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The Effect of Low Intensity Laser on Bone Regeneration of Intrabony Defects Treated by Liquid Phase Platelet Rich Fibrin and Membrane with Bone Graft

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ABSTRACT

Purpose: The current clinical study was done to examine clinical and radiographic changes of intrabony defects in response to LLLT combined with Platelet Rich Fibrin (PRF)system & bovine engraft. **Material and methods**: The study was conducted on twenty (20) sites from ten (10) patients. A spilt-mouth design was used, sites were randomly selected from each patient, into two groups: control group; ten (10) sites received PRF system without irradiation, and laser group; ten (10) sites received PRF system and it was irradiated with low level laser. Clinical parameters included probing pocket depth (PPD), clinical attachment level (CAL), Plaque index (PI), and gingival index (GI).Digital radiograph assessment was done . **Results:** comparison between the groups at 6 months postoperatively showed significant difference between both Probing depth and clinical attachment level, Plaque index and Gingival index. **Conclusion**: The using of low intensity laser with PRF system showed an improvement in the outcome of infra bony defects treatment.

KEYWORDS

Intra-bony defects, LLLT, i-PRF, Xenograft, PRF Membrane

INTRODUCTION

The term "periodontal disease" refers to a group of chronic inflammatory diseases that affect the gums (or the gingiva, the soft tissues round the teeth), the bones, and the ligaments (the connective tissue collagen fibers that connect the teeth to the alveolar bone) which support the teeth. Gingival inflammation is a local inflammation of the gums caused by bacteria in plaque, a biofilm of microorganisms that

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accumulates within the teeth and gums, and is the first stage of periodontitis. During this introduction, the term periodontitis refers to plaque-induced periodontitis. If left untreated, gingival inflammation causes loss of gums, bones, and ligaments, which is characteristic of the disease and leads to deep periodontal "pockets" that can ultimately cause tooth loss. Periodontal disease increases the burden of inflammation throughout the body and may exacerbate conditions like diabetes [1].

Periodontal bone and soft tissue loss are gradual and generally permanent, the first hurdle in treating periodontal disease is a fast and proper diagnosis. This is especially challenging because early periodontal disease is painless, and patients seldom seek early treatment [2].

The ultimate treatment objective is to regenerate missing tissues. Gene-based, protein-based, and cell-based tissue regeneration techniques are combined with scaffolding and guiding biomaterials that may be resorbable or non-resorbable and traditional or 3D printed in new periodontal treatments [3]. These methods are mostly focused on repairing bone in order to support teeth or implants, although soft tissue regeneration is sometimes required, particularly for cosmetic reasons. The membrane barrier is put beneath the soft tissue (and hence is less prone to infection) and utilized as a scaffold or occasionally as a retaining device for bone or bone replacement grafts in guided tissue regeneration [4]. Antimicrobial and growth-stimulating drugs can now be delivered via membranes [5].

Bone grafts or alloplastic materials are utilized in regenerative treatments because they will operate as a scaffold for bone creation (osteoconduction), contain bone-forming cells (osteogenesis), or contain bone-inducing substances (osteoinduction). It should be Osteogenic, meaning it should either provide actual viable osteoblasts or Osteoinductive materials, or it should stimulate primitive mesenchymal cells brought in via the blood supply from adjacent bone or periosteum to differentiate into osteoblasts or Osteoconductive materials, or it should simply act as a lattice or framework for cell growth, allowing osteoblasts from the wound margin to infiltrate the defect and migrate across the graft. A major number of osteoblasts are drawn to the graft site as a result of this [6].

Furthermore, autologous (from the same individual), allogenic (from various people of the same species), and xenogenic grafts are distinguished (originating from different species). Finally, alloplastic materials refer to both synthetic and non-organic materials [7].

Platelet concentration has been used in dentistry for over 30 years as a regenerative therapy capable of releasing super physiological amounts of growth factors required for tissue regeneration from autologous sources [8]. Platelet-rich fibrin (2nd generation PC) is formed by centrifuging blood without the use of chemicals. Both PRP and PRF have high concentrations of transforming growth factor (TGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epithelial growth factor (EGF), and fibroblast growth factor'' (FGF). Platelets are the most abundant source and have the potential to improve wound healing and periodontal regeneration through the regulation of angiogenesis, cell proliferation, migration ,and differentiation [9].

In 2014, injectable platelet-rich fibrin (i-PRF) was created by varying spin centrifugation pressures [10]. Blood centrifuged at lower speeds in plastic centrifuge tubes yielded i-PRF, which is a flowable platelet rich fibrin. In the event of major bone shortages, the administration of bone grafts for bone augmentation may be problematic. To address such issues, i-PRF in conjunction with chopped L-PRF membranes and particle graft biomaterial becomes a feasible solution [11]. As a result, the liquid fibrinogen in the i-human PRF is progressively changed into fibrin, which can act as an autologous fibrin binder (AFB). Clinicians have recently used this strategy to promote biomaterial aggregation or coating in order to facilitate wound healing [12].

Maiman was the first to use the laser in a clinical setting in 1960[13]. The wavelength, power density, and application mode are the three most vital parameters to consider for clinical applications (pulse or continuous, contact or non-contact). The earliest investigations on low-intensity lasers show that low-intensity radiation has a good influence on hair growth and wound healing. Depending on the beam characteristics, low-intensity lasers can produce both stimulatory and inhibitory reactions. In the application of the therapeutic effects of electromagnetic waves, the term "photobiomodulation (PBM)" is now used instead of "low-intensity" lasers, since it has a more specific and complete meaning [13].

LLLT has demonstrated a non-thermally and nondestructively significant increase in fibroblast production and collagen synthesis. These effects on cell exhibit a wide range of benefits on biological tissue and altered pain threshold [14]. The benefits of LLLT in wound healing are multifold as it causes biomodulation effects on different types of cells, such as keratinocytes, fibroblasts, osteoblasts, odontoblasts, and endothelial cells [15].

The semiconductor diode lasers (Gallium arsenide (Ga As), gallium aluminum-arsenide (Ga Al As) are moveable compacted surgical units with effective and dependable benefits. Bone formation may be induced in bony defects by LLLT more than PRF. However, the combination of each LLLT and PRF as a treatment way may induce bone formation within the bone defect better than using LLLT or PRF alone according to an experimental study on rats which underwent tooth extraction showed that this combined use of PRF and LLLT increases new bone formation [16].

MATERIALS AND METHODS

Study design: Ten patients diagnosed as having periodontitis; stage III, grade (A) were selected from the outpatient clinic of Periodontology, Oral Medicine, Radiology & Diagnosis Department, Faculty of Dental Medicine for Girls, Al-Azhar University to be included in this study. All patients signed an informed consent. Research Ethics Committee approval with code **(REC-ME-21-07)** was obtained from the Faculty of Dental Medicine for Girls, Al-Azhar University . The inclusion criteria included patients who are free from systemic diseases with presence of at least seven natural teeth to provide reasonable number of teeth, matched periodontitis with a PD ≥ 5 mm and attachment loss ≥ 4 mm. The patients were non-smoker and non-pregnant (women cases) and they are not taking antibiotics or NSAI drugs within the last three months prior to treatment. Additionally, the selected sites should not have been treated surgically within the last year before the initiation of the study.

Sample Size: The sample size of 10 patients (10 sites in each group) was enough to detect the difference. All patients were divided randomly in two groups. (Lasered group): ten (10) sites side received PRF system and was irradiated with LLLT using 980 nm Diode laser. (Control group): ten (10) sites side received PRF system(i-PRF and PRF membrane) only without using of LLLT.

Study procedures: Phase one therapy, supra & subgingival scaling, debridement also oral hygiene instructions were performed for each patient via manual and ultrasonic instrumentation. Approximately after the initial therapy by 4 weeks, the clinical parameters and plaque control were re-assessed. All the included patients had to have good oral hygiene prior to advancing to the surgical procedures. At the baseline and 2weeks, 3, 6 months, postoperatively the clinical examinations were done to assess the Pocket Probing Depth (PPD), Clinical Attachment Level (CAL), Gingival Index (GI)and Plaque Index (GI)[9].

Surgical procedures: All teeth, with pockets ≥ five mm in the same quadrant as the teeth selected to the study, were surgically treated. In all locations, under local anesthesia, an intra-crevicular incision, full-thickness muco-periosteal flaps were reflected then followed by the elimination of G.T using manual instruments in controlled group and by diode curettage in lased group . Surface of roots were scaled and planed by curettes and flushed by a sterile saline solution.10 ml of blood collected in plastic plain tube and centerfuged in 700 RPM for 3 minutes for preparation of i-PRF then i-PRF mixed with xenograft (UBGEN RE-BONE) to form sticky bone and applied to the defect .Another 10 ml blood collected and centrifuged in 3000 RPM for 10 minutes for PRF membrane to cover the graft (Fig1) . A simple suture of the flap and then covered with periodontal dressing to avoid any infection in all patients. Operations done on patients were almost identical. The teeth on the laser sets, were equal to control sets in numbers, and all procedures were accomplished in 90 minutes. Post-operative instructions were as follows; Soft toothbrush to be used in 1st two weeks, and avoiding flossing, and chewing in the treated area for two weeks. Patients restarted full oral hygiene and function after then.Ibuprofen (200 mg) (Brufen produced in Egypt by Kahira Pharm under licence from Abbott Laboratories) upon need was prescribed , chlorhexidine (2 times/day) for two weeks, Augmentin (1g) tablets for five days to avoid possibility of contamination.

Laser protocol: Diode laser 980nm, one Watt power, contact - continuous mode) applied to the flap. A tip (400-mm-diameter) accustomed eliminating all viable epithelium inside the flap from F.G.M to the end of the flap (Fig 2). The tip was an initiated in type. Treatment was accomplished in parallel manner from the coronal to the apical aspect, and the beam of the laser was intermittent (thirty seconds) /ten seconds irradiation. The resulting char deposit was removed with wet cotton-gauze. Another application with the same WL but, a non-contact continuous mode at 0.1 Watt was used by special bio-stimulation tip. All aspects of the flap, alveolar bone, and the graft material in addition to injectable PRF and PRF membrane were irradiated, resulting in a complete dose of four J/cm2 / surface. Protective eyewear was worn by all to prevent injury from laser wavelength exposure and to comply with safety standards. Laser therapy was applied once at the time of surgery.

Figure (1) Application of PRF membrane

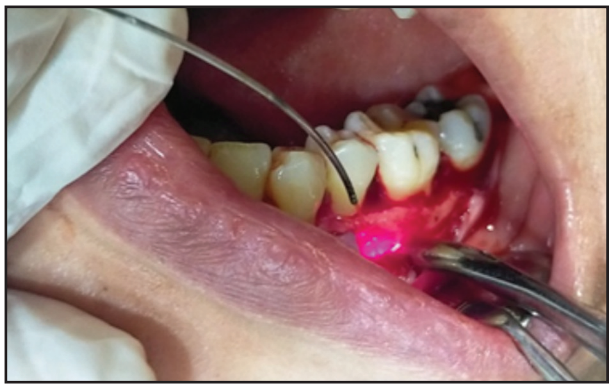

Figure (2) Laser curettage

Statistical Analysis

Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. For parametric data (Pocket depth and VEGF ELISA) independent t-test was used to compare both sets, while ANOVA-test (followed by Tukey's post-hoc test) was used to compare among different observations within the same group. For non-parametric data, Mann Whiney U-test was used to compare both sets, while Wilcoxon-signed rank and Freidman tests were used for comparison between different observations within the same group. Significance level was set at ($p < 0.05$). The statistical analysis was performed with SPSS 18.0 for Windows.

RESULTS

All involved patients completed the study and were re-evaluated at follow up visits. Baseline values were not significant different among the test and control sets for all the assessed variables, suggesting that the initial defect characteristics did not affect the final differences between thee treatment modalities, thus allowing post- treatment results to be compared.

With respect to the PD and CAL; A probing pocket depth showed significant decrease of 3.1mm in laser group and 2.39mm for control group ($P<0.05$), while comparison among groups there was no significant difference at 3 months (P0.261) but were significantly different at 6 months (p 0.035). With respect to CAL, there was a significant gain in CAL of 3.0 mm for laser group and with 2.0mm in control group by 3 months, to reach its maximum gain (almost no CAL) after Six months, without any significance between the groups. (Table 1).

Concerning plaque & gingival indices (PI & GI); There was significant decrease starting from 2 weeks, while the change, 3 and six months was not significant in laser group, while, control group gradually decreased by time, to reach its lowest value after three months, then slightly increased at six months but with no significance between 3 and 6 months; PI (1.340±.163) at baseline for laser group, (1.730 ± 0.133) for control group, and (0.000 ± 0.000) for laser, (0.800 ± 0.200) for control group, postoperatively, GI score was (1.50 ± 0.17) at baseline in laser group, (1.60 ± 0.16) in control group, and (0.00±0.00) in laser, (1.00 ± 0.00) in control group, postoperatively. By comparing between groups there was a significant difference by time in favor to laser group. (Table 2)

Table (1) *Descriptive statistics and comparison of pocket depth (mm) and CAL (mm) among groups*

Variables	Groups	Baseline	3 months	6 months	P
PD (mm)	Laser	4.7 ±0.09	2.13±0.23	1.65±0.08	0.00*
	Control	4.50 ± 0.15	2.53 ±0.20	2.11± 0.10	0.00*
P (between groups)		0.567 ns	0.109 ns	0.025*	
CAL (mm)	Laser	3.03± 0.31	0.17 ±0.021	0.00±0.00	0.00*
	Control	2.12± 0.30	0.23±0.11	0.00 ± 0.00	0.00*
P (between groups)		0.094ns	0.231ns	1.000ns	

Significance level P<0.05, ns=non-significant, Mann Whitney test, Wilcoxon signed Rank test

Table (2) *Descriptive statistics of PI and GI scores comparison intra-group (Friedman test) among groups*

Variables	Groups	Baseline	3 months	6 months	P
PI(scores)	Laser	1.340a±.163	000b±.000	000b±.000	0.00*
	Control	1.730a±.133	0.600c±.163	0.750c±0.20	0.00*
P (between groups)		0.062ns	0.003*	0.001*	
GI(scores)	Laser	1.520a±.16667	.0000b±.000	.0000b±.000	0.00*
	Control	1.430a ±0.15230	0.740b ±0.1227	1.000b±.00	0.00*
P (between groups)		0.634ns	.001*	0.000*	

*Significance level p<0.05, * significant Wilcoxon signed Rank test: means sharing the same superscript letter are not significantly different*

DISCUSSION

Periodontitis is a chief community health problem owing to its huge incidence, and because it possibly will lead to loss of teeth and injury, damagingly affect mastication function and esthetics, and impair quality of life [17]. Periodontitis is a chronic multifactorial inflammatory illness characterized by plaque biofilms and characterized by gradual degradation of the tooth-supporting machinery. Its main consequence is the loss of periodontal tissue support, which is shown as clinical attachment loss (CAL) and radiographically determined alveolar bone loss, as well as the presence of periodontal pocketing and gingival bleeding [18].

Infrabony defects have been found as a long-term risk factor for tooth loss[19]. For these lesions, regenerative periodontal therapy (RPT) has been postulated as a feasible therapeutic idea with positive clinical and radiographic results[20]. For RPT, many flap patterns have been reported. Biomaterials are commonly used in clinical practice to aid periodontal regeneration as barrier membranes, bone replacement grafts (allografts, xenografts, alloplastic materials), and wound modifiers (enamel matrix derivative (EMD)) are the three main categories in this subject. Combinations have also been tried and tested in the clinic. The ultimate objective of RPT is to restore alveolar bone, root cementum, and periodontal ligament regeneration[19].

The capacity to speed up the healing process is the main benefit of employing LLLT in dental and periodontal therapy. Low-level lasers enhanced fibroblast keratinocyte motility, collagen production, angiogenesis, and growth factor release in the treatment of gingivectomies[21].

PRF has also been employed in regenerative dentistry applications. Its application in regeneration periodontics and wound healing has lately been promoted. PRF stimulates periodontal tissue proliferation and differentiation, as well as angiogenesis, according to several in vitro and in vivo investigations [22].

The goal of developing an injectable (liquid) version of PRF (dubbed i-PRF) is to provide doctors with an easy-to-use platelet concentration in a liquid formulation that may be used alone or in combination with other biomaterials. Its ability to connect with biomaterials for bone grafting makes it a viable alternative to platelet-rich plasma (PRP) as a platelet aggregation for bone repair. When compared to alternative PRF formulations that use greater centrifugation speeds, injectable PRF has the benefit of slower and shorter centrifugation rates, resulting in a higher presence of regenerative cells with higher concentrations of growth factors[23].

Low Level Laser Therapy using diode laser has a helpful effect in gingival healing, as it led to increase mRNA production of GFs. So, the best time to apply LLLT is at the stage of cell proliferation, when the secretion of VEGF, BFGF, HGF and SCF GFs is needed to promote the proliferation of fibroblasts and wound healing [14].

Each group showed significantly difference from baseline to 3 and 6 months, this is in accordance to systemic review and meta-analysis to assess and compare studies concerning lasers as an adjunctive or monotherapy for surgical treatment [24].

The utilization of LLLT along with PRF in the site modulated intra-bony defects caused an improvement in the clinical and radiographic outcomes, by means of PD reduction, CAL gain at six months[25] .Plaque index among the two groups showed significant difference indicated that laser groups showed better reduction in PI, indicating the bacterial reduction achieved using laser, as revealed by previous studies evaluated the effect of LLLT on plaque formation .Gingival index' results were significantly different between the groups in favor to laser groups, indicating its greater effect in reduction gingival inflammation. This is in accordance with few previous studies where diode laser was used adjunctive to non-surgical therapy in which no change observed among the groups in gingival index scores.

CONCLUSION

Combination of low level laser in addition to PRF system (i-PRF with bone graft + PRF membrane) system as treatment modality may have a synergetic effect to encourage formation of bone in defects better than LLLT or PRF alone.

RECOMMENDATION

Additional studies are suggested with different wavelengths of laser therapy.

Further investigations on the combined periodontal regenerative technique utilizing platelet rich fibrin system and low level laser with longer follow up period and larger sample size are needed.

CONFLICT OF INTEREST

None declared .

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