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Periodontology, Oral Surgery, Esthetic & Implant Dentistry Open

Volume 1, Issue 3, December 2013

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Table of Contents POSEIDO. 2013;1(3):131-94.

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Aims and Scope of the POSEIDO Journal

The POSEIDO journal focuses on all aspects of the interconnected clinical and research fields of periodontal sciences, oral and cranio-maxillofacial surgery and medicine, esthetic and restorative dentistry, with a particular interest in implant dentistry, and related research.

Most publications are connected to the dental and maxillofacial field, but some are also from orthopedics, material sciences or other scientific disciplines interconnected with the POSEID research topics (e.g. bone implantable materials, bone regenerative medicine strategies), in order to promote transversal translational research.

POSEIDO is organized as an info journal (international forum), and is therefore publishing a significant quantity of editorial material, as a basis of information, debate and discussion for our community. This editorial material takes particularly the form of **clinical case letters** and **research letters**.

The objective of this strong editorial section is to create links between international research teams, to organize our international research community and to develop a neutral international platform for the publication of debates and consensus conferences in the fast-growing and evolving fields of the POSEID disciplines.

The journal is also publishing a classical content with full-length articles (**original articles and reviews**), following a strict double peer-review process. The journal is particularly interested in original research articles and clinical studies about new techniques, biomaterials and biotechnologies with direct clinical applications in the interconnected fields of periodontology, oral surgery, esthetic and implant dentistry. Review articles are also welcome if they make the clear synthesis of debated topics.

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Table of Contents POSEIDO. 2013;1(3):131-94.

Special Theme: New perspectives in dental implant surfaces (Part 1)

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Special review: editorial of the POSEIDO SIREN

In dental implant surfaces, NanoWar has begun… but NanoQuest is still at stake!

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Abstract

Dental implant surface characteristics are defined by a quartet of parameters, respectively at the macro-, micro-, nano- and chemical scale. Many companies are now claiming to use nanofeatures on their implant surface, while in fact only a minority of products really displays significant nanostructures. In this article, the exact terminology of nanostructures was described, and many technologies developed to produce nanostructures on titanium implants were reviewed. Practically, only a few techniques are applicable on dental implants. The most frequent forms of nanofeatures encountered in dental implant surfaces are the nanoroughness (eg Intra-Lock Ossean, AstraTech Osseospeed) and the nanoparticles in various crystalline forms (eg 3I NanoTite, Straumann SLActive). Very little is known about the real impact of these nanocharacteristics, as an element of the quartet of parameters that influence the osseointegration. The first step is to clarify the definitions to avoid the commercial confusion.

Keywords. Dental implant, nanostructure, osseointegration, titanium.

1. NanoWar has begun…

Among the few characteristics that define a dental implant system, the surface treatment and the implant macrodesign are the 2 main parameters that define the bone implant interface, from the early bone peri-implant healing to the long-term stability of the osseointegrated interface **[1]**. Logically, as a key characteristic of each commercially available system, the implant surface is both a very active research topic and a strong commercial argument for companies playing in a very aggressive competitive and growing market **[2]**. Many companies used the arguments of the microtexture of the implant surface topography (microroughness for SLA-Sand-blasted Acid-Etched surfaces, microporosity of anodized surfaces) and/or of the use of chemical modifications (such as Fluoride low impregnation or Calcium Phosphate CaP deposition) in the past, in order to claim better biological interactions and faster and better osseointegration of the implants **[3]**.

In the last years, the use of the term « nano » became a real fashion, and many companies started to refer in their advertisements to the nanocharacteristics of their products, particularly of the dental implant surface. In this sense, on a very competitive

market, NanoWar has begun... The term « nano » started to be used in all circumstances, and it is clearly a source of abuse and confusions, playing on the meaning of this word. However, there is no doubt on the exact definition of nanocharacteristics, as described in engineering and traditional non-dental science, for example for the design of electronic chipsets **[3]**.

In a recent article, a system of characterization of dental implant surface was suggested, and it proposed a clear terminology to characterize the surface topography and patterns at the micro- and nanoscale, following the well defined rules used since many years in general surface science **[3]**. Then, in a following article analyzing in details 14 significant implant systems available on the market **[4]**, it was shown that only 4 of them had really characteristics that may be considered as nano **(Figures 1 and 2)**. Among these 4 systems, one surface is no more promoted due to mixed clinical results (Nanotite, Biomet 3I, Palm Beach Gardens, FL, USA)**[5,6]**, 2 were never copied due to the secret of their production (Osseospeed, AstraTech, Mölndal, Sweden, and Ossean, Intra-Lock, Boca Raton, FL, USA)**[7,8]** and one was considered « nano » because it was covered with dry NaCl crystals (SLActive, ITI Straumann, Basel, Switzerland)**[4]**. Most other dental implant systems available worldwide are often copying the main surfaces analyzed in this first article, what means that in fact only a few percentages of implant systems can claim to have some real nanofeatures.

Are you nano? Between commercialism and misunderstandings, the commercial war has started, but the quest for nanotextured implant surfaces is still uncertain.

2. What is exactly a surface nanostructure?

A nanostructure is an object of intermediate size between molecular and microscopic (micrometer-sized) scales, and measuring between 1 Angström (0.1nm) and 100nm. This range of size is clearly found in all the definitions in the various fields of nanosciences **[3]**.

Nanostructures must be described through their number of dimensions on the nanoscale.

- Structures with one nanometric dimension (a peak height or a layer thickness) are repeated to create a nanotextured surface. A repetitive and homogeneous texture with one dimension on the nanoscale was termed nanoroughness or nanorugosity.
- Structures with 2 nanometric dimensions (nanometric diameter of a repetitive pattern) are repeated to create a nanopatterned surface. Good examples are nanotubes produced by anodization or chemically produced nanopatterned surfaces, where a 2-dimension geometric form is repeated infinitely.
- Structures with 3 nanometric dimensions are nanoparticles, i.e. the particle is nanometric in each spatial dimension. A surface covered with particles of these dimensions is nanoparticled. Although the size of most molecules is within the range of a nanoparticle, individual molecules are not referred as nanoparticles.

The same range of size (between 1 Angström (0.1nm) and 100nm) can be applied to all forms of nanostructures encountered in chemical and material sciences: nanoclusters, nanocrystals, etc. When nanostructures are associated together, they can then create the various forms of nanosurfaces cited above, but also nanopowders and nanomaterials (mostly by association of nanoparticles).

A last form of nanostructure is the nanothickness coating of the surface, for example with a Calcium Phosphate CaP layer. In this situation, only a layer between 0.1nm and 100nm can be considered as really nano.

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These definitions are simple, neutral and based on the terminology used in nanosciences. All surfaces have per definition a topography at the microscale and at the nanoscale, respectively termed microtopography and nanotopography **[4]**. However, this nanotopography may be relatively smooth and may not present clearly identifiable, homogeneous and repetitive nanostructures. Without this kind of nanostructures, a surface can be considered to have no specific nanofeatures or nanocharacteristics, and therefore to not be « nano ». The term « nanosmooth » was advocated recently to describe the surfaces without significant nanofeatures **[3,4]**.

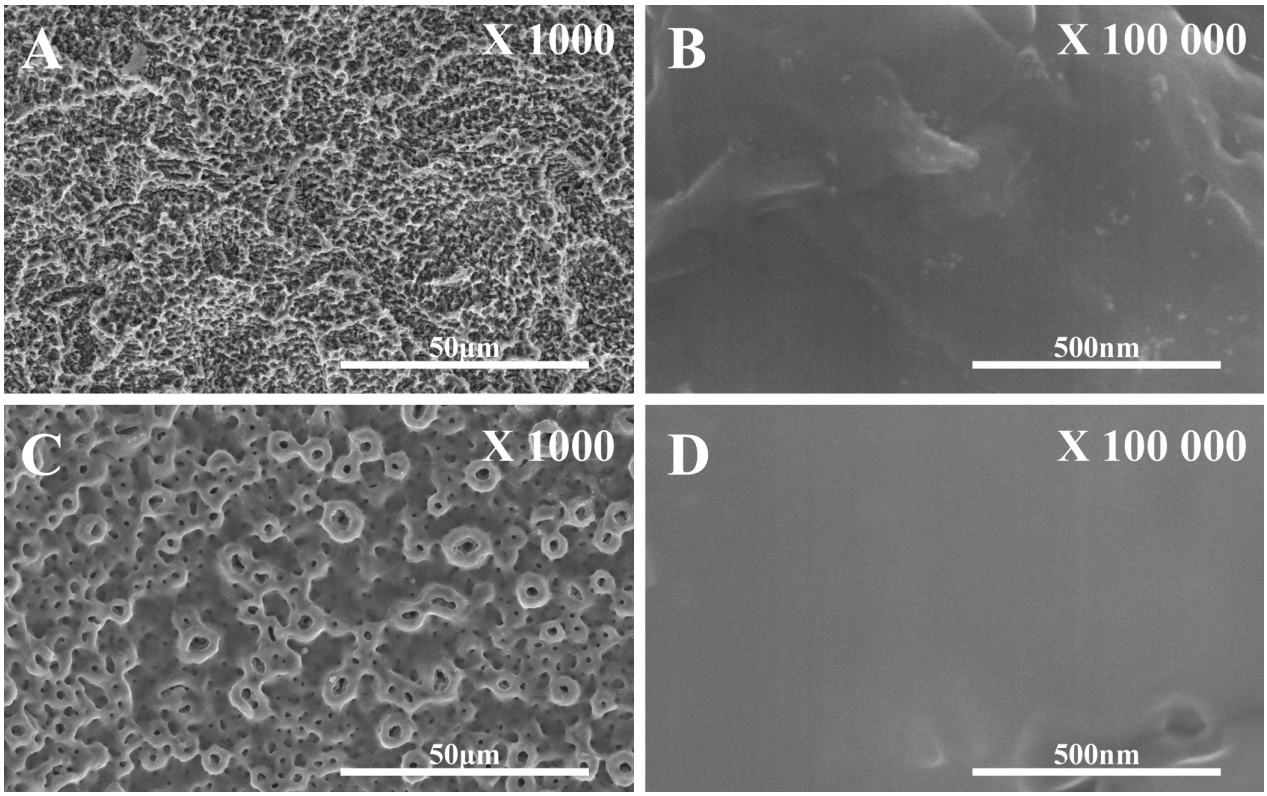

Figure 1. FE-SEM evaluation of some nanosmooth implants available on the market. (A) The Camlog surface (Camlog, Basel, Switzerland) is produced through a classical SLA (sand-blasting acid-etching) protocol. A significant microrough topography is produced. **(B)** At the nanoscale, the Camlog surface is nanosmooth. **(C)** The TiUnite surface (Nobel Biocare, Gothenburg, Sweden) is produced through anodization of titanium. During the process, a high impregnation with Phosphorus is obtained and large microporosities are produced, creating a specific micropatterning and extended cracks on the surface. **(D)** At the nanoscale, the TiUnite surface is completely nanosmooth.

Even with these clear definitions, it remains difficult to observe and characterize properly the nanostructures on dental implant surfaces. There is in fact no real accurate quantitative technique to evaluate the nanotopography on a microtextured surface, due to the interferences between the micro- and nano-architectures **[3]**. Repetitiveness and homogeneity are key parameters to define nanostructures as real nanofeatures, but are difficult to quantify and may be considered as qualitative morphological parameters.

The only instrument that allows to make a careful examination of these surfaces at the nanoscale is the FE-SEM (Field Emission-Scanning Electron Microscope)**[3]**. It offers a

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much higher resolution than a classical SEM with tungsten source **[4,9]**, and allows to get accurate pictures at the nanoscale **(Figures 1 and 2),** even on surfaces with chemical modifications provoking significant charging effects (for example surfaces with Calcium Phosphate impregnation or coating are associated with charging effects and artifacts during the surface mapping using a focused electron beam reflecting across the surface)**[1]**.

There is unfortunately a very common approximation observed in the literature concerning the method of analysis of nanostructures, as the basic Scanning Electron Microscope (SEM) is often used as the gold standard for morphology characterization at the micrometer level **[9]**, but also used to investigate the nanoscale. While in fact, a FE-SEM is required to observe and characterize the nanotopography and associated nanostructures. Finally, coupled with a metrology software, FE-SEM pictures with 3° tilting can be combined and computed to get a three-dimensional reconstruction of the nanostructures for a better evaluation, sometimes advocated as a (semi-)quantitative morphology analysis **[1]**.

Other techniques such as Atomic Force Microscope (AFM) or light interferometer (IFM) are not suitable for this evaluation of the nanostructures on dental implant surfaces. AFM is using a piezoelectric probe that cannot follow the nanotopography within the complex geometry of the implant microtopography. IFM has a similar problem of physical limit related to the wavelengths of light that do not allow to map the nanostructures completely and properly, particularly when it is hidden in the shadow of the microtextures used on dental implant surfaces **[3]**. About morphology, FE-SEM remains the key instrument to perform morphology characterization at the micro- and nanoscale, and topography quantification when using the adequate metrology software **[4]**.

3. What are the effects of the nanostructures?

It is commonly considered that 4 levels of interactions exist during the osseointegration of an implant in the bone: the macrostructures, the microtopography, the nanotopography and the chemical level **[3,10]**. Each level has a different form of interaction with the bone tissue and a different effect for the integration of the implant **[11]**.

The macrodesign and the surface microtopography have first of all a function in the biomechanical interlocking of the implant in bone **[3]**. The macro- and microscale architecture also defines the space available for bone cells to interact with the surface and organize the bone apposition and remodelling: some implants use macrodesigns with larger healing chambers between the implant threads to promote a stronger bone apposition **[12]**. The microtopography was also advocated to influence directly the cell behavior, depending on the kind of microstructures (microroughness, micropores, microparticles) and the spaces between peaks and valleys of the topography **[3]**.

The material chemistry is considered to be responsible of the biochemical interlocking of the implant in bone **[3,13,14]**. If most dental implants use titanium as a core material to promote an ankylosis of the implant, many surface chemical modifications were proposed to increase the bone apposition and remodelling process through cell stimulation and mineral chelation (for example Fluoride or Calcium Phosphate CaP low impregnation, or Calcium high impregnation)**[7,8,13]**. Some implants were even coated with CaP layers, Hydroxyapatite layers or CaP nanocrystals to promote this chemical bone bonding between the implant and the bone **[15]**.

What remains for the nanomodifications? The osseointegration performance of a surface is influenced by its topography at the nanoscale following different biological mechanisms than on the microscale **[16]**. The nanotopography is supposed to influence the

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surface energy and therefore the surface/protein interactions **[17]**. A significant surface energy allows to improve the surface wettability to blood and the adhesion and spreading of fibrin fibers and matrix proteins on the surface, and therefore to improve cell attachment and tissue healing, particularly during the early healing phases on the implant interface **[16]**. Many publications also suggested that a specific nanopatterning of surfaces even promotes cell proliferation and differentiation, through the direct modulation of cell behavior **[18-20]**. Among the many forms of nanostructures (nanoroughness, nanopatterning, nanotubes, nanoparticles)**[4]**, it remains however difficult to determine which nanofeature is the most efficient for the bone implant interface and is practically usable with adequate clinical results in dental implant surfaces, even if some excellent results were already reported with nanorough surfaces available on the market **[6,8]**.

One other specific aspect of nanofeatures is their potential impact in the peri-implant cervical area and the soft tissue attachment and peri-implant gingival sealing **[21]**, and more generally the effects of nanostructures to promote cell growth and reduce bacterial contamination in this competitive oral tissue area **[22,23]**. The implant surface is indeed a key factor of the peri-implantitis risk, peri-implantitis being often considered as a pathology of osseointegration **[24]**. Some recent results showed that nanoroughness applied in the implant cervical region is influencing positively bone maintenance **[21]**. It is interesting to notice that this implant system is commercially available, and using also this nanoroughness, combined with microroughness and chemical modification, on its bone/implant interface **[4,8,11]**. This result may be connected to several experimental results advocating that nanofeatures have a negative effect on bacterial growth and are a method to control bacterial contamination in many medical devices **[22,23]**.

4. Experimental and clinical results of nanofeatures on implant surfaces

There are in the scientific literature many articles describing various forms of nanofeatures prepared on implants and most of them were described as promoting positive effects *in vitro* and *in vivo* **[16]**. However, most of these works remain very experimental, and only few of them have concrete practical applications in products in current daily clinical use.

Among the many techniques that were described, we can for example cite the creations of nanotubes through anodization **[23,25,26]**. The process is quite similar to the anodization used to produce a specific microporous surface (for example the TiUnite system, Nobel Biocare, Gothenburg, Sweden)**[4,13]**, but using different parameters, the surface can be covered with lines of nanoporous structures only. One idea was even to dope the surface by filling these nanotubes with some drugs or minerals to boost chemically the osseointegration process through a slow release of a pharmaceutical preparation **[26]**. The system was considered with interest but finally abandoned for dental implants, as the lines of nanotubes are often considered too fragile to be used on oral implants enduring significant biomechanical constraints during the daily dental function. However, the cell modulation promoted by this kind of surface was described as very interesting to improve cell adhesion **[26]** and to reduce bacterial adhesion and proliferation **[23]** on titanium implantable devices and is still under evaluation in non dental applications like stents **[25]**, where no significant mechanical constraints are applied on the implant, reducing the risk of surface delamination.

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Another form of nanomodification was the discrete calcium phosphate nanocrystalline deposition on a titanium dental implant surface, to enhance the contact osteoconduction on these surfaces **[27]**, following the concept of chemical interlocking described as "bone bonding" **[28]**. Primary results were described as very promising and some famous implant system even used this technology to promote this concept of « bone bonding » (Nanotite, Biomet 3I, Palm Beach Gardens, FL, USA)**[4]**. However, the development of this technology was finally limited due to results inferior to other traditional non nanofeatured surfaces **[5,6]**. This result was never really explained scientifically, but was probably not related to the nanotexturing itself, but it was more probably due to the chemical composition of the nanoparticles (Calcium Phosphate percentage was more than 20% of the surface chemical composition)**[4]**. The same use of calcium phosphates or hydroxyapatites coating (for example nanopolymorphic crystalline hydroxyapatite coating)**[29]** was advocated by other authors, but the general fashion with these coatings is slowly declining nowadays, due to the lack of advantages (and sometimes the failures) observed in practical daily clinical results.

One very commonly tested method to create nanostructures is the chemical nanopatterning (for example oxidative patterning)**[19,30]**. In this domain, many *in vitro* results showed that repetitive nanopatterns created through the action of acidic-basic components are able to modulate positively the cell behavior **[30]**. Many works tried to analyze the cell gene expression in contact with various forms of nanopatterns, following the general concept of tissue engineering through nanoprogramming of the materials **[31]**. Some authors even tried to constitute theoretical libraries of potential nanopatterns and their *in vitro* cell effects **[32]**. The same kind of works can be found with other techniques of nanopatterning such as colloidal lithography **[33]**. These works are all very interesting, but remain quite far from the practical industrial applications, as dental implant surfaces are a 4-level system (macro, micro, nano, chemical) where all levels are interconnected and each level is influencing the effects of the other levels **[11]**.

Another experimental method of nanomodification is to use the crystal phases of titanium (rutile, anatase and amorphous titanium) to create a titanium film with a roughness of about 8-10 nm synthesized by magnetron sputtering **[34]**. First results highlighted that the nanoscale topography created with the anatase phase of titanium promoted the best *in vitro* effects in terms of cell adhesion, proliferation and differentiation **[34]**. This experimental result is difficult to apply on microrough dental implant technologies, but may give some explanation on the good results offered by some forms of nanotexturization **[8,11]**, as all surface treatments are also impacting the external crystal phase of the implant titanium core material **[3]**.

Another example is the coating of dental implant surfaces with various forms of hydroxapatites (HA). If most HA layers are micrometric, some recent developments proposed to cover the implant surface with layers of Calcium Phosphate ranging from 30-50 nm to 300-500 nm, through the use of IBAD (Ion Beam Assisted Deposition)**[15,35,36]**. If it is possible to call « nanocoating » a 30-50 nm layer, the 300-500 nm layer is clearly a micrometric modification **[15]**. Experimental results with these 2 surfaces were reported as different, while in theory they have the same external chemistry. This example demonstrated that the concept of scale of interaction nano/micro is very real when considering the behavior of the cells that will be in contact with the external layer. This kind of surfaces was marketed as a NanoTite or Integra-CP (Bicon, Boston, MA, USA), while in fact the technology is using a layer superior to 100nm thickness, which is therefore not nano **[4]**.

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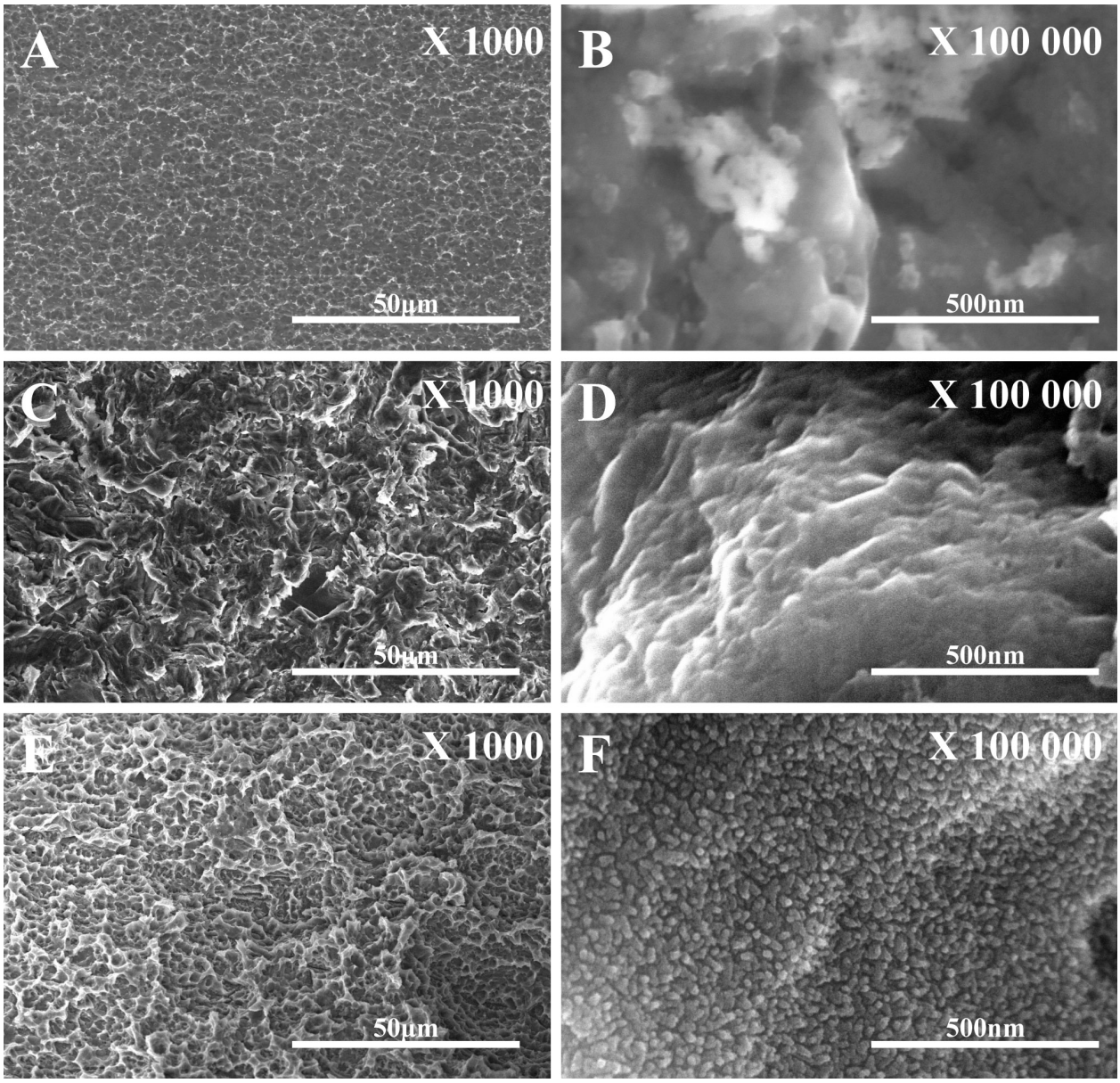

Figure 2. FE-SEM evaluation of some nanotextured implants available on the market. **(A)** The 3I Nanotite surface (Biomet 3I, Palm Beach Gardens, FL, USA) is produced through discrete calcium phosphate nanocrystalline deposition on a titanium implant surface after acid etching. The surface microtopography is quite smooth, and covered by microcrystals of CaP. **(B)** At the nanoscale, the CaP nanocrystals of NanoTite are very visible and covering a significant portion of the surface. **(C)** The Ossean surface (Intra-Lock, Boca Raton, FL, USA) is a microrough nanorough CaP low impregnated surface, obtained through an initial resorbable blasting media treatment followed by specific post-processing. The microtopography presents a moderately rough aspect. **(D)** At the nanoscale, the Ossean surface presents a very significant nanoroughness. **(E)** The SLActive surface (ITI Straumann, Basel, Switzerland) is produced through a classical SLA (sand-blasting acid-etching) protocol, followed by an immersion in a specific physiological solution. A significant microrough topography is produced. **(F)** At the nanoscale, the SLActive surface is covered by an instable soluble layer of crystals of NaCl (Sodium Chloride) deposited by the physiological solution during the drying of the sample, thus giving a nanotextured aspect.

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Many other experimental techniques also reported the production of nanostructures (for example TiO_2 nanoparticles coating to increase surface reactivity and early nucleation of apatite)**[37]**, but most works remain very experimental. Clinical results may be very different from the first published results, and many techniques do not have clear practical and industrial applications for mass-production dental implants.

5. NanoQuest is still at stake

Most of the commercially available implants do not display nanostructures **(Figure 1)[4]**. The first reasons is that most of the experimental methods described in the previous paragraphs were either inadequate for dental implants (fragile nanotubes) or difficult to apply properly and homogeneously on microtextured surfaces because of the interferences with the microtopography (oxidative nanopatterning, colloidal lithography, magnetron sputtering).

The second reason is that some of the technologies used to produce nanostructures did not allow to get the expected good clinical results. This is for example the case of the discrete calcium phosphate nanocrystalline deposition **(Figures 2A and 2B)**, which was finally no more promoted and replaced by a simple acid-etched surface without any nanostructures **[5]**. This experience may have discouraged some companies to move too quickly and for real in the nanotechnologies. Moreover, the use of nanocoating (thickness less than 100nm) remains rare, and much thicker micro-coatings are used by a very limited number of companies using a very specific implant design (Bicon, Boston, MA, USA)**[15]**. As a consequence, most surfaces available on classical screw implants - and giving excellent clinical results - are in fact terribly nanosmooth **(Figure 1)[4]**.

A few rare companies are using real nanoroughness as previously defined (Osseospeed, AstraTech, Mölndal, Sweden and Ossean, Intra-Lock, Boca Raton, FL, USA, particularly) through the development of some specific and secret production processing **(Figures 2C and 2D)[8]**. Several other companies are proposing an apposition of saline soluble nanocrystals as nanomodification (SLActive, ITI Straumann, Basel, Switzerland, and all its copies)**(Figures 2E and 2F)[4,38]**. These 3 systems offer excellent reported results, but their success is related to their combination of macrodesign, microtopography, nanotopography and chemistry – not to their nanofeatures alone. It is probable that a few other systems may present some real nanofeatures, but they still have to be well identified in the future **[39]**. Little is known on what would be the ideal nanostructures to use, and the ideal combination of features at the macro-, micro-, nano- and chemical scale.

Paradoxically, we have never so much heard about « nano » everywhere, while nanostructures are quite absent from the products available on the market, even if many companies are playing on the ambiguity to confuse users. While also we are still very far to really understand how the nanofeatures are influencing healing around dental implants and how they should be combined with other parameters. Yes, the NanoWar is raging, but the Quest for a better understanding and use of these features is just beginning. We can only hope that the commercial interests will not interfere too much with this Quest and to undermine the credibility of this field. In this article, we tried to give a clear vision of the current situation and recall, again, the key definitions of the topic. The non-commercial accurate definition of nanocharacteristics remains the best approach to avoid confusions.

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Disclosure of interests

The authors have no conflict of interest to report.

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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.

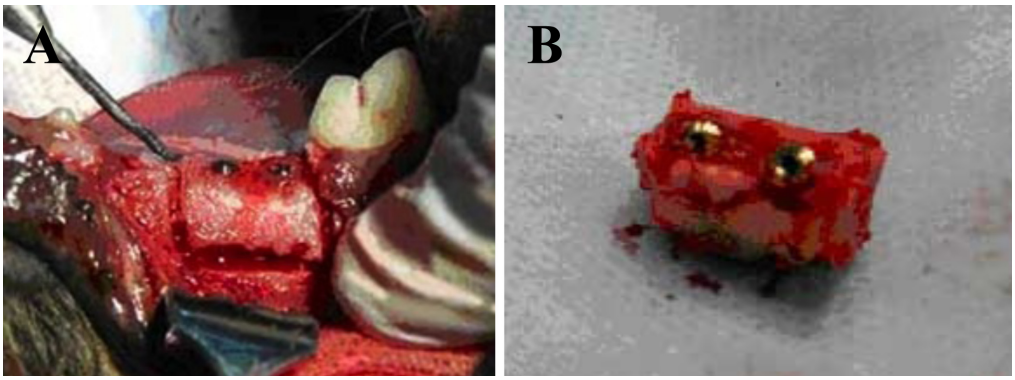

Figure 1. Experimental surgical model. The bone blocks containing the osseointegrated implants were collected from the dog lower jaw, after cutting with a bur and lifting with a chisel.

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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.

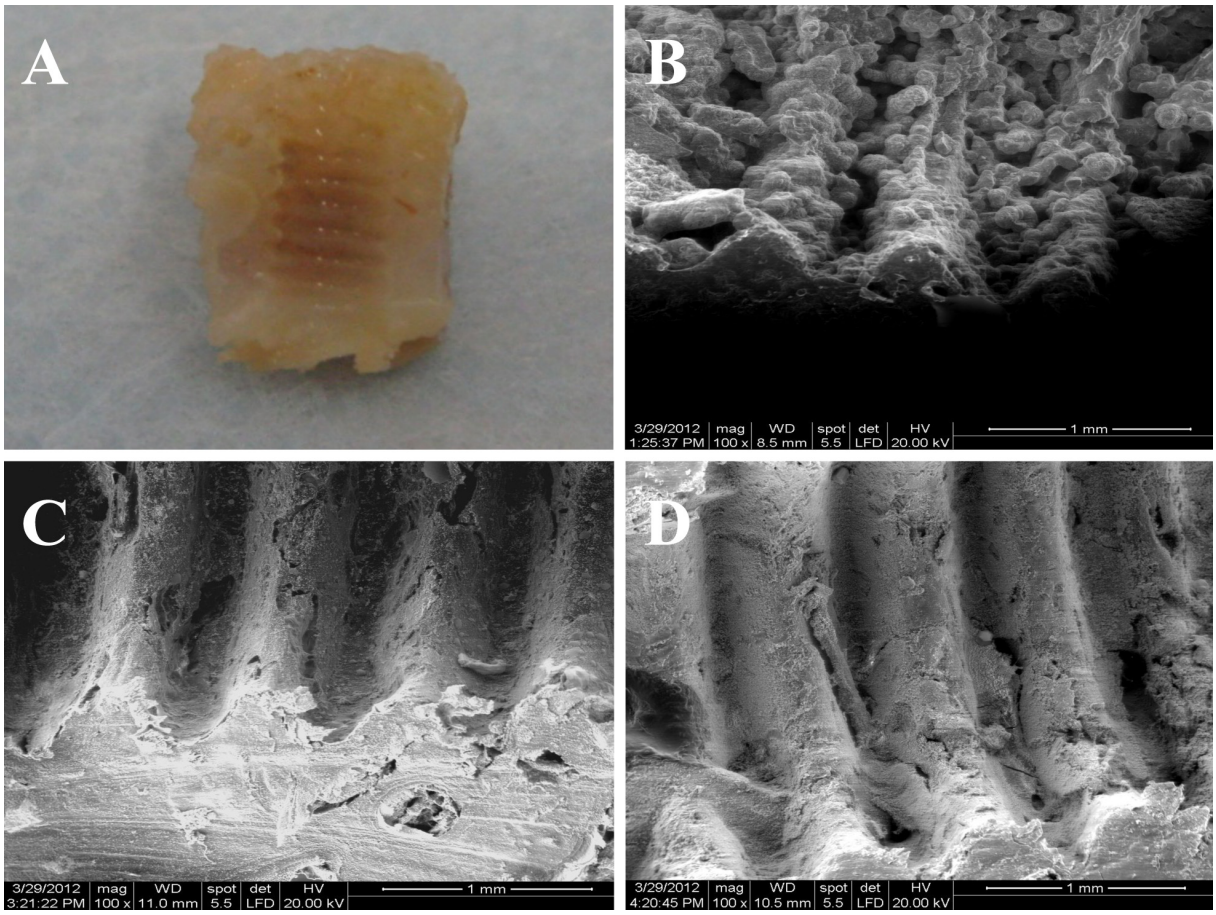

Figure 2. Scanning Electron Microscopy (SEM) of the samples. (A) After etching and removal of the implant materials, the shape of the implant screw threads was distinctly visible on the walls of the bone block and pointed out the area of the implant osseointegrated interface. **(B)** SEM analysis of 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.

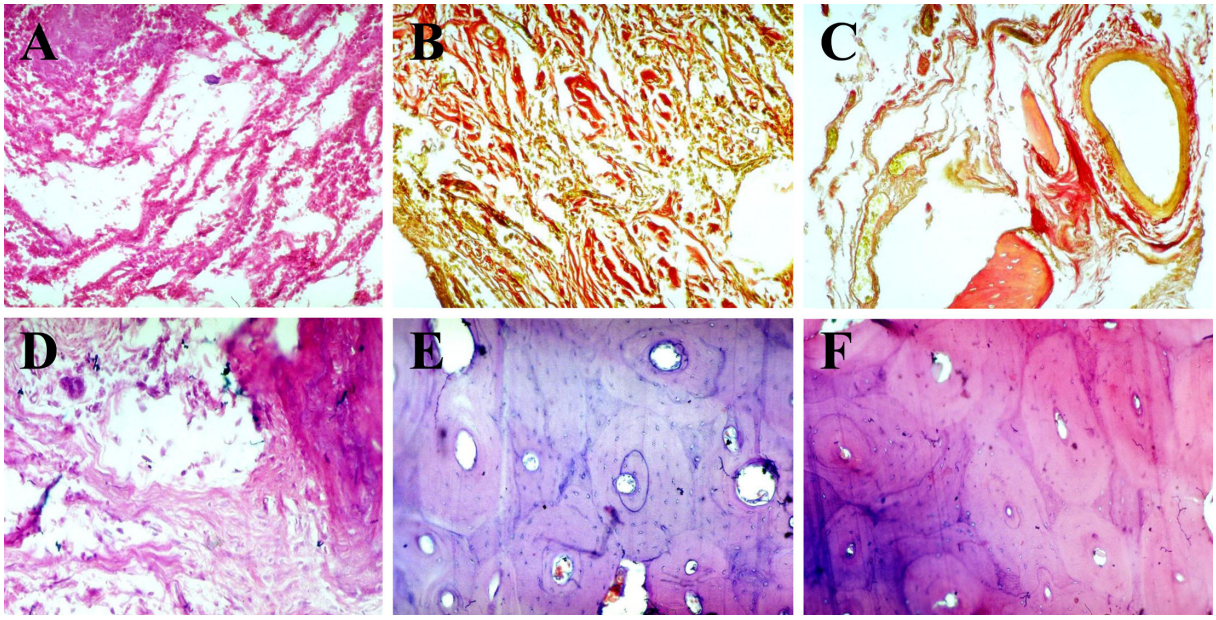

Figure 3. Histological analysis of the bone samples collected 1, 3 or 6 months after implantation (magnification x400). (A, B) One month after implantation, it was observed granulation tissue with lymphoid and histiocyte infiltration of the upper segment (**A**, hematoxylin/eosin staining) and of the 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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Research article

Histomorphometric evaluation of Direct Laser Metal Forming (DLMF) implant surface in the type IV bone: a controlled study in human jaw

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Abstract

Background and objectives. Direct laser metal forming (DLMF) is a procedure in which a high power laser beam is directed on a metal powder bed and programmed to fuse particles according to a CAD file, thus generating a thin metal layer. This surface produces structures with complex geometry and consequently allows better osteconductive properties. This study evaluated the influence of two different implant surfaces on the % bone-to-implant contact (BIC%) and bone density in the human type IV bone after 2 months of unloaded healing.

Materials and Methods. The micro-implants utilized presented DLMF surface and a machined (As-M) surface serving as test and control, respectively. Sixteen subjects (67.5±4.3 years of age) received one implant each during conventional implant surgery in the posterior maxilla. After 8 weeks, the micro-implants and the surrounding tissue were removed and prepared for histomorphometric analysis.

Results. Two As-M implants were found to be clinically unstable at time of retrieval. Histometric evaluation showed significantly higher BIC% and bone density for the test compared to the control surface ($p<0.05$).

Discussion and Conclusion. The histologic data suggests that the DLMF surface implants positively modulated bone healing at early implantation times compared to the As-M, at least after 2 months unloaded healing.

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

1. Introduction

Long-term studies have shown the high predictability of dental implant supported-restorations in edentulous patients **[1,2]**. However, previous data **[3,4]** demonstrated that the survival of these dental implants placed in posterior maxilla, i.e. type IV bone, were inferior to those placed in the anterior mandible, where the bone density is higher. The demand for improved dental implant survival at sites of lower bone density has prompted

researchers to look for implant surface topography alterations that would increase the early host-to-implant response and the system temporal biomechanics.

Since the dental implant surface is the first part of the biomedical device to interact with the host, body fluids and cell interaction to micrometer scale features such grooves, ridges, and wells, as well as different chemistries have been investigated **[5,6]**. Earlier studies **[7-10]** developed by our group have demonstrated that rough implant surface topography at micrometer scale improved osteogenic response compared to machined dental implant surfaces under unloaded conditions.

Traditional methods utilized for manufacturing and processing dental implants resulted in a high-density titanium structure with a micro- or nano-rough surface. However, using these methods, it is not possible to fabricate implants with a functionally graded structure, possessing a gradient of porosity perpendicular to the long axis, a relatively high porosity at the surface and a high density in the core **[11]**.

In the last decades, considerable progress has been made in the development of rapid prototyping techniques, including direct laser metal forming (DLMF)**[7]**. DLMF is a timesaving metal forming procedure in which a high power laser beam is directed on a metal powder bed and programmed to fuse particles according to a CAD file, thus generating a thin metal layer. Apposition of subsequent layers gives shape to a desired 3D form with the need of minimal post-processing requirements **[11]**. This technology allows fabricate dental implants with different shape and texture, directly from CAD models. In addition, laser-forming methods allow the fabrication of functionally graded titanium implants, with a gradient of porosity perpendicular to the long axis. Moreover, with DLMF, a porous surface structure for bone ingrowth is provided **[7,11,12]**. However, there is few human histological information about the behaviour of DLMF implants placed at type IV bone. Therefore, the aim of this histological study was to evaluate the bone-to-implant contact (BIC%) around unloaded DLMF implants retrieved after 2 months healing from human posterior maxilla.

2. Materials and Methods

2.1. Subjects

Sixteen totally edentulous subjects (9 women; 7 men), with a mean age 67.5±4.3 years of age, referred to the Department of Periodontology and Oral Implantology (Dental Research Division, University of Guarulhos, Brazil) for implant therapy were included in this study. Exclusion criteria included pregnancy, nursing, smokers, and any systemic condition that could affect bone healing. The Ethics Committee for Human Clinical Trials at Guarulhos University approved the study protocol (CEP#201/03).

2.2. Experimental Implant Surface Topographies

In this study, screw-shaped micro-implants were prepared with 2 surface morphologies: As - machined (As-M) and direct laser metal forming (DLMF) surface. Each micro-implant was 2.5 mm in diameter and 6.0 mm long. The cpTi micro-implants were made of grade-4 titanium (Conexão Implants, São Paulo, Brazil).

The DLMF was made of master alloy powder, Ti-6Al-4V (Tixos, Leader Implants, Novaxa, Milano, Italy) with a particle size of 25-45 μm as the basic material. Processing was carried out in an argon atmosphere using a powerful Yb (Ytterbium) fiber laser system (EOS GmbH Munchen, Germany) with the capacity to build a volume up to 250 mm × 250 mm × 215 mm using a wavelength of 1054 nm with a continuous power of 200 W, at a scanning rate

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of 7 m/s. The size of the laser spot was 0.1 mm. To remove residual particles from the manufacturing process, the samples were sonicated for 5 min in distilled water at 25°C, immersed in NaOH (20 g/L) and hydrogen peroxide (20 g/L) at 80° C for 30 min, and then further sonicated for 5 min in distilled water. Acid etching was carried out by immersion of the samples in a mixture of 50% oxalic acid and 50% maleic acid at 80°C for 45 min, washing for 5 min in distilled water in a sonic bath.

2.3. Implant Surface Characteristics

The samples were first checked for chemical composition with XPS/ESCA (X-Ray Photoelectron Spectroscopy/Electron Spectroscopy for Chemical Analysis), and no significant pollution was detected **[6]**. The topographies at the microscale were then visualized using routine Scanning Electron Microscopy (SEM) control. At the nanoscale, the SEM confirmed that both surface types were nanosmooth, following the current definition **[6]**. The sole difference between these 2 tested implant types was therefore the specific surface microtopography.

An optical laser profilometer (Mahr GmbH, Brauweg 38 Gottingen, Germany) was used to measure and characterize the dental implant surface topography. Ten micro-implants from both groups (5 micro-implants from each group) were measured 3 times each on the side, top, and bottom. The measured parameters, such as the arithmetic average of all profile point absolute values (Ra), the root-mean-square of all point values (Rq), and the average absolute height values of the five highest peaks and the depths of the five deepest valleys (Rz) were measured in all specimens.

2.4. Implant Surgery

Sixteen experimental implants were used in this study (n=8 DLMF and n=8 As-M). The implants were placed under aseptic conditions as previously described **[7-9]**. After crestal incision, mucoperiosteal flaps were raised and conventional implants were placed in the totally edentulous maxilla in accordance with the surgical/prosthetic plan prepared for each patient. Next, the experimental implant groups were randomly placed in the molar region, i.e. posterior to the most distal conventional implant. The implant recipient sites were prepared with a 2.8 mm diameter twist drill in soft bone. All drilling and implant placement procedures were completed under profuse irrigation with sterile saline solution. If during placement an implant showed low primary stability, a backup surgical site was prepared. The flaps were sutured to cover the micro-implants.

Post-operative medication included Clindamicin administered three times a day (1200mg/day) for 7 days week. The sutures were removed after 10 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine rinses were prescribed, twice a day for 14 days.

After a healing period of 2 months, during the 2-stage surgery of the conventional implants, the experimental implants and surrounding tissues were retrieved with a 4.0-millimeter-wide trephine bur, and the specimens were initially fixed by immersion in neutral formalin at 4%.

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2.5. Specimen Processing and Histomorphometric Analyses

Following retrieval and initial fixation, the implants and surrounding tissues were stored in 10% buffered formalin and processed to obtain thin ground sections (Precise 1 Automated System, Assing, Rome, Italy) as previously described **[13]**. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200, VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally along the implant long axis with a high-precision diamond disc at about 150 μm and ground down to ~30 μm. Two slides were obtained per implant. The slides were stained with acid fuchsin and toluidine blue. Percentage of bone-to-implant contact (BIC%) was defined as the amount of mineralized bone in direct contact with the implant surface. The measurements were made throughout the entire extent of the implant. The bone density in the threaded area (BA%) was defined as the fraction of mineralized bone tissue within the threaded area. All threads were measured and included in the statistical analysis. The specimens were analyzed under a transmitted light microscope that was connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVCs, Yokohama, Japan) and interfaced to a monitor and computer. This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and controlled by a software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milano, Italy).

The mean and standard deviation of histomorphometric variables were calculated for each implant, then for each group. Mann-Whitney test was used to compare the differences of histomorphometric variables between implant surfaces. The significance test was conducted at a 5% level of significance.

3. Results

3.1. Surface Roughness Parameters

Table 1 shows the profilometry measurements. The DLMF surface showed a higher mean value for all parameters (p<0.001). The surface topography of the cpTi surface was well defined, while the DLMF surface topography had no clear orientation **(Figure 1)**.

Implant Surface Topography	Ra (μm)	Rq (μm)	Rz (μm)
As-M	0.32 ± 0.03	0.43 ± 0.02	4.20 ± 3.00
DLMF	66.8 ± 6.56	77.55 ± 11.09	358.3 ± 101.87

Table 1. Mean ± standard deviation of the As-machined (As-M) and direct laser metal forming (DLMF) profilometry. Mann-Whitney test (p<0.05).

Differences statistically significant between the implant surface topographies (p=0.0001), cpTi<DLMF; **Ra** - arithmetic average of the absolute values of all profile points; **Rq** - the root-mean-square of the values of all points; **Rz** - the average value of the absolute heights of the five highest peaks and the depths of the five deepest valleys.

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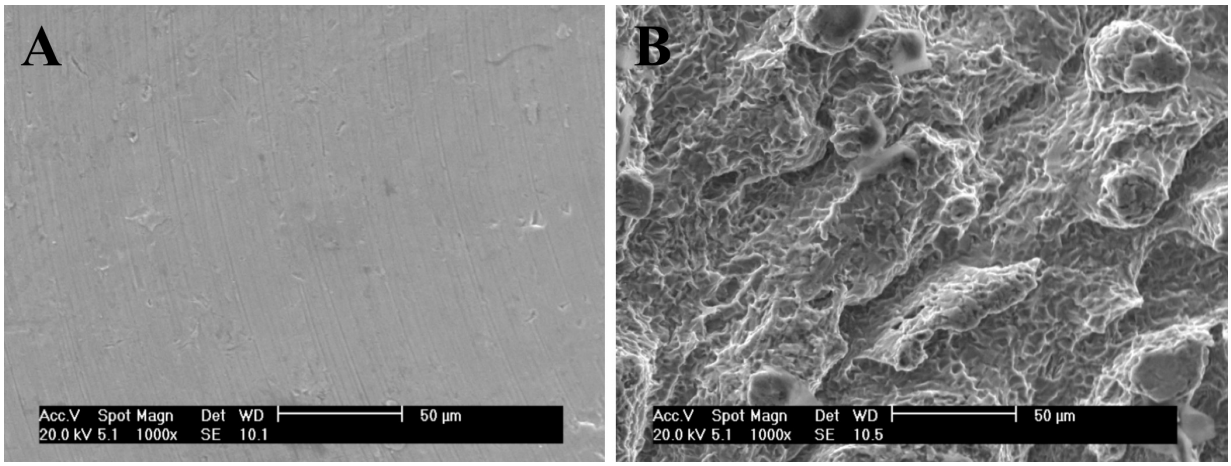

Figure 1. Scanning electron microphotograph of the evaluated implant surface topographies. (A) As-M and **(B)** DLMF.

3.2. Clinical observations

Two As-M micro-implants showed no osseointegration and were not included in the evaluation. The remaining 14 experimental micro-implants were clinically stable at the time of retrieval and did not present clinical evidence of inflammation or infection. Therefore, a total of 14 experimental implants were included in our evaluation: 8 specimens of DLMF group and 6 specimens of As-M group.

3.3. Histological and Histomorphometric Results

The pre-existing bone quality was recorded as D4 **[14]**. At coronal portion, some bone remodelling was observed in both groups. The ground sections showed the presence of remodeling activity in the bone next to DLMF implants **(Figure 2)**. Woven bone with several osteocyte lacunae and preexisting bone were present. The woven newly formed bone was separated from the preexisting bone by cement lines. The newly formed bone showed early stages of maturing and remodeling. Osteoblasts were connected to the newly formed bone, showing ongoing bone formation. Many wide marrow spaces with many capillaries were present in the peri-implant bone. In contrast, smaller amounts of new bone apposition were observed along the As-M implant surface, especially inside the implant threads **(Figure 3)**.

BIC% and BA% were statistically higher for DLMF implant surfaces **(Table 2)**.

Histometric variables	As-M	DLMF	*p*-value	CI 95%
BIC%	10.02 ± 4.53	25.14 ± 1.34	0.0001	8.65 to 25.17
BA%	17.95 ± 7.82	33.36 ± 5.90	0.003	8.58 to 39.66

Table 2. Mean and standard deviation of bone-to-implant contact percentages (BIC%) and bone density in the threaded area (BA%) for machined (As-M) and direct laser metal forming (DLFM) surfaces in posterior maxilla (n= 14 subjects). Two experimental implants from As-M were not evaluated. Mann-Whitney Test (p<0.05).

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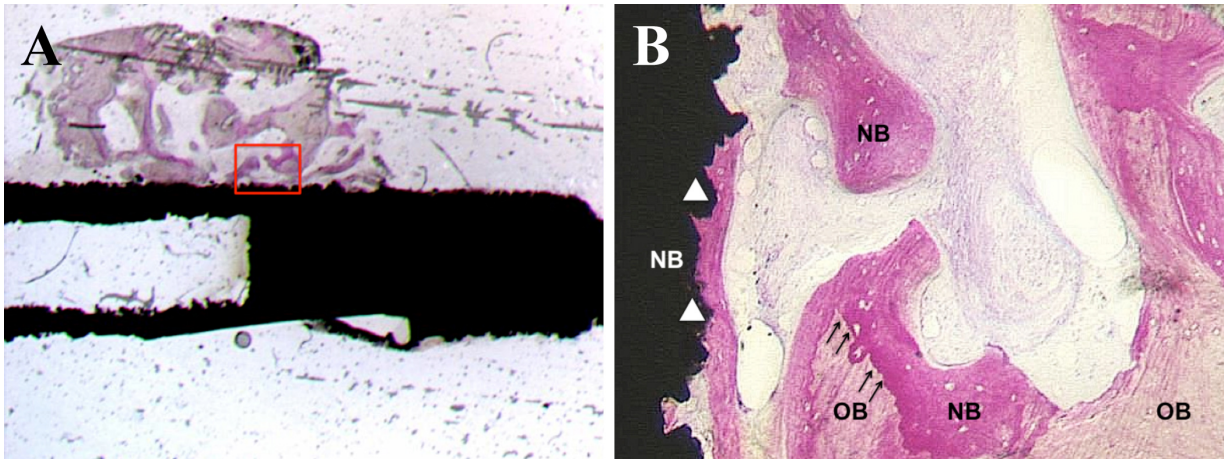

Figure 2. Histologic ground section of DLMF implant. (A) General view. The old bone was mostly lamellar (Basic fuchsin and toluidine blue staining, original magnification x20). **(B)** A larger magnification of the lateral frame area in the section shown in **(A)**. Apposition of new bone (NB) is depicted in close contact (arrow heads) with the implant surface. Reversal lines (arrows) showing the limits between old bone (OB) and new bone (NB)(Basic fuchsin and toluidine blue staining, original magnification x200).

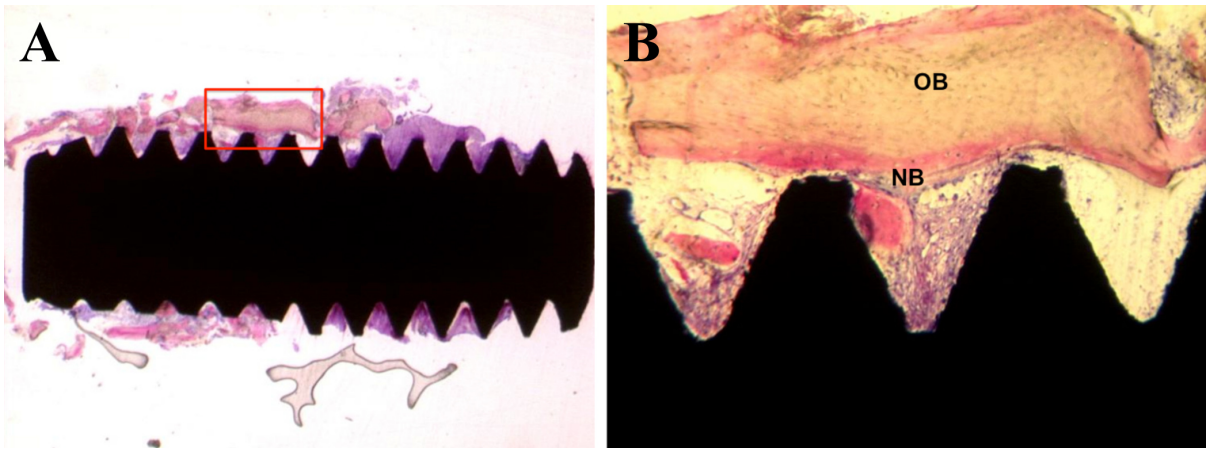

Figure 3. Histological ground section of the As-Machined surface implant. (A) General view after 2 months of healing depicting the newly formed bone showing early maturing and remodeling stages. Note the lack of connecting bridges between the new bone trabeculae and the implant surface (Basic fuchsin and toluidine blue staining, original x20 magnification). **(B)** A larger magnification of the lateral frame area in the section shown in **(A)**. The newer bone (NB) tissue shows no contact with the implant surface with presence of connective tissue (CT)(Basic fuchsin and toluidine blue staining, original x200 magnification).

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4. Discussion

This study demonstrated increased BIC% and BA% values to direct laser metal forming compared to as-machined implant surfaces. Recently, some studies have shown that DLMF influence early bone healing at the tissue/implant interface increasing bone formation in both higher and lower bone density sites **[7,15]**.

A thin bone layer covered a relatively large portion of the DLMF and micro-implant threads. This feature suggests that osteoblasts were activated by direct contact with the DLMF topography, showing contact osteogenesis **[5]**. Osteogenesis at the bone-to-implant interface is influenced by several mechanisms. A series of coordinated events, including protein adsorption, proliferation, and bone tissue deposition might be affected by the different surface topographies. At the micrometer level and beyond, the bone tissue contains complex characteristics of topographic pits, protrusions and fibers, arising in bone tissue from the nanocrystalline-mineralized osteoid. In turn, each of these events is affected by physicochemical interaction between the molecules and cells in the peri-implant area **[16]**. The implant surface chemical and topographical properties as well as the specific properties of individual proteins, determine the organization of the adsorbed protein layer.

The fabrication of dental implants with DLMF technique presents some potential advantages that could be really helpful in bone sites presenting low-density levels. DLMF makes possible to generate implants with a graded elasticity, incorporating a gradient of porosity, from the inner core to the outer surface. The outer surface of this new functionally graded material has an elastic modulus (77 Gpa) closer to that of the surrounding cortical bone (10-26 Gpa), for a more natural transfer of loading stress **[17,18]**. Complementary, extensive body fluid transport through the porous scaffold matrix is possible, which can trigger bone ingrowth, if substantial open pore interconnectivity is established **[10,19]**. Pore interconnectivity as well as pore size play a critical role in bone ingrowth regulating cell growth and function, manipulating tissue differentiation and optimizing scaffold mechanical function **[2,20]**.

5. Conclusion

Within the limits of the present controlled study, the histological data in humans confirmed that the surface topography created on DLMF implants positively influenced early bone tissue response under unloaded conditions in comparison to As-Machined surface. Further research is needed to evaluate the mechanisms of bone interaction of the DLMF surface, and to compare it with other forms of surface modifications studied in the literature.

Disclosure of interests

The authors have no conflict of interest to report.

Author Contributions

JAS was in charge of the elaboration of the study proposal and the financial support of the study, and he participated to the elaboration of the manuscript and the treatment planning of each case. CM and FM were in charge of the statistical analysis, the implant surface characterization and the financial support for the study. OB, BL and FC were in charge of the treatment planning of each case, the implant placement surgery and they participated to the elaboration of the manuscript. ST and AB were in charge of the patients´ monitoring after surgery, the biopsies collection, the oral rehabilitation and they participated to the elaboration of the manuscript. GI and AP were in charge of the laboratory

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processing of the samples, the histology, and participated to the data analyses and elaboration of the manuscript. AP also participated to the elaboration of the study proposal.

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Research article

Soft tissues around an acid-etched healing abutment: a histological and histomorphometrical analysis

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Abstract

Background and objectives. A healthy peri-implant soft tissue has been reported to play a relevant role in the long-term success of a dental implant. The underlying mechanisms of attachment and the factors that affect the integrity of this biological seal are not well understood. The aim of this report was an evaluation of the peri-implant soft tissues around a human submerged acid-etched healing cap.

Materials and Methods. Four implants were inserted in the posterior maxilla. The most distal implant lacked primary stability and, while the other 3 implants were immediately loaded the same day of surgery, it was decided to submerge this implant. An acid-etched healing cap was inserted on this implant to favor the soft tissue attachment. After 6 months, the patient asked, against the advice of the clinicians, to carry out the prosthetic rehabilitation without this implant. The implant, with the surrounding soft tissues was then retrieved after a 6 months healing period.

Results. A tight connection between the soft tissues and the healing abutment was found all around its perimeter. Only in a small portion of the interface a detachment of the tissues was present. Histomorphometry showed a close connection in 97% of the healing abutment perimeter. A close connection was also present at the level of the implant-abutment junction.

Discussion and Conclusion. Roughened surfaces can improve the attachment of the connective tissue to the metal surface. However, further research is required to determine the optimal surface treatment to improve peri-implant soft tissue sealing.

Keywords. Connective tissue, dental implants, epithelium, gingiva.

1. Introduction

A healthy peri-implant soft tissue, with a close contact of the epithelium and of the underlying connective tissue with the implant surface, has been reported to play a relevant role in the long-term success of a dental implant **[1-5]**. These peri-implant tissues are composed of a 2 mm long epithelium and a 1-1.5 mm long connective tissue **[6]**. The underlying mechanisms of attachment and the factors that affect the integrity of this biological seal are not well understood **[2]**.

A promising approach to optimize soft tissue implant integration involves modification of the topography of the implant surface **[7]**. It was hypothesized that

roughened implant surfaces would be effective for soft tissue integration **[7]**. Tissue reactions to implants are determined mainly by surface parameters **[8]**. Implant surfaces with defined characteristics may improve the cell anchoring to the metal surface **[8]**. Cells recognize surface features and react to them, resulting in contact guidance **[9]**. Moreover, the topography of the surface influences the cell adherence and also the cell differentiation, growth and migration **[10]**. The epithelial downgrowth may be stimulated by the disruption of the soft tissue interface induced by micromotion or by cytokines released by cells after stimulation with bacterial-derived products **[11]**. Fibroblasts tend to interdigitate into a rough surface, and to prevent epithelial downgrowth **[7]**.

Most of the histomorphometric studies reported to date in the literature have been done in dogs **[1]**. Human histologic data are valuable to validate and confirm animal models **[4,12]**. Aim of the present report was an evaluation of the peri-implant soft tissues around a human submerged acid-etched healing cap.

2. Materials and Methods

2.1. Clinical procedure

A 59-year-old patient participated in this study. The patient was partially edentulous. Four implants were inserted in the posterior maxilla **(Figure 1A)**. The bone quality of the insertion sites was poor (type 4 bone). The most distal implant lacked primary stability and, while the other 3 implants were immediately loaded the same day of surgery, it was decided to submerge this implant **(Figure 1B)**. An acid-etched healing cap (Dentsply Implants Manufacturing GmbH, Mannheim, Germany) was inserted on this implant to favor the soft tissue attachment. The roughness measure (Ra) of the healing cap was 0.8 μm. After 6 months, the patient asked, against the advice of the clinicians, that to carry out the prosthetic rehabilitation without this implant **(Figure 1C)**. The implant, with the surrounding soft tissues was then retrieved after a 6 months healing period **(Figure 2)**.

2.2. Processing of specimens

The implants and the surrounding tissues were stored immediately in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally along the major axis of the implants with a high-precision diamond disc at about 150 μm and ground down to about 30 μm. Three slides were obtained. The slides were stained with basic fuchsin and toluidine blue.

Histomorphometry of the soft tissues-healing cap contact percentage was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high resolution video camera (3CCD, JVC KY-F55B, JVC, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy). Attachment was determined, according to Kim et al. **[7]**, as the percentage of the implant length in contact with the neighbouring soft tissues.

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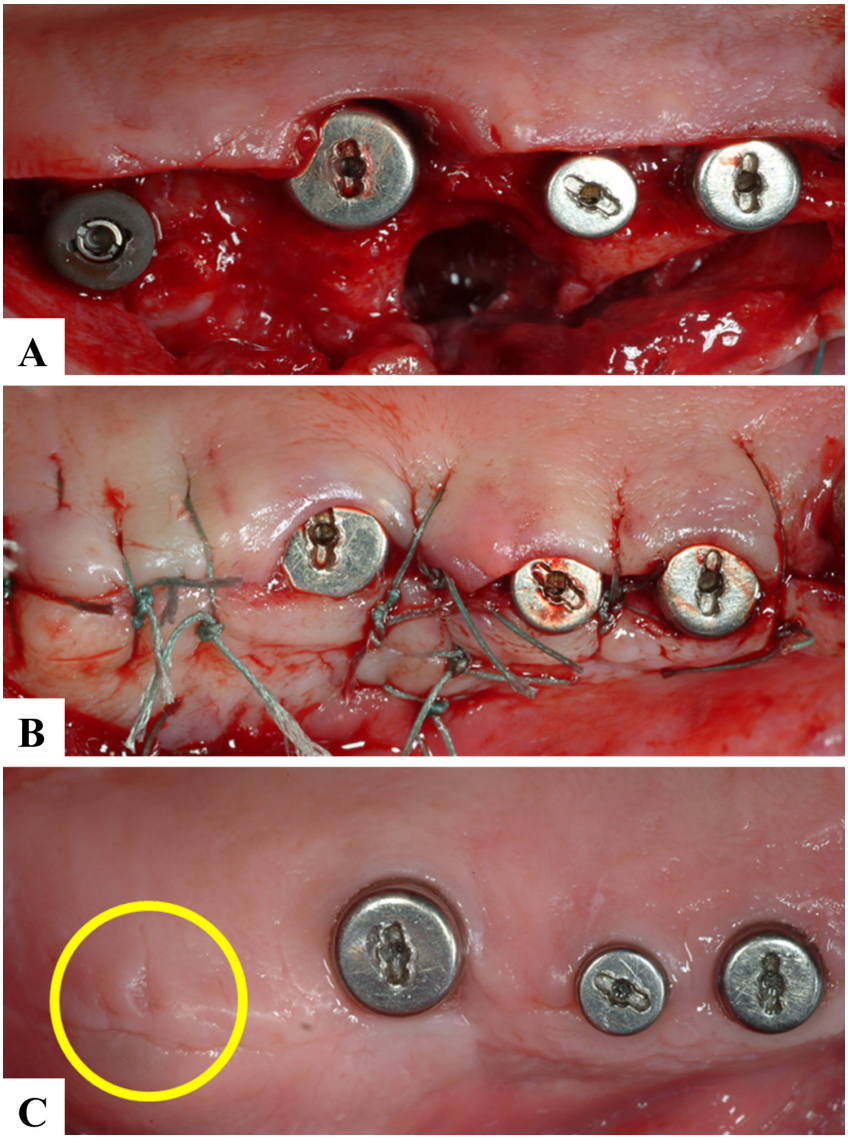

Figure 1. Clinical phases. (A) Four implants were inserted in the posterior maxilla. **(B)** Three implants were immediately loaded the same day of surgery. **(C)** The most distal implant lacked primary stability and it was decided to submerge this implant.

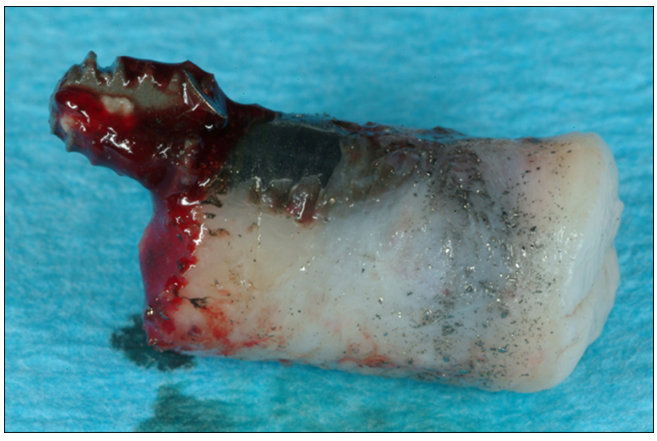

Figure 2. Image showing the retrieved implant with the surrounding soft tissues.

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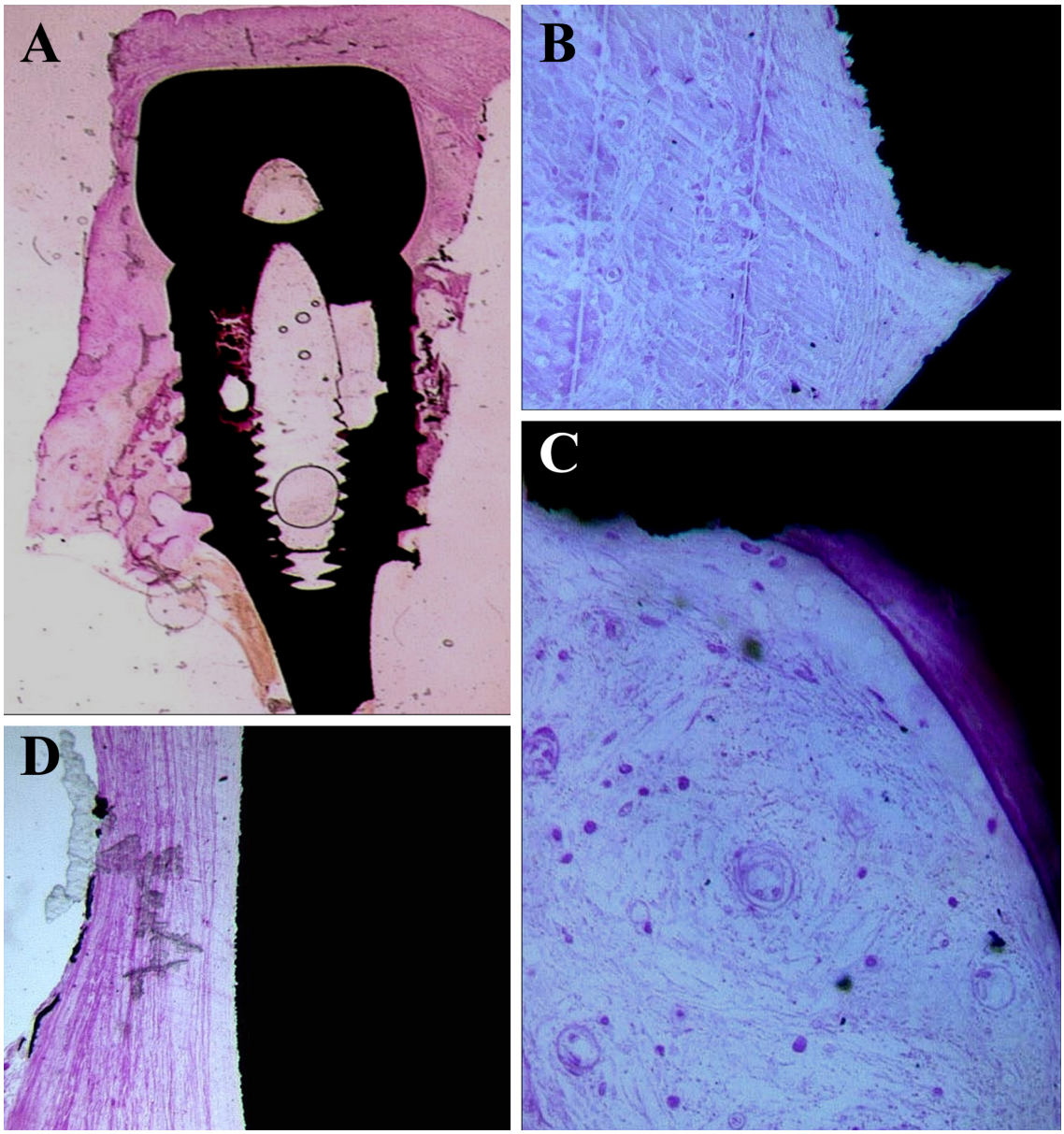

Figure 3. Histological analysis of the sample (staining with Toluidine Blue and Basic Fuchsin). (A) Low power magnification image showing the presence of a dense connective tissue around the healing abutment (magnification 12X). **(B)** Multinucleated giant cells or foreign body reaction cells were not observed (magnification 200X). **(C)** Few, scattered blood vessels were detected (magnification 100X). **(D)** Elongated fibroblasts, with major axis parallel to the long axis of the healing abutment, were seen in contact with the metal surface of the abutment (magnification 200X).

3. Results

At low power magnification, a dense connective tissue was present all around the healing abutment **(Figure 3A)**. At higher magnification, no inflammatory cell infiltrate was present. Multinucleated giant cells or foreign body reaction cells were absent **(Figure 3B)**. Only a few, scattered blood vessels were observed **(Figure 3C)**. Elongated fibroblasts were seen in contact with the metal surface of the abutment; these cells had their major axis parallel to the long axis of the healing abutment **(Figure 3D)**. Near to the abutment surface,

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the tissues presented a denser appearance forming a capsule of 70-150 μm thick. At a distance from the abutment surface, the connective tissue was more loose and more cell-rich. A tight connection between the soft tissues and the healing abutment was found all around its perimeter. Only in a small portion of the interface a detachment of the tissues was present. Histomorphometry showed that a close connection was found in 97% of the healing abutment perimeter. A close connection was also present at the level of the implant-abutment junction.

4. Discussion

A complete understanding of the biology of the peri-implant tissues is still lacking **[3]**. A constant vertical dimension of healthy periodontal tissues is needed to guarantee the esthetics around teeth: this dimension is called Biological Width (BW)**[13]**. The BW is composed by the sulcular epithelium (SE), junctional epithelium (JE) and connective tissue (CT)**[13]**. Around implants the BW represents the dimension of the peri-implant tissues needed to obtain an adequate JE and CT, and to get and maintain a seal around endosseous implants, which provides a protection from mechanical and external biological agents **[13,14]**. The connective tissue shows a close and tight connection to the abutment surface; this connection has been documented to happen through a thin avascular and collagen fiber rich, scar-like tissue of less than 100 μm in width **[4,6,13,15]**. This tissue is surrounded, on the outer side, by an area constituted by connective tissue fibers running in different directions **[6,13,15]**; these fibers appear to be functionally organized **[12]**. Collagen bundles were found to be abundant all around the implant with a maximum density between 200 μm and 800 μm from the abutment surface **[12]**. Collagen fibers were found to be spatially oriented with an inner system dominated by longitudinal fibers and a more external circular system **[12]**. There seems to be a differentiated network of fibers, which might be of clinical relevance as a mechanical protection for the underlying bone **[4]**. In an about 100 to 150 μm wide area adjacent to the implant surface, CT was, in general, free from blood vessels and was dominated by collagen fibers oriented parallel to the longitudinal axis of the implant **[4]**. Adjacent to this area, CT was densely packed with collagen fibers oriented circumferentially around the implant **[4]**. Perpendicularly oriented collagen fibers, directly contacting the implant surface were not observed in any of the sections **[4]**.

While a rough, transmucosal part of an implant will enhance plaque formation, the bony and connective tissue interface requires a porous or microtextured surface to promote tissue ingrowth **[16]**. An increase in the surface roughness of the transmucosal portion will facilitate early plaque formation **[16]**. An ideal transmucosal implant should not only minimize bacterial adhesion, but at the same time allow epithelial and connective tissue abutment **[16]**. Detachment of the peri-implant soft tissues from the implant surface indicates weak tissue attachment **[7]**. The present study showed an almost complete lack of detachment of the soft tissues and this fact, probably, indicates a strong adhesion of the connective tissue **[7]**. In the present case report it was possible to confirm the results of Kim et al. **[7]**, who found in a rat study that, while the coarsely blasted and titanium plasma-spray surfaces showed the highest incidence of complete attachment of the soft tissues, an etched surface produced an integration of the connective tissue that was similar to that observed with much rougher surfaces. It is possible that the unique geometry created by the etching procedure can play a dominant role in promoting the integration of connective tissue **[7]**. Roughened surfaces can then improve the attachment of the connective tissue to the metal surface **[7]**.

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The search for an optimal implant surface able to develop a favorable soft tissue reaction is still ongoing. Even if the literature on the topic is already developed significantly, it is still not possible to draw valid conclusions on the ideal surface for the soft-tissue interface **[17-19]**. The latest approach was to use chemical modifications and nanoroughness to promote this ideal soft tissue attachment and sealing **[20]**, following some patterns that were already discussed for bone integration of dental implant surfaces **[8]**. First results are encouraging **[20]**, but there is still a lack of information on the advantages and disadvantages of the various possible surface modifications, and no consensus on the exact objectives to reach with improved surface treatments.

5. Conclusion

In this report, it was confirmed in this human sample that roughened surfaces can improve the attachment of the connective tissue to the metal surface. This result can be observed in animal studies, but it is still unclear what are exactly the advantages and disadvantages of this kind of surface modifications in human clinical situations. Further research investigations are required to determine the optimal surface treatment to improve peri-implant soft tissue healing and sealing.

Disclosure of interests

The authors have no conflict of interest to report.

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Research article

Immediate versus delayed restorations for implants placed in fresh extraction sockets: a 1-year comparative cohort study

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Abstract

Background and objectives. Immediate implant placement can be considered a predictable protocol, even in esthetic areas. The objective of this study was to compare the clinical outcomes and the total costs of immediate and delayed restoration of implants with a specific design placed into fresh extraction sockets after 1 year from the implant placement.

Materials and Methods. Blossom implants (Ossean, Intra-lock, Boca-Raton, FL, USA) were used. In this prospective cohort study, marginal bone level, facial soft tissue, width of keratinized gingiva and papilla index were compared in both groups; correlations with pristine buccal bone thickness were also investigated.

Results. Marginal bone level records were different in the two groups, while no significant differences were registered in facial soft tissue and keratinized gingiva width. In the delayed group, a loss and reassessment of the papillary tissue was recorded at the time of restoration. The immediate restoration group seemed to show better results in terms of healing time and total costs.

Discussion and Conclusion. The immediate restoration protocol of immediately placed implant seemed to have the same efficiency as the delayed restoration, besides offering other clinical advantages.

Keywords. Bone resorption, dental implants, gingival recession, tooth socket.

1. Introduction

The placement of dental implants for replacing missing teeth is a widely used therapy, also in esthetic areas. In the conventional protocol, implants are placed after the bone healing, providing a highly predictable outcome **[1,2]**. The actual demand for reduced treatment time and simpler protocol led to the immediate placement protocols, where implants are put into fresh extraction sockets. Even if it is a technically demanding procedure, the immediate implant procedure shows to be effective in reducing surgical steps, overall treatment time, morbidity and costs for the patient **[3,4]**.

Several aspects are involved in order to achieve a satisfactory outcome, such as avoiding to raise a flap for controlling the facial bone resorption; leaving the buccal plate

intact; and placing the implant toward the palatal wall of the socket **[5-8]**. An adequate surgical protocol can help to overcome the common difficulties of this technique, such as the tri-dimensional implant positioning, the primary stability and the management of the bone wall remodeling after a tooth extraction **[9]**. In particular, augmentation procedures have shown to be effective in reducing the dimensional changes of the extraction socket and correcting the peri-implant bone defects **[10]**.

The immediate restoration of single maxillary implants placed in fresh extraction sockets is a predictable technique with high survival rates **[11,12]**. The primary implant stability and the lack of occlusal and eccentric contact during the healing phase are necessary to achieve a successful result. Den Hartog at al. in a literature review asserted that there were no differences in terms of survival rates for immediate and conventional load for implants placed immediately after tooth extraction **[13]**.

A crucial aspect in the anterior maxilla is the esthetics, but no or little information are found regarding the soft tissues and the esthetic outcome of the immediate implants. Also, the achievement of a satisfactory esthetics is influenced by the buccal bone remodeling and the soft tissue healing, which could compromise the final result. Another factor that will influence the final treatment plan is the cost-effectiveness of dental implant therapy, which has been studied for more than twenty years **[14,15]**.

The aim of the present study was to compare the overall clinical outcomes of immediate and conventional restorations on immediate implants. The null hypothesis was that there were no differences between the two groups, while the alternative hypothesis was that there was a difference. The study reports the 1-year preliminary data.

2. Materials and Methods

2.1. Patient selection

Consecutively treated patients at Versilia General Hospital, University of Pisa, Lido di Camaiore, Italy, between June 2008 and November 2010, were included in present prospective cohort study. The study was conducted according to the principles outlined in the declaration of Helsinki on clinical research involving human subjects, as revised in 2000. The two operators involved (UC and AB) received a 1-week session training consisting of calibration for surgical and follow-up procedures. Patient were included in and excluded from the study in accordance to the following criteria:

Inclusion criteria:

- 18 years old or older, and able to sign a written informed consent form;
- patients with cuspid/bicuspid failing tooth in the maxillary/mandibular area requiring a tooth extraction and immediate dental implant placement, with either an immediate or delayed restoration; patients whose progress had been followed for at least one year.

Exclusion criteria:

- history of systemic diseases which would contraindicate surgical treatment;
- long-term steroidal and/or amino-bisphosphonate therapy;
- diabetes mellitus;
- pregnant or lactating;

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- uncontrolled periodontal disease;
- patients declaring to smoke more than 10 cigarettes per day. Subjects smoking less than 10 cigarettes per day were requested to stop smoking before and after surgery, even though their compliance could not be monitored;
- absence of adjacent teeth;
- extraction sites with a partial or complete deficiency of buccal bone plate;
- failing tooth with acute infection;
- unwillingness to return for the follow-up examination.

For each patient a financial record including cost for clinical treatment and following aftercare was compiled: total cost for each treatment was calculated **[16]**.

2.2. Surgical Treatment

After an oral hygiene session each patient received clinical evaluation and tooth extraction at baseline. When immediate implant could not be inserted a ridge preservation procedure was performed, the patient was excluded from the study.

Blossom Implants with Ossean surface (Intra-lock International, Boca-Raton, FL, USA) were used **[17]**. Final insertion torque was measured with a calibrated torque wrench (Torque-Lock 2, Intralock International, Boca-Raton, FL, USA; torque measurement range from 20 to 75 Ncm). Implants with an insertion torque of at least 45 Ncm were included in the group of immediate restoration and were temporary restored within 36 hours from implant placement; if the insertion torque was lower than 45 Ncm, the implant was included into the delayed restoration group (with a 4 months provisionalization).

All patients received prophylactic antibiotic therapy (2 g of amoxicillin or 600 mg clindamycin – if allergic to penicillins) 1 hour before the extraction procedure and continued to take the antibiotic postoperatively (1g amoxicillin or 300 mg clindamycin) three times a day for 4 days. All patients rinsed for 1 minute with 0.2% chlorhexidine mouthwash prior to the surgery (and twice a day for the following 3 weeks), and were treated under local anesthesia using lidocaine with adrenaline 1:50.000. A flapless approach was chosen and tooth extractions were carried out with or without elevators to minimize the trauma; great care was taken to maintain the integrity of the buccal bone wall. Ultrasound bone surgery with specific tips was used at the mesial, distal and lingual/palatal sites to allow easier tooth extraction. After extraction, the socket was carefully curetted and, subsequently, the implant bed was prepared according to the standard procedure (with standard drills following the palatal bony wall as a guide, making maximum use of the bone apical to the removed tooth). A periodontal probe was used to verify the integrity of the bone walls and to evaluate the integrity of buccal bone plate after implant osteotomy preparation. The implants (Intra-lock International, Boca-Raton, FL, USA) were placed with the implant platform at the marginal level of the palatal/lingual bone wall.

Cortico-cancellous porcine bone particles (Apatos, Osteobiol-Tecnoss, Coazze, Italy) and a resorbable membrane (Evolution, Osteobiol-Tecnoss, Coazze, Italy) were used to graft the peri-implant bone defect. Impressions were taken and temporary/resin restorations were prepared using prefabricated abutments (Intra-lock International, Boca-Raton, FL, USA) within 36 hours for the immediately restored implants. Secondary soft tissue healing was left with a collagen membrane exposed to the oral cavity for the delayed restored implants.

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Sutures were removed after 10 days and oral hygiene instructions were given. All patients underwent appropriate antibiotic and analgesic anti-inflammatory therapy (generally ibuprofen 600 mg tablets).

The final impressions were made with individual trays using polyvinyl siloxane material (Flexitime, Heraeus/Kulzer, Hanu, Germany) to prepare the metal-ceramic crowns, which were cemented on personally tailored titanium abutments.

From each patient, time spent, clinical and financial records were collected. The mean real salary per hour was obtained from the European Commission website database <http://ec.europa.eu/index_en.htm> which cites labor costs per hour 2013 in Italy at €27.4. The time cost for each patient was calculated by multiplying the time per year spent in the clinic by the mean real salary per hour. The total costs were the costs cumulated during the twelve months of survey.

2.3. Input variables

Variables were taken by one examiner who was not involved in performing the surgical treatment, immediately after implant placement (baseline or time 0, or T_0), at 4 months after placement (time 1, or T_1) and at 12 months after dental implant insertion (time 2 or T_2).

- MBL: peri-implant marginal bone level was evaluated on intra-oral radiographs at the mesial and distal sites ($mMBL_X$ and $dMBL_X$, with X = 0,1,2) and corresponded to the distance between the fixture-abutment interface and the most apical point of the marginal bone level. Digital intra-oral periapical radiographs were taken (70 KVp, 7 mA) using a parallel cone technique with digital sensor (Schick Technologies, Long Island City, NY, USA). A standardization of the x-ray geometry was applied and the known diameter, length or thread-pitch distance of the implants (pitch = 1.0 mm) were used for calibration. Measurements were taken to the nearest mm using computer software (UTHSCSA Image Tool, Version 3.00, University of Texas Health Science, San Antonio, TX).
- WKG: width of keratinized gingiva was the distance between the gingival margin and the mucogingival junction of the interested area, measured midfacially.
- FST: facial soft tissue level was the distance between a reference line, which connected the facial soft tissue level of the adjacent teeth, and the soft tissue at midfacial point amid the two residual teeth adjacent interested area.
- BT: buccal bone thickness was evaluated by means of a surgical caliper at the moment of tooth extraction and represented the thickness of the buccal bone plate at the most coronal point of the marginal crest using a surgical caliper.
- Implant failure: it occurred for any mechanical damaging (fracture) or clinical detriment (peri-implant infection or mobility) which required implant removal.

Cost of the clinical treatment was calculated taking into account the following steps: preoperative consultations and diagnostic tests, stage-one surgery, number of visits during the healing phase, stage-two surgery, and the prosthodontics phase of treatment. Additional clinical costs, including costs for clinical and medical services, and those resulting from time spent by the patient for staged recall visits (oral hygiene program, with a recall visit every 6 months) and other visits required by the patients, were also calculated.

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2.4. Outcome variables

The outcome variables were obtained subtracting from each input variable the respective baseline value: a negative value represented a reduction, whereas a gain was given by a positive value.

- ΔMBL: change at the marginal bone level was calculated for the mesial and distal aspects at 4 and 12 months as reported by the following formula:

$$n\Delta MBL_X = nMBL_0 - nMBL_X, \text{ (with } n \text{ as mesial or distal, and X = 1,2)}$$

- ΔFST: facial soft tissue changes were calculated by subtracting the baseline value from the respective values at T_1 or T_2, according to the formula:

$$\Delta FST_X = FST_X - FST_0 \text{ (with X = 1,2)}$$

- ΔWKG : changes in the width of keratinized gingiva for times T_1 or T_2, according to the formula

$$\Delta WKG_X = WKG_X - WKG_0 \text{ (with X = 1,2)}$$

- IP: the index proposed by Jemt was employed to analyze the status of the interdental papilla **[18]**

  0 = no papilla;

  1 = less than one half papilla is present;

  2 = greater than half of the papilla height is present but not to the full extent of the contact point;

  3 = papilla fills the entire proximal space and is in good harmony;

  4= papilla is hyperplastic.

- SR and CSR: success rates and respective cumulative value were calculated according to the criteria suggested by Buser with a registered radiological peri-implant bone resorption not greater than 1.5 mm, during the first year of loading **[19]**, and 0.2 mm / year, during the following years **[20]**.
- Time of clinical treatment calculated from the surgery time to the definitive prosthesis time.
- Total cost (expressed in labor costs per hour) = Costs for the clinical treatment + Adjunctive clinical cost.

2.5. Statistical analysis

Acquired data were entered into a database for automatic analysis (Database Toolbox, MatLab 7.0.1, The MathWorks, Natick, MA). Matrix laboratory tools package were employed to perform descriptive and statistical analysis (Statistics Toolbox, MatLab 7.0.1, The MathWorks, Natick, MA). A Lilliefor test was employed to confirm normal distribution of the data related to each procedure. For each of the outcome variables pairwise comparisons were performed using the Wilcoxon signed rank test for matched samples and the Wilcoxon rank sum test for unmatched data and *p*-values were obtained. All measurements in the text and tables are described as mean and standard deviation, m±std; for ranked variables the results were given by median and interquartile range (IQR: the difference between the 75th and 25th percentiles). The level of statistical significance was set at .01 for all analyses.

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3. Results

Thirty patients were treated with tooth extraction and immediate implant placement. Immediate prosthetic restoration was performed for 15 patients (age of 44.6±10.2 years within the range 29-62), whereas delayed restoration (3-4 months) was performed for the remaining 15 patients (age of 49.1±11.9 years within the range 31-67). Tooth position, age, gender and patient demographic data were reported in **Table 1**.

Procedure	Immediate prosthetic restoration	Delayed prosthetic restoration
Sample size	15	15
Age (years)	44.6±10.2	49.1±11.9
Age range (years)	29-62	31-67
Time of clinical treatment° (days)	120±8	203±11.5
Two years total cost° (days)	141.9±3.1	179.0±3.2
Genders ratio M/F	7/8	7/8
Cuspid/bicuspid ratio C/B	4/11	2/13
Smoking habit Y/N	6/9	6/9
Buccal plate thickness (mm)	0.7±0.2	0.6±0.2

Table 1. Demographic data for the two different prosthetic loading with description of variable data related to measurements, given by median and interquartile range, $\tilde{m}$ (iqr). For both procedures, a cuspids/bicuspids (C/B) ratio and the thickness value of the buccal plate before dental implant insertion were given.

The outcome variables were reported in **Table 2** for all the time points considered: T_0 was the baseline, T_1 and T_2 the 4- and 12-month follow-up. Changes of the outcome variables were calculated giving mesial or distal ΔMBL, ΔFST and ΔWKG between T_0 and T_1 (marked as Δ_1) and between T_0 and T_2 (marked as Δ_2). The papilla index values for the two procedures were also showed in **Table 2** as median and interquartile range. The dimensional changes of the hard and soft tissues were visualized in **Figure 1**. The comparisons between procedures showed significant differences for the papilla index. The immediate restoration group at 4-month analysis had 2(0), at 1-year analysis the mean value was 2(1.5). The delayed restoration group at 4-month analysis had 0(0), at 1-year analysis the mean value was 2(1) (with significance ranging from .0098 and $2.6 \cdot 10^{-6}$). No significant differences were observed for changes of facial soft tissue level (ΔFST) and of the width of keratinized gingiva (ΔWKG). The marginal bone level showed significant differences between the 2 groups. The immediate restoration group showed significant difference between 4-month (ΔMBL_1) and 1 year analysis (ΔMBL_2) only at the distal sites with values of -0.3±0.5 mm and -0.9±0.4 mm, respectively (p-value = 0.0039). Conversely, the delayed restoration group showed significant changes between 4-month and 12-month analysis at mesial (p-value = 0,0049) and distal (p-value = 0,0098) sites.

No dental implant failure was registered during the considered period of time; moreover, all implants at the final time of the survey were considered successful.

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Procedure	(a) Immediate prosthetic restoration								
	Values for outcome variables					p-value Wilcoxon signed rank test			
Times	T_0	T_1	T_2	Δ_1	Δ_2	T_0 vs. T_1	T_0 vs. T_2	T_1 vs. T_2	Δ_1 vs. Δ_2
mMBL (mm)	0.9 ±0.7	0.5 ±0.9	0.1 ±0.8	-0.3 ±0.5	-0.8 ±0.4				.0156
dMBL (mm)	0.9 ±0.8	0.7 ±0.7	0.1 ±0.7	-0.3 ±0.5	-0.9 ±0.4				**.0039**
FST (mm)	-0.6 ±0.8	-0.3 ±0.5	-0.1 ±0.3	0.3 ±0.6	0.5 ±0.7				.2500
WKG (mm)	3.2 ±0.8	3.3 ±1.1	3.2 ±1.1	0.1 ±0.6	0.0 ±0.5				1
mIP	1(1)	2(0)	3(1)			.0195	**.0012**	**.0078**	
dIP	1(1)	2(1.5)	3(1)			.0703	**$2.4 \cdot 10^{-4}$**	**.0039**	

Procedure	(b) Delayed prosthetic restoration								
	Values for outcome variables					p-value Wilcoxon signed rank test			
Times	T_0	T_1	T_2	Δ_1	Δ_2	T_0 vs. T_1	T_0 vs. T_2	T_1 vs. T_2	Δ_1 vs. Δ_2
mMBL (mm)	0.6 ±0.8	0.6 ±0.7	-0.2 ±0.8	0.0 ±0.9	-0.8 ±0.8				**$4.9 \cdot 10^{-4}$**
dMBL (mm)	0.9 ±0.7	0.7 ±0.8	0.0 ±0.9	-0.1 ±0.4	-0.9 ±0.5				**$9.8 \cdot 10^{-4}$**
FST (mm)	-0.5 ±0.8	-0.2 ±0.9	-0.3 ±0.5	0.3 ±0.7	0.3 ±0.8				1
WKG (mm)	3.5 ±0.7	3.4 ±0.9	3.1 ±0.7	-0.1 ±0.6	-0.3 ±0.6				.2891
mIP	1(1)	0(0)	2(1)			**$1.2 \cdot 10^{-4}$**	.6250	**$1.2 \cdot 10^{-4}$**	
dIP	1(1)	0(0)	2(0)			**$6.1 \cdot 10^{-5}$**	**$9.8 \cdot 10^{-4}$**	**$6.1 \cdot 10^{-5}$**	

Procedure	(c) Comparison between prosthetic restorations	
	p-value Wilcoxon rank sum test	
Times	Δ_1 or at 4 months	Δ_2 or at 12 months
mMBL (mm)	.3115	.3610
dMBL (mm)	.3856	.9762
FST (mm)	.9269	.3110
WKG (mm)	.5505	.1195
mIP	**$2.6 \cdot 10^{-6}$**	**$5.3 \cdot 10^{-4}$**
dIP	**$3.8 \cdot 10^{-6}$**	**.0098**

Table 2. Overall outcome variables given by mean and standard deviation, $\bar{m}$ ± std for continue variables and by median and interquartile range, $\tilde{m}$ (iqr) for ranked variables for both procedures and overall times: baseline (T_0), at 4 (T_1) and 12 (T_2) after implant placement. Differential value (Δ) at 4 (Δ_1) and 12 months (Δ_2) are also given. Costs were given in time (total cost / labor cost per hour) and the significances of the Wilcoxon two-sided signed rank test for matched samples and of the Wilcoxon rank sum test for unmatched samples (type of provisionalization) for overall outcome variables were shown in **bold.**

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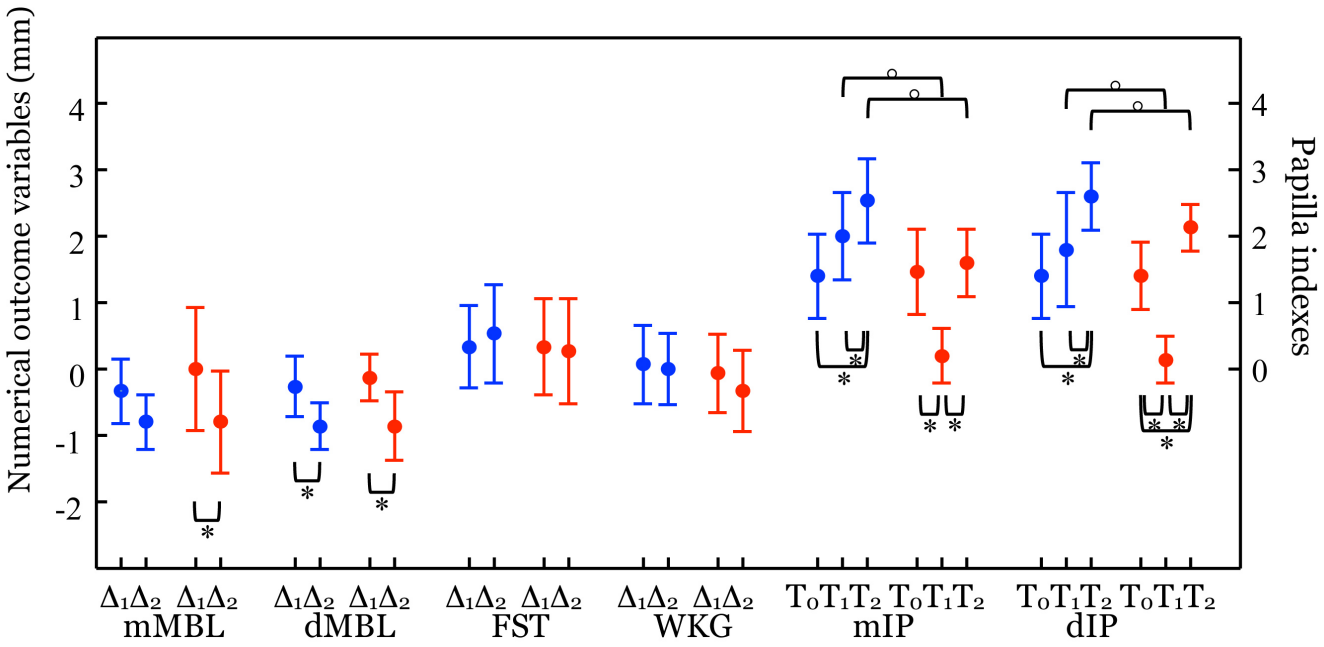

Figure 1. Summary of the overall outcome variables, showed as mean and standard deviation for all the times: immediate loading was represented in blue, whereas delayed loading was showed in red. For the pairwise comparisons by the Wilcoxon two-sided rank sum test for independent samples (°) and the Wilcoxon signed rank test for matched data (*), significant *p*-values are shown as brackets (upper and lower, respectively). For the papilla index were given at baseline (T_0), at 4 (T_1) and 12 months (T_2) after implant placement, and for differential value (Δ) of the remaining outcome variables at 4 (Δ_1) and 12 months (Δ_2).

The mean values for the operating times for the clinical treatments of the two groups were 120 days and 203 days, for immediate and delayed restorations, with a statistically significant ($p = 3.32 \cdot 10^{-6}$) difference. A difference at a significant level was also obtained between the medians of the two groups regarding the total costs at year 1 of the survey (141.9 days and 179.0 days for immediate and delayed prosthetic procedures, with $p = 2.39 \cdot 10^{-6}$) **(Table 2)**.

4. Discussion

In this cohort study, implant survival, peri-implant mucosal changes, marginal bone loss and treatment cost of immediate and delayed restorations of immediate single implants were evaluated and compared. The data were collected after 1 year of function and the two groups were homogeneous, showing similar baseline parameters.

All the implants placed in fresh extraction sockets survived up to one year of function, and no technical or biological complications were recorded. The overall success rate of the immediate implants was 100%, due to the favorable marginal bone changes. The two experimental groups produced similar outcomes also in soft tissue integration. The results of this study showed that immediate restorations of immediate implants are at least as effective and safe as delayed restorations.

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In a recent literature review **[21]**, the authors calculated the overall survival rate of both immediately and delayed restored dental implants, but no conclusive results were obtained. Despite other authors' findings, the present study registered no significant buccal soft tissue recession in the delayed restoration group.

Several factors should be taken into consideration to explain this discrepancy. First of all, the extraction sockets included in the experimental groups presented intact buccal bone plate, adequate soft and hard tissues dimensions and no acute infection. Also, the allocation to the immediate or delayed restoration was guided by the insertion torque value (cut-off value 45 Ncm), excluding a randomization process. Furthermore, an augmentation of the bone contour was performed at all the experimental sites, in order to control the ridge remodeling after tooth extraction. All these surgical steps contributed in managing the risk of the procedure and improving the survival rates and the esthetic results.

All soft tissues outcomes showed no significant differences between the two experimental groups, except for the papilla index. The mesial and distal marginal bone level in the delayed restoration group registered a statistically significant decrease between the 4 months and the 1-year control. The mesial aspect (mMBL) was 0.5 ± 0.9 mm at 4 months and 0.1 ± 0.8 mm at 1 year, the distal aspect (dMBL) was 0.7 ± 0.7 at 4 months and 0.1 ± 0.7 at 1 year. These findings pointed out that the marginal bone loss started at the same time with the delayed restoration. On the other hand, in the immediate restoration group, the bone loss followed a gradual progression, thus resulting in a final value similar to the delayed group.

In a recent review, Lang et al. **[9]** analyzed immediate implant placement studies comparing immediate and conventional loading. The review pointed out that, during the first year of immediate loading, the bone loss is less than 1 mm, while longer-term studies showed a stabilization of the bone level after the first year. On the other hand, a bone loss of 0.05 – 1.16 mm was described in the delayed restoration group, where the baseline measurement was at the time of implant placement.

The soft tissue changes showed similar results in both the immediate and the delayed group. The buccal mucosal margin (ΔFST) and the width of attached mucosa (ΔWKG) changes were registered at baseline and at final time. The baseline facial soft tissue level was positively correlated to the buccal bone thickness, confirming that a buccal plate > 0.5 mm could improve the buccal soft tissues stability, at least in the delayed group. Nevertheless, both procedures showed a small number of patients presenting a slight soft tissue recession in both groups and the final results were similar for the immediate and delayed procedure. The difference between the two groups was connected to the time of the soft tissue remodeling, since in the immediate restoration it started immediately after the surgery, while in the delayed group it started at the time of restoration.

Among the soft tissues values, the papilla index (PI) showed the greatest differences between the two groups. Previous studies observed wider papilla shrinkage in delayed restored implants when compared to immediate provisional restoration procedure. In this study, the difference between the PIs of the two protocols was statistically significant at 4 months. As other authors assessed, most of the papilla shrinkage showed up in the delayed restoration at three months, but after this period a progressive re-growth of the mesial and distal tissue was observed **[9]**. After 1 year, the two PIs were comparable. Moreover the replacement of single tooth seemed to lead to a final positive dimensional change after tooth extraction when compared to the baseline values (median PI = 1) that were registered for impaired teeth.

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Results published in the literature seem to confirm that the most of the hard and soft tissue changes were focused within the first six months following immediate implant placement; afterwards the papillae regardless of the restoration procedure, both at mesial and distal aspects, may undergo a positive remodeling phenomenon leading to a gain in height attested by analysis both of the linear height measurements and of the distribution of papilla scores **[9]**.

The re-growth of the papillae was observed in both groups and reached the original heights. However, adopting an immediate restoration protocol could ensure more predictable soft and hard tissues outcomes. In fact, in the delayed group the soft tissue changes were evident and seemed to follow a loss and restoration process, while in the immediate group the modifications were minimal and slow until a steady state was reached. An immediate provisionalization can improve the patient's compliance and reduce the overall healing time, as found in this paper.

Other Authors compared different strategies to treat partially and totally edentulous patients, reporting long-term costs **[22-26]**. The results of these studies were not comparable with the present work. In the present study, the delayed restoration protocol resulted to be about 26% more expensive due to the adjunctive stage-two surgery and visits required.

One limit of this study could be the treatment selection, which was assigned to the clinician, avoiding a randomization system. Beside this, the differences between the two experimental groups could be so clearly shown because of the absence of a blinded type of measurement. The results of this paper need to be confirmed by a longer period of observation and a larger number of implants studies.

5. Conclusion

Immediate implant's prosthetic restoration could be realized immediately after the implant surgery or after the bone healing period. Both of the protocols showed similar final results regarding the negative bone remodeling, but different timing since in the delayed group the bone resorption was between 4 months and 1 year, while in the immediate group it was slow and gradual during all the follow-up time. No differences were pointed out in the midfacial gingival margin and the width of keratinized gingiva. In the delayed restoration group a loss and regain of the papillae was observed, while slight modifications were recorded in the immediately restored implants until a complete healing. Regarding the healing time and costs of the two procedures, immediate restoration appeared to be a more promising procedure for implant placed in fresh extraction sockets.

Disclosure of interests

The authors have no conflict of interest to report.

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Research article

The Flat One Bridge technique for full-arch edentulism: long term results from a prospective cohort study

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Abstract

Background and objectives. Immediate loading of dental implants was developed in the last years to treat edentulism. In selected clinical situations, the implants can be loaded successfully immediately or just after their placement, although not all clinicians may achieve optimal results. The aim of the present study is to report the success rate of a new technique, the Flat One Bridge (FOB) in a cohort of patients requiring full arch rehabilitation, with a subgroup analysis according to the their clinical status.

Materials and Methods. The study was designed as a prospective cohort study. 48 consecutive patients (24 females), average age 45 years, requiring full arch rehabilitation, were divided into 4 Groups according to their clinical status: 24 in the Native Bone Group (NBG), 8 in the Periodontitis Group (PEG), 2 in the Guided Bone Regeneration Group (GBRG), and 14 in the Fresh Extraction Group (FEG). All patients were treated with FOB on Ossean implants (Intra-Lock, Boca-Raton, FL, USA), and were followed-up for an average of 8 years. The outcome results were measured with the success, survival and failure rates. The statistical analysis was performed with a Fisher's Exact test.

Results. The overall success and implant survival rate of the study population was 95.8%. There were only 2 failures (4.2%), both in the PEG group. Patients in the NBG and GBR groups had a 100% success rate. The PEG showed in majority success. The FEG showed some implant survivals ($p<0.05$).

Discussion and Conclusion. This study shows the effectiveness of the Flat One Bridge to treat full arch edentulism. Most patients had a positive result, that was maintained on the long term.

Keywords. Bone regeneration, dental implants, immediate dental implant loading, maxilla.

1. Introduction

In the recent years, immediate loaded implants were developed to treat edentulism **[1]**. Many publications confirmed that it is possible to load dental implants successfully immediately or just after their placement in selected patients, although not all clinicians may achieve optimal results **[2]**. Osseointegration remains the final objective of all dental implant

materials and implant-supported rehabilitation strategies. For this reason, many researchers have worked during the last decades on protocols and materials to improve it **[3]**. Several techniques based on new implants and prosthetic designs have been proposed throughout the years by different manufacturers **[4]**. Several recent articles proposed new techniques to treat patients in difficult clinical conditions such as fresh extraction sockets and periodontally compromised sites. However, very little data are available on the success rate of many approaches applied to the same materials, and prospective studies are often lacking to validate specific approaches in various clinical situations.

Recently, we published a case report describing a new technique, the Flat One Bridge (FOB), that was developed to provide a simple solution to full-arch immediately loaded rehabilitation **[5]**. This protocol was developed in 2005, and since then it has been routinely applied in our clinical practice **[6]**.

The aim of the present study is to report the success rate of the FOB in a cohort of patients requiring a full-arch rehabilitation with a long-term follow-up. Moreover, we wanted to explore whether different preexisting clinical conditions led to different final therapeutical results.

2. Matherials and Methods

2.1. Population and protocol

This study was planned and designed as a prospective cohort study. Between 2005 and 2007 we included 48 consecutive patients (24 females), average age 45 years, all requiring a full-arch rehabilitation: 42 for the upper arch, 1 lower arch and five complete mouths. Patients were divided into four groups according to the conditions of health of their mouth and bone: 24 with an edentulous alveolar ridge, but large enough to receive the implants, were included in the Native Bone Group (NBG); 8 affected by a severe form of periodontitis in the Periodontitis Group (PEG); 2 requiring sinus-lift, split-crest and regenerative techniques in the Guided Bone Regeneration Group (GBRG); and 14 immediately after a fresh extraction in the Fresh Extraction Group (FEG). All patients were treated with a Flat One Bridge technique.

2.2. Technical details

The procedure called “Flat One Bridge” was developed by Intra-lock International (Boca Raton, FL, USA) during the last years and the research was followed-up especially by an Italian group of clinicians. The concept of this approach allows the creation of a final full-arch bridge within 72 hours from the surgical procedure **[6]**. The implants used have an improved nanorough low Calcium impregnated surface (Ossean) and specific designs adapted to this immediate loading application **[7]**.

The implants were adapted for immediate loading, what means they were placed in function immediately after implantation: eight implants were usually needed for the upper arch, six for the lower arch. The mechanical stress within the first 4 days after the surgical procedure acted during the initial healing phase (inflammation and neoangiogenesis), thus stimulating the following steps **[8,9]**.

The main issue in the treatments of the patients with full-arch rehabilitation was often the problems of axis of the implants. As it can be observed in **Figures 1 and 2**, we often faced damaged alveolar ridges where the implant insertion axes were also guided by the bone morphology and were not ideal for the following prosthetic steps. This could be

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particularly complicated with immediately loaded implants, as the conditions of preparation of an implant-supported bridge just after a significant oral surgery are always more complicated. The particularity of the treatment strategy with flat One Bridge was the use of the flat abutments. Connected to each implants, they allowed to correct immediately and definitively the problems of implant axis prior to the preparation of the implant-supported prosthesis. It was particularly useful in these cases, as immediately loaded implants implied to work in a sensible post-surgical mouth. These flat abutments were used in all these cases, and allow to always use full-arch infrastructures to link and to tighten mutually the implants. With the implant design and surface, they are the main success key characteristics of the materials used in this study. The implant-supported prosthesis was produced using a cobalt-chrome alloy and composite resin, for adequate functional and esthetic results.

2.3. Outcome measurement

The main outcome measurement criteria were the success rate, the implant survival rate and the failure rate.

Success was defined as: no mobility, nor pain at percussion and torsion; the distance between the implant shoulder and the bone ridge occlusal edge equal or lower than 2mm, controlled by a radiological examination; no spontaneous or evoked bleeding - negative probing; a keratinized perimplant gum equal or higher than 3 mm.

Implant survival was defined as: an implant that could support the load even in the presence of conic radiotransparency around the implant collar; a limited number of exposed threads of the implant screws and/or a limited loss of keratinized gingiva.

Failure was defined as the mobility or loss of the implant. The statistical analysis was performed using a Fisher's Exact test to compare results in the different groups.

3. Results

All patients were treated in an outpatients department, and their final prostheses were placed in function 12 to 72 hours after the surgical procedures. No complications or adverse events were noticed at the time of implant-supported prosthesis delivery.

All patients were controlled every 3 months during the first year, and every 6 months from the second year. All the patients were followed-up for a time ranging from 6 to 9 years (average 8). There were no drop out patients.

The overall success and implant survival rate of the study population was 95.8%. There were only 2 failures (4.2%), both in the PEG group **(Table 1)**. Comparing groups, we found that all patients in the Native Bone Group **(Figures 1 and 2)** and GBR group **(Figure 3)** had a 100% success rate. The Periodontitis Group showed in majority success, even if some failures occurred. The Fresh Extraction Group reported several cases of implant survivals **(Table 1)**. All these differences were statistically significant ($p<0.05$). All the failures and the implant survival cases were recorded at the upper arch level.

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	NBG	PEG	GBR	FEG	Total
Success	24	5	2	4	35
Survival	0	1	0	10	11
Failure	0	2	0	0	2
Total	24	8	2	14	48

Table 1. Groups and clinical results. NBG: Native Bone Group, PEG: Periodontitis Group, GBR: Guided Bone Regeneration Group, FEG: Fresh Extraction Group.

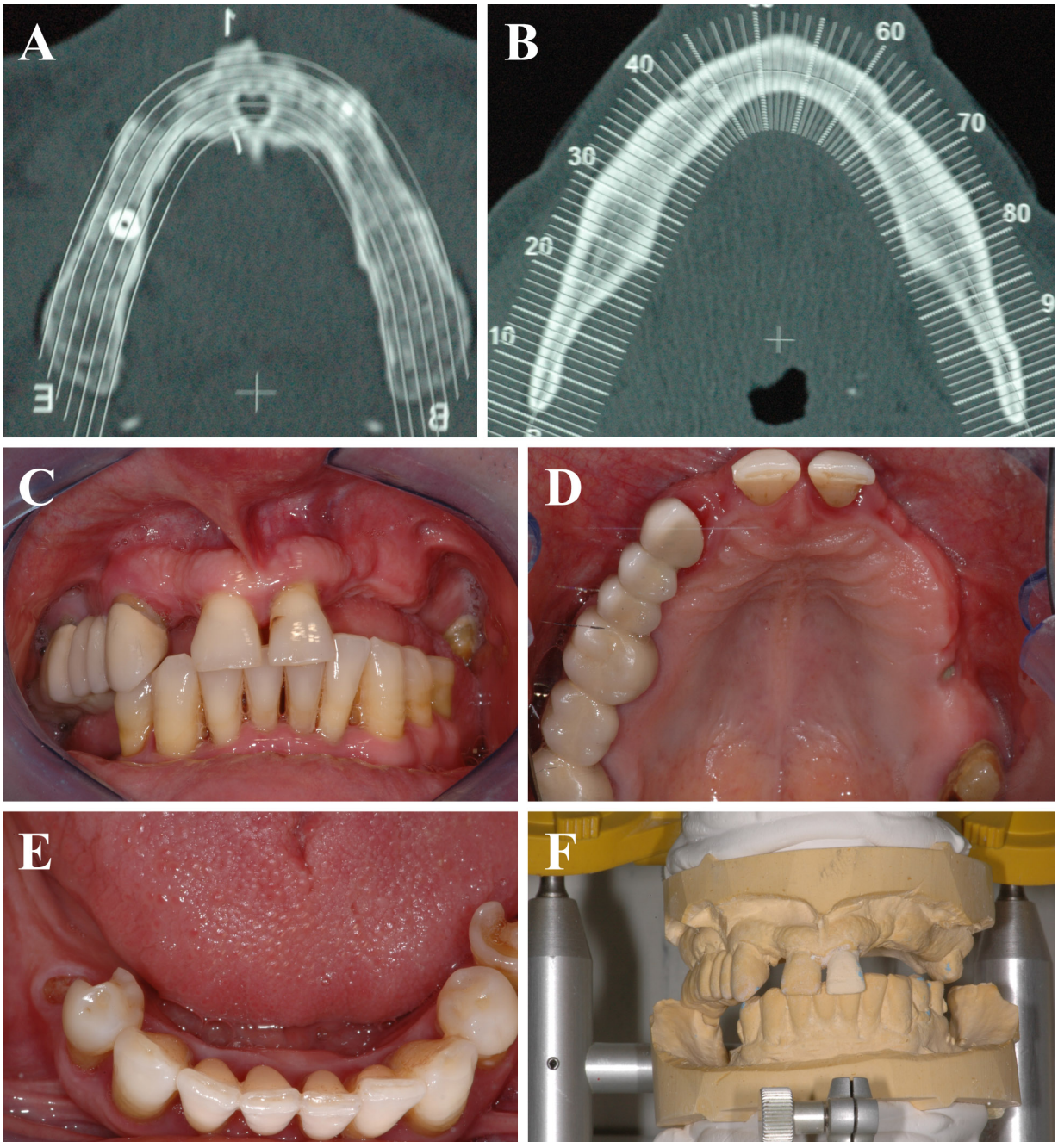

Figure 1. A 40 years old male patient from the Native Bone group. Initial situation. (A) CT scanner of the maxilla before treatment. **(B)** CT scanner of the mandible before treatment. **(C)** General view of the pre-treatment clinical situation. **(D)** Occlusal view of the maxilla before treatment. **(E)** Occlusal view of the mandible before treatment. **(F)** Occlusal analysis of this complex case.

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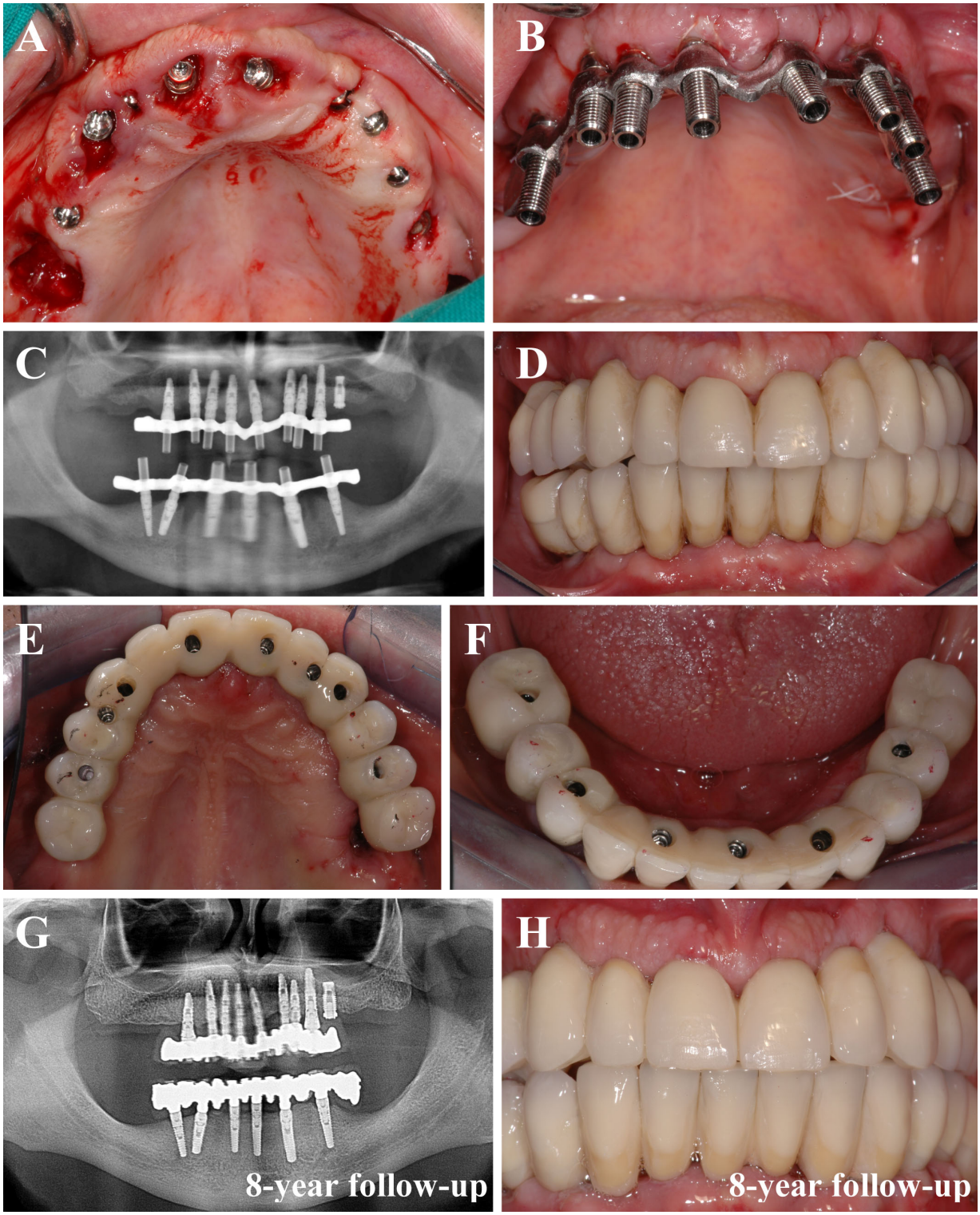

Figure 2. A 40 years old male patient from the Native Bone group. Treatment phases. **(A)** Residual teeth were extracted and implants were placed. **(B)** The prosthetic metallic framework was controlled a few hours after the surgical phase, for adaptation and passive fitting. **(C)** Radiological assessment with panoramic X-Ray a few hours after surgery and with the metallic framework in place. **(D)** Final rehabilitation 72 hours after the surgical phase. **(E)** Occlusal view of the maxillary rehabilitation 72 hours after the surgical phase. **(F)** Occlusal view of the mandibular rehabilitation 72 hours after the surgical phase. **(G)** Radiological control with a panoramic X-Ray after a 8-year follow-up. **(H)** Clinical esthetic aspect after a 8-year follow-up.

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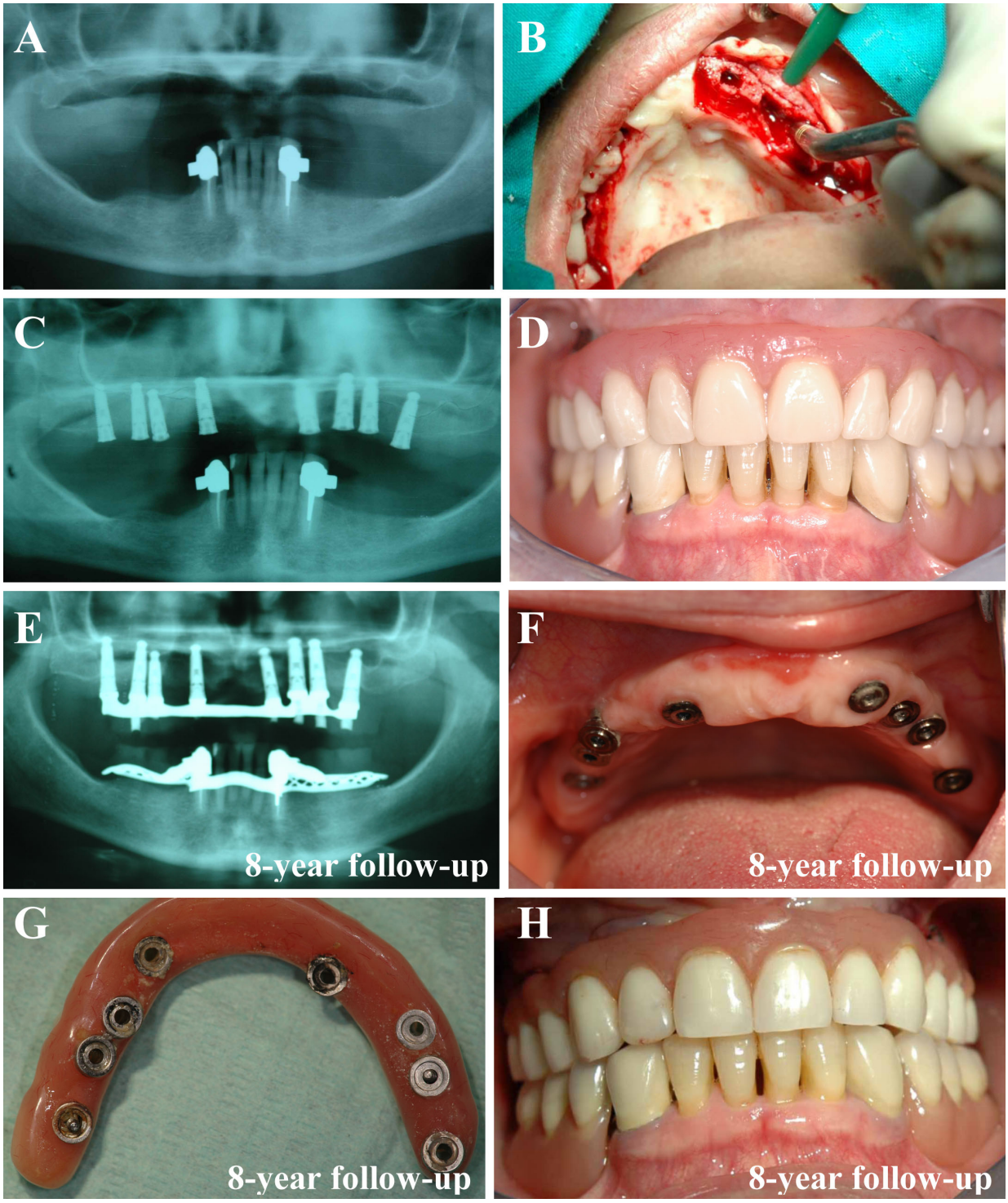

Figure 3. A 70 years old female patient from the GBR group. (A) Pre-treatment radiological assessment. **(B)** Sinus-lift and split crest during the surgical approach. **(C)** Radiological assessment after the surgical treatment. **(D)** Final implant-supported rehabilitation. **(E)** Radiological assessment after a 8-year follow-up. **(F)** Clinical evaluation of the implants, with the prosthesis unscrewed and the flat abutments in position, after a 8-year follow-up. **(G)** Aspect of the implant-supported prosthesis, unscrewed after a 8-year follow-up. Abutments and connections appeared precise and stable after 8 years. **(H)** Clinical esthetic aspect after a 8-year follow-up.

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4. Discussion

Immediately loaded implants are a consolidated technique to treat edentulism, with success rates similar to those of delayed loaded implants, but with the advantage of reduced costs and a shorter clinical protocol **[10,11]**. But in some conditions, there can be technical difficulties in the application of this technique. This is the case in many infectious diseases of the periodontium, systemic diseases like diabetes, hepatic dysfunction and during treatments using bisphosphonates **[12]**. Some reports attributed also a negative impact of smoking on the success rate of this approach **[13]**. However in selected cases, the use of adequate techniques guarantees excellent results **[14]**. The immediate loading approaches are relevant for a single tooth implant treatment, but even more when treating a full arch. In case of a full-arch rehabilitation, the traditional approach was more complex and time-consuming, requiring at least 6 months before the final prosthesis can be positioned. New techniques, like the All-on-Four, reduced these periods signicantly and allowed the fast reconstruction of a functional full-arch **[4,13,15]**. Among these new techniques, the Flat One Bridge was developed at the end of the 1990's - early 2000's to reduce the duration of the provisional prostheses and to help the surgeons in the application of the immediate loading implants **[5,6]**.

The FOB allows an immediate rehabilitation with an esthetic result similar to a traditional delayed fixed prosthesis arch treatment in many clinical conditions. Moreover it allows the use of every residual part of the alveolar space without the need for the crest rectification, thus saving a significant amount of biological tissue and with a similar success rate. Up to now, no direct comparison exists of the different techniques for full-arch immediately loaded rehabilitation, but the comparison of our data with those available in literature shows quite similar success rates **[4,13-18]**.

Our data showed the effectiveness of the Flat One Bridge to treat full arch edentulism. Most of the patients had a functional result, that was maintained during the follow-up period (8 years). Only 2 patients had failures, after 1 and after 3 years respectively. Both of these patients suffered from a periodontitis at the time of the procedure, and this may have had an impact on the final result. Moreover, they were also treated in the early phase of the development of the technique, and this must be considered as a factor which possibly affected the result. Another possible cause of failure is bruxism, as previously reported, but the number of cases in this study is too small to perform an analysis of the impact of this additional pathology **[19]**.

Considering subgroups, we found that in case of native bone, success was achieved in all cases. On the contrary, in the Fresh Extraction Group, a higher rate of implant survivals was reported. This was probably related to the morphology of the implants, which was modified by the manufacturer after the collection of these data to improve the success rate. The implant characteristics, particularly the macrodesign and surface, are key characteristics of an implant system and must evolve and be adapted to each application. The implant system offers now more designs adapted for extraction sockets (particularly the Blossom design). We will soon be able to compare these preliminary data with the results we are now achieving with the updated designs and that we consider even better. In the recent development of the technique, the Flat One Bridge concept became even less invasive and showed an even better esthetic and functional performance, from our experience among the best available for an immediate loading full-arch rehabilitation.

One strength of our study is that our definition of success was much stricter than usually considered, and we added the concept of implant stability, that many authors

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consider as a success **[6,20]**. Moreover, the follow-up from which we considered our results was quite long, making our data more reliable than many short-term studies **[4,15]**.

The present study also has some limitations. First of all, the lack of a control group, but this is not uncommon for first reports of new methods and techniques. Another limit is the small population considered, that made some of the subgroups quite limited. Nevertheless, our statistical analysis showed significant results, so there cannot be any doubt at least about the internal validity. We are still collecting data on the most recent version of the Flat One Bridge implants, and we will soon be able to compare these different implants.

5. Conclusion

Our results show the efficacy of the Flat One Bridge for full-arch rehabilitation. The failure rate was similar to those of other immediate loading implant methods. Further studies including a comparison of different techniques will be useful in the immediate future for a better understanding of the different features of each protocol.

Disclosure of interests

The authors have no conflict of interest to report.

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Research article

Bone characteristics following osteotomy surgery: an *in vitro* SEM study comparing traditional Lindemann drill with sonic and ultrasonic instruments

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Abstract

Background and objectives. Osteotomy surgery is widely used in dental surgery for implant site preparation, bone grafting and GBR. In this study, the characteristics of bone surfaces were examined after bone osteotomy surgery performed with the Lindemann bur, sonic (Komet Sonosurgery) and ultrasonic (Mectron Piezosurgery) instruments.

Materials and Methods. Anatomic integrity and osteotomic precision were analyzed using Scanning Electron Microscopy (SEM) to observe vascular canals, microfractures, exfoliations and bone debris on cortical and cancellous surfaces cut with the 3 types of instruments.

Results. The use of ultrasonic instruments resulted in extremely precise cuts and reduced bone damage. The sonic instrument was precise in cortical bone but showed minor signs of bone damage in cancellous bone. Lindemann bur showed less precision and higher bone damage both in cortical and in cancellous bone. In cortical bone, ultrasonic and sonic cuts showed nicely opened bone vascular canals, while Lindemann bur showed many canals closed by abrasions, exfoliation and cracks by dragging attrition. In cancellous bone, ultrasonic cut showed intact trabeculae and trabecular spaces free of debris, while sonic cut showed more debris accumulation in trabecular spaces. Lindemann bur showed huge quantity of bone debris that filled trabecular spaces.

Discussion and Conclusion. For all parameters, the ultrasonic cut offered the most precise and atraumatic bone cut. Ultrasonic and sonic instruments both showed more precise and less traumatic results than the Lindemann bur.

Keywords. Bone, osteotomy, piezosurgery, surgical instrument.

1. Introduction

Bone cutting technique is a determinant parameter for many applications in neurosurgery **[1]**, as well as orthopedic **[2]**, maxillofacial **[3]** and oral surgery **[4]**. In the past, bone was cut through the use of chisel and mallets or manual saws **[5]**, whereas

rotating instruments, such as bur, rotating disk and saw powered by micromotor, now support this procedure **[6]**. Also, during the last 15 years, surgical bone techniques have undergone a considerable evolution with the introduction of vibrating instruments with sonic-ultrasonic frequencies (Piezosurgery, Mectron s.p.a., Carasco, Italy) and sonic instruments (Sonosurgery, Komet Dental, Lemgo, Germany)**[7,8]**. In dentistry, bone-cutting techniques are commonly used in periodontal and implant surgery **[9]**. Especially in implantology, many bone volume augmentation procedures are based on precise and safe osteotomies **[10]**. Thus, surgical decision-making depends on understanding the advantages and limitations of such surgical techniques as bur powered by micromotor, as well as Piezosurgery and Sonosurgery technologies.

In particular, the cutting action is the result of macro- or micromechanical shocks at different speeds. Saw, bur and disk use high-speed macro vibrations, which may cause bone trauma and damage by producing heat and debris **[11-13]** that may interfere with healing response **[12,14-16]**. Therefore, the cutting characteristics of a traditional instrument (the Lindemann bur) and of vibrating instruments are compared for their bone effects in the present article. More specifically, this *in vitro* study uses Scanning Electron Microscopy (SEM) to analyze the mechanical effects of surgical trauma on cortical and cancellous bone surfaces that result from the cutting action of different surgical instruments. Anatomic integrity and osteotomic precision were evaluated through observation of vascular canals, microcracks and micro-fractures, exfoliations and bone debris.

2. Materials and Methods

Two bovine ribs with dimensions of 25 x 4 x 1 cm were used to prepare 22 bone blocks to be cut with the different technologies. Bovine bone is commonly used as a model in biomechanics because its cortical thickness and cancellous density are similar to human bone. These blocks are characterized by an external dense cortical part, with a thickness of about 2 mm, while the inner part is mainly made of trabecular bone of medium density (D2-D3).

All cuts were performed by the same operator. Each bone rib was sectioned in the longitudinal axis with Piezosurgery 3 with insert OT7 to obtain a first specimen having a length of 25 cm. Each specimen was characterized by an external cortical layer, with a spongious part inside. In order to choose the part of the rib where cortical and cancellous bone have the same thickness in all samples, the specimen obtained was then cut longitudinally in two parts in order to expose internal bone structure. Then the parts of the bone with similar characteristics were chosen in order to obtain the same cutting conditions for each sample. Finally cuts were performed with different instruments, as follows:

- Lindemann Bur (Meisinger 161) powered by W&H handpiece S-11 (W&H GmbH, Bürmoos, Austria): the bone bur is a rotary cylindrical drill powered by a high-speed micromotor with a rotating speed of 20,000 - 40,000 rpm, with external irrigation.
- Piezosurgery: the Piezosurgery 3 system with insert OT7S-4 (Mectron s.p.a., Carasco (GE), Italy). Ultrasonic cut uses linear mechanical microvibrations at both ultrasonic and sonic frequency, ranging from 24 to 36 kHz, depending on the tip used and on the bone quality.
- Sonosurgery: Sonosurgery with insert SFS 101 (Komet Dental, Lemgo, Germany) is a sonic instrument that vibrates at a high frequency (6 kHz).

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Each specimen had dimensions of 1 x 1 x 0.5 cm. During the osteotomy, the cutting device was cooled with profuse physiological solution 0.9%, or water in the case of the sonic device, which was not equipped for saline irrigation. All of the specimens were then inserted in a can containing the physiological solution. Finally, the samples were prepared for SEM analysis. Samples were desiccated and covered with a thin gold layer for conduction, using a Polaron SEM coating system.

3. Results

3.1. Effects of the Lindemann bur on the bone

The cortical part was examined with 100X of magnification **(Figure 1A)**. SEM analysis showed that the cut was not precise, and several signs of extreme bone trauma were seen on the cut surface. Bone surface appeared extremely irregular. Microcracks and exfoliations of bone layers were also visible (15 for field of view). Many bone chips were spread over the bone surface. Cortical bone presented 2 pervious vascular canals for field of view.

The spongious bone showed trabecular fractures and several broken trabeculae. Most bone debris was still linked to the trabeculae and almost completely filled the medullary cavities by an average of 80% **(Figure 1B)**.

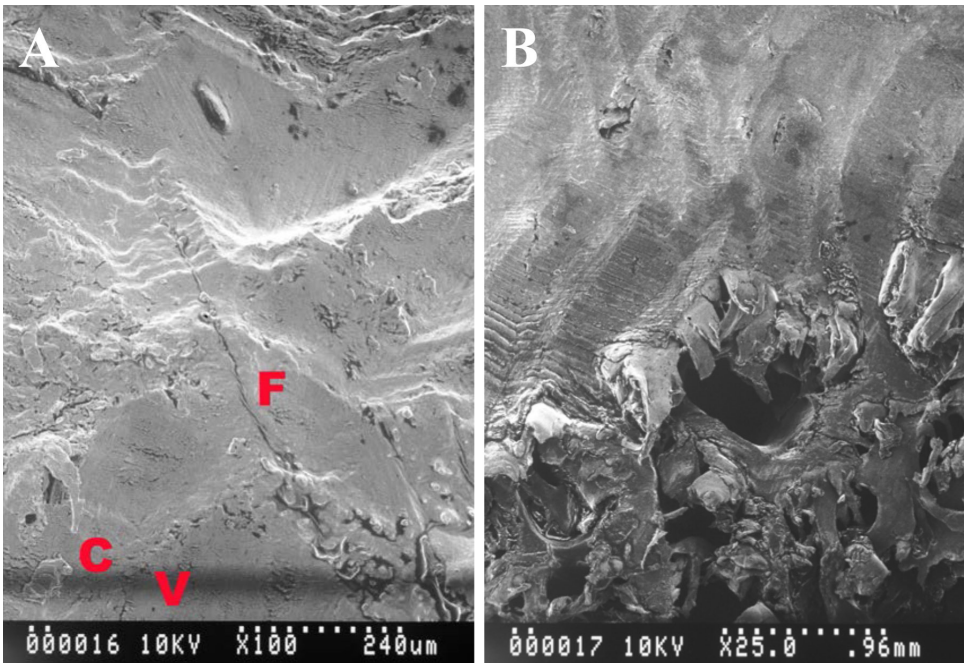

Figure 1. SEM analysis images showing the effects of Lindemann bur on the bone. (A) Effects of Lindemann bur on the cortical bone. The cut was not precise. Several deep abrasions due to the attrition of the cutting edges of the bur corrupted the bone surface, which appeared extremely irregular. Micro-cracks and exfoliations of bone layers were also visible (F). A lot of bone chips (C) were spread over the bone surface, hiding most of the bone vascular canals: only 2 pervious vascular canals for field of view (V) were visible (100X magnification). **(B)** Effects of the Lindemann bur on cortical and cancellous bone. The cortical-spongious junction was still preserved and fairly distinguishable. The deep abrasions of the cortical bone were in continuity with several big bone chips still attached to the bone trabeculae. The chips were mixed with detached bone debris and larger fragments that were almost completely filling the marrow spaces (25X magnification).

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3.2. Effects of the Sonosurgery on the bone

The cortical part, seen with 100X of magnification, showed a precise cut, and the bone surface was smooth and regular **(Figure 2A)**. Microcracks and exfoliations of bone layers were visible (20 for field of view). Few bone chips were visible over the bone surface. Cortical bone presented 10 pervious vascular canals for field of view.

The spongious bone showed few trabecular fractures and unbroken trabeculae. Bone debris occupied medullary cavities with an extremely variable range at a mean of 45% **(Figure 2B)**.

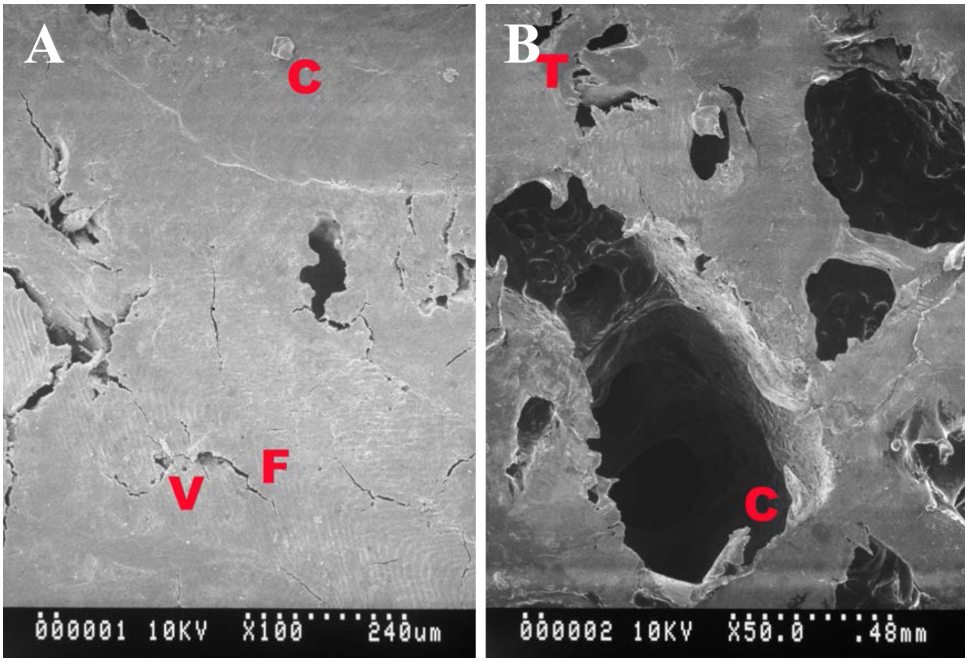

Figure 2. SEM analysis images showing the effects of Sonosurgery on the bone. (A) Effects of Sonosurgery on the cortical bone. The cortical part showed a precise cut, that left bone surface smooth and regular. Microcracks and exfoliations of bone layers were visible (20 for field of view)(F). Few bone chips were visible over the bone surface (C). Cortical bone presented 10 pervious vascular canals for field of view (V)(100X magnification). **(B)** Effects of Sonosurgery on the cancellous bone. The spongious bone showed few trabecular fractures (T) and unbroken trabeculae. Bone debris (C) occupied medullary cavities with an extremely variable range at a mean of 45% (50X magnification).

3.3. Effects of the Piezosurgery 3 with Insert OT7S-4 on the bone

The cortical part, seen with 100X of magnification, showed a precise cut, and the bone surface was well preserved, smooth and regular **(Figure 3A)**. Microcracks were also visible (15 for field of view). Bone debris was almost absent. Cortical bone presented 8 pervious vascular canals for field of view.

The spongious bone showed a very precise cut of the trabecular structure, and bone trabeculae appeared intact. The medullary spaces showed very little debris in the medullary cavities, about 15% on average **(Figure 3B)**.

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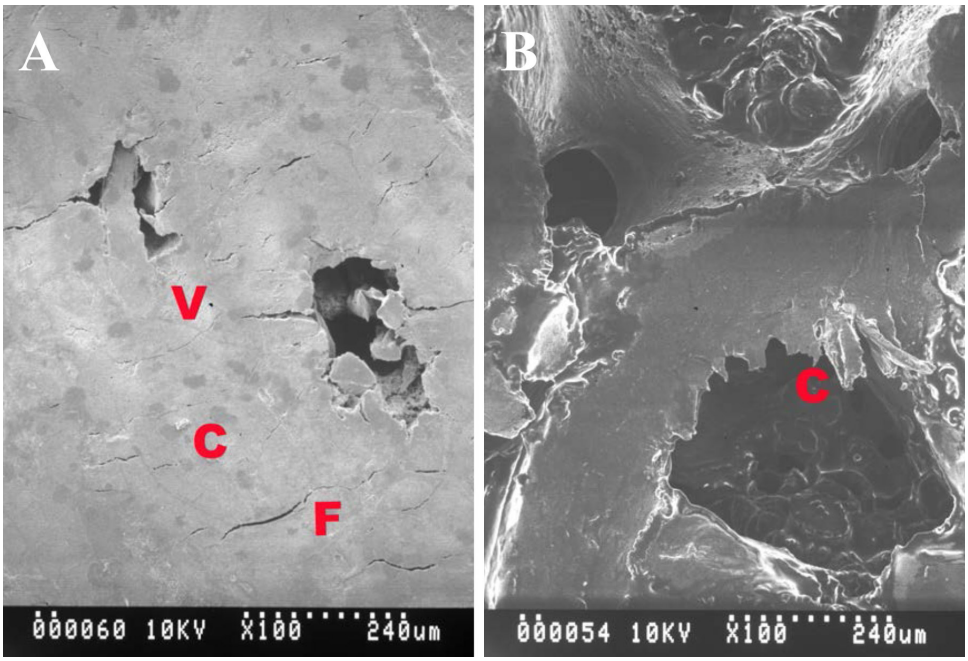

Figure 3. SEM analysis images showing the effects of Piezosurgery on the bone. (A) Effects of Piezosurgery with the insert OT7S4 on the cortical bone. The cortical part, showed a precise cut, and the bone surface was well preserved, smooth and regular. Microcracks (F) were also visible (15 for field of view). Bone debris (C) were almost absent. Cortical bone presented 8 pervious vascular canals (V) for field of view (100X magnification). **(B)** Effects of Piezosurgery with the insert OT7S4 on the cancellous bone. The spongious bone showed a very precise cut of the trabecular structure, and bone trabeculae appeared intact. The medullary spaces showed very little debris (C) in the medullary cavities, about 15% on average (100X magnification).

4. Discussion

Based on the results of the present study, cortical bone exhibits different behaviour in response to the cutting action when compared to trabecular bone. Traumatic damage to cortical bone was limited to microcracks, exfoliations of the bone layers and the formation of deep or superficial abrasions. Abrasions seem to be created by the attrition of the cutting edges on the bone walls. The accumulation of debris was also observed. Two types of debris can be noted: debris still attached to the bone surface and detached debris, which formed a smear layer that completely, or partially, covered the bone surface. The smear layer hid, or closed, most of the vascular canals of the cortical bone. On the other hand, trabecular bone reacted differently to the traumatic cut, probably because of the higher elasticity resulting from the presence of marrow spaces that disrupted the continuity of the mineralized surface of the bone. In spongious bone, this characteristic limited most damage to microcracks and exfoliations, while microfractures, sometimes incomplete, were often seen in trabeculae.

All the cutting devices analyzed in the present study are equipped with irrigation that is aimed to clean surfaces, remove detached debris, improve surgeon visibility and cool the cutting tip. Depending on its efficacy, irrigation seemed to clean most of the cut surfaces. Meanwhile, SEM images showed that both quantity and type of bone damage could be attributed to the cutting technique employed. Based on the amount and type of bone damage, when compared to bur, both cortical and cancellous bone cut with sonic and ultrasonic instruments showed more precision and a cleaner surface with reduced quantity of visible

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damage and lower debris accumulation. Cut surface obtained with the Lindemann bur appeared the most damaged **[17,18]**.

Osteotomies done with the Lindemann bur, showed a considerable accumulation of bone chips on the surfaces, most likely the result of the high kinetic energy used by this instrument and the necessity of applying more pressure during the cut. Among sonic and ultrasonic instruments, the more precise and cleaner cut was achieved by the ultrasonic instrument, while the sonic instrument showed more debris in trabecular bone. The sonic cut appeared more regular in cortical bone, while some lacerations of the trabecular structure were evident in cancellous bone. The ultrasonic instrument showed a more effectively reduced cutting trauma, especially in trabecular bone.

Compared to the bur, the sonic and the ultrasonic instruments showed clean-cut surfaces and the absence of smear layer, in both cortical and cancellous bone. The absence of smear layer was demonstrated by a higher number of visible opened vascular canals in cortical bone and less debris in cancellous bone. Opened vascular canals may improve nutrition during the early healing phase, while clean surfaces may limit inflammation and the need for implementing the cellular cleaning phase of the bone repair sequence. Although studies on ultrasonic implant site preparation and bone healing have shown the possible advantages of clean surfaces and ultrasonic cut **[19,20]**, more biological and clinical studies should be performed in order to clarify the role of bone debris and smear layer on surgically cut bone surfaces. Also, the healing of ultrasonically cleaned bone surfaces should be compared with the healing of surfaces treated with bur, which, in the present study, showed smear layer and a higher amount of bone fragments over bone surfaces and among trabecular spaces.

Sonosurgery seems a promising technique, but still needs technical improvements in order to solve some problems, related to the quality of the cooling solution and sterility. The sonic action, during cutting, produces heat that may cause bone damage, therefore a cooling spray is required. The sonic device is actually equipped of an effective cooling spray, but the cooling solution proposed by the producer is simply normal non sterile drinking water, the same that come from the standard faucet that may be not ideal for surgical applications. Drinking water is not the best for cell preservation, as it is too much hypotonic and also not sterile. *In vivo*, hypotonic solutions does not favorite cells homeostasis, causing a higher risk of tissue suffering and in the meantime the loss of sterility, which may produce contamination of the surgical area. Since it is a common knowledge that in bone surgery a sterile physiologic solution is preferable to non sterile water, the sonic device should be equipped with a sterile spray of isotonic solution. On the contrary the ultrasonic Piezosurgery system and the surgical handpiece for the Lindemann bur, are equipped with a pump which provides an external profuse spray of sterile isotonic saline solution (purified water with 0,9% Na/Cl). This isotonic, purified and sterile solution, without contaminants that can be found in the tap water, is more adapted for the surgical applications of these instruments.

5. Conclusion

In conclusion, the present study illustrates that cortical bone cut with the Piezosurgery ultrasonic device using OT7S-4 may be superior, as it preserves the bone surface and considerably decreases the presence of microfractures and smear layer. Furthermore, cleaning the bone surface with the cavitation effect of the cooling physiological solution should avoid closure of bone vascular canals, which likely occurs in standard cutting techniques by the compression of bone debris between bone surfaces and the cutting device.

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In cancellous bone, ultrasonic cut with the Piezosurgery unit and OT7S-4 insert permits better cutting of the trabeculae and a reduction of 1) fractures that would otherwise weaken bone structure and 2) fragments compressed into the trabecular architecture. Sonosurgery offers also a clean cut with limited tissue trauma. Finally, the cut with the Lindemann bur is the most irregular and traumatic from the 3 instruments. The choice of the adequate instruments during bone surgery should therefore be influenced by these observed results, but also by many other practical considerations (speed of cut, ergonomy, irrigation solution). Further research is needed to understand how these parameters of osteotomy may influence the final bone healing.

Disclosure of interests

The authors have no conflict of interest to report.

Author Contributions

AR wrote the article and, together with GS (Signorini), had the idea to perform the study and prepared the protocol of the work. GS (Sammartino) participated to the elaboration of the design of the study and the revision of the manuscript. GF, FB and MC were in charge of the SEM analysis, morphometric measurements and statistical analysis. MS and GS (Signorini) prepared the samples, performed the cuts, participated to the data collection and to the elaboration of the manuscript.

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