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CLINICAL AND HISTOLOGICAL EVALUATION OF NOVEL APPLICATION OF TETRACYCLINE INCORPORATED PLATELET RICH FIBRIN MEMBRANE AS A GINGIVAL DRESSING IN THE TREATMENT OF GINGIVAL PIGMENTATION - A SPLIT MOUTH STUDY

Periodontology

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ABSTRACT

INTRODUCTION- PRF membrane as a palatal bandage has been described as an efficacious approach to protect the raw wound. It aids in faster epithelialisation neovascularisation and can be used to shield open wound.,

OBJECTIVE – The study was aimed to compare clinically and histologically the efficacy of tetracycline incorporated PRF and Platelet rich fibrin (PRF) alone on wound healing after depigmentation procedure.

METHODOLOGY- In this split mouth study, a total of 12 systematically healthy individuals were divided into two groups after laser depigmentation procedure, where one group received PRF membrane and in second group tetracycline incorporated PRF was placed. The individuals were evaluated for Visual Analogue Scale (VAS), Healing index (HI) on 5th day. Histological Analysis was done on 5th day by taking biopsy.

RESULT- On statistical analysis, Healing index and histological findings were statistically significant between the two study groups with $P < 0.001$. Clinical evaluation of healing index and histological analysis shows better result in tetracycline incorporated PRF group as compared to PRF alone

CONCLUSION-Thus it can be considered that, the application of Tetracycline incorporated PRF or PRF or can be considered to protect the raw wound area of depigmented sites**.** However, tetracycline incorporated PRF group showed faster healing on the basis of healing index and histological analysis

KEYWORDS

Depigmentation, PRF, Tetracycline incorporated PRF

INTRODUCTION

A smile appears to own a favourable influence upon others and makes one more approachable. Gingival health and colour have a very important role in the maintenance of a perfect smile. The gingiva is taken into account to be the foremost frequently pigmented region of the mouth.[1]

The degree of melanin pigmentation varies from one individual to another which is particularly attributed to the melanoblastic activity.[2]

Gingival hyperpigmentation in patients with a gummy smile or excessive gingival display could pose an aesthetic problem. Studies[3] have shown that dark patches of pigmentation on the facial aspects of gingiva might have psychological effects on individuals and that attitudes of people towards pink gingival colour is more favourable as compared to brown, blue-black colorations[4]

Conventional methods of melanin depigmentation such as gingival abrasion and scalpel depigmentation are time-consuming, painful and show recurrence after a short period of time. On the other hand, lasers used in modern dentistry show better results with minimal postoperative complications.[5]

Compared to a conventional scalpel, a diode laser can cut, ablate and reshape oral soft tissues more easily, with none or minimal bleeding and pain; and requiring only a few sutures if necessary.[6]

The depigmentation procedure essentially involves the removal of the gingival epithelium in conjugation with a layer of underlying connective tissue, thereby allowing the denuded connective tissue to heal by secondary intention. The new epithelium that forms is thus entirely devoid of melanin pigmentation thereby providing the patient with desirable results.[7]

The use of platelet concentrates known for its immense reparative potential could aid rapid epithelialisation on the de-pigmented gingival surface.[8] Platelet-rich fibrin (PRF) is a second-generation platelet concentrate, first developed by Choukroun et al.[9] as a membrane with innate wound healing properties that promotes fast cicatricial tissue remodelling; and can be used to shield open wounds.[10] PRF membrane as a palatal bandage has been described as an efficacious approach to protect the raw wound area of the palatal donor site.[11] Platelet-rich gels lacks antibacterial properties, and when producing the gel 30 minutes after intravenous administration of antibiotics rendered the gel with strong antibacterial activity against Enterococcus faecalis. Cieslik- Bielecka et al suggested an approach to combine antibiotics to the PRF.[12]Thus the study was aimed to compare clinically and histologically, the efficacy of Platelet-rich fibrin (PRF) alone and tetracycline incorporated PRF on wound healing after de-pigmentation procedure.

MATERIALS AND METHOD

Twelve patients, within an age range of 18-35 years reporting for the treatment of gingival melanin hyper-pigmentation were selected from the outpatient Department of Periodontology for this split mouth study. Ethical clearance was obtained from the Ethical Committee of the Institute. All participants were verbally informed about the study and written informed consent was obtained before participation.

INCLUSION CRITERIA- Systemically healthy subjects with moderate bands of bilateral physiologic gingival hyper-pigmentation on the facial aspect as given by Dummett[13]; patients who were concerned about their aesthetic appearance patients with a plaque index score of <1 and gingival index score <1, >2 mm of attached gingiva, patients willing to go for surgical treatment, PRF placement and ready to sign the informed consent.

EXCLUSION CRITERIA- Presence of gingival recession, thin

gingival biotype, Individuals on antibiotic or anti-inflammatory drugs for the past 3 months, Individuals who underwent periodontal surgery in the past 6 months, Smokers and tobacco chewers, Pregnant and lactating mothers.

The parameters which were evaluated were
1. Visual analogue scale for pain
2. Healing index [14]
3. Histological healing- histological parameters used to evaluate healing were:

Collagen content was considered into Low/Moderate/Abundant [15] (Table 2)

The density of the inflammatory infiltrate was assessed under 40× magnification. The inflammatory reaction was scored according to the following criteria: minimal/moderate /severe [16](Table 3)

Similarly, 3 fields with high density of blood vessels were identified under 40 × magnification. The mean numbers of blood vessels were calculated and were categorized as containing Scarce/ Moderate/ Abundant vasculature.[16]

SURGICAL PROCEDURE

The de-pigmentation procedure was performed with the Diode Laser technique in the maxillary and mandibular anterior teeth region. The procedure was carried out by applying topical anaesthetic gel over the surgical area. Power setting at 1.5 watt and continuous mode. The fibre optic tip of the diode laser was initiated and the procedure was performed in contact mode. The laser tip was placed at a slight angulation to the surgical area and continuous small lighting brushing strokes were used. The tip was continuously moved to avoid overheating at one point. The surgical area was wiped with a gauze soaked in saline and remnants of ablated tissues were removed. After the de-pigmentation procedure, a PRF membrane was placed in the maxillary or mandibular first quadrant and fourth quadrant respectively. (Figure 1C and 1 D), whereas tetracycline incorporated PRF was placed on the second and third quadrant respectively (Figure 1E). The membrane was stabilized with 4-0 suture (mersilk-4.0) followed by periodontal pack placement.

The patients were refrained from brushing for 5 days following the procedure and were instructed to rinse the mouth with 0.2% Chlorhexidine Digluconate mouthwash twice a day. On the 5th day, the individuals were recalled, and the periodontal pack was removed. The clinical healing index was recorded[13] and biopsies were taken from the group. The histological parameters were evaluated following the surgical procedure. The healing index[14] and Visual Analogue score for pain were checked for each individual on the 5th postoperative day. (Figure 1F)

PREPARATION OF PRF MEMBRANE (GROUP I)

5 ml of blood was taken in glass vacutainer and was centrifuged in REMI 4 (R-8C, REMI Laboratory Instruments, Mumbai, India) centrifugation machine at 3000 rpm for 10 min.[9] The gel obtained following the centrifugation had three layers. The top layer contained platelet poor plasma and the bottom layer consisted of concentrated red blood cells. The middle layer harboured a fibrin network gel. The gel was then compressed in between sterile gauze pieces, and the supernatant was discarded to obtain the PRF membrane.

PREPARATION OF TETRACYCLINE INCORPORATED PRF (GROUP II)

The procedure was similar to group A except, before centrifugation, antibiotics were added to the fresh blood using a syringe at volumes of 0.5ml, i.e. 8 mg/ml tetracycline was added to the blood sample.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software for Windows. (Version.16.0, SPSS Inc., Chicago, IL USA). The Patient data were analysed. The normality of data was tested by the Shapiro-Wilk test. The significant difference between 2 groups was tested by the Non-Parametric test (Mann –Whitney test) and within the Groups by Wilcoxon sign test. The significance of the association (dependence) between 2 qualitative variables was tested by the Pearson chi-square test. The level of significance and confidence interval was 5% and 95% respectively.

RESULTS

VAS FOR PAIN

The VAS score for pain was checked on the 5th day postoperatively. Group I reported a mean VAS Score of 3.00±1.512 as compared to Group II individuals, where the mean VAS score was 2.38±1.061. (Table 6) 62.5% of individuals in Group I reported mild pain whereas 37.5% of individuals reported moderate pain, as compared to Group II where 87.5% reported mild pain and 12.5% reported moderate pain. (Table 4) Although a lower VAS score was found in Group II when compared with Group I, the results were not statistically significant between the groups.

HEALING INDEX

The various scores present in the healing index as per Landry et al[13] were taken on the 5th day after the de-pigmentation procedure. The mean healing score in Group I was 2.25±0.463 as compared to Group II where it was 3.75±0.463. (Table 6) 75% of patients in Group II reported of very good healing whereas 25% patients reported of good healing as compared to Group I where 75% patients reported of poor healing and 25% patients reported of good healing. Inflammation was more in Group I as compared to Group II. (Table 5) Also, the mean healing difference was statistically significant between both the groups $P < 0.001$.

HISTOLOGICAL ANALYSIS

Abundant collagen content (Figure 2) was observed in 9 cases (75%) and moderate collagen content was seen in 2 cases (15%) in Group II as compared to Group I where 7 cases (58%) shows moderate collagen content and 5 cases shows low collagen content. Inflammation was greater in Group I as compared to Group II (1.20±0.447). 8 cases (66.66%) in Group I, demonstrated a moderate inflammatory cell infiltrate and 20% consisted of a severe inflammatory cell infiltrate. In Group II, 7 cases (58.33%) exhibited a distinct parakeratinized stratified squamous epithelium with fibrous connective tissue and minimal inflammatory cell infiltrate; and 8 cases demonstrated a moderate inflammatory infiltrate as compared to Group I. Abundant vessels were seen in 60% cases in Group II in comparison to Group I where 66% cases moderate vasculature and 33% scarce vasculature is seen. The overall healing in Group II was significantly better as compared to Groups I ($p< 0.001$). The histologic healing was well organized in 58.33% individuals of group II compared to the moderate healing seen in 66.66% cases of Group I. (Table 7)

DISCUSSION

Melanin pigmentation of the gingiva does not pose a medical problem, however, the removal of the hyper-pigmented gingival epithelium is required in cases of aesthetic concern. The de-pigmentation leaves behind an exposed connective tissue on the gingival surface for the healing to take place by secondary intention.[4]

Different technique such as scalpel technique, bur method, electrocautery and laser were used to treat gingival hyper-pigmentation and it was seen that postoperative healing and patient comfort were better in laser as compared to other techniques.[17] (The healing process can be enhanced by the use of platelet concentrates. Platelet-rich Fibrin (PRF) has been shown to have several advantages as simplified preparation and lack of biochemical handling of blood requires anticoagulant nor bovine thrombin, which makes the preparation strictly autologous.[18]However, according to Vahabi et al PRF had a negative effect on Human Gingival Fibroblast cell proliferation and caused 38% and 60% decrease in viability.[19]

To the best of our knowledge, this is the first study to evaluate the outcome measures of PRF incorporated antibiotic in wound healing after de-pigmentation. We aimed to compare clinically and histologically, the efficacy of Platelet-rich fibrin (PRF) alone and tetracycline incorporated PRF on wound healing after de-pigmentation procedure. On comparison of VAS, none of the patients reported pain in Group I. Our results were similar to the study reported by Bansal et al [20] wherein no patient-reported of pain at the PRF site after 5 days. Similarly, in a study conducted by Debnath et al [21], the VAS in the individuals on the 3rd day showed 5 cases (45.5%) with moderate pain who received platelet concentrate. Contrastingly, a study by Dahiya et al[22] found that on the 5th day, all 12 (100%) patients in the PRF group reported no pain. Our Group II is first of its kind; however, similar results were obtained by Kumar et al [24] where the VAS score for the pain of PRF incorporated metronidazole group (6.1 ±0.9) was lesser than the PRF group (6.8 ±1.03) on 3rd day after the extraction of a mandibular molar.

The various scores present in the healing index as per Landry et al. were taken on 5th day after the de-pigmentation procedure. The results of group I in terms of inflammation were again in concordance with Bansal et al[20]. The PRF Group demonstrated good healing with no inflammation when compared to the Non-Eugenol dressing Group. Similarly, Debnath et al [21] found that on the 3rd day following de-pigmentation, all cases demonstrated good healing in the PRF group, whereas Dahiya et[22] concluded that on the) 3rd postoperative day, all the individuals of PRF group reported good healing when compared to the Non-Eugenol Dressing Group. As for Group II, healing was better in comparison to Group I. This may be attributed to the protection conferred by the antibiotic against the systemic peri-operative contamination of anaerobic bacteria. Comparably, Shetty et al[25]compared PRF incorporated metronidazole and PRF alone, for changes in bone regeneration and soft tissue healing. They concluded that soft tissue healing was better in the PRF incorporated metronidazole group.

Amongst all the histological parameters assessed in our study, inflammation was the only significant factor found to be statistically significant. The present study is the first to perform histological analysis on tetracycline incorporated PRF. Tetracycline might increase the connective tissue reattachment by enhancing the fibroblast attachment by virtue of its conditioning influence, and also increases the stability of periodontal connective tissues by its anti-collagenase activity. The anti-collagenase activity, in turn, reduces the host-mediated damage to the periodontal tissues, thereby providing stability to the periodontal fiber. Similar results were obtained by Bansal et al [20] and Dahiya[22] et al who found minimal inflammatory infiltrate in a section obtained from the PRF site. Also, Debnath et al[21]reported a moderate inflammatory cell infiltrate in 70% cases of their PRF group. We believe that such a decrease in the inflammatory infiltrate in the lamina propria is the critical morphological trait for the control of a healing process. Antibiotics incorporated PRF has been used in various intrabony defects for socket preservation in numerous studies. Ina similar study by Taneja et al[26], the authors concluded that the combination of PRF+MTZ proved to be better than PRF alone, due to the maximum growth factor concentration and antibiotic action for the first 5–7 days for the treatment of intrabony defects. Kobayashi et al[27] stated that together with the additional benefits of PRF in wound healing, epithelization, and the release of growth factors - makes PRF with antibiotics a suitable device as a dressing for surgical wounds. According to Del Corso et al [28], platelet concentrates are remarkable adjuvants for tissue healing and regeneration because of their growth factor content and sticky nature.

The results of the present research study are in favour of the use of PRF incorporated with tetracycline as a postsurgical dressing, but a small sample size and limited duration of the evaluation of results were its possible limitations. Future studies with larger sample size and a longer follow-up period may extrapolate the role of tetracycline incorporated PRF as a dressing in diverse surgical techniques.

Table 1- Healing Index

Healing Index	Tissue color	Bleeding on palpation	Granulation tissue	Incision margin	Suppuration
1– Very poor: 2 or more signs are present	≥ 50% of red gingiva	Yes	Yes	Not epithelialized, with loss of epithelium beyond incision margin	Yes
2- Poor	≥ 50% of red gingiva	Yes	Yes	Not epithelialized, with exposed connective tissue	No
3– Good	25-50% of red gingiva	No	No	No exposed connective tissue	No
4– Very good	‹25% of red gingiva	No	No	No exposed connective tissue	No
5– Excellent	All pink tissues	No	No	No exposed connective tissue	No

Table 2 – Collagen Content

LOW	MODERATE	ABUNDANT
Thin delicate loosely arranged collagen fibers.	Homogenized bundles of collagen fibres with marked fibroblastic response.	The presence of thick, hypocellular collagen bundles with eosinophilic hyalinization.

Table 3 - The Inflammatory Reaction Was Scored As Minimal/ moderate/severe According To The Following Criteria:

MINIMAL	MODERATE	SEVERE
Sparse distribution of inflammatory cells (up to 10% inflammatory cells per section) in 1 or 2 isolated sites.	Moderate: Moderately dense accumulation of inflammatory cells (between 10% to 30% inflammatory cells per section) in 1 or 2 isolated sites with sparse distribution in other areas	Dense aggregation of inflammatory cells (>30%) throughout the connective tissue.

Table 4 – Percentage Of Visual Analouge Scale For Pain On 5th Day In Group I And Group II

VAS	GROUP I	GROUP II
MILD	62.5%	87.5%
MODERATE	37.5%	12.5%

Table 5 – Percentage Of Healing Index On 5th Day In Group I And Group II

HEALING INDEX	GROUP I	GROUP II
POOR	75%	Nil
GOOD	25%	25%
VERY GOOD	Nil	75%

Table 6- Comparision Of Mean Vas And Healing Index On 5th Day IN GROUP I AND GROUP II

PARAMETERS	GROUP I	GROUP II	P.VALUE
VAS	3±1.512	2.38±1.061	3.00
HEALING INDEX	2.25±0.463	3.75±0.463	**0.01***

Mann whitney test

Table 7- Percentage Of Histologic Healing On 5th Day In Group I And Group II

GRADE OF HEALING	GROUP I	GROUP II
VERY LIGHT HEALING	33.3%	-
MODERATE HEALING	66.6%	-
ADVANCED HEALING	-	41.66%
WELL ORGANISED HEALING	-	58.38%

Figure 1

FIGURE LEGENDS

FIGURE 1 A – Pre operative view

FIGURE 1 B - Immediate postoperative view following depigmentation

FIGURE 1 C - Placement of PRF membrane in 1st quadrant after the procedure

FIGURE 1 D - Suturing of PRF membrane in 1st quadrant (GROUP I)

FIGURE 1 E - Suturing tetracycline incorporated PRF in 2nd quadrant (GROUP II)

FIGURE 1 F- Postoperative view on 5th day

FIGURE 1 G – Postoperative view 3 months after the procedure

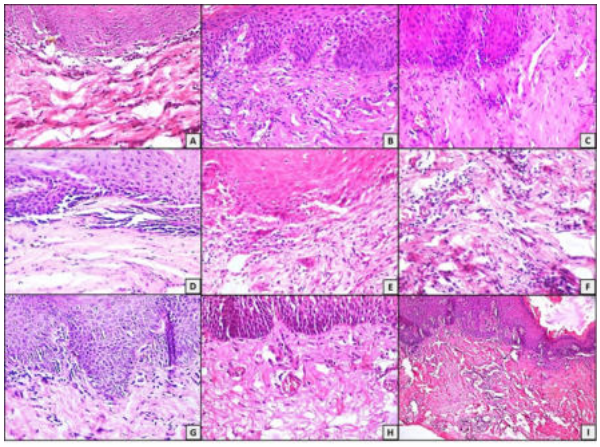

FIGURE 2 - -Haematoxylin– eosin (H&E) stained sections exhibiting (A) Thin, fibrillar collagen with a mild inflammatory cell infiltrate in the connective tissue (40×); (B) Thick bundles of collagen fibres (40×); (C) Dense homogenized connective tissue with abundant collagen content (40×); (D) Minimal inflammatory cell infiltrate within the lamina propria (40×); (E) Moderate chronic inflammatory cell infiltrate (40×); (F) Severe diffuse inflammatory cell infiltrate (predominantly lymphocytic) dispersed throughout the connective tissue stroma (40×); (G) Scarce vasculature consisting of small blood vessels lined by endothelial cells (40×); (H) Multiple blood vessels of varying calibre engorged with RBCs in the juxta epithelial connective tissue stroma (40×); (I) Abundant vasculature composed of numerous blood vessels and haemorrhagic areas (10×)

CONCLUSION

The application of PRF or tetracycline incorporated PRF can be considered to protect the raw wound area of depigmented sites; however, tetracycline incorporated PRF group showed faster healing.

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