International Journal of Biological Macromolecules 269 (2024) 132071

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Rutin-loaded zein gel as a green biocompatible formulation for wound healing application

Agnese Gagliardi [a], Elena Giuliano [a], Silvia Voci [a], Nicola Costa [a], Stefania Bulotta [a], Maria Cristina Salvatici [b], Nicola Ambrosio [a], Donatella Paolino [c], Farhan Siddique [d], Muhammad Majid [e], Ernesto Palma [a], Massimo Fresta [a], Donato Cosco [a,*]

[a] Department of Health Sciences, University of Catanzaro "Magna Græcia", Campus Universitario "S. Venuta", I-88100 Catanzaro, Italy
[b] Institute of Chemistry of Organometallic Compounds (ICCOM), Electron Microscopy Centre (Ce.M.E.), National Research Council (CNR), via Madonna del Piano n. 10, 50019 Sesto Fiorentino, Firenze, Italy
[c] Department of Experimental and Clinical Medicine, University of Catanzaro "Magna Græcia", Campus Universitario "S. Venuta", I-88100 Catanzaro, Italy
[d] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University, 60800 Multan, Pakistan
[e] Faculty of Pharmacy, Hamdard University, Islamabad Campus, Pakistan

ARTICLE INFO

Keywords:
Anti-inflammatory activity
Gels
Rutin
Wound healing
Zein

ABSTRACT

Wound healing is a challenging clinical problem and efficient wound management is essential to prevent infection. This is best done by utilizing biocompatible materials in order to complete the healing in a rapid manner, with functional and esthetic outcomes. In this context, the zein protein fulfills the criteria of the ideal wound dressing which include non-toxicity and non-inflammatory stimulation. Zein gels containing rutin were prepared without any chemical refinement or addition of gelling agents in order to obtain a natural formulation characterized by antioxidant and anti-inflammatory properties to be proposed for the treatment of burns and sores. *In vitro* scratch assay showed that the proposed gel formulations promoted cell migration and a rapid gap closure within 24 h (~90 %). In addition, the *in vivo* activities of rutin-loaded zein gel showed a greater therapeutic efficacy in Wistar rats, with a decrease of the wound area of about 90 % at day 10 with respect to the free form of the bioactive and to DuoDERM®. The evaluation of various markers (TNF-α, IL-1β, IL-6, IL-10) confirmed the anti-inflammatory effect of the proposed formulation. The results illustrate the feasibility of exploiting the peculiar features of rutin-loaded zein gels for wound-healing purposes.

1. Introduction

Wound management is one of the most pressing clinical issues because millions of people experience various types of wounds and injuries, including surgical incisions, burns and ulcers [1,2]. Wound healing could be described as the highly complex physiological response of a living system to physical, chemical, mechanical or thermal injury in which a cascade of cells, matrix components and other biological factors work together to facilitate healing and restore tissue integrity [3]. This is a multistep process which comprises several overlapping phases: (*i*) hemostasis, (*ii*) inflammation, (*iii*) proliferation or granulation, and (*iv*) remodeling or maturation [4,5]. The first two stages, hemostasis and inflammation, begin almost immediately after the appearance of a wound or injury. Cytokines, growth factors, and reactive oxygen species (ROS) accumulate during these two phases in order to recruit and transport cells to the wound site [6–8]. The clot that forms brings about hemostasis and provides a platform for the arrival of inflammatory cells. The proliferative phase lasts 2–4 weeks and begins around the third day after the injury [3]. This stage can be identified by the formation of new tissue by endothelial cells, fibroblasts, and keratinocytes, as well as the formation of granulation tissue [9]. This new tissue provides a support matrix for the epithelial cells to cover the wound surface in a process known as re-epithelialization [10]. The remodeling phase is the last of the wound healing processes and occurs mainly from day 21 for up to a year or even more following the injury [11]. During this stage, the formation of granulation tissue ends through apoptosis or differentiates into myofibroblasts. If any of the phases do not come about correctly, abnormal wound healing may occur. Treatments focused on all healing phases are regarded as optimal strategies for accelerating wound healing [12].

* Corresponding author.
E-mail address: donatocosco@unicz.it (D. Cosco).

https://doi.org/10.1016/j.ijbiomac.2024.132071
Received 3 January 2024; Received in revised form 29 April 2024; Accepted 1 May 2024
Available online 4 May 2024

A suitable dressing must be applied to the wound to protect the site from further external mechanical and microbial stress in order to aid wound care and healing [13]. Traditional wound dressings, such as cotton, bandages, and gauzes, are dry and do not provide a moist and active environment conducive to wound healing [14]. Moreover, because of wound drainage, dressings tend to stick to the wound bed and, when removed, cause pain in patients. In order to address these drawbacks associated with unspecific and only partially effective conventional wound dressings, significant efforts are being directed towards the development of new and effective platforms for wound healing applications [15].

Indeed, wound dressings must fulfill a number of criteria in order to provide a favorable environment for wound closure and tissue regeneration. These criteria include absorbing excess exudate, maintaining the correct humidity around the surface of the wound to allow for better gas exchange, increasing the proliferation of keratinocytes and fibroblasts, as well as protecting from microorganisms and infection. Moreover, dressings should not adhere to the wound and should facilitate painless removal [16]. Additional requirements for wound dressing materials include non-toxicity and biocompatibility, no antigenic or inflammatory stimulation, and a rate of biodegradability directly proportional to the rate of the formation of new tissue. Moreover, in terms of mechanical properties, a wound dressing should be elastic and flexible enough to allow easy handling and comfortable wear. Different types of wound dressings are available on the market such as fiber-based scaffolds, sponges and films and among these, gels and hydrogels have emerged as promising candidates to be utilized as carriers for drug compounds/biomolecules for wound care.

These types of wound dressings are made up of natural or synthetic polymers, but the synthetic ones were only suitable for superficial wounds, as they lacked some important properties such as low adherence, absorption, and permeability [17]. On the other hand, natural polymers such as alginate, silk, fibrin, keratin, and fucoidan have been widely used in skin tissue engineering due to their biocompatibility, biodegradability and ability to repair or regenerate damaged tissue, though an unfortunate drawback is that these are frequently prone to microbial contamination [18–20]. Indeed, several studies describe the combination of plant extract compounds such as terpenoids, alkaloids, essential oils, fatty acids and polyphenols with natural polymers and highlight the acceleration of the healing process due to the anti-inflammatory, antioxidant, antimicrobial and other regenerative properties exhibited by these natural molecules [21,22].

Among the natural polymers, zein (a low-cost amphiphilic prolamine) has drawn attention in the scientific community due its inherent characteristics of biodegradability, biocompatibility, flexibility, high degree of microbial resistance and its antioxidant properties, as well as the GRAS status received from the Food and Drug Administration [23,24]. Moreover, the protein has shown hemostatic and mucoadhesive properties which could be used to accelerate wound healing [25]. In literature there are numerous studies that have been carried out on nanofibrous zein scaffolds containing anti-inflammatory/antioxidant drugs used to treat ulcerative wounds [26,27]. However, despite being effective in many *in vitro* and *in vivo* studies, zein nanofibers were observed to be characterized by scarce mechanical strength and instability. Different experimental approaches have been attempted to improve the mechanical strength of nanofibers, for instance by adding other polymers to the vegetable protein or by using chemical cross-linking agents such as glutaraldehyde or formaldehyde which can, however, present a certain toxicity [28,29].

Recently, our research team used a simple method for the development of zein-based gels that does not require the aid of any complex equipment in order to provide a panel of various formulations useful for alimentary, biomedical and drug delivery applications [30,31]. The main advantages of using gels are their ease of administration and, more importantly, the preparation conditions because they induce only a slight degree of stress for the thermolabile molecules. Moreover, rutin, a flavone glycoside made up of the quercitin flavone and the disaccharide rutinose, showed various therapeutic effects such as cytoprotection, as well as carrying on antitumor, anti-inflammatory and antioxidant activities [32,33]. In addition, the main strength of this gel is related to the pharmacological activities exerted by the protein and the bioactive compound that can address the interdependent variables of wound treatment at all stages. In particular, the association of natural polymers with plant-derived active compounds could accelerate the healing process of skin wounds and avoid the shortcomings of the single elements.

Based on these findings, the main purpose of this investigation was to evaluate the influence of different amounts of rutin on the rheological properties of zein-based gels by using passive and dynamic rheology in order to obtain a conceivable novel formulation to be proposed for the treatment of burns and sores in humans and animals. The morphology as well the release profiles of the compound from the protein network were evaluated and the potential interaction between the drug and zein gels was investigated by Fourier transform infrared spectroscopy (FT-IR). Finally, the "scratch assay" was performed on human keratinocytes (NCTC 2544 cells) and then the wound-healing features of the rutin-loaded zein gels were investigated *in vivo* on a rat model and compared with those of the commercial formulation DuoDerm® Hydroactive® hydrogel.

2. Materials and methods

2.1. Materials

Zein (CAS number 9010-66-62), rutin, ethanol and phosphate-buffered saline (PBS) tablets were purchased from Sigma Aldrich S.r.l. (Milan, Italy). For cell culture studies, minimum essential medium (D-MEM) with glutamine, trypsin/ethylenediaminoacetic acid (EDTA) (1×) solution, fetal bovine serum (FBS) and penicillin–streptomycin solution were supplied by Gibco (Invitrogen Corporation, Life Technologies, Italy). NCTC2544 cells were provided by the Istituto Zooprofilattico of Modena and Reggio Emilia, Italy. All other materials and solvents used in this investigation were of analytical grade (Carlo Erba, Milan, Italy).

2.2. Preparation of zein gels

Zein gels were prepared by the addition of 20 % w/v of the biopolymer to an EtOH/H_2O solution (65:35, %v/v) under constant, slow magnetic stirring to favor the evaporation of the organic solvent as previously reported [30]. Moreover, different amounts of rutin (0.5 %, 1 %, 2.5 %, 5 % w/w with respect to zein) were solubilized in ethanol during the sample preparation in order to obtain zein gels containing the active compound.

The Scanning Electron Microscope Gaia 3 (Tescan s.r.o, Brno, Czech Republic) FIB-SEM (Focused Ion Beam-Scanning Electron Microscope) was used to evaluate the morphology of the zein- and rutin-loaded zein gels, as previously described [30].

2.3. Rheological measurements of zein gels containing rutin

Diffusive wave spectroscopy (DWS) was used to evaluate the rheological properties of the zein gels using a microrheometer Rheolaser Master (Formulaction, I'Union, Toulouse, France). Briefly, freshly prepared gels (20 ml) were placed in suitable cylindrical glass tubes in the Rheolaser chambers and analyzed for 1 h at 37 °C as previously described [34].

In addition, dynamic rheological tests were carried out on the zein-based gels containing different amounts of rutin by means of a Kinexus rheometer (Malvern Panalytical Ltd., Spectris plc, England) using a cone-plate geometry as previously reported [31].

2.4. Evaluation of release profiles and FT-IR analysis

The release profile of rutin from the zein-based gels was evaluated by the dialysis method using cellulose acetate tubes (Spectra/Por with molecular cutoff 10 kDa by Spectrum Laboratories Inc., Eindhoven, The Netherlands). PBS (10 mM) was used as the release medium, which was constantly stirred and warmed (GR 150 thermostat, Grant Instruments Ltd., Cambridge, UK) to 37 ± 0.1 °C throughout the experiment. The samples were analyzed at λ_{max} 362 nm of rutin by a PerkinElmer Lambda 35 ultraviolet-visible (UV–vis) spectrophotometer equipped with PerkinElmer acquisition software (Perkin-Elmer GmbH Uberlingen, Germany) as previously described [35,36]. No interference deriving from the empty zein formulation was observed.

The vibrational spectra of the zein, rutin, their physical mixture and the gel formulation were examined using a Nicolet™ iS5 spectrometer coupled with an iD7 Attenuated Total Reflectance accessory (Thermo Fisher Scientific Inc., Waltham, MA, USA). The FT-IR spectra were acquired through 64 acquisitions with a resolution of 4 cm^{-1} in a wavenumber range between 500 and 4000 cm^{-1}. Spectra analyses were elaborated using OMNIC software, version 9.12.1019.

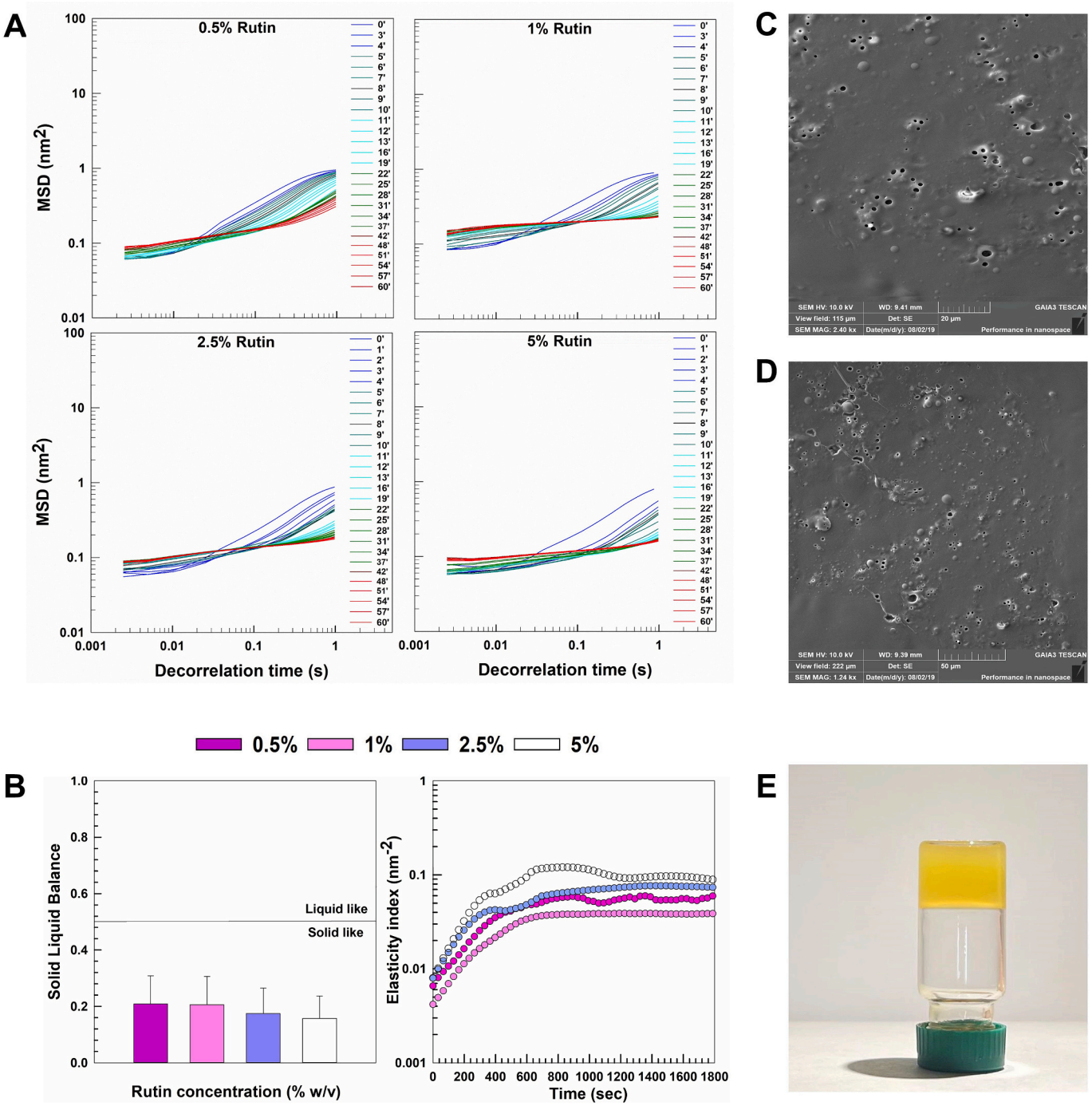

Fig. 1. (A) Mean square displacement (MSD) profiles of zein gels (20 % w/v) prepared with various concentrations of rutin as a function of the decorrelation time; (B) Solid-liquid balance (SLB) and Elasticity index (EI) of rutin-loaded zein gels; (C) SEM micrographs of empty zein gels and (D) systems prepared with 5 % w/w of rutin; (E) Representative picture of zein gels containing 5 % w/w of rutin.

2.5. In vitro wound healing activity

NCTC 2544 cells were cultured as previously described [33].

The scratch assay was used in order to investigate the ability of the rutin-loaded zein gels to promote the proliferation and migration of keratinocytes [37]. In detail, NCTC 2544 cells were seeded into 12-well tissue culture plates in order to obtain a monolayer and then an artificial wound (*i.e.* scratch) was generated using a sterile 20–200 μL pipette tip across the center of the well which can mimic an incision wound. Successively, PBS was utilized to wash the cells to remove cell debris. The cells were then treated with the different formulations as follows: i) control (untreated cells), ii) zein gels (50 μg/ml of protein), iii) rutin as ethanolic solution 65:35 v/v (2.5 μg/ml) or iv) entrapped in zein gels (50 μg/ml of protein, 2.5 μg/ml of active compound). The photographs of the scratch wounds were recorded after 0, 6, 24 and 48 h incubation using a Leica DM IL LED microscope (Leica, Germany) equipped with a Leica DFC 3000G Camera and a HIPLAN 4×/0.10 Objective (Leica Microsystems®, Wetzlar, Germany).

The scratch closure rate (%) area was measured using the Image-J software by using the following equation:

$$\%\text{Scratch Closure} = (A_0 – A_t/A_0) \times 100 \quad (1)$$

where A_0 and A_t are the scratch area at 0 h and after the various incubation times, respectively [38].

2.6. In vivo wound healing assay

All animal experiments were performed following the EU Directive 2010/63/EU for animal experiments guidelines (approval n. EC/716-P/2023, Bahauddin Zakariya University). Briefly, Wistar rats (Harlan, San Pietro al Natisone, Udine, Italy), weighing ~250–300 g, were maintained at standard conditions of both temperature (20 ± 2 °C) and humidity (65 %) following a 12 h light/12 h dark cycle (light on 8:00 a.m.) with food and water *ad libitum*. A full-thickness excision wound model was used to investigate the wound closure after the application of various formulations. The rats were anesthetized through the standard anesthesia procedure with isoflurane. Afterwards, the backs of the animals were shaved and cleaned with ethanol 70 %.

The various formulations were applied once a day for 15 days. In detail, five groups were used in this study as follows: i) control (saline solution), ii) DuoDERM® hydrogel (commercial wound dressing), iii) rutin 5 % w/w (ethanolic solution 65:35 v/v), iv) zein-based gels (empty formulation), v) rutin-loaded zein gels (5 % w/w with respect to the protein). The healing process was assessed by the measurement of the wound region using a digital caliper and the progressive changes were monitored with a camera on predetermined days, *i.e.*, 0, 5, 10, and 15 days. The wound area was calculated according to the following equation:

$$A = \pi R r, \quad (2)$$

where A is the wound area (cm^2), R is the largest radius, r is the smallest radius.

The results were expressed as percentage of wound contraction calculated as follows [39]:

$$\%\text{Wound contraction} = (\text{Initial Wound area} – \text{Final Wound area}/\text{Initial wound area}) \times 100. \quad (3)$$

2.7. Evaluation of pro-inflammatory and anti-inflammatory cytokines

Blood samples were collected from all animals of each group on days 5, 10 and 15 following wound creation. Pro-inflammatory cytokines (TNFα and IL-6, IL-1β) and anti-inflammatory cytokines (IL-10) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen). The assays were performed strictly according to the manufacturer's protocols. The cytokine concentrations were calculated in pg/ml by plotting the graph for the standard [40]. All experiments were done in triplicate to confirm the accuracy of the observations.

2.8. Histopathological study

Tissue biopsies of experimental mice at days 5, 10 and 15 were collected and conserved for histopathology and hydroxyproline content determination at 4 °C and −80 °C respectively. For histological analysis, the samples were fixed in a fixative solution (absolute alcohol; formaldehyde; glacial acetic acid in 7:2:1 ratio) and paraffin embedded. Thin sections (2–5 μm) were cut and stained with Eosin/hematoxylin and Masson Trichrom dyes for observation under light microscope (DIALUX-20 EB). Images were captured by a HDCE (50B) camera at 40× [41].

2.9. Statistical analysis

One-way ANOVA was used for statistical analysis of the various experiments. A p value of <0.05 was considered statistically significant. Results were reported as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Microrheological behavior

Natural zein-based gels are excellent candidates for biomedical applications due to their exceptional network assembly, which offers favorable biological characteristics and tunable physico-chemical properties. In particular, in our previous work, the gelling properties of zein were investigated by means of passive and dynamic rheology. The results showed that the formulations made up of 20 % w/v of protein showed a strong solid-like feature, a pseudoplastic behavior and good spreadability, suggesting their potential application as topical gels and for food coatings/packaging [30]. In detail, protein gelation is based on the perturbation of the native polymer conformation (unfolding), resulting in the exhibition of new reactive sites that are successively exploited in the aggregation step to promote various interactions able to form an ordered network [42]. In this study the effect of different amounts of rutin on the microrheological behavior of zein-based gels was investigated by means of MS-DWS.

The mean square displacement (MSD) is directly related to the viscoelastic properties of the sample, and its change can reflect the gel formation process [43,44]. Fig. 1 showed the representative MSD curves *versus* decorrelation time for the zein gels prepared with various amounts of rutin. In detail, the MSD profiles of all samples are characterized by three sections; at the beginning the MSD curves rise linearly because the particles are free to move during the initial decorrelation time in the continuous medium phase, and the slopes are mainly influenced by the viscosity of the dispersant medium. Successively, they are trapped by their neighboring particles, and the slopes of the MSD curves decrease and become wider until they reach a plateau which indicates the formation of a three-dimensional network structure or a “cage”, suggesting a more pronounced elastic behavior of the formulations. This is a common characteristic of the viscoelastic samples as reported in

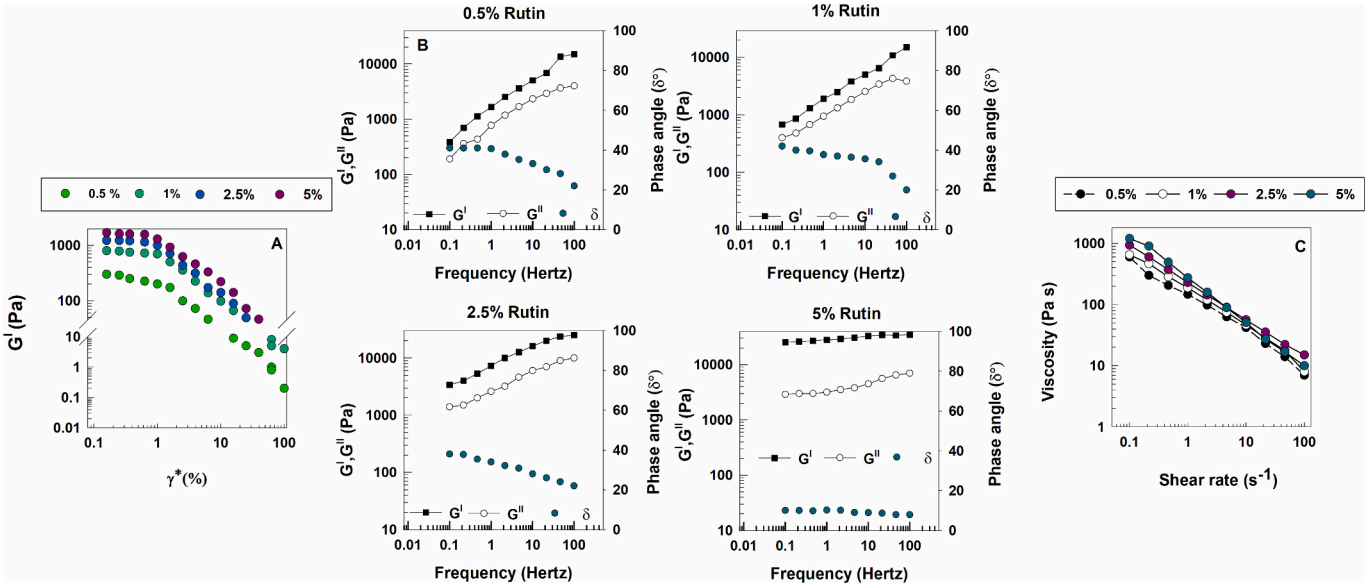

Fig. 2. (A) Correlation between the strain (γ) and the elastic modulus (G′) of zein gels containing rutin. (B) Elastic (G′) and viscous (G″) moduli of rutin-loaded zein gels (20 % w/v of protein) as a function of frequency. (C) Evaluation of viscosity of zein gels containing rutin at 37 °C as a function of the shear rate.

different experimental investigations [45–47]. Moreover, it was interesting to observe that the MSD profiles of the formulations containing rutin were quite different from the empty formulation (Fig. S1). Indeed, as shown in Fig. 1 the addition of increasing amounts of rutin evidenced a more pronounced elastic profile as compared to the empty gels (Fig. 1A, Fig. S1) This phenomenon is probably related to the polyphenol which increased the amount or strength of the intermolecular protein bonds as previously reported in another study [48]. These data can also be confirmed by the Solid-Liquid Balance (SLB) value, which is a parameter regarding the ratio between the solid-like and liquid-like behavior of the samples. Namely, an SLB value between 0.5 and 1 represents a more liquid behavior, whereas an SLB value of <0.5 denotes a solid behavior. As shown in Fig. 1B, all the formulations containing rutin ranging from 0.5 to 5 % w/w, showed SLB values of <0.5, demonstrating a decreasing trend proportional to the increase of the drug concentration. As a result, the gels are mostly dominated by elastic behavior, and the strength of that elasticity is directly related to the amount of rutin used during the sample preparation.

Another parameter that provides more information on the rheological features of gels is the elasticity index (EI) which corresponds to the inverse of the average distance travelled by particles before interacting with the surrounding network [49]. Indeed, since the EI is the inverse of the MSD value, lower MSD plateau values indicate a smaller cage and, hence, a greater degree of elasticity of the systems [35]. As shown in Fig. 1B, the EI values are directly proportional to the amount of rutin present in the formulation, evidencing a slight initial increase, followed by a steady state. This phenomenon is related to the formation of a network able to prevent the movement of the tracers, confirming a gradual and significant increase in both the elasticity and viscosity of the systems over time as was true regarding other gel-based formulations [34]. Also in this case, the SLB and EI showed a more pronounced solid-like characteristic profile with respect to the empty formulation confirming that rutin effectively modulates the rheological properties of zein gel (Fig. S1, Fig. 1).

The morphology of the empty zein gels and of systems prepared with rutin (5 % w/v) was also evaluated by SEM. The formulations obtained using a protein concentration of 20 % w/v (Fig. 1C) appeared dense and characterized by a solid polymeric network as previously reported [30]. The addition of large amounts of rutin did not affect the morphology of the samples (Fig. 1D) demonstrating significant compatibility between the protein molecules and rutin.

3.2. Rheological features of zein-based gels

The influence of various concentrations of rutin on the rheological features of zein-gels under stress is an important aspect to be evaluated. Firstly, strain sweep tests were used to determine the maximum deformation (critical strain) that a sample can withstand and to characterize the linear viscoelastic response (LVR) mechanism. The critical strain for each sample was defined as the limit of LVR, which was expressed as the point at which G′ does not deviate noticeably from a constant value (5 %) [50]. As shown in Fig, 2 A the strain dependence of G′ of the various zein gels was evaluated at a frequency of 1 Hz at 37 °C. The investigated formulations showed a constant profile up to 1 % of strain and then the curves dramatically decreased confirming a partial collapse of the network and a transition from linear to non-linear behavior as reported for empty zein dispersions (Fig. S2). The formulations showed a high G′, a low γ and a short LVR, confirming the ability of these samples to form a gel network.

Based on these data, the frequency sweep test was performed at 1 % strain to better understand the structural strength as well as the response of the elastic and viscous components as a function of different frequencies [51]. In particular, the increasing amounts of rutin added during the preparation of zein gels showed frequency-dependent profiles and a slight, progressive gap between G′ and G″ up to 2.5 % w/w of rutin, demonstrating that the rheological features of the systems are significantly influenced by the concentration of the molecule. This is probably due to the non-covalent physical crosslinks (hydrophobic interaction) of the zein network with low amounts of rutin which are responsible for the frequency dependence of the samples [52].

However, it's interesting to observe that the storage and viscous moduli of the gels are both completely frequency-independent at the highest concentration of the compound (5 % w/w), highlighting a pseudo-plastic or shear-thinning behavior similar to that of the empty formulation (Fig. S2). This data supports our previous study which evidenced that the physico-chemical characteristics of the molecule entrapped in zein networks affect the technological parameters of the polymeric structure [31]. Indeed, it is probable that the peculiar chemical composition of the biopolymer, made up of a predominance of lipophilic aminoacids, promoted a strong hydrogen-bonding interaction with increasing amounts of the poorly water-soluble compound, data consistent with several experimental investigations [53,54]. Moreover, the high storage modulus of the formulation prepared with 5 % w/w of

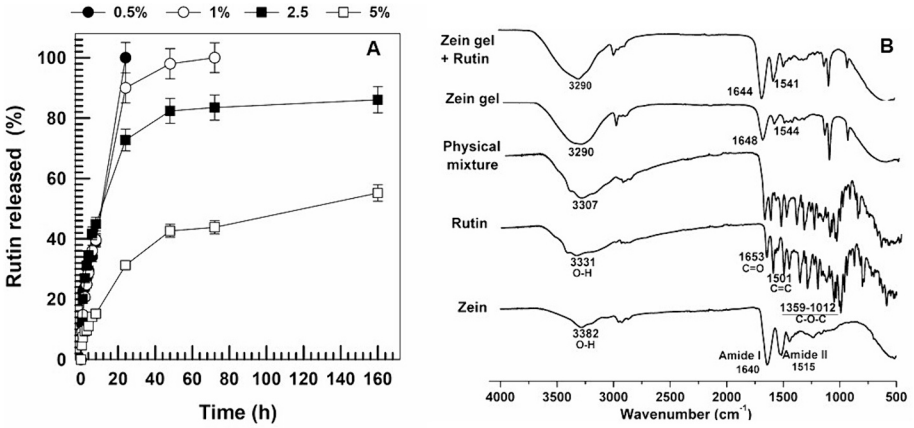

Fig. 3. (A) Release profile of rutin from zein-based gels as a function of the concentration of the active compound used during the sample preparation and incubation time. (B) FT-IR spectra of zein, rutin, their physical mixture, zein-based gels and rutin-loaded zein gels.

rutin confirms that the zein gels had a strong and elastic inner network able to retain the energy of the system [55,56].

The evaluation of the phase angle (tan δ = G″/G′) of the protein gels supported the previous results. Namely, the formulation prepared with 0.5–2.5 % w/w of rutin showed a slightly elastic behavior at low frequencies (δ:40) that became more elastic (δ:22) when this parameter increased. Contrarily, at the highest rutin concentration (5 % w/w), the gels showed a constant value of δ:10, confirming the elastic properties of the formulation (solid nature) (Fig. 2).

In addition, the shear viscosity of the samples was investigated as a function of the shear rate. The resulting data demonstrated that the gels containing rutin were characterized by a non-Newtonian shear thinning behavior similar to the empty formulation which was strongly dependent on the concentration of rutin (Fig. S2). As shown in Fig. 2, the viscosity η of the samples decreased when the shear rate γ increased. In particular, an enhancement of the rutin concentration strengthened the

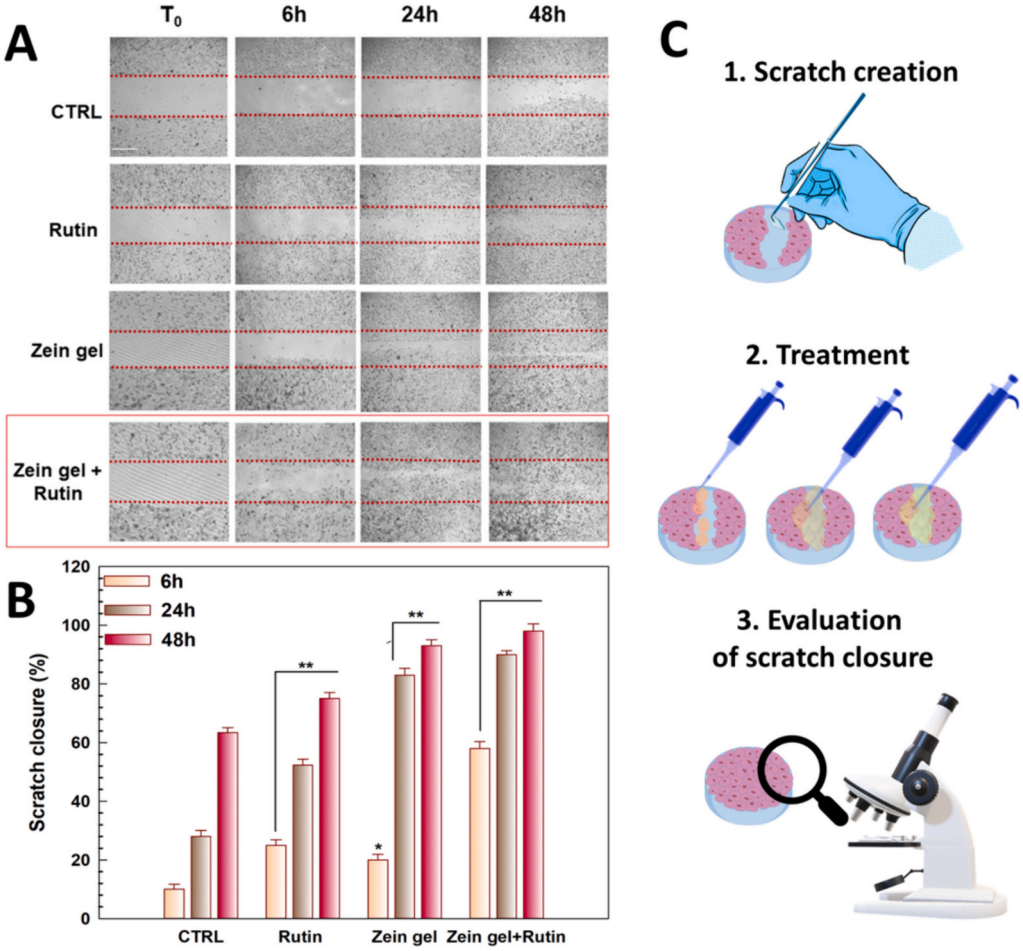

Fig. 4. (A) Representative micrographs of cell scratches treated with the various formulations as a function of the incubation time. Scale bar: 500 μm. (B) Extent of wound closure in scratch assays after 6, 24 and 48 h incubation. (C) Schematic representation of the performed scratch assay. *$p < 0.05$, **$p < 0.001$ (with respect to the untreated cells).

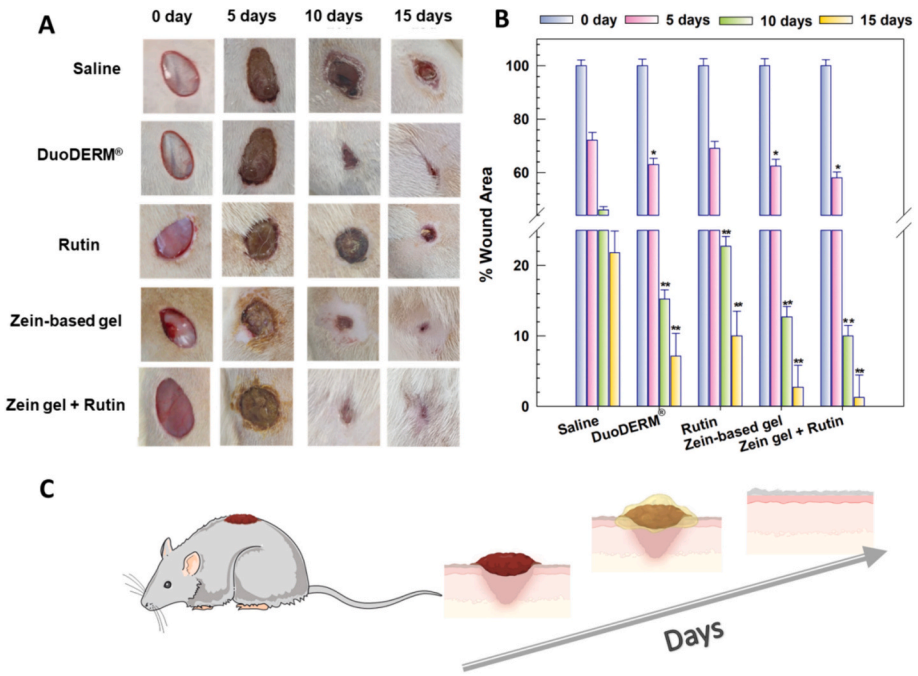

Fig. 5. (A) Representative photographs of rat wounds treated with various formulations as a function of the time. (B) Extent of wound area closure in Wistar rats treated with saline (control), DuoDERM®, zein-based gels and zein gels containing rutin. $*p < 0.05$, $**p < 0.001$ (with respect to the control treated with a saline solution); (C) Schematic representation of the *in vivo* wound healing process.

gel network and provided greater flow resistance. This behavior of the zein gels was due to the reduction of the attractive bonds within the gel network under shear, which caused a decrease in viscosity [25].

3.3. Evaluation of rutin release profiles and FT-IR analysis

The evaluation of the leakage profile of rutin from the zein gels was evaluated as a function of the rutin concentration and the incubation time. As shown in Fig. 3, the drug leakage from the zein gels in a PBS solution was inversely proportional to the concentration of the active compound r, data in agreement with our previous experimental works which demonstrated that the release curves of the bioactive from zein-based systems were modulated by the amount of the entrapped drugs [24]. In detail, as shown in Fig. 3, the release of rutin was complete within 24 h when low concentrations of drug were used (0.5–1 % w/w), while it was more gradual and prolonged when the amount of the active compound increased. In fact, the formulations prepared with 5 % of drug showed a sustained release of about 50 % for up to 160 h. This trend confirms the great affinity of this compound with the protein, highlighting a noteworthy integration of rutin into the polymeric network. Based on these findings, the gels containing 5 % of rutin were selected for further investigations.

Also, in order to corroborate the occurrence of these strong interactions between the protein and the compound, a FT-IR analysis was performed in the range between 400 and 4000 cm^{-1}. As shown in Fig. 3, the gel formulation and its components showed a wide band in the region between 3100 and 3330 cm^{-1}, associated with the stretching vibration of -OH. In particular, zein showed other characteristic bands such as amide I and II, located at 1640 cm^{-1} and 1533 cm^{-1} respectively, due to the vibration and stretching of the amide bonds within the protein structure. In particular, the amide I band was attributed to the stretching of the carbonyl (C=O) of the amide groups belonging to the peptide bonds, while the amide II band was mainly associated with the N—H bending and C—N stretching vibrations [24,57,58]. The spectra of zein gels showed a displacement in both the amide stretching vibration bands, which may be a consequence of a conformational change when the gels are formed [59].

The characteristic peaks of rutin were 3331 cm^{-1} (O – H stretching vibration), 1653 cm^{-1}, (mainly C=O stretching), 1501 cm^{-1} (C=C, aromatic), 1359–1014 cm^{-1} (C–O – C) data in agreement with several studies [53,59,60]. The physical mixture showed almost the same profile as rutin, suggesting the absence of interactions between the raw components.

On the other hand, the spectra of the zein gels containing rutin were quite different. Indeed, the O—H vibration stretching peak of zein shifted from 3382 cm^{-1} to 3290 cm^{-1} and the absorption peaks of the amide I and amide II bands of the protein shifted from 1640 cm^{-1} to 1648 cm^{-1} and from 1515 cm^{-1} to 1541 cm^{-1}, respectively. Additionally, the characteristic rutin peaks between 1000 cm^{-1} and 1500 cm^{-1} almost disappeared due to the overlapping of the characteristic peaks of the drug with the absorption bands of the polymer matrix. This phenomenon is clear evidence of the entrapment of the rutin within the protein network, suggesting the occurrence of strong hydrogen bonds, as well as hydrophobic interactions between the two molecules, results consistent with other experimental studies [21,61].

3.4. Scratch assay

The rate-limiting step for the healing of skin wounds is cell migration [62]. Hence, the use of materials that can promote epidermal keratinocyte migration may aid in the development of targeted therapies for better cutaneous wound healing. The *in vitro* scratch assay is a straightforward method that mimics cell migration during *in vivo* wound healing [37]. In particular, the scratch in a confluent monolayer imitates an injury; then proliferation and migration of keratinocytes play a key role during the granulation and re-epithelialization of wounds [63,64]. In fact, the inhibition of contact during wound healing causes their differentiation into a basal phenotype of stratified squamous keratinizing epidermal cells once the wound area is covered.

In detail, the scratch assay was performed on human keratinocytes (NCTC 2544 cells) in order to evaluate the influence of the zein gels (50 μg/ml of protein), the rutin as ethanol solution 65:35 (2.5 μg/ml) or entrapped in zein gels (50 μg/ml of protein) on their migration. Untreated cells were used as control. As shown in Fig. 4, at time 0 the

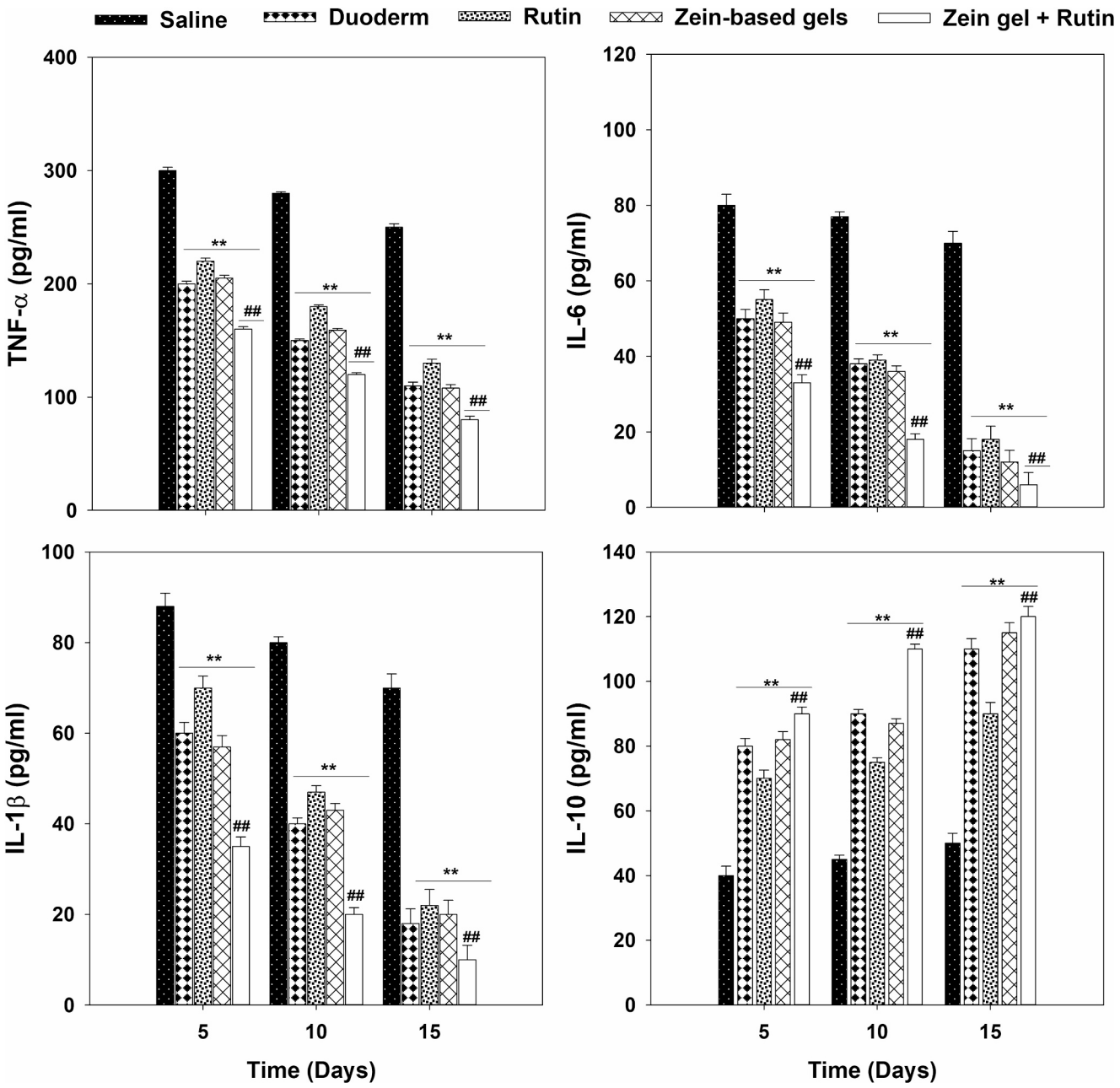

Fig. 6. Evaluation of the serum level of anti-inflammatory and pro-inflammatory cytokines in Wistar rats treated with various formulations as a function of time.

scratch was clear and similar in all samples; all formulations had a similar effect on cell migration after 6 h (~22 %), with the exception of zein gels containing rutin which showed an increased rate of migration (~58 %) (Fig. 4).

However, it's interesting to observe that after 24 h and 48 h the empty gels promoted a faster migration and proliferation of keratinocytes when compared to the control as demonstrated by the high wound closure rates (~83 % and 93 %, respectively). The encapsulation of rutin within the gels improved the cell migration showing a significant reduction in the scratch area after 24 h incubation (~90 %) and a complete closure after 48 h, results significantly better than those obtained when cells were incubated with rutin (rate of wound closure of about 75 % after 48 h, Fig. 4).

These profiles can be related to the peculiar anti-oxidant and anti-inflammatory characteristics of rutin and zein as well as the cell adhesive properties of the protein that are very important for a successful cell proliferation and growth. The described data are consistent with previous studies that demonstrated the promising properties of the protein and the active compound in the acceleration of the *in vitro* and *in vivo* wound healing process [65–67].

3.5. *In vivo wound healing*

In order to determine the *in vivo* wound healing efficacy of the formulations, a full thickness excision was created on the backs of Wistar rats, and then the various samples were applied daily on the wound sites. Fig. 5 shows the results as a function of the time. In detail, among the different formulations, the zein-based gels containing rutin promoted the most rapid wound contraction, followed by the empty zein-based gels, DuoDERM® and then the free drug. During the early stage of healing (first 5 days), the wound areas were significantly reduced by using the zein gels containing rutin (58 %) with respect to the control group (79 %). At day 10, this formulation also demonstrated the best healing effects with respect to the other systems, with a decrease of the

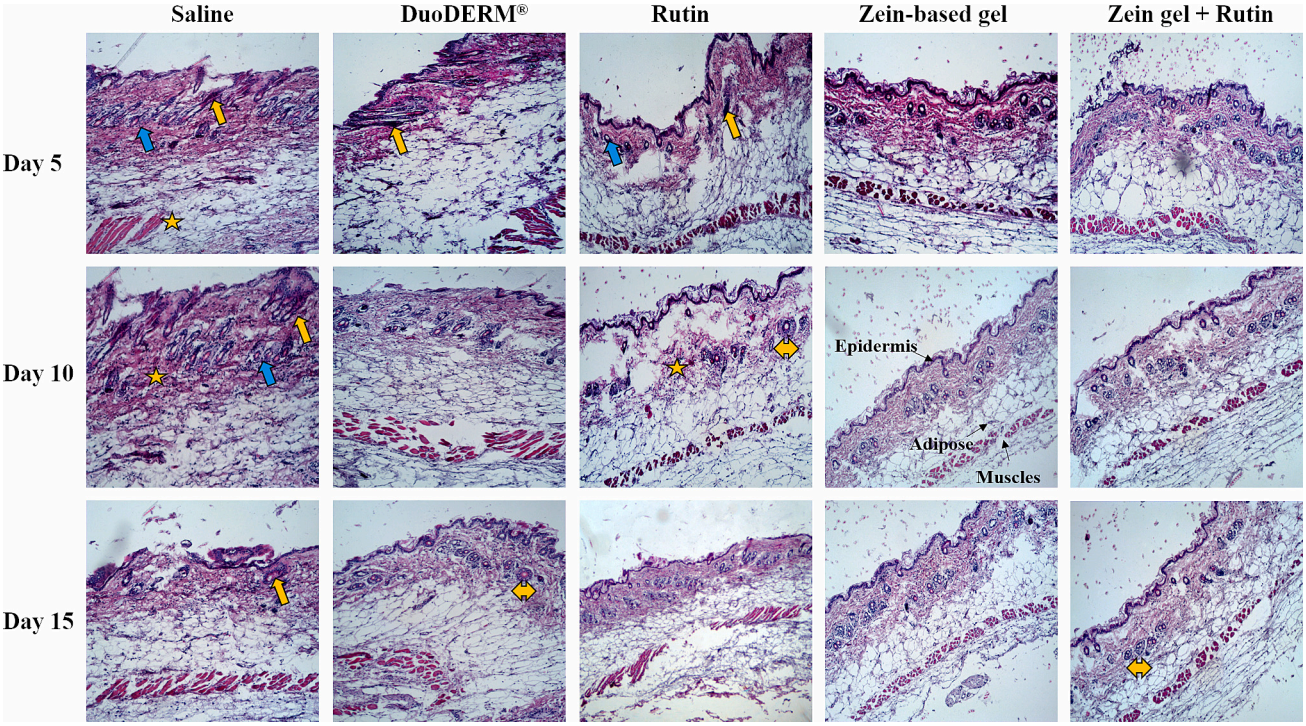

Fig. 7. Effects of saline (control), DuoDERM®, zein-based gels and zein gels containing rutin during wound healing process in Wistar rats (40× magnification with hematoxylin/eosin stain). Yellow arrow (disfigured collagen deposition), blue arrows (macrophages), Esteric: inflammatory cytokines; double-headed arrow: hair follicles with sebaceous glands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

wound area of about 90 % (Fig. 5).

After 15 days of treatment, an almost complete wound closure was observed in all groups (Fig. 5), with the exception of the control group characterized by a statistically large wound area (20 %). It's worth noting that the zein-based gel showed a similar wound healing profile compared to the commercial hydrogel DuoDERM® confirming the *in vitro* results previously discussed. These data are consistent with other studies which showed the ability of zein to improve the *in vivo* wound

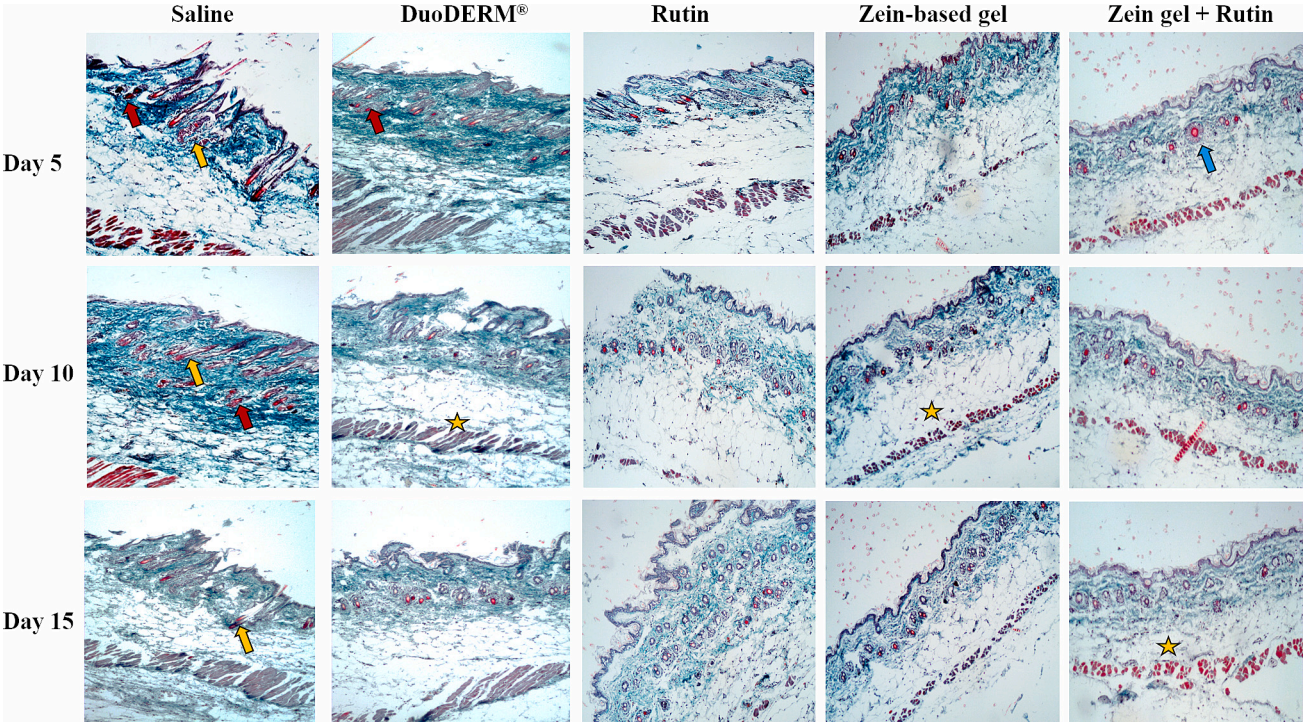

Fig. 8. Effects of saline (control), DuoDERM®, zein-based gels and zein gels containing rutin during wound healing process in Wistar rats (40× magnification with Masson's trichrome stain). Yellow arrow: disfigured collagen deposition; red arrows: macrophages; esteric: muscle cells; blue arrow: angiogenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

healing activity maintaining a physiologically moist microenvironment, promoting cell migration and proliferation and enhancing the wound contraction and closure in the early stages of the process [29,68,69]. Moreover, it is well known that rutin is able to induce a significant cell migration and to decrease the inflammatory response [70].

The results demonstrate that the entrapment of rutin within zein gel could be a plus for the acceleration of the wound healing rate due to the peculiar properties of the two biomaterials. Moreover, the prolonged release of the drug may promote a decrease of the frequency of application of the formulation, prolonging the anti-inflammatory and antioxidant effects of the active compound at the target site for a longer period.

The inflammation markers involved in the healing process were evaluated during the experiment. Pro-inflammatory cytokines are essential in the initiation, maintenance, and control of the post-wound response by the stimulation of the proliferation of keratinocytes and fibroblasts, the modulation of the immune response and chemotaxis to the wound site [71]. However, a prolonged inflammatory phase during the early stages has been identified as one of the major causes of impaired wound healing, abnormal scar formation, and further tissue damage. This is because inflammatory cells secrete numerous toxic mediators, including metalloproteases and reactive oxygen species (ROS), that can degrade the components of the extracellular matrix and growth factors, resulting in damage to the granulation tissue [72]. In particular, the anti-inflammatory properties of the formulations were assessed by measuring the serum levels of the inflammatory cytokines including TNF-α, IL-1β, and IL-6 as well as IL-10 as model of an anti-inflammatory cytokine which can contribute to tissue repair. As shown in Fig. 6, rutin-loaded zein gel showed a remarkable down-regulation of the levels of IL-1β, IL-6, TNF-α in the wounds as compared to untreated groups (all $p < 0.05$), while the expression of IL-10 was highest among all other treated groups. In particular, the expression of TNF-α, IL-6, IL-1β and IL-10 at the early stage (day 5) was (160 ± 1.2 pg/ml, 33 ± 2.1 pg/ml, 35 ± 1.7 pg/ml, 90 ± 3.2 pg/ml, respectively) as compared to the control group (200 ± 2.4 pg/ml, 80 ± 1.9 pg/ml, 88 ± 2.3 pg/ml, 40 ± 2.5 pg/ml, respectively). These results were also confirmed and became more statistically evident after 15 days of treatment. Moreover, also in this case empty zein gels showed results similar to DuoDERM®, confirming once again the peculiar anti-inflammatory properties of the vegetal protein, data in agreement with another study [14].

In addition, histopathological studies at day 5, 10 and 15 showed better epithelialization and improved re-organization of the dermis in comparison to control group (Figs. 7–8). In detail, at day 5, migration of inflammatory cells towards wound regions was observed in groups treated with zein-based gel and zein gel containing rutin. Thickening of the epidermal layer was accompanied by faster migration of fibroblast cells from dermis to epidermis in the treated groups. At day 15, the skin showed a newly-formed keratin and epidermal layer for treated wounds along with blood vessel formation, abundant collagen and hair follicles with sebaceous glands. It's important to observe that the groups treated with zein based gel and rutin-loaded zein gel showed more collagenases with no inflammatory cells compared to DuoDERM® and rutin after 15 days. On the other hand, the use of saline promoted an incomplete healing in wound tissue of rats with less epithelization, disfigured collagen deposition and presence of inflammatory cells.

4. Conclusions

The need to develop biocompatible and efficacious formulations for use in healthcare has been a driving force in technological advancement towards the use of novel biomaterials. Indeed, despite extensive research on novel wound-dressing candidates, only a few products have been approved for human application and are available on the market. As a result, there is a growing demand for the development of novel wound dressings. In fact, the treatment of wounds remains a great challenge for the healthcare system and the related annual costs are over $30 billion [73]. The present study describes an environmentally-friendly and biocompatible protein-based gel containing rutin, which showed a pseudoplastic or shear thinning behavior and good spreadability obtained without the use of toxic solvents or chemical refinement. Zein has been found to be an appealing material for wound-healing applications due to its unique set of biological properties, which include biocompatibility, biodegradability, and low toxicity. Our findings clearly demonstrated that rutin-loaded zein gels improved the healing process both *in vitro* on human keratinocytes and *in vivo* on Wistar rats. This trend is clearly related to the synergic effect of the natural protein and the active compound which brought about improved wound contraction as well as a marked modulation of the levels of pro-inflammatory and anti-inflammatory cytokines. These features open new perspectives concerning the conceivable application of rutin-loaded zein gels in the treatment of various types of wounds or lesions. However, the limitation of this gel is the need for strict operator control over the evaporation of the ethanol as well as the excessive necessary rotation speed which could stiffen or break the system. These issues will be further addressed with upcoming experiments in order to achieve a fast scale-up of the standard laboratory procedure. Obviously, a wound is considered a complex system that can be influenced by various factors including comorbidities, size, physiology and status of lesions and for these reasons the type and nature of dressing should be carefully selected. Diverse experimental investigations are still in progress in order to better evaluate the molecular mechanisms involved in the healing process of the proposed formulation with the aim of increasing its efficacy as a function of the wound status.

CRediT authorship contribution statement

Agnese Gagliardi: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Elena Giuliano:** Writing – review & editing, Software, Formal analysis, Data curation. **Silvia Voci:** Visualization, Software, Investigation. **Stefania Bulotta:** Software, Investigation, Formal analysis. **Maria Cristina Salvatici:** Investigation, Formal analysis, Data curation. **Nicola Ambrosio:** Validation, Investigation, Formal analysis. **Donatella Paolino:** Visualization, Validation, Data curation. **Farhan Siddique:** Methodology, Investigation, Formal analysis. **Muhammad Majid:** Visualization, Software, Methodology, Formal analysis. **Ernesto Palma:** Validation, Funding acquisition, Formal analysis, Data curation. **Massimo Fresta:** Writing – review & editing, Visualization, Validation, Resources. **Donato Cosco:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

Acknowledgments

The authors are grateful to Lynn Whitted for her language revision of this article.

Funding statement

This work was supported by the Italian Ministry of University and Research (PRIN2017, prot.n. 20173ZECCM_003).

Open access funding provided by "Università degli Studi Magna Graecia di Catanzaro" within the CRUI-CARE Agreement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2024.132071.

References

[1] Q. Zeng, X. Qi, G. Shi, M. Zhang, H. Haick, Wound dressing: from nanomaterials to diagnostic dressings and healing evaluations, ACS Nano 16 (2022) 1708–1733, https://doi.org/10.1021/acsnano.1c08411.

[2] M. Farahani, A. Shafiee, Wound healing: from passive to smart dressings, Adv. Healthc. Mater. 10 (2021) 2100477, https://doi.org/10.1002/adhm.202100477.

[3] I. Negut, G. Dorcioman, V. Grumezescu, Scaffolds for wound healing applications, Polymers (Basel) 12 (2020) 2010, https://doi.org/10.3390/polym12092010.

[4] C.R. Choi, E.J. Kim, T.H. Choi, J. Han, D. Kang, Enhancing human cutaneous wound healing through targeted suppression of large conductance Ca2+-activated K+ channels, Int. J. Mol. Sci. 25 (2024) 803.

[5] Y. Liang, J. He, B. Guo, Functional hydrogels as wound dressing to enhance wound healing, ACS Nano 15 (2021) 12687–12722, https://doi.org/10.1021/acsnano.1c04206.

[6] G. Jia, Z. Li, H. Le, Z. Jiang, Y. Sun, H. Liu, F. Chang, Green tea derivative-based hydrogel with ROS-scavenging property for accelerating diabetic wound healing, Mater. Des. 225 (2023) 111452.

[7] K. Wang, R. Dong, J. Tang, H. Li, J. Dang, Z. Zhang, Z. Yu, B. Guo, C. Yi, Exosomes laden self-healing injectable hydrogel enhances diabetic wound healing via regulating macrophage polarization to accelerate angiogenesis, Chem. Eng. J. 430 (2022) 132664, https://doi.org/10.1016/j.cej. 2021.132664.

[8] W. Zhao, Y. Li, X. Zhang, R. Zhang, Y. Hu, C. Boyer, F.-J. Xu, Photo-responsive supramo- lecular hyaluronic acid hydrogels for accelerated wound healing, J. Control. Release 323 (2020) 24–35, https://doi.org/10.1016/j.jconrel.2020.04.014.

[9] Y. Zhao, M. Li, J. Mao, Y. Su, X. Huang, W. Xia, X. Leng, T. Zan, Immunomodulation of wound healing leading to efferocytosis, Smart Med 3 (2024) e20230036, https://doi.org/10.1002/SMMD.20230036.

[10] B. Guo, R. Dong, Y. Liang, M. Li, Haemostatic materials for wound healing applications, Nat. Rev. Chem. 5 (2021) 773–791, https://doi.org/10.1038/s41570-021-00323-z.

[11] S.G. Hameedi, A. Saulsbery, O.O. Olutoye, The pathophysiology and management of pathologic scarring–a contemporary review, Adv. Wound Care (2024), https://doi.org/10.1089/wound.2023.0185.

[12] J. Wang, H. Cheng, W. Chen, P. Han, X. Yao, B. Tang, W. Duan, P. Li, X. Wei, P. K. Chu, An injectable, self-healing composite hydrogel with enhanced near-infrared photo-antibacterial therapeutic effects for accelerated wound healing, Chem. Eng. J. 452 (2023) 139474, https://doi.org/10.1016/j.cej.2022.139474.

[13] B. Sheokand, M. Vats, A. Kumar, C.M. Srivastava, I. Bahadur, S.R. Pathak, Natural polymers used in the dressing materials for wound healing: past, present and future, J. Polym. Sci. 61 (2023) 1389–1414.

[14] R. Yu, H. Zhang, B. Guo, Conductive biomaterials as bioactive wound dressing for wound healing and skin tissue engineering, Nano-Micro Lett. 14 (2022) 1–46, https://doi.org/10.1007/s40820-021-00751-y.

[15] S. Sharifi, M.J. Hajipour, L. Gould, M. Mahmoudi, Nanomedicine in healing chronic wounds: opportunities and challenges, Mol. Pharm. 18 (2020) 550–575, https://doi.org/10.1021/acs. molpharmaceut.0c00346.

[16] B. Azimi, H. Maleki, L. Zavagna, J.G. De la Ossa, S. Linari, A. Lazzeri, S. Danti, Bio-based electrospun fibers for wound healing, J. Funct. Biomater. 11 (2020) 67, https://doi.org/10.3390/jfb110 30067.

[17] S. Prete, M. Dattilo, F. Patitucci, G. Pezzi, O.I. Parisi, F. Puoci, Natural and synthetic polymeric biomaterials for application in wound management, J. Funct. Biomater. 14 (2023) 455.

[18] A. Sadeghianmaryan, N. Ahmadian, S. Wheatley, H.A. Sardroud, S.A.S. Nasrollah, E. Naseri, A. Ahmadi, Advancements in 3D-printable polysaccharides, proteins, and synthetic polymers for wound dressing and skin scaffolding–a review, Int. J. Biol. Macromol. 131207 (2024).

[19] A. Gaspar-Pintiliescu, A.M. Stanciuc, O. Craciunescu, Natural composite dressings based on collagen, gelatin and plant bioactive compounds for wound healing: a review, Int. J. Biol. Macromol. 138 (2019) 854–865, https://doi.org/10.1016/j.ijbiomac.2019.07.155.

[20] M. Pérez-Recalde, I.E.R. Arias, É.B. Hermida, Could essential oils enhance biopolymers performance for wound healing? A systematic review, Phytomedicine 38 (2018) 57–65, https://doi.org/10.1016/j.phymed.2017.09.024.

[21] M. Cedillo-Cortezano, L.R. Martinez-Cuevas, J.A.M. López, I.L. Barrera López, S. Escutia-Perez, V.L. Petricevich, Use of medicinal plants in the process of wound healing: a literature review, Pharmaceuticals 17 (2024) 303.

[22] M. Działo, J. Mierziak, U. Korzun, M. Preisner, J. Szopa, A. Kulma, The potential of plant phenolics in prevention and therapy of skin disorders, Int. J. Mol. Sci. 17 (2016) 160, https://doi.org/10.3390/ijms17020160.

[23] L. Moradkhannejhad, M. Abdouss, N. Nikfarjam, S. Mazinani, V. Heydari, Electrospinning of zein/propolis nanofibers; antimicrobial properties and morphology investigation, J. Mater. Sci. Mater. Med. 29 (2018) 1–10, https://doi.org/10.1007/s10856-018-6174-x.

[24] A. Gagliardi, S. Voci, S. Bonacci, G. Iriti, A. Procopio, M. Fresta, D. Cosco, SCLAREIN (SCLAREol contained in zeIN) nanoparticles: development and characterization of an innovative natural nanoformulation, Int. J. Biol. Macromol. 193 (2021) 713–720, https://doi.org/10.1016/j.ijbiomac.2021.10.184.

[25] A. Raza, Y. Zhang, U. Hayat, C. Liu, J.L. Song, N. Shen, Y. Chao, H.J. Wang, J. Y. Wang, Injectable zein gel with in situ self-assembly as hemostatic material, Biomater. Adv. 145 (2023) 213225, https://doi.org/10.1016/j.bioadv.2022.213225.

[26] A. Akhmetova, G.M. Lanno, K. Kogermann, M. Malmsten, T. Rades, A. Heinz, Highly elastic and water stable zein microfibers as a potential drug delivery system for wound healing, Pharmaceutics 12 (2020) 458, https://doi.org/10.3390/pharmaceutics12050458.

[27] S.A. El-Lakany, A.I. Abd-Elhamid, E.A. Kamoun, E.M. El-Fakharany, W.M. Samy, N. A. Elgindy, α-Bisabolol-loaded cross-linked zein nanofibrous 3d-scaffolds for accelerating wound healing and tissue regeneration in rats, Int. J. Nanomedicine 14 (2019) 8251–8270, https://doi.org/10.2147/IJN.S224315.

[28] M. Ghorbani, P. Nezhad-Mokhtari, S. Ramazani, Aloe vera-loaded nanofibrous scaffold based on Zein/Polycaprolactone/collagen for wound healing, Int. J. Biol. Macromol. 153 (2020) 921–930, https://doi.org/10.1016/j.ijbiomac.2020.03.036.

[29] J. Lin, C. Li, Y. Zhao, J. Hu, L.M. Zhang, Co-electrospun nanofibrous membranes of collagen and zein for wound healing, ACS Appl. Mater. Interfaces 4 (2012) 1050–1057, https://doi.org/10.1021/am201669z.

[30] A. Gagliardi, F. Froiio, M.C. Salvatici, D. Paolino, M. Fresta, D. Cosco, Characterization and refinement of zein-based gels, Food Hydrocoll. 101 (2020) 105555, https://doi.org/10.1016/J.FOOD-HYD.2019.105555.

[31] A. Gagliardi, S. Voci, D. Paolino, M. Fresta, D. Cosco, Influence of various model compounds on the rheological properties of zein-based gels, Molecules 25 (2020) 3174, https://doi.org/10.3390/molecules25143174.

[32] A. Ganeshpurkar, A.K. Saluja, Protective effect of rutin on humoral and cell mediated immunity in rat model, Chem. Biol. Interact. 273 (2017) 154–159, https://doi.org/10.1016/j.cbi.2017.06.006.

[33] D. Cosco, P. Failla, N. Costa, S. Pullano, A. Fiorillo, V. Mollace, M. Fresta, D. Paolino, Rutin-loaded chitosan microspheres: characterization and evaluation of the anti-inflammatory activity, Carbohydr. Polym. 152 (2016) 583–591, https://doi.org/10.1016/j.carbpol. 2016.06.039.

[34] E. Giuliano, M. Fresta, D. Cosco, Development and characterization of poloxamine 908-hydrogels for potential pharmaceutical applications, J. Mol. Liq. 337 (2021) 116588, https://doi.org/10.1016/j.molliq.2021.116588.

[35] E. Giuliano, D. Paolino, M.C. Cristiano, M. Fresta, D. Cosco, Rutin-loaded Poloxamer 407-based hydrogels for in situ administration: stability profiles and rheological properties, Nanomaterials 10 (2020) 1069, https://doi.org/10.3390/nano10061069.

[36] A. Gagliardi, D. Paolino, N. Costa, M. Fresta, D. Cosco, Zein-vs PLGA-based nanoparticles containing rutin: a comparative investigation, Mater. Sci. Eng. C 118 (2020) 111538, https://doi.org/10.1016/j.msec.2020.111538.

[37] C.C. Liang, A.Y. Park, J.L. Guan, In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro, Nat. Protoc. 2 (2007) 329–333, https://doi.org/10.1038/nprot.2007.30.

[38] N. Pettinelli, S. Rodriguez-Llamazares, R. Bouza, L. Barral, S. Feijoo-Bandin, F. Lago, Carrageenan-based physically crosslinked injectable hydrogel for wound healing and tissue repairing applications, Int. J. Pharm. 589 (2020) 119828, https://doi.org/10.1016/j.ijpharm.2020.119828.

[39] X. Zhang, X. Kang, L. Jin, J. Bai, W. Liu, Z. Wang, Stimulation of wound healing using bioinspired hydrogels with basic fibroblast growth factor (bFGF), Int. J. Nanomedicine 13 (2018) 3897 (https://doi:0.2147/IJN.S168998).

[40] T. Shanmugasundaram, M. Radhakrishnan, V. Gopikrishnan, K. Kadirvelu, R. Balagurunathan, In vitro antimicrobial and in vivo wound healing effect of actinobacterially synthesised nanoparticles of silver, gold and their alloy, RSC Adv. 7 (2017) 51729–51743, https://doi.org/10.1039/C7RA08483H.

[41] S. Ali, M.R. Khan, R. Batool, S. Maryam, M. Majid, Wound healing potential of oil extracted from Parrotiopsis jacquemontiana (Decne) Rehder, J. Ethnopharmacol. 236 (2019) 354–365, https://doi.org/10.1016/j.jep.2019.03.018.

[42] S. Voci, A. Gagliardi, R. Molinaro, M. Fresta, D. Cosco, Recent advances of taxol-loaded biocompatible nanocarriers embedded in natural polymer-based hydrogels, Gels 7 (2021) 33, https://doi.org/10.3390/gels7020033.

[43] B. Medronho, A. Filipe, C. Costa, A. Romano, B. Lindman, H. Edlund, M. Norgren, Microrheology of novel cellulose stabilized oil-in-water emulsions, J. Colloid Interface Sci. 531 (2018) 225–232, https://doi.org/10.1016/j.jcis.2018.07.043.

[44] F. Niu, M. Ahmad, J. Fan, C. Ritzoulis, J. Chen, Z. Luo, W. Pan, The application of diffusing wave spectroscopy (DWS) in soft foods, Food Hydrocoll. 96 (2019) 671–680, https://doi.org/10.1016/j. foodhyd.2019.06.011.

[45] D. Yuan, X. Huang, Q. Meng, J. Ma, Y. Zhao, Q. Ke, X. Kou, Recent advances in the application of zein-based gels: a review, Eur. Polym. J. 111557 (2022), https://doi.org/10.1016/j. eurpolymj.2022.111557.

[46] Y. Tan, J. Wang, F. Chen, S. Niu, J. Yu, Effect of protein oxidation on kinetics of droplets stability probed by microrheology in O/W and W/O emulsions of whey protein concentrate, Food Res. Int. 85 (2016) 259–265, https://doi.org/10.1016/j.foodres.2016.05.005.

[47] Y. Zhang, X. Kong, L. Gao, Z. Lu, S. Zhou, B. Dong, F. Xing, In-situ measurement of viscoelastic properties of fresh cement paste by a microrheology analyzer, Cem. Concr. Res. 79 (2016) 291–300, https://doi.org/10.1016/j.cemconres.2015.09.020.

[48] N. Jia, S. Lin, Y. Yu, G. Zhang, L. Li, D. Zheng, D. Liu, The effects of ethanol and rutin on the structure and gel properties of whey protein isolate and related mechanisms, Foods 11 (2022) 3480, https://doi.org/10.3390/foods11213480.

[49] X. Qiao, F. Liu, Z. Kong, Z. Yang, L. Dai, Y. Wang, Q. Sun, D.J. McClements, X. Xu, Pickering emulsion gel stabilized by pea protein nanoparticle induced by heat-assisted pH-shifting for curcumin delivery, J. Food Eng. 350 (2023) 111504, https://doi.org/10.1016/j.jfoodeng. 2023.111504.
[50] M.H. Tunick, Small-strain dynamic rheology of food protein networks, J. Agric. Food Chem. 59 (2011) 1481–1486, https://doi.org/10.1021/jf1016237.
[51] K.D. Mattice, A.G. Marangoni, Evaluating the use of zein in structuring plant-based products, Curr. Res. Food Sci. 3 (2020) 59–66, https://doi.org/10.1016/j.crfs.2020.03.004.
[52] Y. Lu, Y. Ma, Y. Zhang, Y. Gao, L. Mao, Facile synthesis of zein-based emulsion gels with adjustable texture, rheology and stability by adding β-carotene in different phases, Food Hydrocoll. 124 (2022) 107178, https://doi.org/10.1016/j.foodhyd.2021.107178.
[53] C. Li, L. Chen, D.J. McClements, X. Peng, C. Qiu, J. Long, H. Ji, J. Zhao, X. Zhou, Z. Jin, Preparation and characterization of rutin–loaded zein–carboxymethyl starch nano-particles, Foods 11 (2022) 2827, https://doi.org/10.3390/foods11182827.
[54] S. Zhang, Y. Han, Preparation, characterisation and antioxidant activities of rutin-loaded zein-sodium caseinate nanoparticles, PLoS One 13 (2018) e0194951, https://doi.org/10.1371/journal.pone.0194951.
[55] A.M. Salgado, O.K. Ozturk, B.R. Hamaker, O.H. Campanella, Matching textural properties of commercial meat and cheese products using zein as the viscoelastic agent and calcium hydroxide as the textural modifier in plant-based formulations, Food Hydrocoll. 137 (2023) 108323, https://doi.org/10.1016/j.foodhyd.2022.108323.
[56] R. Lai, Y. Liu, J. Liu, Properties of the konjac glucomannan and zein composite gel with or without freeze-thaw treatment, Food Hydrocoll. 117 (2021) 106700, https://doi.org/10.1016/j.foodhyd. 2021.106700.
[57] M. Lenzuni, G. Suarato, D. Miele, R. Carzino, M. Ruggeri, R. Bertorelli, G. Sandri, A. Athanassiou, Development of biodegradable zein-based bilayer coatings for drug-eluting stents, RSC Adv. 11 (2021) 24345–24358, https://doi.org/10.1039/D1RA03748J.
[58] C. Reboredo, C.J. González-Navarro, C. Martínez-Oharriz, A.L. Martínez-López, J. M. Irache, Preparation and evaluation of PEG-coated zein nanoparticles for oral drug delivery purposes, Int. J. Pharm. 597 (2021) 120287, https://doi.org/10.1016/j.ijpharm.2021.120287.
[59] M.K. Remanan, F. Zhu, Encapsulation of rutin using quinoa and maize starch nanoparticles, Food Chem. 353 (2021) 128534, https://doi.org/10.1016/j.foodchem.2020.128534.
[60] J. Zhu, Z. Li, C. Wu, G. Fan, T. Li, D. Shen, J. Dou, Y. Liang, Insight into the self-assembly behavior of α-zein by multi-spectroscopic and molecular simulations: an example of combination with the main component of jujube peel pigments–Rutin, Food Chem. 404 (2023) 134684, https://doi.org/10.1016/j.foodchem.2022.134684.
[61] S. Zhang, H. Zhao, Preparation and properties of zein–rutin composite nanoparticle/corn starch films, Carbohydr. Polym. 169 (2017) 385–392, https://doi.org/10.1016/j.carbpol.2017.04.044.
[62] A.C. Alavarse, F.W. de Oliveira Silva, J.T. Colque, V.M. da Silva, T. Prieto, E. C. Venancio, J.J. Bonvent, Tetracycline hydrochloride-loaded electrospun nanofibers mats based on PVA and chitosan for wound dressing, Mater. Sci. Eng. C 77 (2017) 271–281, https://doi.org/10.1016/j. msec.2017.03.199.
[63] A. Basu, G. Celma, M. Strømme, N. Ferraz, In vitro and in vivo evaluation of the wound healing properties of nanofibrillated cellulose hydrogels, ACS Appl. Bio Mater. 1 (2018) 1853–1863, https://doi.org/10.1021/acsabm.8b00370.
[64] I. Pastar, O. Stojadinovic, N.C. Yin, H. Ramirez, A.G. Nusbaum, A. Sawaya, S. B. Patel, L. Khalid, R.R. Isseroff, M. Tomic-Canic, Epithelialization in wound healing: a comprehensive review, Adv. Wound Care 3 (2014) 445–464, https://doi.org/10.1089/wound.2013.0473.
[65] N.Q. Tran, Y.K. Joung, E. Lih, K.D. Park, In situ forming and rutin-releasing chitosan hydrogels as injectable dressings for dermal wound healing, Biomacromolecules 12 (2011) 2872–2880, https://doi.org/10.1021/bm200326g.
[66] F. Irfan, F. Jameel, I. Khan, R. Aslam, S. Faizi, A. Salim, Role of quercetin and rutin in enhancing the therapeutic potential of mesenchymal stem cells for cold induced burn wound, Regen. Ther. 21 (2022) 225–238, https://doi.org/10.1016/j.reth.2022.07.011.
[67] A. Martin, J. Cai, A.L. Schaedel, M. van der Plas, M. Malmsten, T. Rades, A. Heinz, Zein-polycaprolactone core-shell nanofibers for wound healing, Int. J. Pharm. 621 (2022) 121809, https://doi.org/10.1016/j.ijpharm.2022.121809.
[68] H. Chen, J. Su, C.S. Brennan, P. Van der Meeren, N. Zhang, Y. Tong, P. Wang, Recent developments of electrospun zein nanofibres: strategies, fabrication and therapeutic applications, Mater. Today Adv. 16 (2022) 100307, https://doi.org/10.1016/j.mtadv.2022.100307.
[69] M. Surendranath, R. Rajalekshmi, R.M. Ramesan, P. Nair, R. Parameswaran, UV-crosslinked electrospun zein/PEO fibroporous membranes for wound dressing, ACS Appl. Bio Mater. 5 (2022) 1538–1551, https://doi.org/10.1021/acsabm.1c01293.
[70] L.Y. Chen, C.N. Huang, C.K. Liao, H.M. Chang, Y.H. Kuan, T.J. Tseng, K.J. Yen, K. L. Yang, H.C. Lin, Effects of rutin on wound healing in hyperglycemic rats, Antioxidants (Basel, Switzerland) 9 (2020) 1122, https://doi.org/10.3390/antiox9111122.
[71] M.A. Alsakhawy, D.A. Abdelmonsif, M. Haroun, S.A. Sabra, Naringin-loaded Arabic gum/pectin hydrogel as a potential wound healing material, Int. J. Biol. Macromol. 222 (2022) 701–714, https://doi.org/10.1016/j.ijbiomac.2022.09.200.
[72] H.S. Kim, X. Sun, J.H. Lee, H.W. Kim, X. Fu, K.W. Leong, Advanced drug delivery systems and artificial skin grafts for skin wound healing, Adv. Drug Deliv. Rev. 146 (2019) 209–239, https://doi.org/10.1016/j.addr.2018.12.014.
[73] B.R. Freedman, C. Hwang, S. Talbot, B. Hibler, S. Matoori, D.J. Mooney, Breakthrough treatments for accelerated wound healing, Sci. Adv. 9 (2023) eade7007, https://doi.org/10.1126/sciadv.ade7007.