Acta Biomaterialia 48 (2017) 445–450

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

# The bone-implant interface of dental implants in humans on the atomic scale

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ARTICLE INFO

*Article history:*
Received 9 August 2016
Received in revised form 27 October 2016
Accepted 17 November 2016
Available online 19 November 2016

*Keywords:*
Osseointegration
Atom probe tomography
Transmission electron microscopy
Titanium implant
Dental implant

ABSTRACT

Osseointegration of dental implants occurs on a hierarchy of length scales down to the atomic level. A deeper understanding of the complex processes that take place at the surface of an implant on the smallest scale is of interest for the development of improved biomaterials. To date, transmission electron microscopy (TEM) has been utilized for examination of the bone-implant interface, providing details on the nanometer level. In this study we show that TEM imaging can be complemented with atom probe tomography (APT) to reveal the chemical composition of a Ti-based dental implant in a human jaw on the atomic level of resolution. As the atom probe technique has equal sensitivity for all elements, it allows for 3 dimensional characterizations of osseointegrated interfaces with unprecedented resolution. The APT reconstructions reveal a Ca-enriched zone in the immediate vicinity of the implant surface. A surface oxide of some 5 nm thickness was measured on the titanium implant, with a sub-stoichiometric composition with respect to $TiO_2$. Minor incorporation of Ca into the thin oxide film was also evident. We conclude that the APT technique is capable of revealing chemical information from the bone-implant interface in 3D with unprecedented resolution, thus providing important insights into the mechanisms behind osseointegration.

**Statement of Significance**

Osseointegration of dental implants occurs on a hierarchy of length scales down to the atomic level. A deeper understanding of the complex processes that take place at the surface of an implant on the smallest scale is of interest for the development of improved biomaterials. To date, transmission electron microscopy (TEM) has been utilized for examination of the bone-implant interface, providing details on the nanometer level. In this study we show that TEM imaging can be complemented with atom probe tomography (APT) to reveal the chemical composition of a Ti-based dental implant in a human jaw on the atomic level of resolution. Correlative microscopy ensures the accuracy of APT reconstructions and helps provide both chemical and structural information of the bone-implant interface on the smallest of length scales.



## 1. Introduction

Titanium is widely used for surgical implants in the dental and orthopedic field [1]. The material has unique biocompatible properties also described as osseointegration [2]. Furthermore it provides low toxicity and good long-term mechanical stability [3]. A thin passivizing oxide scale is formed on titanium at ambient conditions, which inhibits release of Ti ions from the implant surface. Therefore, the surrounding biological medium will see and interact with an oxide. The native oxide that forms on titanium surfaces upon exposure to air is $TiO_2$, but lower oxidation states such as $Ti_2O_3$ and TiO are also observed to exist at ambient conditions [4–7]. It is expected that the surface oxide that forms at room temperature is a few nanometers thick, often sub-stoichiometric and contain large amounts of defects if at all crystalline [8–10].

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http://dx.doi.org/10.1016/j.actbio.2016.11.044
1742-7061/

The chemical composition of the oxide is believed to be of importance for the osseointegrating properties as it will influence protein adsorption onto the exposed surface immediately following surgical placement [11,12]. The adsorbed proteins elicit an immunal response to the implant, eventually leading to the recruitment of osteoblasts to the surface region [13–15]. The oxide surface carries a negative charge at physiological pH [16]. It has been suggested that this promotes the formation of an amorphous calcium compound at the implant surface, which acts as a precursor for subsequent apatite nucleation [17]. More generally, biochemical bonding at the bone-implant interface has been proposed to enhance the osseointegrating properties [18]. Such bonding would be strongly dependent on the implant surface chemistry, which has been shown experimentally in comparative *in vivo* studies [19,20]. Some studies suggest incorporation of Ca and P from the bone mineral into the oxide layer after long-term healing *in vivo* [21,22].

The topography of the implant surface plays an important role for the success of an implant; osseointegration occurs on a hierarchy of length scales from clinical parameters i.e. marginal bone levels and resonance frequency analysis down to macroscopic and finally to nanometer level [23]. Analysis on the smallest length scales has customarily been performed with transmission electron microscopy (TEM), where technical advances in recent years has allowed for increasingly sophisticated methodologies to be implemented [24–27]. In order to further expand knowledge of the processes occurring in the contact zone on the nanoscale however, new high-resolution methods for interface analysis are sought [28]. In recent years, a number of studies have employed atom probe tomography (APT) as an analytical tool for nanoscale chemical analysis of various biomaterials, such as the chiton tooth [29], bone [30,31] and enamel [32,33]. Herein, we examine the interface from clinical healing, via histological analysis and finally correlation by means of TEM with atom probe in order to study the interface between a titanium-based implant and human bone tissue. As APT has equal sensitivity for all elements across the periodic table, it has the potential to elucidate the chemistry of the interface, as well as the phases in its vicinity with high precision. However, as the porous nature of bone tissue precludes accurate 3D reconstructions of the APT data, the validity of initial biomaterial studies can be questioned. In addition, interfaces between materials can present challenges as the two phases may exhibit heterogeneous field evaporation behavior, leading to diffuse boundaries. Therefore, correlative imaging of the atom probe samples is necessary to ascertain precision in the APT reconstructions.

In this paper we follow the hierarchy from clinical healing, via histological analysis and finally correlation by means of TEM with atom probe in order to study the interface between a titanium-based implant and human bone tissue. A deeper understanding of this interface is of significant interest. The transition from implants with a traditionally machined surface into modified more rough surfaces i.e. enhanced $TiO_2$ layer, has indicated these surfaces might demonstrate different biological behaviors [34]. However these mechanisms are not yet fully understood. It is also of fundamental interest to describe the interaction at the interface in order to gain a better understanding of the initiation of pathological processes around implants such as marginal bone loss and eventually loss of integration. These mechanisms are not properly understood today [35].

## 2. Materials and methods

A dental implant (3 mm in diameter) (Straumann Standard Plus, Straumann AG, Waldenburg, Switzerland) with a sand-blasted acid etched surface (SLA) was collected from the registered biobank for human retrievals at department of Biomaterials, University of Gothenburg Sweden (Biobank no 513). The analyzed implant was removed due to technical failure of the prosthetic connection, not affecting the osseointegration after 7 years in clinical function. The explantation was conducted by means of a 4.2 mm trephine bur (Straumann AG, Waldenburg, Switzerland). The specimen was stored in 10% buffered formaldehyde. After dehydration in graded series of ethanol, they were embedded in light curing methacrylate (Technovit® 7200 VCL, Kullzer and Co, Wejrheim, Germany). The implant was prepared by using a sawing and grinding technique (Exakt® Apparatebau, Norderstedt, Germany). Ground sections of approximately 10 μm were made and stained with 1% toluidine blue and Pyronin G. The sections were imaged and analyzed in a light microscope (Nikon Eclipse 80i, Tekno Optik AB, Göteborg, Sweden) using ×1.8 to ×100 magnification connected to a personal computer with a software for morphometry (Easy Image Measurement 2000 Tekno Optik AB, Göteborg, Sweden.

Morphometric measurements of the following dimensions were made: The specimen was observed along its full length. The measurements of bone-to-implant contact (BIC) and the bone fill area (BA) within the threads were calculated on the mesial and distal aspect of the specimen. A total mean value was then calculated for the entire specimen. All patients that are donating implants to the biobank are informed that the implant, otherwise disposed, would be used for histological analysis, and has given their signed informed consent. The study followed the principle stated in the Declaration of Helsinki.

## 3. Atom probe tomography

An atom probe is a point projection microscope based on the process 'field evaporation' of surface atoms (and molecules) from a needle-shaped sample [36]. A DC potential on the order of kilovolts is applied to the sample, generating a strong electric field around its surface. Illumination of the tip with short laser pulses will then ionize surface atoms, which are subsequently accelerated towards a position sensitive time-of-flight detector. The chemical identity of each ion is determined from its flight time, the lateral positions in the sample are computed from the impact point at the detector and depth coordinates are calculated from the sequence of detection. This enables 3D reconstructions of the analyzed tip, which in ideal cases reach sub-nanometer resolution. An atom probe analyses typically contain several millions of ions, thus encompassing volumes on the order of $50 \times 50 \times 200\ nm^3$.

TEM foils and APT needles were prepared using conventional *in situ* liftout techniques [37,38] in a combined focused ion beam scanning electron microscope (FIB-SEM) from cross sections of the implant-tissue interface region. Samples were attached to special Si-based grids, compatible with both FEI TEM systems and APT instruments of the LEAP model. Needle-shaped specimen were fashioned using a monoisotopic $Ga^+$ beam operated at 30 kV acceleration voltage, by milling annular patterns with decreasing radii and beam currents. A final polishing step at 2 kV and 8 pA was performed. Atom probe tips and TEM foils were subsequently imaged in an FEI Titan microscope (FEI, the Netherlands) operated at 300 kV acceleration voltage in bright field TEM mode or scanning mode (STEM) using a high angle annular dark field detector (HAADF).

Atom probe analysis was performed in a LEAP 3000X HR (Imago Scientific Instruments, US) instrument equipped with a green laser ($\lambda = 532$ nm). The base temperature of the tips was held at 30 K during analysis. Field evaporation was initiated using laser pulsing at 100 kHz frequency with pulse energies between 0.3 and 0.5 nJ. A pressure below $10^{-9}$ Pa was maintained in the analysis chamber.

The evolution of the geometry of the needle-shaped sample is of great importance for the interpretation of the APT data, as it