口腔インプラント体としての窒化チタンコーティングの有用性

Application of Oral Implants Coated With Titanium Nitride

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[Introduction]

Implants are used in the rehabilitation of edentulous patients. Long-term clinical studies have proven that osseointegrated dental implants can restore the function of missing teeth (Blanes et al. 2007) and improve the quality of life.

In recent years, modifications have been made to machined titanium implants with the aim of optimizing osseointegration (Lang et al. 2009).These modifications to increase the speed and success of osseointegration have included hydroxyapatite coatings, increasing surface roughness and surface free energy (Yoshinari et al. 2002). However, the amounts of bacterial adhesion and oral biofilm accumulation were reportedly accelerated by increasing surface roughness (Amoroso et al. 2006). Especially in case of peri-inplantitis, it is definition an infectious disease and the inflammatory lesion in peri-implant tissues and associated loss of supporting bone develops as a result of accumulation of bacteria on implant surfaces. Thus, the surface roughness and chemical composition of the implant surface are important in bacterial adhesion.

Titanium nitride (TiN) is mainly used as a coating to enhance other materials. It is important to consider TiN for use in implants because of reports about peri-implantitis. However, the utility of TiN-coated dental implants using an experimental infection model has not been investigated in vivo. This study evaluated biological response in bone 2 weeks after placement of TiN-coated commerally-pure (cp) titanium implants using *Porphyromonas gingivalis* (*P. g*), a pathogen in peri-implantitis (Mombelli 1997), in a simplified rabbit model.

[Materials & Methods]

1. Animals

A total 20 adult male Japanese white rabbits weighing approximately 2.5 kg were used in this study.

2. Dental Implants

Forty threaded μ-One HA implants (φ 3.3 mm, length 10.0 mm, identical in size to those in clinical use) were machined from a cp titanium rod (JIS TYPE 2) for use in this study. They were divided into 4 different groups based on surface modification: implant type M, machined; B, sandblasted and acid-etched; HA, radiofrequency magnetron sputter hydroxyapatite (HA)-coated; TiN, TiN-coated using an arc ion plating (AIP) system.

3. Surface Topography and Roughness

Scanning electron microscopy (SEM) was used for an overall picture of the surface topography of implants with each surface modification. Quantitative characterization of the surface topography and roughness was carried out by 3-dimensional (3D) laser microscopy.

4. Bacterial Adherence Test

Bacterial adherence to the M-, B-, HA-, and TiN-coated disks were examined for *P. g*. Each of the 4 kinds of disks were placed in a 24-well plate and incubated with 1 mL of bacterial suspension in BHI broth (OD_{660} = 0.1) for 2 weeks under anaerobic conditions. After incubation, bacterial cells on disks were removed and incubated on BHI blood agar plates to count the colony-forming units (CFU).

5. Surgical Procedure and *P. g* Provision

The procedure of implant placement was according to previously described (Nakanishi et al. 2011). After implant placement, silk threads were twisted 4 times on the abutment-implant junction. *P. gingivalis* suspension 15 μl (OD_{660} = 10.0) was applied to silk threads on the left side as the infection model, *P. g* (+); 15 μl of PBS was applied to the silk threads on the right side as the control, *P. g* (-).

6. Bacterial Sampling, DNA Extraction and Detection of *P. g* by Polymerase Chain Reaction (PCR)

After the rabbit was sacrificed, bacterial samples were collected using sterile paper points (ISO #35) from the sulcus around the implant. Samples were also collected from the control side. Genomic DNA was extracted from individual samples using the InstaGene Matrix Kit. A specific primer set (Bogen and Slots 1999) was used to detect *P. g*.

7. Sample Removal and Specimen Preparation

After bacterial sampling, surrounding bone including the implant in both sides of the femurs was removed. The specimen preparation procedure was previously described by Nakanishi et al. (2011).

8. Observation by Contact Microradiography (CMR) and Measurement of Bone Implant Contact Ratio (BIC)

Pictures of samples with a thickness of 120 μm were taken using a soft X-ray generation device. Bone resorption area (mm^2) around the implant were measured.

BIC was measured by using sections stained with basic fuchsin and methylene blue. Computer analysis with NIH Image® 1.61 was performed with image analysis software. BIC was calculated as the ratio of the contact length of the implant and newly-formed bone adjacent to the implant as peripheral length. Measurements were separately conducted for upside BIC (from the top of the implant to the 3^{rd} thread) and downside BIC (from the 4^{th} thread to the bottom of the implant).

9. Statistical Analysis

Statistical significance was determined using Tukey's analysis after one-way analysis of variance (ANOVA) with SPSS® Statistics 22. A p-value of <0.05 was considered statistically significant.

[Results]

1. Surface Topography and Roughness

According to the results of SEM images, M had the typical topography of machined samples: flat and smooth, with machining grooves. B, HA, and TiN had many surface concavities and convexities, but no drastic vertical interval in the 3D laser microscopy image. In addition, according to the surface roughness measurement, M clearly showed less roughness than B, HA, and TiN. Furthermore, HA showed less roughness than B and TiN.

2. Bacterial Adherence Test

As the results, cells of *P. g* did not tend to adhere to TiN, but the results showed no significant difference with M, B, and HA.

3. Detection of *P. g*

PCR products were only confirmed in *P. g* (+)-affected areas of M, B, and HA. However, there were fewer PCR products from *P. g* (+)-affected areas of M than those of B and HA. No PCR products were confirmed in *P. g* (+)-affected areas of TiN, or in any *P. g* (-)-affected areas.

4. CMR Images and Measurement of Bone Resorption Area around the Implant

Only in the *P. g* (+) group, mean values of the bone resorption areas were M, 0.21; B, 0.86; HA, 1.68; and TiN, 0.36. There were significant differences between HA and M, B, and TiN.

5. Histologic Observation and Measurement of BIC

The mean values of upside BIC in the *P. g* (-) groups were M, 65.6; B, 80.6; HA, 78.8; and TiN, 77.1. There were no significant differences. In the *P. g* (+) group, the mean values of the upside BIC were M, 54.3; B, 56.0; HA, 53.8; and TiN, 77.3. There was a significant difference between TiN and M, B, and HA. The mean values of downside BIC in the *P. g* (-) group were M, 50.7; B, 80.5; HA, 83.5; and TiN, 75.0. There was a significant difference between M and B, HA, and TiN. In the *P. g* (+) group, the mean values

of downside BIC were M, 56.0; B, 74.4; HA, 76.9; and TiN, 70.1. There was a significant difference between M and B, HA, and TiN.

[Discussion]

In this study, B and TiN showed greater surface roughness than M and HA, suggesting that TiN was suitable as an implant surface to achieve osseointegration.

The amounts of bone resorption and BIC with *P. g* infection were evaluated by using 4 kinds of implant surfaces including TiN-coating. In the CMR images, HA in the *P. g* (+) group showed high amounts of bone resorption, whereas M and TiN in the *P. g* (+) group showed low resorption. In addition, PCR results showed that *P. g* was not detected on TiN, and was detected less on M than on B and HA. However, in the bacterial adherence test results, only TiN did not show good adherence by *P. g*. Therefore, the results of our study partly support a report (Persson et al. 2001), and suggest that the results of differences for M occurred in the in vitro and in vivo studies because of totally different conditions.

Despite the surface roughness of TiN, adherence by *P. g* was difficult. In addition, TiN surfaces showed a significant reduction of the presence of bacteria, which could be important in decreasing inflammation in peri-implant soft tissues (Scarano et al. 2003). Furthermore, Ji et al. (2015) reported that their in vitro study evaluated antimicrobial activity against not only *Streptococcus mutans* but also *P. g* by TiN-coating on titanium using an AIP system. The results in this study confirmed that TiN exercised antimicrobial activity against *P. g* both in vivo and in vitro.

The results of the upside BIC in the *P. g* (+) group showed the highest percentage for TiN. It was suggested that TiN induced bone formation and inhibited *P. g* adhesion, thus preventing inflammation. Moreover, Ji et al. (2015) reported that TiN-coated titanium did not influence osteoblast-like cell viability.

It is essential to consider infection in clinical applications. The present study was the first to report that TiN-coated cp titanium implants are excellent as dental implants to induce osseointegration and inhibit *P. g* infection using a simplified animal model. It is expected that TiN-coated cp titanium implants

will be used as dental implants.

[References]

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