2016 Update on new literature review for: L-PRF, A-PRF, I-PRF and CGF

In 2015, Steiner Biotechnology reviewed all published articles relating to PRF and bone. Due to new methodology involving blood preparation’s, we have been asked to update that literature review and include these new methods of producing platelet rich preparations such as: L-PRF, A-PRF, I-PRF and CGF.

At Steiner Biotechnology, our focus and field of expertise has been dedicated to hard tissue regenerative medicine and we do not claim to have any scientific knowledge of soft tissue healing.

The blood is actively involved in soft tissue healing with the presence of factors to aid soft tissue healing including an immune response to pathogens. It would make sense that platelet rich preparations would provide a benefit when used in soft tissue healing. The use of platelet rich preparations for the treatment of periodontal disease is not included in this review because periodontal disease is a soft tissue disease. This literature review is limited to bone growth and regeneration only as this is our area of expertise.

To recap, our review of the PRF literature in 2015, there were 17 controlled human clinical studies on PRF and one controlled animal study. There were 4 human sinus augmentation studies, all of which compared PRF mixed with either, bovine bone or allograft and compared this to using the allograft and bovine bone alone. The results of the studies concluded there was no benefit by adding PRF. One sinus study compared grafting the sinus and compared covering the graft with a PRF membrane to covering the graft with a collagen membrane. The study showed no benefit gained by using the PRF membrane over a collagen membrane.

There were 4 studies on socket healing. Three studies compared PRF to no socket graft and found no difference between the sockets grafted with PRF and no grafting. One study showed PRF performed better than no graft.

There was one study that evaluated the ability of PRF to preserve the ridge after extraction and found no difference between sites grafted with PRF and the sites that received no graft.

There was one interesting animal study that compared PRP, PRF, no graft and what they called PPP (PPP was PRF with the growth factors removed) The preparations were grafted in extraction sockets. PPP significantly outperformed the other preparations and the authors concluded that because the growth factors in PRP and PRF were soft tissue growth factors and not bone growth factors, the growth factors in PRP and PRF inhibited bone formation.
Before we proceed into the recent literature, let's discuss what factors are involved in promoting bone growth.

The following is a list of molecules known to promote bone growth:

- **Runx 2**  Runt related transcription factor 2
- **B-catenin**  β-catenin in postnatal Osx-lineage cells critically regulates bone homeostasis by promoting osteoblast activity and suppressing osteoblast turnover, while restraining osteoclast and marrow fat formation.
- **Osterix**  Regulates calcification and degradation of chondrogenic matrices
- **BMP’s**  Bone morphogenic proteins
- **WNT’s**  Wnt signaling has been shown as an important regulatory pathway in the osteogenic differentiation of mesenchymal stem cells.
- **PTH**  Parathyroid hormone
- **IGF**  Insulin like growth factor

The following are articles that we reviewed. They include both, animal and human studies and present a comprehensive list of related articles published since early 2015. If you do not want to review the articles, you can skip to the end of the list for a summation of the findings presented in the articles.

**Platelets.** 2016 Mar 7:1-5. [Epub ahead of print]

*Cytokine, chemokine, and growth factor profile of platelet-rich plasma.*

The following molecules were found in PRP

IL-1b, IL-1ra, IL-4, IL-6, IL-8, IL-12, IL-13, IL-17, INF-γ, TNF-α, MCP-1, MIP-1a, RANTES, bFGF, PDGF, and VEGF IL-2, IL-5, IL-7, IL-9, IL-10, IL-15 G-CSF, GM-CSF, Eotaxin, CXCL10 chemokine (IP-10), and MIP 1b.

- **Steiner Biotechnology note:** There are no recognized bone growth molecules in PRP preparations.


*Choukroun Platelet-Rich Fibrin as an Autogenous Graft Biomaterial in Preimplant Surgery: Results of a Preliminary Randomized, Human Histomorphometric, Split-Mouth Study.*

Du Toit J, Siebold A, Dreyer A, Gluckman H.

Results: Mean ± SD percent of new mineralized bone was 40.8% ± 10.3% for the PRF specimens and 43.9% ± 16.8% for the control specimens (P = .72, 95% CI, 33.4-55.6 and 19.3-55.5, respectively). Bone derived from PRF histologically did not differ from bone that healed without intervention.

- **Steiner Biotechnology note:** In this study, they compared the bone in human extraction sites grafted with PRF to extraction sites with no graft. There was no difference in the percent of mineralized tissue in sites grafted with PRF as compared to sites that received no graft.
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Experimental Evaluation of the Effectiveness of Demineralized Bone Matrix and Collagenated Heterologous Bone Grafts Used Alone or in Combination with Platelet-Rich Fibrin on Bone Healing in Sinus Floor Augmentation
Elif Peker, DDS, PhD/Inci Rana Karaca, DDS, PhD/Benay Yildirim, DDS, PhD

Conclusions: There is no beneficial effect of the application of PRF in combination with demineralized bone matrix or collagenated heterologous bone graft on bone formation in sinus floor augmentation.

➢ Steiner Biotechnology note: The authors found no benefit of adding PRF to bone graft materials.


Micro-computed tomography and histomorphometric analysis of the effects of platelet-rich fibrin on bone regeneration in the rabbit calvarium.
Acar AH², Yolcu Ü², Gül M³, Keleş A⁴, Erdem NF⁵, Altundag Kahraman S⁶.

In this study, 20 New Zealand white rabbits were used and four calvarial defects were prepared in each animal. PRF, Straumann(®) Bone Ceramic (SBC), or PRF+SBC was applied to the defects; one defect was left untreated as a control. Ten rabbits were sacrificed at week 4 (T1) and 10 at week 8 (T2). After micro-computed tomography (micro-CT) scanning, the samples were sent for histological and histomorphometric analysis to evaluate and compare the volume and area of regenerated bone.

Results: Histomorphometric and micro-CT analysis showed that both PRF and SBC significantly increased bone regeneration at T1 and T2 (P<0.01). When PRF was used in combination with HA/βTCP, a further significant increase in new bone formation was observed at T1 and T2 compared with that when PRF or SBC was used alone (P<0.01).

Conclusions: PRF has a positive effect on bone formation when used alone and in combination with HA/βTCP.

➢ Steiner Biotechnology note: In this animal study, the authors found that there was a benefit to adding PRF to bone graft materials.


Sheet of osteoblastic cells combined with platelet-rich fibrin improves the formation of bone in critical-size calvarial defects in rabbits.
Wang Z¹, Hu H², Li Z¹, Weng Y³, Dai T¹, Zong C¹, Liu Y⁴, Liu B⁵.

Techniques that use sheets of cells have been successfully used in various types of tissue regeneration, and platelet-rich fibrin (PRF) can be used as a source of growth factors to promote angiogenesis. We have investigated the effects of the combination of PRF and sheets of mesenchymal stem cells (MSC) from bone marrow on the restoration of bone in critical-size calvarial defects in rabbits to find out whether the combination promotes bony healing. Sheets of MSC and PRF were prepared from the same donor. We then implanted the combined MSC and
PRF in critical-size calvarial defects in rabbits and assessed bony restoration by microcomputed tomography (microCT) and histological analysis. The results showed that PRF significantly increased bony regeneration at 8 weeks after implantation of sheets of MSC and PRF compared with sheets of MSC alone (p=0.0048). Our results indicate that the combination of sheets of MSC and PRF increases bone regeneration in critical-size calvarial defects in rabbits, and provides a new way to improve skeletal healing.

➢ Steiner Biotechnology note: In this animal study, the authors found that there was a benefit to adding PRF to sheets of mesenchymal stem cells.

Since previous literature reviews did not cover L-PRF, we reviewed all of the published studies on L-PRF and bone.

Leukocyte-platelet-rich plasma (L-PRP) induces an abnormal histophenotype in craniofacial bone repair associated with changes in the immunopositivity of the hematopoietic clusters of differentiation, osteoproteins, and TGF-β1.
Giovannini AF¹, Grossi JR, Gonzaga CC, Zielak JC, Göhringer I, Vieira Jde S, Kuczera J, de Oliveira Filho MA, Deliberador TM.
Results: These results coincided with the lower bone matrix deposited and larger medullary area, which were composed of fibrosis, when treated with only L-PRP, or intense adiposity on defects filled with L-PRP mixed with autograft.
Conclusions: From this study, it can be concluded that the L-PRP used alone or mixed to autograft hindered the osteoneogenesis due to suppression of immunoexpression of BMP2, while the immunopositivity of TGF-β1 was intense. When used alone, the L-PRP induced a fibrotic condition associated with TGF-β1 presence and lack of osteoproteins, but when L-PRP was mixed to autograft, it induced the presence of the osteolineage cells (BMPR1B (+) Runx2(+)), but also inhibited the terminal osteoblastic maturation associated with the lack of BMP2 and the presence of TGF-β1(+), a fact that contributed to cellular transdifferentiation into fat cells.

➢ Steiner Biotechnology note: This animal study found that L-PRF inhibited bone formation and resulted in fat formation when mixed with an autograft.

Effects of leukocyte-platelet rich fibrin on postoperative complications of direct sinus lifting.
Gurler G¹, Delilbasi C.
Pain, swelling, sleeping, eating, phonetics, activities of daily living, missed work days and soft tissue healing were evaluated postoperatively.
Results: Data of 24 patients were evaluated. Improvements were seen in the studied parameters in the L-PRF group; however, the difference was not significant between the two groups (P>0.05).
Conclusions: The use of L-PRF and allogenous bone graft in combination with L-PRF membrane does not significantly improve postoperative complications following direct sinus lifting.
**Steiner Biotechnology note:** L-PRF does not improve post-operative healing in humans.

**Effect of leukocyte- and platelet-rich fibrin (L-PRF) on bone regeneration: a study in rabbits.**
A total of 72 hemispheres were implanted on the calvaria of 18 rabbits and filled with three different space fillers: L-PRF, bovine hydroxyapatite (BHA), BHA + L-PRF, and an empty hemisphere was used as control. Six rabbits were sacrificed at three distinct time points: 1 week, 5 weeks, and 12 weeks. Histological and histomorphometrical analyses were carried out.
**Results:** No statistical differences were found within the four groups in terms of bone quantity and quality at each timepoint (p = .3623).
**Conclusion:** According to the present study, L-PRF does not seem to provide any additional effect on the kinetics, quality, and quantity of bone in the present model of guided bone regeneration.

**Steiner Biotechnology note:** In this rabbit study, L-PRF did not improve healing over no graft or when mixed with bone graft materials.

**The Effect of Concentrated Growth Factor on Rat Bone Marrow Cells In Vitro and on Calvarial Bone Healing In Vivo.**
Takeda Y, Katsutoshi K, Matsuzaka K, Inoue T.
Results: Cell proliferation and osteoblastic differentiation were significantly higher in cells cultured on the CGF-coated disks than on the PPP gel-coated disks. In vivo, more new bone had formed in defects treated with CGF than in defects treated with PPP gel.
Conclusion: In this preliminary study, fibrin and soluble factors in CGF promoted initial cell stretching, proliferation, and osteoblastic differentiation of RBM cells in vitro and bone regeneration in rat calvarial bone defects in vivo.

**Steiner Biotechnology note:** In this animal study, the authors found that CGF improved bone healing in rabbits.

**Comparison of removal torques of SL.Active® implant and blasted, laser-treated titanium implant in rabbit tibia bone healed with concentrated growth factor application.**
Park SH¹, Park KS¹, Cho SA¹.
Conclusions: It was found that BLT surface modification exhibited excellent osseointegration. In addition, CGF application did not affect the insertion and removal torque of the implant.

**Steiner Biotechnology note:** CGF produced no clinical benefit to implant integration.

**Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing.**
Kim TH¹, Kim SH¹, Sándor GK², Kim YD³.

Results: In micro-CT analysis, bone mineral density and bone volume were greater in the experimental group than in controls at both 6th and 12th week, but not among the experimental groups. The histomorphometric examination showed no significant difference between the bone formation at 12 weeks among control or experimental groups.

➢ Steiner Biotechnology note: PRP, CGF and PRF showed improved bone formation over no graft when evaluated using Micro CT scan. However, histologically there was no difference between PRP, CGF, PRF and no graft. There was no statistic difference in the bone formed by PRP, PRF and CGF.


Comparative release of growth factors from PRP, PRF, and advanced-PRF.
Kobayashi E¹,², Flückiger L³, Fujioka-Kobayashi M¹,⁴, Sawada K¹,², Sculean A³, Schaller B¹, Miron RJ⁵,⁶.

The results from the present study indicate that the various platelet concentrates have quite different release kinetics. The advantage of PRP is the release of significantly higher proteins at earlier time points whereas PRF displayed a continual and steady release of growth factors over a 10-day period. Furthermore, in general, it was observed that the new formulation of PRF (A-PRF) released significantly higher total quantities of growth factors when compared to traditional PRF.

➢ Steiner Biotechnology note: The growth factors released from the platlette prepertrions were PDGF-AA followed by PDGF-BB, TGFB1, VEGF, and PDGF-AB. None of these growth factors promote bone growth.


Early Bone Formation at a Femur Defect Using CGF and PRF Grafts in Adult Dogs: A Comparative Study.
Park HC¹, Kim SG, Oh JS, You JS, Kim JS, Lim SC, Jeong MA, Kim JS, Jung C, Kwon YS, Ji H.

Results: At 4 weeks, the comparisons of each experimental group showed a significant difference between the CGF group and the synthetic bone graft group. When comparing the CGF and allograft material groups, the allograft group showed significantly more new bone formation. In the case of vascular endothelial growth factor, CGF had 1.5 times more than PRF. CGF showed a fibrinogen structure with a constant diameter.

Conclusions: When applied to a clinical case, CGF is predicted to show better results than PRF.

➢ Steiner Biotechnology note: As is the case with some articles published in Implant Dentistry, it appears there is little editing or peer review. The results and conclusions make no sense so we will summarize the findings. They evaluated the concentration of growth factors and they could not find any TGF-B in either PRF or CGF. They did find VEGF but there was no difference in concentration between PRF and CGF. The amount of new bone formation and the amount of bone in contact with the implant was significantly higher in the synthetic bone graft group than the control, PRF or CGF groups.
Bone tissue engineering with bone marrow-derived stromal cells integrated with concentrated growth factor in Rattus norvegicus calvaria defect model. Honda H, Tamai N, Naka N, Yoshikawa H, Myoui A.

In the in-vivo study, the CGF group regenerated bone better than the control group, and combined therapy with CGF and BMSCs almost completely repaired critical-size bone defects within 12 weeks after surgery. In the in-vitro study, the CGF extract, at concentrations between 1 and 10%, promoted proliferation, osteogenic maturation, and mineralization of hTERT-E6/E7 human MSCs in a dose-dependent manner but had an inhibitory effect at higher concentrations. In conclusion, a CGF extract promoted the proliferation, osteogenic maturation, and mineralization of mesenchymal stem cells in vitro, and combination therapy with CGF and BMSCs resulted in excellent healing of critical-size bone defects in vivo.

➢ Steiner Biotechnology note: Whenever we see words like “excellent” used in an abstract we know the authors are trying to sell something and that is the case with this study. This is the first study we have reviewed that did a quantitative real-time PCR analysis on mesenchymal stem cells to evaluate which genes are activated by the platelet rich preparations. In this case, CGF was added to stem cells and the authors found that CGF had no effect on the genes that are involved in osteogenesis. CGF had no effect on the expression of RunX2 or osterix. These are the master regulators of osteogenesis and if they are not increased, they will likely not stimulate bone formation.

PDGF-regulated miRNA-138 inhibits the osteogenic differentiation of mesenchymal stem cells.
Qu B, Xia X, Wu HH, Tu CQ, Pan XM.

Abstract
Differentiation-specific microRNAs may play a critical role in MSC differentiation, and they can be altered by PDGF signaling. We propose that PDGF modulates MSC differentiation by regulating microRNA expression. Therefore, we investigated whether PDGF treatment could alter the expression profile of miRNAs in MSCs. Furthermore, we assessed the osteoblast phenotype of MSCs after inducing osteogenic differentiation. We found that PDGF treatment significantly inhibits the osteogenic differentiation of MSCs and that miR-138 gene transcription is controlled by PDGF signaling. Our results confirm that miR-138 inhibits the osteogenic differentiation of MSCs and suppresses the phosphorylation of FAK, ERK1/2, and Runx2. Furthermore, our study clearly demonstrates that downregulation of Runx2 by miR-138 is critical for the PDGF-mediated inhibition of osteogenic differentiation of MSCs.

➢ Steiner Biotechnology note: PDGF is a main growth factor in platelet preparations. This study clearly outlines how this molecule inhibits bone formation.
Tumor Necrosis Factor-alpha Attenuates the Osteogenic Differentiation Capacity of Periodontal Ligament Stem Cells by Activating Protein Kinase Like Endoplasmic Reticulum Kinase Signaling.

Tan J1,2, Zhou L3, Xue P1, An Y1, Luo L1, Zhang R1, Wu G1,4, Wang Y1, Zhu H1, Wang Q1

Results: Higher concentrations of TNF-α (10 ng/ml and 20 ng/ml) impaired the osteogenic differentiation of PDLSCs, but activated the PERK pathway. Pretreatment of PDLSCs with lower concentrations of 4-PBA prevented the TNF-α-induced upregulation of GRP78, PERK, and ATF4, and recovered differentiation ability. Finally, PERK knockdown also restored osteogenic differentiation.

Conclusion: TNF-α attenuates the osteogenic differentiation ability of PDLSCs through activation of the PERK pathway.

➢ Steiner Biotechnology note: TNF is another significant growth factor in platelet preparations and again, this study shows how it inhibits bone formation.

Literature review summation:

In approximately 50% of the animal studies platelet preparations showed improved bone growth.

In approximately 50% of the animal studies the platelet preparations showed no improvement in bone growth.

Platelet preparations do not contain bone growth factors and do not stimulate the production of bone growth factors.

There were no human studies that showed any benefit for bone growth when the preparations were mixed with bone grafts.

There were no human studies that showed any benefit for bone growth when the platelet preparations were used alone.

Steiner Biotechnology note: It is very common for products and procedures to show promise in treating animals but very often show no benefit in humans. The research shows that is the case with platelet derived preparations and bone.

We invite any comments or the presentation of any literature that contradicts the findings of our literate review.

This literature review was produced by Steiner Biotechnology