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Host Responses in Peri-Implant Tissue in Comparison to Periodontal Tissue

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Abstract**—**The host response in peri-implant tissue may differ from that in periodontal tissue in a healthy individual. The purpose of this study is to investigate the expression of inflammatory cytokines in peri-implant crevicular fluid (PICF) from single implant with different abutment types in comparison to healthy periodontal tissue. 19 participants with healthy implants and teeth were recruited according to inclusion and exclusion criteria. PICF and gingival crevicular fluid (GCF) was collected using sterile paper points. The expression level of inflammatory cytokines including IL-1α, IL-1β, TNF-α, IFN-γ, IL-6, and IL-8 was assessed using enzyme-linked immunosorbent assay (ELISA). Paired t test was used to compare the expression levels of inflammatory cytokines around natural teeth and peri-implant in PICF and GCF of the same individual. The Independent t-test was used to compare the expression levels of inflammatory cytokines in PICF from titanium and UCLA abutment. Expression of IL-6, TNF-α, and IFN-γ in PICF was not statistically different from GCF among titanium and UCLA abutment group. However, the level of IL-1α in the PICF from the implants with UCLA abutment was significantly higher than GCF (P=0.030). In addition, the level of IL-1β in PICF from the implants with titanium abutment was significantly higher than GCF (P=0.032). When different abutment types was compared, IL-8 expression in PICF from implants with UCLA abutment was significantly higher than titanium abutment (P=0.003).

Keywords**—**Abutment, dental implant, gingival crevicular fluid and peri-implant crevicular fluid.

I. Introduction

THE use of dental implant has been increased steadily due to its high predictability and survival rates. According to the systematic review by Moraschini et al., many longitudinal studies revealed high survival rate of more than 90% with a follow up period of up to 20 years [1]. It has been proposed that the inflammatory cells surrounding an osseointegrated implants are the result of host immune responses to the foreign body. The term foreign body equilibrium was first introduced by Albrektsson et al.which stated that osseointegration is the result of a foreign body reaction that with the right intensity in the inflammatory response, will balance itself out by hormones and cytokines and allow for bone to grow on the implant surface. On the other hand, the disturbance of this equilibrium will result in peri-implant bone loss. There are many factors that can compromise the equilibrium which are trauma, occlusal loading, microgap, but one of the main etiologic factors is the accumulation of dental plaque on the transmucosal or abutment surface of the dental implant [2]-[4]. However, the accumulation of dental plaque on the implant surface depends on the transmucosal part or the abutment material that comes in contact with the soft tissue. Therefore, the abutment surface or transmucosal part of implant must provide integration of marginal soft tissue to protect the implant body from the microbial community and thereby maintaining a healthy connective tissue [5].

Inflammatory cytokines play a primary role in tissue homeostasis. They are important modulators of both normal and pathologic processes within the periodontium [6]. For example, Interleukin (IL)-1 β, tumor necrosis factor (TNF-α), receptor activator for nuclear factor kB ligand (RANK- L), IL-6, IL-8 are all released during normal bone turnover and during inflammatory reactions [6]. A recent study has shown that an increased release of IL-12, TNF-α and RANK-L is associated with an increase in the inflammatory reaction, thereby affecting the severity of peri-implant disease [7]. The level of these biomarkers can be detected in the fluid around dental implant or PICF.

PICF like GCF is an osmotically mediated inflammatory exudate originating from the vessels of the gingival plexus. PICF and GCF contains host-derived enzymes and their inhibitors, inflammatory mediators and host response modifiers and tissue breakdown products Therefore, PICF can be used as diagnostic tools in monitoring early changes to any adverse peri-implant tissue reaction [7].

The purpose of this study is to compare the levels of six inflammatory cytokines, including IL-1α, IL-1β, IL-6, IL-8, IFN-*y*, and TNF-α, surrounding natural teeth and dental implant in healthy state. It is postulated that the level of these inflammatory cytokines in PICF are similar to GCF from healthy tissue. The differences in the level of expression of these cytokines between two types of abutment material were also studied.

II. Materials and Methods

The study was started after obtaining ethical clearance from the Ethics Subcommittee, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (HREC-DCU 2016-046).

Nineteen participants who received implant-supported fixed partial prostheses from Esthetic restorative and implant dentistry clinic, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand from January 2010 to December 2016 were included in the study. Following the inclusion and exclusion criteria, individuals with a healthy

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single implant placed in the anterior sextant with controlled oral hygiene and with positive informed consent were enrolled. Individuals with smoking, systemic disease, immunosuppressed status, current corticosteroid therapy, on antibiotics within three months, pregnancy, and inflammatory and autoimmune diseases of the oral cavity were excluded.

Periodontal parameters such as bleeding on probing and probing depth were evaluated on the mesial, buccal, distal, and lingual, aspects of each implant and tooth by plastic periodontal probe (Periowise, Premier Dental, Playmouth Meeting, PA,)

Intraoral radiographs were taken on implant with periapical technique to measure the distance from the mesial and distal margins of fixture-abutment interface to the most coronal point where the bone appears to be in contact with the implant.

For PICF and GCF collection, the tooth that has the same probing depth as the peri-implant were chosen for the collection of GCF. The site at which the PICF was collected was isolated using a cotton roll and air dried to ensure there is a good moisture control. Supragingival calculus or plaque was removed prior to sample collection. PICF and GCF were collected by using four sterile absorbent paper points size M (Kerr, CA, USA), as shown in Fig. 1. The absorbent paper point were marked at 15 mm and then placed in the sulcus for 30 seconds. After 30 seconds, they were removed and cut with scissors at the marked length (15 mm). PICF absorbed from each strip were stored in 1.5 mL plastic tube containing 100 µL of phosphate buffer saline (PBS), pH 7.2, supplemented with 1% protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The paper points were then discarded, and the tube containing PBS were centrifuged at 8000 rpm for three minutes, and then supernatant were stored at -80°C until used.

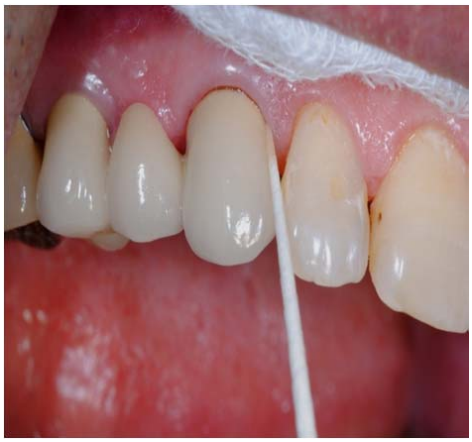

Fig. 1 Collection of PICF from peri-implant using paper point

For biochemical analysis, the level of IL-1α, IL-1β, IL-6, IL-8, IFN-*y* and TNF-α were determined by using specific sandwich ELISA for each cytokines (R&D systems, Co Ltd). The standard curve was plotted as described in the ELISA kit manual, and the levels of IL-1α, IL-1β, IL-6, IL-8, IFN-*y* and TNF-α were determined in PICF samples accordingly.

The data generated were subjected to statistical analysis, SPSS program (SPSS version 16.0, SPSS Inc., Chicago, IL) was used to analyze all data. Paired sample t-test was used for the comparison of cytokines concentration between peri-implant and natural teeth. Independent t-test was used to compare the concentration of cytokines between UCLA and titanium abutment.

III. Results

Among the 19 participants with a healthy single implant, 10 participants had their implants restored with UCLA abutment while nine participants had a titanium abutment installed. The demographic data is shown in Table I. The mean age of the participants was 47.5±11.12. The average months of implant in function were 45.42±16.9 months. The implant sites appeared to be healthy with approximately 4 mm to 5 mm probing depth without any bleeding on probing whereas the adjacent tooth that were used for sample collection was also in a healthy state with a probing depth of ≤ 3 mm and absence of bleeding on probing. The adjacent tooth that had a restoration was not used for sample collection; a pristine tooth in the same sextant was used instead for the sample collection.

TABLE I
Demographic Data

Sex	Age	Abutment	Months of loading
F	46	Titanium	56
M	56	UCLA	50
F	59	UCLA	22
M	23	UCLA	46
M	27	UCLA	22
F	54	UCLA	20
F	23	UCLA	22
M	45	UCLA	20
M	56	UCLA	48
M	24	UCLA	40
F	32	Titanium	54
F	45	UCLA	42
M	53	Titanium	56
M	31	Titanium	42
F	58	Titanium	61
F	46	Titanium	63
M	45	Titanium	66
F	58	Titanium	66
F	33	Titanium	67

TABLE II
Cytokines Level between Teeth and Peri-Implant with Titanium Abutment (pg/ml)

Cytokine	Titanium abutment	Teeth
IFN- *y*	48.9(28)	57.3(40.9)
IL-1 α	181.7(103)	175.2(51.4)
IL- β *	14.6(8.7)	7.9(7.1)
IL-6	7.6(5.2)	7.3(3.9)
TNF- α	10.3(3.3)	9.7(3.4)
IL-8	216.6(3.8)	219.3(7.8)

Mean (SD) values of the six inflammatory cytokines around natural teeth and peri-implant with titanium abutment (Table

II) and UCLA abutment (Table III) are shown. Paired sample t-test demonstrated a significant difference in the level of IL-1β (P-value=0.032) between natural teeth and peri implant with titanium abutment, and IL-1α (P-value=0.030) between natural teeth and peri implant with UCLA abutment.

TABLE III
CYTOKINES LEVEL BETWEEN PERI-IMPLANT WITH UCLA ABUTMENT AND TEETH (PG/ML)

Cytokine	UCLA abutment	Teeth
IFN- *y*	75.4(4.2)	69.2(25.7)
IL-1 α*	189.5(76.2)	114(71.3)
IL- β	18.2(20.2)	16.6(11.8)
IL-6	6.9(3.7)	8.2(3.5)
TNF- α	12.1(5.4)	11.9(5.7)
IL-8	223.4(4.6)	222.3(2.9)

IV. DISCUSSION

PICF analysis may be used in monitoring the osseointegration process and bone response to occlusal loading and infection, thereby improving the long-term success of implants [8]. To study the host response to microenvironment surrounding natural teeth or dental implants, the inflammatory cytokines in GCF or PICF was assessed in this study. Similar level of cytokine expression in natural teeth and dental implant is reported except for the level of IL-1α and IL-1β. Moreover, on comparing the level of expression between two abutment groups, only IL-8 showed a significantly higher expression around UCLA abutment compared to titanium abutment. Early detection of inflammatory reactions might be clinically beneficial, simultaneously with other clinical signs and symptoms, for disease prevention.

The results of this study are in agreement with previous studies that showed an elevated level of IL-1β in PICF as compared to GCF [9], [10]. However, the level of IL-1β may be associated with the use of titanium abutment. This result supports a study by Pettersson et al., which proposed that Ti particles act as a stimulus, activating the inflammasome in the macrophages, resulting in the release of active IL-1β from cells [11]. IL-1β is a potent stimulator of bone resorption by up-regulating RANKL in stromal/osteoblastic cell as Ti ions at a concentration of 9 ppm increased the ratio of RANKL/ osteoprotegerin and resulted in osteoclastogenesis [12], [13].

Another similar study was conducted using multiplex proteomic immunoassays that assess the difference between the levels of interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17A, tumor necrosis factor (TNF)-α, C-reactive protein, osteoprotegerin, leptin, and adiponectin in GCF and PICF of 73 healthy individual. All cytokines were present in PICF and GCF; However, Only IL-17A and TNF-α were present in significantly higher concentrations in PICF compared with their levels [14]. Differences in study design, number of participants, selected biomarkers panel and the inclusion criteria between our study and theirs could have influenced the outcomes.

This study uses a single ELISA array kit for the assessment of each cytokines level instead of a multiplex assay. The use of single array for each cytokine is more reliable in terms of generating a desirable standard curve unlike in multiplex assay. Moreover, in multiplex assay problems may arise from presence of a broad and varying dynamic range of concentrations of the different proteins being assayed together.

In addition, this study showed a similar expression of IL-1β, IL-1α, IL-6, TNF-α, IFN-*y* between the two types of abutment except for the expression of IL-8, which was significantly higher in the UCLA abutment group. Differences in the materials surface roughness could have an influence on the adhesion of plaque and thereby, resulting in an increased in the level of inflammatory cells. However, from the clinical standpoint, both the abutment materials performed well in terms of soft tissue seal around the dental implant and none of them showed gingival recession or bone loss after 48 months of service. In terms of clinical performance, if only the soft tissue response is considered, the choice between using gold or titanium abutment is merely up to the clinician's preference [15]. The gold and titanium were shown to form and maintain an appropriate soft tissue response within this human study [15].

V. CONCLUSION

The expression of some inflammatory cytokines in healthy peri-implant tissues, including IL-1α and IL-1β, may be different from those in healthy periodontal tissue. The implant abutment materials and design also influence on the expression of the inflammatory cytokines in PICF.

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