

## RADIOGRAPHIC EVALUATION OF BONE DENSITY AFTER BONE GRAFTING ASSOCIATED WITH DIFFERENT LASER BIO-MODULATION TECHNIQUES IN RAT SAMPLE

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### ABSTRACT

**Objective:** The objective of the present study was to evaluate the regenerative power of sticky bone in guided tissue regeneration alone compared to sticky bone with different biomodulation techniques in terms of bone density at the regenerated site.

**Materials and methods:** 40 rats were randomly assigned into 5 groups (8 rats each), the control group (CG), the sticky bone group (SB), the sticky bone with LASER (SB-L), the sticky bone with PRF membrane, (SB-P), and sticky bone with LASER and PRF (SB-L-P). A rectangle shaped bone defect (3ml height and 4 mml length) was created in the diastema between the incisors and molar area with preservation of the lingual plate of bone at the mandible of all rats. The defects were filled according to the assigned test groups. 60 days after surgery the rats were radiographically examined to obtain the bone density measurements.

**Results:** The measurement of bone density in the defect has shown that the SB-L group and SB group had the highest mean ( $172.5 \pm 15$ ), ( $169.9 \pm 14.9$ ) respectively. The difference between the two groups were not statistically significant. However, both groups were statistically significant when compared to all three groups, CG, SB-P group and SB-L-P group. All three groups had the lowest mean ( $72.5 \pm 4.7$ ), ( $74.1 \pm 8.4$ ), ( $88.2 \pm 14.5$ ). There were no statistically significant differences between these three groups.

**Conclusions:** The sticky bone augmented with LASER biomodulation has demonstrated higher bone density compared to sticky bone alone as graft material. However, the addition of PRF, or LASER and PRF has shown to be non-significant.

**KEY WORDS:** Sticky bone, low level Laser, Platelet rich fibrin, Guided bone regeneration, bone density and laser Biomodulation.

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## INTRODUCTION

Bone regeneration represents a challenge to oral surgeons <sup>(1)</sup>. The achievement of restoring bone defects with the best grafting material seems a dream that still hard to achieve.

The osteogenic, biocompatible, readily available graft is the material of choice for successful regeneration. However, and until now, the grafting material which has succeeded to meet all our requirements is the autogenous graft despite the fact that some obstacles are still present when it comes to clinical practice <sup>(2)</sup>. Many complications are involved and considered prior to selecting this graft. Some of these complications are related to the donor site, pain and impaired healing and the other complications could be related to the recipient site such as large bone defects <sup>(2)</sup>. These factors enhanced the existence and surveillance of xenografts as readily available and cost-effective graft material with least possible complications <sup>(3)</sup>. Tissue engineering clinical concepts introduced new materials that could accelerate and modulate the healing process opened the door in front of many research trials to examine the capacity of these new concepts to accelerate bone healing and even to enhance the bone quality <sup>(4)</sup>. Sticky bone is one of the new concepts developing the concept of grafting along with growth factors and better bone consistency along with better graft handling <sup>(5)</sup>. The concept introduced the combination between the platelet- rich fibrin (PRF) gel and injectable PRF to form a coherent graft with bone particles sticking together facilitating graft handling and promoting accelerated healing. The presence of platelet derived growth factors will play a crucial role in the healing process through its ability to signal more stem cells to the defect site. The augmentation of sticky bone with PRF membrane will further increase the growth factors and expected to enhance the quality of the regenerated bone <sup>(6)</sup>.

Low-level laser therapy (LLLT) or photo biomodulation (PMB) can affect key cellular

pathways of life-forms. Results in an open to argument cellular communication pathway, which is mediated by ATP, ROS, or calcium, and which leads to the manipulation of energetic cellular metabolism. PMB can, by this manner, regulate tissue inflammation, improve growth factor expression and cell proliferation, and enhance the healing processes <sup>(7)</sup>. Therefore, PMB offers a good method to support bone regeneration.

Digital radiographs used to evaluate bone density via periapical sensor to detect quantitative change occur in bone <sup>(2,3)</sup>.

The present study compares the quality of regenerated bone with sticky bone xenograft augmented by three different techniques for biomodulation, PRF membrane, LASER, and a combination of both techniques together.

## SUBJECTS AND METHODS

**Study design:** This is an in vivo animal study

**Ethical committee approval:** REC-FDB-SU/12052022-02/SW

### Preparation of the materials

**Isolation of PRF membrane, gel and injectable PRF:** 20 deeply anaesthetized rats were used to obtain blood samples directly by cardiac puncture. The blood samples were placed in 10-ml glass tubes, without addition of any anticoagulant, and immediately centrifuged as follow:

**PRF membrane preparation and PRF gel preparation:** Blood samples were centrifuged for 12 min. at 2700 rpm <sup>(8,9)</sup>. After the three layers were formed in the test tube, PRF gel was obtained. The gel was collected in sterile metal dish, the gel was minced with sterile scissors and immediately mixed with the bone particles. The PRF membrane was easily separated from the tube by a sterile tweezer and by gentle squeezing between 2 sterile glass slab to transform it into a thin membrane.

For injectable PRF preparation (iPRF), blood samples were centrifuged for 3 min. at 2300 rpm <sup>(5)</sup>

In the iPRF tubes, after two layers were separated the aliquot was aspirated with sterile syringe and placed in metal bowel.

*Sticky bone preparation:* The xeno-bovine bone graft particles were mixed with the minced PRF then the iPRF was added to add fibronectin to the mixture rendering the bone graft particles coherent as one block of bone particles (8). The mixture is then used to fill the surgical defects.

### Surgical procedures

After the acclimatization period, the animals were sedated by placing them in a container with a cotton soaked in ethylic ether then received an intramuscular injection of 25 mg/kg xylazine chl oride to attain muscular relaxation and they were anesthetized intramuscularly with 75 mg/kg Ketamine chloride.

The surgical field was shaved and the skin was disinfected by povidone-iodine. 1-cm incision was performed overlying and parallel to the inferior border of the mandible. a Blunt dissection was performed to retract the muscles and expose the lower boundary of the mandible. using a fissure surgical bur with copious irrigation, a rectangle shaped bone defect (3ml height and 4 mml length) was created in the diastema between the incisors and molar area with preservation of the lingual plate of bone, at the upper and lower border of the mandible.

### LASER Application

A red diode LASER (SmartM, Lasotronix, Poland) was used in the study; at 635 nm wavelength with biomodulating handpiece with following set parameters: output power: 100mW, handpiece diameter: 14mm, spot area: 0.5024cm<sup>2</sup>, average power density: 199.04mW/cm<sup>2</sup>, contin- uous mode, dose: 4J per point (8J/cm<sup>2</sup>), time: 40 sec per point, points of irradiation on a surgical side of the wound

and total energy per session 8 J (Figure 1). The diode laser was used in contact mode with wound soft tissue only for the laser groups (G3 and G5) according to the following irradiation protocol: 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days after operation . The total dose after all therapeutic sessions was 24J. <sup>(11)</sup>



Fig. (1) 635 nm diode laser used in the study (Smart M, Lasotronix, poland)

### Study groups

60 adult male albino rats (250–300 gm) were used in the study; 20 were used for PRP and PRF isolation and 40 were subjected to surgical procedure. The animals were obtained, housed and all the experimental procedures were performed in the animal house of NAHDA University, Beni Suef, Egypt. The animals were housed under standard conditions and fed on a standard diet with free access to water.

After the acclimatization period, the surgical procedures were carried out and the rats were randomly assigned into 5 groups (8 rats each) according to the treatment protocol as follow;

Group 1, the control group (CG): control –ve as the bone defects were irrigated with saline only

Group 2, the sticky bone group (SB): the bone defects were filled with sticky bone

Group 3, the sticky bone with LASER (SB-L): the bone defects were filled with sticky bone and the wounds were subjected to LASER application postoperatively

Group 4, the sticky bone with PRF (SB-P): the bone defects were filled with sticky bone and covered with PRF membrane

Group 5, the sticky bone with LASER and PRF (SB-L-P): the bone defects were filled with sticky bone and covered with PRF membrane the wounds were subjected to LASER application postoperatively.

**Sample size calculations**

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference would be found between tested groups. By adopting an alpha level of (0.05) a beta of (0.2) i.e. power=80% and an effect size (f) of (0.584) calculated based on the results of a previous study by Kökdere et al. in 2015 (12); the predicted sample size (n) was found to be (40) samples (i.e. 8 samples per group). Sample size calculation was performed using PASS 2021 software for Windows.

**Radiographic measurements**

After 60 days, the mandibles were radiographed, Imaging the specimens were performed by digital periapical radiograph with Nanopix sensor size 2 .

The images were introduced to imaging J software (1.53 wayne Rasband and contributor, National Institutes of Health, USA) for reading and measuring as linear and area density.

**Densiometric measurements of the bone defect**

To assess the bone density at the bone defect, after sacrifice, a “Region of Interest “(ROI) was chosen just opposing to the surgical site on the rat skin. This ROI was assessed for density measurements twice using the same software by two different blinded radiologists.

Two different measures were recorded, the area and Linear density at the defect site. For linear measurements, sex lines were determined according to the size of each defect. The mean of lines was calculated and pooled into further statistical analysis

**Statistical analysis:** All data of linear and area density were tabulated and statistically analyzed by Statistical Package for the Social Sciences (IBM, SPSS, version 22) in a form of means and standard deviations. The Shapiro-Wilk tests did not show a significance departure from the normality for each of the five tested groups. Data were normally distributed.

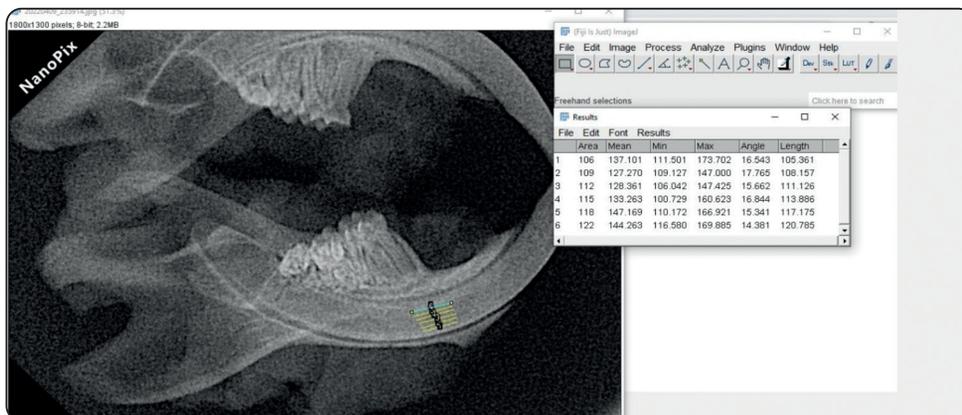


Fig. (2): A digital radiograph illustrating the line density measurements for the same extraction defect: the radiograph demonstrating 6 lines drawn parallel to each other and starting parallel to each other in the defect.

**RESULTS**

The significant differences between different groups were determined by One-way Anova test followed by Post-Hock test. The mean differences were considered statistically significant at  $P < 0.05$ .

The measurement of bone density in the defect has shown that group 3 (The sticky bone group with LASER) and group 2 (the sticky bone group) had the highest mean  $(172.5 \pm 15)$ ,  $(169.9 \pm 14.9)$  respectively. The difference between the two groups were not statistically significant. However, both groups were statistically significant when compared to all three groups, group 1 (The control group), group 4 (The sticky bone and PRF membrane group) and group 5 (The sticky bone with LASER and PRF membrane). These three groups had the lowest mean  $(72.5 \pm 4.7)$ ,  $(74.1 \pm 8.4)$ ,  $(88.2 \pm 14.5)$ . There were no statistically significant differences between these three groups. (Figure 3)

The measurement of the defect area bone density in the defect has shown that group 3 (The sticky bone with laser group) and group 2 (the sticky bone group) had the highest mean  $(181.4 \pm 9.8)$ ,

$(179.2 \pm 9.5)$  respectively. The difference between the two groups were not statistically significant. However, both groups were statistically significant when compared to all three groups, group 1 (The control group), group 4 (The sticky bone and PRF membrane group) and group 5 (The sticky bone with LASER and PRF membrane). These three groups had the lowest mean  $(71.9 \pm 5.1)$ ,  $(81.1 \pm 4.9)$ ,  $(87.6 \pm 8.7)$  respectively. There were no statistically significant differences between these three groups.

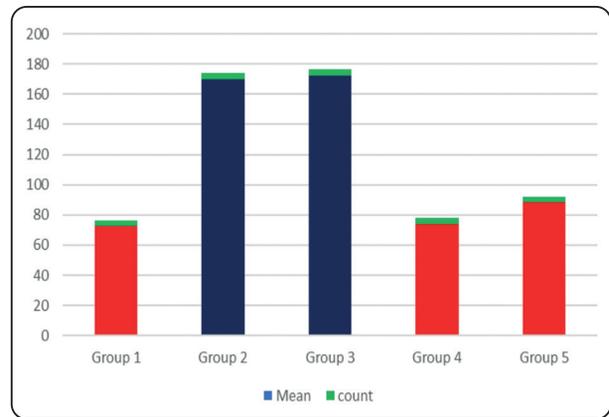


Fig. (3): Histogram showing the count and mean density for each group

TABLE (1): The One-way Anova test results comparing the mean bone density in between the test groups.

Group	Control	Sticky bone	Sticky bone and LASER	Sticky bone and PRF	Sticky bone, LASER and PRF	p-value
Mean	72.5075 <sup>a</sup>	169.9275 <sup>b</sup>	172.53 <sup>b</sup>	74.105 <sup>a</sup>	88.2225 <sup>a</sup>	1.72 *
SD	4.75745	14.98652	15.09326	8.42587	14.59777	

\*: Significant at  $P- 0.621$ , Means with the same letter within each column are not significantly different. Different superscripts are statistically significantly different.

TABLE (2): The defect area bone density

Group	Control	Sticky bone	Sticky bone and LASER	Sticky bone and PRF	Sticky bone, LASER and PRF	p-value
Mean	71.9 <sup>a</sup>	179.2 <sup>b</sup>	181.43 <sup>c</sup>	81.1 <sup>a</sup>	87.6 <sup>a</sup>	1.089 *
SD	5.1	9.5	9.8	4.9	8.7	

\*: Significant at  $P- 0.806$ , Means with the same letter within each column are not significantly different. Different superscripts are statistically significantly different.

## DISCUSSION

Wound healing is a complex process involving cell proliferation, angiogenesis, matrix formation and induction of tissue repair. The use of growth factors will have a positive role in matrix formation, angiogenesis and recruitment of native tissue cells to induce repair. The PRF contents of growth factors are immediately released into the wound area. Epidermal growth factor (EGF) along with Colony stimulating- growth factor (CSGF) will induce new matrix formation, followed by vascular endothelial growth factor (VEGF) inducing new genesis. Tumor growth factor (TGF), Platelet derived growth factor (PDGF), Fibroblastic growth factor (FGF) all will act on cell signaling to recruit and proliferate. The addition of LASER to induce biomodulation by enhancing the vascularity directly correlates to the acceleration of healing process<sup>(13)</sup>.

In order to estimate the effect of biomodulation on sticky bone with xenograft, the present article compared the sticky bone as sole bone graft material to sticky bone with three different biomodulation techniques. The sticky bone with PRF membrane, with LASER and with combination of PRF and LASER. The study compared the regenerative capacity in terms of bone density assessed by digital periapical radiographs. Linear measurements of the defect area were analyzed and compared at all the test groups.

The results of the present study have shown that, the sticky bone augmented with LASER biomodification had the highest bone density, followed by the sticky bone alone. The other two groups of sticky bone where PRF were used either alone or augmented with LASER biomodification, didn't show significant difference. The use of PRF had no significant results when added to the sticky bone.

The use of sticky bone as a coherent material mixing the bioactive PRF membrane and iPRF with Xenograft has been developed by **Sohn et al. in 2015**<sup>(9)</sup>. The technique offered the clinician a

less invasive and less critical procedures with one surgical site when compared to the autogenous on-lay grafts. The results of graft bone density and implant stability after bone grafting with sticky bone were promising. Although the published data are case reports and case series<sup>(14,15)</sup> the results has shown to be clinically significant compared to xenograft alone.

A case report by Sohn et al in 2015 demonstrated the ability of sticky bone to regenerate the alveolar bone in socket preservation and ridge augmentation. The article described favorable bone formation at the graft site.

In 2020 Rupawala et al.<sup>16</sup> investigated the bone regeneration capacity of sticky bone graft placed in extraction socket of lower wisdom tooth. In a split mouth study on 47 patients, the study compared the pain, swelling and stated a rapid bone healing with better bone density after 3 months. However, the study didn't clarify the exact results and the statistical difference at the 3 months follow up time.

Barbu et al. in 2021<sup>15</sup> compared the bone graft with bone shell versus sticky bone around dental implant in 127 implants. The results have shown that the amount of bone formed was comparable in both groups with no statistically significant difference in bone density.

In 2018, Skondra et al. published a systematic review to investigate the efficacy of low-level LASER on bone regeneration and concluded a positive effect of low-level LASER on bone regeneration. The results of the -present study were in accordance with the current literature supporting the LASER biomodulation in bone regeneration.<sup>(17-18)</sup>

On the other hand, the use of PRF membrane for guided bone regeneration was a point of debate. Some authors demonstrated no clinical difference were observed in regards to bone density. These factors may result in unsatisfactory clinical outcomes regarding bone regeneration. These findings were demonstrated by the study by **Oliveira et al. in 2015**.<sup>(20)</sup> The study compared the guided

bone regeneration with Bio-oss alone and Bio-oss with PRF. The study used histometric analysis to assess bone formation. Both groups had the same results with insignificant difference in regards to bone formation after 60 days. **Engler-Pinto et al. in 2019** <sup>(21)</sup> studied the regeneration by bovine bone graft with and without PRF in rat models. The study assessed the bone trabeculation as indicator for bone density at the defect site with micro-computed tomography (C.T). The results have shown that no significant difference was observed between the two techniques after 30 days of the grafting procedures. These findings raised the debate about using collagen membrane in Contact with bone surface and to add the PRF in contact with the soft tissue. <sup>(22)</sup>

## CONCLUSIONS

The sticky bone augmented with LASER biomodulation has demonstrated higher bone density compared to sticky bone alone as graft material. However, the addition of PRF, or LASER and PRF has shown to be non-significant.

## Conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

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