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Using Niosomes and Solid Lipid Nanoparticles to Encapsulate Vitamin A: A Green Assessment of Wound Healing and Histopathological Evaluation

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Abstract

Background: This study set out to use ultrasonic technology to create vitamin A (Vit A) niosomes and solid lipid nanoparticles (SLNs), and then assess how well they facilitated wound healing. A process called ultrasonication was used to create the nanoparticles. They were then analyzed using dynamic light scattering (DLS) and transmission electron microscopy (TEM). In addition, the nanoparticles were tested for a number of characteristics, including stability, pH, viscosity, spreadability, in-vitro cytotoxicity, and in-vivo wound healing. The results showed that the Vit A-niosome and SLN were indeed spherical, as predicted by TEM. In the accelerated stability test, which involves a freeze-thaw cycle, both the niosome and SLN vitamin A formulations remained stable. The release of vitamin A from SLN gel and niosome gel was much greater than that of plain vitamin A gel, at almost 70% and 80%, respectively. The animal research found that the Vit A niosomal gel and Vit A SLN gel had the best wound healing closure compared to the other groups 21 days after surgery ($P < 0.05$). Additionally, these two groups were quite close to each other throughout the trial and on day 21. Wounds treated with Vit A niosome gel or Vit A-SLN gel produced more collagen than wounds treated with other groups, according to histological data. Following 21 days, there was a notable decline in MDA malondialdehyde (an end-product of lipid peroxidation) in the Vit A-niosome and Vit A-SLN gel groups, with levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), an endogenous antioxidant, and hydroxyproline showing an increase.Results: The generated vitamin A nano-formulations showed promise as a safe nano-vesicle for vitamin A cutaneous administration, which might lead to new possibilities in wound disease therapy, according to this study's conclusion.

Introduction

The subject of cutaneous wound healing has garnered significant attention in recent years, prompting researchers to investigate the fundamental processes involved and design several types of treatment strategies. The fundamental understanding of wound healing has been clarified, while there are still several particular systems that need clarity.1 The process of wound healing is a vital and dynamic biological phenomenon that consists of four interconnected phases: hemostasis, inflammation, proliferation, and remodeling.2,3 In the prevention of peroxidation of skin lipids. This process facilitates the acceleration of cell renewal and epidermal turnover, resulting in a more youthful appearance characterized by increased freshness and smoothness.7 Previous research on wound healing in animals has shown that the use of topical retinoids resulted in improved healing of full-thickness skin wounds.8 Nevertheless, it is important to note that Vit A is very susceptible to UV light and oxygen, both of which may accelerate its destruction and subsequently result in a reduction in Vit A levels at the site of the wound.9 Hence, it is essential to use a suitable drug delivery order to repair the shape and function of injured cells and tissues, it is essential that the processes and their corresponding bio-physiological aspects take place in the appropriate sequence.4,5 The principal objectives of wound care include quick wound closure, turnover of the epidermis, and the attainment of a scar that is aesthetically pleasing.6 Vit A is a lipid-soluble micronutrient that plays a crucial role in the development and maintenance of optimal skin health. Exfoliation of the outermost layer of the skin aids in mechanism in order to maintain the efficacy of this lipophilic molecule. Strategies aimed at conserving materials to ensure long-term stability and preservation of their inherent properties have garnered significant attention as a means to address these challenges. Numerous encapsulation techniques have been investigated in order to enhance the stability of retinyl acetate against various potential degrading factors.10 The investigation of niosomes and solid lipid nanoparticles (SLN) as drug-delivery vehicles has received significant attention in the

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last several decades.11-14 Niosomes and SLNs are well recognized for their safety profile, cost-effectiveness, and capacity to maintain stability over an extended period.15,16 Niosomes and SLNs have been shown to have the ability to extend the duration of medication release in the context of skin injury, resulting in a potential reduction in the frequency of required dressing changes throughout the day.17-20 Several studies have shown the advantageous effects of niosomes and SLNs in the context of wound healing in animal models.21-23 In their study, Motaal *et al*.22 demonstrated the enhanced characteristics of a niosomal gel containing *Hypericum perforatum* L. in the healing of skin wounds in a rat model. In a study conducted by Farmoudeh *et al*.24, it was shown that the application of methylene blue-loaded noisome vesicles proved to be efficient in wound healing and demonstrated positive outcomes in the treatment of skin injury. Gad *et al*.23 showed that the use of SLN for encapsulating Chamomile oil resulted in a notable enhancement of wound healing acceleration. To the best of our knowledge, there is not any study on a green topical gel of Vit A–niosomes and Vit A-SLNs developed as a wound healing agent via the ultrasonic method for local delivery in the rat. Moreover, the *in-vitro* drug release, stability, rheological, wound closure, and histo-pathological features of the formulation were evaluated.

Methods

Materials

Vit A was acquired from Osve Pharmaceutical Company, located in Tehran, Iran. Tween 80, span 80, ethanol, methanol, cholesterol, and tri-ethanolamine were obtained from Merck (Merck Company, Germany). The beeswax (BW) used in this study was obtained from John's Laboratory Chemicals, located in India. Subsequently, a Human Power 2 system, developed by Human Co. in Korea, was used for the purpose of purifying deionized water. The selection of Carbopol 941 was made from the product offerings of B.F Goodrich (B.F Goodrich Chemical Co., UK).

Preparation of Vit A-niosome and Vit A-SLN

Vit A-niosome was prepared by ultrasonic processing technique.25 The mixture containing Vit A (0.5%), cholesterol (2%), span 80 (1%), and tween 80 (1%) was introduced into a glass vial using magnetic stirring at a temperature of 70 °C. Water is then heated to uniform heating. The two phases were homogenized using a hot plate magnetic stirrer to generate a pre-emulsion. Subsequently, a probe sonicator (Bandelin; UW3100; Germany, with a 20 % amplitude for 2 min) was used to subject the blend to sonication, which was promptly followed by chilling the mixture by immersion in an ice bath. This process was carried out to obtain niosomes enriched with Vit A, as shown in Table 1.

In the preceding investigation, the synthesis of Vit A nanoparticles was conducted using the probe ultra-sonication technique. In summary, a combination of BW (2.5%), span 80 (0.375%), and Vit A (0.5%) was subjected to a heating and stirring process, with the temperature maintained below 70 °C. The surfactant solution, consisting of 3.013% of tween 80 and water up to a total mass of 80 g, was subjected to heating at a temperature range of 75- 80 °C. The surfactant solution, which had been pre-heated, was combined with the mixture of lipids and Vit A that had also been heated. This combination was achieved by using a hotplate magnetic stirrer to create a pre-emulsion. The combination underwent sonication with a 20 % amplitude for 2 minutes using a probe sonicator. Following the completion of the sonication procedure, the mixture was promptly placed in an ice bath and subjected to stirring at a rate of 300 rpm.16

Characterization of nanoparticle

The diameter and poly-dispersity index (PDI) of nanoparticles were determined using DLS using a Zetasizer Nano ZS system (Malvern Instruments Worcestershire:

Table 1. Properties of both formulations.

Formulation	Particle size	PDI*	Zeta potential (mv)	EE%(%)
Vit A-SLN	275.00±29.860	0.321±0.015	-3.16±0.44	61.36±6.700
Vit A-niosome	239.43 ± 13.25	0.516 ± 0.014	-9.70 ± 0.700	82.16 ± 2.40

*Polydispersity index
$Entrapment efficiency

UK). The morphology of the nanoparticle was evaluated using an EM 208S TEM manufactured by Philips in the Netherlands. The quantification of encapsulated Vit A in nanoparticles was conducted using centrifugation (SIGMA 3-30KS chilled centrifuge, Germany). Next, the liquid portion was gathered and analyzed at a wavelength of 325 nm using a high-performance liquid chromatography (HPLC) instrument manufactured by Knauer. The HPLC system was outfitted with a Knauer XDBC18 column, which had a particle size of 5 µm and dimensions of 4.6×250 mm. The mobile phase consisted of methanol (100 %) at a flow rate of 1 ml/min.16

Preparation of Vit A–niosome and Vit A-SLN gel

In order to create the gel base, a dispersion of Carbopol 941 (1.5%) was prepared by mixing it with preserved water and allowing it to stand overnight. Subsequently, the Carbopol dispersion underwent neutralization by the use of triethanolamine. In order to produce a Vit A-niosome gel, a mixture consisting of 12.5 g of Vit A niosome, containing 0.5% Vit A, and 12.5 g of gel base was subjected to homogenization using a propeller homogenizer operating at a speed of 400

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rpm. In order to create Vit A-SLN and Vit A simple gels, we consulted our prior study.16

In vitro release experiments

The *in vitro* release investigation was conducted using immersion cells equipped with an acetate cellulose membrane having a molecular weight cut-off (MWCO) of 12 kDa. The Vit A niosome gel, Vit A-SLN gel and Vit A simple gel were introduced into the immersion cells, then safeguarded by an acetate cellulose membrane. Subsequently, the compartments were sealed with a cover. Subsequently, the cells were introduced into the United States Pharmacopeia (USP) dissolving Apparatus II.25,26 The dissolving jars were filled with 900 mL of a dissolution environment consisting of Phosphate buffer pH 6.8 and ethanol at a ratio of 20:80. A volume of 5 mL of the dissolving medium was extracted at various time intervals (2, 4, 6, 8, and 24 hours) and then filtered using a 0.22 μm syringe filter. The concentration of Vit A in the collected samples was analyzed using a High-Performance Liquid Chromatography (HPLC) system set at a wavelength of 325 nm, as described in the procedure outlined in section 2.3.

Stability studies

The stability of the Vit A-niosome and Vit A-SLN were evaluated at 4 and 25 °C for up to three months according ICH guideline. Physical stability was monitored, and the effects of temperature and time on the size, zeta potential, EE%, and PDI, as well as the changes in the color of the formulation and the creation of precipitate aggregation or lipid ingredients.

Centrifugation and spreadability test

In order to conduct the centrifugation test, a quantity of 10 grams of the formulation was introduced into a tapered test tube. The test was carried out using a Sigma centrifuge model 3-30K from Germany, operating at a speed of 3000 revolutions per minute for a duration of 30 minutes, with the temperature maintained at 25 °C. In order to assess the spreadability of the Vit A-niosome gel and Vit A-SLN gel, a quantity of 0.5 g of each gel formulation was evenly distributed onto a glass plate, forming a circular area with a diameter of 2 cm. A mass of 0.5 kilograms was allowed to remain stationary on the top surface of the glass for a duration of 5 minutes. The diameter of the circle was determined subsequent to the application and distribution of the gel.

$$\% \text{ Spreadability} = (A2/A1) \times 100$$

$A1 = 2$ cm and $A2$ = after spreading

Accelerated stability and physicochemical analysis of vit A-niosome gel and vit A-SLN gel

The Vit A-niosome (30 grams) and Vit A-SLN (30 grams) were subjected to accelerated stability testing. The gel was maintained at ambient temperature (20–25°C) to assess its color, odor, pH, and viscosity.27 To investigate the resistance of Vit A-niosome and Vit A-SLN to freeze-thaw cycles, the samples were subjected to temperatures of 4°C and 40°C over a period of 12 days. The pH and conductivity of the Vit A-niosome and Vit A-SLN formulations were measured using a digital pH meter manufactured by MSTecnopon equipment. Special LTDA. The viscosity of the Vit A-niosome gel and Vit A-SLN gel was assessed by using the S-94 spindle on the Brookfield viscometer (DV-II Pro Viscometer, Middleboro, MA). A series of experiments were conducted to investigate the viscosity of a sample at varying speeds, ranging from 0.5 to 100 rpm, at a temperature of 25 ± 1.0 °C.

In vitro non-specific cytotoxicity

The study design included conducting in vitro tests to examine the cytotoxicity effect of formulations. HFF (human foreskin fibroblast) cells were used as the model for these investigations. Subsequently, the cells were cultured on 96-well plates (Nunclon), with a cell density of 105 cells per well. Various concentrations of the formulation, blank SLNs, and free Vit A (10, 5, 2.5, 1, and 0.5 μM) were added to the wells, along with vehicle control. The cells were then incubated for a duration of 24 hours. After the removal of chemicals, the cells were subjected to PBS washing, and their cell viability was assessed by colorimetry formazan (MTT). The compound 3[4,5-di-methylthiazol-2-yl]-2,5 diphenyl-tetrazolium bromide (MTT) has been widely recognized as a simplified, dependable, and replicable colorimetric method for assessing the decrease in mitochondrial metabolism. This method involves the conversion of the yellow tetrazolium salt into insoluble formazan crystals within the aqueous solution of viable cells. Subsequently, the MTT (0.5 mg/mL) and cells were incubated at a temperature of 37°C for a duration of 4 hours. In the subsequent phase, the supernatant was extracted and the formazan crystals were solubilized in 100 μL of dimethyl sulfoxide (DMSO). In addition, the agitation of the plates was conducted for a duration of 20 minutes, and the optical density was measured using a multi-wall spectrophotometer set to operate at a wavelength of 560 nm. The concentrations were subjected to three repetitions, each accompanied by six control samples consisting of cells in a medium.28

Animals models

The research used male Wistar rats, obtained from the laboratory animal facility of Mazandaran University of Medical Science (MAZUMS), Sari, Iran. The rats weighed between 200 and 250 g. The study was conducted in accordance with ethical guidelines, using the ethical code IR.MAZUMS.REC.1398.1690. The animals were kept in separate enclosures maintained at a consistent temperature of 22 °C and subjected to a light/dark cycle of 12 hours each. During the

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specified time frame, individuals were granted unrestricted access to water and sustenance.

Full-thickness wound model

To induce temporary anesthesia, a solution was prepared by diluting xylazine (20 mg/kg) and ketamine (100 mg/kg) in 100 µL of normal saline, which was then administered through intraperitoneal injection. The dorsal hairs were removed by shaving, and the skin in the designated region was sanitized using a 70% ethanol solution. The experimental procedure included doing full-thickness surgical excisions on the dorsal trunk skin of rats, wherein a square area of 2 × 2 cm was surgically removed. Following the surgical procedure, a silicone ring was applied to the wound site to impede wound contraction (murine model). Subsequently, the rats were subjected to random allocation, resulting in their division into seven groups, each consisting of five individuals. The animals were then randomly divided into several groups.29 The groups were designated as follows: group I served as the negative control, group II received a gel base containing Carbopol 1.5%, group III received a niosomal gel without any drug, group IV received a solid lipid nanoparticle (SLN) gel without any drug, group V received a simple gel containing Vit A, group VI received a niosomal gel containing Vit A, and group VII received a Vit A-SLN gel. A topical application of one gram of gel formulations was administered daily for three weeks (21 days) to each experimental group.

Histology

On the twenty-first day after the surgical procedure, all rats within each experimental group were euthanized in order to conduct a histological analysis. Surgical excision was performed to obtain tissue samples from the incision region, which were promptly immersed in a 10% formalin solution for fixation. Then, the aforementioned materials were incorporated into paraffin blocks, and thin slices measuring 4-7 µm in thickness were then generated. The samples underwent sectioning and subsequent staining using hematoxylin and eosin (H&E) as well as Masson's trichrome (MT) techniques. Pathological alterations were examined with a light microscope, and stained sections were viewed. This research used a scoring system as a semi-quantitative approach to assess and evaluate the rate of wound healing across different groups. Six histological parameters were assessed, which included epidermal regeneration, granulation tissue quantity, infiltration of inflammatory cells, angiogenesis, fibroblast cell proliferation, and collagen deposition.

Lipid peroxidation

In order to measure malondialdehyde (MDA) as a final outcome of lipid peroxidation in tissue homogenate, a commercially available Nalondi lipid peroxidation (MDA) test kit (Navand Salamat Company, Urmia, Iran) was used, in conjunction with the thiobarbituric acid technique.30 In each experimental group, a total of three rats were euthanized on days 7, 14, and 21, respectively. A total of 40 mg of skin biopsy specimens were collected from the periphery of the incision at a distance of 2-3 mm. Subsequently, the tissue specimens were subjected to homogenization using a mixture of butylated hydroxytoluene and lysis buffer. The resulting homogenate was then subjected to a series of washes, including potassium chloride (1.15%) and cold physiological saline. The removal of debris was achieved by subjecting it to centrifugation at a speed of 13,000 revolutions per minute for a duration of 3 minutes. The mixture was subjected to heating at a temperature of 95 °C using a water bath for a duration of 45 minutes after the combination of 800 µL of 0.67% thiobarbituric acid with 200 µL of tissue homogenate. Following this, the mixture was cooled in an ice bath for a period of 10 minutes. The absorbance of the sample was measured using spectrophotometry at a wavelength of 550 nm. The UV absorbance values of the specimens were acquired and afterward compared to the standard curve generated using the MDA concentrations in the tissue. In conclusion, the normalization of lipid peroxidation was performed by considering the protein content determined using the Bradford test, and the quantity of MDA expressed in nanomoles per microgram of protein.

Glutathione peroxidase (GPx) assay

The substrate used for the measurement of GPx activity was tert-butyl-hydroperoxide. In addition, the regulation of NADPH elimination occurred within the wavelength range of 330–350 nm.31 The substrate used for the measurement of glutathione peroxidase (GPx) activity was tert-butyl-hydroperoxide. In addition, the regulation of NADPH elimination occurred within the wavelength range of 330– 350 nm. The measurement of GPx activity was conducted using a Nagpix GPx activity assay kit, manufactured by Navand Salamat Company in Urmia, Iran. A total of three rats were selected from each experimental group and were euthanized on days 7, 14, and 21. Skin biopsy specimens weighing 100 mg were collected at a distance of 2–3 mm from the edge of the lesion. The tissue samples underwent a rinsing process using a solution of potassium chloride (1.15%) and cold physiological saline. Subsequently, the samples were homogenized in assay buffer and subjected to centrifugation at 9,000 rpm for a duration of 15 minutes at a temperature of 4 °C in order to eliminate any debris. The homogenized samples were prepared in accordance with the instructions provided in the kit, and the measurement of absorption was conducted at a wavelength of 340 nm using spectrophotometry. The UV absorption values of

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the specimens were obtained by interpolating from the standard curve, which was used for measuring the tissue. The normalization of enzymatic activity was performed by using the measured protein quantity obtained from the Bradford assay. The calculation of GPx activity per microgram of protein was performed, where one unit of glutathione peroxidase activity is defined as the use of 1 nanomole of NADPH per minute.

Superoxide dismutase (SOD) activity

The assessment of the overall SOD activity of the supernatants was conducted by evaluating their ability to block the pyrogallol autoxidation, as described by Marklund.24 The SOD activity was determined using a Nasdox SOD activity test kit (Navand Salamat Company, Urmia, Iran). The blank and reaction mixes were prepared in accordance with the instructions provided by the manufacturer. The enzymatic activity was assessed at a wavelength of 405 nm relative to the blank and subsequently determined.

Hydroxyproline test

The researchers performed hydroxyproline assays to ascertain the density dependency of collagen turnover.32 The hydroxyproline concentration was determined using a hydroxyproline assay kit manufactured by Kiazist Company, located in Hamedan, Iran. Concisely, a quantity of 40 mg of formulations was subjected to homogenization using deionized water. Next, a 12 M hydrochloric acid (HCl) solution was subjected to incubation for a duration of 4 hours at a temperature of 120 °C. Subsequently, the provided sample was subjected to centrifugation with a force of 12,000 rcf for a duration of 15 minutes. A volume of 20 µL of the supernatant was extracted and afterward subjected to the protocol provided by the kit. The absorbance of the sample was then determined at a wavelength of 560 nm using spectrophotometry. Subsequently, the UV absorbance values of the specimens were obtained using interpolation from the standard curve, enabling measurements inside the tissue.

Data analysis and statistics

As previously stated, the data were presented in the form of the mean ± standard deviation (SD). The identified variables were subjected to statistical analysis using analysis of variance (ANOVA) and the Tukey test for post-hoc analysis.33 The statistical significance of the P-value < 0.05 was cited.

Results

Characterization of niosome and SLN

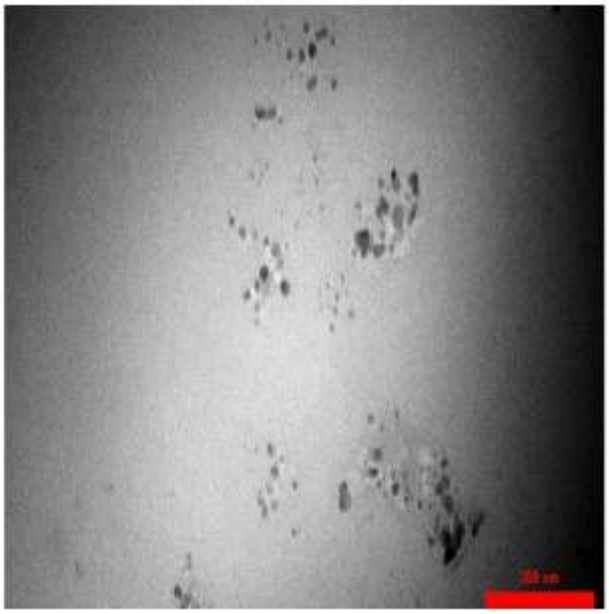
DLS was used to evaluate the average particle size, PDI, and zeta potential of niosome and SLN preparations and results are shown in Table 1. The EE% of Vit A in SLN and niosome were 61.30±6.70% and 82.16±2.40%, respectively, and EE percent of Vit A in niosome was higher than Vit A in SLN significantly ($p<0.05$) . TEM images of SLN and niosome preparation demonstrated practically spherical forms of both preparations with similar diameters and forms (Figure 1).

Stability studies

Stability was conducted based on zeta potential, entrapment efficiency, particle size, and PDI for three months at 4 and 25 °C (Table 2). The results indicated that Vit A-niosome and Vit A-SLN kept at both temperatures were within the same colloidal nanometer range. No pronounced alterations in size, PDI, EE% and zeta potential were found ($P>0.05$) for Vit A-niosome and Vit A-SLN kept at 4 and 25 °C, confirming that 4 and 25 °C could be a suitable storage

Table 2. Stability information of the preparations dispersion followed by storage for 3 months.

Storage condition	Time (month)	Particle size (nm)	PDI	Zeta potential (mV)	EE%	Organoleptic
Initial (Vit A niosome)	-	239.43 ± 13.25	0.516 ± 0.014	-9.70 ± 0.70	82.16 ± 2.40	Stable-milky
4°C	1	239.99 ± 11.15	0.516 ± 0.020	-9.87 ± 0.72	81.87 ± 2.10	Stable-milky
	2	240.12 ± 14.20	0.517 ± 0.018	-9.99 ± 0.79	81.5 ± 2.22	Stable-milky
	3	240.78 ± 14.91	0.517 ± 0.014	-10.00 ± 0.90	81.09 ± 2.44	Stable-milky
25°C	1	240.79 ± 10.17	0.517 ± 0.024	-9.92 ± 1.00	81.02 ± 3.10	Stable-milky
	2	241.00 ± 11.00	0.518 ± 0.021	-10.20 ± 0.99	81.00 ± 3.46	Stable-milky
	3	241.08 ± 14.99	0.518 ± 0.029	-10.21 ± 0.75	80.88 ± 2.88	Stable-milky
Initial (Vit A-SLN)	-	275.00±29.66	0.321±0.015	-3.16±0.44	61.30±6.70	Stable-milky
4°C	1	276.41±24.60	0.322±0.010	-3.82±0.42	61.10±6.21	Stable-milky
	2	276.45±27.96	0.322±0.011	-3.89±0.64	61.02±6.00	Stable-milky
	3	277.10±25.73	0.322±0.017	-4.17±0.40	60.80±5.74	Stable-milky
25°C	1	277.98±22.00	0.323±0.017	-3.99±0.25	61.00 ±5.12	Stable-milky
	2	278.10±21.99	0.324±0.018	-4.19±0.24	60.80±5.79	Stable-milky
	3	279.50±22.56	0.324±0.012	-4.21±0.22	60.23±5.00	Stable-milky



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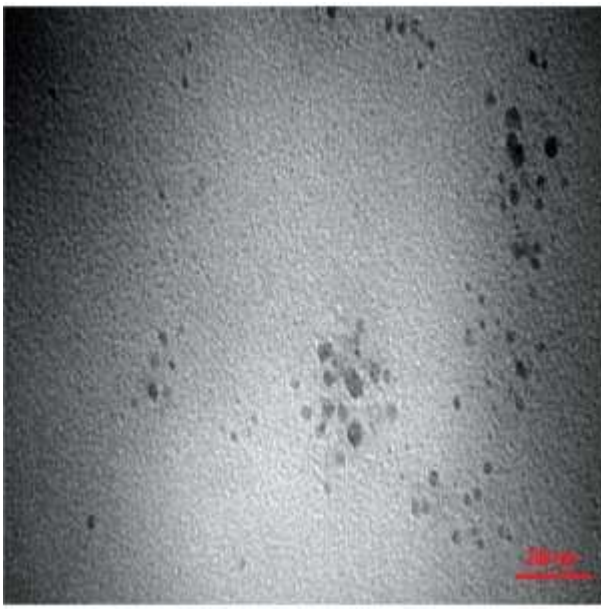

Figure 1. TEM image of Vit A-niosome and Vit A-SLN. temperature for Vit A-niosome and Vit A-SLN.

In vitro drug release

Physicochemical analysis of vit A-niosome gel and vit A-SLN gel

The macroscopic properties and attributes, such as color and aspect, of the Vit A-niosome gel and Vit A-SLN gel formulations were evaluated. The formulations of Vit A-niosome gel and Vit A-SLN gel have a uniform texture with a smooth consistency, displaying a weak yellow tone. Furthermore, these formulations possess a homogenous composition and emit a typical odor. The features of the Vit A-niosome gel and Vit A-SLN gel were consistent for a period of 6 months after their production. Additionally, there was no discernible variation in the appearance of the Vit A-niosome gel and Vit A-SLN gel before and after undergoing a freeze-thaw cycle. The pH values of the Vit A-niosome gel and Vit A-SLN gel were seen to vary from 6.13 ± 0.8 to 6.09 ± 0.54 and 5.99 ± 0.15 to 6.04 ± 0.37, respectively. The pH measurements indicate that the Vit A-niosome gel and Vit A-SLN formulations are unlikely to cause skin irritation since their pH values are within an acceptable range. The pH values of the Vit A-niosome gel and Vit A-SLN were steady throughout the experiment. No statistically significant variation in pH values was observed before and after the freezing-thaw cycle. Therefore, the Vit A-niosome gel and Vit A-SLN gel that was synthesized are appropriate for use in topical applications. Table 3 presents the physical and chemical characteristics of Vit A-niosome gel and Vit A-SLN gel both before and during a freeze and thaw cycle lasting 12 days. The spreadability of Vit A-niosome gel and Vit A-SLN gel was considered high by having a low spread of time. The spreadability percent of the Vit A-niosome gel and Vit A-SLN gel was 306.25% and 272.25% respectively.

In vitro, release tests were conducted to investigate the release behavior of Vit A from SLN and niosome gels in contrast to Vit A simple gel, and their release profiles were displayed in Figure 2. The findings indicated that SLN gel and niosome gel released approximately 20% of the substance within the first two hours of the test, followed by an increasing drug dissolution rate of about 70 and 80% respectively during 24 h. Also, niosome gel of Vit A had higher drug release percentage than Vit A SLN gel during 24 significantly ($p<0.05$).

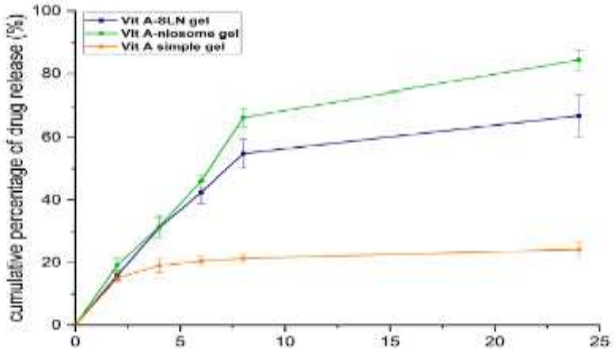

Figure 2. *In vitro* drug release of Vit A simple gel, Vit A niosome gel and Vit A SLN gel.

Table 3. Results of evaluation of the preliminary stability of Vit A-niosome 4 gel and Vit A-SLN gel before and after the freeze-thaw cycle.

sample	Appearance	pH	Centrifugation
Vit A-niosome gel-before	Homogeneous, mild yellow, odorless	6.13 ± 0.8	No noticeable instability in the formulation
Vit A-niosome gel- after	Homogeneous, mild yellow, odorless	6.09 ± 0.54	No noticeable instability in the formulation
Vit A-SLN gel- before	Homogeneous, mild yellow, odorless	5.99 ± 0.15	No noticeable instability in the formulation
Vit A-SLN gel -after	Homogeneous, mild yellow, odorless	6.04 ± 0.37	No noticeable instability in the formulation

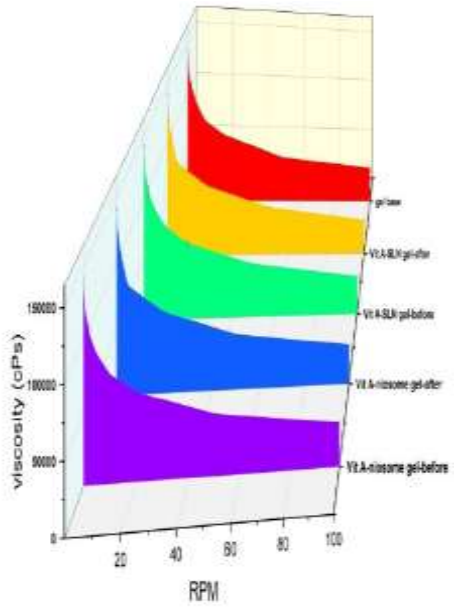

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Figure 3. Effect of shear rate on the viscosity of Vit A-niosome gel, Vit A SLN gel before and after the freeze-thaw cycle, and Carbopol 941 gel formulation.

The behavior of the Vit A-niosome gel was assessed by measuring its viscosity at different rpm, as seen in Figure 3. The gels exhibit a drop-in viscosity as the rate of shear increases, indicating non-Newtonian flow characterized by shear thinning. This decrease in viscosity is also found in the formulation of Vit A-niosome and Vit A-SLN, suggesting the presence of probable pseudoplastic habits.

In vivo wound healing

As shown in Figures 4a and 4b, it can be seen that by the 21st day, wounds treated with Vit A (in the form of niosomal gel or SLN gel) exhibited a notable closure, but wounds treated with Vit A simple gel, niosome placebo gel, SLN placebo gel, gel base, and negative control did not exhibit significant closure. The closure of wound healing was measured at various time intervals. The findings of the study indicated that the wound healing closure seen in the Vit A niosomal gel and Vit A SLN gel groups were significantly greater than that of the other groups throughout the course of 21 days after the surgical procedure ($P < 0.05$). The wound healing process exhibited similar patterns in both the Vit A niosomal gel and Vit A SLN gel throughout the duration of the study ($p>0.05$). On the 21st day, there was no statistically significant difference seen between the two groups ($P>0.05$).

Histopathological assessment

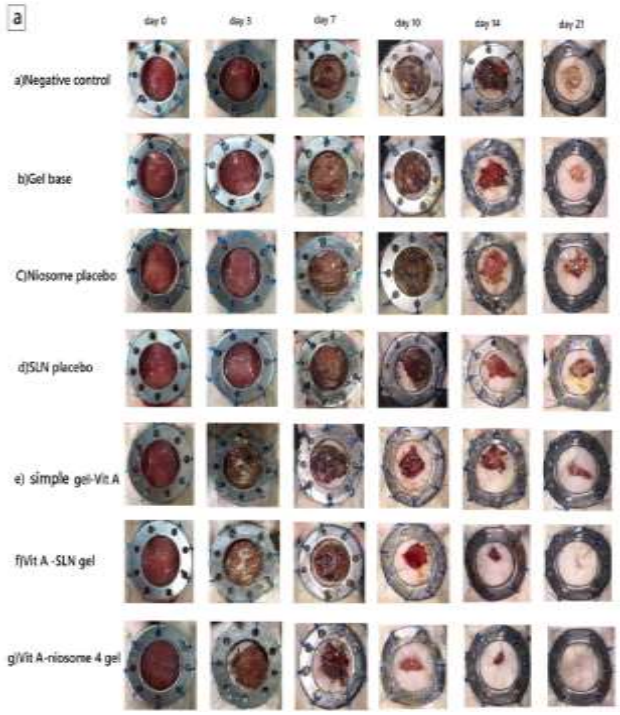
The investigation of cutaneous structure healing included the use of H&E and MT staining techniques on tissue slices. On the 21st day, the treatment groups receiving Vit A niosomal gel and Vit A SLN gel exhibited an increase in the thickness of collagen fibers arranged in organized parallel bundles, a reduction in inflammatory infiltration, and an increase in the presence of fibroblasts, as seen in Figure 5. During the same time frame, the wounds in the control group exhibited the presence of sparse granulation tissue, a significant number of inflammatory cells, and disorganized collagen fibers. The best ratings in the wound healing process were seen in the treatment groups that received Vit A niosomal gel and Vit A SLN gel, as shown by this comparison. The findings of the study indicated that the use of the nanoparticle resulted in a favorable impact on the wound-healing characteristics of Vit A.

MTT assay

To assess the cellular viability potential of Vit A-SLN and Vit A-niosome, various concentrations of free Vit A, blank SLN, blank niosome, Vit A-niosome, and Vit A-loaded SLN ranging from 0.5 to 15 μM were subjected to a 24- hour incubation period with HFF normal fibroblast cell line obtained from Pastor Institute in Tehran, Iran (refer to Figure 6). In solutions with concentrations ranging from 0.5 to 2.5 μM, the inclusion of Vit A, blank solid lipid nanoparticles (SLN), blank niosomes, Vit A-niosomes, and Vit A-SLN did not result in a significant decrease in cell viability during 24 hours ($P > 0.05$). Following exposure to a concentration of 15 μM of Vit A-niosome and Vit A-SLN for 24 hours, a noteworthy decrease in cell viability was seen. Specifically, 92% and 86% of cells remained viable after the aforementioned treatments, respectively ($p>0.05$). It is worth noting that the percentage of surviving cells was statistically equivalent to that of the control group treated just with Vit A ($P < 0.05$).

Lipid peroxidation status of wound tissue

The levels of MDA in the tissue homogenate of cutaneous specimens were evaluated to determine the extent of lipid peroxidation (Figure 7a). The statistical analysis using two-way ANOVA demonstrated that both the formulation and time variables had a substantial impact on the levels of MDA, as shown by the low p-values ($P < 0.001$) for both factors. Furthermore, a significant interaction was seen



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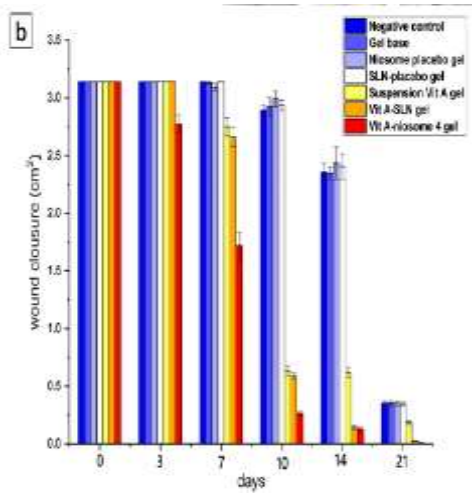

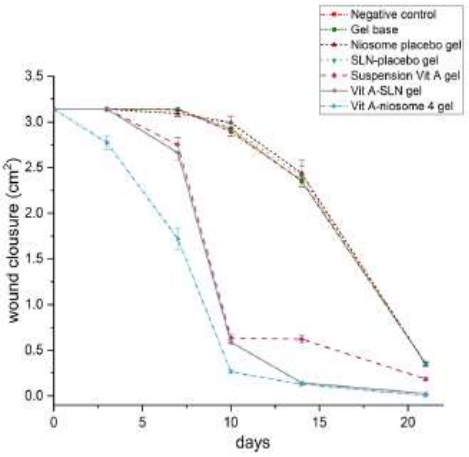

Figure 4. (a) Pictures of wound closure via all treatment groups at several intervals after surgery. (b) Wound closure of several treatment groups at different day intervals.

between the variables of time and formulation ($P < 0.001$). As per the findings, after doing multiple comparisons across various groups, it was observed that the levels of MDA were significantly lower in the Vit A-niosome and Vit A-SLN groups compared to the Vit A-simple, simple gel, niosome placebo gel, and SLN placebo gel groups ($p < 0.001$). Also, in the present study, it was shown that there was no statistically significant difference between the Vit A-niosome gel and Vit A-SLN gel ($p>0.05$).

Glutathione peroxidase activity in wound tissue

Figure 7b illustrates the impact of GPx activity in the skin specimens from different experimental groups. The results indicate that both time ($P < 0.001$) and formulation ($P < 0.001$) had a substantial impact on GPx levels. Moreover, a significant association was observed between formulation and time about the amount of Gpx ($P < 0.001$). Statistical analysis including multiple comparisons across several groups demonstrated significantly elevated levels of GPx in both the Vit A-niosome gel and Vit A-SLN gel as compared to the Vit A simple gel, SLN placebo gel, niosome placebo gel, and gel base ($P < 0.001$). In the present study, it was shown that there was no statistically significant difference between the Vit A-niosome gel and Vit A-SLN gel ($P = 1$).

Superoxide dismutase activity in wound tissue

Figure 7c illustrates the impact of SOD activity in the skin samples of the different experimental groups used in the investigation. The statistical analysis using a two-way ANOVA demonstrated that both the formulation and time variables had a substantial impact on the levels of SOD ($P < 0.001$ for both variables). Additionally, the results of the two-way analysis of variance (ANOVA) indicated a significant interaction between the formulation and time variables ($P < 0.001$). Consequently, after doing multiple

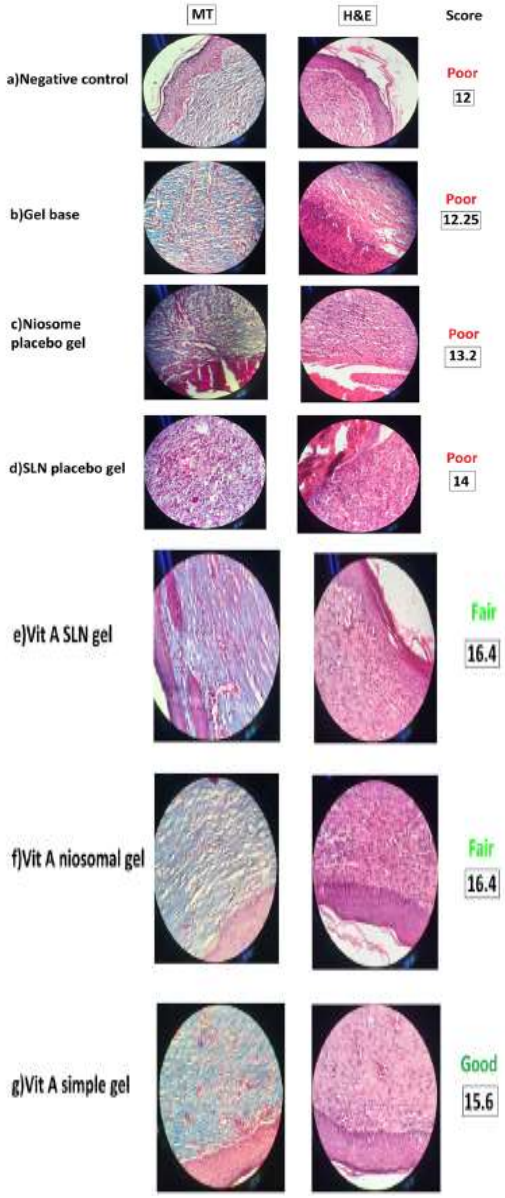

Figure 5. Histological characteristics of hematoxylin and eosin (H&E) and Masson's

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trichrome (MT) of cutaneous tissue specimens from various treatment groups on days 21 after surgery.

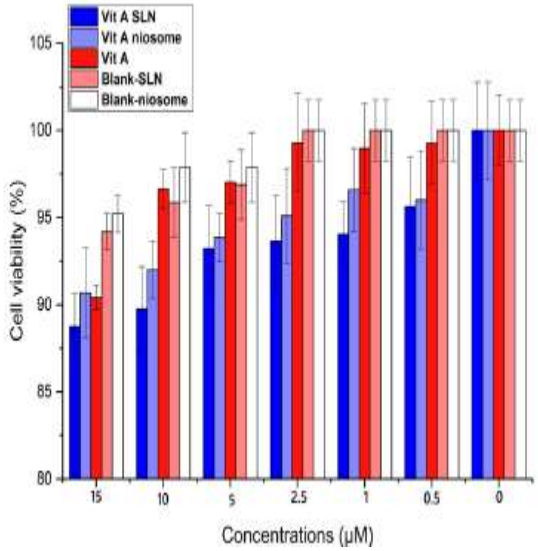

Figure 6. Cell safety of Vit A-SLN, Vit A-niosome, Vit A, blank-SLN, and blank-niosome on HFF cell line.

comparisons across several groups, it was shown that the levels of SOD were significantly greater in both the Vit A-niosome gel and Vit A-SLN gel compared to the Vit A simple gel, SLN placebo gel, niosome placebo gel, and gel base ($P < 0.001$). Similarly, there were no discernible differences seen between the Vit A-niosome gel and Vit A-SLN gel ($P = 1$).

Hydroxyproline production in wound tissue

Figure 7d demonstrates the hydroxyproline level in the skin specimens of the respective groups. The findings from the two-way ANOVA indicate that both the formulation ($P < 0.001$) and time ($P < 0.001$) variables had a significant impact on the amounts of hydroxyproline. Additionally, a significant interaction was seen between time and formulation ($P < 0.001$). Consequently, the analysis of hydroxyproline levels across the several groups revealed significantly elevated levels in both the Vit A-niosome gel and Vit A-SLN gel, in contrast to the Vit A simple gel, SLN placebo gel, niosome placebo gel, and gel base ($P < 0.001$). No significant changes were seen between the Vit A-niosome gel and Vit A-SLN gel ($P = 0.9$).

Discussion

Treatment for cutaneous wounds is an essential issue during human life.34 Innovative drug delivery methods are one of the most important ways to improve the therapeutic effect of wound recovery. SLN and niosome are both interesting and prospective vehicles for wound healing agents.24,35 The purpose of this study was to produce an SLN and niosome utilizing ultrasonication to improve the therapeutic effect of Vit A for wound recovery. According to the findings, the majority of niosome and SLN particles were smaller than 300 nm (Vit A niosome and SLN diameters were 239.43 ± 13.25 nm and 275.00 ± 29.86 nm, respectively). Also, the PDI values of both nanoparticles were under 0.6 (0.516 ± 0.014 for Vit A niosome and 0.321 ± 0.015 for Vit A-SLN), and these values were acceptable. The high lipid concentration of the SLNs and niosomes likely contributed to their medium homogeneity. The energy released throughout the sonication procedure probably impacted the lipids, leading to the aggregation of some particles and hence greater PDI values and diameter.36 The niosome and SLN with zeta potentials below ±10 mV is typically regarded as physically stable.16,37 It is notable that the utilization of non-ionic surfactants resulted in the generation of a negative zeta potential covering the colloidal particles. The dipole character of the ethoxy groups of non-ionic surfactants may be responsible for this phenomenon.38 Vit A Niosome and SLN with a zeta potential of -9.70 ± 0.700 mV and -3.16±0.44 could be desirable as it provides more stability to niosomes and SLN over storage and when it is mixed with water due to electrostatic stabilization of the formulation.37

High encapsulation efficiency of Vit A was observed in all formulations (more than 60%). It is obvious that a change in formulation (SLN versus niosome) causes significant alterations in the EE of Vit A. The niosome demonstrated more Vit A encapsulation than the corresponding SLN formulations. The niosome and SLN formulations showed EE%, 82.16 ± 2.40 % and 61.30 ± 6.70 %, respectively. The encapsulation efficacy of solid lipid nanoparticles (SLNs) and niosomes can vary based on several factors, and there are several reasons why SLNs might have lower encapsulation efficacy compared to niosomes in some cases.39 The encapsulation efficacy of SLNs and niosomes can vary due to a combination of parameters related to the properties of the drug, the lipid or surfactant composition, and the manufacturing process. The choice of lipids for SLN formulation can significantly impact encapsulation efficacy. Lipids with high crystallinity may limit the loading of lipophilic or large molecules. In contrast, niosomes can incorporate a wider range of lipophilic compounds due to their lipid bilayer structure, which provides a more flexible environment for encapsulation.40 Also, in comparing the two studies conducted by Shahraeini *et al*.41 and Abootorabi *et al*.42 on solid lipid nanoparticles and niosomal formulation of atorvastatin, it has been demonstrated that niosomal formulations exhibited a higher EE% than solid lipid nanoparticles.

The size determined by TEM pictures was consistent with DLS findings (Figure 1). TEM pictures revealed that niosomes and SLN have a spherical form. No pronounced alterations in size, PDI, EE% and zeta potential were found ($P>0.05$)

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for Vit A-niosome and Vit A-SLN kept at 4 and 25 °C, confirming that 4 and 25 °C could be a suitable storage temperature for Vit A-niosome and Vit A-SLN.

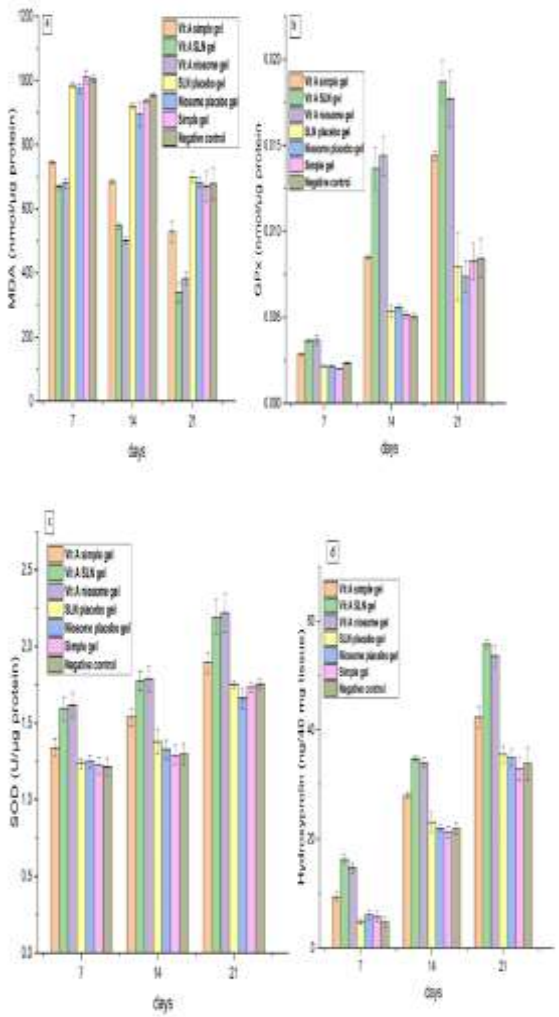

Figure 7. Effect of Vit A simple gel, Vit A-niosome gel, Vit A-SLN, blank niosome gel, blank SLN gel, simple gel, and negative control on (a) MDA (lipid peroxidation product); (b) GPx; (c) SOD; and (d) hydroxyproline content and in excision wound model.

All freshly made formulations exhibited pH values between 5.9 and 6.13, which is deemed safe for topical administration because the pH of the skin ranges from 4 to 6.43 Spreadability% of the Vit A-niosome gel and Vit A-SLN gel was 306.25% and 272.25% respectively. The effectiveness of gels in therapy is contingent upon their ability to disperse. Gel spreading is a crucial process that facilitates the even distribution of gel onto the skin. Consequently, it is essential that the formulated gels possess desirable spreadability characteristics in order to meet the optimal quality standards for topical application. Moreover, this aspect is regarded as a significant determinant of patient adherence to therapeutic regimens.44 The viscosity of topical formulations is a critical factor in determining their effectiveness, particularly when applied to the delicate layers of the skin. Therefore, the gel's consistency plays a significant role in regulating the penetration of drugs. The observed decrease in viscosity in the formulations of Vit A-niosome and Vit A-SLN indicates a potential pseudoplastic behavior. This decrease in viscosity, when subjected to a certain force, confirms the high spreadability characteristic. Additionally, these formulations possess the property of remaining at the application site without draining, as illustrated in Figure 3. The *In vitro* drug release finding showed the nano vehicle caused enhance the rate of dissolution of drug (Figure 2). Previous study demonstrates that niosome and SLN enhanced drug solubility and therapeutic effect.15,45 Based on the release graph the burst release of the active compound (Vit A) from niosome and SLN formulation gels within the initial 8-hour period maybe is attributable to the change in the physical state occurring in both surfactants and lipids at phase transition temperature.46 The sustained release from 8 hours to the end of the test is often related to the metastable polymorph of surfactants and lipids.47 In the case of innovative delivery forms such as nanoparticles that do not have established regulatory or compendial standards, the evaluation of drug release *in vitro* becomes even more important as it serves as an indication of the quality and effectiveness of the product. Numerous methods have been employed for this purpose, each with their own advantages and disadvantages in terms of their ease of setup, sampling, and the speed of buffer replacement. It is desirable for an in vitro release method to mimic the conditions and mechanisms of drug release *in vivo*, and also facilitate the establishment of an *in vitro-in vivo* correlation (IVIVC). In the future, research should prioritize the development of appropriate mathematical models that can predict drug release behavior, as well as the identification of release mechanisms that can be applied to a broad range of nano-sized dosage forms.48 The findings of the study indicate that the wound healing closure seen in both the Vit A niosomal gel and Vit A solid lipid nanoparticle (SLN) gel groups was significantly greater compared to the other groups during the 21-day post-surgery period ($P < 0.05$). Furthermore, the wound healing closure rates in the two aforementioned groups were similar to each other across time and particularly on day 21, as shown in Figure 4a and 4b. The application of retinoids to the skin results in an augmentation of muco-polysaccharide, collagen, and fibronectin synthesis, while concurrently suppressing collagenase production.49 The aforementioned benefits are contingent upon the presence of particular binding receptors for retinoids in the skin, which have been shown to reduce the duration of healing for aesthetic invasive treatments.50 Previous research

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has shown that the use of SLNs (solid lipid nanoparticles) and niosomes has resulted in accelerated wound healing as compared to control groups.24,51

The investigation of cutaneous structure recovery included the use of H&E and MT staining techniques on tissue specimens. After an injury, the process of angiogenesis and the subsequent expansion of blood vessels are regulated by the actions of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).52 Angiogenesis is accelerated during the inflammatory phase. The number of newly formed vessels surpasses the standard amount seen during the proliferation stage. A significant reduction in the quantity of vessels occurred as a consequence of the remodeling phase, which was accompanied by a substantial loss of endothelial cells.53,54 The histopathological analysis demonstrated that the development of new blood vessels in the granulation tissue occurred 21 days post-surgery and had a positive correlation with the presence of inflammatory cells and fibroblasts. After a period of three weeks post-surgery, the wound margins in all three groups exhibited the emergence of new epithelium. However, this occurrence was seen only in the therapy group. Collagen, as the predominant structural protein inside the extracellular matrix, assumes the crucial role of providing a framework for cellular attachment, development, and differentiation.55 Collagen rearrangement occurs during the remodeling period, and the development of cross-links between fibers enhances wound tensile strength.56 The collagen fiber remodeling can be highlighted by MT labeling (the protein is stained blue).57 After 21 days of treatment, tissue specimens colored with MT and Vit A niosome gel and Vit A-SLN gel treated wounds presented more collagen than the other groups. These data suggest that SLN and niosome formulations of Vit A are more effective at wound healing than other treatment groups.

Conclusion

In this study, niosome and SLN formulations of Vit A were prepared and characterized. Our results showed that encapsulation Vit A in the niosome and SLN increased the release of Vit A from niosome gel and SLN gel formulations compared to conventional gel. This impact appears to be very reliant on the physicochemical characteristics of the niosomes and SLNs. Topical use of Vit A-niosome gel and Vit A-SLN gel to full-thickness slashed wounds of Wistar rats considerably improved the speed and extent of wound healing compared to groups treated with plain Vit A gel, gel base, placebo and control groups. Histological examination of the wounds revealed that the Vit A-niosome gel and Vit A-SLN gel treated group had a more rapid rate of epithelialization and collagen production compared to other groups. Also, the use of lipidic nano colloidal carriers with Vit A has shown promising results in improving the wound healing process by modulating the oxidative stress and antioxidant defense systems in the wound tissue. The use of lipid-based drug delivery systems on a nanoscale has displayed potential in addressing challenges associated with wound treatment, particularly in increasing the efficacy of drugs with low solubility and low bioavailability. These systems have been found to enhance medication stability in wound healing therapy, resulting in improved effectiveness and reduced adverse effects compared to conventional formulations. Furthermore, the current investigation may propose a unique approach for efficient wound healing employing the topical application of Vit A SLN gel and niosomal gel.

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