Research article

Interfaces in osseointegrated dental implants and a new inverted approach to their microscopic and histological study

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Abstract

Background and objectives. The various techniques for the analysis of the bone/implant interface *in vivo* are incomplete and do not allow to have a full vision of the osseointegration process. In this article, we present a new inverted approach for the study of the osseointegration of dental implants, based on the chemical deep etching of titanium-made implants prior to microscopic and histological evaluation.

Materials and Methods. The method was tested on 18 implants placed in 6 dogs. Bone/implant blocks were collected at 1, 3 and 6 months after implantation respectively. The titanium was chemically removed from the interface, leaving bone tissue intact. Once metal was removed, bone tissue was analyzed macroscopically and microscopically with a Scanning Electron Microscope, and then decalcified and used for histological analysis.

Results. The process of implant integration into the bone tissue was followed and analyzed, and clear patterns were observed at 1 month, 3 months and 6 months after implantation respectively. After 1 month, the bone/implant interface was still very immature. After 3 months, the bone was already quite mature and organized. After 6 months, the external bone layer on the bone/implant interface appeared in its final osseointegrated form.

Discussion and Conclusion. This inverted method of analysis of osseointegration offers interesting results and a new insight in the illustration of the healing of the bone/implant interface after implantation. Further research is needed to use this approach for a quantitative evaluation of different implant surfaces and designs.

Keywords. Dental implants, materials testing, maxilla, titanium.

1. Introduction

Despite the clinical success in using dental implants made of titanium and its alloys, there is still a great need for the improvement of implant materials and designs **[1]**. One important field of research is the development of new surfaces and macrodesigns, in order to promote a stronger and quicker osseointegration, i.e. an optimization of the bone/implant

interface **[2]**. In the broad sense, an interface represents a border between interacting independent objects. From this perspective, the term is appropriate to describe dental implant interactions with the jaw bone, oral mucosa, abutments, prosthetic superstructures and also teeth surrounding the implant-supported rehabilitations. Interfaces are everywhere in the oral cavity, and many of them are much more complex than the implant/bone interface.

The evaluation of the bone implant interface parameters seems to be very well documented in the literature **[2]**, as the development of new implant surfaces and design is an important research topic sponsored by many dental companies. The techniques to characterize a surface are already well known, even if their use remains not frequent enough **[1,3,4]**, and *in vitro* analyses are also widely used **[5]**. However, the number of techniques to evaluate this interface *in vivo* is actually very small. It is mostly implant torque removal (biomechanical evaluation of the strength needed to break the bone/implant interface)**[6]** and bone/implant histology through the use of undecalcified specific histological procedures **[7]**. Both systems are incomplete and need to be combined to reach reasonable scientific conclusions **[2]**. On one hand, the torque removal gives interesting information on the biomechanical characteristics of the interface, but the results are too often of relatively weak statistical significance and the method does not allow to examine and understand the reasons of the observed results **[6]**.

On the other hand, the bone/implant undecalcified histological analysis is intrinsically of limited analytical relevance: the cutting-grinding histological technique used to cut bone and implant together only allows to obtain 1 or 2 good histological slides for each analyzed implant **[7]**. It means that researchers can only observe one axis of the osseointegrated implants, while the osseointegration process may be very different in other area of the implant periphery. In fact, most of the data are lost with this histological technique, but this is the only method available. Even with many samples and a good theoretical statistical significance, the concept itself of this histological method is a limitation for the interpretation of these data.

To analyze the osseointegration on the whole implant periphery, some authors suggested to use physical non destructive techniques such as synchrotron radiations **[8]** and micro CT scanners **[9]** in order to reconstruct the whole osseointegrated interface around the implant. However, these techniques have also their limits, related to the physical behavior of the implant material itself (particularly its absorbance). Artifacts are numerous and make the accurate analysis of the whole interface difficult **[10]**.

Finally, it is always recommended to combine these various techniques in order to improve the significance of any study about the bone/implant interface **[2]**. Even if the literature about dental implant surfaces is wide, it remains very contradictory and difficult to interpret, due to these technical limitations to investigate the interfaces with quantitative analysis. However, even with their limits, these techniques are needed to explore the characteristics of the interface parameters, and to assess the reliability and effectiveness of these interfaces for the purpose of manufacturing implants suitable for clinical use.

In this first article, we present a new approach for the study of the osseointegration of dental implants. This approach is based on chemical deep etching of Titanium-made implants. In this method, the titanium is chemically removed from the interface, leaving bone tissue intact. Once metal is removed, bone tissue can be decalcified and used for microscopic study. Using this method we were able to follow the implant integration into the bone tissue for up to 6 months.

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2. Materials and Methods

Here we utilized a new concept to study the bone/implant interfaces, where the interface is analyzed after the non-traumatic removal of the implant material from the test bone sample.

The essence of this method is to remove the titanium without damaging the bone tissue. Each bone block containing an osseointegrated titanium implant was washed in Phosphate Buffer Saline (PBS, pH 7.4) and placed in a special solution (19.6% hydrofluoric acid, 8.9% metallic zinc, 71.5% ethylene glycol). The composition of the solution was specifically designed to remove titanium-made implants from the bone tissue blocks. Titanium reacts readily with weak acids in the presence of complexing agents. Each bone block was incubated in this solution for 30 days allowing chemical etching of the titanium. At the end of the chemical etching, the titanium implant was removed from the contact interface, leaving surrounding bone tissue preserved (patent number 2464646 from October 20th, 2012). Remaining bone tissue could then be further processed to remove the bone mineral component (decalcified samples) and utilized for an extended histological evaluation.

In this preliminary study, this method of analysis of the osseointegration of titanium implants into the bone tissue was tested in a dog model. A total of 6 dogs were involved in this study according to the local research ethics committee (protocol 6, 07/26/2012). Eighteen experimental grade 4 titanium implants were installed in the lower premolar regions of 6 dogs. All procedures were performed under general anesthesia. Six implant/bone samples were collected and analyzed at each experimental time, respectively after 1, 3 and 6 months of healing. At each time, bone blocks with the integrated implants were cut out of the dog mandible under general anesthesia **(Figure 1)**. Then each sample was cut individually, washed in PBS and prepared for the deep etching process of the implant titanium material. After etching, the analysis of the bone blocks was performed in 3 phases, including:

- Phase 1: the macroscopic evaluation **(Figure 2A)**,

- Phase 2: Scanning Electron Microscopy (SEM) evaluation of each sample, to analyze the microscopic aspects of the bone interface **(Figures 2B to 2D)**,

- Phase 3: histological examination, after decalcification of the bone samples with a 10% EDTA (pH 7.4) solution **(Figure 3)**. Samples were embedded in the paraffin and 10 micrometers thick histological cuts were stained with hematoxylin/eosin solution or Van Gieson's staining.

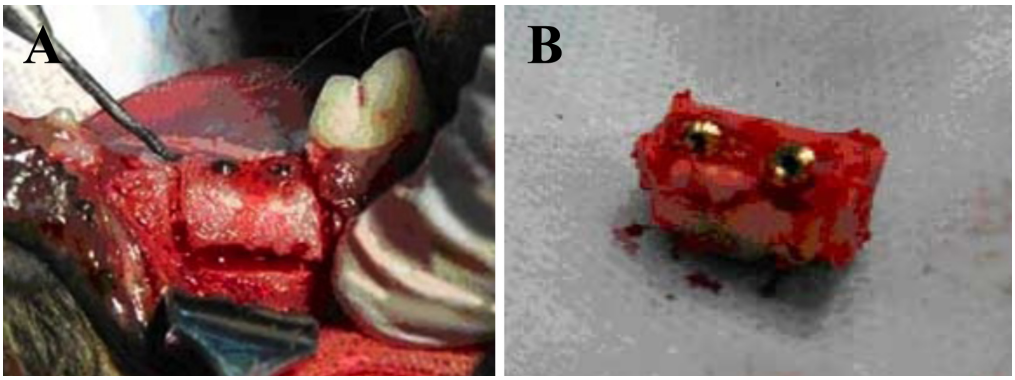

Figure 1. Experimental surgical model. The bone blocks containing the osseointegrated implants were collected from the dog lower jaw, after cutting with a bur and lifting with a chisel.

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3. Results

In the first phase of this sample analysis, the macroscopic evaluation revealed the general aspect and patterns of the osseointegrated interface between the threaded surface of the implant and the bone tissue **(Figure 2A)**. We can consider at a macroscopic level that the clear imprint of a screw implant shape within the bone block is a characteristic feature of its osseointegration.

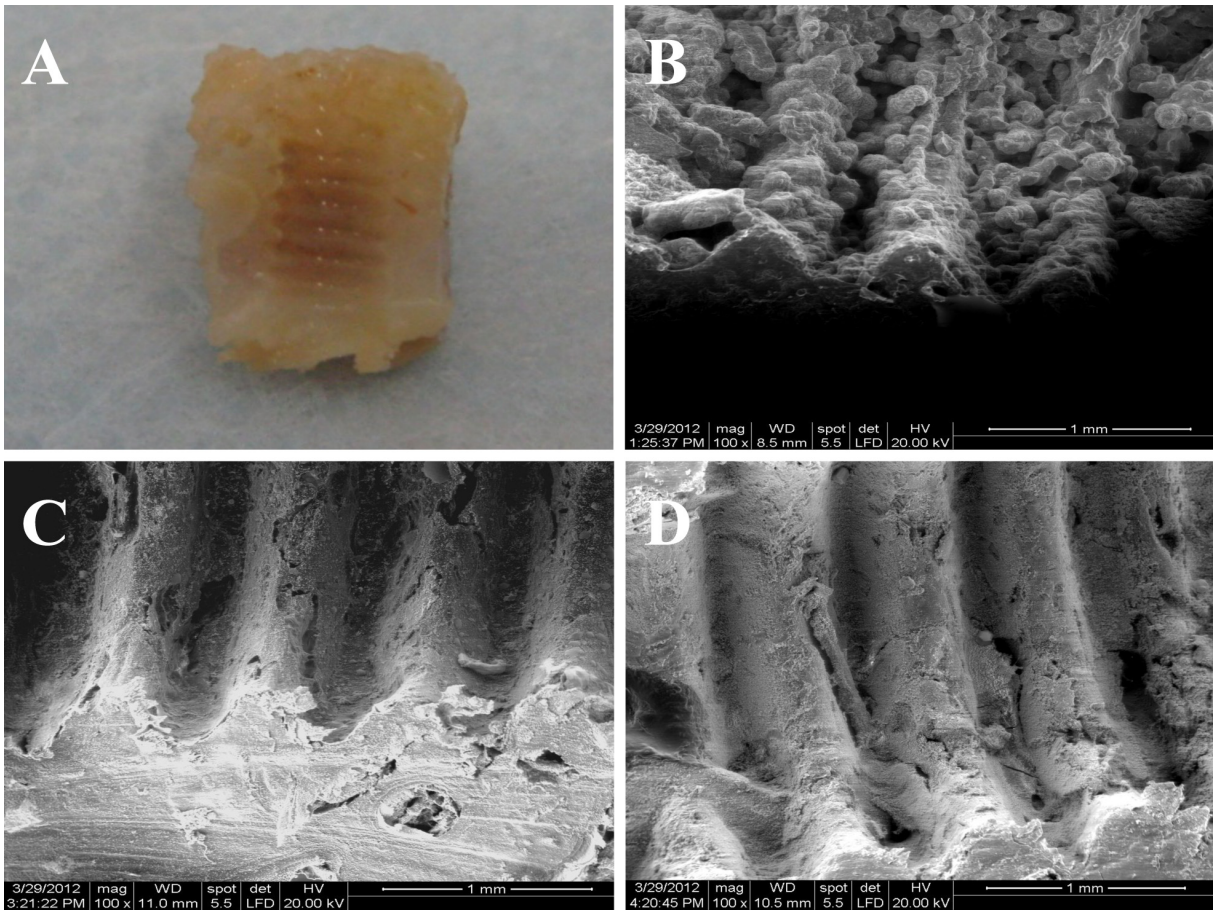

Figure 2. Scanning Electron Microscopy (SEM) of the samples. (A) After etching and removal of the implant materials, the shape of the implant screw threads was distinctly visible on the walls of the bone block and pointed out the area of the implant osseointegrated interface. **(B)** SEM analysis of the bone tissue collected 1 month after implantation. Early shape of the bone growth and remodeling between the implant threads was already visible on the sample. **(C)** SEM analysis of the bone tissue collected 3 months after implantation. After 3 months of healing, a complete bone volume was built between the implant threads and was observed as an imprint of the screw pattern of the test implant. At this time, the osseointegrated interface appeared already quite continuous. **(D)** SEM analysis of the bone tissue collected 6 months after implantation. The bone tissue at the osseointegrated interface appeared homogeneous and repeating exactly the shape of the implant threads.

In the second phase of this sample analysis, we conducted a scanning electron microscopy evaluation of the bone blocks collected at 1, 3 and 6 months after the implantation. One month after implantation, the bone tissue interface started to follow the general shape of the implant threads, but the "bone carving" was still incomplete. Bone was

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growing between the implant threads but patterns of the implant design were still not fully reproduced **(Figure 2B)**. Three months after implantation, the bone interface appeared like an exact imprint of the implant macrodesign, and the patterns of the screw threads were fully visible on the bone surface **(Figure 2C)**. The bone external surface appeared compact, proving that a dense cortical bone was formed at the bone/implant interface to create a continuous osseointegrated interface. Six months after implantation, the bone interface appeared even more cortical and homogeneous than after 3 months, but the general characteristics of maturity were very similar between the 3 months and the 6 months experimental times **(Figure 2D)**.

The histological analysis of the samples confirmed the same evolutions of the peri-implant bone remodeling **(Figure 3)**. During the first month, the peri-implant bone in the upper and middle segments of the implant was disorganized as a fibrous and granulation tissue with lymphoid and histiocyte infiltration, while the presence of connective tissue and separate bone beams was identified in the lower segment of the peri-implant bone tissue. Three months after implantation, the substitution of fibrous bone tissue for organized bone tissue was observed in the peri-implant area. Six months after implantation, the peri-implant bone tissue was organized as a mature lamellar bone.

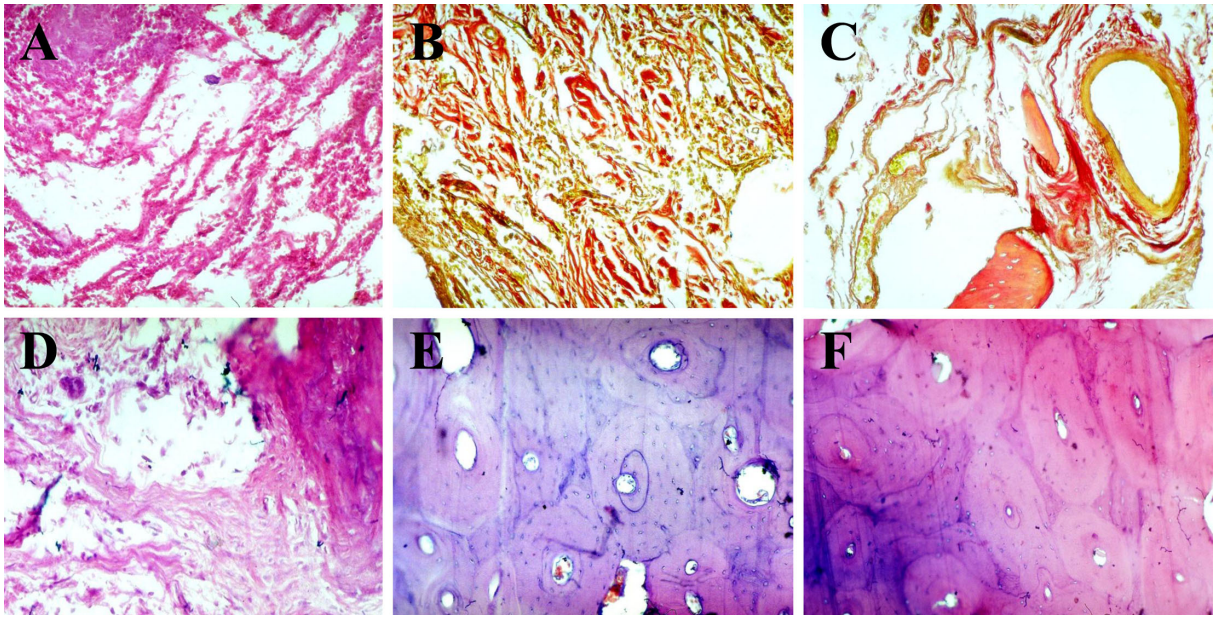

Figure 3. Histological analysis of the bone samples collected 1, 3 or 6 months after implantation (magnification x400). (A, B) One month after implantation, it was observed granulation tissue with lymphoid and histiocyte infiltration of the upper segment (**A**, hematoxylin/eosin staining) and of the middle segment (**B**, Van Gieson's staining) of the implant. **(C)** One month after implantation, microscopic analysis of the lower segment of the peri-implant bone tissue revealed the presence of connective tissue and separate bone beams (Van Gieson's staining). **(D)** Three months after implantation, microscopic analysis of the upper segment of the peri-implant bone tissue revealed the substitution of fibrous bone tissue for organized bone tissue (hematoxylin and eosin staining). **(E, F)** Six months after implantation, mature lamellar bone was detected through microscopic analysis of the peri-implant bone tissue (hematoxylin and eosin staining).

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4. Discussion

The results of this study illustrate the steps of the osseointegration of screw implants and also a new inverted approach to analyze this process. In this method, the osseointegration of a screw implant can be defined as the step when all the space between the implant and the osteotomy walls (particularly the space between the threads) is filled with newly formed mature bone tissue, and when the bone tissue accurately repeats the geometry of the implant, like a mirror image of the implant shape. When osseointegration is reached, an exact imprint of the screw design and a continuous and compact external bone surface can be observed at the interface on the bone samples.

Osseointegration was initially defined as an experimental observation of ankylosis of titanium implant in bone **[1]**. In this study, we illustrate a new concept that defines osseointegration of screw implants as an experimental observation of complete bone growth and remodeling along the bone/implant interface. This definition remains quite theoretical, as the most important parameter remains the clinical evaluation of implant stability that allows to load it with a crown and to place it in function.

In this study, the tested samples needed 6 months to be fully osseointegrated following this concept, in the sense of obtaining a mature compact bone all along the implant surface. It is important to notice that this result is not exactly following the most recent advances in implant surfaces technologies and design, where osseointegration can be quicker. In this conceptual study, we used a simple screw-designed titanium implant and surface, to test the basic mechanisms of the analytical protocols, and it could be interesting to validate this method with various forms of surfaces **[11]** and designs, as it is commonly done with torque removal and bone/implant undecalcified histology **[7]**.

5. Conclusion

As a conclusion, the experimental morphological study of the integration of implants using the chemical etching method revealed some features of the bone regeneration around the threaded implant. This technique gives an original insight allowing to visualize the formation of mature compact bone all over the implants during the osseointegration process. This approach requires now to be validated as a comparative experimental tool between different implant designs and surfaces.

Disclosure of interests

The authors are the inventors of this technique and the related patent.

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