EVALUATION OF FIBRIN NETWORK PATTERN CHANGES OF PLATELET RICH FIBRIN AND TITANIUM PREPARED PLATELET RICH FIBRIN OF INDIVIDUALS WITH AND WITHOUT PERIODONTITIS - A CELL BLOCK CYTOLOGY

Dissertation submitted to

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In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTICS

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THE TAMILNADU Dr MGR MEDICAL UNIVERSITY, CHENNAI.

DECLARATION BY THE CANDIDATE

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Introduction

Introduction

Periodontitis is a chronic infectious disease of the supporting tissues of the teeth affecting approximately 10% of the population which may lead to loss of teeth[1].The pocket formation and ulceration of the epithelial lining which is formed during inflammation of periodontal tissues form ports of entry which may lead to transient bacteremia[2, 3]. Periodontal disease is a low grade systemic inflammation leading to increase in number of platelet activation, [4] and platelet numbers decrease after periodontal therapy[5]. Interestingly Porphyromonas gingivalis and Streptococcus sanguis induce platelet activation and aggregation in vitro and in animal studies[6].

The ultimate goal of periodontal treatment is halting periodontal disease progression and regeneration of tissues which was destroyed as a result of periodontal disease. Attaining a complete periodontal regeneration with this current regenerative procedures offer a limited success.[4]Various biomaterials based on endogenous regenerative technology (ERT) have been used for periodontal tissue regeneration in addition to autogenous[5] and allogenic bone grafts. Platelets have important role in hemostasis and wound healing along with various growth factors. Hence lead to the evolution of platelet concentrates into existence for periodontal regeneration. Initially fibrin glue was originally described in 1970[7] and these platelet rich plasma by ***Marx et al 1998***, contains fibrin, fibronectin and vitronectin which leads a cell to osteoconduction& epithelial migration, but it is least favorable to cytokine and cellular migration.

Thus second generation platelet concentrates Platelet Rich Fibrin was developed by ***Choukroun et al 2001***, It is a polymerized fibrin matrix in a tetra molecular structure with >97% of the platelets, cytokines, leuckocytes & circulating stem cells.[13] It is based on simple strategy of enhancing healing capacity of natural blood clot by supplementing the natural blood clot with growth factors. The third

Introduction

generation of platelet concentrate Titanium PRF was introduced by ***Tunali et al 2013*** by using biocompatible material titanium. In titanium prepared PRF, fibrin network pattern are well organized and appeared thicker compared to PRF.

Various factors that influence the fibrin network pattern of PRF & T-PRF are genetic factors, acquired factors like variations in XIII & thrombin in plasma hyperchromocystemia, blood flow, platelet activation, hypertension, strain hyperglycemia, medication, oxidation, and cigarette smoking[9].Thus the fibrin network pattern of this platelet concentrate can be changed due to periodontal disease. The aim of present study is to evaluate the various fibrin network patterns of PRF & T-PRF in patients with & without periodontitis.

Aim and Objectives

AIM

- To compare fibrin network pattern changes of platelet rich fibrin and titanium prepared platelet rich fibrin in healthy, gingivitis, generalized chronic periodontitis and generalized aggressive periodontitis patients.

OBJECTIVES

- Preparation of platelet rich fibrin and titanium prepared platelet rich fibrin by centrifugation.
- To compare the variation in the fibrin network pattern of platelet rich fibrin and titanium prepared platelet rich fibrin clot by histological analysis.
- To compare the variation in the fibrin network pattern of healthy, gingivitis and generalized chronic periodontitis and generalized aggressive periodontitis patients.

Review of literature

Tomoyuki Kawase et al (2003)[10] investigated PRP's action on extracellular matrix production in periodontal ligament (PDL) and osteoblastic MG63 cell cultures and found Gel-like material rapidly (<30 minutes) formed in cultures of either PDL or osteoblastic MG63 cell cultures after addition of PRP (≥0.5%). PRP changed cell shape and up-regulated type I collagen at 24 hours. Fibrinogen was detected in the PRP preparations and insoluble fibrin networks were found in the newly formed gel-like material. PRP's action on collagen synthesis was mimicked by purified fibrinogen and blocked by thrombin inhibitor. Thrombin was expressed both in PDL and MG63 cells and concluded these findings demonstrated that the gel-like material formed in cell cultures of either PDL or MG63 cells is fibrin clot that helps in collagen synthesis in the extracellular matrix and promote wound healing.

David M. Dohan et al (2006)[11] evaluated the biochemical properties of generations of surgical additives, fibrin glues, (cPRP) and PRF. The 3-dimensional fibrin architecture is dependent on artificial clinical polymerization processes, such as massive bovine thrombin addition. The fibrin network of PRF preparation was similar to the natural one which leads to organized cell migration and proliferation.

David M. Dohan et al (2006)[12] investigated the PRF, platelets activation and their cytokine release. He quantified TGFb-1, PDGF-BB and IGF-I within PPP (platelet-poor plasma) and PRF clot exudate serum. The incorporation of platelet cytokines and glycanic chains in the fibrin meshes were found during PRF processing. Theses cytokine release during PRF preparation will explain the healing properties of PRF.

David M. Dohan et al (2006)[13] investigated the immune features of PRF and quantified proinflammatory cytokines (IL-1b, IL-6, and TNF-a), (IL-4) an anti-inflammatory cytokine, and (VEGF). This explains the immune regulating properties of PRF which helps in reducing postoperative infections.

Joseph Choukroun, et al (2006)[14] investigated biology of PRF to determine the potential fields of clinical applications. The fundamental events angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization were concentrated. It plays an important role in neovascularization, wound closing, tissue remodelling. This initial research helps in future PRF applications, including bone surgery.

Joseph Choukroun et al (2006)[15] nine sinus floor augmentations were performed. In 6 sites, PRF with FDBA particles, and FDBA without PRF was used in 3 sites. After 4 months and 8 months, bone specimens were harvested and treated for histologic analysis. It revealed the newly formed bone and connective tissue. The results were same for both groups. PRF with FDBA for Sinus floor augmentation leads to a reduction of healing time prior to implant placement. This healing time could be reduced to 4 months.

Hyeon-Jung Lee et al (2007)[16] evaluated the use of autogenous bone in combination with platelet-enriched fibrin for maxillary sinus augmentation with simultaneous implant placement 12 sinuses in dogs bilaterally. In the right sinus, autogenous bone mixed with platelet-enriched fibrin glue was grafted. In the left sinus, autogenous bone alone was grafted in the control group. 2 dental implants were inserted into the grafting material. After 6 months the animals were killed and examined. He concluded that it showed enhanced Osseo integration of dental implants and increased height of new bone.

Antoine Diss et al (2008)[17] Implants were placed using Platelet rich fibrin in the bone-added osteotome sinus floor elevation (BAOSFE) technique. The survival rate at abutment tightening (6 to 12 weeks of healing) and at 1 year was calculated. The radiographic analysis determined the mean residual bone height (RBH) at implant

placement, the change in bone level. He assessed the endosinus bone changes in the mesial and distal implant sides at 1 year. The BAOSFE procedure with Platelet rich fibrin can lead to an endosinus bone gain. Despite a limited RBH, a healing period of 2-3 months was found to be sufficient during abutment tightening. At 1 year, formation of a new recognizable bone structure delimiting the sinus floor was identified.

Chung-Hung Tsai et al (2009)[18]investigated the biologic effects of PRF on human gingival fibroblasts (GFs), periodontal ligament (PDL) cells, oral epithelial cells, and osteoblasts in 10 healthy volunteers. Blood collected and centrifuged at 3000 rpm for 12 minutes with a PC-02 table Centrifuge. The U2OS osteoblast cell line was examined by trypan blue and tetrazolium bromide reduction assays to view the cell viability and proliferation. PRF did not interfere with cell viability,but stimulated cell proliferation of osteoblasts (135% of the control), PDL cells (130% of the control), and GFs (120% of the control) during a 3-day culture period. It suppressed oral epithelial cell growth to as low as 80% of the control. In phase-contrast microscopy, GFs, PDL cells, and osteoblasts were attached at the margins of PRF. He suggested that PRF modulates cell proliferation in a cell type specific manner and its actions may be beneficial for periodontal regeneration.

David M. Dohan Ehrenfest et al (2010)[19] examined the processed PRF by light microscopy and scanning electron microscopy. Depending on the centrifugation forces approximately platelets 97% and leukocytes>50% were concentrated and showed a specific three-dimensional distribution, beyond the red blood cell base, in first few millimeters, Platelets and fibrin formed large clusters of coagulation. The fibrin network was very mature and dense. Moreover, the PRF architecture between groups using the different tested collection tubes and compression techniques no

difference was found, even if these two parameters could have influenced the growth factor content and biologic matrix properties.

Lekovic V et al (2011)[20] used 17 paired intrabony defects were treated either with PRF or with PRF–Bovine porous bone mineral (BPBM) combination. Changes in defect fill, pocket depth, attachment level and were examined. The results showed that PRF can improve clinical parameters associated with human intrabony periodontal defects, and BPBM reinforces effect of PRF in periodontal regeneration.

Anuj Sharma et al (2011)[21] used 36 mandibular grade II furcation defects were randomly treated either with autologous PRF and OFD or OFD. Sulcus bleeding index, relative vertical and horizontal clinical attachment level Plaque index, probing depth, gingival marginal level, and radiographic bone defect were recorded at baseline and after 9 months. All clinical and radiographic parameters showed statistically significant improvement at the treated sites.

Thorat MK et al (2011)[22] examined thirty-two intra-bony defects (one site/patient) and treated either with PRF or a conventional open flap debridement alone. Clinical parameters and radiographic features were recorded at baseline and after 9 months. In both the groups, intra-bony defect fill was calculated on standardized radiographs by software. Clinical parameters were improved and greater intra-bony defect fill at sites treated with PRF group.

Kai-Chiang Yang et al (2011)[23] in a miniatures wine unerupted second molar tooth buds were obtained and cultured in vitro for 3 weeks to obtain dental bud cells (DBCs). Platelet rich fibrin and fibrin glues were prepared from blood before surgery. The original alveolar sockets were filled with the DBC-fibrin glue-PRF composite. After 36 weeks of implantation, radiographic and histological examinations and Immunohistochemical staining were used to examine the regenerated tooth structure

and to detect tooth regeneration specific proteins. Complete crown, root, pulp, enamel, dentin, odontoblast, cementum, blood vessels, and periodontal ligaments in indiscriminate shape were developed in one pig. Dentin matrix protein, Cytokeratin 14, vascular endothelial growth factor, and osteopontin were found in another animal with an unerupted tooth. This study showed a complete tooth regenerated by DBCs seeded into fibrin glue-PRF.

Y-C Chang et al (2011)[24] evaluated the effects of PRF on PDLFs were determined by measuring the expression of osteoprotegerin (OPG),phosphorylated extracellular signal-regulated protein kinase (p-ERK), and alkaline phosphatase (ALP) activity. He studied the feasibility and safety of regeneration of infrabony defects. PRF increased p-ERK, OPG and ALP expression which helps in periodontal regeneration.

Sashwati Roy et al (2011)[25] showed the kinetics of the viability of platelet-embedded fibrin matrix. PRFM released growth factors was observed. The endothelial mitogenic response via extracellular signal-regulated protein kinase activation pathway by vascular endothelial growth factor released from PRFM. This study showed that chronic wounds were with proliferation of endothelial cells and enhanced wound angiogenesis.

Gulnihal Eren et al (2012)[26] assigned CAF +PRF (test) or CAF+SCTG (control) sites in a 23-year-old female patient with bilateral gingival recessions in maxillary cuspids. Clinical parameters and clinical photographs were taken at baseline; 1, 3, and 6 months; and 1 year. Improved root coverage, gingival thickness, and keratinized tissue width were resulted in both sites and remained stable for 1 year. PRF seems to be eliminates the requirement of a donor site.

C-L Wu et al (2012)[27] determined the effects on cell attachment, proliferation, phosphorylated Akt, heat shock protein 47 (HSP47) and lysyl oxidase (LOX)

expression on human osteoblasts measured with WST-1 and alamar blue respectively. PRF stimulate U2OS cell attachment compared with untreated controls and increase osteoblast proliferation during a 5-day incubation period ,increase Akt phosphorylation in a time-dependent manner, Collagen-related proteins HSP47 and LOX were significantly elevated by stimulation with PRF compared with untreated controls. He suggested that PRF is capable of enhancing osteoblast attachment, proliferation and simultaneously upregulating collagen-related protein which promoteregeneration of bone.

A. R Pradeep et al (2012)[28] 90 intrabony defects were treated either with PRF with open flap debridement or autologous PRP with open flap debridement or open flap debridement alone. After 9 months probing depth (PD), clinical attachment level (CAL), intrabony defect depth and % defect fill were recorded. Both groups showed similar results.

Mustafa Tunali et al (2013)[29] [90]developed a new titanium-prepared, platelet-rich fibrin (T-PRF) with the protocol for preparation, which is based on the presumption that platelets can be activated by titanium tubes may be more effectual than the glass tubes used by Choukroun in his platelet-rich fibrin (PRF) method and within 30 days of treatment with T- PRF induced the new bone formation with connective tissue which helps in wound healing.

Bajaj P et al (2013)[30] investigated mandibular degree II furcation defects with either Platelet Rich Fibrin with open flap debridement (OFD; 24defects) or autologous PRP with OFD (25), or OFD alone (23) in seventy-two patients. All clinical and radiographic parameters relative vertical clinical attachment level and horizontal clinical attachment level, probing depth, along with gingival marginal level showed

statistically significant improvement at both the test sites compared to open flap alone group.

Jayant R Ambulgekar et al (2014)[31] stated that Aggressive periodontitis causes rapid destruction of the periodontal attachment apparatus & the supporting alveolar bone. In aggressive periodontitis early diagnosis and treatment is critically important because preventing further destruction is often more important than attempting to regenerate lost supporting tissues. An organised treatment plan is required. A critical aspect of periodontal regeneration is the stimulation of the series of events & cascades at some point, which can result in the coordination & completion of integrated tissue formation. The growth factors in platelet rich fibrin are involved in wound healing & which helped in tissue regeneration. It is both nontoxic & non-immune reactive.

Ashish Agarwal et al (2015)[32] determined the addition of PRF with a DFDBA in the treatment of intrabony periodontal defects in sixty patients. After 12 months, resulted in significant changes in all clinical and radiographic parameters and concluded that a combination of PRF and DFDBA is more effective than DFDBA with saline for the treatment of infrabony periodontal defects

Mustafa Tunali et al (2015)[33]developed platelet-rich product- titanium prepared platelet-rich fibrin (T-PRF). T-PRF is based on the presumption that platelets can be activated by titanium more effectively than the silica activators used with glass tubes in Chouckroun's platelet-rich fibrin (PRF) and decided that T-PRF membrane can be successfully used for augmentation of bone.

Pradeep et al (2015)[34] evaluated the efficacy of PRF, 1% Metformin gel and PRF+1%MF gel, with open flap debridement (OFD), in the treatment of intrabony defects in chronic periodontitis (CP) patients and found Platelet Rich Fibrin, 1%MeforminF and Platelet Rich Fibrin+1% Metfromin groups showed significant

probing depth reduction and relative attachment loss gain than open flap debridment group. In PRF+1% MF group showed probing depth reduction and mean RAL gain as compared to PRF alone or MF alone at 9 months. He concluded PRF+1% MF group showed greater enhancements in clinical parameters with higher percentage intrabony defect depth reduction in CP patients.

Ana B. Castro et al (2016)[35] analyzed the regenerative potential of Leucocyte- and Platelet Rich Fibrin (L-PRF) during periodontal surgery. Databases were collected from an electronic and hand search and reviewed the randomized clinical trials only. Pocket depth (PD), clinical attachment level (CAL), bone fill, and keratinized tissue width (KTW), recession reduction, and root coverage (%) were considered as outcome. When possible, meta-analysis was performed. Twenty-four articles fulfilled the inclusion and exclusion criteria. Meta-analysis was performed in these three subgroups intrabony defects (IBDs), furcation defects, and periodontal plastic surgery. Significant PD reduction (1.1±0.5mm, $p<0.001$), CAL gain (1.2±0.6mm, $p<0.001$), and bone fill (1.7±0.7mm, $p<0.001$) were found when comparing L-PRF to open flap debridement (OFD) in IBDs. For furcation defects, significant PD reduction (1.9±1.5mm, $p=0.01$), CAL gain (1.3±0.4mm, $p<0.001$), and bone fill (1.5±0.3mm, $p<0.001$) were reported when comparing L-PRF to OFD. When L-PRF was compared to a connective tissue graft, were recorded for PD reduction (0.2±0.3mm, $p>0.05$), CAL gain (0.2±0.5mm, $p>0.05$), KTW (0.3±0.4mm, $p>0.05$), and recession reduction (0.2±0.3mm, $p>0.05$). PRF enhances periodontal wound healing.

Jane K. Chadwick et al (2016)[36]Thirty-six patients completed the study protocol. Each patient contributed a single intrabony defect, which was randomized to receive either DFDBA or PRF. Clinical and standardized radiographic data were collected at baseline and 6 months after treatment. Primary outcomes measures included (1)

radiographic bone fill as measured from the CEJ to base of bony defect, and (2) change in clinical attachment level (CAL). Both treatment groups had significant gains in CAL as well as bone fill, with no significant differences in outcomes between groups. Treatment of intrabony defects with either DFDBA or PRF resulted in a significant gain in CAL as well as bone fill after 6 months of healing, with no significant difference between materials.

Santosh S. Martande et al (2016)[37] used PRF with Atrovastatin in bone defects of ninety six individuals and they were grouped as OFD with PRF, OFD with PRF+1.2% Atrovastatin and OFD alone. Clinical parameters probing depth (PD), relative attachment level (RAL),site specific plaque index (PI), modified sulcus bleeding index (mSBI), and gingival marginal level (GML) were examined at baseline before surgery and 9 months post-operatively. Intra-bony defect depth reduction was evaluated radio graphically at baseline and 9 months.PRF+1.2% ATV and PRF alone showed significantly greater PD reduction and RAL gain as compared to OFD alone at 9 months. Platelet Rich Fibrin+1.2% ATV showed same refinement in clinical parameters with greater reduction in percentage radiographic defect depth as compared to PRF alone in treatment of intrabony defects in CP individuals. Thus 1.2% Atrovastatin was unsuccessful to augument the regenerative potential of PRF alone inintrabony defects.

A R Pradeep et al (2016)[38] investigated total of 90 Intrabony defects and were randomly assigned to one of the 3 treatment groups: 1) OFD alone, 2) OFD + PRF and 3) OFD + PRF + 1.2% Rosuvastatin (RSV) gel placement. Periodontal parameters: probing depth (PD), clinical attachment (CA) level, Plaque index (PI), modified sulcus bleeding index and IBD depth were recorded at baseline and at 9 months. Significant PI and mSBI reductions were observed in all the 3 groups. PRF

placement significantly enhanced the improvements in periodontal parameters than OFD alone. 1.2% RSV with PRF results in significantly higher periodontal regeneration compared to OFD alone or with PRF.

Mustafa Tunali et al (2016)[39] conducted preliminary study of a new centrifugation method, which aimed to change the direction of fibrin formation during the platelet aggregation and make T-PRF much denser and more resistant. According to this hypothesis, it is possible to use in guided bone, and guided tissue regeneration more successfully and concluded that the pilot study defines 10 min MT-PRF as a new autogenous product with superior fibrin network and results showed that, fibrin formation was made more organised and denser with 2-way direction centrifugation.

Gulbaharustaoglu et al (2016)[40] determined the clinical effects of titanium prepared, platelet rich fibrin on human palatal mucosal healing (PMWH) and to identify its effect on time –dependent changes in palatal soft tissue thickness(PSTT) in terms of histoconduction**,** free gingival graft donor sites were treated with T-PRF and compared with an untreated control group. Colour match, complete epithelisation were recorded on days 3, 7, 14, and 21. Pain level and bleeding status were recorded for the first 7 days. PSTT was measured at baseline and after 1 and 6 months.

Humeyra Aydemir Turkal et al (2016)[41] treated infrabony (IBD) defects in chronic periodontitis with enamel matrix derivative alone and with enamel matrix derivative with platelet rich fibrin using split mouth design. Clinical and radiographic measurements were recorded at baseline (BL) and at 6 months following therapy. Both therapies showed in significant improvement in Intra bony defect treatment. Addition of PRF did not improve the clinical and radiographic outcomes.

Masako Fujioka-Kobayashi et al (2017)[42] studied characterizes how centrifugation speed (G-force) along with centrifugation time influence growth factor release from fibrin clots, as well as the cellular activity of gingival fibroblasts exposed to each PRF matrix and found the low-speed concept (A-PRF, A-PRF+) demonstrated a significant increase in growth factor release of transforming growth factor (TGF)-b1, platelet-derived growth factor (PDGF), epidermal growth factor, and insulin-like growth factor, with A-PRF+ being highest of all groups. Although all PRF formulations were extremely biocompatible due to their autogenous sources, both A-PRF and A-PRF+ demonstrated significantly higher levels of human fibroblast migration and proliferation compared with L-PRF. Furthermore, gingival fibroblasts cultured with A-PRF+ demonstrated significantly higher messenger RNA (mRNA) levels of PDGF, TGF-b, and collagen1 at either 3 or 7 days and concluded the modifications to centrifugation speed and time with the low-speed concept favor an increase in growth factor release from PRF clots. This, in turn, may directly influence tissue regeneration by increasing fibroblast migration, proliferation, and collagen mRNA levels. Future animal and clinical studies are now necessary.

Kazushige Isobe et al (2017)[43] studied the mechanical and chemical characterization of fibrin clot membranes prepared from advanced platelet-rich fibrin (A-PRF) or concentrated growth factors (CGF). They have been used as bioactive barrier membranes for regeneration of alveolar bone tissue because of their rapid biodegradability which have and found out when A-PRF and CGF are prepared by essentially similar mechanisms. Both membranes have identical mechanical and chemical properties. It was mechanically weaker and degradable which was prepared by a different mechanism.

Pavan Bajaj et al (2017)[44] explored the efficacy of PRF in treatment of 54intrabony defects in 17 patients with aggressive periodontitis and compared with Open Flap Debridment technique alone. After 9 months, Clinical and radiological parameters such as probing depth, clinical attachment level, intrabony defect depth and % defect were significantly greater in PRF as compared to control group. Greater bone fill was observed in PRF group than conventional OFD alone.

David M. Dohan Ehrenfest et al (2017)[45] evaluated the mechanical vibrations appearing during centrifugation in four models of commercially available table-top centrifuges used to produce L-PRF and studied the influence of the centrifuge characteristics on the cell and fibrin architecture of a L-PRF clot and membrane and also evaluated how changing some parameters of the L-PRF protocol may influence its biological signature, independently from the characteristics of the centrifuge and concluded that the centrifuge characteristics and centrifugation protocols have a very significant effect on the cell, growth factors and fibrin architecture of a L-PRF clot and membrane and that any modification of the original L-PRF material and method shall be clearly investigated and identified separately from the original methods, in order to avoid creating confusion and inaccurate results in the literature.Thus emphasized that periodontitis can be a contributing factor in the development of cardiovascular disease.

Taner Arabacı et al (2017)[46] evaluated benefaction of platelet-rich fibrin (PRF) with conventional flap surgery on growth factor levels in gingival crevicular fluid (GCF) and periodontal healing and concluded PRF membrane combined with open flap debridement OFD provides significantly higher GCF concentrations of angiogenic biomarkers for 2 to 4 weeks and better periodontal healing in terms of conventional flap sites.

J. Du et al (2017)[47]studied the efficacy and influence of aspirin in local defects and the use of platelet-rich fibrin (PRF) in periodontal defects were investigated. He studied delivery system to carry sustained-release aspirin/salicylic acid to improve regeneration of alveolar bone and can be used as suitable scaffold a was determined and found the PRF/aspirin complex provided a sustained-release aspirin/salicylic acid. High concentrations were found at 4 hours after transplantation and were maintained to 48 hours at 37°C; the total concentration of aspirin/salicylic acid of 83.5 mg/mL was released, respectively. Periodontal ligament mesenchymal cells proliferation and migration were enhanced. Micro-computed tomography and histological data showed that both groups enhanced formation of bone. Moreover, the new bone formation was two times greater in the PRF/aspirin complex group than the PRF group and concluded Aspirin/salicylic acid could be sustained-released from PRF/aspirin complex, which could increase the role of mesenchymal cells and hamper inflammation. This study might provide a new safe and easy clinical treatment procedure to improve periodontal bone regeneration.

Anirban Chatterjee et al (2017)[48] compared the variation in fibrin network pattern of titanium platelet-rich fibrin and platelet-rich fibrin in smoker participants and hypertensive patients through histological analysis. The fibrin clot pattern of PRF and TPRF in hypertensive and smoker participant varied as compared to healthy participants, but showed a better organization of network and increased entrapment of cellular components were seen in TPRF clot.

Umesh Pratap Verma et al (2017)[49] gathered systematic reviews from January 2006 to August 2016 highlighting PRF for soft and hard tissue regeneration and/or wound healing, in various studies like in vitro, animal, and clinical studies utilizing PubMed electronic database. He confirmed that PRF is a remedial regenerative biomaterial that

has vast clinical applications in general surgeries as well as dental perspectives. The use of PRF alone or in combination with other biomaterials (such as bone grafts, soft tissue grafts, and pharmacologic agents) given safe and guaranteed results in the enhancements of clinical and radiographic parameters in the treatment of periodontal osseous defects, PRF also provided an added advantage in enhancing in gingival tissue width and thickness (gingival biotype).

Eva Dohle et al (2017)[50]presented autologous PRF-based matrices generated by LSCC a very beneficial therapeutic tool by simply profiting from the natural conditions in the human body. Using PRF containing blood plasma, platelets and leukocytes for tissue engineering purposes results in the initiation of wound healing processes in the established co-culture system of primary osteoblasts (pOBs) and outgrowth endothelial cells (OECs)*in vitro* with special attention to the improvement of the process of angiogenesis.

Makoto Horimizu1 et al (2017)[51] evaluated the combined use of hCP human – cultured alveolar bone derived periosteal sheets with PRF complex could facilitate bone regeneration synergistically was evaluated in nude mice. At 4 weeks post implantation, new bone formation was evaluated by using μCT. Cell growth and neovascularization were evaluated by histochemical and immunohistological methods. In the subcutaneous tissue, mineral deposit formation, collagen deposition, and number of vessels were higher in the hCP + PRF group than in the hCP alone group. At 4 weeks post implantation, new bone formation was evaluated by using μCT. Cell growth and neovascularization were evaluated by histochemical and immunohistological methods. In the subcutaneous tissue, mineral deposit formation, collagen deposition, PCNA-positive cells and number of vessels were higher in the

hCP + PRF group than in the hCP alone group. In the calvarial defect models, new bone formation was significantly higher in the hCP + PRF group.

Shantipriya Reddy et al (2018)[52] examined the clinical and radiographic outcomes of two intrabony defects treated with titanium prepared platelet rich fibrin, after 6 months followup revealed a notable reduction in Probing Depth (PD) and Relative Attachment Level (RAL) gain as well as radiographic intrabony defect depth reduction and increase in bone density helping the role of various growth factors present in the T-PRF in hasten the soft and hard tissue healing.

Taner Arabaci et al (2018)[53] investigated the periodontal outcomes in chronic periodontitis patient treated with titanium-prepared platelet-rich fibrin (T-PRF) combined with open flap debridement (OFD) on biological markers in gingival crevicular fluid (GCF) and clinical outcomes in twenty-nine participants. At baseline and 2, 4, and 6 weeks postoperatively, GCF growth factor levels and relative receptor activator nuclear factor kappa-B/osteoprotegerin (RANKL/OPG) ratio were analysed, and after 9 months, RAL gain, mean PD reduction, and GML change were notably higher in the OFD+T-PRF sites. After 2 weeks, both groups showed increased growth factor levels, followed by reductions at weeks 4 and 6. After 6 week post-surgery, GCF growth factor levels in the test group were seen at higher concentrations with respect to control group. Relative RANKL/OPG ratio was found significantly decreased in the OFD+T-PRF group compared to the OFD group at 6-week period.

Anil Kumar et al (2018)[54] explored the effect of PRF/BCP on differentiation and survival of osteoclasts obtained from peripheral blood of Chronic Periodontitis patients and compared with healthy patients. He assessed the number of osteoclasts by Tartrate acid resistant acid phosphatase (TRAP)-positivity. The mechanism of apoptosis was studied with reference to expression of Bcl-2, Bax, Bcl-xL, Nuclear

Factor Kappa-light chain enhancer of activated B cells (NF-κB), caspase 3/9 and Deoxyribonucleic acid (DNA) fragmentation. The results showed PRF/BCP displayed an inhibitory role in osteoclasts formation and its molecular mechanism of action was related to the apoptosis induction through intrinsic mitochondrial pathway.

Ishita Wanikar et al (2018)[55] treated 40 bilateral grade II furcation defects with 1% Alendronate gel in combination with Platelet Rich Fibrin (Platelet Rich Fibrin+ Alendronate) and Platelet Rich Fibrin alone and at baseline, 3 and 6 months clinical and radiographical parameters were measured, in PRF with 1% Alendronate gel treated defects exhibited better outcomes suggestive of enhanced periodontal regeneration.

Materials and Methods

Materials and Methods

A comparative study of histological features of platelet rich fibrin and titanium prepared platelet rich fibrin in healthy, gingivitis and generalized chronic and aggressive periodontitis patients. This study was assessed and accepted by institutional ethics committee. The entire study and related agendas were explained to the patients and written informed consent was obtained from all the patients before the commencement of the study. A total of 60 patients were enlisted from the Department of Periodontics of Vivekanandha Dental College for Women, Tamil Nadu, depending on the following criteria.

INCLUSION CRITERIA

- Systemically healthy patients.
- Age 20-60years.
- Normal platelet count of 150,000–450,000 per microliter
- Varying degree of periodontal health status (gingivitis and periodontitis).

EXCLUSION CRITERIA

- Tobacco Smoking or chewing habits.
- Platelet & coagulation disorder.
- Systemic diseases/conditions & medications affecting blood.
- H/O antibiotics use or periodontal therapy within past 6 months.
- Pregnancy or lactating women.

CRITERIA FOR GROUPING

GROUP A: HEALTHY – Probing Depth 2-3 mm, No Clinical Attachment Loss, Gingival index = 0.

GROUP B: GINGIVITIS – Probing depth 2-3 mm, No Clinical Attachment Loss, Gingival Index ≥ 1, positive Gingival Bleeding Index, Plaque Index.

GROUP C: GENERALISED CHRONIC PERIODONTITIS- Pocket depth ≥ 4mm and Clinical Attachment Loss involving more than 30% of the sites.

GROUP D: GENERALISED AGGRESSIVE PERIODONTITIS - Pocket depth ≥ 4mm, interproximal attachment Loss at least three permanent teeth other than first molars and incisors.

CLINICAL PARAMETERS

The following variables were measured at baseline

1. Plaque index

2. Gingival index

3. Gingival bleeding index

4. Number of sites with probing depth ≥ 4mm

PLAQUE INDEX (PI): (Turskey et al modification of Quigley Hein index, 1970)[56]

With the PI, mesial, distal, and mid surfaces of facial and lingual aspects were scored. Scoring was as follows:

0= No plaque/debris

1= Separate flecks of plaque at the cervical margin of the tooth [56]

2 = A thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth.[56]

3= A band of plaque wider than 1 mm but covering less than one third of the crown of the tooth.[56]

4= Plaque covering at least one third but less than two thirds of the crown of the tooth.[56]

5= Plaque covering two thirds or more of the crown of the tooth.[56]

GINGIVAL INDEX (GI): (Loe and Silness 1964)[57]

The Gingival Index records qualitative changes in the gingiva. The marginal and interproximal gingival scored separately. The criteria are:

0= Normal gingiva;

1= Mild inflammation – slight change in color and slight edema but no bleeding on probing[57]

2= Moderate inflammation – redness, edema and glazing, bleeding on probing[57]

3= Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding.[57]

The scores of the four areas of the tooth can be summed and divided by four to give the GI for the tooth[57]. A score from

0.1-1.0 = Mild inflammation;

1.1-2.0 = Moderate inflammation

2.1-3.0 = Signifies severe inflammation.[57]

GINGIVAL BLEEDING INDEX (GBI): (Ainamo and Bay, 1975)[58]

Gingival bleeding index was based on recordings from all four tooth surfaces of all teeth. Percentage of affected sites was calculated by gingival bleeding index. It was recorded after 10 seconds of probing as - Bleeding present (+), Bleeding absent (–) [58]

METHOD

A blood sample of each volunteer was obtained from the antecubital vein of the subject's right or left arm, (a total of 10 ml blood, was collected from patients with 10 ml injectors). 5ml blood was transferred to disposable vacutainer, centrifuged for 2800 for 12 min[59]and 5 ml in grade IV titanium tube at 3000 for 10 min[60] in REMI centrifuge machine. ***Figure-3***

The PRF and T-PRF clots were removed from the respective tubes using sterile tweezers after centrifugation, RBC base layer were separated using scissors, and placed on sterile gauze. Both clots were left over 20 minutes on sterile gauze to release their serum. This PRF and T-PRF clots were used for the light microscopy analysis.

Histological Procedures for Light Microscopy

- Step 1: Fixing – Both the PRF and T-PRF clots were transferred into a perforated stainless steel cassette and identification of patient was written and was placed in 10% formalin-containing container. The PRF and T-PRF clots were then fixed for 24 hrs to prevent cell death and to preserve the biological tissues.
- Step 2: Tissue processing – After 24 hrs of fixation, the cassette-containing clots were subjected to dehydration, clearing, and infiltration of wax into the clots as they passed through various processing solutions such as 10% formalin, 60%, 70%, 80%, 90%, and 100% isopropanol alcohol, xylene (two changes), and paraffin wax in an orderly manner. This process was continued in an automated tissue processor which enabled the removal the water from

tissue for 16 h and replaced in a medium that after solidification allow thin sections to be cut. ***Figure-7,8***

- Step 3: Embedding – Paraffin was used for tissue embedding. ***Figure-9***
- Step 4: Tissue sectioning – the PRF and TPRF clots were sliced using microtome about 4 μm thickness of tissue. ***Figure- 10,11***
- Step 5: Dewaxing – The deparaffinization of the slides was done by heating it for about 55°C, to eliminate wax enabling the tissues to be stained followed by dropping into xylene. ***Figure-12,13***
- Step 6: Tissue staining – Hematoxylin and eosin stain sections were used for staining. ***Figure-14***
- Step 7: Slide numbering – The slides were numbered based on the order for record
- Step 8: Histological slide analysis – Following the preparation of stained section of PRF and T-PRF clot from Group A (healthy patients), Group B (gingivitis patients), and Group C (generalized chronic periodontitis patients); Group D (generalized aggressive periodontitis patients), the slides were assessed with compound microscope at ×20 and ×40 magnifications to evaluate: ***Figure-15-22***

1. Presence of dense fibrin network
2. Presence of loose fibrin network
3. Enmeshment pattern of platelets and WBC cells within the dense and loose type of fibrin network.

PROFORMA

Name: Date:

O.P.No: Age/Sex:

Address: Occupation:

HISTORY

Chief Complaint:

Past Medical History:

Past Dental History:

Personal History:

RADIOGRAPHIC FINDINGS:

INTRAORAL EXAMINATION

PLATELET COUNT:

INDICES

MODIFIED QUIGLEY HEIN PLAQUE INDEX :(Turesky et al 1970)

17	16	15	14	13	12	11	21	22	23	24	25	26	27

47	46	45	44	43	42	41	31	32	33	34	35	36	37

Score: Interpretation:

GINGIVAL INDEX : (Loe&Silness 1963)

16	12	24	36	32	44

Score: Interpretation:

GINGIVAL BLEEDING INDEX (Ainamo&Bay 1975)

17	16	15	14	13	12	11	21	22	23	24	25	26	27

47	46	45	44	43	42	41	31	32	33	34	35	36	37

No of sites: Percentage of sites:

PROBING DEPTH AND CLINICAL ATTACHMENT LEVEL:

CAL																	CAL
PD																	PD
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	
PD																	PD
CAL																	CAL

DIAGNOSIS	**PLATELET RICH FIBRIN**	**TITANIUM PREPARED PLATELET RICH FIBRIN**
HEALTHY		
GINGIVITIS		
GENERALISED CHRONIC PERIODONTITIS		
GENERALISED AGGRESSIVE PERIODONTITIS		

HISTOLOGICAL INTERPRETATION

INFORMED CONSENT FORM

Patient Name: **Age/Sex:**

Address:

I have been explained the nature and purpose of the study in which I have been asked to participate. I understand that I am free to withdraw my consent and discontinue at any time without prejudice to me or effect on my treatment. I have been given the opportunity to question about the study and other procedures. I have also given consent for photographs to be taken at the beginning, during and end of the study. I agree to participate in the study.

I hereby given the consent to be included in "EVALUATION OF FIBRIN NETWORK PATTERN CHANGES OF PLATELET RICH FIBRIN AND TITANIUM PREPARED PLATELET RICH FIBRIN OF INDIVIDUALS WITH AND WITHOUT PERIODONTITIS – A CELL BLOCK CYTOLOGY

Place:

Date:

Signature of patient

Materials and Methods

தகவல் ஒப்புமை படிவம்

நோயாளி பெயர்: ஆண்/பெண்.

வயது: முகவரி

நான் பங்கேற்க கேட்கப்பட்ட ஆய்வுப் படிவத்தில் இயல்பு மற்றும் நோக்கம் பற்றி விளக்கப்பட்டுள்ளது. என் சம்மதத்தை நீக்கிவிட்டு, எந்த நேரத்திலும் என்னைத் தாங்கிக் கொள்ளாமல் அல்லது என் சிகிச்சையில் தாக்கத்தை ஏற்படுத்துவதற்கு நான் சுதந்திரமாக இருக்கிறேன் என்பதை புரிந்துகொள்கிறேன்.

ஆய்வு மற்றும் பிற நடைமுறைகளைப் பற்றி கேள்வி கேட்க எனக்கு வாய்ப்பு கிடைத்தது.ஆரம்பத்தில், ஆய்வின் முடிவிலும் எடுக்கப்படும் புகைப்படங்களுக்கு நான் ஒப்புதல் அளித்திருக்கிறேன். ஆய்வில் பங்கேற்க நான் ஒத்துக்கொள்கிறேன். **எனது இரத்த மாதிரி எடுப்பதற்கும் பரிசோதனைகளுக்கும் முழுமனதோடு சம்மதிக்கிறேன்.**

இடம்: நோயாளி கையொப்பம்:

தேதி:

ARMAMENTARIUM

MATERIALS AND INSTRUMENTS USED:

- Gloves
- Mouth mask
- Patient apron
- Chair apron
- Head cap
- Sterile cotton rolls
- Gauze
- Kidney tray
- Syringe
- Mouth mirror
- Straight probe
- Explorer
- William's probe
- Tweezers
- Tourniquet
- Scissors

MATERIALS AND INSTRUMENTS FOR PREPARATION OF PRF AND T-PRF:

- Sterile grade IV titanium tubes[29]
- Sterile vacutainer.
- 10 ml syringe.
- REMI centrifuge machine for centrifugation.

Photographs

Armamentarium

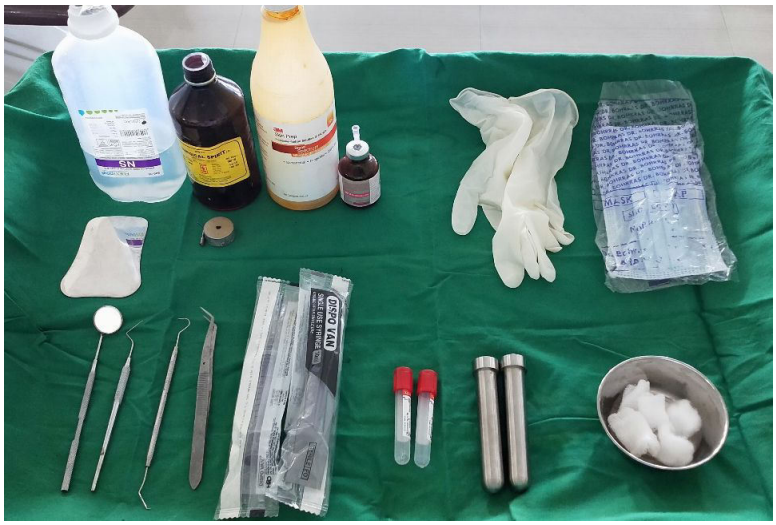


Figure-1

Blood Collection

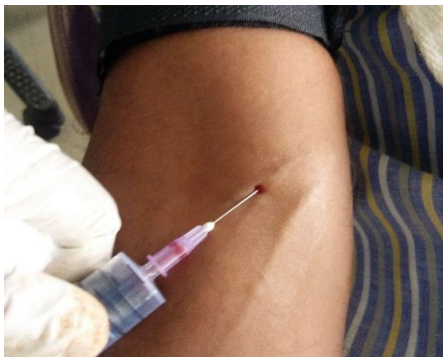


Figure-2

Centrifugation Machine

Figure-3

Vacutainers

Figure -4

Titanium Tubes

Figure-5

Platelet Rich Fibrin And Titanium Platelet Rich Fibrin Clot Obtained

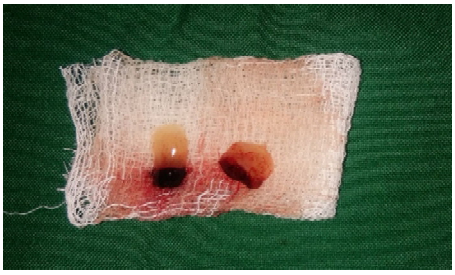


Figure-6

Tissue Processing

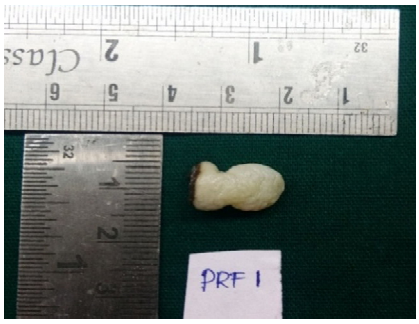


Figure- 7

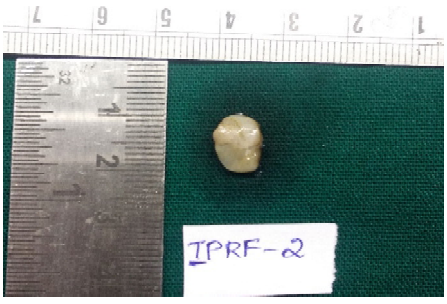


Figure-8

Embedding

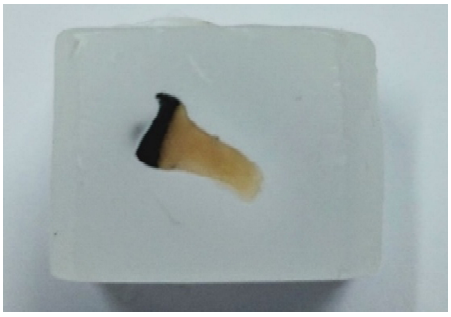


Figure-9

Tissue Sectioning Of PRF And T PRF Clot

Figure-10 ***Figure-11***

Dewaxing Done Tissue Floating Bath

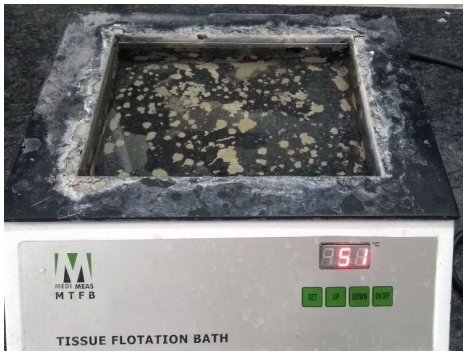


Figure-12

After Dewaxing

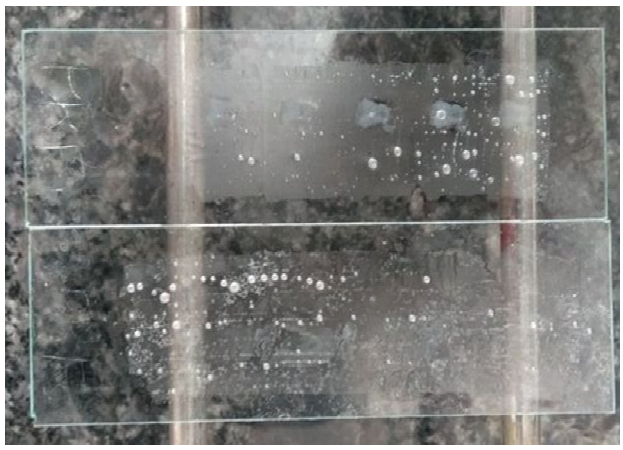


Figure-13

PRF And T PRF Clot Slides Prepared

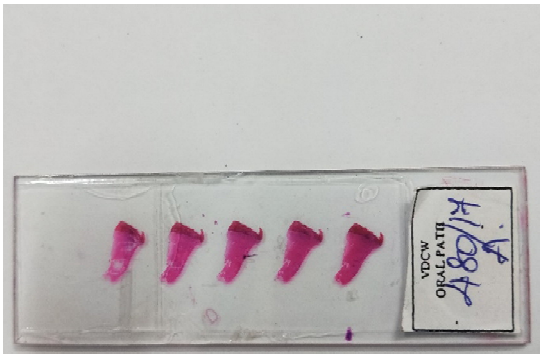
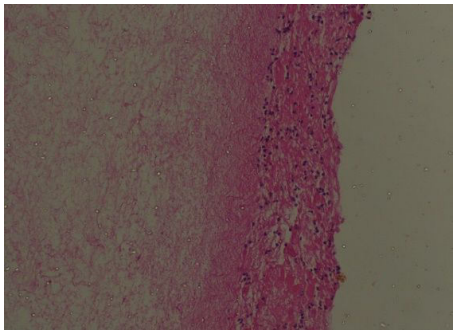


Figure-14

Microscopic view of PRFand TPRF clot under manification

Healthy PRF and T PRF Clot



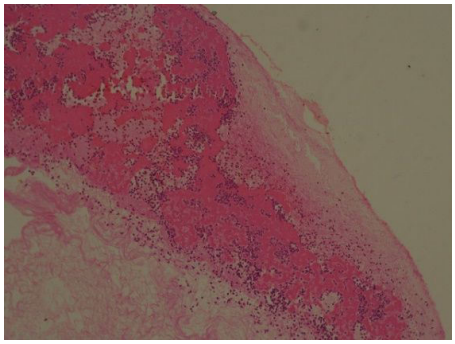
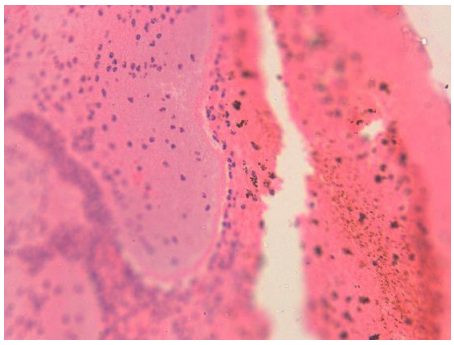


Figure-15 Figure-16

Gingivitis PRF and T PRF Clot



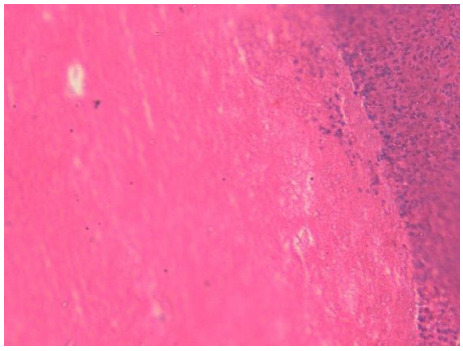
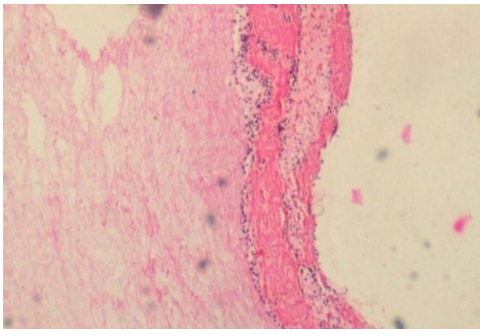


Figure-17 ***Figure-18***

Generalized Chronic Periodontitis PRF and T PRF Clot



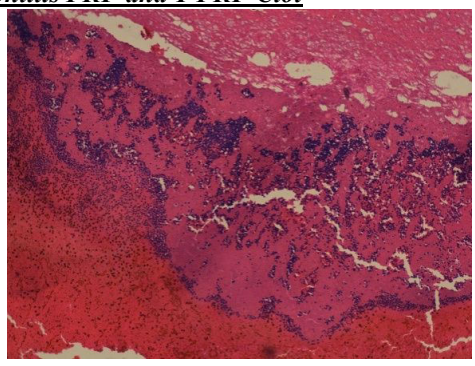


Figure-19 ***Figure-20***

Generalised aggressive periodontitis PRF and TPRF

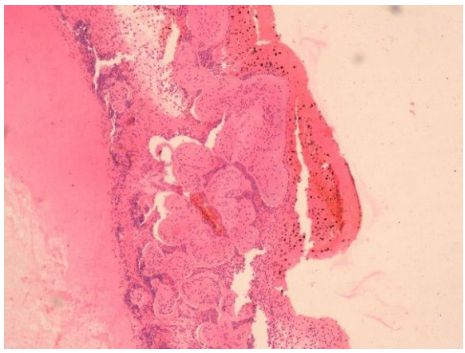


Figure-21

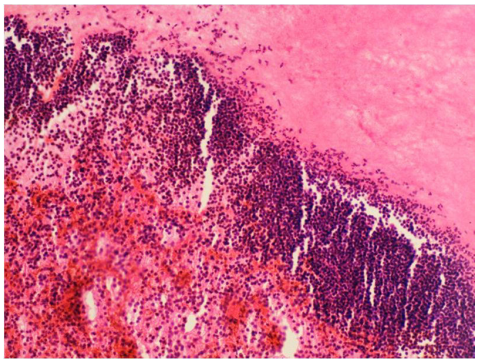


Figure-22

Results

Results

The results obtained in this study were statistically analyzed and the normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal that the variable (Age) follows Normal distribution. Therefore, to analyses the data parametric methods are applied. To compare mean age in years between the groups independent samples t-test is applied. To compare the mean age between groups one way ANOVA is applied followed by Tukey's HSD post hoc tests for multiple pairwise comparisons. To compare proportions between study and control groups Chi-Square test is applied, if any expected cell frequency is less than five then Fisher's exact test is used. To analyze the data SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp. Released 2015) is used and 5 % ($\alpha = 0.05$) fixed as significance level.

The histological section of PRF and T-PRF of all participants showed outermost layer of RBC with dense fibrin network layer with more platelets and WBCs entrapment and inner layers with loose fibrin network with entrapment of platelet and WBC.

The presence of dense and loose fibrin network were analyzed and its percentage difference was investigated and the difference between the PRF and T-PRF fibrin matrix compared. The differences and percentage variation were compared in periodontal disease patients. Healthy, gingivitis and chronic periodontitis and aggressive periodontitis patients were excluded because of less sample size than other groups.

Platelet rich fibrin and titanium Platelet rich fibrin in healthy individuals

Both loose fibrin network and dense network were found and entrapment of platelet and WBC cells in both layers. The percentage of loose fibrin network was

more as compared to dense fibrin network, and entrapment of platelets and WBC cells was aggregated within the dense fibrin network whereas fewer cells were diffusely arranged in loose fibrin network. There was no well-defined demarcation between the loose fibrin network and dense fibrin network

The mean age groups of healthy patients were 27.85 years with a standard deviation of 4.24.Table -1. The percentage of loose fibrin network was about 85 % with mean age of 27.18 and dense fibrin network was about 15% in mean age of 31.67 with less percentage of platelet and WBC entrapment. ***Table -4***

The T-PRF clot showed highly organized dense fibrin network with 100% with mean age of 27.85 with standard deviation of 4.24 as in ***Table -5*** as compared to healthy PRF clot with less percentage of loose fibrin network. There was a well-defined demarcation between loose fibrin network and fibrin network pattern in this group.

Platelet-rich fibrin and titanium platelet-rich fibrin clot in gingivitis patients

The histological section of both PRF and T-PRF clot showed the following. Both layers were found. The PRF clot occupied higher percentage of loose fibrin clot with about total of 86% with mean age difference of 30.53 and dense fibrin network about 14% in the gingivitis group with mean age of 30.33 with standard deviation of 8.59 which with less amount of cellular component of platelet and WBC but compared to healthy individual more entrapment were found. ***Table -1***

In T-PRF clot, loose fibrin network was less, dense fibrin network was present in an increased quantity with mean age of 30.50 and standard deviation about 8.59 which showed an increased amount of cellular components were diffusely arranged

all through the clot. The demarcation between loose and dense fibrin were well defined.

Platelet-rich fibrin and titanium platelet-rich fibrin clot in periodontitis patients

With chronic periodontitis patients mean age was 39.75 with standard deviation of 8.22. In PRF clot, both dense and fibrin network was present compared to the dense fibrin mesh, the loose fibrin mesh was dominant with the percentage of 87% and 13 % of dense fibrin network. The loose fibrin network the mean age were 39.35 and dense fibrin network with 42.00.The WBC and platelet aggregates were seen higher in the dense fibrin network and the rest part of the clot; the entrapment of cells was scattered.

In the T-PRF clot, the mean age was 39.75 with standard deviation of 8.22 the presence of both dense and light fibrin networks was homogeneously present. However, as compared to PRF clot of the same chronic periodontitis individual, T-PRF clot showed thick dense fibrin layer more amount of entrapment of platelets and WBC in the dense fibrin network as compared to loose network and the presence of a fragile fibrin border which was completely absent in PRF clot.

In 10 Generalized Aggressive Periodontitis patients the fibrin network patterns of PRF have both loose and dense fibrin network were seen with loose fibrin were more and increased aggregation of platelet and WBCs were seen and in T-PRF group showed thicker dense layer and increased cells compared to PRF group which similar to chronic periodontitis patients. ***Figure -21, 22***.

Tables

Tables

TABLE-1

ONE-WAY ANOVA TO COMPARE MEAN AGE BETWEEN GROUPS

Group	N	Mean Age (years)	Std. Dev	95% CI for Mean		F-value	p-value
				LB	UB		
Healthy	20	27.85	4.246	25.86	29.84	14.679	<0.001
Gingivitis	20	30.50	8.593	26.48	34.52		
Periodontitis	20	39.75	8.226	35.90	43.60		
Total	60	32.70	8.823	30.42	34.98		

TABLE-2

TUKEY HSD POST HOC TESTS FOR MULTIPLE COMPARISONS

Group		Mean Difference	p-value
Healthy	Gingivitis	-2.65	0.488
	Periodontitis	-11.90	<0.001
Gingivitis	Periodontitis	-9.25	0.001

TABLE- 3
CHI-SQUARE TEST TO COMPARE PROPORTIONS BETWEEN GROUPS

Group	PRF		
	Loose	Dense	Total
	%	%	%
Healthy (20)	85.0%	15.0%	100.0%
Gingivitis(20)	85.0%	15.0%	100.0%
Periodontitis(20)	83.0%	17.0%	100.0%
Total(100)	85.0%	15.0%	100.0%

Chi-Square Test	Value	p-value
Fisher's Exact Test	0.151	1.000

	TPRF					
	Dense		Thick dense		Total	
Group	N	%	N	%	N	%
Healthy	20	100.0%	0	0.0%	20	100.0%
Gingivitis	20	100.0%	0	0.0%	20	100.0%
Periodontitis	0	0.0%	20	100.0%	20	100.0%
Total	40	66.7%	20	33.3%	60	100.0%

Chi-Square Test	Value	p-value
Pearson Chi-Square	60.000	<0.001

TABLE-4

INDEPENDENT SAMPLE T-TEST TO COMPARE MEAN AGE BETWEEN LOOSE AND DENSE PRF (OVERALL)

PRF	N	Mean Age (years)	Std. Dev	t-value	p-value
Loose	51	32.35	8.944	0.722	0.473
Dense	9	34.67	8.307		

INDEPENDENT SAMPLE T-TEST TO COMPARE MEAN AGE BETWEEN LOOSE AND DENSE PRF (GROUP WISE)

Group	PRF	N	Mean Age (years)	Std. Dev	t-value	p-value
Healthy	Loose	17	27.18	3.795	1.783	0.091
	Dense	3	31.67	5.508		
Gingivitis	Loose	17	30.53	9.063	0.035	0.972
	Dense	3	30.33	6.658		
Periodontitis	Loose	17	39.35	8.329	0.504	0.621
	Dense	3	42.00	8.888		

ONE-WAY ANOVA TO COMPARE MEAN AGE BETWEEN GROUPS AMONG LOOSE PRF

Group	N	Mean Age (years)	Std. Dev	95% CI for Mean		F-value	p-value
				LB	UB		
Healthy	17	27.18	3.795	25.23	29.13	12.161	<0.001
Gingivitis	17	30.53	9.063	25.87	35.19		
Periodontitis	17	39.35	8.329	35.07	43.64		
Total	51	32.35	8.944	29.84	34.87		

Tables

TUKEY HSD POST HOC TESTS FOR MULTIPLE COMPARISONS

Group		Mean Difference	p-value
Healthy	Gingivitis	-3.353	0.394
	Periodontitis	-12.176	<0.001
Gingivitis	Periodontitis	-8.824	0.003

ONE-WAY ANOVA TO COMPARE MEAN AGE BETWEEN GROUPS AMONG DENSE PRF

Group	N	Mean Age (years)	Std. Dev	95% CI for Mean		F-value	p-value
				LB	UB		
Healthy	3	31.67	5.508	17.99	45.35	2.388	0.173
Gingivitis	3	30.33	6.658	13.79	46.87		
Periodontitis	3	42.00	8.888	19.92	64.08		
Total	9	34.67	8.307	28.28	41.05		

TABLE-5

INDEPENDENT SAMPLE T-TEST TO COMPARE MEAN AGE BETWEEN DENSE AND THICK DENSE T-PRF (OVERALL)

PRF	N	Mean Age (years)	Std. Dev	t-value	p-value
Loose	40	29.18	6.823	5.281	<0.001
Dense	20	39.75	8.226		

INDEPENDENT SAMPLE T-TEST TO COMPARE MEAN AGE BETWEEN DENSE AND THICK DENSE T-PRF (GROUP WISE)

Group	TPRF	N	Mean Age (years)	Std. Dev	p-value*
Healthy	Dense	20	27.85	4.246	-
	Thick dense	0	.	.	
Gingivitis	Dense	20	30.50	8.593	-
	Thick dense	0	.	.	
Periodontitis	Dense	0	.	.	-
	Thick dense	20	39.75	8.226	

* p-value cannot be computed because at least one of the groups is empty.

Tables

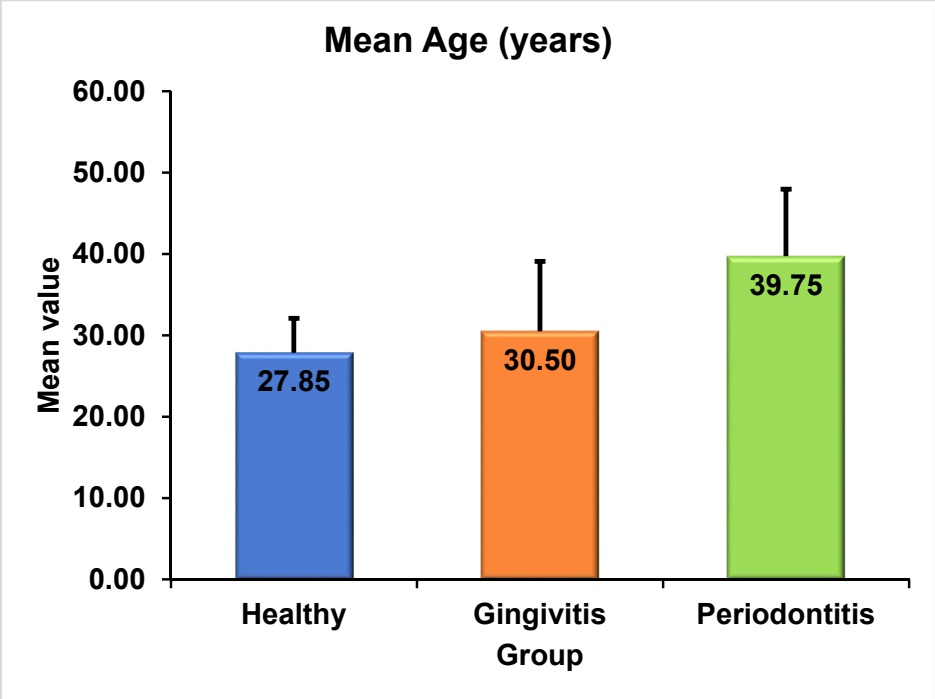
ONE-WAY ANOVA TO COMPARE MEAN AGE BETWEEN GROUPS AMONG DENSE T-PRF

Group	N	Mean Age (years)	Std. Dev	95% CI for Mean		F-value	p-value
				LB	UB		
Healthy	20	27.85	4.246	25.86	29.84	1.529	0.224
Gingivitis	20	30.50	8.593	26.48	34.52		
Total	40	29.18	6.823	26.99	31.36		

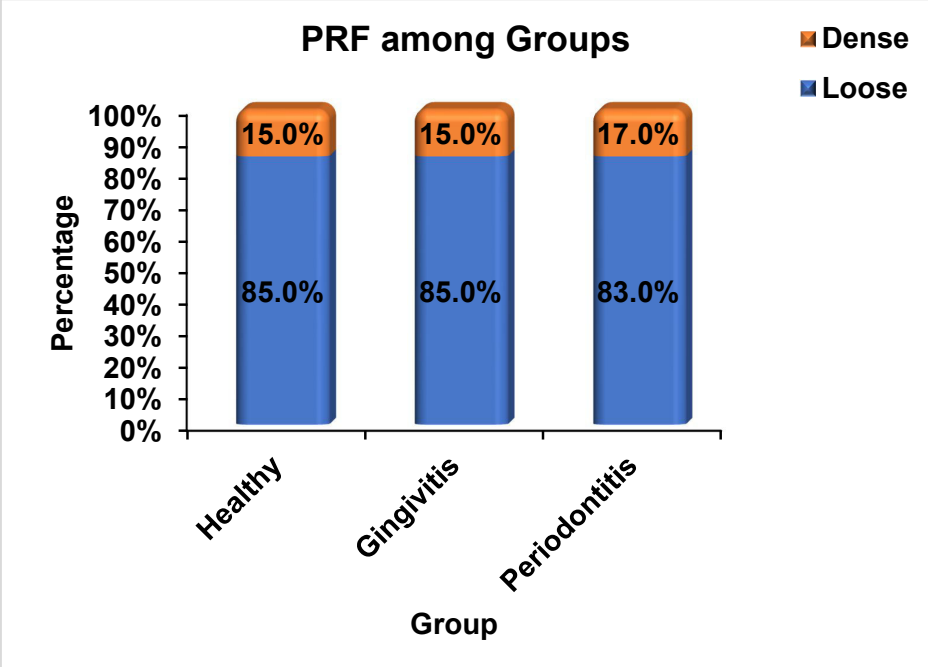
Group	TPRF	PRF					
		Loose		Dense		Total	
		N	%	N	%	N	%
Healthy	Dense	17	85.0%	3	15.0%	20	100.0%
	Thick dense	0	0.0%	0	0.0%	0	0.0%
	Total	17	85.0%	3	15.0%	20	100.0%
Gingivitis	Dense	17	85.0%	3	15.0%	20	100.0%
	Thick dense	0	0.0%	0	0.0%	0	0.0%
	Total	17	85.0%	3	15.0%	20	100.0%
Periodontitis	Dense	0	0.0%	0	0.0%	0	0.0%
	Thick dense	17	85.0%	3	15.0%	20	100.0%
	Total	17	85.0%	3	15.0%	20	100.0%

Graphs

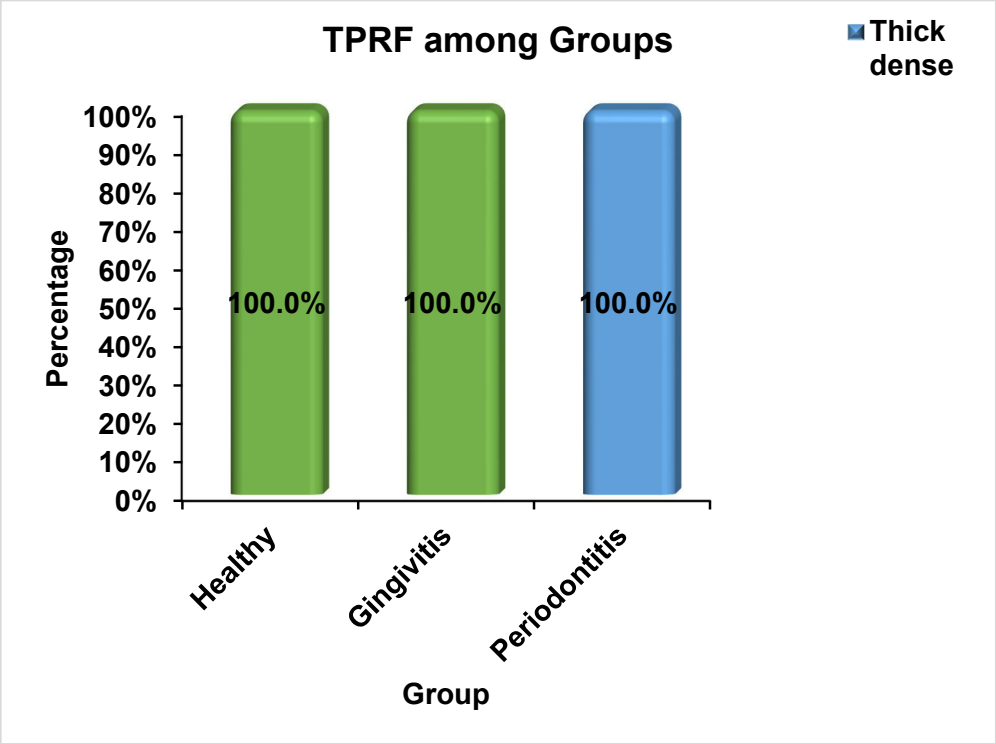
GRAPH-1



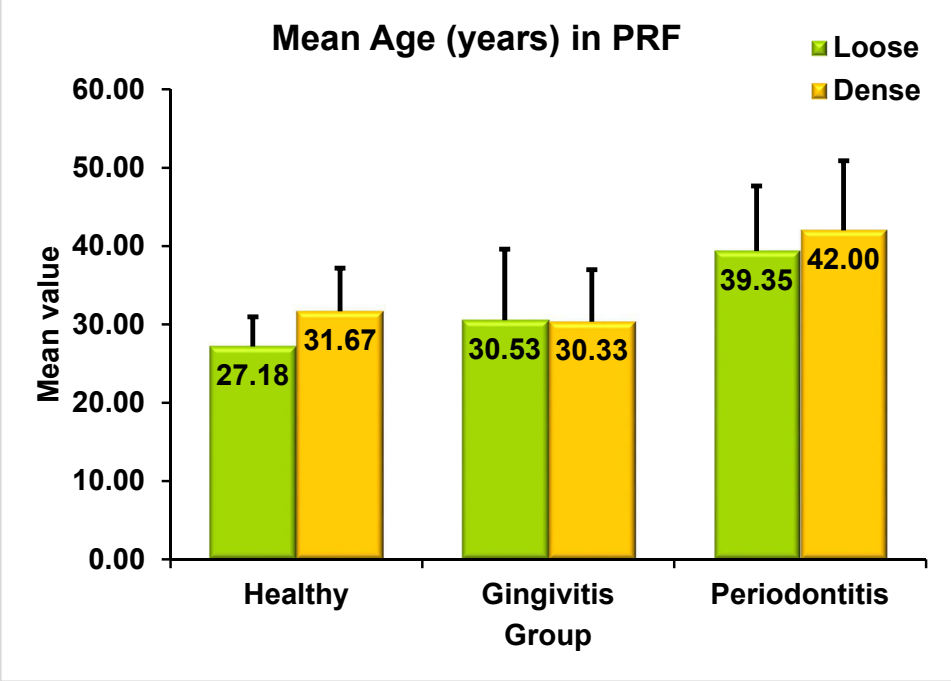
GRAPH-2



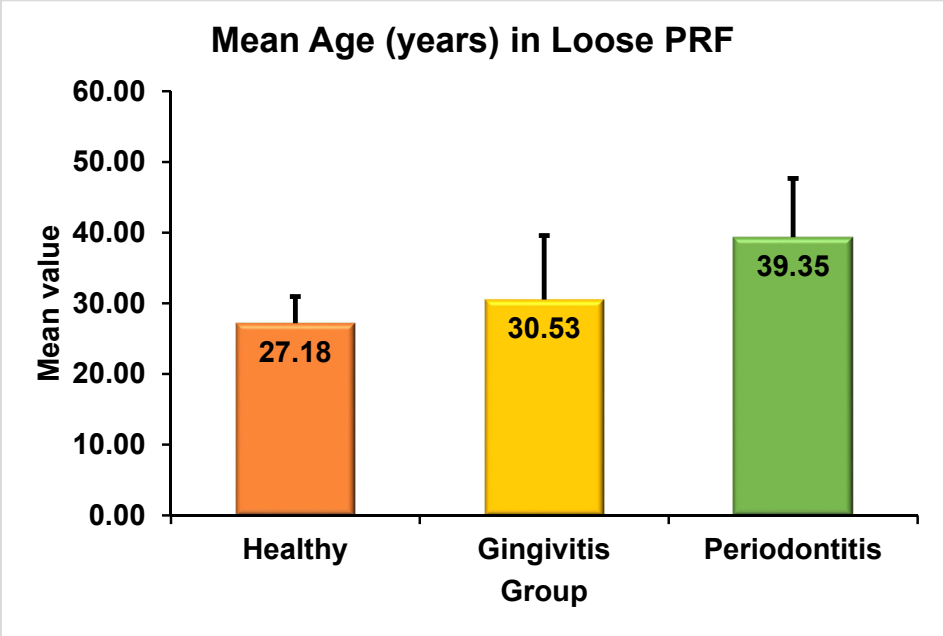
GRAPH-3



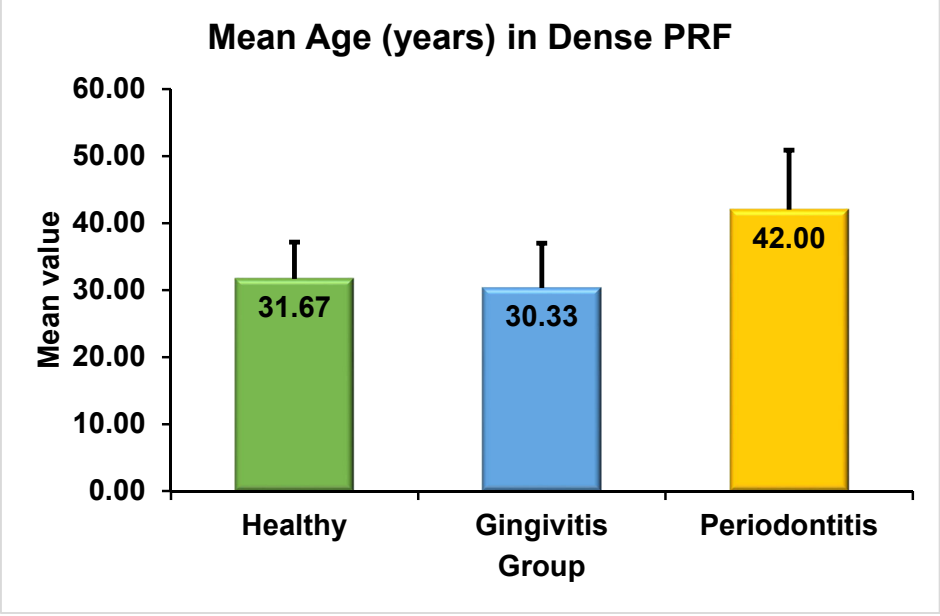
GRAPH-4



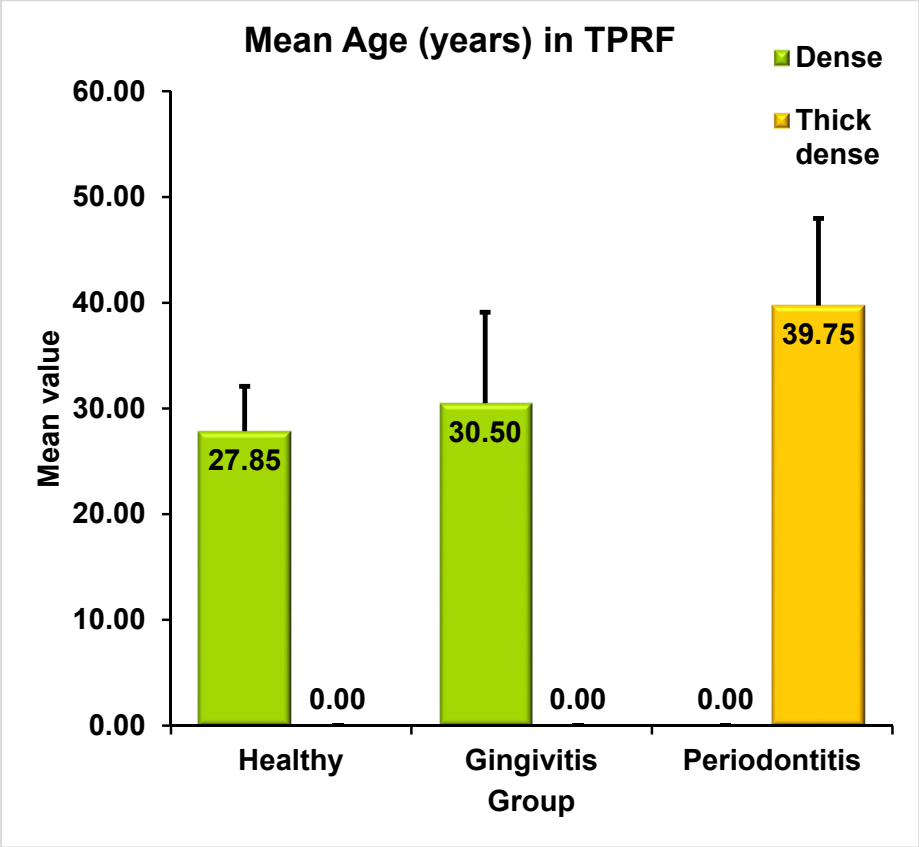
GRAPH-5



GRAPH-6



GRAPH-7


Mean Age (years) in TPRF
Dense
Thick dense
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Mean value
27.85
0.00
30.50
0.00
0.00
39.75
Healthy
Gingivitis
Periodontitis
Group

Discussion

Discussion

In this study, fibrin network patterns of platelet rich fibrin and titanium prepared platelet rich fibrin of patients with gingivitis, chronic periodontitis, and aggressive periodontitis were compared with healthy patients through histological analysis. As per our knowledge of the literature, this study was the first conducted on the variation of fibrin network pattern in periodontal disease conditions. The importance of understanding these variations in periodontal disease patients in the platelet concentrates could be because it contains higher concentrations of growth factors which could play an important role in the regeneration of the lost tissues. Platelet glycoprotein binds to fibrinogen which reliant the platelet thrombus on formation and helps in cross-linking of the platelets[61], and the fibrin collagen component triggers this platelet thrombus formation.

Platelet concentrates were used initially for the treatment of severe thrombopenia which was characterized by hemorrhage. They were also used for wound healing which lead to introduction of fibrin glues contains concentrated fibrinogen 40 years ago. These adhesives were procured commercially or obtained from the patients because of the cost of production and risk in transmission of diseases.

A newer platelet concentrate was identified and it was called platelet rich plasma (PRP) in the early 1990s. It was obtained by means of plasmapheresis and PRP sequestration. It contains 95% of platelets, 4% of RBCs and 1 % of WBCs. This platelet secretes growth factors which initiates wound healing process and the protein present in it helps in cell adhesion and act as matrix for bone, connective tissue and epithelial migration. Advantages of PRP are safe, cost effective, auotologous product, no risk in disease transmission or immunogenic reactions and remains stable and sterile in the anticougulated state for 8 hours. Hence it can be used for longer

surgeries. Disadvantages of PRP were difficult to handle and requires secure implantation in specific site. ***Saluja et al 2011*** showed that PRP has limited potential to induce bone regeneration because growth factors are released quickly before the surrounding cell outgrowth.

To overcome the limitations of PRP, ***Choukroun et al 2001***developed platelet rich fibrin PRF widely used to induce soft and hard tissue healing. The PRF production protocol attempts to accumulate cytokines and platelets in a fibrin clot. It can be used as a clot or as a membrane after compression. ***Toffler et al 2009, Dohan et al 2010.*** There are two types of PRF- Leucocyte-poor or pure platelet rich fibrin (P-PRF), Leucocyte-rich platelet rich fibrin (L-PRF) or **Choukroun's PRF**.

Leucocyte-poor or pure platelet rich fibrin (P-PRF) prepared by collecting blood is in a tube containing anticoagulant and centrifuged for 6 minutes. The obtained buffy coat and PPP are transferred to second tube containing calcium chloride which helps in triggering the clotting process and centrifuged for 15 minutes. The fibrin matrix is denser and more stable than PRPs.

Choukroun's PRF was prepared by centrifuging the collected blood without anticoagulant. The absence of anticoagulant allows activation of majority of platelets and increased fibrinogen in the upper part of the tube, until the thrombin transforms to fibrin network. Middle of the tube contains fibrin clot with platelets. It does not dissolve quickly after application unlike the PRPs.

Advantages of PRF are ease of preparation, lack of biochemical modification, increased incorporation of cytokines in the fibrin meshes (intrinsic cytokines), and minimal expense. Quick handling to get a clinically usable PRF clot was the only disadvantage.

Discussion

During gelling of PRF, the fibrin fibrillae arranged in two different biochemical architectures bilateral junctions or condensed tetramolecular junctions and connected trimolecular or equilateral junctions. The strong thrombin concentrations in the bilateral junctions allows thickening of fibrin polymers leading to rigid network, unfavorable to cytokines enmeshment and migration of cells. Weak thrombin in equilateral junctions leads to a fine and flexible fibrin network favorable to cell migration and cytokines.

Platelet rich fibrin has to be considered as a fibrin biomaterial and a favorable loose and dense fibrin matrix for migration of endothelial cells and fibroblasts which helps in rapid angiogenesis and remodeling of tissues. The dense fibrin matrix in clots have more entrapment of inflammatory cells and platelets which helps in enhanced wound healing and or regeneration of periodontal tissue. Therefore, these PRF clots can be used for all types of superficial cutaneous and mucous healing.

The glass evacuated collection tubes with silica activators are used during preparation of PRF. These silica particles found in sediment with red blood cells, fibrin, and buffy coat and might reach the patient when product used for the treatment. In order to overcome this, Titanium prepared platelet rich fibrin was identified, where grade IV titanium tubes are used for preparation at 3500rpm for 15 minutes. Platelet activation was found to be similar to glass tubes and showed increased biocompatibility

New products titanium prepared platelet-rich fibrin (T-PRF), advanced platelet-rich fibrin (APRF), and concentrated growth factors (CGF)have been developed in recent years to improve PRF and to get rid of the limitations.

Recently, ***Ghanaati et al***.[62]developed a new fibrin structure (A-PRF) with a decrease in centrifugation speed and an increase in centrifugation time. The

centrifugal force also influences the specific cell types scattered differentially in the clot.

Periodontitis is a chronic infectious disease, and this inflammation of the supporting periodontal tissues results in pocket formation and epithelial ulceration. This lead to transient bacteremias[2, 3] which in turn lead to the production of pro inflammatory mediators like interleukin (IL-1β), IL-6, tumor necrosis factor TNF-α, C - reactive protein and resulting in platelet binding to leucocytes and endothelial cells.

These make platelet important factor in both thrombotic and inflammatory reactions in the vasculature.[63]this activation of platelet is responsible for the development of atherosclerosis, atherothrombosis and subsequent coronary vascular and cerebrovascular disease.

Dimitris Papapanagiotou et al[64]investigated the soluble P-selectin and sCD40 ligand and surface-exposed P-selectin and the ligand-binding conformation of the glycoprotein IIb–IIIa complex (binding of PAC-1 antibody) in the plasma, it was used as marker for platelet activation in periodontitits patients and healthy patients. He found activation of platelet was more pronounced in patients with more severe periodontal disease.

Mario Romandini et al 2018[65] found that severe periodontitis displayed more platelets for µl of blood and has shown to be highlighted in older patients, females, non- smoker and with normal HDL blood levels.

In this study, generalized aggressive periodontitis patients the PRF clots with predominant dense fibrin layer and TPRF clots showed both loose and dense fibrin matrix with increased platelets and WBCs entrapment when compared to healthy and gingivitis patients this may be because of the oral bacteria Aggregatibacter actinomycetemcomitans (Aa) causing periodontitis responsible for the increase

platelet and increased formation of platelet –leukocyte complexes with elevated capacity of for bacterial clearance.[66]

The platelets are hyper-reactive in patients with periodontitis and forms more platelet- leucocyte complexes compared to healthy patients. In response to P.gingvalis lead to the formation of platelet -leukocyte complexes and reduced at recall after periodontal therapy.[67]

Chronic periodontitis patients demonstrated a significantly higher WBC count $7.22 \pm 1.42 \times 10^9$ cells/L and also platelet count (290.73 ± 56.56 · 109 cells/L) systemically higher than the healthy patients.[68]Hence the influence of systemic and periodontal diseases was a necessary to assess the quality of fibrin network pattern of platelet rich fibrin and titanium prepared platelet rich fibrin.

The variation in fibrin network pattern changes of PRF clot at various age groups was evaluated, wherein older age group period, loose fibrin network and scanty entrapment of WBC and platelets were found which correlates with this study[8]. On histological sectioning, larger fibrin network area was observed in TPRF fibrin than leukocyte- and platelet-rich fibrin network and also, fibrin seemed thicker in the TPRF samples. Thus, platelet activation by titanium tubes has shown higher characteristics.[29]

Dohan et al. observed the cytokines and fine flexible fibrin network and stabilization of the wound by using platelet concentrate in regeneration process.[12] Laurens *et al*. stated that the fibrin structure, such as the thickness of the fibers, the number of branch points, the porosity, and the permeability of platelet rich fibrin greatly influences the wound healing. It also depends on platelet concentrates and their functions.[64]

Discussion

We excluded smoking patients and platelet & coagulation disorder patients, systemic diseases because of various factors affect the fibrin matrix formation, and the entrapment of WBC and leukocytes changes due to change in vascularity which may be due to genetic factors and acquired factors (abnormal concentration of factor XIII and thrombin in plasma, hypertension, blood flow, platelet activation, oxidative stress, hyperglycemia, hyperhomocysteinemia, medications, and cigarette smoking).[65]

Titanium is a non-corrosive material which is compatible to cell and tissue (cytocompatibility) and blood (hemocompatibility). The medical grade titanium showed variation in the platelet and leucocyte, reduction in platelet number and surface variations has no thrombogenic variations[69].

Hence the present study examined the histological assessment of fibrin network pattern and its interaction with platelets and WBC in healthy, gingivitis and periodontitis.The healthy patients with 27.85 years with a standard deviation of 4.24.Table -1. The percentage of loose fibrin network was about 85 % with mean age of 27.18 and dense fibrin network was about 15% in mean age of 31.67with less percentages of platelet and WBC entrapment. Table -4.The TPRF clot showed highly organized dense fibrin network with 100% with mean age of 27.85 with standard deviation of 4.24 as in Table -5 which was correlated with the histological section evaluation done by ***Tunali et al 2014***.

In Gingivitis patients, it also showed both loose and dense fibrin network with the PRF clot occupied higher percentage of loose fibrin clot with about total of 86% and decreased platelet aggregation. But TPRF showed denser matrix and increased platelet aggregation than PRF gingivitis group as in figure16, 18, and 20.

Discussion

In Generalized Chronic Periodontitis patients, The loose fibrin mesh was dominant with the percentage of 87% and 13 % of dense fibrin network in PRF group and TPRF clot showed thick dense fibrin layer maximum amount of entrapment of platelets both PRF and TPRF clots showed both loose fibrin network and dense fibrin network with more platelet aggregation in dense fibrin network and thick fibrin border was also appreciated in periodontitis patients when compared to healthy patients as in Table -5 and chart-3.

In Generalized Aggressive Periodontitis patients showed predominant loose fibrin network in the PRF group and in TPRF group showed more of dense layer and increased inflammatory cells and platelets. In this study all the patients of TPRF group healthy, gingivitis and chronic and aggressive periodontitis patients have showed well organized fibrin mesh structure with continuous integrity with 100% of dense fibrin matrix and100% of thick dense pattern in periodontitis patients as in chart -3. The RBC and leucocytes are easily detectable when compared to PRF groups. Here one the factor platelet activation could be the cause for the variation of PRF and TPRF in fibrin matrix and platelet aggregation in this study. In periodontal disease low grade systemic inflammation which triggers the platelet formation more.

Anirban Chatterjee et al 2017[48]evaluated fibrin clot pattern in hypertensive and smoker participant varied as compared to healthy participants in PRF and TPRF clot, but a better organization of meshwork and increased entrapment of cells were appreciated in TPRF clot. Since both systemic condition causes changes in the network pattern of platelet rich fibrin and titanium prepared platelet rich fibrin. Based on this article this study was performed to see variation of these clot in gingivitis and periodontitis patients.

Discussion

This study accentuated the influence of diseased oral condition like gingivitis and periodontitis which could recast the fibrin clot formation, fibrin network contrast and its interaction of cellular component. From this study we interpret that using titanium to prepare PRF enhances its quality of the fibrin network which was found have better regenerative capacity[52].To certain variations and limitations of this study, variation in age groups, platelet count distribution evaluation of fibrin clot of individual having gingivitis and periodontitis patients compared with healthy patients could have yielded a better validation. Histologically, correct appreciation of the changes in the individual fibrin thickness, morphology and, hindrance in recognizing and isolating out the platelets and WBC cells by hematoxylin staining, and precise counting of the platelets entrapped within the fibrin meshwork were difficult.

Summary & Conclusion

Summary and Conclusion

This study defined and evaluated the fibrin network pattern changes of Platelet rich fibrin and Titanium prepared platelet rich fibrin clot by histological method in patients with periodontal diseases which varied compared to healthy individuals. Total of 60 patients were divided into 4 groups Healthy, Gingivitis, Generalized Chronic Periodontitis and 10 Generalized Aggressive Periodontitis patients. Baseline clinical parameters were assessed to confirm the diagnosis like probing depth, clinical attachment loss, plaque index, gingival index, bleeding index. In each patient, 10 ml blood was drawn and divided for centrifugation to obtain PRF and T-PRF clot and the specimens were obtained by fixing, tissue processing, embedding, tissue sectioning, dewaxing, tissue staining and finally slide preparation. Then histological analysis was done by compound microscope at 20X and 40X magnification.

On analysis, the healthy and Gingivitis patients showed no significance variations in fibrin matrix formation in the PRF group, but in Generalized Chronic Periodontitis and Generalized Aggressive Periodontitis patients more cellular entrapment, matrix formation was found and in T-PRF on the whole it showed thick dense fibrin matrix and well organized fibrin border and increased entrapment of cellular components and this was more exaggerated in generalized chronic and aggressive periodontitis patients. With limitations in this study, more research on clinical parameters such as resorption time, clinical outcome and other systemic factors that influences the fibrin network are required.

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VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

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Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

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Dr. R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
Dr. B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Dr. Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/36/2016

Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. A.S.Abarnashree

Title: Evaluation of fibrin network pattern changes of PlateletRich Fibrin and Titanium prepared - Platelet Rich Fibrin of individuals with and without periodontitis.

Institutional ethics committee thank you for your submission for approval of above proposal .It has been taken for discussion in the meeting held on 25.10.16.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

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