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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 *A. vera* content (5%, 15% and 25%, v/v) on water absorption was evaluated by the incubation of the films into a 0.1 M HCl solution (pH 1.0), acetate buffer (pH 5.5) and simulated body fluid solution (pH 7.4) during 24 h. Results show that the water absorption is significantly higher for films containing high *A. vera* contents (15% and 25%), while no significant differences are observed between the alginate neat film and the film with 5% of *A. vera*. The *in vitro* enzymatic degradation tests indicate that an increase in the *A. 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 *A. vera* content.

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, 2013). 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, 2013; 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, Carvalho, Vaz, Gil, Mendes, & Bártolo, 2013).

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, Carvalho, et al., 2013; 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

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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 *A. vera* leaves, like a bitter yellow juice called *A. vera* latex or *Aloe* juice and a clear mucilaginous *A. 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, Sajithlal, & Chandrakasan (1998), Chithra, Sajithlal, & Chandrakasan (1998) showed that *A. 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 *A. 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 *A. vera* gel, as well to the synergy established between the compounds (Hamman, 2008; Pellizzoni et al., 2012).

Recently, we developed novel *A. vera*-Ca-alginate hydrogel films, combining the occlusive and haemostatic properties of calcium alginate hydrogels with the therapeutic properties of *A. vera* (Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011; Pereira, Mendes, et al., 2013; Pereira, Carvalho, et al., 2013). In this system, alginate hydrogel acts as a vehicle for the incorporation and release of *A. 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 *A. 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 *A. vera* on both the water absorption and the *in vitro* degradation behaviour of *A. vera*-Ca-alginate hydrogel films. It is expected that the incorporation of *A. 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 *A. vera* (ACTIValoe®, *A. 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, $K_2HPO{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, 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 *A. 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 *A. vera* (1.0%, w/v), in order to obtain final alginate/*A. 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 = 9.5 cm) and left to dry in room at 25 °C, under 50% of humidity. After drying, the films were immersed into a calcium chloride aqueous solution (5.0%, w/v) for 5 min 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 54.4–66.0 μm, into 20 mL of solution during 24 h. 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) 0.1 M HCl solution at pH 1.0 simulating the gastric fluid, (ii) acetate buffer at pH 5.5 (10 mM) simulating the pH of the skin, and (iii) SBF solution at pH 7.4 (50 mM of trishydroxylaminomethane and 45 mM hydrochloric acid),