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Research Article

Evaluation of Effectiveness of Honey-Based Alginate Hydrogel on Wound Healing in Rat Model

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Abstract

Alginate hydrogel and honey have a notably effect on wound healing process, therefore assessment of combined impact of those are essential. In this study, the effect of honey-based alginate hydrogel, alginate hydrogel and commercial alginate dressings in recovery of wound in a rat model was studied. In this study, 20Wistar rats were divided into four groups of five. One wound of 1×1 cm square was marked using a template and the skin, on either side of the vertebral column between the ears, excised. One of the rats in each group was euthanized on the 4th, 7th, 14th, and 21th days and skin samples were taken for histopathological analysis. Findings showed that the average total time of wound healing in group of treated with honey-based alginate hydrogel dressing was the best treatment as opposed to the other groups. With respect to all information obtained of the study we found that the honey-based alginate hydrogel is much convenient for wound dressing and treatment of surface wounds. Therefore, outcomes of the treatment make our dressing highly promising as an alternative wound healing system for the treatment of wounds and certainly opening new path for future research and development.

Keywords: Alginate, Honey, Alginate Hydrogel, Dressing, Wound Healing

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Introduction

Wound healing exhibits a dynamic physiological process initiated and influenced by a lot of agents. The process can be divided into four stages: haemostasis, inflammation, proliferation, and finally remodeling [1]. Honey has long been used for treating wounds and other skin conditions [2-6], attributed to its antibacterial, anti-inflammatory, and antioxidant properties [7]. Honey is composed of sugars including mainly about 40% fructose, 30% glucose, and 10% maltose with other compounds such as oligosaccharides, minerals, carbohydrates, enzymes, and phytochemicals such as flavonoids, and ferulic and caffeic acids [8]. The specific composition depends on the flowers available to the bees that produce the honey [9, 10]. Antibacterial activity of honey is because of its high osmolarity, pH, H_2O_2 production, and presence of other phytochemical components that originate from the nectar of plant [11]. There are several reports on the antibacterial activities of honey against a large number of microorganisms that result in acceleration of wound healing process [11–13]. Antioxidant activity of honey arises from its phytochemical component which protects cells from the damage caused by free radicals thus decreasing the inflammation process [14]. The free radical scavenging activities of honey is mainly due to the contents of flavonoids and phenolic acids [15].

Bangroo et al., [16] have shown previously that the usage of honey can enhance the thickness of the granulation tissue and the area of re-epithelization, and Suguna et al., [17] have demonstrated that honey can enhance collagen metabolism during wound healing. Subramanian [18] exhibited that honey was superior to sulfadiazine in producing early subsidence of acute inflammatory changes, better control of infection and quicker wound healing. Several authors have reported on hydrogel technologies providing products suitable for biomedical application especially in wound management [19–21]. Hydrogels appear to be optimal media to increase wound healing due to its features including moist wound healing, good fluid absorbance as well as having high water retention capacity [19]. Thus, developing hydrogel-based wound dressings from a wide range of biomaterials as well as biologically active substances including chitin, chitosan, alginate, and sea cucumber is in the spotlight [22–25].

Various dressings and gels containing honey are now available [26, 27], but in the current study, the dressing derived from honey and alginate hydrogel was introduced as a novel dressing that have reservoir capacities for the sustained delivery of honey. The development of a sustained release system would substantially increase the utility of incorporated honey in tissue repair and thus facilitate healing and we was therefore undertaken to assess its wound-healing activity in rat model, using topically applied gel formulations.

Materials and Methods

Honey formulations

For topical at a concentration of 70% were made.

Animals

A total of 20 male wistar rats (weight 180–200 g) were used in the current study. The animals were acclimatized to the laboratory conditions for one week prior to the onset of the experiment. The rats were individually caged, fed with commercial rat chow, and allowed water ad libitum.

The experimental protocol was approved by Institutional Animal Ethics Committee of Pasteur institute of Iran.

Experimental group

The animals were randomly divided into four experimental groups of five rats in each group. Group 1 animals (NC: Negative Control) were not topically treated. Animals in Group 2 (PC: Positive Control) were treated topically with commercial gel. Animals in Group 3 and 4 were treated topically with the formulations of alginate hydrogel and 70% honey-based alginate hydrogel, respectively.

Experimental Designs

The rats were anesthetized intraperitoneally with Ketamine hydrochloride (100 mg/kg body weight) and Xylazine (10 mg/kg body weight) prior to production of the excision wound [28]. Briefly, the skin area had been shaved one day prior to the experiment. One full-thickness wound of 1×1 cm square was marked using a template and the skin, on either side of the vertebral column between the ears, excised using dissecting scissors and forceps. Treatments continued till 21th day and every two days the dressing was changed. One of the rats was euthanized on 4, 7, 14, and 21 days after an overdose of ether inhalation, and skin samples were taken for histopathological analysis.

Histopathological Analysis

In order to macroscopic assessment, the wound area was measured by taking photo every day from the outline of the wound and the time required for healing was measured [29]. For microscopic assessment, skin samples were fixed in 10% formalin solution and embedded in paraffin. Tissue sections of 4-5 μm thickness were cut, and then Hematoxylin-Eosin staining (H&E) and Masson's trichrome staining were performed. The wounds were evaluated for the extent of re-epithelialization, inflammation, angiogenesis, fibroblast, collagen and hair follicle.

Statistical analysis

The analysis was performed using the SPSS software, Version 22.0. Due to the low sample size in each group, we used non-parametric tests including the Kruskal-Wallis test to compare the target groups and the Mann-Whitney test to compare pairs of groups. P values of less than 0.05 were considered to be significant.

Results and Discussion

The prepared honey-based alginate gel and control alginate gel represented uniform, transparent sheets of three-dimensional networks with a thickness of 3-4 mm. They showed good transparency to allow monitoring of the healing progression as well as ensuring timely dressing alterations. Honey-based alginate gel demonstrated a golden yellow due to the original color of the honey itself which is yellow. Honey has long been documented as having healing properties [30-32]. The increased rate of healing could also be attributed to the osmotic action of honey drawing out lymph, and thus providing a constant flow of nutrients from the function in capillaries deeper down [33]. The time required for wound healing in negative control, positive control, alginate hydrogel and honey-based alginate hydrogel were 10.2, 8.1, 8.3 and 7 days, respectively. The results showed that there were a significant difference between the honey-based alginate hydrogel and the other groups ($p<0.05$, Fig. 1).

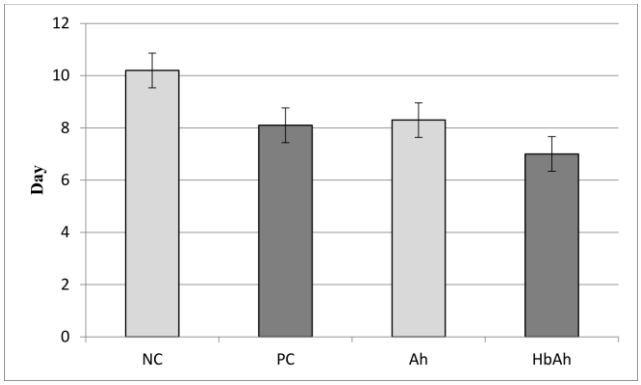

Figure 1. Comparison of the time required for wound healing among treatments. Treatments are Positive Control (PC), alginate hydrogel (Ah) and Honey-based alginate hydrogel (HbAh). NC: Negative Control.

Many researchers found that honey stimulates development of new blood vessels in the bed of wounds [34-39]. Honey gives a "slow-release delivery" of innocuous concentrations of hydrogen peroxide, a substance which at low levels can stimulate angiogenesis and stimulate the growth of fibroblasts [40]. Histopathological comparisons showed that angiogenesis on 7 day ($p<0.05$, Fig. 2) and the growth of fibroblast on the 14th day ($p<0.05$, Fig. 3) was the greatest in honey-based alginate hydrogel dressing as opposed to the other treatments.

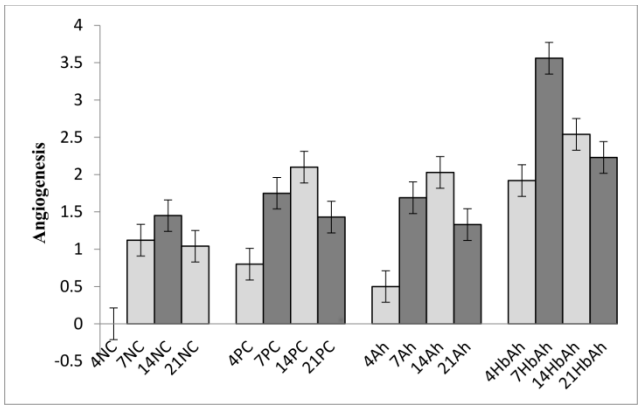

Figure 2. Comparison of the angiogenesis on the 4th, 7th, 14th and 21th days in the different groups. Treatments are Positive Control (PC), Alginate hydrogel (Ah) and Honey-based alginate gel (HbAh).

One of the indices of the quality of wound healing is the presence of hair follicle. On the 21th day, hair follicles were observed in some rats. The average of observed number of hair follicle in negative control, positive control, alginate hydrogel and honey-based alginate hydrogel were 1, 3, 2 and 4 (data not shown). Re-epithelialization in wound location is one of the effective solutions in determination of wound healing rate.

A lot of studies showed that honey promotes epithelialization of wounds [41-46].

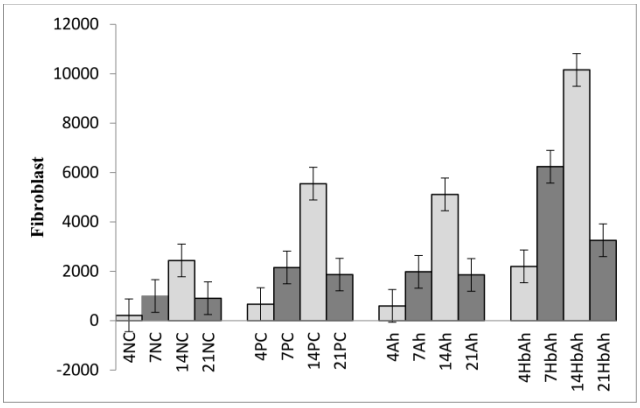

Figure 3. Comparison of the growth of fibroblast on the 4th, 7th, 14th and 21th days in the different groups. Treatments are Positive Control (PC), Alginate hydrogel (Ah) and Honey-based alginate hydrogel (HbAh).

The mentioned studies were consistent with our study and microscopic evaluation demonstrated that there was a significant acceleration of re-epithelialization in wounds treated with honey-based alginate hydrogel dressings as compared to other experimental groups (Fig. 4). On the 4th day, the thickness of epiderm was not computable because epiderm can't be complete untill this day. The results showed that honey-based alginate hydrogel on the 21th day had the greatest epiderm growth as opposed to the other groups ($p<0.05$, Fig. 4 and Fig. 6).

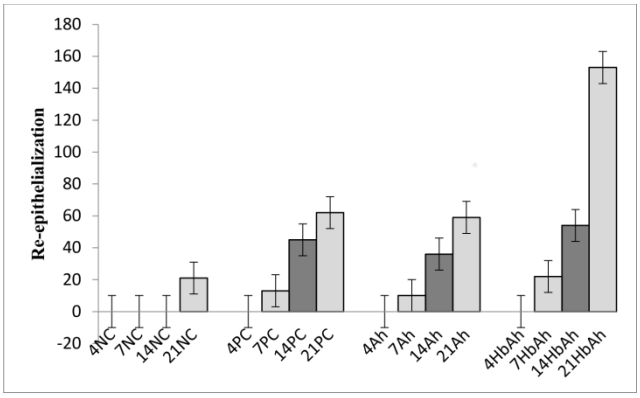

Figure 4. Comparison of the re-epithelialization on the 4th, 7th, 14th and 21th days in the different groups. Treatments are Positive Control (PC), alginate hydrogel (Ah) and Honey-based alginate hydrogel (HbAh).

In several studies, the results showed that honey has increased significantly the quantity of collagen synthesized and stimulates tissue growth [34-39]. Since collagen formation is a curtail step for the wound healing, the skin tissue was stained with Masson's trichrome stain that can highlight the collagen remodeling and maturation. The results indicated that honey-based alginate gel-treated wounds exhibits abundant mature and compact collagen comparing with other treatments (Fig. 6). Honey-based alginate hydrogel on the 14th day had the greatest collagen as opposed to the other groups ($p<0.05$, Fig. 5).

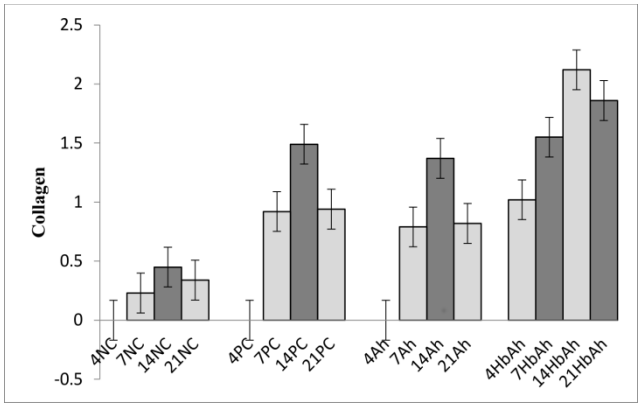

Figure 5. Comparison of the collagen on the 4th, 7th, 14th and 21th days in the different groups. Treatments are Positive Control (PC), Alginate hydrogel (Ah) and Honey-based alginate hydrogel (HbAh).

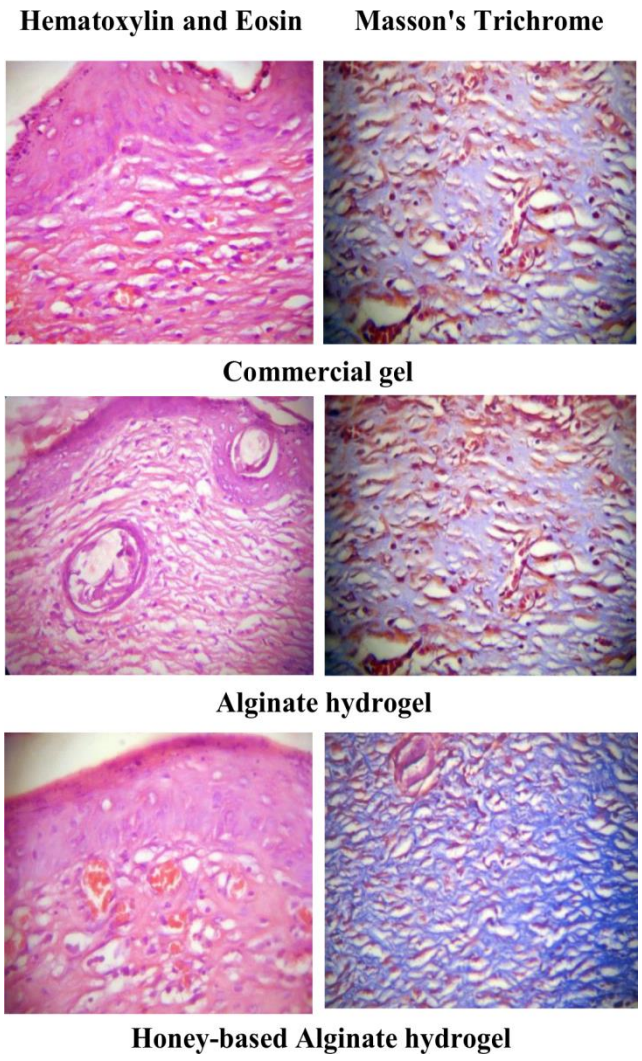

Figure 6. Histopathology by using H & E staining and Masson's Trichrome staining on day 21.

In several studies were shown that honey reduces inflammation [47-49]. Also, we observed the same result regarding inflammation in honey-based alginate hydrogel. Skin samples on the 4th day had much inflammation and there wasn't a significant difference among treatments. Moreover, we observed edema in most of the rats in the mentioned day and edema continued till the 7th day only in negative control. Histopathological comparisons showed that on the 21th day the lowest inflammation rate was observed in honey-based alginate hydrogel as opposed to other dressings (data not shown).

Conclusion

In conclusion, honey-based alginate hydrogel represents a feasible and productive approach to support dermal wound healing by regulation of multiple events that are central to the process of healing. The resultant wound healing influences were attributed to the synergistic effect of the alginate hydrogel and the incorporated honey. Outcomes of the treatment make our dressing highly promising as an alternative wound healing system for the treatment of wounds and certainly opening new path for future research and development.

Acknowledgements

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