Elucidation of the Relationship between Peri-Implantitis and Fluoride: A Correlation Study

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Abstract: Fluoride has recently been indicated as a risk factor for peri-implantitis. However, no reports have confirmed this intraorally in humans or in experiments using large animals. Thus, in the present study, we used beagles to verify the effects on surrounding tissue when dental implants were implanted and peri-implantitis was induced. To elucidate any possible correlation with peri-implantitis, we also quantitatively examined titanium corrosion and elution due to fluoride. Subjects were divided into three groups, namely, (1) no fluoride, no pressure thread; (2) fluoride, no pressure thread; and (3) fluoride, pressure thread. All the total 12 implants survived, indicating an implant survival rate of 100%. Dental X-ray measurement of bone resorption and measurement of bone destruction volume with μCT indicated significantly more bone resorption and bone destruction in group (3) than in group (1). There was no significant difference between group (1) and group (2). In addition, there was no significant difference between group (2) and group (3). Scanning electron microscope measurement of titanium in gingiva around the implants did not reveal any significant differences among the three groups. Based on quantitative data, our results suggested that fluoride exacerbates peri-implantitis.

Key words: Bone resorption, Fluoride, Peri-implantitis, Titanium

Introduction

Dental implant treatment is indicated for various types of cases ranging from partially edentulous to edentulous jaws. In recent years, it has been widely performed as a means of treatment offering excellent functional, long-term stability. Meanwhile, various complications have also been reported, including sensory nerve paralysis, implant body deviation or loss, peri-implantitis, and prosthetic appliance failure. Of these, peri-implantitis is considered the most serious as it causes a decreased quality of life caused by the surrounding gingival inflammation, esthetic impairment resulting from resorption of surrounding bone, pain and swelling that make it difficult to eat, and unpleasant odors. However, as no method of treatment has currently been established for peri-implantitis, its prevention is highly important.

Peri-implantitis is widely known to be caused by plaque buildup resulting from a lack of regular maintenance or poor self-care or factors such as smoking. It has also been reported that the surface corrosion of titanium implants caused by fluoride may encourage the adhesion of plaque or intraoral bacteria, putting the patient at risk of developing peri-implantitis. Although there have been some reports describing the effects of fluoride on titanium, most of these involve in vitro studies using a titanium plate. To the best of our knowledge, we were not able to determine any in vivo studies. Additionally, although a small number of reports indicated that fluoride can promote peri-implantitis were observed, no studies used quantitative evaluation. Thus, we considered that quantitatively measured titanium corrosion and elution should be investigated.

Hence, in the present study, we implanted dental implants and induced peri-implantitis to examine its effects on the surrounding tissue. Moreover, to elucidate any correlation with peri-implantitis, we quantitatively investigated titanium corrosion and elution caused by fluoride.

Materials and Methods

Three adult beagles (12–15 kg, female) were used in the experiment. Institutional review board committee of the Faculty of Medicine, Tokyo Medical University has approved this study protocol (approval No. H29-1003). The protocol for animal experiments comply with the provisions of the Helsinki Declaration. The animal experiment was started once the protocol was approved. First, they were sedated followed by extraction of the left and right mandibular postmolar teeth under local anesthesia. They were sedated with intramuscular injections of 0.01–0.08 ml/kg of medetomidine hydrochloride (DOMITOR; Nippon Zenyaku Kogyo Co., Ltd., Aichi, Japan). The local anesthetic used on the operated site was an injection of lidocaine hydrochloride/adrenaline (Dental XYLOCAINE; Dentsply Sirona, Pennsylvania, USA). The tooth extractions were conducted by segmenting the root and then removing the teeth with dental forceps and an elevator.

After bone healing was confirmed with a dental X-ray at 6 months after tooth extraction, two dental implants were placed on the left and right sides at the sites of the left and right mandibular postmolar teeth (bone level 3.3 × 8 mm; Institut Straumann AG, Basel, Switzerland) with standard procedure, under sedation and using local anesthesia.
Plant stability quotient (ISQ) was 60–75, indicating favorable initial fixation. A healing screw was affixed to each of the dental implants, and we then waited for 1.5 months, by which time, osseointegration was obtained. The dental implants were implanted in the buccolingual center of the beagles’ mandible with even mesiodistal intervals between them (Fig. 1).

Peri-implantitis was induced using the method outlined by Monje et al. (Fig. 2). Fluoride was applied by brushing the area around the healing cap three times per week with acidulated phosphate fluoride (APF: 9,000 ppm). Once osseointegration was confirmed with dental X-rays and ISQ measurement, subjects were divided into three groups, namely, (1) no fluoride, no pressure thread; (2) fluoride, no pressure thread; and (3) fluoride, pressure thread (Fig. 3). Subjects in all groups underwent tooth brushing every other day to control plaque. Once 3 months had passed, the animals were euthanized by intraperitoneal administration of an overdose of pentobarbital sodium. The left and right common carotid arteries and veins were cut open. While removing blood from the venous side, 1,000 ml of the physiological solution followed by 1,000 ml of 10% formalin solution were infused from the arterial side to achieve perfusion fixation, after which the mandible was extracted.

The extracted mandibles underwent immersion fixation for 3 weeks with 10% formalin, followed by tissue fixation.

For all experiments, an animal experiment protocol was prepared based on applicable laws including Article 41 of the Act on Welfare and Management of Animals (Method to Be Applied, Subsequent Measures, etc. in the Case of Providing Animals for Scientific Use), Guidelines for Animal Experiments of the Tokyo Medical University Animal Research Center, and the Tokyo Medical University Animal Experiment Implementation Manual. The protocol was submitted to the university animal experiment committee to undergo screening. The animal experiment was started once the protocol was approved. Following Japan’s Law Concerning the Protection and Control of Animals, as well as the animal experiment implementation rules and manuals of Tokyo Medical University, the humane treatment of all animals has been considered. We also carefully considered the 3Rs (Refinement, Replacement, Reduction) proposed by Russell and Burch in 1959 prior to starting the experiment.

**Evaluation methods**

**Measurement of bone resorption by dental X-rays**

Bone resorption was measured with dental X-rays to comparatively examine the amount of resorption of bone around the implants. To

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**Figure 1.** (a) Bilateral retromandibular posterior extraction socket healing model, (b) Implant placement position. The dental implants were implanted in the buccolingual center of the beagles mandible with even mesiodistal intervals between them.

**Figure 2.** Peri-implantitis induction model. The healing cap ligated with 3-0 silk thread.

**Figure 3.** Study population and its division into three groups.
standardize the X-ray images, imaging was conducted with the long cone paralleling technique and a film holder. The X-ray film was positioned parallel to the long axis of the implant body. X-rays were taken using portable dental X-ray imaging equipment (PORT-X III) with a focal distance of 20 cm. Output was set at a rated capacity of 60 kV ± 2 mA.

X-ray images were taken twice—once the healing period of 1.5 months had passed after dental implant surgery and after the animals were euthanized. The amount of bone resorption was measured as the difference in bone resorption measured at these two timepoints. Two measurement points were set around the implants. We measured the lowest point of bone resorption adjacent to the implant body from the platform (Fig. 4). A one-way analysis of variance was used for data analysis, and results for bone resorption were compared among the three groups.

**Residual bone volume measurement with micro-computed tomography (μCT)**

To compare the state of bone destruction around the implants, we conducted a three-dimensional measurement of destroyed bone volume. Imaging was conducted using DELPet μCT100 equipment (DELBio, Taoyuan County, Taiwan) and dedicated DELPet software (conditions: scan mode: ex vivo; continuous or step and shoot: continuous; tube voltage (kV): 40; tube current (µA): 375; additional filter type: FT_0p5_mmAl; binning mode: one by one; 1 pixel: 60 µm). Data were then reconstructed into DICOM images.

Next, to calculate the volume of bone destruction around the implants within a specified range, VivoQuant 4.0 patch 1 (INVICRO, Massachusetts, US) was used.

The specified range was a box-shaped range of 6.6 mm × 3.9 mm × 6 mm with the implant body as the central axis (Fig. 5). We determined the specified range considering metal artifacts caused by the implant bodies, the distance between implants, and bone volume changes due to the cavity in the subalveolar canal area. A one-way analysis of variance was used to study these values, and data were used to compare the volume of destroyed bone among the three groups.

**Scanning electron microscope (SEM) measurement of titanium within gingiva surrounding implants**

To compare titanium corrosion and elution in gingiva around the implant bodies, quantitative measurement with an SEM was conducted.
The specimens were prepared using UltraCut UCT Type 706200 (Leica Microsystems, Wetzlar, Germany). For observation, we used a JSM-7200F microscope (JEOL, Tokyo, Japan), with an acceleration voltage of 10 kV, detection signal backscattered electron images and observation mode set at low vacuum mode.

We then used JED-2300 analysis equipment (JEOL) to calculate the titanium area ratio in the observation zone.

In the specified range, the floating titanium volume was measured by thinly slicing the area around the gingival surface of the anterior portion of the implant body using the bulk section observation technique. A one-way analysis of variance was used to study these values, and data were used to compare the volume of titanium particle area the three groups.

**Results**

A total of 12 dental implants were implanted, and all survived, garnering an implant survival rate of 100%.

**Dental X-ray measurement of bone resorption volume**

Mean bone resorption in the no fluoride, no pressure thread group was 0.83 mm.

Mean bone resorption in the fluoride, no pressure thread group was 1.83 mm.

Mean bone resorption in the fluoride, pressure thread group was 2.33 mm.

Thus, a significant difference (P < 0.05) was noted between the no fluoride, no pressure thread group and the fluoride, pressure thread group (Fig. 6).

**Measurement of bone resorption volume in the specified range with \( \mu \text{CT} \)**

The mean bone resorption volume around the implant in the no fluoride, no pressure thread group was 24.94%.

The mean bone resorption volume around the implant in the fluoride, no pressure thread group was 37.13%.

The mean bone resorption volume around the implant in the fluoride, pressure thread group was 53.36%.

Thus, a significant difference (P < 0.05) was noted between the no fluoride, no pressure thread group and the fluoride, pressure thread group (Fig. 7).

**SEM measurement of titanium in gingiva around the implant**

Although titanium particles were observed in the gingival surface areas of all the implant body interfaces (Fig. 8), no titanium particles were identified in any of the deep gingival sections. No titanium ions were detected in any of the observed ranges.

The mean titanium particle area in the no fluoride, no pressure thread group was...
The mean titanium particle area in the fluoride, no pressure thread group was 5.92%.

The mean titanium particle area in the fluoride, pressure thread group was 3.59%.

Therefore, no significant differences were observed (Fig. 8).

Discussion

The present study quantitatively demonstrated that fluoride tends to aggravate peri-implantitis. It was previously shown in an in vitro study that with a fluoride concentration of 400–1,000 ppm and in an acidic environment (pH 4.0–5.0 or below), titanium corrosion and elution are observed [17]. In this study, although no significant differences were observed in terms of titanium particle area, more titanium particles were observed in the gingiva around the implants in the fluoride, no pressure thread group than in the no fluoride, no pressure thread group. This suggested that the destruction of the implant body titanium surface was aggravated by fluoride. However, the APF used in this study was a high concentration paste that is often used clinically (pH 3.5–4.0, fluoride concentration: 9,000 ppf). Because it has been reported that corrosion occurred, the surface became rough and titanium elution was also noted when the paste was applied to the titanium plate and left for 3 days [18, 20]. We expected to see a greater difference in titanium elution depending on whether fluoride was used. Despite this, although we observed some differences, they were not statistically significant.

It has been reported that, even in periodontal pockets with a depth of 10 mm, the pH is 7.0 or higher [21]; that the adhesion of protein to titanium improves its corrosion resistance [22]; that subgingival plaque contains large amounts of bacterial types that feed on protein; and that the basic substances produced as metabolites by such bacteria raise pH [23]. Thus, it seems possible that, at the sites of bone resorption caused by peri-implantitis, the pH that was lowered by APF may have been neutralized by a similar mechanism, allowing pH levels to rise to a level at which titanium corrosion would not occur.

It has also been reported that intraoral pH decreases to approximately pH 4.0 when carbohydrates or sugar are consumed because of acid being produced by Streptococcus mutans and that this situation lasts for approximately 30 min [23, 24]. Thus, bone resorption caused by peri-implantitis expands horizontally as well as vertically and the implant body titanium surface may be particularly susceptible to corrosion when it is completely exposed within the mouth. The significant difference noted between favorable and poor conditions on X-ray and µCT evaluation of bone destruction around the implants appeared to be caused by the corrosion and elution of titanium promoted by fluoride causing a large amount of plaque to attach to the roughened titanium surface and the pressure thread to further exacerbate the intraoral environment.

When using our APF in a clinical setting, physicians must avoid contact between fluoride and the titanium surface in peri-implantitis where titanium is exposed due to bone resorption progression.

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Conflict of Interest

The authors have declared that no COI exists.

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