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Alginate/Aloe vera hydrogel films for biomedical applications

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Abstract

This paper describes a methodology to produce hydrogel films, composed of alginate and *Aloe vera*, for wound healing and drug delivery applications. The films were prepared through the solvent-casting method and subsequently submitted to an additional cross-linking step to improve their properties. Alginate films with different *Aloe vera* contents (5, 15 and 25%) were prepared and its properties evaluated in terms of thickness, transparency, swelling behavior and *in vitro* degradation. Results show a positive influence of *Aloe vera* on the transparency of the films, in both dry and wet state. Films were immersed in acetate buffer at pH 5.5 simulating the value of the skin, to evaluate its water absorption capacity. It was found that water absorption increases as the *Aloe vera* content increases, suggesting that *Aloe vera* enhances the hydrophilic properties of the films. The *in vitro* degradation tests were performed through the incubation of the films, for 10 weeks, in a simulated body fluid at 37°C. After this period, films kept its structure integrity exhibiting a weight loss in the range of 14.1-18.6%.

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1. Introduction

Biodegradable films based on natural origin polymers have been widely investigated for several biomedical applications, including tissue engineering [1], drug delivery [2] and wound healing [3], due to its biocompatibility, biodegradability and properties similar to the human tissues [4,5].

Hydrogels are 3D hydrophilic polymer networks that can swell in aqueous solutions without dissolution, maintaining its structure [6-8]. Natural hydrogels are attractive materials for the treatment of different types of wounds, due to its properties such as smoothness, high water content, elasticity, malleability and ability to provide a moist environment protecting the wound from desiccating [5,8,9].

The healing of a wound is a complex, dynamic and continuous process aiming at the repairing of damaged tissue. The efficient treatment system of a wound is very

important to improve the healing process, in terms of both quality and time, as well to reduce the costs associated with the treatment.

Currently, there is a great variety of wound-care products, available in the market, including creams, solutions, dressings or skin tissue engineering substitutes [10,11]. Among these products, polymeric dressings represent an effective method for wound treatment, presenting a good relationship between clinical efficacy and manufacturing cost. However, for some types of wounds, such as infected wounds, the isolated use of polymeric dressings cannot be sufficient to promote the healing process, as many of these materials do not present therapeutic activity (e.g. antibacterial and anti-septic characteristics). In order to solve this limitation, some dressings, based on natural polymers, were developed incorporating different synthetic drugs [12,13] to reduce the growth of microorganisms in wounds [14]. The continuous administration of synthetic drugs in infected wounds, though associated with the

development and spread of antibiotic-resistant strains of bacteria [14], present satisfactory clinical results. To address this challenge, the potential use of biodegradable films is being investigated, consisting of alginate and *Aloe vera* for biomedical applications [15,16].

Alginate is a natural polymer widely used for the treatment of several types of wounds, due to its biocompatibility, biodegradability and ability to form hydrogels, for example by the interaction with calcium ions [17,12]. Calcium alginate gels partly dissolve when in contact with the wound fluids, as a result of ion exchange, creating a moist environment that protects the wound and promotes the healing [9]. In addition, it has been reported that alginate gels are able to incorporate and release different types of therapeutic agents [12,18], which is very important for wound healing. In this research work, we include *Aloe vera* gel with the alginate film, to explore its therapeutic properties, which includes antibacterial, anti-septic, anti-inflammatory and ability to stimulate the fibroblast proliferation and the collagen synthesis [19-21]. We hypothesized that the *Aloe vera* compounds incorporated within the alginate hydrogels can diffuse to the wound bed during the swelling or, as result of the film dissolution, stimulate the healing process. Furthermore, as the *Aloe vera* gel contains antimicrobial properties [19], it can be used as an alternative to synthetic drugs commonly administrated to treat infected wounds.

This paper presents a methodology for the development of thin hydrogel films, composed of alginate and *Aloe vera*, which can be applied to the wounds in both dry and wet state, according to the level of exudate present in the wound (Fig. 1).

Dry films are appropriate for the treatment of superficial exuding wounds [10,14], in which they will absorb the excess of exudate to avoid maceration, facilitating the healing. These films are also very useful for applications in bleeding wounds, due to the haemostatic properties of the calcium ions released during the use [9]. When the films are applied into the wound, the calcium ions of the film are released, as a result of the ion exchange with the sodium ions present in the exudate, resulting in the formation of a hydrophilic gel that maintains a moist environment and protects the wound.

Wet films, used in the form of hydrogel, are suitable for the treatment of dry and painful wounds [10,14]. Firstly, the films are hydrated by the immersion into appropriate solutions (e.g. water, saline solution, solutions containing therapeutic agents, etc.), and then applied in the wound site. When placed in the wound, films release the absorbed water, providing a moist environment that rehydrates the dry wound bed, and promotes the debridement and cleaning of the wound [9]. If some therapeutic agents are previously

incorporated within the films, they can be released directly into the wound bed, increasing their effectiveness.

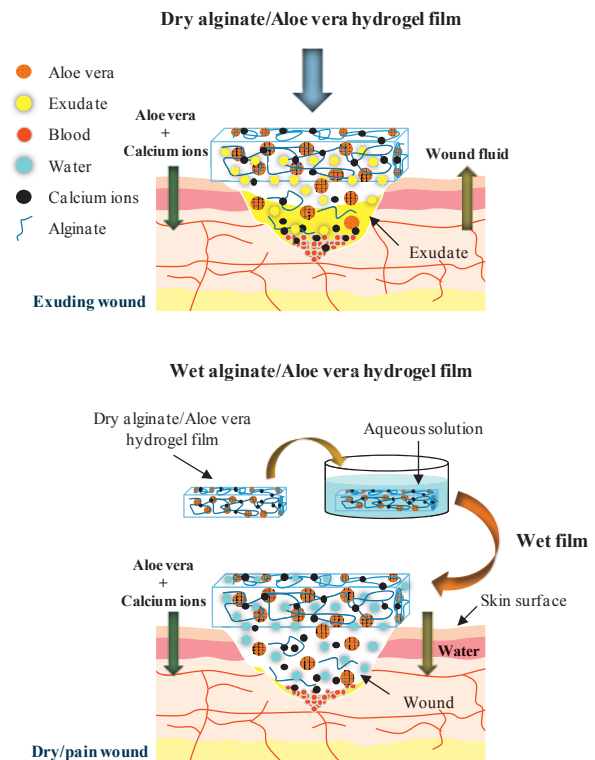


Fig. 1. Application of the alginate/*Aloe vera* hydrogel films into exuding and dry wounds

The aim of this study is to investigate some relevant properties of the alginate/*Aloe vera* films to apply in wound dressing, including the thickness, transparency, swelling behavior and *in vitro* degradation properties.

2. Materials and methods

2.1. Materials

The sodium alginate used in this study was obtained from BDH Prolabo (VWR International, UK), containing 54% of acid mannuronic units [16]. *Aloe vera* (ACTIValoe®, *Aloe vera* Gel Qmatrix 200X Flakes) was kindly offered by Aloecorp (Broomfield, U.S.A.) and glycerol was purchased from Scharlau (Spain). Acetate buffer was prepared using sodium acetate dihydrate and acid acetic, while simulated body fluid (SBF) was prepared using the following reagents: NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, HCl, CaCl₂ and (CH₂OH)₃CNH₂.

2.2. Methodology for the preparation of hydrogel films

The hydrogel films were prepared through the solvent-casting method as illustrated in Fig. 2.

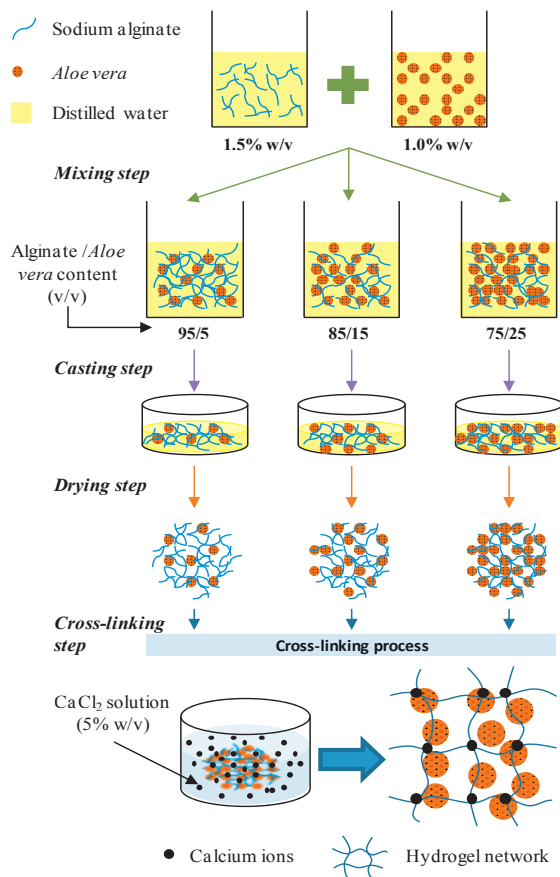


Fig. 2. Production of alginate/Aloe vera hydrogel films

Solutions of sodium alginate (1.5% w/v) and *Aloe vera* (1.0% w/v) were initially prepared through the dissolution in distilled water. During the preparation of the alginate solution, glycerol was added at a percentage of 15% (w/w, based on the mass of the alginate), in order to increase the flexibility of the films. Subsequently, the alginate and *Aloe vera* solutions 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). Afterwards, 25 mL of each mixture was casted into petri dishes ($\phi=9.5\text{cm}$) and left to dry at room temperature (25°C) and controlled humidity (50%). After drying, the films were immersed into a calcium chloride (CaCl₂) aqueous solution at 5.0% (w/v) for 5 minutes to obtain the hydrogel films. Finally, the resulting films were washed with distilled water and dried at room temperature before use.

2.3. Film thickness and transparency

The thickness of the cross-linked films was determined in both dry and wet state, using a manual micrometer (Model 102-301, Mitutoyo). In order to evaluate the thickness of the wet films, they were previously immersed into 10mL of SBF solution at pH 7.4 and physiological temperature (37°C), for 1 week to reach the equilibrium. After this period, the films were collected from the solution, the excess of water removed with a filter paper and the thickness measured.

The transparency of the films was evaluated by spectrophotometry. Film samples of 10 x 35 mm were placed on a spectrophotometer cell (UV-Visible Cary 50 C, Varian) and analyzed at 600 nm [22]. Tests were performed in triplicate. The transparency was determined by the following equation:

$$\text{Transparency} = \frac{\text{Abs}_{600}}{\alpha} \quad (1)$$

where, Abs_{600} and α correspond to the value of absorbance at 600 nm and to the film thickness (mm), respectively.

2.4. Swelling behavior

To determine the swelling behavior of the films, pre-weighed cross-linked samples (20 mm of diameter) were immersed in 20 mL of acetate buffer pH 5.5 (10mM) at 37°C, simulating the skin pH. At predetermined periods of time, the samples were removed, the excess of water withdrawn with a filter paper and its wet weight determined. The swelling was accessed gravimetrically using the following equation:

$$\text{Sw} (\%) = \left(\frac{W_h - W_d}{W_d} \right) \times 100 \quad (2)$$

where W_h corresponds to the hydrated weight of the sample, and W_d represents the sample dry weight.

2.5. In vitro degradation tests

The *in vitro* degradation tests were performed by the immersion of dry and cross-linked film samples (50 x 15 mm²) in 10mL of SBF solution (pH 7.4) at 37°C, during 10 weeks. 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 with a filter paper. Then, the wet weight of the samples was immediately accessed to determine the water absorption. Samples were placed in an oven and dried at 37°C until a constant mass was achieved, to evaluate the weight loss. The degradation medium was

replaced weekly, and nine samples were used for each condition. The degradation was accessed by determining the weight loss (WL), as follows:

$$WL (\%) = \left(\frac{W_i - W_f}{W_i} \right) \times 100 \quad (3)$$

where W_i and W_f represent the initial weight of the samples before the degradation and the dry weight of the degraded samples, respectively.

3. Results and discussion

An ideal wound dressing should be able to create a favorable environment to promote the healing process. The dressings must present high transparency, flexibility, durability, permeability to water vapor, adequate mechanical properties and adherence to the tissue. Additionally, they should have the ability to hydrate or dehydrate the wound bed, according to the needs of each wound type (dry or exuding wounds) and protect the wound from infections [14]. In wound care, it is widely recognized that there are no dressings suitable for the treatment of all types of wounds, and only few of them present the required properties to be used in the different phases of the healing process [9]. In this work, we developed hydrogel films composed of alginate and *Aloe vera*, for application in both exuding and dry wounds. Key properties were tested to evaluate the potential of these films for wound dressings, such as thickness, transparency, swelling behavior and *in vitro* degradation.

3.1. Film thickness and transparency

The thickness of the films was evaluated at both dry and wet state, as illustrated in Fig. 3. The dry films present thickness values comprised between 59.7 μ m and 75.3 μ m, while the wet films exhibited values in the range of 139.0-211.0 μ m. Results show that an increase in the *Aloe vera* content leads to a slight decrease in the thickness of the dry films, which can be attributed to the reduction on the alginate content. In wet films, the increase in *Aloe vera* leads to a significant increase in the thickness, which may be related with differences in terms of the water absorption capacity.

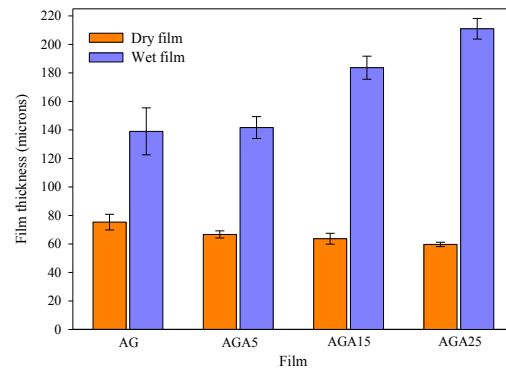


Fig. 3. Influence of *Aloe vera* content on the thickness of the films in dry and wet state

Light transmission works were performed to evaluate the transparency of the films and the influence of *Aloe vera* in this property. Results show that an increase in the *Aloe vera* content increases the transparency of the films: AG: 1.28 \pm 0.28, AGA5: 1.60 \pm 0.16, AGA15: 1.90 \pm 0.25, and AGA25: 2.11 \pm 0.09. As indicated in Fig. 4, the developed films exhibit high transparency in both dry and wet state, which is of great importance for the proposed applications. The AGA25 film was applied to a human finger (Fig. 4c), and it was possible to observe its ease application and removal, with a good conformity, allowing the visualization of the skin. These data show the positive influence of *Aloe vera* on the film's transparency, which is very important for wound healing, allowing the visual inspection of the injury without the removal of the film.



Fig. 4. Transparency of the film AGA25 in dry (a) and wet state (b), and its appearance on the human finger of a volunteer (c)

3.2. Swelling behavior

It is fundamental to investigate the swelling behavior of the hydrophilic films for wound dressings, as it determines the capacity of the films to absorb exudate from the wound, avoiding maceration. Additionally, it is also widely recognized that water absorption affects the release rate of the entrapped molecules and the *in vitro* degradation of hydrophilic polymers [23].

Fig. 5 shows the water absorption profiles of the films, when immersed into acetate buffer pH 5.5 at 37°C. Results show that an increase on the percentage of *Aloe vera* significantly increases both the film's water absorption and the necessary time to reach the

equilibrium. This behavior can be attributed to the hydrophilic properties of *Aloe vera*, which may improve the hydrophilicity of the films' surface and enhance the affinity with water [16]. It was also found that the neat alginate film (AG) and the alginate film containing 5% of *Aloe vera* (AGA5) present a quickly absorption of water along the first 30 minutes after immersion, reaching the equilibrium in approximately 90 minutes. Similarly, the films with high *Aloe vera* contents, AGA15 and AGA25, exhibit a rapid absorption of water after immersion. However, after this stage, the films present a progressive and slower absorption of water, not reaching the equilibrium during the 24 hours of the test. These results show that both the film's water absorption properties and the removal of exudate from the wound are dependent on the *Aloe vera* content. Thus, based on these results, it is possible to prepare films with a specific capacity to absorb the wound exudate.

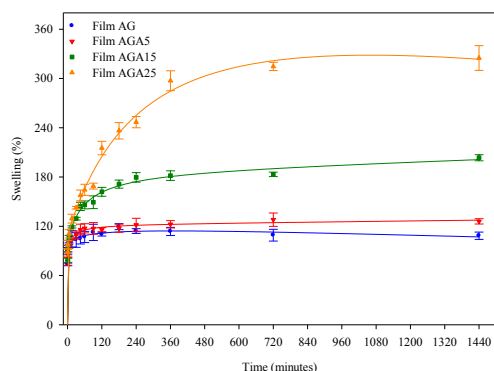


Fig. 5. Swelling behavior of the films immersed in acetate buffer pH 5.5

3.3. *In vitro* degradation tests

The *in vitro* degradation tests were performed to evaluate the influence of *Aloe vera* on the degradation behavior of the films, as well to investigate the durability of the films when immersed in an aqueous solution simulating the body fluids.

Fig. 6a displays the weight loss profiles of the films along the degradation period. All films present a quick loss of weight during the first week of degradation, which is followed by a slower and gradual loss of weight during the following weeks. The quickly weight loss verified at the first week of degradation can be related to the leaching of the plasticizer agent (glicerol) and/or the release of *Aloe vera* compounds from the film. As observed from Fig. 6a, the films AG and AGA5 exhibit a very similar weight loss profile, which suggests that 5% of *Aloe vera* do not have significant influence on the *in vitro* degradation, as the one observed for the swelling behavior. For the films AGA15 and AGA25, it is possible to observe a significant loss of weight from the

first to the sixth week of degradation. After this period, it seems that the film's weight loss tends to stabilize, reaching a maximum of $16.7 \pm 0.9\%$ and $18.6 \pm 0.5\%$ for the films AGA15 and AGA25, respectively. The weight loss data clearly shows that an increase in the *Aloe vera* content improves the *in vitro* degradation behavior of the films.

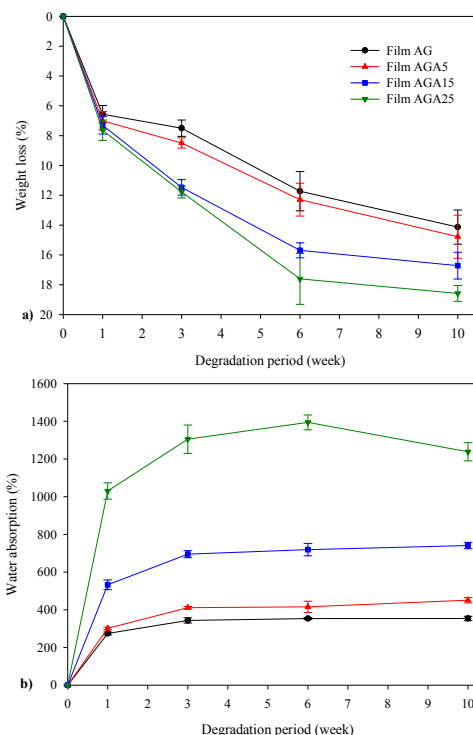


Fig. 6. Weight loss (a) and water absorption (b) profiles of the alginate/*Aloe vera* films, during the hydrolytic degradation

It is possible to observe from Fig. 6b that an increase in the water absorption of the films during the degradation, as a function of the increase in the *Aloe vera* content, agrees with the results obtained for the swelling study. The changes in the film's thickness during the degradation (Fig. 7) reflect the differences observed for the water absorption. As expected, after 10 weeks of degradation, the wet thickness of the films increases. However, the thickness variation depends on the *Aloe vera* content, reaching a minimum of $87.8 \pm 7.4\%$ for the alginate neat film (AG) and a maximum of $308.3 \pm 11.8\%$ for the film AGA25.

The abovementioned results clearly show that the *Aloe vera* improves the film's weight loss (Fig. 6a) and water absorption (Fig. 6b). Thus, it is possible to assume that the films with high *Aloe vera* content are more susceptible to the hydrolytic degradation, due to the further penetration of water within the hydrogel network [23], which leads to the cleavage of degradable linkages and raises the film degradation.

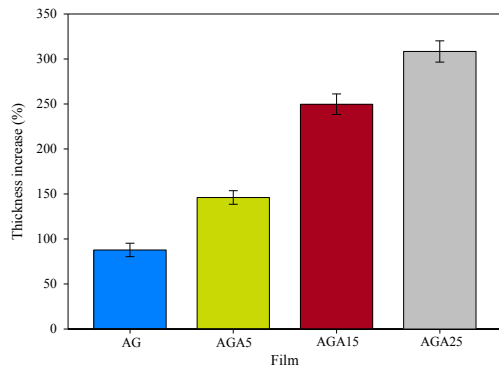


Fig. 7. Change of the thickness of the films after 10 weeks of degradation

4. Conclusions

Hydrogel films, composed of alginate and *Aloe vera*, were prepared for wound healing applications. The films were developed by combining the haemostatic properties of calcium alginate gels and the therapeutic properties of *Aloe vera*. These films exhibit high transparency and can be applied into the wound, in either dry or wet state, according to the level of exudate and the specific needs of each wound type. Results show that the swelling behavior of the films depends on the *Aloe vera* content, while a similar behavior was observed for the *in vitro* degradation. The films with high percentage of *Aloe vera* present both improved water absorption and *in vitro* degradation. These results also suggest that alginate/*Aloe vera* hydrogel films can be potentially explored as wound dressing for dry and exuding wounds.

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References

- [1] Rosellini, E., Cristallini, C., Barbani, N., Vozzi, G., Giusti, P., 2009. Preparation and characterization of alginate/gelatin blend films for cardiac tissue engineering, *Journal of Biomedical Materials Research Part A* 91A, p. 447.
- [2] Guo, R., Du, X., Zhang, R., Deng, L., Dong, A., Zhang, J., 2011. Bioadhesive film formed from a novel organic-inorganic hybrid gel for transdermal drug delivery system, *European Journal of Pharmaceutics and Biopharmaceutics* 79, p. 574.
- [3] Thu, H.-E., Zulfakar, M.H., Ng, S.-F., 2012. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing, *International Journal of Pharmaceutics* 434, p. 375.
- [4] Sionkowska, A., 2011. Current research on the blends of natural and synthetic polymers as new biomaterials: Review, *Progress in Polymer Science* 36, p. 1254.
- [5] Huang, S., Fu, X., 2010. Naturally derived materials-based cell and drug delivery systems in skin regeneration, *Journal of Controlled Release* 142, p. 149.
- [6] Pasqui, D., De Cagna, M., Barbucci, R., 2012. Polysaccharide-Based Hydrogels: The Key Role of Water in Affecting Mechanical Properties, *Polymers* 4, p. 1517.
- [7] Hoffman, A.S., 2002. Hydrogels for biomedical applications, *Advanced Drug Delivery Reviews* 43, p. 3.
- [8] Bartolo, P., Kruth, J.-P., Silva, J., Levy, G., Malshe, A., Rajurkar, K., Mitsuishi, M., Ciurana, J., Leu, M., 2012. Biomedical production of implants by additive electro-chemical and physical processes, *CIRP Annals - Manufacturing Technology* 61, p. 635.
- [9] Weller, C., Sussman G., 2006. Wound Dressings Update, *Journal of Pharmacy Practice and Research* 36, p. 318.
- [10] Wild, T., Rahbarnia, A., Kellner, M., Sobotka, L., Eberlein, T., 2010. Basics in nutrition and wound healing, *Nutrition* 26, p. 862.
- [11] Boateng, J.S., Matthews, K.H., Stevens, H.N.E., Eccleston, G.M., 2008. Wound Healing Dressings and Drug Delivery Systems: A Review, *Journal of Pharmaceutical Sciences* 97, p. 2892.
- [12] Kim, J.O., Park, J.K., Kim, J.H., Jin, S.G., Yong, C.S., Li, D.X., Choi, J.Y., Woo, J.S., Yoo, B.K., Lyoo, W.S., Kim, J.-A., Choi, H.-G., 2008. Development of polyvinyl alcohol-sodium alginate gel-matrix-based wound dressing system containing nitrofurazone, *International Journal of Pharmaceutics* 359, p. 79.
- [13] Sriprya, R., Kumar, M.S., Sehgal, P.K., 2004. Improved Collagen Bilayer Dressing for the Controlled Release of Drugs, *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 70B, p. 389.
- [14] Abdelrahman, T., Newton, H., 2011. Wound dressings: principles and practice, *Surgery* 29, p. 491.
- [15] Pereira, R., Tojeira, A., Vaz, D., Mendes, A., Bártolo, P., 2011. Preparation and Characterization of Films Based on Alginate and *Aloe vera*, *International Journal of Polymer Analysis and Characterization* 16, p. 449.
- [16] Pereira, R., Carvalho, A., Vaz, D.C., Gil, M.H., Mendes, A., Bártolo, P., 2013. Development of novel alginate based hydrogel films for wound healing applications, *International Journal of Biological Macromolecules* 52, p. 221.
- [17] Lee, K.Y., Mooney, D.J., 2012. Alginate: Properties and biomedical applications, *Progress in Polymer Science* 37, p. 106.
- [18] Jeon, O., Powell, C., Solorio, L.D., Krebs, M.D., Alsborg, E., 2011. Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels, *Journal of Controlled Release* 154, p. 258.
- [19] Pellizzoni M., Ruzickova, G., Kalhotka, L., Lucini, L., 2012. Antimicrobial activity of different *Aloe barbadensis* Mill. and *Aloe arborescens* Mill. leaf fractions. *Journal of Medicinal Plants Research* 6, p. 1975.
- [20] Atiba, A., Nishimura, M., Kakinuma, S., Hiraoka, T., Goryo, M., Shimada, Y., Ueno, H., Uzuka, Y., 2011. *Aloe vera* oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor- β and fibroblast growth factor production, *American Journal of Surgery* 201, p. 809.
- [21] Choi S., Chung, M.-H., 2003. A review on the relationship between *Aloe vera* components and their biologic effects, *Seminars in Integrative Medicine* 1, p. 53.
- [22] Norajit, K., Kim, K.M., Ryu, G.H., 2010. Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract, *Journal of Food Engineering* 98, p. 377.
- [23] Göpferich, A., 1996. Mechanisms of polymer degradation and erosion, *Biomaterials* 17, p. 103.