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The Effect of Honey, Aloe Vera, and Hydrocolloid Dressing on the Healing Process of Murine Excisional Wounds

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José Enrique Hernández-Rodríguez, PhD[1], José Luis Martín-Barrasa, PhD[2], J. Aragón-Sánchez, PhD[3], Maximina Monzón-Mayor, PhD[4], José Manuel Pérez-Galván, MD[5], Pedro Saavedra-Santana, PhD[6], and María del Mar Romero-Alemán, PhD[4]

Abstract

Chronic ulcers are a major health problem associated with high costs and a loss of quality of life. Because of this, the search for products that accelerate wound healing is a constant, given the need for alternatives that help to alleviate this serious health problem. We analyzed the efficacy of 2 natural products—honey and aloe vera—versus hydrocolloid (HC) dressings as a control group in healing full-thickness wounds. For this purpose, we performed full-thickness excisions of the skin, including the *panniculus carnosus*, in mice. We inserted a nitrile ring into the subcutaneous cellular tissue simulating the second-intention wound healing course. We found that aloe vera reduced the diameter of the wounds compared to honey ($p < .001$) and the control group ($p < .001$).

Keywords

wound healing, pressure ulcers, honey, aloe, murine model wounds and subcutaneous ring

Chronic ulcers are a major health problem associated with high costs and a loss of quality of life.[1,2] Because of this, the search for products that accelerate wound healing is a constant, given the need for alternatives that help to alleviate this serious health problem. Different clinical studies endorse the successful use of bee honey and aloe vera in the healing process of different cutaneous lesions because of their physical properties, the synergy of which bestows several traits, with the antibacterial, anti-inflammatory, antioxidant, and healing process stimulation properties standing out due to their clinical effects.[3–5]

The high osmolality and low water (Aw) activity of honey, along with its low pH (3.2-4.5), is partly responsible for these effects,[6] as is the presence of organic acids (hexadecanoic, formic, propionic, gluconic, acetic, and benzoic acids), phenolic and flavonoid compounds and the combination of hydrogen peroxide and benzoic acid which generates very stable peroxide compounds in the presence of endogenous catalase with a strong antibacterial effect.[7–13]

Moreover, the effects of aloe vera and its main component, acemannan, have been described as a healing agent for superficial wounds[5] and full-thickness wounds.[14] It has angiogenic[5] and anti-inflammatory[15–17] effects, which favors the proliferation and migration of keratinocytes and collagen deposition,[5,16,17] triggering the different healing phases and reaching sufficient epithelialization.

In recent years, empirical evidence has shown the use of these natural products on different types of partial-thickness and full-thickness wounds to be successful on their own or combined with other treatments.[4,11,15,18,19]

[1]Department of Nursing, University of Las Palmas de Gran Canaria, University Institute for Biomedical and Health Research, Las Palmas de Gran Canaria, Las Palmas, Spain
[2]Dr. Negrin University Hospital Research Unit and University Institute for Animal Health, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas, Spain
[3]Medical Director of the Diabetic Foot Unit, La Paloma Hospital, Las Palmas de Gran Canaria, Las Palmas, Spain
[4]Department of Morphology, University of Las Palmas de Gran Canaria, University Institute for Biomedical and Health Research, Las Palmas de Gran Canaria, Islas Canarias, Spain
[5]SIMACE– Facility Research in Advanced Confocal and Electron Microscopy, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Islas Canarias, Spain
[6]Department of Mathematics, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Islas Canarias, Spain

Corresponding Author:
José Enrique Hernández Rodríguez, Nursing Department, Health Sciences Faculty, Institute for Biomedical and Healthcare Research, Las Palmas de Gran Canaria University, Las Palmas de Gran Canaria, Paseo Blas Cabrera Felipe, s/n, 35016, Las Palmas, Canary Islands, Spain.
Email: joseenrique.hernandez@ulpgc.es

The effectiveness of honey on its own in the healing of partial-thickness wounds has been compared with the use of a hydrocolloid dressing. One study did not report any significant difference between the use of honey and the use of hydrocolloid dressing.[20] Similarly, studies have shown that a combination of honey, aloe vera and milk is effective in healing partial-thickness wounds in rats within 28 days, with positive outcomes observed.[16]

However, no studies compare honey with aloe vera and hydrocolloid dressing. We aim to study the role of these natural products in healing full-thickness wounds by secondary intention compared to using hydrocolloid dressing.

We hypothesize that aloe vera and honey are associated with complete healing by secondary intention at 50 days in a murine animal model. We also hypothesize that wounds treated with aloe vera and honey will have less slough during the healing process.

Methods

A small subcutaneous nitrile ring was inserted into the animals' dorsal area to study the tissue repair mechanisms in this type of wound and regulate and control the contraction that occurs to these animals' skin during the healing process. Ten days later, the skin within the ring's interior was excised, creating a full-thickness wound by removing the subcutaneous cellular tissue and the *panniculus carnosus*.[20–22] From a macroscopic point of view, complete wound healing was assessed at 50 days. Complete healing has been considered the process in which healthy granulation tissue is generated, without slough or cellular detritus, non-exudative with non-erythematous and clean edges, with healthy perilesional skin and with complete closure covered by epithelial tissue with abundant hair follicles.[23]

Animals

In line with the proposal to reduce the number of animals required without altering statistical significance,[24] 15 eight-week-old male CD15 *Swiss* mice ($n = 15$), with an average weight of 42.8 g, were randomly selected for this experiment. The mice were placed in individual cages equipped with food and water dispensers to which they had unrestricted access. They were cared for according to the European Directive 2010/63/EU on protecting animals used for scientific purposes.[25] The animals were randomly assigned to 2 experimental groups and 1 control group, with 5 animals per group.

The Animal Ethics and Wellbeing Committee (CEBA) at the University of Las Palmas de Gran Canaria (ULPGC) approved the experimental procedures (Ref 004/2013CEBA ULPGC).

Before and after the surgical procedure, measurements were taken for *weight*; *glycemia*, measured with a standard glucometer (FreeStyle Optium Neo de Abbott®) with the sample extracted from the lateral tail vein (coccygeal vein); *body temperature*, measured with an infrared thermometer (TZL-801A); and *wound surface area*, measured with digital calipers (Vernier Calipers® RS PRO 243-6615, UK). These measurements were supplemented by photographs taken from the same distance using a smartphone (Samsung S5) camera, with the animals photographed on millimeter graph paper for scale. Observations were made at different moments ([t_0]: immediately following surgery; and on days 2[t_2], 5[t_5], 15[t_{15}] and 50[t_{50}] of treatment,[16,20,26,27] measuring the size of the wounds based on the average of 2 measurements, taken perpendicular (d1) and parallel (d2) to the mouse's backbone. Therefore, 20 observations were obtained per mouse, with a total of 260 (see Supplemental data).

Furthermore, it was noted whether the granulation tissue formed was pink and clean, whether the wound bed presented total or partial slough or cellular detritus and whether the wound presented complete closure covered by epithelial tissue with abundant hair follicles.

Image analysis was carried out with the image analysis software package ImageJ®.[28]

Surgical Procedure

The animals were anesthetized intraperitoneally with 0.03 mL of metomidine (1 mg/kg; 0.03 mg) and 0.06 mL of ketamine (100 mg/kg; 3 mg); 0.01 mg/kg of atipamezole was used for revival. Oral Tramadol was administered *ad libitum* (25 mg/kg) to control postoperative pain.[29,30]

A sterile nitrile ring, 10 mm in diameter (Figure 1A), was inserted and fixed subcutaneously in each animal. Subsequently, 10 days after insertion and using the same anesthetic procedure described above, a full-thickness wound was caused to the skin within the ring, including the *panniculus carnosus*. A sterile skin biopsy punch, 8 mm in diameter, was used (Ref 94158BP-80-FA) (Figure 1B) and the internal edge of the ring was sutured to fix the wound and prevent the skin's contraction, as per the experimental model of Davidson et al[21] and Ren et al[22] (Figure 1C).

Control and Experimental Treatments

The wounds of the first experimental group (5 mice) were treated with saline (SF) and multifloral bee honey (Cuevas de Guayadeque® RGSEAA [Spanish General Food Safety Register of Food Businesses and Foodstuffs]: E23.03229/GC/CEE). The honey was previously sterilized through exposure to ultraviolet radiation for 30 min.[30] The second experimental group (5 mice) was given SF and Canary

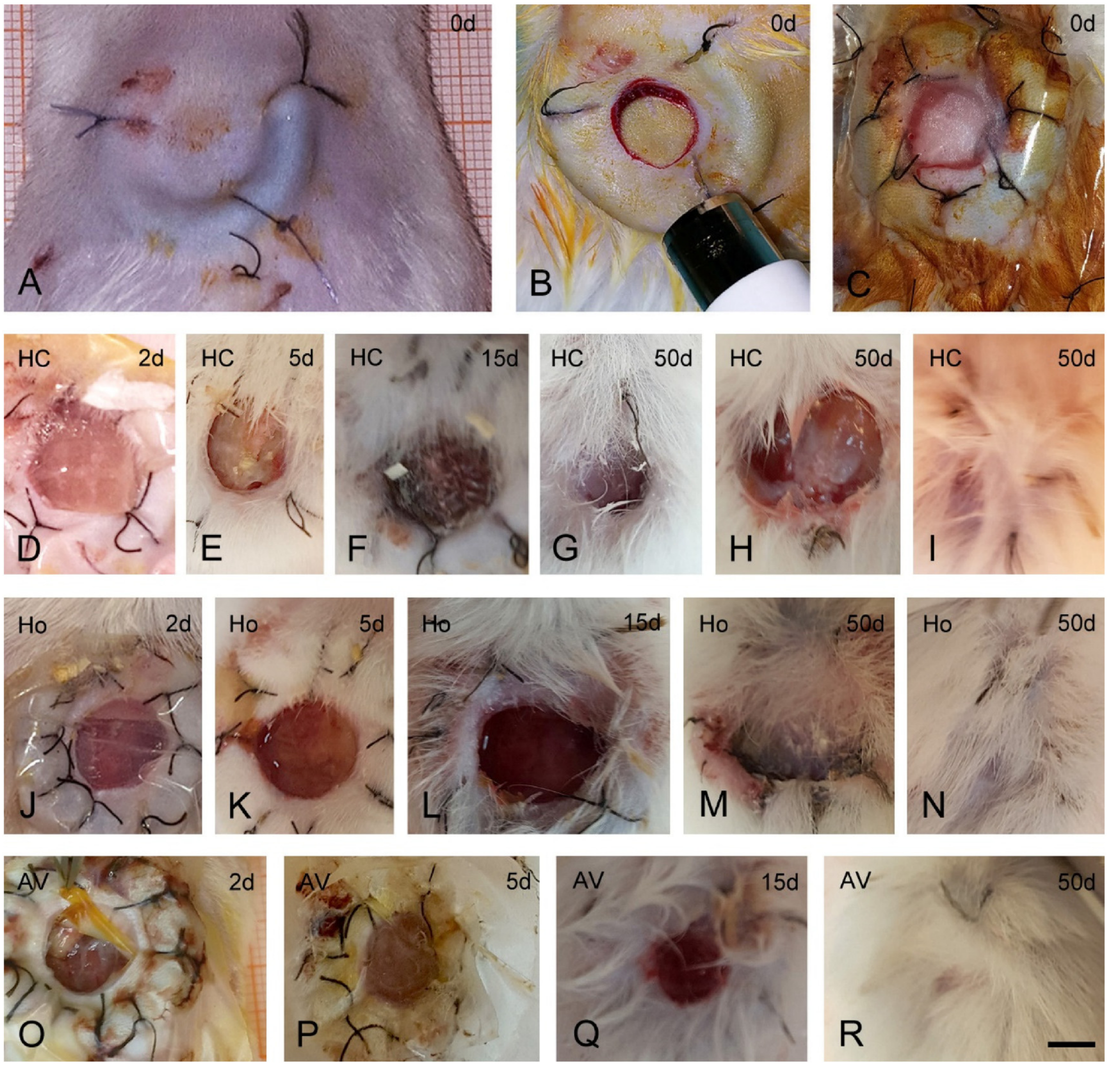

Figure 1. Anatomical views of mice dorsal skin during the surgical procedure (A-C) and representative images of the wound healing process at 2, 5, 15, and 50 days of treatment with HC dressing (D-I), natural honey (Ho) (J-N), and commercial AV (O-R). The rostral side of the animals is shown in all images. (A-C) Note the subcutaneous nitrile ring (10 mm diameter) before removing the *full*-thickness skin with a *trucut* (B). The nitrile ring was fixed with additional sutures before the beginning of the corresponding topical treatment (C). (D-I) Note a torpid and uneven evolution of the wound healing in the HC (control) group, showing dark wound beds at 15 days (F). Wounds were closed in the same specimens (I) but not in others (G, H) at 50 days postlesion. (J-N) The honey group showed large ulcers with granulation tissue from 2 (J) (K) to 15 days (L). Many hair follicles were closed and covered the wounds at 50 days postlesion. (O-R) The AV group R showed a gradual reduction in wound size. Note the granulation tissue at 15 days (Q). The wounds were closed and covered by many hair follicles at 50 days postlesion (R). Animals were photographed on a laminated millimeter grid paper for scale reference. The scale bar in R (5 mm) applies to all images. Abbreviations: AV, aloe vera; HC, hydrocolloid.

Island aloe vera gel (99.6% aloe vera gel Aloveria® from Pejoseca SL). Saline and a conventional hydrocolloid dressing (Skin-fixe® Hydrocare Bimedica-HiC) were applied to the control group (5 mice). The wounds of the 2 experimental groups were covered with a Tegaderm® transparent dressing. Images were processed with a specific computer software (Image J®). Topical treatments were applied at different moments ([t_0]: immediately following surgery; [t_2] 2 days; [t_5] 5 days; [t_{15}] 15 days; [t_{50}] 50 days) with weight, temperature, glycemia, and wound size also measured (Figure 1D-R).

Statistical Analysis

Measurements were taken for the animals (weight, glycemia, temperature, and diameter) at the time of surgery (t_0)

and on days 2, 5, 15, and 50 of treatment. Therefore, 20 observations were obtained per mouse, with a total of 260 (see Supplemental data).

The study design leads to a data set in the form of:

$$\{(ID, Treatment, Day, Weight_{ID,Day}, Wound_{ID,Day,Treatment})\}$$

where *ID* is the code for the mouse, $Treatment_{ID}$ is the treatment assigned ($Treatment \in \{HC, Honey, Aloe\}$), *Day* is the day of observation and $Weight_{ID,Day,}$ and $Wound_{ID,Day}$ are the weight of mouse *ID* and the diameter of their wound on day *Day*. For this data set, we consider the following mixed-effects models[31]:

$$Wound_{ID,Day,Treatment} = \alpha + \beta_{Treatment} \cdot Day + \gamma \cdot Weight_{ID,Day} + ID + e_{ID,Day,Treatment}$$

The intercept is represented by α, $\beta_{Treatment}$ indicates the slope corresponding to the treatment received (1 per treatment), ID is the randomly assigned mouse and $e_{ID,Day,Treatment}$ are the error terms, which may be established as independent and normally distributed. Note that the slopes assess the rate of decrease in wound size. Thus, the best treatment will be that represented by the slope showing the greatest decrease. The model was estimated by means of the restricted maximum likelihood (REML). Statistical significance was set at $p < .05$. Data were analyzed using the R package, version 3.6.1.[32] The statistical model created for analyzing the variables studied was developed by the Department of Mathematics of the University of Las Palmas de Gran Canaria.

Results

The 3 groups of mice were homogeneous in terms of weight, temperature, and glycemia. The final sample comprised 13 eight-week-old animals (n = 13), with an average weight of 42.3 g (SD 1.05). Two were removed (1 from the control group and another from the aloe experimental group) as they did not meet the experimental criteria (both suffered from subcutaneous ring intolerance).

In the control group (HC), wound evolution was slow and irregular from day 2. On day 15, a dark wound bed was present with no discharge and a reddish wound bed was observed alongside the formation of incomplete epithelial tissue (Figure 1D-F). A moderate amount of hair follicles were in the partially healed area (Figure 1G, I). The average size of the wounds for this group was 10 and 9.2 mm, respectively, on days 2 (t_2) and 5 (t_5) (Figure 1D-E), 9.1 mm on day 15 (t_{15}) (Figure 1F) and 7.4 mm on day 50 (t_{50}) (Figure 1G-H) (Graph 1).

The evolution of wounds treated with honey was different, because we observed an increase in the initial diameter of the wounds. This began on the second day ($t_2 = 8.74$ mm) and continued gradually on days 5 ($t_5 = 9.62$ mm) and 15 ($t_{15} = 10.2$ mm) before subsequently falling to 5.1 mm on day 50 (Graph 1) (Figure 1M). From day 2 to day 15, the wound was kept clean, free of debris and had a pinkish appearance with a moderate amount of granulation tissue (Figure 1J-L). In the already healed areas, there were many hair follicles present (Figure 1N). The average weight was 41.2 g, the average temperature was 34.6 °C and glycemia was 121.2 mg/dL.

In the aloe vera experimental group, the wound size gradually decreased ($t_2 = 9.55$ mm; $t_5 = 8.5$ mm; $t_{15} = 7.85$ mm; $t_{50} = 0$ mm) with no secretions observed, presenting healthy granulation tissue without slough (Figure 1O-R). Total healing was reached on day 50 with abundant hair follicles in all animals of this group (Figure 1R). The average weight of the animals was 38.8 g, the average temperature was 34.7 °C and the average glycemia was 127.7 mg/dL.

Results of Statistical Analysis

Figure 2 shows the individual trajectories of the size of the wound of each mouse. Figure 3 overlays the trajectories based on the treatment applied. Table 1 summarizes the mixed model projections for wound sizes. All covariables showing statistical significance of $p < .1$ were included in the model. Glycemia and temperature did not show statistical significance and were therefore not included in the model. Weight was shown to be a contributing factor in accelerating wound closure (the greater the weight, the smaller the size of the wound; $\gamma = -0.103$: 95% CI = [−0.190; −0.016]). The slope for HC did not show statistical significance ($p = .756$). This result is clearly compatible with the trajectories shown in Figure 2. The slope for the honey group showed a significant decrease [($\beta_{Honey} = -0.07$); $p = .002$]. Therefore, it did not show a significant difference compared to β_{HC} ($p = .381$). The slope for the aloe vera group showed a clear significant decrease [($\beta_{Honey} = -0.181$); $p < .001$] (Figure 3). This means the daily reduction expected in the wound size was 0.181 mm. In addition, a statistically significant difference was shown between the other 2 slopes, both having *p*-values <.001.

Discussion

We have demonstrated that aloe vera is the only treatment that achieves total healing at day 50. The healing process was faster than that seen in wounds treated with honey and the control group. Furthermore, wounds treated with honey decreased in diameter faster than those treated with hydrocolloid dressings.

It can be seen, in general, that the scar tissue across the 2 experimental groups on day 50 was better in the aloe vera group than the honey group. In comparison, the quality

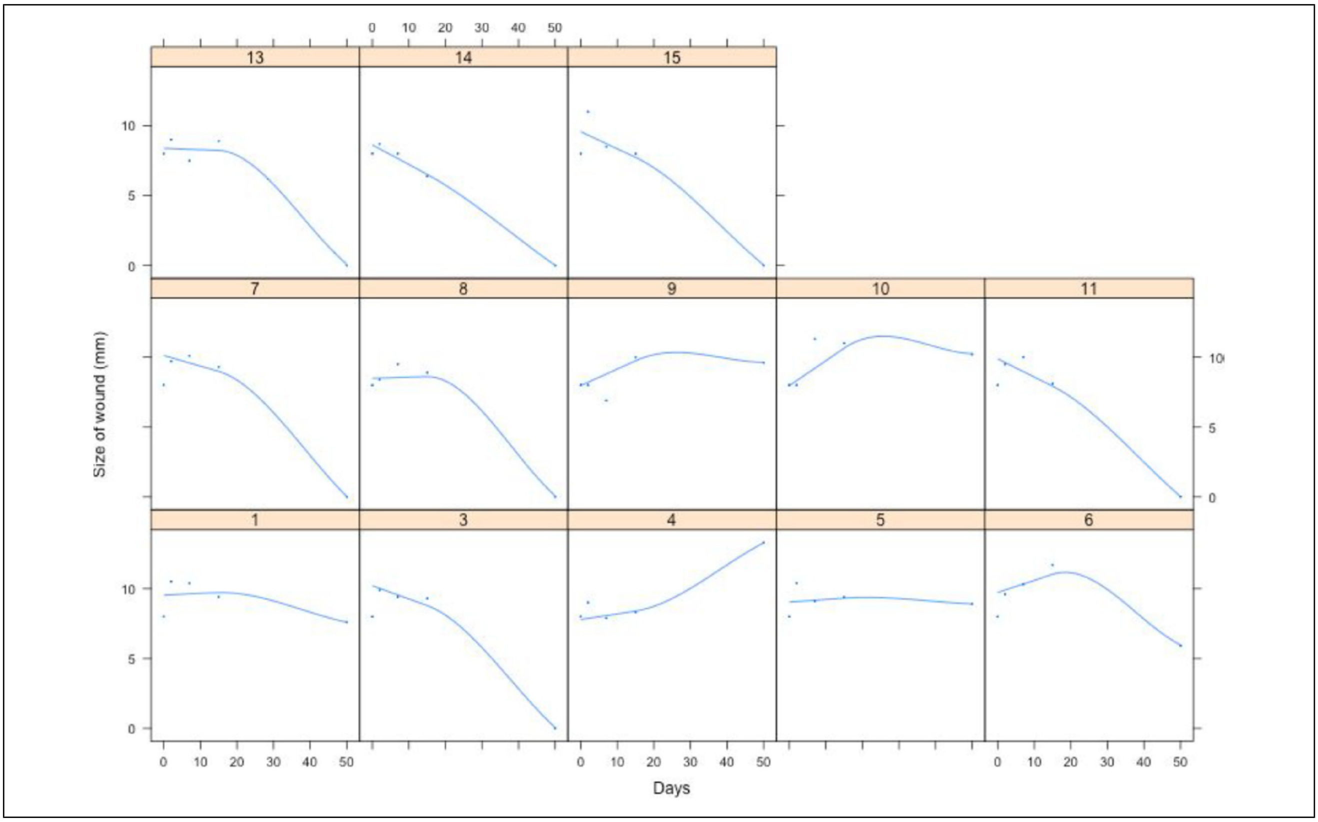

Figure 2. Individual trajectories of wound sizes throughout the follow-up period. Mice 1 to 5 were treated with HiC, 6 to 10 with Honey, and 11 to 15 with aloe vera. Trajectories were fitted using cubic splines. Abbreviation: HiC, hydrocolloid.

was poorer in the control group. Moreover, it can be seen that the glycemia, temperature, and weight parameters fall within physiologically normal limits.[33]

The current study presents evolutionary data that exceeds the typical 7 to 15 day range discussed in the existing literature.[20,25,26]

From a macroscopic perspective, and based on the initial data, we can observe that the honey group's wounds did not show the presence of slough.

This could be related to its antibacterial and granulation tissue-promoting properties, as described by other authors.[3,8,10,15] As for the increase in the surface area of the wound treated with honey (Figure 4), it could be related to the enzymatic and autolytic properties highlighted by various authors,[4,6,11,12,19,34–39] who have observed this feature in mouse wounds, which enhances the formation of granulation tissue.

Aloe vera showed an apparent greater efficacy in the healing process compared to honey or the use of saline with a hydrocolloid dressing, with the difference between honey and aloe vera being significant. Mukai et al[20,38] note that the combination of honey and the HC dressing does not promote better healing than using only the HC dressing. Our study concurs with this, showing no significant differences between the use of honey and an HC dressing. In our case, using HC made the wound evolution slow and irregular, with a reddish and friable wound niche after almost 2 months of treatment. The fact that the HC dressing reduces the immune response during the first days of the healing process may explain this evolution.[39] However, the tissue repair process was stimulated in both the honey and aloe vera experimental groups, although aloe vera did present a better clinical response with the synthesis of quality granulation and epithelial tissue at 50 days, with a pink epidermis and abundant hair follicles. This was consistent with the results of Singh *et al*,[40] whose study achieved complete healing after 20 days using aloe vera, although a subcutaneous ring was not used.

From a preclinical and macroscopic perspective, this preliminary study provides interesting and encouraging data which are in line with other results from animal models[6,16,17,20,41–43] in terms of the efficacy of the honey and aloe vera as effective healing agents, given their ability to generate active granulation tissue and keep the wound bed clean and free of exudate, thus creating an epithelial tissue covered in an abundance of hair follicles.

The literature reviewed[3–5,8,9,14–16,18,19,44] supports using these 2 natural alternative products in treating wounds. The

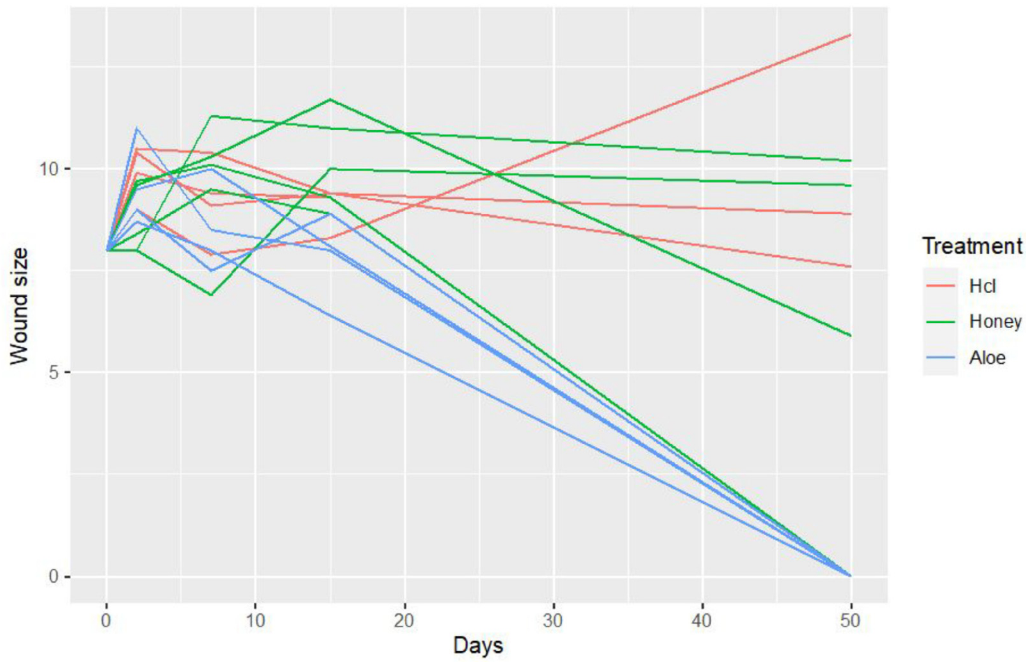

Figure 3. Simultaneous graphics of the trajectories according to the treatment applied. The slope for the honey group showed a significant decrease [(βHoney = −0.07); $p = .002$]. Therefore, it did not show a significant difference compared to β_HC ($p = .381$). The slope for the aloe vera group showed a clear and significant decrease [(βHoney = −0.181); $p < .001$].

Table 1. Estimation of the Mixed Model for the Wound Sizes.

	Coefficient (95% CI)	*p*-Value
Intercept (α)	13.6 (10.0; 17.3)	<.001
Slopes		
HiC (β_{Hcl})	−0.024 (−0.068; 0.020)	.756
Honey (β_{Honey})	−0.070 (−0.108; −0.031)	.002
Aloe vera (β_{Aloe})	−0.181 (−0.223; −0.138)	<.001
Weight (γ)	−0.103 (−0.190; −0.016)	.095
Comparison of slopes		
Honey vs HiC ($\beta_{Honey} - \beta_{Hcl}$)	−0.046 (−0.098; 0.007)	.381
Aloe vs HiC ($\beta_{Aloe} - \beta_{Hcl}$)	−0.157 (−0.213; −0.101)	<.001
Aloe vs Honey ($\beta_{Aloe} - \beta_{Honey}$)	−0.111 (−0.163; −0.059)	<.001

The slope corresponding to Honey did not show significant differences from that of HiC. Aloe vera showed a significant difference between HiC and Honey.
Abbreviation: HiC, hydrocolloid.

present study has been able to confirm this, observing a better clinical result for aloe vera when compared with honey on day 50 of treatment (Figure 1O-R) and also when compared to the conventional treatment with hydrocolloid (Figure 1D-I). Introducing these products into the care of chronic wounds is therefore feasible, either separately or jointly, at distinct phases of the healing process by swapping one product for another. Honey would be primarily used in the inflammatory and proliferative phases as a fibrinolytic and antimicrobial agent to clean the wound bed, creating stable granulation tissue.[4,6,11,20,44] In contrast, aloe vera would be used as a gel in the maturation phase, given that its acemannan would stimulate collagen deposition and enable the remodeling of scar tissue.[5,16,17,44,45] To succeed in managing the exudate generated, in particular, due to the osmotic effect of the honey in the wound bed, an absorbent alginate dressing should be used.[45] The aim of this would be to avoid the maceration of wound edges and maintain a specific level of humidity. However, for greater safety in clinical use, it would be necessary to sterilize both honey and aloe vera. In the case of honey, this would be through gamma irradiation with a range of between 10 and 25 kGy, ensuring the elimination of possible spores such as *Clostridium botulinum* and *Bacillus subtilis* without altering the product's antibacterial and anti-inflammatory properties.[46–48] In the case of aloe vera, sterilization would be through irradiation with intervals oscillating between 10 and 100 kGy, without altering its biological activity and increasing its antioxidant activity.[49] Therefore, in line with the studies previously cited in this article, these 2 products are a valid alternative worthy of consideration.

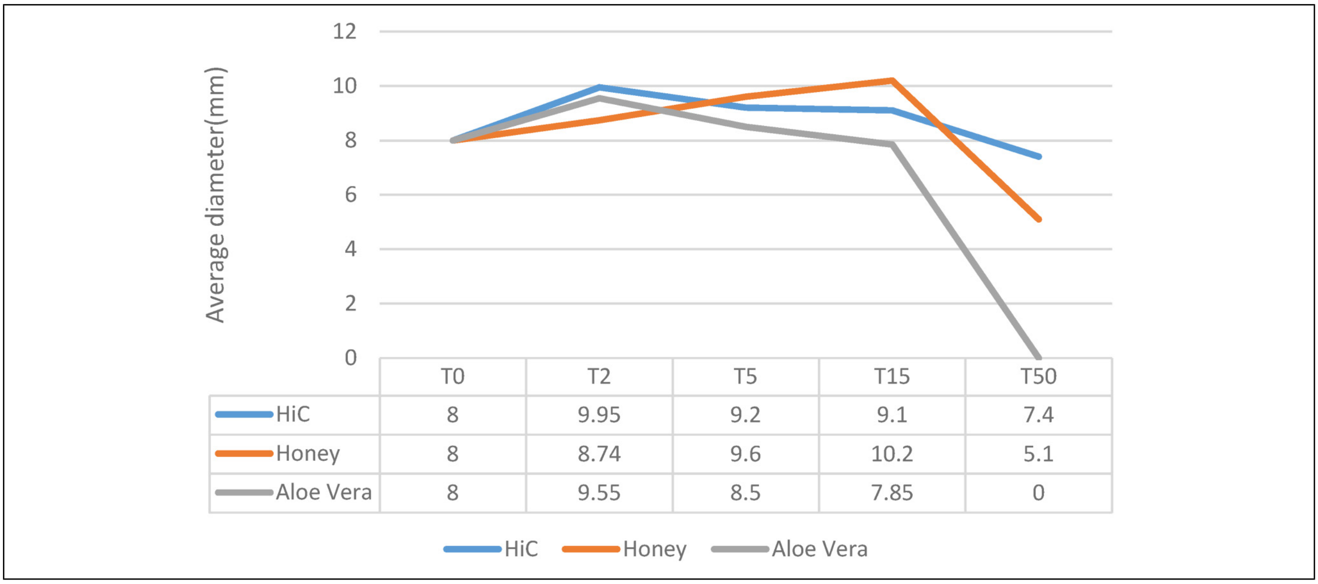

Figure 4. Wound healing evolution. Wound healing evolution (mm) with hydrocolloid (HiC) dressing, honey, and aloe vera. Time of measurement: T0: immediately after surgery; T2: 2 days; T5: 5 days; T15: 15 days; T50: 50 days.

Although the present study was developed with a small sample size following the recommendations of applying the 3Rs in animal experimentation, this possible limitation is compensated for by the number of observations made (260), which allows the statistical power of the analyses to be maintained.

Furthermore, there may be chance of biasness on pictures and in the study of wound healing efficacy observation, because the camera position was not fixed. However, and with the aim of reducing the possible measurement error, all the images were taken by resting the 2 elbows of the same person on the surgical table, on a millimeter paper. On the other hand, images were processed with a specific computer software that used graph paper as a reference to calculate the dimensions of the wound.

Conclusions

In conclusion, aloe vera seems to be more effective than the other 2 treatments in reducing the size of the wound. Weight is a factor that contributes to increasing the speed of wound closure: the greater the weight, the smaller the size of the wound. Both honey and aloe vera are effective alternative products in attaining suitable epithelial tissue.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Disclosures

The experimental procedures were approved by the Animal Ethics and Wellbeing Committee (CEBA) at the University of Las Palmas de Gran Canaria (Ref 004/2013CEBA ULPGC).

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ORCID iD

José Enrique Hernández-Rodríguez https://orcid.org/0000-0002-5437-1902

Supplemental Material

Supplemental material for this article is available online.

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