Van Med J 33 (1):112 -119, 2026
DOI: 10.5505/vmj.2026.69875

The Effect of Hypochlorous Obtained From Different Salts on Keratinocyte and Fibroblast Cells

Elvan Alkaya[1], Elif Önder[1], Doğukan Mutlu[2], Nazlı Çil[1], Ahmet Koluman[3], Şevki Arslan[4], Gülçin Abban Mete[1]

[1]*Pamukkale University, Faculty of Medicine, Department of Histology and Embryology, Denizli, Türkiye*
[2]*Üsküdar University, Faculty of Engineering and Natural Science, Department of Molecular Biology, Istanbul, Türkiye*
[3]*Pamukkale University, Faculty of Technology, Biomedical Engineering, Denizli, Türkiye*
[4] *Pamukkale University, Faculty of Science, Department of Biology, Denizli, Türkiye*

Abstract

Introduction: Hypochlorous acid (HOCl) is a physiological molecule synthesised by neutrophils during phagocytosis and plays an active role in the destruction of all microorganisms. In general, HOCl has been demonstrated to function as an antiseptic and an agent that induces apoptosis or cell necrosis in cells, depending on the ambient concentration. The aim of this study was to examine the impact of sodium hypochlorite (NaOCI) and zinc hypochlorite (ZnOCI), derived from diverse chlorine salts, on cellular proliferation and migration in keratinocyte and fibroblast cell lines in vitro.

Materials And Methods: The effects of different concentrations of NaOCl and ZnOCl on keratinocyte (HaCaT) and fibroblast cell lines(L929) were compared using cell viability, colony formation and wound healing assays. In addition, the presence of apoptosis in the cells was determined using the TUNEL method.

Results: The application of NaOCI and ZnOCI resulted in a substantial increase in colony formation in keratinocyte cell lines (HaCaT) in comparison to the control group, while this effect was not observed in fibroblast cell lines (L929). The results of the wound healing migration assay demonstrated that both NaOCI and ZnOCI were effective in the fibroblast cell line. However, when we compared the results of the two solutions in terms of wound healing and colony formation, ZnOCI was more effective than NaOCI in terms of colony formation in HaCaT cells and wound healing in L929 cells. NaOCI and ZnOCI reduced apoptosis in the HaCaT and L929 cell lines compared to the control group. NaOCI was more effective than ZnOCI in HaCaT cells. No difference was observed between the two applications in the L929 cell line.

Conclusion: Further advanced research, including molecular studies, is required to understand the properties of NaOCI and ZnOCI.

Key words: NaOCI; ZnOCI; fibroblast cell line; keratinocyte cell line.

Introduction

Hypochlorous acid (HOCl) is a physiological molecule that is synthesised by neutrophils during phagocytosis. It is involved in the active elimination of all microorganisms. HOCl is a weak acid and undergoes decomposition into hypochlorite ions (OCI-) and protons (H+) in accordance with the pH of the solution. (1) It is evident from extensive research that HOCI has been shown to have antiseptic properties. Furthermore, it has been determined that HOCI can cause apoptosis or cell necrosis in cells, depending on the ambient concentration (2, 3). Due to these characteristics, HOCl is employed as a disinfectant and antibacterial agent. Disinfectants based on HOCl affect all the vital functions of microorganisms, resulting in their death within seconds (4). The process of biofilm destruction prevents the development of antibiotic resistance amongs microorganisms. Furthermore, it ensures that harmful microorganisms present beneath this layer are prevented from entering the body from the external environment. In particular, it facilitates the process of wound healing and tissue regeneration by eliminating harmful microorganisms present in the wound area (5). In contrast to alcohol or iodine antiseptics, which have been demonstrated to damage keratinocytes and fibroblasts in healthy tissue, HOCl disinfectants have been shown to accelerate wound healing by increasing the proliferation (migration) of these cells to the damaged area. The product provides 99.99% antisepsis in less than a minute and is fully biodegradable, turning to water in 2-3 minutes. As it does not enter the systemic

*Corresponding Author: Gülçin Abban Mete Pamukkale University, Faculty of Medicine, Department of Histology and Embryology, Denizli, Turkiye **Email:** gabban@.pau.edu.tr **Orcid:** Gülçin Abban Mete 0000-0001-6794-3685, Elvan Alkaya 0000-0002-7057-2544, Elif Önder 0000-0002-7187-1669, Doğukan Mutlu 0000-0003-3259-5822, Nazlı Çil 0000-0002-2164-8688, Ahmet Koluman 0000-0001-5308-8884, Şevki Arslan 0000-0002-4215-5006

Received: 28.07.2025, Accepted: 01.12.2026

circulation, it can be used in conjunction with other preparations required for treatment. It does not interact with any systemic product (6). For this reason, HOCl disinfectants are used to treat ulcers and wounds of various origins, including acute and chronic wounds, partial- and full-thickness skin wounds, contusion wounds, post-operative wounds, graft beds and skin grafts, burns (first- and second-degree), skin ulcers (stages I–IV), arteriovenous problems, neurological conditions, diabetes, trauma and other conditions. HOCl disinfectants are also used to treat various wounds, including small tears and cuts, abscesses, wound irrigation and debridement, and reduction of wound moisture and odour (7). It plays a pivotal role in the healing of these injuries by combining antimicrobial activity with non-cytotoxic action on the surrounding tissues (8-10). The aim of this study was to examine the impact of hypochlorous acid solutions, sodium hypochlorite (NaOCI) and zinc hypochlorite (ZnOCI) on the proliferation of keratinocytes (HaCaT) and fibroblasts (L929) in cell monolayers. The effects of these substances on cell proliferation, cell migration and apoptosis were investigated comparatively.

Materials and Methods

The ZnOCl and NaOCl salts used in the study were prepared in the laboratory of the Department of Biomedical Engineering, Faculty of Technology, Pamukkale University

Sodium hypochlorous (NaOCl) and Zinc hypochlorous (ZnOCl):

Chemicals: 0.9% NaCl solution, zinc salt ($ZnCl_2$ or $ZnSO_4$), deionised water.

Instrument and Equipment: Electrolysis cell, power supply (DC), platinum and titanium electrodes, magnetic stirrer, pH meter, conductivity meter, UV-Vis spectrophotometer, ICP-MS device

Obtaining sodium hypochlorous (NaOCl):

1. The electrolysis cell was filled with 100 mL of 3% NaCl solution.
2. Titanium coated platinum electrode was used as anode and stainless steel electrode was used as cathode.
3. 4-6 V DC voltage and 200-300 mA current were applied to the cell.
4. Electrolysis time was determined as 5 minutes.
5. The pH and conductivity of the resulting sodium hypochlorous solution were measured.
6. The concentration of active chlorine was determined using a UV-Vis spectrophotometer with a wavelength of 292 nm.

Obtaining zinc hypochlorous (ZnOCl):

1. The electrolysis cell was filled with 100 mL solution containing 3% $ZnCl_2$.
2. Titanium coated platinum electrode was used as anode and zinc electrode was used as cathode.
3. Electrolysis conditions: 6-8 V DC voltage, 250-350 mA current, 5 min process time.
4. The pH and conductivity of the resulting ZnOCl solution were measured.
5. Zinc concentration in the solution was analysed by ICP-MS.

Analysis and Results

pH values:

NaOCl solution: pH 7.2 ± 0.3

ZnOCl solution: pH 6.8 ± 0.2

Conductivity:

NaOCl: 18.5 ± 0.5 mS/cm

ZnOCl: 17.2 ± 0.4 mS/cm

Active Chlorine Concentration (Chlorine measuring strips):

NaOCl: 120 mg/L

ZnOCl: 105 mg/L

Cell culture: In this study, fibroblast (L929) and keratinocyte (HaCaT) cell lines were employed in a two-dimensional monolayer cell culture (11,12). The L929 and HaCaT cells were cultured at 37°C, with 5% CO_2 and 95% humidity. The cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were passaged at intervals of between two and three days, contingent on the density of the cells. Once the cells had reached a density of approximately 80-90% in the 100 mm culture plates, they were detached using trypsin-EDTA, centrifuged and resuspended in fresh medium.

Cell viability test: The XTT test was employed to investigate the impact of NaOCI and ZnOCI on the viability of the HaCaT cell line, while the MTT test was utilised to examine their influence on the L929 cell line.

XTT assay: The impact of ZnOCl and NaOCl on the proliferation of HaCaT cells were evaluated through the XTT assay, following the manufacturer's instructions (Cell Proliferation Assay with XTT Reagent-Cell Proliferation Kit, Lot: ≠213190). The HaCaT cells were seeded in 96-well plates at a concentration of $2x10^3$ cells. The medium was replaced with solutions of NaOCI and ZnOCI at varying concentrations, which were then incubated for a further 24 hours. At the conclusion of the 24-hour incubation period, 100 µL of medium was added to each well,

followed by the addition of 50 µL of activated XTT solution (comprising 49 µL of XTT reagent solution and 1 µL of activator solution) (12). The determination of the absorbance values absorbance values (OD) was conducted spectrophotometrically at 450 nm using a microplate reader (Biotek), with the results then being subjected to analysis.

MTT assay: The MTT assay was employed to ascertain the impact of NaOCI and ZnOCI salts on the viability of the L929 cell line. The cells were seeded in 96-well plates at a concentration of $2x10^3$ cells/well and incubated for 24 hours. Following the incubation period, the medium was replaced with solutions of varying concentrations and incubated for a further 24 hours. Subsequently, the culture medium was removed and 10 µL of MTT (5 mg/mL) and 100 µL of medium were added to the cells, which were then incubated for four hours. Subsequently, the medium containing MTT was removed and the resulting formazan crystals were dissolved in 100% DMSO. The absorbance of the resulting colour was determined at 590 nm using a microplate reader spectrophotometer (Epoch, BioTek) and the results were evaluated (13).

Colony formation test: The control and dose groups were seeded in a six-well plate at a density of 10^3 cells per well. The cells were incubated at 37°C with 5% CO_2 for 14 days, with medium changes performed every two days. At the conclusion of the 14-day period, the cells were fixed with cold methanol stored at -20°C for a duration of 10 minutes. Subsequently, the fixed colonies were stained with crystal violet for a period of 15 minutes. The number of stained colonies was counted and compared with that of the control group.

Wound healing assay: HaCaT and L929 cells were seeded in cell culture dishes at a density of 10^6 cells per well and incubated for 24 hours. Once the cells had reached a confluence of 90%, a scratch was made in the cell layer using a 200 microlitre pipette tip. The migration status of the cells was then observed at 0, 16 and 24 hours, with photographs taken under an inverted microscope.

TUNEL saining: The apoptotic effect of NaOCl and ZnOCl on HaCat and L929 cells was assessed using the TUNEL assay. Cells from the groups cultured in chamber slides were fixed with 4% (w/v) paraformaldehyde for 20 minutes at room temperature. The cells were then washed with 0.5% Triton X for 30 minutes and incubated in blocking solution for a further 30 minutes. The TdT-mediated dUTP nick-end labelling (TUNEL) kit protocol was used to determine the occurrence of apoptosis in the cells. The number of cells in total and the number of TUNEL-positive cells were counted in 10 randomly selected fields of view under the microscope. The apoptotic index (API) was calculated as the ratio of apoptotic cells to the total number of cell.

Ethical Consent: As this study was conducted within a laboratory setting and used cell lines, approval from an ethics committee was not a compulsory requirement. The study used two cell lines: The HaCat cell line Applied Biological Materials (ABM) Cat. No. ≠T9145. L929: European Collection of Authenticated Cell Cultures (ECACC) Cat. No.≠85011425

Statistical analysis: The data obtained were analysed using SPSS 21.0 for Windows. Shapiro Wilk test was used to examine the suitability of the data for normal distribution.One-way Analysis of Variance (ANOVA,) was used to compare independent groups and Tukey was used as post-hoc variance analysis were employed to analyse independent groups. Statistical differences were considered significant at the 0.05 level or less.

Results

Cell viability: In HaCaT cells, the highest cell viability rate in the NaOCI application was determined at 1.44 ppm, while the lowest cell viability rate was determined at 23 ppm. In the ZnOCI application, the highest cell viability rate was determined at 3 ppm, while the lowest cell viability rate was determined at 44 ppm (Figure 1).

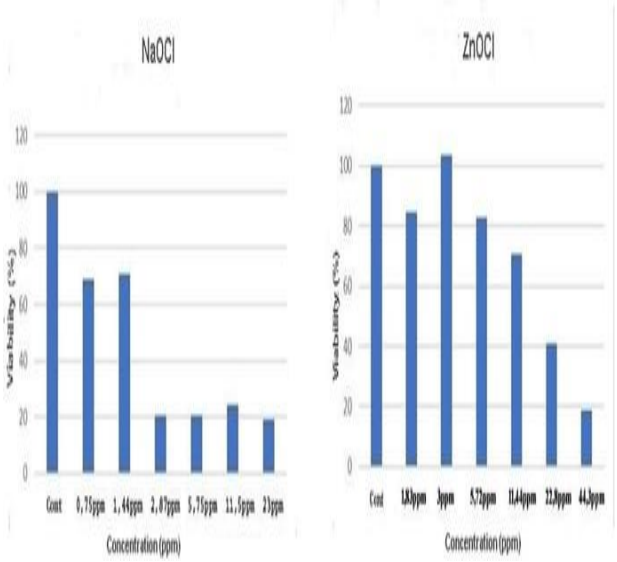

Figure1: Effect of NaOCl ve ZnOCl On HaCaT cells. Percentage cell viability of HaCat cells induced by NaOCl and ZnOCl in different concentrations calculated with XTT assay.

In L929 cells, the highest cell viability rate was observed in the NaOCI application at 30 ppm, while the lowest cell viability rate was determined at 50 ppm. In the ZnOCI application, the highest cell viability rate was determined at 3 ppm, whereas the lowest was determined at 44.3 ppm (Figure 2).

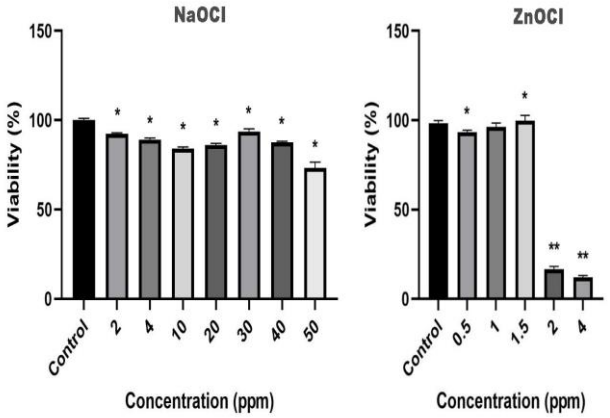

Figure 2: Effect of NaOCl ve ZnOCl on L929 cells. Percentage cell viability of L929 cells induced by NaOCl and ZnOCl in different concentrations calculated with MTT assay.

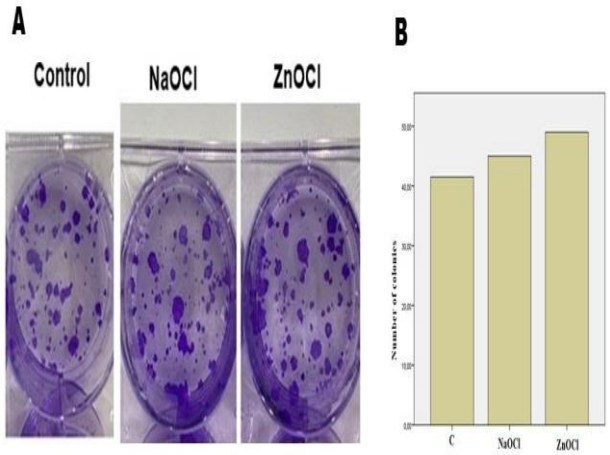

Figure 3: A. This image represents colony formation in HaCaT cells that have been treated with NaOCl And ZnOCI. Colonies were stained with crystal B: Colony numbers were counted in the control and traeted groups

Colony formation: The evaluation of the colony formation of the cells revealed a significant discrepancy in the number of colonies observed in the control group and those in the dose groups. Furthermore, the number of colonies in the HaCaT cell line demonstrated a notable increase following treatment with NaOCl and ZnOCI ($p < 0.05$). Furthermore, a significant difference was observed between the two dose groups, with ZnOCI demonstrating a greater capacity to form colonies in the HaCaT cell line compared to NaOCI ($p < 0.05$) (Figure 3). The observation of L929 cell line revealed the absence of colony formation.

Wound healing assay: Wound healing migration assay was performed to detect of effect of NaOCl and ZnOCl on migration in L929 cells. It was observed that the application of 20 ppm NaOCI resulted in approximately 1.2 times the number of cells migrating to the wound area in comparison to the control group at the 48-hour mark. Furthermore, the use of 40 ppm NaOCI led to the coverage of 2.7 times the wound area in comparison to the control group (Figure 4). A significant difference in wound area was observed between the treatment and control groups ($p<0.05$). The 0.5 and 1.5 ppm ZnOCI applications resulted in the wound area being covered 1.21 and 9.7 times more, respectively, than in the control group (Figure 4.3.4). A significant difference was observed in the wound area of the treated group in comparison to the control group ($p<0.05$) (Figure 4). In the HacaT cell line, no cell proliferation was observed in either the experimental or control groups.

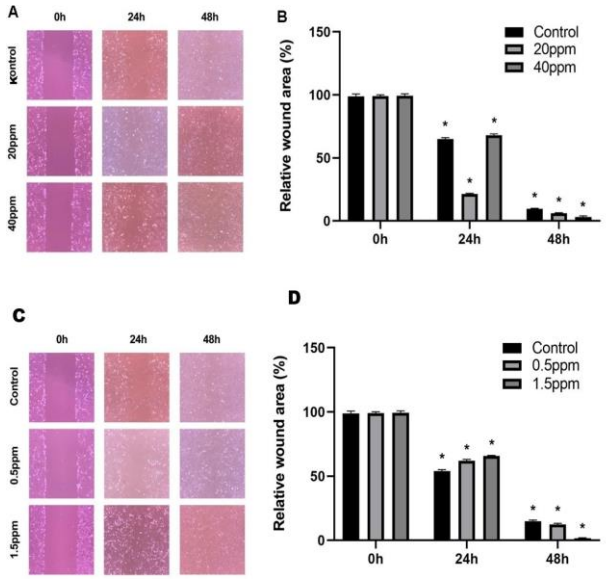

Figure 4: The images show the wound healing in the control group and NaOCl (A,B) and ZnOCl (C,D) treated groups. The results showed that NaOCl and ZnOCl administration increased cell migration. Representative images of cells in the control and dose groups at 0h, 24h and 48h are given.

TUNEL staining results: Compared to the control group, application of NaOCI and ZnOCI resulted in a significant reduction in apoptosis in the HaCaT and L929 cell lines ($p<0.05$) (Figure 5). However, it was demonstrated that NaOCI was

more effective than ZnOCI at reducing apoptosis in HaCaT cells ($p<0.05$). In the L929 cell line, the apoptotic index was observed to be lower in the treatment groups than in the control group. Nevertheless, no significant difference was identified between the NaOCI and ZnOCl - treated groups ($p\geq0.05$).

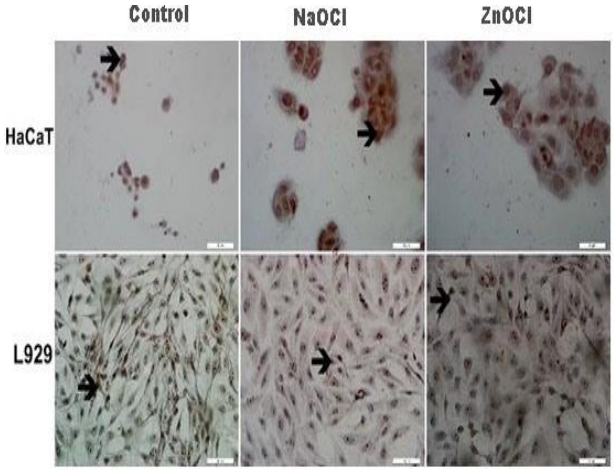

Figure5: The image presents the results of TUNEL staining in the HaCat and L929 cell lines. The arrow indicates TUNEL-positive cells. Immunoperoxidase & Haematoxylin, X400, Bar=50μM

Discussion

The objective of this study was to examine the healing effects of NaOCl and ZnOCl, derived from different chlorine salts, on keratinocyte (HaCaT) and fibroblast (L929) cell lines in vitro. The impact of NaOCl and ZnOCl on keratinocyte and fibroblast cell lines was examined through the utilisation of cell viability, colony formation and wound healing assays. Furthermore, the occurrence of apoptosis in the cells was ascertained through the utilisation of the TUNEL methodology. The results demonstrated that NaOCI and ZnOCI solutions were more effective in colony formation in HaCaT cells compared to the control group. Furthermore, the data indicated that ZnOCI was more effective than NaOCI among the two different hypochlorite acids. It is noteworthy that no colony formation was observed in L929 cell lines. The results of the wound healing migration assay demonstrated that both NaOCI and ZnOCI were effective in the fibroblast cell line. The data indicate that the 40 ppm NaOCI solution resulted in approximately 2.7 times the cell migration observed in the control group. The 1.5 ppm ZnOCI solution demonstrated a 9.7-fold increase in cell migration compared to the control group. Our findings indicated that the ZnOCI solution exhibited superior efficacy in wound closure compared to the NaOCI solution. A review of the literature on the role of zinc in wound healing indicates that zinc levels increase significantly during the early inflammatory phase of wound healing (14). In a study conducted by Lonsdown et al., it was demonstrated that within the initial 24 hours following injury, there was a 15-20% increase in zinc levels at the wound edge. This increase subsequently reached up to 30% during the subsequent stages of granulation tissue development and epidermal proliferation. It has been reported that the early influx of zinc is due to an increase in the expression of the metallothionein (MT) protein family in keratinocytes, macrophages and dermal fibroblasts at the wound edge. Conversely, in the later stages of wound healing, a reduction in cell division and scar maturation is associated with a decline in zinc levels (15). In this study, colony formation was observed in HaCaT cells; however, migration was not observed in wound healing experiments, including the control group. This phenomenon may be attributed to the inherent biological characteristics of the specific cell lines in question. A comprehensive review of the current literature has revealed studies showing that strong intercellular connections in epithelial-derived HaCaT cells promote colony formation while inhibiting migration (16,17). In their study, Jiang and colleagues observed that fibroblast-derived L929 cells exhibited minimal tendency for colony formation and high migration activity on the extracellular matrix (ECM) (18). Studies using sodium hypochlorite (NaOCl) have demonstrated that the application facilitates colony formation in HaCaT cells and simultaneously causes migration loss. This is likely attributable to oxidative damage and its disruptive effects on the cytoskeleton, focal adhesions, and protease signalling (16-20)

Another potential explanation pertains to the impact of the doses and application times of the solutions employed on the cells. A study by Jiang et al. reported that sublethal doses of these agents may facilitate colonisation, depending on the concentration and exposure time. However, they may also disrupt the actin remodelling and cellular energy mechanisms necessary for migration. It has been hypothesised, in particular, that NaOCl may alter the activities of MMP-2 and MMP-9, which are necessary for extracellular matrix (ECM) degradation. This alteration could result in the formation of a microarchitecture that hinders cell migration (18). Another study indicates that applying NaOCl (sodium hypochlorite) and zinc (Zn/ZnO or Zn salts) to L929 cells may promote short-term cell survival and motility; however,

long-term proliferation (colony formation) may be inhibited (21). In our study, no colonisation was observed in L929 cells at any of the application times. In some cases, it has been observed that cellular stress increases migration while simultaneously inhibiting proliferation (19). However, it is important to note that in some cases, a lack of proliferation may be observed due to the dominance of migration. In this particular case, it has been documented that cells can migrate to the wound but cannot proliferate, thereby preventing colony formation. As noted in most research findings, sodium hypochlorite (NaOCl) exhibits significant toxicity on fibroblast cells (22,23). Despite the paucity of direct studies analysing colony formation in L929 cells with the specific compound ZnOCl, it has been reported that ZnO nanoparticles may exhibit dose-dependent toxicity in L929 cells (24,25). We found that apoptosis was lower in both solutions and cell lines than in the control group. This led us to conclude that neither NaOCl nor ZnOCl has a toxic effect on cells. The passage number is a critical factor in both proliferation and migration. Research has indicated that L929 cells which have undergone extensive passage may retain their migratory capacity for a certain period, whilst concomitantly losing their proliferation capacity (21). The L929 cells used in this study were at passage 26, which may have inhibited colony formation. It has also been shown by research that the passage number is an important factor in HaCaT cells. For example, a 2021 study on HaCaT cells concluded that changes in passage number could affect morphology, thereby influencing cell proliferation and wound healing experiment (26). The HaCaT cell passage used in this study was 15, and this could affect the efficacy of NaOCI and ZNOCI. Another factor that affects cell behaviour is microRNAs (miRNAs). Studies conducted to date suggest that miRNAs have the potential to modulate cellular behaviour. For instance, a 2021 study found that microRNA-185 (miRNA-185) reduced the proliferation and migration of HaCaT cells, while also altering their oxidative stress conditions (27). More recently, Chang et al. reported that increased expression of microRNA-126 promoted migration and colonisation in HaCaT cells. While there has been no direct study investigating the relationship between ZnOCI and miRNAs, it has been hypothesised that the combination of Zn and hypochlorite may modulate cellular behaviour via ROS and miRNAs (28). Wound healing is an important and complex process in both humans and animals. It involves several phases, including haemostasis/inflammation, proliferation, and remodelling (29). In their 2007 study, Werner et al. examined keratinocyte-fibroblast interactions in vitro using HaCaT and fibroblast cultures. They demonstrated that, in the early stages after injury, proinflammatory mediators such as IL-1, which are derived from keratinocytes, regulate interactions between keratinocytes and fibroblasts. They also showed that TGF-β activity is strongly up-regulated in co-cultures of keratinocytes and fibroblasts. They also found that IL-1-responsive genes such as keratinocyte growth factor (KGF), IL-6, endothelin-1 (ET-1), heparin-binding EGF and GM-CSF were up-regulated in fibroblasts, and that these factors could stimulate keratinocyte proliferation and differentiation. The researchers also observed that components of the epidermal basement membrane are expressed by both keratinocytes and, to a lesser extent, fibroblasts. They noted that, in the middle stage of keratinocyte-fibroblast interactions, the balance of the effects of keratinocyte-derived IL-1 and TGF-β begins to shift, prompting fibroblasts to increase their synthesis of basement membrane components. It has been demonstrated that basal membrane components are strongly expressed in fibroblasts during the final stage of wound healing (30). In the present study, HaCaT and L929 cells were not co-cultured; therefore, it is possible that keratinocytes and fibroblasts did not interact, which would explain the absence of colony formation and cell migration.

Study Limitations: The present study has several limitations. Not being able to determine the effects of NaOCI and ZnOCI on cells at the molecular level and not being able to perform co-cultures of the HaCaT keratinocyte cell line and the L929 fibroblast cell line are significant limitations of our research.

Conclusion

NaOCI and ZnOCL were found to enhance colony formation in Hacat cells. In contrast, NaOCI and ZnOCL did not induce cell migration in Hacat cells NaOCI and ZnOCL were found to be ineffective in inducing colony formation in L929 cells .Whereas NaOCI and ZnOCL were found to enhance cell migration in L929 cells. The underlying reasons for these outcomes may be attributable to the varying doses of the solutions employed, which exhibited cell-specific disparities. A further rationale pertains to the passage numbers of the cells utilised, which have the capacity to influence colony formation and cell migration. Alternatively, the absence of interaction between keratinocytes and fibroblasts is a possibility. Nonetheless, a comparative analysis of

NaOCI and ZnOCI has demonstrated that ZnOCI is more effective in promoting colonisation and enhancing cell growth. The present study also demonstrated that both NaOCI and ZnOCL reduced apoptosis in both Hacat and L929 cell lines. In contrast, the results of our study indicated that NaOCI reduced apoptosis in HaCaT cells to a greater extent than ZnOCI. Considering the effect of hypochlorous acid on microorganisms, the results of our research will be beneficial for developing new treatment methods through research on skin and connective tissue cells. We believe that developing these results for use in biomedical engineering and product design will lead to the introduction of new, effective medical products. HOCl is at the forefront of the search for environmentally friendly products that leave little residue, act quickly and are easily obtainable for use in treating injuries. It is evident that further research is required to comprehensively understand the molecular effects of these solutions.

Ethical Statement: As this study was conducted within a laboratory setting and used cell lines, approval from an ethics committee was not a compulsory requirement. The study used two cell lines: The HaCat cell line Applied Biological Materials (ABM) Cat. No. ≠T9145. L929: European Collection of Authenticated Cell Cultures (ECACC) Cat. No.≠85011425

Conflict of interest: The authors declare no conflict of interes.

Statement of financial support: This study was supported by the Scientific Research Projects Coordination Unit of Pamukkale University. Project no: 2021SABE005

Author contributions:

Concept/Design: EA, GAM, AK.
Data acquisition: EA, GAM, EÖ, DM, SA.
Data analysis and interpretation: GAM, EA; EÖ; DM, NÇ, SA, AK
Drafting manuscript: EA, GAM, EÖ;
Critical revision of manuscript: GAM, EA; EÖ; DM, NÇ, SA, AK ,
Final approval and accountability: GAM, EA; EÖ; DM, NÇ, SA, AK ,
Supervision: GAM

References

1. Foote CS, Goyne TE, Lehrer T. Assessment of chlorination by human neutrophils. Nature. 1983; 301:715– 716.
2. Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Science. 2006; 11(4): 147-57.
3. Güngör N, Knaapen AM, Munnia A, Peluso M, Haenen GR, Chiu RK, Godschalk RW, van Schooten FJ. Genotoxic effects of neutrophils and hypochlorous acid. Mutagenesis. 2009; 25(2): 149-54.
4. Robaszkiewicz A, Pogorzelska M, Bartosz G, Soszyński M. Chloric acid (I) affects antioxidant defense of lung epitelial cells. Toxicol In Vitro. 2011; 25(7): 1328-34.
5. Romanowski EG, Stella NA, Yates KA, Brothers KM, Kowalski RP, Shanks RMQ. In vitro evaluation of a hypochlorous acid hygiene solution on established biofilms. Eye contact lens. 2018; Suppl 2: S187-S191.
6. Morris JC. The acid ionization constant of hypochlorous acid from 5 to 35 degrees. J Phys Chem. 1966; 70:3798– 3805.
7. Sakarya S, Gunay N, Karakulak M, Ozturk B, Ertugrul B. Hypochlorous Acid. An Ideal Wound Care Agent With Powerful Microbicidal, Antibiofilm, and Wound Healing Potency. Wounds. 2014; 26:342-350.
8. Qadir Tantry I, Ali A, Mahmood R. Hypochlorous acid decreases antioxidant power, inhibits plasma membrane redox system and pathways of glucose metabolism in human red blood cells. Toxicol Res. 2021; 10:264-271.
9. Gold MH, Andriessen A, Bhatia AC, Bitter P Jr, Chilukuri S, Cohen JL, Robb CW. Topical stabilized hypochlorous acid: the future gold standard for wound care and scar management in dermatologic and plastic surgery procedures. J Cosmet Dermatol. 2020;19:270-277.
10. Day A, Alkhalil A, Carney BC, Hoffman HN, Moffatt LT, Shupp JW. Disruption of biofilms and neutralization of bacteria using hypochlorous acid solution: an in vivo and in vitro evaluation. Adv Skin Wound Care .2017; 30:543-551.
11. Şahin B, Koluman BÜ, Seçme M, Dodurga Y, Mete GA, Köse M, Njjar MS, Koluman A. Determination of The Effects of Red And Infrared Light On Keratinocyte (HaCaT) And Fibroblast Cell Line (Human Dermal Fibroblast HDFa) Combined With Hypochlorous Acid Application. JSTER. 2023;4:17-29
12. Önder E, Çil N, Seçme M, Mete GA. Effect of alpha lipoic acid on epithelial

mesenchymal transition in SKOV-3 cells. Gene. 2024;20;892:147880.
13. Mutlu D, Gunal B, Secme M, Bozbeyoglu KNN, Abban MG, Mercan DN et al. Biological activities of Liquidambar orientalis: Antibiofilm, cytotoxicity, apoptosis, and miRNA expressions. Botanica Serbica. 2024;48: 17-26
14. Kogan S, Sood A, garnick MS. Zinc and Wound Healing: A review of zinc physiology and clinical applications. Wounds. 2017; 29(4):102-106
15. Lansdown AB, Sampson B, Rowe A. Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds. J Anat. 1999; 195(Pt3):375–386.
16. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, and Fusenig NE. Normal Keratinization in a Spontaneously Immortalized Aneuploid Human Keratinocyte Cell Line. J Cell Biol. 1988;106: 761-771
17. Lång E, Poleć A, Lång A, Valk M, Blicher P, Rowe AD, Tønseth KA, Jackson CJ, Utheim TP, Janssen LMC, Eriksson J, Bøe SO. Coordinated collective migration and asymmetric cell division in confluent human keratinocytes without wounding. Nat Communm. 2018; 10:3665.
18. Jiang L, Qi Y, Kong X, Wang R, Qi J, Lin F, Cui X, Liu Z. Activin A as a Novel Chemokine Induces Migration of L929 Fibroblasts by ERK Signaling in Microfluidic Devices. Front Cell Dev Biol. 2021; 21;9:660316.
19. Friedl P and Wolf K. Plasticity of cell migration: a multiscale tuning model J Cell Biol. 2010; 188:11–19.
20. Hidalgo E, Bartolome R, Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chem Biol Interact. 2002; 20;(139):265-82.
21. Banerjee M, Al-Eryani L, Srivastava S, Rai SN, Pan J, Kalbfleisch TS, States JC. Delineating the effects of passaging and exposure in a longitudinal study of arseniciInduced squamous cell carcinoma in a HaCaTcell line Toxicol Sci. 2022;185:184-196.
22. Heling I, Rotstein I, Dinur T, Szwec-Levine Y, Steinberg D. Bactericidal and cytotoxic effects of sodium hypochlorite and sodium dichloroisocyanurate solutions in vitro. J Endod. 2001;27:278-80.
23. Gomes-Filho JE, Sivieri-Araujo G, Sipert CR, da Silva Santos LM, de Azevedo Queiroz ÍO, Men Martins C, do Carmo Maia NK, Cintra LTA, Dezan-Junior E, Bagnato VS, Chaves-Neto AH, de Oliveira SHP. Evaluation of photodynamic therapy on fibroblast viability and cytokine production. Photodiagnosis Photodyn Ther. 2016;13:97-100.
24. Zheng Y and Ranzhong LI. In Vıtro And In Vıvo Bıocompatıbılıty Studıes Of Zno Nanopartıcles. Int J Mod Phys B. 2009; 23:566–1571
25. Jowkar Z, Moaddeli A, Shafiei F, Tadayon T, Hamidi SA. Synthesis and characterization of mesoporous zinc oxide nanoparticles and evaluation of their biocompatibility in L929 Clin Exp Dent Res. 2024;10:e844.
26. Raha S, Ahmaruzzaman MD. Raha S, Ahmaruzzaman M. ZnO nanostructured materials and their potential applications: progress, challenges and perspectives. Nanoscale Adv. 2022;4:1868-1925.
27. Yang J, Deng P, Qi Y, Feng X, Wen H, Chen F. MicroRNA-185 inhibits the proliferation and migration of HaCaT keratinocytes by targeting peroxisome proliferator-activated receptor β. Exp Ther Med. 2021;21:366.
28. Chang L, Liang J, Xia X, Chen X. miRNA-126 enhances viability, colony formation, and migration of keratinocytes HaCaT cells by regulating PI3 K/AKT signaling pathway. Cell Biol Int. 2019; 43(2):182-191.
29. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and biomarkers for wound healing. Plast Reconstr Surg. 2016; 138: 18-28.
30. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiological reviews. 2003; 83(3):835-70.