Effectiveness of I - PRF in Periodontal Regeneration - A Systematic Review And Metanalysis

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Abstract
Objective: The purpose is to systematically review the current literature as to whether I-PRF is effective in regeneration of periodontal procedures, in adults in comparison to a control of traditional treatment.
Methods:
A systematic electronic search of Medline, Scopus, Web of Science, and Embase databases was performed from 2019 to December 2022 using a combination of keywords. All in vitro and in vivo studies, written in English and concerning the potential role of I-PRF in regenerative dentistry were considered.
Results:
Probing pocket depth and Clinical attachment level was decreased in subjects treated with injectable platelet rich fibrin as compare to the control group. Periodontal Index though was decreased in the study group, was not significantly different. Overall, it can be inferred that I – PRF was better in periodontal regeneration amongst the studies analysed in combination with surgical procedures
Conclusion:
Current literature approves that i-PRF has shown to be a modern technology that produces dependable results in the field of dentistry. To ascertain the cell differentiation activity of i-PRF component, more investigation should be needed.

Keywords: Platelet-rich fibrin, Injectable platelet-rich fibrin, Periodontitis, Periodontal therapy, Periodontal regeneration.

INTRODUCTION:

Periodontitis is an inflammatory disease of the periodontal tissues, which is characterized by loss of support of the affected teeth, specifically periodontal ligament fibers and the bone into which they are inserted.
The goal of periodontal therapy includes arrest of periodontal disease progression and the regeneration of structures lost due to pre-existing disease process. Conventional surgical techniques offer only limited potential towards recovering the lost periodontal structures. Successful periodontal reconstruction comprises of regeneration of multiple tissues of the periodontium. It is a complex biological process in itself which is intricately regulated between cells, locally acting growth factors and the extracellular matrix components. The key to periodontal regeneration is to stimulate the progenitor cells to re-occupy the defect [1].
Earlier attempts to achieve regeneration included denudation of interdental bone to treat intrabony defects and use of autografts to fill the surgical site. Also, favourable results have been gained in treatment of such defects using a combination of graft material and collagen membranes. [2] However, recently, the attention has shifted to the use of growth factors which are the biologic mediators that can regulate the proliferation, chemotaxis and differentiation of the locally derived progenitor cells in the defect site. [3] Among the rich sources of autologous growth factors the various generations of platelet concentrates are currently in use. Platelet Rich Plasma, first generation concentrate, has been used alone and in combination with grafting materials and barrier membranes in treatment of periodontal and surgical defects. [4,5] However, the effects of Platelet rich plasma on bone regeneration have been limited. The second and latest generation

of platelet concentrates is Platelet Rich Fibrin. It is a promising, completely autologous leukocyte and platelet concentrate which is being successfully used in various fields of dentistry and medicine. PRF has shown successful results when used as a sole agent in the treatment of periodontal intrabony defects. [6]
Regeneration or repair following periodontal therapy depends on two crucial events: availability of cell types needed and presence or absence of signals necessary to recruit and stimulate the cells. The cascade of healing of any wound is initiated by clot formation, followed by proliferative stage and maturative stage. Growth factors favor wound healing by promoting proliferation of cells (mitogenesis), migration of cells (chemotaxis), and stimulation of new blood vessel formation (angiogenesis).
The use of blood-derived products to heal wounds began in 1970 when fibrin glues or fibrin sealants, which were formed by polymerizing fibrinogen with thrombin and calcium, were introduced. Fibrin glue had clinical applications such as topical hemostasis and tissue sealing, soft tissues, and melting agents for particulate bone substitutes. As the concentration of fibrinogen in plasma is less, the stability and quality of fibrin glue were low.
Variation in its composition and characteristics, lower resistance to physical stresses, costly processing and associated with a risk of viral transmission are its drawbacks. Platelets contain biologically active proteins that bind on to a developing fibrin mesh or to the extracellular matrix. The proteins thus create a chemotactic gradient for recruitment of stem cells. These stems cells undergo differentiation, and promote healing by regeneration. Hence, the use of autologous platelet concentrates opens a promising treatment option in the field of periodontal regeneration, especially in clinical situations demanding rapid healing.[4] The regenerative potential of platelets was initially introduced in 1974 by Ross et al.[5] It was proposed that platelet-derived growth factor (PDGF) serves as growth factor on fibroblasts, smooth muscle cells, and glial cells. The application of platelet concentrates was initially limited to treatment and prevention of hemorrhage due to severe thrombopenia. As its scope in medical application expanded, an idea to combine the fibrin sealant properties with the growth factors in platelets was tried for wound healing and regeneration of tissues.

METHODOLOGY:

SELECTION CRITERIA OF THE STUDIES.

SEARCH METHODS AND STRATEGY PROTOCOL

Objective: The purpose is to systematically review the current literature as to whether PRF is effective in regeneration of periodontal procedures, in adults in comparison to a control of

Research question:
The research question is,
"What is the difference in treatment outcome of periodontally affected teeth treated with injectable platelet rich fibrin and a conventional / surgical therapy?

Protocol and Registration
The PRISMA checklist, used for reporting systematic reviews and meta-analysis (Moher *et al.*, 2009) was employed for this review and was not registered with PROSPERO (Centre for Reviews and Dissemination, 2019).

Ethical Approval
There was a submission of a completed checklist to the Peoples College of Ethics Committee for deliberation before the systematic review was undertaken.
The committee confirmed that there was not a need for formal University Ethics Approval in view of the fact there was no physical human or animal participation.

Data Sources: The electronic search strategy included searching the PubMed / MEDLINE database, Scopus, Journal of web, the Cochrane Central Register of Controlled Trials database and manually searching the bibliographies of included articles and journals relating to dentistry and periodontology published upto September 2022: Periodontology 2000; Journal of Clinical Periodontology; Journal of Periodontology; Journal of Periodontal Research; Journal of Adhesive Dentistry; International journal of Periodontics and restorative dentistry; Clinical advances in periodontics; Journal of Indian society of Periodontics; AAP journal of periodontology; Journal of Cranio-Maxillo-Facial Surgery, Journal of Craniofacial Surgery, Dental Traumatology, Orthodontics & Craniofacial Research, Head & Face Medicine, Journal of Oral Pathology & Medicine, European Journal of Oral Sciences, Oral Diseases, British Dental Journal, The Journal of the American Dental Association, Journal of Dentistry, Journal of Dental Research, Oral Surgery Oral Medicine Oral Pathology Oral Radiology.
Search terms included "platelet rich fibrin"; "injectable platelet rich fibrin"; "periodontitis"; "periodontal therapy"; "periodontal regeneration"; "i-prf";

Study selection: Only randomised controlled trials with a comparator or control group conducted on human, with gradable clinically evaluated outcomes of i- PRF usage in periodontal regenerative procedures in adults were included. Only articles published in the English language were included. Randomised controlled trials without a comparator group, case reports, case series, review articles, abstracts, discussions, interviews, editorials and opinions were excluded.
PICO format employed for the systematic review and meta-analysis is;

Population: Systemically healthy human individuals with periodontal disease

Intervention: Surgical treatment of condition through the use of I - PRF alone or in combination with other biomaterials with a follow-up period

Comparison: Any other intervention apart from I - PRF
Outcomes: The outcome variable and data collection included clinical parameters such as change in pocket depth (PD), clinical attachment level (CAL), gingival index and plaque index

Study design: Randomised controlled trials

Search Limits
Searches incorporating literature from the year 2000 up until 2019 as the concluding year for the search. Only sources in English were used.

Process of Study Identification
Endnote X8 was used to import the results of the search data and to remove the duplicates. The screening of abstracts was carried out by the use of the eligibility criteria and for those not excluded, full text articles were searched for. These were, then, assessed for inclusion and upon acceptance, underwent data extraction and quality assessment. Articles, failing to meet inclusion criteria, were omitted.

Data collection
All the title and the extracts were independently screened by the reviewer and upon a meticulous review of the full text articles, the data was extracted and documented in a data extraction table, which shows the various data items evaluated for the review.

Data extraction:
Data extraction was performed for studies that fulfilled the inclusion criteria. Data extraction included participant and intervention characteristics, reported data on the efficacy of the outcomes using standard data extraction templates.

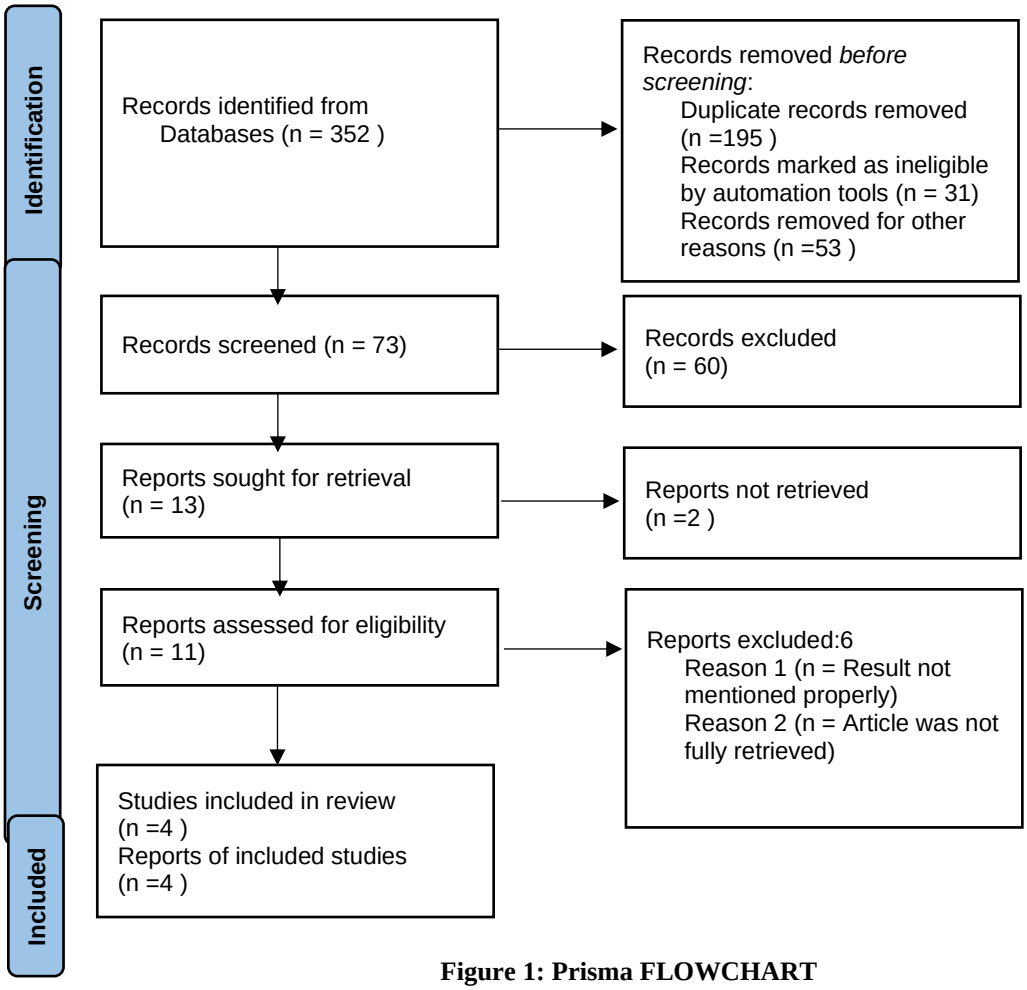


Figure 1: Prisma FLOWCHART

RISK OF BIAS EVALUATION

The risk of bias of the articles was assessed according to the Cochrane Risk of Bias Tool. A total of seven domains were evaluated: random sequence generation, allocation concealment, blinding of patients and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting risk and other potential threats to validity. The risk of bias was evaluated both at the study level and outcome level.

RESULTS:

Study selection:

Identification:

Database and hand searches of the reference list yielded 352 articles after a comprehensive literature search. Two writers who conducted large scale systematic reviews in the past, with works published on the periodontal regeneration with i-prf , were emailed for provision of any information or additional papers meeting the eligibility criteria, but neither responded. Hand searching did not identify any further paper.

Screening:

After title and abstract screening, 279 articles were removed. The 73 articles that remained underwent an appraisal for eligibility of inclusion, of which 60 were excluded. The overwhelming factors for exclusion, at this point, were; studies done to assess whether outcome measures were clearly defined.

Risk of bias:

Two studies had random sequence generation, the remaining studies did not mention the method of sequence generation. Two studies had mentioned allocation concealment. Only one study mentioned blinding of participants and personnel. None of the studies mentioned blinding of outcome data, the others did not. All studies had no issues relating to incomplete outcome data and all articles discussed patient attrition where relevant. All the Four studies had pre-specified outcomes, meaning a low risk regarding selective outcome reporting.

Study characteristics:

Study details are presented in Table 3. The four articles included were from different countries. Three articles were from India, Belgrade, Egypt and Turkey. All articles were randomised controlled trials with bilateral treatment in adult subjects.

Table 1: Risk of bias of included studies:

Study ID	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Mila Vuckovic et al, 2020 (7)	+	?	+	?	+	+	+
Ahmed Elbarbary et al 2022 (8)	+	+	+		+	+	+
Uma P Nair et al 2022 (9)	?	?	?	?	+	+	+
Onur Ucak Turer et al, 2019 (10)	+	-	?	?	+	+	+

+ = Low risk of bias

-- = High risk of bias

? = Unclear risk of bias

Table 2: Data characteristics of study included:

Study ID	Location	Sample	Groups	Parameters measured	Follow up	Results / Inference	Study design
Mila Vuckovic et al, 2020 (7)	Belgrade	24 patients with Chronic generalised periodontitis and confirmed 2 sites of PPD > 5 mm in the mean age of 37.29 $\pm$ 10.23 years with 10	Study group treated with Scaling and root planning along with Injectable Platelet rich fibrin Control group treated with Scaling and root planning	Periodontal parameters of Clinical attachment level, GML, Probing pocket depth, Bleeding on probing and	3 months	Both interventions showed improvement in periodontal parameters. When compared between the groups, study	Split mouth design

		mean and 14 women				group was significantly better than control group for CAL, GML and PPD	
Ahmed Elbarbary et al 2022 (8)	Cairo, Egypt	12 patients in each group with stage III periodontitis with atleast one intrabony defect, PPD > 5mm and radiographical evidence of cortical bone loss in the age range of 36 – 59 years	Study group treated with Injected Platelet fibrin and Xenograpft while control group treated with xenograft alone	Probing depth (PD), clinical attachment level (CAL.), bone defect depth and bone density	6 months	Though significant difference in CAL and PD was noted, no significant difference was found in bone fill between study and control group	Concurrent parallel study design
Uma P Nair et al 2022 (9)	Mysuru, India	12 patients in each group, in the age range of 30 – 55 years	Group I – Open flap debridement with bone graft Group II – Open flap debridement with sticky bone (i PRF + bone graft)	PI, GI, PPD, CAL, HPD and VPD	3, 6 and 9 months	At the baseline, there was no statistically significant difference between the tested parameters. After 9 months all the clinical parameters, PI, GI, PPD, CAL, HPD and VPD as well as radiographic bone fill showed a significant increase in both the groups ($p < 0.05$) (PI-TGr; CGr–VPD—3.5 ± 0.54 to 0.66 ± 0.51; 3.3 ± 0.81 to 2 ± 0.63/BAF—2.9 ± 0.88 to 5.6 ± 1.10; 3.4 ± 1.39 to 3.9 ± 1.4). On comparison the test group (i- PRF group) showed better results for each clinical parameter	Randomised control trial, parallel design
Onur Ucak Turer et al, 2019 (10)	Cukurova University	36 patients per treatment arm in ages above 19 years were	Test Group intervened with CAF+CTG+i-PRF and control group treated with	GR depth (RD), GR width (RW), probing	6 months	This randomized clinical study showed that CAF+CTG+i-	Double-masked, randomized, controlled clinical trial,

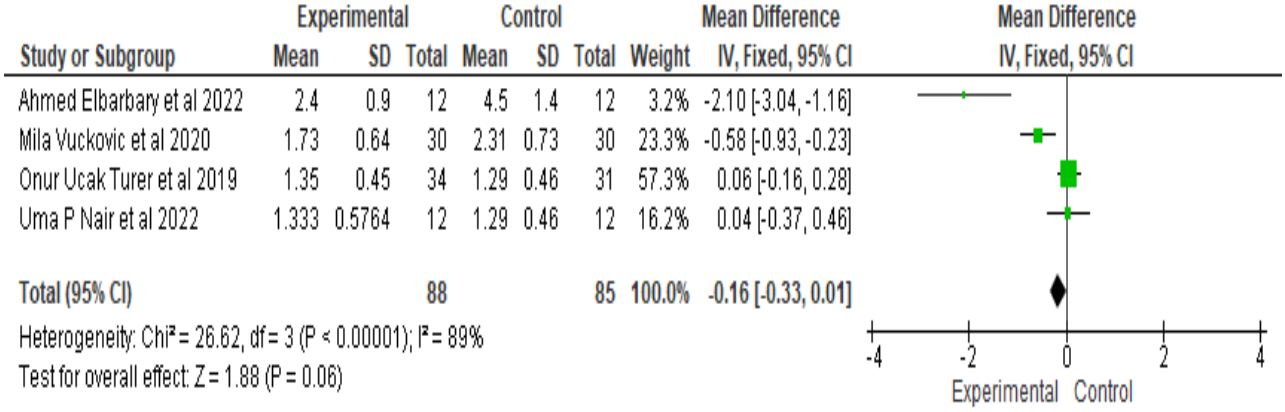
			CAF+CTG for the treatment of gingival recession	depth (PD clinical attachment level (CAL), keratinized tissue height (KTH), gingival thickness (GT)		PRF technique can provide additional benefit in terms of KTH, RD reduction and the probability of obtaining CRC for the treatment of deep Miller class I and II gingival recessions.	with parallel design,

Table 3: Periodontal Parameters assessed:

Study ID	Test group	Control group
Mila Vuckovic et al, 2020 (N=30)		
GML	0.62 ± 0.49	0.99 ± 0.57
PPD	1.73 ± 0.64	2.31 ± 0.73
BOP	0.15 ± 0.18	0.33 ± 0.12
PI	0.19 ± 0.23	0.20 ± 0.89
Ahmed Elbarbary et al 2022 (N=24)		
PD	2.4 ± 0.9	4.5 ± 1.4
CAL	3.0 ± 0.8	5.1 ±1.9
Uma P. Nair et al , 2022 ; N=12		
PI	0.440 ± 0.16982	0.5483 ± 0.22807
GI	0.3233 ± 0.1504	0.5783 ± 0.3210
PPD	1.333 ± 0.5764	2.1667 ± 0.4082
CAL	1.000 ± 0.0000	1.333 ± 0.516
HPD	0.6667 ± 0.516	2.666 ± 1.2110
Onur Ucak Turer et al 2019		
PD	1.35 ± 0.48	1.29 ± 0.46
CAL	1.5 ± 0.6	1.6 ± 0.70
RW	0.2 ± 0.6	0.4 ± 1.2
RD	0.4 ± 0.7	0.1 ± 0.3
KTH	4.8 ± 1.2	4.0 ± 1.3
GT	1.7 ± 0.6	1.6 ± 0.7
MRC	97.1 ± 8.3	94.6 ± 11.9

Metanalysis Results:

Figure 2: Comparative evaluation of Probing Pocket depth between groups



Forest plot of Figure 2 shows that the mean pocket depth in the Experimental group was much lesser than the control group, with a mean difference of -0.16, which was statistically significant at p=0.04 as seen in Figure 6. PPD was analysed

amongst a total of 88 patients in test group and 85 in the control group. Heterogeneity levels of 89% was noted for the PPD analysis, suggesting higher methodological and clinical differences between the four studies included.

Figure 3: Funnel plot for Comparative evaluation of Probing Pocket depth between groups

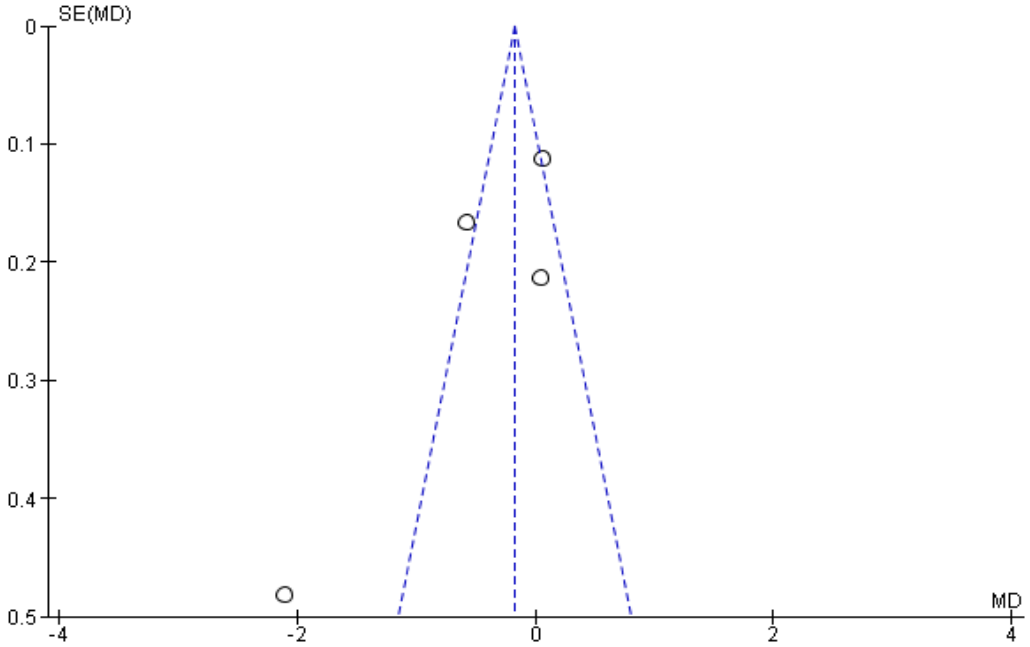
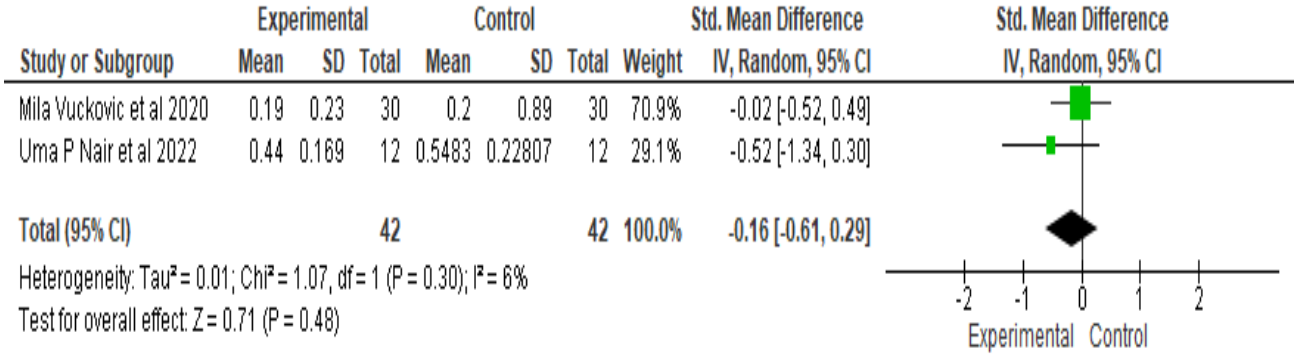


Figure 4: Comparative evaluation of Periodontal Index between groups



Two studies were analysed for the Periodontal Index between test and control groups, with 42 patients in both. Periodontal Index was higher in the control group after a follow up period as compared to the test group intervened with i- prf, but was not statistically significant at p=0.48 as seen in Figure 8.

Figure 5: Funnel plot for Comparative evaluation of Periodontal Index between groups

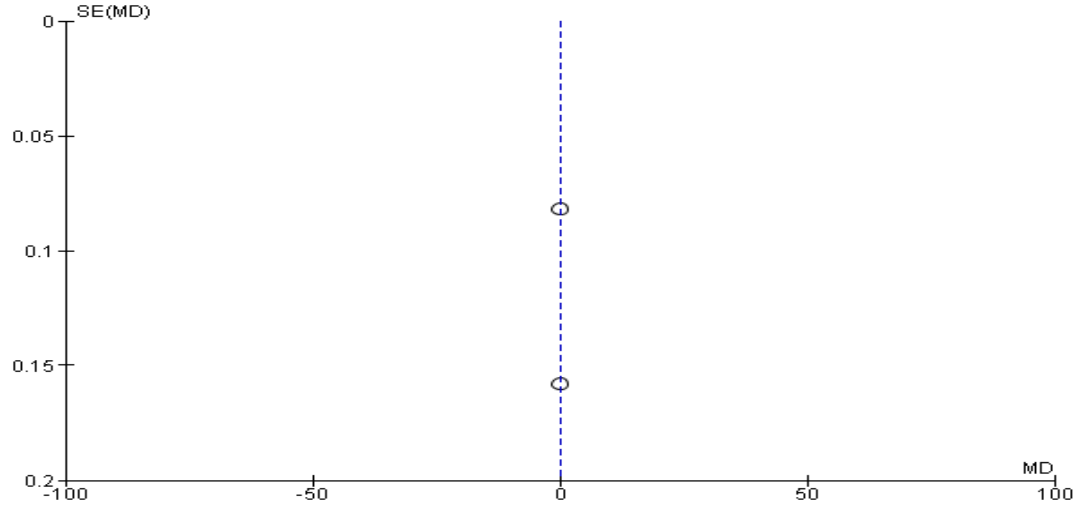
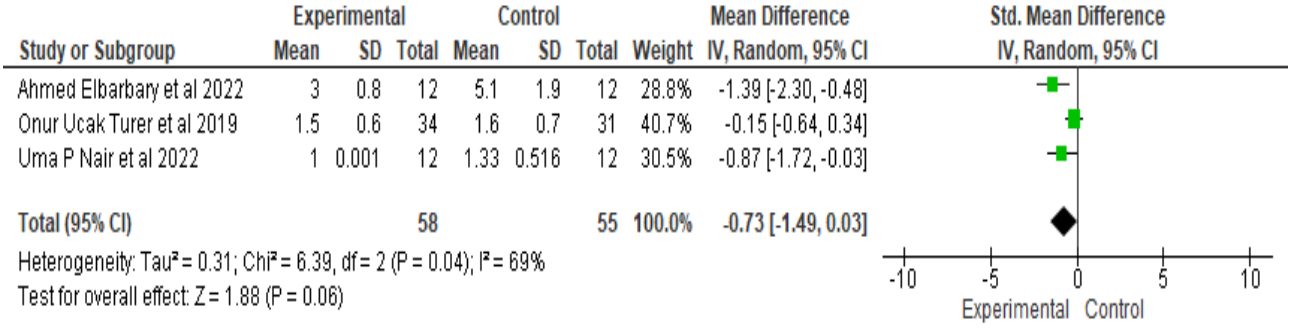
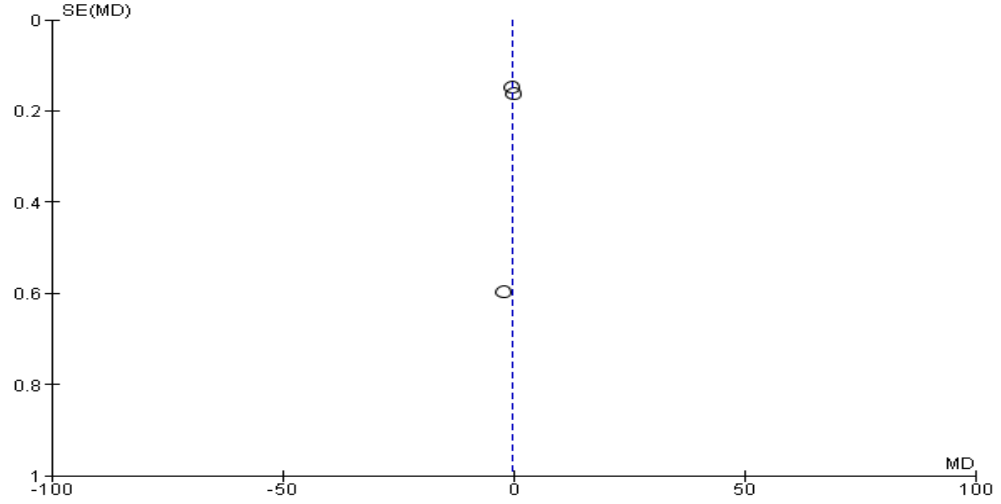


Figure 6: Comparative evaluation of Clinical attachment level between groups



Three studies were analysed for Clinical attachment level between groups. CAL scores showed a mean difference of -0.73 with experimental group having lesser scores than the control group, which was statistically significant at p=0.06 as seen in Figure 10 with 58 subjects in Experimental group and 55 in the control group.

Figure 7: Funnel plot for Comparative evaluation of Clinical attachment level between group



Cumulative results:

Probing pocket depth and Clinical attachment level was decreased in subjects treated with injectable platelet rich fibrin as compare to the control group. Periodontal Index though was decreased in the study group, was not significantly different. Overall, it can be inferred that I – PRF was better in periodontal regeneration amongst the studies analysed in combination with surgical procedures.

Discussion:

One of the greatest challenges that researchers are facing today is producing a biomaterial, which can be employed to enhance tissue regeneration with maximum predictability. Though the knowledge on tissue healing process is still insufficient, it is clear that platelets can play a significant role in the tissue regenerative procedures.

PRF was first introduced in 2001 by Choukroun et al. PRF is produced without biochemical polymerisation of the patient's blood. Choukroun's protocol allows for a greater chance of reproducible and reliable results to be obtained as it consists of a single centrifugation step. As PRF preparation uses only the patient's own blood, the possible risk of interspecies disease transmission is eliminated. Venous peripheral blood is collected into 9ml glass coated plastic tubes and then centrifuged at about 400g (3000 rpm) for 12 minutes. The fibrinogen in the blood is polymerised by centrifugal forces. Three layers are formed in the tube; red blood cells at the bottom, a PRF clot, and an acellular plasma on the surface. The PRF clot is removed and compressed to form a plateletrich fibrin membrane. Delayed preparation of the blood sample may result in diffuse polymerised fibrin within the glass tube and produce only a small clot. There are various methods of PRP production mentioned in the literature though it is frequently not possible to determine whether the protocol is producing pure platelet-rich plasma or leucocyte-rich platelet-rich plasma. The most basic outline of the PRP protocol includes centrifugation twice, at two different centrifugation forces. The centrifugation forces for the first centrifugation step vary from 160g to 3000g from 3 to 20 minutes, producing three layers: red blood cells followed by a "buffy coat", and a platelet-poor plasma layer on the surface. The platelet-poor plasma layer and the (7) "buffy coat" are transferred to a second tube and are centrifuged at a high centrifugal force. Most of the platelet-poor plasma is discarded. The fibrinogen polymerisation is aided using bovine thrombin and calcium chloride.(2) The PRGF protocol involves centrifuging the collected venous blood from the patient, in small tubes, producing three layers: red blood cells, a "buffy coat" and an upper layer of acellular plasma. The uppermost layer of acellular plasma is discarded using a pipette. The

remaining plasma in the tube is removed using eyeballing as a method of measurement. The polymerisation of the fibrin in the obtained PRGF is chemically induced with a 10% calcium chloride solution. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and leucocytes from the patient's blood sample. PRF is a reservoir of platelets, leukocytes, cytokines and immune cells. It has been found that PRF releases growth factors for at least seven days, as well as cytokines over seven days. It has also been found that PRF contains heparin and hyaluronic acid.(7) The increased density of fibrin fibres found in PRF (100 times the normal amount), may provide additional stability of the wound and promote rapid neoangiogenesis. PRF contains platelets contain α-granules which release growth factors such as platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF) and epidermal growth factor which are thought to play a vital role in angiogenesis and tissue healing. PDGF receptors are present on the gingival, the periodontal ligament and cementum. Upon activation, these receptors activate fibroblasts and osteoblasts. PRF also contains cytokines such as Interleukin-1 (IL-1), Interleukin-4 (IL-4), Interleukin-6 (IL-6) and tumor necrosis factor (TNF-α. It has also been reported that PRF enhances angiogenesis and supports immunity. The growth factors present in platelet-rich fibrin stimulate stem cells and attract them to the injury site, inducing angiogenesis and osteogenesis. PRF traps stem cells that are circulating in the blood. The low level of thrombin in PRF allows for optimal migration of endothelial cells and fibroblasts, promoting angiogenesis. PDGF induces migration and proliferation of mesenchymal stem cells, and an angiogenic effect. TGF-β stimulates osteoblast proliferation, the production of collagen type 1 and woven bone, and stimulates angiogenesis. Insulin like growth factor (IGF-1) and fibroblast growth factor (FGF) both encourage the proliferation of osteoblasts and enhance wound healing. PDGF originates from platelets and macrophages, TGF-β originates from platelets and lymphocytes, and IGF-1 originates from osteoblasts and macrophages.

Various platelet-rich concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have been proposed and utilized for tissue regeneration in several *in vitro* and *in vivo* studies. Nevertheless, PRF has many benefits over PRP, including easy handling, low cost, and the lack of anticoagulant or bovine thrombin, which reduces biochemical alteration and risks associated with the use of bovine thrombin. For about three decades, PRF has been used for regenerative purposes in dentistry. Additionally, PRF has the potential to be used in fields other than dentistry, such as maxillofacial surgery and orthopedic surgery.

In 2014, by adjusting spin centrifugation forces, injectable platelet-rich fibrin (I-PRF) was developed. The blood centrifuged in non-glass centrifugation tubes at lower centrifugation speeds resulted in a flowable PRF called I-PRF. I-PRF is a newly formed platelet concentrate enriched with leukocytes which can promote both soft and hard tissue regeneration phenomena . Since I-PRF remains liquid for roughly 15 min, it will provide dental practitioners with a further practical form of PRF . Following application, the human liquid fibrinogen in I-PRF is gradually transformed to a growth factor-rich PRF clot, which releases continuously over 10–14 days.

Up to now, several *in vitro* and *in vivo* studies have been carried out concerning the role of I-PRF in the enhancement of wound healing, the acceleration of orthodontic tooth movement, and the regeneration of bone, periodontal, cartilage, and pulp tissues. On this basis, I-PRF is able to enhance the potential of intrinsic tissue regeneration by inducing human mesenchymal stem cells (MSCs) proliferation and migration, and by triggering osteogenic differentiation of MSCs. I-PRF has also been reported to have greater anti-inflammatory and anti-microbial activity against many pathogens, which can contribute to faster tissue regeneration. On the other hand, I-PRF is commonly used in regenerative dentistry as an injectable biomaterial, as a carrier for various biomolecules, or in conjunction with other biomaterials for a variety of clinical applications. Clinicians have recently used this method to facilitate the agglomeration or coating of biomaterials in order to improve the healing process of both soft and hard tissue.

Strengths and limitations of evidence:

Due to the limited number of papers and small sample sizes, no pre-operative factors (gender, dental arch type, gradation of periodontal diagnosis) were found to have statistical impact on tooth survival.

But the greatest strength of this metanalysis being the inclusion of randomised clinical trials done with good quality studies.

Conclusion:

The development of PRF innovations like i-PRF has made it possible to utilise platelet concentrates for various applications. A significant release of growth factors, either alone or in conjunction with biomaterials, affects osteoblastic behaviour. It has the ability to efficiently transform an osteoconductive graft into an osteopromotive one due to the presence of growth factors and platelets. As a result, i-PRF has shown to be a modern technology that produces dependable results in the field of dentistry. To ascertain the cell differentiation activity of i-PRF component, more investigation should be needed. Undetermined is the exact methodology for ideal preparation and how to apply it to mucogingival surgery.

References:

1. Gottlow J, Nyman S, Lindhe J, Karring T, Wennström J. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. J Clin Periodontol. 1986 Jul;13(6):604-16. doi: 10.1111/j.1600-051x.1986.tb00854.x. PMID: 3462208.

2. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. Ann Periodontol. 2003 Dec;8(1):227-65. doi: 10.1902/annals.2003.8.1.227. PMID: 14971256.
3. Chen FM, Shelton RM, Jin Y, Chapple IL. Localized delivery of growth factors for periodontal tissue regeneration: role, strategies, and perspectives. Med Res Rev. 2009 May;29(3):472-513. doi: 10.1002/med.20144. PMID: 19260070.
4. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol. 2010 Apr;81(4):546-55. doi: 10.1902/jop.2009.090531. PMID: 20373539.
5. Everts, Peter & Knape, J.t.a & Weibrich, Gernot & Schönberger, Jacques & Hoffmann, Johannes & Overdevest, Eddy & Box, Henk & Van Zundert, André. (2006). Platelet-Rich Plasma and Platelet Gel: A Review. The Journal of extra-corporeal technology. 38.
6. Tang, Y., Yeaman, M.R., & Selsted, M.E. (2002). Antimicrobial Peptides from Human Platelets. *Infection and Immunity*, *70*, 6524 - 6533.
7. Vučković, M., Nikolić, N., Milašin, J., Đorđević, V., Milinković, I., Asotić, J., Jezdić, Z., Janković, S., & Aleksić, Z. (2020). The effect of injectable platelet-rich fibrin use in the initial treatment of chronic periodontitis. Srpski Arhiv Za Celokupno Lekarstvo, 148(5–6), 280–285
8. Elbarbary, Ahmed & Reda, Ahmed & ELaziz, Ahmed. (2022). Evaluation of the Addition of Injectable Platelet Rich Fibrin to Xenograft in Management of Periodontal Intraosseous Defects. "Randomized Controlled Trial". Al-Azhar Dental Journal for Girls. 9. 321-330. 10.21608/adjg.2022.111166.1461.
9. Nair, Uma & Shivamurthy, Ravindra & Nagate, Raghavendra & Chaturvedi, Saurabh & Al-Qahtani, Saad & Magbol, Mohammad & Gokhale, Shankar & Tikare, Shreyas & Chaturvedi, Mudita. (2022). Effect of Injectable Platelet-Rich Fibrin with a Nano-Hydroxyapatite Bone Graft on the Treatment of a Grade II Furcation Defect. Bioengineering. 9. 602. 10.3390/bioengineering9110602.
10. Turer, Onur & Ozcan, Mustafa & Alkaya, Bahar & Surmeli, Seren & Saydaoglu, Gulsah & Haytac, Mehmet. (2019). Clinical evaluation of injectable platelet rich fibrin with connective tissue graft for the treatment of deep gingival recession defects. A controlled randomized clinical trial. Journal of Clinical Periodontology. 47. 10.1111/jcpe.13193.