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Research Article

Investigation of the wound healing effects of castor oil-based biocompatible greases on the HaCaT cell line

Reyhan Hocaoğlu[1,2], Cüneyd Yavaş[1,2], Nermin Akçalı[1,2], Mırsajjad Eslamkhah Taghizad[1,2], Lütfiye Karcıoğlu Batur[1,2]

[1]*Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Biruni University, İstanbul, Türkiye*
[2]*Biruni Research Center (B@MER), Biruni University, İstanbul, Türkiye*

ABSTRACT

Aim: Castor oil has recently gained attention for its wound-healing properties due to its rich phytochemical composition. In particular, the ricinoleic acid exhibits anti-inflammatory, antibacterial, and skin barrier-supporting effects. In addition, the development of environmentally sustainable and biodegradable materials has increased the interest in plant-based formulations. This study aimed to evaluate the wound healing potential of castor oil-based biocompatible greases at different concentrations using an in vitro scratch assay on human keratinocyte (HaCaT) cells.

Methods: HaCaT cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin–Streptomycin under standard incubation conditions (5% CO_2, 37 °C). Once confluent, a scratch was created in the cell monolayer using a sterile 200 µL pipette tip. Castor oil-based biocompatible greases at different concentrations (0.1%, 1%, 5%, and 10%) were applied to the experimental groups, while standard culture medium served as the negative control. The term "castor oil-based biocompatible grease" refers to castor oil applied at different concentrations in culture medium, representing a component of a proposed grease formulation. Cells were incubated for 0, 12, 24, and 48 hours. Wound closure was evaluated by inverted microscopy and analyzed quantitatively using ImageJ software.

Results: Quantitative analyses demonstrated a concentration-dependent effect of castor oil-based grease on wound closure. The 5% concentration group showed the greatest wound closure rate at 12 hours, comparable to the negative control. Lower concentrations (0.1% and 1%) exhibited slower closure rates, whereas the 10% group showed reduced cell migration and morphological deterioration, suggesting possible cytotoxicity. The order of wound closure effectiveness was 5% castor oil > negative control > Tween control > 10% > 0.1% > 1%.

Conclusion: Castor oil-based biocompatible greases influenced wound healing in a dose-dependent manner, with optimal effects observed at 5% concentration. These findings highlight the potential of castor oil-based greases as biocompatible, biodegradable, and environmentally sustainable biomaterials for wound healing applications, warranting further preclinical and clinical investigations.

Keywords: castor oil, biocompatible grease, wound healing, anti-inflammatory properties

✉ Lütfiye Karcıoğlu Batur ▪ lbatur@biruni.edu.tr

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Introduction

Traditionally, the primary function of grease—similar to mineral oils—is to provide lubrication to machine components by reducing friction and wear. Lubricating greases are particularly preferred in mechanisms that are difficult to access or in systems that remain idle for extended periods (1,2). In these materials, soap molecules are dispersed in oil and organize themselves into a three-dimensional lattice structure, preventing the free flow of oil and allowing the grease to function as a semi-fluid material (3). Greases generally consist of mineral oil, soap thickeners, and additives, with the desired consistency achieved through various combinations of oils, alcohols, and bases (4,5).

A wide variety of such greases are available on the market, including soap-based, non-soap, synthetic, and bio-based types. Modern industrial formulations commonly contain mineral oils combined with metallic soaps, silica, bentonite, or polyurea derivatives (6,7). However, limited petroleum resources and growing environmental concerns have prompted researchers to explore biologically degradable and environmentally friendly alternatives, such as vegetable oils, waste oils, and ionic liquids (8). These formulations typically contain 75–95% base oil, 5–20% thickener, and 0–20% additives, with thickeners most often composed of metallic salts of long-chain fatty acids (9).

Among these alternatives, castor oil-based greases have attracted particular attention due to their biodegradability and environmental sustainability. Gallego et al. developed a castor oil-based grease using chemically modified biopolymer thickeners, and demonstrated properties comparable to those of conventional lithium- and calcium-based greases (10). Castor oil is rich in ricinoleic and linoleic acids, exhibits bactericidal activity, and may support wound healing by maintaining skin moisture balance (11). In addition, as a traditional herbal remedy, castor oil contains various phytochemical compounds, flavonoids, alkaloids, and vitamins, which contribute to its multiple health benefits (12,13).

In recent years, studies have demonstrated the skin-supporting effects of castor oil in wound healing processes. Topical application of castor oil can reduce skin infections and keratosis, alleviate skin problems such as acne, and contribute to wound healing (14). Furthermore, when combined with biocompatible and biodegradable synthetic polymers, castor oil-based greases can serve as potential biomaterials for wound healing patches and drug delivery systems (15-17).

This study aimed to develop an environmentally sustainable, biodegradable, and plant-based castor oil grease formulations and to explore the wound healing potential of these formulations from both biomedical and environmental perspectives.

Methods

The study was performed according to the Helsinki Declaration. Ethical approval for this study was obtained from the Biruni University Scientific Research Ethics Committee (Decision No: 2024-BİAEK/11-07, Date: 30.06.2025). All experimental procedures were conducted after obtaining the relevant ethical approval and in accordance with the principles of the Declaration of Helsinki.

Cell culture preparation

In this study, the human keratinocyte cell line HaCaT was used. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin. The cells were maintained under standard incubation conditions (5% CO_2, 37 °C) and grown in culture flasks until reaching approximately 90% confluency.

Cell culture

Prior to the experiment, cells were cultured at an appropriate suspension prepared at a concentration of 3×10^5 cells/mL and seeded into 6-well culture plates. The plates were incubated for 24–48 hours to allow the cells to reach approximately 90% confluency.

Wound creation (scratch assay)

Once a confluent monolayer was formed, a straight scratch (wound line) was created in the center of the cell layer using a sterile 200 μL pipette tip to initiate the wound healing assay. The wells were then washed with phosphate-buffered saline (PBS) to remove detached cells generated during scratching.

Application of castor oil-based biocompatible grease

Different concentrations (0.1%, 1%, 5%, and 10%) of castor oil-based biocompatible formulations were applied to the experimental groups. The selected concentrations were determined based on preliminary dose-finding experiments conducted to identify non-cytotoxic ranges that did not induce overt cell death or severe morphological alterations. Due to technical limitations, a fully homogeneous grease formulation could not be obtained under in vitro laboratory conditions. Therefore, in the present experimental setup, the term "castor oil-based biocompatible grease" refers to castor oil dispersed in culture medium at the indicated concentrations. The complete grease system, including thickener and additive components, could not be evaluated in this study and remains to be tested in future investigations.

For the negative control group, standard culture medium without any grease was used. In addition to the negative control group, a Tween control group was included to assess the independent effect of the surfactant used as a carrier. Tween 80 (polysorbate 80) was prepared at the same final concentration used to disperse castor oil in the culture medium and was applied to the cells under identical experimental conditions. All experimental groups were performed in triplicate.

Incubation and imaging

Following wound creation, cells were incubated in grease-containing or control media at different time points (0, 12, 24, and 48 hours). At each time point, wound closure was observed under an inverted microscope and digitally imaged.

Wound area analysis

Microscopic images obtained at different time points were analyzed using ImageJ software. The wound closure rate was calculated by measuring the wound area at baseline and at subsequent time intervals. The rate (%) was calculated using the following formula according to the literature (17): Wound closure (%) = $[(A_0 - A_t) / A_0] \times 100$; where A_0 represents the initial wound area and A_t represents the wound area at a specific time point. For comparative analysis, percentage changes relative to the negative control were calculated, where negative values indicate lower wound closure compared to the negative control.

Statistical analysis

The effects of different concentrations of castor oil on wound healing were evaluated in comparison to the control group. Quantitative wound area measurements were evaluated descriptively based on mean values obtained from independent experiments. Given the exploratory nature of the study and the absence of sufficient replicates for formal statistical testing, no inferential statistical comparisons were performed.

Results

In this study, wound healing outcomes reflect the biological effects of castor oil applied at different concentrations in culture medium, as a fully homogeneous grease formulation could not be prepared under the experimental conditions. Scratch assay tests performed on HaCaT cells demonstrated that castor oil-based biocompatible greases at different concentrations produced distinct effects on the wound healing process. Accordingly, negative percentage values reflect impaired wound closure compared to the negative control. Quantitative analyses revealed that at the 12th hour, the lowest wound area percentage was observed in the 5% castor oil group. This group showed a wound closure rate similar to that of the negative control. At lower concentrations (0.1% and 1%), the wound area remained larger, whereas at higher concentration (10%), cell morphology deterioration and reduced cell migration were detected microscopically. In the Tween control group, the wound area was also larger than that of the negative control (Table 1).

These findings indicate that the wound closure rates in descending order were as follows:

5% castor oil > Negative control > Tween control > 10% castor oil > 0.1% castor oil > 1% castor oil.

Microscopic examination revealed that in the 5% castor oil group, cells exhibited intensive migration toward the wound area, re-establishing the monolayer and largely closing the wound gap (Figure 1). In contrast, both low and high concentrations showed weaker cell migration and decreased cell density at the

Table 1. The results of the quantitative analysis, based on wound area measurements

Sample Group	Wound Area (%)	Relative wound closure compared to negative control (%)
Negative Control	24.05	0.0
Tween control	34.32	−42.7
Castor oil 10%	41.69	−73.3
Castor oil 5%	24.53	−2.0
Castor oil 1%	47.95	−99.3
Castor oil 0.1%	44.91	−86.7

Negative values indicate reduced wound closure relative to the negative control group. Data are presented as mean values from independent experiments. Quantitative comparisons are descriptive, and no formal statistical analysis was performed.

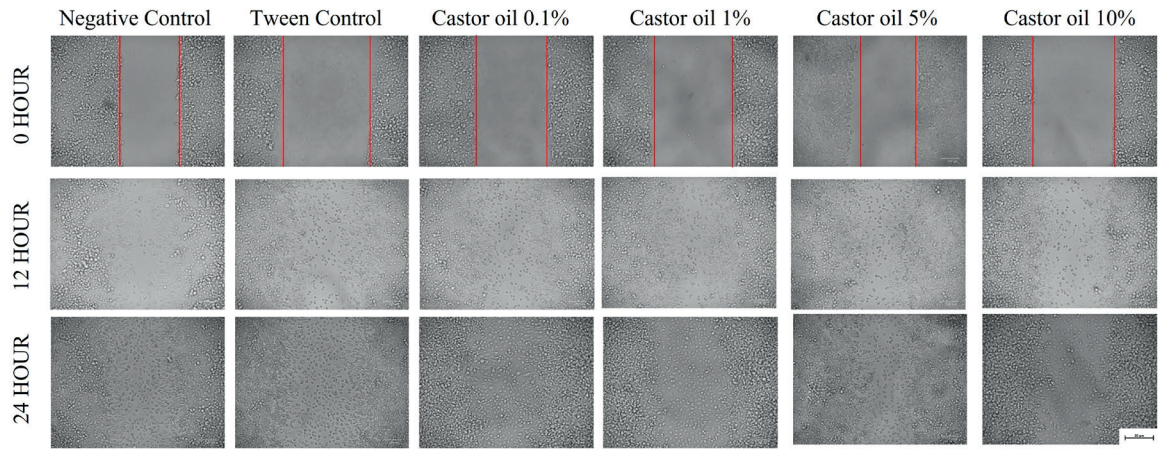

Figure 1. Microscopical Images of Scratch test at 0, 12th and 24th hours of incubations of HaCaT cells from the Negative Control, Tween Control, Castor oil 0.1%, Castor oil 1%, Castor oil 5%, Castor oil 10%

wound edges. The high concentration (10%) was associated with altered cell morphology and reduced migration rates, suggesting a possible cytotoxic or irritant effect.

These data indicate that castor oil-based formulations influence wound healing in HaCaT cells in a dose-dependent manner, with the optimal effect achieved at 5% concentration. The results are consistent with the model described in the Materials and Methods section and confirm the biocompatible, anti-inflammatory, and wound healing potential of this grease formulation.

Discussion

Wound healing is a complex, multi-stage process that requires coordinated cellular and molecular responses. Disruption of this process may delay tissue repair and contribute to the development of chronic wounds, which remains a significant global health problem. Accordingly, there is growing interest in identifying natural and biocompatible materials capable of supporting and accelerating wound repair. In this context, plant-derived oils have attracted considerable attention due to their antioxidant, anti-inflammatory, and regenerative properties (18-20).

In the present study, an in vitro wound healing model using the HaCaT keratinocyte cell line was employed to investigate the effects of different concentrations of castor oil-based formulations on cell migration. Scratch assays results demonstrated a clear dose-dependent response. The 5% castor oil group showed marked cell migration toward the wound area, effective reformation of the epithelial monolayer, and substantial wound closure. In contrast, lower concentrations (0.1% and 1%) resulted in weaker migratory responses, while exposure to a higher concentration (10%) was associated with altered cell morphology and reduced migration rates, suggesting a potential cytotoxic or irritant effect at elevated doses. However, as no dedicated cell viability assays (such as MTT or LDH) were performed, definitive cytotoxicity cannot be concluded and should be interpreted with caution.

These findings can be interpreted in the light of the biological properties of castor oil, which is rich in ricinoleic acid - a fatty acid known for its anti-inflammatory and tissue-regenerative effects (18,19). Consistent with our findings, previous studies have demonstrated that ricinoleic acid attenuates inflammatory processes and supports tissue regeneration, thereby promoting wound repair (18). Similarly, excessive exposure to ricinoleic acid has been reported to exert irritant effects on cell membranes, potentially disrupting membrane permeability and cellular integrity (20), which may explain the impaired migration and morphological deterioration observed at the highest concentration tested in this study. Together, these observations underscore the importance of identifying an optimal concentration range when considering castor oil for wound healing applications.

The inclusion of a Tween-containing control group further highlighted the importance of evaluating carrier substances independently. The relatively low wound closure rate observed in this group suggests that surfactants alone may negatively influence cellular behavior. In line with this observation, Godugu et al. (21) reported that emulsifiers can alter cell membrane permeability and reduce cell viability, emphasizing the need to account for potential confounding effects of formulation components in in vitro wound healing assays.

Our results are in agreement with previous studies investigating plant oil-based materials in wound healing contexts. Lacatusu et al. (16) demonstrated that lipid carriers derived from natural oils support tissue regeneration, while Jaganathan et al. (14) reported enhanced cell proliferation and accelerated wound closure in biomaterials containing castor oil. These studies support the notion that plant-derived oils can positively influence key cellular processes involved in wound repair.

However, an important limitation of the present study is that a fully homogeneous grease formulation could not be prepared under in vitro laboratory conditions. Consequently, the observed biological effects should be attributed solely to castor oil applied at different concentrations in culture medium, and the potential modulatory contributions of other grease components, such as thickeners or additives, could not be assessed. This limitation should be taken into consideration when interpreting the results, particularly with respect to the adverse effects observed at higher concentrations.

Future studies should focus on the development and standardization of fully homogenized grease formulations to enable more comprehensive evaluation of their biocompatibility and wound healing efficacy. Such investigations should include additional assessments of cytotoxicity (e.g., MTT or LDH assays), inflammatory responses, collagen synthesis, and in vivo validation. Similarly, other plant-derived oils, including virgin coconut oil and olive oil, have been reported to enhance wound healing through antioxidant, antibacterial, and pro-regenerative mechanisms (22-24), suggesting shared biological pathways that merit comparative evaluation. Collectively, these findings highlight the potential of natural oil-based formulations as promising, environmentally sustainable candidates for wound healing applications, while emphasizing the need for further systematic investigation.

Conclusion

This study demonstrated that castor oil applied at defined concentrations can influence wound closure and epithelial cell migration in an in vitro HaCaT scratch assay model. The enhanced wound closure observed at the 5% concentration suggests a concentration-dependent effect of castor oil on keratinocyte migration under controlled in vitro conditions. In contrast, the morphological alterations observed at higher concentrations underscore the importance of identifying safe and effective dose ranges.

Given the in vitro nature of this study and the absence of a fully formulated homogeneous grease system, these findings should be interpreted as preliminary. Future studies should focus on the development of standardized formulations and their evaluation using complementary in vitro assays and relevant in vivo models to more comprehensively assess biocompatibility, toxicity, and wound healing efficacy. Overall, the present results provide initial evidence supporting the potential of castor oil-based biomaterials as component of environmentally friendly biomaterial formulations for wound healing applications, warranting further investigation.

Ethical approval

This study was approved by the Biruni University Scientific Research Ethics Committee (Decision No: 2024-BİAEK/11-07, Date: 30.06.2025).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: RH, CY, NA; data collection: RH, CY, NA, META, LKB; analysis and interpretation of results: RH, CY, NA, META, LKB; draft manuscript preparation: RH, CY, NA. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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