Kocatepe Veterinary Journal

Kocatepe Vet J. (2024) 17(2): 162-168

DOI: 10.30607/kvj.1432296

CASE REPORT

The Effect of Platelet-Rich Fibrin (PRF) on Wound Healing in a Dog with Comorbidities

Osman BULUT[1], Doğan Can HANEY[2*], Tolga Meriç DÜMBEK[1], Neslihan SÜRSAL ŞİMŞEK[3]

[1]Department of Surgery, Faculty of Milas Veterinary Medicine, Mugla Sıtkı Koçman University, Milas, Mugla, Türkiye
[2]Department of Internal Medicine, Faculty of Milas Veterinary Medicine, Mugla Sıtkı Koçman University, Milas, Mugla, Türkiye
[3]Department of Parasitology, Faculty of Milas Veterinary Medicine, Mugla Sıtkı Koçman University, Milas, Mugla, Türkiye

ABSTRACT

Platelet-rich fibrin (PRF) is an autogenous material produced from the patient's own platelets, used to improve wound healing and tissue regeneration. In this case report, the effect of PRF on wound healing is investigated in a dog with tissue damage associated with infection by different pathogens (Anaplasma, Canine Coronavirus, Pneumonia, and Citruvite Crystals). PRF was locally applied to the wound area for 21 days, and it was observed that the wound area and the underlying bone tissue healed without necrotic tissue. It has been concluded that PRF accelerates wound healing and tissue regeneration in material loss tissue injuries accompanied by a multifactorial disease in this case.

Keywords: Anaplasma, canine, coronavirus , multifactorial disease, platelet-rich fibrin

***

Komorbidli bir Köpekte Plateletten Zengin Fibrin'in (PRF) Yara İyileşmesi Üzerindeki Etkisi

ÖZ

Platelet zengin fibrin (PRF) hastanın kendi trombositlerinden üretilen otojen bir materyaldir ve yara iyileşmesini ve doku rejenerasyonunu iyileştirmek için kullanılır. Bu vakada, farklı patojenlerle (Anaplasma, Canine Coronavirus, Pnömoni ve Sitrüvit Kristalleri) ilişkili doku hasarı olan bir köpekte PRF'nin yara iyileşmesi üzerindeki etkisi araştırılmıştır. PRF, yara bölgesine lokal olarak 21 gün boyunca uygulanmış ve yara bölgesi ile altta yatan kemik dokuda herhangi bir nekrotik doku olmadan iyileştiği gözlemlenmiştir. Bu durumda PRF'nin, multifaktöriyel bir hastalık tablosu ile birlikte oluşan doku kayıplı yaralanmalarda yara iyileşmesini ve doku rejenerasyonunu hızlandırdığı sonucuna varılmıştır.

Anahtar kelimeler: Anaplazma, köpek, koronavirus, multifaktöriyel hastalık, platelet zengin fibrin

To cite this article: Bulut O. Haney D.C. Dümbek T.M. Sürsal Şimşek N. The Effect of Platelet-Rich Fibrin (PRF) on Wound Healing in a Dog with Comorbidities. Kocatepe Vet J. (2024) 17(2):162-168

Submission: 19.01.2024 **Accepted**: 23.04.2024 **Published Online**: 14.06.2024

ORCID ID; *OB: 0000-0003-2773-8243, DCH: 0000-0001-9737-6722, TMD: 0000-0003-1734-349, NSŞ: 0000-0002-4144-9520*
*Corresponding author e-mail: dogancanhaney@mu.edu.tr

INTRODUCTION

The wound is called to disrupt tissue integrity in the skin, subcutaneous tissues, muscles, and bones (Campanholi et al., 2022). The wound-healing process involves a complex but important sequence of interrelated stages, including hemostasis/inflammation, proliferation, and remodeling (Wang et al., 2018). Adequate wound healing in animals and humans depends on various variables, including circulation, wound size, width between wound edges, mobility, infection, and underlying tissue type (Iacopetti et al., 2012). Due to its frequent occurrence in different breeds and species of animals, wound healing is a comprehensive area of study in veterinary medicine. In this context, other treatments are being developed to accelerate the healing process and reduce the risk of secondary infection (Porsani et al., 2016).

Platelet-rich fibrin (PRF) is an autogenous material produced from the patient's own platelets, used to improve wound healing and tissue regeneration (Fan et al., 2020). Autologous platelet therapy became popular in the 1990s with the use of platelet-rich plasma (PRP). However, it has been said to have problems, including low effectiveness, the need for thrombin from animals for clotting, and differences between individuals (Karimi and Rockwell, 2019). In contrast, PRF does not require any additional substances during preparation, does not contain anticoagulation factors known to inhibit wound healing, guides natural clot formation, and supports growth factors and stem cell formation, among other advantages (Fan et al., 2020). These advantages led to the term PRF being first coined by Joseph Choukroun in 2001 (Choukroun et al., 2001).

PRF forms a three-dimensional fibrin network containing live cells that can serve as a scaffold in the early stages of wound healing. At this stage, platelets interact with the fibrin matrix to create a hemostatic plug and stimulate cell migration and proliferation (Pitzurra et al., 2020). The interaction between these cells and the fibrin matrix simulates the slow release of growth factors which can result in better wound healing in the early stages of this process (Davis et al., 2014).

CASE HISTORY

The material of the study was a 3-year-old mixed breed stray dog brought to the Milas Veterinary Faculty of Muğla Sıtkı Koçman University. During the initial examination, an open wound covering the left metacarpal and phalanx regions and reaching the depth of the bone tissue was observed. Subsequently, during a clinical examination, it was determined that the rectal body temperature value, mucous membrane colour, capillary refill time, and lung sounds in auscultation were normal. It was also found that the patient's vital signs were stable, and there was no orthopedic problem during the orthopedic examination. Open wound treatment with PRF was started immediately on the first day of admission.

During the clinical examination after the patient's general condition deteriorated in the following week, a rectal body temperature of 40.2 °C was measured, and reactive lymph nodes were palpable. The patient developed anorexia and weakness two days later. Hematological measurements revealed a decrease in lymphocytes, eosinophils and platelets. Suspecting an infection caused by blood parasites, a rapid diagnostic test kit named 'Vet Diagnostix Ehrichia + Lyme + Anaplasma + Heartworm Ag' was used (Canivet Tick-4 Combo Test, a lateral flow immunochromatographic test for qualitative detection of *Ehrlichia canis* antibodies (EHR), *Borrelia burgdorferi* antibodies (LYM) and *Anaplasma* spp. (ANA) antibodies and Heartworm (CHW) antigens in dogs' serum, plasma, and whole blood samples). The result of this test showed that *Anaplasma* spp. was positive and treatment was started accordingly. On the same day, another rapid test kit named 'VET Diagnostix Parvo (CPV) Ag + Corona (CCV) Ag + *Giardia*' showed that Canine Coronavirus was positive (Canivet CPV-CCV-*Giardia* Ag Combo Test, a lateral flow immunochromatographic test for differential diagnosis of canine parvovirus antigen (CPV Ag), canine coronavirus antigen (CCV Ag) and *Giardia lamblia* antigen (Giardia Ag) in the dog's feces or vomit sample). In addition, dysuria and stranguria were observed in the patient, and an abdominal ultrasound examination was performed. This examination revealed thickening of the bladder walls with accompanying cystitis, as well as the presence of bleeding and struvite crystals in the sediment, and treatment was initiated for this condition as well.

The hematological evaluation was conducted in the following days to monitor the patient's condition, lymphopenia, neutropenia, monocytopenia, and thrombocytopenia were detected. An increased respiratory rate, loss of appetite, and a high fever were observed. Pathological sounds were detected in the lung auscultation, and treatment was started. As a result of this picture, a pneumonia diagnosis was made. In the following days, a severe anemia picture accompanied by thrombocytopenia developed in the patient, and blood transfusion was performed with the help of another donor. On the second day following the transfusion, the patient passed away.

Preparation and Application of PRF

After the clinical examination, mechanical cleaning of the wound was performed. To regain the vitality of the wound, irrigation was carried out with isotonic 0.9% NaCl solutions. The purification of PRF from the blood samples was done according to the method of Dohan et al. (2006) with some modifications. For

preparing PRF, a blood sample (15ml) was collected from the cephalic vein of a clinically healthy dog into red blood tubes from another healthy dog. The tube was centrifuged at 3000 rpm for 10 minutes.

After the centrifugation, three layers were observed in the blood tube (Figure 1). On the bottom layer, which is not used, contains the part rich in red blood cells. The middle layer includes the clotting PRF layer, which is rich in platelets. The top layer consists of acellular plasma (Fan et al., 2020). A fibrin clot taken from the middle part with sterile forceps (Figure 2) was homogenized by mixing it with 10 grammes of vaseline pomade in sterile sample containers without delay for its application to the wound area.

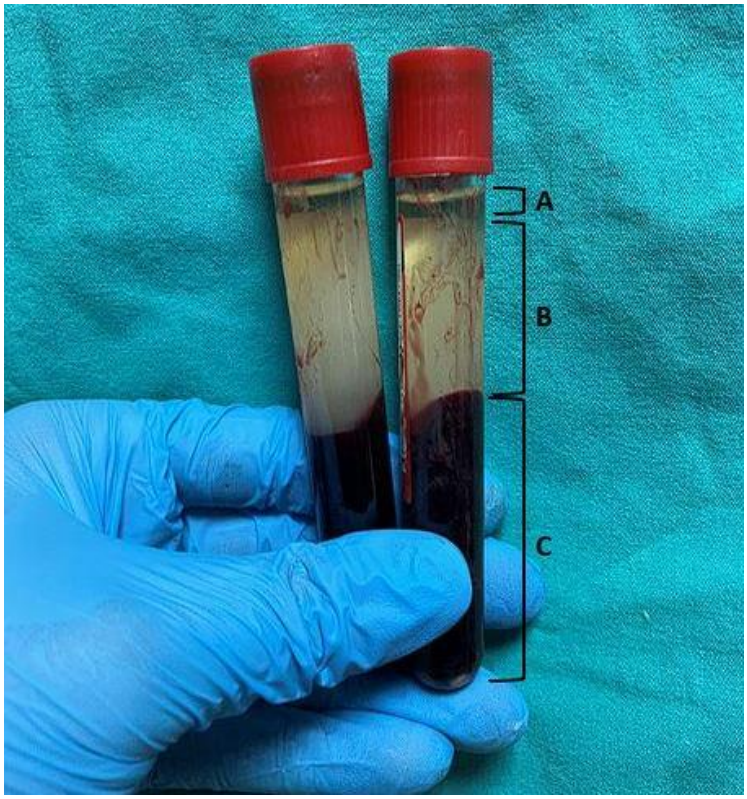

Figure 1: The three layers formed in the blood tube after the centrifugation process are as follows: **Figure 1A:** The acellular plasma portion, **Figure 1B:** The clotted PRF layer, **Figure 1C:** The layer rich in red blood cells.

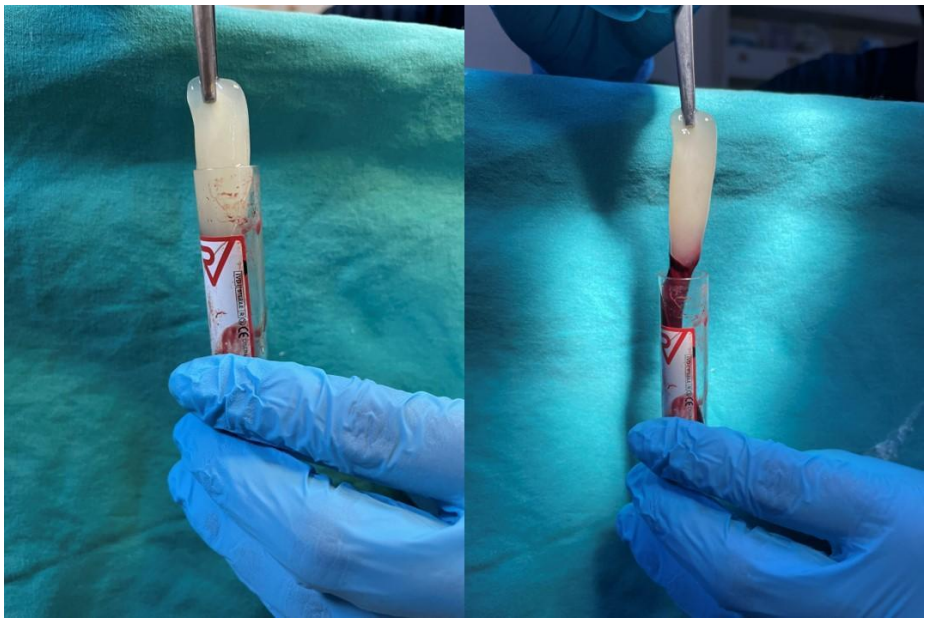

Figure 2: The fibrin clot obtained from the middle section with the help of sterile forceps.

The homogeneous mixture obtained was applied to the wound area homogeneously and then covered with sterile materials (Figure 3). The patient was also given systemic antibiotics (Synulox,

Amoxicillin/Clavulanate Acid 15mg/kg) and systemic analgesic treatments (Bavet Meloxicam, Meloxicam 0.2mg/kg) during the same period. The wound was covered with the same dressing bandage by repeating the same procedures every day for the first week and three times a week for the next two weeks, 21 days (Soares et al., 2021).

Figure 3: Application of PRF mixed with vaseline ointment to the wound area and dressing.

In this case report, the effect of PRF on wound healing is investigated in a dog with tissue damage associated with infection by different pathogens.

Comorbidity Management: Treatment Strategies for Other Diseases

The term comorbidity refers to the presence of any additional disease or disease in an individual with a particular disease that is not a complication of that main disease (Magyari and Sorensen, 2020). In this case report, the dog was referred to as a dog with comorbidities' due to its multifactorial disease profile. For the treatment of identified comorbidity pathogens; doxycycline 5-10 mg/kg/12 hours PO and enrofloxacin 5 mg/kg/12 hours SC were used, and fluid therapy, B vitamin complex, and amino acid solutions were used IV for supportive treatment. The sediment and crystal appearance seen on ultrasound examination of the bladder were determined to be struvite crystals on microscopic examination. Therefore, a preparation containing vitamin C was included in the prescription. Lavage was applied by resisting the bladder with a sterile dog urinary catheter 'Buster' brand, and thus, sediment and crystals within the bladder were removed. An unaffected male dog was used as a donor for the blood transfusion performed on the patient. A blood transfusion was performed at 20 ml/kg/4 hours.

DISCUSSION

Wound cases have found a comprehensive field of study in the veterinary medicine area from the past to the present due to their frequent occurrence in different animals. Various treatment methods have been tried to speed up the healing process and reduce the risk of secondary infections, such as platelet concentrates like PRF, which are used for regenerative procedures in various medical fields including dentistry, reconstructive surgery, plastic surgery, and dermatology (Porsani et al., 2016; Soares et al., 2021). In this case, the effect of PRF on wound healing in a dog with multifactorial disease has been examined. PRF has been reported as an effective treatment procedure in wound healing as a primary and complementary technique due to the long-term release of fibrin matrix, cellular components, and growth factors (Karimi and Rockwell, 2019).

Wound healing occurs through a series of events, starting with hemostasis and followed by inflammatory, proliferative, and finally remodeling phases. Hemostasis begins with platelet aggregation and continues with thrombus formation. The inflammatory phase starts with the accumulation of neutrophils and macrophages at the wound site within 24-48 hours (Scopelliti et al., 2022). The proliferative phase encompasses re-epithelialization, angiogenesis, collagen accumulation, and granulation tissue formation. Cytokines such as interferon (IFN) and transforming growth factor (TGF) are stimulated to synthesize factors such as collagen and fibronectin,

which facilitate fibroblast proliferation, the closure of tissue gaps, and the restoration of mechanical strength. Epidermal growth factor (EGF), fibroblast growth factor (FGF), and TGF stimulate keratinocyte proliferation and re-epithelialization by promoting migration on the wound bed. Simultaneously, angiogenesis is induced by various growth factors such as vascular endothelial growth factor (VEGF), FGF, and platelet-derived growth factor (PDGF). Neo-angiogenesis is further facilitated by releasing proteolytic enzymes and metalloproteinases (MMP) by endothelial cells that degrade the basement membrane and surrounding tissues (Scopelliti et al., 2022). PRF is a three-dimensional structure containing 97% platelets and >50% leukocytes, and it contains cytokines and growth factors (TGF-β1, IGF-1 and 2, PDGF, VEGF, IL-1, 4, and 6) that play a role in tissue regeneration and wound healing (Kızıltoprak, 2019). It has been stated that PRF positively affects and accelerates wound healing thanks to these components (Lektemur Alpan and Torumtay Cin, 2020). Lundquist et al. (2008) emphasized in their study the importance of fibrinogen factors contained in PRF for wound healing and tissue regeneration. Clipet et al. (2012) stated in their study that growth factors found in PRF induce cell viability and proliferation differentiation. In an in vivo study conducted by Roy and colleagues (2011), it was reported that PRF stimulated angiogenesis and accelerated wound healing at the end of 14 days (Roy et al., 2011). In a study conducted by Tunali et al. (2013), it was reported that PRF accelerates wound healing in oral mucosal injuries in rabbits. Desai et al. (2013) followed the wound healing process with the PRF obtained after applying a centrifuge process for 10 minutes at 3000rpm in a 30-year-old individual. They emphasized that PRF is a successful innovative technique in wound healing. In line with the studies conducted, the effects of PRF on wound healing have been examined in this case, and it has been observed that the wound area and the underlying bone tissue healed without any necrotic tissue.

What sets this condition apart from other illnesses is a multifactorial disease profile. Among these diseases, is Anaplasmosis, which is caused by the obligatory intracellular pathogen *Anaplasma* spp. transmitted by ticks and belonging to the Anaplasmataceae family, is of great importance (Dahmani et al., 2017). In a recent study, it was shown that *Anaplasma* spp. infection increases the activity of interferon and neutrophil chemotaxis pathways in the skin, while interestingly decreasing the expression of genes involved in extracellular matrix (ECM) organization and wound healing responses (Underwood et al., 2022). However, in this case, it was observed that there was a significant macroscopic improvement in the open wound of the dog infected with *Anaplasma* spp. after 21 days. Therefore, further studies are needed to clarify this situation more clearly.

During the 21-days for wound healing, PRF was locally applied to the wound area and no signs of infection were detected during the application. During the first week, when dressing and PRF application were repeated daily, the wound tissue was constantly monitored . At the end of the week, a significant demarcation area was observed on the wound lips, and rapid wound healing was seen. In the following two-week period, dressing and PRF application were repeated three times a week. Throughout the 21-days, no signs of infection, dead tissue, or aggressive wound lips were found in the wound area. On the contrary, it was observed that the boundaries of the wound area progressed significantly and the underlying bone tissue healed without any necrotic tissue formation (Figure 4).

Figure 4: The macroscopic appearances of the wound area from day zero to day twenty-one.

At the end of the 21-day treatment, it was determined that healing was still ongoing at the site of the wound, however, the patient, who had some systemic diseases and was infected with different pathogens, passed away in the following period.

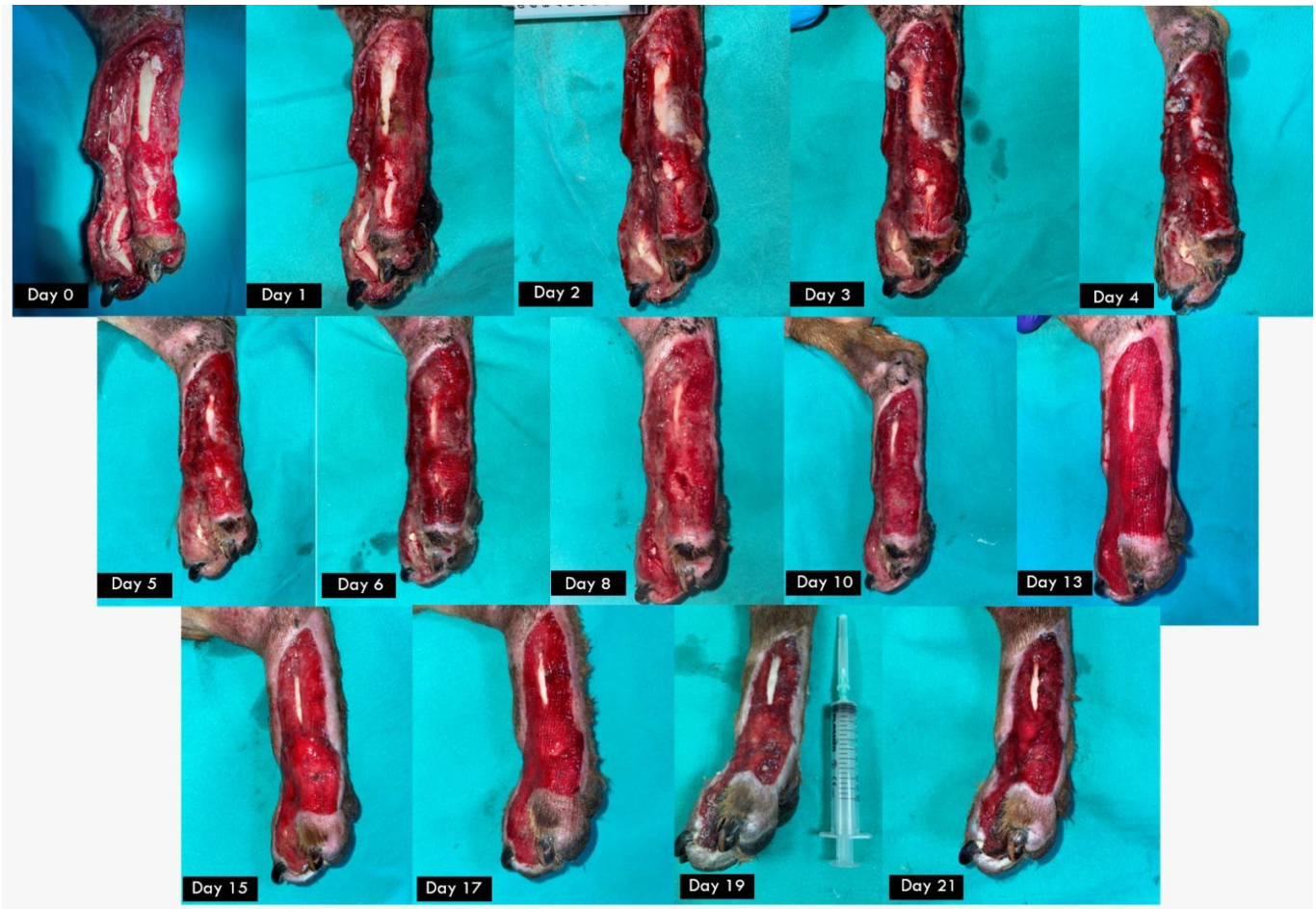

Figure 4: The macroscopic appearances of the wound area from day zero to day twenty-one.
At the end of the 21-day treatment, it was determined that healing was still ongoing at the site of the wound, however, the patient, who had some systemic diseases and was infected with different pathogens, passed away in the following period.

CONCLUSION

As a result, it has been concluded that PRF accelerates wound healing and tissue regeneration in material loss tissue injuries accompanied by a multifactorial disease presentation in this case. However, further studies are needed to demonstrate the effectiveness of PRF in large material loss open wounds.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: All authors contributed to the project idea, designing and conducting the study, obtaining data, analyzing data, drafting and writing the manuscript, and critically reviewing the manuscript. All authors have read and approved the final manuscript.

Ethical approval: This study was carried out in Muğla Sıtkı Koçman University Milas Veterinary Faculty Training and Practice Hospital. This study was approved by the Ethics Committee of Muğla Sıtkı Koçman University Experimental Animals Application and Research Center Animal Experiments Local Ethics Committee (MUDEM-HADYEK, Ref No: E-40051172-100-622678, Date: 21/06/2023)

Explanation: Presented as an oral presentation at the 8th International Congress on Advances in Veterinary Sciences and Techniques (ICAVST) (2023).

REFERENCES

Campanholi, K. da S. S., Silva Junior, R. C. da, Gonçalves, R. S., Bassi da Silva, J., Pedroso de Morais, F. A., Said dos Santos, R., Vilsinski, B. H., Oliveira, G. L. M. de Pozza, M. S. dos S., Bruschi, M. L., Saraiva, B. B., Nakamura, C. V., & Caetano, W. (2022). Design and Optimization of a Natural Medicine from Copaifera reticulata Ducke for Skin Wound Care. *Polymers*, *14*(21). https://doi.org/10.3390/polym14214483

Choukroun, Joseph. , F. Adda. , C. Schoeffler. , A. Vervelle. (2001). Une opportunite´ en paro-implantologie: Le PRF. *Implantodontie*, *42*, 55–62.

Clipet, F., Tricot, S., Alno, N., Massot, M., Solhi, H., Cathelineau, G., Perez, F., De Mello, G., & Pellen-Mussi, P. (2012). In vitro effects of Choukroun's platelet-rich fibrin conditioned medium on 3 different cell

lines implicated in dental implantology. *Implant Dentistry*, *21*(1),51–56. https://doi.org/10.1097/ID.0b013e31822b9cb4

Dahmani, M., Davoust, B., Tahir, D., Raoult, D., Fenollar, F., & Mediannikov, O. (2017). Molecular investigation and phylogeny of Anaplasmataceae species infecting domestic animals and ticks in Corsica, France. *Parasites and Vectors*, *10*(1). https://doi.org/10.1186/s13071-017-2233-2

Davis, V. L., Abukabda, A. B., Radio, N. M., Witt-Enderby, P. A., Clafshenkel, W. P., Cairone, J. V., & Rutkowski, J. L. (2014). Platelet-rich preparations to improve healing. Part II: Platelet activation and enrichment, leukocyte inclusion, and other selection criteria. In *Journal of Oral Implantology* (Vol. 40, Issue 4, pp. 511–521). https://doi.org/10.1563/AAID-JOI-D-12-00106

Desai, C., Kini, Y., Mahindra, U., & Bakshi, M. (2013). Use of platelet-rich fibrin over skin wounds: Modified secondary intention healing. *Journal of Cutaneous and Aesthetic Surgery*, *6*(1), 35. https://doi.org/10.4103/0974-2077.110096

Fan, Y., Perez, K., & Dym, H. (2020). Clinical Uses of Platelet-Rich Fibrin in Oral and Maxillofacial Surgery. In *Dental Clinics of North America* (Vol. 64, Issue 2, pp. 291–303). W.B.Saunders. https://doi.org/10.1016/j.cden.2019.12.012

Iacopetti, I., Perazzi, A., Ferrari, V., & Busetto, R. (2012). Application of Platelet-Rich Gel to Enhance Wound Healing in the Horse: A Case Report. *Journal of Equine Veterinary Science*, *32*(3), 123–128. https://doi.org/10.1016/j.jevs.2011.08.012

Karimi, K., & Rockwell, H. (2019). The Benefits of Platelet-Rich Fibrin. In *Facial Plastic Surgery Clinics of North America* (Vol. 27, Issue 3, pp. 331–340). W.B. Saunders. https://doi.org/10.1016/j.fsc.2019.03.005

Lektemur Alpan, A., & Torumtay Cin, G. (2020). PRF improves wound healing and postoperative discomfort after harvesting subepithelial connective tissue graft from palate: a randomized controlled trial. *Clinical Oral Investigations*, *24*(1), 425–436. https://doi.org/10.1007/s00784-019-02934-9

Lundquist, R., Dziegiel, M. H., & Ågren, M. S. (2008). Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. *Wound Repair and Regeneration*, *16*(3), 356–363. https://doi.org/10.1111/j.1524-475X.2007.00344.x

Magyari M, Sorensen PS. Comorbidity in Multiple Sclerosis. Front Neurol. 2020 Aug 21;11:851. doi: 10.3389/fneur.2020.00851. PMID: 32973654; PMCID: PMC7473304.

Pitzurra, L., Jansen, I. D. C., de Vries, T. J., Hoogenkamp, M. A., & Loos, B. G. (2020). Effects of L-PRF and A-PRF+ on periodontal fibroblasts in in vitro wound healing experiments. *Journal of Periodontal Research*, *55*(2), 287–295. https://doi.org/10.1111/jre.12714

Porsani, M. Y. H., Carvalho, L. A. R., Pereira, C. S., Paludetti, M., Zangeronimo, M. G., & Pereira, L. J. (2016). The use of papain gel cream and sunflower oil in promoting healing in a wound in dogs: Three case reports. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, *68*(5), 1201–1206. https://doi.org/10.1590/1678-4162-8561

Roy, S., Driggs, J., Elgharably, H., Biswas, S., Findley, M., Khanna, S., Gnyawali, U., Bergdall, V. K., & Sen, C. K. (2011). Platelet-rich fibrin matrix improves wound angiogenesis via inducing endothelial cell proliferation. *Wound Repair and Regeneration*, *19*(6), 753–766. https://doi.org/10.1111/j.1524-475X.2011.00740.x

Scopelliti, F., Cattani, C., Dimartino, V., Mirisola, C., & Cavani, A. (2022). Platelet Derivatives and the Immunomodulation of Wound Healing. In *International Journal of Molecular Sciences* (Vol. 23, Issue 15). MDPI. https://doi.org/10.3390/ijms23158370

Soares, C. S., Dias, I. R., Pires, M. A., & Carvalho, P. P. (2021). Canine-origin platelet-rich fibrin as an effective biomaterial for wound healing in domestic cats: A preliminary study. *Veterinary Sciences*, *8*(10). https://doi.org/10.3390/vetsci8100213

Tunali, M., Özdemir, H., Küçükodaci, Z., Akman, S., & Firatli, E. (2013). In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. *British Journal of Oral and Maxillofacial Surgery*, *51*(5),438–443. https://doi.org/10.1016/j.bjoms.2012.08.003

Underwood, J., Harvey, C., Lohstroh, E., Pierce, B., Chambers, C., Guzman Valencia, S., & Oliva Chávez, A. S. (2022). Anaplasma phagocytophilum Transmission Activates Immune Pathways While Repressing Wound Healing in the Skin. *Life*, *12*(12). https://doi.org/10.3390/life12121965

Wang, P. H., Huang, B. S., Horng, H. C., Yeh, C. C., & Chen, Y. J. (2018). Wound healing. In *Journal of the Chinese Medical Association* (Vol. 81, Issue 2, pp. 94–101). Elsevier Ltd. https://doi.org/10.1016/j.jcma.2017.11.002