**ORIGINAL ARTICLES**

# Study on matrix metalloproteinase-2, 9 in peri-implant sulcular fluid

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## Abstract

**Objective:** To study the expression of matrix metalloproteinases-2, 9 (MMP-2, MMP-9) of healthy implant and peri-implant sulcular fluid (PISF) by enzyme-linked immunosorbent assay (ELISA) method, and evaluate the level of MMP-2 and MMP-9 in sulcular fluid as an objective indicator of tissue inflammation around implants.
**Methods:** A total of 40 implants were selected from 30 patients who were treated with dental implants and were divided into two groups: the inflammatory group and the healthy control group with 20 pieces respectively. ELISA double antibody sandwich method was used to detect the levels of MMP-2 and MMP-9 in PISF.
**Results:** The MMP-2 and MMP-9 expressions were significantly different between the healthy implant group and the peri-implant group ($p < .05$). The concentration of MMP-2, MMP-9, and the amount of sulcular fluid in the inflammatory implant group were positively correlated with the clinical parameters (probing depth [PD], modified sulcus bleeding index [mSBI]).
**Conclusions:** Under physiological conditions, the levels of MMP-2 and MMP-9 were low. When the periodontal tissue was stimulated by inflammation, the expression levels of MMP-2 and MMP-9 were increased, which could reflect the severity of inflammation. The increase levels of MMP-2 and MMP-9 in PISF could better reflect the health status of peri-implant tissues, which could be used as an objective indicator to assist in the diagnosis of peri-implant inflammation.

**Key Words:** Peri-implant inflammation, Gingival crevicular fluid, Matrix metalloproteinase-2, Matrix metalloproteinase-9, Enzyme linked immunosorbent assay

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Implant denture has been widely used in clinic, and is becoming a "predictable" oral repair technology. Implant denture theory and technology has been recognized as one of the biggest breakthroughs in stomatology in $20^{th}$ Century. It establishes a model of perfect implementation of system engineering, and verifies the decisive role of the correct theoretical system guidance. Peri-implant inflammation usually leads to the loss of implant bone interface, alveolar bone resorption and implant loosening. Inflammation is caused by the interaction between the pathogen and the immune system. Effective defense against pathogens enables the host in healthy state or the disease is in a quiescent state, while the abnormal immune response results in tissue destruction and disease progression. Based on a large number of studies, interleukin-1 beta (IL-1$\beta$), IL-6, higher content of TNF-alpha (TNF-$\alpha$), intercellular adhesion molecule, osteocalcin, prostaglandin E2 and matrix metalloproteinase can be detected in the peri-implant sulcular fluid (PISF) when there is inflammation. To date, most studies have focused on the inflammatory factors that stimulate the host cells to produce excessive matrix metalloproteinases, which can destroy and degrade periodontal tissues. Matrix metalloproteinase (MMP) and peri-implant inflammation, therefore, have been introduced to the non-inflammatory system.

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# 1 Material and method

## 1.1 Collection of PISF

In our study, the patients received conventional crown repair treatment for more than 12 months in our hospital from August 2011 to August 2012 were chosen and their sulcular fluid was collected. They were divided into two groups: 20 cases in peri-implant inflammation group, and another 20 cases in healthy implant group. The patients were 20-45 years old, with an average age of 35.5 ± 3 years, including 18 males and 12 females, with a total of 30 cases. The consents of all cases were obtained from the patients who met the following conditions: non-diabetes, liver disease, heart disease, hypertension, systemic autoimmune diseases, abnormal bite; non-use of long-term antibiotics, cyclosporine, phenytoin drugs; non-smoking; no bad habits; non-pregnant, no-contraceptive pill-taking.

## 1.2 Collection methods

The sampling site was cleaned, saliva isolated and dried. A Whatman-3 mm-filter in a 2 mm × 10 mm size was inserted into the gingival margin below and was taken out after 30 seconds by the same doctor. The sample must be discarded and recollected if saliva or blood contamination was included. Each implant was taken from 2 sites near the buccal (lip) side, and the length of the filter paper was measured by vernier caliper. In order to figure out the comparison, same length (2 mm) of the filter was taken and placed in the Eppendorf tube with PBS and shaked for 30 min. Centrifuged at 4°C for 10 min (10,000 r/min), and then put the supernatant into the refrigerator at minus 70°C for preservation.

## 1.3 Collection of serum

The amount of serum (0.1 $\mu$l, 0.2 $\mu$l, 0.3 $\mu$l,... 1.5 $\mu$l) was collected by trace sampling gun and dropt to the filter paper in the same specifications. The length and area were mearsured and calculated, which showed linear correlation, $r = 0.997$ ($p < .01$), and could be converted to the amount of PISF (see Figure 1).

## 1.4 Extraction of MMP-2 and MMP-9 in PISF

ELISA double antibody sandwich method was used. Samples were taken from -70°C and melted in room temperature for 20 min. Standard and sample wells were set. The standard well was added with different concentrations of 50 $\mu$l of standard substance respectively, the sample well was added with 40 $\mu$l of sample diluent and 10 $\mu$l of tested samples, while the blank well was not added. In the standard well and the sample well, each well was added with 100 $\mu$l of horseradish peroxidase (HRP)-labeled antibody, sealed with the sealing membrane and set in water bath at 37°C for 1 h; then washed in the washing plate for 6 times. 50 $\mu$l of substrate A, B was added into each well and incubated for 15 min without light at 37°C. Within 15 min, the OD value at the wave length of 450 nm was measured. Standard curve: a standard concentration as the abscissa and the corresponding OD as ordinate were drawn in Excel, as well as the standard curve equation of the linear regression line. The sample concentration was calculated according to the curve equation.

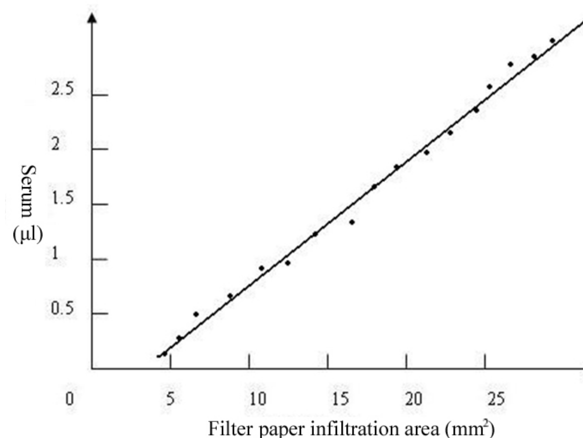


**Figure 1:** Standard curve of relationship between serum and filter paper infiltration area

## 1.5 Experimental data

The experimental data were input into SPSS 11.5 for statistical analysis. Single factor analysis of variance was used to compare the differences among the groups; meanwhile, correlation analysis was performed, $p < .05$ was statistically significant.

# 2 Results

The expressions of MMP-2 and MMP-9 in PISF of healthy group and peri-implant group were as follows.

## 2.1 Comparison of periodontal probing depth (PD) in each group

The depth of exploration in the healthy group was less than that in the peri-implant group, and the difference was statistically significant ($p < .05$) (see Table 1).

**Table 1:** Comparison of periodontal PD between experimental groups

| Groups | n | PD (mm) |
|---|---|---|
| Healthy group | 20 | 2.09 ±0.36 |
| Peri-implant group | 20 | 4.79 ±0.50△ |

*Note*. △ Comparison with the control group, $p < .05$

**Table 2:** Comparison of periodontal improved bleeding index between experimental groups

| Groups | n | mSBI |
|---|---|---|
| Healthy group | 20 | 0.52 ±0.13 |
| Peri-implant group | 20 | 2.73 ±0.35△ |

*Note*. △ Comparison with the control group, $p < .05$

### 2.2 Comparison of periodontal modified sulcus bleeding index (mSBI) between groups

The mSBI in the healthy group was less than that in the peri-implant group, the difference was statistically significant ($p < .05$) (see Table 2).

### 2.3 Comparison of gingival crevicular fluid (GCF), MMP-2 and MMP-9

According to Table 3, GCF, MMP-2 and MMP-9 values in the healthy group were less than those in the peri-implant group, and the differences were statistically significant ($p < .05$).

**Table 3:** Comparison of GCF, MMP-2 and MMP-9

| Groups | n | GCF (µl) | MMP-2 (ng/ml) | MMP-9 (ng/ml) |
|---|---|---|---|---|
| Health group | 20 | 0.72 ±0.22 | 5.88 ±1.54 | 30.35 ±3.87 |
| Peri implant group | 20 | 2.01 ±0.45△ | 13.81 ±3.08△ | 44.94 ±10.88△ |

*Note*. △ Comparison with the control group, $p < .05$

### 2.4 Correlation analysis between PD, mSBI and MMP-2, MMP-9

In Table 4, the correlation coefficient $r$ between MMP-2, MMP-9 concentration and clinical index of PD were 0.83 and 0.74 separately ($p < .05$); the correlation coefficients between MMP-2, MMP-9 concentration and mSBI were 0.84, 0.72 separately ($p < .05$). The results showed that the concentrations of MMP-2 and MMP-9 were correlated with PD and mSBI.

**Table 4:** Correlation analysis of PD, mSBI and MMP-2, MMP-9

| | | MMP-2 | MMP-9 |
|---|---|---|---|
| PD | *r* | 0.83 | 0.74 |
| | *p* | .00 | .00 |
| mBIS | *r* | 0.84 | 0.72 |
| | *p* | .00 | .00 |

## 3 Discussion

### 3.1 Comparison and analysis of GCF

The amount of GCF in the peri-implant group was significantly higher than that in the healthy group ($p < .05$), and there were significant differences of PD value and mSBI value between the two groups ($p < .05$), which were completely consistent with Jiao Yanjun's study.[1] It further verifies the role of GCF in reflecting the degree of inflammation of the implant. It may be the evaluation index of peri-implantitis. Gingival sulcus around implant is similar to natural teeth in the field of the shape and its physicochemical properties.[2] The pathogenesis of peri-implantitis is an extremely complex pathological process. Peri-implant inflammation is the result of the interaction between host and bacterial microorganism. Peri-implant microorganisms and their toxic metabolites are widespread in gingival sulcus, resulting in inflammatory response. The presence of plaque and aggregation is a major etiological factor of inflammation, and GCF volume increases with the aggravation of inflammation.

### 3.2 Comparison and analysis of MMP-2 and MMP-9 in GCF

This study showed that the concentrations of MMP-2 and MMP-9 in the peri-implant group were significantly higher than those in the healthy implant group and the natural tooth control group, and the differences were statistically significant. As bacterial infectious diseases, periodontitis is similar to peri-implantitis. In the study of periodontal, most scholars believe that MMP-2 and MMP-9 have a certain relationship with periodontitis, and the analysis of MMP-2 and MMP-9 in GCF confirms highly positive correlation with periodontitis. Soell et al.[3] indicated that the MMP-1, MMP-2, MMP-3, MMP-9 levels in GCF of dentition periodontitis were higher than those of the healthy periodontal control group.

### 3.3 Analysis of concentration of MMP-2 and MMP-9 in PISF with periodontal clinical parameters

According to the results of this study, the correlation coefficient ($r$) between the concentration of MMP-2, MMP-9 and PD were 0.83, 0.74 respectively ($p < .05$); and the

correlation coefficients between MMP-2, MMP-9 and mSBI were 0.84 and 0.72 ($p < .05$), which indicated correlation. It further demonstrated that the concentration of MMP-2 and MMP-9 increased with the growth of PD and mSBI. In other words, the concentration of MMP-2 and MMP-9 in GCF was proportional to the inflammation around the implant. Ingman et al.[4] showed that the PISF of healthy implants had lower MMP-2, MMP-9 levels in the study of PISF enzyme, and the healthy implant PISF was different from periodontitis. It further indicated the degree of inflammation via MMP-2 and MMP-9.

In a word, MMP-2 and MMP-9 play a significant role in tissue inflammation around the body. As a gelatinase, MMP-2, MMP-9 impose complex effects on other enzymes and cytokines. The specific molecular mechanism of MMP-2 and MMP-9 in the process of peri-implant inflammation remains to be further studied. To study the expression of MMP-2 and MMP-9 in the surrounding tissues of the implants, we can decide the severity of inflammation and the severity of destruction by the concentration changes of MMP-2 and MMP-9. It is also helpful to evaluate the therapeutic effect of peri-implant infection, and to provide a reference for the success of implant repair. MMP-2, MMP-9 could serve as clinical markers of peri-implantitis according to results of this experiment. By detecting the concentration of gelatinase MMP-2 and MMP-9, effective methods can be used to interfere with the production of MMP-2, MMP-9, or to make the enzyme lose its activity to eliminate peri-implant inflammation, which may provide clinical indicators for the success of implants.

## Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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