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RESEARCH ARTICLE

COMPARATIVE ASSESSMENT OF T-PRF ALONE AND T-PRF INJECTED WITH ANTIBIOTIC GELS, HERBAL PRODUCT SEPARATELY: A HISTOLOGICAL STUDY

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ABSTRACT

Back ground: Antibiotics and herbal product incorporation into T-PRF are scant. Hence present study aimed to assess the fibrin network pattern, cellularity, cell distribution for T-PRF injected with metronidazole gel (MTZ), amoxicillin+ clavuanic acid (Amox-clav) gel and Neem (N) gel with T-PRF alone histologically through light microscope (LM) and scanning electron microscope (SEM).

Materials and Method: Present histological study utilizes 24 healthy volunteers where 20 ml of blood was drawn; transferred to titanium tubes; centrifuged; T-PRF clots prepared; MTZ, Amox-clav and Neem gels were incorporated, compared with T-PRF alone by preparing histological slides and observed under LM and SEM. Data was subjected to statistical analysis using one-way anova, fishcer test, chi-square test and frequency distribution analysis for all the group comparisons.

Results: all the groups in the present study showed non-significant results. For fibrin network pattern MTZ and Amox-clav group had shown greater percentages of thick and dense but values were non-significant (p=0.555#), cell distribution shown wide range pattern, presence of cells, all showed non-significance. Regarding mean score of cell distribution there was a range of 26-50% (score 1-2) with non-significance (p=0.386#). Values were non-significant for SEM examination for all groups (p>0.05)

Conclusion Thus within limitations T-PRF can be a sustained a drug delivery carrier system by incorporating antibiotics or herbal extract because of its thicker fibrin meshwork and greater cellular entrapment. This opens a gateway to give a new treatment protocol for treating a periodontal disease.

Keywords: Histology, Microscopy, Neem, Periodontitis, Platelet Rich Fibrin, Titanium

INTRODUCTION

Periodontitis is a painless multi-factorial disease that challenges a dental surgeon or periodontist with its attachment loss and bone loss.[1] To treat this periodontal disease, surgical and non-surgical periodontal therapies have been employed. Biomaterials like bone grafts, guided tissue regeneration materials (resorbable or non-resorbable) have been utilized to treat the periodontal soft & hard tissue defects to achieve promising results.[2] Platelet concentrates (PC's) usage was started in 1970's and has become a boon to dentistry. Because of their autologous nature and rich presence of growth factors (GF) they became a cost effective treatment strategy. First generation PC's like Platelet Rich Plasma (PRP), Leukocyte Platelet Rich Fibrin (L-PRF), Advanced Platelet Rich Fibrin (A-PRF) were used back and forth in all the fields of dentistry and medicine.[3] Various therapies like intra bony defect (IBD), gingival recession, sinus lift, socket preservation treatments have advocated these PC and achieved adequate results. But because of their shorter resorption time, rapid growth factor releases and addition of anti-thrombin (for PRP) lead to the search for better biomaterials of autologous nature. During this quest, titanium has attracted the researchers.[4] Because of its hemo-compatibility, non-corrosive nature and regular use of this metal in implant dentistry & medical surgical field led to the formulation of Titanium- Platelet Rich Fibrin (T-PRF).[5] To this support, O' Connell[6] also stated the possible contamination of silica particles in silica coated plastic tubes. Breakage of glass tubes within or outside centrifuge also increased the usage of titanium. In order to prepare T-PRF clots, Grade IV titanium tubes were utilized, by Tunali M et al.,[7] 2013. Various authors like Bhattacharya HS et al.,[8, 9] 2020, 2022 and Gummaluri SS et al.,[10] 2020 utilized medical grade titanium tubes for the preparation of T-PRF clots and achieved good treatment outcomes. In all these quoted studies, there was thicker fibrin meshwork, with higher cellular entrapment and packeting pattern of T-PRF was recorded than that of L-PRF when checked for light & scanning electron microscopy and immunohistochemical analysis. Chatterjee A et al.,[11] 2017 and Mitra DK et al.,[12] 2019 also stated that T-PRF had a better meshwork pattern than L-PRF in their histological studies.

T-PRF was used as a biomaterial to treat IBD and achieved a decrease in probing pocket depth (PPD), gain in clinical attachment level (CAL) and bone fill when compared from baseline to 9 months post-operative.[13] Since ages, antibiotics usage was recorded in literature for both surgical and non-surgical periodontal therapies as a purpose of control of post-operative infection and pain. But because of adverse systemic effects of antibiotics, researchers started their search for a better delivery of drugs.[14] Materials like carbopol polymer, collagen membranes, polyvinyl pyrrolidone, carboxy methyl cellulose etc. were also tried as carrier systems but their efficiency of positivity on the body mainly depends upon the interaction of immune system of body and the material used.[15] Tihan et al.,[16] 2019 utilized collagen sponge (CS) incorporated with doxycycline and oxytetracycline as a local drug delivery system and concluded that oxytetracycline loaded CS had a greater sustained release than Doxycycline loaded CS. They also concluded that oxytetracycline and 0.5% glutaraldehyde will help in tissue regeneration by preventing infections at application site.[16] Various drugs like Tetracycline, Doxycycline, Minocycline, Metronidazole, Azithromycin etc. have been used as Local drug delivery (LDD) in periodontitis patients in the form of fibers, gels, chips, microspheres, films, nano particles and so on.[17] Esra ercan et al.,[18] 2022 conducted a study incorporating doxycycline hyclate liquid in T-PRF & Collagen sponge was incorporated with Doxycycline was used as control group and concluded that T-PRF+Doxy group had a greater sustained drug release than Collagen sponge, thus indicating T-PRF as a sustained drug delivery system.

In both non-surgical and surgical treatments, several other antibiotics, including clindamycin, minocycline, lincomycin, and neem gels, were utilized as LDD or as topical applicants and produced good drug concentration levels in the pocket.[19] But to the authors knowledge no study was performed utilizing Amoxicillin+clavulanate (Amox-clav) gel, metronidazole (Metz) gel and neem (N) gel incorporating in the T-PRF membrane separately. Assessment of membrane structure and cellularity histologically is an indirect way of predicting the ability of restoration of soft and hard tissues. It is not possible to perform a surgical re-entry to assess the amount of regeneration in humans because of their ethical concerns. Hence present study aimed to evaluate the fibrin network, meshwork pattern, cellularity and cell distribution among T-PRF incorporated with amox+clav, metz and N gels

separately and compared with plain T-PRF using light microscopy and SEM for surface morphology.

Materials and Method

Sample Size Estimation
A sample of 20 per group was estimated with 80% power and 5% alpha error and effect size of 0.25, hence 80 samples (4 groups= 20 samples per group) were considered. G Power software version 3.0 was used for estimation of sample.

Design, Enrolment and Ethics
In this present histological analysis, 24 healthy volunteers (12 males (mean age of 25.2 years) and 12 females (mean age of 24.6 years)) were considered after taking proper informed consent. Initially 30 healthy volunteers were recruited but 6 people were not interested and declined to participate in the study. All protocols were clarified to them and only attentive people were recruited. Prior to the start of study ethical clearance was obtained with protocol number 71086061123 from institutional ethics. Study was performed at Department of Periodontics, GITAM Dental College and Hospital where healthy volunteers were recruited. Slides preparation & assessment for Light microscopy (LM) were done in Department of Oral pathology & Microbiology GITAM Dental College and Hospital, Visakhapatnam and Scanning electron microscopy (SEM) at Saveetha Dental College and Hospital, Chennai. Study had followed the Helsinki declaration 1975 that was modified in 2013 in the study.

Inclusion and Exclusion Criteria
Volunteers who were >18 years of age, systemically healthy, having > 2,00,000 lakhs per cubic millimetres platelet count, who were not under any sought of medications that would alter the systemic health, not having any sought of inflammatory diseases etc. were included. Whereas volunteers who were not ready to participate, not fascinated in the study, pregnant and lactating females, smoking, having any sought of systemic health complications were excluded.

T-PRF clots and antibiotic gels preparations
Twenty millilitres of blood was drawn and transferred to sterile medical grade titanium tubes (Supra Alloys Company, Camarillo USA), using modified Tunali M et al.,[20] 2014 and Bhattacharya HS et al.,[8] 2020 protocol (3500rpm for 15 minutes). T-PRF clots were prepared in a centrifuge (Remi R 8C, New Delhi, India). Further, using sterile tweezers, clots were carefully retrieved from the tubes, placed in a sterile area and injected with respected Amoxiclav/ MTZ and Neem gels respectively. Twenty grams of neem leaves were boiled in one litre of water to produce ten grams of liquid azadirachta indica extract. At 25µg/ml, the minimum inhibitory concentration must be achieved. To make a basic gel, 20g of carbopol 934P was soaked in one liter of water. To prepare the corresponding gel, additional calcium chloride (0.02%) and sodium chloride (NaCl) (0.9%) were added. In order to get a final mixture with a 2.5% potency, 2.5% neem extract (N) was added to this mixture.[21] Metronidazole gel (MTZ gel) of 15% concentration was prepared by combining poloxamer 123 30% w/w, propylene glycol 5% w/w, methyl-isothiazolinone 0.15% w/w, metronidazole 15% w/w and adding distilled 100%.[22] Amoxicillin + clavulanate acid gel (Amox-clav gel) was prepared by procuring the powders with a weight ratio of 7:3. To this 3 parts of sodium carboxy methylcellulose and 33% of magnesium stearate was added. All the materials were blended into a uniform single mixture. Further 0.5% polycarbophil powder and glycerine were added to form a 25% concentration.[23] All the gels that were prepared were properly sterilized and stability tested. They were procured from Periobiologics^{TM} lab, Hyderabad, India.

Drug injections into T-PRF clots
Based on Esra Ercan et al.,[18] 2022 protocol, all the prepared the clots that were retrieved, placed on sterile kidney tray separately, filled with 5 units of respective antibiotic gels and herbal extract. individually Later these were transferred to 10% formaldehyde solution and subjected to microscopic slide preparation and 2.5% glutaraldehyde for surface morphological analysis.

Light Microscopy method and slides preparation
Light microscopy and slides preparation were done based on Bancroft's manual steps.[24] After the obtainment of clots from respective groups, they were subjected to fixation in 10% formaldehyde solution for at least 24 hours. Later they were washed and subjected to different concentrations of alcohol such as 25%, 50%, 75% and 100% to remove the excess water. Later these clots were embedded individually into wax blocks using paraffinization technique. Then the cytology blocks that were prepared are sectioned into small sections which were transferred to slides, excessive paraffin was eliminated by subjecting the slides to heat at 55^{o}C and under xylene to remove remnants of paraffin. Then these fixed slide sections were stained with

haematoxylin and eosin stains to observe under the penta-head microscope. Slides examination was done by an experienced oral pathologist who has a previous experience of visualizing the slides and images of T-PRF. A total of 80 slides (20 per each group) were prepared and to avoid confusion slides were numbered according to patients and groups (Figure 1 and 2).

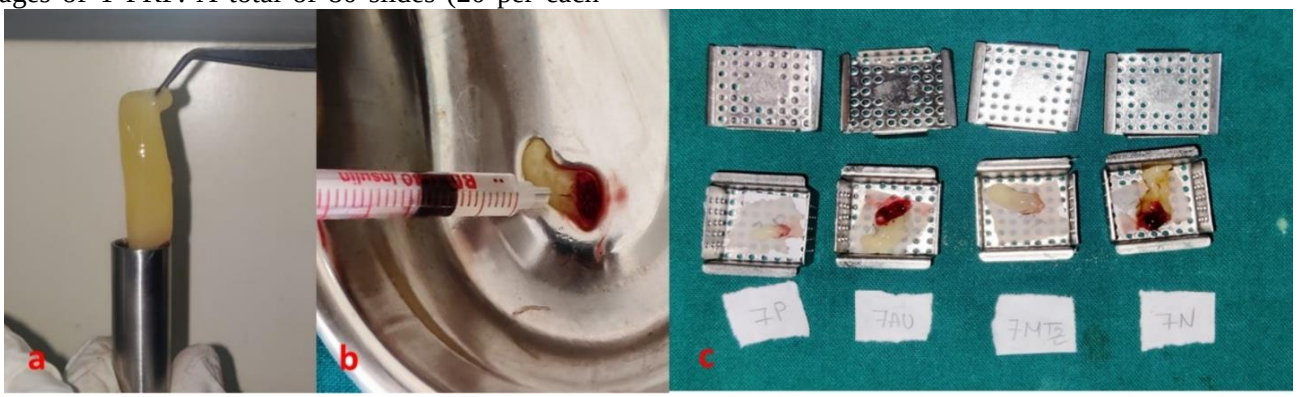

Figure 1 (a) depict the retrieval of T-PRF clot from titanium tubes, (b) depict the method of injecting Amox- clav/ Metronidazole (MTZ)/ Neem gels and (c) depict the paraffinization of samples of all the groups

Procedure for Scanning Electron Microscopy

T-PRF clots were prepared from additional three volunteers by centrifuging and injected with antibiotic gels or herbal extract. These 12 samples were transferred to 2.5% glutaraldehyde solution for tissue fixation. Further, samples were subjected to various graded concentration of alcohol (25%, 50%, 75%, 90% and 100%) for about 20 seconds. Later, alcohol treated T-PRF injected clots were transferred to a desiccation chamber and desiccated using calcium chloride ($CaCl_2$). For SEM examination platinum sputtering done and checked the surface morphology of injected T-PRF clots under scanning electron microscope (model JSM-IT800 ultra high-resolution field emission microscope, JOEL, USA) (Figure 3).[9] For checking the semi quantification of elemental composition of T-PRF, Energy Dispersive X-ray (EDX) (Figure 4) was performed for all the groups at the mid membrane region using the same SEM machine which contains additional. This would help in identification of chemical elements.

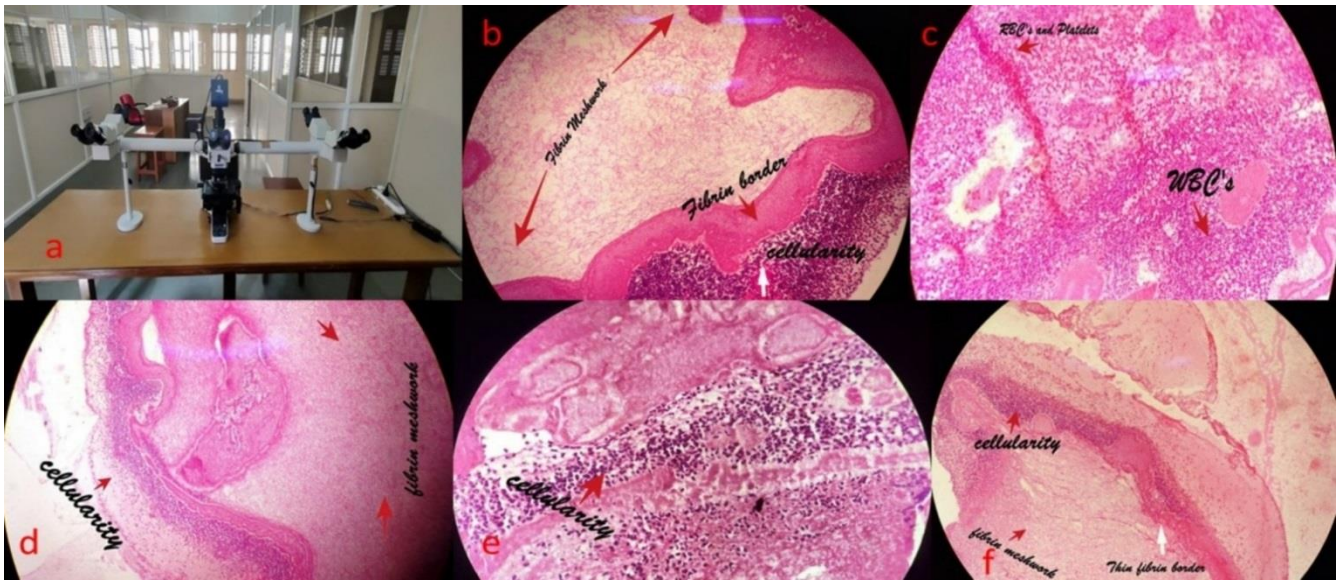

Figure 2 (a) depict the image of a penta head microscope, (b, c) depict the image of plain T-PRF at 10x and 20x magnification where fibrin meshwork, cellularity, border are shown; (d) shows the histological image of T-PRF Metronidazole (MTZ) group; (e) depict the image of T-PRF Amox+ clav gel; (f) depict the histology of T-PRF Neem gel.

Parameters Assessed

Parameters such as Fibrin network pattern- thick and dense or thin and loose; meshwork whether packeting, layered or scattered; cellularity whether present or absent; cell distribution whether narrow or wide; presence or absence of red & white blood cells (RBC, WBC) and platelets. Mean cells percentage scores that ranged from 1-4 (score 1 <25%, score 2 26-50%, score 3 51-75%, score 4 >75% of cells distribution).[9] For SEM, fibrin thickness, fibrin structure, cellular entrapment and presence of RBC,WBC and platelets were assessed at 1000x and 5000x magnification. All the parameters were assessed by experienced oral pathologist and came for conclusion along with a periodontist who has previous experience of assessing the slide sections of T and L-PRF clots.[9]

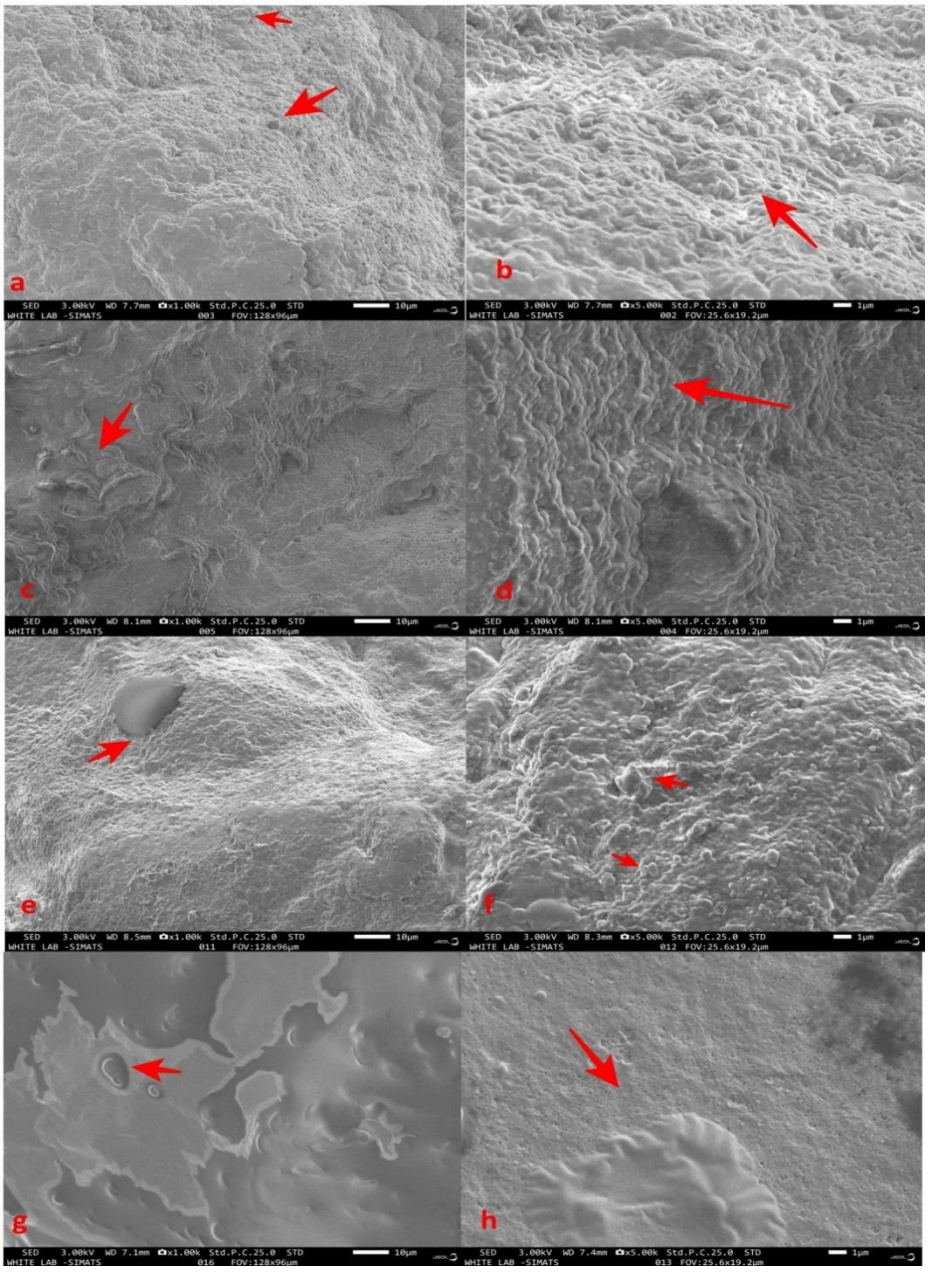

Figure 3 depicts the scanning electron microscopic images of T-PRF plain (a, b), T-PRF +Amox+clav gel (c, d), T-PRF+ MTZ gel (d, e), T-PRF+ Neem gel (f, g) at 1000x and 5000x magnifications. Red arrow depicts the presence of thicker fibrin mesh work and cellular entrapment for 3a to 3d), Red arrows in 3e and 3f report the presence of red blood cells (RBC) and white blood cells (WBC) with haziness showing the coating of drug on the

surface. Red arrows on 3g depict the RBC & fibrin coated with neem and 3h depict the surface porosity present indicating a looser fibrin meshwork in neem group.

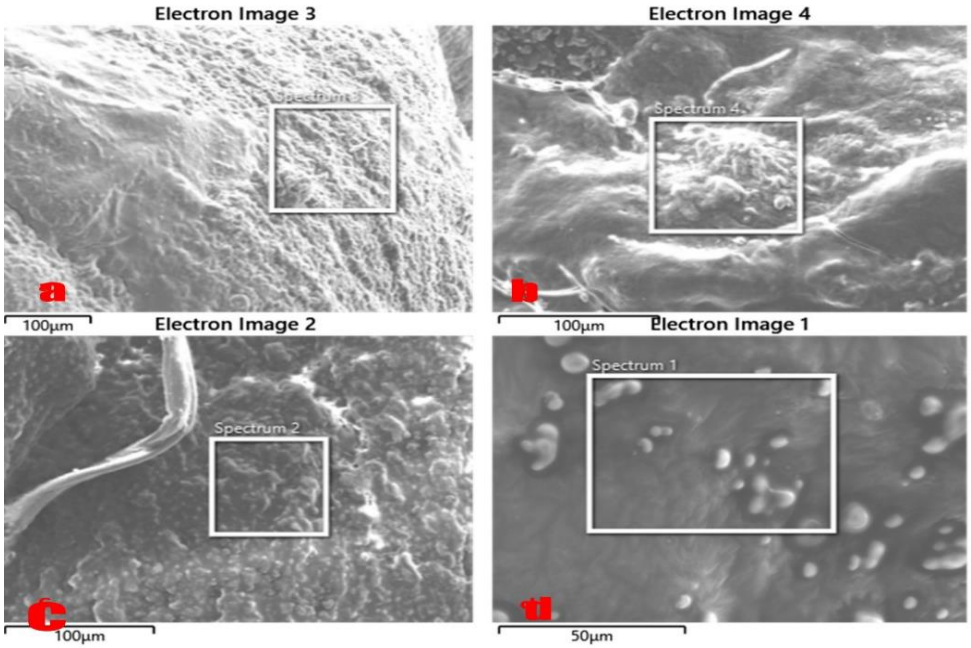

Figure 4 depict the image of energy dispersive X-Ray (EDX) of Plain T-PRF (a), T-PRF+ Amox+ clav gel (b), T-PRF + MTZ gel (c) and T-PRF+ Neem gel (d)

Statistical analysis

Entire data was transferred into Microsoft excel spread sheet and analysed using statistical package for social sciences (SPSS) version 23.0, IBM Pvt Ltd, Chicago, Illinois, USA. Frequency distribution analysis was done to qualitative data, mean and standard deviation was expressed for amount of cellularity across the clot. Fisher exact test was employed to find association in different groups, one Way ANOVA test was employed to find significant difference in score in different groups. P value <0.05 was considered statistically significant. Peason's chi-square test was performed to check the parameters of all the groups in SEM examination

RESULTS

Regarding fibrin network patterns in all the groups, T-PRF plain had an equal distribution of 50% of both Thick & Dense and Thin & loose. In case of T-PRF+ MTZ gel group 80% was thick & dense fibrin network pattern while 20% was thin & loose. For T-PRF+ neem gel group there was 60% of thin & loose fibrin network pattern and 40% was thick & dense pattern. Further T-PRF + Amoxiclav gel group showed 70% thick& dense and 30% showed thin & loose pattern. All the values were statistically non-significant (p=0.555#) (Table 1).

Table 1. The significant comparisons of fibrin network patterns among all the study groups

FIBRIN NETWORK	T-PRF Plain N (%)	T-PRF MTZ N (%)	T-PRF Neem N (%)	T-PRF Amox clav N (%)	P-Value
Thick and Dense	10 (50)	16 (80)	8(40)	14 (70)	0.555#
Thin and Loose	10(50)	4 (20)	12 (60)	6 (30)	

Statistically not significant, N- Number, %- percentages, Amox clav- Amoxicillin+Clavulanic acid gel, p value <0.05 was considered statistically significant, MTZ- Metronidazole, T-PRF- Titanium Platelet Rich Fibrin

Table 2. The significant comparisons of all the groups regarding fibrin pattern, cell distribution, presence of red blood, white blood cells and platelets

PARAMETERS	T-PRF Plain N (%)	T-PRF MTZ N (%)	T-PRF Neem N (%)	T-PRF Amox clav N (%)	P-Value
Fibrin network- pattern					
PACKETING	12 (60%)	8(40%)	8(40%)	18(90%)	0.221#
LAYERED	0(0%)	6(30%)	6(30%)	0(0%)	0.371#
SCATTERED	8(40%)	6(30%)	6(30%)	2 (10%)	0.436#
Cell Distribution					
NARROW	6(30%)	6(30%)	8(40%)	6(30%)	0.990#
WIDE	14(70%)	14(70%)	12(60%)	14(70%)	
Cellularity					
PRESENT	20 (100%)	20 (100%)	20 (100%)	20 (100%)	1.000
RBC'S					
PRESENT	20(100%)	20(100%)	20 (100%)	20(100%)	1.000
PLATELETS AND WBC'S					
PRESENT	20(100%)	20(100%)	20(100%)	20(100%)	1.000

#Statistically not significant, N- Number, %- percentages, Amox clav- Amoxicillin+Clavulanic acid gel, p value <0.05 was considered statistically significant, MTZ- Metronidazole gel, T-PRF- Titanium Platelet Rich Fibrin

Regarding fibrin network pattern, for T-PRF plain group 60% showed packeting pattern and 40% showed scattered pattern. For T-PRF MTZ group 40% showed packeting and 30% layered and remaining three slides couldn't be assessed. Further T-PRF+ Neem group showed similar pattern to that of metronidazole group with 30% percent scattered pattern additionally. In case of T-PRF + Amoxiclav group reported 90% packeting pattern and 10% showed scattered pattern. When all the groups were statistically compared, they reported a statistical non-significance. While coming to cell distribution all the groups showed non-significant (p=0.990#) 70 % of wide distribution except for T-PRF+ Neem group where it recorded 60% and 30% of all groups showed narrow distribution. Further regarding presence of cells, all the test and control groups reported non-significant (p=1.00#) presence of cells including platelets, RB & WB cells (Table 2). Regarding the mean score of cellularity, all the groups reported a statistical non-significance (p=0.386#). In this, T-PRF+ MTZ and T-PRF Amoxiclav group reported a mean of 1.2±0.78, 1.2±1.03; while T-PRF plain reported 0.9±0.9 and T-PRF+ Neem gel group reported a mean of 0.6 ± 0.69 (Table 3). Regarding SEM images all the groups showed similarity regarding fibrin thickness, fibrin structure, cell entrapment, red blood cells, white blood cells, platelets with no statistical significance (p>0.05). Thus, indicating injecting drugs or herbal extract didn't affect the morphology of T-PRF (Table 4).

Table 3. The mean significant scores of all the groups for light microscopy.

GROUPS	N	Mean	Std. Deviation	p-Value
T-PRF Plain	20	0.900	0.99	0.386#
T-PRF MTZ	20	1.200	0.78	
T-PRF Neem	20	0.600	0.69	
T-PRF Amox clav	20	1.200	1.03	

#statistically not significant, N- Number, p value< 0.05 was considered statistically significant, MTZ- Metronidazole, Amox clav- Amoxicillin +Clavulanic acid, Std- Standard

In case of semi quantification of EDX, highest carbon (C) % was reported in T-PRF+ Neem followed by T-PRF+ MTZ group then T-PRF+Amoxiclav group and T-PRF plain. Highest Oxygen (O) % was reported in T-PRF Plain followed by T-PRF+Amoxiclav gel then T-PRF+MTZ and T-PRF+ Neem. Sulphur (S) was also reported in all the groups in traces. Silicon (Si) was reported only in T-PRF+Amoxiclav gel sample (Table 5).

Table 4. Depict the frequency distribution percentages of scanning electron microscopy of T-PRF clots injected with Augmentin or Metronidazole or Neem gels with T-PRF alone

GROUPS		T-PRF Amoxi-Clav gel	T-PRF MTZ gel	T-PRF NEEM gel	T-PRF PLAIN	P value
		Count (%)	Count (%)	Count (%)	Count (%)	
Fibrin thickness	Thick	2 (50%)	3 (75%)	2 (50%)	3 (75%)	0.785#
	Thin	2 (50%)	1 (25%)	2 (50%)	1 (50%)	
Fibrin Structure	Organized	4 (100%)	4 (100%)	2 (50%)	3 (75%)	0.211#
	Disorganized	0	0	2 (50%)	1 (25%)	
Cell Entrapment	Present	4(100%)	4(100%)	4(100%)	4(100%)	-
	Absent	0	0	0	0	
Red Blood Cells	Present	2(50%)	2(50%)	2(50%)	3 (75%)	0.859#
	Absent	2(50%)	2(50%)	2(50%)	1 (25%)	
White Blood Cells	Present	4(100%)	4(100%)	3 (75%)	4(100%)	0.362#
	Absent	0 (0%)	0 (0%)	1 (25%)	0 (0%)	
Platelets	Present	3 (75%)	2 (50%)	2(50%)	4(100%)	0.362#
	Absent	1 (25%)	2(50%)	2(50%)	0 (0%)	

%- Percentages, # non-significant, P >0.05 was considered statistical non-significance, Amoxi-Clav- Amoxicillin+ Clavulanic acid, MTZ- metronidazole

Table 5. Depict the mean values of Energy Dispersive X-ray (EDX) of Titanium Platelet Rich Fibrin (T-PRF) plain and incorporated with Amoxiclav gel/ Metronidazole gel/ Neem gel

Elements	T-PRF Plain (%)	T-PRF+ Amoxclav gel (%)	T-PRF+ MTZ gel (%)	T-PRF+ Neem gel (%)
C	68.6 ± 0.4	71.40± 0.31	77.5± 0.4	87± 0.4
O	30.9 ± 0.4	26.65± 0.30	22.1± 0.4	12.6± 0.4
S	0.5 ± 0.0	0.84± 0.03	0.4± 0.0	0.4± 0.0
Si	-	1.11± 0.03	-	-

C-Carbon, O- Oxygen, S- Sulphur, Si- Silica, Amoxi-Clav- Amoxicillin+ Clavulanic acid, MTZ- metronidazole, %- Percentages

DISCUSSION

Mixing antibiotics to the L-PRF was done by Bernardo F et al.,[25] 2023 where they concluded that drugs mixed with L-PRF in the oral surgery treatment helped in reduced post-operative pain and enhanced therapeutic effect of systemic antibiotics. This mixing of antibiotics in PRF preparation didn't affect the structural properties of PRF. Many antibiotics like metronidazole, amoxicillin, clindamycin, alendronate, metformin etc. in gel forms were used as local drug delivery systems in surgical and non-surgical treatments. To our

knowledge, no study was performed till now by injecting these antibiotics and herbal extract gels in T-PRF and assessed for histological analysis. All the participants in the study were voluntarily willing and participated in the study. No complications and pain were reported at the blood drawing site. Due to scarce comparisons, present study results will be compared with the existent literature of any platelet concentrate.

Various drugs like vancomycin, ampicillin, diclofenac sodium have been incorporated into PRF and reported that there was no loss in structure, had greater anti-microbial efficacy for antibiotic studies while lower post-operative pain was recorded as diclofenac sodium study concerned in oral surgery 3rd molar extraction.[26, 27] But injecting antibiotics prior to centrifugation is always a issue of concern as they might disrupt the centrifugation process and coagulation process might hampered. Apart from this, Polak D et al.,[28] in their study injected different concentrations of antibiotics and concluded that injecting more than 0.5 ml of antibiotic into PRF might alter the structure, make it fragile and interfere with PRF maturation.[28] In the present study we had used T-PRF, injected antibiotics and herbal extract after centrifugation which had thicker fibrin meshwork, carried an inherent property of swelling and holding of antibiotics within itself.

Recently Ercan et al.,[18] 2022 used T-PRF for sustained drug delivery system (SDDS) where they have incorporated doxycycline hyclate liquid within T-PRF and observed that T-PRF acted as a SDDS and kinetically released the drug for longer time duration than doxycycline incorporated collagen sponge. Hence, present study was conducted to check whether incorporation of N/Amoxiclav/MTZ in T-PRF led to any change in fibrin meshwork, cell pattern, cell distribution etc. and check histologically under light microscope.

Regarding fibrin network though there was no significant difference among the groups thick and dense pattern was reported with MTZ and Amox-clav group than Neem gel group. This is in accordance for MTZ and Amox-clav groups and contrast to Neem gel group with studies conducted by Tunali M et al.,[7] 2013 and Chatterjee A et al.,[11] 2017 where they stated a thick and dense fibrin network pattern. Present study results for fibrin network was similar to Bhattacharya HS et al.,[9] 2022 specified that T-and L-PRF shared a like arrangement with non-significance and thicker network found in middle membrane region with statistical significance. This thick and dense pattern that was recorded in the present study might be due to inherent thick membrane that was formed during centrifugation.

Regarding fibrin pattern all the groups showed greater percentages of packeting pattern which is in harmony with Tunali M et al.,[20] 2014 and Chatterjee A et al.,[11] 2017 where they stated a packeting pattern with thicker fibrin and contrast with Bhattacharya HS et al.,[9] 2022 where they described a scattered pattern. This might be due to stimulation of platelets by the passivated titanium tube (inner titanium dioxide layer) lead to thicker meshwork with packeting pattern.

While coming to cell distribution, all the groups showed wider distribution of cells over the length of the clot. This is in harmony with Chatterjee A et al.,[11] 2017, Tunali M et al.,[7] 2013, Bhattacharya HS et al.,[9] 2022 and 2020[8] where they also reported cells had a wider distribution. This shows that incorporation of antibiotics or herbal extract didn't alter the distribution of cells over the clot.

All T-PRF clots incorporated with antibiotics or herbal extract showed the presence of RBC's, WBC's and Platelets. While coming to mean scores of presence of cells, all the groups showed non-significant 26-50% cells distribution. These parameters are in harmony with Bhattacharya HS et al.,[9] 2022; Tunali M et al.,[7] 2013 & Chattarjee A et al.,[11] 2017 where they also conveyed the wider distribution of cells. This may be due to greater centrifugation time of 15 minutes and higher rotations of 3500 rpm which had helped in entrapment of higher percentage with wider distribution over the clot. Hence present study results shared similarity with that of Esra ercan et al.,[18] 2022 where T-PRF holded Doxycycline hyclate whereas in the present study T-PRF holded MTZ, Amox-clav and Neem gels separately without disturbing the cell distribution, fibrin network and distribution pattern. This might be due to inherent swelling property; soft nature of the clots makes it a viable treatment modality for making a ray of hope in utilizing this T-PRF as SDDS.

While checking for SEM comparison present study results were in accordance with Ercan E et al.,[18] 2022; Bhattacharya HS et al.,[9] 2022 and Mitra DK et al.,[12] 2019 where they have concluded that T-PRF had greater cellular entrapment with thicker fibrin

structure and presence of RBC, WBC and platelets. Because of T-PRF clot inherent property injecting drugs didn't alter the morphology and under SEM surface haziness (whitish or dark) was noted in all the test groups indicating the adherence and presence of drug gels within and outside the clots. Further it can be understood that T-PRF had a greater holding capacity within itself. Present study had a greater fibrin thickness and organized pattern even after injecting drugs or herbal extract and were much

better than Ravi S et al.,[29] 2020 for SEM while accordant results were reported for light microscopy, where they stated that T-PRF had looser fibrin pattern that help in greater angiogenesis and also concluded that T-PRF had 0.1% titanium that got incorporated when checked under energy dispersive X-ray spectroscopy (EDX).

Current study results of semi-quantitative elemental analysis showed similarity regarding the elements of C, O, and S with that of Carey and Giuliano[30] 2016 and Dias F J et al.[31] 2020 where they recorded additional elements like Nitrogen (N), Chlorine (Cl), Fluorine (F) Phosphorus (P) and Sodium (Na) apart from C, O and S. This might be due to the presence of only fibrin structure without red cell component in the present sample. Presence of S in the samples depict the amino acid composition, N, Cl, P and Na were absent in the current study samples and this variation might be due to the absence of red part in the T-PRF structure, influence of added drugs on the clot and EDX was performed at mid membrane region. In T-PRF +Amoxclav gel sample there was a detection of silica (Si) and this might be due to the minor contamination of sample or some sought of artifacts.

Present study histological results were also in accordance with a recent study done by Gummaluri SS et al., [32] 2024 for neem gel incorporation in T-PRF where they shared a loose thin layered pattern of T-PRF+ neem gel clot. This finding may indicate that T-PRF had a proper space to incorporate antibiotic gels or herbal extract to become a standard SDDS.

Limitations of present study might be smaller sample size, performing drug kinetics (using spectrophotometric analysis), antimicrobial efficacy, animal trial or a human randomized clinical trial might be better way of assessing the clinical outcome of this newer treatment strategy making T-PRF as a SDDS. Titanium tubes are costly but it is a sought of one-time investment, non-breakable and re-sterilisable without cross contamination. This article opens up a path way to newer surgical treatment protocol for treating periodontal disease.

CONCLUSION

Within limitations, present study concluded that T-PRF incorporated with MTZ, Amox-clav and Neem gels separately had maintained an intact fibrin network structure and pattern to most extent without disturbing the cell distribution. SEM showed that incorporation of antibiotic gels or herbal extract didn't alter the surface structure and also able to identify the coating of drug on to the clots. Hence present study opened a way that T-PRF can be used as a SDDS by incorporating antibiotics or herbal extract for treating periodontal disease.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Conflict of interest and Financial Disclosures

The authors declare no conflicts of interest related to this study and no source of external funding.

Ethical Approval

This study was approved by the Institutional Ethical Committee of GITAM Dental College and Hospital with protocol number 71086061123. All participants signed an informed consent form.

Informed Consent

All participants signed an informed consent form. Informed consent in regional language Telugu or English was received from the patients included in the study and a patient information sheet in regional language Telugu or English explaining about the study was also given to the patients. After receiving the informed consent from the patients, the samples were collected

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