Liposomal stem cell extract formulation from Coffea canephora shows outstanding anti-inflammatory activity, increased tissue repair, neocollagenesis and neoangiogenesis

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Abstract

Coffea canephora plant stem cells can have bioactive compounds with tissue repairing and anti-inflammatory action. This study aimed to develop a liposomal stem cell extract formulation from *C. canephora* (LSCECC) and to investigate its capacity to contribute to the dynamic mechanisms of tissue repair. The liposome was developed and characterized through the dynamic light scattering technique, atomic force microscopy, and transmission electron microscopy. The excisional full-thickness skin wound model was used and daily topically treated with the LSCECC formulation or vehicle control. On days 2, 7, 14, and 21 after wounding, five rats from each group were euthanized and the rates of wound closure and re-epithelialization were evaluated using biochemical and histological tests. LSCECC resulted in faster re-epithelialization exhibiting a significant reduction in wound area of 36.4, 42.4, and 87.5% after 7, 10, and 14 days, respectively, when compared to vehicle control. LSCECC treated wounds exhibited an increase in granular tissue and a proper inflammatory response mediated by the reduction of pro-inflammatory cytokines like TNF-α and IL-6 and an increase of IL-10. Furthermore, wounds treated with LSCECC showed an increase in the deposition and organization of collagen fibers at the wound site and improved scar tissue quality due to the increase in transforming growth factor-beta and vascular endothelial growth factor. Altogether, these data revealed that LSCECC may have remarkable applications in skincare cosmetic formulations to support healthy skin.

Highlights

- A liposomal stem cell extract formulation from *C. canephora* (LSCECC) was developed
- The LSCECC emerged as a potential approach for skincare applications.
- The LSCECC promotes wound healing resulting in enhanced epithelization.
- The LSCECC accelerated wound closure and neo-dermal tissue formation.
- The LSCECC increased the deposition and organization of collagen fibers.

Introduction

Plants are responsible for synthesizing a wide range of bioactive compounds, such as secondary metabolites, described as small molecules with biological action, offering huge potential as antioxidants, anti-inflammatory, and nutraceuticals, with applications in the pharmaceutical and cosmetic industries [1]. *Coffea canephora* Pierre ex A. Froehner emerges as a natural source of several bioactive compounds, such as antioxidants, flavonoids, and phenolic compounds, such as chlorogenic acids, caffeic acid, caffeine, and melanoidins [2]. Indeed, the antioxidant capacity of *C. canephora* is related to the presence of both natural constituents and compounds formed during coffee processing [3]. However, the content of bioactive compounds in fruits may vary considerably from one crop to another due to climatic and water variations for example [4]. Plant cell culture technology emerges as an innovative way in the search for bioactive compounds, as plant stem cells have been widely adopted in cosmetics a few years ago [5]. Products made from a cultivated plant the cells are considered "new cosmeceuticals" because they offer

the possibility of standardization of preparation, with an increase in the biological activity of the secondary compounds produced by the cultures [1].

These bioactive compounds produced by plant cultures are water-soluble, that is, they have polar characteristics, presenting great difficulties in penetrating the skin through the stratum corneum [6]. In this context, liposomes are vesicles made up of one or more bilayers that allow the entry of water-soluble compounds into the skin. In these vesicles, phospholipids are oriented concentrically around an aqueous compartment and serve as drug carriers, biomolecules, or diagnostic agents [7]. Water-soluble compounds occupy the aqueous core of liposomes, while reduced-affinity molecules for water are trapped in the lipid bilayers [8]. The stability of liposomes can be affected by chemical, physical, and biological processes [8]. The stability assessment must include the characterization of the final product and monitoring during storage of the liposomal formulation, to guarantee its physicochemical properties [8].

Wound healing or tissue repair consists of a well-coordinated cascade of cellular events, tissue restructuring, and reconstitution [9]. The wound healing complex process is divided into three stages, which take place in an overlapping way. Initially, occurs an inflammatory stage, followed by a proliferation stage which is ending with repair in the remodeling stage [10]. The inflammation phase depends, in addition to numerous chemical mediators, on inflammatory cells, such as polymorphonuclear leukocytes (PMN), macrophages, and lymphocytes [11]. This phase is crucial for the proliferation and remodeling phase to happen concisely [12]. In addition, the proliferation phase includes fibroplasia and matrix formation, which is extremely important in the formation of granulation tissue. The formation of granulation tissue depends on the fibroblast, a critical cell in matrix formation [13]. Far from being just a collagen producer, fibroblasts produce elastin, fibronectin, glycosaminoglycan, and proteases, which are responsible for physiological debridement and remodeling [14]. The last phase of proliferation is angiogenesis, which is essential for the supply of oxygen and nutrients for healing [15].

Plant extracts have been used for years in tissue repair treatment [16]. Emulsions containing marigold (*Calendula officinalis*) oil showed an improvement in the recruitment of inflammatory cells to the wound site, decreasing healing time [17]. A pharmaceutical preparation containing 5% of the ethanolic extract *Struthanthus vulgaris* can accelerate the closure of skin wounds in rats and increase collagen synthesis in the remodeling phase, in addition to decreasing the inflammatory process [18]. In addition, methanol extract from the leaves of *Hibiscus micranthus* exhibited a potential antibacterial activity against the tested microorganisms that often infect the skin and wound healing activity [19].

Therefore, for innovative cosmetic scientists, the goal is to connect the traditional medicinal herbal knowledge and practices to the evidence in modern science and see where the application of plant stem cell extracts in cosmetics can improve the delivery to the skin and be more effective. Thus, this study aimed to develop a liposomal stem cell extract formulation from *C. canephora* (LSCECC) and to investigate its capacity to contribute to the dynamic mechanism of tissue repair.

Material And Methods

Chemicals and biochemical

IL-6, IL-10, TNF-, TGF-β, VEGF, ELISA kits were purchased from eBiosciense (San Diego, CA, USA). Bradford and N-acetyl-beta-D-glucosaminidase (NAG) were purchased from Thermo Scientific (Rockford, IL, USA). Hydrated chloride was purchased from Vetbrands (Paulinia, SP, Brazil). Other's reagents used were of analytical grade and were obtained from various commercial sources.

Preparation of the stem cells extract from Coffea canephora

Leaves of *C. canephora* were harvested from 10 individuals, located at the Espírito Santos Institute for Research, Technical Assistance and Rural Extension (INCAPER), Marilândia - ES - Brazil (latitude 19°23'56"S, longitude 40°32'07"O). The leaves were washed with distilled water followed by immersion in 70% ethanol and 3.5% NaOCl solution for surface disinfestation. Subsequently, leaves were washed three times in sterile distilled water into a laminar flow cabinet. Leaf explants were inoculated in Petri dishes containing an induction culture medium for callus and incubated at 24°C in the dark for 30 days [20]. 0.5 g of the friable callus were transferred to Erlenmeyer flasks containing 30 mL liquid culture medium for establishment and multiplication of cell aggregate suspensions [20]. The culture was kept under stirring, at 110 rpm, in the dark at 24°C, and subcultivated every 14 days for two months to increase biomass. In the end, cell aggregates in suspension and culture medium were processed using an ultrasonic probe followed by lyophilization. For extraction, the lyophilizate was resuspended in ultrapure water (10% w/v), homogenized, and centrifuged at 10,000 rpm at 4°C. The supernatant obtained (extract) was named stem cell extract from *C. canephora* (SCECC).

Liposome preparation

Phosphatidylcholine, cholesterol, polyethylene glycol (PEG 400), and soy lecithin were dissolved in a solvent mixture of chloroform and methanol (2:1 v/v) in a 500 mL round bottom flask. Organic solvents were evaporated at 50°C. This mixture was added in the proportion of 20% (v/v) of the SCECC and stirred at room temperature (25°C) at 15000 rpm for 15 min [6]. The liposome of stem cell extract from *C. canephora* (LSCECC) was stored in a refrigerator at 4°C until final preparation and application. A similar void liposome (without the sample) was also prepared and stored for further experiments. The schematic LSCECC is represented in Fig. 1.

Figure 1.

Formulation stability testing

To carry out the *in vivo* experiments, two emulsions in a non-ionic base were prepared. The positive control with 10% by weight of LSCECC and the negative control with 10% by weight of the void liposome. To determine the stability of emulsions and the shelf life, the accelerated stability method according to

the National Health Surveillance Agency of Brazil was used [21]. Both emulsions were stored in a tightly closed glass bottle, protect from light, and submitted to the heating and cooling cycles. For 28 days, the emulsions were heated to 45°C ± 2°C for 24 h and cooled to 4°C ± 2°C for another 24 h. After this period, the organoleptic characteristics of the emulsions were evaluated: appearance, odor, color, viscosity, and pH.

Particle size analysis for dynamic light scattering

The synthesized emulsions were taken to the Microtrac device to determine the diameter of the particles, and thus characterize the formed liposome through the technique of the dynamic light scattering (DLS). The colloid was inserted into the reading vat, through which the light-emitting laser passes. The result was given in a table and a particle size histogram [6, 7]. The equipment used was an NPA152 Zetatrac, Microtrac, USA.

Atomic force microscopy

The atomic force microscopy (AFM) was performed according to Ruozi et al., (2011)[7]. The sample preparation occurred through the deposition of a drop of the emulsion on a cleaved mica slide. Subsequently, a second blade was used to spread the liquid. The sample was decanted for 30 min and was then taken to the AFM. The intermittent contact mode was used, where the tip vibrates at a resonant frequency, practically all the time interacting with the sample surface.

Transmission electron microscopy

The transmission electron microscopy (TEM) was performed according to Ruozi et al., (2011)[7]. The equipment used was a Jeol JEM1400 microscope, with tungsten filament and acceleration up to 120kV. The surface on which samples are deposited on a copper device is called a Grid. Grids are covered with a carbon film called Formvar. One drop of the diluted emulsion is added and stored overnight in a Petri dish to dry. The contrast used was phosphotungstic acid.

Animals

In vivo experiments were conducted by the National Council for the Control of Animal Experimentation and were approved by the Ethical Committee, Bioethics and Animal Welfare of the Universidade Vila Velha (UVV) (ID n.575–2020). A total of 40 adult male Wistar rats (*Rattus norvegicus*) weighing 240–310 g and aged 8–9 weeks were used. All animals were kept under standardized conditions and monitored at a controlled temperature of 24°C ± 2°C with 12 h light-dark period in the bioterium of Universidade Vila Velha. Access to water and food was free during the entire experimental period.

In vivo skin regeneration experiment

All animals were anesthetized, shaved, and had their back cleaned with 70% ethanol. Then, four full-thickness excisional wounds were created on the back region of each rat with a sterile 15-mm punch biopsy. The rats were separated into 2 distinct groups (n = 20). Rats were daily treated with the developed liposomal formulation containing 10% LSCECC (positive control) or with the emulsion containing 10%

void liposome. The wounds were covered with gauze to keep them protected. Five animals were euthanized on days 2, 7, 14, and 21 according to a standardized protocol. The biopsies were collected and stored for future histological and biochemical investigations [18, 22].

Evaluation of wound healing index

The morphometric analysis of the wounds was performed using images of the wounds at 0, 2, 5, 7, 10, 14, and 21-days post-wounding. The wound areas were calculated using ImageJ software (*Wayne Rasband do Research Services Branch, National Institutes of Health* – NIH, Bethesda, Maryland, EUA). The determination of wound area size reduction was calculated by comparing the current size and the initial size in percentage using the following formula: (wound area day 0 – wound area days 2, 5, 7, 10, 14, and 21)/wound area day 0) x 100. Values were expressed as the percentage of healed wounds [22].

Histological analysis

From each animal on each corresponding day, two wounds were collected, conditioned for 24 h in 3.7% formaldehyde phosphate buffer, followed by processing and paraffin inclusion. Serial histological sections with approximately 3–6 μm thicknesses were mounted on glass slides and stained with hematoxylin and eosin (H&E) for evaluation and quantification of the inflammatory infiltrate and with a Picrosirius Red for quantification of collagen [18].

Evaluation of inflammatory infiltrate

The semi-quantitative evaluation of the inflammatory infiltrate was performed as previously described in the literature [18, 22], and the results were reported as the average of the total number of inflammatory cells per group.

Evaluation of collagenesis by imaging

The semi-quantitative study of collagenesis was performed as previously described [23], using the histomorphometric image analysis of the filled areas corresponding to stained collagen fibers, using picrosirius staining. The image analyses were determined by digital densitometry recognition using computer-aided ImageJ software. The results were reported as the average distribution of collagen per treatment.

Cytokines and growth factors measurements

The biopsies collected from each animal on days 0, 2, 7, 14, and 21 post-wounding were homogenized on ice using Lysing Matrix A tubes and a Fast Prep-24 homogenizer (MP Biomedicals, Santa Ana, CA) and centrifuged (1500g) for 10 min. The homogenate obtained from the ulcers was used to measure pro and anti-inflammatory cytokines IL-6, IL-10, TNF-α, and the growth factors TGF-β and VEGF using the immunoenzymatic assay (ELISA - "Enzyme Linked Immuno Sorbent Assay") following the manufacturers' specifications for each assay (eBioscience®). Optical densities were measured at 450 nm in a microplate reader device. The cytokine and growth factors quantitation were expressed in pg; sensitivities were > 10 pg/mL.

Total protein quantification

The total protein concentration for each tissue biopsy (homogenate) was determined by colorimetric method using a commercial Pierce® kit, as per the manufacturer's specifications (Labtest Diagnóstica - Lagoa Santa, MG, Brazil). Experiments were performed in 96-well plates, and protein concentrations were calculated by regression analysis using a standard curve of a solution of bovine serum albumin by colorimetric measurements at 595 nm in an ELISA plate reader.

Measurement of neutrophilic infiltrate

The neutrophilic infiltrate into the wound site was determined through the colorimetric myeloperoxidase (MPO) assay, as previously described [18, 22]. Quantitation of neutrophils was determined against a standard neutrophil curve. The results were described as the total number of neutrophils per mg of tissue.

Measurement of macrophage tissue accumulation

The presence of macrophages in the wound site was determined by N-acetyl-β-D-glycosaminidase (NAG) assay as previously described [24]. Thus, 100 µL of the supernatant from the biopsy samples was added to a 96-well plate. To the samples, 100 µL of the substrate (p-nitrophenyl-N-acetidyl-β-D-glucosamine) 2.23 mM diluted in citrate-phosphate buffer pH 4.5 was added. It was incubated at 37°C for 60 min. To stop the reaction, 100 µL of glycine buffer pH 10.6 was added to each well. Absorbance was measured by spectrophotometry in a microplate reader at 405 nm. Results were expressed as NAG activity in optical density (OD/mg tissue).

Statistical analysis

Statistical analyses were performed using GraphPad software (San Diego, CA, 176 USA). Results are presented as the mean ± standard deviation (SD). Comparisons between two groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey’s post-test or two-way ANOVA when appropriate. The level of significance was $p < 0.05$.

Results

Stability of LSCECC

The results of the accelerated stability test evaluating different physicochemical properties of LSCECC shows that there were no changes in the color, odor, appearance, pH, and viscosity of the liposome. These results elucidate the great stability of the liposome during the test period.

Size determination of LSCECC

The results are originally represented in Fig. 2. The average size of the empty liposome was 40 nm, while the average size of the liposome containing the sample was 50 nm (Table 1). This difference in size observed between the full and void liposome shows the presence of actives and molecules inside the

liposome prepared from SCECC. On the other hand, the absence of bioactive compounds in the empty liposome decreases its size when compared to the liposome containing SCECC, but without a statistically significant difference between them.

Table 1
Size of void liposome and LSCECC recorded by dynamic light scattering (DLS).

	Dynamic light scattering (nm), ± SD	
	Void liposome	**LSCECC**
Size	38.90 ± 6.4	50.54 ± 8.21

Figure 2

Table 1.

Atomic force microscopy (AFM)

The AFM analysis showed moderate stability of the liposome maintained a spherical well-defined shape (Fig. 3).

Figure 3.

Transmission Electron Microscopy

The morphology, architecture, and dispersion of the liposomes obtained from TEM images showed that the particles are distributed in a polydisperse way, have different sizes, and have an approximate shape of a sphere (Fig. 4).

Figure 4.

Evaluation of regeneration in the skin wounds

The topical application of LSCECC allowed a better and dynamic skin regeneration reflecting in a faster wound closured when compared to the vehicle control group (see Fig. 5). In addition, to accelerate the closure of the skin wound, the LSCECC led to a rapid recovery from the epidermal and dermal structure, with the lesions becoming progressively less inflamed and producing a normotrophic scar (Fig. 5A). Furthermore, the rate of wound regeneration of lesions treated with LSCECC was greater than the vehicle control, especially during the re-epithelialization stage between days 10 and 21. A significant reduction in the wound area of 36.4, 42.4, and 87.5% was observed in the group treated with LSCECC compared to the vehicle control group after 7, 10, and 14 days post-injury, respectively ($p < 0.05$).

Figure 5.

Measurement of MPO and NAG activities

The activity and involvement of neutrophils and macrophages during the healing process were investigated by studying the marker enzymes myeloperoxidase (MPO) and N-Acetyl-β-D-glucosaminidase (NAG), respectively. Low levels of these enzymes were detected in intact skin (day zero) (Fig. 6). On day 2 and day 7 post-wounding the concentration of MPO and NAG respectively were significantly decreased in wounds treated with LSCECC compared to vehicle control-treated wounds. No significant effects in the following days of the experiment were observed (Fig. 6).

Figure 6.

Total protein quantification

The total protein content was analyzed in tissue biopsy homogenates. Wounds from the LSCECC treated group exhibited an apparent reduction in total protein content after 7 days when compared to the vehicle control group (Fig. 7). After 2, 14, and 21 days no differences were observed between the experimental groups.

Figure 7.

Cytokines and growth factor analysis

The influence of LSCECC on the production and release of different cytokines and growth factors, such as TNF-α, IL-10, IL-6, and TGF-β, in wound biopsy homogenates, was examined (Fig. 8). LSCECC was able to modify the production and release of cytokines at the site of tissue regeneration. As observed in Fig. 8A, the TNF-α concentration detected in the homogenized tissues prepared from wound biopsies after treatment with LSCECC for 7 days was significantly reduced compared to the vehicle control group ($p < 0.05$). In turn, IL-6 concentration was significantly reduced on day 2 (Fig. 8B). In contrast, on day 7 there was a significant increase in IL-10 production in the LSCECC-treated group compared to the vehicle control group (Fig. 8C). On day 14 a significant increase in the production of TGF-β in the LSCECC treatment group was observed (Fig. 8D).

Figure 8.

Inflammatory infiltrate

Histological analysis of wound biopsies stained with H&E was used for quantitative analysis of cell density at the wound site (Fig. 9). Inflammatory analysis of cellular infiltrate in wound biopsies treated with LSCECC showed a proportional reduction in the inflammatory infiltrate at the wound site after 2, 7, and 14 days when compared to the vehicle control (Fig. 9). The photomicrograph shows the migration of inflammatory cells from the bloodstream to the site of injury.

Figure 9.

Collagen measurement

The LSCECC significantly increased collagen production and deposition in the wound site on day 7 during the most important phase of fibroblast cell proliferation (Fig. 10). During the experiment, we observed that the skin wounds showed a marked and robust increase in the deposition and organization of collagen fibers, detected after staining with picrosirius red. The photomicrograph shows the deposition and organization of collagen fibers during tissue repair (Fig. 10A). The density of collagen fibers on day 7 post-injury was greater than on day 2, increasing further after 14 days.

Figure 10.

Measurement of VEGF

The VEGF was analyzed in the wound tissues (Fig. 11). The LSCECC significantly increases VEGF levels on day 7 compared to the negative control group (Fig. 11).

Figure 11.

Discussion

Thanks to the unlimited capacity to divide or transform into different types of cells and create new organs and tissues, plant stem cells are responsible for the regeneration processes in the plant and can produce an unlimited number of bioactive compounds [5]. Thus, plant stem cell culture has emerged as a promising tool for large-scale and sustainable production of secondary metabolites, offering a continuous source of bio extracts and compounds for the development of health care formulations [1, 25].

Thus, this study aimed to formulate an innovative stem cell liposome from *C. canephora* (LSCECC) and to investigate it is *in vivo* capacity in contributing to the dynamic mechanisms of tissue repair and healing. This biotechnology has been widely used to obtain plant extracts enriched with primary and secondary metabolites, in concentrations that cannot be obtained by traditional methods, such as from parts of the plant extraction or chemical synthesis. For example the presence of a high concentration of verbascoside in the extract *Syringa vulgaris* and the *trans*-resveratrol in grape wines plants (*Vitis vinifera)* [26, 27]. These bio extracts have been demonstrating greater biological effects. *Rubus ideaus* stem cell culture extract exhibited high anti-inflammatory activity by decreasing the expression of inducible nitric oxide synthase 2 (iNOS2) and the activity of cyclooxygenase 2 (COX-2). These effects were addressed by the authors to the presence of high flavonoids and anthocyanins content. In addition, the extract provided strong antioxidant power due to the presence of phenolic components [28]. A formulation obtained from tobacco cell cultures (*Nicotiana sylvestris*) showed robust anti-aging properties in keratinocytes and fibroblasts, as this extract was rich in amino acids such as glycine, proline, and hydroxyproline, the most abundant in the collagen present in the skin [29].

Associating plant extracts with liposome techniques improves the topical action and biological effects of bioactive compounds. The human skin barrier is an important part of skin integrity, and its functionality is a precondition for healthy skin. However, cosmetic formulations with extracts and/or compounds of

natural origin generally have greater difficulty in penetrating through the stratum corneum, due to their hydrophilic characteristics. [30]. Liposoming a plant extract is an interesting way to increase the penetration capacity of plant extracts. The liposome is a technology employed to increase the penetration of polar water-soluble actives into the skin, allowing action in the deeper layers of the dermis. Another important aspect of these formulations is their ability to remain stable after preparation [6]. Our work has shown that it is possible and practicable to develop a liposome encapsulated with stem cell extracts obtained from *C. canephora*. The developed liposomal formulation showed a stable oval shape during liposome formation, maintaining this stability after being incorporated into the formulation. The LSCECC did not alter its physicochemical characteristics during the accelerated stability test. This demonstrates the stability of the sample against environmental stress. Another important fact to point out is the format and size (lower than 60 µm) which allows greater dispersibility and greater topical penetration capacity [7]. TEM analyses elucidated the morphology, architecture, and dispersion of the liposomes which indicates that the LSCECC production process is reproducible and scalable [7].

A proof-of-principle study on the local and systemic efficacy and therapeutic activity of the liposomal stem cell formulation was carried out using the classical full-thickness excision wound model in rats [18, 22]. The topically applied LSCECC significantly accelerated wound closure and neodermal tissue formation. In general, the inflammatory process must be short and resolute, and the inflammatory cells must play their role dynamically. When inflammation evolves to chronicity, tissue repair is seriously compromised [14]. The LSCECC reduced the infiltration of inflammatory cells, especially in the initial phase of tissue repair. The lower presence of neutrophils on day 2 and macrophages on day 7 favored a more homeostatic evolution of healing [31]. This happens because LSCECC can decrease the release of inflammatory cytokines such as IL-6 and TNF-α and increase the production and release of anti-inflammatory interleukins such as IL-10. These LSCECC properties could modulate the inflammatory process in the skin and can be interesting not only for wound healing but also for other disorders in which the inflammation process is present as for example in melasma [32], rosacea [33], vitiligo [34], dermatitis, lupus erythematosus and photoaging [35]. Similarly, a vegetable oil blend formulation modulated the inflammatory response by inhibiting the production and release of pro-inflammatory cytokines, especially IL-1, IL-6, and TNF-α in the wound [22]. Thus, the effect of LSCECC on the enhancement of wound closure can be attributed to the downregulation of proinflammatory cytokines and upregulation of anti-inflammatory cytokines.

Together with the cytokines, the growth factor is the essential player in different stages of tissue repair [36]. Transforming growth factor (TGF-β) is known to promote wound healing and regeneration by stimulating dermal fibroblast proliferation and extracellular matrix deposition [37]. The LSCECC increased TGF-β release and consequently stimulated collagen production at the injury site. Cutaneous wound healing was impaired in transforming growth factor-β inducible early gene knockout mice proven by delay in wound closure related to an impairment in wound contraction, granulation tissue formation, collagen synthesis, and reepithelialization [38].

LSCECC also stimulated the production and release of another important growth factor, the VEGF. The VEGF is the most important signaling growth factor in angiogenesis and vasculogenesis. VEGF is secreted by macrophages, platelets, fibroblasts, and keratinocytes and is involved in wound healing [39]. It has been demonstrated that VEGF is one of the most potent proangiogenic molecules in the skin during physiological and pathological promoting neovascularization and inducing proliferation of fibroblasts and endothelial cells promoting re-epithelialization, and collagen deposition [40].

The synthesis of extracellular matrix, especially collagen, is fundamental during tissue repair. Collagen plays an important role in all phases of wound healing. Collagen has a chemotactic nature, attracting mainly fibroblasts to the wound site. Collagen also supports the formation of new blood vessels and the formation of granulation tissue, increasing the wound's ability to re-epithelialize [41]. Thus, in general, the effects observed with LSCECC, evidenced by the increase in TGF-β and VEGF and modulation of the inflammatory process, altogether could contribute to the production of collagen and consequently many important events that occur during skin wound healing.

Conclusion

This is the first study to demonstrate the efficacy and therapeutic activity of the stem cell liposome from *C. canephora* (LSCECC) as a new treatment for cutaneous wound healing as well as improvement of skin disorders related to decreased the granulation tissue and extracellular matrix formation. Moreover, the formulated LSCECC exhibited sustained-release behavior and stability. In summary, LSCECC has emerged as a promising bio extract that can have much applicability by acting as a tissue regenerator, with anti-inflammatory action, stimulator of collagen production, and neovascularization in the skin. Altogether, these results evidenced that LSCECC could have remarkable applications in skincare cosmetic formulations to support healthy skin. However, the encouraging results must be confirmed in extended clinical studies.

Declarations

Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical standards. This work follows ethical standards and all experiments involving the use of animals were conducted by the National Council for the Control of Animal Experimentation and were approved by the Ethical Committee, Bioethics and Animal Welfare of the Universidade Vila Velha (UVV) (ID n.575-2020).

Informed consent. Informed consent was obtained from all individual participants included in the study.

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Figures

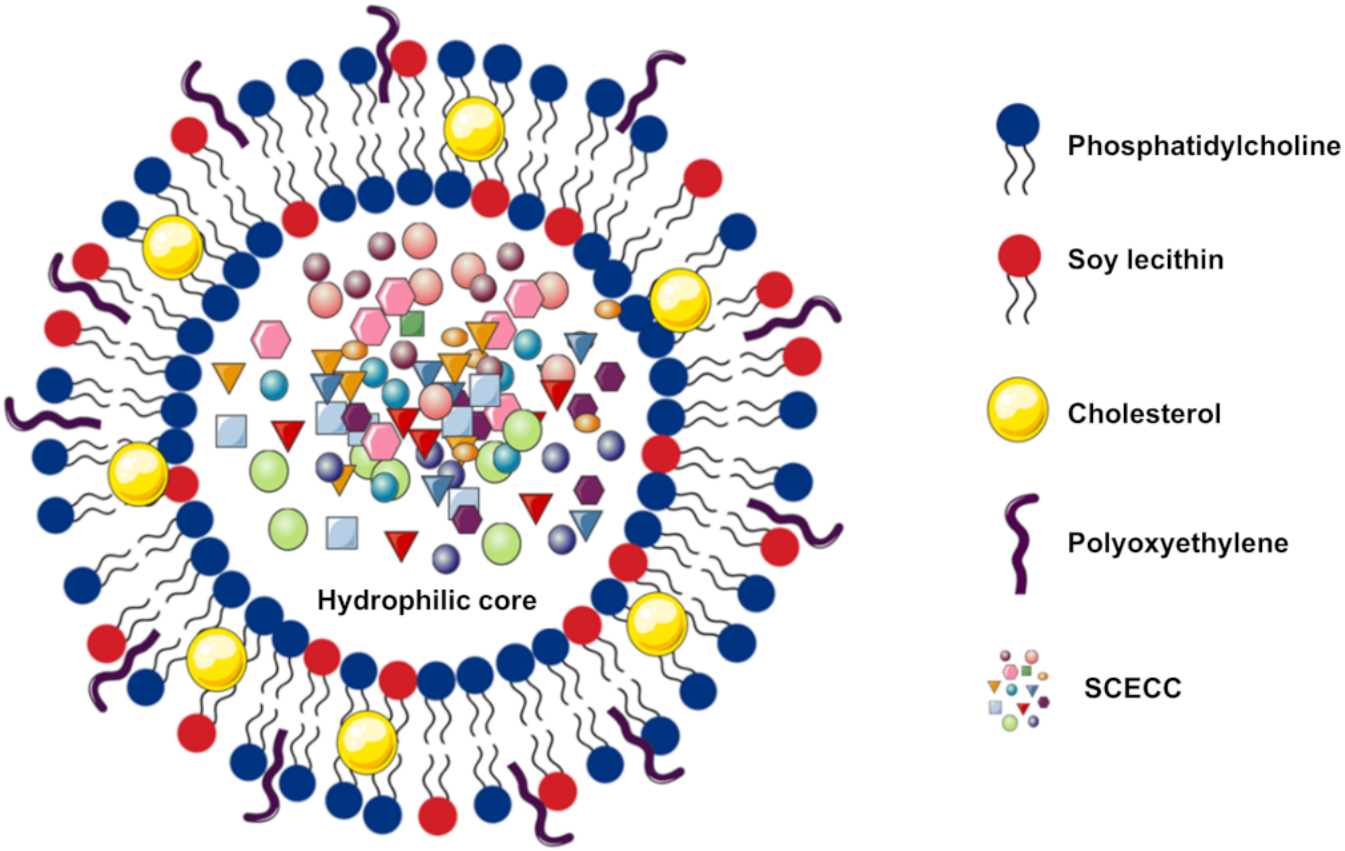

Figure 1

Schematic diagram of liposome of stem cell extract from *C. canephora* (LSCECC).

Figure 2

Size in nm of void liposomes (**A**) and LSCECC (**B**) recorded by dynamin light scattering (DLS).

Figure 3

Atomic force microscopy images of liposome of stem cells extract from *Coffea canephora* (LSCECC) in 3D format.

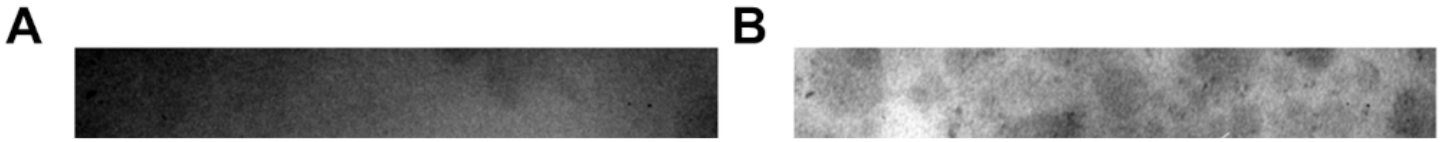

Figure 4

Void liposome (**A**) and LSCECC (**B**) were visualized by transmission electron microscopy at 100,000 times magnification. The arrows in panels A and B represent the void liposome and LSCECC, respectively.

Figure 5

Wound healing effects of LSCECC over 21 days of treatment. **(A)** Macroscopic images of the wounds on the indicated days post-wounding. **(B)** Percentage of wound closure at 2, 5, 7, 10, 14, and 21days after daily topical application of LSCECC or vehicle control. Data are expressed in percent of area reduction from the original wound size (day 0). Values represent mean ± SD (n = 20 wounds/group). * Significant ($p < 0.05$) compared to vehicle control.

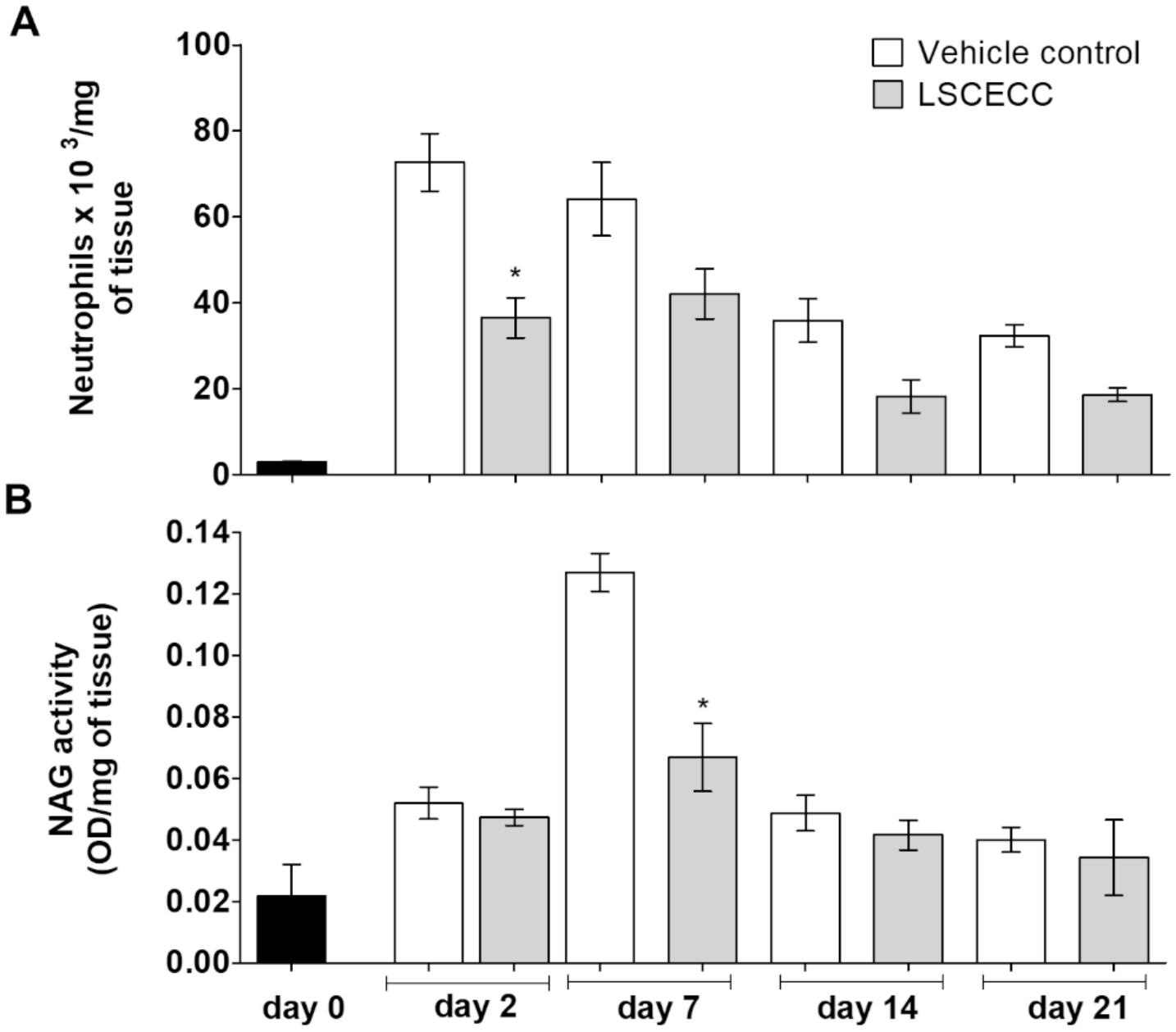

Figure 6

Myeloperoxidase (MPO) activity (neutrophil marker) (**A**) and N-acetyl-β-d-glucosaminidase (NAG) activity (macrophage marker) (**B**) in the wounds at day zero and after 2, 7, 14, 21 days of treatment with LSCECC or vehicle control. Results are shown as mean ± SD (n = 10 wounds/group). * Significant ($p < 0.05$) compared to vehicle control.

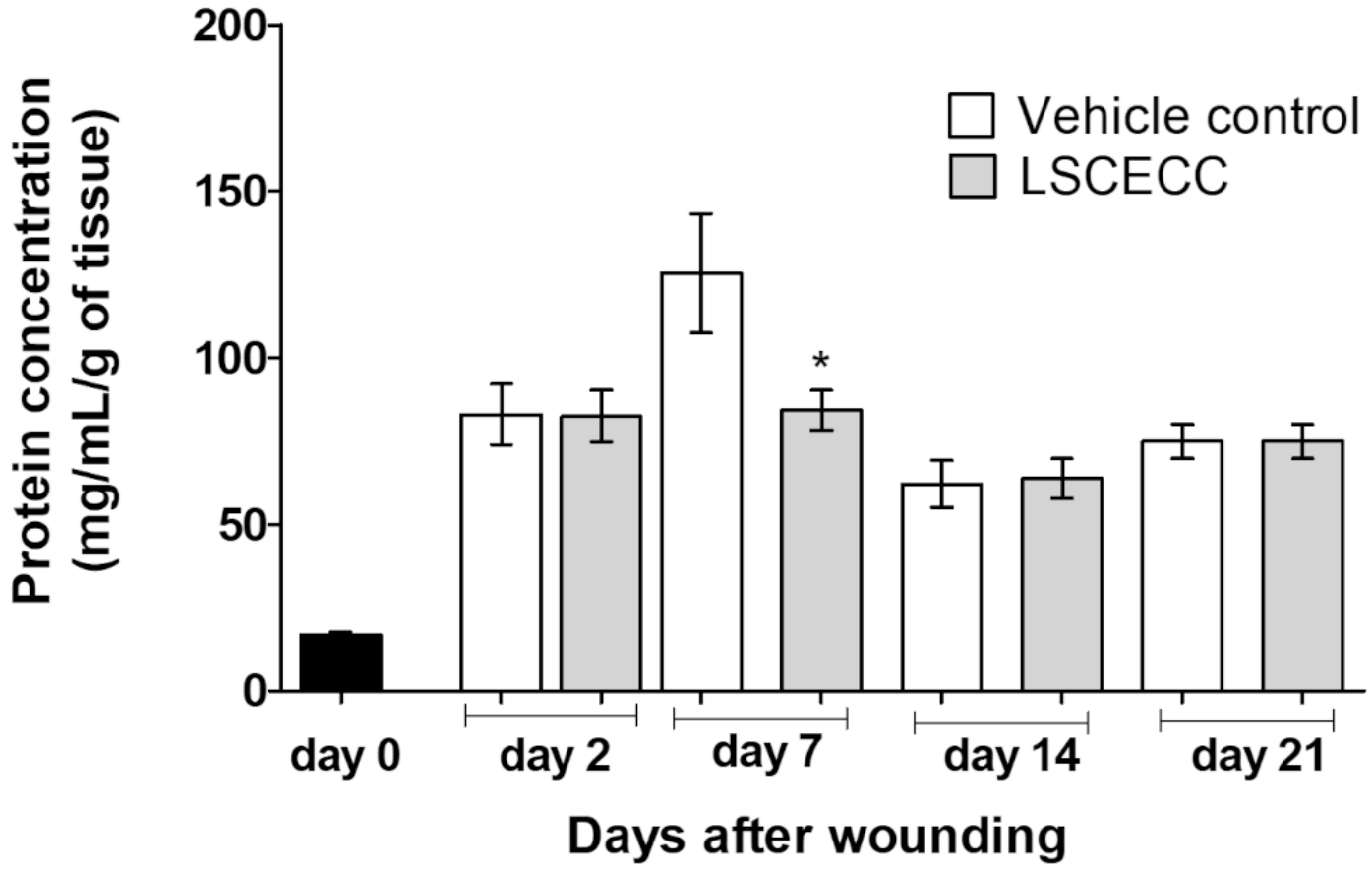

Figure 7

Total protein content in the wound tissue's biopsies treated with LSCECC or vehicle control at days 0, 2, 7, 14, 21. Total protein was measured according to the Coomassie assay in homogenates prepared from the wound biopsies. Values represent mean ± SD (n = 10 wounds/group). * Significant ($p < 0.05$) compared to vehicle control.

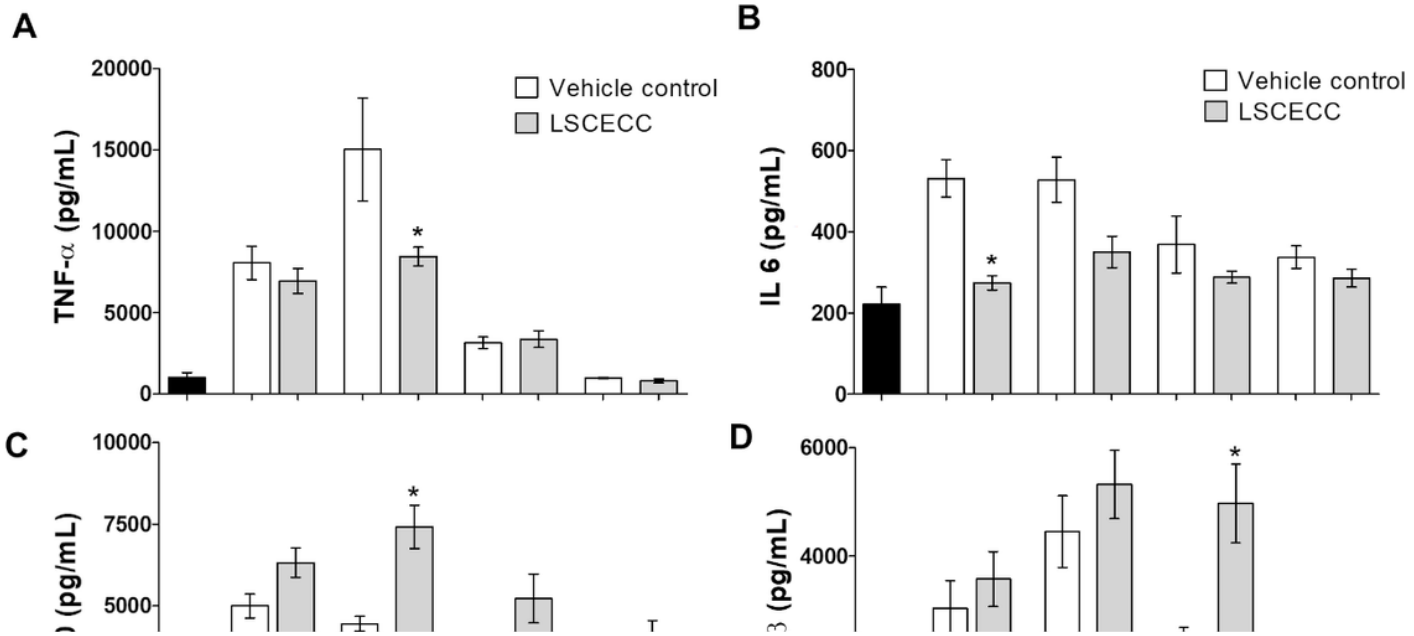

Figure 8

LSCECC modulates cytokines production in the skin wound biopsies. Tissue homogenates were prepared from the wound biopsies obtained from animals treated with LSCECC or vehicle control on days 0, 2, 7, 14, and 21. Values were expressed in pg/mL. TNF- a (A), IL-6 (B), IL-10 (C), and TGF-β (D) were assayed by ELISA. Values represent mean ± SD (n = 20 wounds/group). *Significant ($p < 0.05$) compared to vehicle control.

Figure 9

LSCECC affects inflammatory cells recruitment at the wound site. (A) Representative photomicrography of the wound sections stained with H&E (X400). (B) Quantitative analysis of inflammatory infiltrate. Data represents the mean ± SD (n=20 wounds/group) * Significant ($p < 0.05$) compared to vehicle control.

Figure 10

Effects of LSCECC on the collagen content in the wound tissue biopsies at days 0, 2, 7, 14, 21 after injury. (A) Representative photomicrograph of wound tissue sections stained with Picro-Sirius red (x400). (B) Collagen content determined by digital densitometry using ImageJ software. Data represent the mean ± SD (n=20 wounds/group) * Significant ($p < 0.05$) compared to vehicle control.

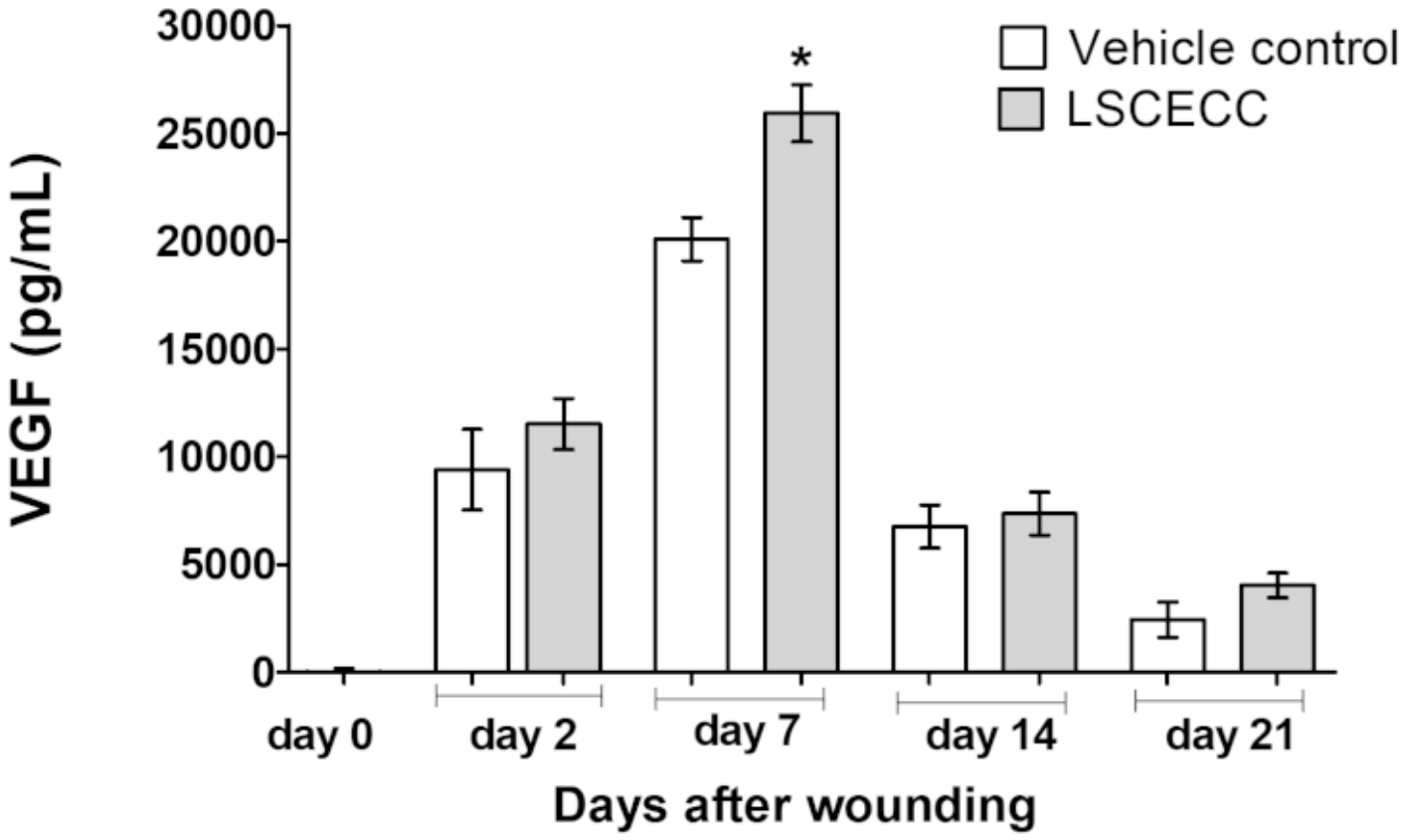

Figure 11

Total contented of Vascular Endothelial Growth Factor (VEGF) in the wound tissues treated with LSCECC and in the negative control, at days 0, 2, 7, 14, 21. VEGF was assayed by ELISA. Values were expressed in pg/mL. Values represent mean ± SD (n = 20 wounds/group). * Significant ($p < 0.05$) compared to vehicle control.

Supplementary Files

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