



Microbiota Associated with Osseointegrated Implants Restored with Sloping Shoulder and Platform Switched Implants

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Authors' contributions

This work was carried out in collaboration between all authors. Author JM designed the study, wrote the protocol and managed the experimental process. Author SS wrote the first draft of the manuscript.

Author SE drafted the manuscript managed the literature searches. Authors VJ and KR did the analyses of the study and performed the spectroscopy analysis. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The implant abutment connection is an important factor regarding peri-implant bone remodeling as the highest number of inflammatory cells has been observed at the implant-abutment interface. Supracrestal implant position favours the establishment of biological width at the crest sweeping away the microgap and bacterial contamination at the bone crest thereby

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reduces inflammatory infiltrate. When supracrestal implant installation is done absence of microgap at the bone crest level and reduced inflammatory peri implant cells with minimal bone loss is seen. Among the parameters that can influence crestal bone levels around implants restored with sloping shoulder and platform switching, the composition of the submucosal peri-implant microbiota has been seldom investigated. Therefore, the aim of the present study is to evaluate the bacterial colonization in dental implants inserted in crestal or supracrestal position and correlated it to radiographic measurements of bone remodeling.

Materials and Methods: A prospective, randomized parallel clinical trial with 24 subjects who required single-tooth rehabilitations were enrolled in the Group 1 which included 12 subjects with sloping shoulder implants placed 2 mm subcrestal level & Group 2 included 12 subjects with platform switched implants placed crestal level. Radiographic examination was performed at baseline (implant installation) and after 6 months. Clinical and microbiological data were collected after 6 months. Digital radiography was used to assess bone remodeling (marginal bone loss and optical alveolar density). Bacterial profile was analyzed by checkerboard DNA- DNA hybridization, including a panel of 40 bacterial species.

Results: We found higher counts of *P. gingivalis*, *T. denticola*, *T. forsythia* (red complex) at crestal level when compared to subcrestal position and it was least at tooth site. The values of bone remodeling at baseline and after 6 months was statistically significant ($p < 0.05$) in both the groups, also there was statistically significant difference in bone levels at T2 between crestal and subcrestal groups.

Conclusion: The present study results shows that sloping shoulder design with subcrestal implant insertion had significantly less red bacterial complex and bone loss at 6 months post insertion. Platform switched implants showed significantly higher red complex and bone loss.

Keywords: *Sloping shoulder implant; subcrestal position; bacterial complex; bone remodeling.*

1. INTRODUCTION

Biofilm is described as relatively undefinable microbial community associated with tooth surface or any hard nonshedding material. Biofilms are ubiquitous and they form on virtually all surfaces immersed in natural aqueous environment, e.g., water pipes, living tissue, tooth surface, implanted medical devices, dental implants [1].

Osseointegrated dental implants to replace lost teeth in edentulous and partially edentulous patients have become a predictable treatment modality in prosthetic dentistry. Favourable long-term results of dental implant systems have been reported [2]. The success of the prosthetic treatment is widely affected by a various factors which can change the bio mechanical coupling between implant and bone, such as implant location, mechanical and morphological properties of bone, mechanical and geometrical features of implant, and type and magnitude of the load transferred by the implant to the bone, as well as by host factors such as smoking and bacterial environment [3].

The implant abutment connection is considered to be an important factor regarding peri-implant bone remodeling, as the highest number of inflammatory cells has been observed at the implant-abutment interface [4].

In addition to successful treatment, low amounts of plaque and low levels of marginal inflammation have been identified at the implants [5]. Despite the predictable treatment results, with most failures occur during initial healing and the first year of loading, complications do arise during maintenance and retention of implants. The tissues supporting osseointegrated dental implants are susceptible to disease that may lead to implant loss [5].

Sloping shoulder concept was introduced in 1985 with an unique characteristic that facilitates appropriate transfer of occlusal loads to the bone when positioned below the bony crest and it provides room for the bone over the implant which provides support for the inter dental papillae enabling esthetic gingival contours to be easily and consistently achieved. It also provides sensible biological width and provides impressive bone maintenance [6].

The platform switching concept is based on the use of an abutment smaller than the implant neck resulting in a horizontal offset at the top of the implant that separates the crestal bone and the connective tissue from the interface. The biomechanical rationale proposed that by platform switching the stress-concentration zone (from the forces of occlusal loading) is directed from the crestal bone– implant interface to the

axis of the implant and so reduces the stress level in the cervical bone area [7].

At the time of implant placement, the implant surfaces are devoid of any local microflora. Soon after the implant insertion the colonization of implant supported restorations leads to increase in peri implant infection, altering the habitat. Microflora adjacent to implant and implant site is influenced by local environment at the interface between peri implant mucosa and implant surface [8].

Many clinical studies have been evaluated about the peri implant microbial composition and few studies have shown that an increase in proportions of spirochetes and motile organisms is associated with increase in probing depth around the implants [9,10]. Studies have also found increased periodontal pathogens around implants with marginal bone loss [11,12].

Supracrestal implant position favours the establishment of biological width at the crest and sweep away the microgap and bacterial contamination from the bone crest and reduces inflammatory infiltrate. Studies have shown that when supracrestal implant installation is done absence of microgap at the bone crest level and reduced inflammatory peri implant cells with minimal bone loss [13,14].

Among the parameters that can influence crestal bone levels around implants restored with sloping shoulder and platform switching, the composition of the submucosal peri-implant microbiota has been seldom investigated. Therefore, the aim of the present study is to evaluate the bacterial colonization in dental implants inserted in crestal or Subcrestal position and to assess the changes in the bone remodeling at baseline and 6 months.

1.1 Objectives of the Study

- To evaluate the bacterial colonization in dental implants inserted in crestal and subcrestal position.
- To assess the bone remodelling at baseline and 6 months at crestal and subcrestal implant position.

2. MATERIALS AND METHODS

2.1 Study Population and Study Design

The population was composed of 24 subjects selected from the OPD of private clinics of Dubai.

2.1.1 Design

Prospective randomized parallel clinical trial.

2.2 Sampling Method

Convenience sampling technique.

- Subjects were selected as and when they visited the clinic fulfilling the selection criteria.

2.2.1 Inclusion criteria

1. Good general health.
2. Absence of oral and dental disorders.
3. Aged 40 - 70 years.
4. Partially Edentulous patients willing for implant replacement in mandibular first molar region.
5. Healed osseous architecture to receive an implant with the diameter of at least 4.2 mm and length of 10 mm.
6. No history of bone augmentation procedures at the implant site.
7. No smoking habit.

2.2.2 Exclusion criteria

1. Uncooperative patients.
2. Those who did not give informed consent.
3. Presence of active periodontal disease as expressed by probing pocket depths > 4mm and attachment loss of >2 mm.
4. Presence of periapical lesions or any other abnormalities or infections at the implant site.
5. History of radiotherapy to head and neck region.
6. Uncontrolled diabetics, Immunologically & medically compromised patients and with systemic disorders.
7. Patients with known bleeding disorders, Cardiac problems

2.3 Duration of Study

The study was conducted for a period of six months.

2.4 Study Procedure

This study was a prospective, randomized parallel clinical trial. 24 subjects who required single-tooth rehabilitations were enrolled at the clinic. All subjects received detailed information about the study and provided written

informed consent before the start of the treatment.

In brief, 24 healthy adult subjects with a single missing tooth were randomly allocated to one of the following treatment groups.

Group 1: 12 subjects with sloping shoulder implants placed 2 mm subcrestally.

Group 2: 12 subjects with platform switched implants placed crestally.

Randomization protocol was introduced from a computer generated list to distribute the subjects into 2 groups. Treatment assignments were stored in sealed envelopes for each subject and the envelopes was opened at the time of procedure. Biotech implants (platform switched 4.2 diameter & 8 mm length), Drive implants Sloping shoulder (4.2 mm diameter & 8 mm length) implants was placed in all the subjects. Patients were evaluated at baseline (T1) and after 6 months (T2). In T1 radiographic examination was performed. In T2 radiographic, and microbiologic data were gathered.

2.5 Implant Treatment

The implants (Drive and Bicon) were used in the current study with diameters of 4.2 mm and length of 8 mm. The implant size was selected based on existing bone dimensions. Immediately after local anesthesia crestal incisions was used and full-thickness flaps was elevated to expose the bone. The recipient sites were enlarged according to the protocol of the manufacturer.

Osteotomy procedure was carried out using 10 mm drills with stopper. Subsequent to osteotomy preparation, implants of 8 mm depth was placed in the prepared site. In group 1 dental implants was placed in the edentulous segments with 2 mm below the buccal aspect of the alveolar ridge and group II implants was placed crestally and the flaps were closed with interrupted sutures. Patient was advised to follow standard postoperative instruction, which included ice packs, soft high nutrient diet, postoperative medications were prescribed.

Patients were instructed not to brush the surgical site, but rather to rinse with 2% chlorhexidine gluconate. After about 7-10 days, sutures were removed. After 3 months of surgical procedure, second-stage surgery was initiated, standard gingival former was placed under normal surgical protocol. The patients were then followed-up

postoperatively after 2 weeks and closed tray impression technique was taken with additional silicone rubber based impression. Care was taken to avoid any soft tissue injury during the impression procedure. Followed by zirconia crown was delivered to every subject, occlusion was verified and all discrepancies were removed. Post treatment oral hygiene instructions and follow up check ups was provided.

2.6 Microbiological Analysis

Two sites for each implant were selected. After removing the supragingival plaque, the most apical subgingival biofilm was collected using sterile Teflon curettes Samples were placed in separate microtubes containing 0.15 mL TE (10 mM Tris-HCl and 1 mM EDTA, pH 7.6). Freshly prepared 0.5 M NaOH was added to each tube so that the bacterial DNA remained viable. Samples were kept under -2°C until analysis. Counts of 40 bacterial species were determined in each sample using checkerboard DNA- DNA hybridization. The analyses were performed at the private Laboratory of Dubai. A single blinded examiner performed radiography films readings twice in two different days [15].

2.7 Radiographic Evaluation

Radiographic assessment was performed in the mesial and distal sites of each implant. Periapical radiographies were obtained at baseline and after 6 months, by paralleling technique, The X-ray device was calibrated with 70 kV and 7 mA. The exposure time was 0.077 for all implants. Two radiographic parameters were assessed: linear bone loss and optical alveolar density.

All assessments were performed by the same examiner. Intra-examiner concordances were 93.7% for linear measurements within 90.1 mm and 99% for optical density assessments within 93 pixels. Linear measurements corresponded to the distance from the crest of the bone to the base of the implant. and were obtained in millimeters. The bone regions of interest (ROIs), approaching 1 mm^2 , were positioned laterally to each implant, at the mesial and distal most coronal point of the implant/bone contact, in the alveolar bone crest (without touching the implant). For the ROIs confection, a radiodense net with 1 mm^2 was positioned above the digital sensor and was used as a calibrator to the X-ray system. Optical alveolar density was determined by the average intensity of the grayscale in a diagonal line from the left inferior vertex to the

right superior vertex of the ROI. The grayscale varied from 0 to 256 pixels, where 0 corresponded to black and 256 to white [15].

2.8 Statistical Tests

The data was tabulated in microsoft excel sheet (2007), ANOVA was used to compare the bacterial complexes in dental implants inserted in crestal and subcrestal position. Tukey's Post Hoc analysis was performed for intragroup comparison.

Bone remodelling in baseline and after 6 months was assessed using student ' t ' test.

3. RESULTS

From the 24 patients who were included, 22 subjects were assessed.

3.1 Microbiological Profile

In the present study 40 microbial species were examined. The mean values of microbial species at the tooth site, subcrestal and the crestal level are presented in the Fig. 1.

3.2 Bacterial Complexes

Difference in the mean bacterial count in green, orange and red complexes of crestal, tooth site and subcrestal groups was analysed using one way ANOVA. There was a statistically significant difference in the bacterial counts of red complex only ($p < 0.05$), further Tukey,s post hoc analysis for intragroup comparison in red complex showed that there was statistically significant difference between crestal and subcrestal, crestal and tooth site groups ($p < 0.05$) (Table 1).

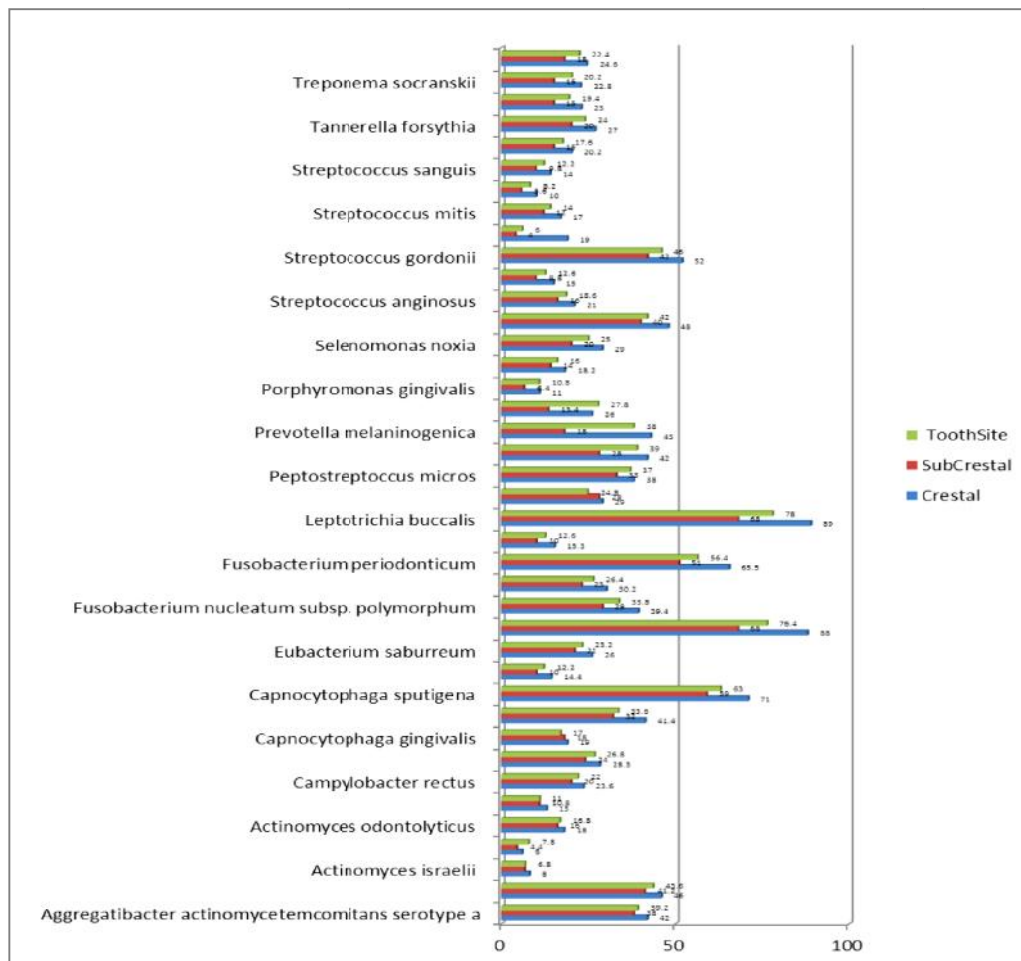


Fig. 1. Bacterial profile of implant positioned at subcrestal and crestal and tooth site

Table 1. One way ANOVA analysis for inter-group comparison of bacterial complexes and Tukey’s Post Hoc analysis for intra-group comparison

	Groups	Mean	Std Dev	df	F value	P value			
Yellow/ green complex	Crestal	34.97	20.48						
	Tooth site	27.75	19.19	2	0.315	0.73			
	Sub - Crestal	29.86	19.88						
Orange complex	Crestal	45.44	24.07						
	Tooth site	34.54	19.19	2	0.453	0.643			
	Sub - Crestal	40.22	20.72						
Red Complex	Crestal	20.33	8.32				P	Crestal-Tooth	0.033
	Tooth site	13.80	6.87				O	Site	
	Sub - Crestal	15.06	6.70	2	1.913	0.047	S	Tooth Site-	0.56
						T	Sub Crestal		
						H	Sub Crestal-	0.043	
						O	Crestal		
						C			

Table 2. Bone remodelling in baseline (T1) and after 6 months (T2) in subcrestal and crestal groups

		Sub crestal group (n=12)	Crestal group (n=12)
Linear measurement	T1	4.20 (±0.31)	4.23 (±0.20)
	T2	4.88 (±0.42)	4.46 (±0.38)*
	Bone loss (T2-T1)	0.66 (±0.28)*	0.23 (±0.21)*
Optical alveolar density	T1	56 (±21.2)	59 (±19.8)
	T2	63 (±26.6)	67 (±32.3)

t - test was applied to analyse the difference between the means of the groups

*- *P* value <0.001 (i.e. highly significant difference between the sub-crestal and crestal group)

3.3 Radiographic Bone Analysis

Bone loss and optical alveolar density at T1 (Baseline) and T2(6 Months) in subcrestal and crestal are presented in Table 2 above. There was a slight change in optical alveolar density from T1 – T2 in both the groups, but the difference was not statistically significant (*p* > 0.05). The values of bone remodeling at baseline and after 6 months were subjected to paired ‘*t*’ test, it was seen that there was statistically significant bone loss (*p* < 0.05) in both the groups, also there was statistically significant difference in bone levels at T2 between crestal and subcrestal groups.

4. DISCUSSION

The Dental implant success rates show that most of the implant failure following insertion and after attachment of implant abutment is attributed to factors like surgical trauma during insertion, occlusal overload, supporting bone quality, habits

and location of microgap between implant abutment interface [8].

There is a disagreement regarding implant insertion level and its relation to bone crest and its influence on periimplant bone remodeling. Thus, this study was performed to evaluate the effect of the implant insertion level on bacterial profile and peri-implant bone remodeling.

We found higher counts of *P. gingivalis*, *T. denticola*, *T. forsythia* (red complex) at crestal level when compared to subcrestal position and it was least at tooth site. There was a statistically significant difference in the bacterial counts of red complex. The findings are contradictory to studies conducted by Canullo et al. [8] Mariano Sego et al. [15] this could be due to tooth selection and implant design and positioning. Canullo et al. [8] study was done in completely edentulous patients whereas the present study was done in partially edentulous patients. In mariano et al. [15] study supracrestal positioning

of implant was done whereas this study subcrestal position was done.

There was a slight change in optical alveolar density from T1 – T2 in both the groups, but the difference was not statistically significant ($p > 0.05$). The values of bone remodeling at baseline and after 6 months were subjected to paired 't' test, it was seen that there was statistically significant bone loss ($p < 0.05$) in both the groups, also there was statistically significant difference in bone levels at T2 between crestal and subcrestal groups. The changes in reduced bacterial complex and bone loss is due to the implant design and subcrestal positioning.

The present study used sloping shoulder (Bicon) implants were used. Sloping shoulder (Bicon Implants) is a screwless implant system. The implant and implant-abutment unit connect by means of a 3.0° locking taper. Assembly is achieved by tapping the abutment into then matching socket in the implant. A high clamping force between abutment and implant is generated through elastic deformation of both parts. During engagement, the high friction force resulting from the relative slip between the friction surfaces yields high contact forces. This interaction results in the surface oxide layers breaking down and the asperities fusing, commonly referred to as cold welding. The locking-taper connection provides a frictional bacterial seal with excellent clinical reliability [16] Bicon has demonstrated the ability of their connection to provide an adequate microbial seal. The cold weld formed between the implant and abutment has been shown to create a hermetic seal keeping bacteria from colonizing the implant. Because there is no retaining screw with the Bicon system, there are no concerns about screw loosening. Generally in the posterior part of the mouth, occlusal forces decrease the preload of the retaining screw, but with the Bicon implant the occlusal forces strengthen the connection between the implant and abutment [17].

Meanwhile, other studies done by Buser et al and Huang et al. have found advantages with subcrestal placement of dental implants [18,19]. study found statistically significant difference when bone remodeling was evaluated in two-piece implants inserted 2 mm subcrestally and at the bone level. This result is in contradiction with studies by Heijdenrijik et al. and Todescan et al. which found no effect of the microgap location in bone remodeling [20,21,22].

Several factors may account for the conflicting results, as interface implant/abutment and healing time. In our study, the interface implant/abutment in the subcrestal group was located 2 mm. Boynueğri et al. [23] have located the microgap 2.8 mm above the bone crest. Piattelli et al. [24] located the interface implant/abutment 1 - 2mm above the bone crest and found that this position was favorable to a minor bone loss in this group. However, Guruprasada et al. [25] and Piattelli et al. [24] found no significant differences in peri-implant bone remodeling comparing conventional loading and immediate loading protocols.

A reduced sample and short term follow up for bone remodeling is limitation of our study. Studies with greater sample sizes and longer follow-ups are needed to investigate if these results and their clinical implications will be maintained.

5. CONCLUSION

The present study results shows that sloping shoulder design with subcrestal implant insertion had significantly less red bacterial complex and bone loss at 6 months post insertion. Platform switched implants showed significantly higher red complex and bone loss.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wilderer PA, Charakalis WG. Structure and function of biofilms. In: Charakalis PA, Editor. Structure and function of biofilms. Chichester, UK: John Wiley. 1989;5-17.
2. Albrektsson T. A multicenter report on osseointegrated oral implants. J. Prosthodontic Dentistry. 1988;60:75-84.

3. Telleman G, Raghoobar GM, Vissink A, Henny Meijer JA. Impact of platform switching on peri - implant bone remodelling around short implants in the posterior region, 1 year results from a split mouth clinical trial. *Clinical Implant Dentistry and Related Research*. 2014; 16(1):70-80.
4. Mombelli A, Van Oosten MAC, Schurch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiology Mand. Immunology*. 1987;2:145-51.
5. Lekholm V, Adell R, Lindhe J, Branemark PI, Eriksson B, Lindvall AM, Yonoyama T. Marginal tissue fractions at osseointegrated titanium fixtures (II) a crosssectional retrospective study. *Int. J. Oral Maxillofac. Surg*. 1986;15:53-61.
6. Marincola M, Perpetuini AP, et al. Time proven clinical success of SHORT™ IMPLANT. *CAD/CAM*. 2013;2(1):16-18.
7. Koutouzis T, Neiva R, Nonhoff J, Lundgren T. Placement of implants with platform-switched taper connections with the implant-abutment interface at different levels in relation to the alveolar crest: A short-term (1-Year) randomized prospective controlled clinical trial. *The International Journal of Oral & Maxillofacial Implants*. 2013;28:1553-63.
8. Canullo L, Quaranta A, Teles RP. The microbiota associated with implants restored with platform switching: A preliminary report. *J Periodontol*. 2010;81: 403-11.
9. Papaioannou W, Quirynen M, Nys M, van Steenberghe D. The effect of periodontal parameters on the subgingival microbiota around implants. *Clin Oral Implants Res*. 1996;197-204.
10. Renvert S, Roos-Jansaker AM, Lindahl C, Renvert H, Rutger Persson G. Infection at titanium implants with or without a clinical diagnosis of inflammation. *Clin Oral Implants Res*. 2007;18:509-16.
11. Hultin M, Gustafsson A, Klinge B. Long-term evaluation of osseointegrated dental implants in the treatment of partly edentulous patients. *J Clin Periodontol*. 2000;27:128-33.
12. Quirynen M, Alsaadi G, Pauwels M, Haffajee A, van Steenberghe D, Naert I. Microbiological and clinical outcomes and patient satisfaction for two treatment options in the edentulous lower jaw after 10 years of function. *Clin Oral Implants Res*. 2005;16:277-87.
13. Broggin N, McManus LM, Hermann JS, Medina RU, Oates TW, Schenk RK, et al. Persistent acute inflammation at the implant-abutment interface. *J Dent Res*. 2003;82:232-7.
14. Broggin N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Peri-implant inflammation defined by the implant-abutment interface. *J Dent Res*. 2006;85:473-82.
15. Mariana Ribeiro de Moraes Rego, et al. Osseointegrated implants placed at supracrestal level may harbour higher counts of *A. gerencseriae* and *S. constellatus* a randomized, controlled pilot study. *Journal of Oral Microbiology*. 2015;7:1-6.
16. Dibart S, Warbington M, Fan Su M, et al. *In vitro* evaluation of the implant-abutment bacterial seal: The locking taper system. *Int J Oral Maxillofac Implants*. 2005;20: 732-737.
17. Gross M, Abramovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegrated implants: A comparative study. *Int J of Oral Maxill Impl*. 1999;14:94-100.
18. Buser D, von Arx T. Surgical procedures in partially edentulous patients with ITI implants. *Clin Oral Implants Res*. 2000; (Suppl 1):83-100.
19. Huang B, Meng H, Piao M, Xu L, Zhang L, Zhu W. Influence of placement depth on bone remodeling around tapered internal connection implant: A clinical and radiographic study in dogs. *J Periodontol*. 2012;83:1164-71.
20. Heydenrijk K, Raghoobar GM, Meijer HJ, van der Reijden WA, van Winkelhoff AJ, Stegenga B. Two-stage IMZ implants and ITI implants inserted in a single-stage procedure. A prospective comparative study. *Clin Oral Implants Res*. 2002;13: 371- 80.
21. Heijdenrijk K, Raghoobar GM, Meijer HJ, Stegenga B, van der Reijden WA. Feasibility and influence of the microgap of two implants placed in a non-submerged procedure: A five-year follow-up clinical trial. *J Periodontol*. 2006;77:1051-60.
22. Todescan FF, Pustigliani FE, Imbrono AV, Albrektsson T, Gioso M. Influence of

- the microgap in the peri-implant hard and soft tissues: A histomorphometric study in dogs. *Int J Oral Maxillofac Implants*. 2002; 17:467-72.
23. Boynueğri AD, Yalim M, Nemli SK, Erguder BI, Gokalp P. Effect of different localizations of microgap on clinical parameters and inflammatory cytokines in peri-implant crevicular fluid: A prospective comparative study. *Clin Oral Investig*. 2012;16:353-61.
24. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibaldi S, Scarano A. Role of the microgap between implant and abutment: A retrospective histologic evaluation in monkeys. *J Periodontol*. 2003;74:346-52.
25. Guruprasada Thapliyal GK, Pawar VR. A comparative analysis of periimplant bone levels of immediate and conventionally loaded implants. *Med J Armed Forces India*. 2013;69:41-7.

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