World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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The effect of topical zinc oxide gel on scratch wound closure in fibroblast culture: A preliminary study and therapeutic design for traumatic ulcers

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World Journal of Advanced Research and Reviews, 2026, 29(01), 768-773

Publication history: Received on 05 December 2025; revised on 12 January 2026; accepted on 14 January 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.29.1.0085

Abstract

Traumatic ulcers are common oral lesions that can cause discomfort and impair oral function. Topical therapies such as corticosteroids are effective in suppressing inflammation; however, excessive use may inhibit wound healing. Zinc oxide (ZnO) has long been used in topical preparations and is known to play a role in tissue regeneration. This study aimed to evaluate the effect of zinc oxide gel at varying concentrations on scratch wound closure in fibroblast cell cultures. This in vitro experimental study was conducted using BHK-21 fibroblast cell cultures with a scratch assay method. Cells were divided into an untreated control group, a triamcinolone acetonide control group, and treatment groups receiving zinc oxide gel at concentrations of 6.5%, 13%, and 26%. Wound closure was observed at 24 and 48 hours. The results showed that zinc oxide gel influenced the scratch wound closure process in fibroblasts, with different response patterns at each concentration. The 13% concentration demonstrated more stable wound closure at later observation compared to the 26% concentration. These findings indicate that fibroblast responses to zinc oxide are not linearly related to increasing concentration.

Keywords: Zinc Oxide; Fibroblasts; Wound Healing; Scratch Assay

1. Introduction

A wound is defined as a break in tissue continuity due to tissue damage or loss caused by trauma, medical procedures, or other factors[1]. In the oral cavity, one of the most common types of wounds is traumatic ulcers. Traumatic ulcers can occur due to mechanical, thermal, or chemical trauma, and often cause pain and functional impairment such as difficulty speaking, eating, and swallowing[2]. Although most traumatic ulcers heal spontaneously, slow or suboptimal healing can increase the risk of malignancy[3].

Topical medication therapy is a commonly used approach in the treatment of traumatic ulcers. Topical corticosteroids, such as triamcinolone acetonide, are effective in reducing inflammation and clinical symptoms[4]. However, prolonged use of corticosteroids has been reported to inhibit fibroblast proliferation, decrease growth factor expression, and potentially slow wound healing[5]. Therefore, an alternative topical therapy is needed that not only suppresses inflammation but also supports the tissue regeneration process.

Zinc oxide is an inorganic compound that has long been used in topical preparations due to its anti-inflammatory, antioxidant, and tissue-protective properties[6],[7]. Zinc, as an essential micronutrient, plays a role in various cellular processes, including protein synthesis, cell division, and DNA repair[8]. In the context of wound healing, zinc is known to support cell migration and proliferation, as well as extracellular matrix formation[9]. However, the cellular response to zinc oxide can be influenced by concentration and cellular environmental conditions[10].

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The wound healing process is highly dependent on cellular activity, which can be systematically studied through cell culture approaches, particularly fibroblast culture. Fibroblasts are the main mesenchymal cells that play a role in the proliferation phase of wound healing and are responsible for migration to the wound area, cell proliferation, and extracellular matrix synthesis and deposition[11]. Therefore, fibroblast cell culture has been widely used in various studies, particularly to study the wound healing process. One of the most commonly used methods is the scratch assay, which simulates mechanical injury to the cell layer and allows observation of the wound closure process[12].

Although various studies have reported the benefits of zinc oxide in wound healing, studies on the response of fibroblasts to varying concentrations of zinc oxide, particularly in the context of in vitro closure of abrasions, are still limited. Therefore, this study aims to evaluate the effect of zinc oxide gel with varying concentrations on the closure of abrasions in BHK-21 fibroblast cell cultures.

2. Material and methods

2.1. Study Design

This study was an in vitro experimental study with a post-test only control group design using BHK-21 fibroblast cell cultures.

2.2. Fibroblast Cell Culture

BHK-21 fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with serum and antibiotics. Cells were incubated at 37°C in a 5% CO_2 atmosphere until approximately 80% confluence was achieved

2.3. Scratch Assay

After reaching confluence, a mechanical scratch was created using a sterile yellow pipette tip on the fibroblast cell monolayer. This scratch produced a cell-free area simulating a wound condition.

2.4. Treatment Groups

The cell cultures were divided into five groups:

- Untreated control group
- Triamcinolone acetonide control group
- Zinc oxide gel 6.5% treatment group
- Zinc oxide gel 13% treatment group
- Zinc oxide gel 26% treatment group

2.5. Wound Closure Observation

Observations were conducted at 24 and 48 hours after treatment. Wound distances were measured using ImageJ software based on microscopic images.

2.6. Data Analysis

Data were analyzed statistically using parametric tests (One-Way ANOVA) or nonparametric tests (Kruskal–Wallis), depending on data distribution, with a significance level of $p < 0.05$.

3. Results

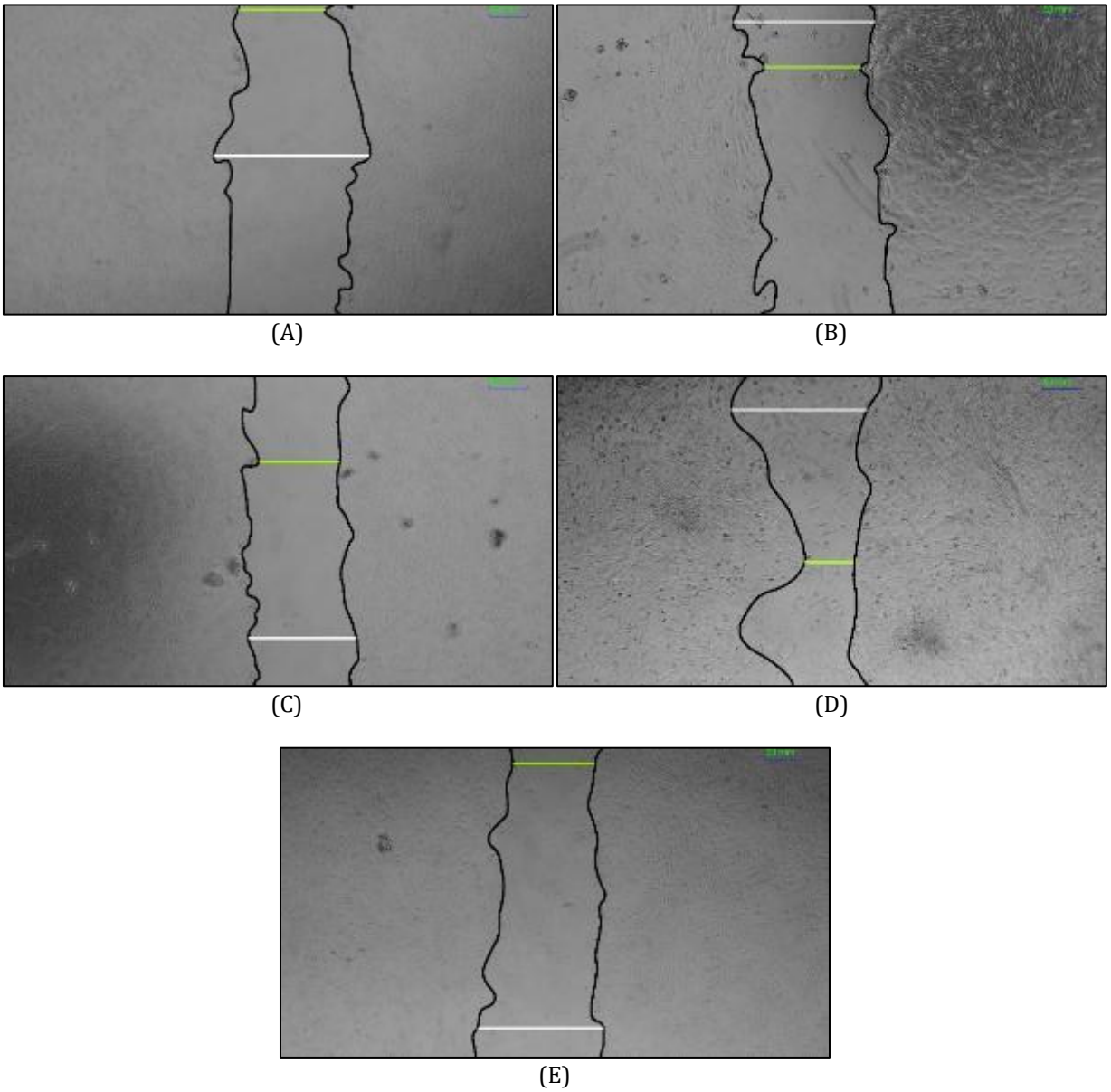

Figure 1 Microscopic observation of the scratch healing time test of BHK-21 fibroblast culture at 24 hours. (A) Negative control without treatment. (B) Positive control with TCA application. (C) Treatment 1 with 6.5% topical zinc oxide gel application. (D) Treatment 2 with topical zinc oxide gel 13% application. (E) Treatment 3 with topical zinc oxide gel 26% application

Notes:

- Black line: imaginary line from the edge of the scratch
- White line: furthest distance (μm)
- Green line: the closest distance (μm)

Table 1 Mean values and standard deviations of wound closure distances in BHK-21 fibroblast cell cultures at 24-hour observation, measured using the ImageJ application

Time	Group	Wound closure distances (µm)			Mean±SD
		Replication I	Replication II	Replication III	
24 hours	K-	163.425	162.116	162.488	162.676±0.674
	K+	161.960	159.813	156.160	159.311±2.932
	K1	141.2035	138.164	143.9455	141.1043± 2.892
	K2	129.6295	130.244	130.3745	130.0827±0.397
	K3	127.022	125.132	126.762	126.305±1.024

K- = No treatment; K+ = Application of TCA medication; K1 = Application of 6.5% zinc oxide topical gel; K2 = Application of 13% zinc oxide topical gel; K3 = Application of 26% zinc oxide topical gel

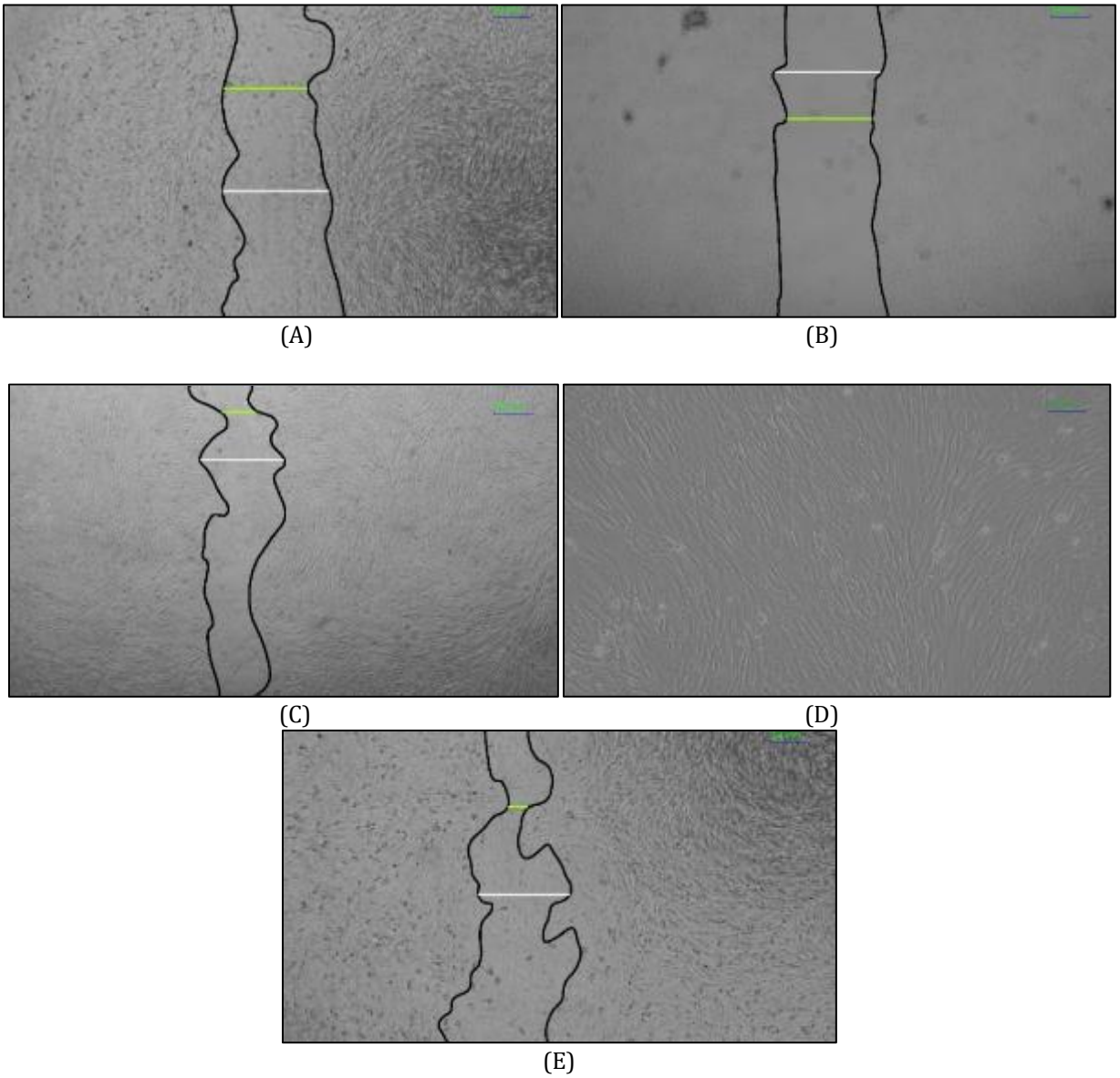

Figure 2 Microscopic observation of the scratch healing time test of BHK-21 fibroblast culture at 48 hours. (A) Negative control without treatment. (B) Positive control with TCA application. (C) Treatment 1 with 6.5% topical zinc oxide gel application. (D) Treatment 2 with topical zinc oxide gel 13% application. (E) Treatment 3 with topical zinc oxide gel 26% application

Notes:

- Black line: imaginary line from the edge of the scratch
- White line: furthest distance (μm)
- Green line: the closest distance (μm)

Table 2 Mean values and standard deviations of wound closure distances in BHK-21 fibroblast cell cultures at 48-hour observation, measured using the ImageJ application

Time	Group	wound closure distances (μm)			Mean ± SD
		Replication I	Replication II	Replication III	
48 hours	K-	150.000	148.291	150.504	149.598±1.160
	K+	82.407	77.820	84.054	81.427±3.230
	K1	75.000	75.580	73.827	74.802±0.893
	K2	0	0	0	0
	K3	123.611	122.942	120.455	122.3360±1.662

K- = No treatment; K+ = Application of TCA medication; K1 = Application of 6.5% zinc oxide topical gel; K2 = Application of 13% zinc oxide topical gel; K3 = Application of 26% zinc oxide topical gel

The results showed that all zinc oxide treatment groups experienced a reduction in wound distance compared to the control group. At the 24-hour observation, the group with a 26% zinc oxide concentration showed a relatively shorter wound distance compared to the other groups. However, at the 48-hour observation, the group with a 13% zinc oxide concentration showed more stable wound closure, while the 26% concentration group showed less than optimal wound closure. Not all scratch wounds closed completely at 48 hours. This is thought to be related to cellular stress due to mechanical trauma from the scratch assay method and the cellular response to the test material concentration.

4. Discussion

The reduction in wound distance in the zinc oxide treatment group shows that zinc oxide can affect fibroblast activity in the wound closure process. The role of zinc as an enzyme cofactor supports protein synthesis and cell division, which are important in the proliferation phase of wound healing[13]. In addition, Zn^{2+} ions are known to facilitate fibroblast migration and proliferation to the wound area.

However, findings at higher concentrations of zinc oxide indicate that the cellular response does not always increase with increasing concentration. At high concentrations, zinc oxide has been reported to interact with cellular components and disrupt membrane function, as well as potentially increase oxidative stress[7]. This condition can inhibit the continuity of the proliferation and migration response of fibroblasts in the later stages of observation.

The difference in wound closure response between the 24- and 48-hour observation times shows that the effect of zinc oxide on fibroblasts is dynamic and influenced by cellular adaptation to the environment. This response does not show a linear relationship with increasing concentrations, but rather reflects a certain concentration that better supports the balance between cell stimulation and cellular tolerance.

5. Conclusion

Zinc oxide gel influences the scratch wound closure process in BHK-21 fibroblast cell cultures. Wound closure responses varied across concentrations, with the intermediate concentration demonstrating more stable wound closure at later observation compared to higher concentrations. These findings indicate that fibroblast responses to zinc oxide are not linearly related to increasing concentration.

Compliance with ethical standards

Disclosure of Conflict of interest

The Authors declare that they have no conflict of interest

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