UDC [615.454.1:577.112.6]+[616-08-031.84:616-001.4]
https://doi.org/10.15407/biotech18.01.055

PRODUCTION AND *in vitro* EVALUATION OF RECOMBINANT HUMAN HB-EGF FOR WOUND HEALING AND TARGETED THERAPY

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Received 2024/11/28
Revised 2025/02/06
Accepted 2025/02/28

Aim. The goal of the study was to evaluate the biological activity of recombinant human heparin-binding EGF-like growth factor (rhHB-EGF) on mouse fibroblasts *in vitro* and the possibility of its use as a potential agent for promoting wound healing and tissue regeneration.

Methods. The study employed a scratch assay to evaluate the migration of mouse fibroblasts (L929 and NIH-3T3), the MTT test to assess cell proliferation and/or metabolic activity, MALDI-TOF mass spectrometry for protein identification, and flow cytometry to determine cell viability.

Results. In the concentration range of 500–1000 ng/ml rhHB-EGF, no cytotoxic effect was recorded, but an increase in proliferation and/or metabolic activity, as well as migration of fibroblasts, was detected, with a maximum effect at 500 ng/ml rhHB-EGF in the cell incubation medium. A 30% overgrowth of the wound surface of fibroblasts was demonstrated in the scratch assay test under the influence of rhHB-EGF compared to the corresponding control.

Conclusions. rhHB-EGF at a concentration of 500 ng/ml can be used in preparations to stimulate wound healing and tissue regeneration due to its ability to stimulate proliferation/metabolic activity and migration of fibroblasts, as well as the lack of cytotoxicity. Further, in vivo studies are needed for a comprehensive evaluation of this possibility.

Key words: human heparin-binding EGF-like growth factor (rhHB-EGF), recombinant protein, cell culture, fibroblasts, proliferation, migration, cytotoxicity, wound healing.

Citation: Vovk, I., Didan, A., Zhukova, D., Dronko, L., Rebriev, A., Rybalko, A., Legach, E., Gorbatiuk, O., Usenko, M., Skvarchynskyi, A., Dovbymchuk, T., Siromolot, A., Romaniuk, S., Kolybo, D. (2025). Obtaining and cell culture testing of recombinant human hb-egf for wound healing and targeted therapy. *Biotechnologia Acta*, 18(1), 55–67. https://doi.org/10.15407/biotech18.01.055

Therapy using growth factors holds significant potential to overcome the limitations of traditional wound treatment methods. Heparin-binding epidermal growth factor-like growth factor HB-EGF are critical for controlling wound healing by acting as extracellular signals that regulate re-epithelialization, reduce infection risks, and shorten hospital stays [1]. In chronic wounds, these factors can provide the necessary stimuli to promote closure, which would otherwise be impossible [2]. HB-EGF has been shown to stimulate keratinocyte proliferation, migration, and granulation tissue formation [3].

HB-EGF is a member of the Epidermal Growth Factor family known for its potent mitogenic potential and involvement in cell proliferation, migration, and tissue repair [4]. HB-EGF primarily exerts its biological effects through activation of the Epidermal Growth Factor Receptor (EGFR and ErbB4), making it a critical mediator in processes such as wound healing, inflammation, and oncogenesis [5, 6]. HB-EGF was first identified in the conditioned medium of cultured human macrophages [7]. Beyond its role in tissue repair, HB-EGF has been implicated in various developmental processes, such as blastocyst implantation and angiogenesis, with critical contributions in mediating cellular proliferation, migration, and differentiation during pregnancy [8, 9]. Its activity is particularly significant in orchestrating the interactions between neurons and neuronal progenitor cells [10].

HB-EGF signaling is essential in several organ systems, including skin, heart, brain, liver, etc., where it promotes cell migration and matrix protein secretion and supports cell survival and proliferation [10, 11]. So, HB-EGF takes part in neurogenesis, angiogenesis, and immune response regulation. Additionally, HB-EGF plays a pivotal role in cardiovascular development by supporting endothelial cell survival and vascular remodeling [12]. It also contributes to the formation and maintenance of the extracellular matrix, thereby influencing tissue integrity and repair mechanisms [13].

In pathological contexts, dysregulation of HB-EGF expression has been associated with tumorigenesis, particularly in promoting tumor cell proliferation, invasion, and metastasis [14, 15]. Its role as a ligand for the EGF receptor (EGFR) and ErbB4 highlights its importance in signaling pathways that control cellular responses to environmental stimulus. Understanding the functions of HB-EGF continues to provide valuable insights into both developmental biology and therapeutic interventions for various diseases [16].

Widely studied fibroblast cell lines, L929 and 3T3, used as complementary models, were chosen to understand cell-specific responses to HB-EGF. L929 cells are a fibroblast cell line derived from the mouse subcutaneous areolar and adipose tissue. They were initially established from a mouse tumor [17].

3T3 cells are derived from mouse embryonic firoblasts. The NIH-3T3 variant is the most widely studied subline and is used primarily to study cellular transformation, cancer research, and growth factor signaling. The NIH-3T3 cell line was initially immortalized by the introduction of the Simian Virus 40 (SV40) large T antigen [18]. NIH-3T3 cells maintain the ability to respond to growth factors like HB-EGF and exhibit oncogenic transformation when exposed to certain mutagens or transfected with oncogenes, making them valuable for studying cancer-related pathways [19].

Both L929 and 3T3 cells express the Epidermal Growth Factor Receptor (EGFR), a tyrosine-kinase receptor that mediates cellular responses to various growth factors, including HB-EGF. Upon activation by HB-EGF, EGFR undergoes dimerization and phosphorylation of tyrosine residues, which in turn activates several downstream signaling cascades. These include the MAPK/ERK, PI3K/AKT, and PLCγ pathways, which regulate key cellular processes such as proliferation, survival, and migration [20].

In contrast, 3T3 cells exhibit a different pattern of EGFR-mediated signaling. NIH-3T3 cells have potent contact inhibition, and their EGFR signaling is tightly regulated to prevent uncontrolled proliferation. Upon HB-EGF treatment, NIH-3T3 cells activate the MAPK pathway [21]. In addition, 3T3 cells activate the PI3K/AKT pathway, which regulates cell survival and metabolic processes [22].

In chronic wounds, proactive treatment is essential, as standard therapies are often ineffective. The use of controlled delivery systems for HB-EGF, such as those developed with coacervates, has shown promise in accelerating wound closure through rapid re-epithelialization and granulation tissue formation. In murine models, this approach significantly accelerated healing compared to free HB-EGF, suggesting that controlled release can enhance the efficacy of growth factor therapy [23]. Delivery systems designed for the controlled release of growth

factors like EGF have faced challenges, such as low efficiency and rapid degradation. However, incorporating extracellular matrix (ECM) components such as hyaluronic acid, collagen, and vitronectin can improve growth factor bioactivity and stability, enhancing therapeutic outcomes [24]. One promising approach involves using heparin, a highly sulfated glycosaminoglycan, which binds growth factors to prolong their activity and protect against degradation [25]. Coacervate-based delivery systems using heparin and polyethylene arginine aspartate (PEAD) offer a way to localize growth factor delivery at the wound site and control its release, improving therapeutic outcomes [23].

This study aimed to investigate the effects of HB-EGF on fibroblast migration proliferation and/or metabolic activity, and cell viability in L929 and 3T3 cell lines. By comparing these responses, the study seeks to elucidate the differential and cell-specific roles of HB-EGF, contributing to a deeper understanding of its potential applications in tissue repair, inflammation modulation, and metabolic regulation.

Materials and Methods

Cell cultures

A fibroblast cell line isolated from a mouse NIH/Swiss embryo (3T3, ATCC number CRL-1658) and a fibroblast cell line isolated from the areolar adipose tissue of a 100-day-old male mouse (L929 ATCC number CRL-2648) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, USA) supplemented with 10% fetal bovine serum (Sigma) according to manufacturer's protocol. Cells were from the cell line collection of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences (NAS) of Ukraine.

Expression of recombinant proteins

The recombinant protein rhHB-EGF was expressed in *E. coli* BL21(DE3) Rosetta with a C-terminal His-tag. Target protein expression was induced by adding 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 3 hours at 30 °C. It was purified using a His-Trap affinity column per the manufacturer's protocol, and the residual imidazole was removed by dialysis against phosphate-buffered saline (PBS, pH 7.4). The purity of the target protein was analyzed by SDS-PAGE in 10% polyacrylamide gel at 60 V for 20 min and 120 V for 60 min. Coomassie Brilliant Blue 40 was used as the protein-dye [26]. Obtained gels were scanned and protein concentrations were calculated by FiJi ImageJ Software.

Cell Migration and Scratch Assays

Cells 3T3 and L929 were seeded into 6-well plates ($2 \cdot 10^4$ per well) and grown in the DMEM supplemented 10% FBS up to 80–90% confluency. A sterile pipette tip was then used to create a linear "scratch" across the cell monolayer in each well with further rinsing by PBS. Cells were growing in the DMEM-supplemented rhHB-EGF (500 ng/ml or 1000 ng/ml) without FBS for 24 hours. PBS was used as a negative control. The plates were then incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for the duration of the experiment. Cell migration was monitored and recorded at regular intervals (e.g., 0, 6, 12, and 24 hours) by capturing images of the scratch [27]. Cells were counted using the MRI Wound Healing Tool of FiJi ImageJ Software.

MTT-Assay for Cell Proliferation and/or Metabolic Activity.

The cells 3T3 and L929 were seeded in 96-well plates ($2 \cdot 10^4$ per well) in the DMEM supplemented 10% FBS and, after 24 hours of growing, were treated with rhHB-EGF (range of concentration 5–1000 ng/ml) in the absence of FBS for 24 hours. PBS was used as a negative control. The cell proliferation/metabolic activity was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) based colorimetric assay [28].

MALDI-TOF mass spectrometry

Analysis of the purity of recombinant protein rhHB-EGF was performed with a MALDI-TOF Voyager DE Pro mass spectrometer (Applied Biosystems, USA), serial number 6393. Mass spectra were obtained in the linear mode, applied voltage of 25 kV, with positive ionization of the studied samples. Results were analyzed by Data Explorer 4.0.0.0. Sinapinic acid was used as a matrix (Applied Biosystems) [29].

Flow Cytometry

Cells 3T3 and L929 were seeded in 6-well plates ($2 \cdot 10^5$ per well) in the DMEM supplemented 10% FBS and, after 24 hours of growing, were treated with rhHB-EGF (500 ng/ml or 1000 ng/ml) in serum-free medium for 24 hours. After incubation, cells

were harvested, stained with Propodium Iodide and Annexin V fused to an enhanced green fluorescent protein (eGFP), and analyzed by the DxFlex flow cytometer (Beckman Coulter, USA). The signal for eGFP was measured in the FL1 (555–725 nm) channel. Cytometry data were analyzed by Kaluza Analysis Software.

Statistical Analysis

Statistical calculations and analysis were made by MS Office (2010) and GraphPad Prism software using the Kruskal-Wallis ANOVA and Unpaired *t*-test methods (differences between indicators with a value of $P < 0.05$ were considered statistically significant). Mean values ± standard deviations (M±SD) are indicated for 12-fold experiments.

Results and Discussion

1. Physical properties of the recombinant human rhHB-EGF

Recombinant proteins are essential tools in biotechnology, research, and therapeutic applications, as they allow the production of specific protein molecules with predictable properties. This study focuses on the recombinant protein rhHB-EGF (recombinant human heparin-binding epidermal growth factor-like growth factor), expressed in *E. coli* strain BL21 Rosetta (DE3), transformed with the plasmid vector pET-32a-rhHB-EGF.

The studied recombinant protein rhHB-EGF was expressed in *E. coli* strain *BL21 Rosetta (DE3)*, which was transformed with the *pET-32a-rhHB- EGF* plasmid vector. The method of isolation and purification of the rhHB-EGF recombinant protein in this case was described in detail [29]. The results of the purification of expressed rhHB-EGF protein are presented in Fig. 1. The Co^{2+}-NTA affinity resin was selected for protein purification due to its superior efficiency compared to Ni^{2+}-NTA resin in achieving higher purity of His-tagged recombinant human HB-EGF (rhHB-EGF). In our case, the Co^{2+}-based sorbent demonstrated enhanced binding specificity, making it a more compelling choice for isolating the target protein in high yields and purity.

The molecular mass of the recombinant protein rhHB-EGF, as determined by electrophoresis, was 28 kDa. This can be attributed to the presence of the TRX tag and His tag included in the pET32a construct, separated from the target sequence by thrombin and enterokinase cleavage sites. The observed value aligns closely with the theoretically calculated molecular weight, confirming the expected size and composition of the tagged recombinant protein. The alternative method for the determination of molecular mass and identification of proteins by MALDI-TOF mass spectrometry. Due to its information content, accuracy, and high productivity, it has become an indispensable method for the identification of proteins, nucleic acids, and lipids. To identify the recombinant protein rhHB-EGF using MALDI-TOF mass spectrometry, after performing SDS-electrophoresis from a polyacrylamide gel, a subsoil was collected and

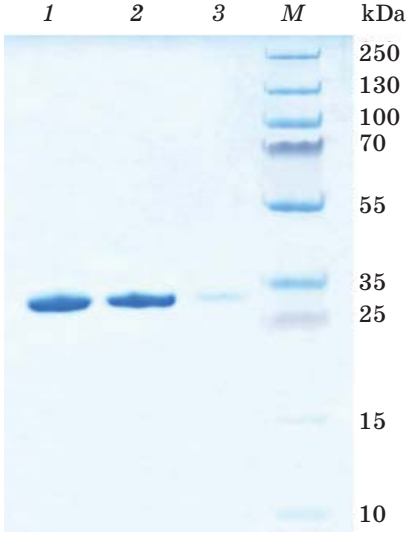

Fig. 1. **SDS-PAGE of purified protein rhHB-EGF**
1, *2*, and *3* — different fractions of extracted protein, *M* — protein molecular weight markers (PageRuler™ Prestained Protein Ladder, 10 to 250 kDa, Thermofisher). The estimated molecular mass of rhHB-EGF is ~28 kDa

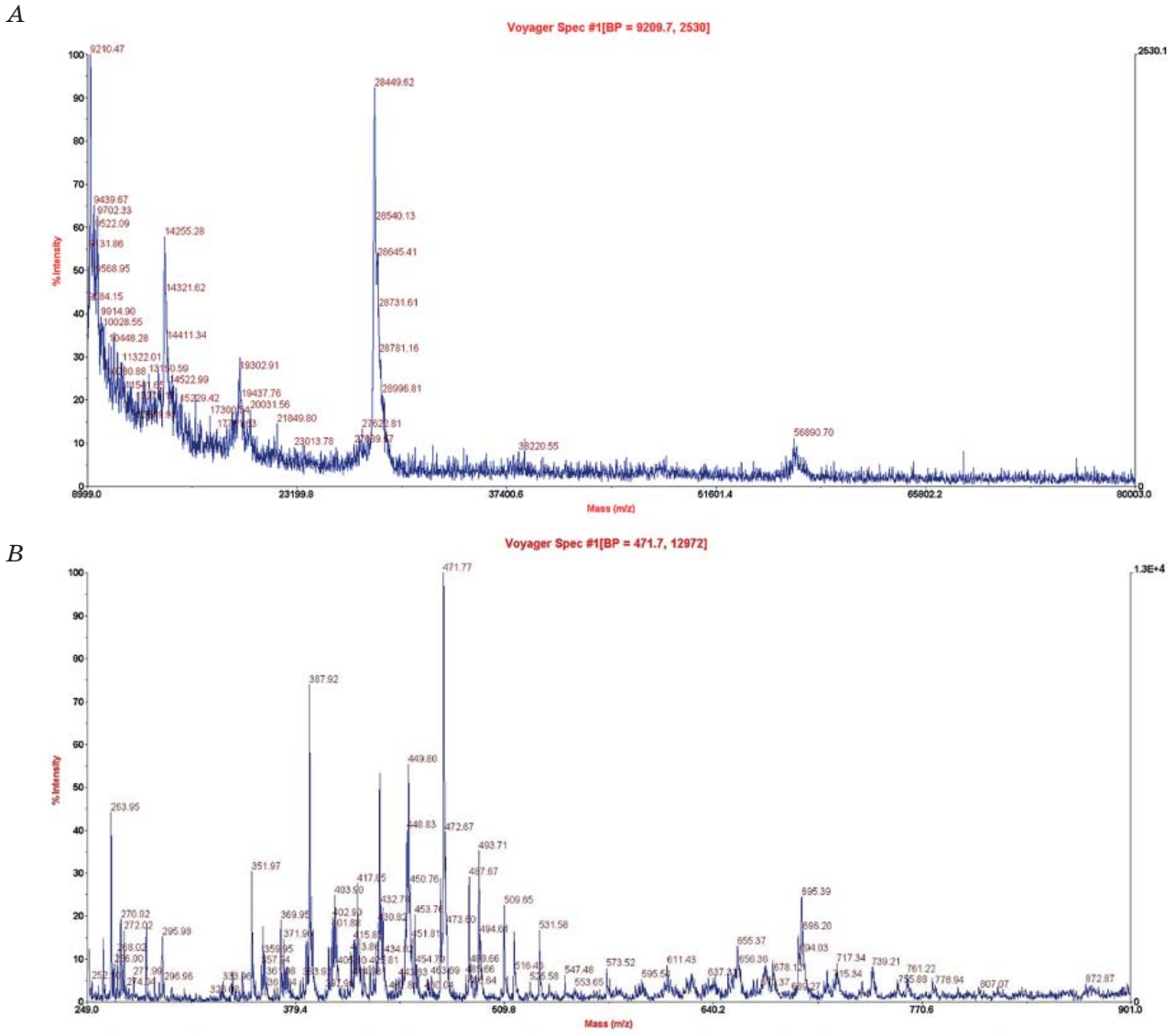

Fig. 2. **Spectra obtained from MALDI-TOF mass spectrometry of the recombinant protein rhHB-EGF**
A — with TRX- tag, *B* — using short tryptic peptides

mixed with recombinant rhHB-EGF proteins and tryptic peptide samples were prepared for MALDI-TOF mass spectrometry. Figure 2, *A* shows the spectra obtained by MALDI-TOF mass spectrometry of various variants of the recombinant protein rhHB -EGF, which were suspected of having a TRX tag. As can be seen from Fig. 2, *A*, the head peaks with the maximum mass, which are unbroken rhHB -EGF molecules, show a molecular mass of 28.5 kDa, which appears in the warehouse of the TRX– tag molecule.

To identify the amino acid sequence of the obtained recombinant protein rhHB-EGF, MALDI-TOF mass spectrometry was carried out with short-term tryptic peptides to the rhHB-EGF protein. The results of this study demonstrated that the entire sequence of the rhHB-EGF protein was overlapped by peptides identified using MALDI-TOF mass spectrometry (Fig. 2 B), which is consistent with the similarity sequences of the acquired recombinant rhHB -EGF (without tags) and natural protein sHB-EGF (soluble HB-EGF).

2. Assessment of the influence of the growth factor rhHB-EGF on the metabolic activity of sensitive cells in vitro

The aim of this part of the present study was investigation of the proliferation/ metabolic activity of sensitive cells of two fibroblast cell lines (L929 and 3T3) during cultivation *in vitro* in the presence of different concentrations of the recombinant growth factor rhHB-EGF. For this purpose, the MTT test was used. The principle of action of the

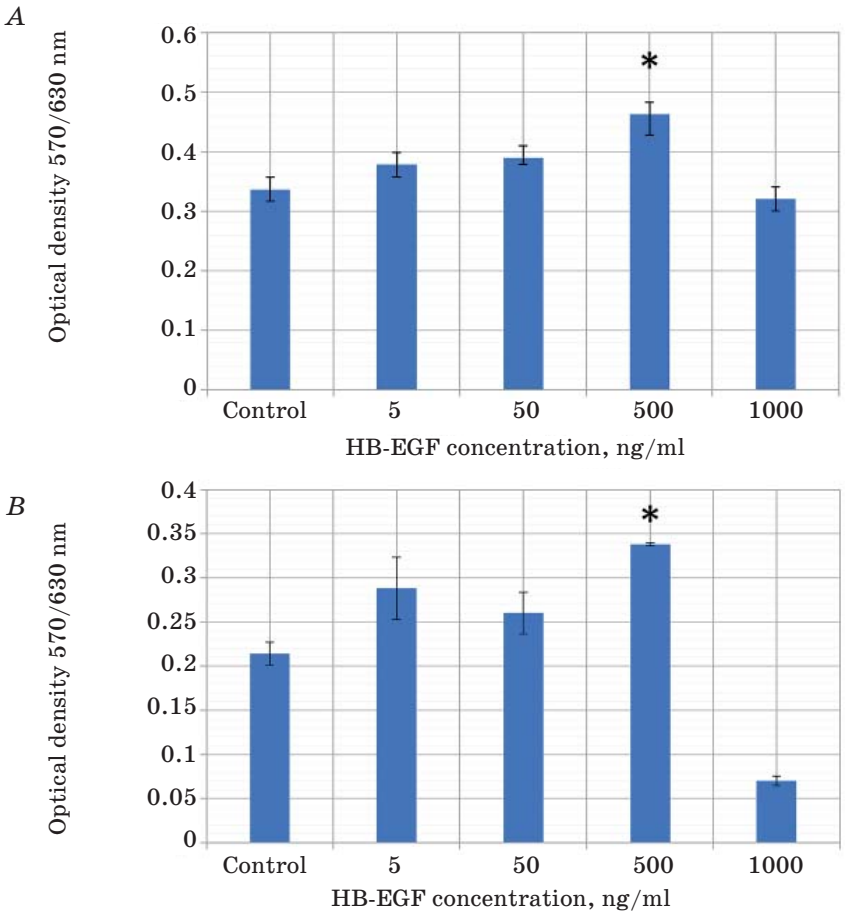

Fig. 3. **Effect of the recombinant human HB-EGF on the proliferation and/or metabolic activity of sensitive cells**

* – differences were considered to be significant when $P < 0.01$. *A* — L929 cells; *B* — 3T3 cells

MTT test is based on the ability of viable cells to convert the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) into an insoluble substance — formazan, which has the appearance of blue crystals (and can be registered spectrophotometrically).

Fig. 3, *A*, B MTT results of L929 and 3T3 cells, respectively under the treatment of different concentrations of the rhHB-EGF protein (from 5 ng/ml to 1000 ng/ml).

The data obtained demonstrates statistically significant increase in the proliferation/metabolic activity of both studied cells is observed at a rhHB-EGF concentration of 500 ng/ml. Cells of line 3T3 had a lower level of metabolic activity compared to line L929. However, as in the previous experiment, a statistically significant increase in the metabolic activity of 3T3 cells was observed at a rhHB-EGF concentration of 500 ng/ml. Concentration 5000 ng/ml demonstrates inhibition effect on the 3T3 fibroblasts.

3. Assessment of the influence of the growth factor rhHB-EGF on the migration activity of sensitive cells in vitro

The objective of the third part of this study was to examine the migratory activity of fibroblast cell lines L929 and 3T3 during in vitro cultivation in the presence of varying concentrations of recombinant rhHB-EGF. The investigation used the scratch test (or wound healing assay), a widely used method for classical analysis of cell migration in monolayer cultures. This method involves the dynamic measurement of the "scratch" area created on a cell monolayer, with the rate of scratch closure serving as an indicator of cellular migratory activity. Figures 4 and 5 illustrate a representative view of a cross-scratched monolayer of L929 cells in the control group and 24 hours after the addition of rhHB-EGF recombinant growth factor at concentrations of 500 ng/ml and 1000 ng/ml.

Both tested concentrations of rhHB-EGF demonstrated a stimulatory effect on

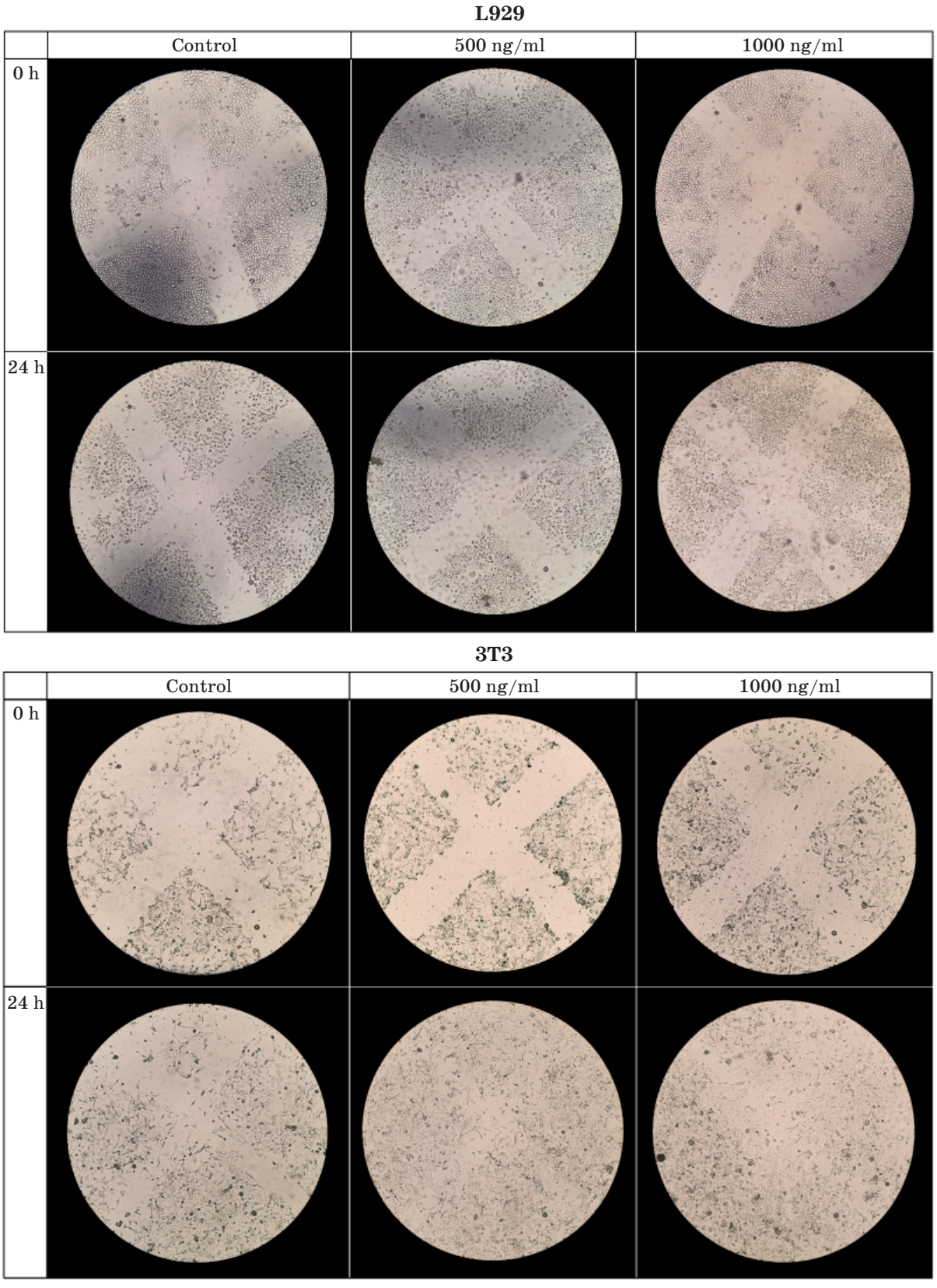

Fig. 4. **Effect of rhHB-EGF at concentrations of 500 ng/mL and 1000 ng/mL on the migratory activity of L929 and NIH 3T3 fibroblasts**
Exposure time: 24 hours

the migratory activity of L929 fibroblasts, indicating that this growth factor enhances cellular motility in a dose-independent manner. Analysis of the data derived from imaging, as presented in Figure 5, revealed a statistically significant increase in migratory activity in the rhHB-EGF-treated groups compared to the control. These results highlight the efficacy of rhHB-EGF in enhancing fibroblast migration, supporting its potential role in promoting wound closure under in vitro conditions.

Notably, no significant difference in migration activity was observed between the two concentrations studied (500 ng/ml and 1000 ng/ml). This suggests that the lower concentration (500 ng/ml) is sufficient to elicit a maximal response in fibroblast motility, thereby making it a more economically viable option for applications requiring enhanced cell migration. The economic implications of using a lower effective concentration are particularly relevant for large-scale applications in tissue engineering, regenerative medicine, and wound healing research.

The obtained results highlight the importance of dose optimization in experimental and clinical settings, as unnecessarily high concentrations of growth factors may not yield additional biological benefits while increasing costs and the potential for unintended effects. These findings provide a foundation for future studies aimed at exploring the mechanistic basis of rhHB-EGF-induced migration and its applicability in therapeutic contexts.

The 3T3 cell line exhibited results consistent with those observed in the L929 fibroblast cell line, demonstrating a comparable activation of migratory activity in response to rhHB-EGF treatment (Fig. 4–5).

This finding suggests that rhHB-EGF exerts a broadly similar pro-migratory effect across different fibroblast cell lines, reinforcing its role as a potent modulator of cell motility.

Quantitative analysis of the migration

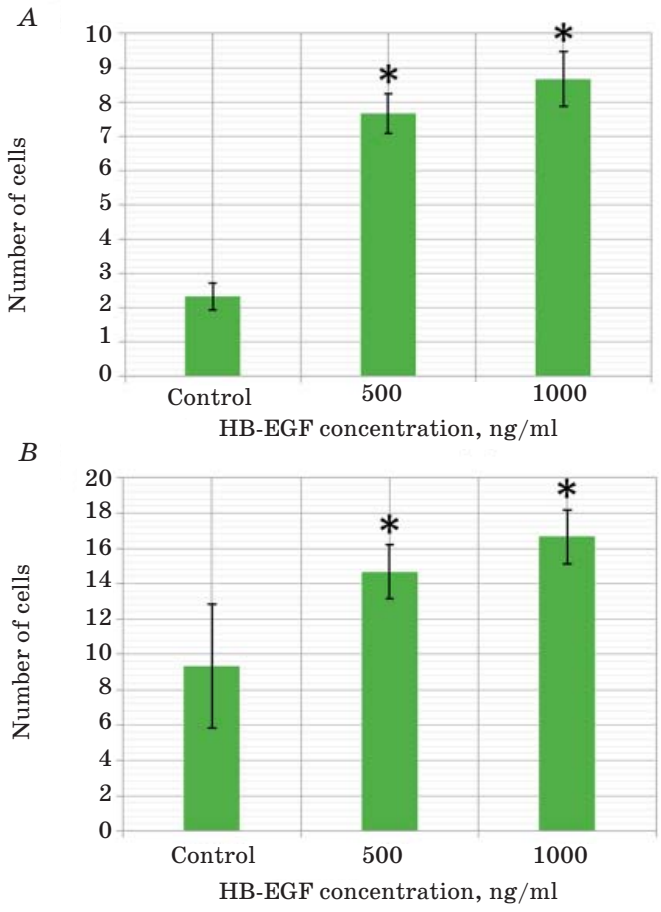

Fig. 5. **Migratory activity of L929 fibroblasts (*A*) and NIH 3T3 fibroblasts (*B*) in the control group and after 24 hours of treatment with rhHB-EGF at concentrations of 500 ng/ml and 1000 ng/ml**
* — differences were considered to be significant when $P < 0.05$.

patterns revealed that the stimulation induced by rhHB-EGF was significant when compared to untreated controls. This effect underscores the universal capability of rhHB-EGF to enhance cellular responses involved in migration, a critical process in tissue repair and regeneration.

The aim of the studies examining the cytotoxic effects of rhHB-EGF is crucial for the application of this protein in the design of gels for wound-healing applications. These studies have demonstrated promising results. Specifically, the number of viable 3T3 fibroblasts not only remained unaffected but also increased, indicating that rhHB-EGF at concentrations of 500–1000 ng/ml stimulates cell proliferation. These findings underscore the significant potential of rhHB-EGF in promoting wound healing and cellular regeneration, as evidenced by its effects on fibroblast cell lines L929 and 3T3. The apoptotic response of L929 fibroblasts to rhHB-EGF treatment was analyzed using flow cytometry, as depicted in Fig. 6.

The flow cytometry analysis revealed that 0.77% of the cells underwent apoptosis under treatment with rhHB-EGF at a concentration of 500 ng/ml, as indicated by the red-shaded region in the histogram. This low apoptotic percentage suggests that at this concentration, HB-EGF has little to no pro-apoptotic effect on L929 fibroblasts. When the concentration of HB-EGF was increased to 1000 ng/ml, apoptosis slightly increased to 1.05%. Despite the doubling of the HB-EGF concentration, the apoptotic response remained minimal, indicating that the factor does not induce significant apoptosis, even at higher doses. Ionomycin, a well-known inducer of apoptosis, triggered a markedly higher apoptotic response, with 23.51% of cells in the apoptotic phase. This serves as a positive control, confirming that the L929 cells are capable of undergoing apoptosis under appropriate conditions.

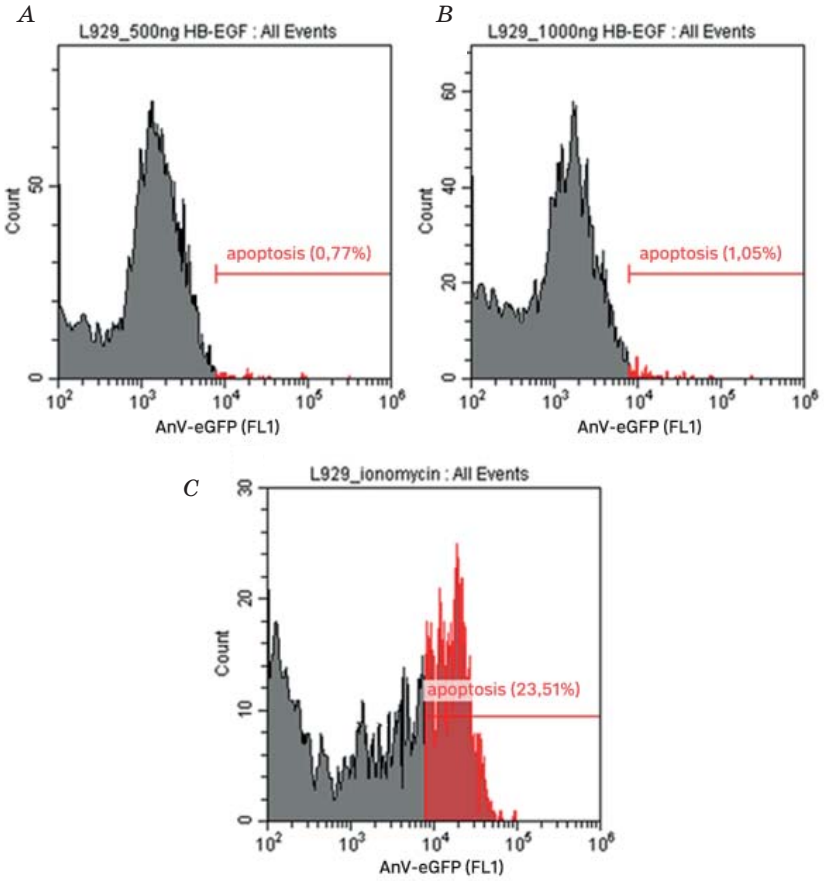

Fig. 6. **The apoptotic response of L929 fibroblasts to rhHB-EGF treatment after 24 hours of factor application**
The fluorescence intensity on the X-axis (Annexin V-eGFP) (FL1) indicates apoptotic cells, while the Y-axis represents the count of cell events. L929 cells were treated with 500 ng/ml HB-EGF (*A*), 1000 ng/ml (*B*), and ionomycin (*C*) as positive control for cell death

These data suggest that HB-EGF is not an inducer of apoptosis in L929 fibroblasts. Even at a high concentration (1000 ng/ml), the apoptotic response remains negligible. In contrast, ionomycin effectively induces apoptosis, validating the experimental conditions. HB-EGF might instead play a role in other cellular processes, such as proliferation or survival, rather than triggering cell death pathways. These findings support the therapeutic potential of rhHB-EGF in wound-healing applications, where its ability to enhance fibroblast viability and proliferation can contribute to tissue regeneration. Further studies are warranted to explore its broader biological effects and mechanisms of action.

Conclusion

The ability of rhHB-EGF to stimulate cellular proliferation and migration, as evidenced by the MTT and scratch tests, highlights its potential application in acute and chronic wound management. The results support the hypothesis that HB-EGF, through its interaction with EGFR, promotes re-epithelialization and granulation tissue formation — the key processes in wound healing. Furthermore, the minimal cytotoxicity observed for fibroblasts treated with rhHB-EGF validates its biological activity and suggests its possible integration into advanced wound care formulations. The incorporation of HB-EGF into delivery systems, such as coacervates or hydrogels, can improve its stability and localized therapeutic efficacy, addressing challenges like rapid degradation and low bioavailability. Future studies should focus on optimizing these delivery systems and exploring the long-term effects of rhHB-EGF in clinical settings.

In conclusion, rhHB-EGF emerges as a promising candidate for targeted wound-healing therapies, combining effective stimulation of fibroblast activity with minimal cytotoxic effects. This study paves the way for its application in regenerative medicine and underscores the importance of cell-specific responses in developing personalized treatment strategies.

Funding

This research was funded by the Ministry of Education and Science of Ukraine project under the state order for scientific and technical developments and products (0123U104137).

Conflicts of Interest

The authors declare no conflicts of interest.

This article does not contain any human or animal studies conducted by any of the authors.

Author Contributions

I. Vovk — figure preparation, A. Didan — data analysis, D. Zhukova — paper writing, data analysis, L. Dronko — data analysis, A. Rebriev — editing, A. Rybalko — contributed to the methodology of the review, E. Legach — data analysis, O. Gorbatiuk — editing, M. Usenko —- assisted in the critical revision, A. Skvarchynskyi — figure preparation, T. Dovbymchuk — literature review, A. Siromolot — conceptualized the study, S. Romaniuk — contributed to the design, D. Kolybo — project leading.

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ОДЕРЖАННЯ ТА ОЦІНЮВАННЯ *in vitro* РЕКОМБІНАНТНОГО HB-EGF ЛЮДИНИ ДЛЯ ЗАГОЮВАННЯ РАН І ТАРГЕТНОЇ ТЕРАПІЇ

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Мета. Оцінювання біологічної активності рекомбінантного гепарин-зв'язувального EGF-подібного фактора росту людини (rhHB-EGF) на фібробластах миші *in vitro* та можливості його застосування як потенційного агента для стимулювання загоєння ран та регенерації тканин.

Методи. У дослідженні застосовували міграційний тест (*scratch assay*) для вивчення міграції фібробластів миші (L929 та NIH-3T3), МТТ-тест для оцінювання проліферації та/або метаболічної активності клітин, MALDI-TOF мас-спектрометрію для ідентифікації протеїнів і проточну цитометрію для визначення життєздатності клітин.

Результати. У діапазоні концентрацій 500–1000 нг/мл rhHB-EGF не зафіксовано цитотоксичного ефекту. Натомість виявлено підсилення проліферації та/або метаболічної активності, а також міграції фібробластів з максимальним ефектом при 500 нг/мл rhHB-EGF у середовищі інкубації клітин. Продемонстровано 30% заростання раневої поверхні фібробластів у *scratch assay*-тесті за впливу rhHB-EGF порівняно з відповідним контролем.

Висновки. rhHB-EGF у концентрації 500 нг/мл може використовуватися у складі препаратів для стимулювання загоєння ран і регенерації тканин завдяки здатності стимулювати проліферацію/метаболічну активність та міграцію фібробластів, а також відсутності цитотоксичності. Для докладного вивчення цієї можливості надалі є необхідним проведення відповідних досліджень *in vivo*.

Ключові слова: гепарин-зв'язувальний EGF-подібний фактор росту людини (rhHB-EGF), рекомбінантний протеїн, культура клітин, фібробласти, проліферація, міграція, цитотоксичність, загоєння ран.