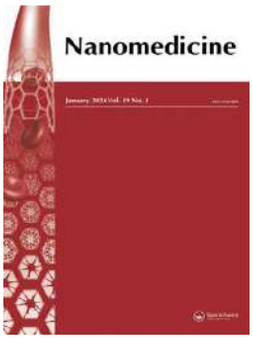

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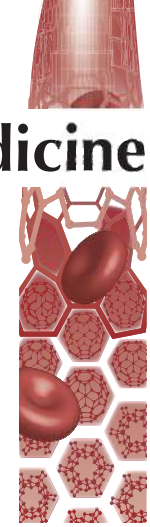

Antibacterial and wound-healing potential of PLGA/spidroin nanoparticles: a study on earthworms as a human skin model

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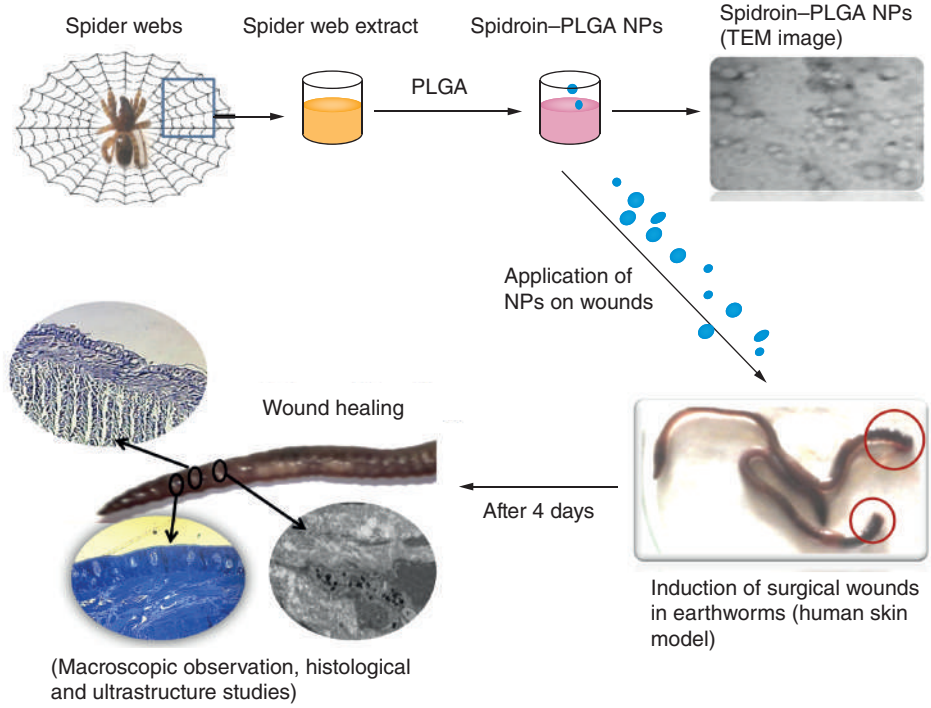
Aim: The essential protein element of spider silk 'spidroin' was used to assess its impact on the wound-healing process. **Methods:** Spidroin nanoparticles (NPs) were prepared using poly(lactic-co-glycolic acid) polymer (PLGA/spidroin NPs) at different weight ratios (5:1, 10:1, 15:1) and were *in vitro* characterized. The optimized NPs were tested *in vitro* for release and antibacterial activity. To assess wound-healing effects, NPs were topically applied on surgically induced injuries in *Allolobophora caliginosa* earthworms as a robust human skin model. **Results:** Optimized NPs (173 ± 3 nm) revealed considerable antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*. After 4 days of NPs application on wounds, macroscopical and histological examinations revealed a significant reduction in wound and re-epithelialization times. **Conclusion:** PLGA/spidroin NPs may represent a promising option for wound repair.

Graphical abstract:



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Keywords: earthworms • PLGA/spidroin nanoparticles • spider silk • spidroin • wound healing

Future Medicine

Spiders are the arthropods' pre-eminent silk craftsmen, known for their aerial orb nets used to trap flying insects [1]. Spiders develop up to seven various forms of silk from various abdominal glands [2]. This spider silk is characterized by strength and flexibility. Spiders' silk is a proteinaceous fiber. Spidroin is the predominant ampullate silk protein that spiders produce [3]. Spider silk has been used in several applications, including wound healing and military applications [4–6].

Nanoparticles (NPs) are a delivery system applied to most administration routes and can be used as potential carriers for various drugs, including proteins. NPs can be used for topical application on injuries to accelerate regenerative properties and promote healing. In general, NP-based wound healing can be attributed to cell adhesion, increased penetration at the wound sites and controlled and sustained drug release from NPs [7,8].

In this study, spidroin was encapsulated in NPs delivery system to be applied directly on the wound. Polymer-based NP systems (polymeric NPs) represent a popular platform in drug delivery due to the availability of different kinds of polymers, biocompatibility, biodegradability and the ability to prolong drug release and protect the drug against degradation [9–12].

PLGA is one of the major commonly used polymers in the fabrication of NPs to encapsulate the drug [13]. PLGA is a biodegradable polymer; it is biologically hydrolyzed into the quickly metabolized components (lactic and glycolic acid). PLGA NPs can be developed using a nano-precipitation technique, which depends on the interfacial deposition of polymers [14,15]. This technique is characterized by reproducibility and ease of preparation. The use of PLGA in drug delivery is confirmed by the US FDA and the EMA [16].

This work aims to develop PLGA NPs of spidroin to be applied on injuries, which are surgically induced in earthworms, and to continuously release the drug.

Earthworms belong to the phylum Annelida and are the major controller of soil organic matter dynamics and soil structure [17]. Earthworms are invasive species worldwide; there are more than 4000 species of earthworms [18], with only 120 of them being widely distributed [19]. In many soils, earthworms are the primary source of animal biomass [20].

In medicine, earthworms have been used for different purposes. Earthworm paste was shown to provide promising gastric cytoprotection [21]. Additionally, phototoxic effects of solar UV radiation were studied on the skin of earthworms due to the existence of similar photosensitive biomolecules as in human skin [22,23]. Importantly, our previous work shows that earthworms can be efficiently and economically used as an alternative human skin model for studying wound-healing efficiency of formulations such as spidroin/carbopol-based gel [5]. The use of earthworms as a human skin model is based on the presence of triene and tetraene sterols in the skin of both earthworms and human [22].

Materials & methods

Spidroin was extracted from the collected spider web. Bradford protein assay and bovine serum albumin (BSA) reagents were obtained from Sigma Chem. Co. (MO, USA). PLGA (25,000–35,000 Da; lactic acid:glycolic acid ratio = 60:40) was purchased from Polyscitech-Akina Inc. (IN, USA). All other chemicals were of pharmaceutical grade and were used in their original state.

Collection of spider webs & extraction of spidroin

The collection of spider webs and extraction of the protein content (spidroin) have been previously described [5]. Briefly, spider silks of *Tegenaria domestica* and *Araneus diadematus* were collected from various locations and gently washed. The extraction process was performed according to the modified NaOH method [24], where the dried spider silks (1 g) in NaOH solution (0.1 M, 10 ml) were heated for 1 h at 90°C under magnetic stirring. After cooling, the dispersions were centrifuged (5000 r.p.m., 30 min), and the supernatant was filtered and kept at 4°C for quantification, formulation and evaluation (*in vitro* and *in vivo*).

Protein content quantification

Bradford reagent (2.5 ml) was used to assess total protein content [25]. Bradford reagent was added to the samples and incubated at room temperature for 15 min. The protein content of the obtained blue solution was analyzed colorimetrically at 595 nm against a blank and determined using BSA calibration curve.

Fabrication of spidroin nanoparticles

PLGA was completely dissolved in dichloromethane and acetone (1:4 solvent ratio). The PLGA solution was diluted with acetone and dropped gradually into an aqueous phosphate-buffered solution (pH 6) containing spidroin extract and 0.5% of pluronic F-68 (PF-68) under continuous stirring for 3 h. NPs at different weight ratios of PLGA:Spidroin (5:1, 10:1, 15:1) were obtained via centrifugation (16,000 r.p.m., 4°C for 20 min).

Characterization of spidroin nanoparticles

Particle size & zeta potential

The PLGA/spidroin particles were dispersed in distilled water or phosphate buffer (pH 6.8) to determine the size and zeta potential, respectively, with Zetasizer Nano-ZS (Malvern, UK).

Entrapment efficiency & drug loading

The protein content entrapped inside NPs was indirectly measured by Bradford assay as mentioned earlier [25], and the following equations are used to calculate entrapment efficiency (EE%) and drug loading (DL%):

$$EE\% = \frac{\text{Entrapped protein in NPs}}{\text{Total amount}} \times 100$$

$$DL\% = \frac{\text{Entrapped protein in NPs}}{\text{Weight of NPs}} \times 100$$

Transmission electron microscopy

The morphology of the selected NPs (PLGA/spidroin weight ratio of 15:1) was examined using transmission electron microscopy (TEM). NPs were deposited on carbon-coated copper grids and negatively stained with uranyl acetate (2%). The TEM image was taken with a transmission electron microscope (JEM-100CX II, JEOL, Tokyo, Japan).

In vitro release

The *in vitro* release of proteins from optimized NPs was studied using standard semipermeable cellophane membrane (Spectra/Por, molecular weight cutoff of 12–14 kDa). Briefly, NPs were dispersed in 1 ml water and transferred to the release tube, which was one side-capped with the membrane. Release tubes were immersed in a beaker containing 25 ml of release medium (phosphate buffer pH 6.8). Beakers were then placed into a shaking water bath (37 ± 0.2°C). At predetermined time points, samples were withdrawn and the protein content was colorimetrically measured at 595 nm using Bradford assay.

In vitro antibacterial activity

The antibacterial efficacy of the selected NPs (PLGA/spidroin weight ratio of 15:1) was studied on both Gram-positive and Gram-negative bacteria using the agar-well diffusion method as previously described [5,26,27]. Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA), and Gram-negative bacteria such as *Escherichia coli* were used in this study to determine the inhibition zones.

In vivo evaluation of spidroin nanoparticles

The effect of the selected spidroin NPs (15:1) on wound healing was studied *in vivo* on earthworms.

Earthworms

The earthworms were obtained from the garden of Faculty of Science, Assiut University, Egypt. Worms were transported to the laboratory of the Zoology Department, Faculty of Science. Worms of approximately the same length and weight were selected and adapted for 2 weeks under laboratory conditions (25–28°C with a 12 h light:12 h darkness) to make them habitual of the laboratory conditions. The earthworms were nourished with dried cow manure applied to the soil surface (1% of the total weight of soil) and contained in plastic containers filled with soil.

Earthworms were placed in four groups (10 earthworms each). One group (group I) was used as a control (untreated worms). In groups II, III and IV, earthworms were cut in the frontal part (first 10 segments) using a sterile disposable scalpel piercing the earthworm's skin.

Treatment of worms

Earthworms of group I were not cut or treated. The induced cut in worms of group II was kept without treatment and subjected to natural healing. Plain PLGA NPs (no spidroin) were applied to the cut of group III worms, whereas the injuries induced in group IV were treated with PLGA/spidroin NPs. In groups III and IV, the treatment was applied on the cut three times daily for 4 days. During the experiment, the worms were kept in a Petri dish containing pieces of moistened filter paper to ensure a humid environment.

Measurement of wound area

Wound region was measured after every day. The reduction rate in wound area was quantified in millimeters.

Histological investigations

Tissue parts (5 μm) from various groups were placed on slides and dried overnight at 37°C. They were then dewaxed in xylene and hydrated in measured sequences of alcohols before being stained with hematoxylin and eosin [28].

Ultrastructural study

Preparation for semithin sections

After fixation in 4% cold glutaraldehyde, the earthworms were rinsed three or four times (20 min each) in phosphate buffer (pH 7.2), and postfixed in 1% osmium tetroxide (OsO_4) for 2 h, then rinsed in the same buffer four times. Dehydration was accomplished by gradually increasing the concentration of ethyl alcohol. Tissue specimens were immersed in propylene oxide for 30 min to eliminate alcohol residues and then immersed in propylene oxide plus Epon 812 (1:1, v/v) for another 30 min before being immersed in Epon 812 for 4 h. After inserting the tissue blocks into capsules, including the embedding mixture, they were polymerized in an oven at 60°C for 2 days. The LKB ultramicrotome was used to cut semithin parts of 0.5-μm thickness that were then stained with toluidine blue [29].

Preparation for TEM

Semithin parts were tested for tissue localization, and accordingly ultrathin parts were prepared as required. Ultrathin parts (50–80 nm) were cut by Leica AG ultramicrotome, stained with uranyl acetate and lead citrate. Parts from different groups of earthworms were examined under TEM (JEOL, 100 CXII) operated at 80 kV. For the chosen semithin areas, electron micrographs were obtained, reconstructed, and analyzed using Photoshop software to analyze the chosen samples. The results were presented as micrographs.

Results

The protein content (spidroin), which was extracted from the spider silks, was formulated as NPs to evaluate its wound-healing effect. As previously mentioned, the simple and effective modified NaOH method was used to extract the protein content of spider silks.

PLGA/spidroin nanoparticles

At various weight ratios of PLGA/spider web protein (5:1, 10:1 and 15:1), NPs were fabricated and optimized. Dropping of PLGA organic solution resulted in the encapsulation of the spider silks protein inside the polymer. The prepared PLGA/spidroin particles showed different sizes in the range of 173 ± 3 nm to 212 ± 14 nm (Figure 1). The particle size of these NPs decreased with increasing the content of PLGA, reaching a size of 173 ± 3 nm at the highest PLGA/spidroin ratio (15:1). Additionally, all the fabricated NPs showed a good polydispersity index (PDI), which is less than 0.5.

The surface of the different fabricated PLGA NPs was negatively charged (Figure 1). The surface charge was not significantly different in all the formulations (-21 ± 1 mV). All the formulations showed nonsignificantly different EE%, which was >60% (Figure 2). However, DL% decreased with increasing the PLGA/spider web protein weight ratio.

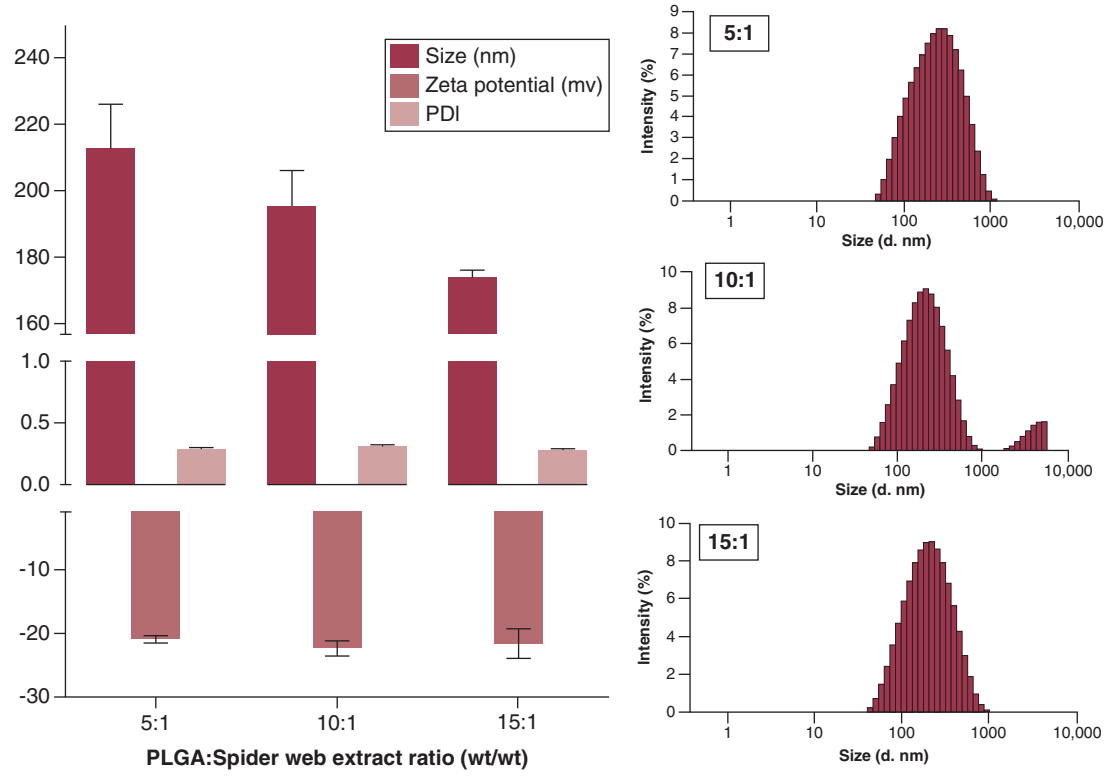

Figure 1. Characterization of poly(lactic-co-glycolic acid)/spider web protein nanoparticles at different weight ratios.
PDI: Polydispersity index; PLGA: Poly(lactic-co-glycolic acid).

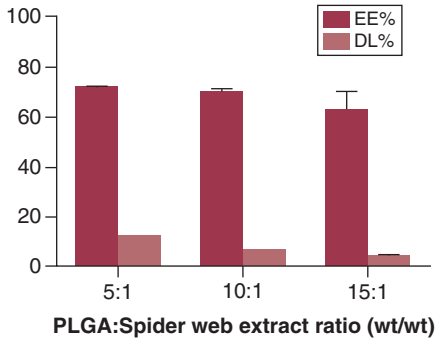

Figure 2. Entrapment efficiency and drug loading of poly(lactic-co-glycolic acid)/spider web protein nanoparticles.
DL: Drug loading; EE: Entrapment efficiency; PLGA: Poly(lactic-co-glycolic acid).

On the basis of previous studies, PLGA/spider web protein NPs, which were formed at weight ratio of 15:1, were selected for the further *in vitro* and *in vivo* studies. The morphology of these selected NPs formulation was revealed from the TEM micrograph (Figure 3A). It appeared as spherical particles. Figure 3B shows the initial burst release of the protein content from PLGA matrix at the first 15 min, followed by slow release over 6 h.

Antibacterial effect of PLGA/spidroin nanoparticles

PLGA/spidroin NPs (weight ratio of 15:1) produced a considerable antibacterial effect against some of the tested bacteria (*S. aureus* and *E. coli*). Table 1 shows the inhibition zones produced by the optimized PLGA/spidroin NPs. The entire antibacterial effect was attributed to spider silk extract because blank PLGA NPs had no antibacterial effect. This promising antibacterial effect of PLGA/spidroin NPs reveals its suitability as a promoter for wound healing.

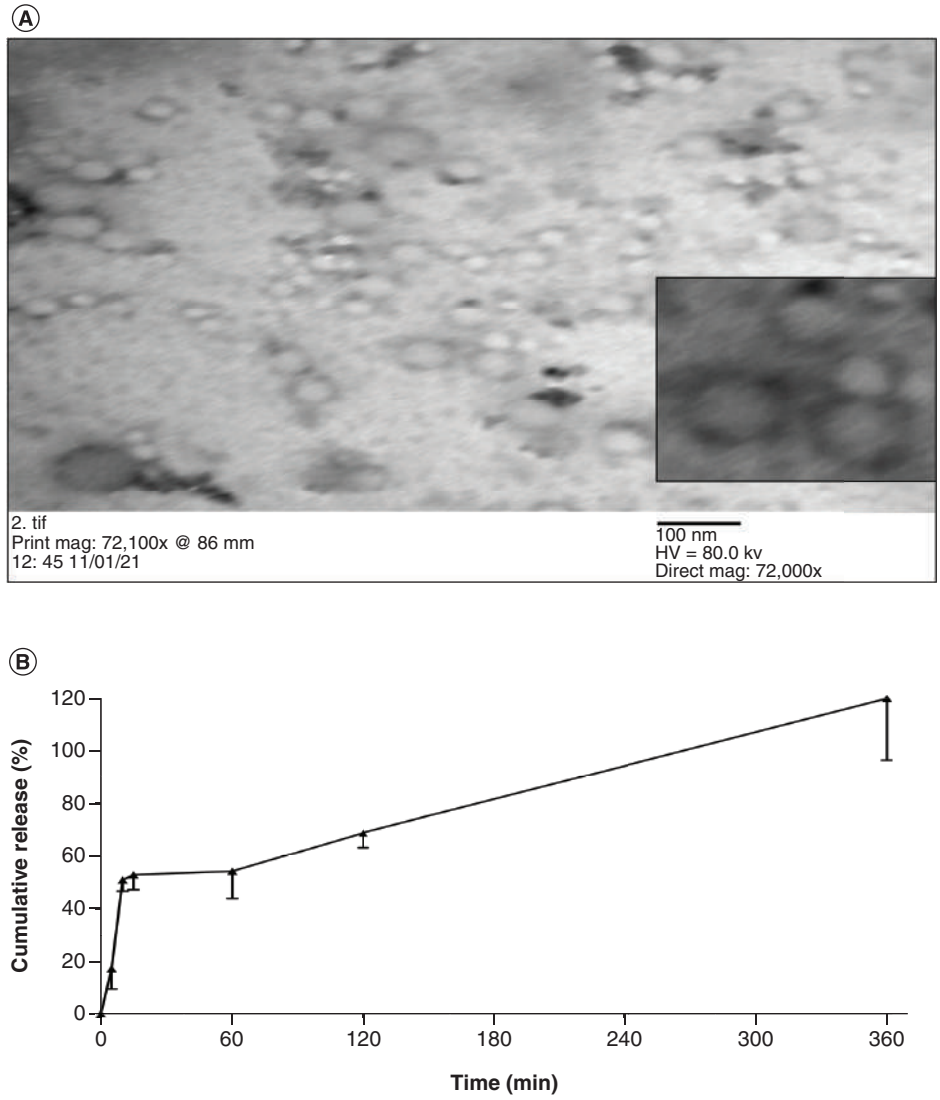

Figure 3. Characterization of poly(lactic-co-glycolic acid)/spider web protein nanoparticles. (A) Transmission electron micrograph shows spherical poly(lactic-co-glycolic acid)/spidroin nanoparticles. **(B)** Percentage release of loaded protein from poly(lactic-co-glycolic acid) nanoparticles.

Table 1. Antibacterial activity of poly(lactic-co-glycolic acid)/spidroin nanoparticles on Gram-positive and Gram-negative bacteria.

Bacteria	Inhibition zone diameter (mm)
Methicillin-sensitive *Staphylococcus aureus*	22 ± 1
Methicillin-resistant *Staphylococcus aureus*	18 ± 2
Escherichia coli	15 ± 2

Macroscopic observation of wound healing

All the earthworms groups with the surgically induced cut (II, III and IV) experienced hemorrhage, redness, edema and exudation around the wound region on the first day. Group II (untreated control) and group III (received plain PLGA NPs; no spidroin) took longer than 12 days to heal normally. However, the group receiving the selected PLGA/spidroin NPs (group IV) was coagulated the fastest and showed the best results (Figure 4). The spider silk proteins, which have effective antimicrobial activity against a wide spectrum of pathogenic bacteria (Table 1) and can endure proliferation of the cell, are responsible for the rapid healing of injuries. In addition, compared with

Figure 4. Macroscopic observation of the different groups of earthworms (*Allolobophora caliginosa*) after induction of surgical wounds and examination of wound healing. (A) Worms were left untreated to heal naturally. **(B)** Worms received PLGA nanoparticles (no spidroin). **(C)** Worms received spidroin-PLGA NPs (injury to the earthworms [square] and cut in the first six segments [circle]).
PLGA: Poly(lactic-co-glycolic acid); NP: Nanoparticle.

natural healing, spider silk treatment resulted in a significant reduction in wound closure and re-epithelialization time.

Histological study

Epidermal, longitudinal and circular layers are indicative of three layers of earthworm skin. On the fourth day of the experiment, the transverse part moving through the body wall of control worms (group I) revealed the usual epidermal, longitudinal and circular muscle structure (Figure 5A).

Transverse sections of groups II and III that were left untreated or treated with plain PLGA NPs showed vacuolization and epithelial cell hypertrophy, as well as degeneration of circular and longitudinal muscles (Figure 5B & C). There was no alteration in the epidermal layer throughout healing. In comparison, the thickness of the longitudinal and circular muscles was decreased (Figure 5B & C). In the case of group IV, where the worms treated with PLGA/spidroin NPs, the transverse sections revealed that the inflammatory cells had almost completely vanished, and the body wall was composed of circular, epidermal and longitudinal muscles (Figure 5D).

Semithin sections (electron microscopical observation)

Earthworms in the control group had normal architecture and intact longitudinal and circular muscles, as seen in a photomicrograph of a semithin segment (Figure 6A & B). Semithin sections of groups II (untreated control) and III (treated with plain PLGA NPs) showed a loss of design and a proclivity for excessive glandular epithelium, as well as the destruction of the ectodermal layer, cuticular membrane and a widening of the gaps among longitudinal muscles, based on the impact of the cut, which may be due to necrosis (Figure 6C & D). Wound and inflammatory cells vanished in semithin portions of group IV, which received the PLGA/spidroin NPs. The circular, epidermal and longitudinal muscles in the body wall were all intact after the wound was closed (Figure 6E & F).

TEM observation

The TEM micrographs of the skin of *A. caliginosa* of the control group are obsessive (Figure 7A & B). Additionally,

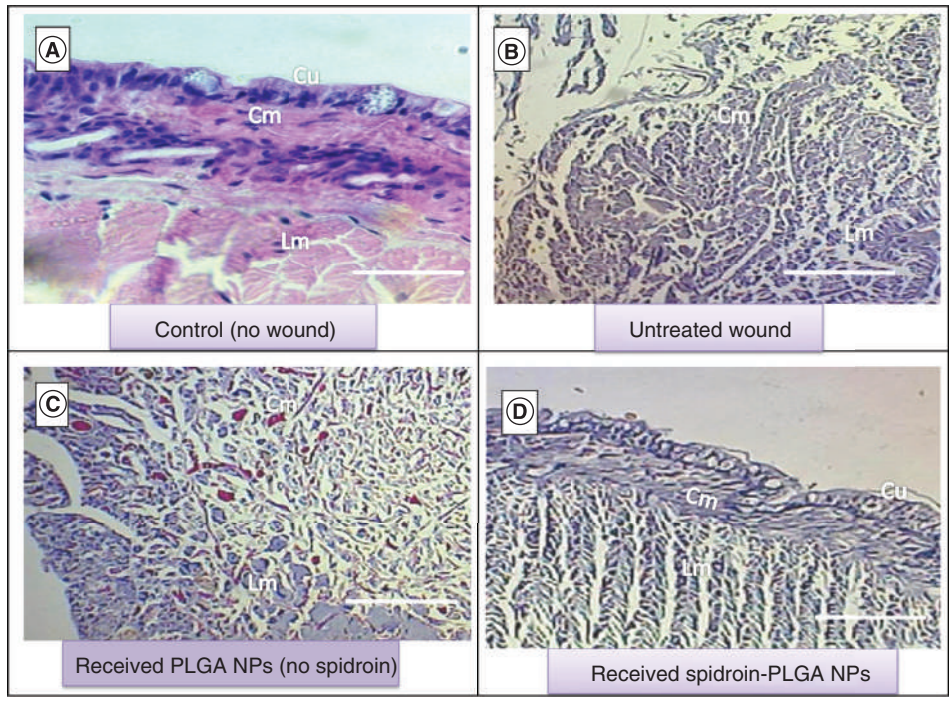

Figure 5. Photomicrographs of cross section of the earthworms (*Allolobophora caliginosa*) for all four groups. (A) Earthworm section in control group (no wound). **(B)** Worms in which wounds were left untreated. **(C)** Worms that received PLGA nanoparticles (NPs; no spidroin). **(D)** Worms that received spidroin-PLGA NPs (hematoxylin and eosin). Scale bar = 50 μm.
Cm: Circular muscle; Cu: Cuticle; Ep: Epidermis; Lm: Longitudinal muscle; PLGA: Poly(lactic-co-glycolic acid); NP: Nanoparticle.

clear damage was observed into the earthworm group that received plain PLGA NPs (Figure 7C & D), with the cuticle beginning to degrade the epidermis and circular muscles severely necrotic. Circular muscles likewise were totally damaged. The intercellular matrix was edematous and loose, allowing for minute vessel extension and the creation of new capillaries. Furthermore, the proliferation of fibroblasts was observed. However, the skin of *A. caliginosa* earthworms that were treated with PLGA/spidroin NPs showed no significant difference compared with the control group (Figure 7E & F). Capillaries, fibroblasts and collagen all develop in response to a wound, forming granulation tissue.

Discussion

In the modified NaOH method, heating spider silks in NaOH removed the outer shell of spider silks, exposing the protein content. This protein was collected and quantified with Bradford assay, then the protein was encapsulated in the matrix of PLGA. PLGA is a commonly used synthetic, biocompatible and biodegradable polymer. PLGA NPs were fabricated, optimized and applied on the surgically induced wounds in the skin of earthworms as a human skin model. The use of NPs as a topical delivery system for treatment of wounds offers several advantages [7,8].

The particle size of the fabricated NPs decreased with increasing the PLGA/spidroin ratio. The decrease of particle size could be attributed to effective condensation of spider silk proteins, and the presence of pluronic decreased the particle aggregation, improving the polydispersity index (PDI). The reduction of particle aggregation could be attributed to the presence of pluronic F-68, which act as a stabilizer. The size and morphology of PLGA NPs were also demonstrated on the TEM image (Figure 3A). PF-68 is a neutral low molecular weight polymer. Its presence as a stabilizer causes slight reduction of the PLGA negative charge [30]. %EE of PLGA formulations were nonsignificantly different (>60%) with different PLGA/spider web protein weight ratios. This high %EE might be due to the high viscosity of the PLGA solution, which reduces the drug diffusion into surrounding aqueous solutions. However, the increase in polymer content resulted in significant reduction of %DL. The entrapped drug in the optimized PLGA NPs showed slow release of the drug from PLGA matrix over 6 h; however, the initial burst release could be attributed to any adsorbed proteins on the surface of NPs.

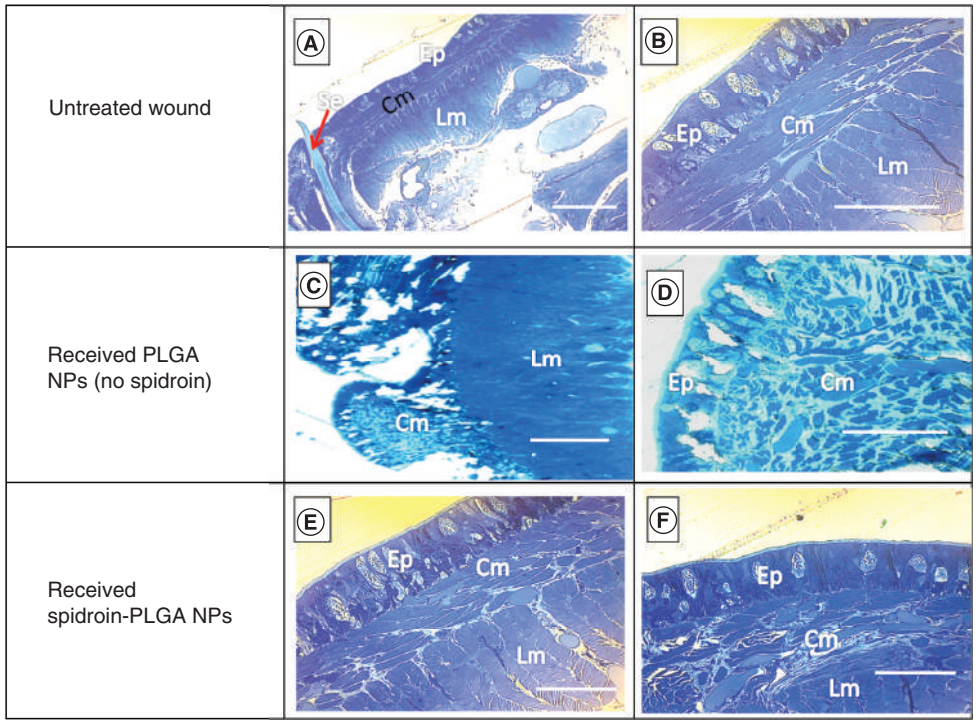

Figure 6. Photomicrographs of semithin sections of the earthworms (*Allolobophora caliginosa*) of different groups. (A & B) The control group received no wounds. **(C & D)** Worms received plain PLGA nanoparticles (NPs; no spidroin). **(E & F)** Worms received PLGA/spidroin NPs. Toluidine blue stain: scale bar = 50 μm.
Cm: Circular muscle; Ep: Epidermis; Lm: Longitudinal muscle; PLGA: Poly(lactic-co-glycolic acid); NP: Nanoparticle; Se: Setae.

The antibacterial effect of these optimized spidroin NPs revealed an effective antibacterial efficacy against the tested bacterial strains; this is attributed mainly to the protein content of NPs [4,5]. These antibacterial properties of PLGA/spidroin NPs are expected to potentiate the wound-healing ability of spider web extract by keeping the wound clean from infections. These positive effects of NPs could be attributed to the enhanced cell adhesion, increased wound penetration, controlled and sustained drug release from NPs [7,8]. PLGA NPs were tested *in vivo* in this study as a promoter of wound healing using earthworms as a skin model.

Wound healing is a necessary, self-sustaining response to tissue injury, but only when the tissue has been seriously damaged and cannot recover independently. The prepared PLGA/spidroin NPs demonstrated a significant improvement in wound-healing action. Plant and animal-derived natural biomaterials have beneficial medicinal properties [31]. During the experiment and throughout the procedure, no earthworms died. In this study, we selected earthworms as an alternative skin model because they contain the same triene and tetraene sterols as human [5,22,32]. Additionally, they are less expensive and more sensitive than other animal models [23]. Accordingly, we used earthworms, particularly *A. caliginosa*, as an excellent animal model for this research.

Wound dressings, compression bandaging, debridement, negative pressure wound treatment, ultrasound, electrical stimulation, phototherapy and skin replacements are all available for effective wound rehabilitation. Despite the vast array of treatment options currently available, none meet any of the criteria for rapid and optimal cutaneous regeneration [33].

Following wound therapy, group IV coagulated the quickest, and full wound healing was obtained in 4 days compared with 12 days, which was required for typical healing in both group II and III (Figure 4).

In another study, the earthworm *Eudrilus eugeniae* required 24 days to heal its posterior segment [34]. Spider silk proteins had a high antimicrobial efficacy and were capable of maintaining the cell proliferation against a wide variety of pathogenic bacteria [35]. According to Kumari *et al.*, spider silk treatment resulted in a significant reduction in the time required for wound closure and re-epithelialization, taking just 24 days as opposed to the more than 36 days for normal healing [36].

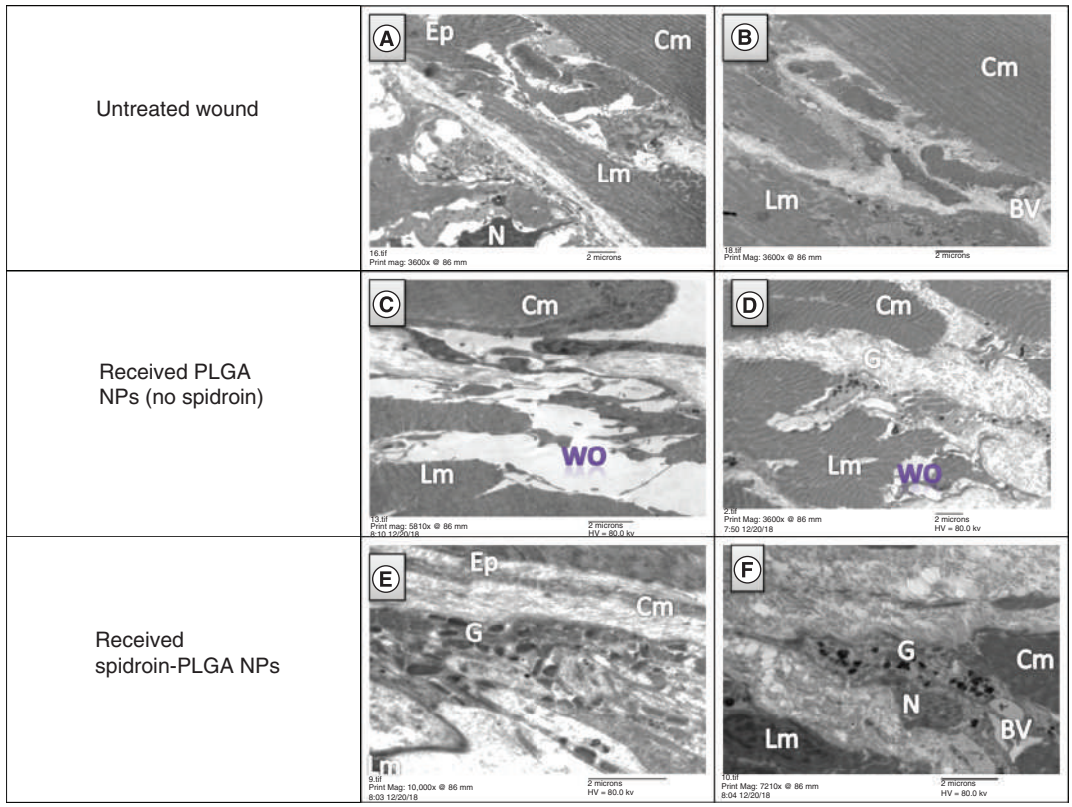

Figure 7. Transmission electron microscopy micrographs of earthworms (*Allolobophora caliginosa*) at the end of the wound-healing study. (A & B) Control group showing Ep, Cm and Lm. **(C & D)** Worms that received plain PLGA nanoparticles (NPs; no spidroin) show Wo, granules of remaining gel and Lm. **(E & F)** Group that received PLGA/spidroin NPs show circular muscle and Lm with granules of NPs. Note complete healing of wound.
BV: Blood vessel; Cm: Circular muscle; Ep: Epidermis; G: Granules; Lm: Longitudinal muscle; PLGA: Poly(lactic-co-glycolic acid); NP: Nanoparticle; N: Nucleus; Wo: Wound.

According to Cinar *et al.*, the annelid epidermis is composed of a monolayered epithelium that comprises glandular, behind, ciliated and sensory cells and is encased in a collagen fiber-based cuticle [37]. *A. caliginosa* has shown vacuolization and hypertrophy of the epithelial cells in the current cross-part of group II and III and shows that circular degeneration occurs, with significant amounts of inflammatory cell infiltration and longitudinal musculation as well as vacuolization and cell hypertrophy. The epidermal layer remained unchanged throughout healing. In comparison, the thickness of the circular and longitudinal muscles decreased [34]. In the present work, we observed that the earthworm peristalsis is normally accomplished by mutual contractions of the circular and longitudinal muscles. Without synchronized muscle contractions, crawling movement is impaired; this is in accordance with Mizutani *et al.* [38], who worked on the worms and described the 'fictive locomotion'. Çinar *et al.* demonstrated the presence of epidermal mucous cells of the skin of *Allolobophora chlorotica* [37]. Likewise, we observed this in all groups.

Semithin sections revealed that the wound and inflammatory cells almost vanished in the *A. caliginosa* population (group IV) and that the spider webs were more functional in terms of skin regeneration and enhanced wound condition through wound-healing time. The rate of wound closure on the circular, epidermal and longitudinal muscles revealed their structure. The ultrastructure of group I presented the cuticle, circular, epidermal and longitudinal muscles. In groups II and III, obvious damage was observed; the cuticle had started to degrade, and the epidermis and circular muscles were severely necrotic; circular muscles were destroyed; the intercellular matrix was edematous and loose; vessel expansion, new capillary development and fibroblast proliferation occurred within a few minutes. The skin of *A. caliginosa* (group IV) exhibited no apparent deviations from that of control group (group I). Capillaries, fibroblasts and collagen all develop in response to a wound. This results in granulation tissue formation [39].

Several studies demonstrated the efficacy of spidroin in enhancing wound healing [4,5], the results of these studies were less than those of PLGA/spidroin NPs, which promotes the wound healing over 4 days restoring skin structure. This study observed more capillaries and fibroblasts in group IV than in groups II and III on the fourth day.

Conclusion

PLGA/spidroin NPs were fabricated and topically applied on the wound. *In vitro* antibacterial activity of the NPs was demonstrated against certain clinical bacterial isolates (MRSA, MSSA and *E. coli*). When NPs were applied to the induced injuries in *A. caliginosa* earthworms, the effects were observed on the fourth day where the injuries fully closed, and re-epithelialization took less time than usual healing. Furthermore, in case of earthworms treated with spidroin NPs, the histological and TEM studies revealed the absence of inflammatory cells and the existence of more fibroblasts and capillaries. These findings demonstrated the efficacy of PLGA/spidroin NPs in promoting wound healing; however, future research using mammalian experimental models is required to validate these findings.

Summary points

- Poly(lactic-co-glycolic acid) (PLGA)/spidroin particles have small sizes from 173 ± 3 nm to 212 ± 14 nm with a negative surface charge.
- Pluronic F-68 is used as a stabilizer, decreasing the particle aggregation.
- Spider silk is a biomaterial with the potential to heal injuries because of its regenerative, biocompatible and antimicrobial properties.
- *Allolobophora caliginosa* earthworms are used as a robust human skin model.
- The entrapped protein in optimized PLGA NPs showed slow release over 6 h with initial burst release.
- PLGA/spidroin nanoparticles (NPs) have a considerable antibacterial effect against some of the tested bacterial strains.
- The antibacterial properties of PLGA/spidroin NPs are expected to potentiate the wound-healing ability of spider web extract.
- Using PLGA/spidroin NPs, wounds coagulated quickly and wound healing was obtained in 4 days.

Financial & competing interests disclosure

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