Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Short-Term Stability of Growth Factors in Umbilical Cord Blood Platelet Lysate: Implications for Corneal Cell Culture and Regenerative Applications

Ivana Vidović

ivana.vidovic@kbc-zagreb.hr

University Hospital Centre Zagreb

Marta Himelreich-Perić

School of Medicine, University of Zagreb

Branka Golubić Ćepulić

University Hospital Centre Zagreb

Mirna Golemović

The Agency for Medicinal Products and Medical Devices (HALMED)

Davor Ježek

School of Medicine, University of Zagreb

Kristina Kralik

The Faculty of Medicine Osijek, Josip Juraj Strossmayer University

Koraljka Gojčeta

University Hospital Centre Zagreb

Ines Bojanić

University Hospital Centre Zagreb

Research Article

Keywords: umbilical cord blood, platelet lysate, corneal regeneration, growth factor stability, tissue banking

Posted Date: May 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9438093/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations:

No competing interests reported.

Tables are available in the Supplementary Files section.

Title page

Article title

Short-Term Stability of Growth Factors in Umbilical Cord Blood Platelet Lysate: Implications for Corneal Cell Culture and Regenerative Applications

Author names

Author Full Name: Ivana Vidović, MD, PhD
Affiliation: University Hospital Centre Zagreb
Department: Clinical Department of Transfusion Medicine and Transplantation Biology, Tissue Bank
Address: Kišpatićeva 12, 10000 Zagreb
Country: Croatia
E-mail address: ivana.vidovic@kbc-zagreb.hr
Phone: +385995976402
Corresponding Author: Yes

Author Full Name: Marta Himelreich-Perić, MD, PhD
Affiliation: School of Medicine, University of Zagreb; Health Centre Zagreb – West
Department: Centre of Excellence for Reproductive and Regenerative Medicine; Health Centre Zagreb – West
Address: Prilaz baruna Filipovića 11, 10000 Zagreb
Country: Croatia
E-mail address: marta.himelreich@gmail.com

Author Full Name: Assoc. Prof. Branka Golubić Ćepulić, MD, PhD
Affiliation: University Hospital Centre Zagreb
Department: Clinical Department of Transfusion Medicine and Transplantation Biology
Address: Kišpatićeva 12, 10000 Zagreb
Country: Croatia
E-mail address: branka.golubic@gmail.com

Author Full Name: Mirna Golemović, MSc in Biology, PhD
Affiliation: The Agency for Medicinal Products and Medical Devices (HALMED)
Address: Ksaverska cesta 4, 10000 Zagreb
Country: Croatia
E-mail address: mgolemov@gmail.com

Author Full Name: Prof. Davor Ježek, MD, PhD
Affiliation: School of Medicine, University of Zagreb
Department: Centre of Excellence for Reproductive and Regenerative Medicine; Department of Histology and Embryology
Address: Šalata 2, 10000 Zagreb
Country: Croatia
E-mail address: davor.jezek@mef.hr

Author Full Name: Assoc. Prof. Kristina Kralik, PhD
Affiliation: The Faculty of Medicine Osijek, Josip Juraj Strossmayer University
Department: Department of Medical Statistics and Medical Informatics
Address: Josipa Huttlera 4, 31 000 Osijek
Country: Croatia
E-mail address: kristina.kralik@mefos.hr

Author Full Name: Koraljka Gojčeta, specialist in medical biochemistry and laboratory medicine
Affiliation: University Hospital Centre Zagreb
Department: Clinical Department of Transfusion Medicine and Transplantation Biology
Address: Kišpatićeva 12, 10000 Zagreb
Country: Croatia
E-mail address: koraljka.gojceta@kbc-zagreb.hr

Author Full Name: Assoc. Prof. Ines Bojanić, MD, PhD
Affiliation: University Hospital Centre Zagreb
Department: Clinical Department of Transfusion Medicine and Transplantation Biology
Address: Kišpatićeva 12, 10000 Zagreb
Country: Croatia
E-mail address: ines.bojanic@kbc-zagreb.hr

Abstract

Purpose

Platelets are a major source of growth factors essential for hemostasis, angiogenesis, and tissue regeneration, including in the human cornea. This study evaluated the stability of key growth factors in umbilical cord blood–derived platelet lysate (UCB-PL) during six months of storage at −20 °C to assess its suitability for corneal cell culture and regenerative applications.

Methods

UCB-PL was prepared from ten umbilical cord blood units unsuitable for allogeneic hematopoietic stem cell transplantation. Concentrations of TGF-β, PDGF-BB, EGF, VEGF-A, VEGF-D, BDNF, and HGF were measured immediately after preparation and after 3 and 6 months of storage at −20 °C using a microsphere-based assay performed on a Luminex 200 fluoroanalyzer.

Results

Most growth factors involved in corneal repair, including BDNF, EGF, HGF, VEGF-A, VEGF-D, and PDGF-BB, remained stable during short-term storage, with peak concentrations observed after three months. In contrast, TGF-β levels declined significantly over time and fell below the detection limit after six months of storage. These findings indicate that storage at −20 °C preserves the biological activity of most growth factors for up to three months, whereas prolonged storage adversely affects less stable proteins.

Conclusion

UCB-PL maintains biologically relevant levels of key growth factors and represents a promising animal-free supplement for corneal cell culture, regenerative therapies, and wound-healing applications. Short-term storage at −20 °C is recommended to ensure optimal growth factor stability and bioactivity for tissue banking and clinical use.

Keywords: umbilical cord blood; platelet lysate; corneal regeneration; growth factor stability; tissue banking

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Platelets are fundamental mediators of primary hemostasis, wound repair, and tissue regeneration. Their lysosomes and diverse granule populations contain a variety of bioactive molecules, including energy substrates, enzymes, coagulation factors, adhesion molecules, chemokines, immune mediators, and growth factors, all of which contribute critically to hemostatic processes, angiogenesis, and tissue restoration (Chaudhary et al., 2022). Among the growth factors contained within platelets, platelet-derived growth factor AB (PDGF-AB), transforming growth factor β1 (TGF-β1), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) are particularly relevant to ocular tissue regeneration (Alió et al., 2019). The principal growth factors implicated in ocular tissue repair are summarized in Table 1.

Platelet α-granules are enriched with mitogenic and chemotactic growth factors, as well as inactive molecular precursors essential for tissue repair and regeneration (Burnouf et al., 2016, Graca et al., 2023). The release of these bioactive molecules may occur physiologically via thrombin activation and subsequent clot formation or may be induced artificially through platelet lysis methods, including repeated freeze–thaw cycles, sonication, or chemical treatment. Among these, repeated freeze–thaw cycles are widely regarded as the most accessible, efficient, and cost-effective approach to induce platelet lysis and release trophic factors, thereby generating a platelet lysate.

Human platelet lysate (hPL) has therefore emerged as a viable, animal component–free supplement that is rich in growth factors and, in most instances, derived from expired platelet units. hPL represents a safe and effective alternative to recombinant growth factors or animal-derived supplements for both in vitro and in vivo applications. Platelet concentrates obtained from healthy donors provide a secure source for hPL production; however, their routine availability for laboratory purposes is constrained by logistical limitations (Schallmoser et al., 2020).

Umbilical cord blood (UCB), collected post-delivery via venipuncture of umbilical cord vessels, constitutes a rich source of hematopoietic stem and progenitor cells (HSPCs). UCB has been extensively utilized for HSPC transplantation in patients with hematologic malignancies, immunodeficiencies, and metabolic disorders. Notably, approximately 80% of UCB units stored in cord blood banks are deemed unsuitable for allogeneic transplantation due to insufficient cellularity. Nevertheless, these units retain significant regenerative and anti-inflammatory potential, in addition to low immunogenicity and minimal risk of disease transmission (Bień et al., 2024; Wang et al., 2023).

UCB units unsuitable for transplantation can be employed in the production of umbilical cord blood platelet lysate (UCB-PL). UCB-PL has demonstrated utility in the treatment of inflammatory disorders and tissue injuries requiring enhanced regenerative support. Moreover, it has been shown to facilitate the expansion and proliferation of mesenchymal stromal cells in vitro (Christou et al., 2018; Samarkanova et al., 2020; Giannaccare et al., 2020; Valentini et al., 2019).

In the field of ophthalmology, UCB-PL has been applied to the management of dry eye syndrome, corneal burns, and ulcerative lesions. Its high concentration of growth factors involved in cellular migration, proliferation, and corneal repair underscores its potential for maintaining corneal integrity and positively modulating the biological characteristics of human corneal endothelium during in vitro storage and culture (Christou et al., 2018; Farag et al., 2022; Tovar et al., 2021).

The objective of this study was to evaluate the concentration of growth factors involved in ocular tissue repair in UCB-PL and to investigate the stability of these growth factors during six months of storage at −20 °C.

2. Materials and Methods

2.1. Umbilical Cord Blood Collection and Selection

Umbilical cord blood (UCB) units were collected immediately after delivery by venipuncture of the umbilical vein under standard aseptic conditions. Units were included in the study if they did not meet the criteria for allogeneic hematopoietic stem cell transplantation, specifically total nucleated cell count < 14×10^8/L, collected volume ≥ 100 mL, or platelet count ≥ 150×10^9/L.

A total of ten UCB units were obtained from healthy donors following written informed consent. All procedures were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Review Board (approval number 02/21 AG, dated 20 December 2021). Donor serological screening was performed in compliance with the guidelines of the European Directorate for the Quality of Medicines & HealthCare.

2.2. Preparation of UCB-PL

UCB-PL was prepared from the ten selected UCB units using the protocol described by Golubić Ćepulić et al. (2009). Platelet-rich plasma was separated from each unit and subsequently subjected to controlled freeze–thaw cycles to induce platelet lysis, releasing growth factors and other bioactive molecules. The lysate was clarified by centrifugation and stored in sterile aliquots at −20 °C until further analysis. All procedures were carried out under sterile conditions to maintain sample integrity.

UCB-PL was produced in two steps: first, UCB platelet concentrate (UCB-PC) was prepared using double-spin centrifugation, and then UCB-PL was produced through freeze-thawing (-80/+37 ºC) (Samarkanova et al., 2020). A closed bag system (BioNest-CB, Meditalia, Italy) was used for UCB-PC production, with platelet counts ranging from 800 to 1200 x 10^9/L (Samarkanova et al., 2020).

2.3. Quality Control and Volume Determination

Initial quality control of each UCB unit included complete blood count analysis using a hematology analyzer (ADVIA 2120i, Siemens, Germany) and volume determination by gravimetric methods. The weight of each blood component was measured using an electronic scale (PGL 3002, Adam Equipment Co. Ltd., United Kingdom), and volume was calculated based on the specific gravity of blood components as outlined in the Standards for Blood Banks and Transfusion Services.

The absolute number of total nucleated cells was calculated using the formula:

TNC ($\times 10^8$) = white blood cell count ($\times 10^9$/L) × collected blood volume (mL) ÷ 100.

2.4. Storage of UCB-PL

UCB-PL samples were aliquoted into 1.8 mL cryoampoules (CryoPure, Sarstedt, Germany) and stored at −20 °C (Arctic 600, Fiocchetti, Italy) for up to six months.

2.5. Growth Factor Stability Analysis

Concentrations of TGF-β, PDGF-BB, EGF, VEGF-A, VEGF-D, BDNF, and HGF were measured immediately after UCB-PL preparation and after 3 and 6 months of storage at −20 °C. Growth factor levels were determined using a microsphere-based immunoassay on a Luminex 200 fluoroanalyzer (Luminex Corporation, Austin, TX, USA). Commercially available multiplex panels were used according to the manufacturers' instructions. TGF-β measurements included a sample activation step using NaOH/HEPES pretreatment. All samples were analyzed in duplicate, and growth factor concentrations are reported as mean values of the two measurements.

2.6. Statistical Analysis

Categorical variables are presented as absolute and relative frequencies. Continuous variables are described using median values and interquartile ranges. Due to the small sample size and paired design, non-parametric statistical methods were applied. Differences between paired measurements were analyzed using the Wilcoxon signed-rank test with Hodges–Lehmann estimates of median differences and corresponding 95% confidence intervals. All statistical tests were two-sided, with significance set at $P < 0.05$. Statistical analyses were performed using MedCalc® software (version 23.3.4).

3. Results

3.1. Characteristics of Umbilical Cord Blood and Platelet Lysate

Ten UCB units were included in the analysis. The median collected UCB volume was 105 mL (interquartile range [IQR] 91.4–118.3 mL). The median absolute platelet count was 22.2 × 10^9 (IQR 17.3–33.5), the median absolute leukocyte count was 1.0 × 10^9 (IQR 0.8–1.3), and the median absolute erythrocyte count was 0.4 × 10^{12} (IQR 0.3–0.4). Following double-spin centrifugation, UCB platelet concentrates (UCB-PC) had a median volume of 8.4 mL (IQR 7.12–11.91 mL). The median absolute platelet count in UCB-PC was 7.3 × 10^9 (IQR 4.66–13.51), while leukocyte and erythrocyte contamination was minimal, with median values of 0 × 10^9 (IQR 0–0.01) and 0.1 × 10^{12} (IQR 0.07–0.14), respectively. Detailed characteristics of UCB and UCB-PC are summarized in Table 2.

3.2. Growth Factor Stability in UCB-PL

Concentrations of TGF-β, PDGF-BB, EGF, VEGF-A, VEGF-D, BDNF, and HGF were measured in ten UCB-PL preparations at three time points: immediately after preparation (day 0), after three months, and after six months of storage at −20 °C. Growth factor concentrations are reported as mean values of duplicate measurements (pg/mL). The results are summarized in Table 3.

3.2.1. Transforming Growth Factor-β (TGF-β)

TGF-β concentrations were highest at the initial measurement, with a mean value of 2205.9 pg/mL. After three months of storage at −20 °C, TGF-β levels decreased by approximately 31%. After six months, TGF-β concentrations declined below the detection limit of the assay. Statistically significant differences were observed between all measurement time points ($P < 0.001$). The results are presented in Table 3 and Figure 1.

3.2.2. Brain-Derived Neurotrophic Factor (BDNF)

BDNF concentrations increased markedly during early storage. After three months at −20 °C, BDNF levels increased by approximately 196%, reaching the highest mean concentration observed during the study period (2296.2 pg/mL). After six months, BDNF concentrations decreased by approximately 52% compared with baseline values. Differences between all measurement points were statistically significant ($P < 0.001$). Results are shown in Table 3 and Figure 2.

3.2.3. Epidermal Growth Factor (EGF)

EGF concentrations increased during storage. After three months at −20 °C, EGF levels increased by approximately 317%, while after six months they remained elevated, showing a 93% increase compared with baseline values. The highest EGF concentrations were observed after three months of storage (488.4 pg/mL). Differences between all measurement points were statistically significant ($P < 0.001$). Data are presented in Table 3 and Figure 3.

3.2.4. Hepatocyte Growth Factor (HGF)

HGF concentrations increased substantially after three months of storage, with a mean increase of approximately 348% compared with baseline values. After six months, HGF levels decreased by approximately 83.2% relative to initial concentrations. Statistically significant differences were observed between all time points ($P < 0.001$). Results are summarized in Table 3 and Figure 4.

3.2.5. Platelet-Derived Growth Factor-BB (PDGF-BB)

PDGF-BB concentrations increased progressively throughout the storage period. After three months of storage at −20 °C, PDGF-BB levels increased by approximately 2247% compared with baseline values. After six months, concentrations further increased, reaching the highest observed mean value (2248.5 pg/mL), corresponding to an increase of approximately 3521%. Statistically significant differences were detected between baseline and both subsequent time points ($P < 0.05$). Results are shown in Table 3 and Figure 5.

3.2.6. Vascular Endothelial Growth Factor-A (VEGF-A)

VEGF-A concentrations increased modestly during storage. After three months at −20 °C, levels increased by approximately 40%, while after six months they remained elevated by approximately 24% compared with baseline values. VEGF-A concentrations at three months were significantly higher than both baseline and six-month values ($P < 0.05$). Results are presented in Table 3 and Figure 6.

3.2.7. Vascular Endothelial Growth Factor-D (VEGF-D)

VEGF-D concentrations increased by approximately 26% after three months of storage at −20 °C. After six months, VEGF-D levels declined markedly, decreasing by approximately 93% compared with baseline values. Statistically significant differences were observed between all measurement points ($P < 0.05$). Results are summarized in Table 3 and Figure 7.

4. Discussion

Platelets play a central role in hemostasis, tissue repair, and regeneration owing to their high content of biologically active molecules stored within cytoplasmic granules (Chaudhary et al., 2022; Bieback et al., 2019). Among these, platelet-derived growth factors such as PDGF-AB/BB, TGF-β1, EGF, VEGFs, FGF, HGF, and neurotrophic factors including BDNF regulate key cellular processes involved in proliferation, migration, extracellular matrix synthesis, angiogenesis, and wound healing (Alió et al., 2019; Burnouf et al., 2016). In the present study, we investigated the stability of growth factors in UCB-PL during storage at −20 °C, with particular emphasis on factors relevant to corneal tissue regeneration. UCB-PL was produced from UCB units deemed unsuitable for allogeneic hematopoietic stem cell transplantation but retaining significant regenerative and anti-inflammatory potential (Bień et al., 2024; Wang et al., 2023). Platelet concentrates were prepared using double-spin centrifugation and lysed through repeated freeze–thaw cycles, a widely applied and cost-effective method for releasing intracellular growth factors (Burnouf et al., 2016; Samarkanova et al., 2020a). The characteristics of the resulting UCB platelet concentrates were consistent with previously reported data, supporting the feasibility and reproducibility of this production approach (Rebulla et al., 2016; Valentini et al., 2019).

Analysis of growth factor concentrations revealed distinct stability profiles during storage. Most growth factors, including BDNF, EGF, HGF, VEGF-A, VEGF-D, and PDGF-BB, demonstrated increased concentrations after three months of storage at −20 °C. This transient increase is most likely attributable to delayed platelet lysis and continued release of growth factors from intracellular granules during early storage, as previously described for platelet-derived preparations (Zimmermann et al., 2003; Strandberg et al., 2017). In contrast, TGF-β exhibited marked instability, with concentrations declining progressively and falling below the assay detection limit after six months of storage. These findings indicate that growth factor stability in UCB-PL is factor-specific and influenced by intrinsic protein properties, storage temperature, and duration (Düregger et al., 2016; Liu et al., 2024). Among the analyzed factors, PDGF-BB demonstrated the greatest stability, with concentrations remaining elevated throughout the entire six-month storage period. PDGF-BB is known to stimulate corneal endothelial cell proliferation and migration and plays a critical role in tissue repair processes (Alió et al., 2019; Li et al., 2023). Sustained availability of PDGF-BB may therefore be particularly advantageous for regenerative ophthalmic applications. VEGF-A and VEGF-D exhibited moderate stability, with peak concentrations observed after three months followed by partial declines at six months, consistent with previous reports describing their sensitivity to prolonged storage (Schär et al., 2015; Wu et al., 2023). BDNF and EGF also showed early increases in concentration followed by subsequent decreases, suggesting that short-term storage optimizes their availability while limiting degradation. BDNF plays an important role in corneal nerve maintenance and epithelial integrity, while EGF is a key regulator of epithelial proliferation and wound healing (Strandberg et al., 2017; Tovar et al., 2021). These dynamics further support the use of UCB-PL within a defined storage window to preserve maximal biological activity. The pronounced instability of TGF-β during prolonged storage represents a critical limitation for extended shelf life of UCB-PL. Although TGF-β is essential for extracellular matrix regulation and tissue remodeling, its excessive or prolonged activity has been associated with fibrotic responses (Parnell, 2011; Li et al., 2023). Consequently, the observed decline of TGF-β during storage may have both limiting and potentially beneficial implications, depending on the intended therapeutic application.

Collectively, the growth factors identified in UCB-PL; PDGF-BB, VEGF-A, VEGF-D, HGF, EGF, BDNF, and TGF-β are known to support key aspects of corneal endothelial cell biology, including proliferation, migration,

angiogenic signaling, and tissue repair (Alió et al., 2019; Giannaccare et al., 2020; Farag et al., 2022). The presence and short-term stability of these factors support the use of UCB-PL as an animal-free alternative to fetal bovine serum for corneal cell culture and regenerative applications.

From a practical standpoint, the findings highlight the importance of optimized production and storage protocols. While three freeze–thaw cycles effectively released a broad spectrum of growth factors, the observed concentration dynamics suggest that additional optimization of lysis and storage conditions may further improve product consistency (Passaretti et al., 2014; Palombella et al., 2022; Oeller et al., 2023). Based on the present data, short-term storage at −20 °C, limited to approximately three months, appears optimal for preserving the biological activity of most growth factors in UCB-PL.

5. Conclusions

This study demonstrates that umbilical cord blood–derived platelet lysate can be reliably produced from cord blood units unsuitable for allogeneic hematopoietic stem cell transplantation and provides a biologically relevant profile of growth factors associated with corneal tissue regeneration. UCB-PL contains detectable levels of PDGF-BB, VEGF-A, VEGF-D, HGF, EGF, BDNF, and TGF-β, supporting its potential application as an animal-free supplement for corneal cell culture and regenerative therapies. Short-term storage at −20 °C preserves the stability of most growth factors for up to three months, whereas prolonged storage leads to selective degradation, most notably of TGF-β. These findings underscore the need for carefully optimized production and storage protocols to maintain bioactivity and therapeutic potential. Overall, UCB-PL represents a promising, ethically acceptable alternative to animal-derived supplements in ophthalmic and regenerative medicine applications.

Acknowledgement

This research was funded by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials”.

Refrences

Alió JL, Alió del Barrio JL, Arnalich-Montiel F (2019) Corneal regeneration therapy and surgery. 1st edn. Springer, Cham

Andresen JL, Ledet T, Ehlers N (1997) Keratocyte migration and peptide growth factors: the effect of PDGF, bFGF, EGF, IGF-I, aFGF and TGF-beta on human keratocyte migration in a collagen gel. Curr Eye Res 16:605–613. https://doi.org/10.1076/ceyr.16.6.605.5081

Bień A, Vermeulen J, Bączek B, Pięta M, Pięta B (2024) Cord blood banking: balancing hype and hope in stem cell therapy. Eur J Midwifery 8:192930. https://doi.org/10.18332/ejm/192930

Bieback K, Fernandez-Muñoz B, Pati S, Schäfer R (2019) Gaps in the knowledge of human platelet lysate as a cell culture supplement for cell therapy: a joint publication from the AABB and the International Society for Cell & Gene Therapy. Cytotherapy 21(9):911–924. https://doi.org/10.1016/j.jcyt.2019.05.001

Borzini P, Mazzucco L (2015) Tissue regeneration and in loco administration of platelet derivatives: clinical outcome, heterogeneous products and heterogeneity of the effector mechanisms. Transfusion 45:1759–1767. https://doi.org/10.1111/trf.13226

Brecher ME (ed) (2005) Technical manual. 15th edn. AABB Press, Bethesda

Burnouf T, Strunk D, Koh MBC, Schallmoser K (2016) Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials 76:371–387. https://doi.org/10.1016/j.biomaterials.2015.10.001

Chandrasekher G, Kakazu AH, Bazan HE (2001) HGF- and KGF-induced activation of PI3K/p70 S6 kinase pathway in corneal epithelial cells: relevance in wound healing. Exp Eye Res 73:191–202. https://doi.org/10.1006/exer.2001.1026

Chaudhary PK, Kim K, Kim S (2022) An insight into recent advances on platelet function in health and disease. Int J Mol Sci 23(11):6022. https://doi.org/10.3390/ijms23116022

Christou I, Mallis P, Michalopoulos E, Chatzistamatiou T, Mermelekas G, et al (2018) Evaluation of peripheral blood and cord blood platelet lysates in isolation and expansion of multipotent mesenchymal stromal cells. Bioengineering 5(1):19. https://doi.org/10.3390/bioengineering5010019

Daniels JT, Khaw PT (2000) Temporal stimulation of corneal fibroblast wound healing activity by differentiating epithelium in vitro. Invest Ophthalmol Vis Sci 41:3754–3762

Delabie W, Boretti G, Groot SA, Ardanary D, Sigurjónsson Ó, Klei TRL, Vandekerckhove P, Feys HB (2025) Human platelet lysate standardization across three independent European blood establishments. Stem Cell Res Ther 16:329. https://doi.org/10.1186/s13287-025-03729-8

Denk PO, Knorr M (1997) The in vitro effect of platelet-derived growth factor isoforms on the proliferation of bovine corneal stromal fibroblasts depends on cell density. Graefes Arch Clin Exp Ophthalmol 235:530–534. https://doi.org/10.1007/BF00947012

Dunn SP, Heidemann DG, Chow CYC, Crockford D, Turjman N, Angel J, Allan CB, Sosne G (2010) Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta 4. Ann N Y Acad Sci 1194:199–206. https://doi.org/10.1111/j.1749-6632.2010.05471.x

Düregger K, Peng A, Eblenkamp M (2016) Influence of storage conditions on the release of growth factors in platelet-rich blood derivatives. Curr Dir Biomed Eng 2(1):311–314. https://doi.org/10.1515/cdbme-2016-0037

European Directorate for the Quality of Medicines & HealthCare (2020) Guide to the preparation, use and quality assurance of blood components. 5th edn. Council of Europe Publishing, Strasbourg

Farag R, Dawood M, Ele M (2022) Safety and efficacy of eye drops from umbilical cord blood platelet lysate to treat resistant corneal ulcer. Med Hypothesis Discov Innov Ophthalmol 11(4):189–202

Giannaccare G, Carnevali A, Senni C, Logozzo L, Scorcia V (2020) Umbilical cord blood and serum for the treatment of ocular diseases: a comprehensive review. Ophthalmol Ther 9:235–248. https://doi.org/10.1007/s40123-020-00239-9

Golubić Ćepulić B, Bojanić I, Mazić S (2009) Banke krvi iz pupkovine. Acta Med Croatica 63(3):245–250

Graca F, Minden Birkenmaier B, Stephan A, Demontis F, Labelle M, et al (2023) Signaling roles of platelets in skeletal muscle regeneration. BioEssays 45(12):e230013

Haber M, Cao Z, Panjwani N, Bedenice D, Li WW, Provost PJ (2003) Effects of growth factors (EGF, PDGF-BB and TGF-beta1) on cultured equine epithelial cells and keratocytes. Vet Ophthalmol 6:211–217. https://doi.org/10.1046/j.1463-5224.2003.00296.x

Immalaraju S, Goyal S, Jonnalagadda R (2025) Towards the standardization of human platelet lysate production and its comparison to fetal bovine serum for human hematopoietic cell culture: a scoping review. Front Toxicol 7:1496231. https://doi.org/10.3389/ftox.2025.1496231

Joo MJ, Yuhan KR, Hyon JY, Lai H, Hose S, Sinha D, O'Brien TP (2004) The effect of nerve growth factor on corneal sensitivity after laser in situ keratomileusis. Arch Ophthalmol 122:1338–1341. https://doi.org/10.1001/archopht.122.9.1338

Kamiyama K, Iguchi I, Wang X, Imanishi J (1998) Effects of PDGF on the migration of rabbit corneal fibroblasts and epithelial cells. Cornea 17:315–325. https://doi.org/10.1097/00003226-199805000-00013

Kay EP, Lee MS, Seong GJ, Lee YG (1998) TGF-betas stimulate cell proliferation via an autocrine production of FGF-2 in corneal stromal fibroblasts. Curr Eye Res 17:286–293. https://doi.org/10.1076/ceyr.17.3.286

Lee JG, Kay EP (2006) FGF-2-induced wound healing in corneal endothelial cells requires Cdc42 activation and Rho inactivation through the phosphatidylinositol 3-kinase pathway. Invest Ophthalmol Vis Sci 47:1376–1386. https://doi.org/10.1167/iovs.05-1223

Li J, Zhang Y, Zhang H, et al (2023) Growth factor content in umbilical cord blood–derived platelet preparations: implications for regenerative medicine. Sci Rep 13:45747. https://doi.org/10.1038/s41598-023-45747-0

Liu W, Liu Y, Li T, Liu L, Du M, Du J, et al (2024) Long-term stability of frozen platelet-rich plasma under −80 °C storage conditions. Regenerative Ther 26:826–830

Nakamura Y, Sotozono C, Kinoshita S (2001) The epidermal growth factor receptor: role in corneal wound healing and homeostasis. Exp Eye Res 72:511–517. https://doi.org/10.1006/exer.2000.0979

Oeller M, Laner-Plamberger S, Krisch L, Rohde E, Strunk D (2023) Human platelet lysate standardization across three independent European blood establishments. Stem Cell Res Ther 14:1–15

Palombella S, Perucca Orfei C, Castellini G, Gianola S, Lopa S, Mastrogiacomo M, Moretti M, de Girolamo L (2022) Systematic review and meta-analysis on the use of human platelet lysate for mesenchymal stem cell cultures. Stem Cell Res Ther 13:142

Pancholi S, Tullo A, Khaliq A, Foreman D, Boulton M (1998) The effects of growth factors and conditioned media on the proliferation of human corneal epithelial cells and keratocytes. Graefes Arch Clin Exp Ophthalmol 236:1–8. https://doi.org/10.1007/s004170050034

Parnell LKS (2011) Protein degradation and protection observed in the presence of novel wound dressing components. J Funct Biomater 2:338–354. https://doi.org/10.3390/jfb2030338

Passaretti F, Tia M, D'Esposito V, De Pascale M, Del Corso M, Sepulveres R, et al (2014) Growth-promoting action and growth factor release by different platelet derivatives. Platelets 25:252–256

Rebulla P, Pupella S, Santodirocco M, Greppi N, Villanova I, Buzzi M, et al (2016) Multicentric standardisation of the clinical grade procedure for the preparation of allogenic platelet concentrates from umbilical cord blood. Blood Transfus 14(1):73–79. https://doi.org/10.2450/2015.0122-15

Samarkanova D, Rodríguez L, Vives J, Coll R, Tahull E, Azqueta C, et al (2020a) Cord blood-derived platelet concentrates as starting material for new therapeutic blood components. Blood Transfus 18(3):208–216. https://doi.org/10.2450/2020.0305-19

Samarkanova D, Cox S, Hernandez D, Casaroli-Marano RP, Madrigal A, Querol S (2020b) Cord blood platelet-rich plasma derivatives for clinical applications in non-transfusion medicine. Front Immunol 11:585005. https://doi.org/10.3389/fimmu.2020.585005

Schär MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D (2015) Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro. Clin Orthop Relat Res 473:1635–1653

Schallmoser K, Henschler R, Gabriel C, Koh MBC, Burnouf T (2020) Production and quality requirements of human platelet lysate. Trends Biotechnol 38(1):13–23. https://doi.org/10.1016/j.tibtech.2019.06.002

Sosne G, Rimmer D, Kleinman HK, Ousler G (2016) Thymosin beta 4: a potential novel therapy for neurotrophic keratopathy. Vitam Horm 102:277–306. https://doi.org/10.1016/bs.vh.2016.04.012

Strandberg G, Sellberg F, Sommar P, Ronaghi M, Lubenow N (2017) Standardizing the freeze–thaw preparation of growth factors from platelet lysate. Transfusion 57:1058–1065. https://doi.org/10.1111/trf.14043

Thakkar B, Acevedo EO (2023) BDNF as a biomarker for neuropathic pain. Brain Behav 13:e2903. https://doi.org/10.1002/brb3.2903

Tovar A, White I, Sabater A (2021) Use of acellular umbilical cord-derived tissues in corneal and ocular surface diseases. Medicines 8(2):12. https://doi.org/10.3390/medicines8020012

Trosan P, Svobodova E, Chudickova M, Krulova M, Zajicova A, Holan V (2012) The key role of insulin-like growth factor I in limbal stem cell differentiation. Stem Cells Dev 21:3341–3350. https://doi.org/10.1089/scd.2012.0180

Valentini CG, Nuzzolo ER, Bianchi M, Orlando N, Iachininoto MG, Pinci P, Teofili L (2019) Cord blood platelet lysate: in vitro evaluation. Mediterr J Hematol Infect Dis 11(1):e2019021. https://doi.org/10.4084/MJHID.2019.021

Wang J, Metheny J (2023) Umbilical cord blood-derived cellular therapy. Front Oncol 13:1167266. https://doi.org/10.3389/fonc.2023.1167266

Wilson SE, Walker JW, Chwang EL, He YG (1993) Hepatocyte growth factor, keratinocyte growth factor and their receptors in the cornea. Invest Ophthalmol Vis Sci 34:2544–2561

Wu W, Ma H, Huang X (2023) Effects of different storage conditions on the content of key growth factors in platelet-rich plasma. Chin J Blood Transfus 36(8)

Yazdani M (2023) Tear film lipid layer and corneal oxygenation. Eye (Lond) 37:3534–3541. https://doi.org/10.1038/s41433-023-02557-1

You L, Kruse FE, Völcker HE (2000) Neurotrophic factors in the human cornea. Invest Ophthalmol Vis Sci 41:692–702

Zagon IS, Sassani JW, McLaughlin PJ (1995) Opioid growth factor modulates corneal epithelial outgrowth. Am J Physiol 268:R942–R950. https://doi.org/10.1152/ajpregu.1995.268.4.R942

Zieske JD, Takahashi H, Hutcheon AEK, Dalbone A (2000) Activation of epidermal growth factor receptor during corneal epithelial migration. Invest Ophthalmol Vis Sci 41:1346–1355. https://doi.org/10.1167/iovs.41.6.1346

Zimmermann R, Arnold D, Strasser E, Ringwald J, Schlegel A, Wiltfang J, Eckstein R (2003) Sample preparation technique and white cell content influence growth factor levels. Vox Sang 85:283–289. https://doi.org/10.1046/j.1423-0410.2003.00524.x

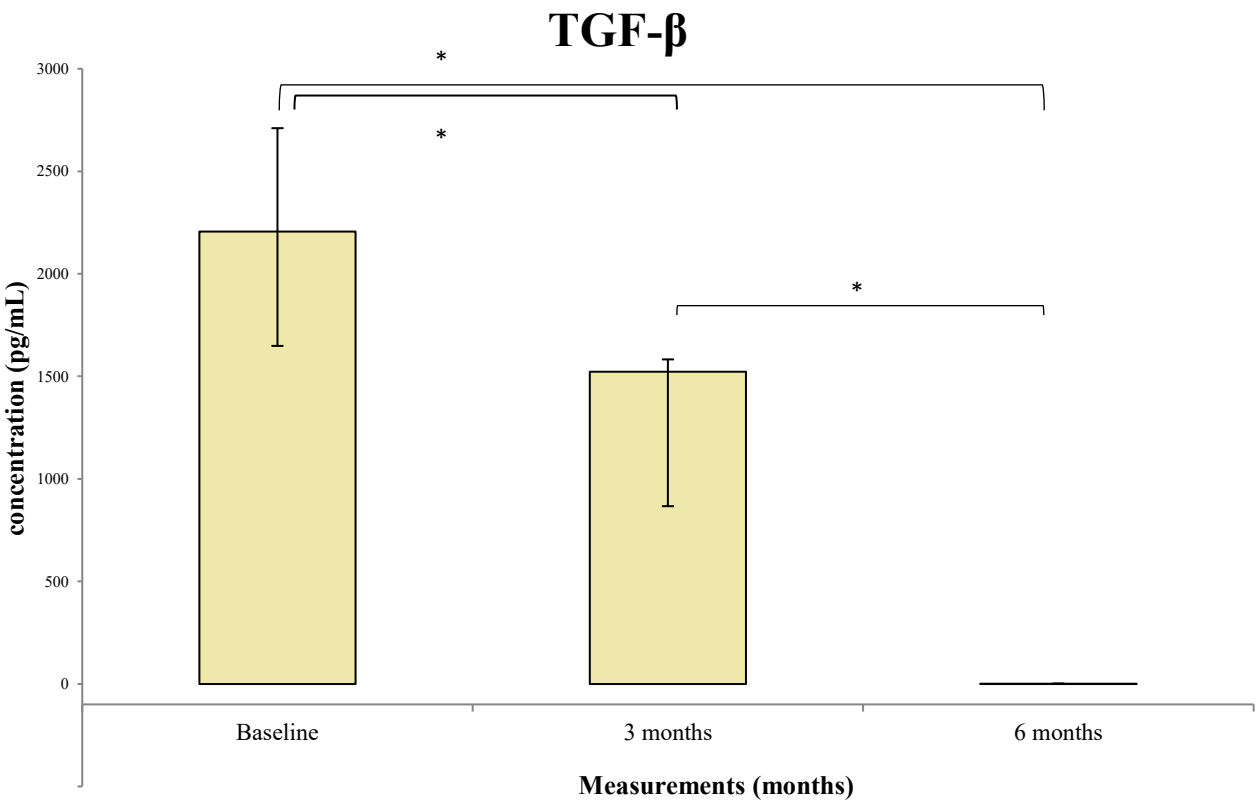


Figure 1. Differences in TGF-β concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

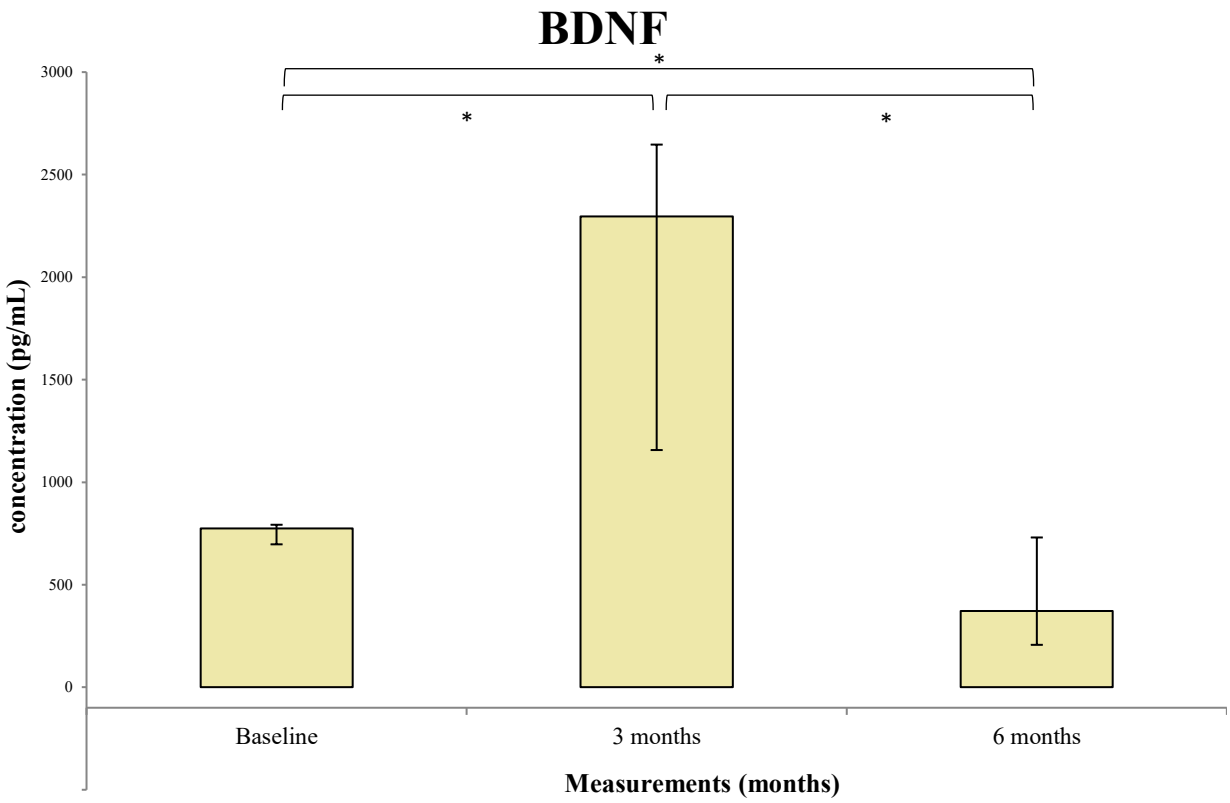


Figure 2. Differences in BDNF concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

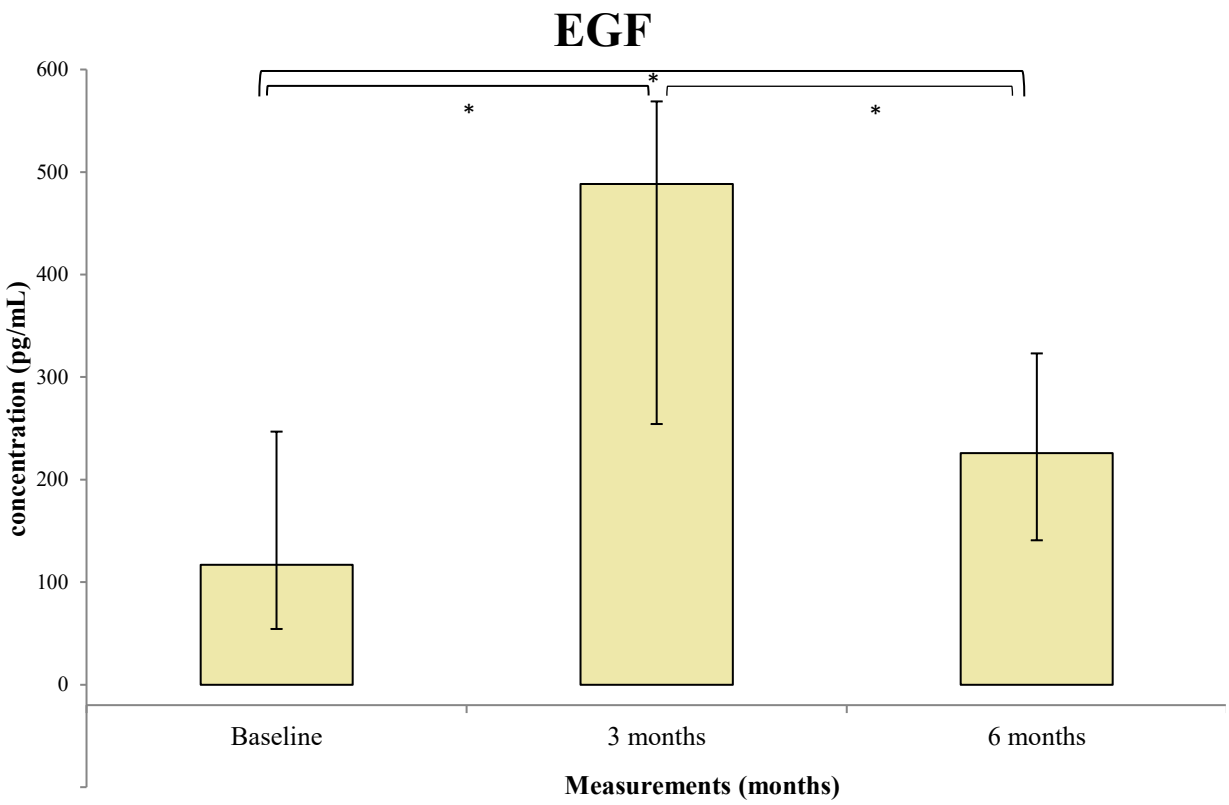


Figure 3. Differences in EGF concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

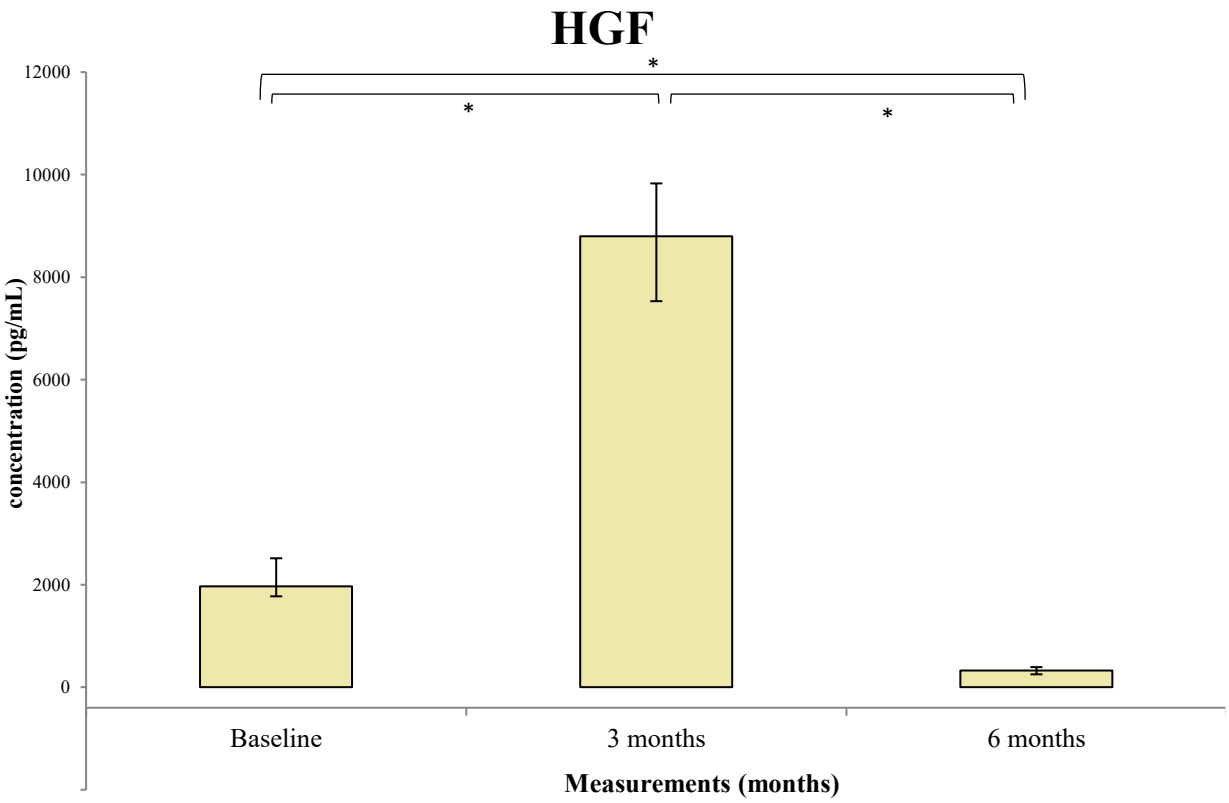


Figure 4. Differences in HGF concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

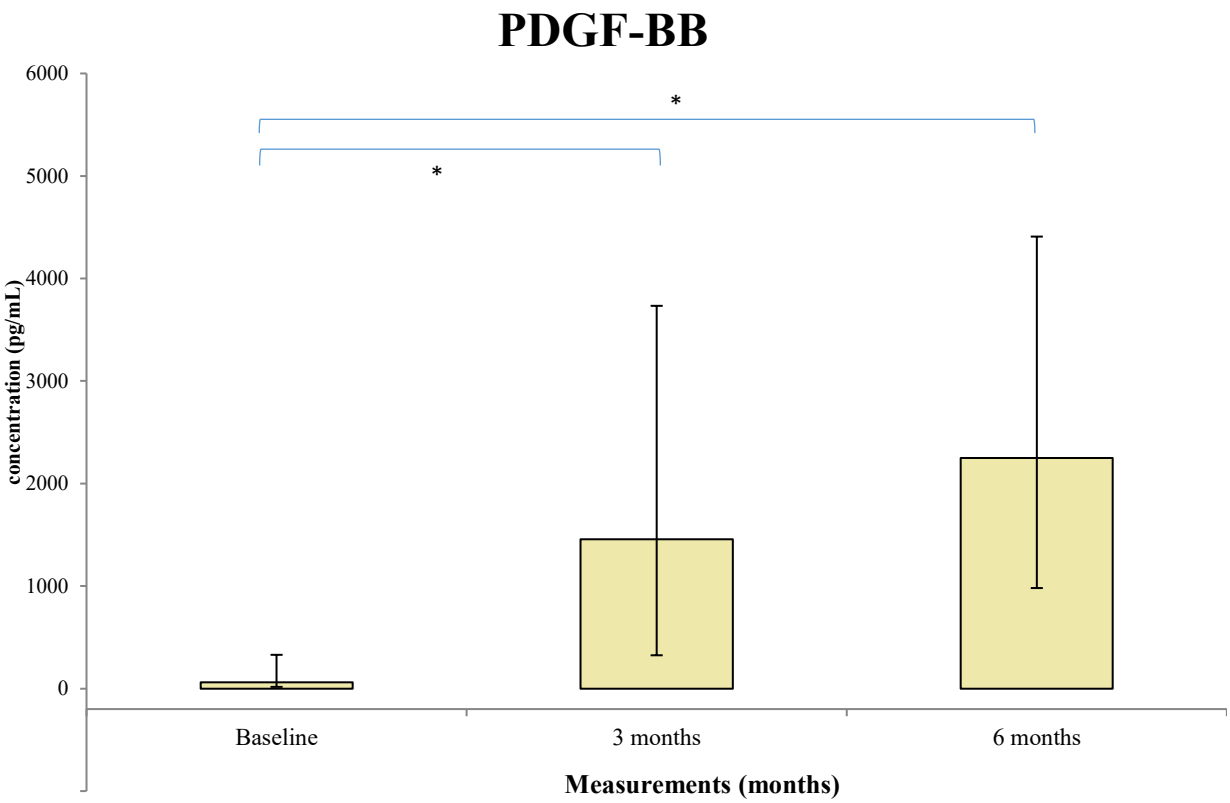


Figure 5. Differences in PDGF-BB concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

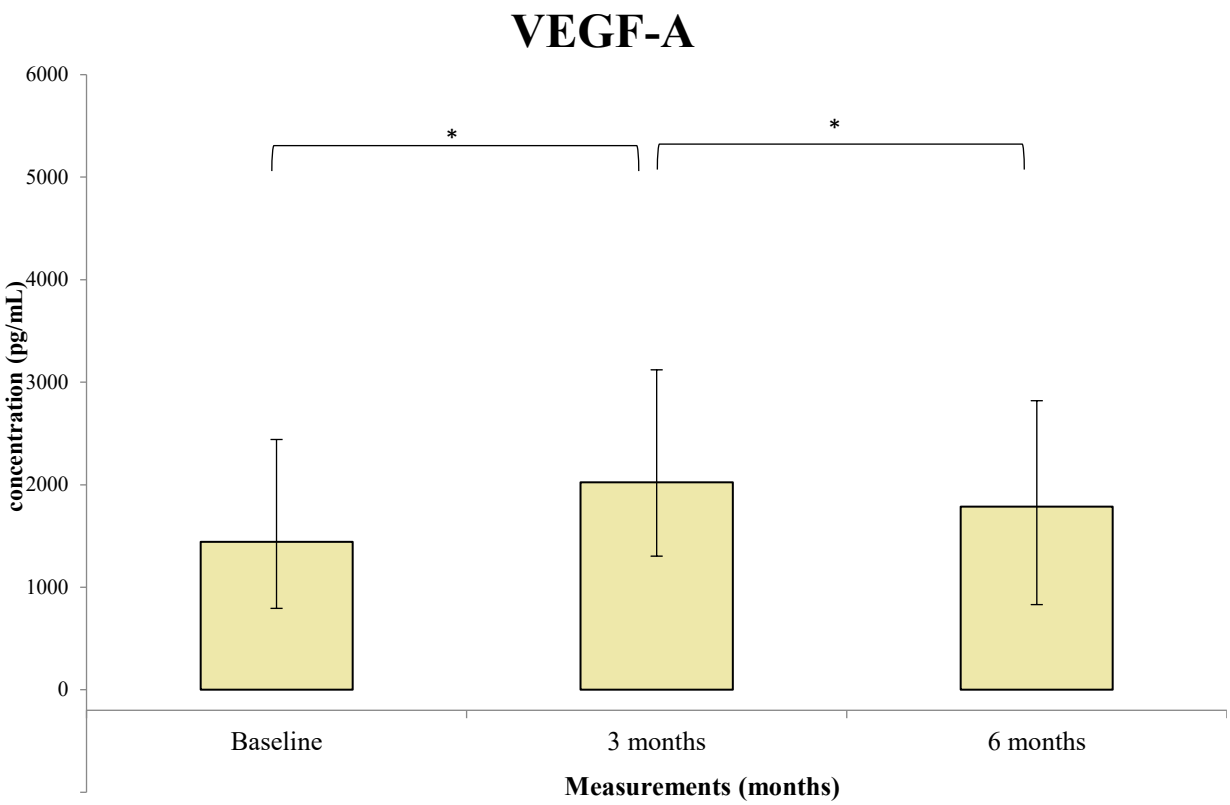


Figure 6. Differences in VEGF-A concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

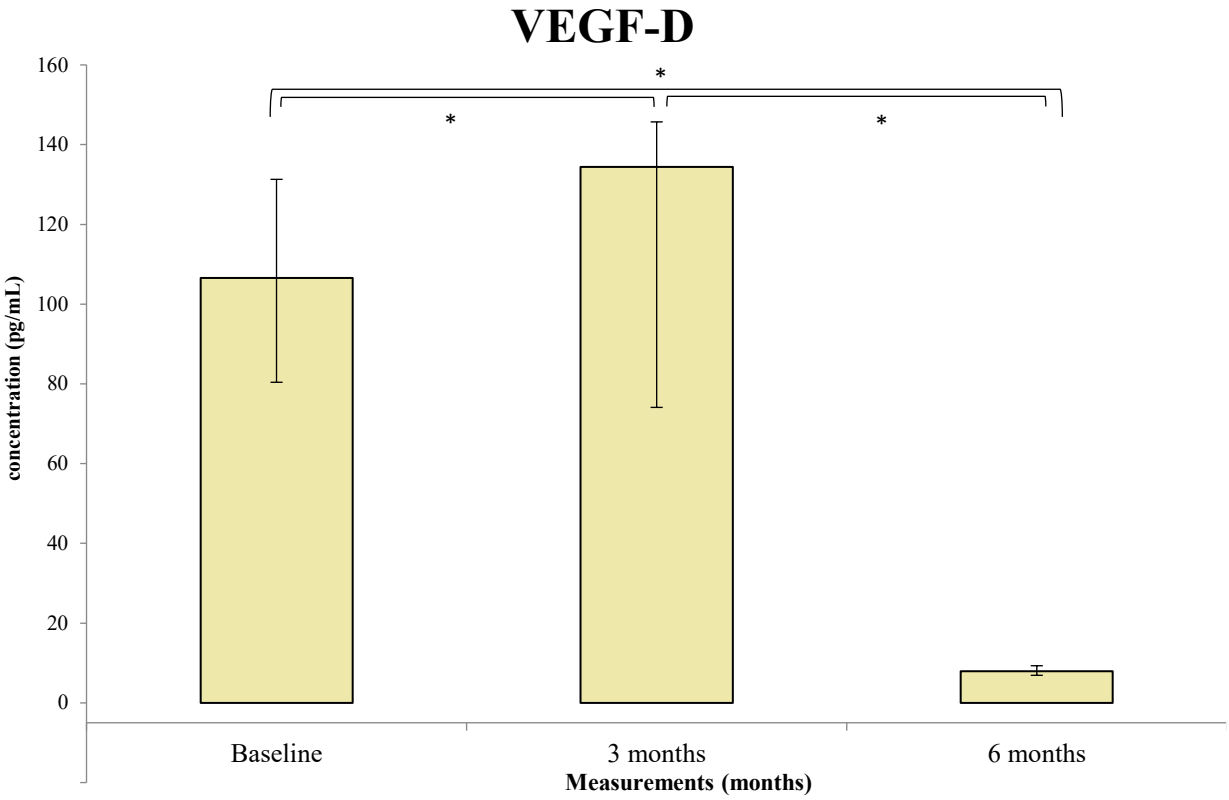


Figure 7. Differences in VEGF-D concentrations between time points.

Data are presented as median with 95% confidence intervals. Statistically significant differences are indicated (*$P < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx