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RESONANCE FREQUENCY ANALYSIS OF GROWTH FACTORS AND CYTOKINES IN IMMEDIATE IMPLANT PLACEMENT OVERDENTURES

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ABSTRACT

Objectives: This study was conducted to measure the effect of growth factors and cytokines present in platelet rich fibrin (PRF) on the stability of immediately placed implants as measured by resonance frequency analysis for implant overdentures.

Materials and Methods: Seven patients with one remaining cuspid or bicuspid indicated for extraction on each side of the mandibular arch were selected for the study. After atraumatic extraction, the first implant was inserted without grafting material while the second implant was placed with PRF grafting material. Implant stability was measured using resonance frequency analysis by the Osstelle device at the time of implant placement and at 2, 4, 6 and 8 weeks after implant placement. An implant overdenture using Locator attachments was fabricated and delivered to each patient.

Results: The results of this study showed higher ISQ values for the second implant than the first implant at the time of implant placement, but the difference was not statistically significant. A statistically significant increase in implant stability for both implants was found at all follow-up appointments. On comparing the two implants, the second implant had significantly higher implant stability quotient (ISQ) values at every follow-up appointment than the first implant. At the end of the 8-week follow-up period, the second implant showed significantly higher ISQ values than the first implant.

Conclusions: Growth factors and cytokines present within PRF grafting material can be used with immediate implant placement to enhance implant stability of during the early stages of healing.

INTRODUCTION

Tooth extraction is usually followed by bony changes in the alveolar socket that eventually lead to a decrease in residual bone height and width[1,2]. A systematic review by Tan et al[3] showed that the alveolar ridge undergoes up to 63% horizontal bone loss and 22% vertical bone loss in the subsequent 6 months following tooth extraction. Implant placement into fresh extraction sockets has gained popularity as a reliable technique that

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provides patients with an immediate replacement of extracted teeth. This technique was first introduced in 1978 by Schulte et al[4] in an attempt to prevent collapse of the alveolar socket and preserve soft and hard tissue architecture. It was defined in 2004 by Hammerle et al[5] as type 1 implant placement; "placement immediately following tooth extraction and as part of the same surgical procedure". This does not only reduce the overall treatment time and number of surgical procedures, it also enhances overall esthetics as it reduces the time between tooth extraction and prosthesis delivery[6–8].

However, the success of this procedure depends to a great extent on the stability of the soft and hard tissues surrounding the implant [2,5]. Some studies have shown that immediate implant placement was unable to prevent bone remodeling that occurred 3 to 4 months after installation resulting in vertical and horizontal reduction in the alveolar bony walls surrounding the implant. Botticelli et al[9] reported a 50% bone loss on the buccal wall combined with 30% loss on the lingual wall after 4 months of healing. Studies have also reported that almost 40% of immediately placed implants demonstrate gingival recession due to bone loss on the buccal side. These changes - occurring more commonly in the buccal wall - may compromise the prognosis of the implant both functionally and esthetically[1,7,10,11].

Furthermore, the consensus statement regarding immediate implant placement by Hammerle et al[5] stated that, "The implant should not be placed at the time of tooth removal if the residual ridge morphology precludes attainment of primary stability of an appropriately sized implant in an ideal restorative position". Lack of bone of sufficient quality and quantity at the extraction socket could affect the primary stability of the implant and in turn have a negative effect on osseointegration [12,13].

Recent studies have shown that there is usually a need for ridge augmentation with immediate implant placement in order to counteract bone changes that occur during the first year of placement. Augmentation also fills the gap between the implant and the alveolar bony wall and protects the wound during healing. In addition, augmentation aids in maintaining adequate horizontal and vertical ridge dimensions as well as improving bone quality at the site of implant placement. This enhances primary stability as well as functional and esthetic outcomes[14–17].

Augmentation procedures involving the use of autogenous bone grafts are highly reliable and well documented[18–20]. However, their use usually involves additional surgical procedures at bone harvesting sites resulting in more patient discomfort and longer healing time[21]. Bone substitutes such as xenografts and allografts have also been successfully used for ridge augmentation[22], although slowly resorbing grafts may interfere with the process of osseointegration when immediate implant placement is performed [23,24].

Platelet rich fibrin (PRF) is one of the most recent innovations in oral implantology and a rich source of autogenous growth factors and cytokines[25]. PRF is mainly a concentration of platelets that are trapped in a fibrin matrix [26,27]. These platelets provide a gradual and sustained release of growth factors and cytokines. Growth factors include bone morphogenetic proteins, platelet derived growth factor, vascular endothelial growth factor and transforming growth factor. Growth factors attract stem cells to the site of the wound promoting tissue regeneration through cell mitosis, angiogenisis, and osteogenisis. This occurs in the first few weeks resulting in considerably faster healing as well as soft and hard tissue regeneration [28–30].

Dohan et al also pointed out that during the centrifuging process of the PRF material, leucocytes secrete cytokines in response to the artificially induced haemostatic and inflammatory situation. The authors added that this could be the explanation for the reduction in post operative infections when PRF was used as a surgical additive[31]. Other studies also demonstrated that cytokines have a direct

immunological and antibacterial effect during inflammation, thereby promoting wound healing[32,33].

PRF has been used successfully during various surgical procedures. Shaarawy and Fahmy[34] reported that PRF provided a higher rate of bone regeneration and healing when compared to bone graft substitutes around immediately placed implants. It has also been used for socket preservation after extraction, especially in situations where buccal bone was resorbed [35,36]. Cortese et al[37] reported that PRF grafting is a minimally invasive technique that can be used with elderly patients requiring bone regeneration to decrease the risk of implant failure and complications. Faster bone healing and regeneration was also reported with the use of PRF with or without bone grafts during sinus floor elevation [33,38]. PRF was also used to decrease marginal bone loss surrounding immediately loaded implants[39]. A histological animal study by Oncu et al[40] showed that PRF enhanced bone-to-implant contact and increased the amount of new bone formation during the early stages of healing.

Implant stability has been clinically assessed by several methods. Resonance frequency analysis (RFA) using the Osstelle device is a simple, reliable and non-invasive technique for measuring implant stability. The Osstelle device measures RFA in ISQ values (implant stability quotient) on a scale from 0 to 100. Higher ISQ values indicate higher stability[41,42].

This study was conducted to measure the effect of growth factors and cytokines released by PRF on the stability of immediately placed implants as measured by RFA using the Osstelle device.

MATERIALS AND METHODS

This was a split-mouth study where each patient received two immediate implants. The first implant was placed without a grafting material on one side of the mandible, while the second implant was placed with PRF material on the other side of the mandible.

Seven patients from the outpatient clinic of the Department of Prosthodontics, Faculty of Dentistry, Cairo University were selected for this study. Inclusion criteria comprised patients ranging from 40 to 60 years of age. They all had an edentulous mandible except for one remaining cuspid or bicuspid on each side that was indicated for extraction. The width of the alveolar ridge surrounding the teeth was 4-5 mm, as measured by the pre-operative cone beam image*, to provide sufficient bone for implant placement. Selected patients also had a fully dentulous maxillary arch. Patients with periapical pathosis related to the remaining teeth, maxillo-mandibular discrepancies or poor oral hygiene were excluded from the study. Exclusion criteria also included heavy smokers, uncontrolled diabetes, and other systematic condition that could have a negative effect on wound healing and osseointegration. All patients were informed about the nature of the research and asked to sign a consent form before proceeding with the study.

Primary and secondary impressions were made and the master casts were modified by cutting off the remaining teeth till the level of the alveolar ridge. Conventional steps of prosthesis fabrication were continued and a mandibular denture was made for each patient.

Local anesthesia was administered and the first tooth was extracted atraumatically using periotomes and forceps (fig. 1). The implant bed was prepared by sequential drilling to a depth that is 3mm apical to the original socket depth. The preoperative cone beam image was used for planning and selecting an implant** of a suitable diameter and length. The implant was threaded 2 mm deeper to the bone level of the osteotomy (fig.2). No grafting material was

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placed for the first implant. The smart peg of the implant system was carefully threaded onto the implant until it reached its final position. The implat staibility was measured using the Osstelle* device. The Osstelle Sensor was placed 2 mm away and at a 45 degree angle to the surface of the smart peg. Four readings were taken for each implant; buccally, lingually, mesially and distally. The smart peg was then removed and the healing abutment was screwed onto the implant. Finally, suturing of the flap around the healing abutments was performed.

Fig (1) atraumatic extraction of the first tooth

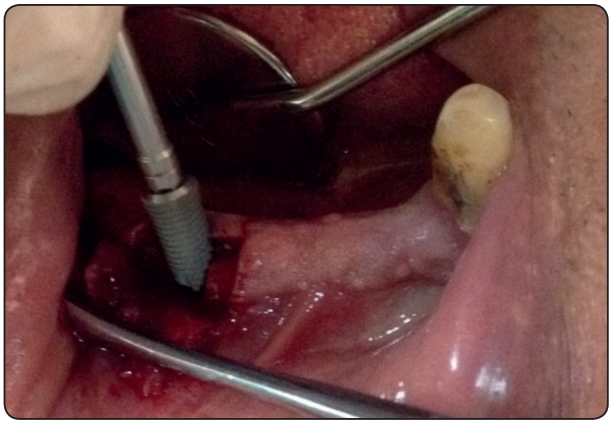

Fig (2) Installation of the first implant

Preparation of the PRF grafting material for the second implant was then commenced as described by Choukroun[25]. A 10 mm blood sample was drawn from each patient into a sterile test tube. The test tube was then centrifuged** at 3000 rpm for 12 minutes. The PRF clot was handled and separated from the red blood cell clot (fig.3). It was then placed on the metal grid in the PRF Box**. Using the condenser apparatus, the clot was compressed to produce the PRF membrane.

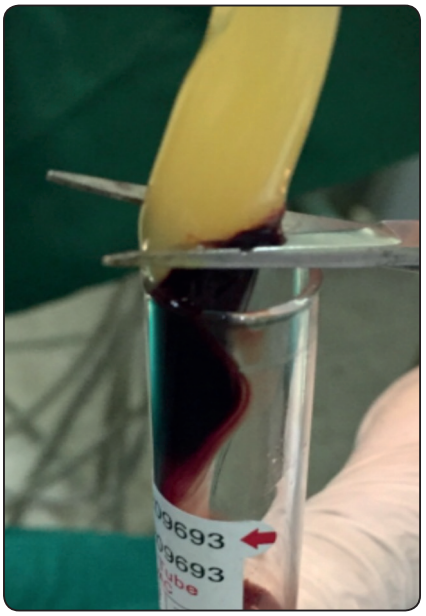

Fig. (3) separation of the PRF clot from the red blood cell clot

The same surgical procedure was repeated for the second tooth on the other side of the arch. after atraumatic extraction, two small buccal vertical incisions on each side of the socket were made to create a partial thickness flap for proper socket closure after placement of the PRF material. then, the second implant was placed followed by the PRF grafting material. Stability measurement was

* Integration Diagnostics AB, Gothenburg, Sweden
** Process, Nice, France

done in the same manner described earlier. Flap repositioning and suturing was done carefully around the healing collars and the grafting material. The dentures were relieved at the site of the healing collars using pressure indicating paste to avoid any loading of the implants during the healing period. Occlusal adjustments were also performed and the dentures were delivered.

Patients were recalled at 2, 4, 6, and 8 weeks after implant installation. At each follow-up visit, the healing collars were removed and smart pegs were placed to measure implant stability. The readings were tabulated for statistical analysis.

Patients were given a strict oral hygiene regimen during the follow-up period. After 3 months, the healing collars were replaced with locator attachments*. The denture was prepared for pick-up by making 2 windows to correspond with the location of the Locators. The attachment spacers were placed to block the undercuts in the attachment (fig.4). Next, the nylon cap and the metal housing were placed on the attachments. A soft mix of self-cure acrylic resin was added to the fitting surface of the denture and injected around the attachments. The denture was placed in the patient's mouth and the pick-up was commenced under the patient's centric occlusion. After polymerization of the resin, the denture was inspected to ensure proper pick-up of the housings and the nylon caps. Patients were recalled weekly for one month following denture delivery for any complaints and readjustments. They were instructed on the after care of the attachments and dentures and oral hygiene measures were reinstated.

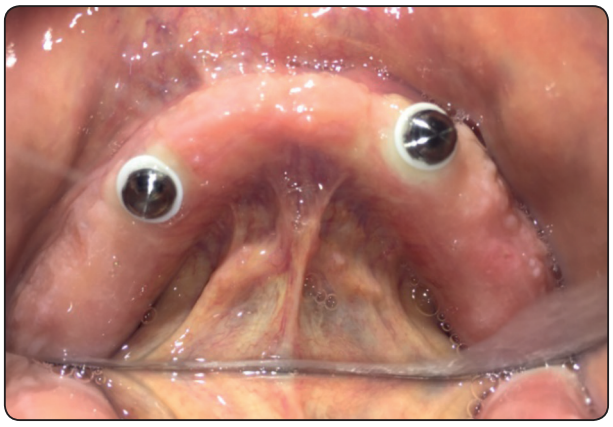

Fig (4) locator attachment ready for overdenture pick-up

Statistical Analysis

The mean and standard deviation values were calculated for each implant in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric (normal) distribution.

Independent sample t-test was used to compare between two implants in non-related samples. Repeated measure ANOVA was used to compare between more than two groups in related samples.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

There was a statistically significant increase in implant stability at each follow up appointment for both implants; with the lowest mean ISQ values at time of implant placement and the highest mean values after 8 weeks of placement (table 1, fig 5).

At the time of implant placement, ISQ values of the second implant were higher than the first implant, but the difference was not statistically significant. ISQ values were significantly higher in the second implant than the first implant at all five follow-up appointments (table 1, fig 6 & 7).

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TABLE (1) ISQ measurements for the two implants

Variables	ISQ measurements				*p-value*
	First implant		Second implant		
	Mean	SD	Mean	SD	
At time of implant placement	57.25 ^{aA}	1.71	61.10 ^{aA}	1.55	**0.173ns**
At 2 weeks	61.75 ^{bA}	1.26	69.75 ^{bB}	2.63	**0.001***
At 4 weeks	66.00 ^{cA}	0.82	75.00 ^{cB}	2.22	**≤0.001***
At 6 weeks	75.50 ^{dA}	1.29	78.50 ^{dB}	0.82	**0.017***
At 8 weeks	79.10 ^{eA}	0.52	82.70 ^{eA}	0.93	**0.031***
p-value	**≤0.001***		**≤0.001***		

Superscripts with different small letters indicate statistically significance difference within the same column. Superscripts with different capital letters indicate statistically significance difference within the same row. *; significant (p≤ 0.05)

ns; non-significant (p>0.05),

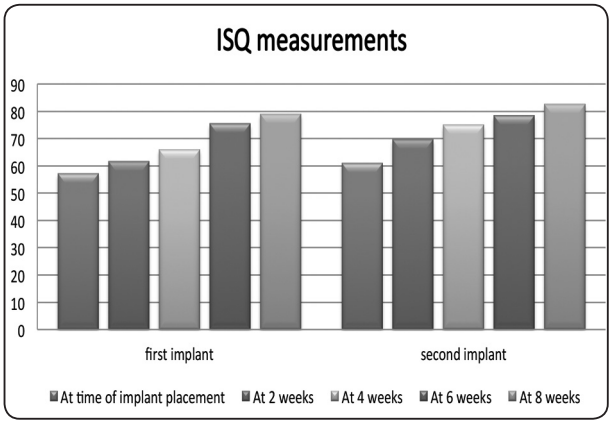

Fig. (6) Bar chart showing ISQ measurements for each implant at every follow-up appointment

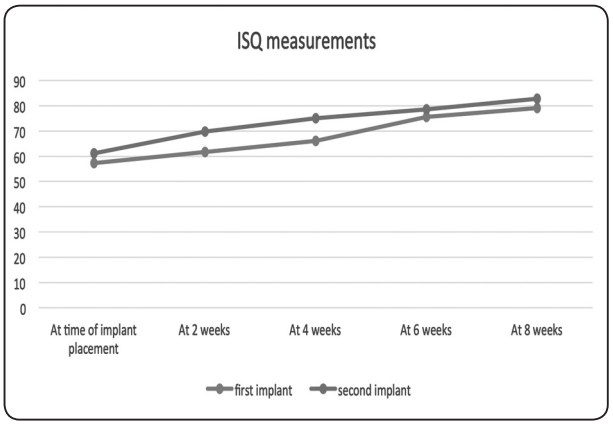

Fig. (7) line graph showing the effect of time on ISQ measurements for the two implants

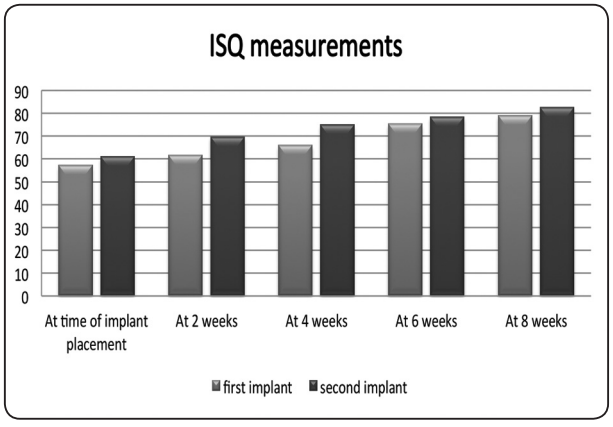

Fig. (8) bar graph showing comparison of ISQ values between the two implants

DISCUSSION

In the past, it was possible to wait for up to 12 months after extraction before an implant could be placed. The idea behind this was to establish sufficient bone quality and quantity for implant placement and osseointegration. However, the prolonged waiting time and unpredictable final esthetic results were problematic to both patient and practitioner. Today, with the recent advances in implant technology, patients expect superior esthetic as well as functional results in the least possible time. Immediate implant placement following tooth

extraction has shown successful results with respect to osseointegration as well as esthetic and functional outcomes, especially when combined with grafting materials. PRF is a relatively economic, minimally invasive autogenous material that has a high healing and regenerative capacity which results in faster healing and an increase in bone to implant contact; factors that are increasingly important with immediate implantation cases.

One of the detrimental factors for predicting the success of osseointegration is implant stability. RFA is a well documented, commonly used method of measuring implant stability during various stages of prosthetic treatment [43,44]. The literature seems to be lacking sufficient evidence regarding the effect of PRF on the stability of immediately placed implants.

The results of the present study show that growth factors and cytokines present in the PRF material have a positive effect on implant stability during the first two months of healing. The increase in ISQ values over a period of 8 weeks was significantly higher with the second implant than the first implant. Results have shown that PRF significantly increases implant stability after implant placement and continues to increase implant stability at the biweekly follow up appointments. These results are supported by the strong evidence in the literature regarding the effect of PRF on wound healing and tissue regeneration, especially in the early healing phase [32,37,39,40]. The results also come in accordance with a study by Oncu and Alaaddinoglu[45], which showed that PRF had a significant effect on the stability of implants placed 6 months after extraction. The present study also agrees with the findings of Shaarawy and Fahmy[34] that showed a significant increase in peri-implant bone density after PRF grafting of immediately placed implants when compared to synthetic bone substitutes. They also agree with the results by Oncu et al[40] which demonstrated that PRF increased the amount and rate of new bone formation and enhanced bone to implant contact during the early stages of healing.

CONCLUSIONS

Within the limitations of this study, it can be concluded that the growth factors and cytokines present in PRF make it a reliable grafting material that can be used with immediately placed implants following tooth extractions. They have a positive effect on implant stability of immediately placed implants.

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