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ORIGINAL PAPER

Study of hybrid alginate/gelatin hydrogel-incorporated niosomal *Aloe vera* capable of sustained release of *Aloe vera* as potential skin wound dressing

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Abstract

Nowadays, wound dressings serve as advanced skin products, which mainly aim to accelerate the wound healing process. The wound dressings with a potential of localized and prolonged release of therapeutic agents have attracted tremendous attention. In this study, synthesis of a sustained release of niosomal *Aloe vera* (AV) loaded in alginate/gelatin (AG) hybrid hydrogel is aimed at improving skin regeneration as wound dressing. For this purpose, AV-loaded niosomes are synthesized and incorporated in the hybrid AG hydrogel. The size and polydispersity index (PdI) of niosomes, AV entrapment efficacy and AV in vitro release are characterized. In addition, the hydrogel characteristic, such as swelling ratio, degradation behavior and mechanical property, are studied. MTT assay is utilized to evaluate the effect of AV incorporation and release on the proliferation of fibroblast cells. Results demonstrate that size, PdI and EE% of AV-loaded niosomes are 270.080 nm, 0.108 and $42.039 \pm 4.090\%$, respectively, and in vitro release of AV is about 20% after 7 days. AG hybrid hydrogel loaded by niosomal AV shows an extended sustained release manner, where its swelling ratio and percentage of degradation are 60 wt% after 72 h and 70 wt% after 6 days, respectively. The average Young's modulus of the hybrid hydrogel is measured around 12.64 ± 1.3 kPa, which seems suitable as a wound dressing. Finally, MTT assay confesses an increased fibroblast proliferation in the presence of AV, particularly in the niosomal AV-loaded hydrogel. Concludingly, alginate/gelation hybrid hydrogel incorporated with niosomal AV, with

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sustained release potential can be suggested as a promising candidate for wound dressing applications.

Graphical abstract

Keywords Niosome · *Aloe vera* · Sustained release · Alginate · Gelatin · Wound dressing

Introduction

Skin, as the largest single organ in the body, encompasses 15–20% of total body weight. Epidermis, dermis and hypodermis are the three main layers of skin tissue [1]. The dermal extracellular matrix (ECM) is mainly made of collagen, elastin and reticular fibers, and the main cell in dermis is fibroblast that secretes collagen and proteoglycan matrix [2]. Skin defects, usually named wounds, are mainly originated from physical, thermal and chemical damages as well as physiological conditions [3], such as venous stasis and diabetic ulcers [4]. Wound healing is a very complex process, including hemostasis, inflammation, migration, proliferation and

maturation. A number of factors affect skin regeneration, such as keeping the environment around the wound moist [3, 5] and having effective oxygen circulation and low bacterial load [3].

Nowadays, wound managing products are widely utilized for acceleration of wound healing. Since 1980, numerous wound dressings have been developed, which satisfy critical characteristics such as non-cytotoxicity, non-antigenicity, biocompatibility, supporting migration and proliferation of epithelial, fibroblasts and endothelial cells [5]. In addition, an ideal wound dressing should absorb exudates from the wound surface, preserve humidity on the wound surface and provide proper oxygenation or angiogenesis conditions [6].

Among various wound dressing classes, hydrogel-based wound dressing attracts much more attention in the market and research as well [4]. Hydrogels are polymeric networks, which are hydrophilic and have a water absorption range from 10–20% up to thousands of times of their dry weight [7]. Hydrogel-based wound dressings could keep the moist environment of the wound and provide a cool surface of the wound, while they do not adhere to the wound surface that reduces the pain of the patient, particularly when dressing is removed. Furthermore, it could improve re-epithelialization of the wound and enhance autolytic debridement, making these products ideal as dressing materials [3].

Alginate is a linear polysaccharide copolymer, extracted from algae, which is a biocompatible, readily available and non-expensive biomaterial [8]. Alginate-based hydrogels are widely used in wound dressing, tissue engineering and drug delivery applications [5]. However, due to the absence of cell interactive ligands, alginate is a biologically inactive (inert) biopolymer [9]. Blending the alginate with other bioactive hydrogels could improve its inertness. For instance, the hybrid hydrogel derived from alginate and gelatin has advantageous properties for making wound dresses [10]. Gelatin can be obtained by controlled hydrolysis of collagen fibers [11] which has natural cell binding motifs such as RGD, which cause excellent biological characteristics such as cell adhesion and cell elongation, making it an attractive material for tissue engineering applications [12]. Blends of alginates/gelatin have been successfully utilized for drug delivery and wound dressing applications [10, 11, 13–15].

In addition to blending, some researchers suggest the use of natural components such as plant extracts as therapeutic agents, which could be incorporated into the scaffolds to improve bioactivity [16]. The *Aloe vera* (AV) plant has a wide variety of applications in therapeutic fields. It composes sugars, enzymes, vitamins and minerals, which interestingly shows anti-bacterial, antioxidative and antiviral activities [17]. In addition, extracts or components of AV effect the proliferation of several cell types [18, 19]. The glucomannan, one of the AV plant compounds, has a healing property that affects the fibroblast growth factor secretion, stimulates the proliferation of cells and improves collagen production [20]. AV increased transforming growth factor (TGFβ-1) expression in the wound area that accelerated the wound healing process via [21, 22] stimulation of angiogenesis, proliferation of fibroblasts and formation of the extracellular matrix (ECM) [23–25].

Numerous studies have investigated the desirable effects of AV extract on cell proliferation and wound healing. Hajian et al. [26] investigated hydrogel films composed of polyvinyl alcohol (PVA) and AV-extracted gel that demonstrated

AV-improved surface morphology, water absorption capacity and fibroblast proliferation of the PVA film as wound dressing. Suganya et al. [27] studied a polycaprolactone (PCL)—AV 10% electrospun nanofibrous scaffold and results demonstrated that AV was able to promote the regenerative process of the skin. Collagen–chitosan scaffolds supplemented with different concentrations of AV were investigated by Jithendra et al. as tissue engineering scaffold. The findings showed the significant enhancement of growth and proliferation of fibroblasts in the presence of AV [28]. In another work, Majid Salehi et al. synthesized a nanofibrous poly(L-lactic acid)/collagen (PLLA/COL) scaffold coated with chitosan layer, which could release AV in a sustained manner. The results confirmed that AV-incorporated scaffold supported attachment, viability and proliferation of mouse fibroblast cells and is suggested as a desirable scaffold for skin tissue engineering [29].

In addition to the incorporation of therapeutic agents into wound dressing or skin scaffolds, sustained exposure of the wound to these agents could also enhance their positive effects, particularly the agents with short half-life such as growth factors. Some researchers have focused on sustained release of such therapeutic agents in the wound site [30–33]. The release rate, as an important parameter, is commonly achieved by incorporation of particle-based carrier, nano- and microcarrier, loaded by desired therapeutic agents [34, 35].

Among various drug delivery carriers, niosome is one of the nanocarrier subcategories, which has been considered because of their biocompatibility and ability in high drug loading efficiency. Niosome is a vesicular nanocarrier madding from nonionic surfactant, which is used in different applications such as targeted and sustained drug delivery systems, growth factor delivery in tissue engineering and controlled release of biomolecules such as peptides, proteins or genes [36, 37].

Considering the good features of the alginate/gelation (AG) hybrid hydrogel in skin tissue engineering and wound healing, as well as remarkable effects of AV extract on the wound healing process, in this study a sustained release of AV from niosomal nanocarriers, incorporated into the AG hydrogel, is proposed. Based on our knowledge, AV-loaded niosomes are incorporated into the AG hybrid hydrogel in order to localize, and sustaining the release of AV has not been reported as skin wound dressing.

Materials and methods

Materials

Sodium alginate, calcium chloride, gelatin, MTT dye, sorbitan monostearate (Span 60) and cholesterol were purchased from Sigma Chemical Co. (Germany); diethyl ether was from Merck Chemical Co. (Germany). Fetal bovine serum (FBS), trypsin/EDTA solution and dimethyl solfoxid (DMSO) were purchased from Goya Biotechnology Co. (Iran). Phosphate-buffered saline (PBS) and AV were prepared in the laboratory. The human skin fibroblast cells and Dulbecco's modified Eagle's medium (DMEM) were purchased from Pasteur Institute of Iran. All other chemicals were of the highest grade commercially available.

Preparation of AV-loaded niosomes

AV extraction

Leaves of AV were washed with deionized water to clean their external pollutants. The edges of the leaves were cut, and their internal gel was separated, homogenized and centrifuged at 10,000 rpm for 30 min at 4 °C to separate the gel from the fibers. Then, the supernatant was lyophilized and stored at − 20 °C until use [28].

Calibration of AV concentration

Freeze-dried AV was dissolved in deionized water with different concentrations (0, 50, 100, 250, 500, 750 and 1000 µg/ml). AV solution optical density (OD)—concentration standard curve was made by measuring absorbance at 205 nm with NanoDrop 2000/2000c spectrophotometer (Thermo scientific. USA).

AV encapsulation in niosome

AV-loaded niosomes were prepared by the reverse-phase evaporation method [38]. Briefly, Span 60 and cholesterol were weighted with 1:1 molar ratio and dissolved in diethyl ether. This mixture was emulsified in aqueous solution containing AV (5 mg/ml) using vortexing (1 min) and water bath sonication (10 min, 10 °C). The volume ratio of organic solution to the aqueous solution was 1:1.5. Finally, the organic solvent was evaporated by rotary evaporator (Buchi model R-114, Switzerland, 40 °C, 60 rpm). Scheme 1 represents a schematic illustration of AV-loaded niosomes synthesis.

Characterizations of AV-loaded niosomes

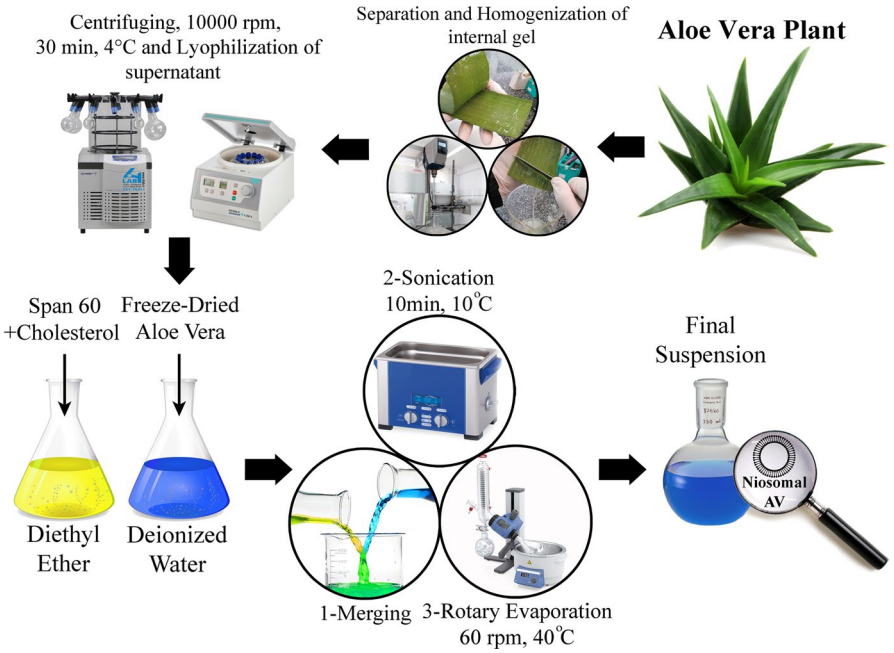
Size and polydispersity index analysis

The size and polydispersity index (PdI) of niosomes were studied by dynamic light scattering (DLS) analysis, using a submicron particle analyzer zetasizer (Malvern instruments Ltd., Worcestershire, UK).

AV entrapment efficiency

The prepared AV-loaded niosomes were centrifuged at 13,000 rpm for 30 min at 4 °C using a refrigerated ultracentrifuge to precipitate the vesicles. After obtaining the optical absorption of supernatant and the final suspension before centrifugation in NanoDrop 2000/2000c spectrophotometer (wavelength = 205 nm), entrapment efficiency of AV-loaded niosomes (EE%) is obtained using Eq. 1:

$$\mathrm{EE}\% = \frac{W_t - W_f}{W_t} \times 100; \tag{1}$$



Scheme 1 Schematic illustration of reverse-phase evaporation method for preparation of AV-loaded niosomes

where W_t is the total amount of AV in the suspension (loaded and free) and W_f is the free AV in the suspension.

Evaluation AV in vitro release from niosomes

AV-loaded niosomal suspension was immersed in 15 ml PBS (37 °C). The concentration of the released AV from niosomes was measured after 1, 3, 5 and 7 days by a Nanodrop instrument, as described.

Preparation of AV-loaded niosome-incorporated AG hydrogel

Synthesis of AG hydrogels To prepare the alginate 4% solution, the powder of alginate was dissolved in a cell culture medium under vigorous shaking at 60 °C for 4 h. Cross-linker $CaCl_2$ solution (40 mg/ml) was added to the alginate solution [39–42]. To prepare the blend alginate 4%—gelatin 1% (AG) hydrogel, after weighing the gelatin and alginate, they were dissolved in a cell culture medium under vigorous shaking at 70 °C for 1 h and then vigorously shook at 60 °C for 3 h. The homogenized solution was casted in a suitable mold and kept at 4 °C until use.

Preparation of AV-loaded niosomal AG hydrogel

The final AV-loaded niosomal suspension was centrifuged (13,000 rpm, 30 min), and the pellet was resolved in the AG solution. Then, $CaCl_2$ solution was added to the niosomal AV incorporated into AG solution and permitted to be cross-linked thermally and ionically at 4 °C for 15 min. The prepared niosomal AV-loaded AG was identified as AG–nAV. For comparison, a formulation by directly adding the same amount of AV (non-niosomal) to the AG hydrogel was prepared by the same procedure and the sample was identified as AG–AV.

Hydrogel characterizations

Swelling ratio analysis

To investigate swelling of the samples including alginate, AG, AG–AV and AG–nAV, the cross-linked samples were washed with PBS and then freeze-dried. The dried samples were weighed and then immersed in PBS and incubated at 37 °C.

The swelling ratio of each sample at different incubation times (0.5, 1, 2, 3, 24, 72 and 144 h) is measured based on Eq. 2:

$$\text{Swelling ratio} = \frac{W_s - W_d}{W_d} \times 100; \tag{2}$$

where W_s is the swollen weight and W_d is the dry weight of each sample [13].

Degradation analysis

To analyze the degradation rate of samples, the freeze-dried hydrogels were weighed and subsequently immersed in PBS (37 °C) for 24, 72 and 144 h. At each time point, the samples were removed from PBS and dried under freeze drying. The dry weights of the samples were measured at each time point. The degradation percentage of the hydrogels is estimated according to Eq. 3:

$$\text{degradation percent} = \frac{W_{d_2} - W_{d_1}}{W_{d_2}} \times 100; \tag{3}$$

where W_{d_1} is first dry weight and W_{d_2} is second dry weight of the samples at each time point.

Mechanical evaluation

The mechanical properties of the AG–nAV sample were measured using an uniaxial compression testing machine (Cesare Galdabini S.p.A, Italy) equipped with a 50 N load cell (TM–SM, Instron, England). The cylindrical samples (1 cm

diameter, 2 cm height) were examined at a constant strain rate of 1 mm/min until failure. Young's modulus of the sample was calculated over the linear part of the stress–strain curve based on Eq. 4. The results are presented as the average ± standard deviation obtained from triplicate measurements.

$$E = \frac{\sigma}{\varepsilon}; \tag{4}$$

where E, σ and ε are Young's modulus, uniaxial stress and strain, respectively.

AV release study

For evaluation of AV release from AV-loaded niosomes, AG–nAV and AG–AV hydrogels, 2 ml of the samples were immersed in 6 ml PBS (37 °C). After 1, 3, 5 and 7 days of incubation, 500 µl of supernatant was removed and replaced by the same amount of fresh PBS. The released AV absorption at 205 nm was measured by a Nanodrop instrument. The release profile was drawn by using the following equations.

$$W_1 = \frac{(\mathrm{EE}\%) \times V_s}{100}; \tag{5}$$

$$\text{AV released}\,(\%) = \frac{W_r}{W_1} \times 100; \tag{6}$$

where W_1 is the total amount of loaded AV in the samples, V_s is the total volume used in the samples and W_r is the released amount of AV in the suspension.

Cell culture

The human skin fibroblast cells were obtained from Pasteur Institute of Iran (Iran, Tehran) and were cultured in the standard incubator (37 °C; 5% CO_2). DMEM supplemented by 10% FBS, 100 IU/ml penicillin and 100 µg/ml streptomycin was prepared, and the medium of cells was replaced every 2 days.

Cell proliferation study (MTT assay)

The cells proliferation was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay [43]. The samples of alginate, AG and AG–nAV hydrogel were prepared in sterile conditions in the solution state. The cells were mixed with the sample (9×10^5 cells/ml), and $CaCl_2$ solution was added to the cross-link and hydrogel formation. After washing with PBS, the cell culture medium was added to each well containing hydrogel-encapsulated cells. The encapsulated cells were cultured in the standard incubator (37 °C; 5% CO_2) for 3, 5 and 7 days. At each time point, 20 µl of MTT solution (5 mg/ml) was added into each well and incubated at 37 °C and 5% CO2 for another 4 h. During this period, the formazan crystals were produced from reducing MTT via viable

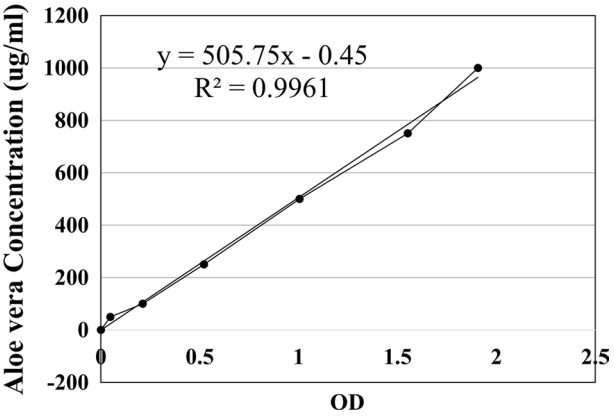


Fig. 1 The standard curve representing AV concentration OD (205 nm)

Table 1 The size, PdI and EE% of AV-loaded niosomes

Sample	Size (nm)	PdI	EE%
AV-loaded niosome	270.080	0.108	42.039 ± 4.090

cells. The supernatant of wells was removed, and 100 µl DMSO was added into each well to dissolve the formazan pigment and incubated overnight at 37 °C. The absorbance of each sample at 570 nm was recorded by a microplate reader (URIT-660, China).

Results and discussion

AV concentration standard curve

The standard curve representing AV concentration OD (205 nm) plotted for seven concentrations and a linear graph were precisely ($r^2 = 0.993$) fitted to the points (Fig. 1). The fitted formula was used for estimation of AV concentration in this study.

Characterization of AV-loaded niosomes

The size and PdI of AV-loaded niosomes were obtained by DLS analysis (Table 1). The PdI < 0.2 represented an almost sharp size distribution and low tendencies for particle aggregation [44]. The measured EE% of AV loading in niosomal carrier was 42.039 ± 4.090%, which is in a suitable range compared with similar cases reported by other studies [38, 45–48].

AV release study

The release profile of AV from naked noisome, AG–nAV hydrogel and AG–AV hydrogel, during 7 days of incubation is depicted in Fig. 2. By comparing the curves of AG–AV hydrogel and AG–nAV hydrogel, it was clear that the encapsulation of AV in niosomes resulted in a more sustained release of AV, where AV-loaded AG hydrogel released about 30% of the AV content compared to AV-loaded niosomal AG hydrogel, which released around 20% of the loading content after 7 days. As shown, the AV-loaded noisome showed a burst release of AV at the first day of incubation compared to the hydrogel-encapsulated formulations. According to the results, both AV encapsulated hydrogels could provide a sustained release of AV, suitable for wound dressing applications. However, the niosomal one seems potential to more prolonged release during the skin regeneration.

Swelling ratio characterization

Clearly, excessive fluid in the wound site is not suitable for the wound healing process, which may lead to complications in the healing process. Therefore,

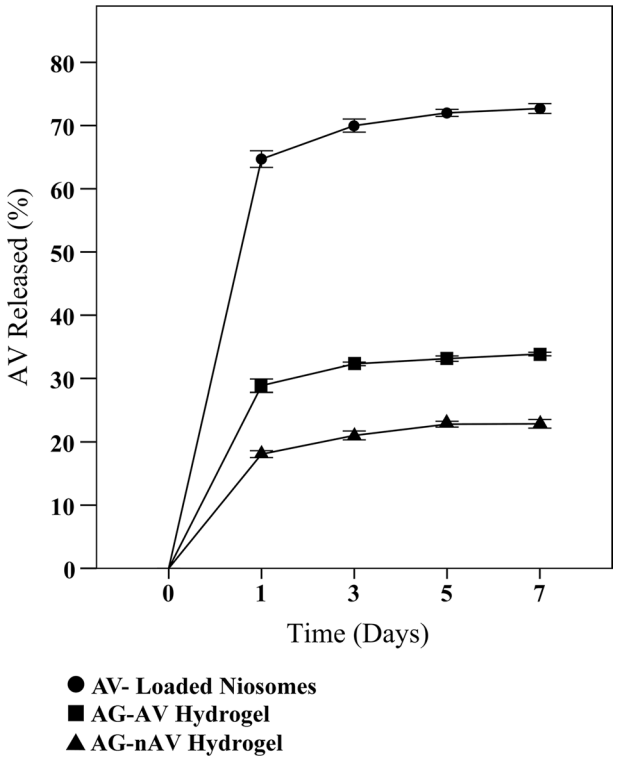


Fig. 2 Release profile of AV from various formulations

there is an essential need for wound dressings that enable absorbing the exudate [49]. Swelling ratios of the samples during 72 h of incubation in PBS at 37 °C are depicted in Fig. 3. All of the three samples showed almost a constant rate of water absorption of up to 24 h. During this time, water absorbance of AG was higher than alginate. This is due to the presence of gelatin that can improve the water absorption of alginate. As Fan et al. investigated the AG blend fibers, the water retention of blend fibers was higher than pure alginate and the value of water retention increased as the amount of gelatin was raised. They expressed that the alginate's hydrophilicity decreased after cross-linking due to the presence of Ca^{2+}, but adding gelatin can improve the hydrophilic property of the blends structure [50]. The swelling ratio of AG–nAV was lower than the AG hydrogels. It is anticipated that due to interactions between polar heads of niosomes and carboxyl groups in the gelatin structure, the free hydrophilic groups of hydrogel were fewer than non-niosomal hydrogel for binding with water molecules. Then, the water absorption rate of alginate and AV-loaded niosomal AG–nAV hydrogel showed an increasing trend up to 72 h, but water absorption of the AG hydrogel reduced. This could be attributed to the dissolution of gelatin in water and gradual removal of the hydrogel structure. As expected, since gelatin was thermally and ionically cross-linked, increasing the temperature to 37 °C and interaction with water as well as media ions leaded to gradual loosing of the physical cross-links between the chains. Although alginate also ionically cross-links, it interacts with Ca^{+2} stronger than gelatin, so it keeps the stability longer than gelation [39–42]. This is while in AG–nAV hydrogel, due to the presence of niosomes in the structure and possible interaction among them, the dissolution of the gelatin in water showed reduction.

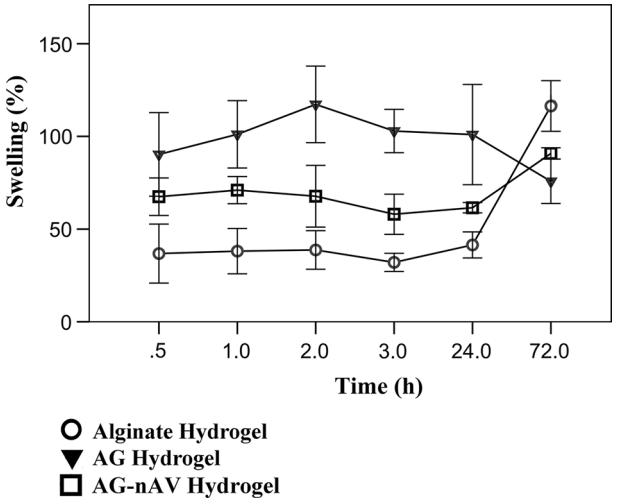


Fig. 3 Swelling profile of alginate hydrogel, AG hydrogel and AG–nAV hydrogel

Degradation evaluation

The results of the degradation study are depicted in Fig. 4. As demonstrated, the samples degradation rate was gradually raised by time. Alginate and gelatin are both physically cross-linked hydrogels, which could dissolve slowly during incubation. Since the incorporated AV and its release amount were very low, incorporation of niosomal AV in the AG hydrogel did not significantly affect the degradation behavior of the sample.

Mechanical analysis

For cell protection in the 3D structure, it is required that the hydrogel has good mechanical properties. The stress–strain curve of AV-loaded niosomal AG hydrogel under compressive tension is depicted in Fig. 5. The average Young's modulus (n = 3) was calculated at about 12.64 ± 1.3 kPa. Based on the other reports, this value seems suitable for the skin substitute, which can be a good protector for fibroblast cells. For instance, Jachowicz et al. found that Young's modulus of forearms and face skin was in the range of 7–33 kPa [51].

Cell viability assay

The MTT analysis was utilized to evaluate the viability and proliferation potential of cells encapsulated in alginate, AG and AG–nAV hydrogels. Figure 6 shows the absorbance values (at 570 nm) after 3, 5 and 7 days of culture. According to the

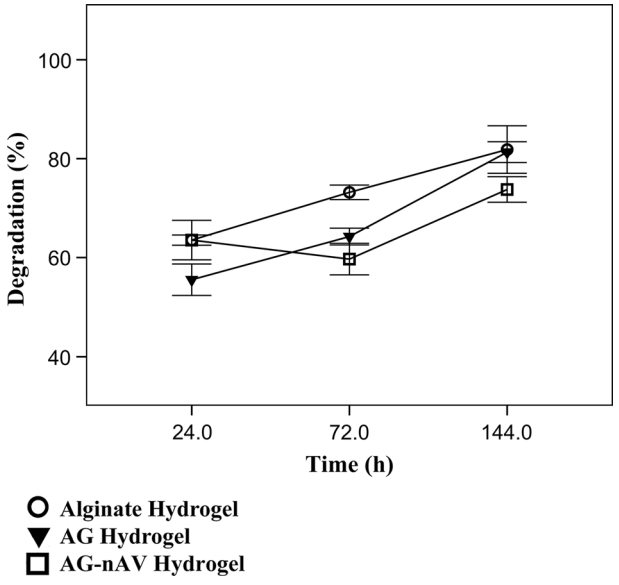


Fig. 4 Degradation profile of alginate, AG and AG–nAV hydrogels

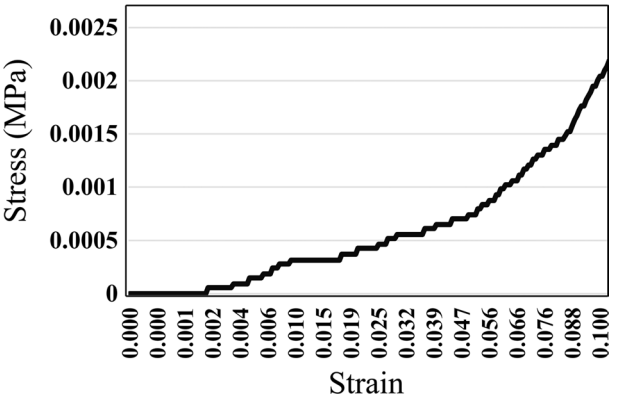


Fig. 5 A graph of mechanical test results for AG–nAV hydrogel

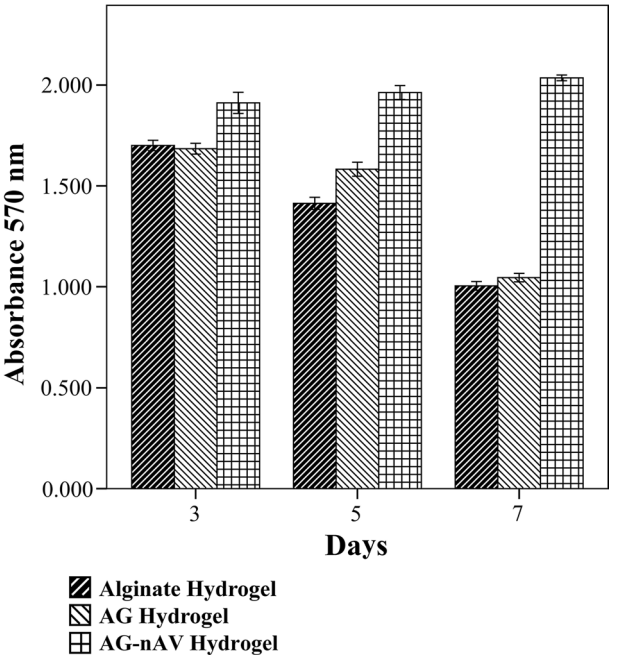


Fig. 6 Proliferation analysis via MTT assay of fibroblast cells cultured in alginate, AG and AG–nAV hydrogels

results, although fibroblast cells encapsulated in alginate and AG hybrid hydrogels showed almost the same viability during incubation time, AV-loaded niosomal hydrogel provided remarkable viability and proliferation. In addition, the cell growth in alginate and AG samples were gradually reduced, while the niosomal hydrogel kept an increasing trend. As expected, the sustained release of AV from niosomal hydrogel could stimulate fibroblast cells to keep their proliferation during culture

time. Finally, according to this analysis, it could be concluded that incorporation of niosomal AV in the alginate/gelation hybrid hydrogel with the potential of sustained release seems a good candidate for skin tissue engineering and wound healing applications.

Conclusion

Hydrogel-based wound dressings serve as an ideal candidate for wound management because they provide a moist environment surrounding the wound and effectively absorb exudates, thus accelerating the healing process. Stimulation of skin cells proliferation via localized exposure to the suitable therapeutic agents could further support this process. AV extract can stimulate proliferation of a variety of cells, such as fibroblast, and be commonly used in the skin products as supplement. Herein, a hybrid natural hydrogel composed of gelatin and alginate was utilized as a substrate to incorporate the niosomal AV releasing system and be evaluated as wound dressing. Where AV extract consists of a water-soluble component, which could be blithely released in the wound site, it was hypothesized that incorporation of AV extract in the niosomes as sustained release nanocarrier could improve and prolong AV effects. The obtained results supported that the synthesized nanosize niosome with 42% encapsulation efficacy provided a suitable sustained release system (30% release during 7 days) when incorporated in the blend hydrogel without any undesired effect on the other hydrogel characteristics. Cell viability analysis also confirmed the greater effectiveness of AV incorporation in the form of a niosomal system, rather than direct incorporation in the blend hydrogel. However, to more precisely conclude that extra cellular evaluations are needed, the results of this study demonstrated that alginate/gelation hybrid hydrogel incorporated with niosomal AV, with sustained release potential, could be suggested as a promising candidate for hydrogel-based wound dressing applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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