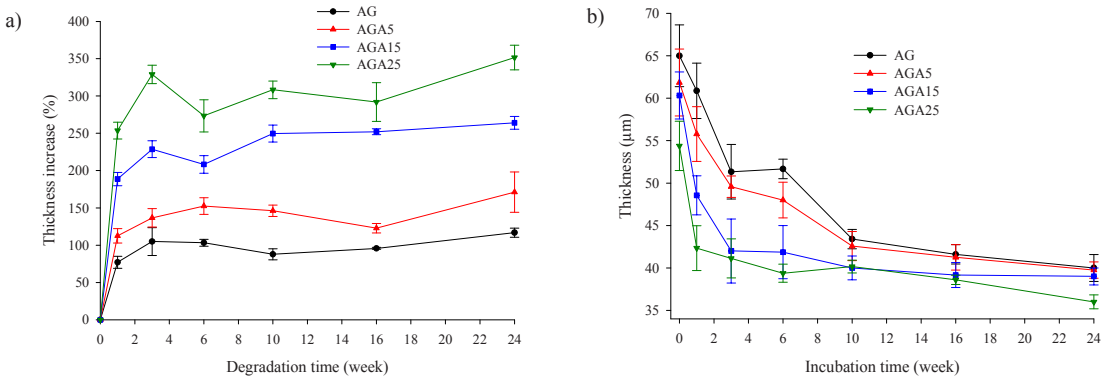

Fig. 2. Changes in the wet (a) and dry (b) thickness of the membranes at different periods of degradation.

3.2. Drug release studies

The ability of the membranes to act as a drug delivery system is very important for skin tissue engineering, enabling the topical release of aloe vera compounds and other therapeutic agents directly into the lesion site. The drug release tests were performed using nitrofurazone as a model drug and acetate buffer to simulate the skin pH.

The drug-loaded membranes exhibit a yellowish coloration due to the incorporation of nitrofurazone, maintaining a good transparency. During the crosslinking process, the drug diffused from the membranes to the crosslinking solution, leading to a decrease in the incorporation efficiency. The amount of drug lost during the crosslinking process is comprised between 3.61% and 7.95%, being dependent on the film composition (membrane AG/NFZ: 3.61 ± 0.64%; AGA5/NFZ: 3.66 ± 0.10%; AGA15/NFZ: 6.39 ± 3.99%; AGA25/NFZ: 7.95 ± 2.12%). Fig. 3 shows the influence of the membrane composition in the drug release kinetics. From the results, it is possible to observe that the drug release increases as the aloe vera content within the membranes also increases. All profiles are characterized by an instantaneous release of drug (burst release) due to the diffusion of the particles located at the surface of the membrane. The control membrane (AG/NFZ) and the membrane containing 5% of aloe vera (AGA5/NFZ) present a reduced percentage of drug released along the experiment, which is due to the differences in the water absorption. The increase in aloe vera content leads to a significant increase in the water absorption, causing a higher diffusion of the drug for the medium. The membranes containing 15% and 25% of aloe vera release the incorporated drug in approximated 120 min, while the remaining films release about 80% of the drug at the same period of time.

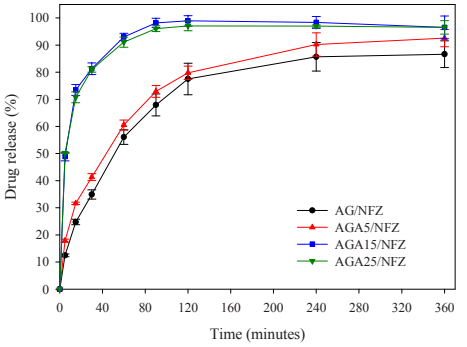

Fig. 3. Release profiles of the drug-loaded membranes immersed into acetate buffer at pH 5.5.

4. Conclusions

Biopolymer-based membranes, composed of alginate, aloe vera and glycerol, were prepared and its degradation behavior investigated. Long term *in vitro* degradation tests performed in SBF solution show that the membranes are resistant to the hydrolytic degradation, while aloe vera slightly improves the loss of weight during the incubation time. Preliminary *in vitro* drug release tests suggest that the membranes are able to incorporate and release drugs.

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