Seco-Calvo *et al.*
Translational Medicine Communications (2022) 7:14
https://doi.org/10.1186/s41231-022-00120-w

Translational Medicine Communications

RESEARCH Open Access

Successful healing of non-healing surgical wound based on the release of platelet-derived growth factors from single donor allogeneic platelet-RICH plasma with one freeze-thaw cycle: a case report after a 1-year follow-up

Jesús Seco-Calvo[1,2*], Julia Vidán-Estévez[3,4] and Sergio Sánchez-Herráez[5]

Abstract

Background: Non-healing surgical wounds is a risk in certain patients. Recently, allogenic plasma-rich-platelet (PRP) is used such as regenerative treatment of different non-healing surgical wounds.

Purpose: We explore the potential role of using platelet-derived growth factors from single donor PRP, with a freeze-thaw process, for the treatment of surgical scar ulcer.

Methods: We have used a PRP preparation protocol that involved a single cycle of centrifugation at a mean speed of 2400–2800 rpm of donor blood taken with an apheresis machine.

Results: To our knowledge, this is the first study using the platelet-derived growth factors (PDGF) from single donor apheresis, with a freeze-thaw process. Four weeks after daily application of al-PRP, the ulcer progressed satisfactorily; at six weeks, the ulcer had healed.

Conclusion: We concluded that the healing of a surgical wound observed in our case, is promising and suggests that al-PRP might play a role in treating surgical scar ulcers. This must be confirmed in future studies.

Keywords: Non-healing surgical wound, Platelet-derived growth factors (PDGF), Freeze-thaw process, Allogenic plasma-rich-platelet (al-PRP), Single donor apheresis

Introduction

Non-healing surgical wounds (NHSWs) caused by various diseases, trauma or un-known reasons tend to be difficult to manage [1] and may require advanced treatments [2]. The treatment of NHSWs is a great clinical challenge. Current treatments include debridement and offloading as well as other adjuvant therapies. Nonetheless, treatment response is usually poor and result disappointing. These wounds carry a high risk of infection, amputation and even death. As optimal wound healing requires the complex bio-logical and molecular events involved in cell migration and proliferation, as well as extracellular matrix deposition and remodelling, to occur in a well-coordinated way [3], various novel approaches for the treatment of NHSWs have been proposed [4], and these include the use of platelet-rich plasma (PRP). Obtained from whole blood, PRP can be either autologous or allogeneic (au-PRP and al-PRP respectively).

*Correspondence: jesus.seco@unileon.es; dr.seco.jesus@gmail.com; jasecc@unileon.es

[2] Visiting Professor and Researcher of Department of Physiology, University of the Basque Country, Leioa, Spain
Full list of author information is available at the end of the article

Finally, PRP has been applied to chronic non-healing wounds and acute wounds at different times, depths and frequencies using various doses and delivery methods, with markedly different results [2]. In particular, while PRP has been shown to be effective, improving healing rates, reducing pain and surgical site infection (SSI) rates in open and closed surgical wounds, results in terms of pain and SSI incidence remain inconsistent likely due to a lack of robustness in the research methods and outcome measures [2]. For this reason, it would be interesting to improve the treatment of this common clinical problem, novel approaches are required. In this context, the description of this case report is strategic to provide insights for clinical practice. Thus, we explore the potential role of using plate-let-derived growth factors from single donor PRP, with a freeze-thaw process, for the treatment of NHSWs, based on the case of a patient with a surgical wound after total knee replacement, after a 1-year follow-up.

Case presentation

Our patient was a 68-year-old widower, working in the catering sector, who was an ex-smoker and ex-drinker, with no known medical allergies. The patient had a scheduled admission for total knee replacement surgery involving the placement of a cemented prosthesis. He presented with septic arthritis in the right knee, with local redness, heat, pain, joint effusion, and cellulitis with fistula and ulcer on the front of the right knee. He underwent anterior arthrotomy of the right knee for joint lavage and change of the polyethylene insert and received IV antibiotic therapy for 14 days.

At two months later, the patient had no signs or symptoms of infection but a persistent approximately 35 × 18-mm non-healing surgical wound on the front of the right knee (Fig. 1). At this stage, we proposed starting treatment based on al-PRP. Baseline blood results were: Hb level of 15.3 g/dl; haemtocrit of 45.5%, leukocyte count of 5.0×10^3/μL, platelet count of 174×10^3/μL, erythrocyte sedimentation rate of 10 mm/h, C-reactive protein level of 2.5 mg/L and glucose level of 102 mg/dL. Ulcer cultures were negative.

The patient was referred to the Complejo Asistencial Universitario de León (CAULE) for assessment for treatment with PRP (22 august 2021). First, he was seen in the Transfusion Service, where the lesion to be treated was cleaned (Fig. 1), dressing changes, specified, and measured. The procedure was explained to him, and it was decided that he would be able to reliably carry out the daily treatment himself, independently.

After signing the informed consent form, he underwent a full blood test to obtain a complete blood count, biochemical parameters, and his blood group, the indirect Coombs test (ICT) to screen for irregular antibodies and serological tests (hepatitis B surface antigen, anti-hepatitis C virus, anti-HIV, and syphilis, as well as polymerase chain reaction for hepatitis B and C viruses and HIV). Having assessed the blood results and confirmed that the patient was eligible, we opted for treatment with ABO-identical single donor (apheresis) platelets: 200 to 400×10^3/μL platelets in 200 to 300 ml.

For the plateletpheresis, as well as the aliquoting and cryopreservation of the product, we followed the procedure described in the preliminary study by Vidán-et al., (2013) [5]. In brief, this involved the use of PDGFs from

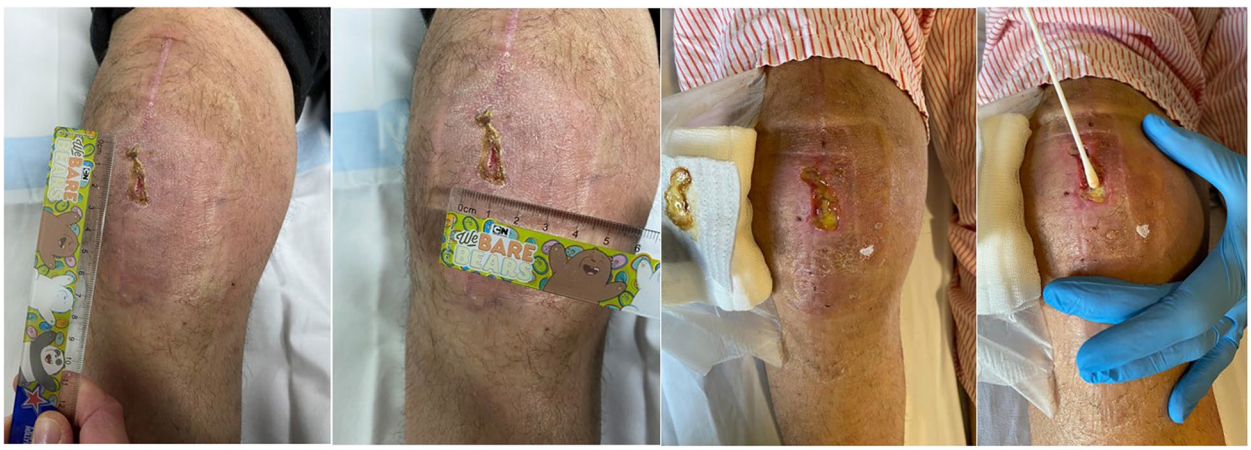

Fig. 1 A persistent approximately 35 × 18-mm ulcer on the front of the right knee (**A, B**). The ulcer before the treatment: Appearance when removing dressing (**C**). Wound cleaning and dressing changes (**D**). After debridement, the al-PRP was administrated immediately

single-donor platelets (al-PRP) obtained by one centrifugation at 2400–2800 rpm in an apheresis system, allowing us to obtain a platelet concentration 2- to 3-fold higher than baseline.

For the patient, ABO-identical single donor platelets were prepared, aliquoted into 2.5-ml syringes under sterile conditions in a laminar flow cabinet and stored in an ultra-low freezer at −80 °C. Each week, a batch of product was delivered to the patient, one aliquot of PRP to be thawed each day and applied to the wound. This freeze-thaw process was assumed to be sufficient to cause platelet activation and release of granules carrying PDGF that would stimulate tissue repair and wound healing.

Using the syringe, the thawed product was applied across the entire wound, and this was then covered with a clean gauze.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the University of Leon (n° COD: ETICA-ULE-004-201). Written in-formed consent has been obtained from the patient to publish this paper.

Results

Four weeks after daily application of al-PRP, the ulcer progressed satisfactorily (partially healed) (Fig. 2, left). Six weeks after daily application of al-PRP, the ulcer had healed (Fig. 2 [middle]); the patient was asymptomatic, not requiring any analgesic/anti-inflammatory treatment, and had normal lab results. His knee range of motion was a flexion of 100° with a loss of extension of 5°. After his last check-up in the Trauma and Orthopaedic Surgery Department, on 7 September 2021, the patient was discharged (Fig. 2, right).

Discussion

To our knowledge, this is the first study using the PDGF from single donor apheresis, with a freeze-thaw process, for the treatment of a non-healing surgical wound. Indeed, we have yet to find any previous studies in the scientific literature regarding the use of single donor PRP for this type of condition.

Further, there is no agreement on the best PRP activation procedure. Anitua et al. [6] used thrombin and calcium chloride, and Lacoste et al. [7] reported that thawing frozen platelets by mechanical means activates platelet growth factors. More recently, Steller et al. [8] compared PRP activation by Ca2+ and a freeze-thaw method in terms of the release of growth factors, assessing the levels of vascular endothelial growth factor, PDGF-BB and transforming growth factor-β1. They concluded that the freeze-thaw method was sufficient to release growth factors and that calcium activation was not necessary. In line with this, in our study, the platelets were not activated by adding calcium chloride or similar agents, rather a simple freeze-thaw process was used seeking to damage the platelets and trigger the release of granules carrying PDGF, which would lead to tissue repair.

As well as the centrifugation step [9] and the activation methods, the volume of whole blood processed and the distance of the sample from the centrifuge rotor axis are other important factors involved in PRP variability [10]. In our case, we aliquoted the ABO-identical single donor (apheresis) PRP into 2.5- or 5-ml syringes, which were stored at −80 °C. It is known that patients with chronic non-healing wounds have difficulties healing due to the loss of growth factors associated with wound healing and an inflammatory imbalance in the wound bed [11]. In this context, new biological therapies may be able to

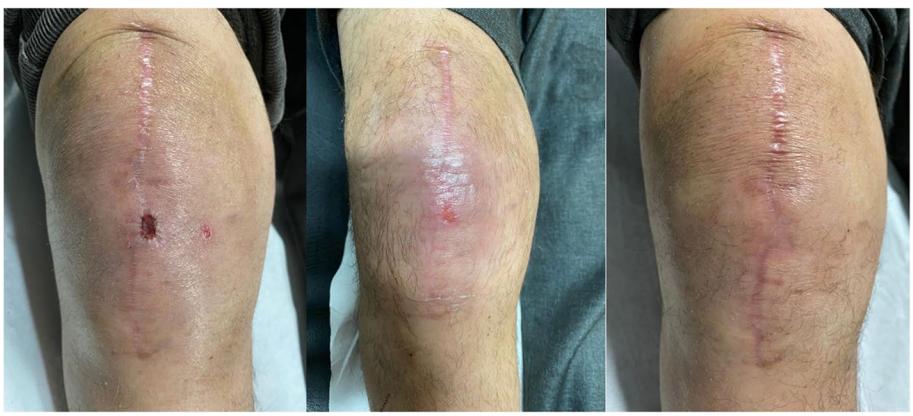

Fig. 2 The ulcer after the treatment. Four weeks after the treatment, the wound was partially healed (left). Six weeks after the treatment, the wound was complete closure (complete healing) (middle). The wound complete closure at one-year follow-up (right)

regulate or reverse possible mechanisms of imbalance in the wound [12]. Moreover, past year, He et al. [13] published what we believe to be the first study showing the feasibility, efficacy and safety of using al-PRP in the treatment of chronic non-healing wounds in place of its autologous counterpart.

To overcome the clinical limitations of treatment with au-PRP [14], they [13] found that both al- and au-PRP can be enriched by the separation of accumulated autologous and allogenic platelets respectively. Most notably, they found no significant differences between al- and au-PRP in the platelet concentrations obtained, concentrations in al-PRP reaching equally effective levels of approximately 6-fold higher than in whole blood [13]. Earlier studies indicated that satisfactory results would be achieved with an adequate PRP concentration of approximately 5-fold higher than baseline [15].

Hence, al-PRP from well-characterised donors has been considered a solution that is ready for application as it avoids the need to collect large quantities of whole blood and no valuable blood resources are wasted [16]. Moreover, given the wide range of problems associated with the use of different protocols, au-PRP quality could be compromised, which would result in lower clinical efficacy [13]. In relation to this, in the aforementioned pioneering study by He et al. [13], and similarly in a preliminary study [5] and the case presented herein, the al-PRP was prepared from whole blood from a healthy donor using a blood cell separator. Hence, it can be considered a biological therapy product that is based on a standard preparation protocol. On the other hand, regarding the use of allogenic platelet gel vs hydrogel in the treatment of chronic wounds, a systematic review and meta-analysis on acute and chronic skin wounds found that PRP therapy improved partial and complete wound healing rates compared to standard wound care [17]. The advantages are notable: units of allogeneic platelets are available through transfusion services, and are safe, affordable and highly standardized in terms of platelet count, as well as residual leukocyte and red cell content.

For all these reasons, considering the data currently available, together with the findings in the case we present, it may be suggested that the use of al-PRP in wound healing shows promising results [13, 18–21]. If confirmed in future studies, it could become the gold standard for the treatment of non-healing surgical wounds or slow-healing skin ulcers.

Similarly, regarding PRP preparation protocols, numerous variations have been reported, these differing in centrifuge cycles and the concentration of platelets used. We agree with the findings of Mazzocca et al. [22], who after analysing three protocols with eight healthy individuals, using short low velocity (1500 rpm for 5 min) and long high velocity (3200 rpm for 15 min) centrifugation as well as the combination of gentle and strong centrifugation (1500 rpm for 5 min and 6300 rpm for 20 min) obtained a higher platelet concentration ($873.8 \pm 207.2 \times 103/\mu L$) with a single centrifuge cycle at 3200 rpm for 15 min (compared to that obtained with a lower velocity or a double cycle).

Hence, in our case, we have used PDGF from single donor apheresis (al-PRP), with a freeze-thaw process, and with a PRP preparation protocol that involved a single cycle of centrifugation at a mean speed of 2400–2800 rpm of donor blood taken with an apheresis machine. This approach enabled us to obtain a platelet concentration of 2- to 3-fold baseline.

The current study is a case report after a 1-year follow-up,only with one patient. Therefore, at the present time of knowledge, it ispreliminary to say whether the application of PDGF in the treatment of surgicals-car ulcers will be adequate in clinical settings. Future research is needed toperform in vitro characterization, in addition, more studies with a largecohort of patients to validate our findings are required.

Conclusions

The healing of a surgical wound observed in our case, with the use of PDGFs from single donor apheresis (al-PRP), with a freeze-thaw process, and the PRP preparation protocol described, is promising and suggests that al-PRP might play a role in treating NHSWs. Further studies with a larger sample size in different populations are required to validate our findings.

Abbreviations

al-PRP: Allogenic plasma-rich-platelet; au-PRP: Autologous plasma-rich-platelet; ICT: Indirect Coombs test; NHSWs: Non-healing surgical wounds; PDGF: Platelet-derived growth factors; PRP: Plasma-rich-platelet; SSI: Surgical site infection.

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization, JV-E, SS-H and JS-C; Data curation, JV-E; Formal analysis, JV-E and JS-C; Funding acquisition, JV-E, SS-H and JS-C; Investigation, Sergio Sanchez-Herraez and Jesús Seco-Calvo; Methodology, JV-E and SS-H; Project administration, JV-E and JS-C; Resources, JV-E; Software, JV-E; Supervision, JV-E and JS-C; Validation, JV-E; Visualization, JV-E and JS-C; Writing – original draft, JV-E, SS-H and JS-C; Writing – review & editing, JV-E, SS-H and JS-C. The author(s) read and approved the final manuscript.

Funding

This research received no external funding.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Universidad de León (n° COD: ETICA-ULE-004-201). Written informed consent has been obtained from the patient to participate in this study.

Consent for publication

Written informed consent has been obtained from the patient to publish this paper.

Competing interests

The authors declare no conflict of interest.

Author details

[1]Institute of Biomedicine (IBIOMED), University of León, 24071 León, Spain. [2]Visiting Professor and Researcher of Department of Physiology, University of the Basque Country, Leioa, Spain. [3]Servicio de Transfusión. Complejo Asistencial Universitario de León (CAULE), 24008 León, Spain. [4]Cooperative Working Group on Lymphomas and Lymphoproliferative Disorders of the Castilla y León Society of Hematology and Hemotherapy (SCLHH), Department of Haematology, 47007 Valladolid, Spain. [5]Servicio de Cirugía y Traumatología Ortopédica, Complejo Asistencial Universitario de León (CAULE), 24008 León, Spain.

Received: 18 January 2022 Accepted: 12 June 2022
Published online: 20 June 2022

References

1. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453(7193):314–21. https://doi.org/10.1038/nature07039.
2. Bolton L. Platelet-rich plasma: optimal use in surgical wound care. Wounds. 2021;33(8):219–21. https://doi.org/10.25270/wnds/2021.219221.
3. Harding KG, Morris HL, Patel GK. Science, medicine and the future: healing chronic wounds. BMJ. 2002;324(7330):160–3. https://doi.org/10.1136/bmj.324.7330.160.
4. Riordan NH, George BA, Chandler TB, et al. Case report of non-healing surgical wound treated with dehydrated human amniotic membrane. J Transl Med. 2015;13:242. https://doi.org/10.1186/s12967-015-0608-8.
5. Vidán Estévez J, Escalante F, Escribano P, Cechini C, Moro MJ, Ahmadi A, et al. Uso de los factores de crecimiento derivados de las plaquetas en úlceras cutáneas y osteonecrosis mandibular secundarias: una terapia eficaz. In: Proceedings of LV Congreso Nacional de la Sociedad Española de Hematología y Hemoterapia. Sevilla: SEHH-SETH Publisher; 2013.
6. Anitua E, Sánchez M, Orive G. The importance of understanding what is platelet-rich growth factor (PRGF) and what is not. J Shoulder Elb Surg. 2011;20(1):e23–4. https://doi.org/10.1016/j.jse.2010.07.005.
7. Lacoste E, Martineau I, Gagnon G. Platelet concentrates: effects of calcium and thrombin on endothelial cell proliferation and growth factor release. J Periodontol. 2003;74(10):1498–507. https://doi.org/10.1902/jop.2003.74.10.1498.
8. Steller D, Herbst N, Pries R, Juhl D, Hakim SG. Impact of incubation method on the release of growth factors in non-ca(2+)-activated PRP, ca(2+)-activated PRP, PRF and A-PRF. J Craniomaxillofac Surg. 2019;47(2):365–72. https://doi.org/10.1016/j.jcms.2018.10.017.
9. Jo CH, Roh YH, Kim JE, Shin S, Yoon KS. Optimizing platelet-rich plasma gel formation by varying time and gravitational forces during centrifugation. J Oral Implantol. 2013;39(5):525–32. https://doi.org/10.1563/AAID-JOI-D-10-00155.
10. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy. 2010;26(2):269–78. https://doi.org/10.1016/j.arthro.2009.11.015.
11. Xu F, Othman B, Lim J, Batres A, Ponugoti B, Zhang C, et al. Foxo1 inhibits diabetic mucosal wound healing but enhances healing of normoglycemic wounds. Diabetes. 2015;64(1):243–56. https://doi.org/10.2337/db14-0589.
12. Everts PA, Brown Mahoney C, Hoffmann JJ, Schönberger JP, Box HA, van Zundert A, et al. Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. Growth Factors. 2006;24(3):165–71. https://doi.org/10.1080/08977190600821327.
13. He M, Guo X, Li T, Jiang X, Chen Y, Yuan Y, et al. Comparison of allogeneic platelet-rich plasma with autologous platelet-rich plasma for the treatment of diabetic lower extremity ulcers. Cell Transplant. 2020;29:963689720931428. https://doi.org/10.1177/0963689720931428.
14. Anitua E, Prado R. Addressing reproducibility in stem cell and PRP therapies. Trends Biotechnol. 2019;37(4):340–4. https://doi.org/10.1016/j.tibtech.2018.11.010.
15. Tambella AM, Attili AR, Dupré G, Cantalamessa A, Martin S, Cuteri V, et al. Platelet-rich plasma to treat experimentally-induced skin wounds in animals: a systematic review and meta-analysis. PLoS One. 2018;13(1):e0191093. https://doi.org/10.1371/journal.pone.0191093.
16. Anitua E, Prado R, Orive G. Allogeneic platelet-rich plasma: at the dawn of an off-the-shelf therapy? Trends Biotechnol. 2017;35(2):91–3. https://doi.org/10.1016/j.tibtech.2016.11.001.
17. Carter MJ, Fylling CP, Parnell LK. Use of platelet rich plasma gel on wound healing: a systematic review and meta-analysis. Eplasty. 2011;11:e38 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3174862/.
18. Zhao Q, Ma Y, Lu Y, Chai Y, Zhou Y. Successful treatment of chronic lower extremity ulcers with allogeneic platelet-rich plasma and artificial dermis: a case report. Adv Skin Wound Care. 2019;32(12):550–2. https://doi.org/10.1097/01.ASW.0000604176.47082.60.
19. Liao X, Liang JX, Li SH, Huang S, Yan JX, Xiao LL, et al. Allogeneic platelet-rich plasma therapy as an effective and safe adjuvant method for chronic wounds. J Surg Res. 2020;246:284–91. https://doi.org/10.1016/j.jss.2019.09.019.
20. van der Bijl I, Vlig M, Middelkoop E, de Korte D. Allogeneic platelet-rich plasma (PRP) is superior to platelets or plasma alone in stimulating fibroblast proliferation and migration, angiogenesis, and chemotaxis as relevant processes for wound healing. Transfusion. 2019;59(11):3492–500. https://doi.org/10.1111/trf.15535.
21. Vidán-Estévez J, Sánchez-Herráez S, Escalante-Barrigón F, Seco-Calvo J. Healing of chronic wounds with platelet-derived growth factors from single donor platelet-rich plasma following one freeze-thaw cycle. A Cross-Sectional Study. J Clin Med. 2021;10(24):5762. https://doi.org/10.3390/jcm10245762.
22. Mazzocca AD, McCarthy MB, Chowaniec DM, Cote MP, Romeo AA, Bradley JP, et al. Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am. 2012;94(4):308–16. https://doi.org/10.2106/JBJS.K.00430.

Publisher's Note