Effects of Four Different Crown Materials on the Peri-Implant Clinical Parameters and Composition of Peri-Implant Crevicular Fluid

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This study aimed to identify the preferred crown material by measuring the peri-implant clinical parameters and the concentrations of receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG), and calcium in peri-implant crevicular fluid (PICF) with 4 different crown materials. A total of 196 patients with a single missing posterior tooth received crown restoration with cobalt-chromium (Co-Cr) porcelain-fused-to-metal (PFM; n = 50), aurum platinum (Au-Pt) PFM (n = 48), titanium (Ti) PFM (n = 52), or zirconia (Zi) all-ceramic crown (n = 46). Fifty-one natural counterpart teeth served as controls. Before and 12 months after restoration, the PICF was collected, and the concentrations of RANKL, OPG, and calcium were quantified. The peri-implant clinical parameters (plaque index, bleeding on probing, and probing depth [PD]) and gingival crevicular fluid (GCF) volumes were assessed. Twelve months after restoration, the PD and GCF volumes for the 4 experimental groups were significantly greater than those for the control group and before restoration. The Co-Cr group showed the greatest PD, GCF volume, RANKL/OPG, RANKL, and calcium ion concentration, followed by the Au-Pt group. The Ti group had the highest OPG concentration, followed by the Zi group. The RANKL and calcium ion concentrations of the Ti and Zi groups were the smallest. The Ti group had the smallest RANKL/OPG ratio, followed by the Zi group. Different crown materials differentially affected the PD, volume, RANKL/OPG ratio, OPG, RANKL, and calcium concentration. Among the 4 tested crown materials, Zi and Ti are preferred. However, some limitations of the present study should be considered.

Key Words: osteoprotegerin, receptor activator of nuclear factor-κB ligand, peri-implant crevicular fluid, gingival crevicular fluid, calcium

INTRODUCTION

Dental implantation has become the preferred method for restoring chewing function, improving appearance, and supporting the mental health of patients with missing teeth.1–3 Upper crown restoration, as an important part of implant restoration, often requires patients and doctors to choose crown restoration materials. It is well known that different crown materials vary in appearance, metal-porcelain binding force, or hardness, but there have been no related reports on whether the long-term implant health is related to the crown material. Therefore, the present study was conducted to provide some reference values for clinical work.

Subsequent morbidity and failure of implants pose great economic and psychological burdens to both patients and clinicians.4 The long-term success of an implant is inextricably linked to the health of the hard and soft tissues in proximity to the implant. Several studies have demonstrated that changes in the composition of peri-implant crevicular fluid (PICF) are reflective, to some extent, of the health status of hard and soft tissues in the areas of implants.5–7 In recent years, increasing esthetic requirements have led to the more widespread application of bone level implants,8 for which the crown edge is placed 1–2 mm below the gum line, putting the crown material in close contact with the PICF. A previous study showed that differences in gingival crevicular fluid (GCF) were observed when comparing fixed crown restorations with different levels of crown margin placement.9 The components of GCF of natural teeth are derived from patients’ serum, local tissues, and structural cells of the periodontium in addition to oral bacteria.10,11 Although previous studies have reported similar mechanisms and rates for the production of PICF and GCF for natural teeth, differences in the structure and vascularity in the peri-implant area and gingival mucosa may lead to differences in the amounts of these fluids produced.12,13 Because both PICF and the GCF of natural teeth essentially represent wound fluids, changes in their composition may be used to evaluate healing progress after the repair of a local
tissue, including tooth repair via dental implantation. Although the volumes of PICF and GCF produced in response to varying grades of inflammation show similar trends, changes in the volume of PICF show the strongest correlation with clinical conditions. Therefore, investigations into the changes in PICF specifically are warranted beyond characterization of GCF associated with natural teeth. However, little research has studied the GCF surrounding the implant.

Porcelain-fused-to-metal (PFM) or zirconia all-ceramic crowns are ideal crown restorations for the upper tooth structure of implants and thus have been widely used in implant dentistry. Now, in clinical work, cobalt-chromium (Co-Cr) PFM, aurum-platinum (Au-Pt) PFM, titanium (Ti) PFM, and zirconia (Zi) all-ceramic crowns are the 4 most commonly used crown restoration materials. Although Co-Cr dental alloys are routinely used in dental applications, the biocompatibility of Co-Cr alloys is controversial because it induces cytotoxicity and inflammatory responses in human gingival fibroblasts and osteoblasts.

Titanium alloys, gold alloy, and Zi are believed to have excellent biocompatibility and have become the most popular metallic biomaterials in dental applications, with no adverse effects on the in vitro capacity of preosteoblasts in marrow to differentiate and form mineralized bone nodules, despite the differences between them.

We hypothesized that different crown materials will induce different changes in the PICF that may affect the health of the hard and soft tissues in the implant area.

Various proteins have been demonstrated to play critical roles in bone remodeling and to be differentially expressed under conditions of oral disease. For example, receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) are known to directly regulate osteoclasts and osteoblasts and thus are important factors in alveolar bone resorption. In periodontitis, infected periodontal tissue expresses significantly elevated levels of RANKL protein and significantly reduced levels of OPG. These findings indicate that RANKL and OPG are important factors in alveolar bone resorption. Moreover, soluble (s)RANKL, RANK, and OPG levels in PICF were shown to be altered with bone resorption and tissue damage around dental implants, differently from the changes associated with periodontitis. Calcium is an important element for human health, with critical functions in the regulation of cell growth, differentiation, apoptosis, secretory functions, and so forth. Changes in calcium concentration directly affect the bone-remodeling process. In addition, the growth and adhesion of periodontal ligament cells are affected by changes in calcium ion concentration, with the greatest impact on cell growth. The calcium content of GCF in periodontitis patients is higher than that in patients with gingivitis. Therefore, in this study, the RANKL, OPG, and calcium ion concentrations in PICF were measured and used to evaluate the peri-implant soft tissue and bone tissue. Although it has been reported that the Co-Cr alloy showed a significant influence on GCF indexes when compared with the preseration values and the Au-Pt alloy group, the interleukin-8 concentration in GCF did not differ significantly among the Co-Cr and gold alloy groups at different time periods. However, there are few reports on the effects of pure Ti and zirconium dioxide on the GCF around the implant.

Based on the absence of an investigation into the effects of crown restoration materials on PICF, and thus, to learn whether long-term implant health is related to the crown material, the aim of our study was to compare 4 types of crown materials by investigating peri-implant clinical parameters and the composition of the peri-implant crevicular fluid (RANKL, OPG, and calcium concentrations) to predict the best crown material option for long-term healthy implants.

**MATERIALS AND METHODS**

**Study design and patient population**

The present study was a prospective, randomized, single-blind, preliminary clinical trial carried out between December 2011 and November 2015 on patients requiring single implant (ITI)-supported posterior crown restorations. Crowns had been implanted 12 weeks previously at the implanting center of the Jinan Military General Hospital of the People’s Liberation Army (PLA), China. Random group assignment was performed by a professional statistician using predefined randomization tables. The patients were selected by convenience sampling. Ethical approval of the study was obtained from the medical ethics committee of the Jinan Military General Hospital of the PLA, and before participation in this study, patients provided written informed consent.

In total, 196 patients (99 men and 97 women) with ages ranging from 19–55 years (mean age, 32.31 ± 12.56 years) were included in this study. These patients were divided into 4 groups according to the type of crown material used: the Co-Cr PFM group (50 cases), the Au-Pt PFM group (48 cases), the Ti PFM group (52 cases), and the Zi all-ceramic crown group (46 cases). From the 196 implantation cases, 51 implant natural contralateral counterpart teeth were randomly selected as controls. The characteristics of the 5 groups did not differ significantly (P > .05). At time points before crown restoration and after 12 months, the PICF or GCF was collected and clinical indicators were evaluated by a very experienced implant restoration doctor. The implantation surgery was performed by a specialized doctor. The elution of GCF from filter paper strips and the measurement of RANKL, OPG, and calcium concentrations were completed by the same experienced technicians. The experimental operators in addition to the experimental design staff were not aware of the grouping.

**Inclusion criteria and exclusion criteria**

The inclusion criteria were as follows: (1) age greater than 18 years, (2) well-healed bone and soft tissues around implants, (3) normal occlusion, and (4) presence of natural teeth near the implant and a natural contralateral counterpart tooth, (5) good oral hygiene, and (6) implantation with one of the specific implant systems investigated in this study, and (7) ability to follow the doctor’s instructions for treatment and referral. The following exclusion criteria were considered with respect to preimplantation conditions: age less than 18 years, ≤10 natural teeth, periodontal disease, smoking, pregnant or lactating, use of antibiotics or nonsteroidal anti-inflammatory drugs, poor
healing of bone and soft tissues around implants, abnormal occlusion, history of drug allergy or atopy, and systemic metabolic disease. Also, individuals were excluded if they had a previous history of implant therapy or their medical records revealed any local or systemic contraindications to dental implant therapy. Patients were included only if they completed implant therapy procedures successfully. If any sign of failure occurred during any stage of crown restoration treatment, including crown detachment or chipping of the porcelain, the patient was excluded from the study.

**Instruments and materials**

ITI implants, upper solid abutments, the internal base of the restoration crown, and other implant-related tools were all supplied by the ITI implantation system in Switzerland. The RANKL enzyme-linked immunosorbent assay (ELISA) kit (BioVendor, Modrice, Czech Republic) and OPG ELISA kit (Bender Medsystems, Vienna, Austria) were purchased and well preserved. The versatile fluorescent light analyzer (FLUOstar Omega, BMG Labtech, Offenberg, Germany) and the atomic absorption spectrometer (ICE3500, Thermo Fisher Scientific, Waltham, Mass) were used according to standard procedures, and the test results are reliable. The instruments used in the experiment are very mature and reliable and have been calibrated specifically for patients by professional technicians.

The Co-Cr alloy (Bego Co, Bremen, Germany) was composed of 60.2% Co, 25% Cr, 4.8% molybdenum, 6.2% wolfram, 2.9% gallium, and 0.9% other materials. The gold alloy (Grikin Co, Beijing, China) was composed of 86.2% Au, 11.5% Pt, and 2.3% zinc, and 2.3% manganese. The titanium content of the Ti PFM was 99.99% (Grikin Co). The Zr (Cercon Co, Germany) was composed of 92% Zr, <5% Yttria, and 2% hanium oxide. Porcelain powder and binder (Shofu Inc, Kyoto, Japan), impression materials (Heraeus, Hanau, Germany), and plaster powder (Shanghai Dental Materials Factory, China) were used.

**Implant installation**

Before the implant installation, all patients received appropriate examinations, treatment, and oral hygiene instruction. The soft-tissue level ITI implants were inserted according to the recommended procedures for the ITI implant system. No complications occurred during surgery or the postsurgical healing period in any of the patients, and all of the inserted implants had primary stability. Postsurgical panoramic radiographs were performed to evaluate implant placement and showed that the implant shoulders were at the crestal bone level. In addition, no guided bone regeneration procedure was performed in any of the cases.

Patients were instructed not to wear any type of prosthesis that could come in contact with the surgical area following the surgical procedure. Patients were seen at 1 and 2 weeks after the 1-stage procedure to monitor healing and postsurgical complications. Sutures were removed after 7–10 days.

**Restorative procedure and the detection of clinical indicators**

After 3 months of healing, the patients were recalled for the restorative treatment. A panoramic radiograph was taken to examine whether radiolucency existed around the implant body. The corresponding solid abutments were attached to the implants with a force of 35 N. The casting bases of crowns were prepared by the manufacturer. Crowns were bonded by glass ionomer adhesive after being highly polished.

All implants and natural teeth were evaluated using the plaque index (PI) and bleeding on probing. The probing depth (PD) around the implant was determined according to 4 sites (mesial, distal, facial, and palatal) using a conventional periodontal probe (Hu-Fridy, Chicago, Ill) at baseline and 12 months after crown restoration. All of these indicators were assessed by the same experienced dentist.

**Sample collection and GFC volume determination for natural teeth and PICF for implants**

Patients were asked to rinse their mouths. After gently air drying each implant site and careful isolation, a 2- × 8-mm strip of Whatman 3-mm chromatography paper (Whatman Industries, Kent, UK), which had been preweighed in an Eppendorf (EP) tube, was gently placed into the gingival buccal and lingual sulcus until slight resistance was encountered and kept there for 30 seconds before removal. The removed filter strip was returned to the EP tube, which was weighed immediately. The change in the weight of the filter paper strip before and after sample collection represented the volume of PICF or GCF. If stained with blood or saliva, the filter paper strip was discarded, and sampling was repeated 24 hours later. After PICF and GCF collection, filter paper strips were returned to EP tubes and stored at −70°C.

**PICF and GCF elution from filter paper strips**

First, 350 μL of 0.9% NaCl solution was added to each EP tube containing a PICF or GCF sample. The tubes were placed on a shaker thermostat and shocked for 60 minutes, followed by centrifugation at 10 000 r/min and 4°C for 15 minutes. Finally, 100-μL aliquots of supernatant was transferred to EP tubes for RANKL, OPG, and calcium ion detection.

**Measurement of RANKL and OPG concentrations**

RANKL and OPG concentrations were determined using commercial ELISAs according to the manufacturers’ instructions (total sRANKL ELISA kit, BioVendor, and Human OPG ELISA kit, Bender Medsystems). The minimum detection limit was 0.2 pmol/L for sRANKL and 2.5 pg/mL for OPG. Absorbance values were measured at 450 nm using a versatile fluorescent light analyzer (FLUOstar Omega, BMG Labtech).

**Measurement of calcium concentration**

The calcium content in PICF and GCF was detected via atomic absorption. Ten-microliter samples of PICF or GCF were added to 990 μL of 1% nitric acid solution in deionized water, and then the calcium level was assessed by trace element analysis using an atomic absorption spectrometer (ICE3500, Thermo Fisher Scientific).

**Statistical analysis**

Statistical analysis of the experimental data was performed using SPSS 16.0 software (SPSS, Inc, Chicago, Ill). All data are
presented as mean ± SD values. The normality of the distribution for each variable was measured using the Smirnov-Kolmogorov test. Comparisons between all groups were performed using the Kruskal-Wallis test. The level of statistical significance was set at \( P < 0.05 \).

**RESULTS**

The aim of this study was to predict the best crown material option for long-term implant health. The present study first determined the effects of different materials on clinical parameters (PI, bleeding index [BI], PD, and volume) and then the levels of cytokines (OPG, RANKL, and calcium ion).

In this study, the PI score for each tooth was less than 2, which means that a small amount of plaque or no plaque could be seen in the gingival margin or tooth surface; the bleeding index score for each tooth was less than 1, which meant that no serious or active gingivitis was found. The results in Figure 1a and b showed that no significant differences were observed in average values for the PI or the BI among the groups whether before or after crown restoration \( (P > 0.05) \). For each specific crown restoration, there also were no significant differences in PI or BI between before and 12 months after crown restoration \( (P > 0.05) \).

As shown in Figure 2, the average PDs for the experimental groups were significantly less than that for the control group before crown restoration. However, the average PDs in the experimental groups at 12 months after crown restoration were significantly greater than that of the control group and those before crown restoration \( (P < 0.05) \). At 12 months after crown restoration, as seen in Figure 2, the PD of the Co-Cr PFM group was significantly greater than those of the other 3 experimental groups \( (P < 0.05) \), and there were no statistically significant differences in the PD for the Au-Pt PFM, Ti PFM, and Zi all-ceramic crown groups \( (P > 0.05) \).

Figure 3 shows the results for the volume of GCF, which is influenced by many factors, such as inflammation, mechanical stimulation, chemical stimulation, and so on. The results show that before crown restoration, the PICF volumes in the experimental groups were significantly less than GCF volume in the control group \( (P < 0.05) \) but did not differ significantly among the experimental groups \( (P > 0.05) \). After crown restoration, the PICF volumes in the experimental groups were significantly greater than the GCF volume in the control group, and the volume of PICF in the Co-Cr PFM group was greater than that in the Au-Pt PFM group, which was greater than those in the Ti PFM and Zi all-ceramic crown groups \( (P > 0.05) \). The PICF volume for each of the 4 crown restoration materials after crown restoration was significantly larger than that before crown restoration \( (P < 0.05) \).

The OPG levels are shown in Figure 4. After crown restoration, the OPG concentrations in the Au-Pt PFM, Ti PFM, and Zi all-ceramic crown groups were increased, with the greatest increase observed for the Ti PFM group and the smallest increase observed for the Au-Pt PFM group. The OPG...
increase in the Zi group was between those of the Au-Pt PFM and Ti PFM group ($P < .05$). The OPG concentration did not change significantly between the experimental time points in the Co-Cr PFM group and the control group ($P > .05$).

Figures 5 and 6 show that the RANKL expression and RANKL/OPG ratio in each experimental group before crown restoration were greater than those in the control group ($P < .05$), but there were no significant differences among the 4 experimental groups ($P > .05$). At 12 months after crown restoration, in the Co-Cr PFM and Au-Pt PFM groups, the RANKL/OPG ratio and RANKL concentration were increased ($P < .05$), and those in the Co-Cr PFM group were higher than those in the Au-Pt PFM group ($P < .05$). However, in the Zi all-ceramic crown group and Ti group, the RANKL/OPG ratio and RANKL expression were not the same. The RANKL in the Zi all-ceramic crown group and Ti group after crown restoration did not differ significantly from those measured before crown restoration ($P > .05$) or between each other ($P > .05$). However, the RANKL/OPG ratio in the Zi all-ceramic crown group was larger than that in the Ti group or control group ($P < .05$). The surprising result is that the RANKL/OPG ratio in the Ti group after crown restoration was less than before crown restoration ($P < .05$), and there was no obvious difference between the Ti group and the control group ($P > .05$).

The measurement results for calcium ion concentration as an important component of bone are shown in Figure 7. There were no significant differences between the 5 groups before crown restoration. After crown restoration, there were no significant differences between the Zi all-ceramic crown group, Ti group, and control group, and there was no significant change from before crown restoration. The calcium ion concentrations in the Co-Cr PFM and Au-Pt PFM groups after crown restoration were significantly larger than those in the other 3 groups and were greater than those before crown restoration. More importantly, the Co-Cr PFM group showed the greatest increase in calcium concentrations, followed by the Au-Pt PFM group.

**Discussion**

Because the chemical and morphological characteristics of implant surfaces available on the market are different,\textsuperscript{31} which may influence the PICF composition, the implants included in the study were standardized as ITI and from the same implant company. The patient inclusion criteria and exclusion criteria were strictly enforced. The experimenter’s experience and use of the double-blind method ensured the reliability of the data. At present, dental implants have become the preferred method for replacing missing teeth. The health of soft and hard tissue surrounding implants plays a role in the long-term success of implants. In addition to the acquisition of clinical data such as PI, BI, PD, and volume, the composition of PICF...
such as OPG, RANKL, and calcium concentration is also very important, as it reflects changes in the soft and hard tissue around the implants. Dental alloys that are placed in the mouth release metal ions and cause adverse reactions within the oral tissue. Gold, palladium, and Ti release less ions, but Co-Cr alloy releases more ions. However, in this particular environment surrounding the implant, which is also a type of metal, the material used for the crown restoration, in conjunction with the implant itself, can cause microenvironmental changes around the implant and may affect the long-term health of the implant. No other clinically relevant studies were identified.

The PI and BI were used to evaluate the oral hygiene condition and gingivitis activity of the patients in this study. If there had been any patients with poor oral hygiene or gingivitis, they would have been unsuitable to participate in this study and excluded. For the 4 specific crown restoration materials, there were also no significant differences in PI or BI before and 12 months after crown restoration or among them, which may indicate that neither the crown restoration material itself nor the crown shape will affect the cleanliness around the implants or cause active gingivitis.

The results obtained in the present study demonstrate that before crown restoration, the PICF volume differed significantly with GCF volume but did not differ significantly among groups with different crown materials. This observation may be supported by the results of PD in the study over the same period. Although it was previously reported that there were no significant differences in the GCF volume between teeth with implants and neighboring natural teeth, another previous study also reported that the PICF volume was higher than the GCF volume at healthy sites. However, the gingival sulcus fluid volume or PD for the implant and natural teeth taken before crown restoration were not evaluated. In this study, before crown restoration, the PICF volumes in the crown restoration groups were lower than that in the control group. This result may be related to the shallow gingival groove of implants before crown restoration. Crown restoration led to remodeling of soft tissue around the implant with deepening of the gingival sulcus and an increase in the PICF volume. After crown restoration, the Co-Cr PFM group showed the smallest changes in PD. These differences in the PICF volume and PD may reflect the better biocompatibility of the Ti PFM crown restoration, the Co-Cr PFM group showed the greatest bone tissue metabolism around the implants was stronger than around the natural tooth. After crown restoration, each experimental group showed some differences in RANKL, OPG, RANKL/OPG, or calcium ions, indicating that the metabolic activity in the tissue around the implant was still different from that around natural teeth, and different material crown restorations had different effects on the tissues around implants. Perhaps in the presence of the implant, the remodeling activities will always occur in the surrounding tissues. The Co-Cr PFM group had the largest increase in RANKL, RANKL/OPG, and calcium ions, and the Au-Pt PFM group had the second largest increases compared with the other groups. The OPG of the Co-Cr PFM group showed no change compared with before or that in the control group and was the smallest compared with the other 3 experimental groups. The OPG in the Au-Pt PFM group increased compared with that in the control group but was less than that in the Ti and Zi all-ceramic crown groups. For RANKL and calcium ions, the Ti group and Zi group had the smallest levels among the 4 experimental groups, but for OPG, the Ti group had the largest concentration, and the Zi group had the second largest. For the RANKL/OPG, the Ti group had the smallest, and a key point is that the Ti group had a lower RANKL/OPG ratio than the control group; also, the RANKL/OPG ratio for the Zi group was greater than that for the control group and Ti group but smaller than those for Co-Cr PFM group and Au-Pt PFM group. Higher RANKL levels correlate with bone loss around the implant and the retention time of the implant. Based on the changes in the markers assessed in this study, different crown materials had different effects on the concentrations of these markers in PICF. Overall, the results of this study indicate that Zi all-ceramic crowns and Ti PFM implants showed greater biocompatibility than the other materials, and thus, Co-Cr PFM and Au-Pt PFM are not top choices for crown implants. One study found that epithelial cells and fibroblasts in peri-implant tissues have a stronger negative response to Co-Cr than Ti, which can only be explained by the material itself. When exposing human gingival fibroblasts to permanent prosthetic materials, Ti proved to be nontoxic, whereas Au alloy and feldspathic ceramic short-term were found to be cytotoxic and a Co-Cr alloy had the highest cytotoxic effect. This finding in this study may also be related to the metal material itself, in that the Co-Cr PFM and Au-Pt PFM materials exhibited relatively
poor biocompatibility, especially the Co-Cr alloy, and further in-depth study of these materials is still needed. At the same time, this study suggests that PICF detection may be used as a valuable clinical test for the assessment of the health of an implant.

Some limitations of the present study should be considered in the interpretation of the findings, such as the lack of long-term follow-up assessments. Also, the potential effects of patient demographic factors such as gender, education level, health condition, and income were not analyzed in this study. The levels of additional relevant cytokines also should be measured in future studies, as the small volume of gingival fluid available in this study limited the number of assays that could be applied. To reduce experimental error and increase the credibility of the results, in this study, the same implant system was applied. To reduce experimental error and increase the overall effect would likely be the same, and this point will be addressed in future experiments.

CONCLUSIONS

Different crown materials differentially affected the PD of surrounding periodontal tissue, the PICF volume, and the concentrations of RANKL, OPG, and calcium in PICF. According to the results of this study, Zirconia all-ceramic crowns and Ti PFM implants showed greater biocompatibility than the other materials, and thus, Co-Cr PFM and Au-Pt PFM are not top choices for crown implants.

ABBREVIATIONS

Au-Pt: aurum platinum
Bi: bleeding index
Co-Cr: cobalt-chromium
ELISA: enzyme-linked immunosorbent assay
EP: Eppendorf
GCF: gingival crevicular fluid
OPG: osteoprotegerin
PD: probing depth
PFM: porcelain-fused-to-metal
Pl: plaque index
PICF: peri-implant crevicular fluid
PLA: People’s Liberation Army
RANKL: receptor activator of nuclear factor-κB ligand
Ti: titanium
Zi: zirconia

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NOTE

None of the authors have any conflicts of interest to declare, and all authors approved submission of this article.

REFERENCES


