

## EVALUATION OF THE EFFICACY OF FLOWABLE LEUKOCYTE AND PLATELET –RICH FIBRIN VERSUS HYALURONIC ACID AS BIOACTIVE IMPLANT COATINGS (RANDOMIZED CONTROLLED CLINICAL TRIAL)

Ghada Bassiouny\* , Mahmoud Abu Brika\*\* , Mohamed Ezzat\*\*\*  and Ahmed Hommos\*\*\* 

### ABSTRACT

**Background:** osseointegrated dental implants are often left load-free during the healing period. Many trials have been conducted to improve osteointegration to minimize loading time and ultimately raise patient satisfaction.

**Aim of the Study:** to assess the effectiveness of flowable L-PRF and hyaluronic acid as bioactive coatings for dental implants, to decrease loading time.

**Material and Methods:** 30 sites indicated for implant placement in the mandibular posterior region were selected and divided into 3 groups. In test group I; 10 implants were coated with flowable L-PRF and in test group II; 10 implants were coated with Hyaluronic acid while in the negative control (group III) the remaining 10 implants were left uncoated. After osteotomy; all implants were installed. Clinical assessment of implant stability was carried out immediately post-insertion (primary stability) and then 2 and 3 months post-insertion (secondary stability) using Resonance Frequency Analysis. Radiographic assessment of marginal bone level was evaluated immediately post insertion and 3 months later before loading.

**Results:** The results of this study revealed that implants coated with flowable L-PRF showed less marginal bone loss than those coated with hyaluronic acid or the uncoated ones (median of marginal bone loss at 3rd-month post insertion was 0.35mm, 0.37mm and 0.51mm respectively). On the other hand, implants coated with HA showed better secondary stability at 3rd-month post insertion with a median of (89.50) compared to (82.50) for flowable PRF and (79.00) for the uncoated control group.

**Conclusion:** bioactive implant coatings can enhance osseointegration and decrease loading time.

**KEYWORDS:** Bio coatings, liquid PRF, Hyaluronic Acid, Marginal bone level, Implant Stability

\* Associate Professor of Periodontology, Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology, Faculty of Dentistry, Alexandria University, Egypt

\*\* B.D.S, clinical master of oral implantology , Faculty of Dentistry Alexandria University, Egypt

\*\*\* Professor of Periodontology, Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology, Faculty of Dentistry Alexandria University, Egypt

## INTRODUCTION

Dental implants are regarded as an effective and acceptable therapeutic technique for lost teeth.<sup>(1)</sup> Osseointegration is the direct interaction of bone with an inert alloplastic surface.<sup>(2)</sup> Multiple factors, notably implant design, surface topography, bone condition, surgical approach, and implant loading circumstances, are required for reliable osseointegration to occur.<sup>(3)</sup> For better osseointegration, physiochemical features of implant surfaces like surface topography, wettability, and surface energy have been widely researched and adjusted.<sup>(4)</sup> The adoption of micro-rough surface has improved the implant-bone interface's biomechanical qualities. Multiple approaches have been explored to boost implant biocompatibility and osteogenic potential, varying from surface modification with mineral coatings to implant surface biocoatings in order to modulate peri-implant tissue responses.<sup>(5)</sup>

Biocoating substances such as, bone morphogenetic proteins (BMPs), and growth factors have been employed to enhance osteoconduction, osteoinduction, and osteogenesis through various biomimetic approaches for implant surfaces functionalization.<sup>(6,7)</sup>

Several growth factors are expressed throughout healing phases and consequently could be employed as therapeutic agents to promote peri-implant hard and soft tissue repair. However, their application is limited by their costly price and short lifespan.<sup>(8)</sup>

Platelet concentrate is a highly concentrated autologous suspension of platelet growth factors in a small volume of plasma.<sup>(9)</sup> Now it encompasses numerous products, frequently addressed as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF). Unique formulae incorporate as well leucocyte and platelet-rich fibrin (L-PRF) products<sup>(9)</sup>. L-PRF is the recent innovation of various platelet concentrates as first characterized by Choukroun et al in 2001<sup>(10)</sup>. L-PRF are able to release growth factors such as platelet-derived growth factor-AB (PDGF-

AB) transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), and vascular endothelial growth factor (VEGF) with their roles in stimulating angiogenesis, cell migration, and differentiation in an autologous and safe biocomplex.<sup>(11)</sup> Thus providing all the blood components conducive to healing and immunity, along with the concentrated growth factors within the surgical site to facilitate and promote wound healing.<sup>(11)</sup>

L-PRF encompasses two main variants namely the solid L-PRF and the liquid L-PRF. The solid variant is the one used for the production of PRF plugs and membranes. On the other hand liquid L-PRF is the variant referred to as the flowable or injectable PRF or sometimes named liquid fibrinogen because it is rich in both growth factors together with vitronectin and fibronectin; mainly responsible for platelet adhesion, aggregation, and activation and subsequent cell adhesion to the extracellular matrix in the healing process.<sup>(12,13)</sup>

Another bioactive material with increasing interest and promising healing potentials nowadays is Hyaluronic acid (HA). It is a high molecular weight glycosaminoglycan that is expressed in almost all body tissues and fluids.<sup>(14)</sup> It sends out a lot of biological signals to the cells and tissues around it.<sup>(15)</sup> HA also has crucial viscoelastic qualities that reduce viral and bacterial penetration into the tissue.<sup>(16)</sup> By its involvement in various biological processes linked to morphogenesis and tissue repair, as well as its biocompatibility, biodegradability, and non-immunogenicity, HA has been extensively explored as a promising biomaterial for tissue engineering over the last decades. It plays a critical role in bone healing by boosting cell migration, adhesion, and proliferation of undifferentiated mesenchymal cells, encouraging differentiation into osteoblastic cells.<sup>(17)</sup>

Currently, the evidence about the use of flowable L-PRF and Hyaluronic acid as biomimetic implant coating materials are very limited, requiring further studies.

The aim of this study is to evaluate the efficacy of flowable L-PRF versus Hyaluronic acid as bioactive implant coatings in comparison to the uncoated implants via assessment of both clinical implant stability and radiographical marginal bone level.

The null hypothesis was that the use of either flowable PRF or Hyaluronic Acid as bioactive implant coatings would not affect the implant stability or radiographical marginal bone level in comparison to the uncoated implants.

**MATERIALS AND METHODS**

**Study design**

A randomized controlled clinical trial was conducted following CONSORT guidelines <sup>(18)</sup>

Clinical and radiographic evaluation		
Case selection according to Inclusion& exclusion criteria N=30		
Pre-surgical preparation &implants coating		
Surgical osteotomy		
Test group I N=10 10 biocoated implants with flowableL- PRF were installed	Test group II N=10 10 biocoated implants with Hyaluronic acid were installed	Control group N=10 10 uncoated implants were installed
Clinical assessment of implant stability immediately post insertion (primary stability) 2 and 3 months post insertion (2ry stability) using Resonance Frequency Analysis RFA		
Radiographic assessment of marginal bone level immedi- ately post insertion and 3 months later		
Statistical analysis of result		

**Study sample and setting**

The current study was conducted at the Department of Oral medicine, Periodontology, Oral diagnosis and Oral Radiology, Faculty of Dentistry, Alexandria University, Egypt. Ethical clearance was gained and the study protocol was accepted by the institute review board, Faculty of

Dentistry, Alexandria University (IRB No. 001056 – IORG0008839). The study protocol is inaccordance with the Helsinki Declaration 2013. <sup>(19)</sup>

Patients seeking implant placement were screened for this study. All eligible patients should meet the need. for implant-supported crowns on both sides of the mandibular posterior region. All subjects were informed of the study’s aims and signed informed consent.

**Sample Size Estimation**

Sample size was estimated assuming 5% alpha error and 80% study power. The mean (SD) marginal bone loss after 3 months was 2.27 (1.13) mm for the control group, 0.26 (0.065) mm for liquid PRF <sup>(20, 21)</sup> and 0.02 (1.68) mm for Hyaluronic Acid. <sup>(22)</sup> By using F test and the highest SD=1.68mm to ensure study power, the minimum sample size was calculated to be 10 implants per group. Total sample size = number per group xnumber of groups = 10 x 3 = 30 implants.

Software Sample size was based on Rosner’s method <sup>(23)</sup> calculated by Gpower 3.1.9.7. <sup>(24)</sup>

**Inclusion and exclusion criteria:**

Participants were selected according to the following inclusion criteria: Age over 20 years, No serious systemic disorders that affect bone metabolism, Nonsmoker, Good oral hygiene and controlled periodontal disease, and keratinized tissue with a thickness of at least 2 mm. The exclusion criteria were: active infection at implant sites, severe bruxism or clenching behaviors, uncontrolled periodontal disease or poor oral hygiene, pregnancy, and the necessity for bone augmentation.

**Randomization and allocation concealment**

All included patients were randomly allocated to the three arms, the allocation sequence were the permuted block randomization approach, and the block size varied<sup>(25)</sup> The allocation sequence/code

was masked from the person allocating patients to the intervention arms by utilizing sealed opaque envelopes..<sup>(26,27)</sup>

### Blinding

A double-blind strategy was applied on the patients, and the statistical analysis team who was blinded to the group allocation of patients..<sup>(28)</sup>

### PICO question

Following the PICO framework, the population was 30 patients in need for implant-supported crowns on both sides of an edentulous mandibular posterior region and met the inclusion exclusion criteria. Intervention: 10 biocoated implants with flowable PRF and 10 biocated implants with hyaluronic acid were installed after successive osteotomy, compared to 10 uncoated implants. The outcome measures of this study were carried out clinically via assessment of implant stability immediately post insertion, 2 months and 3 months post insertion using the Resonance Frequency Analysis with OSTELL. Radiographic assessment of marginal bone level was evaluated immediately post insertion and 3 months later before loading using the On Demand software (OnDemand 3D software (Cybermed Inc).

### Materials and equipment

- Hyaluronic acid (gengigel.co.uk)
- Centrifugation machine: 80-1 Electronic Centrifuge- Delta lab Egypt.
- Implant fixtures (Strauman group (neodent) Australia 7cyaturay court, port melbourne VIC 3207, Australia).
- CBCT machine (I-CAT FLX v17 Cone beam computered tomography machine USA)
- Ostell (info@ostell.com.) Osstell®

### Intervention

#### Pre-surgical phase

Patients were evaluated following a designed diagnostic chart. Patients underwent radiographic

assessment using Cone Beam Computed Tomography (CBCT) to evaluate alveolar bone width and height, and to insure absence of any pathology. Phase I therapy was carried out for all patients. Patients signed a written informed consent after having a sufficient explanation about the intended surgery, prosthetic procedures, and evaluation process.

### Preparation of biocoating materials

#### First group (flowable L- PRF)

Liquid (flowable) L- PRF Preparation. Venous blood sample were collected in 9 mL noncoated vacutainer tubes with no anticoagulants (white caps). The samples were spun in a table centrifuge at 2700 rpm for 3 minutes, following the methodology described by Andrade et al.<sup>(29,30)</sup>

Immediately after centrifugation, the upper yellow fluid (liquid fibrinogen) was collected by sterile syringes, (avoiding red blood cells). Half of the collected amount of liquid PRF was kept aside to be injected into the osteotomy before implant placement and the other half was used for implant coating (immersion technique)<sup>(30,31)</sup> by injecting it into the implant container for one hour to avoid implant contamination. Fig. (1)

#### Test group 2 (Hyaluronic acid)

Coating of the implant with Hyaluronic acid was carried out one hour before starting the surgery (immersion technique)<sup>(30,31)</sup>. HA was injected into the implant container for one hour to avoid implant contamination.

### Surgical procedures

#### Patient preparation

Systemic antibiotic (1g amoxicillin-clavulanic acid, orally) was given to the patients one hour prior to the procedure. The patients instructed to rinse with chlorhexidine digluconate mouth wash 0.2% for 2 minutes.

### Surgical procedure

- Local anesthesia was injected with mepivacaïne using inferior alveolar nerve block technique for anesthetizing the site of the surgery.
- After anesthesia, mid crestal incision was made and the mucoperiosteal flap was fully reflected, and then sequential drilling was carried out according to manufacture to obtain a perfect osteotomy allowing for primary stability. Fig. (2)

### Grouping:

Test Group I: (Flowable L-PRF) where liquid PRF bio coated implant was installed in prepared osteotomy.

Test Group II: (hyaluronic acid) where hyaluronic acid bio coated implant was installed in prepared osteotomy.

Group III: (negative control group) where un-

coated implant was installed in prepared osteotomy.

### Post-surgical phase

Immediately after implant placement post insertion evaluation parameters were taken. Clinically by measuring the primary stability using ISQ (Ostell) and Radiographically by CBCT.

Post-surgical instructions and medications were prescribed to the patients ;antibiotic (amoxicillin-clavulanic acid (augmentin, 1 g every 12 hours ,GlaxoSmithkline (gsk), Hungary) and analgesic (ibuprofen 600 mg) for 6 days( Cataflam, Novartis Pharma, Cairo, Egypt). The patients were asked to rinse with chlorhexidine gluconate 0.2% 2-3 times a day for 4-5 days following the surgery. Patients were seen after 10 days for sutures removal and assessment of healing. A follow up was scheduled two and three months for clinical and 3 months for radiographical assessment.

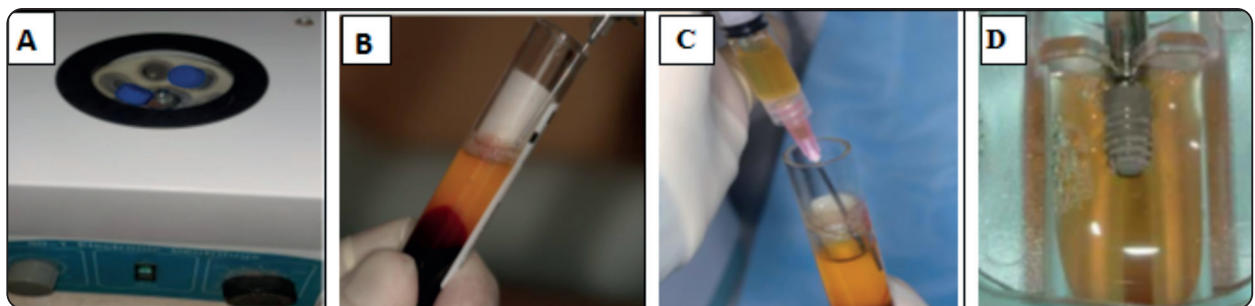


Fig. (1) Illustrating the steps of liquid L-PRF preparation. A: the centrifugation machine. B&C formation and aspiration of liquid L-PRF. D: implant coating with immersion technique.

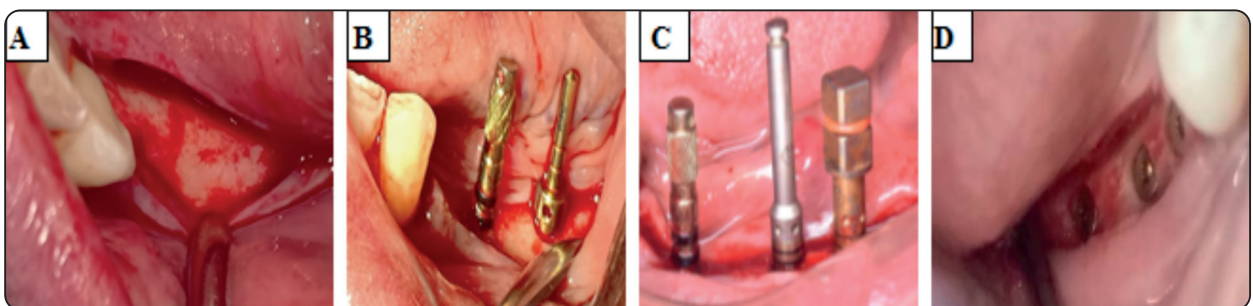


Fig. (2) Illustrating the steps of surgical procedure. A: incision and flap reflection. B&C: osteotomy and paralling pins. D: implant insertion one hour after coating

### Post insertion evaluation parameters

#### Clinically

##### Implant Stability Quotient

The Osstell Mentor (Integration Diagnostics AB, Göteborg, Sweden) was utilized to track Resonance Frequency Analysis (RFA) immediately post-insertion for primary ISQ. For recording data, the implant fixture was fitted with a Smartpeg (Integration Diagnostics AB) sensor. The RFA values are reported in a quantitative unit called the implant stability quotient (ISQ), which ranges from 1 to 100. A high ISQ value suggests great stability, whereas a low one indicates poor implant stability. Each implant was examined from four different angles (mesial, distal, buccal, and lingual) and the average of the four values was recorded. (Fig.3).

#### Radiographically

##### Marginal bone level (MBL)

Panoramic radiographs and CBCT scans were taken immediately following implant placement (base line) for assessment of marginal bone level (MBL). Radiographic magnification has been measured using the image/actual length of the implant fixture fitted. The distance between the implant shoulder and the marginal bone was measured (figure 4). At the planned follow-ups, the MBL was determined by taking the average of the mesial and distal values for each fixture.

#### Follow up phase

- A- Clinical evaluation: the installed implants were evaluated through comparing the primary stability obtained immediately post insertion versus secondary stability 2 and 3 months later (before loading) by measuring the Resonance Frequency Analysis (RFA) with Ostell.
- B- Radiographic evaluation: CBCT scan was performed 3 months post-insertion. The images were analyzed using On Demand 3D software (Cybermed Inc) and compared to the immediate post -insertion scan (base line) for marginal bone level evaluation. All exposures were carried out with the same dental X-ray machine at the same kilovoltage, milliampere and exposure time.

#### Measurements were taken as follow

- The saved radiographic DICOM files were "Opened" by OnDem and 3D software (Cybermed Inc)

The distance from the implant shoulder to the first visible bone-to- implant contact was measured mesially and distally by the linear measurements. The mean of the mesial and distal measurements of each implant was calculated in millimeters immediately following implant placement (baseline) and after 3 months for analysis

#### Data management and statistical analysis:

The study groups were compared using appropriate parametric and non-parametric tests of significance based on the distribution of the



Fig. (3) base line ISQ values of RFA using OSTELL for the three studied groups; A: group I (flowable L-PRF coated implants), B: group II (HA coated implants) and C: group III (uncoated control)

	Group I	Group II	Group III
Base line			
2 month			
3 month			

Fig. (4) ISQ values of RFA using OSTELL for the three studied groups at base line, 2 months and 3 months post insertion (group I (flowable L-PRF coated implants), group II ( HA coated implants) and group III uncoated control)

gathered data. Statistical analysis was performed on Windows using the statistical package for social studies (SPSS 23, SPSS, Inc., Chicago, IL, USA). The significance level was set at 5% (P<0.05).

**RESULTS**

The current study was conducted on 30 adult patients with an age range of 30 to 65 years having mandibular posterior edentulous ridge. They were selected from the department of oral Medicine, Periodontology, Oral diagnosis and Oral radiology, Faculty of Dentistry, Alexandria University. The patients were divided into three groups; two test groups composed of 10 patients for each group in need for implant placement .In test group I implants coated with flowable L-PRF and in test group II implants coated with HA, while in control group the implants left un coated. Patients were evaluated clinically and radiographically

immediate post-operative and after 3 months. After three months all patients received zircon crowns or bridges restoration.

**Statistical analysis**

Normal distribution of all variables was checked using normality test. Implant stability was normally distributed and presented mainly by mean and standard deviation. Percent change was not normally distributed and presented mainly using median, minimum, and maximum values. Percent change was calculated according to the following formula: [(Follow up values – baseline follow up) / baseline follow up] x 100. Pearson Chi square was used to compare gender between groups. One Way ANOVA was used to compare the study groups regarding age and implant stability followed by Tukey’s post hoc test when results are significant. Differences in implant stability across time were assessed using

Repeated Measures ANOVA. Kruskal Wallis was performed to compare percent change among the study groups, followed by Dunn's post hoc test with Bonferroni correction. Significance level was set at p value 0.05. Data was analyzed using IBM SPSS version 23.

### Follow-up results for the three groups

#### Postoperative healing

Surgical area was examined clinically on the second day, after 10 days, two and three months later. All implants were successfully osseointegrated showing a success rate 100%. In the post-healing period, all patients were evaluated as follows:

#### Implant stability quotient (ISQ)

The results on the stability of implants at 2<sup>nd</sup> and 3<sup>rd</sup> month revealed that, the stability of implants on test group I and II and for the control group was improved with increasing the period of implantation compared to base line (table 1). For test group 2 it was noted that hyaluronic acid was very viscous and due to its viscosity, the reading of Osstell was low immediately post insertion compared to group 1 and 3 but it increased rapidly at 2<sup>nd</sup> and 3<sup>rd</sup> month post insertion. On the other hand, the stability of non-coated implants showed slow improvement on the stability with increasing the period after implant insertion.

#### B) Radiographic evaluation

#### Marginal bone level results, fig. (5)

Marginal bone level was measured immediately post-operative and after 3 months table (5) & graph (3)

#### Test group 1 (Liquid -PRF)

The mean marginal bone level scores for the test group I at baseline was  $0.20 \pm 0.01$ . After 3 months the mean marginal bone level scores were  $0.36 \pm 0.02$ .

The difference between MBL scores at baseline and after 3 months was found to be statistically significant ( $p < 0.001^*$ ).

#### Test group 2 (hyaluronic acid)

The mean of marginal bone level scores for the test group II at baseline was  $0.26 \pm 0.01$ . After 3 months the mean marginal bone level scores were  $0.37 \pm 0.02$ .

The difference between MBL scores at baseline and after 3 months was found to be statistically significant ( $p < 0.001^*$ ).

#### Group 3 (negative control)

The mean MBL scores for the control group at baseline was  $0.39 \pm 0.01$ . After 3 months the mean marginal bone level scores were  $0.57 \pm 0.05$ .

The difference between MBL scores at baseline and after 3 months was found to be statistically significant ( $p < 0.001^*$ ).

\*: Statistically significant at  $p \leq 0.05$

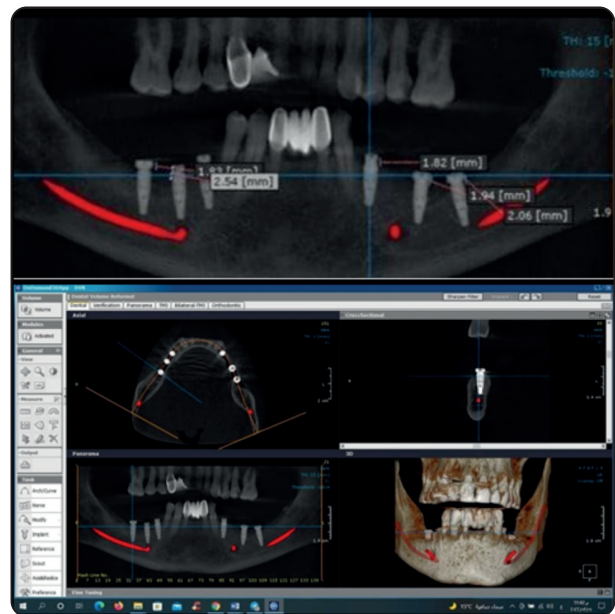


Fig (5) : Panoramic view from CBCT 3month post-operative showing marginal bone level for test group I (flowable L-PRF coated implants) ,test group II (HA coated implants) and uncoated control (group III) arranged from anterior to posterior respectively on both sides.



TABLE (1) Comparison of implant stability among the study groups at different time intervals.

		Group I (n=10)	Group II (n=10)	Group III Control (n=10)	P value
Baseline	Mean (SD)	64.90 (6.15)	61.00 (6.50)	63.90 (7.11)	0.271
	Median	66.00	61.50	62.50	
	Min - Max	56.00 – 73.00	51.00 – 71.00	51.00 – 73.00	
2 Months	Mean (SD)	74.50 (5.13)	75.30 (4.40)	72.80 (4.64)	0.604
	Median	73.50	76.00	72.00	
	Min - Max	64.00 – 84.007	66.00 – 80.00	67.00 – 80.00	
3 Months	Mean (SD)	82.20 (4.85)	88.40 (7.89)	79.00 (7.05)	0.018*
	Median	82.50	89.50	79.00	
	Min - Max	76.00 – 89.00	68.00 – 96.00	70.00 – 89.00	
P value		<0.0001*	<0.0001*	<0.0001*	
Post hoc test		P1=0.001*, P2<0.0001*, P3=0.002*	P1<0.0001*, P2<0.0001*, P3<0.0001*,	P1=0.001*, P2<0.0001*, P3<0.0001*,	

\*Statistically significant at  $P$  value  $\leq 0.05$ ,  $P_1$ : comparison between baseline and 2 months,  $P_2$ : comparison between baseline and 3 months,  $P_3$ : comparison between 2 months and 3 months.

TABLE (2) Pairwise comparisons regarding implant stability between the study groups after 3 months.

Groups	Compared to	P value
Group I	Group II	0.149
	Group III	0.677
Group II	Group III	0.011*

\*Statistically significant at  $P$  value  $\leq 0.05$

TABLE (3) Comparison of percent change in implant stability among the study groups from baseline.

		Group I (n=10)	Group II (n=10)	Control (n=10)	P value
2 Months	Mean (SD)	13.43 (6.86)	24.15 (8.62)	14.74 (9.83)	0.054
	Median	12.46	23.52	11.20	
	Min - Max	3.95 – 28.07	11.27 – 43.14	4.48 – 33.33	
3 Months	Mean (SD)	23.64 (8.82)	45.71 (14.50)	24.25 (10.13)	0.004*
	Median	22.53	42.67	19.45	
	Min - Max	13.04 – 43.86	29.41 – 74.51	13.43 – 43.10	

\*Statistically significant at  $P$  value  $\leq 0.05$ .

TABLE (4) Pairwise comparisons regarding change in implant stability between the study groups after 3 months.

Groups	Compared to	P value
Group I	Group II	0.010*
	Group III	1.00
Group II	Group III	0.015*

\*Statistically significant at  $P$  value  $\leq 0.05$

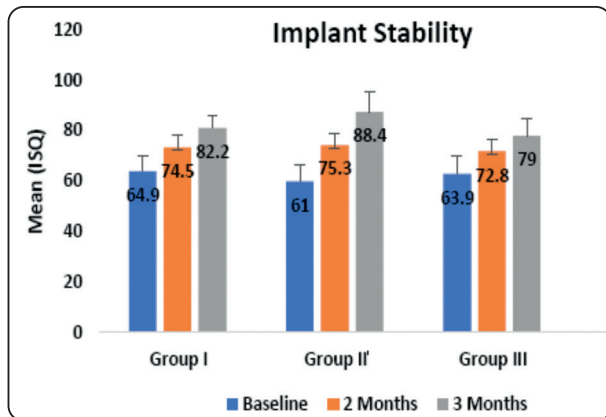
Table (5): Comparison of percent change in marginal bone level (MBL) among the two- study groups and control group.

MBL		Immediate Postoperative	3 months
Test group I (liquid fibrinogen)	Min. – Max.	0.10 – 0.20	0.30-0.39
	Mean ± SD. Median	0.20±0.01	0.36±0.02
		0.13	0.35
	<b>Sig. bet. periods</b>		<b>P &lt;0.001**</b>
	<b>t-test</b>	–	<b>3.501*</b>
Test group II (hyaluronic acid)	Min. – Max.	0.11 – 0.21	0.32 – 0.42
	Mean ± SD. Median	0.26 ± 0.01	0.37 ± 0.02
		0.13	0.37
	<b>Sig. bet. periods</b>		<b>P &lt;0.001**</b>
	<b>t-test</b>	–	<b>5.103*</b>
Group III (negative control)	Min. – Max.	0.23 – 0.36	0.49 – 0.58
	Mean ± SD. Median	0.39 ± 0.01	0.57 ± 0.05
		0.24	0.51
	<b>Sig. bet. periods</b>		<b>P &lt;0.001**</b>
	<b>t-test</b>	–	<b>5.103*</b>

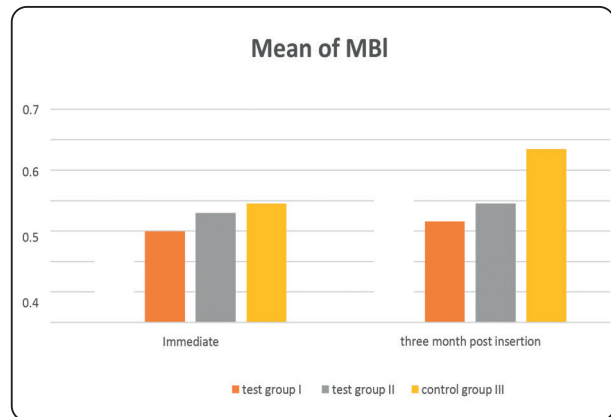
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\* = Significant at (P < 0.5)

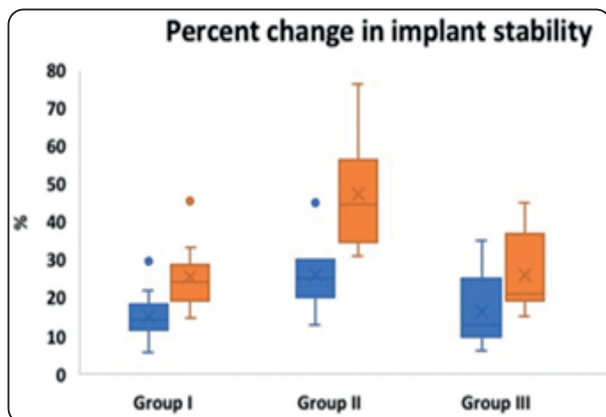
\*\* = Significant at (P < 0.01)



Graph (1): Comparison of implant stability among the study groups at different time intervals.



Graph (3): Comparison between the two studied groups and control group according to MBL.



Graph (2): Comparison of percent change in implant stability among the study groups from baseline.

The comparison between the three groups regarding the decrease in marginal bone loss 3 month post insertion versus the base line marginal bone level revealed that ; group I ( liquid fibrinogen) showed the least marginal bone loss (mean 0.36mm) followed by group II (hyaluronic acid) (median 0.37mm) compared to (0.57mm) for the uncoated control group. These results revealed the superiority of liquid fibrinogen coated implants and hyaluronic coated implants over the uncoated ones with the decrease in marginal bone loss in favor to liquid fibrinogen group more than that of hyaluronic

acid however this difference was not statistically significant.

## DISCUSSION

Biomimetic functionalization of implant surfaces is an emerging biotechnology that carries promising benefits to enhance osseointegration aiming to shortening loading time and subsequently gain patient satisfaction.<sup>(32)</sup>

The present study was carried out to evaluate the effectiveness of Liquid L-PRF and Hyaluronic Acid as implant bioactive coatings. A total of 30 cases 10 cases for each group (including the control group) with an age range of 30 to 65 years and having mandibular posterior edentulous ridge participated in this study.

The implants used for this study were Straumann® implants with SLA® surface. This is based on a large-grit sandblasting method followed by acid etching, resulting in micro-roughness on the titanium surface. The resulting topography provides the perfect framework for cell adhesion.<sup>(33)</sup>

The interaction of various implant surfaces with flowable L-PRF was investigated to determine whether implant topography, wettability, and coating influenced the fibrin mesh properties to eventually use it as an effective biomimetic coating for dental implants. They concluded that, all implant surfaces studied (including the SLA® surface) created a stable fibrin mesh. However, Macroscopic and microscopic variations were identified.<sup>(30)</sup>

Acquiring and sustaining appropriate implant stability is crucial for effective and satisfying treatment outcomes. It has been considered a reliable parameter to measure implant-bone anchoring and osseointegration. Implant stability is typically separated into two stages: primary stability, which results from mechanical contact with bone and can be altered by bone status, surgical procedures, and implant geometry.<sup>(34)</sup> Secondary stability develops from regeneration of peri-implant bone.<sup>(35)</sup>

In the current investigation, resonance frequency analysis (RFA) was used to identify the implant stability quotient (ISQ). The Osstell Mentor (Integration Diagnostics AB, Göteborg, Sweden) was used to record ISQ values immediately post-insertion (primary ISQ) and 2&3 months later for secondary ISQ. According to studies, the noninvasive, quantifiable, repeatable, and reliable qualities of RFA have significantly expanded its appeal in clinical use.<sup>(36)</sup>

The findings of the current study regarding implant stability revealed that the stability of implants at 2<sup>nd</sup> and 3<sup>rd</sup> month on test group I (liquid L-PRF) and test group II (hyaluronic acid) and for the control group was improved with increasing the period of implantation compared to base line (table 1). Comparing the 3 groups with each other at the end of the observation period (3<sup>rd</sup> month) revealed a significant increase in implant stability in favor to the two test groups compared to the control group (table 2). For test group II it was noted that hyaluronic acid was very viscous and that is why, the RFA reading was low immediately post insertion compared to group I and III but it increased rapidly at 2<sup>nd</sup> and 3<sup>rd</sup> month post insertion. On the other hand, the stability of non-coated implants showed slow improvement on the stability with increasing the period after implant insertion.

Our findings regarding implant stability for liquid PRF coated implants revealed improved ISQ with increasing the period of implantation. It was 64.90 at base line then improved to 74.50 at the 2<sup>nd</sup> month and 82.20 at the 3<sup>rd</sup> month after insertion compared to (63.90 at base line, 72.80 at 2<sup>nd</sup> month and 79.00 at 3<sup>rd</sup> month) for control group. Table (1, 2, 3)

These outcomes are in accordance. with of Öncü, 2019<sup>(37)</sup> who reported that the Mean of implant stability quotients (ISQs) of the liquid PRF coated implants were  $69.3 \pm 10.5$ , versus  $64.5 \pm 12.2$  for the uncoated implants at the end of the first week. The mean ISQs at 4<sup>th</sup> weeks postoperatively were  $77.1 \pm 7.1$  for the liquid PRF coated group versus

70.5±7.7 for the uncoated group. They found that using L-PRF boosted implant stability throughout the early healing period, as shown by higher ISQ values. They determined that the simple application of this material appears to promote osseointegration and early loading by its ability to release growth factors, vitronectin and fibronectin leading to cellular proliferation, collagen synthesis, and osteoid formation.<sup>(38,39)</sup>

In test group II (Hyaluronic acid); the results of the current study regarding Implant stability revealed that, the stability was 61.00 at base line, increases to 75.30 at 2nd month and 88.40 at 3rd month after insertion

Elhadidi, et al 2023 conducted a clinical and experimental study to evaluate the effect of hyaluronic acid (HA) as a coating material on the stability of immediately loaded implants in the posterior maxilla. They found There were no statistically significant differences between the two groups in terms of implant stability. However, the HA group had a substantial statistical advantage in bone density from the buccal aspect.<sup>(40)</sup>

Maintaining peri-implant bone level is crucial for implant functionality, aesthetics, and long-term success. Given how the marginal bone level affects the gingival level, implant placement in patients with a high smile line is tricky, particularly in the aesthetic zone.<sup>(41)</sup>

Regarding the radiographic findings of the current study ,the comparison between the three groups regarding the decrease in marginal bone loss 3 month post insertion versus the base line marginal bone level revealed that ; group I ( liquid fibrinogen) showed the least marginal bone loss (mean 0.36mm) followed by group II (hyaluronic acid) (median 0.37mm) compared to (0.57mm) for the uncoated control group. These results revealed the superiority of liquid fibrinogen coated implants and hyaluronic coated implants over the uncoated ones, with the decrease in marginal bone loss in favor of liquid fibrinogen group more than that of

hyaluronic acid however this difference was not statistically significant. (Table 5)

These findings were supported and explained by the study by Li et al., 2008<sup>(42)</sup> who reported that TGF-β1, PDGF-AB, PDGF-BB, BMP-2, FGF-2, and VEGF in liquid fibrinogen from donors were released up to 14 days following collection. Moreover Wang et al., 2017<sup>(43)</sup> found that Fibrin clots, such as liquid fibrinogen, have been shown to release a considerable number of growth factors over time from platelet alpha granules.

Furthermore, Varela et al<sup>(44)</sup> identified an elevated concentration of platelets and lymphocytes in liquid fibrinogen than in whole blood. Lollobrigida et al. (2018)<sup>(45)</sup> reported similar results. They examined Fibrin formation on titanium discs with modified nanosurfaces (Ossean®) and machined surfaces immersed in liquid fibrinogen and L-PRF exudate and revealed that utilizing liquid fibrinogen instead of L-PRF exudate resulted in a denser fibrin network along with more blood cells. However, micro/nano-rough samples retained more fibrin than machined surfaces, resulting in a more dense coating.<sup>(45)</sup>

In 2021 Andrade et al<sup>(30)</sup> investigated the interaction of various implant surfaces with liquid fibrinogen. They reported that when exposed to liquid fibrinogen, all implant surfaces in their investigation produced a stable fibrin mesh. However, macroscopic and microscopic discrepancies were found. They concluded that liquid fibrinogen could be an affordable and effective modality to achieve an autologous biomimetic functionalization of implant surfaces.

Recognizing how crucial it is to establish a fibrin clot to stimulate chemotaxis, proliferation and differentiation during wound healing, platelet concentrates might provide all necessary blood elements to promote these events including platelets, leucocytes, fibrin mesh, and growth factors, as well as fibronectin and vitronectin.<sup>(46)</sup>

Our findings regarding Hyaluronic acid are agreed with Yazan<sup>(47)</sup> who reported that hyaluronic

acid had a favorable effect on osseointegration, revealed by the presence of extensive osteoid tissue and new bony tissue seen in the HA group<sup>(47)</sup>

Furthermore, a clinical and animal study by Elhadidi et al 2023<sup>(40)</sup> found a substantial statistical difference in bone density from the buccal aspect between hyaluronic-coated and uncoated implants. Their results revealed that the newly produced bone in the HA group was of higher quantity and quality, with denser bone trabeculae and smaller marrow gaps than the uncoated implants. They concluded that hyaluronic acid treatment improved buccal bone density around immediately loaded implants while also having a Complementary influence on the quality and quantity of peri-implant bone.

Nasr et al. (2022) evaluated the influence of a melatonin and hyaluronic acid combination on hard tissue dimensional changes around immediate implants and found that hyaluronic acid significantly reduced buccal and palatal bone resorption.<sup>(48)</sup> This suggests that hyaluronic acid has strong biomimetic characteristics that could aid in bone healing and implant osseointegration.

Furthermore, Carvino et al. 2021<sup>(49)</sup> investigated several surface modifications in titanium implants, revealing that the topography and surface biomodification can influence host response. They reported that adding HA to the implant surface can promote chemotaxis, adhesion, proliferation, and differentiation of cell precursors on titanium implants by enhancing the link between implant and bone. They determined that HA has osteoinductive properties. As a result, it can accelerate early loading phase, thereby meeting the patients' expectations.

Therefore, flowable L-PRF and Hyaluronic acid could be affordable and effective modalities for biomimetic functionalization of implant surfaces with a simple, economic and clinically applicable protocol.

This approach may be particularly beneficial in individuals with bone healing issues or who require immediate implant placement and have a gap between the alveolar bone and the implant surface.

A limitation of the current study could be that only one type of implant surfaces was evaluated (SLA surface). Other implants with special nanosurfaces should be considered in future research.

However, to the best of our knowledge this study is the first randomized controlled clinical trial to evaluate the efficacy of liquid L-PRF and Hyaluronic acid as bioactive coatings versus uncoated dental implants with a most realistic scenario.

## CONCLUSION

Implant surface biomimetic coatings have a lot of benefits compared to the traditional uncoated implants. They are promising osteoinductive biomaterials rich in proteins and growth factors. The use of biomimetic coating has resulted in improved biological characteristics and enables successful site-directed bone regeneration therapy.

## REFERENCES

1. Douglass CW, Shih A, Ostry L. Will there be a need for complete dentures in the United States in 2020? *J Prosthet Dent.* 2002;87:5–8. [PubMed] [Google Scholar]
2. Brånemark R, Brånemark PI, Rydevik B, Myers RR. Osseointegration in skeletal reconstruction and rehabilitation: A review. *J Rehabil Res Dev.* 2001;38:175–81. [PubMed] [Google Scholar]
3. Misch CE, Qu Z, Bidez MW. Mechanical properties of trabecular bone in the human mandible: implications for dental implant treatment planning and surgical placement. *J Oral Maxillofac Surg.* 1999; 57:700–706.
4. Dohan Ehrenfest D. M., Vazquez L., Park Y.-J., Sammartino G., Bernard J.-P. Identification card and codification of the chemical and morphological characteristics of 14 dental implant surfaces. *The Journal of Oral Implantology.* 2010;37(5):525–542.
5. Moradian-Oldak J, Wen HB, Schneider GB, Stanford CM. Tissue engineering strategies for the future generation of dental implants. *Periodontol* 2000. 2006;41:157–76. [PubMed] [Google Scholar]
6. Boyan B. D., Lotz E. M., Schwartz Z. roughness and hydrophilicity as osteogenic biomimetic surface properties. *Tissue Engineering Parts A.* 2017;23(23–24):1479–1489. doi: 10.1089/ten.tea.2017.0048

7. Narasimha Rao G., Pampana S., Yarram A., Suresh Sajjan M. C., Ramaraju A. V., Bhee-malingeswara rao D. Surface modifications of dental implants: an overview. *International Journal of Dental Materials*. 2019;1(1):17–24.
8. Peck MT, Marnewick J, Stephan LX, Singh A, Patel N, Majeed A. The use of leucocyte- and platelet-rich fibrin (L-PRF) to facilitate implant placement in bone deficient sites: a report of two cases. *SADJ*. 2012;67(2):54–49.
9. Lee JW, Kim SG, Kim JY, Lee YC, Choi JY, Draços R, et al. Restoration of a peri implant defect by platelet-rich fibrin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2012;113(4):459–63
10. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet rich fibrin (PRF): A second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):56–60.
11. Castro A., Cortellini S., Temmerman A., Li X., Pinto N., Teughels W., Quirynen M. Characterization of the Leucocyte- and Platelet-Rich Fibrin Block: Release of Growth Factors, Cellular Content, and Structure. *Int. J. Oral Maxillofac. Implant*. 2019;34:855–864.
12. Wu, Y. P., Bloemendal, H. J. , Voest, E. E. et al., “Fibrin-incorporated vitronectin is involved in platelet adhesion and thrombus formation through homotypic interactions with platelet-associated vitronectin,” *Blood*, vol. 104, no. 4, pp. 1034–1041, 2004.
13. Pankov, R. (2002): “Fibronectin at a glance,” *Journal of Cell Science*, vol. 115, no. 20, pp. 3861–3863, 2002.
14. Strauss, F.J.; Stähli, A.; Gruber, R. The use of platelet-rich fibrin to enhance the outcomes of implant therapy: A systematic review. *Clin. Oral Implant. Res*. 2018, 29 (Suppl. 18), 6–19. [CrossRef] [PubMed].
15. Manuela G. Neuman, Radu M. Nanau , Loida Oruña-Sánchez , Gabriel Coto (2015) “Hyaluronic Acid and Wound Healing” 18(1) 53 – 60
16. L. Tettamanti, C. Andrisani, M. Andreasi Bassi, R. Vinci, J. Silvestre-Rangil, A. Tagliabue (2017) “Immediate Loading Implants: Review of the Critical Aspects” *Oral & Implantology*, 10(2), 129.
17. Ahmed R Hemed, Mohamed A Mohamed, Ahmed A El Feki, “Evaluation of the Stability of Immediate Placed Dental Maxillary Implant in Fresh Extracted Socket Versus Delayed Placed Implant”, *Al-Azhar Journal of Dental Science*, Vol. 21- No. 5- 533:538- December 2018.
18. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.
19. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* 2013 27; 310:2191–4.
20. Shahbaz Alam M, Dhiman A, Jain V, Bhutia O, Pruthi G. Vertical bone implant contact around anterior immediate implants and their stability after using either alloplast or L- PRF or both in peri-Implant gap: A prospective randomized trial. *J Maxillofac Oral Surg* 2020;21:533–41.
21. Boora P, Rathee M, Bhorla M. Effect of Platelet Rich Fibrin (PRF) on Peri-implant Soft Tissue and Crestal Bone in One-Stage Implant Placement: A Randomized Controlled Trial. *J Clin Diagn Res* 2015;9:Zc18–21.
22. Sánchez-Fernández E, Magán-Fernández A, O’Valle F, Bravo M, Mesa F. Hyaluronic acid reduces inflammation and crevicular fluid IL-1 $\beta$  concentrations in peri-implantitis: a randomized controlled clinical trial. *J Periodontal Implant Sci* 2021;51:63–74.
23. Rosner B. Hypothesis Testing: Two-Sample Inference. In: *Fundamentals of biostatistics*. 7<sup>th</sup> ed. Boston: Brooks/Cole: Nelson Education; 2015. pp. 269–301.
24. Heinrich-Heine-Universität Düsseldorf (HHU). G\*Power Statistical Power Analyses for Mac and Windows. HHU. 2019. Available from: <https://www.psychologie.hhu.de/arbeitsgruppen/allgemeinepsychologie-und-arbeitspsychologie/gpower> [Accessed on: Nov, 2021]
25. Doig GS, Simpson F. Randomization and allocation concealment: a practical guide for researchers. *J Crit Care* 2005;20:187–91; discussion 91–3.
26. Schulz KF, Grimes DA. Generation of allocation sequences in randomised trials: chance, not choice. *Lancet* 2002;359:515–9.
27. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. *Lancet* 2002;359:614–8.
28. Karanickolas PJ, Farrokhyar F, Bhandari M. Practical tips for surgical research: blinding: who, what, when, why, how? *Can J Surg* 2010;53:345–8.
29. C. Andrade, J. Camino, M. Nally, M. Quirynen, B. Martínez, and N. Pinto, “Combining autologous particulate dentin, LPRF, and fibrinogen to create a matrix for predictable ridge preservation: a pilot clinical study,” *Clinical Oral Investigations*, vol. 24, no. 3, pp. 1151–1160, 2020.

30. Catherine X. Andrade ,Marc Quiryneen , David R. Rosenberg and Nelson R. Pinto. Interaction between Different Implant Surfaces and Liquid Fibrinogen: A, Pilot In Vitro Experiment, *BioMed Research International* Volume 2021, Article ID 9996071, 8 pages <https://doi.org/10.1155/2021/9996071>
31. Serafini G, Lopreiato M, Lollobrigida M, et al. Platelet Rich Fibrin (PRF) and Its Related Products: Biomolecular Characterization of the Liquid Fibrinogen. *J Clin Med*. 2020;9(4):1099. Published 2020 Apr 12. doi:10.3390/jcm9041099
32. Zafar MS, Farooq I, Awais M, Najeeb S, Khurshid Z, Zohaib S. Bioactive surface coatings for enhancing osseointegration of dental implants. In: *Biomedical, therapeutic and clinical applications of bioactive glasses*: Woodhead Publishing; 2019. pp. 313-29.
33. Emmert M, Spille J, Behrens E, Ayna M, Karayürek F, Wiltfang J, et al. Comparative Assessment of the Primary Stability of Straumann BLX Implant Design Using an In Vitro Sinus Lift-Simultaneous Implant Insertion Model. *J Oral Implantol* 2022;48:269-75.
34. Meredith N. Assessment of implant stability as a prognostic determinant. *Int J Prosthodont (IJP)* 1998;11:491e501.
35. Sennerby L, Roos J. Surgical determinants of clinical success of osseointegrated oral implants: a review of the literature. *Int J Prosthodont (IJP)* 1998;11:408e20.
36. Rowan M, Lee D, Pi-Anfruns J, Shiffler P, Aghaloo T, Moy PK. Mechanical versus biological stability of immediate and delayed implant placement using resonance frequency analysis. *J Oral Maxillofac Surg* 2015;73:253e7.
37. Öncü E, Erbeyoğlu AA. Enhancement of Immediate Implant Stability and Recovery Using Platelet-Rich Fibrin. *Int J Periodontics Restorative Dent* 2019;39:e58-63.
38. Öncü E, Alaaddinoğlu EE. The effect of platelet-rich fibrin on implant stability. *Int J Oral Maxillofac Implants* 2015;30:578-82.
39. Öncü E, Bayram B, Kantarci A, Gülsever S, Alaaddinoğlu EE. Positive effect of platelet rich fibrin on osseointegration. *Med Oral Patol Oral Cir Bucal* 2016;21:e601-7.
40. Elhadidi, Merna Hosny / Yousef, Eman Abdel Salam / Elhindawy, Menatalla M. / Ahmed, Wael Mohammed Said, The Effect of Topical Application of Hyaluronic Acid on the Stability of Immediate Loaded Dental Implants in the Posterior Maxilla: Clinical and Animal Study *The International Journal of Oral & Maxillofacial Implants*, 04/2023 DOI: 10.11607/jomi.10100, PubMed ID (PMID): 37669513Pages 775-783,
41. P.-S. Fu, T.-H. Lan, P.-L. Lai et al. Implant stability and marginal bone level changes: A 2-year prospective pilot study *Journal of Dental Sciences* 18 (2023) 1272e1279
42. Li MO, Flavell RA. TGF-beta: a master of all T cell trades. *Cell* 2008;134:392-404.
43. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Behavior of Gingival Fibroblasts on Titanium Implant Surfaces in Combination with either Injectable-PRF or PRP. *Int J Mol Sci* 2017;18.
44. Varela HA, Souza JCM, Nascimento RM, Araújo RF, Jr., Vasconcelos RC, Cavalcante RS, et al. Injectable platelet rich fibrin: cell content, morphological, and protein characterization. *Clin Oral Investig* 2019;23:1309-18.
45. M. Lollobrigida, M. Maritato, G. Bozzuto, G. Formisano A. Molinari, and A. De Biase, “Biomimetic implant surface functionalization with liquid L-PRF products: in vitro study,” *BioMed Research International*, vol. 2018, Article ID 9031435, 7 pages, 2018.
46. D. M. Dohan Ehrenfest, T. Bielecki, R. Jimbo et al., “Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF),” *CPB*, vol. 13, no. 7, pp. 1145–1152, 2012.
47. Yazan, Mürüde, et al. Effect of hyaluronic acid on the osseointegration of dental implants. *British Journal of Oral and Maxillofacial Surgery*, 2019, 57.1: 53-57.
48. Nasr A, Shoeib M, Darhous M, El Barbary A. The Effect of Melatonin and Hyaluronic Acid Mixture on Hard Tissue Dimensional Changes Around Immediate Post-Extraction Implants. *Egypt Dent J* 2022;68:565-77.
49. Cervino, G.; Meto, A.; Fiorillo, L.; Odorici, A.; Meto, A.; D’Amico, C.; Oteri, G.; Cicciù, M. Surface Treatment of the Dental Implant with Hyaluronic Acid: An Overview of Recent Data. *Int. J. Environ. Res. Public Health* 2021, 18, 4670. <https://doi.org/10.3390/ijerph18094670>