The Effect of Leukocyte Platelet Rich Fibrin (L-PRF) Membrane in The treatment of Localized Gingival Recession (Randomized Controlled Clinical Study)

Methodology

Date:3/11/2020

Sample selection and assignment:

Twenty patients who were diagnosed with Miller class I and II gingival recession, participated in this prospective clinical trial.

The patients were recruited from the outpatient clinic of Oral Medicine, Periodontology, oral Diagnosis and Radiology Department, Faculty of Dentistry, Ain Shams University. The purpose of the study was explained to all patients and an informed consent was signed before the conduction of the study. The proposal was presented to the faculty of Dentistry Ain Shams University Research Ethics committee and was approved before starting the research with number (453).

Patient selection:

Inclusion Criteria:

1- Systemically healthy patients (***American Society of Anesthesiologists I; ASA I***).
2- Age range (20 -40) years.
3- Good compliance with the plaque control instructions following initial therapy.

4- Patients diagnosed with localized gingival recession ***Miller*** class I or II in anterior and premolar teeth (maxillary or mandibular)

5- Availability for follow up and maintenance program.

Exclusion criteria:

1. Patient suffering from periodontitis
2. Presence of smoking habit.
3. Presence of occlusal interferences.
4. Pregnant and lactating females.
5. Vulnerable group of patients (handicapped, mentally retarded and prisoners) (according to the recommendation of esthetical committee of Faculty of Dentistry Ain Shams University).
6. Carious teeth and teeth with periapical infection.

Calculation of Power analysis:

The study consisted of two groups; **group (I) and group (II)** each group included 10 patients. The number of patients in each group was determined by sample size calculation based upon the results of ***Tunali et al. (2015)***. Using alpha level of 0.05 (5%) and β level of 0.20 (20%) i.e. power = 80%; the estimated minimum required sample size was approximately 4 cases in each group. Patients were randomly selected using computer generated randomization (www.randomizer.org). Allocation concealment was achieved using a sealed coded opaque envelope containing treatment of the subject.

Grouping Criteria:

Group I: (L-PRF+ coronally repositioned flap):

Included 10 patients who received L-PRF membrane with coronally repositioned flap for the treatment of gingival recession

Group II: (Sub epithelial connective tissue+ coronally repositioned flap):

Included 10 patients who received sub-epithelial connective tissue graft with coronally repositioned flap for the treatment of gingival recession

III- Clinical parameters Assessments:

For the selected sites, the following clinical parameters were assessed using periodontal probe (UNC-15) * preoperative (baseline), 3, 6 and 9 months after the surgical procedure.

1. Plaque index ***(Silness and Löe 1964)***.
2. Gingival Index (***Löe and Silness 1963)***.
3. Probing depth (PD) ***(Glavind and Loe, 1967)***.
4. Clinical attachment level (CAL) ***(Glavind and Loe 1967)***.
5. Gingival recession depth.
6. Keratinized tissue width.

* UNC15 Single-ended, color-coded probe. ergonomic handle. University of North Carolina, Hu Friedy, USA.

- **Pre surgical therapy:**

  - Full Conventional periodontal treatment including supra, sub-gingival scaling and root planning were performed using ultra sonic[†] and hand scalers [‡] and hand curettes[§].
  - Oral hygiene instructions (regular brushing 2 times/day and flossing) were repeated until patients had achieved a proper level of oral hygiene where plaque index (PI) and gingival index (GI) ≤ 1.

[†] Woodpecker UDS-K LED Ultrasonic Scaler EMS Compatible, Faye dental professional supplies, China

[‡] Sickle scaler, posterior jacquette, Hu-Friedy ,USA

[§] Universal Curettes(2R/2L),(4R/4L), Hu-Friedy ,USA

IV-Surgical protocol:

Group (I)

1) Anesthesia (Articaine HCL 4% containing epinephrine at a concentration of 1:100,000)[**] was administrated at the recession area.

2) An intrasulcular incision was made with a surgical blade (15c)[††] on the buccal aspect of the involved tooth. The incision was extended horizontally to dissect the facial aspect of the adjacent papillae both mesially and distally.
3) Two oblique releasing incisions were made mesial and distal to the horizontal incision beyond the mucogingival junction.
4) A full-thickness flap was raised with a periosteal elevator up to the mucogingival junction. A partial-thickness dissection was done apically, leaving the underlying periosteum in place.
5) A mesio-distal and apical dissection parallel to the vestibular lining mucosa was performed to release residual muscle tension and facilitate the passive

[**] Artinibsa 40mg/0.01mg/ml solution for Sc/IM injection, epinephrine 1:100000,cartridges of 1.8ml ,inibisa dental, Spain
[††] Trinon15c sterile scalpel Blades ,Germany

coronal displacement of the flap. The papillae adjacent to the involved tooth were de epithelialized.

6) After flap reflection debridment[‡‡] was done to the root surface for removal of necrotic cementum from the root surface.
7) Just prior to surgery a 10 ml intravenous blood was obtained from the median antecubital vein of the patient, put in glass test tube[§§] without additives and centrifuged[***] immediately at 2700 revolutions per minute for 12 minutes at room temperature for L-PRF preparation.
8) Coagulation started immediately and three parts quickly appear in the tube: a packed red blood cell at the bottom, acellular plasma at top and the L -PRF clot in between.
9) The L-PRF clot was extracted from the tube and it was cut from the blood clot using a surgical blade (15c)[†††] , the clot was then placed in the PRF box to obtain a membrane.

[‡‡] Universal Curettes(2R/2L),(4R/4L), Hu-Friedy ,US
[§§] Dry Vacutube, Biocon®, Brazil
[***] PRF CENTRIFUGE (Duo machine), UNSPSC code 41100000 (Ostralos Ltd, New Zealand)
[†††] Trinon sterile scalpel Blades ,Germany

10) The L-PRF membrane was fixed at the site then the flap was coronally repositioned by sling suturing technique using 5-0 polypropylene sutures[‡‡‡].

Fig (1): PRF centrifugation machine

[‡‡‡] Assut Sutures, non Absorbabale (Polypropylene) 5-0 USP, braided violet coated 3/8 rev.cutt.17mm, AM trading, Swithezland

Group (II)

As group I from step number 1 to step number 6

7) Infiltration anesthesia was given in the palate anteriorly and posteriorly to the area of sub epithelial connective tissue graft harvesting.

8) Sub epithelial connective tissue graft was obtained from the palate using surgical blade 15c [§§§]through a single incision technique approximately 3 mm apical to the gingival margin of the maxillary teeth. An incision was made perpendicular to the palatal tissue until reaching the bone in a horizontal direction. Partial thickness incision was done to separate the sub epithelial connective tissue from the covering palatal epithelium, then using a small mucoperiosteal elevator the tissue was dissected from the palatal bone and the graft was cut from both sides. The palatal tissues were sutured with x suture using 5-0 polypropylene [****]sutures.

9) The sub epithelial connective tissue was fixed at the recipient site using resorbable 5-0 vicryl

[§§§]Trinon sterile scalpel Blades ,lot0483,Germany

[****] Assut Sutures, non Absorbabale (polypropylene) 5-0 USP, braided violet coated 3/8 rev.cutt.17mm, AM trading, Swithezland

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[††††] Assut Sutures, Absorbabale (PGA) 5-0 USP, braided violet coated 3/8 rev.cutt.17mm, AM trading.

[‡‡‡‡] Assut Sutures, non Absorbabale (Polypropylene) 5-0 USP, braided violet coated 3/8 rev.cutt.17mm, AM trading, Swithezland

V-Postoperative care:

- All patients received antibiotics for 1 week (Augmentin 1 gm every 12 hours[§§§§], metronidazole[*****] 500gm every 12 hours) and analgesics (Brufen [†††††]400 every 8 hours) were prescribed for the patients.
- Patients were instructed to avoid brushing at the surgical site. They were instructed to rinse twice daily with chlorohexidine[‡‡‡‡‡] mouthwash the day after surgery.
- Sutures were removed after 2 week.
- Patients were instructed not to brush for another 2 weeks after suture removal and to continue using chlorohexidine mouthwash.
- Patients were instructed to use modified bass technique for tooth brushing after one month from surgery.

[§§§§] Augmentin 1gm,Medical union pharmaceuticals(MUP) Egypt under license from ,Glaxosmithkline

[*****] Flagyl 500 mg, sanafi Aventis Egypt under license of sanafi Aventis French.

[†††††] Brufen 400mg, Al kahira pharm. and chem..Ind.Co. under license from abbott laboratories

[‡‡‡‡‡] Hexitol(Chlorohexidine HCL 125mg/100ml)the Arab drug company(ADCO)A.R.E

VI-Postoperative evaluation and assessment:

The patients were recalled every month for examination of the surgical area and for plaque removal when necessary

- Clinical and esthetic parameters were evaluated and scored by a separate blinded operator at 3, 6 and 9 months postoperative.

Esthetic outcome evaluation was done using Root coverage esthetic score (RES) system *(Cairo et al. 2009*) the RES include the following parameters

- Level of the gingival margin **(GM)**: Zero points = failure of root coverage (gingival margin apical or equal to the baseline recession); 3 points = partial root coverage; 6 points = complete root coverage **(CRC)**.
- Marginal tissue contour **(MTC)**: Zero points = irregular gingival margin (does not follow the CEJ); 1 point =proper marginal contour/ scalloped gingival margin (follows the CEJ).
- Soft tissue texture: Zero points = scar formation and/or keloid like appearance; 1 point = absence of scar or keloid formation.

- Mucogingival junctional aligment **(MGJ)**: Zero points =MGJ not aligned with the MGJ of adjacent teeth; 1 point = MGJ aligned with the MGJ of adjacent teeth.
- Gingival color **(GC)**: Zero points = color of tissue varies from gingival color at adjacent teeth; 1 point = color of the tissue is similar to the gingival color of the adjacent teeth

These parameter were measured 3, 6 and 9 post-surgical

Volumetric assessment after 9 months :

Impressions were done using addition silicon impression[§§§§§] material at baseline and 9 months.

These impressions were poured using extra hard stone[******] material. All the stone cast models were digitized using optical scanner. The digital surface models were imported as STL files into the software for volumetric analysis (plastycad, 3diemme, Como, Italy). On each cast 4 fixed points were determined and remained untouched during the observation time. These 4 fixed points defining a

§§§§§ Elite HD+ Putty Soft addition silicon Manufacturer Part No: ZHC203000. Zhermack SpA, Italy

******Rock extra hard stone, Manufacturer Part No: C410339, Zhermack SpA, Italy

base plane for one series of measurements. The relevant area for the measurements of volume changes was defined by the mesial and distal surfaces of adjacent teeth.

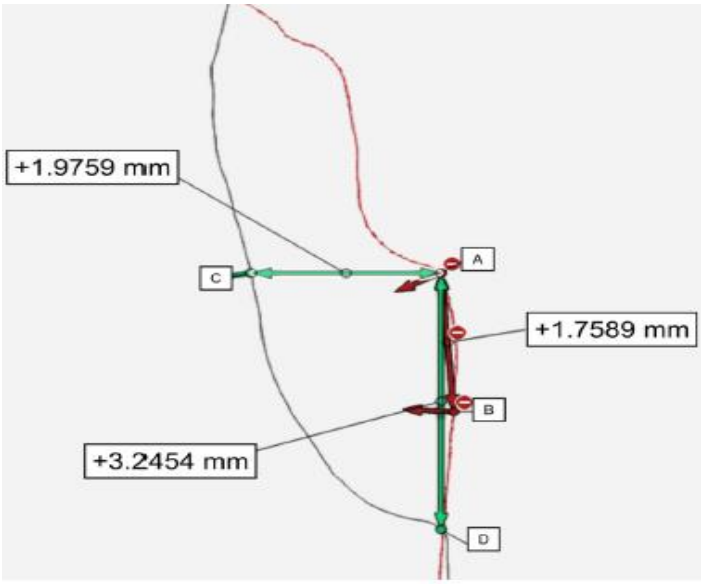
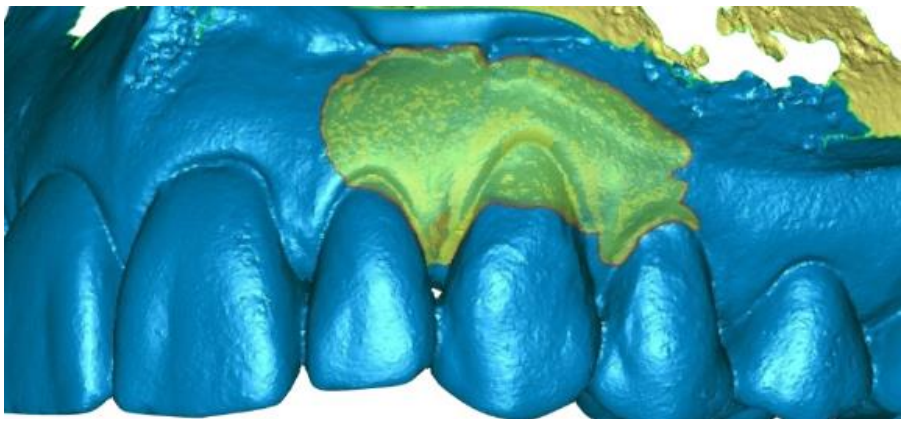


Fig (2): Four fixed points defining a base plane for one series of measurements

The digital images obtained were transferred into digital imaging software, which was used to superimpose and match the images obtained in one coordinate system. For superimposition of different images, the buccal surfaces of the adjacent teeth were used as a reference. The scanned cast images were superimposed and subtracted and the soft tissue volume was determined for any volume changes

To evaluate the differences in form between the preoperative and postoperative casts, the variation between images was calculated by subtracting the corresponding phase images, leaving a phase difference image.



Fig(3): Diagram showing the superimposition for the volumetric assessment of the soft tissue thickness

- All data were collected and statistical analysis was done.

VI-Statistical analysis:

Categorical data were presented as frequencies and percentages and were analyzed using chi square test. Numerical data were tested for normality using Shapiro-Wilk test and were presented as mean and standard deviation values. Parametric data were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and one-way repeated measures ANOVA followed by Bonferroni post hoc test for intragroup comparisons. Non-parametric data were analyzed using Kruskal-wallis test followed by pairwise comparisons utilizing Mann Whitney U test with Bonferroni correction for intergroup comparisons and Friedman's test of repeated measures followed by multiple pairwise comparisons utilizing Wilcoxon signed-rank test with Bonferroni correction for intragroup comparisons. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with IBM ††††††SPSS‡‡‡‡‡‡ Statistics Version 26 for Windows

†††††† IBM Corporation, NY, USA.
‡‡‡‡‡‡ Statistical Package for Social Science, Inc., an IBM Company.