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Lab-scale Production of Recombinant Platelet-derived Growth Factor-BB Homodimer (PDGF-BB) in Microbial Expression System

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ABSTRACT

Background: In recent years, the number of recombinant proteins used for therapeutic applications has increased dramatically. Among the growth factors, platelet-derived growth factors (PDGFs) are effective in the management of injury and involve many cellular events in the healing process, including inflammatory cell recruitment, fibroblast proliferation and migration, collagen deposition, and granulation tissue formation. During the process of natural wound healing, platelets are one of the first cells that respond at the wound site or around it and play an essential role in wound healing. Platelets are a rich source of growth factors, including PDGF. PDGF is the first and only recombinant growth factor approved by the US Food and Drug Administration (FDA) for topical administration to treat diabetic foot ulcers.
Methods: In this study, we've built a microbial expression vector containing the coding sequence for optimized PDGF-BB and transformed it into an E. coli Strain. Then we developed a complete production process that includes: expression, purification using affinity chromatography, and biological activity assay by evaluation of NIH-3T3 cell growth in response to the produced recombinant human PDGF-BB.
Results: The purified PDGF-BB (15 mg/L of culture) was well-folded and biologically active. The whole procedure is fast and scalable.
Conclusion: The chosen E. coli Strain can produce and fold recombinant human PDGF-BB. The produced PDGF-BB has biologically active, and could be considered for further investigation.

Keywords: Recombinant human PDGF-BB; Recombinant DNA Technology; Microbial Expression System; Wound Healing

Introduction

Wound repair is a dynamic and complex process, and multiple events, including homeostasis, inflammation, proliferation, and remodeling, occur in a regular and overlapping fashion [1-3]. Failure of one or more cellular processes leads to poor wound healing. Although some treatments are available for wound healing, their effects are not very satisfactory, especially for the treatment of chronic wounds, such as diabetic foot ulcers. Treatment with growth factor is promising in wound healing, it has multiple functions and promotes cell proliferation, migration, angiogenesis, and other processes that are disordered in the wound healing process [2, 3].

Growth factors are intrinsic signaling molecules that regulate cellular responses to the wound-healing process. These proteins are regulated in response to tissue damage and are secreted by platelets, leukocytes, fibroblasts, and epithelial cells. When growth factors are secreted, they act through autocrine, paracrine, or endocrine mechanisms by binding to membrane or cytoplasmic receptors. Binding to the receptors leads to a cascade of events that activate cellular machinery to facilitate wound healing. Even at low concentrations, growth factors can have a significant effect on the wound microenvironment, leading to a rapid increase in cell migration, proliferation, and differentiation [3, 4].

Among these factors, platelet-derived growth factors (PDGFs) play a crucial role in managing injuries by regulating various cellular activities during healing, such as recruiting inflammatory cells, promoting fibroblast activity, and collagen deposition[2, 3, 5].

Platelets are among the first responders at the wound site and are vital for initiating the healing process. They are a significant source of growth factors like PDGF,

which is also produced by macrophages, endothelial cells, fibroblasts, and keratinocytes [4, 6, 7]. PDGF is the first recombinant growth factor approved by the FDA for topical use in treating diabetic foot ulcers[4, 6, 7]. PDGF contributes to several repair activities, including soft tissue and bone repair, as well as epithelial regeneration [5, 8]. PDGF functions may be enhanced through interactions with other growth factors that regulate cellular activities either additively or synergistically [8]. Furthermore, PDGF influences cell growth and division while also playing a role in angiogenesis [4, 9, 10]

FDA approved a recombinant human PDGF-BB gel (Regranex) for treating diabetic neurogenic foot ulcers in 1997 [2, 11]. In a randomized controlled trial (RCT), a topical gel containing PDGF-BB (Regranex®) was compared with a placebo in 118 patients with non-healing diabetic ulcers enrolled from 10 different centers. Patients were treated for 20 weeks or until complete wound closure. 48% of PDGF-treated patients improved compared to 25% of placebo-treated patients. A combined analysis of three other clinical trials reached similar results regarding the efficacy of PDGF-BB[4, 12]. Studies indicate that a daily dose of 100 µg/g of PDGF-BB enhances recovery rates by 39% relative to placebo. Given its favorable safety profile and ease of use, PDGF-BB is recommended for treating diabetic foot ulcers resistant to standard therapies[4, 13]. However, topical applications of PDGF for pressure and venous ulcers have shown limited effectiveness, potentially due to challenges in growth factor penetration or patient age[4, 14, 15]

Numerous studies have reported the successful production of growth factors and cytokines, including PDGF[16], interleukin-1 [17], interleukin-4 [18], epidermal growth factor [19, 20], FGF-2[20, 21], and leukemia inhibitory factor[22-24] in *E. coli* for both research and clinical purposes. In this study, we describe a straightforward strategy to produce biologically active recombinant human PDGF protein in E. coli. The method offers a rapid and cost-effective means to purify soluble human PDGF-BB protein that retains biological activity and functionality. Additionally, this protocol may be applicable for producing other growth factors.

The recombinant human PDGF produced in this study shows promising potential for further research investigations. It could be applied in developing innovative wound dressings enriched with this recombinant growth factor, thereby contributing to improved wound healing outcomes.

Methods

Cloning of PDGF-BB Fragment from cDNA

Total RNA was isolated using NucleoSpin RNA II (MN, Germany) from human Embryonic Stem Cells. Total RNA was treated with RNase-free DNase (Invitrogen, Carlsbad, CA, USA) to ensure the complete removal of genomic DNA. The first strand of cDNA synthesis was performed using Super Script III reverse transcriptase (Invitrogen, Carlsbad, CA, USA), oligo dT primer, and 2 µg of purified total RNA. The primers used to amplify PDGF-BB were designed from Gen Bank Accession No.: NM_002608.4, Ser82-Thr190, and remaining amino acids excluded. Generated cDNA was amplified with the following set of primers: F: 5' aaa aaa cca tgg ctg ggt tcc ctg acc att gc 3' and R: 5' aaa aaa ctc gag cta ggt cac agg ccg tgc agc 3'.

For fragment amplification, pfx DNA polymerase (Invitrogen, USA) and a Mastercycler® Gradient PCR (Eppendorf Netheler-Hinz GmbH, Germany) were used. Amplification steps included: pre-incubation at 95°C for 4 min; 35 cycles at 95°C for 30 s, 61°C for 30 s, and 68°C for 45 s; followed by one incubation step at 68°C for 8 min. Next, the PCR products were analyzed by electrophoresis on 1.5% agarose gel and visualized by gel green (parstous, IR Iran) staining under ultraviolet (UV) light.

Construction of pET 32a/PDGF Expression Vector

The PCR product was digested with NcoI and XhoI restriction enzymes (Roche Applied Science, Basel, Switzerland), cloned in a pET 32a vector, and digested with the same restriction enzymes. Expression of PDGF-BB - His, cloned in pET 32a, was under the direct control of the T7 promoter and transcription terminator. The recombinant expression vector construct that carried the PDGF-BB gene (pET 32a /PDGF-BB) was transferred into an *E. coli* strain competent cells (Novagen, Madison, WI, USA) by the heat shock method as described by the manufacturer (User Protocol TB 009 Rev. F 0104). The transgene nucleotide sequence in pET 32a /PDGF-BB was analyzed by DNA sequencing.

Expression and Purification of Recombinant Human PDGF-BB Protein

The pET32a/PDGF-BB that encoded the His6-PDGF-BB protein was transferred into an *E. coli* Strain. Different IPTG concentrations (0.1, 0.5, or 1.0 mM) and induction temperatures (30°C to 37°C) were used. Expression of the His6-PDGF-BB protein after 6 hours of induction yielded more proteins at the 30°C induction temperature compared to the 37°C temperature. We determined the best concentration of IPTG to be 0.1 mM concerning expression level and inclusion body formation. Expressed recombinant PDGF was purified by the Ni-NTA Agarose (ABT, Spain), impurities were washed with 25 mM imidazole, and the target protein was eluted with 250 mM imidazole.

Most contaminant bacterial proteins were eliminated in the flow-through and washing steps. None of the specifically bound proteins were further reduced by washing the resin or by increasing concentrations of imidazole. The PDGF-BB recombinant protein (approximately 37 kDa) was obtained in the 250 mM imidazole fractions.

Determination of the Purified PDGF-BB

The identity of the purified PDGF-BB, proteins was confirmed by SDS-PAGE gel and Western Blotting. The results indicated that our proteins matched PDGF-BB.

SDS-PAGE gel

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was utilized to investigate the production and identify the protein structure. Protein samples were prepared by mixing with sample buffer on both reduced and unreduced conditions, denaturing at 75°C, and loading onto a 12% polyacrylamide gel. Electrophoresis was performed at 120 V until the dye front reached the bottom of the gel, followed by Coomassie Brilliant Blue G250 staining for visualization. The results indicate of production of PDGF-BB (Figure 1) and formation of a dimeric structure, as illustrated in Figure 2.

Western Blotting

As the primary antibody, Purified anti-His Tag (Cat#: 652501, Biolegend Inc, USA) was used to carry out western blot analysis. The secondary antibody was Goat Anti-Mouse IgG (HRP) (cat#:DB9571, DNABiotech Inc., IR IRAN. Anti-His Tag antibody was used at 1:1000, and Goat Anti-Mouse IgG (HRP) at 1:1000 final dilutions. For western blotting, the ECL Detection Kit was obtained from Cyto Matin Gene (CMG, IRI). Polyvinylidene fluoride transfer membrane (PVDF) and complete protease inhibitor cocktail tablets were purchased from Roche (Life Science, USA).

Determination of the Concentration of Recombinant PDGF

After purification, the concentration of recombinant human PDGF-BB was analyzed by Bradford assay test with BSA as standard. This colorimetric assay is based on the binding of Coomassie Brilliant Blue G-250 dye to protein molecules under acidic conditions, which induces a shift in the dye's absorbance maximum. The resulting absorbance change—typically measured at 595 nm—correlates with protein concentration. A calibration curve constructed using protein standards was utilized, allowing for precise quantification of the protein content in the purified samples [25].

Analysis of the Effects of Produced PDGF-BB on NIH-3T3 Cell Line

To investigate our recombinant human PDGF-BB product functionality, we applied it to the NIH-3T3 culture, which is a Fibroblast-like cell line derived from NIH Swiss mice, and its growth curve was drawn.

We divided our experiment into different groups that were treated with 0.06, 0.12, 0.47, 0.94, 1.87, 3.75, 7.5, 15, and 30 ng/Well concentrations of recombinant PDGF-BB.

Results

SDS-PAGE Gel

An SDS-PAGE analysis of the expressed protein was conducted to evaluate the production, purification, and structural characteristics of recombinant human PDGF-BB. The results indicate of production of PDGF-BB (Figure 1) and formation of a dimeric structure, as illustrated in Figure 2.

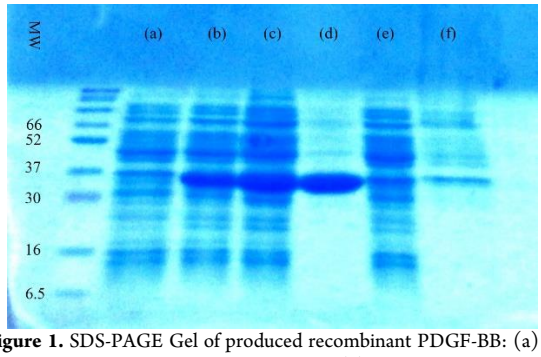


Figure 1. SDS-PAGE Gel of produced recombinant PDGF-BB: (a) bacterial proteome prior to IPTG induction (b) soluble proteins after induction with IPTG (c) precipitated proteins in plate after solubilization by urea (d) purified proteins with Ni-NTA (e) waste proteins of Ni-NTA chromatography (f) proteins present in the culture medium.

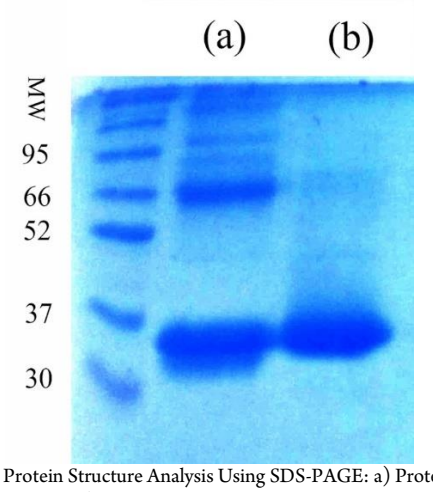


Figure 2. Protein Structure Analysis Using SDS-PAGE: a) Protein in its unreduced form b) Protein after reduction. In the unreduced form, a protein band was detected at about 66 kD, while in the reduced form, the band was found at approximately 37 kilodaltons. Trimers, tetramers, etc. are observed to a lesser extent at the top of the gel in unreduced form

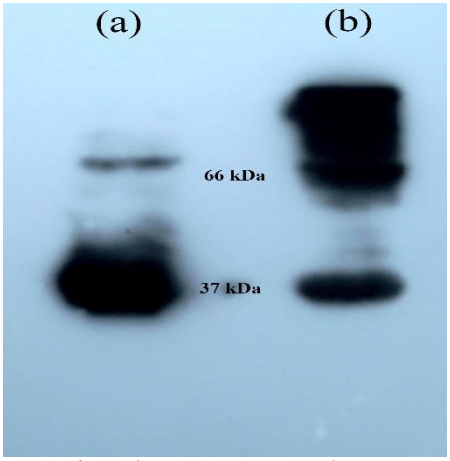


Figure 3. Recombinant human PDGF-BB Analysis Using Western blotting: (a) PDGF after reduction. PDGF exists as a monomer with a molecular weight of approximately 37 kDa (Trx-tag+ His-tag), along with a small quantity of dimeric PDGF at 66 kDa (Trx-tag+ His-tag).

(b) PDGF-BB in its unreduced form. . Trimers, tetramers, etc. are observed to a lesser extent at the top of the gel in unreduced form.

Western Blotting

A Western blotting analysis of the expressed protein was conducted to evaluate the production and structural characteristics of recombinant human PDGF-BB. Western blotting results show the production of recombinant human PDGF-BB as illustrated in Figure 3. Recombinant human PDGF-BB is found as a mixture of various oligomeric states, including monomers, dimers, trimers, and tetramers.

Recombinant PDGF Concentration

Bradford method was employed to determine the concentration of the purified protein. The concentration of eluted recombinant human PDGF-BB was 15 $^{mg}/_{L}$ of culture. Bradford's standard curve is shown in Figure 4.

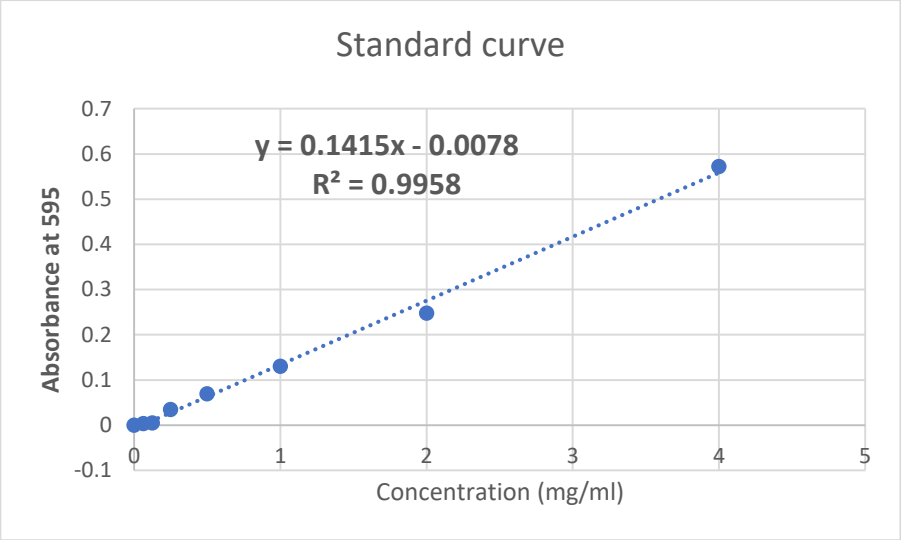


Figure 4. Bradford's standard curve

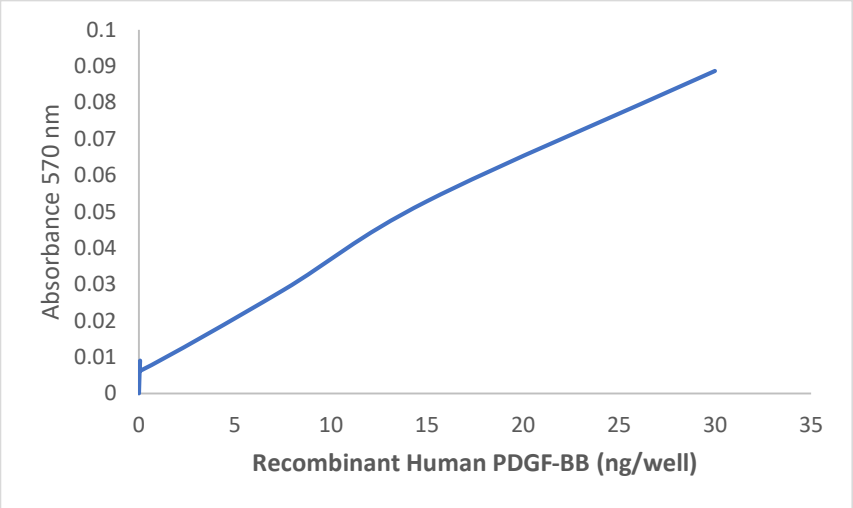


Figure 5. Evaluation of NIH-3T3 cell growth in response to the produced recombinant human PDGF-BB. NIH-3T3 cells were treated with the indicated concentrations of recombinant human PDGF-BB (ng/well). Cell proliferation was measured over time by optical density and plotted. Data show a dose-dependent modulation of cell growth with a clear exponential growth phase

Recombinant Human PDGF-BB Bioassay

As detailed in the Methods section, the growth curve analysis was employed to assess biological activity. This approach involved tracking the kinetics of the culture over a designated period by measuring optical density at regular intervals. The results showed an increasing growth curve, proportional to the increase in growth factor concentration (Figure 5). This finding is indicative of cellular proliferation in the presence of the growth factor, thereby reflecting its stimulatory bioactivity.

Discussion

The process begins with gene isolation, where the PDGF-B coding sequence is amplified from genomic or cDNA sources. This is followed by cloning into an expression vector, in which the amplified gene is inserted into a plasmid (pET32a) designed for bacterial expression. Next, the construct is introduced into a

suitable *E. coli* host in the transformation and expression step, leading to the production of recombinant PDGF-BB. In the formulation and application step, the expressed protein is purified using nickel-affinity chromatography to isolate His-tagged PDGF-BB. Subsequent quality control procedures include analyses of protein purity, structural integrity, and biological activity to confirm functionality. In the final stage, the active protein is considered for practical applications, such as incorporation into topical formulations, wound dressings, or regenerative therapy platforms (shown in Schematic Figure 6).

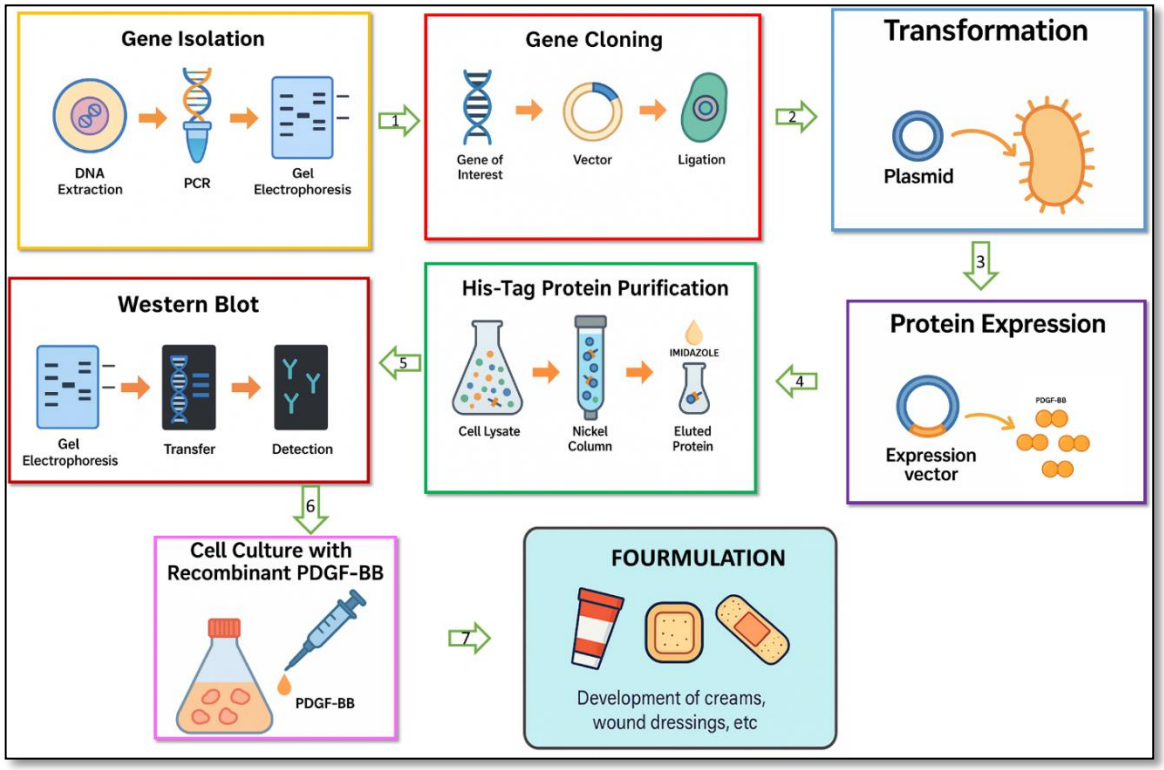


Figure 6. Schematic overview of the recombinant PDGF-BB production workflow

In the present study, we successfully cloned and expressed the human PDGF-BB gene using the pET32a vector in an *E. coli* expression system. The production yield reached 15 mg/L, and the recombinant protein showed proper folding, dimer formation, and strong biological activity, as demonstrated in NIH-3T3 fibroblast proliferation assays. These findings emphasize the feasibility of using microbial systems, particularly E. coli, for efficient and cost-effective production of recombinant growth factors such as PDGF-BB.

Our results align with previous studies that reported successful expression of growth factors, including PDGF and others like FGF and EGF, in bacterial systems. However, a major challenge in bacterial expression systems is protein misfolding and formation of inclusion bodies, which often compromise bioactivity. We addressed this issue by optimizing induction conditions (low IPTG concentration and reduced temperature), which facilitated the expression of biologically active PDGF-BB in a soluble form with minimal aggregation. The presence of dimeric PDGF-BB, confirmed by SDS-PAGE and Western blotting, further supports the correct folding of the protein, which is essential for its bioactivity.

Importantly, the functional assay demonstrated that even low concentrations of our recombinant PDGF-BB could stimulate fibroblast proliferation, confirming that the protein retains its physiological function. The biological activity observed in NIH-3T3 cells was dose-dependent, which strengthens the potential application of the recombinant protein in regenerative medicine, particularly wound healing.

A minor fraction of monomers and higher-order oligomers (trimers, tetramers) was also observed, which could reflect either transient intermediates or artifacts from sample handling. Nevertheless, the dominant active form remained the dimer, which is the physiologically relevant structure.

Compared to commercially available PDGF-BB products such as Regranex®, our system offers a more cost-effective and scalable solution, especially suitable for research and possibly future therapeutic development. However, further purification refinements and formulation studies would be needed before considering clinical-grade production.

Future directions may include expression of PDGF-BB in other hosts (e.g., yeast or mammalian cells) for glycosylated forms, structural characterization using mass spectrometry or circular dichroism, and incorporation of the recombinant protein into biomaterials such as hydrogels or wound dressings. Testing the product in animal models of wound healing would also be a crucial next step to validate its therapeutic efficacy and safety.

Conclusion

This study demonstrates a robust, efficient, and scalable method for the production of biologically active recombinant human PDGF-BB using a bacterial expression system. The successful expression in *E. coli* using the pET32a vector resulted in a high protein yield (15 mg/L), with the majority of the product correctly folded in its active dimeric form.

The biological functionality of the produced PDGF-BB was confirmed through in vitro bioassays, showing significant stimulation of fibroblast proliferation. These findings not only support the utility of microbial systems in producing therapeutic-grade growth factors but also highlight the cost-effectiveness and simplicity of this platform for potential commercial or clinical applications.

Given the critical role of PDGF-BB in wound healing, this recombinant protein may serve as a promising candidate for inclusion in advanced wound care products or regenerative therapies. With further optimization, purification, and validation in preclinical models, this approach may contribute meaningfully to the development of affordable biotherapeutics for chronic wound management and tissue repair.

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Practical Implications

The successful lab-scale production of biologically active recombinant human PDGF-BB in a microbial expression system presents important practical implications for both industrial biotechnology and therapeutic protein manufacturing. First, the use of an optimized gene construct in E. coli provides a cost-effective and scalable platform for producing PDGF-BB compared to more complex eukaryotic expression systems. This could significantly reduce production costs and improve accessibility of PDGF-based therapies, particularly in developing countries where advanced wound care treatments may be limited. Second, the demonstrated biological activity of the purified PDGF-BB confirms the feasibility of microbial systems for producing functionally active growth factors. The relatively high yield obtained (15 mg/L of culture) suggests that further process optimization and scale-up strategies could support pilot- and industrial-scale manufacturing. Third, this production platform may facilitate local or regional manufacturing of recombinant PDGF-BB for research, preclinical studies, and potential formulation development for wound healing applications, including diabetic foot ulcers. The availability of an efficient expression and purification workflow may also accelerate translational research focused on growth factorbased regenerative therapies. Finally, the developed methodology can serve as a framework for the recombinant production of other disulfide-bonded growth factors in microbial systems, contributing to broader biopharmaceutical development efforts.

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Author's Contributions

Study designed by Hassan Rassouli, Armin Nazemi Zadeh, Fereshteh Sarafrazi, and Masoud Habibi Fozon Abadi. Experiments performed by Armin Nazemi Zadeh, and Fereshteh Sarafrazi. All Author's contributed to data analysis. All Author's contributed to draft the manuscript. All authors reviewed and approved the final version of the manuscript.

Ethical Considerations

Ethical Approval ID:
IR.ACECR.IBCRC.REC.1403.021.

Conflicts of Interest

The authors declare no conflict of interest, financial or otherwise.

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