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Evaluation of bone loss in antibacterial coated dental implants: An experimental study in dogs

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ABSTRACT

The aim of this study was to evaluate the *in vivo* effect of antibacterial modified dental implants in the first stages of peri-implantitis. Thirty dental implants were inserted in the mandibular premolar sites of 5 beagle dogs. Sites were randomly assigned to Ti (untreated implants, 10 units), Ti_Ag (silver electrodeposition treatment, 10 units), and Ti_TSP (silanization treatment, 10 units). Coated implants were characterized by scanning electron microscopy, interferometry and X-ray photoelectron spectroscopy. Two months after implant insertion, experimental peri-implantitis was initiated by ligature placement. Ligatures were removed 2 months later, and plaque formation was allowed for 2 additional months. Clinical and radiographic analyses were performed during the study. Implant-tissue samples were prepared for micro computed tomography, backscattered scanning electron microscopy, histomorphometric and histological analyses and ion release measurements. X-ray, SEM and histology images showed that vertical bone resorption in treated implants was lower than in the control group ($P < 0.05$). This effect is likely due to the capacity of the treatments to reduce bacteria colonization on the implant surface. Histological analysis suggested an increase of peri-implant bone formation on silanized implants. However, the short post-ligature period was not enough to detect differences in clinical parameters among implant groups. Within the limits of this study, antibacterial surface treatments have a positive effect against bone resorption induced by peri-implantitis.

1. Introduction

Peri-implantitis is an inflammatory disease that affects soft and hard tissues around dental implants and is characterized by bleeding upon probing and progressive peri-implant bone loss [1,2]. If left untreated, it may cause progressively increased implant mobility and eventually implant failure. Peri-implantitis inflammatory reactions have been detected in about 10 to 45% of dental implants within 10 years after implantation [3]. Therefore, peri-implantitis creates a persistent clinical problem without an easy treatment [4].

Bacterial biofilm can be decisive in the formation and progression of peri-implantitis, so inhibiting or decreasing the bacterial colonization of the implant surface in order to reduce biofilm formation is important for the treatment of peri-implantitis. Many studies have focused on the incorporation of antibacterial agents such as silver on titanium surfaces. Silver and silver-based compounds are highly effective at inhibiting bacteria growth [5] as they damage the DNA of both Gram-positive and Gram-negative bacteria [6]. Different techniques have been explored to add silver in different states to titanium surfaces (*e.g.*, ion implantation [7], physical vapor deposition (PVD) [8], magnetron sputtering [9] and micro arc oxidation [10]). Another strategy to confer antibacterial properties to titanium surfaces involves using silanes as an anchoring platform for active molecules with different effects on cells and bacteria, such as induction of cell proliferation, cell differentiation, or antibacterial properties. Silanes may also induce such biological effects by

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themselves. In a previous study, the antibacterial effect of TESPSA silane was detected *in vitro* when compared to control and surface-treated titanium samples without eliciting cytotoxic effects on human cells [11].

Antibacterial coatings may significantly influence the progress of peri-implantitis. Different antibacterial coatings have been tested *in vitro*, achieving a decrease in biofilm formation and bacterial adhesion. Few studies, however, have provided *in vivo* information on the early effect on peri-implantitis of antibacterial coatings of dental implants [12,13]. Most studies have focused on the effect of bonding antibiotics [14,15] or antiseptic molecules [16] onto the implant surface. Other investigations were based on doping titanium with a photosensitizing surface [17], metallic ions with antibacterial properties [18] or studying the effect of distinct titanium oxide microstructures and thicknesses [13].

The hypothesis of the present study was that dental implants treated with antibacterial coatings (silver electrodeposition and 3-(triethoxysilyl)propyl succinic anhydride (TESPSA) silane) would reduce bone resorption caused by ligature-induced peri-implantitis and enhance osseointegration.

2. Material and methods

EU Directive 2010/63/EU and Spanish RD 1201/2005 regulations for the care and use of laboratory animals for scientific purposes have been observed. The protocol was approved by the Ethics Committee for Animal Research of the Rof Codina Veterinary Hospital, University of Santiago de Compostela, Spain. In order to minimize the effect of performance bias when allocating the implants in animals and assessing results, samples were identified with a code unknown by any person relevant to the study. All the details of the study are described in accordance with the ARRIVE guidelines [19].

2.1. Animals

Five adult female beagle dogs about 2.3 ± 0.05 years old and 11.6 ± 1.3 kg were used. The number of animals was determined by considering previous studies, the 3 Rs (replacement, refinement, and reduction) for the use of animals in research and performing a sample size calculation for a statistical power of 0.9 [12,13,17,20,21]. All the animals had normal mandibles, no generalized occlusal trauma, no viral or fungal lesions, good overall health, and no systemic compromises according to veterinary exams. During the experiment, the 5 animals were housed separately in kennels at Rof Codina Veterinary Hospital in 100% fresh air with ambient temperature of 25 ± 0.1 °C and humidity of 40–70%. They were fed a soft diet twice daily and given free access to fresh water.

2.2. Surface treatments

Thirty commercial dental implants with cylindrical threaded geometry (3.5 mm diameter and 8 mm length) were provided by Klockner (Soadco S.A., Escaldes-Engordany, Andorra). The implants are manufactured with the threaded body chemically etched and sandblasted, while the implant head is untreated (machined).

Each implant group consisted of 10 implants. Groups were coded as: (i) **Ti_Ag**: Implants with silver electrodeposition, (ii) **Ti_TSP**: Implants with TESPSA silanization and (iii) **Ti**: Dental implants without further processing (control group).

Electrodeposition of silver on dental implants was carried out as previously described for titanium surfaces [22,23]. Briefly, the anodizing process was controlled with a potentiostat (PARSTAT 2273, Princeton Applied Research, Oak Ridge, TN, USA) that generated a rectangular voltage pulse. The electrolyte consisted of a solution of $AgNO_3$ 0.1 M and $Na_2S_2O_3$ 0.2 M. The treatment was applied to the head of the implant. All implants were sonicated in ethanol, distilled water and acetone for 15 min each and dried with nitrogen.

TESPSA bonding to titanium surfaces has been described elsewhere [11]. Succinctly, dental implant surfaces were activated with 5 M NaOH for 24 h at 60 °C. Implants were thoroughly cleaned twice by immersion in distilled water for 30 min, washed with acetone and dried with nitrogen gas. Pretreated implants were silanized with TESPSA (0.5%, v/v) in anhydrous toluene for 1 h at 70 °C in nitrogen atmosphere. The silanization was applied in a solution of 3% (v/v) N,N-diisopropylethylamine (DIEA) to maintain a basic environment. After completion of the reaction, the silanized implants were sonicated with toluene for 10 min. Afterwards, substrates were thoroughly washed with isopropanol, ethanol, distilled water, and acetone for 15 min each and dried with nitrogen. All implants were individually packaged and sterilized with ethylene oxide (Soadco S.A., Escaldes-Engordany, Andorra) and stored at room temperature.

2.3. Physicochemical characterization of the surfaces

Implant surfaces were analyzed with scanning electron microscopy (SEM) (Zeiss Neon40, Carl Zeiss NTS GmbH, Oberkochen, Germany) and white-light interferometry (Wyko NT1100, Veeco Instruments, NY, USA). Surface elemental analyses (3 per group) were performed by X-ray photoelectron spectroscopy (XPS) with a Mg anode XR50 operating at 150 W (D8 advance, SPECS Surface NanoAnalysis GmbH, Zurich, Switzerland). Binding energies were referred to the C 1s signal at 284.8 eV.

2.4. Surgical and clinical procedures

A modified ligature-induced peri-implantitis model in beagle dogs was used, because of their likeness with human bone in relation to their size and ease of handling [20,21]. An outline of the experiment is presented in Fig. 1. Mandibular premolars were extracted from the animals. Implant insertion surgeries were performed 3 months later. Lateral incisions were made to avoid tension in the area of implantation, and mucoperiosteal flaps were elevated on both sides of the mandible (Fig. 2). All surgeries were done under general inhalation anesthesia with a mix of isofluorane, nitrous oxide and oxygen (5%).

Once the sites were prepared and cleaned of debris, the implants were placed with a torque wrench (maximum torque: 35 Ncm) following the manufacturer's surgical guide. Six implants (2 per implant group) were inserted in each animal mandible. A permuted random block design was used to allocate each implant position avoiding

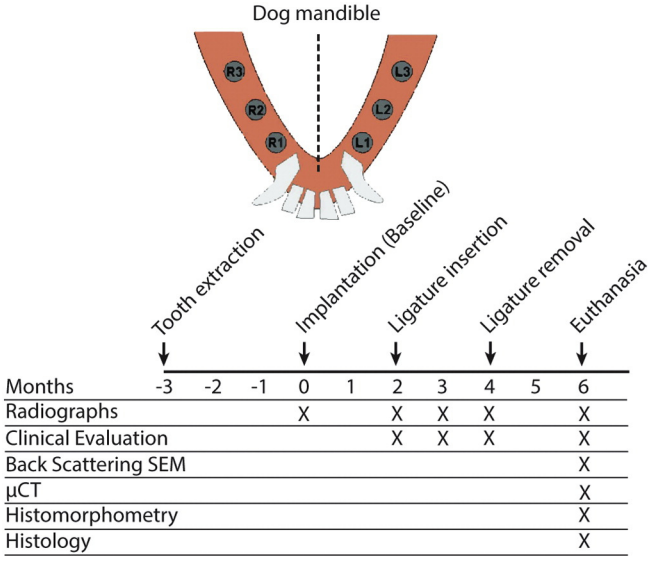

Fig. 1. Study outline: L1-L3 and R1-R3 are the left- and right-mandible implant sites. Ligatures were placed 2 months after implant insertion and removed at month 4. Animals were euthanized 2 months later. 'X' indicates that a given analysis/evaluation was conducted at the corresponding milestone.