Pesquisa Brasileira em Odontopediatria e Clinica Integrada 2018, 18(1):e4123
DOI: http://dx.doi.org/10.4034/PBOCI.2018.181.82
ISSN 1519-0501

ORIGINAL ARTICLE

Effect of Consecutive Removal and Placement of Abutments during Prosthetic Procedures on an Increase in Proinflammatory Cytokine Levels around Dental Implants

Ramin Negahdari[1], Mahdi Rahbar[2], Fatemeh Dabaghi-Tabriz[2], Alireza Pour-Nasrollah[1], Sepideh Bohluli[3], Hosein Eslami[3], Vahid Fakhrzadeh[1]

[1]Department of Prosthodontics, Dental and Periodontal Research Center, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Department of Operative and Esthetic Dentistry, Dental and Periodontal Research Center, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Department of Oral Medicine, Dental and Periodontal Research Center, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran.

Author to whom correspondence should be addressed: Mahdi Rahbar, Department of Operative and Esthetic Dentistry, Dentistry Faculty, Tabriz University of Medical Sciences, Tabriz, Iran. Phone: +98 914 154 3176. E-mail: mahdirhbr@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 20 May 2018 / Accepted: 06 August 2018 / Published: 14 August 2018

Abstract

Objective: To evaluate the effect of repeated removal and placement of abutments during prosthetic stages on increasing proinflammatory cytokine levels around dental implants. **Material and Methods:** All the patients with dental implants, referring to the prosthodontics private office during a 3-month period, were examined in relation to the health of the implants and included in the present study based on inclusion criteria; the patients had a multi-unit abutment on one side of the jaw and a conventional healing abutment on the implant on the other side of the jaw. Samples of gingival crevicular fluid were taken from all the eligible subjects for immunological analyses. The samples were taken from the sulcus around each implant in each subject. The samples were sent to the immunology laboratory for determination of IL-6 and IL-1β proinflammatory cytokines with the use of an ELISA kit. Data were analyzed with SPSS 16 and Descriptive statistics and T-test was used. Statistical significance was set at $p<0.05$. **Results:** The results showed that the mean concentrations of IL-1β and IL-6 in the group with multi-unit abutment were less than those in the conventional abutment group. In addition, comparison of the means of IL-6 and IL-1β concentrations showed that in both groups the concentration of IL-6 was higher than that of IL-1β. **Conclusion:** The use of multi-unit abutments resulted in less inflammation compared to the use of conventional two-segment abutments, which require repeated removal and placement during the prosthetic stage.

Keywords: Gingival Crevicular Fluid; Inflammation; Dental Abutments; Cytokines.

Introduction

Increasing of dental implants usage and the high percentage of success rate of their osseointegration is amplified the demand for the replacement of the lost teeth with implants [1,2]. However, retrospective studies have shown the prevalence of peri-implant conditions [3,4]. Franson et al showed that over 90% of peri-implant tissues exhibit some degree of inflammatory response [5]. In addition, researchers have reported a prevalence rate of 28% for peri-implantitis [3,4].

It is necessary to manage peri-implantitis in order to decrease bone loss around the implant neck, maintain the health of the adjacent soft tissues and increase the efficacy and longevity of the implants [6]. Various factors play a role in the induction of inflammation around dental implants, including absence of attached gingiva around the implant, excessive thickness of gingiva around the implant neck, the material of the implants used and the position of the margin of the implant-supported crown relative to the free gingival margin [6,7].

A study reported that the main etiologic factor for inflammation around dental implants is the infection resulting from contamination by anaerobic bacteria. Other factors might include inadequate plaque control and incomplete tightening of the abutment [8]. In the standard protocol of the fabrication of implant-supported prostheses, the healing abutment is removed during the various stages of the procedure and returned to its location again. Some studies have reported that repeated removal and placement of healing abutments result in frequent injuries to the soft tissues, finally leading to bone loss [7]. A new technique, which is believed to be an alternative method and results in less soft tissue injuries is the use of multi-unit abutments. In this technique, after the second-stage surgery the multi-unit abutment is placed and the final torque is applied. Then an impression is taken at the abutment level, with no removal and re-placement of the abutment during prosthetic sessions [7].

Studies on the inflammation in gingival tissues resulting from the effect of implant have shown that inflammation results in the activation of innate immune receptors, affecting the expression of proinflammatory cytokines [10]. Cytokines are peptide mediators that have a role in the regulation of immune responses, local and systemic inflammatory reactions and reparative responses in the face of invading agents. They exert their effect through induction of proliferation and difference of cells or by inhibiting their proliferation and difference [11]. The most important proinflammatory cytokines are IL-1β and IL-6, with similar and synergistic effects [12-14].

The aim of the present study was to determine the concentrations of proinflammatory cytokines in the gingival crevicular fluid around implants with the use of two different techniques for the fabrication of implant-supported prostheses, i.e. the standard technique (impression taking at fixture level) and a new protocol (impression taking at the level of multi-unit abutments) under controlled conditions in an attempt to answer the question whether or not repeated removal and re-placement of abutments during prosthetic procedures or its absence can help decrease inflammation in tissues around dental implants.

Material and Methods

Study Design and Data Collection

The present split-mouth clinical trial was done in Dentistry Faculty of Tabriz University of Medical Science from June to August 2016. The results of a previous study were used to determine the sample size [18]. By considering a mean difference of 3.37 in the means of proinflammatory cytokine levels between the two techniques of fabrication of implant-supported prostheses, with standard deviations of 3.73 and 8.55, respectively, and at α=0.05 and a study power of 80%, the sample size was calculated at n=22 in each group but in order to increase the validity of the study the sample size in each group increased up to 10% to have 25 samples in each group. The subjects were selected from those that had undergone the second implant surgery and referred to the dental office for at least two weeks for prosthetic procedures of the implant, by considering the inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

The inclusion criteria consisted of the following: signing an informed consent form to be included in the study, presence of at least two titanium implants on two contralateral locations in one jaw, placement of the implants based on the two-stage surgical procedure, requiring the exposure in the second stage of the surgery, presence of attached gingiva around the implants (at least 2 mm), a maximum thickness of 3 mm of gingiva around the implant neck, age≥18 years [13,18,19].

The exclusion criteria consisted of the following: subjects receiving scaling and root planing in the previous three months, subjects requiring bone augmentation and those with periodontitis, subjects undergoing radiotherapy in the head and neck region during the previous year, pregnant and breastfeeding women, patients receiving antibiotics during the previous 6 months, any systemic condition such as diabetes affecting the oral health, drug abusers and smokers (smoking ≥10 cigarettes a day), a history of taking biphosphonates, and subjects taking systemic antiinflammatory agents [13,18,19].

In the present study, implant-supported prostheses were fabricated using a multi-unit abutment on one side and the patients received their final abutment at this stage; on the other side of the jaw, a healing abutment was placed on the implant (Daegu, Dentis Co., Ltd. South Korea) and an impression was taken at the fixture level. On the healing abutment side, the abutment was removed and placed again at least three times (proving of crown frame, proving of crown porcelain and cementation of final crown) and each time the inside of the implant and the transmucosal area were rinsed with 0.2% CHX (chlorhexidine) to eliminate foreign agents that induced inflammation. Only in the prosthesis delivery session, it was not necessary to rinse and the Gingival Crevicular Fluid (GCF) was used to measure the proinflammatory cytokines in the GCF around the implants.

The subjects were instructed about suitable oral hygiene and effective plaque control around the healing abutment with the use of a toothbrush, water jet and CHX for 2 weeks after placement of healing abutments. After two weeks the subject were recalled for checking the control of plaque. All

the subjects eligible for being included in this study underwent a sampling procedure for immunological analyses.

Collection of Immunologic Samples

The samples were collected from the gingival sulcus around the implants in each patient. To this end, sampling from the GCF was carried out half an hour after thorough toothbrushing. Each area was isolated with cotton rolls and dried with an air current for 5 seconds. The samples were collected with paper points (Meta Biomed Co,. Ltd, Chungbuk, Korea), which were placed in four specific areas (mesiobuccal, distobuccal, mesiolingual and distolingual) up to a point where some resistance was encountered, and kept there for 30 second. Any paper point with blood contamination was excluded from the study. Each paper point was placed in a small sterile tube and frozen at -80°C until all the samples were collected. The samples were sent to the immunology laboratory for the determination of IL-6 and IL-1β proinflammatory cytokine levels with the use of an ELISA kit [13,18].

Laboratory Procedures

The immunological tests were carried out in the Immunology Laboratory of the Immunology Research Center, Tabriz University of Medical Sciences. After defrosting the samples, 100 mL of PBS (phosphate-buffered saline) were added to each sample, followed by centrifugation for 5 minutes at 3000 g at 4°C. Human Platinum ELISA kit (Northamerica/EBiosciences) was used to determine the concentrations of IL-1β and IL-6 proinflammatory cytokines in the resultant solutions. The procedural steps were similar to those with the Sandwich ELISA technique.

In all the kits, the antibody against the relevant cytokine had been plate-coated at the bottom of the wells. When the samples were added to the plates along with the standards, the relevant cytokines in the samples were considered as the antigen and coated with the anti-cytokine at the bottom of the plate. After irrigation and a definite time after incubation, the relevant biotin-conjugated monoclonal anti-cytokine was added to bond to antigen if present. After a specific duration of time of incubation of biotin-conjugated anti-cytokines, which had been bonded to the relevant cytokines, they were eliminated during irrigation. Then streptavidin bonded to peroxidase enzyme (HRP) was added and bonded to biotin-conjugated anti-cytokine.

After incubation, unbounded HRP did not bond to the relevant biotin-conjugated anti-cytokine and was not eliminated after irrigation. In the next stage, tetramethyl benzidine substrate (TMB) solutions were added, which reacted with HRP and after incubation for a definite period of time, a blue color appeared depending on the concentration of cytokine. At this stage, the reaction was stopped by adding an inhibitory solution and the intensity of the color was read at a wavelength of 450 nm and a frequency of 620 nm in an ELISA reader (State Fax 3200). The equipment drew a standard curve and based on the intensity of standard colors and their concentration, these curves were used to determine the concentrations of cytokines.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows Software, version 20 (IBM Corp., Armonk, NY, USA) and descriptive statistic and T-test was used. Statistical significance was set at $p<0.05$. The normal distribution of data was evaluated with the use of Kolmogorov-Smirnov test.

Ethical Aspects

The protocol of the study was approved by the Ethics Committee of Tabriz University of Medical Science under the code IR.TBZMED.REC.1395.302.

Results

In the present study of 50 samples selected for analysis, 7 samples failed during the tests and were excluded from the study. Mean age of the patients participated in the study was 22.2±6.43 years. The study was respectively consisted of 35% and 65% male and female patients.

The mean concentration of IL-6 in both groups was mentioned in Table 1 and Figure 1. T-test showed significant differences between these two groups ($p<0.001$) (Table 1 and Figure 2).

Table 1. The minimum and maximum values, mean and standard deviation (SD).

Groups	Minimum	Maximum	Mean±SD
IL1β HA*	26.46	38.09	34.04± 3.29
IL1β MA**	12.95	23.87	18.85 ± 2.83
IL6 HA	35.25	45.31	40.68±2.69
IL6 MA	19.32	23.06	21.44 ±1.25

*Healing Abutment; **Multi-unit Abutment.

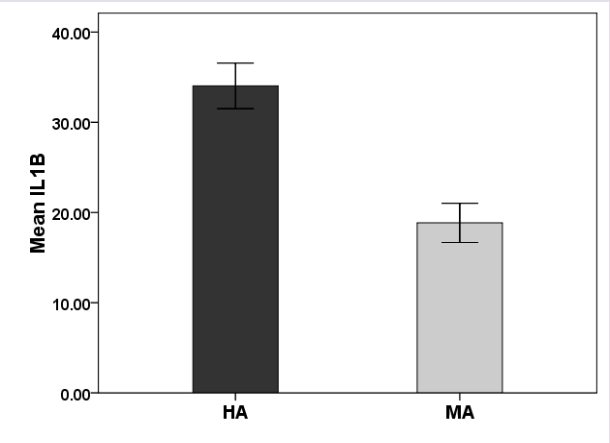

Figure 1. Comparison of concentrations of IL-6 proinflammatory cytokine in the GCF of the neck area of the implant in the different protocols.

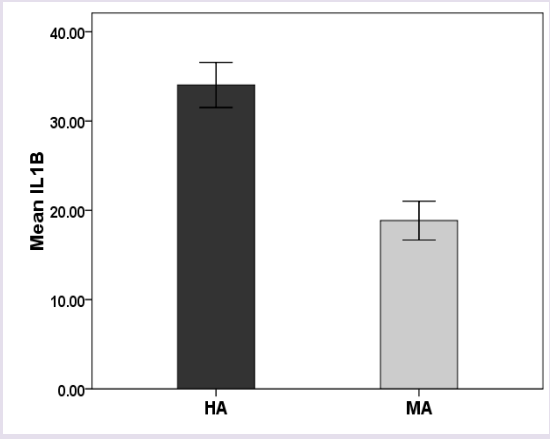

Figure 2. Comparison of concentration of IL-1β proinflammatory cytokine in the GCF of the neck area of the implant in the different protocols.

The mean concentration of IL-1β in both groups was mentioned in Table 1 and Figures 1 and 3. There was significant difference between these two groups (p<0.001). Comparison of the means of IL-1β and IL-6 showed that the concentration of IL-6 was higher in both study groups compared to that of IL-1β (Figures 3 and 4). Comparison of the mean concentrations of IL-1β an IL-6 in the healing abutment group showed that the concentration of IL-6 was higher than that of IL-1β; and this difference was significant (p<0.001) (Figure 3).

Comparison of the mean concentrations of IL-1β an IL-6 in the multi-unit abutment group showed that the concentration of IL-6 was higher than that of IL-1β; and the difference was significant (p=0.023) (Figure 4).

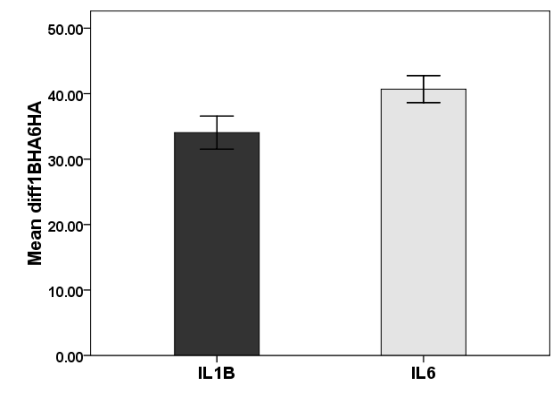

Figure 3. Comparison of concentrations of IL-6 an IL-1β proinflammatory cytokine in the GCF in the implant neck area with the use of conventional healing abutment.

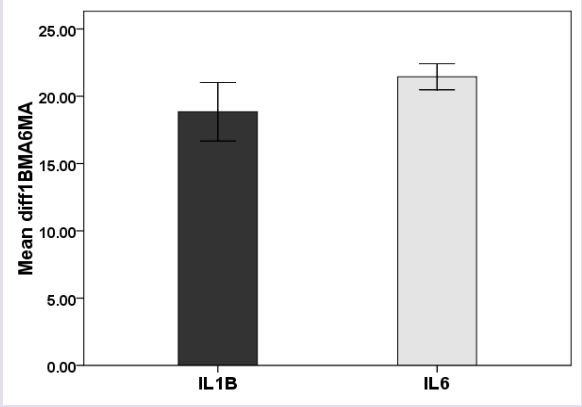

Figure 4. Comparison of concentrations of IL-6 an IL-1β proinflammatory cytokine in the GCF in the implant neck area with the use of multi-unit abutment.

Discussion

In dental implant treatments, stability and preservation of the health of soft tissues around the implants are important factors in the long-term success of implants. Various factors play a role in the preservation of such stability around implants; one of these important factors is the number of times an abutment is placed and removed and the trauma resulting from it. Based on the results of previous studies, repeated placement and removal of abutments in the standard protocol leads to injuries to the soft tissues and increasing of cytokines, finally resulting in bone loss [7]. A new technique has been introduced as an alternative method that decreases traumas to soft tissues is a multi-unit abutment and was used in the present study.

The results of this study showed that repeated removal and re-placement of the abutments increases the chances of tissue irritation, and increases TH1 and TH17 cell counts in the tissue, increasing the concentrations of IL-6 and IL-1β proinflammatory cytokines.

In the majority of studies, clinical evaluations have been used to evaluate the success of implants and the health of tissues around the implants. In a previous study on the effects of placing the abutment in one session and not removing it during the prosthetic steps on the healing of bone

around the implant; 6 months after surgery in 12 patients the standard protocol of prosthetic procedures was used. In 12 other patients, the "one abutment one time" protocol was used. Based on the results of the study, the new protocol resulted in a significant decrease in horizontal resorption of bone around the implant; however, other indices were not significantly different from each other [9].

Various studies have shown an increase in the concentration of other cytokines such as IL-6 in the GCF (Gingival Crevicular Fluid) of patients exhibiting failure of implant treatment [11,15-17]. IL-1β, too, is a chief proinflammatory cytokine, with a relationship with implant-supported prostheses and in fact it is a key molecule in the pathogenesis of implants [15].

In another research, two protocols for fabrication of implant-supported prostheses were compared: placement of an abutment and preserving it until the last stage [the new protocol, i.e. use of a multi-unit abutment) and removal and placement of the abutment during different stages [the conventional protocol, i.e. use of a healing abutment). After 3 years, bone loss around the implant in the conventional technique was greater than that in the new protocol. In that study, proinflammatory cytokine were not evaluated [9].

As reported in various studies, in dental implant treatments, the tissues surrounding the implant should not be irritated and the secretion of proinflammatory cytokines should decrease for the long-term homeostasis of the mucosa around the implant. Analysis of the gingival crevicular fluid around the implant is a non-invasive process for the evaluation of secretion of these cytokines and the inflammatory process around the implant [7].

A previous study was developed with 28 patients with partial edentulism in the posterior area [20] and reached the same conclusion. By considering the limitations of their study the authors suggested that the placement of the final abutment in one stage results in a significant decrease in the resorption of crestal bone around the dental implants in partial edentulism, although a difference of 0.3 mm was not deemed to have an important clinical effect [20]. Other authors evaluated this hypothesis in patients receiving implants immediately after extraction of teeth in 32 patients with 32 hopeless maxillary premolars in 3 private offices and concluded that similar to the studies mentioned above definitive placement of an abutment in one session resulted in a significant decrease in the loss of crestal bone around the implants placed immediately after tooth extraction. However, these researchers suggested further clinical trials with larger sample sizes to reach a definitive conclusion [21].

Based on evaluation of previous studies, it appears only a limited number of studies have evaluated the health of the mucosa around dental implants by analyzing the GCF around dental implants, although it is a non-invasive procedure for the evaluation of secretion of these cytokines and inflammatory conditions around implants. As shown in previous studies, in dental implant treatments, the mucous cells around dental implants that are responsible for secretion of cytokines should not be irritated so that the long-term homeostasis in the mucosa around implants can be achieved [7]. Studies on the inflammatory reactions of the gingival tissue as a result of implants have shown that the inflammatory process results in the activation of innate immune system receptors, affecting the expression of proinflammatory cytokines [10].

The effect of removing and re-placing the abutment on the secretion of cytokines around implants was evaluated, using immunological techniques at different intervals after placement of implants by collecting GCF and determining the concentrations of IL-1β and TNF-α with the use of ELISA. Based on the results of the study, the test group (that received the definitive abutment after the surgical stage), the concentrations of IL-1β and the probing depths were lower than those in the control group; however, there were no significant differences between TNF-α concentrations and bone height between the two groups. However, based on the results, placing the final abutment during the second surgical procedure resulted in lower inflammatory reactions in tissues around the implant [7]. This finding is consistent with the results of the present clinical trial. Based on the results of the present study, too, the mean concentrations of IL-1β and IL-6 in the multi-unit abutment group were less than those in the conventional healing abutment group and statistical comparisons between IL-6 and IL-1β showed that the concentration of IL-6 in both study groups was higher than that of IL-1β.

It should be pointed out that only one of the cytokines evaluated in the present study was similar to that previously reported [7]. However, some of the disadvantages of that study were a lack of attention to the possible contamination of the transmucosal area during removal and re-placement of the abutment in the conventional protocol, the old age of the subjects (over 60 years of age) and the small sample size [7].

Finally, it might be pointed out that under the limitations of the present study, a decrease in IL-6 and IL-1β concentrations in the test group (placement of the final abutment in the surgical session) was comparable to that in the control group, resulting in a decrease in bone loss around the implant, which has been confirmed in short-term and medium-term follow-ups in similar studies.
Limitations of this study were the people who were referred to dental practice and mostly had good oral hygiene. Future studies will be proposed to be done on people with different social and oral hygiene level and also, other inflammatory cytokines survey in studies e.g. IL-27 and IL-23.

Conclusion

The use of multi-unit abutments (with no need for removing and re-placing them during the prosthetic procedures) results in less severe inflammatory reactions compared to conventional two-segment abutments (that should be removed and re-placed several times during the prosthetics procedures).

References

1. Foley CH, Kerns DG, Hallmon WW, Rivera-Hidalgo F, Nelson CJ, Spears R, et al. Effect of phosphate treatment of Acid-etched implants on mineral apposition rates near implants in a dog model. Int J Oral Maxillofac Implants 2010; 25(2):278-86.

2. Dabaghi-Tabriz F, Fakhrzadeh V, Babaloo AR, Tagilu H, Eslami H, Rahbar M, Khadem-neghad S, Panahi P. Evaluation of periodontal status of adjacent teeth to posterior single-tooth implant during a one-year period after restoration: A cross-sectional study. Pesq Bras Odontoped Clin Integr 2018; 18(1):3872. doi: 10.4034/PBOCI.2018.181.17.

3. Roos-Jansåker AM, Lindahl C, Renvert H, Renvert S. Nine-to fourteen-year follow-up of implant treatment. Part II: Presence of peri-implant lesions. J Clin Periodontol 2006; 33(4):290-5. doi: 10.1111/j.1600-051X.2006.00906.x.
4. Chung DM, Oh T-J, Lee J, Misch CE, Wang H-L. Factors affecting late implant bone loss: A retrospective analysis. Int J Oral Maxillofac Implants 2007; 22(1):117-26.
5. Fransson C, Lekholm U, Jemt T, Berglundh T. Prevalence of subjects with progressive bone loss at implants. Clin Oral Implants Res 2005; 16(4):440-6. doi: 10.1111/j.1600-0501.2005.01137.x.
6. Berglundh T, Lindhe J, Ericsson I, Marinello C, Liljenberg B, Thornsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991; 2(2):81-90. doi: 10.1034/j.1600-0501.1991.020206.x.
7. Kuppusamy M, Watanabe H, Kasugai S, Kuroda S. Effects of abutment removal and reconnection on inflammatory cytokine production around dental implants. Implant Dent 2015; 24(6):730-4. doi: 10.1097/ID.0000000000000330.
8. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 2000 1998; 17(1):63-76. doi: 10.1111/j.1600-0757.1998.tb00124.x.
9. Degidi M, Nardi D, Piattelli A. One abutment at one time: non-removal of an immediate abutment and its effect on bone healing around subcrestal tapered implants. Clin Oral Implants Res 2011; 22(11):1303-7. doi: 10.1111/j.1600-0501.2010.02111.x.
10. Hosseini M, Kleven E, Gotfredsen K. Fracture mode during cyclic loading of implant-supported single-tooth restorations. J Prosthet Dent 2012; 108(2):74-83. doi: 10.1016/S0022-3913(12)60110-3.
11. van Brakel R, Cune MS, van Winkelhoff AJ, de Putter C, Verhoeven JW, van der Reijden W. Early bacterial colonization and soft tissue health around zirconia and titanium abutments: An in vivo study in man. Clin Oral Implants Res 2011; 22(6):571-7. doi: 10.1111/j.1600-0501.2010.02005.x.
12. Döring K, Eisenmann E, Stiller M. Functional and esthetic considerations for single-tooth ankylos implant-crowns: 8 years of clinical performance. J Oral Implantol 2004; 30(3):198-209. doi: 10.1563/1548-1336(2004)30<198:FAECFS>2.0.CO;2.
13. Negahdari R, Rahbar M, Fakhrzadeh V, Eslami H, Akbari T, Bohluli S. Comparison of proinflammatory cytokine levels in gingival crevicular fluid around dental implants with ceramic and titanium abutments. J Contemp Dent Pract 2017; 18(9):831-6.
14. Watkin A, Kerstein RB. Improving darkened anterior peri-implant tissue color with zirconia custom implant abutments. Compend Contin Educ Dent 2008; 29(4):238-40, 242.
15. Linkevicius T, Apse P. Influence of abutment material on stability of peri-implant tissues: A systematic review. Int J Oral Maxillofac Implants 2008; 23(3):449-56.
16. Nothdurft F, Pospiech P. Prefabricated zirconium dioxide implant abutments for single-tooth replacement in the posterior region: evaluation of peri-implant tissues and superstructures after 12 months of function. Clin Oral Implants Res 2010; 21(8):857-65. doi: 10.1111/j.1600-0501.2009.01899.x.
17. Sailer I, Zembic A, Jung RE, Hämmerle CHF, Mattiola A. Single-tooth implant reconstructions: Esthetics factors influencing the decision between titanium and zirconia abutments in anterior regions. Eur J Esthet Dent 2007; 2(3):296-310.
18. Barwacz CA, Brogden KA, Stanford CM, Dawson DV, Recker EN, Blanchette D. Comparison of pro-inflammatory cytokines and bone metabolism mediators around titanium and zirconia dental implant abutments following a minimum of 6 months of clinical function. Clin Oral Implants Res 2015; 26(4):35-41. doi: 10.1111/clr.12326.
19. Bonino F. The impact of peri-implant soft tissue properties on patient-reported and clinically assessed outcomes: A prospective study (Thesis). Tufts University School of Dental Medicine, 2015.
20. Grandi T, Guazzi P, Samarani R, Garuti G. Immediate positioning of definitive abutments versus repeated abutment replacements in immediately loaded implants: Effects on bone healing at the 1-year follow-up of a multicentre randomised controlled trial. Eur J Oral Implantol 2012; 5(1):9-16.
21. Canullo L, Bignozzi I, Cocchetto R, Cristalli MP, Iannello G. Immediate positioning of a definitive abutment versus repeated abutment replacements in post-extractive implants: 3-year follow-up of a randomised multicentre clinical trial. Eur J Oral Implantol 2010; 3(4):285-96.