Intraoral versus Extraoral Cementation of Implant-supported Single Crowns; Clinical, Biomarker and Microbiological Comparisons

Running title: Crown cementation technique and implants

Begüm Kıran[1] DDS, Resident

Muhittin Toman[1] DDS, PhD, Professor

Nurcan Buduneli[2] DDS, PhD, Professor

David F. Lappin[3] PhD

Suna Toksavul[1] DDS, PhD, Professor

Nejat Nizam[2] DDS, PhD, Associate Professor

[1] Department of Prosthetic Dentistry, School of Dentistry, Ege University, İzmir, Turkey.

[2] Department of Periodontology, School of Dentistry, Ege University, İzmir, Turkey,

[3] Oral Sciences Research Group, Dental Hospital and School, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

Corresponding author:

Dr. Nejat Nizam

Ege University, School of Dentistry

Department of Periodontology

35100-Bornova, İzmir, Turkey

E-mail: nejat.nizam@ege.edu.tr

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Author contribution statement: Begüm Kıran recruited eligible patients, did the clinical recordings, collected the GCF samples and did the clinical work of prosthetic reconstruction. Muhittin Toman and Suna Toksavul took part in study design, recruited eligible patients and partially drafted the manuscript. David Lappin performed all laboratory and statistical analyses. Nurcan Buduneli took part in study design, critically read and edited the manuscript. Nejat Nizam took part in study design, placed the implants and drafted the manuscript. All authors substantially contributed to study design, acquisition, analysis, interpretation of data, and drafting or critically revising the manuscript and approved the final version.

Abstract

Objectives: Implant supported single metal-ceramic crowns cemented either extraorally or intraorally, were comparatively evaluated by clinical, radiologic, biomarker and microbiological data.

Materials & Methods: Twelve patients with one missing tooth at each side of the maxilla received 2 locking-taper implants; 4.5 mm width, 8 mm length. Selection of intraoral cementation (IOC) or extraoral cementation (EOC) using screwless titanium abutments was done randomly. Peri-implant crevicular fluid (PICF), gingival crevicular fluid (GCF) samples were collected from the implants and adjacent teeth in the same individuals before starting the prosthetic procedures (baseline) and 3, 6 months after implant loading. Radiographs were taken immediately after and 6 months after cementation and crestal bone loss was measured. Cytokine levels, amounts of bacteria were determined in PICF/GCF samples. Data were tested by appropriate statistical analyses.

Results: All clinical parameters were similar in the crowns cemented extraorally or intraorally at all times ($p<0.05$). PICF data were similar to GCF data. Lower amounts of IL-17E and OPG levels were observed in the intraorally cemented crowns.

Conclusion: EOC and IOC implant supported metal-ceramic single crowns exhibited similar crestal bone loss after loading. However, at 3 months, differences in anti-inflammatory processes and stimulation might be suggested at IOC crowns.

MeSH Key words: dental implant, extraoral cementation, intraoral cementation

Introduction

Intraosseous dental implants are frequently preferred treatment choice for rehabilitation of partial or total edentulous patients to reduce the physical and cosmetic consequences of tooth loss. Implants restored with fixed dental prosthesis (FDPs) have high survival rate in spite of the fact that mechanical, aesthetical or biological complications may occur in clinical use. Biological complications can be categorized as surgery-related implant loss, bone loss and peri-implant soft tissue diseases.[1] Bacteria play a major role in the etiology of peri-implant diseases, which can be restricted to the soft tissues in terms of mucositis or progress to the supporting bone and induce bone destruction; namely peri-implantitis.

Implant supported FDPs can be fixed on implants either by screws or by cementation. Survival rates for single-unit screw retained and cemented prosthesis were shown to be 89.3% (95% CI: 64.9%-97.1%) and 96.5% (95% CI: 94.8%-97.7%), respectively without any statistical significant difference.[2] Both techniques have their own advantages and disadvantages, cemented restorations have less technical complications (24.4% vs. 11.9%), whereas biological complications are more frequently associated with cemented crowns (2.8% vs. 0%).[3,4] It is known that excess cement left in the peri-implant mucosa/sulcus promotes the formation of biofilm leading to inflammation in the peri-implant tissues and cause biological complications.[5] A clinical study by Wilson[6] reported a positive relation between residual cement and development of chronic peri-implant diseases. In order to eliminate the biological complications of cemented reconstructions, screw retained prostheses can be preferred in certain cases. However, an implant with locking taper connection does not have a retention screw and single crowns could be extra-orally cemented and therefore, may eliminate the negative consequences of both screw retained and cemented prosthesis while preserving the positive aspects of both techniques.

Inflammation affecting the peri-implant tissues causes mucositis and peri-implantitis via similar inflammatory mechanisms acting in the pathogenesis of gingivitis and periodontitis. There are studies reporting similar content of the pathogenic bacteria in the biofilm around dental implants and natural teeth[7,8,9] while differences in bacterial species in the same individuals was demonstrated recently suggesting distinct pathogenic mechanisms.[10] It is also shown that presence of *A. actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *F. nucleatum* at the implant sulcus was affected by the

presence or absence of these microorganisms in the gingival crevice of adjacent teeth rather than other sites in the dentition. [9]

Interactions between the three members of the tumor necrosis factor (TNF) superfamily; receptor activator of NF-κB ligand (RANKL), RANK, and osteoprotegerin (OPG) are important in coordination of osteoclastogenesis and alveolar bone resorption.[11] RANKL binds directly to RANK on the surface of preosteoclasts, osteoclasts and stimulates both the differentiation of osteoclast progenitors and activity of mature osteoclasts. OPG is the naturally occurring inhibitor of osteoclast differentiation. It is a soluble molecule that binds to RANKL with high affinity and blocks RANKL from interacting with RANK.[12]

On the other hand, interleukin-17 (IL-17) synergizes with other cytokines, including IL-1β, TNF-α. IL-17A increases RANKL expression and concomitantly decreases OPG expression in osteoblastic cells *in vitro* and *in vivo*, thereby enhancing osteoclast formation and bone erosion.[13] In contrast with the other IL-17 family cytokines IL-17E is an anti-inflammatory cytokine, which opposes the functions of IL-17A.[14] The levels of inflammatory biomarkers are expected to change in periimplantitis[15] and periodontits[16] sites and therefore, their levels could be informative for the inflammatory status of periimplant and periodontal tissues.

It was hypothesized that due to the reduced risk of excess cement, extraorally cemented implant-supported crowns exhibit less crestal bone loss and microbiological load, and smaller amounts of inflammatory biomarkers than intraorally cemented crowns. Therefore, the aim of this clinical study was to comparatively evaluate the implant supported metal-ceramic crowns cemented either intraorally or extraorally by clinical, biomarker, and microbiological parameters during the 6-months period after implant loading. Moreover, the biomarker and microbiological data were compared with those around the natural adjacent teeth in the same individuals in an attempt to compare the process around natural teeth and implants.

Materials and Methods

Study population and design

This study was carried out at Ege University, School of Dentistry between May 2014 and June 2016. Detailed medical and dental histories were obtained from all volunteer participants and clinical, radiographical examinations were performed. The procedures related to the study was reviewed and approved by the Ethical Board of Ege University School of Medicine with the protocol number of 14-5/18.

Clinical evaluations including occlusion, interocclusal distances, parafunctions, endodontic and periodontal lesions, and soft tissues surrounding the edentulous space were made. Panoramic radiographs were evaluated for bone pathologies, vertical dimensions of the available bone, and distances to the anatomical structures. Nonsurgical periodontal therapy consisting motivation and instruction on oral hygiene, scaling and root-planing was provided as required. Patients were informed about all prosthetic rehabilitation alternatives with or without implant-supported restorations and all individuals signed the informed consent form before enrolment in the study.

Inclusion criteria were; presence of bilateral single tooth gap in the maxillary posterior region with the extractions performed at least 6 months ago, presence of adjacent teeth on both sides, presence of sufficient bone height and width at the alveolar crest, at least 2-mm vestibular keratinized mucosa width and 3 mm mucosa thickness, and with no history of periodontitis. Furthermore, a full-mouth plaque score of < 20 % was a prerequisite for implant placement. Patients with known systemic diseases, conditions or those using medications with potential to impair surgery and wound healing dynamics and also smokers were excluded from the study. Moreover, patients with bone height < 8 mm, presenting ridge and soft tissue deficiencies that require augmentation procedures, and having endodontic or periodontal lesions neighboring the edentulous sites were also excluded.

Twelve patients (7 males and 5 females, age range 35-45 years) fulfilling the inclusion criteria were scheduled for surgical procedures.

Surgical procedures

All the surgical procedures were carried out at the Department of Periodontology, School of Dentistry, Ege University. Following local anesthesia (2% lidocaine with 1:100 000 epinephrine), the soft tissue thickness corresponding to mid-crestal incision line was measured by bone sounding at mesial, middle and distal aspects. A mid-crestal incision was then placed and continued intrasulcularly to mid-facial and mid-palatal area of adjacent teeth. A full thickness flap was then elevated to visualize the alveolar crest and the vestibulo-palatal thickness was measured to validate the width of the crest. Following the initial osteotomy with a pilot drill at 800 rpm under saline irrigation, the consecutive drills were used at 50 rpm without irrigation in line with the manufacturers' instructions.

The depth of the osteotomy slightly exceeded the length of the implant and the width of the osteotomy was the same as the implant diameter. Implants with locking-taper that allows

extraoral cementation of single crowns were placed by the same periodontist (NN). All the implants (Bicon LLC, Boston, MA, USA) had the same diameter and length (4.5 x 8 mm).

After the implants were placed into the osteotomy by slight vertical tapping a healing cap of the same size (3 mm height, 5 mm diameter) was installed. Non-absorbable 5-0 silk sutures were placed (Medipac SA, Kilkis Greece). Surgeries on both sites were carried out in a single session. Antibiotics were not prescribed following the implant surgery. Patients cleaned the surgical area with a postoperative dental brush (GUM Delicate Post-surgical toothbrush, Sunstar Americas Inc, Chicago, IL, USA) and avoided flossing at the surgical area. The sutures were removed at the first week, and the patients were evaluated monthly thereafter. During the osseointegration period the patients strictly complied with oral hygiene measures.

Prosthetic procedures

Following the implant surgery a 3-month period was allowed for osseointegration and single PFM crowns were fabricated for each patient. The implant used in the current study has a locking taper connection, and the abutment of the system fits into the well of the implant without a retentive screw. This structure enables cementation of a crown prior to installation of the abutment into the implant. At the first prosthetic stage, a closed-tray technique with transfer posts was used for taking the impressions. Impression (Virtual, Ivoclar Vivadent, Schoon, Lichtenstein) and bite registration material was polyvinyl siloxane (Memoreg 2, Heraeus Kulzer, Germany). The impression posts and implant analogs were installed and placed into the impression as described by the manufacturer. After the master cast is obtained in the laboratory, the titanium abutments of the same size were chosen and the shoulders of the abutments were prepared 1mm subgingivally at all sites so that the crown margins were located 1 mm below the gingival margin. When the metal-ceramic crowns were fabricated following standard procedures the cementation steps were initiated. The intra-oral cementation (IOC) and extra-oral cementation (EOC) groups were randomly assigned at the final prosthetic stage by a researcher blinded to the study by toss of a coin. The abutments in the IOC group were cleaned, sterilized, and placed into the implants, and after the shoulders of the abutment was confirmed to be 1mm subgingivally, slight vertical tapping was performed to assure the cold weld between the implant and the abutment. The crowns were then cemented over the abutments using zinc polycarboxylate cement (Poly-F Plus; Dentsply International, York, PA, USA). After the setting, maximum care was taken to remove excess cement around the abutments. The abutments in EOC group were also placed into the

implants to confirm the position of the shoulder and then removed back to perform EOC procedures. The crowns were cemented extra-orally using the same material and the excess cement was thoroughly cleaned before the final installation of the abutment into the implants as described for IOC group. After completion of the prosthetic stage, patients were recalled for clinical evaluation, at 3 and 6 months follow-up sessions. During this period, patients were motivated and instructed for optimum plaque control but flossing around the implants was avoided.

Clinical and radiographic recordings

Clinical evaluations were performed at baseline and also at 3, 6 months after placement of implants and construction of single unit porcelain fused to metal crowns (PFM). Soft tissue inflammation around the implants and natural teeth were evaluated by presence or absence of bleeding on probing (0=absent, 1=present). Soft tissue thickness at the implant sites was measured on the day of surgery by bone sounding using a straight periodontal probe and a rubber stop. The keratinized tissue width was measured as the distance between the gingival/mucosal margin and the mucogingival junction. All the measurements were rounded up to the nearest 0.5 mm.

Standardized periapical radiographs were taken immediately (baseline) and 6 months after cementation using the long-cone paralleling technique (Kodak 2100 230 V; Carestream Health Inc Rochester, NY) and appropriate position holders (Rinn XCP, Dentsply Corporate, York, PA, USA) and parameters set at 0.125 sec exposure time, 60 kV, and 70 mA. Images were transferred to computer by a photostimulatable phosphor plate scanner (Digora Optime, Soredex, Milwaukee, WI, USA) and radiographs were used to measure the crestal bone loss at the mesial and distal surfaces of the implants. The radiographs were stored as digital images and on each image the implant length and implant diameter were used as the reference for measurements. Implant-abutment interface and first bone to implant contact was depicted on each image and the vertical distance between these two points were noted as the crestal bone level (CBL). The bone loss at 6 months was calculated subtracting the CBL at baseline from the CBL at 6-months. The follow-up radiographs were randomly numbered by a periodontist not involved in the study and all radiographic assessments were performed following the numeric order by the same investigator (BK) in a blinded manner.

Collecting biofluid and microbiological samples

Peri-implant crevicular fluid (PICF), gingival crevicular fluid (GCF) samples and microbiological samples were obtained from two sites of each implant and adjacent tooth in the same individuals before starting the prosthetic procedures (baseline) and also 3, 6 months after cementation of crowns. If the implant was a premolar the neighboring premolar, and if the implant was a molar the adjacent molar tooth was chosen for sampling. All samples were collected by the same researcher (BK). First, the sampling sites were isolated by cotton rolls and healing abutment surfaces were air-dried gently. Paperstrips (Periopaper; ProFlow, Inc., Amityville, NY, USA) were inserted 1 mm into the crevice and left in place for 30 sec. Care was taken to avoid mechanical injury and those samples with visual blood contamination were discarded. The PICF/GCF volume absorbed on each paper strip was determined by a specific electronic impedance device (Periotron 8000, ProFlow, Inc., Amityville, NY, USA) and both strips from each implant or tooth were placed in one sterile precoded polypropylene tube and kept at -40°C until the laboratory analysis. The readings from the Periotron 8000 were converted to actual volumes (µl) by reference to the standard curve.

Microbiological samples were collected using paperpoints (ISO 45). .....

Elution of PICF/GCF

The PICF/GCF samples were eluted from paperstrips into 500 µl PBS by vortexing the sample vigorously for 30 sec, the paperstrip was removed and microorganisms in the sample were pelleted by centrifugation at 14 000 g for 10 min and the supernatant was transferred to a clean tube.

Measurement of RANKL, OPG, IL-1β, IL-17A, IL-17E levels in PICF/GCF samples

Commercial ELISA kits were purchased for the measurement of OPG (R&D Systems Abingdon, UK), sRANKL, IL-17A, IL-17E (Peprotech London, UK), IL-1β (Thermo Fisher Paisley, UK). The ELISA assays were carried out according to the manufacturers' recommendations. The optical densities were read at 450 nm with a background subtraction at 570 nm and the samples were compared with the standards. The minimum detection limits for the assays were as follows OPG: 7.56pg/ml; RANKL: 7.56 pg/ml; IL-17A, IL-17E: 0.79 pg/ml; IL-1β: 0.79 pg/ml. The PICF/GCF data were expressed as total amounts per sampling time (pg/30sec) and also as concentrations (pg/µl).

Microbiological analyses

Preparation and assessment of genomic DNA

The Epicentre Masterpure Gram positive DNA isolation kit (Cambio, Cambridge, UK) was used to prepare genomic DNA from the pelleted microbes present on paper points and from known quantities of laboratory strains of the target microorganisms. The amount of DNA and the purity was checked at 260 nm and 280 nm (260/280 nm ratio = 1.8-2.0 = good DNA purity) using a NANODROP 1000 spectrophotometer (Thermo Renfrew, UK). The DNA from laboratory strains was measured by fluorimetric analysis using the CYquant assay system (Invitrogen Paisley, UK) and the value plotted against the copy number bacteria use as standards for each bacterial assay.

Real-time quantitative polymerase chain reaction (RT-QPCR)

A RT-QPCR assay using TaqMan chemistry (ABI/Invitrogen Paisley, UK) was used for the detection and quantification of bacterial cell copy numbers. The primers and probes selected for the following bacteria were as published in the sources shown: *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum*[17,18,19,20] *Treponema* denticola,[21] *Streptococcus salivarius*,[22] and were purchased from Invitrogen (Paisley, UK).

Statagene MRX III thermal cycler (Agilent Edinburgh, UK), the TaqMAN assay PCR cycling parameters used in the study were 10 min at 95°C, and 40 cycles of 30 sec at 95°C and 1 min at 60°C. All primer sets were validated by running four serial 1/10 dilutions of the standard DNA and calculating the efficiency of the reaction (E) where E= (10 -1/slope)-1.

All reaction efficiencies calculated were acceptable (between 91% and 104%). All primer sets failed to amplify the DNA purified from different microbial standards.

Statistical analyses

Microsoft Excel and commercially available statistical software (SPSS Inc. version 21 IBM, Chicago, USA and Graphpad Prism version 5, La Jolla, USA) were used to analyze the data. Statistical power calculation indicated that to exceed 80% statistical power where the effect size =1 a minimum of 23 individuals should be sampled for a non-parametric analysis using independent sample tests and for paired (dependent) sample tests the minimum number of participants N=12. With effect size =1.5, 80% statistical power was achievable in independent sample tests with N=12.

After assessing that the data distribution was skewed as determined by a Q-Q plot, the data were analyzed by nonparametric tests. The Friedman test with a post hoc Dunn's test was

used to determine significance levels between baseline and the sample time points. Differences between the treatment sites were assessed by the Mann-Whitney U-test. Correlations between the clinical parameters and the microbial analysis, and between biomarker data were evaluated with Spearman rho rank correlation test. All tests were performed at $\alpha = 0.05$ significance level.

Results

Clinical and radiographic findings

All patients included in the present study completed the study protocol. The number of molars and premolars was 8 and 4, respectively. Clinical and radiographic findings are shown in Table 1. No excess cement was seen at any of the x-rays. According to the statistical analysis, no significant differences were found between control teeth (C), EOC and IOC groups in terms of crestal bone loss, soft tissue inflammation or PICF/GCF sample volumes ($p<0.05$).

Biomarker findings

Biomarker findings are shown in Table 2 and Table 3. There were no significant differences in RANKL, OPG, IL-17A, IL-17E, IL-1β levels or RANKL/OPG ratio between the study groups at baseline or at the 3-, 6-month evaluations (Table 2). RANKL, IL-1β levels and RANKL/OPG, IL-17A/IL-17E ratios were similar within each study group between baseline and follow-up evaluations (Table 2). The total amounts of OPG and IL-17E were significantly lower at the 3-month evaluation than the baseline values in the intraoral cementation group ($p=0.03$, $p=0.013$, respectively). IL-17A/IL-17E ratio was significantly higher in the intraoral cementation group than the extraoral cementation group ($p=0.03$) at the 3-month evaluation (Table 2). At 6 months concentrations of sRANKL, OPG and IL-17A were lower in the EOC group than in the control group ($p=0.013$, $p=0.024$ and $p=0.031$, respectively) but no significant differences were seen between the IOC group and the EOC group or the IOC group and the control group (Table 3).

Microbiological findings

Microbiological findings are shown in Table 4 and Table 5. Levels of *A. actinomycetemcomitans, P. intermedia, T. forsythia, T. denticola, S. salivarius* as well as the total bacterial load were similar in the study groups at all times (Table 2). *F. nucleatum* level

(Table 4) and percentage (Table 5) were significantly lower in the samples of the intraorally cemented implants than those around the natural teeth at the third month (p=0.03). At baseline, *P. gingivalis* levels were significantly higher (p=0.02) in the control teeth than those around the extraorally cemented implants (Table 4).

Discussion

Peri-implantitis is usually diagnosed by clinical measures such as bleeding on probing, suppuration and radiographic findings of bone resorption.[23] Local risk factors such as excess cement seem to play an important role in the etiology of peri-implant diseases.[24,25] Various studies have focused on the relationship between excess cement and peri-implantitis.[6,26,27,28] There are published clinical protocols for the cementation procedure[29] but it has proven difficult to completely eliminate excess cement risk at the cement retained implant crowns. Therefore, EOC technique in particularly single implant crowns with taper locking implant systems may be a good alternative to eliminate excess cement risk. The present study was undertaken in order to compare IOC and EOC techniques and their potential impact on clinical, biochemical, and microbiological parameters. The two different cementation techniques were used in the same patients to minimize individual differences that may act on the host response to cement remnants, cement material itself and resident bacteria. Moreover, only those patients with bilateral single tooth gaps were included in the study in order to limit possible patient-related confounding factors.

Published studies mostly suggest that biomarker and microbiological data are similar around natural teeth and intraosseous dental implants as long as the clinical health state is similar.[7,30] Accordingly, the present study revealed similar findings in the PICF and GCF samples. This finding can be regarded as a further support for the similarity of the milieu/media of peri-implant and periodontal environments.

The prevalence of peri-implant diseases around implants with cemented restorations were reported to be 75 % and 64 % of those being positive for cement excess.[22] Excess cement without inflammation of the peri-implant tissue was rarely detected with permanent cement and amounted 8.5-8.8 %.[31,32] In a recent review investigating the role of excess cement as a possible risk indicator for peri-implant diseases, it was found that undetected excess cement occurred irrespective of the cement medium or type of abutment used, and the prevalence was higher with short soft tissue healing periods. In order to eliminate that risk the healing

abutments were placed at the day of implant placement and a 3-month soft tissue healing period was allowed in the current study. Moreover, in the same review it was suggested that an early detection of the disease and accompanied excess cement up to 5 month after restoration placement was more often related to the diagnosis of peri-implant mucositis as the early stage of the disease process than with peri-implantitis.[33] Even though the follow-up period in the current study slightly exceeded the time limit suggested by Staubli et al.,[33] the crestal bone change in IOC and EOC groups are more likely to be a consequence of physiologic bone remodeling rather than an outcome of the cementation technique.

Screw-retained prostheses may be considered as alternatives for cement-retained ones to eliminate problems arising from the use of excess cement. However, screw-retained prostheses have major drawbacks such as screw loosening, esthetic problems, difficulty in fabrication and in providing a passive fit, etc. In order to provide an acceptable final result with screw retained prosthesis the implant requires an optimum 3D positioning, which is not always achievable. Since the cemented prostheses are generally the treatment of choice in the daily practice, we compared the IOC and EOC techniques.

In a recent study, Canullo et al.[34] compared EOC and IOC techniques with "traditional" chamfer abutment design or feather-edge abutment design. The authors suggested that EOC technique could minimize the presence of cement remnants, but they also observed that there were little cement remnants in the IOC group. They considered the possibility of cement-induced pathology to be very low. These findings are supported by the current findings since excess cement related peri-implant pathology was not detected in the present study. Apart from the cementation technique, the type of cement and accurate cement cleaning are the other determining factors for the presence or absence of excess cement and related pathology.

A major limitation of the present study is that the study included a rather small population and the conditions of a conservative *a priori* power analysis were difficult to achieve. This was due to the difficulties in recruiting patients that fulfilled the inclusion criteria; particularly where the split-mouth design rather than a parallel arm study design was chosen. However, sufficiently large effect sizes were achieved for some of the chosen biomarkers: OPG, sRANKL, Il-17A and IL-17E and for the microbes *F. nucleatum* and *P. gingivalis.*

Inflammation in the peri-implant tissues may be latent in the early stages and not readily detected by clinical measures. Therefore, PICF analysis might provide valuable information

for the early diagnosis of inflammatory changes and might even serve as a tool for determination of patient susceptibility.[35] Although differences were observed with the control site measurements at 3 and 6 months, particularly for the EOC sites the present study revealed similar biomarker and microbiological data in the PICF samples obtained from the EOC and IOC sites. This finding can be explained by the efficacy of cement removal from 1 mm depth of crown margins in IOC techniques, thus, it should be noted that pushing the crown margin deeper than this threshold may change the results obtained in the current study. Moreover, the periodontal and peri-implant tissues were healthy at baseline leading to similarly low levels of inflammatory biomarkers in the biofluid samples. The patients were followed for 6 months after loading of implants. One has to concede that this period may be too short for detection of some of the changes in the inflammatory cytokine content of PICF/GCF sample. IL-1β and IL-17A, IL-17E are more related to soft tissue inflammation and their levels are known to increase during the early stages of inflammation around teeth or implants. A transient change in these markers could have been missed by the sampling protocol. Although a difference in the amount of the anti-inflammatory cytokine IL-17E was observed to be greater at the EOC sites than the IOC sites. The importance of this finding remains unclear although this cytokine is a key player in tissue healing.

However, most of the cytokine levels were similar at the different study sites and this might be explained by appropriate oral care provided by the patients. On the other hand, RANKL and OPG levels and particularly the ratio of RANKL/OPG is regarded as a reliable biomarker for bone loss in periodontitis or peri-implantitis. A higher amount of OPG was seen at 3 months at the EOC sites compared to the IOC sites. Whether this is an important finding needs further clarification, however, one is tempted to speculate that this might bode well for good osseointegration. The present findings revealed similar levels and proportions of several bacteria in the samples obtained from sites at EOC or IOC implants as well as their adjacent teeth with the following exceptions: *F. nucleatum* at 3 months and lower *P. gingivalis* levels at baseline than at the control tooth sites. Previous studies that compared screw-retained abutments with cement-retained abutments reported that cement-retained abutment implants offered better results relating to fluid and bacterial permeability when compared to screw-retained abutment implants.[36,37] Our microbiological data is in line with these previous studies and further emphasizes the need for complete removal of the fixation cement particularly after intraoral cementation. Presence of an inflammatory cell infiltrate at the implant-abutment junction has been described even around implants with very good hygiene and healthy peri-

implant tissues.[38] This appears to be accompanied by bacterial plaque accumulation that cannot be completely prevented. Accordingly, the present study indicated presence of various bacteria, even of several putative periodontal pathogens at the implant sites without appearing to cause clinically detectable inflammation.

Conclusion

EOC and IOC implant-supported metal-ceramic crowns exhibited similar clinical performance during the 6-month follow-up period. Differences in microbiological and biomarker data were few between the two cementation techniques. At 3-month evaluation, a transient decrease in anti-inflammatory processes and an increase in osteoclast stimulation might be suggested at IOC sites and a higher carriage of *F. nucleatum* was observed at EOC sites. It may be suggested that EOC is a safe and reliable method for the single crowns on the implants with locking taper and the clinical outcome is similar to that obtained in IOC sites with 1mm subgingival margins. Further clinical studies comparatively evaluating the EOC and IOC techniques particularly for sites with subgingival margins >1mm are warranted.

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Table 1. Clinical and radiographic parameters of the study sites

Clinical Parameter		Evaluation times	Control Mean ± SD	IOC Mean ± SD	EOC Mean ± SD
PICF/GCF Volume (µl)		**T0**	1.38±0.53	1.06±0.6	1.21±0.66
		T3	1.55±0.37	1.51±0.63	1.41±0.48
		T6	1.51±0.3	1.57±0.49	1.93±0.09
BOP (+/-)		**T0**	0±0	0±0	0±0
		T6	0.06±0.10	0.19±0.40	0.03±0.09
Crestal Bone Loss	**Mesial**	**T0-T6**	0.00-0.00	0.14±0.42	0.14±0.46
	Distal	**T0-T6**	0.00-0.00	0.19±0.47	0.20±0.68
STT	**Mesial**	**T0**	-	3.50±0.52	3.75±0.58
	Middle	**T0**	-	3.17±0.33	3.21±0.45
	Distal	**T0**	-	3.79±0.50	3.58±0.51
KTW		**T0**	3.0±0.56	2.92±0.56	2.96±0.84
		T6	2.92±0.42	2.75±0.66	2.88±0.57

T0: Baseline, T3: 3rd month, T6: 6th month, IOC: Intraoral cementation group, EOC: Extraoral cementation sites, STT: Soft Tissue Thickness (mm), KTW: keratinized tissue width (mm).

Table 2. Median (innerquartile range) total amounts of biomarkers in GCF/PICF at study sites

Biomarker	Evaluation Time	Control Median (q1-q3)	IOC Median (q1-q3)	EOC Median (q1-q3)
RANKL (pg)	**T0**	163 (147-206)	156 (111-220)	159 (104-246)
	T3	188 (87.6-222)	173 (131-208)	153 (103-205)
	T6	177 (140-203)	159 (104-184)	137 (112-181)
OPG (pg)	**T0**	114 (101-128)	149 (97.9-182)	118 (72.1-141)
	T3	108 (72.2-152)	79.5 (43.3-153)*	120 (84.7-147)†
	T6	112 (87.8-141)	88.7 (46.6-140)	98.8 (85.7-146)
IL-17A (pg)	**T0**	15.0 (12.0-16.7)	15.1 (11.3-43.4)	15.4 (13.6-17.7)
	T3	15.7 (13.2-21.0)	14.5 (11.5-16.3)	12.8 (9.79-16.0)*
	T6	13.7 (12.5-15.5)	14.2 (13.2-18.4)	12.8 (10.4-17.6)
IL-17E (pg)	**T0**	27.9 (16.7-46.6)	48.0 (31.1-69.2)	23.8 (17.1-37.9)
	T3	31.8 (19.6-44.7)	12.2 (8.44-40.2)*	36.8 (24.7-47.6)†
	T6	30.0 (20.2-44.4)	20.2 (15.8-31.4)	28.3 (22.0-38.53)
IL-1β (pg)	**T0**	7.96 (6.48-9.38)	6.81 (4.01-9.60)	6.18 (4.17-12.6)
	T3	7.09 (4.35-9.62)	8.55 (6.90-10.8)	7.44 (4.98-9.14)
	T6	7.7 (6.75-9.97)	7.24 (2.96-8.82)	7.00 (4.79-9.62)
RANKL/OPG	T0	1.33 (1.08-2.11)	1.00 (0.69-3.13)	1.72 (0.96-2.84)
	T3	1.59 (1.32-2.03)	2.01 (1.25-3.31)	1.15 (0.82-1.58)
	T6	1.93 (1.31-2.75)	1.38 (1.26-2.44)	1.47 (1.13-1.72)
IL-17A/IL-17E	T0	0.68 (0.52-0.87)	0.56 (0.23-1.00)	0.54 (0.37-1.06)
	T3	0.48 (0.41-1.22)	0.80 (0.48-1.63)	0.32 (0.23-0.68)*†
	T6	0.71 (0.43-1.08)	0.78 (0.58-1.2)	0.52 (0.27-0.87)

T0: Baseline. T3: 3rd month. T6: 6th month. IOC: Intraoral cementation sites. EOC: Extraoral cementation sites. *Statistically different from the control sites. †Statistically different from the IOC sites.

Table 3. Median (innerquartile range) concentrations of biomarkers in GCF/PICF at study sites

Biomarker	Evaluation Time	Control Median (q1-q3)	IOC Median (q1-q3)	EOC Median (q1-q3)
RANKL (pg/µl)	**T0**	113 (104-190)	124 (81.9-330)	193 (92.3-252)
	T3	102 (61.4-168)	105 (72.0-217)	106 (71.9-163)
	T6	108 (97.3-126)	98.1 (82.3-128)	71.9 (57.3-94.3)*
OPG (pg/µl)	**T0**	80.3 (53.5-171)	163 (124-208)	95.0 (60.9-147.6)
	T3	81.9 (47.8-117)	65.9 (22.8-135)	85.6 (51.5-100)
	T6	75.8(69.5-95.9)	74.9 (33.5-103)	53.7 (42.9-75.4)*
IL-17A (pg/µl)	**T0**	9.93 (6.76-34.0)	22.6 (8.63-59.3)	13.6 (8.49-19.1)
	T3	11.4 (7.53-14.7)	7.71 (6.32-25.7)	9.42 (7.60-11.5)
	T6	8.89 (8.28-13.3)	10.7 (7.13-12.9)	6.62 (5.68-8.80)*
IL-17E (pg/µl)	**T0**	19.9 (11.3-70.0)	54.7 (27.5-81.7)	22.5 (16.2-41.9)
	T3	24.1 (13.1-40.4)	11.3 (4.98-27.1)	25.8 (15.7-45.0)
	T6	17.0 (12.66-27.4)	14.5 (9.07-21.0)	15.1 (11.0-19.6)
IL-1β (pg/µl)	T0	4.97 (4.23-10.3)	5.47 (4.59-9.03)	5.16 (4.53-12.6)
	T3	4.87 (2.80-6.49)	5.23 (4.42-9.77)	5.20 (3.83-7.77)
	T6	4.78 (4.24-6.76)	4.65 (2.51-5.85)	3.74 (2.45-5.00)

T0: Baseline. T3: 3rd month. T6: 6th month. IOC: Intraoral cementation sites. EOC: Extraoral cementation sites. *Statistically significantly different from the control site.

Table 4. Median (innerquartile range) 16S gene copy number of putative periopathogens at study sites.

Putative pathogen	Evaluation time	Control Median (q1-q3)	IOC Median (q1-q3)	EOC Median (q1-q3)
	T0	1814 (633-5409)	986 (483-1715)	992 (373-2610)
A.a.	T3	6952 (1657-21571)	1010 (491-8153)	2203 (541-9248)
	T6	1623 (1016-3090)	618 (301-4299)	3840 (480-8847)
	T0	117822 (86164-152086)	78933(22483-158432)	98417(30406-218439)
F.nucleatum	T3	189604 (81068-418548)	43026(18481-126448)*	111925(40730-349994)†
	T6	338670(149893-1316507)	141810(66558-261812)	150072(68296-411439)
	T0	460 (244-787)	186 (82.5-323)	111 (0.00-234)*
P.gingivalis	T3	431 (200-1405)	529 (151-1384)	198 (55.5-1303)
	T6	150 (85-536)	455 (40-1049)	333 (115-1447)
	T0	25.3 (9.38-46.6)	14.5 (0.00-46.0)	13.0 (0.00-52.8)
P.intermedia	T3	42.5 (22.4-71.3)	72.0 (0.00-216)	34.0 (0.00-148)
	T6	44.0 (0.00-95.5)	39.5 (0.00-115)	35.0 (.000-177)
	T0	29.8 (17.3-63.1)	36.5 (0.00-277)	21.5 (0.00-88.3)
T.denticola	T3	14.5 (0.00-125)	0.00 (0.00-97.3)	0.00 (0.00-294)
	T6	46.0 (0.00-182)	207 (73.5-586)	40.5 (0.00-207)
	T0	33.5 (15.8-55.4)	35.0 (0.00-447)	26.5 (0.00-87.5)
T.forsythia	T3	18.8 (0.00-91.6)	12.5 (0.00-92.0)	0.00 (0.00-347)
	T6	56.0 (0.00-189)	191 (58.5-287)	53.0 (0.00-388)
	T0	29663 (3617-66735)	5588 (2653-96748)	4985 (1388-40436)
S.salivarius	T3	4328 (1105-37359)	5457 (409-75945)	1032 (175-6061)
	T6	27000 (4976-58335)	11044 (4909-39548)	5398 (482-7501)
	T0	146125(119479-1171681)	129179(718035-530179)	148047(70600-322569)
Total	T3	279618(164692-487707)	134948(43288-317958)	124429(66175-357859)
	T6	493917(227335-2037433)	296357(102390-413909)	203631(110961-421209)

T0: Baseline, T3: 3rd month, T6: 6th month, IOC: Intraoral cementation group, EOC: Extraoral cementation group. *Statistically different from the control sites. †Statistically different from the IOC sites.

Table 5: Median (innerquartile range) percentage of putative periopathogens at study sites.

Putative pathogen	Evaluation Time	Control Median (q1-q3)	IOC Median (q1-q3)	EOC Median (q1-q3)
A.a. (%)	T0	1.38 (0.68-9.07)	0.89 (0.10-2.75)	0.90 (0.12-3.06)
	T3	4.07 (1.4-7.59)	2.01 (0.23-10.3)	1.12 (0.18-12.2)
	T6	0.74 (0.17-2.39)	0.49 (0.14-3.14)	0.69 (0.39-5.55)
F.nucleatum (%)	T0	70.5 (49.8-88.4)	87.5 (0.11-95.2)	79.4 (57.6-96.0)
	T3	80.5 (71.4-92.2)	51.1 (18.4-81.4)*	92.3 (81.5-98.8)†
	T6	68.9 (60.1-85.3)	73.8 (54.8-94.1)	93.5 (79.6-96.8)
P.gingivalis (%)	T0	0.26 (0.16-0.64)	0.09 (0.01-0.48)	0.10 (0.00-0.23)*
	T3	0.53 (0.11-0.73)	0.52 (0.08-2.04)	0.23 (0.02-0.67)
	T6	0.19 (0.03-0.68)	0.21 (0.01-0.79)	0.13 (0.05-0.44)
P.intermedia (%)	T0	0.01 (0.00-0.02)	0.00 (0.00-0.04)	0.01 (0.00-0.05)
	T3	0.05 (0.01-0.11)	0.05 (0.00-0.31)	0.02 (0.00-0.08)
	T6	0.01 (0.00-0.03)	0.01 (0.00-0.09)	0.00 (0.00-0.03)
T.denticola (%)	T0	0.03 (0.00-0.05)	0.00 (0.00-0.13)	0.00 (0.00-0.04)
	T3	0.00 (0.00-0.10)	0.00 (0.00-0.06)	0.00 (0.00-0.14)
	T6	0.01 (0-0.17)	0.04 (0.02-0.51)	0.03 (0.00-0.12)
T.forsythia (%)	T0	0.02 (0-0.04)	0.00 (0.00-0.07)	0.00 (0.00-0.06)
	T3	0.00 (0.00-0.16)	0.00 (0.00-0.07)	0.00 (0.00-0.16)
	T6	0.01(0.00-0.14)	0.07 (0.01-0.20)	0.03 (0.00-0.21)
S.salivarius (%)	T0	17.09 (5.5-28.3)	8.93 (3.40-93.1)	11.0 (1.15-29.2)
	T3	6.37 (1.62-17.0)	7.08 (0.54-64.0)	0.52 (0.21-1.62)
	T6	29.5 (5.62-38.6)	5.82 (1.41-37.2)	1.43 (0.23-13.4)

T0: Baseline, T3: 3rd month, T6: 6th month, IOC: Intraoral cementation sites, EOC:Extraoral cementation sites. *Statistically different from the control sites. †Statistically different from the IOC sites.