Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Development of curcumin-loaded liposomes in lysine–collagen hydrogel for surgical wound healing

Ibilola Mary Cardoso-Daodu*, Margaret Okonawan Ilomuanya and Chukwuemeka Paul Azubuike

Abstract

Background: A surgical wound is an incision made by a surgeon. Slow surgical wound healing may lead to chronic wounds which may be a potential health problem. The aim of this study is to formulate curcumin-loaded liposomes in lysine–collagen hydrogel for enhancing surgical wound healing. Curcumin-loaded liposomes were prepared using thin-film hydration method. The liposomal formulation was characterized by analysing its size, morphology, encapsulation efficiency, and in vitro release. The hydrogel base was prepared, and then, curcumin-loaded liposomes were infused to give formulation (F1). Curcumin-loaded liposomes were infused into the hydrogel base after which lysine and collagen were incorporated to give (F2), while (F3) comprised lysine and collagen incorporated in hydrogel base. All formulations were characterized by evaluation of the safety, stability, swelling index, pH, rheological properties, and in vivo wound healing assay. Histology and histomorphometry of tissue samples of wound area treated with formulations F1, F2, and F3 and the control, respectively, were examined.

Results: Curcumin-loaded liposomes were 5–10 µm in size, and the values for encapsulation efficiency and flux of the loaded liposomes are 99.934% and 51.229 µg/cm^2/h, respectively. Formulations F1, F2, and F3 had a pH of 5.8. F1 had the highest viscosity, while F2 had the highest swelling index indications for efficient sustained release of drug from the formulation. The in vivo wound healing evaluation showed that curcumin-loaded liposomes in lysine–collagen hydrogel had the highest percentage wound contraction at 79.25% by day three post-surgical operation. Histological evaluation reflected a normal physiological structure of the layers of the epidermis and dermis after surgical wound healing in tissue samples from wound areas treated with formulations F1 and F2. The histomorphometrical values show highest percentage of collagen, lowest inflammatory rates, highest presence of microvessels, and re-epithelization rates at wound site in wounds treated with formulation F2 (curcumin-loaded liposomes in lysine–collagen hydrogel).

Conclusion: Curcumin-loaded liposomes in lysine–collagen hydrogel was found to be the most effective of the three formulations in promoting wound healing. Hence, this formulation can serve as a prototype for further development and has great potential as a smart wound dressing for the treatment of surgical wounds.

Keywords: Surgical wounds, Curcumin, Liposomes, Re-epithelization, Hydrogel

1 Background

Surgical wounds heal by primary intention; however, the longer the process of wound healing, the higher the chances of the wound becoming chronic. Slow surgical wound healing may lead to chronic wounds which is a potential health problem. The overall outcome of an elective or emergency surgery may be affected by slow

*Correspondence: icardoso@unilag.edu.ng

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, PMB 12003, Lagos, Nigeria

surgical wound healing in healthy or immune-compromised patients [1]. Factors that slow down wound healing include, disease conditions, smoking, alcoholism, and nutritional deficiencies. These factors contribute to the hindering of physiological and biochemical processes that are important for the advancement of wound healing. Wound dressing techniques have been researched with the aim of tackling the issue of poor surgical wound healing. The National Institute for Healthcare Excellence (NICE) defined an ideal dressing as one that allows for a conducive environment for wound healing through moisture control, minimizing exposure of the wound, and enhancing absorption of medicament [2]. Modern new generation dressings like films, foams, hydrocolloids, and hydrogels may fit this description, but only to some extent [3].

Hydrogels are three-dimensional networks which are made up of polymeric chains that are hydrophilic in nature, these colloidal systems serve as a barrier to protect wounds from sepsis, and they function to provide an ideal environment for tissue regeneration through scaffolding. Their ability to absorb water is attributed to their polymeric chains and structural matrix which mimic the body's structural tissue component and extracellular matrix, on a molecular level [4]. Hydrogels play a pivotal role in the transition towards smart wound dressings by allowing tissue regenerative strategies through its three-dimensional structures which act as a framework for tissue regeneration [5]. The hydrogel's structure helps to limit exposure of the wound, regulate moisture at the wound bed, and house bioactive materials and medicaments. Hydrogels offer protection of the wound from infections through coverage and absorption of wound exudates [6]. Hydrogels are biocompatible and biodegradable, they are of incredibly low cytotoxicity, and these properties make them a very vital part of the second-generation wound dressings for wound healing. A step further would involve incorporating an active medicament within the hydrogel matrix. The structural framework of the hydrogel which accommodates the bioactive molecules plays a vital role in the process of wound healing [7].

Currently, in the process of wound healing, unremitting accumulation of free radicals has been suggested as one of the primary reasons for continuous stimulation of the inflammatory system, leading to delayed wound healing. Other factors that may slow wound healing include the lack of collagen deposition and lysine deficiency. Proper control of free radical accumulation at the wound site, sufficient deposition of collagen, and adequate systemic levels of lysine could be key to an ideal microenvironment for healthy wound healing. The design and fabrication of curcumin-loaded liposomes infused in lysine–collagen hydrogel as bioactive dressing proposes an ideal environment for proper wound healing. It introduces the concept of the smart wound dressing by embedding bioactive molecules in a system (hydrogel) that already demonstrates core functions of conventional dressings, thereby taking advantage of the synergetic effect gained from developing a formulation containing curcumin, lysine, and collagen [8].

Curcumin is a natural polyphenol found in the rhizome of *Curcuma Longa* (Turmeric). It is also known as diferuloyl methane. It is the main curcumoid present in turmeric and is responsible for its yellow colour. Curcumin has been shown to possess relevant wound healing properties, as it enhances granulation tissue formation, collagen deposition, tissue remodelling, and wound contraction. In a study by Emiroglu et al., the effects of curcumin on the wound healing of rat models were studied and it was observed that in rats treated with curcumin there was reduced inflammation at the later stages of angiogenesis and overall, there was accelerated wound healing [9]. Liposomes are spherical vesicles with bilipid layers, and they can encapsulate both lipophilic and hydrophilic drugs and hence their suitability as an ideal drug delivery system for curcumin. To enhance liposomal stability, liposomes are infused in hydrogel. The major reason for infusion of liposomes into a lysine–collagen hydrogel is to control the release of curcumin and to stabilize the liposomal bilayer structure by the creation of a protective film around surface of the liposomal vesicles. Liposomes infused in lysine–collagen hydrogel potentially serve as an excellent carrier for curcumin as this improves the solubility, sustained release property, and dermal contact time at the surgical wound site [10].

Figure 1 shows the structural mechanisms of wound healing resulting from curcumin, collagen, and lysine. Lysine is an essential cationic amino acid that must be available in sufficient amount in the body; however, it is not synthesized by the body. Lysine influences a composition of cellular and molecular processes aimed at restoring and remodelling the original architecture of damaged tissues. In a study by Amato et al., Hyaluronan/Poly-L-lysine/Berberine nanogel was formulated and its affinity for enhancing wound healing was evaluated using *in vitro* wound healing assessment. It was found that wound contraction rate at the wound site was higher in the developed formulation containing lysine compared to the control formulation [14]. Collagen is a structurally and functionally vital protein in the extracellular matrix. The main function of collagen is to act as a scaffold in the connective tissue. Collagen deposition and remodelling contribute to the increase in tensile strength of the wound during healing. In chronic wounds, impaired collagen synthesis leads to poor tissue scar formation. The

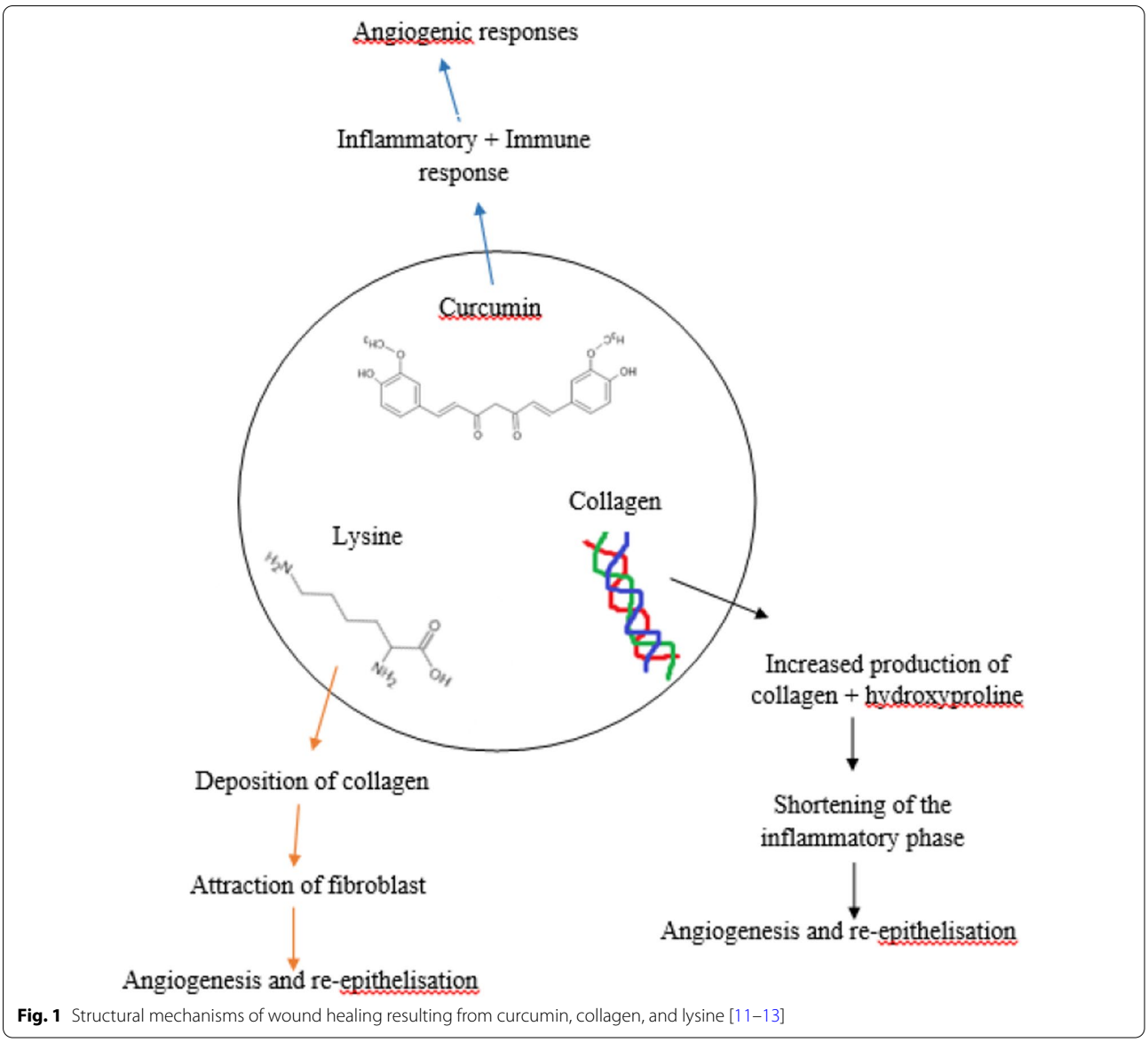

Fig. 1 Structural mechanisms of wound healing resulting from curcumin, collagen, and lysine [11–13]

presence of collagen increases fibroblast production and aids the uptake of fibronectin and the maintenance of the chemical and thermostatic microenvironment of the wound. Collagen plays a pivotal role in wound healing and tissue engineering because of its low antigenicity and inherent biocompatibility with most endogenous tissues [15].

The aim of the study is to fabricate curcumin-loaded liposomes infused in lysine–collagen hydrogel for the management of surgical wounds. This study proposes a bioactive, functional, and smart formulation that offers 100% support to the biochemical, and pathophysiological shortcomings of slow wound healing by ensuring that the journey to regeneration proceeds through every stage within the expected time phase, in the right order. Successful fabrication of this functional bioactive formulation will improve healing, and improvement is associated with enhanced vascularization, regulation, and prevention from wound infection. This study will also bridge the gap existing in the field of wound healing due to a lack in the presence of ideal formulations for enhancing surgical wound healing as well as preventing manifestation of the chronic state. The entire system of this developed formulation is designed with the intent of actively encouraging biological wound healing processes through the sustained release of a combination of medicaments (curcumin, lysine, and collagen) that act on a molecular and cellular level to upgrade biochemical pathways and

cues, thereby promoting prompt and timely wound healing [16].

2 Methods

2.1 Materials

The following materials were used: Curcumin (Sigma-Aldrich Co., St. Louis®, MO, USA), Phosphatidylcholine (Sigma-Aldrich Co., St. Louis®, MO, USA), Methanol (Merck, Darmstadt, Germany), Phosphate buffer (Loba Chemie, Colaba, Mumbai, India), 1% Cremophor (RH 40) (Macklin Biochemical, Shanghai, China), Alloxan (Merck, Germany), Urethane (Sigma-Aldrich Co., St. Louis®, MO, USA), Carbopol Ultrez (Surfachem, U.K), Deionised water, Lysine (Sigma-Aldrich Co., St. Louis®, MO, USA), Collagen (Neocell, USA).

2.2 Preparation of curcumin-loaded liposomes

Liposomes were prepared using the thin lipid film hydration method [17]. Exactly 100 mg of phosphatidylcholine was weighed and transferred into 25 ml of methanol and allowed to dissolve, while 435 mg of curcumin was then weighed and added to the mixture. The mixture was dried using a rotary evaporator to give a thin lipid film which was then hydrated with 25 ml of phosphate buffer (pH 7.4). Afterwards, sonication commenced for 15 min, and then, the liposomal formulation was vortexed for 10 min. Liposomes were stored in the refrigerator at 2–8 °C [18].

2.3 Characterization of curcumin-loaded liposomes

2.3.1 Scanning electron microscopy of curcumin-loaded liposomes

Scanning electron microscopy (SEM) was utilized to characterize the size, morphology, and structure of the liposome [19].

2.3.2 Encapsulation efficiency

The ultracentrifugation method was employed to determine the entrapment efficiency. Exactly 1 ml of liposomal suspension was transferred into a centrifuge tube and sealed with a screw cap. The samples were centrifuged at 30,000 rpm for 10 min at room temperature, and the supernatants were used to ascertain the concentration of non-encapsulated curcumin. The concentration of curcumin was obtained using UV–visible spectrophotometer at a peak wavelength of 425 nm [20].

The efficiency of encapsulation (EE %) was calculated using the equation:

$$\text{Ecapsulation Efficiency} = \frac{\text{Total concentration of curcumin} - \text{Non encapsulated concentration of curcumin}}{\text{Total concentration of curcumin}} \text{x}100 \quad (1)$$

Table 1 Formulations F1–F3 and their varying constituents

Ingredients	F1	F2	F3
2% w/v Carbopol	400 ml	400 ml	400 ml
Curcumin-loaded liposomes	5 ml	5 ml	–
Lysine	–	5 mg	5 mg
Collagen	–	1000 mg	1000 mg

2.3.3 In vitro drug permeability (Flux)

The *in vitro* drug permeability was performed utilizing an independent vertical modified Franz® diffusion cell. The diffusion cell was made up of a donor and receptor compartment. The volume of the receptor compartment was 50 ml, and the effective area for dissolution was 3.147 cm^2. Exactly 1 ml of the curcumin-loaded liposomal formulation was applied. Under conditions of stirring at 100 rpm, 37 ± 1.0 °C, 1 ml aliquots were taken from the sampling port of the apparatus and replaced with same volume of media (pH 7.4 phosphate buffer containing cremophor). This was done at varying time lapse, and the concentration of curcumin was determined at 425 nm via UV–visible spectrophotometry [21].

2.3.4 Preparation of formulations (F1, F2, and F3)

At room temperature, the hydrogel was prepared by weighing 8 g of Carbopol Ultrez and dispersing it in 400 ml of distilled water. It was then stirred at 40 rpm on a magnetic stirrer for 60 s and then allowed to soak for 24 h. The pH was adjusted with the cross-linking agent triethanolamine and was further adjusted to pH 5.8 using NaOH. The final pH of the hydrogel was maintained at pH 5.8. The constituents of the varying formulations are shown in Table 1. Exactly 5 ml of curcumin-loaded liposomal formulation was infused into 400 ml of polymer hydrogel to give formulation F1. To prepare formulation F2, 5 ml of curcumin-loaded liposomal formulation was infused into 400 ml of polymer hydrogel and then 5 mg of lysine and 1000 mg of collagen were incorporated into the formulation. Finally, formulation F3 was prepared by incorporating 5 mg of lysine and 1000 mg of collagen into 400 ml of polymer hydrogel. The formulations were stored in the refrigerator at 2–8 °C [22].

2.4 Physicochemical characterization of formulations F1–F3

2.4.1 Physical assessment and pH determination

Physical assessment and pH determination of the formulations were ascertained. The formulations were visually assessed for colour, homogeneity, and consistency. The pH of the formulations F1–F3 was measured using a pH meter. The electrode was in contact with the formulation for 45 s to permit equilibration. Measurements were performed in triplicate [22].

2.4.2 Rheology test

The viscosity of the formulations F1–F3 was determined at 25 °C at 20–100 rpm using a Spindle 2.0, cone and plate viscometer (DV-E Digital viscometer, Brookfield Engineering Laboratories, Middleboro, USA). Measurements were performed in triplicate [23].

2.4.3 Skin irritancy test

Exactly 0.5 g of each of the formulations (F1–F3) was applied to the shaved dorsal surface (2.5 cm^2) of male Wistar rats ($n=3$/treatment). The skin was visually checked for erythema or oedema 1 h after application [24].

2.4.4 Swelling test

The level of water absorption by the formulations was assessed by incubating 100-mg-dry thick membrane samples in 50 ml of phosphate-buffered saline pH 7.4 at 37 °C. Initial dry weights of the formulations were represented as Wa and equilibrium swelling weight as Wb. All measurements were carried out in triplicate [23].

The swelling ratio was expressed as

$$\%\ \textbf{Swelling ratio} = \frac{(\mathbf{Wb} - \mathbf{Wa})}{\mathbf{Wa}} \text{x } 100 \tag{2}$$

2.4.5 Formulation stability testing

To assess the stability of the formulations F1–F3, a stability test was performed after storage of the formulations for one month in a refrigerator at 2–8 °C. The texture and bio-adhesiveness of the formulations were determined after storage on (day 1, days 5, 10, 15, and 30) [8].

2.4.6 In vivo wound healing studies

Fifteen male Wistar rats, weighing 350–400 g each, were purchased at the commencement of the study from Komad Farms®. The rats were allowed to be acclimatized in their new environment for seven days before the experiment started. Standard housing, diet, and feeding conditions were made available. The mice were accommodated, in an individual polypropylene cage (one animal per cage). The animals were kept under regulated temperature (25 ± 2 °C), relative humidity (45 ± 10%), and a twelve-hour light and dark cycle with the lights out at 7 pm. Animals were fed the standard Harlan Teklad Diet (2016) with clean water for drinking ad libitum. Ethical approval for the study was covered by approval number CMUL/ACUREC/08/21/923. Twelve male Wistar rats were randomly selected and anaesthetized by intraperitoneal injection of 0.03 mL urethane/kg body weight of each rat. The anaesthetized rats were positioned flat with head down on a surgical table, their dorsal skin was disinfected with Dettol® antiseptic (10%), and the hairs of an area to be surgically operated were completely shaved with a razor. A 20 mm incision by means of a No. 24 scalpel was made with the depth of incision including both epidermis, dermis, and hypodermis. Four stitches at 1 cm intervals were made using 3.0 Nylon suture, the wound area was disinfected again, and the animals were kept at room temperature (25 ± 2 °C) until consciousness. The pictures of the wound were taken, and wound contraction was measured on days 0, 3, 7, and 14 to monitor wound healing progression post-treatments. The percentage wound closure was determined using the formula

$$\%\text{wound closure} = \frac{A_0 - A_1}{A_0} \text{ x } 100 \tag{3}$$

where A_0 = Wound area on day 0, and A_1 = Wound area on days 3, 7, and 14 after treatment [19].

2.4.7 Histological examination

Fourteen days after surgical operation, the area of skin around the surgical incision site of representative rats containing the dermis and hypodermis was sampled and fixed in 10% neutral buffered formalin. After paraffin embedding, 3–4 μm sections were prepared and stained with haematoxylin and eosin (H&E) and Masson's trichrome. Light microscopic examination on histological profiles of skin cross-section was performed utilizing a Leica Microsystems microscope (Mannheim, Germany) [23].

2.4.8 Histomorphometry

The numbers of microvessels in granulation tissues (vessels/mm^2 of field), numbers of infiltrated inflammatory cells in granulation tissues (cells/mm^2 of field), percentages of collagen-occupied regions in granulation tissues (%/mm^2 of field), thicknesses of central regions of granulation tissues (mm from epidermis to dermis), and percentage re-epithelization rates were measured on the histological rat skin samples using a digital image analyser (DMI-300, DMI, South Korea) [25].

2.4.9 Statistical analysis

To determine the level of significance ($p < 0.05$), statistical analyses were performed using a one-way ANOVA test followed by Bonferroni's multiple comparisons test (if applicable) performed on GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA) [26].

3 Results

3.1 Liposome size and morphology using scanning electron microscope

Considering the results of the scanning electron microscopy in Fig. 2, liposomes prepared via thin-film hydration method were observed under a scanning electron microscope to characterize the morphology and shape. The liposomes were spherical with a uniform size distribution.

3.2 Encapsulation efficiency

The percentage encapsulation efficiency of the curcumin-loaded liposome was 99.934%, indicating that the liposomes encapsulated the compound curcumin efficiently, there was also minimal leakage and curcumin-loaded liposomes were stable.

3.3 In vitro release studies of curcumin-loaded liposomes (flux) and the release kinetics

In order to obtain quantitative and qualitative information of the release properties of liposomal formulations, the flux was determined using a modified Franz diffusion cell and plain buffer saline (pH 7.4). Figure 3A shows a gradual increase in the permeation of curcumin with a peak after thirty minutes. The determined slope of the plot gives the flux in μg/cm^2/h. Figure 3B gives a plot of the Higuchi release model. The Higuchi release model can help to better understand the underlying drug release mechanisms. The graph of percentage drug release against square root of time had a positive trend for the curcumin-loaded liposome formulation.

3.4 Physicochemical properties of formulations F1–F3

The viscosity of the various hydrogels F1–F3 at 20 rotations per minute using a Spindle 2.0, cone and

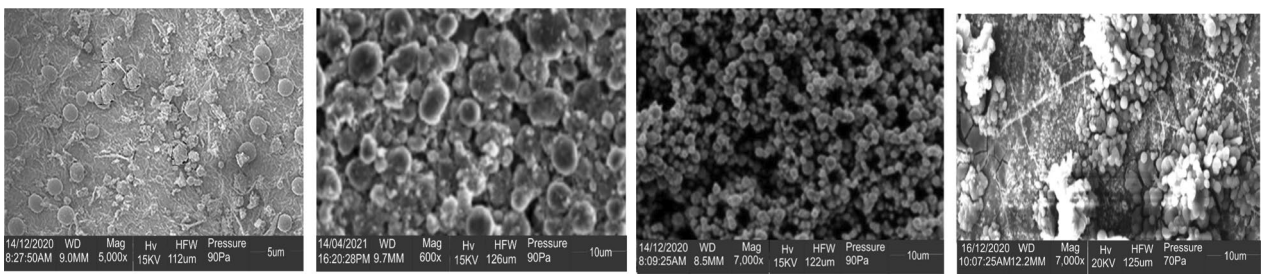

Fig. 2 Scanning electron microscopy images of stable curcumin-loaded liposomes, size range from 5 to 10 μm

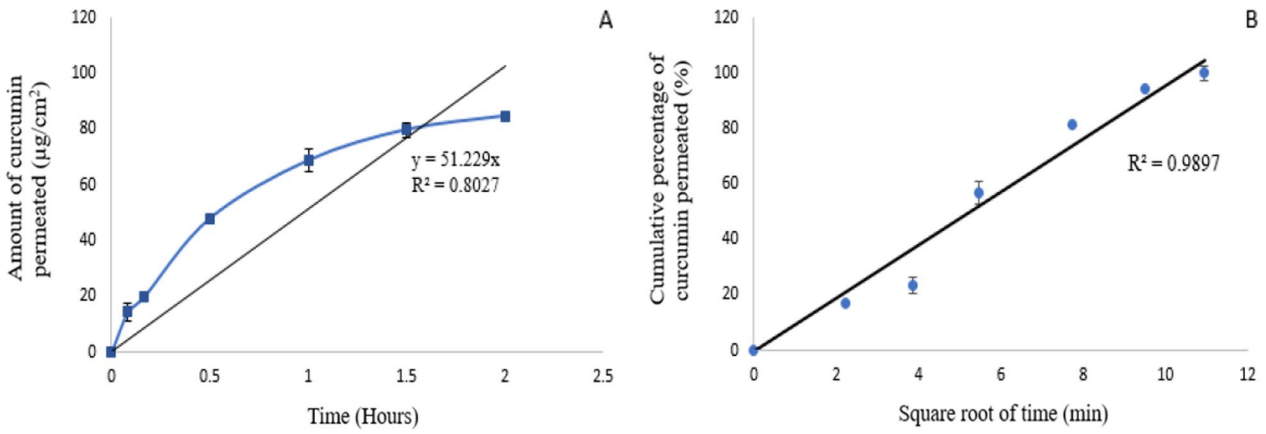

Fig. 3 Graph of amount of curcumin released against time in hours (**A**), graph of Higuchi release kinetics with percentage cumulative of drug released against square root of time (**B**) (All data sets ($p < 0.05$))

Table 2 Physicochemical properties of formulations F1–F3

Hydrogel formulation	Dynamic viscosity (Pas) (20 rpm)	pH	Swelling index %	Skin irritancy
F1	526 ± 2.97**	5.8 ± 1.88	81.7 ± 1.21**	Nil
F2	312 ± 2.01**	5.8 ± 2.11	82.2 ± 1.76**	Nil
F3	426 ± 1.07**	5.8 ± 2.40	84.4 ± 0.82**	Nil

Results are expressed as mean ± S.D (n = 3)

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

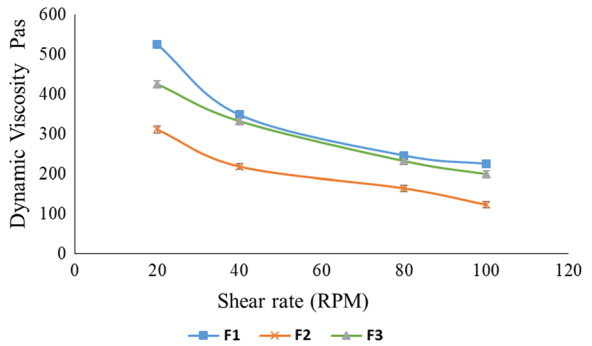

Fig. 4 Graph of dynamic viscosity plotted against shear rate of formulations F1–F3. (All data sets ($p < 0.05$))

plate viscometer was determined. Formulation F1 had the highest dynamic viscosity (Table 2). On the physical assessment of all formulations, F1 and F2 were translucent and pale orange in colour, while F3 as whitish and translucent. The pale orange colour in F1 and F2 was due to the presence of curcumin, the main natural polyphenol which has a yellowish-orange colour**.** The pH of the formulations was slightly acidic and close to the pH of the skin which is within 5.5–5.7. The swelling index was highest in F3, and then F2, but lowest in F1.

The swelling action of the hydrogel formulations is controlled by the rate of water uptake into its matrices. An inverse relationship exists between drug release rate and matrix swelling rate. The lower the swelling index, the faster the drug release rate. The skin irritancy test showed that the formulations are safe for the dermal use, and there was no give sign of erythema and oedema on the dermal application in all formulations (Table 2).

3.5 Formulation stability testing

Formulations were stored in the refrigerator at 2–8 °C, and appearance, texture, and bio-adhesiveness were evaluated 24 h after preparation and on days 1, 5, 10, 15, and 30. The formulations prepared were stable, there was no colour change, and they maintained their original texture and bio-adhesiveness.

3.6 Rheological evaluation

The curves in Fig. 4 depict the rheological properties of the formulations in terms of their flow tendencies. Figure 4 shows a plot of viscosity against shear rate, the graph reflects that an increase in shear rate leads to a decrease in viscosity, and this implies that the formulation is easily spreadable on dermal application. The curve shows that the flow behaviour is non-Newtonian with evidence of shear thinning.

3.7 In vivo *wound healing, histological and histomorphometrical evaluation*

Wound healing is composed of four levels known as the haemostasis, inflammatory, proliferative, and remodelling phases. For healthy wound healing to occur, each wound will go through these group of complex-overlapping phases. The early stage of a wound typically involves constriction of blood vessels which occurs to reduce bleeding. The sole target becomes sealing of the damaged blood vessels and coagulation, and this is facilitated by the inflow and accumulation of platelets at the wound bed. In Fig. 5A, relative wound contraction was highest in formulation F2 with a percentage wound reduction of 79.25% by day three. There were no scarring and 100% wound closure by day seven post-surgery. The reduction in wound size was significant ($p < 0.05$) when compared with the control (non-treated). Control showed complete closure by day 14 post-surgery with obvious scarification, while formulation F3 had the lowest percentage wound reduction of 23.70% by day seven but attained full wound closure by day 14 with minor scarification.

The graph of percentage relative wound size reduction over a period of two weeks as shown in Fig. 5B shows that most of the wound contraction took place after the initial inflammation and proliferative phases by days 5–7, allowing commencement of the remodelling phase of wound healing. All the rats used for the investigation survived through the 14-day period (post-surgical operation) till euthanization. Figure 5C shows representative images of the haematoxylin and eosin staining, and all formulations showed the normal architecture of the skin with the five layers of the epidermis intact except tissues treated with F3 which was abnormal. It showed a severe distortion in the five layers of the epidermis. There was no sign

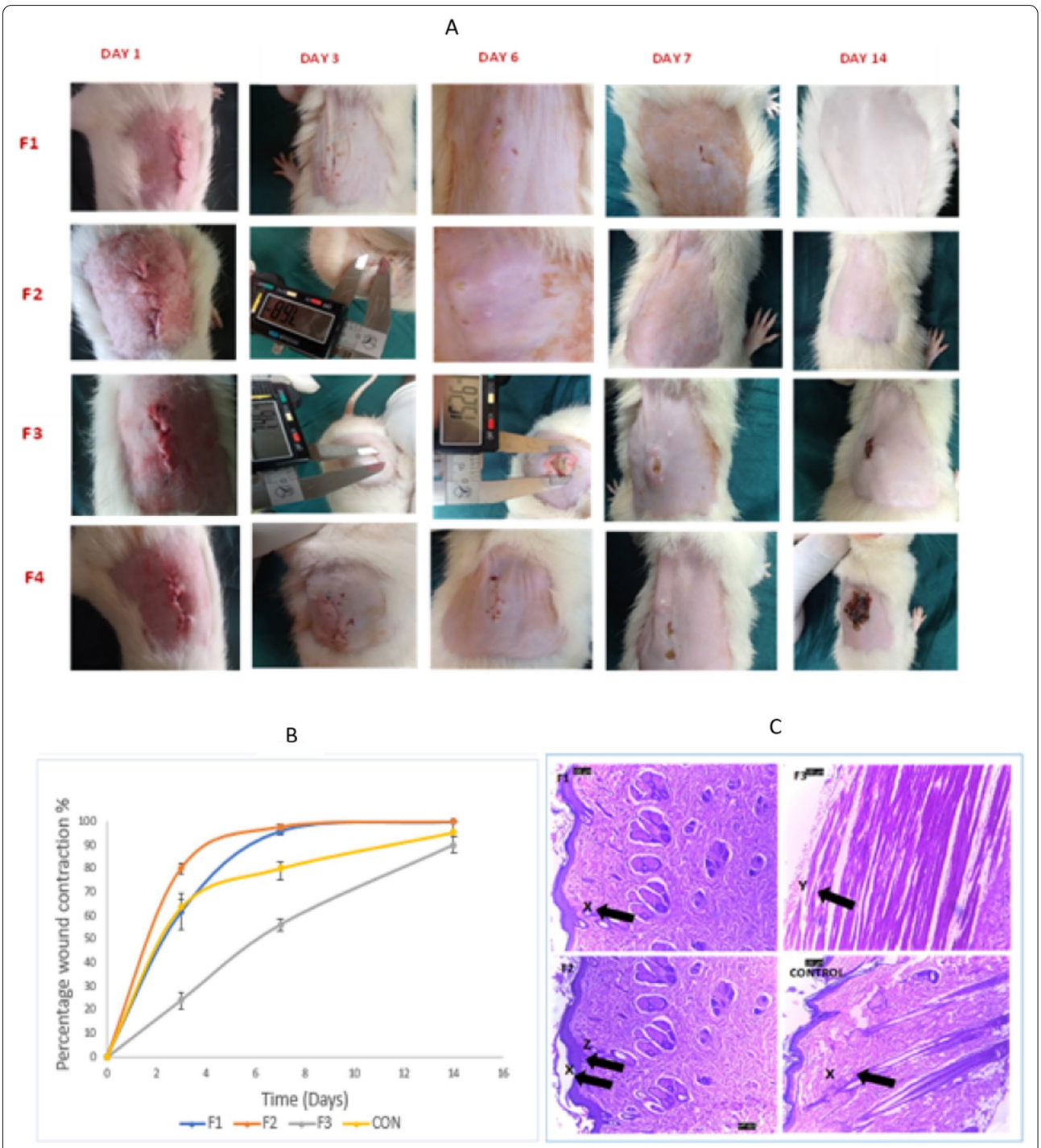

Fig. 5 The effect of formulations F1–F3 on the rate of wound healing in the surgical wound model (**A**). Percentage relative wound size reduction from days 1 to day 14 (post-surgery) for surgical wounds (**B**). Skin tissue section of wound area from a representative rat in (F1–F3, and control) stained with haematoxylin and eosin 14 days post wound incision (**C**)

of tissue necrosis observed on the surface of the wounds. There was visible inflammation taking place in rats treated with formulation F3 and rats in the control group showing a dense wound area with scabbing as shown in Fig. 5A.

Figure 6A–E shows that the number of the microvessels in granulation tissue, the thickness of the central region between the dermis and epidermis, percentage re-epithelization rates, and the percentage occupied

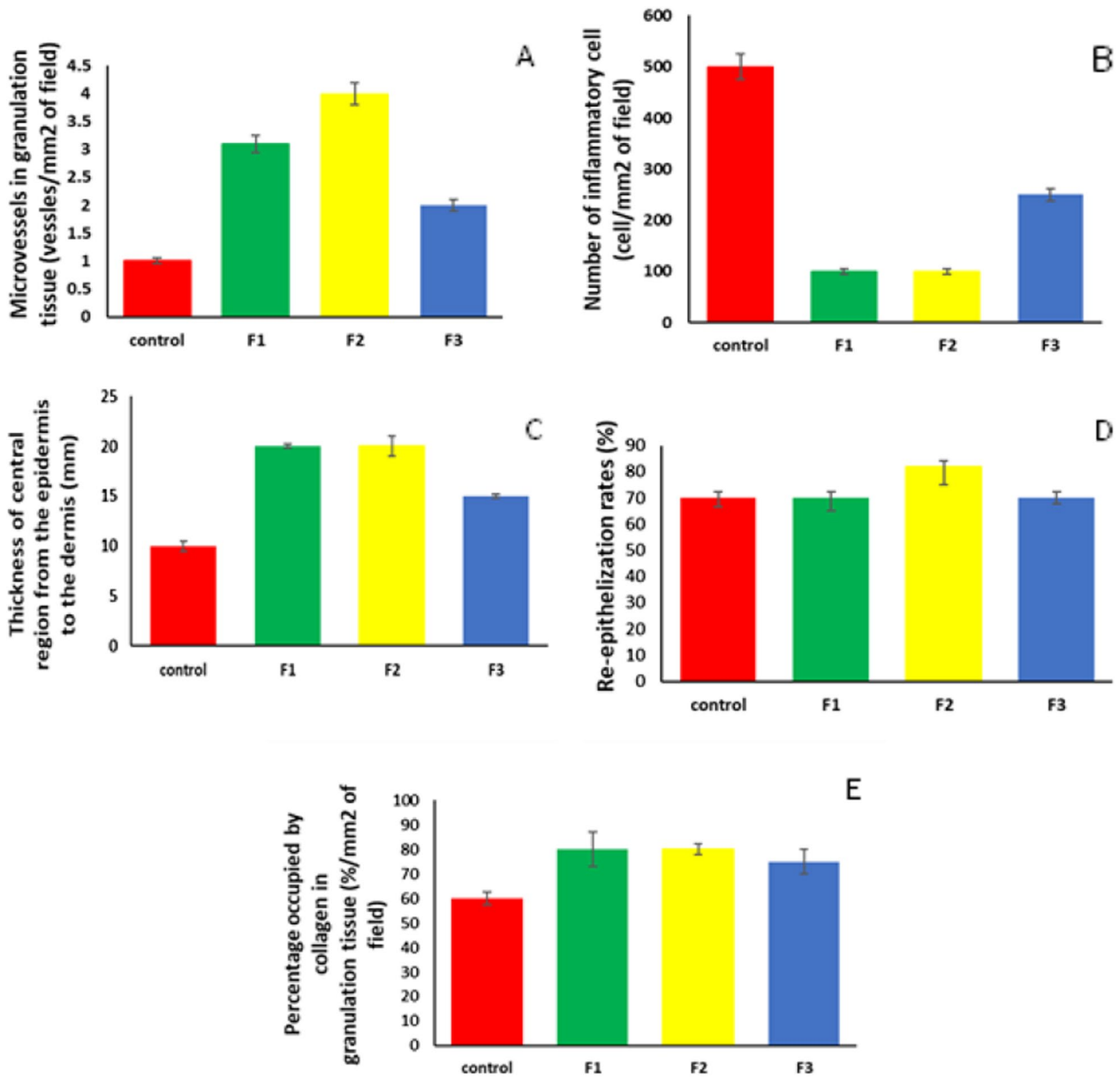

Fig. 6 Histomorphometrical values for surgical wounds showing the number microvessels in granulation tissue (**A**), number of inflammatory cells in the tissue percentage (**B**), thickness of the central region between the dermis and epidermis (**C**), re-epithelization rates (**D**), percentage occupied by collagen in granulation tissue (**E**)

by collagen in granulation tissue were higher in treated groups. The number of inflammatory cells is shown in Fig. 6B with the control group having the highest number of inflammatory cells indicating possibilities of excessive inflammation in animals not treated with any formulation as well as rats treated with formulation F3.

4 Discussion

The scanning electron microscopic image proved that stable liposomes were formed. Liposomes were spherically shaped with smooth surface and homogenous size distribution of a mixed population of uni-lamellar small- and medium-sized vesicles [27, 28]. Liposomes were

5–10 μm in size. The loaded liposome size distribution is vital to its performance in terms of drug delivery as it influences its stability, encapsulation efficiency, and drug release [29]. The percentage encapsulation efficiency of the developed curcumin-loaded liposome formulation was 99.934 ± 1.85% (mean ± S.D, $n = 3$). The high percentage of drug-loaded content buttresses that fact that the liposomes are stable and that the drug molecule curcumin was well loaded in the vesicular dispersions. High encapsulation efficiency suggests good stability and also indicates negligible leakage of the curcumin from the liposomes.

In a study by Liu et al., biodegradable liposomes containing Madecassoside were formulated, and it was proven that the higher the encapsulation efficiency of the formulation, the higher the drug release and hence permeability of the drug at the target site [30]. In regard to permeability (flux), liposomes improved the solubility and permeability of curcumin at the wound site. Infusion into hydrogel polymer matrices conferred a sustained release system because though we desire high permeability of curcumin at the wound site, it should not be so high as to attain a toxic level systemically [31]. Flux is the process of flow of a substance through a surface (permeable barrier). Liposomal size distribution, encapsulation efficacy, morphology, lipid composition, and preparation technique directly affect the flux.

A larger phospholipid/drug (curcumin) ratio means more surface area at the bilipid layer for the accommodation of the lipophilic curcumin. This also means that a higher amount of drug is available for membrane permeation during *in vitro* release. Figure 3A shows the amount of curcumin released that permeated the membrane (51.229 μg/cm^2/h). The maximum drug released was obtained in two hours with phosphate buffer and cremophor as the diffusion medium. The sustained release of the drug may also be due to the synergistic lipophilic behaviour of the phospholipid and curcumin. The slope of the plot of amount of drug released in μg/cm^2 against time in hours was 51.229 μg/cm^2/h which is high as a result of high encapsulation efficiency 99.934%. The regression coefficient (R^2) indicates the drug release model if it is close to unity (1). Based on the data the curcumin release followed Higuchi model of release kinetics as $R^2 = 0.8027$ (Fig. 2B) [31]. This means that the release of the curcumin from liposomes involved both dissolution and diffusion mechanisms [32].

The pH of all the formulations is shown in Table 2, with all formulations having the pH 5.80 slightly above the skin's pH of 5.5–5.7 indicating that the formulations are dermatologically safe for use. A pH below 6.0 is found to be ideal as it creates an environment to enhance wound healing. An alkaline pH in the wound bed would contribute to creating an unsuitable environment for healing by encouraging the growth of pathogenic bacteria. In contrast, an acidic wound bed will stunt pathogenic bacteria growth and keep the wound bed and microenvironment healthy towards the expected healing progression. The skin irritancy test showed that the hydrogels were tolerable to the skin, and no sign of erythema and oedema was observed showing the safety of all the formulations. Swelling index can be explained as the amount of water taken up by the swelling hydrogel at specific environmental conditions (37∘C, 9.8 m/s2). The rate and degree of hydrogel swelling are vital parameters which control the release patterns of solvents and drugs from polymeric networks. Therefore, the precise account of hydrogel behaviour as well as mathematical description of equilibrium swelling is affected by fluid uptake (in this case curcumin-loaded liposomes), de-sorption, or release. F1 showed the lowest swelling index followed by F2. This reflected on the *in vivo* wound healing investigation, as healing was improved in F1 and F2 due to lower swelling ratios which allowed for facilitated release of the drug molecules from the hydrogel matrixes and hence more efficient delivery of curcumin at the wound site and faster wound healing [33].

For formulations F1–F3, Spindle 2.0 (Brookfield Rheometer) was used to determine the dynamic viscosity at shear rate 20–100 rpm. F1 had the highest viscosity at 526 ± 2.97 Pas in Table 2. This can be attributed to the fact that lysine, which mildly tends to breakdown hydrogel polymer chains by forming reversible imine bonds to give poly-l-lysine, was absent. It was observed that the higher the shear rate, the lower the dynamic viscosity for all formulations suggesting that the formulations are easily spreadable on dermal application. Hydrogels were stable all through days 1–30 at room temperature. Over the period of 30 days, no formulation ingredient interaction was observed as the appearance, texture, and bio-adhesiveness remained intact [34].

Surgical wounds are often a therapeutic problem as acute wounds, if exposed to infection they have the possibility of becoming chronic. Curcumin interferes favourably with pharmacological, biochemical, and cellular events that take place in the inflammatory and proliferative stages of wound healing. Curcumin acts by recruiting macrophages into translucent fatty tissues, and thus, cytokines production is vital for inflammatory response. Curcumin subsequently reduces inflammation by the activation of the signal pathway linked with associated light chain enhancers of activated B cells. Curcumin acts as an antioxidant scavenger by scavenging reductive–oxidative species [35].

Lysine, which was incorporated in to the hydrogel base, can improve wound healing, at the onset of a wound, as

it becomes more active at the site of a wound and helps speed up the repair process. Lysine itself may also act as a binding agent, thereby increasing the number of new cells at a wound and promoting the formation of new blood vessels. Lysine is necessary for the formation of collagen, which acts as a scaffold and helps support and give structure to skin and bones. Collagen is another vital component for wound healing. The production of collagen in the wound bed by proliferating fibroblasts signifies the next phase allowing the wound edges to contract and wound closure. Usually, this stage may last for a minimum of 5 days to a maximum of 3 weeks during which time inflammation is totally resolved and re-epithelization occurs [36].

In the present study, we investigated the effect of a synergistic combination of collagen, lysine, and curcumin on the wound healing in animal models (rats). Formulation one (F1) is composed of curcumin-loaded liposomes infused in hydrogel, while formulation two (F2) is curcumin-loaded liposomes in lysine–collagen hydrogel, formulation three (F3) lysine–collagen embedded in hydrogel, and control (no treatment on the wound of the animal models). Wound contraction and re-epithelization were most rapid in F1 and F2 with no scarring at all. This could be due to the presence of curcumin which serves as a powerful wound healing facilitator. F3 and control showed much slower contraction and poor re-epithelization as scarring occurred in both cases. Dermal wound healing consists of four overlapping stages, namely haemostasis, inflammation, proliferation, and remodelling. The three latter phases primarily determine whether the wound heals normally or whether an abnormal healing process will lead to the excessive production of extracellular proteins and to fibrosis (scarring) [37].

Scar overgrowth is controlled by the inflammation in the reticular dermis, with accumulation of inflammatory cells and fibroblasts to the scar area. In addition, neovascularization and the formation of collagen fibres are highly active. The constant presence of αSMA-positive contracting myofibroblasts is typical for scar formation. Fibroblasts and myofibroblasts, stimulated by several growth factors such as TGFβ, platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF), produce high amounts of collagen and other extracellular matrix components, thereby accelerating the formation of abundant fibrotic tissue in pathological scars. Collagen synthesis is estimated to be sevenfold higher in hypertrophic scars compared with normal skin. Curcumin interferes to facilitate wound healing at every phase in the absence of curcumin in formulation F3, and control is reflected in the rate and quality of tissue regeneration at the wound site [38].

In a healthy skin, there are five layers of the epidermis and two layers of the dermis. This forms a barrier to protect the body from the surrounding environment. In the process of wound healing, complex processes that involve inflammation, epithelization, angiogenesis, granulation tissue formation, deposition of interstitial matrix, and biochemical events are carried out by keratinocytes, fibroblast, inflammatory cells, and endothelial cells. The regeneration of the epidermis, matured granulation tissue, and lower infiltration of inflammatory cells was observed in groups treated with formulations F1 and F2 and control. In Fig. 5C, the arrow pointing to the spot marked x shows the completely regenerated epidermal layer, while spot z shows the matured granulation tissue. Spot y shows an abnormal tissue for F3 with deformed epidermal layer and dermis indicating poor wound healing and a lack of regeneration of the epidermal and dermal layers [39].

The formulation-treated rats F1–F3 all showed re-epithelization rates above 70%, with those containing curcumin (F1 and F2) being significantly higher than the other groups. Thus, the optimal formulation, which facilitated re-epithelization, contained curcumin, lysine, and collagen (F2). They showed the highest re-epithelization rates. The number of infiltrated inflammatory cells, however, was significantly higher in the control than any of the groups. Rat groups treated with formulations F1–F3 contained more collagen tissues and less inflammatory cells compared with the control. The formulations containing curcumin gave more rapid tissue regeneration and less inflammation. This was primarily because the curcumin interferes progressively with the cellular events that take place in the inflammatory and proliferative stages of wound healing and hence its importance in skin regeneration and wound healing. Curcumin also demonstrates anti-inflammatory properties by blocking the TLR4 and NF-KB pathways [38]. Lysine facilitates the formation of collagen which is vital for wound healing, and other compounds similar to lysine such as proline and hydroxyproline are amino acid components of collagen. In a study by Aydin et al., wounds were inflicted at the back of three groups of Sprague Dawley rats and then each group was treated with proline, topically and intra-peritoneally, while the last group served as the control. Although it was confirmed that proline had some positive effects on wound healing, systemic administration of proline yielded more viable results with regard to enhanced wound healing than topical administration of the compound [39].

In another study by de Aquino et al., 10% Methyl-(2R, 4R)-trans-4-hydroxy-L-proline gel was formulated and used to treat wounds induced on mice. It was observed that while collagen deposition increased on days 7 and 12, only 58% wound reduction was observed by day

12 suggesting that the effect of hydroxy-L-proline in enhancing wound contraction may not be as pronounced as that of lysine in this current study [40]. Collagen is also known to increase the tensile strength of the tissue during healing at the wound area [39]. The presence of collagen in formulation F2, and F3, is evident by fact that the percentage of collagen in the granulation tissue was higher for these formulations when compared with the control. The number of microvessels in the granulation tissue and the thickness of the central region of the epidermis to dermis indicate the depth of structural wound healing taking place as formation of microvessels is necessary for vascular function at the wound site. Formulations F1–F3 showed higher numbers of microvessels and more thickness of the central region of the epidermis and the dermis compared to the control [40–42].

5 Conclusion

The liposome-in-hydrogel formulation F2 had highest number of microvessels per mm^2 of granulation tissue (4.1 vessel/mm^2), thickness of the central region from the epidermis to the dermis (22.5 mm). It also has the highest re-epithelization rate (80%) and percentage collagen occupied in granulation tissue (81%). The in vivo wound healing evaluation showed that F2 had the highest percentage wound contraction at 79.25% by day three post-surgical operation with 100% wound contraction by day seven with no scar confirming its superior performance to the other formulations. Complete regeneration of the epidermal layer was achieved showing the matured granulation tissue as evidence of healthy wound healing. This highlights and establishes the wound healing enhancing capabilities of the curcumin-loaded liposome in lysine–collagen hydrogel (F2) in surgical wounds, providing an viable and affordable formulation for filling the gap of a pertinent need for an ideal formulation for dressing surgical wounds, and also serves as evidence of a contribution to knowledge. This formulated novel dermal delivery system shows great potential as wound dressing for surgical wounds resulting from elective or emergency surgery and can serve as a prototype for future development as a pharmaceutically marketable formulation for clinical use.

Abbreviations

EE: Encapsulation efficiency; F1: Curcumin-loaded liposomes in hydrogel; F2: Curcumin-loaded liposomes in lysine–collagen hydrogel; F3: Lysine–collagen hydrogel; H&E: Haematoxylin and eosin.

Acknowlegdements

Authors would like to acknowledge the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria, for allowing use of equipment during the course of this research.

Author contributions

IC, CA, and MI designed this study. IC performed the experiments and statistical analysis. IC, CA, and MI involved in data interpretation and discussion. All authors contributed to the manuscript generation. All authors read, reviewed, and approved the final manuscripts.

Funding

This research received no external funding**.**

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethics approval for animal studies was obtained and approved by the Health Research Ethics committee of College of Medicine of the University of Lagos. Ethics approval number is CMUL/ACUREC/08/21/923.

Consent for publication

Not applicable.

Competing interest

Authors declare the have no competing interests.

Received: 2 June 2022 Accepted: 8 August 2022
Published online: 17 August 2022

References

1. De Leo V, Milano F, Agostiano A, Catucci L (2021) Recent advancements in polymer/liposome assembly for drug delivery: from surface modifications to hybrid vesicles. Polymers. https://doi.org/10.3390/polym13071027
2. Powell K, Pujji S, Jeffery S (2021) Wound healing: what is the NICE guidance from the UK? J Wound Care 30:172–182
3. Hussey L, Stocks J, Wilson P, Dumville C, Cullum N (2019) Use of antimicrobial dressings in England and the association with published clinical guidance: interrupted time series analysis. BMJ Open 9:1–9
4. Hao C, Ruoyu C, Xin Z, Yuhui Z, Allison T, Yufei Y, Haokai S, Yu Shrike Z, Jin Q, Yonghai F, Lei L, Guoqing P, Wenguo C, Lianfu D (2019) An injectable self-healing coordinative hydrogel with antibacterial and angiogenic properties for diabetic skin wound repair. NPG Asia Mater. https://doi.org/10.1038/s41427-018-0103-9
5. Wang X, Yang Y, Shi Y, Jia F (2020) Editorial: smart hydrogels in tissue engineering and regenerative medicine. Front Chem. https://doi.org/10.3389/fchem.2020.00245
6. da Silva L, Reis L, Correlo M, Marques A (2019) Hydrogel-based strategies to advance therapies for chronic skin wounds. Annu Rev Biomed Eng 21:145–169
7. Okur E, Karantas D, Şenyiğit Z, Üstündağ Okur N, Siafaka I (2020) Recent trends on wound management: new therapeutic choices based on polymeric carriers. Asian J Pharm Sci 15:661–684
8. Ternullo S, Schulte Werning V, Holsæter M, Škalko-Basnet N (2019) Curcumin-in-deformable liposomes-in-chitosan-hydrogel as a novel wound dressing. Pharmaceutics. https://doi.org/10.3390/pharmaceutics12010008
9. Emiroglu G, Ozergin Coskun Z, Kalkan Y, Celebi Erdivanli O, Tumkaya L, Terzi S, Özgür A, Demirci M, Dursun E (2017) The effects of curcumin on wound healing in a rat model of nasal mucosal trauma. Evid Based Complement Alternat Med 9452392:1–6
10. Sharifi-Rad J, Butnariu M, Ezzat M, Adetunji O, Imran M, Sobhani R, Tufail T, Hosseinabadi T, Ramírez-Alarcón K, Martorell M, Maroyi A, Martins N (2020) Mushrooms-rich preparations on wound healing: from nutritional to medicinal attributes. Front Pharmacol. https://doi.org/10.3389/fphar.2020.567518

11. Ayavoo T, Murugesan K, Gnanasekaran A (2021) Roles and mechanisms of stem cell in wound healing. Stem Cell Investig. https://doi.org/10.21037/sci-2020-027
12. Tejada S, Manayi A, Daglia M, Nabavi S, Sureda A, Hajheydari Z, Gortzi O, Pazoki-Toroudi H, Nabavi S (2016) Wound healing effects of curcumin: a short review. Curr Pharm Biotechnol 17:1002–1007
13. Wilkinson N, Hardman J, (2020) Wound healing: cellular mechanisms and pathological outcomes. Open Biol. https://doi.org/10.1098/rsob.200223
14. Amato G, Grimaudo A, Alvarez-Lorenzo C, Concheiro A, Carbone C, Bonaccorso A, Puglisi G, Musumeci T (2020) Hyaluronan/Poly-L-lysine/Berberine nanogels for impaired wound healing. Pharmaceutics 13:1–34
15. Tracy E, Minasian A, Caterson J (2016) Extracellular matrix and dermal fibroblast function in the healing wound. Adv Wound Care 5:119–136
16. Koehler J, Brandl F, Goepferich A (2018) Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. Eur Polym 100:1–11
17. Umbarkar M, Thakare S, Surushe T, Giri A, Chopade V (2021) Formulation and evaluation of liposome by thin film hydration method. J Drug Deliv Ther 11:72–76
18. Torres-Flores G, Gonzalez-Horta A, Vega-Cantu I, Rodriguez C, Rodriguez-Garcia A (2020) Preparation and characterization of liposomal everolimus by thin-film hydration technique. Adv Polym. https://doi.org/10.1155/2020/5462949
19. Lujan H, Griffin C, Taube H, Sayes M (2019) Synthesis and characterization of nanometer-sized liposomes for encapsulation and microRNA transfer to breast cancer cells. Int J Nanomed 14:5159–5173
20. Abd E, Gomes J, Sales C, Yousef S, Forouz F, Telaprolu C, Roberts S, Grice E, Lopes S, Leite-Silva R, Andréo-Filho N (2020) Deformable liposomes as enhancer of caffeine penetration through human skin in a Franz diffusion cell test. Int J Cosmet 43:1–10
21. Salamanca CH, Barrera-Ocampo A, Lasso JC, Camacho N, Yarce CJ (2018) Franz diffusion cell approach for pre-formulation characterisation of Ketoprofen semi-solid dosage forms. Pharmaceutics 10:1–10
22. Ilomuanya M, Adeyinka O, Aghaizu C, Cardoso-Daodu I, Akhimen T, Ajayi T, Odimegwu J (2019) Co-formulation and characterisation of gentamicin-loaded alkyl acrylate cross polymer hydrogel infused with ethanol extract of Tetracarpidium conophorum impregnated on gauze sponge for wound dressing. WHSA 12:8–14
23. Elegbede D, Ilomuanya O, Sowemimo A, Nneji A, Joubert E, de Beer D, Koekemoer T, van de Venter M (2020) Effect of fermented and green Aspalathus linearis extract loaded hydrogel on surgical wound healing in Sprague Dawley rats. Wound Med. https://doi.org/10.1016/j.wndm.2020.100186
24. Ilomuanya M, Ekerebe Z, Cardoso-Daodu I, Sowemimo A (2020) Formulation and evaluation of sunscreen cream using detarium senegalense oil as base. Trop J Nat Prod Res 4:141–145
25. Ilomuanya MO, Adebona AC, Wang W, Sowemimo A, Eziegbo CL, Silva BO, De Beer D (2020) Development and characterization of collagen-based electrospun scaffolds containing silver sulphadiazine and Aspalathus linearis extract for potential wound healing applications. SN Appl Sci. https://doi.org/10.1007/s42452-020-2701-8
26. Sinjari B, Pizzicannella J, D'Aurora M, Zappacosta R, Gatta V, Fontana A, Trubiani O, Diomede F (2019) Curcumin/Liposome nanotechnology as delivery platform for anti-inflammatory activities via NFkB/ERK/pERK pathway in human dental pulp treated with 2-HydroxyEthyl Meth-Acrylate (HEMA). Front Physiol. https://doi.org/10.3389/fphys.2019.00633.eCollection
27. Heydari P, Zargar Kharazi A, Asgary S, Parham S (2021) Comparing the wound healing effect of a controlled release wound dressing containing curcumin/ciprofloxacin and simvastatin/ciprofloxacin in a rat model: a preclinical study. J Biomed Mater Res A 2:341–352
28. Peretz Damari S, Shamrakov D, Varenik M, Koren E, Nativ-Roth E, Barenholz Y, Regev O (2018) Practical aspects in size and morphology characterization of drug-loaded nano-liposomes. Int J Pharm 547:648–655
29. Maja L, Željko K, Mateja P (2020) Sustainable technologies for liposome preparation. J Supercrit Fluid. https://doi.org/10.1016/j.supflu.2020.104984
30. Liu M, Li Z, Wang H, Du S (2016) Increased cutaneous wound healing effect of biodegradable liposomes containing madecassoside: preparation optimization, in vitro dermal permeation, and in vivo bio-evaluation. Int J Nanomed 11:2995–3007
31. Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y (2020) Liposomes: Advancements and innovation in the manufacturing process. Adv Drug Deliv. https://doi.org/10.1016/j.addr.2020.07.002
32. O'Callaghan S, Galvin P, O'Mahony C, Moore Z, Derwin R (2020) "Smart" wound dressings for advanced wound care: a review. JWC 29:394–406
33. Kowalski G, Kijowska K, Witczak M, Kuterasiński Ł, Łukasiewicz M (2019) Synthesis and effect of structure on swelling properties of hydrogels based on high methylated pectin and acrylic polymers. Polymers. https://doi.org/10.3390/polym11010114
34. Lopez Mora N, Owens M, Schmidt S, Silva F, Bradley M (2020) Poly-epsilon-lysine hydrogels with dynamic crosslinking facilitates cell proliferation. Materials. https://doi.org/10.3390/ma13173851
35. Xu Y, Meng X, Li S, Gan Y, Li Y, Li B (2018) Bioactivity, health benefits, and related molecular mechanisms of curcumin: current progress, challenges, and perspectives. Nutrients. https://doi.org/10.3390/nu10101553
36. Landén X, Li D, Ståhle M (2016) Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci 73:3861–3885
37. Marshall D, Hu S, Leavitt T, Barnes A, Lorenz P, Longaker T (2018) Cutaneous scarring: basic science, current treatments, and future directions. Adv Wound Care 7:29–45
38. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, Hu C, Xu R (2021) Anti-inflammatory effects of curcumin in the inflammatory diseases: status, limitations and countermeasures. Drug Des Devel Ther 15:4503–4525
39. Aydin H, Tatar C, Savas A, Karsidag T, Ozer B, Dursun N, Bekem A, Unal A, Tuzun S (2018) The effects of local and systemic administration of proline on wound healing in rats. J Invest Surg 32:523–529
40. de Aquino A, Lustosa R, de Sousa S, Chaves-Filho M, Lima V, da Conceição SD, Gramosa V, Silveira R, de Barros VS (2020) The N-Methyl-(2S, 4R)-trans-4-hydroxy-L-proline-Enriched methanol fraction from sideroxylon obtusifolium shows an anticonvulsant activity associated with its anti-inflammatory/antioxidant actions. Planta Med 7:158–169
41. Mathew-Steiner SS, Roy S, Sen CK (2021) Collagen in wound healing. Bioengineering (Basel) 11:1–15
42. Karppinen M, Heljasvaara R, Gullberg D, Tasanen K, Pihlajaniemi T (2019) Toward understanding scarless skin wound healing and pathological scarring. F1000Research. https://doi.org/10.12688/f1000research.18293.1

Publisher's Note