Proceedings of the 12th World Congress on Electrical Engineering and Computer Systems and Sciences (EECSS 2026)
August 16 – 18, 2026 – London, United Kingdom
Paper No. ICBES 127
DOI: 10.11159/icbes26.127

Endothelial Wound Repair Dynamics Under Nerve Growth Factor and Oligopeptide-215 Treatment in a Standardized HUVEC Scratch Assay

Shenggang Yan, *Isabella Fahy, *Sylvie Brown, *Shibo Zhu, *Audrey Zhao, Phu Nguyen
Linda Shi, Veronica Gomez-Godinez
Institute of Engineering in Medicine, University of California, San Diego
9500 Gilman Drive, La Jolla, CA 92093, USA
s7yan@ucsd.edu; izzyfahy8@gmail.com; sylviebrown305@gmail.com; kingswordtiger@gmail.com;
audrey.zhao06@gmail.com; p8nguyen@ucsd.edu; zshi@ucsd.edu; vgomez.godinezserf@gmail.com

Abstract **-** Human umbilical vein endothelial cells (HUVECs) provide a widely used model for investigating endothelial repair and angiogenesis following vascular injury. Rapid re-endothelialization is critical for restoring vascular barrier function and preventing thrombosis. Nerve growth factor (NGF) has been reported to promote endothelial survival and migration through TrkA-mediated signaling pathways, while the synthetic peptide Oligopeptide-215 (O-215) has been suggested to possess barrier-repair and anti-inflammatory properties. In this study, we evaluated the effects of NGF, O-215, and their combined treatment on endothelial wound closure using a HUVEC scratch assay model. Confluent monolayers were subjected to controlled scratch injury and treated under multiple conditions while varying initial cell density (5,000–40,000 cells per well). Time-lapse imaging was performed every two hours for up to 114 hours using the Agilent xCELLigence RTCA eSight system, and wound closure was quantified using the automated analysis module integrated within the Agilent RTCA eSight software, which calculates wound area at each time point from time-lapse brightfield images. NGF produced the most rapid early wound closure, whereas O-215 exhibited delayed but substantial acceleration beginning approximately 30 hours post-injury. Combined treatment did not produce additive effects and displayed increased variability. Higher initial seeding densities significantly enhanced wound closure across all conditions. These findings demonstrate distinct kinetic mechanisms for NGF and O-215–mediated endothelial repair and highlight the importance of cell density in scratch assay interpretation.

Keywords: HUVEC, nerve growth factor, Oligopeptide-215, endothelial repair, wound healing, scratch wound assay, angiogenesis

1. Introduction

The vascular endothelium plays a central role in maintaining cardiovascular homeostasis by regulating barrier integrity, vascular tone, and inflammatory signaling. Disruption of the endothelial barrier contributes to the development and progression of many cardiovascular diseases, including atherosclerosis, thrombosis, and chronic inflammatory conditions [1]. Recent studies have further clarified the molecular pathways controlling endothelial permeability and repair, highlighting the importance of coordinated cellular migration and junctional remodeling during vascular injury and regeneration [2].

Among the signaling molecules involved in tissue repair, nerve growth factor (NGF) has emerged as a multifunctional regulator that extends beyond its classical role in neuronal survival and differentiation. NGF has been shown to influence the behavior of several non-neuronal cell types, including fibroblasts, keratinocytes, and endothelial cells that participate in wound healing processes [3]. Previous work from our group reported that NGF, in combination with oligopeptide-215 (O-215), accelerated wound closure in HUVEC scratch assays, suggesting that NGF signaling may enhance endothelial repair dynamics under injury conditions [4]. Scratch wound assays have long been used as a reliable experimental approach for studying cell migration and wound repair mechanisms in vitro, allowing quantitative evaluation of collective cell movement during tissue regeneration [5].

At the cellular level, NGF signaling through the TrkA receptor pathway has been shown to promote cell survival, migration, and adhesion under stress conditions, including hypoxia [6]. In parallel, increasing attention has been directed toward bioactive peptides that exhibit anti-inflammatory and antioxidant activities capable of modulating wound healing pathways. Certain peptides have been shown to regulate signaling networks such as MAPK and NF-κB, which are closely

associated with inflammatory responses and tissue regeneration [7]. Notably, Oligopeptide-215 was found to reduce inflammation through suppression of NF-κB dependent cytokine expression [8]. These findings suggest that peptide-based molecules may represent promising therapeutic agents for improving vascular repair.

Recent quantitative and computational studies have also emphasized the importance of understanding the kinetics of endothelial proliferation and migration in response to environmental stimuli and biochemical regulators [9]. In addition, impaired endothelial repair is a key contributor to pathological wound healing conditions such as chronic diabetic wounds and excessive scar formation, highlighting the need for improved strategies to enhance vascular regeneration [10].

Human umbilical vein endothelial cells (HUVECs) provide a widely used and well-characterized in vitro model for investigating endothelial behavior and vascular repair mechanisms. These cells preserve many morphological and functional properties of vascular endothelium and are therefore commonly employed in experimental studies of endothelial migration and barrier function [11].

Despite increasing interest in NGF signaling and peptide-based therapeutics, the dynamic effects of NGF combined with synthetic oligopeptides on endothelial wound repair remain incompletely characterized. In particular, little is known about how these molecules influence the temporal kinetics of endothelial migration during wound closure.

In the present study, we investigated the effects of NGF and oligopeptide-215 (O-215) on endothelial wound healing using a standardized scratch assay generated with the AccuWound96 system. Time-lapse imaging was performed using the Agilent xCELLigence RTCA eSight platform, which allows automated monitoring and quantification of wound closure dynamics over extended time periods. By analyzing wound area reduction under different treatment conditions, this study aims to characterize how NGF and O-215 influence endothelial migration and repair kinetics.

2. Materials and Methods

2.1. Cell Culture

Human umbilical vein endothelial cells (HUVECs) were used as an in vitro model to study endothelial wound repair dynamics. Wells were seeded at four initial densities (5,000, 10,000, 20,000, and 40,000 cells per dish), grown to approximately 90% confluence, and incubated for 24 hours before any analysis or experimentation.

Cells were cultured in an endothelial growth medium supplemented with growth factors according to the manufacturer's protocol. Cultures were maintained in a humidified incubator at 37 °C with 5% CO_2.

For scratch wound experiments, cells were seeded into 96-well plates and allowed to grow until a confluent monolayer was formed. To investigate the influence of cell density on wound closure kinetics, cells were plated at varying densities prior to reaching confluence.

2.2. Generation of Standardized Wounds

Endothelial wounds were generated using the Agilent AccuWound96 system, which produces highly uniform and reproducible wound gaps across multi-well plates. The AccuWound device simultaneously generates scratches in all wells using a standardized pin array, minimizing variability associated with manual scratch techniques. Cells at the wound edge exhibited polarized morphology and initiated migration and proliferation to close the wound, enabling quantification of wound closure in this study [5].

Following wound generation, wells were gently washed with fresh culture medium to remove detached cells and debris. Plates were then prepared for treatment and time-lapse imaging.

2.3. Treatment Conditions

After wound generation, HUVEC monolayers were exposed to the following treatment conditions:

- Control group: no treatment
- NGF group: nerve growth factor (100 ng/mL)
- O-215 group: oligopeptide-215 (100 ppm)
- Combination group: NGF and O-215 applied simultaneously

Treatments were applied immediately following wound formation to evaluate their effects on endothelial migration and wound repair dynamics. Each treatment group was assessed across all four seeding densities, resulting in a factorial experimental design that allowed assessment of both treatment and density effects

2.4. Time-Lapse Imaging

Wound closure was monitored using the Agilent xCELLigence Real-Time Cell Analysis (RTCA) eSight system, which enables automated imaging of cell cultures under controlled environmental conditions.

Brightfield images were acquired at two-hour intervals for a total observation period of 114 hours. Images were captured every 2 hours, allowing continuous monitoring of endothelial migration and gap closure over time without disturbing the cultures.

2.5. Image Analysis and Wound Quantification

Wound closure measurements were obtained using the RTCA eSight analysis software. The software performs automated segmentation of the wound region in each image and calculates the corresponding wound area at each time point.

The automated analysis pipeline, indicated in Fig. 1 (A), provided consistent identification of wound boundaries and minimized user-dependent variability in wound segmentation.

2.6. Statistical Analysis

All experimental conditions were performed in triplicate to allow statistical assessment of variability. Quantitative results are presented as **mean ± standard deviation**, stratified by condition and seeding. Differences between treatment groups were evaluated using **Student's t-test**, and the statistical significance was defined as $\mathbf{p < 0.05}$. For each well, wound area measurements were normalized to the initial wound area recorded immediately after scratch generation. The percentage of wound closure was then calculated based on the reduction in wound area over time.

3. Results

3.1. NGF Accelerates Early Endothelial Wound Closure

To evaluate the effects of NGF and O-215 on endothelial repair, wound closure dynamics were monitored using the RTCA eSight time-lapse imaging system following scratch generation with the AccuWound96 device.

Under control conditions, endothelial cells gradually migrated into the wound region, resulting in progressive reduction of the wound area over time. Treatment with NGF markedly accelerated this process. Within the first 24–48 hours after injury, as shown in Fig. 1 (A), NGF-treated cultures exhibited a substantially greater reduction in wound area compared with untreated controls. The early phase of endothelial migration appeared particularly responsive to NGF stimulation, suggesting that NGF primarily enhances the initial migratory response following mechanical injury. The enhanced early kinetics of NGF-treated cultures, more so than any other conditions, were reproducible across triplicates and most pronounced at higher initial seeding densities (20,000 and 40,000 cells per dish), where the proliferative capacity was greatest.

These findings indicate that NGF promotes rapid endothelial migration during the early stages of wound repair. These findings were statistically significant. Student's t-test, ***P = 0.000047, Stdev = 0.000454

3.2. O-215 Produces Delayed but Sustained Repair Activity

In contrast to NGF, cultures treated with O-215 showed a different temporal pattern of wound closure. During the first 24 hours after scratch generation, wound closure rates in O-215–treated wells were comparable to those observed in control cultures.

However, beginning approximately 30 hours after injury, a noticeable increase in wound closure rate was observed in the O-215 treatment group. This delayed response persisted throughout the later stages of the experiment, eventually resulting in wound closure levels comparable to those observed in NGF-treated cultures. O-215 also demonstrated a similar, though markedly weaker, association between seeding density and enhanced wound closure.

The distinct temporal profile suggests that O-215 may influence endothelial repair through mechanisms that become active during the later phases of wound healing rather than immediately following injury. These findings were statistically significant. Student's t-test, *** P = 0.00001, Stdev = 0.0004

3.3. Combined Treatment Does Not Produce Additive Effects

To determine whether NGF and O-215 act synergistically to promote endothelial repair, both agents were applied simultaneously following wound generation.

Although combined treatment produced accelerated wound closure compared with untreated controls, the closure kinetics were not substantially greater than those observed with NGF treatment alone. In several experiments, the combined treatment group exhibited greater variability in closure rates during intermediate time points.

These observations suggest that the repair processes stimulated by NGF may already approach maximal migratory activity, limiting the additional effect of O-215 under the conditions tested. These findings were statistically significant. Student's t-test, *** P = 0.00006, Stdev = 0.0005

3.4. Initial Cell Density Influences Wound Repair Kinetics

Because collective migration plays an important role in endothelial repair, the effect of initial cell density on wound closure dynamics was examined. Increasing the number of seeded cells prior to scratch generation significantly accelerated wound closure.

Cultures with higher initial cell densities exhibited faster reduction of wound area across all treatment groups. This effect was most pronounced during the early stages of repair, when densely seeded cultures rapidly populated the wound region.

These results as shown in Fig. 1 (C) indicate that cell density is an important experimental variable in scratch wound assays and can strongly influence the observed kinetics of endothelial migration. Quantitative analysis as summarized in Table 1. confirmed that NGF treatment produced the shortest time to 50% wound closure, while O-215 exhibited delayed but measurable enhancement of endothelial migration relative to untreated controls.

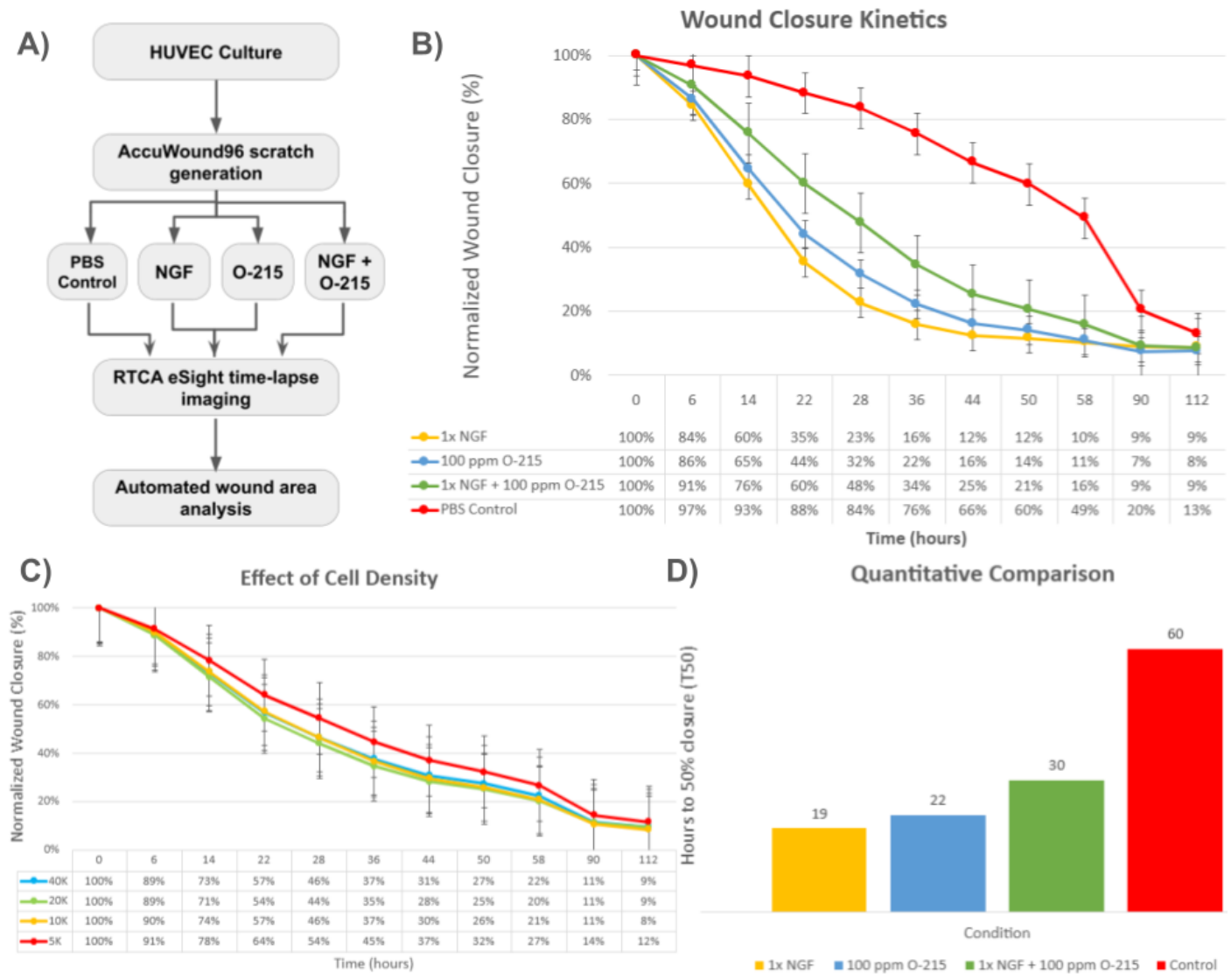

Fig. 1: Endothelial wound repair dynamics under NGF and O-215 treatment.
A) Experimental workflow illustrating HUVEC culture, wound generation using the AccuWound96 system, treatment conditions, time-lapse imaging with the RTCA eSight system, and automated wound area quantification.
B) Time-course analysis of wound closure following treatment with NGF, O-215, or combined therapy. Results are presented as mean ± standard deviation of all cell densities per condition.
C) Influence of initial cell density on endothelial migration dynamics.
D) Quantitative comparison of repair kinetics measured as time required to achieve 50% wound closure (T50).

Table 1: Time required to reach 50% wound closure (T50)

Treatment	Time to 50% Closure (hours)
Control	60
NGF	19

Treatment	Time to 50% Closure (hours)
O-215	22
NGF + O-215	30

4. Discussion

Endothelial repair following vascular injury requires coordinated cell migration, proliferation, and restoration of barrier integrity. In this study, we investigated the effects of nerve growth factor (NGF) and oligopeptide-215 (O-215) on endothelial wound closure using a standardized scratch assay generated with the AccuWound96 system and monitored with the RTCA eSight imaging platform. The results demonstrate that NGF and O-215 influence endothelial repair through distinct temporal patterns of wound closure.

NGF treatment produced the most pronounced acceleration of wound closure during the early stages following injury. This observation is consistent with previous reports indicating that NGF signaling can enhance cell migration and survival through activation of the TrkA receptor pathway and downstream regulatory mechanisms [3,6]. NGF has been shown to promote cytoskeletal coupling and cell adhesion, both of which are essential processes for directed cell migration during wound healing [12, 13, 14]. The early enhancement of wound closure observed in the present study suggests that NGF primarily stimulates the initial migratory response of endothelial cells following mechanical injury.

In contrast, treatment with O-215 resulted in a delayed but sustained increase in wound closure activity, suggesting engagement with alternative signaling cascades involving barrier-reinforcing mechanisms within integral endothelization stages, which may take longer to manifest as measurable reductions in wound gap width. During the first 24 hours after scratch generation, wound closure rates were similar to those observed in untreated controls. However, a noticeable acceleration of repair became apparent at later time points. One possible explanation for this pattern is that O-215 may influence cellular pathways associated with inflammation, oxidative stress regulation, or extracellular matrix remodeling. Bioactive peptides have been reported to modulate signaling networks such as the MAPK and NF-κB pathways, which are known to regulate inflammatory responses and tissue regeneration [7]. Although the precise mechanism of O-215 activity requires further investigation, the delayed repair profile observed here suggests that the peptide may contribute to later phases of endothelial wound healing.

Interestingly, the combined treatment of NGF and O-215 did not produce a substantial additive effect on wound closure compared with NGF alone, raising the possibility of an antagonistic interaction under the conditions tested. Several factors may contribute to this observation. First, NGF-mediated stimulation of endothelial migration may already approach a maximal response under the experimental conditions used in this study. Alternatively, the two molecules downstream signalling may partially overlap on shared pathways such as MAPK and NF-κB, limiting the magnitude of their combined effect [8, 12, 15]. Additional studies examining dose-dependent responses and signaling pathway interactions may help clarify the relationship between NGF and peptide-mediated repair mechanisms.

The results also highlight the importance of initial cell density as an experimental variable in scratch wound assays. Higher cell densities consistently yield faster, more consistent repair and lower densities showing slower, more variable closure across all treatment conditions. In the present study, the density-dependent effect was most pronounced in NGF-treated cultures, highlighting that strong early-acting treatments amplify density-related differences. This finding is consistent with the collective migration behavior commonly observed in endothelial monolayers, where cell-cell interactions and mechanical coupling can strongly influence migration dynamics. Previous modeling studies have similarly demonstrated that environmental conditions and cell population density can significantly affect endothelial proliferation and migration behavior [9]. These observations emphasize the need for careful experimental standardization when interpreting wound closure assays.

Several limitations should be noted. The present study focuses on morphological measurements of wound closure and does not directly evaluate the underlying molecular signaling pathways involved in NGF- or peptide-mediated repair. Future studies incorporating molecular assays, such as gene expression analysis or pathway inhibition experiments, may provide further insight into the mechanisms responsible for the observed repair dynamics. Additionally, in vitro scratch assays cannot fully replicate the complexity of wound healing in vivo, where multiple cell types and biochemical signals interact during tissue repair [16].

Despite these limitations, the experimental approach used in this study provides a reproducible platform for investigating endothelial migration and repair dynamics. The combination of the AccuWound96 standardized scratching system and RTCA eSight automated imaging and analysis enables consistent wound generation and quantitative monitoring of repair processes over extended time periods.

5. Conclusion

This study examined the effects of NGF and oligopeptide-215 on endothelial wound repair using a standardized HUVEC scratch assay. The results demonstrate that NGF significantly accelerates early endothelial migration and wound closure in vitro and in vivo alike [12], while O-215 produces a delayed but sustained enhancement of repair activity. The distinct temporal patterns observed for the two treatments suggest that they may influence different phases of the endothelial healing process. Combined treatment did not produce additive effects and introduced increased variability.

In addition, the experiments reveal that initial cell density was a significant modulator in determining wound closure kinetics, particularly at higher initial densities, highlighting the need for careful control of experimental conditions in migration assays. Together, these findings contribute to a better understanding of molecular factors that regulate endothelial repair and may inform future investigations of therapeutic strategies aimed at improving vascular regeneration. These results support the pro-endothelialization potential of both agents and underscore the need for replicated, dose-response, and mechanistically focused investigations to characterize their therapeutic utility in vascular wound repair fully.

Acknowledgements

The authors gratefully acknowledge Winkey Technology for providing *WKPep® Salaregen S* (INCI: Oligopeptide-215) and Agilent Technologies, Inc. for providing the *Agilent xCELLigence RTCA eSight* system. This project was supported by a generous gift from the Beckman Laser Institute Inc. to the UCSD Biophotonics Technology Center. The authors also extend our sincere gratitude to the donors of the UCSD OPALS Program—including the Leighton Foundation Inc., Ming-Wei Wu, Inc., Dr. Shu Chien (UCSD Bioengineering), Dr. Lizhu Chen (CorDx Inc.), Dr. Xinhua Zheng, David & Leslie Lee, and Mingwei Hu & Wen Shi—for their invaluable support and generosity.

References

[1] D. A. Chistiakov, A. N. Orekhov, and Y. V. Bobryshev, "Endothelial barrier and its abnormalities in cardiovascular disease," *Frontiers in Physiology*, vol. 6, pp. 365, 2015.

[2] R. Wakasugi, K. Suzuki, and T. Kaneko-Kawano, "Molecular mechanisms regulating vascular endothelial permeability," *International Journal of Molecular Sciences*, vol. 25, no. 12, 2024.

[3] N. Gostynska, M. Pannella, M. L. Rocco, L. Giardino, L. Aloe, and L. Calzà, "The pleiotropic molecule NGF regulates the in vitro properties of fibroblasts, keratinocytes, and endothelial cells: implications for wound healing," *American Journal of Physiology – Cell Physiology*, vol. 318, pp. C360–C371, 2020.

[4] S. Yan, I. Fahy, S. Brown, S. Zhu, A. Zhao, V. Gomez-Godinez, and L. Shi, "Nerve growth factor and oligopeptide-215 accelerate endothelial wound closure in human umbilical vein endothelial cells scratch assays," in *Proceedings of the American Society of Cell Biology Annual Meeting,* Philadelphia, PA, 2025.

[5] M. E. Alishahedani, M. Yadav, K. J. McCann, P. Gough, C. R. Castillo, J. Matriz, and I. A. Myles, "Therapeutic candidates for keloid scars identified by qualitative review of scratch assay research for wound healing," *PLoS One*, vol. 16, e0253669, 2021.

[6] C. N. Fang, H. Q. Tan, A. B. Song, N. Jiang, Q. R. Liu, and T. Song, "NGF/TrkA promotes the vitality, migration, and adhesion of bone marrow stromal cells in hypoxia by regulating the Nrf2 pathway," *Metabolic Brain Disease*, vol. 37, pp. 2017–2026, 2022.

[7] J. Q. Zhan, J. X. Wu, J. J. Fu, G. S. Li, F. Wu, and Y. W. Chen, "Antioxidant synergistic anti-inflammatory effect in the MAPK/NF-κB pathway of peptide KGEYNK (KK-6) from giant salamander (*Andrias davidianus*)," *Journal of the Science of Food and Agriculture*, vol. 104, pp. 8613–8620, 2024.

[8] Zhang Q, Hu C, Chen Y, Chen Y, Guo C, Liu Z, Ding W. Dual-Targeting Oligopeptide-215 Regulates Skin Barrier Homeostasis Through Concurrent Modulation of JAK-STAT and NF-κB Signaling. J Cosmet Dermatol. 2026 Mar;25(3):e70704. doi: 10.1111/jocd.70704. PMID: 41858149; PMCID: PMC13003196.

[9] H. Helmholz, R. Resuli, M. Tacke, J. Nourisa, S. Tomforde, R. Aydin, R. Willumeit-Römer, and B. Zeller-Plumhoff, "Regression models for predicting the influence of magnesium ions on primary endothelial cell proliferation and migration," *Computational and Structural Biotechnology Journal*, vol. 27, pp. 2711–2718, 2025.
[10] N. Dasari, A. Jiang, A. Skochdopole, J. Chung, E. M. Reece, J. Vorstenbosch, and S. Winocour, "Updates in diabetic wound healing, inflammation, and scarring," *Seminars in Plastic Surgery*, vol. 35, pp. 153–158, 2021.
[11] H. Duranova, L. Kuzelova, P. Borotova, V. Simora, and V. Fialkova, "Human umbilical vein endothelial cells as a versatile cellular model system in diverse experimental paradigms: an ultrastructural perspective," *Microscopy and Microanalysis*, vol. 30, pp. 419–439, 2024.
[12] Liu Z, Wu H, Huang S. Role of NGF and its receptors in wound healing (Review). Exp Ther Med. 2021 Jun;21(6):599. doi: 10.3892/etm.2021.10031. Epub 2021 Apr 11. PMID: 33884037; PMCID: PMC8056114.
[13] Turney SG, Ahmed M, Chandrasekar I, Wysolmerski RB, Goeckeler ZM, Rioux RM, Whitesides GM, Bridgman PC. Nerve growth factor stimulates axon outgrowth through negative regulation of growth cone actomyosin restraint of microtubule advance. Mol Biol Cell. 2016 Feb 1;27(3):500-17. doi: 10.1091/mbc.E15-09-0636. Epub 2015 Dec 2. PMID: 26631553; PMCID: PMC4751601.
[14] Li Y, Zhang D, Jin B, Xia L, Zhang A. Proteomic Analysis of Uterine Tissues During Peri-Implantation Period in Mice with Experimentally Induced Adenomyosis that Treated with anti-Ngf: Implications for Cell-Cell Adhesion and Metabolic Processes. Reprod Sci. 2021 Jan;28(1):207-217. doi: 10.1007/s43032-020-00262-y. Epub 2020 Jul 16. PMID: 32676925; PMCID: PMC7782370.
[15] El Baassiri M, Mjahed K, Ahid S, et al. Nerve growth factor and burn wound healing: Update of molecular interactions with skin cells. Pharmacol Res. 2023;188:106656. doi:10.1016/j.phrs.2022.106656.
[16] Sarian MN, Zulkefli N, Che Zain MS, Maniam S, Fakurazi S. A review with updated perspectives on in vitro and in vivo wound healing models. Turk J Biol. 2023 Aug 10;47(4):236-246. doi: 10.55730/1300-0152.2659. PMID: 38152620; PMCID: PMC10751087.