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Corrosion behavior of dental implants immersed into human saliva: preliminary results of an *in vitro* study

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Abstract. – OBJECTIVE: Over the years, different implant surfaces have been used to try to maximize bone to implant contact. The aim of this study was to compare levels of metallic ions and particles dissolution collected from two different dental implants surfaces immersed into human saliva.

PATIENTS AND METHODS: A total of 60 dental implants were tested. Group A: sanded with aluminium oxide medium grade particles and acid-etched; Group B: micro-sanded with calcium phosphate powders and acid-etched. Forty implants were immersed in 20 ml of human saliva, twenty, as a control, in sterile saline solution. ICP-MS was performed to detect any metallic ions released from dental implants at T0, on day 1 (T1), on day 3 (T2), after one week (T3), on day 14 (T4), after 3 months (T5) and after 6 months (T6).

RESULTS: Dissolution of metallic particles of titanium and nickel, absent in human saliva (T0), were found after one week (T3) for Group B and after 3 months (T5) for Group A. Vanadium was already detected in small concentrations in either group after 1 day, with an exponential growth for Group B.

CONCLUSIONS: Preliminary results reported significant values of Ti, Ni and V released by Group B, showing for the first time statistically significant values of vanadium.

Key Words
Corrosion, Peri-implantitis, Vanadium, Titanium, Tribocorrosion, ICP-MS.

Introduction

Over the years, several materials have been used for dental implants. In 1947 the Italian dentist Formiggini proposed a new "self-tapping screw" made of tantalium[1,2]. However, due to its production cost, the material was soon replaced by titanium and Branemark coined the term "osseointegration" in 1977, marking the advent of modern implant dentistry[3,4]. Titanium and titanium-based alloy TiAl6V4 are, nowadays, the most common materials used, due to their biocompatibility, mechanical proprieties and excellent corrosion resistance[5-7]. The corrosion behavior of titanium alloys depends on an oxide film mainly composed of TiO_2, called 'passive layer' which spontaneously covers the titanium surface and its alloys in the presence of oxygen. This oxide film is very stable, continuous, highly adherent and protective of the metal's surface. Furthermore, its chemical proprieties play an important role in the biocompatibility of titanium implants and the surrounding tissues[8-10]. However, dental implants, when placed in oral cavity, are exposed to several adverse mechanical, chemical and microbiological events, leading to complex degradation processes[11,12]. During mastication, implants are subjected to both axial and oblique forces, which create micro-movements and may induce wear. In addition, dental implants are exposed to an aggressive environment including agents such as bacterial biofilm and saliva[13]. Several studies[14-16] have demonstrated the ability of bacteria to get trapped in the micro-gaps of the implant-abutment interface. A relationship between early/primary-colonizing bacteria, bacteria biofilm, and the ability of bacteria to generate acidic environments, release lactic acid and decrease pH, triggering surface oxidation, has been hypothesized[17]. These simultaneous actions of wear and corrosion are know as 'tribocorrosion' defined as: 'a degradation phenomenon that is subjected to the combined action of tribological (wear and fretting) and corrosive (chemical and/or electrochemical) events influenced by the variation in the

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mechanical contact conditions (load and relative velocity) and in nature of the environment (pH, humidity and biochemistry)' and may influence the overall performance of dental implants[18-20]. In such situations, metallic ions dissolution may lead to toxicity, reddening and allergic reaction of the skin or tissue inflammation, which could lead to early failure of the implant[21]. Moreover, today, numerous implant surfaces have been used to try to increase the bone to implant contact (BIC) and to accelerate the osseointegration process[22]. The improvement in surface roughness of dental implants may be obtained using several processes: acidification, etching, sandblasting, nano-texturing, coating with hydroxyapatite (HA) or combining them. This surface processing can alter the titanium and expose it more to corrosion[23,24]. Several studies[25-27] have focused their attention on dental implant corrosion, but few have used human saliva and have compared different implant surface types. The aim of this study was to compare the levels of metallic ions dissolution collected from two different dental implants surfaces immersed into human saliva. Authors hypothesized that a different implant surface may influence the metallic corrosion process.

Patients and Methods

Study Design

To address the research purpose, authors designed and implemented an *in vitro* study conducted at the Department of Oral and Maxillo-Facial Sciences at "Sapienza" University of Rome (Rome, Italy). The inductively coupled plasma mass spectrometry (ICP-MS) analysis was instead carried out by the Department of Earth Sciences. The study was approved by the Institution Review Board.

Saliva collection

Human saliva was collected from 16 healthy patients, 8 male and 8 female, with a mean age of 33±4.5 years (range = 24-45 years), who met specific inclusion and exclusion criteria, and signed the informed consent form according to the World Medical Declaration of Helsinki (Table I). From each patient, 40 ml of saliva were collected via the non-stimulated drainage method, or alternatively by leaving the saliva flow passively from the lower lip directly into designated sterile containers. Patients were instructed to avoid food or beverage ingestion an hour prior to saliva collection, and no mechanical cleaning and/or mouth washing was allowed in the same amount of time. All the saliva collected was then mixed, to have the same composition in each sample, and redistributed within the designated sterile test tubes. Prior to sample collection, supramucosal plaque was collected from the upper and lower first molars using teflon curettes, and was added to test tubes every five days, in order to keep an active culture medium.

Table I. Inclusion and exclusion criteria.

No clinical signs of oral mucosal diseases
No clinical signs of inflammation
Maximum gingival sulcus depth of 3 mm
No cavities or active white spots
Not pregnant or lactating
No antibiotic treatment in the previous 3 months
Non smokers
Absence of uncontrolled systemic diseases
Absence of dental implants
Absence of metal reconstructions within the mouth
Signed the informed consent form

Sample

A total of 60 dental implants were tested, 30 for each type of implant, divided into 2 macro groups:

- Group A: implants with surfaces sanded with aluminium oxide medium grade particles (250 μm) and etched with a solution of hydrofluoric acid at 3% and nitric acid at 30%, with a fixture's core made by commercially pure titanium grade 4.
- Group B: implants with surfaces formed by micro-sanding with calcium phosphate powders, followed by acidification and cleaned in a clean room. All samples were immersed, using sterile materials in a sterile environment under a hood, in 20 ml of human saliva. Twenty implants, ten for each group, were used as control and were immersed in 20 ml of sterile saline solution (0.9% NaCl). All samples were then placed in an incubator at 37°C for 6 months. Macroscopic variations of sediment and liquid were recorded before the constitution of the samples (T0), on day 1 (T1), on day 3 (T2), after one week (T3), on day 14 (T4), after 3 months (T5) and after 6 months (T6). Using sterile pipettes, 3 ml of liquid and material contained were collected on day T0, T1, T2, T3, T4, T5, T6 and the presence of metal particles was evaluated via inductively coupled plasma mass spectrometry (ICP-MS). The analysis on day T0 (saliva only) was aimed at detecting any

metallic ions already present in saliva before the experiment. Finally, two test tubes were filled with 20 ml of saliva each, left in the incubator at 37°C for the entire duration of the experiment and analyzed via ICP-MS on the following six months, to evaluate any variation in the sediment and saliva composition.

ICP-MS Analysis

The saliva samples were defrosted at room temperature. 250 µL of saliva was retrieved for removal of organic content using 15 µL of ultrapure nitric acid, 69.0-70.0% Sigma-Aldrich (St. Louis, MO, USA). The mixture was agitated until it presented a homogeneous aspect on visual examination. The Eppendorf tube (Eppendorf, Hamburg, Germany) was opened and put in an oven at 60°C for two hours. After heating and consequent evaporation of the organic part, the Eppendorf tubes (Eppendorf, Hamburg, Germany) presented various levels of volume. To maintain the original concentration proportional to volume, ultra-pure deionized water (Milli-Q water, Merck KGaA, Darmstadt, Germany), acidified with nitric acid at 3%, was added to all samples, until they had the same volume of 5 mL. Standard solutions were prepared in 5% v/v nitric acid, from a 100 mg/L ICP-multi element standard solution (XVI Sigma Certipur R, Merck KGaA, Darmstadt, Germany) containing 21 elements at 100 mg l-1 (100 ppm). The final calibration range was 0.05-10 mg/ L. A 0.5 mg/L standard was run at the beginning and the end of the process in order to check for drift. A volume of Rh-standard solution was added as internal standard to have 10 g l-1 (10 ppm) Rh concentration in every sample and reference solutions. Quality control (QC) samples were prepared using blank saliva, analyzed either as a blank or spiked with 10 mg/L standard solution. For "Device" QCs, 1 mL of saliva was sampled from a plastic beaker using the sampling device. The device was stored overnight at 20°C and then prepared. For "Fresh" QCs, 1 mL spiked saliva was added to 1 mL ultrapure water, in order to replicate the volume of buffer in the device, and, then, mixed. "Fresh" and "Device" QCs (blank and 10 mg/L spike) were analyzed at the beginning and the end of the analysis and every 10 samples. The diluted saliva samples were analyzed using a Thermo Scientific XSERIES2 ICP-MS instrument (Thermo-Fisher Scientific, Waltham, MA, USA) at the Department of Earth Sciences at "Sapienza" University of Rome (Rome, Italy). The instrument was tuned on a daily basis to ensure optimization. The instrument was set up with direct nebulization in normal mode with optimized conditions. Extraction voltage was set at -100 V, Rf Power 1400 W, focus voltage 12.0V and nebulizer gas flow rate 0.83 L/min. Dwell times were 100 ms for trace elements. Concentrations of Ni, Ti and V were quantified in µg/L.

Statistical Analysis

Descriptive statistics (mean, frequency, range, standard deviations) were computed for each variable of the study. LSD post-hoc test was used for Analysis of Variance (ANOVA), which was performed examining differences by group and time for primary outcome variables. A p-value <0.05 was considered significant. Specific statistical software (IBM SPSS V10 Statistics, IBM, Armonk, USA) was used to analyze data.

Results

A total of forty dental implants immersed in human saliva were tested and divided in two groups, Group A and Group B, while twenty dental implants were immersed in physiologic solution: CGA (control Group A) and CGB (control Group B). Results showed the presence of particles of Al, Mn, Cr, Fe, Co, Cu, Zn, As, Rb, Sr, Mo, Sb, Cs, Hg in human saliva at T0; titanium and nickel levels were <0.001 µg/l, while vanadium was detected in small quantity (0,109 µg/L) (Table II). In the experimental groups, dissolution of metallic particles of titanium and nickel, absent in human saliva (T0), were found after one week (T3) for Group B and after 3 months (T5) for Group A (Table III). Vanadium was already detected in small concentrations both in Group A and Group B after 1 day (0,103 *vs.* 0,126 µg/l) with no statistically significant difference (p>0.05). However, while for Group A the quantity tended to remain stable at every interval of time, for Group B there was an exponential growth (p<0.05). Complete absence of metallic particles was showed in both control groups.

Table II. Quantity of titanium, nickel and vanadium in human saliva at T0 (time of samples constitution) and at T6 (after 6 months). Values in µg/L.

	Ti	Ni	V
T0 s	<0.001	0.001±0.001	0.109±0.002
T6 s	<0.001	0.001±0.002	0.118±0.003

Table III. Inductively coupled plasma mass spectrometry (ICP-MS) analysis; titanium (Ti), vanadium (V), nickel (Ni). Values in μg/L.

	Ti	Ni	V
GCT1A	<0.001	<0.001	<0.001
GCT1B	<0.001	<0.001	<0.001
T1A	<0.001	<0.001	0.103±0.002
T1B	<0.001	<0.001	0.126±0.003
GCT2A	<0.001	<0.001	<0.001
GCT2B	<0.001	<0.001	<0.001
T2A	<0.001	<0.001	0.142±0.012
T2B	<0.001	<0.001	0.137±0.021
GCT3A	<0.001	<0.001	<0.001
GCT3B	<0.001	<0.001	<0.001
T3A	<0.001	<0.001	0.125±0.023
T3B	0.125±0.003	0.249±0.034	0.236±0.032
GCT4A	<0.001	<0.001	<0.001
GCT4B	<0.001	<0.001	<0.001
T4A	<0.001	<0.001	0.137±0.033
T4B	0.186±0.012	0.301±0.047	0.358±0.041
GCT5A	<0.001	<0.001	<0.001
GCT5B	<0.001	<0.001	<0.001
T5A	<0.001	<0.001	0.108±0.0573
T5B	0.216±0.054	0.518±0.059	0.587±0.0531
GCT6A	<0.001	<0.001	<0.001
GCT6B	<0.001	<0.001	<0.001
T6A	<0.001	<0.001	0.331±0.0632
T6B	0.253±0.032	0.726±0.067	0.674±0.0642

Discussion

Although titanium alloys are highly corrosion-resistant because of the stability of the TO2 oxide layer, *in vitro* they are not completely inert to corrosive attack[7,18,19]. According to our results, all implants immersed in human saliva already released metallic particles of titanium, nickel and vanadium after 7 days, with Group B showing statistically significant higher values than Group A at all intervals of time ($p<0.05$). Dental implants immersed in sterile saline solution showed no significant ($p>0.05$) dispersion of metallic ions, therefore assuming an active role of natural organic and inorganic constituents of human saliva in the corrosion process. Martin-Camean et al[28,29] in investigated the *in vivo* dissolution of different metallic ions from orthodontic mini-implants in oral mucosa, using ICP-MS. They detected only traces of V, while Cu, Al and Ti showed statistically significant higher values. The same authors conducted a review of *in vivo* and *in vitro* studies[30] to asses the cytotoxic and genotoxic effects of metallic ions dissolution of orthodontic devices; they highlighted lack of *in vivo* studies, necessity to conduct further research on the field, focusing on oxidative damage. To the best of the author's knowledge, this is the first article to report statistically significant dissolution levels of V ions in human saliva by ICP-MS: implants of group B, with a microsanded and acid-etched surface, showed increasing values of V at all intervals of time. Vanadium is ubiquitous in fresh water and soil, representing the 21st most abundant element on Earth's crust, and its systemic effects are still controversial and debated in medical and pharmacological literature: several authors have reported use of V for diabetes mellitus, revealing encouraging results in phase II studies, while others have correlated exposition to vanadium compounds with lung cancer[31-34]. Research on corrosion behavior of dental implants is being extensively conducted, nowadays, to assess its role in peri-implantits pathogenesis: in a recent case-control study, Safioti et al[35] hypothesized for the first time an association between higher levels of titanium dissolution and dental implants affected by peri-implantitis. They correlated their findings with the work of several authors, who investigated the immunogenic effects of titanium corrosion[36-38] showing an increased release of inflammatory cytokines (TNFα, IL-1 and IL-6) from the surrounding host cells. This may lead researchers to conduct further and extensive investigations to demonstrate the association between titanium and vanadium dissolution and peri-implantitis and its mechanisms, to develop long-term successful treatment strategies.

Conclusions

Preliminary results of our *in vitro* study reported significant values of Ti, Ni and V released by micro-sanded and acid-etched dental implants immersed in human saliva, showing for the first time statistically significant values of V. Authors speculate, in accordance with recent studies, that might be an association between metallic ions dissolution and peri-implantitis pathogenic mechanisms. However, further investigations with an *in vivo* design, larger sample and long-term follow-up are necessary to validate this hypothesis.

Conflict of Interests:

The authors declare they have no conflict of interest; they gave their final approval to the manuscript and agreed to be accountable for all aspects of the work.

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