The Level of Interleukin-1 Beta in Peri-implant Crevicular Fluid around Titanium, Zirconium Oxide and Gold Alloy Implant Abutments: a Pilot Study

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Abstract

Objectives: Stable peri-implant soft tissue around implant abutments are the essential factor for long-term success and survival of dental implants. The aim of this study was to describe the level of IL-1ß around 3 types of abutment materials : gold alloy, titanium and zirconium oxide.

Methodology: The soft tissue characteristics were evaluated using plaque assessment score and mucosal conditions score. 12 patients were enrolled in this study. Clinical parameters and peri-implant crevicular fluid (PICF) were collected 4 weeks later. Cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). Nonparametic statistics were used to describe the cytokine levels.

Results and Discussion: At 4-week of healing period, gold alloy abutments induced the highest level of IL-1ß cytokine compared with zirconium oxide and titanium abutments. There were statistical differences in cytokine levels between gold alloy and titanium abutment groups. (p=0.005) The PICF findings supported the observed clinical appearances of abutment materials.

Conclusion: Gold alloy abutment exhibited increased level of IL-1ß and gingival plaque index score compared with zirconium oxide and titanium in early healing period of dental implant. Oral hygiene instructions should be given and maintained in patients when using gold alloy abutments.

Keywords**:** *Abutment materials, Peri-implant crevicular fluid, Proinflammatory cytokines, Enzyme-linked immunosorbent assay (ELISA), Clinical study*

1. Introduction

The osseointegrated implants was first described in 1981 and become the effective treatment of choice in oral rehabilitation. Due to high survival and success rates, the dental implant has been used in order to restore functions, esthetics and health in partially or fully-edentulous patients (Adell, 1981; Albrektsson, 1998; Moraschini, Poubel, Ferreira, & Edos, 2015). The success and sustainability of dental implant depend upon both mechanical and biological effects toward hard and soft tissue (Lekholm, 1999; Mombelli & Lang, 2000; Rompen, 2006). Therefore, the dental implant has to be placed in the correct position in bone and surrounded by suitable mucosal tissue. The anatomy of soft tissue around dental implant is different from natural tooth. A biological seal in the transmucosal zone of dental abutment comes from the circular and parallel oriented connective tissue fibers. It is an important structure to protect the bacterial invasion (Abrahamsson & Cardaropoli, 2007; Abrahamsson, 1998; Berglundh, Abrahamsson, Welander, Lang, & Lindhe, 2007; Kawahara, 1998; Moon, 1999).

Implant abutment is considered as the connection between intraosseous and prosthetic part. Several factors including surface roughness, surface chemistry, surface free energy, designs and connections which influence the transmucosal segment (Han, Tsoi, Rodrigues, Leprince, & Palin, 2016). Commercially pure titanium demonstrated excellent survival rates and biocompatibility for implant restoration (Rompen, 2006). However, the disadvantage of the titanium abutment is dark grey color that may shine through the peri-implant soft tissue. It is an esthetic concern. Another type of metal abutment material has been widely used since 1988 is a customized gold alloy known as UCLA abutment (Lewis, 1988). Nevertheless, the animal studies have been shown that no proper mucosal seal around gold alloy abutment (Abrahamsson & Cardaropoli, 2007; Berglundh et al., 2007). Over the past few years, the use of

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zirconium oxide abutment has increased to solve this problem especially in anterior region. The milling machine nowadays can fabricate precise components using CAD-CAM technology (Bidra & Rungruanganunt, 2013; Lops, Bressan, Chiapasco, Rossi, & Romeo, 2013; Zembic, Sailer, Jung, & Hammerle, 2009). Consequently, the anatomical shaped abutment can be created with suitable emergence profile and supported with peri-implant soft tissue. Several studies have been shown the reaction of soft and hard tissue around zirconium abutment similar to titanium abutment (Linkevicius, 2008).

The gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) are comprised of host-derived immune elements, tissue degradation components and bacterial byproducts (Bostanci & GN., 2018). The numerous biomarkers in GCF including proinflammatory cytokines [e.g. TNFα, IFNγ, IL-1, IL-6, IL-12, IL-17 and RANKL], anti-inflammatory cytokines [e.g. IL-4, IL-10 and IL-1ra] and chemokines [e.g. IL-8] have been suggested to be crucial mediators of inflammation(Stadler et al., 2016). These mediators can reflect the activity of cells and the level of tissues destruction. Furthermore, it is an uncomplicated and noninvasive technique in collecting GCF (Griffiths & S., 2003). Several studies used the immunological analysis to determine the state of health and disease (Champagne, 2003; Zani et al., 2016). Hence, the higher level of inflammatory cytokines in PICF, the higher loss of hard and soft tissue. One of the most widespread proinflammatory cytokines which has been studied in peri-implantitis and peri-implant mucositis is IL-1ß (Ataoglu, 2002; Duarte et al., 2016; Kao, Curtis, Richards, & Preble, 1995; Masashi et al., 2002). This cytokine is produced mainly by macrophage. The main functions of IL-1ß are to increase the inflammatory cell migration and osteoclastogenensis (Stadler et al., 2016). IL-1ß and TNFα are also related to prepare blood vessels for the diapedesis of PMNs, monocyte and T cells within connective tissues and through the sulcus. The concentration of IL-1ß has been found to correlate to disease activity (Nowzari, Phamduong, Botero, Villacres, & Rich, 2012). 30 days after the operation, the implant surface areas covered by newly formed bone which have the process of bone apposition and deposition. Bone to implant contact (BIC) has reached nearly 30% of the implant surface under microscopic level. IL-1ß serves as the biomarkers of peri-implant status releasing during late healing (Bielemann, Marcello-Machado, Cury, & Faot, 2018).

It is a key to control inflammation around dental implant in order to preserve the crestal bone as well as maintain the peri-implant tissue and adjacent structure. The abutment materials which can provoke any inflammation reaction of mucosal around implant should be avoided. At present, there are few prospective human studies in this matter. Recent histological sections of mucosal tissue around dental abutment showed differences in the amount of inflammatory cells and the percentage of attachment in various abutment types (Sampatanukul, Serichetaphongse, & Pimkhaokham, 2017). Nevertheless, there are a few studies in biological mediators released by cells to describe the response of implant abutment materials.

2. Objectives

The objective of the study was to evaluate the level of IL-1ß of 3 implant abutment materials, which were titanium, zirconium oxide and gold alloy at 4 weeks after implant placement using an immunological method and clinical examinations. The clinical parameters included plaque index and peri-implant soft tissue condition.

3. Materials and Methods

The clinical trial was designed as a randomized controlled trial, single blinded assessment and was conducted in one center to describe the level of proinflammatory cytokine (IL-1ß) around 3 different abutment materials which were Gold alloy, Titanium and Zirconium oxide. The protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. The study approval number was HRE-DCU 2018-036. The patients with posterior edentulism seeking the implant treatment were enrolled in this research based on inclusion and exclusion criteria. Before joining the study, all participants were explained about the methods of trial and signed the consent form. Following the

inclusion criteria, patients with missing first or second molar teeth, adequate quantity and quality of bone for 4.8 mm diameter implants, inter-arch space > 5 mm, sufficient keratinized mucosa ≥ 2mm and gingival thickness 3 mm and a minimum age of 21 years were recruited. Patients who were smoker, disabled person, pregnant and lactating women, had immunosuppressant or antibiotic within 3 months and had systemic diseases were excluded. All participants had to be done a dental cone beam computed tomography (CT scan) to diagnose the placement of implants.

3.1 Surgical procedures

Osteotomy sites were prepared under local anesthesia for OsseoSpeed™ EV bone level implants (Astra Tech, Dentsply, Mölndal, Sweden) diameter 4.8 mm according to the manufacturer's manual. The surgical protocol was performed by postgraduate students who studied in the Esthetic Restorative and Implant Dentistry program, Chulalongkorn University under the supervision of an experienced surgeon. The flap opening was designed depending on keratinized tissue width. Then, the implant fixture was positioned in the proper 3 dimensions and fully submerged in the bone. The randomization of abutment types was created by picking up the envelope. Each envelope was opened and abutment (Gold alloy, Titanium, Zirconium dioxide) was immediately installed instead of a healing abutment. The flaps were sutured. The abutment was reduced to avoid the contact with the opposing teeth in all directions. Then, the hole was covered with teflon tape and resin composite. All subjects were prescribed antibiotic for 7 days and advised to rinse 0.2% chlorhexidine mouthwash for 2 weeks. The oral hygiene instructions were informed.

3.2 Immunology procedures

Peri-implant crevicular fluid collection (PICF) and Enzyme-linked immunosorbent assay (ELISA)
All participants were recalled at 4 weeks post-operation and the peri-implant crevicular fluid was collected. The implant site was isolated and dried with cotton roll. Size M paper points were introduced into sulcus for 30 seconds. The contaminated blood and/or saliva strips were discarded. All strips were stored in 1.5 ml plastic tube containing 100 µl of phosphate buffer saline (PBS), supplemented with protease inhibitor cocktail. The samples were frozen at -80 ºc for later analysis.

Proinflammatory cytokine (IL-1ß) was assessed using a single ELISA array kits (R&D system Inc., USA). This kit contained human capture antibody, human detection antibody, human standard streptavidin-HRP, reagent diluent concentrates, substrate solution, stop solution, wash buffer and 96 well microplates. The assessment was performed in regard to the manufacturer's instructions. The well plates determined the optical density using spectrophotometry 450 nm. Concentrations in each sample were calculated by generating of a standard curve. Finally, the total amount of IL-1ß was defined as pictograms per milliliter.

3.3 Data collection and Statistical analysis

After abutment connection, a plaque control program was initiated and maintained for 4 weeks. At the visit of examination, the oral hygiene levels were evaluated according to a 3-point scale which was proposed by Lindquist and associates (Lindquist, 1988) (Table 1). Additionally, the mucosal conditions around dental implant which was a simplified GI index proposed by Apse were assessed (Apse, 1991) (Table 2). Two outcomes, the plaque score and the mucosal condition, were appraised before PICF collection and performed. The sample size in this study was calculated as a pilot study. With small sample size of total of 12 participants, nonparametric test was selected for analysis of this study. Kruskal-Wallis statistical test was used to determine the differences of the median between three groups. Dunn's test is taken into account to perform pairwise comparison across three groups.

Table 1 Plaque assessment

Score	Descriptive
0	No visible plaque
1	Local plaque accumulation
2	General plaque accumulation greater than 25%

Table 2 Mucosal conditions around dental implant

Score	Description
0	Normal mucosa
1	Minimal inflammation with color change and minor edema
2	Moderate inflammation with redness, edema and glazing
3	Severe inflammation with redness, edema, ulceration and spontaneous bleeding without probing

4. Results and Discussion

Table 3 described the demographic data of the study sample. A total of 12 participants, 7 males (58.33%) and 5 females (41.67) were examined. 12 Asta Tech OsseoSpeed™ EV implants with diameter of 4.8 mm were placed in the first and second molar regions. The number of abutments in each group was divided equally by randomization technique. The mean age of the subjects who received gold alloy, titanium and zirconium oxide abutments were 48.5, 59 and 64.25, respectively.

Clinical parameters and cytokine expression in peri-implant crevicular fluid (PICF) were investigated at healing period of 4 weeks. Plaque index and mucosal score were depicted in Table 4.

4.1 Evaluation of oral hygiene

According to plaque assessment index, score of 3 was found only in gold alloy abutment group (25%) whereas 50% of gold alloy abutment group had no visible plaque (score 0) and 25% demonstrated some local plaque accumulation (score 1). 75% of titanium abutment group showed plaque assessment score of 0 while 25% depicted plaque accumulation score of 1. On the contrary, zirconium oxide group showed 75% of plaque assessment score of 1 and 25% has score of 0.

Regarding the mucosal conditions around dental implant, none of the groups showed moderate to severe inflammation of mucosal tissue at 4 weeks healing period. However, gold alloy abutment group is the only group that received a mucosal tissue condition score of 1 (50%) which the peri-implant tissue presented inflammation with slightly red and edema. The Titanium and Zirconium oxide groups demonstrated the same mucosal tissue condition score results. Nevertheless, the zirconium oxide group had higher percentage of plaque accumulation than the titanium group.

4.2 Peri-implant crevicular fluid (PICF) analysis

The present study compared the level of pro-inflammatory cytokine (IL-1ß) among different types of abutment materials. An overall trend for higher values was observed in the Gold alloy group. The mean concentrations of IL-1ß in the Gold alloy, Zirconium oxide and Titanium were 133.87, 70.47 and 20.48 pg/ml respectively. There was statistical significance of the IL-1ß levels among groups of abutment material based on the result of the Kruskal-Wallis with Dunn test (p=0.007277) (Figure 1).

Table 3 Demographic data of study sample

Parameter	Subjects
Number	12
Age, mean ±SD	57.25 ± 12.04
Gender	Male 7, Female 5
Edentulous region	Lower left 4, Lower right 2, Upper left 4, Upper right 2
Abutment type	Gold alloy 4 Titanium 4 Zirconium oxide 4

Table 4 Descriptive results of the plaque index and mucosal tissue condition score

Type of abutment	Plaque assessment			Mucosal tissue condition	
	0	1	2	0	1
Gold alloy	2	1	1	2	2
Titanium	3	1	0	4	0
Zirconium oxide	1	3	0	4	0

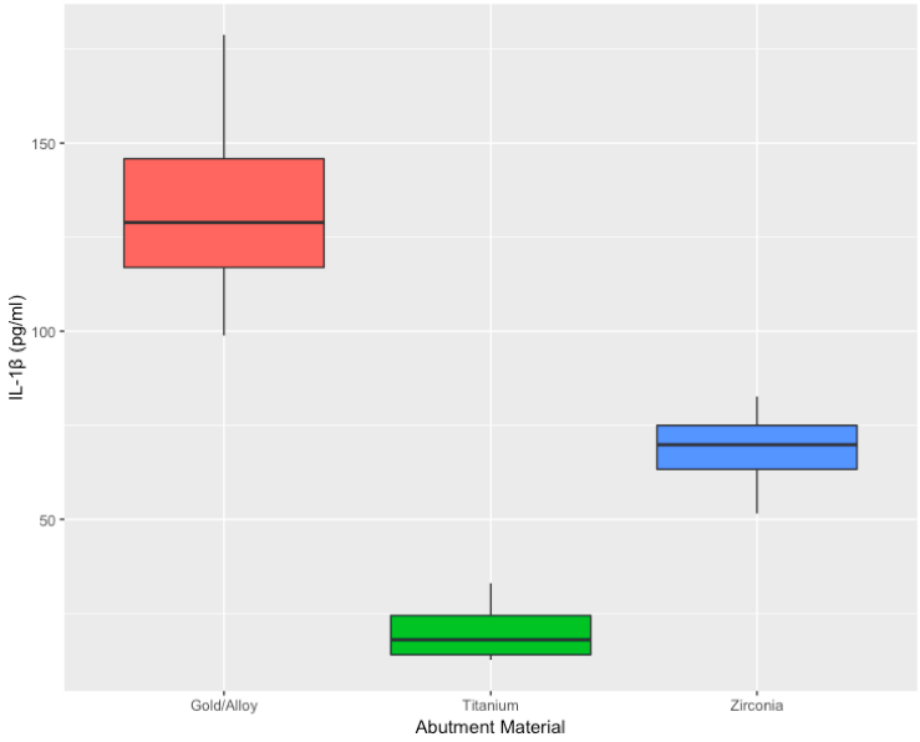

Figure 1 Effect of implant abutment material on cytokine concentration in peri-implant crevicular fluid. IL-1ß levels were significantly elevated in gold alloy abutment compared with zirconium oxide abutment. (p=0.005)

The analysis of the peri-implant crevicular fluid (PICF) has been used to assess the inflammatory mediators. This diagnostic tool is reliable and can be used as an early detector of periodontal disease. A number of research indicated the crevicular fluid collection is a noninvasive method to measure the immune function. IL-1β is a major of proinflammatory cytokine secreted by macrophages, neutrophils, epithelial cells and endothelial cells. IL-1β plays an important role in osteoclastogenesis. It promotes the fusion of osteoclast and prolongs the survival of mature osteoclasts (Braun & Zwerina, 2011). Many studies were carried out on the expression of IL-1β to compare health and disease conditions. There were significantly higher levels of IL-1β around implants with peri-implantitis than healthy implants (Casado et al., 2013; Masashi et al., 2002; Silva et al., 2008; Yaghobee, Khorsand, Ghohroudi, Sanjari, & Kadkhodazadeh, 2014). Dental implant abutments have been considered as a transmucosal component because they exhibit the relationship between the implant fixture and the prostheses. Several studies claimed that soft tissue around transmucosal zone provides the protective barrier from microbial invasion.(Abrahamsson , 1998; Kawahara, 1998; Moon, 1999).

This study examined the concentration of IL-1β proinflammatory cytokine in PICF among different types of implant abutment materials. The level of IL-1β in gold abutment was significantly higher than zirconium oxide and titanium abutment at 4 weeks after implant placement. Higher level of IL-1β

concurred with higher plaque assessment score and mucosal tissue condition found in gold abutment group. These results suggested that gold abutment materials induce more inflammation of soft tissue around dental implant compared with zirconium oxide and titanium. Moreover, the titanium group showed the least level of IL-1β proinflammatory cytokine in PICF and also lower plaque assessment score and mucosal tissue condition. In spite of no statistically difference found in IL-1β proinflammatory cytokine in PICF between titanium and zirconium oxide groups, raw data showed that the titanium group had lower IL-1β levels than zirconium oxide. This finding could be further elucidated with the study of surface properties of abutment and the immunological processes.

With respect to surface topography and plaque retention, a recent literature review showed that surface roughness can affect the biofilm formation and maturation. The rougher surface promoted more cell adhesion and microbial colonizers. The most common surface roughness parameters were a linear profile (R_a) and a surface (S_a) (Han et al., 2016). Bollen and associates concluded the surface roughness below $R_a \leq 0.2$ µm had no or lesser influence of plaque accumulation (Bollenl, 1997). Titanium surface with $R_a \leq 0.088$ µm exhibited the lower plaque retention on early 24-hour of healing (Rimondini, 1997). To date, no conclusive value of surface roughness has been introduced as a guideline for plaque deposit. In our study, some of zirconium oxide and titanium implant abutment roughness value were measured but not calculated in the statistical model. The value of R_a in titanium and zirconium oxide were 0.022 and 0.041 µm, whereas for S_a, was 0.047 and 0.084 µm respectively. These results were in agreement with previous studies. An in vitro study about the effect of implant abutments on the bacterial profile and biofilm formation showed that the titanium disk demonstrated lower biofilm mass and density than the zirconium oxide disk. However, type of materials did not affect the bacterial profile around abutment (de Avila et al., 2017). The study of Zhao and colleagues showed zirconium oxide appeared with more biofilm formation than titanium and titanium-zirconium alloy because of the roughness of its surface. They concluded that smooth titanium surface is suitable for soft tissue seal around implant abutment (Zhao et al., 2014).

The immunological processes drive the series of events following implant placement. The results of the current study pointed to the highest concentration of IL-1β in gold alloy abutment at 4-week healing period. These data were correlated with the histological section studies. An animal study by Abrahamsson and colleagues reported that the mucosal attachment around gold alloy abutment was smaller in dimensions after 6 months of healing (Abrahamsson, 1998). Another animal study revealed that soft tissue healing to titanium and zirconium oxide abutment were stable, while gingival recession and bone loss were found in gold alloy after a 5-month healing period. Additionally, the connective tissue zone of gold alloy abutment showed lower amounts of collagen and fibroblasts and larger fractions of leukocytes than that of titanium and zirconium oxide abutments (Welander, Abrahamsson, & Berglundh, 2008). A recent human study demonstrated that the amount and location of the inflammatory cells with the highest percentage were found in the gold alloy group. Titanium and zirconium oxide presented similar mean histological attachment percentages while gold alloy had a significantly lower percentage (Sampatanukul et al., 2017).

Within the limits of this clinical study, the results can explain particularly in this stud. A larger sample size is required to determine the effect of abutment materials to the production of inflammatory cytokines. Moreover, other mediators should be studied in order to understand the biological process responding to dental implant abutment materials. A further study with longer time points and more types of abutment should be conducted.

5. Conclusion

Within limitation of this study, at 4 weeks healing period gold alloy abutments induced the highest level ofIL-1β proinflammatory cytokine in PICF compared with zirconium oxide and titanium abutments along with highest plaque index and mucosal tissue condition scores. Therefore, careful oral hygiene care and instruction should be given to patients when using gold alloy abutment.

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