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Development of Zinc-loaded Hydrogel Infused with *Aloe barbadensis* Mucilage for Wound Healing

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Abstract

This study aims to formulate and characterize zinc-loaded hydrogel infused with *Aloe barbadensis* mucilage for wound dressing. Five formulations containing varying proportions of carbopol, zinc, Aloe and water (as vehicle) were developed via physical crosslinking using triethanolamine. All formulations had a translucent off-white colour while the control gave a transparent gel. The viscosity was the highest in the control, 30000.00 ± 2.07 PaS. The pH of the formulations was between 5.7 and 5.8. formulation 2 which was composed of 30 mg of Zinc and 1.4 mg of *Aloe barbadensis* incorporated into 1% w/v Carbopol Ultrez hydrogel polymer had the lowest swelling index of 79.2 ± 1.95% implying that it had the fastest drug release rate. The wounds treated with formulation 2 had the most rapid healing with no sign of scars in the wound area. Histomorphometric evaluation reflected a high re-epithelisation rate of 70%, a significant percentage occupied by collagen in granulation tissue of 85%. The thickness of the tissue's central region was 10 mm. The inflammatory cells /mm^2 tissue was 200 cells/mm^2 while the number of microvessels in granulation tissue was 1.0 microvessels/mm^2. Zinc-loaded hydrogel infused with *Aloe barbadensis* mucilage shows great potential as a modern wound dressing.

Keywords

Aloe barbadensis, wound healing, hydrogel, zinc.

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INTRODUCTION

A wound is a break in the epithelial lining or mucosa in any anatomical part of the body due to trauma (Dhivya, 2015; Sarp *et al.,* 2021). Wound dressings are fabricated to aid healing by creating an optimal microenvironment by shielding the wound from bacteria and other microorganisms (Ilomuanya *et al.,* 2019). Hydrogels are Hydrogels are biocompatible, biodegradable, and water-soluble. Moreover, the structural network of hydrogels imitates that of the body's extracellular matrix making them ideal for wound dressing (Liu *et al.,* 2022). Hydrogels provide an ideal microenvironment for wound healing by allowing proper penetration of oxygen while shielding the wound from dust, particles, and bacteria. Hydrogels show moderate attachment to the wound bed allowing easy separation from the wound surface while removing the wound dressings making it less painful for the patient (Soleimanpour *et al.,* 2022). One prominent advantage of hydrogels is the fact that they can house bioactive medicaments in their polymer matrices and provide sustained release of the medicaments into the wound bed over a specific period (Avila-Salas *et al.,* 2019). well-established examples of second-generation wound dressings. They are three-dimensional polymer networks that can absorb large amounts of water into their matrices whilst retaining their structure due to chemical and physical crosslinking of polymer chains (Yiyang *et al.,* 2016; Bahram *et al.,* 2016).

Zinc is an important micronutrient that should be present in minute quantities in the human body. It plays a vital role in growth, development, bone formation, immune function and wound healing. Low zinc levels in the body have been associated with poor wound healing. Zinc influences wound healing positively through extracellular matrix regulation and antioxidant defense (Lin *et al.,* 2017). Antioxidants like zinc are important in proper wound therapy because they help to reduce the excessive presence of oxidants at the wound site and clear the accumulation of products of inflammation. Zinc participates in extracellular matrix re-modeling through the regulation of matrix metalloproteinases and interacts with the tripartite motif family of proteins to facilitate membrane repair and angiogenesis (Ilomuanya *et al.,* 2019). Zinc can be incorporated into the hydrogel matrix to allow sustained micronutrient activity at the wound bed facilitating wound healing. It is vital that while facilitating wound healing, the wound bed is also actively protected from microorganisms. Further incorporation of a bioactive material with antibacterial properties into the polymer hydrogel matrices is essential (Arab *et al.,* 2020).

Aloe barbadensis is a tropical plant that is about 1-2 feet in height. It is perennial and can be described as a spiky cactus-like xerophytes clump with a thick fibrous root belonging to the *Asphodelaceae* family. Aloe gel consists of about 98.5% - 99.5% water (Adom *et al.,* 2020). Its solid component contains more than 200 different phyto-constituents with polysaccharides being the most abundant compound. Other phytochemical constituents present include chemical compounds such as soluble sugar, glycoprotein, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, sterols, saponins, and vitamins (Zeng *et al.*, 2020). Aloe is a popular plant with well-established health benefits. It acts as an antioxidant, reduces constipation and increases insulin sensitivity thereby reducing blood sugar levels in type 2 diabetes. Aloe also possesses antibacterial properties and can be applied to wounds to facilitate healthy wound healing. Aloe possesses anthraquinones which exhibit antibacterial properties via direct interference with solute transport through the cellular membrane of a bacterium (Zeng *et al.,* 2020).

This study aims to co-formulate and characterize zinc-loaded hydrogel infused with *A. barbadensis* for wound dressing. Based on the current trend in the field of wound healing there is a need for an ideal dressing that is cost-effective (Okur *et al.*, 2020). This study bridges the gap between conventional dressing and second-generation dressing as hydrogel possesses all the functional properties of a conventional dressing. A step further would be the advancement of the hydrogel polymer by the inclusion of the bioactive materials such as zinc and *A. barbadensis* mucilage into its polymer matrices upgrading it to function as a smart wound dressing. The study particularly takes advantage of the antimicrobial and antioxidant properties of zinc and *A. barbadensis* by fabricating a potent hydrogel formulation that can serve as an excellent wound dressing for trauma wounds when impregnated on a gauze sponge (Rasli *et al.,* 2020).

MATERIALS AND METHODS

Materials

The materials used in this study include *A. barbadensis* mucilage (grown and harvested in Lagos South-west Nigeria), zinc (Hebei Co Ltd, China), phosphate buffer (Nanjing Biotech, China), 1% cremophor (RH 40) (Shandong Ltd, China), triethanolamine (Xingtai Dakun Technology Ltd, China), urethane (Selleck, USA), Carbopol® Ultrez (Qingdao Co Ltd China).

Plant collection and authentication

The leaves of *A. barbadensis* was obtained from Yaba, Lagos state, Nigeria in August 2022. They were identified taxonomically and authenticated at a Herbarium in the Department of Botany, University of Lagos. The leaves were designated voucher number LUH 5977.

Preparation of *A. barbadensis* mucilage

Mature leaves cut from the *Aloe barbadensis* were rinsed with an adequate quantity of water to remove dirty particles. The plant leaves were allowed to dry at room temperature without direct contact with sunlight, to make the gel thicker. The rind was removed gently after it was cut open transversely and the mucilage was carefully scrapped out (Saleem *et al.*, 2022).

Preparation of zinc and *A. barbadensis*-loaded hydrogel

The hydrogel polymer was prepared at 24°C by dissolving Carbopol® Ultrez in distilled water using a mechanical stirrer at 100 rpm. Then it was left to soak for 24 hours. The required quantities of zinc (Table 1) were incorporated into the hydrogel. The stipulated quantity of *Aloe* mucilage was incorporated using the mechanical stirrer (Chang Bioscience CA, U.S) at a constant stirring rate of 100 rpm to achieve proper mixing. The pH was modified by adding the cross-linking agent triethanolamine. Distilled water was used to make up the final volume (Ilomuanya *et al.*, 2019).

Table 1: Composition of alkyl acrylate cross polymer hydrogels and control.

Ingredient	F1	F2	F3	F 4	F5	Control
Carbopol® (g)	1.5	1	0.75	1.5	1	1
Triethanolamine (ml)	0.4	0.4	0.4	0.4	0.4	0.4
Zinc (mg)	40	30	25	10	-	-
***Aloe babadensis* mucilage (mg)**	-	1.4	1.4	1.4	1.4	-
Water (ml) to	100	100	100	100	100	100

Physical examination and pH assessment of the hydrogels

Physical examination of the formulations was carried out after preparation and pH assessment was done using a pH meter (Mettler Toledo, Columbus USA). The hydrogels were optically evaluated for color, consistency and homogeneity. The pH of the formulations was determined by immersing the electrode in the formulation (Ilomuanya *et al.*, 2020).

Rheology test

The viscosity of the formulations was determined at 24°C at 20 rpm using Spindle 7.0 cone and plate viscometer (BYK Instruments Shanghai, China) (Elegbede *et al.,* 2020).

Skin patch test

This study is covered by ethical approval number CMUL/HREC/0974/19. The formulations (0.4 g) were applied to the shaved dorsal surface (1.5 cm^2) of male Wistar rats. The skin appearance was visually examined for redness and swelling 1h post application (Ternullo *et al.,* 2019).

Swelling test

The degree of water absorption by hydrogel formulations was evaluated by incubating 100 mg of hydrogel in 50 ml of phosphate buffer saline (pH 7.4) at 37 °C for 1h. Initial dry weights of the formulations were recorded as "SWa" and equilibrium swelling weight as "SWb". Measurements were carried out in triplicate (Elegbede *et al.,* 2020).

The swelling ratio was expressed as

$$\%\ \text{Swelling ratio} = \frac{(\text{SWb} - \text{SWa})}{\text{SWa}}\ x\ 100$$

(Equation 1)

Formulation stability testing

To assess the stability of the formulations, stability testing was carried out after storage for one month at room temperature and relative humidity (24 °C and 40%, respectively). The appearance, texture properties and bio-adhesiveness of the formulations were determined before (one day after the formulation was prepared) and after storage (on days 1, 3, 7, 14 and 30) (Ilomuanya *et al.*, 2020b).

***In-vivo* wound healing studies**

Eighteen male Wistar rats, each weighing 350–400 g, were purchased at the start of the experiment from Komad Farms® Lagos, Nigeria. The rats were allowed to adapt to their new environment for one week before commencing the experiment. The required feeding, housing and diet conditions were provided. Ethical approval for the study was obtained with approval number CMUL/ACUREC/02/24/1387. Rats were divided into six sets of three rats each. All rats were anaesthetized intraperitoneally with urethane (0.03 mL/kg). The dorsal area was completely shaved and cleaned with 70% ethanol. A 20 mm excision wound was incised on the upper back of each animal with a scalpel. The bioactive dressing containing formulations F1-F5 and control was used to dress the wounds of the rat groups. The pictures of the wound surface were taken, and wound contraction was measured on days 0, 3, 7, and 14 post-treatment. The wound dressing was changed on days 3, 7, and 14 (wound size was measured using a caliper). Data was reported as a percentage of wound contraction against time. The percentage of wound contraction was calculated using equation (2) (Ilomuanya *et al.*, 2020, a)

$$\% \text{ wound closure } = \frac{A_0 - A_1}{A_0} \text{ x } 100$$

(Equation 2)

A_0 = Wound area on day zero A_1 = Wound area on day 3, 7, 14 and 21 after-treatment.

Histological examination

Fourteen days after the operation, tissues from the wound area (containing the dermis and hypodermis) of rats representing each group were sampled and fixed in 10% neutral buffered formalin. Paraffin embedding was carried out after which, 3–4 µm sections were prepared and stained with haematoxylin and eosin (H&E) and Masson's trichome. Light microscopic examination on histological profiles of individual skin was performed using a Leica Microsystems microscope (Mannheim, Germany) (Elegbede *et al.*, 2020).

Histomorphometry

The thicknesses of central regions of granulation tissues (in mm from the epidermis to dermis), numbers of infiltrated inflammatory cells in granulation tissues (cells/mm^2 of field), numbers of micro-vessels in granulation tissues (vessels/mm^2 of field), percentages of re-epithelization rates (%/mm^2 of field), and percentage collagen-occupied regions in granulation tissues, were measured on the histological Wistar rat skin samples using a digital image analyzer (Image Pro, Media Cybernetics, U.S) (Ilomuanya *et al.*, 2020 a).

Statistical analysis

Measurements were carried out in triplicates. The statistically significant difference was determined using a one-way ANOVA test ($p < 0.05$). Bonferroni's multiple comparisons test was carried out, when necessary (Sinjari *et al.*, 2019).

RESULTS

The physicochemical properties of the hydrogel formulations are presented in Table 2.0. All formulations had a pH within the range of 5.6 to 5.8 which is close to the natural pH of the skin 5.5. The viscosity was the highest in the control formulation. This may be due to the absence of Aloe mucilage and zinc in the formulation. The second highest was formulation F4 which did not contain Aloe mucilage as well. The swelling index that indicates the release rates of the formulations is indirectly proportional to the release rate.

Table 2: Physicochemical properties of formulations, F1-F5 and control.

Hydrogel Formulation	Dynamic Viscosity (PaS) (20 rpm)	pH	Swelling Index %	Skin Irritancy
F1	21250 ± 1.9	5.7 ± 1.7	80.7 ± 1.7	Nil
F2	22900 ± 1.1	5.8 ± 1.1	79.2 ± 1.9	Nil
F3	20500 ± 2.8	5.6 ± 1.3	83.4 ± 0.8	Nil
F4	28860 ± 1.2	5.8 ± 1.7	82.3 ± 1.4	Nil
F5	22950 ± 1.0	5.7 ± 1.1	80.4 ± 1.0	Nil
CONTROL	30000 ± 2.1	5.8 ± 1.3	84.5 ± 1.2	Nil

A pictorial representation of the progress in wound healing in rats treated with the different formulations (F1-F5) and control is displayed in Figure 1. Wounds treated with formulations F2-F4 showed 100 % contraction, complete re-epithelization, and tissue remodeling by day fourteen. Healing was also without any scarification. Scars were observed on wounds treated with F1, F5 and control after wound healing on day fourteen.

Figure 1: The contraction and re-epithelialization of wounds in rat groups treated with formulations (F1- F5) and control.

The histological evaluation of the skin tissues in the wound area post-wound healing is displayed in Figure 2A. Figure 2A provides an overview of the anatomical state of the epidermis, dermis and hypodermis after angiogenesis. The black two-way arrows show the thickness of the epidermis, while the blue arrows point the mature pink granulation tissues which is evidence that the wound healing pattern is healthy. The red arrows indicate the micro-vessels in granulation tissue. The percentage of wound closure over fourteen days is displayed in Figure 2B. The graph of percentage wound contraction against time showed that healing occurred over fourteen days for most of the formulation-treated wounds. The wound contraction phase took place after the prior inflammation and proliferative phases by the end of the first week allowing the commencement of the tissue re-modeling phase of wound healing which was within the second week for the treated wounds. Figures 3A-E display the number of microvessels in granulation tissue, the thickness of the central region between the dermis and epidermis, re-epithelization percentage, the percentage occupied by collagen in granulation tissue and the number of inflammatory cells.

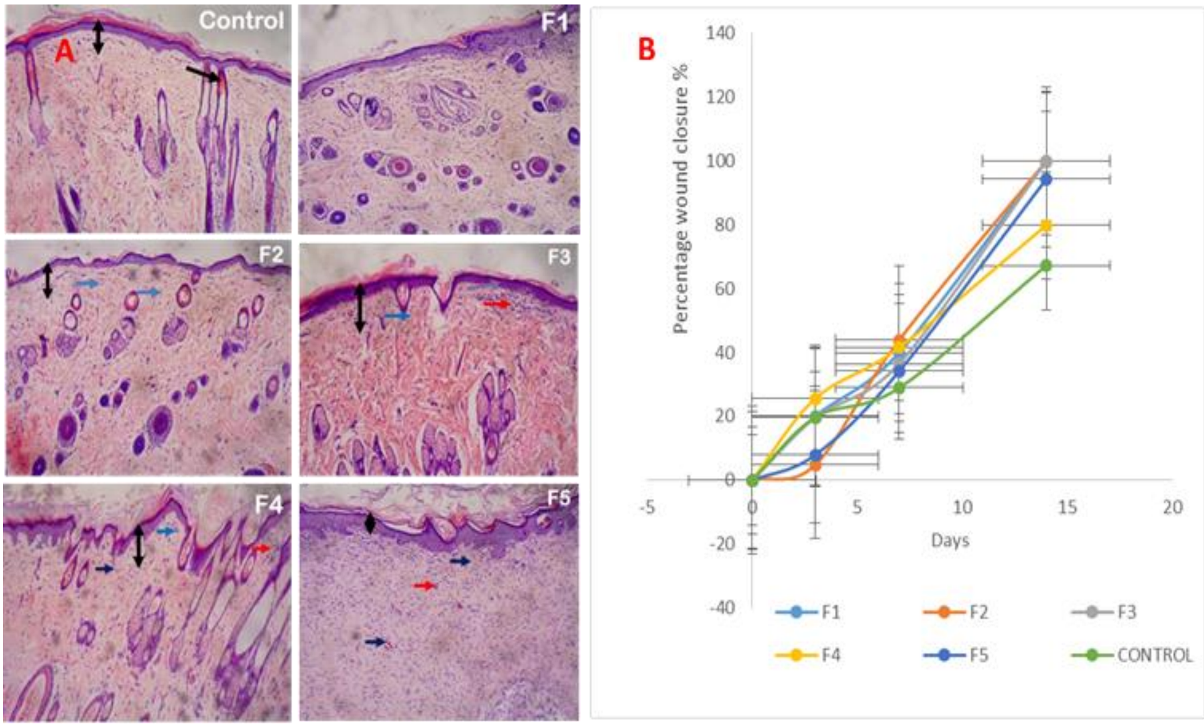

Figure 2A and B: The histological images of the tissue sections from the wound area (A). The percentage relative wound contraction from day 1 to day 14 (post-surgery) (B).

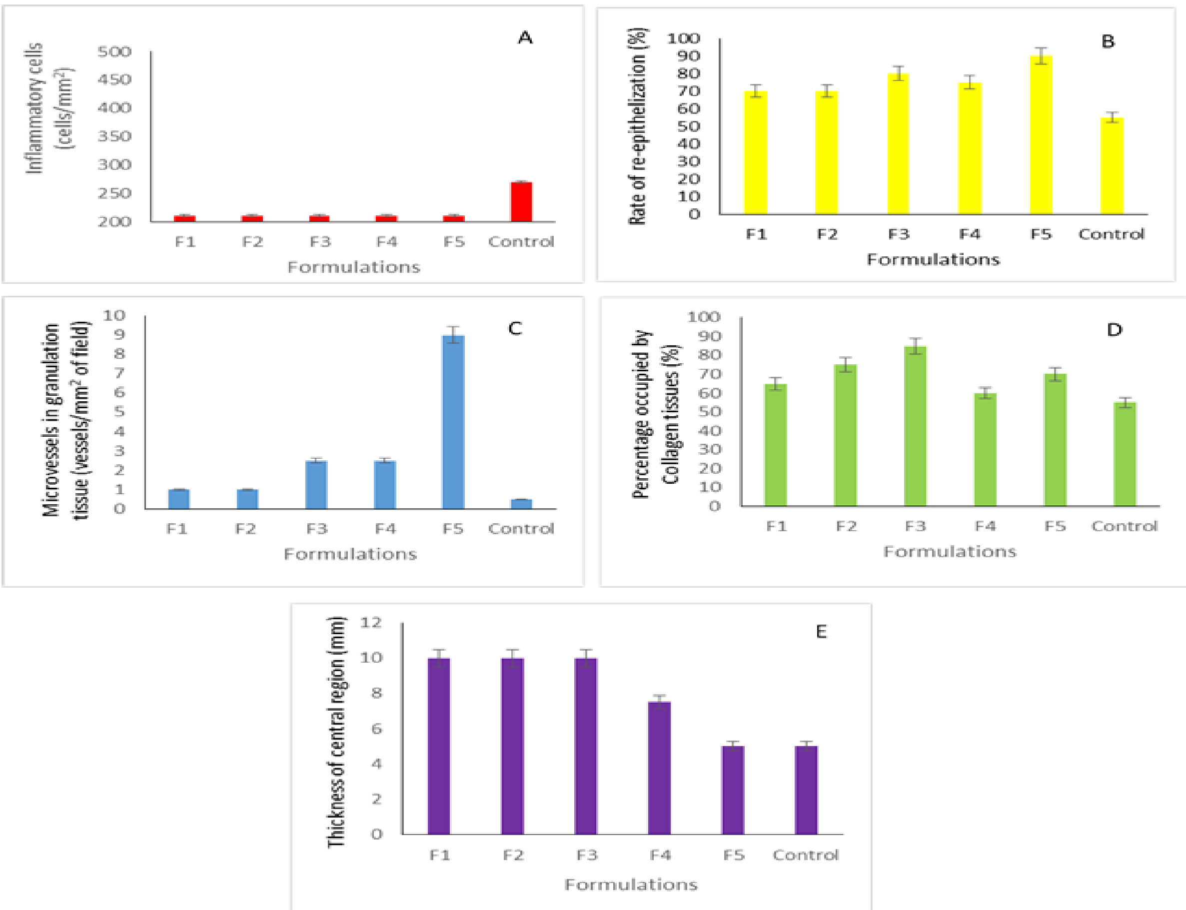

Figure 3 (A - E). Histomorphometrical values for diabetic wound tissues 14 days post wound incision. Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

DISCUSSION

Hydrogels have reflected great potential as wound dressings. They can control the moisture level in the wound environment while serving as a barrier thereby preventing exposure of the wound's microenvironment to microorganisms (Parhi *et al.*, 2017). Hydrogels have acquired some level of prominence because of their similarity to the extracellular matrix. Their ability to house bioactive compounds in their polymer matrix has placed them in an indispensable position. In this present study, hydrogel polymer was loaded with *A. barbadensis* and zinc. The wound-enhancing potential of developed and characterized *A. barbadensis* and zinc-loaded alkyl acrylate cross-polymer hydrogel was investigated (Ilomuanya *et al.*, 2020b).

Formulations F1-F5 and control were physicochemically examined. All formulations gave no characteristic odor. Formulations F1-F4 had a translucent off-white color while F5 and control gave a transparent gel. No erythema or edema was observed on the application of the formulations to the bare skin of the rat model after observation for one hour. Hence, all formulations were safe for dermal application (Niu *et al.*, 2022). The pH of the wound's microenvironment is usually basic (7.2-8.5) and a basic pH encourages the growth of microorganisms. The pH of the formulations as seen in Table 2, was between 5.7-5.8 (slightly acidic) (Rippke *et al.*, 2018). An acidic pH is necessary to cause a regression in the growth of bacteria in the wound environment to allow a healthy healing pattern and proper wound re-epithelisation and tissue remodeling (Chen *et al.*, 2019).

Hydrogels are non-Newtonian and display pseudoplastic behaviour with thixotropy reflecting the fact that the hydrogel remains thick when static but becomes thinner on the introduction of shear stress. The components of a formulation influence its rheological properties. The control had the highest dynamic viscosity of 30000.00 ± 2.07 PaS. This may be because it did not contain zinc or *A. barbadensis* in its formulation because the inclusion of these bioactive materials affects the thickness and texture of the hydrogel formulation. A good swelling property indicates that the hydrogel is an ideal medium for prompt release of the loaded medicaments. The swelling index has an inversely proportional relationship with the release rate (Gupta and Kumar, 2015). The formulation with the highest swelling index was the control, 84.5 ± 1.21 %. This shows that the release rate will be the slowest in the control formulation. Formulation 2 had the lowest swelling index of 79.2 ± 1.95 % which means that it had the fastest release rate. All formulations were stable throughout the storage. There was no physical or chemical decomposition nor changes in the appearance or odor (Tsumura *et al.,* 2016). Angiogenesis starts with homeostasis at the point of injury and proceeds to an inflammatory phase. Subsequently, proliferation of the epithelial and extracellular matrix components occurs. Finally a scar tissue that is marked by an array of a highly organized collagen matrix is formed. Figure 1 portrays a pictorial representation of the wound healing progression in rat models. The wounds treated with formulations F2, F3, F4 had a healthy wound healing trajectory as the wound healed completely by day 14 with no sign of scars in the wound area. This may be due to the presence of both bioactive components, zinc and *A. barbaensis*, in these formulations creating a synergistic wound healing enhancing effect. F1, F5 and control showed a less healthy pattern of angiogenesis that can be due to the absence of either of the main active ingredients, zinc or *A. barbadensis* (Alves *et al.,* 2018).

Histology can be described as the evaluation of tissue structure in relation to its function through microscopic evaluation. The tissues of the healed wound site were observed microscopically 14 days post-surgical incision. The anatomy and structural integrity of the tissues are showen in Figure 2A, as an indication of the depth and quality of wound healing. As in all formulations, the presence of matured granulation tissues, an intact epidermis featuring its five main layers as well as the presence of the dermis and micro-vessels indicate that proper and healthy wound healing had taken place at the wound bed. However, the tissue architecture was less defined in the control sample compared to the wound tissues treated with formulations F1-F5. Figure 2B shows a positive trend for the wound healing progression with the control having the slowest wound contraction (Wynn and Vannella, 2016). The histomorphometric evaluation can be described as a quantitative study of the microscopic array and architecture of a tissue especially by computer-assisted analysis of images formed by a microscope (Zhong *et al.,* 2022). In tissues sampled from wound sites, histomorphometric evaluation indicated the nature of wound healing processes, cues and pathways. Macrophages are the most important inflammatory cells during wound healing. They may function to assist cell proliferation and tissue restoration, but their primary function is to provide a host defense against foreign invasion, remove cellular debris and microorganisms (Rouselle *et al.,* 2019). A high level of inflammatory cells at the latter stages of wound healing may indicate that the wound is infected. All treated formulations showed a lower number of inflammatory cells at the first stages of wound healing compared to the control (Lin *et al.,* 2022). The high number of inflammatory cells in the control at day 14 may be because of bacterial infection (Heras *et al.,* 2022).

Re-epithelisation can be described as the layering of a wound surface by the formation of new epithelium. The molecular and cellular pathways that initiate the stimulation, maintenance and resolution of epithelisation are important for complete wound contraction and healthy wound healing. The re-epithelialisation rate was higher in wounds treated with formulations F1-F5 compared to the control. The thickness of the central region from the epidermis to the dermis that was the least in the control group and the highest in wounds treated with formulations F1, F2, and F3 indicates the level of advancement of angiogenesis (Reilly and Lozano, 2021). Granulation tissue is newly formed connective tissue and micro blood vessels on the surface of the wound bed during the wound repair. The number of micro-vessels in granulation tissue indicates the structural depth of wound healing (Sadoyu *et al.*, 2020). The treated wound had a higher number of micro-vessels when compared to the control. The percentage of collagen tissue was the highest in the wounds treated with formulation F2 and F3 but the lowest in the control (Wallace *et al.,* 2019). Collagen is a vital building block of the cutaneous layer that initiates the recruitment of fibroblast. It is also involved in the degradation of matrix metalloproteinase allowing preservation of the extracellular matrix structure that faciliates wound healing (Mei *et al.,* 2022). It is important to note that the formulations developed in this study are to be applied on a wound surface and not on an intact skin. This means that the dermal layer which is to act as a semi-permeable membrane for absorption of the bioactive molecules is already compromised. One of the limitations of the present study is that, Franz cell may not be a perfect model as the membrane is still intact. Future studies will be conducted towards developing an ideal model for drug release on wound surfaces.

CONCLUSION

Formulation, F2, containing both *A. barbadensis* and zinc gave the best performance regarding the enhancement of wound healing with an ideal pH, good viscosity and an ideal swelling index that brings about rapid release of bioactive agents enhnancing wound-healing. Histomorphometrical evaluation of wound dressing of F2, reflected a re-epithelisation rate of 70 %, percentage occupied by collagen in granulation tissue of 85 %, thickness of the tissue central region from the epidermis to dermis 10 mm. The inflammatory cells /mm^2 tissue was 200 cells/mm^2 while the number of microvessels in granulation tissue was 1.0 microvessel/mm^2. This spotlights and establishes the wound healing enhancing abilities of ‘F2’ zinc-loaded alkyl acrylate cross polymer hydrogel infused with *A. barbadensis* mucilage, providing a useful and affordable formulation for filling the gap of an urgent need for a novel and smart formulation for dressing wounds evidently. The findings of the present study fills an existing knowledge gap in the field of wound healing.

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