Wound Medicine 25 (2019) 100155

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

Original research article

A Keratin-based biomaterial as a promising dresser for skin wound healing

Saeid Vakilian[a], Fatemeh Jamshidi-adegani[a], Sulaiman Al-Shidhani[a], Muhammad U. Anwar[a], Rashid Al-Harrasi[a], Nasar Al-Wahaibi[b], Asim Qureshi[c], Sausan Alyaqoobi[d], Issa Al-Amri[d], Ahmed Al-Harrasi[a,**], Sulaiman Al-Hashmi[a,*]

[a] Laboratory for Stem Cell & Regenerative Medicine, Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman
[b] Department of Allied Health Sciences, College of Medicine & Health Sciences, Sultan Qaboos University, P. O. Box: 35, Alkoudh, 123, Oman
[c] Department of Pathology, College of Medicine & Health Sciences, Sultan Qaboos University, P. O. Box: 35, Alkoudh, 123, Oman
[d] DARIS Centre for Scientific Research and Technology Development, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman

ARTICLE INFO

Keywords:
Snake
Shed
Skin
Keratin
Wound healing

ABSTRACT

Keratin-based biomaterials can be considered as the beneficial platform for designing suitable wound dressers. One of the most common natural materials that are composed of keratin and lipid is snakes shed skin. In this new study, shed skin of two different Omani snakes; Bitis arietans snake (Puff adder, "P") and Telescopus dhara snake (Arabian Cat snake, "C"); were examined as wound dresser. The presence of lipid and keratin were assessed using Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR). 3- Layered filamentous morphology of the P shed skin and the 3-layered compact structure of C shed skin were analyzed by Scanning Electron Microscopy (SEM). X-Ray powder Diffraction (XRD) analysis proved two main peaks corresponding to the α-helix and β-sheet of the protein. Crystallinity index (CI) of P and C shed skins were obtained from XRD peaks 42.85 and 28.57%. The in vivo and histopathological results indicated that skin reconstruction was effectively improved under P shed skin treatment as compared with negative and positive control (PC) and C groups. Superior histopathological scores were the beneficial properties of P group over the positive control. The synergistic effect of natural extracellular matrix-mimicking structure and keratin beneficial proteins for wound healing could develop a natural substrate for wound healing in the clinical setting.

1. Introduction

Immobilized patients suffer much from chronic non-healing wounds [1]. This situation resulted in the extension of treatment time and costs. Since there are limited effective cures to improve the clinical treatment of these chronic lesions, several researchers focused on finding the optimal wound dresser with minimal side effects [1]. Treatment of chronic wounds is still one of the most challenging issues in the field of regenerative medicine. To treat these wounds there are several difficulties such as necrotic tissue, infection and scar formation [2]. To overcome these issues, natural or synthetic wound dressers such as honey, cotton, and linen gauze have been commonly applied [3,4]. Although these wound healing products are accessible worldwide, their outcomes still limited and novel materials should be solicited especially for chronic wounds. Among these novel products, biomaterial-based matrixes, which are mostly biomimetic to the natural tissue, are broadly applied for skin treatment. These matrixes provided a promising solution for wound healing [5,6].

The acute wound healing process consists of three overlapping steps of inflammatory reaction, proliferation, and remodeling [7]. The inflammatory phase is a vascular or cellular response to the injury, which generally results in tissue regeneration [8]. Tissue injury leads to blood vessel disturbance, loos of blood, and platelets release many mediators to accelerate coagulation [9]. Platelets are also playing a vital role by releasing antimicrobial agents, cytokines and growth factors for initiating granulation tissue formation [10]. In parallel, blood clotting acts as a cellular matrix for the settlement of cells to the injured area [3,11]. Proliferative phase is the simultaneous formation of epithelium and granulation tissue to cover and fill the wound space by the proliferation of fibroblasts, collagen deposition and neovascularization [12]. When the new tissue covers the wound, the remodeling phase restores tissue structural integrity and functional competence. All the wounds that heal in a timely manner are called acute wounds which include a clean and uninfected surgical incision wound created by

* Corresponding author at: Laboratory for Stem Cell & Regenerative Medicine, Natural and Medical sciences Research Center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman.
** Corresponding author at: Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman.
E-mail addresses: arrasi@unizwa.edu.om (A. Al-Harrasi), sahashmi@unizwa.edu.om (S. Al-Hashmi).

https://doi.org/10.1016/j.wndm.2019.100155
Received 18 March 2019; Accepted 25 April 2019
Available online 26 April 2019
2213-9095/

surgical tools [13]. Wound healing progression from one phase to the next is supported by cellular, matrix, cytokine and growth factors. This process can be controlled by a suitable wound dresser to reduce the wound healing delay [12,14,15].

One of the wildly used and biocompatible biomaterials is keratin. Keratin proteins have 3D fibers, which are the main component of horns, hair, wool, feathers, and nails [16]. Keratins are composed of cysteine-rich structural amino acids, which contain a huge number of disulfides that are responsible for good mechanical properties due to their hard and fibrous structures [17]. The natural presence of cell adhesion sequences makes Keratin suitable as a biomaterial for tissue engineering applications [18,19]. Moreover, keratin is biocompatible and biodegradable and is able to accelerate the skin regeneration. A large number of researches have been studied the characteristics and application of keratin-based biomaterials [20–22]. Keratin was presented for the first time by Blanchard et al. as one of the most effective hydrogels for wound healing. In this study, the effect of keratin hydrogel on improving cell proliferation and healing was studied [23]. A few years ago, several articles have also demonstrated keratin-based biomaterials for improving wound healing. For instance, it has proven that keratin film improved cellular proliferation and migration which are critical for successful epithelial wound healing [16,24]. Furthermore, Blood loss resulting from lethal critical injuries should be prevented, urgently. The current therapies for this purpose have failed to stop bleeding efficiently and they are costly too [25]. Various types of naturally based hydrogels with unique hemostatic properties provide a novel platform to manage hemorrhagic trauma [26]. By this way, keratin-based wound dresser was a candidate as an efficient hemostatic agent [22,27]. Hemostatic properties of keratin were proved for the first time by Wake Forest University researchers [22]. One of the major sources of keratin protein could be snakes shed skin.

The snake's skin consists of scales interrupted by hinge parts (thinner-scale areas) with no attachments. The shedding of the old epidermal film of the snakeskin usually happens as a complete molt during a complicated mechanism [28,29]. The shed skin of snakes is made of four layers: the outer layer Oberhautchen, the β-layer (mainly protein), the Mesos layer (lipid-rich) and thinner α-layer (mainly protein). The Oberhautchen layer contains special types of β-cells that are mainly responsible for the shedding process [30]. Combination of the Oberhautchen and the β-layer, which both consist of β-keratins are considered as a distinctive β-layer in the mature epidermis. The α, β and Mesos layers play different barrier functions. The lipid connection with proteins, particularly in the Mesos-layer, is not identified. It has been demonstrated that the hinge parts of the skin consist of A-layers and a thin Oberhautchen layer, made of proteins and lipids. This keratinized skin is responsible for the beneficial properties of reptilian skin. These epidermal scales cover protect Lepidosaurians from abrasion and dehydration [30].

Because of the vital role of the skin in protecting of the body from the outside environment, it is greatly essential to repair the wound in the shortest time. Due to keratin beneficial properties, keratin-based wound dressers are mostly recommended by researchers to promote wound healing dramatically. Moreover, it was evaluated that keratin can act as a synthetic extracellular matrix (ECM) which has fibronectin-like cell binding domains responsible for cell adhesion. Since keratin based scaffolds can support and promote cell proliferation, numerous studies demonstrated its beneficial properties for wound healing. In addition to the beneficial properties of keratin, the lipid backbone of snake shed skin will help making the membrane stronger and provide structural cues required for clotting cascade initiating.

Our current study is willing to evaluate the possibility of using the protein-lipid framework of the shed snake epidermis membrane as a natural wound dresser. The aim of this study is to examine Puff and Cat Snakes shed skins for in vivo wound healing in rat model. To prove this hypothesis, snakes shed skins were structurally and morphologically identified, as well as wound closure, histology and pathology scores were evaluated.

2. Experimental

Shed snakeskins were collected from the snakes hosted at the Natural and Medical Sciences Research Center of the University of Nizwa. Ventral portions isolated from Puff Adder (P) and Cat (C) snakes shed skin. For further characterization, strips of snakeskin were cut from the middle region of the scale. For in vivo studies, the shed skins were washed with deionized water and sterilized using 70% ethanol solution.

Scanning electron microscope (SEM, Jeol Ltd., Japan) was used to characterize the surface and cross-section morphologies of the snake shed skins. The sample was platinum coated with a sputter-coater and analyzed at different magnifications.

The chemical compositions of snakes shed skin were examined using Attenuated Total Reflection-Fourier Transform Infrared spectroscopy (ATR-FTIR) and the spectra were recorded on a Bruker, ATR-Tensor 37 spectrophotometer. The spectra were collected in the range of 4000–500 cm^{-1}.

In order to examine crystallinity of the samples, the X-ray diffraction (XRD) scan was done using a Bruker D8 Discover instrument was operated at 40 kV and 40 mA with 2θ ranging from 5 to 50 at a scan speed of 0.02 s^{-1}. in the direction to the snake shed scale surface. The peak integration and treatment were carried out with the Diffracrt EVA software package.

The crystallinity index (C.I.), which represents the grade of crystallinity, was calculated using the following equation reported by L. Segal et.al.;

$$\text{C.I.} = (I_{10} - I_{20})/I_{10} \times 100$$

Where C.I. is the crystallinity index, I_{10} is the maximal intensity of the crystal at around 10, whereas I_{20} is the minimal intensity at around 20. It has proven that the higher C.I. value is responsible for more crystallinity of the structure.

In this study, we used the male Wistar rats (Provided by Animal House, University of Nizwa, Oman) for full-thickness skin wound model. All animal experiments were approved by the University of Nizwa Animal Ethics Committee. Rats were individually anesthetized via intraperitoneal injection of Ketamine (70 mg/kg) and Xylazine (10 mg/kg). The dorsal surface of rats was shaved and sterilized with alcohol and povidone-iodine solutions. A round full thickness wound excision with a diameter of 1.5 cm was created with a curved blade and surgical scissors. The depth and the diameter of the wound in all groups were created to be as similar as possible in all groups. All rats were then divided randomly into three groups as follow: group 1, positive wound control (PC) where the wounds wrapped with Solcoseryl ointment: group 2, wounds were covered with Puff snake shed skin (P) and served as wound dressing sample; group 3, wounds were covered with Cat snake shed skin (C) and served as another wound dressing sample. Additionally, negative control (NC) wound without treatment was made in each rat. Wound closure was calculated at the specific time point (t) using the following equation:

$$\text{Wound closure } (\%) = (\text{A-Ai})/\text{Ai} \times 100, \quad (1)$$

where the Ai represents the initial wound area and A is wound area at time t.

For histopathological evaluation, a group of rats were euthanized after one week to check the immune response for wound healing, while the other group euthanized after two weeks to evaluate pathological criteria for desired healing. From both groups, the wounds surrounding tissues were separated and the isolated tissues were soaked in 10% buffered formalin (BF). The fixed samples were immersed in the paraffin. Using the microtome, several paraffin sections were numerous paraffin sections were cut by microtome for Haematoxylin-Eosin (H&E)

Table 1
Wound-healing histologic scoring system.

		0	1	2	3
Hematoxylin and eosin staining	Acute inflammation	None	Scant	Moderate	Abundant
	Chronic inflammation	None	Scant	Moderate	Abundant
	Granulation tissue amount	None	Scant	Moderate	Abundant
	Hair follicles development	None	Scant	Moderate	Abundant
	Reepithelization	None	Partial	Complete but immature or thin	Complete and mature
	Neovascularization	None	Up to five vessels per HPF*	6-10 vessels per HPF	More than 10 vessels per HPF

* HPF: High Power Fields.

and Masson's trichrome staining.

The histology of the dissected tissues around the wounds has been evaluated by expert pathologists in a blind manner. The main histologic criteria such as the amount of acute and chronic inflammatory infiltration, the quantity and evolution of granulation tissue, re-epithelialization, hair follicles development, and neovascularization were evaluated. This scoring system was modified based on Greenhalgh et.al publication, which assessed each parameter autonomously and give a score between 0 and 3 (Table 1) [31]. Moreover, the additional scoring system presented by Abramov et al. was applied to evaluate three properties of epidermis and five criteria of dermis as described in Table 2. The higher score represents a more desirable result [14].

Masson's trichrome staining was used for collagen deposition assay. In this assay, collagen and nuclei stained blue while the muscle and keratin stained red. The extension and the morphology of collagen bundles were assessed in this assay Table 3 [14].

Each physical characterization was done in triplicate while in vivo studies were done in sextuplicate. Data were represented as the mean ± SD. One-way analysis of variance ANOVA with Tukey's post hoc was applied to compare between the results. All comparison analyses were performed using Minitab software (Minitab 17). P-values of less than 0.05 were assumed as statistically significant.

3. Results

3.1. SEM images of surface and cross-section

The snake-shed skin is a valuable source of the epidermis, which is frequently renewed. The surface morphology of ventral regions of snakes-shed skin was shown in Fig. 1.1 (P & C). As shown in this figure, wide-scale areas are connected with narrow hinge regions. Fig. 1.2 represented the multilayered structure of shed skin from P and C snakes represented by cross SEM images. The cross-section of the P demonstrated three distinctive layers containing filamentous structure with large voids, whereas the cross images of C in the TS only shows three intact layers with compact structures.

3.2. FTIR analysis

FTIR was applied to evaluate the structures of shed skins as shown in Fig. 2. The characteristic absorption bands related to the amide I, II and III are observed between 1200 and 1700 /cm. wavelength whereas lipid bands are seen in the range of 3000–2800 /cm. The spectra of snakes shed skins obtained from different snakes are mainly similar and they are mostly composed of keratin and lipid.

3.3. Crystallinity analysis

The crystallinity of the snake-shed skins was examined using X-ray diffraction. As illustrated in Fig. 3, two major crystal structures were detected in the XRD pattern of P and C shed skins. According to previous studies, the first peak around 10 is related to the α-helix structure of the keratin and the next peak, which is close to the 20 is responsible for the β-sheet structure of the protein [30,32,33]. Additional diffraction peaks corresponding to the superficial lipids were seen in the XRD figure are not significant possibly due to their low amount and non-crystalline form.

It was clearly seen that in the C sample the first peak around 10 is significantly weaker than the similar peak in P sample, while the second peak is vice versa and this peak in the C sample is dramatically stronger than that of P sample. This fact demonstrated that the C sample has relatively a lower ratio of α-helix structure and higher amount of β-sheet in comparison with P sample, that later will be discussed by crystallinity index (CI) values.

3.4. Evaluation of wound closure

Generally, two weeks' observation of wounds revealed a remarkable improvement. The wound healing rate in the animal treated with P shed skin is significantly higher compared to negative control, Positive control and the group treated with C shed skin. Fast wound healing would prevent the invasion of pathogens which may lead to the chronic wounds. As shown in Fig. 4, Both P and C snake shed skin could dramatically accelerate wound closure with the minimum cosmetic scar. As shown in Fig. 5, P shed could dramatically accelerate wound closure. About 55.4 ± 5.6% wound area was reduced by this wound dresser after 7 days while positive control leads to just 18.1 ± 5.2% regeneration of wound's area. Moreover, C shed skin is responsible for 39.2 ± 1.8% wound healing after 7 days. After 14 days both snake shed skins showed a significant decrease in wound area compared to the positive control.

Table 2
. Pathology scoring system.

			0	1	2	3
Hematoxylin and eosin staining	epidermis	Dermal-epidermal separation	Diffuse	Focal	None	–
		Hypergranulosis	None	Focal	Diffuse	–
		Crust formation	Present	Absent	–	–
	Dermis	Edema	Severe	Moderate	Mild	None
		Fibroblast maturation	None	Mild	Moderate	Full
		Collagen amount	None	Scant	Moderate	Abundant
		PMN amount	Abundant	Moderate	Scant	None
		Depth of inflammation	Deep myonecrosis	Superficial myonecrosis	Lower dermis	Upper dermis

Table 3
Masson's trichrome scoring system.

Masson's trichrome staining		0	1	2	3
	Extension	Scant	Moderate	Abundant	–
	Morphology	Mostly amorphous	Mostly thin wavy	Mostly thick wavy	–

3.5. Histopathological examination

As shown in Figs. 6 & 7 .A, acute inflammation, tissue granulation, re-epithelization, neovascularization, hair follicle development and collagen deposition were detected mostly in the Puff shed skin group on a postoperative day 7. With the histological examination of wounds on day 7, the P group was significantly better than the C and positive control. Moreover, P group had the finest normalized pathologic score at the end of the experiment due to focal hyper granulation, absent of crust formation, mild fibroblast maturation, scant collagen amount and superficial myonecrosis (Fig. 6 & 7.B).

Mason's Trichrome staining was applied to investigate the collagen deposition in the wound area. As shown in Fig. 8, at day 14 PC group contained dense wavy collagen fibers and total collagen deposition in this group was extensively higher in comparison with the C and P groups. Furthermore, a noticeable difference was detected in the Masson score of P and C group with their negative controls. As well as noticeable keratin deposition was seen in the P sample.

4. Discussion

The snake shed skin is a multilayered membrane composed of bioactive keratin and self-assembles lipids that provide mechanical support. The FTIR spectrum has indicated the presence of both lipid and protein in the structure of the snake shed skin. Based on the X-ray diffraction results, keratin protein has been presented in two forms of α-helix and β-sheet. The relative amount of crystallinity between the snakes' skin was compared by CI calculation. CI amounts of the C and P samples were obtained at 28.57 and 42.85, respectively. By this way, P snake shed skin has higher crystallinity compared to the C skin, which suggests that the P sample can absorb water faster than C sample which is beneficial for wound healing application. Moreover, it was concluded that the P sample is mechanically stronger and stable, whereas the lower crystallinity of the C sample is responsible for a higher degradation rate of the shed skin [34].

To the best of our knowledge, there is no report on the application of snakes shed skin on wound healing. The in vivo experiment of this study has presented snake-shed skin as a novel wound dressing material to accelerate wound healing. In comparison with the positive wound control wrapped with the Solcoseryl ointment that is commonly used as clinical treatments of wounds, P-shed skin proved more effective in wound closure (55.4 ± 5.6%) after one week which is remarkable compared to the wound closure of positive control (18.1 ± 5.2%). Park et al. have reported similar wound closures by using keratin-based hydrogels [21].

Acute wounds which have timely mannered healing are significantly regenerated by several growth factors and cytokines released at the wound site. Even though the appropriate final healing of acute wound would be the skin regeneration with a biomimetic structure and functions, any cues that accelerate controlled release of growth factor would improve tissue repair in the inflammatory phase [7].

Higher initial acute inflammation in the P sample is responsible for the faster release of growth factors and cytokine, which can accelerate the wound healing process. This acute inflammatory response of the body may retain within 2 weeks in some cases. Besides platelets adhesion, aggregation, and release of coagulation mediators, platelets are playing a vital role in facilitating the healing process via the release of chemoattractant and growth factors. In addition, the clot itself can merge as an extracellular matrix for the recruitment of cells. In the result of these beneficial factors, neutrophils and macrophages, penetrate inside the wound to remove tissue debris and foreign particles. Whenever activated, macrophages release specific growth factors and cytokines and hence granulation tissue formation is started leading to neovascularization, angiogenesis, and fibroblasts accumulation. Moreover, the early inflammatory phase provides a suitable platform to create a permeable barrier for re-epithelialization and providing

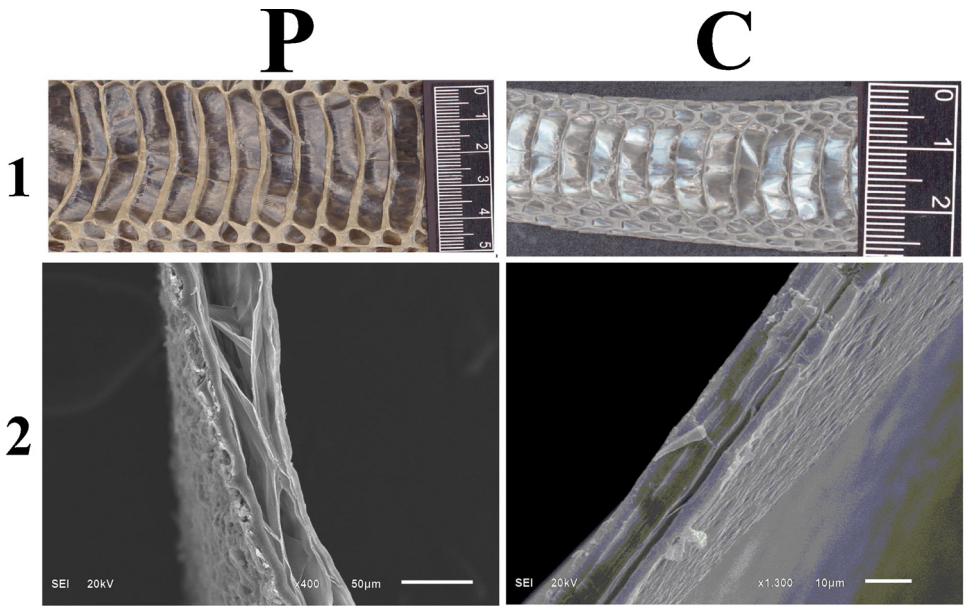

Fig. 1. Surface morphology of ventral regions of snakes-shed skin (P1 & C1) and scanning electron microscope cross morphology of the ventral regions of P and the C snake shed skin (P2 & C2).

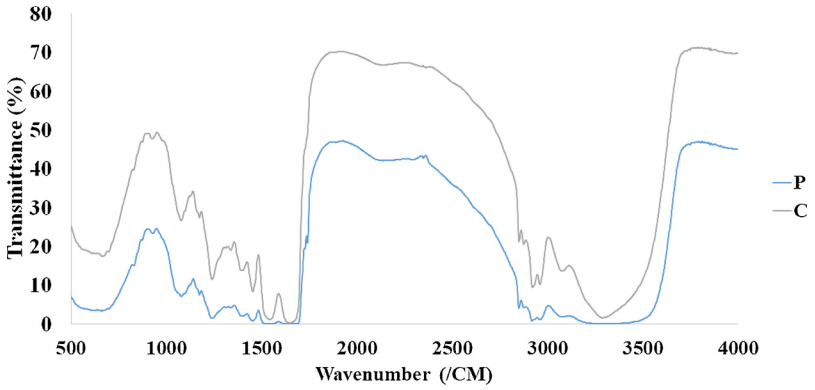

Fig. 2. ATR-FTIR spectrum of the P and the C snake shed skin.

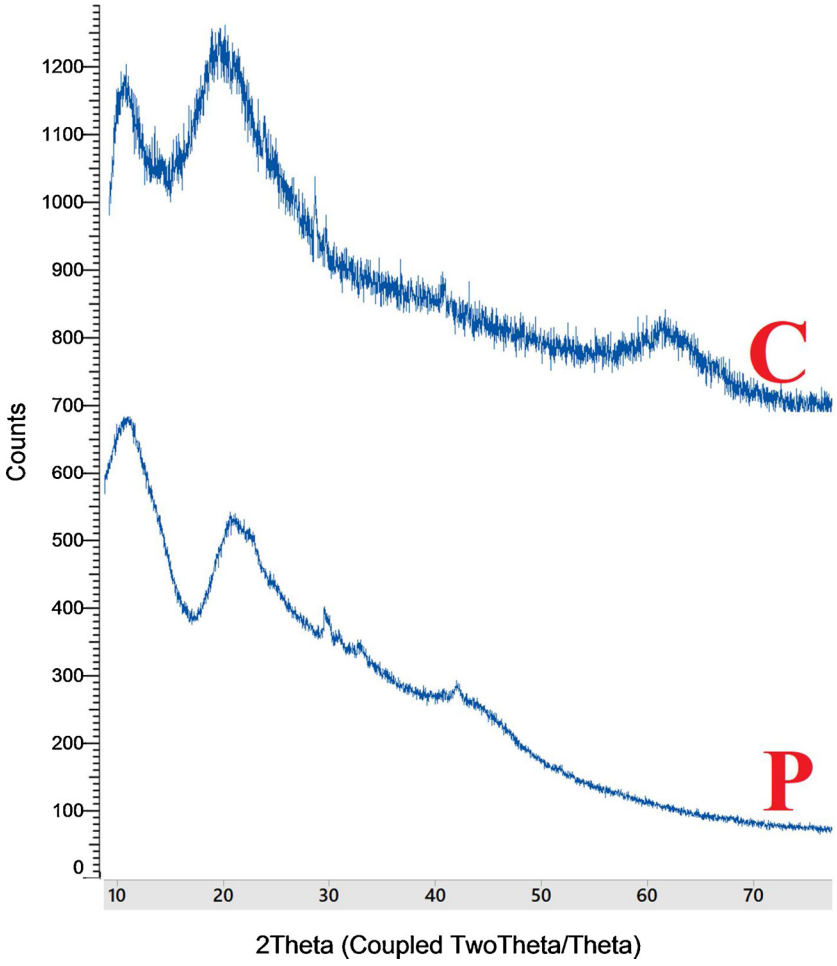

Fig. 3. XRD pattern of the P and the C snake shed skin.

adequate blood supply essential for angiogenesis, as well as reinforcement of the injured dermal tissue required in fibroplasia that is mostly seen in the P-treated wounds. Higher hair follicles development observed in the P group is beneficial because the hair follicles as a reservoir for keratinocytes in wound healing, can be considered as a major source of epithermal stem cells which play an important role in skin regeneration [3,7].

P treated rats represented higher normalized pathological score in comparison with positive wound control. Mason's Trichrome staining after 14 days demonstrated that Puff and Cat shed-snake skins revealed enhanced wavy collagen formation in comparison with their negative controls, whereas there is no significant difference between them. All of these showed the priority of the P group over the C and positive control. Higher crystallinity of P compared to the C group is responsible for faster water absorption that may also help to accelerate and improve wound healing. Although Solcoseryl Ointment as the positive control is widely applied for clinical wound healing and skin grafting, P snake shed skin provides better biomimetic ECM-like structure with superior environment for cells migration and proliferation resulted in better wound dressing [35,36].

Several natural based biomaterials such as collagen, alginate, chitosan, gelatin, silk, and cellulose derivatives have been broadly applied for wound healing application [6,37–44]. The natural assembled keratin based snakes shed skin can act as a potential platform for chronic

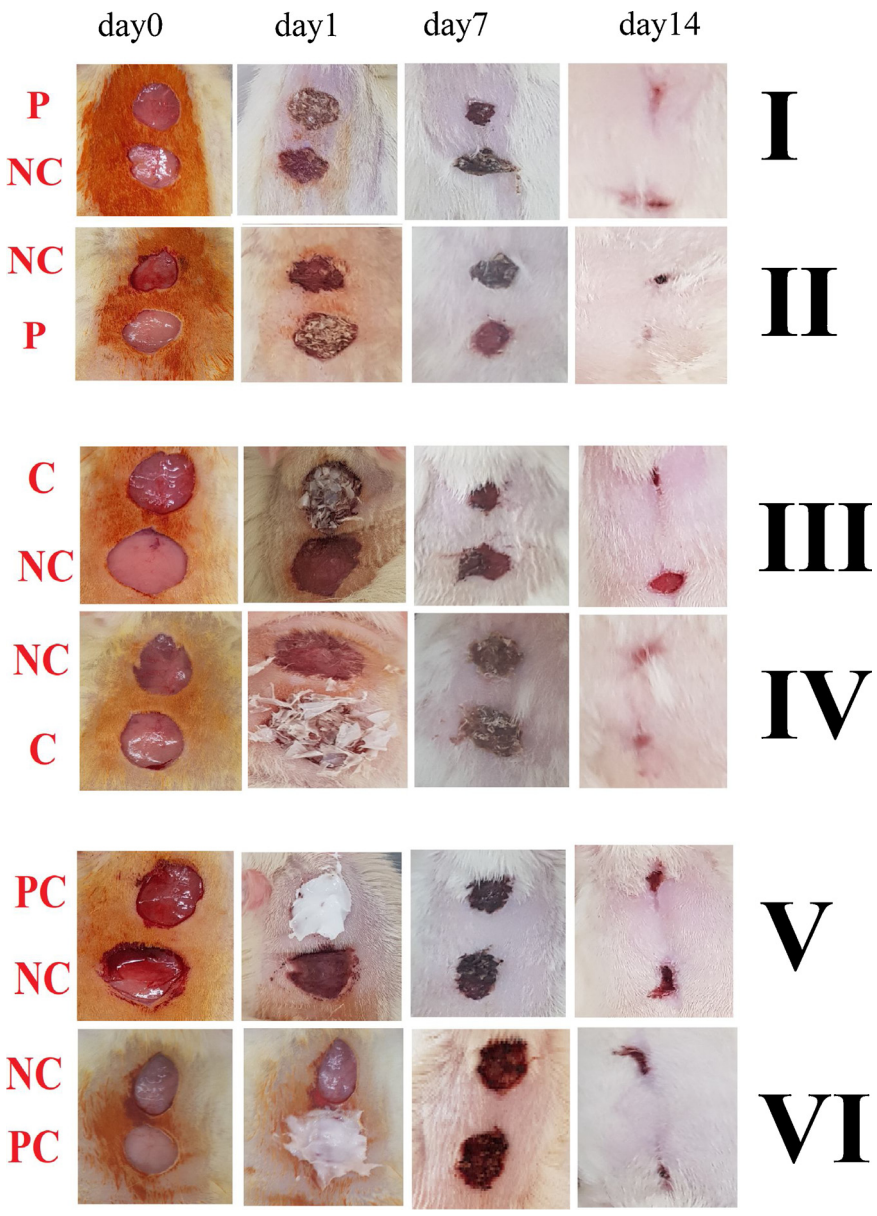

Fig. 4. Morphological evaluation of wounds healing during 14 days: P: Wound treated with Puff shed skin, C: Wound treated with Cat shed skin, PC: Positive control, NC: Negative wound control created in the same treated rat. In I, III and V the treated wounds are located in upper position while in II, IV and VI in downer position.

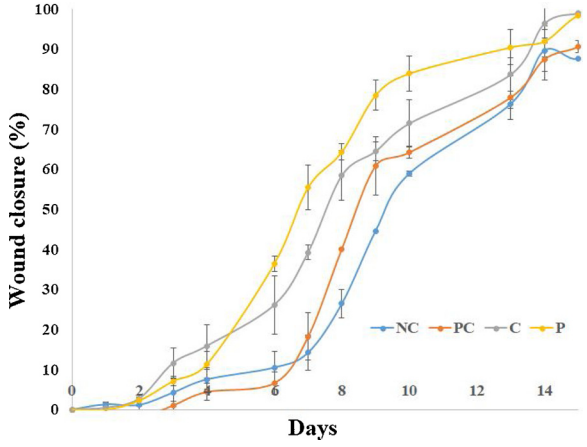

Fig. 5. Wound closure percentage during 14 days.

wound healing. The most outstanding success of this research was the acceleration of wound healing by P snakes shed skin as a natural ECM that include keratin protein. Moreover, histological normalized scores of the one week treated wounds in the criteria such as acute inflammation, granulation tissue amount, re-epithelization, neovascularization and hair follicles development were improved remarkably in the P sample compared to other groups. Pathology evaluation of wounds after two weeks revealed focal hyper granulation, no crust formation, mild fibroblast maturation, scant collagen amount and superficial myonecrosis in the P sample which lead to higher relative pathology score in comparison with positive and C group. Albeit the production of new collagen is not higher than the positive group, the amount of wavy collagen deposited in the P group was greater than the negative control. Wound healing process may also benefit from the capability of the keratin-based membrane as a hemostatic agent to stop bleeding in the opened wounds. Ongoing works will focus on purification and modification of keratin hydrogel derived from snakes shed skin for wound dressing applications.

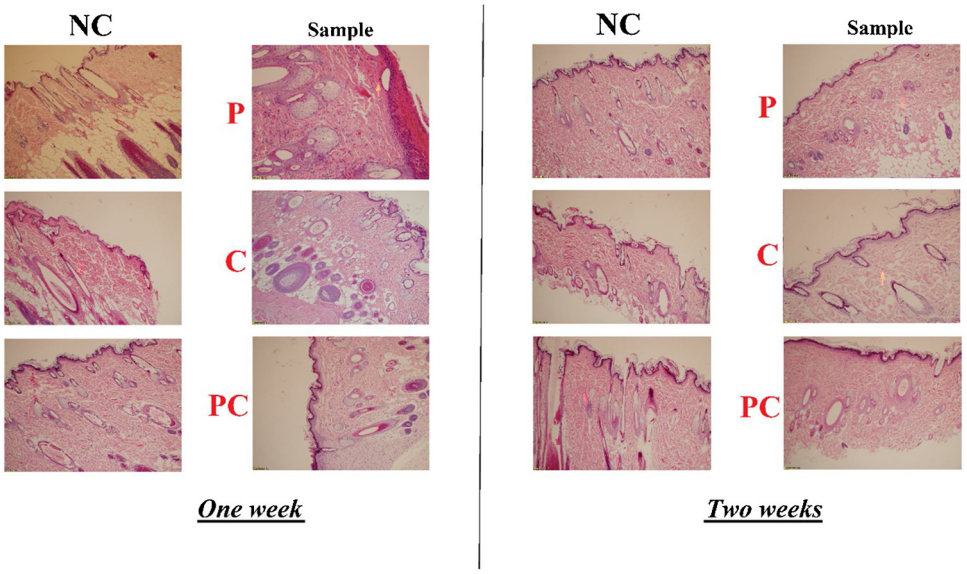

Fig. 6. Histological evaluation of wounds healing during 7 and 14 days.

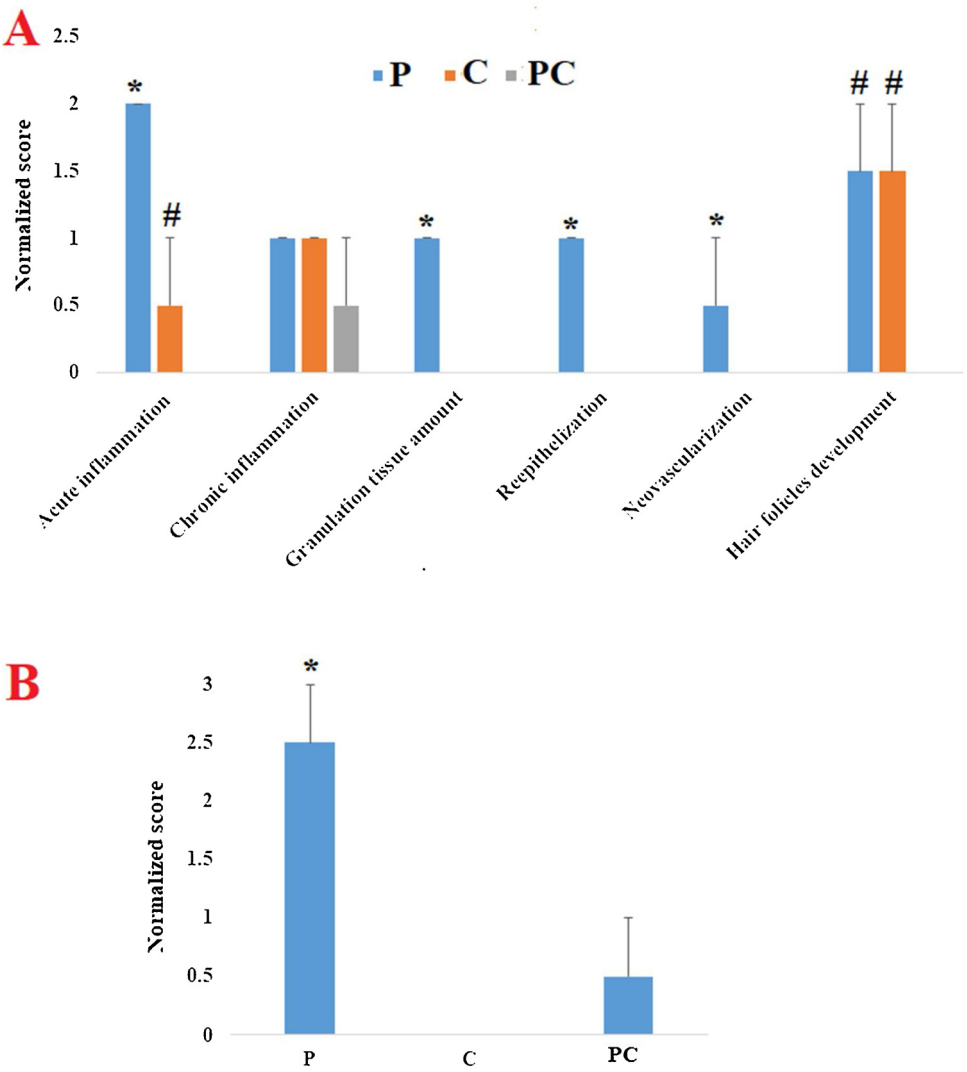

Fig. 7. Histopathological examination of wounds based on Greenhalgh (A) and Abramov (B) scoring systems. * indicates significant difference in comparison with all other groups. # indicates significant difference in comparison with positive control.

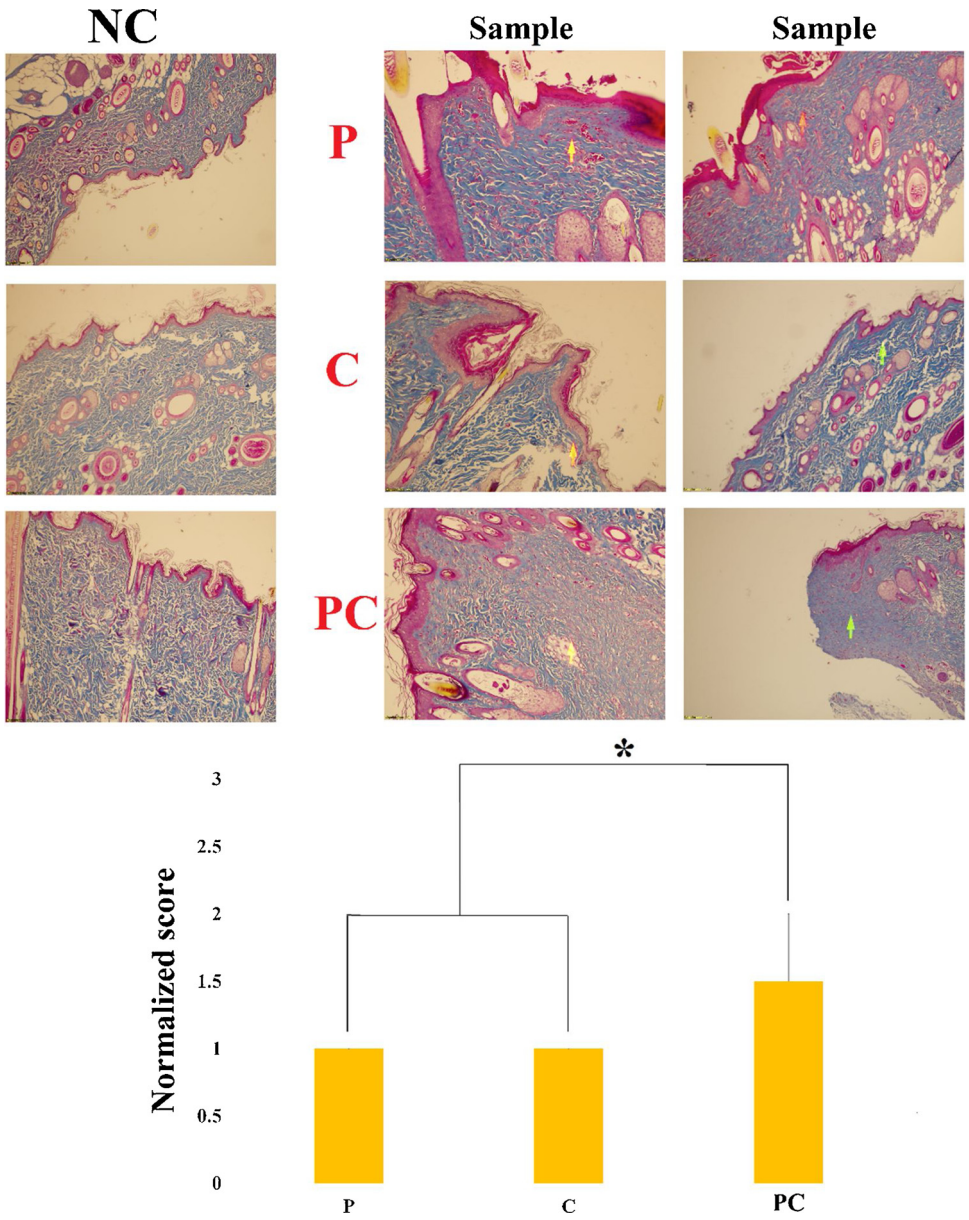

Fig. 8. Masson's trichrome staining and score on day 14 at 400× magnification.

5. Conclusion

The possibility of using biocompatible keratin-based shed skins collected from two different Omani snakes was examined as a wound dressing material in this study. Wound closure assessment of the negative and positive control as well as C and P treated wounds clearly demonstrated that the wounds treated with P shed skin healed faster. These outcomes demonstrated that P snake shed skin might heal acute wounds either directly or indirectly via the release of inflammatory cytokines, granulation tissue formation resulted in reepithelization, neovascularization, and fibroblast expansion. In addition, the remodeling phase of wound healing was improved via collagen formation. Further studies warranted for evaluating the shed skin of different snake types to optimize the wound healing efficiency.

Acknowledgment

This research was supported by University of Nizwa internal grant and the Research Council (TRC) open grant (ORG169). We would like also to thank Mr. Sayed Farook for his technical assistant.

References

[1] T. Bjarnsholt, K. Kirketerp-Møller, P.Ø. Jensen, K.G. Madsen, R. Phipps, K. Krogfelt, N. Høiby, M. Givskov, Why chronic wounds will not heal: a novel hypothesis, Wound Repair Regen. 16 (1) (2008) 2–10.
[2] I. George Broughton, J.E. Janis, C.E. Attinger, Wound healing: an overview, Plast. Reconstr. Surg. 117 (7S) (2006) 1e-S-32e-S.
[3] P. Martin, Wound healing–aiming for perfect skin regeneration, Science 276 (5309) (1997) 75–81.
[4] J.A. Sherratt, J.C. Dallon, Theoretical models of wound healing: past successes and future challenges, C. R. Biol. 325 (5) (2002) 557–564.
[5] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (7193) (2008) 314.
[6] S. Tanha, M. Rafiee-Tehrani, M. Abdollahi, S. Vakilian, Z. Esmaili, Z.S. Naraghi, E. Seyedjafari, H.A. Javar, G-CSF loaded nanofiber/nanoparticle composite coated with collagen promotes wound healing in vivo, J. Biomed. Mater. Res. A. 105 (10) (2017) 2830–2842.
[7] J. Li, J. Chen, R. Kirsner, Pathophysiology of acute wound healing, Clin. Dermatol. 25 (1) (2007) 9–18.
[8] T.J. Koh, L.A. DiPietro, Inflammation and wound healing: the role of the macrophage, Expert Rev. Mol. Med. 13 (2011).
[9] A.J. Singer, R.A. Clark, Cutaneous wound healing, N. Engl. J. Med. 341 (10) (1999) 738–746.
[10] S. Barrientos, O. Stojadinovic, M.S. Golinko, H. Brem, M. Tomic-Canic, Growth factors and cytokines in wound healing, Wound Repair Regen. 16 (5) (2008)

585–601.
[11] J. Gailit, R.A. Clark, Wound repair in the context of extracellular matrix, Curr. Opin. Cell Biol. 6 (5) (1994) 717–725.
[12] T. Velnar, T. Bailey, V. Smrkolj, The wound healing process: an overview of the cellular and molecular mechanisms, J. Int. Med. Res. 37 (5) (2009) 1528–1542.
[13] Y. Yamaguchi, K. Yoshikawa, Cutaneous wound healing: an update, J. Dermatol. 28 (10) (2001) 521–534.
[14] Y. Abramov, B. Golden, M. Sullivan, S.M. Botros, J.J.R. Miller, A. Alshahrour, R.P. Goldberg, P.K. Sand, Histologic characterization of vaginal vs. Abdominal surgical wound healing in a rabbit model, Wound Repair Regen. 15 (1) (2007) 80–86.
[15] J.L. Monaco, W.T. Lawrence, Acute wound healing: an overview, Clin. Plast. Surg. 30 (1) (2003) 1–12.
[16] J.G. Rouse, M.E. Van Dyke, A review of keratin-based biomaterials for biomedical applications, Materials 3 (2) (2010) 999–1014.
[17] P. Hill, H. Brantley, M. Van Dyke, Some properties of keratin biomaterials: kerateines, Biomaterials 31 (4) (2010) 585–593.
[18] A. Tachibana, Y. Furuta, H. Takeshima, T. Tanabe, K. Yamauchi, Fabrication of wool keratin sponge scaffolds for long-term cell cultivation, J. Biotechnol. 93 (2) (2002) 165–170.
[19] F. Loschke, K. Seltmann, J.-E. Bouameur, T.M. Magin, Regulation of keratin network organization, Curr. Opin. Cell Biol. 32 (2015) 56–64.
[20] A. Shavandi, T.H. Silva, A.A. Bekhit, A.E.-D.A. Bekhit, Keratin: dissolution, extraction and biomedical application, Biomater. Sci. 5 (9) (2017) 1699–1735.
[21] M. Park, H.K. Shin, B.-S. Kim, M.J. Kim, I.-S. Kim, B.-Y. Park, H.-Y. Kim, Effect of discarded keratin-based biocomposite hydrogels on the wound healing process in vivo, Mater. Sci. Eng. C 55 (2015) 88–94.
[22] T. Aboushwareb, D. Eberli, C. Ward, C. Broda, J. Holcomb, A. Atala, M. Van Dyke, A keratin biomaterial gel hemostat derived from human hair: evaluation in a rabbit model of lethal liver injury, J. Biomed. Mater. Res. Part B Appl. Biomater. 90 (1) (2009) 45–54.
[23] C.R. Blanchard, S.F. Timmons, R.A. Smith, Keratin-based hydrogel for biomedical applications and method of production, Google Patents (1999) Patent Number: 5,932,552, Date of Patent: Aug. 3, 1999.
[24] A. Vasconcelos, A. Cavaco-Paulo, The use of keratin in biomedical applications, Curr. Drug Targets 14 (5) (2013) 612–619.
[25] M.B. Witte, A. Barbul, General principles of wound healing, Surg. Clin. North Am. 77 (3) (1997) 509–528.
[26] P. Hangge, J. Stone, H. Albadawi, Y.S. Zhang, A. Khademhosseini, R. Oklu, Hemostasis and nanotechnology, Cardiovasc. Diagn. Ther. 7 (Suppl 3) (2017) S267.
[27] T. Luo, S. Hao, X. Chen, J. Wang, Q. Yang, Y. Wang, Y. Weng, H. Wei, J. Zhou, B. Wang, Development and assessment of kerateine nanoparticles for use as a hemostatic agent, Mater. Sci. Eng. C 63 (2016) 352–358.
[28] H. Abdel-Aal, R. Vargiolu, H. Zahouani, M. El Mansori, IOP PublishingA Study on the Frictional Response of Reptilian Shed Skin, Journal of Physics: Conference Series2011, A Study on the Frictional Response of Reptilian Shed Skin, Journal of Physics: Conference Series (2011) p. 012016.
[29] M.-C.G. Klein, S.N. Gorb, Epidermis architecture and material properties of the skin of four snake species, J. R. Soc. Interface 9 (76) (2012) 3140–3155.
[30] A. Ripamonti, L. Alibardi, G. Falini, S. Fermani, M. Gazzano, Keratin-lipid structural organization in the corneous layer of snake, Biopolymers 91 (12) (2009) 1172–1181.
[31] J.G. Pickering, D.R. Boughner, Quantitative assessment of the age of fibrotic lesions using polarized light microscopy and digital image analysis, Am. J. Pathol. 138 (5) (1991) 1225.
[32] X. Liu, Y. Nie, X. Meng, Z. Zhang, X. Zhang, S. Zhang, DBN-based ionic liquids with high capability for the dissolution of wool keratin, RSC Adv. 7 (4) (2017) 1981–1988.
[33] S. Duangjit, L.M. Mehr, M. Kumpugdee-Vollrath, T. Ngawhirunpat, Role of simplex lattice statistical design in the formulation and optimization of microemulsions for transdermal delivery, Biol. Pharm. Bull. 37 (12) (2014) 1948–1957.
[34] B. Chapman, 15—a review of the mechanical properties of keratin fibres, J. Text. Inst. 60 (5) (1969) 181–207.
[35] A. Mohanty, C. Das, S. Prusty, P. Sahu, Wound healing potential of medicinal plants: a review, Res. Rev. A J. Pharm. 4 (2) (2018) 17–40.
[36] S. Saghazadeh, C. Rinoldi, M. Schot, S.S. Kashaf, F. Sharifi, E. Jalilian, K. Nuutila, G. Giatsidis, P. Mostafalu, H. Derakhshandeh, Drug delivery systems and materials for wound healing applications, Adv. Drug Deliv. Rev. 127 (March) (2018) 138–166.
[37] S. Vakilian, M. Norouzi, M. Soufi-Zomorrod, I. Shabani, S. Hosseinzadeh, M. Soleimani, L. Inermis-loaded nanofibrous scaffolds for wound dressing applications, Tissue Cell 51 (2018) 32–38.
[38] M. Piran, S. Vakilian, M. Piran, A. Mohammadi-Sangcheshmeh, S. Hosseinzadeh, A. Ardeshirylajimi, In vitro fibroblast migration by sustained release of PDGF-BB loaded in chitosan nanoparticles incorporated in electrospun nanofibers for wound dressing applications, Artif. Cells Nanomed. Biotechnol. (2018) 1–10.
[39] W. Paul, C.P. Sharma, Chitosan and alginate wound dressings: a short review, Trends Biomater. Artif. Organs 18 (1) (2004) 18–23.
[40] R. Montesano, L. Orci, Transforming growth factor beta stimulates collagen-matrix contraction by fibroblasts: implications for wound healing, Proc. Natl. Acad. Sci. 85 (13) (1988) 4894–4897.
[41] C. Vepari, D.L. Kaplan, Silk as a biomaterial, Prog. Polym. Sci. 32 (8-9) (2007) 991–1007.
[42] W. Czaja, A. Krystynowicz, S. Bielecki, R.M. Brown Jr, Microbial cellulose—the natural power to heal wounds, Biomaterials 27 (2) (2006) 145–151.
[43] M. Norouzi, S.M. Boroujeni, N. Omidvarkordshouli, M. Soleimani, Advances in skin regeneration: application of electrospun scaffolds, Adv. Healthc. Mater. 4 (8) (2015) 1114–1133.
[44] M. Norouzi, I. Shabani, H.H. Ahvaz, M. Soleimani, PLGA/gelatin hybrid nanofibrous scaffolds encapsulating EGF for skin regeneration, J. Biomed. Mater. Res. A. 103 (7) (2015) 2225–2235.