POSEIDO Journal

Periodontology, Oral Surgery, Esthetic & Implant Dentistry Open

Volume 1, Issue 1, June 2013

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An official publication of the POSEIDO Academic network

in partnership with:

ABROSS (Academia Brasileira de Osseointegração)

AFI (Association Française d'Implantologie)

SENAME Association (South European, North African, Middle Eastern)

SICOI (Societa Italiana di Chirurgia Orale ed Implantologia)

TAO (Taiwan Academy of Osseointegration)

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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.

Detailed guidelines for authors can be found on http://www.poseido.info

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ABROSS (Academia Brasileira de Osseointegração), Brazil
APPO (Asociacion Peruana de Periodoncia y Oseointegracion), Peru
AFI (Association Française d'Implantologie), France
International Piezosurgery Academy, International
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Editorial

The Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization (POSEIDO) and Open Journal: an international academic and scientific community for a new approach of open-access publishing

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1. What is open-access publishing?

Scientific open-access publishing is an important movement today in the academic and scientific communities, as a collateral consequence of the recent evolutions in information and communication technologies and globalization of higher education, research and knowledge **[1]**.

The concept of this approach is to give unrestricted online access to the content of a scholarly journal to all readers, in the online electronic version, as the cost of maintenance of the journal are covered by the payment of publication fees by the authors (the author-pays model) after acceptance of their manuscript **[1,2]**. In some rare cases, the costs of publication are covered by a scientific society (through the yearly membership fees paid by their members), a Foundation or Universities.

This approach was largely promoted by academic people who considered abnormal and immoral the classical functioning of academic publishing, where the journals exist thanks to the free work of authors, reviewers and sometimes editors, while the publishing companies require the payment of huge fees for the access to an article or to their journal databases **[1,3]**. In short, academic people are working for free for a journal/publisher, are giving for free their research works for publication, and their academic library pays a lot of money for the access to this work, all that for the huge benefits of the publishing private companies.

The biggest issue in this situation was that most published research works are funded with public money. And what is funded by public money should logically be available for the public. Because of this situation, the policies for copyrights in scholarly publishing have started to evolve **[1,3]**. Many major Universities (such as Harvard, Princeton and many others) have now policies to push their researchers to not give their copyrights to publishing private companies and to publish their works in an open-access platform **[4]**. Several major funding bodies - for example the Wellcome Trust in the UK, the Howard Hughes Medical Institute (HHMI) and the National Institutes of Health (NIH) in the US, and the National Health and Medical Research Council (NHMRC) in Australia - have established strong public

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access policies requiring an open-access publication for all the research they are funding **[4]**. This phenomenon has reached a national policy scale in several Western Countries, particularly Australia and UK **[5]**, and is extending in Europe **[6]** and the USA **[4]**. The forms and paths taken by these changes are different in each environment, but the general evolution is the same. Most traditional publishers are trying to adapt to this tendency and offer several options to help authors to comply with the policies of their university or funding body. One solution is that authors can pay open-access fees for their article, even if the journal is globally not open-access.

The concept of open-access publishing is therefore very attractive and evolving very quickly nowadays.

2. The dark side of open-access publishing

Even if a part of the traditional journals migrated to open-access publishing, this movement remains limited, as the major publishing companies still make significant profits with the traditional publication format. Nowadays most of the open-access journals were in fact created very recently by newly established publishers. The quantity of new open-access journals is considerable, and it is very difficult to determine the real value of these many new items **[7]**.

This situation allowed the development of a large quantity of "predatory open-access publishers" described in the literature as artificial publishing companies or entities that exploit the author-pays model of open-access publishing to make quick and easy profit **[8]**. All Academic people are nowadays massively spammed by these predatory publishers (as our emails are often available in the contact details in our publications) with confusing call for papers, unprofessional publishing operations and a nonexistent peer-review and editorial process. Many of these entities are real fraud, while the exact profile of many other publishers is more debatable **[8]**. In fact, this strange situation raised a much wider concern and philosophical debate of what is scholarly publishing and what kind of value can be given to the various publications.

Indeed, even with open-access publishers that can be considered credible, the open-access electronic publishing strategy has created a new form of journals: the publication without academic and scientific community. Many new journals do not represent anyone anymore, they are not the emanation of a scientific society or scholarly network, but only a platform assembled artificially by a publisher around a publication project. Electronic only open-access journals are slowly becoming a huge list of items (the articles) to satisfy the huge global demand for publication.

This is the natural consequence of the globalization and massification of higher education and research, where all academic people have now the obligation to publish something in international peer-reviewed journals to develop their career, whatever the quality or interest of what is published. The journal becomes just a platform, a database for mass publication without soul, without opinion, without search for debate and consensus. In short, in our opinion, a publication with limited interest.

3. Open-access publishing in dentistry

Open-access publishing started to develop in dentistry, even if these new journals have still a limited reputation in our field. Many of them have already failed, and only 2 of them (in general dentistry) have succeeded to be indexed in PubMed/Medline. None of them

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has impact factor yet, and the general impact of these publications is very limited in our community. The situation is therefore quite different from what we can observe in other domains.

Among all scientific fields, dentistry is a quite specific case, where dentists are more attached to their community (organizing congress and meetings) than to a publication. Many dental publications are already available and all of them are funded through the membership to various scientific societies and academies (and the support of commercial advertisement), and this current functioning seems to have found its own equilibrium. Therefore in dentistry, the pressure for evolution towards open-access seems to be very limited at this time. Even if most readers and authors would prefer open-access publishing for a better dissemination of the publication work, this publication model does not seem to be actively promoted in our community.

In our opinion, this situation will evolve, as in the times of globalization of higher education and research, knowledge will be more and more open-access. But this situation raised the major question on what should be the future of scientific publishing in our field. Do we have only the choice between locked journals of private scientific societies or large open-access publication platform without community?

4. The POSEIDO initiative, the third way

The POSEIDO Foundation was created to support the POSEIDO Organization (Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization), an international network of academic departments and scientific societies. The Organization is managing and publishing the POSEIDO journal (Periodontology, Oral Surgery, Esthetic and Implant Dentistry Open journal).

4.1. The Concept

The POSEIDO initiative was developed to offer a new approach of open-access publishing. The concept is to develop a new scientific journal with an unrestricted online access to the content to all readers, and to give the full control of the content to an international dental community where opinions and debates can be organized and published.

But this approach is not enough. In our times of globalization of research and higher education and of extremely quick communication, it is also needed to move beyond the limited patterns of open-access publishing and to reach the ultimate objective of this movement: global collaboration, global democracy, particularly the freedom to express scientific opinions without the pressure of the major commercial companies. A journal can not only be a list of scientific data compiled by specialists, a scientific publication must first of all be a platform for the development and exchange of ideas and debates, at a global scale.

This is the concept of POSEIDO: global collaborative publication.

4.2. Functioning

In a scholarly journal, most of the work is done for free by academic people, while most of the costs of functioning are generated by a publishing private company. The POSEIDO Foundation was created and generously funded by academic people and non-commercial sponsors with this idea of supporting the functioning of an independent open-access journal. The Foundation supports the logistics for the Organization. Most of the work

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is done for free by a network of scholars – what is similar with a traditional journal – except that the editorial offices are in fact academic departments on each continent or areas. Thanks to this organization, the journal works with no publication fees for all members of the community (members of registered academic departments or partner scientific societies).

POSEIDO is an international academic network and community managing and publishing its own independent open-access international scientific peer-reviewed journal. This is a collaborative international publication platform, trying to give the same representation to all the partners whatever their country. The system is also completely independent from commercial pressure or conflicts of interest, as the Foundation covers all the fees of functioning and the journal does not require any commercial advertisement to exist.

This journal and organization may also be an efficient instrument to promote inter-university collaborations and help to develop links between the many national scientific societies partnering in this platform. But first of all, this journal wants to be an instrument of international debates and a platform of education for all the members of our community.

4.3. Aims and Scope

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.

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5. Perspectives

As a conclusion, the POSEIDO initiative is opening the third way for dental scholarly publishing, and we hope that this community will largely develop in the coming years through an increasing number of academic members of the POSEIDO network and the partnership with many scientific societies. This is the first step of a necessary evolution, and we hope that it will open the way for the development many new international projects. This

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first issue was designed to give some guidelines and examples of the functioning of the journal, and the debates are now officially opened!

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Special Review: Consensus Conference

Guidelines for the publication of articles related to implant surfaces and design from the POSEIDO: a standard for surface characterization

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Abstract

Dental implant surface engineering is a very active field of research, however the abundant literature on the topic is often difficult to sort and interpret. Indeed there is a significant lack of homogeneity in the methods to describe the various surfaces available on the market or tested in experimental studies, resulting in confusions in the literature and difficulties to compare the numerous published results. In this article, the POSEIDO (Periodontology, Oral Surgery, Esthetic & Implant Dentistry Organization) is developing and promoting a validated concept for the characterization and description of the implant surface characteristics. The objective of these guidelines is to help researchers to standardize their studies and to promote clarity in this field of research. Illustrated by the description of 2 types of implant surfaces (TiUnite, Nobel Biocare, Gothenburg, Sweden, and Ossean, Intra-Lock, Boca-Raton, FL, USA), these guidelines describe some standardized tools of analysis and terminology that can be used to characterize and define a dental implant surface, particularly its chemical composition (core material, such as titanium, and chemical or biochemical modification through impregnation or coating) and its topography at the micro- and nanoscale (such as microroughness, microporosity, nanoroughness, nanotubes, nanoparticles, nanopatterning and fractal architecture). These POSEIDO guidelines are an important step for the clarification of knowledge and standardization of experiments in this field.

Keywords. Dental implants, osseointegration, titanium.

1. Introduction

The development of implantable materials is an important field of research in medicine in general, and in dentistry in particular **[1]**. Dentals implants are mostly defined by their macrodesign (which is a significant parameter in the clinical indications of the implants)**[2]**, by their mechanical parts (prosthetic components and their accuracy)**[3]** and by their surface **[4]**. Implant design (macroscale) and surface (micro- and nanoscale) of the implants are 2 interconnected parameters that define the interactions of the implanted material with the host tissue, and therefore these characteristics must be well investigated **[4]**.

The literature about dental implant surfaces is currently abundant **[5]**. Many teams and companies around the world are making financial investments to study this topic **[6]**. However, in fact there is very little defined knowledge about what should be an « ideal surface ». The literature is controversial and the published results are difficult to sort and interpret **[1,5]**. The presence of conflicts of interests between researchers and companies may help explain a portion of these problems. However, the true reason of this lack of clarity and consensus is probably more simple: the absence of a relevant standard for the characterization of the studied surfaces **[1]**. In short, researchers are testing many surfaces in vitro (with cells)**[7]** and in vivo (in patients or animals)**[5,8,9]**, but very often they do not accurately describe the surface they are testing. When examining the articles published in the international literature during the last 20 years **[5]**, we can see that researchers often describe their surface by the method of production (sand-blasted acid-etched, blasting with resorbable blasting media, anodization, etc.)**[10]** and not by the detailed characteristics of the surface **[1]**.

For this reason, the POSEIDO (Periodontology, Oral Surgery, Esthetic & Implant Dentistry Organization) intended to define a simple standard to use in surface science and associated publications, so that these works can constitute a more reliable and valuable database for the scientific community. Additionally, this would make these research works easier to understand by the clinician readership **[4]**. This need for well defined classification and terminology exists in all fields **[11,12]**, but it is particularly obvious in surface science. The first step of this strategy was published in 2010 as a general classification and codification system **[1]**. This initiative was followed in 2011 with the publication of the Identification Cards of 14 implant surfaces available on the market **[4,13]**, where these surfaces were fully characterized following the complete codification system previously described.

2. Chemistry and topography, the key parameters

Two levels of characterization can be defined for a dental implant surface **[1,4]**: chemistry and morphology/topography. Both are deeply interconnected and define together the biological properties of a surface **[14-17]**, but they have to be analyzed separately.

The first level is based on the chemical composition of the surface, i.e. the composition of the core material (commercially pure titanium grade 2 or 4, titanium-aluminiun-vanadium alliage Ti6Al4V i.e. grade 5 titanium, zirconia, hydroxyapatite, etc.)**[1,18]** and its eventual chemical (or sometimes biochemical)**[19]** modifications (for example a fluoride or a Calcium Phosphate CaP low impregnation)**[20,21]**. As shown previously, this chemical modification can often be an inorganic or an organic pollution **[4]**. The chemical composition and architecture is a key parameter for the biochemical interlocking between the implant surface and the bone tissue **[1,22-24]**.

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The second level is based on the surface topographical characteristics, i.e. the general morphology and structures at the microscale (microrough, microporous, microparticles, presence of cracks or large particles) and at the nanometer scale (nanosmooth, nanorough, nanopatterned, nanoparticled)**[25]**. Several morphological parameters (height deviation amplitude Sa, developed area ratio Sdr%) can be used to quantify this morphology on the microscale **[1]**. The microtopography is a key parameter for the biomechanical interlocking between the implant surface and the bone tissue **[1]**.

The investigation of the nanostructures on the implant surfaces is a recent approach, with potential applications in bone tissue engineering **[25,26]**. The production of surface features at the nanoscale is a new method to control the cell-surface interactions **[27-30]**.

The definition of each characteristic can sometimes be sensitive, and for this reason a classification system and terminology was suggested **[1]**. In the articles about the codification and classification of implant surfaces **[1,4]**, a detailed protocol of characterization was proposed and can be considered as a relevant basic standard. However, many different protocols and instruments exist and allow to gather similar informations.

3. Many techniques of analysis, one objective

Most relevant surface parameters can be characterized using standard analytical tools. We illustrate here these characteristics and analyses with two different commercially available implant surfaces: TiUnite (Nobel Biocare, Göteborg, Sweden)**[10]** and Ossean (Intra-Lock, Boca-Raton, Florida)**[21,31]**.

For the evaluation of the surface chemistry, the use of X-ray Photoelectron Spectroscopy (XPS), also called Electron Spectroscopy for Chemical Analysis (ESCA), can be considered as a gold standard **[32,33]**. XPS is used to determine accurately the quantitative mean atomic composition (in %) and chemistry of a wide and thin surface area (typically 300µm in diameter, less than 20 nm in depth)**[1]**. XPS provides the chemical state of the detected elements, such as the binding forms of phosphorus in phosphates (**Figure 1**). The data provided by this technique may be difficult to understand for a non-physicist, but it is in fact very simple to summarize them in a table with percentages of atomic composition **[4]**.

Auger Electron Spectroscopy (AES) is less accurate than XPS, but it can analyze very small areas and is ideal for checking surface chemical homogeneity, using several repetitive analyses. AES can perform a quick and accurate in-depth chemical profiling of the surface (**Figure 2**)**[32]**. It is thus particularly useful to characterize a core material **[4]**.

A complementary technique called Energy Dispersive X-ray Spectroscopy (EDX) is a simple elemental analysis coupled with the Scanning Electron Microscope (SEM) and allows the identification of particles or structures observed with the SEM (**Figure 3**). The reality is that a wide range of tools can be used to perform the chemical analysis of a surface, for example Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), Raman Spectroscopy, or even Transmission Electron Microscopy (TEM) after Focused Ion Beam (FIB) cross sectioning of a sample **[34]**. However, most of these techniques require a high degree of calibration to get relevant quantitative data, and do not truly fit to the requirements of osseointegrated surface standardized evaluation.

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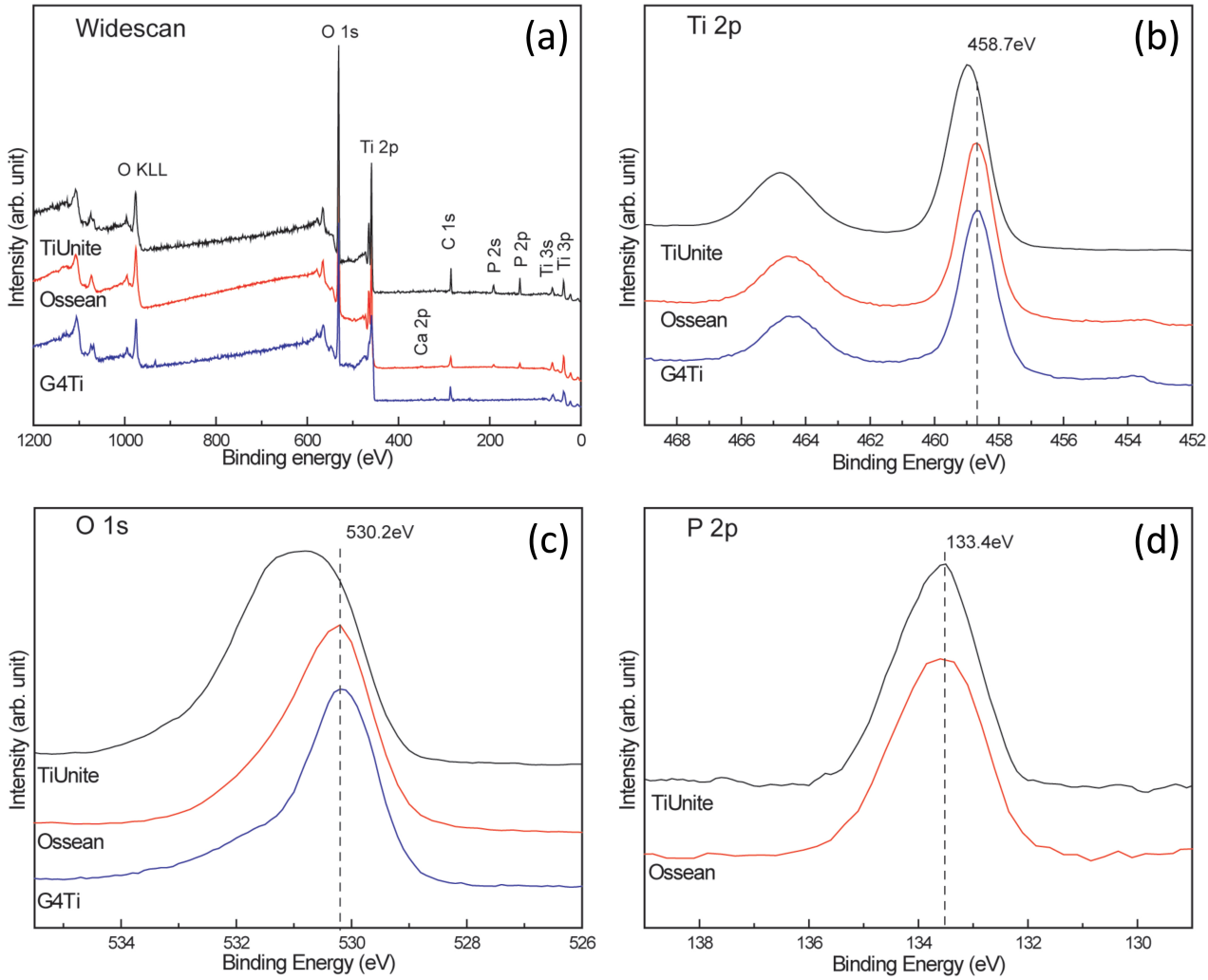

(e)	Ti	O	C	N	P	Ca
TiUnite	17.5	55.1	17	0.9	9.5	-
Ossean	19.8	57	18.6	1.5	2.8	0.3

Figure 1. XPS data of TiUnite, Ossean and G4Ti (grade 4 titanium) surfaces: **(a)** survey XPS spectra; **(b)** high resolution Ti 2p spectra; **(c)** high resolution O 1s spectra; **(d)** high resolution P 2p spectra. Survey XPS data showed major peaks of O 1s, Ti 2p and C 1s for all the samples and minor peaks of P 2p for TiUnite and Ossean. In P 2p high resolution spectra, there was no significant difference in peak position and spectra shape between TiUnite and Ossean. On the contrary, Ti 2p and O 1s spectra of TiUnite showed higher peak positions and wider peak shape than the spectra of G4Ti and Ossean. TiUnite is indeed an anodized surface, with phosphorus high impregnation within a micrometer thick titanium oxide TiO_2 layer, and with thus formation of titanium phosphates. On the other hand, Ossean shows a calcium phosphate low impregnation that negligibly altered the surface chemistry of TiO_2. The results of these XPS analyses are also reported in a more simple and reader-friendly way as percentages of atomic composition for each element **(e)**.

(O as oxygen, Ti as titanium, C as carbon, N as nitrogen, Ca as calcium, P as phosphorus)

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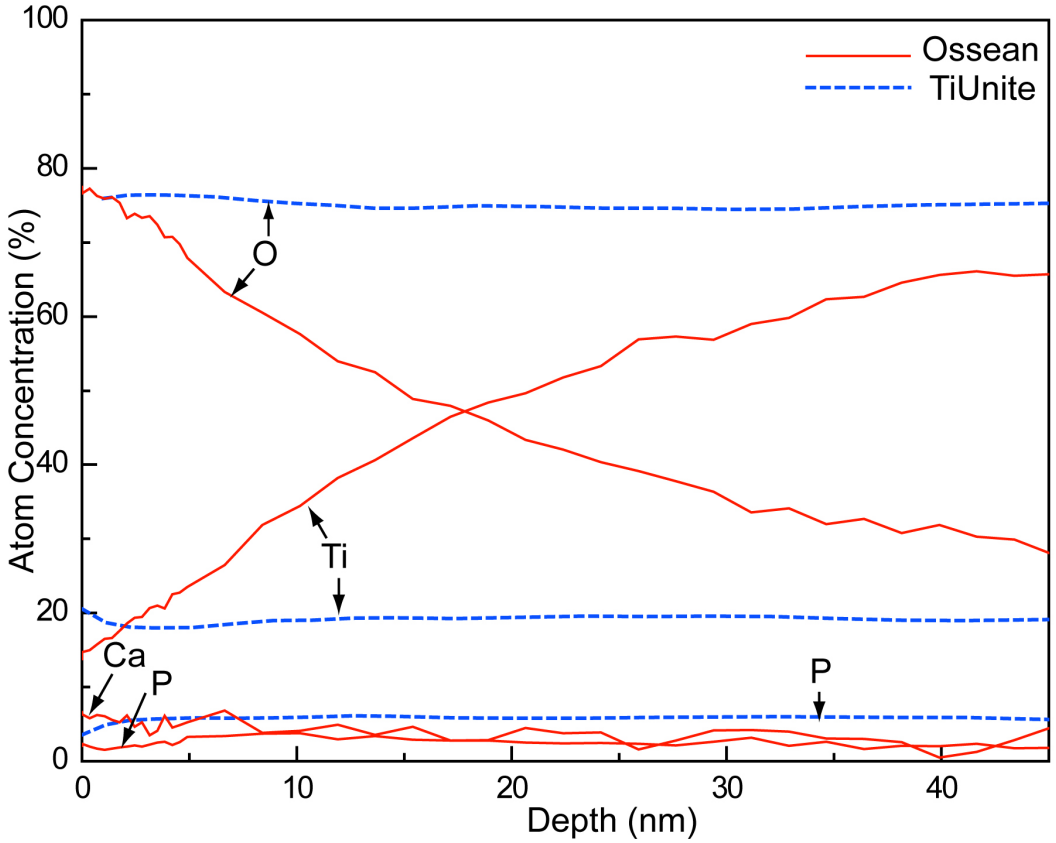

Figure 2. AES in-depth profiling of TiUnite and Ossean surfaces down to 45nm. The two surfaces show completely different patterns. TiUnite is anodized and thus presents a thick and homogeneous TiO_2 layer highly impregnated with phosphorus. Ossean is based on another technology, with a decreasing proportion of TiO_2 and a stable CaP low impregnation along the in-depth profile of the surface. (O as oxygen, Ti as titanium, C as carbon, Ca as calcium, P as phosphorus)

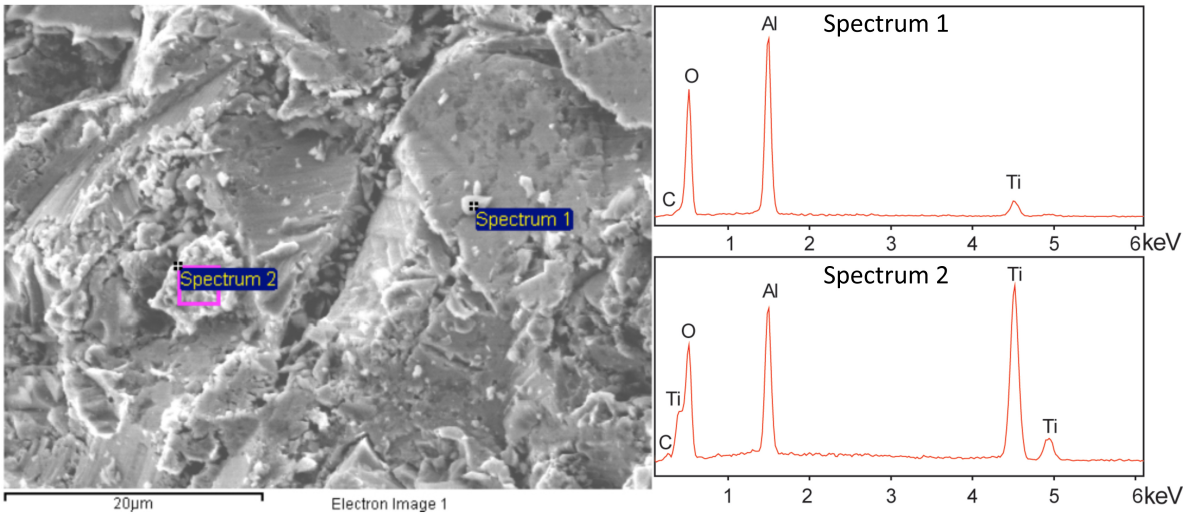

Figure 3. Composition analysis with EDX probe of particles observed with SEM. The surface of this early version of this Ankylos implant (Friadent, Mannheim, Germany) is covered with microparticles. The EDX analysis allows to identify these particles as Aluminium Oxide blasting residues. Spectrum 1 was acquired in a very small area, showing clear Al and O signals. Spectrum 2 was acquired using a larger interaction volume, resulting in clear signals of both the AlO residual particles and the Titanium oxide below.

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The topography can be assessed with many different tools, but two are particularly adapted and common. Scanning Electron Microscopy (SEM) is the gold standard for morphology characterization at the micrometer level (SEM with tungsten source)**[32]**. However, Field Emission-SEM (FE-SEM) is required to increase the analytical resolution, and to observe and characterize the nanotopography and associated nanostructures (**Figure 4**)**[4]**. Without FE-SEM, the analysis of the nanostructures should be considered as incomplete and inadequate, even if the authors may have the feeling to observe something relevant **[1]**. This is a problem of resolution, and using the wrong instruments simply creates artefacts.

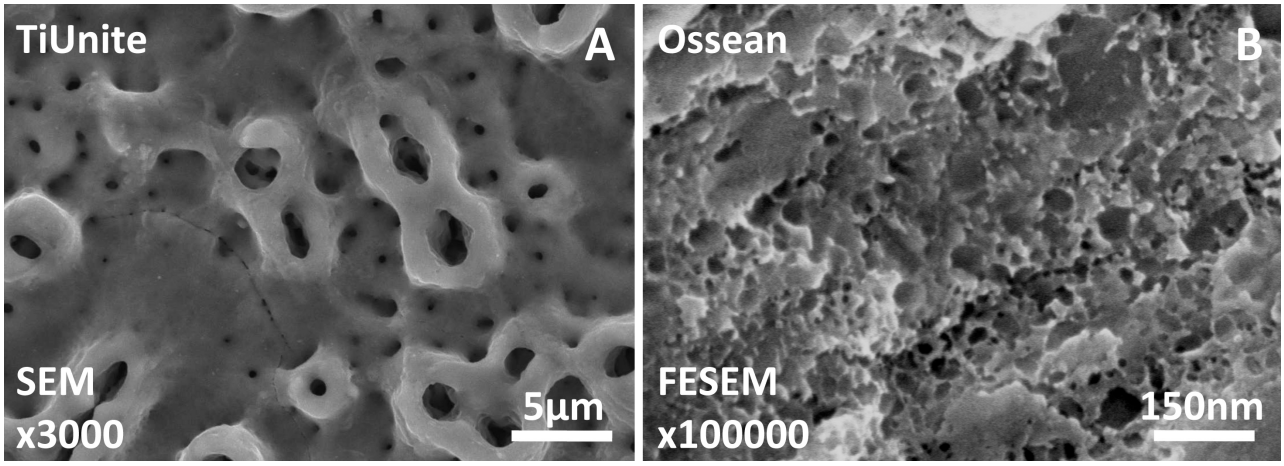

Figure 4. Scanning Electron Microscopy (SEM) examination. A. TiUnite is an anodized surface with a typical microporous topography and cracks observable with the classical SEM at low magnification. **B.** Ossean is a microrough surface presenting a typical nanoroughness observed at higher magnification. However without the use of a Field Emission-Scanning Electron Microscope (FE-SEM), it would be impossible to observe so clearly the nanostructures, particularly in this environment rich in CaP.

Interferometer (IFM) or optical profilometry (OP) is an efficient tool for the evaluation of the microtopography general aspect and quantitative parameters on wide areas (**Figure 5A**)**[4,8]**. A FE-SEM can also be coupled with a metrology software to produce 3D reconstructions of the surface (stereo SEM) and to perform a quantitative morphology analysis, both at the micrometer and nanometer level (**Figure 5B**).

All these techniques have their advantages and limitations. This list of instruments is not exhaustive, and all these analyses are not required to publish an article about surfaces. However, it should be now mandatory for the authors to provide a clear and detailed chemical and topographical characterization of the tested surfaces if they want to have their article considered for further review in an international journal. The POSEIDO suggested characterization system offers a strong coherence and an easy way to clarity, even if all protocols offering similar information are acceptable. This endeavour is an important step for the development of a high quality database about dental implant surfaces, and also to simplify the understanding of basic science surface articles by the clinician readership.

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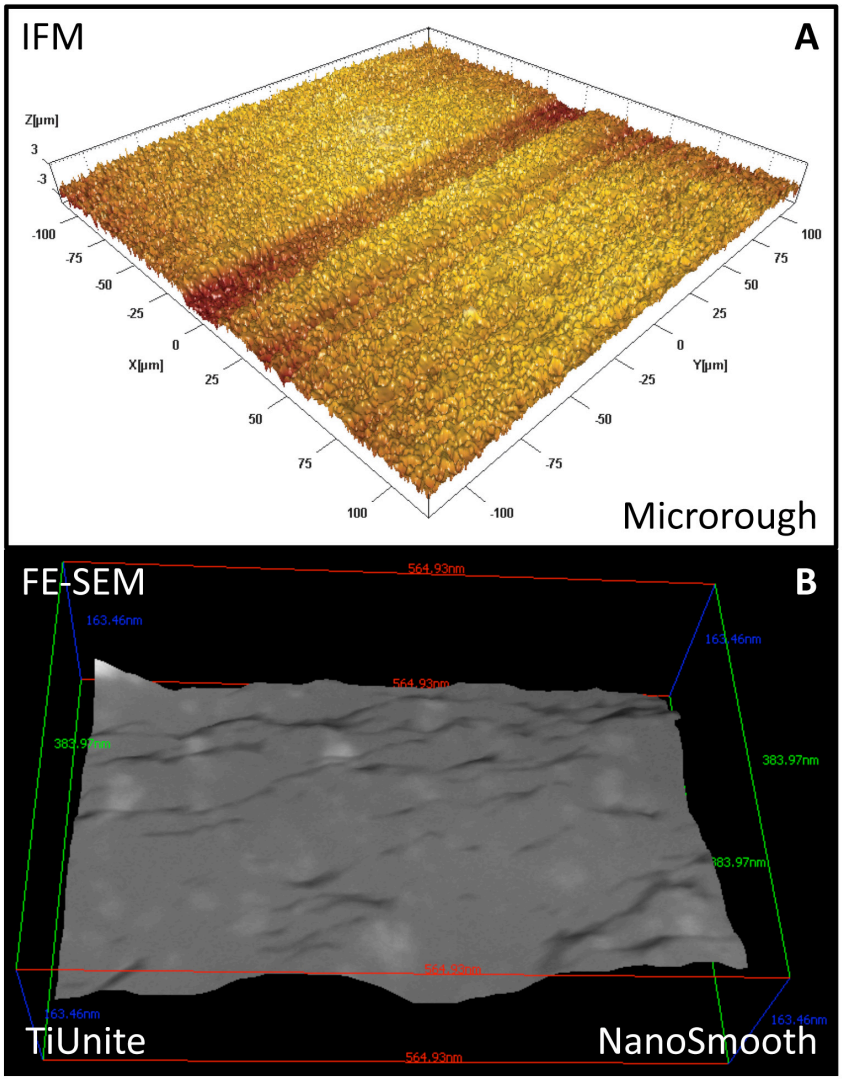

Figure 5. IFM and computed FE-SEM evaluation of implant surfaces. A. IFM is an easy and powerful tool for quantitative evaluation of the microroughness values on wide surface areas (typically 230µm x 230µm). **B.** FE-SEM analysis coupled with a metrology software allows to perform a quantitative morphology down to the nanoscale. This TiUnite nanometric square surface shows an almost flat nanotopography, and is considered as nanosmooth.

This protocol for surface has also to be considered for all articles about implant macrodesign. Indeed, testing a new design always implies to rule out the potential bias related to surface. The first step is therefore to characterize carefully the surfaces, to be sure that they are strictly the same between the samples, before proceeding further for the new design testing. In the literature, the surfaces are rarely checked before testing different designs, and this may explain why the published results in the international literature are so difficult to sort and interpret.

4. Conclusion and Perspectives

This consensus article is a first step of the POSEIDO initiative to develop common standards in the field of implantable biomaterials. These general guidelines for surface characterization offer a simple standard method for the research in this field, to improve the

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quality of the experiments and to clarify the literature. When more results will be published using this approach, it will be possible to sort and interpret more easily the data on this topic, and to refine our knowledge. These general guidelines are a first important instrument, and should be completed in the future with the feedback of experience.

Disclosure of interests

The authors have no conflict of interest to report.

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Special Review: Consensus Conference

Guidelines for the publication of articles related to platelet concentrates (Platelet-Rich Plasma - PRP, or Platelet-Rich Fibrin - PRF): the international classification of the POSEIDO

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Abstract

Platelet concentrates for surgical use are autogenous regenerative preparations, produced by the centrifugation of the patient own blood sample. Most techniques are often regrouped inappropriately under the historical term of Platelet-Rich Plasma (PRP). Since 15 years, their use dramatically increased in many surgical fields, particularly in oral and maxillofacial surgery. The literature on this topic is considerable, but the published results are often contradictory. It is very difficult to sort and interpret the available data, due to a large number of preparation techniques, terminologies and forms of these materials, and the endless list of potential applications. This consensus conference of the Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization (POSEIDO) was established to support a classification system of these products, in order to improve and clarify the publications on this topic. Four main families of preparations can be defined, depending on their cell content and fibrin architecture: Pure Platelet-Rich Plasma (P-PRP), such as cell separator PRP, Vivostat PRF, PRGF-Endoret or E-PRP; Leukocyte- and Platelet-Rich Plasma (L-PRP), such as Curasan, Regen, Plateltex, SmartPReP, PCCS, Magellan or GPS PRP; Pure Plaletet-Rich Fibrin (P-PRF), such as Fibrinet; and Leukocyte- and Platelet-Rich Fibrin (L-PRF), such as Titanium-prepared PRF and Intra-Spin L-PRF System. P-PRP and L-PRP exist in an inactivated liquid form, and can be activated and transformed respectively into a P-PRP gel and a L-PRP gel. This terminology will serve as a basis for future works to be published in the POSEIDO journal and as a first step for further research on the topic.

Keywords. Fibrin, fibrin tissue adhesive, Platelet-Rich Plasma, platelet, leukocyte.

1. What are platelet concentrates for surgical use?

Platelet concentrates for surgical use are autogenous products prepared through the centrifugation of a blood sample of the patient **[1]**. The concept of these technologies is to collect and gather the most active components from the blood sample - platelets (rich in growth factors), fibrin and sometimes leukocytes - and to prepare them in a clinically usable form. These preparations can be solutions or gels and can be injected or placed in a surgical site, on a wound or in an injured area, in order to regenerate the damaged tissues **[2,3]**.

In most of these techniques, blood is collected with anticoagulant and then processed following a 2-step centrifugation **[4]**. The first step of centrifugation is used to separate the blood in 3 layers following a gradient depending on their weight: red blood cells at the bottom of the tube, acellular plasma (called platelet-poor plasma, PPP) at the top of the tube, and a whitish layer (sometimes called buffy coat, like in transfusion science) rich in platelets and cells between the 2 other layers. The red blood cells are then discarded and the second step of centrifugation is used to collect only this buffy coat and some acellular plasma. The final liquid platelet suspension is called Platelet-Rich Plasma (PRP) in transfusion medicine, and the term was used to regroup the many families of platelet concentrates for surgical use **[1]**.

This platelet suspension can be injected in an injured site (for example in tendons or articulations in sport medicine)**[5,6]** or activated with bovine thrombin (or calcium chloride, or equivalent platelet activator)**[7,8]**. The activation of the suspension provokes the platelet growth factors release and the polymerization of fibrinogen into fibrin, to form a platelet gel similar to a fibrin glue that can be used on a surgical site or a wound **[9]**. This is the general description of the production of platelet concentrates, but many variations of the production exist. Particularly for the subfamily called Leukocyte- and Platelet-Rich Fibrin (L-PRF), blood is taken without anticoagulant, processed with a one step centrifugation and no platelet activator is needed **[10]**.

The philosophy of these treatments is in fact to concentrate and use the positive effects of the actors of the coagulation process. Platelets, fibrin and leukocytes act naturally in synergy in order to promote the wound healing and tissue regeneration, and the concept of platelet concentrates for surgical use is to multiply this coagulation/regeneration effect on a surgical site or wound. In the history of these techniques, researchers have focused alternately on the fibrin matrix, the platelets, the growth factors and more recently on the leukocytes and circulating stem cells **[3]**, and the terminology of these materials has evolved following these trends **[11]**.

2. History of the terminology

2.1. Early history

The history of these technologies starts in fact with the fibrin adhesives developed more than 40 years ago **[12]**. The need of surgical adjuvants in order to improve healing and control diffuse bleeding promotes the development of fibrin glues. As first matrix of coagulation, fibrin is indeed a key element of the healing process, and these glues are still used nowadays **[13]**.

In a second time, some researchers tried to improve their fibrin adhesives preparations by combining it with the other natural key actors of coagulation. These autologous preparations were termed « platelet-fibrinogen-thrombin mixtures » and were used with success in ophthalmology **[14,15]**, general surgery **[16]** and neurosurgery **[17]**.

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Other authors called it « gelatin platelet (gel foam) » **[18]**. In these applications, these new preparations were used as fibrin tissue adhesives and the role of the platelets was advocated to serve only to reinforce the fibrin matrix architecture. The presence of platelet growth factors and the potential direct healing properties were not advocated or even considered.

It took several more years before the concept evolved and these preparations were considered to have direct healing properties. In 1986, Knighton et al. **[19]** developed an efficient clinical application for the treatment of chronic non-healing cutaneous ulcers, using a preparation using a 2-step centrifugation procedure and named "platelet-derived wound healing factors" (PDWHF). In other articles in 1988 and 1990 **[20,21]**, the same technique was named "platelet-derived wound healing formula (PDWHF)". In that time, the term "platelet-rich plasma" was only used as a technical term and was not the name of the final usable product. A few years later, Whitman et al. **[22]** published their clinical results in oral and maxillofacial surgery, using a platelet concentrate termed "platelet gel".

2.2. PRP and the craze for growth factors

The craze for "growth factors" and the use of the term "Platelet-Rich Plasma" (PRP) really started with the article of Marx et al. in 1998 **[7]**, in a study about the effect of a platelet-rich preparation during maxillofacial bone reconstruction. The platelet suspension was then activated into a gel using bovine thrombin. The use of the term PRP by these authors was in that time quite correct, as the preparation was produced using a cell separator from the hematology laboratory (and therefore was similar to a PRP used for transfusion). The "platelet-rich plasma (PRP)" term was initially developed in 1954 by Kingsley to designate thrombocyte concentrate **[23]**, used for the treatment of patients suffering from severe thrombopenia.

After this article, the term of PRP – associated with the concept of growth factors - widespread and soon was used to name all kinds of preparations and techniques **[24,25]**. A huge number of new experimental or commercial techniques were proposed during the last 15 years **[26-31]**. This is at this time that started a significant confusion in the literature, as in most articles about platelet concentrates, many different protocols (commercially available or "home-made") were tested under the name "PRP", but in most cases without a proper characterization of the content and architecture of the tested concentrates **[32]**. Moreover, as the concept of "regeneration through growth factors" seduced many authors **[33-35]**, the key role of the fibrin was almost completely neglected during many years, as if 30 years of research in fibrin-based surgical adjuvants had almost not existed.

As a result, even if these PRPs were largely investigated in vitro and in vivo in many applications, the literature is very contradictory and controversial, and the data are difficult to sort and interpret. In dentistry, it led to the general feeling that PRPs are not so useful **[24,25]**. After the initial craze, dental clinicians using these PRP preparations in their daily practice became very scarce.

2.3. Leukocyte- and Platelet-Rich Fibrin (L-PRF)

In parallel of the PRP history, a second family of materials initially called Platelet-Rich Fibrin was developed a few years later **[10]**, and started to replace the PRP in oral and maxillofacial surgery. In this simple technique, blood is taken without anticoagulant and is immediately centrifuged with moderate forces during 12 minutes. Three layers appear then in the tubes: the red blood cells are gathered at the bottom, acellular plasma is at the top of the

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tube and a strongly polymerized fibrin clot called PRF is formed between **[36]**. This PRF clot gathers most of the platelets and half of the leukocytes (mostly the lymphocytes) of the blood sample **[36]**, and it was therefore called Leukocyte- and Platelet-Rich Fibrin (L-PRF)**[1]**. It can be used clinically as a clot or as a membrane **[37]**. In comparison with PRP gels, this PRF gel is particularly strong, and releases significantly during more than 7 days large quantities of key coagulation and healing molecules (thrombospondin-1, fibronectin, vitronectin) and growth factors - particularly the platelet growth factors TGFβ1 (Transforming Growth Factors β1), PDGF (Platelet-Derived Growth Factors) and VEGF (Vascular Endothelial Growth Factor)**[38,39]**.

This clot is produced without blood modification, and can be considered as an optimized natural blood clot, prepared in a clinically usable form **[36]**. It is a solid biomaterial and not a liquid suspension: therefore it can not be injected like the various PRPs **[6]** and it only exists in an activated gel form. Reported in vitro and in vivo experimental effects were very positive and significant **[40-42]**. This family of platelet concentrates developed nowadays very strongly with excellent results in periodontology **[43-45]**, oral surgery **[46]** and implant dentistry **[47-51]**. This strong fibrin membrane/clot form is particularly adapted to oral clinical applications **[24,25]**, even if other applications in orthopedic and sports medicine **[52]** and for the treatment of chronic skin ulcers are also advocated **[53]**.

After several years of experimental use by clinicians at the borderline of the local regulations **[54]**, the production system and kit are now marketed and available as a CE-marked and FDA-approved inexpensive system called Intra-Spin (Intra-Lock, Boca Raton, Florida, USA), as shown in the **Figure**.

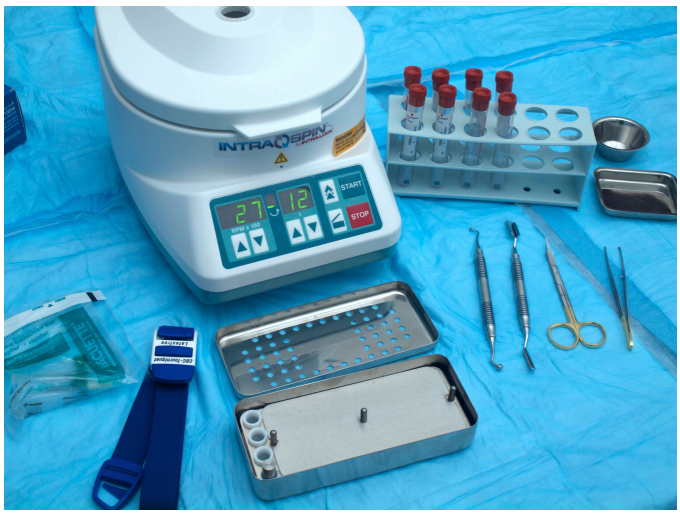

Figure. The centrifuge and kit for the preparation of Intra-Spin L-PRF (Intra-Lock, Boca Raton, FL, USA). This system is the CE-marked FDA-approved version of the well-known open-access technique for the production of L-PRF clots and membranes. All the systems for the production of platelet concentrates on the market require a specific centrifuge (the model on the photo is one of the most compact) and an adapted collection and preparation kit (in this case, mostly tubes and a box of collection). The ergonomics of the final system is an important parameter for the development of these techniques in daily use.

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2.4. Evolutions of the terminology

While the literature about PRPs developed with all these contradictions, several authors started to point out the need for a more accurate terminology and the importance of some neglected parameters, such as the leukocyte contents and the fibrin architecture.

In an opinion published in 2006, Bielecki et al. **[55]** insisted on the different forms of PRP used in clinical practice: PRP can be injected without activator (for example in injured tendons or articulations)**[6]**, but is more often used after activation resulting in a gel formation. It was therefore proposed to call Platelet-Rich Plasma the suspension, and "Platelet-Rich Gel" (PRG) the activated fibrin gel. The 2 forms are not the same products. The authors also pointed out the presence of leukocytes in these preparations, and the need to take them into consideration. In 2008, Everts et al. **[56]** insisted on the importance of the leukocytes and the activation in the biology of the PRPs. These authors suggested to name the inactivated suspension "platelet-leukocyte rich plasma (P-LRP)", and the activated gel "platelet-leukocyte gel (PLG)". These two terminologies were used in a few articles **[57-60]**.

However, these suggested terminologies remained incomplete, as not all PRPs have leukocytes **[61]**, and PRPs do not require to be activated prior to injection to be active (they activate in a different way after injection in the host tissue)**[6]**. Moreover, after activation of a PRP, the gels never reach the strength of natural fibrin polymerization obtained in the PRF subfamily **[36,62]**. The definition of a more global terminology for all platelet concentrates was needed, in order to integrate all the potential configurations and components of these preparations. A classification system was finally published **[1]** and confirmed through a first international consensus article **[11]**. This system will serve as a basis of the POSEIDO recommendations.

3. Current POSEIDO terminology

3.1. Classification system

The POSEIDO recommendations are based on the previously published classification of platelet concentrates for surgical use **[1]**, and will serve as a basis for future evolutions of the terminology and recommendations for clinical use.

First, all the products of this category are regrouped under the general term of "platelet concentrates", whatever their form or cell content.

Second, it is important to highlight the key influence of the leukocyte content **[63-65]** and fibrin architecture **[66,67]** in the potential clinical or experimental effects of these products, and that each product refers to a specific biological imprint **[39,68,69]**.

Four families can be highlighted, based on their leukocyte and fibrin content. Liquid platelet concentrate suspensions (before activation) are termed Platelet-Rich Plasma (PRP): "Pure Platelet-Rich Plasma" (P-PRP) without leukocytes, "Leukocyte- and Platelet-Rich Plasma" (L-PRP) with leukocytes. On the other side, solid platelet concentrate biomaterials, with a strong fibrin architecture (therefore existing only in this activated form), are termed Platelet-Rich Fibrin (PRF): "Pure Platelet-Rich Fibrin" (P-PRF) without leukocytes, "Leukocyte- and Platelet-Rich Fibrin" (L-PRF) with leukocytes. The activated versions of a P-PRP and a L-PRP are respectively a « P-PRP gel » and a « L-PRP gel ». The 2 PRF subfamilies only exist in the gel form, per definition. The main described technologies are classified in the **Table**.

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This basic terminology has the advantage to be simple and to avoid commercial interference **[70]**. It may not be enough to avoid the many possible experimental bias detected in the literature **[45,71-73]**, but it is a first important step to create a minimal common basis for terminology and characterization of marketed or experimental products.

Platelet Concentrate Class and terminology	**Methods of production** (generic name, detailed appellation when existing, company, city, country)**[references]**	
P-PRP (Pure Platelet-Rich Plasma), before activation (**P-PRP gel**, after activation)	**AP**	- Cell separator PRP (experimental)**[7]** - Vivostat PRF (Vivolution, Alleroed, Denmark)**[31]**
	MP	- PRGF/Endoret (Preparation or Plasma Rich in Growth Factors, BTI BioTechnology Institute, Vitoria, Spain)**[61,70]** - E-PRP (Eye Platelet-rich Plasma, experimental)**[8]** - Nahita PRP (Nahita, Navarra, Spain)**[28]**
L-PRP (Leukocyte- and Platelet-Rich Plasma), before activation (**L-PRP gel**, after activation)	**AP**	- PCCS PRP (Platelet Concentrate Collection System, 3I, Palm Beach Gardens, FL, USA)**[26,31]** - SmartPReP PRP (Harvest Corp, Plymouth, MA, USA)**[27,31]** - Magellan PRP (Magellan APS (Autologous Platelet Separator), Medtronic, Minneapolis, MN, USA)**[30]** - Angel PRP (Angel Whole Blood Processing System (AWBPS), Sorin Group, Mirandola, Italy) - GPS PRP (Gravitational Platelet Separation System, Biomet Biologic, Warsaw, IN, USA)**[69]**
	MP	- Friadent PRP (Friadent-Schütze, Vienna, Austria)**[27]** - Curasan PRP (Curasan, Kleinostheim, Germany)**[26]** - Regen PRP (Regen Laboratory, Mollens, Switzerland)**[32]** - Plateltex PRP (Plateltex, Prague, Czech Republic)**[29]** - Ace PRP (Surgical Supply and Surgical Science Systems, Brockton, MA, USA)**[28]**
P-PRF (Pure Platelet-Rich Fibrin)	**MP**	Fibrinet PRFM (Cascade Medical, Wayne, NJ, USA)**[31,32]**
L-PRF (Leukocyte- and Platelet-Rich Fibrin)	**MP**	- Intra-Spin L-PRF (Intra-Lock, Boca Raton, FL, USA)**[36,37]** - Titanium-prepared PRF (experimental)**[42]**

Table. Classification of the main available methods of production of platelet concentrates, in the 4 main families of products. In each category, many marketed or experimental custom-made protocols exist. Even if all techniques use similar concepts and fall within the limits of this classification system, the possible variations of production techniques are endless and this table regroups only some significant well-defined products. Some techniques require little handling and are considered as Automated Procedures (AP), while others require more handling steps and are considered as Manual Procedures (MP).

3.2. Potential evolutions of the classification

At this point of our knowledge, three last parameters are still kept outside of this classification system: the platelet concentration rate, the leukocyte concentration rate, and the proportion of the various sorts of leukocytes. Indeed, even if these parameters may have

some impact, their exact clinical influence remains still too vague, particularly in oral and maxillofacial applications.

Platelet concentrations can be very different between the various systems **[35,61,74]** but the immediate effects of dilution undermine the impact of this parameter in vivo. Mishra et al. **[75]** suggested a specialized sub-classification for injectable PRPs in sports medicine, where a 5-fold platelet concentration rate may be a relevant baseline for the definition of PRP subfamilies (concentrations higher than 5-fold often gave better clinical results). However, this baseline is probably not universal and therefore not valid for all clinical applications. This issue does not exist in the PRF family, where all the platelets of the blood sample are activated and integrated in the fibrin matrix of the clot **[36]**.

The leukocyte concentration and formula may also have an impact **[63,68]**, but they were often neglected in the literature. Their influence should be investigated carefully in the future, as their presence or not may explain many contradictory results that were observed, particularly in sports medicine and orthopedic surgery **[75]**.

There is a very last parameter that remains even more unclear than the others: the global cell content of the L-PRP and L-PRF **[3]**. Indeed, the products containing leukocytes in fact also contains a large and diverse population of circulating cells, all of them interacting and influencing their environment **[40]**. The control and adequate management of these cells may open new therapeutic opportunities.

All these parameters should be assessed carefully now, in order to develop with accuracy our knowledge and maybe improve this first classification system in the future.

4. Perspectives

It is important to notice the current evolution of the use of platelet concentrates in the interconnected fields of periodontology, oral surgery, esthetic and implant dentistry (POSEID disciplines). Even if they are used with some success for the treatments of chronic skin ulcers and in sports medicine, PRPs are slowly disappearing from the POSEID fields, due to their complexity of use, costs of production, and mixed clinical results. On the other hand, the development of the L-PRF in the POSEID fields is accelerating, as it can be observed in the number of publications appearing recently. The reasons are very simple and pragmatic: the L-PRF is inexpensive, easy to use and efficient in many oral applications. In short, the technology meets the criteria of daily use of the specialists. This is now an important topic of research in the POSEID disciplines.

As a conclusion, this consensus conference was designed to help both authors and readers to understand the current situation and perspectives in the field of platelet concentrates for surgical use. For authors, this classification system should be considered as guidelines for preparation of research works on this topic. This is also for our community a first step to develop research projects on this theme and improve our knowledge of these preparations. Platelet concentrates are playing and will play even more a significant role in our therapeutic strategies in the coming years, and this classification will probably be completed and improved in the future.

Disclosure of interests

The authors have no conflict of interest to report.

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Clinical case letter

"M" flap design for promoting implant esthetics: technique and cases series

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1. Introduction

The objectives of modern implant dentistry are no more to reach only a stable osseointegration, but are now focusing on the quality of the final esthetic result. In the anterior maxillary area, the reconstructions have to be indistinguishable from the natural teeth. Factors such as a thin gingival biotype, a high lip line, triangular shaped teeth, and high patient esthetic demand may affect the final outcome of the treatment in the maxillary anterior region, and many techniques are developed to improve this final esthetic outcome **[1,2]**.

The management of soft tissues during the second-stage of implant surgery (implant uncovering surgery) is an important parameter to improve the final esthetic aspect around the implant-supported restoration. Traditionally, a tissue-punch or a full thickness flap opening prior to abutment connection have been used at this stage. This may lead to bone loss resulting in soft tissue recession, and causes unesthetic implant restorations **[3]**. Many different flap designs have been advocated to reduce these negative consequences. This includes, but is not limited to: split finger technique **[4]**, by splitting the soft tissue flap in two halves and place them respectively on the mesial and distal sides; roll technique, by moving tissue from palatal side to the buccal area; palatal roll technique, by rotating the palatal tissue after removing the epithelium layer to the buccal side **[5]** and inlay connective tissue graft **[6]**.

In this article, a simple surgical approach, called "M" flap design, is described and evaluated in a series of 58 cases, to prevent buccal marginal recession and to achieve an esthetic peri-implant soft tissue remodeling and predictable implant-supported gingiva-prosthetic integration, particularly during the single tooth rehabilitations.

2. Materials/methods and results

In this article, we illustrate this technique with 2 clinical cases among a series of 58 patients. A.N (Case 1, **Figure 1**) and P.M (Case 2, **Figures 2 and 3**) were referred to the Department of Oral Surgery, Faculty of Medicine, University of Naples Federico II, and were expecting a fixed rehabilitation of their missing upper lateral incisor. An implant-supported prosthesis was planned (**Figures 1A, 2A**). Three months after the placement of a sand-

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blasted acid-etched implant (Thommen Medical AG, Waldenburg, Switzerland), the fixtures exposures were performed following the "M" flap surgical technique.

Briefly, an intrasulcular inner beveled incision (Micro-blade M6900, Advanced Surgical Technologies, Sacramento CA, USA) was performed around the distal aspect of the adjacent teeth, rounding buccally and palatally (**Figures 1B, 1C, 2B**). A horizontal slightly palatal M-shaped incision connected the vertical incisions (**Figures 1B, 1C, 2B**). A full thickness flap was then raised in order to visualize the implant head (**Figure 1D**). A healing cap was placed, and a monofilament mattress suture at the gingival papillae stabilized the flap around the healing cap. Furthermore, single suture knots assured a tension free wound closure (**Figures 1E, 1F, 2C**). Ten days after surgery soft tissue was almost completely healed (**Figure 2D**). After 6 weeks, soft tissue modeling was apparently complete (**Figures 1G, 2E-2H**). A Zirconia abutment was placed and soft tissue integration was controlled (**Figures 3A, 3B**). A metal-free crown rehabilitation was finally achieved (**Figures 1H, 3C, 3D**).

The same technique was applied successfully in a series of 58 cases of lateral maxillary incisors, using the exact same protocol, and showed the same outcomes during a two-year period. The accurate evaluation and scoring of the benefit of this approach is difficult, as all cases are different and difficult to standardize. However the experience on this case series confirmed that this simple incision line has no notable side-effects or unexpected negative consequences.

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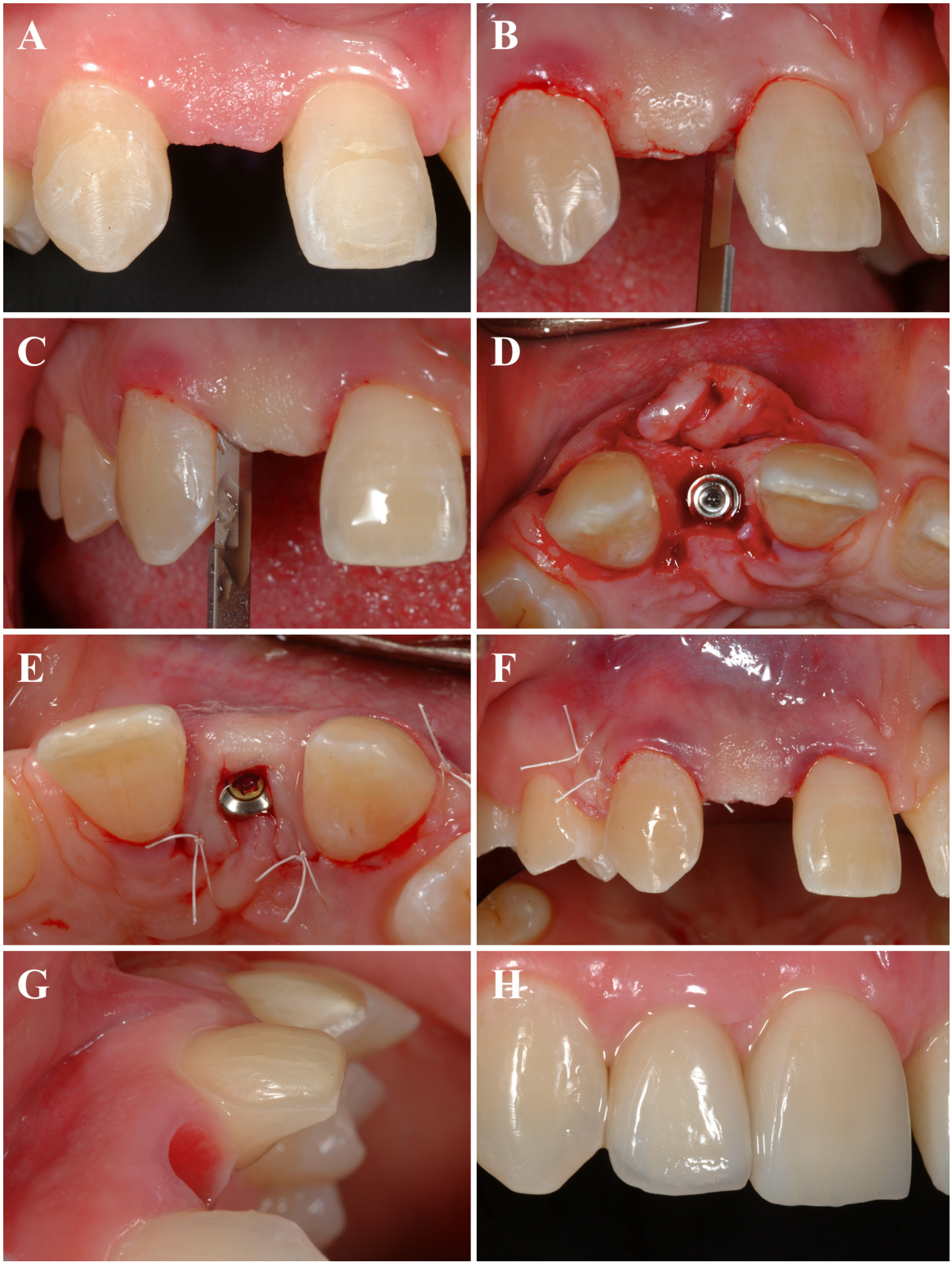

Figure 1. First case. (A) Preoperative view: the right maxillary lateral incisor was missing in a thick gingival biotype case. **(B, C)** An intrasulcular inner beveled incision was performed around the distal aspect of the adjacent teeth, rounding buccally and palatally and connecting with a M-shaped incision. **(D)** The full thickness "M" flap was raised to visualize the bone surface and connect the implant abutment. **(E, F)** The flap was closed and sutured with a mattress monofilament suture at the gingival papilla to stabilize the flap around the healing cap. Single knots were used to assure a tension-free wound closure. **(G)** After 6 weeks, a complete soft tissue healing was apparently achieved. **(H)** The final zirconia-based implant-supported crown offered an excellent esthetic outcome.

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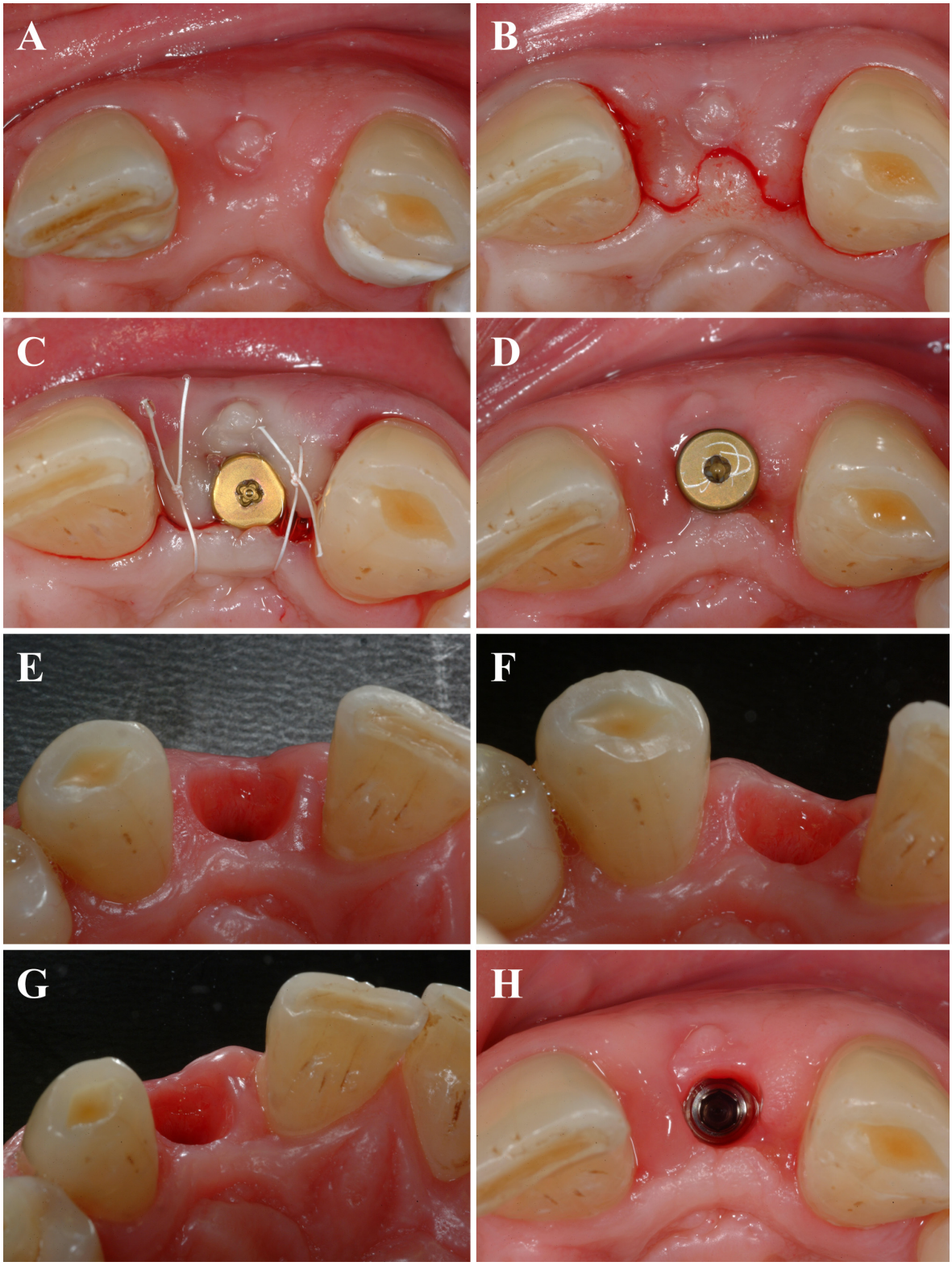

Figure 2. Second case, surgical step. (A) Preoperative view: the left maxillary lateral incisor was missing. **(B)** A M-shaped flap was performed. **(C)** A mattress monofilament suture was used at the gingival papilla to stabilize the flap around the healing cap. **(D)** After ten days, the healing was good and uneventful. **(E, F, G, H)** After six weeks, the healing was almost complete with a stable contour around the temporary crown.

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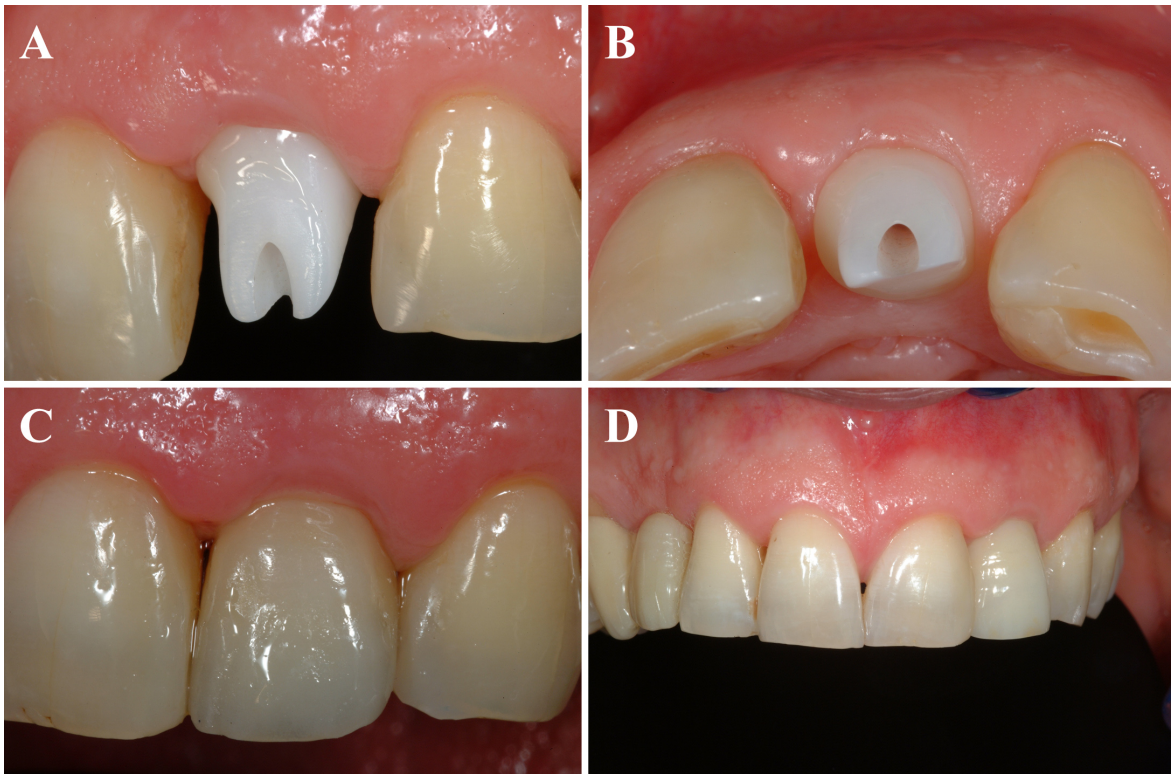

Figure 3. Second case, prosthetic step. (A, B) A zirconia abutment was placed and presented a correct peri-implant soft tissue integration. **(C, D)** The final zirconia implant-supported crown was placed and showed a proper esthetic aspect and contour.

3. Discussion

The incisions are critical parameters in all periodontal and implant surgeries **[7,8]**, particularly for the wound closure after a bone reconstruction and for the management of a natural soft-tissue contour in complex rehabilitations **[1,2,9]**.

The second implant surgical stage could be a challenging procedure, especially in the anterior maxilla where the esthetic expectations are always very high. Gingival recession and implant shoulder exposure can seriously compromise the final esthetic outcome of incisor rehabilitations, especially in immediate postextractive cases **[10]** and when an adequate architecture of the surrounding papilla is still present. High lip line smile, thin gingival biotype, triangular tooth shape, high patient expectation represent risk factors for the proper management of the prosthetic implant-supported rehabilitation in the esthetic anterior area **[11]**.

In immediate postextractive cases, the buccal bone resorption can affect the esthetic outcome. The thin buccal bone plate resorption, related to the tooth loss and past infections, may cause a wide marginal recession, with the implant shoulder exposure **[10,12]** and sometimes the beginning of an implant contamination **[13]**. In such cases, a slightly palatal implant placement via a flapless approach allows an adequate primary fixture stability and reduces the buccal plate stress **[11,14]**. The reported "M" flap technique represents a low risk approach to the implant shoulder, especially when natural adjacent teeth are present. As the

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case 2 shows, a more palatal incision allows to get a thicker buccal soft tissue, reducing the risk of gingival recession even in thin biotype cases. The M-shaped flap technique needs microsurgical devices in order to minimize soft tissue inflammation. By this way, it assures a better flap vascularization with a tension-free flap healing, and thus reduces the risk of buccal gingival recession **[15]**. The internal vertical mattress suture at the papilla level (each suture for each papilla) assures a better soft tissue modeling around the implant healing cap and the adjacent teeth. By this way, the esthetic results are more predictable, especially in more demanding cases.

The M-shaped incision offers good results, but this approach could also be combined with some healing biomaterials such as platelet concentrates for surgical use, in order to promote a supplementary stimulation of the periosteum and gingival maturation **[7-9]**.

As a conclusion, in anterior implant rehabilitation, the M-shaped flap offers excellent esthetic outcomes, especially in single tooth restorations and in immediate postextractive cases. With the "M" flap design, the gingival architecture is preserved, peri-implant soft tissue healing during the immediate postoperative period is more predictable (particularly around temporary crowns) and consequently soft tissue-crown integration is improved. The reported technique allowed to achieve these results in all of the 58 surgical cases performed.

Disclosure of interests

The authors have no conflict of interest to report.

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Clinical case letter

Esthetic management of the maxillary anterior region with multi-discipline approaches

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1. Introduction

The management of anterior implant-supported rehabilitations is always challenging. Patients have logically high expectations from the restorative treatments. In order to achieve a predictable and stable, functional and esthetic final result, both hard and soft tissue managements are often required **[1,2]**. In the case of a complete maxillary anterior region rehabilitation, there are many steps to respect and disciplines to combine in order to reach an adequate outcome. Among the key endeavors of this kind of treatments, some steps are of particular importance, such as: to prevent the alveolar ridge resorption, to augment the bone and soft tissue thickness during implant placement, to construct provisional prosthesis to model the soft tissue profile and crown/gingiva integration, and to adapt the abutment/restoration contour to further enhance the final esthetic aspect **[3-5]**.

The concept of this multi-discipline approach is frequently advocated in modern restorative dentistry **[4,5]**, but its proper application remains quite seldom. The objective of this article is to discuss and illustrate the relevance of this systematic multi-discipline approach for the treatment of the severe anterior maxilla atrophy, in order to achieve a successful, predictable and stable long-term esthetic restoration in this challenging area.

2. Materials/methods and results

The patient was referred to the department of oral surgery of the University of Naples Federico II for an upper anterior fixed partial rehabilitation. This patient was healthy, however he smoked more than 7 cigarettes/day. The upper teeth had a thin tissue biotype (<1.5mm), severe periodontitis with significant gingival recessions and severe tooth mobility. After a preliminary periodontal treatment, an implant-supported rehabilitation of the frontal region was planned (**Figures 1A to 1C**).

A prosthetic guide was fabricated based on the final prosthetic rehabilitation project, in order to guide the next surgical steps. In the first surgical stage, upper central and lateral incisors were removed and socket filling using collagen sponges was performed, to stabilize the clot and to promote wound healing. A fixed provisional bridge was then placed (**Figures 1D to 1G**). It quickly appeared that the position and strength of the median frenum was high and could compromise the next surgical steps of the treatments, by tearing on the future flaps (**Figure 1H**).

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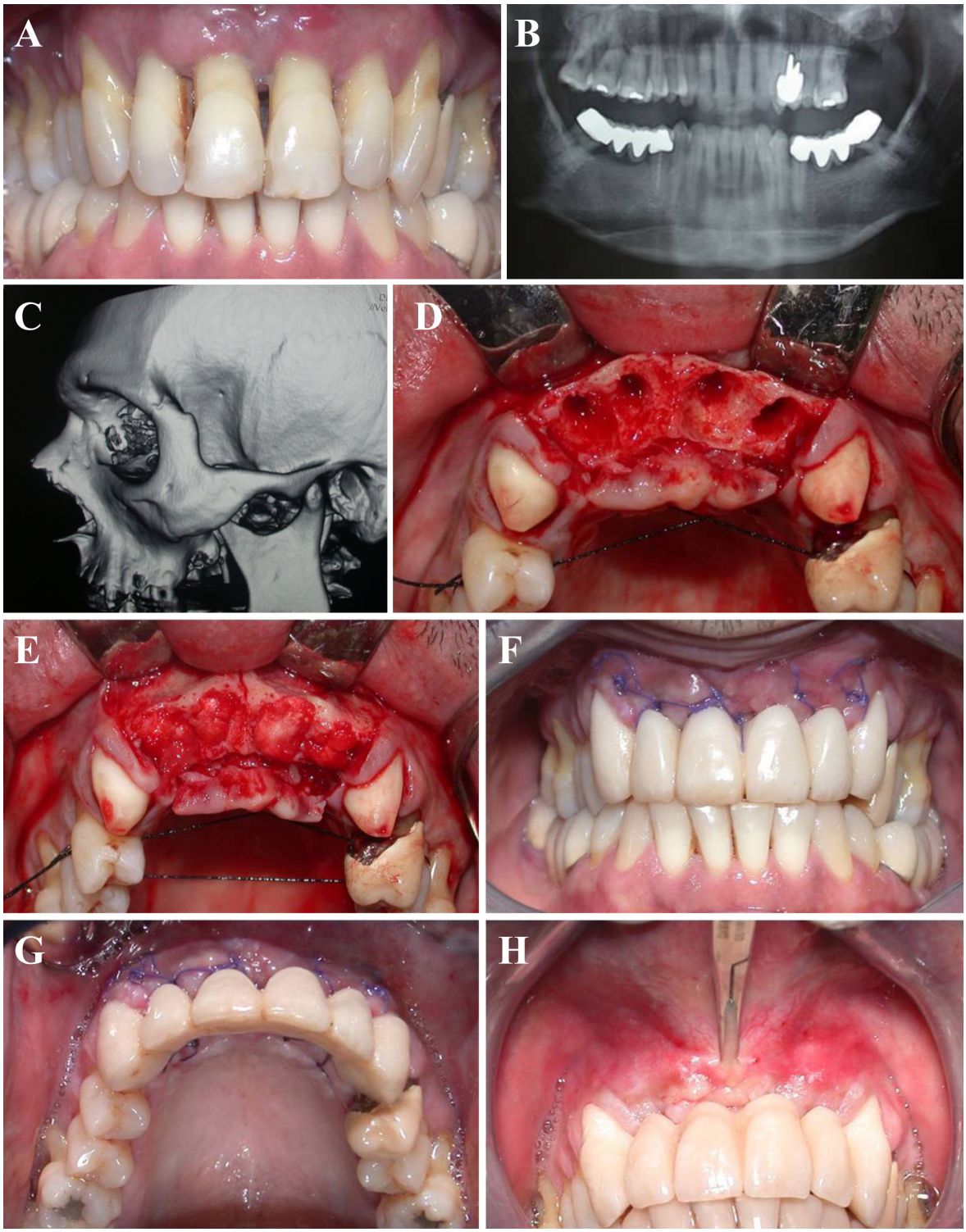

Figure 1. First steps. (A, B) Preoperative view and panoramic X-Ray showing the horizontal alveolar bone loss due to the periodontal disease. **(C)** The preoperative CT scanner revealed the maxillary bone atrophy. **(D, E)** During the first surgical step, teeth were removed, the fresh sockets were filled with synthetic collagen and covered with a full thickness flap. **(F, G)** A temporary resin bridge between the canines was placed to model the future rehabilitation. **(H)** After 4 weeks of healing, it was confirmed that a frenectomy was needed before the bone augmentation procedure, to avoid the flap retraction after the next surgery.

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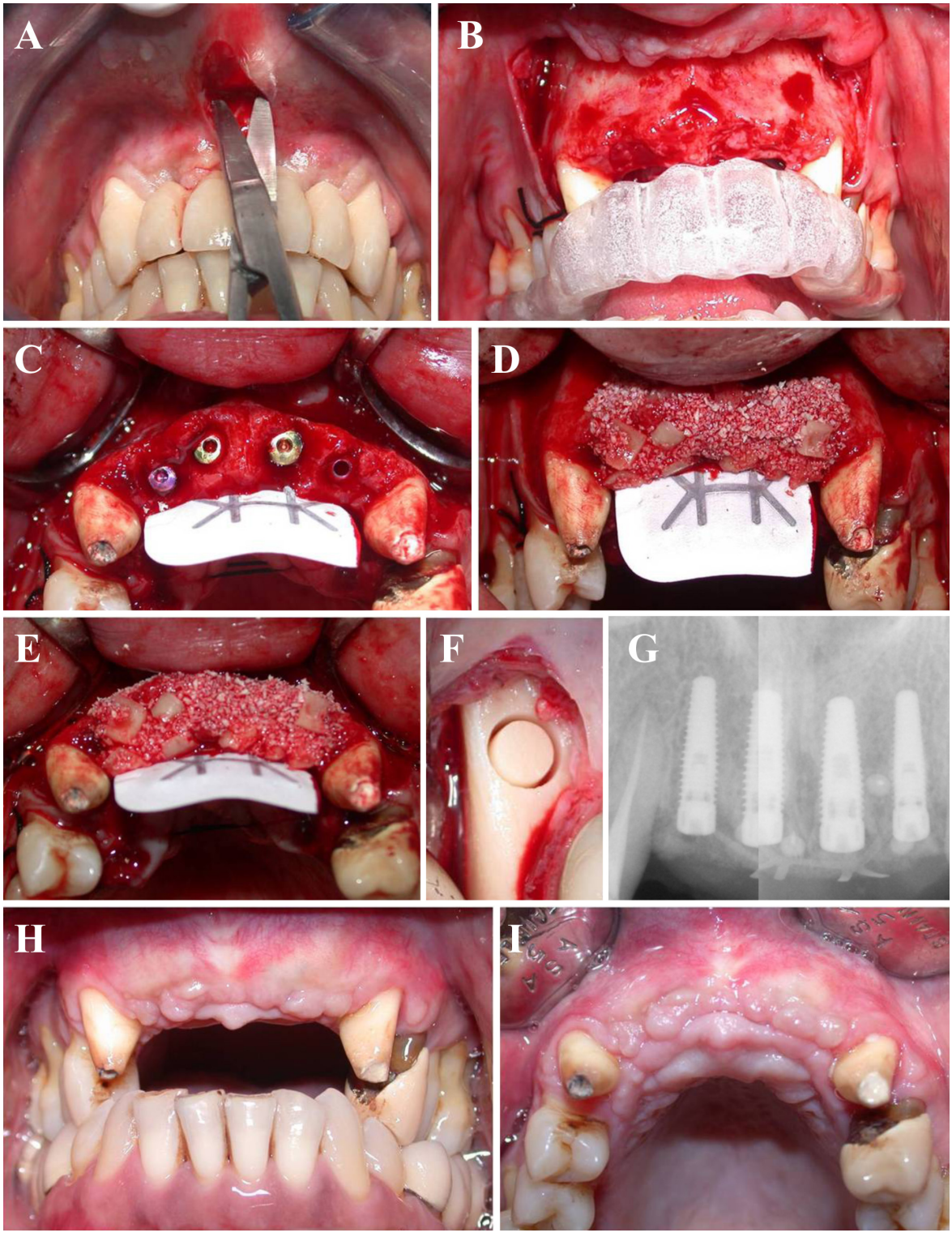

Figure 2. Multiple surgical steps. (A) Four weeks after the teeth extractions, a frenectomy was performed. **(B, C)** Four weeks after the frenectomy, a full-thickness gingival flap was raised, the alveolar ridge was prepared and 4 implants were placed according to the prosthetic guide. **(D, E, F)** The bone augmentation procedure was performed using a 50/50 mix of autologous bone (from ramus in F) and xenograft material. **(G, H, I)** After 6 months, the retroalveolar X-Rays **(G)** showed a stable aspect of the implant and bone volumes, and the gingival tissues were healed and matured **(H, I)**.

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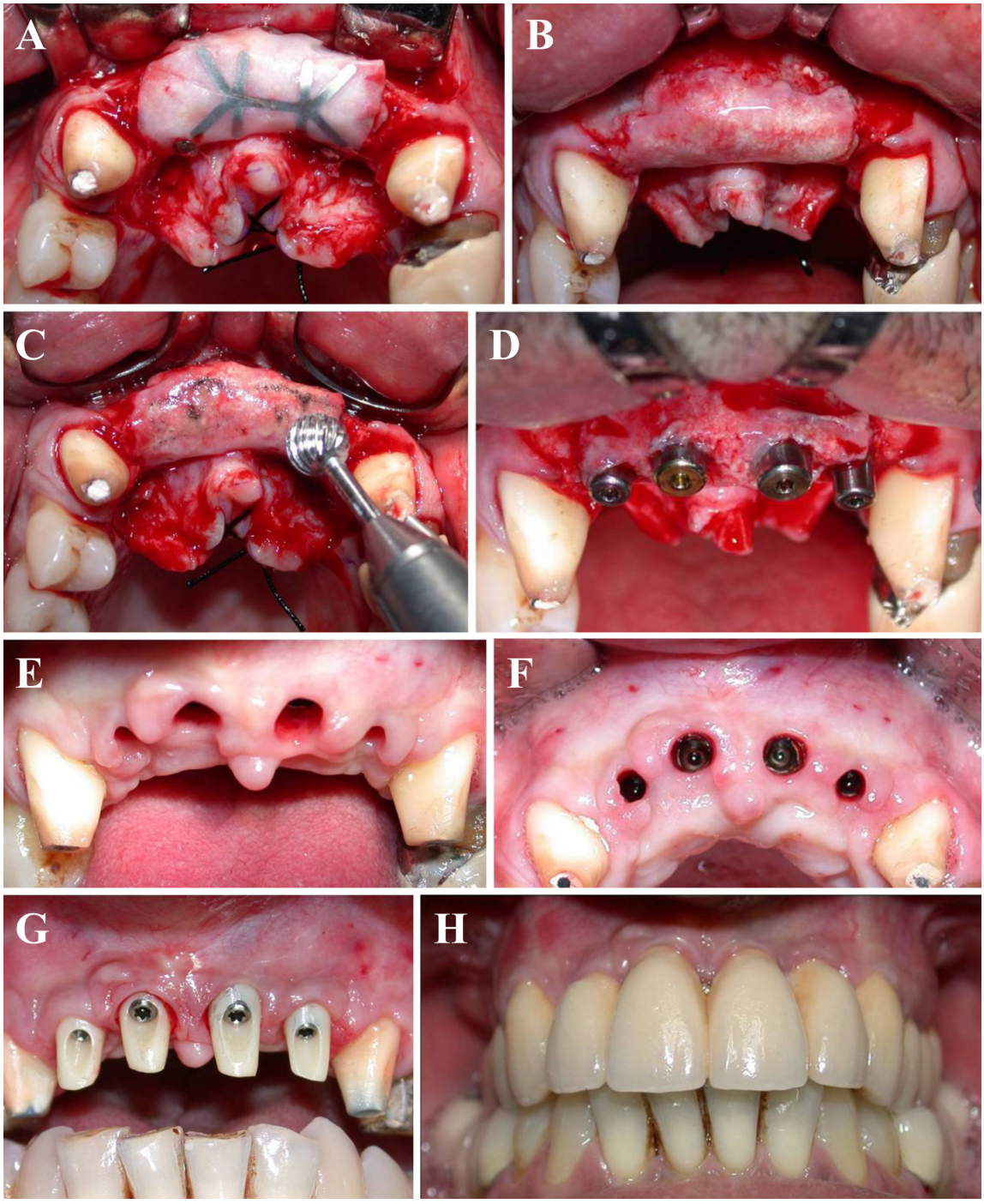

Figure 3. Final surgical and prosthetic steps. (A, B) Six months after the previous surgery, the non resorbable membrane was removed and the regenerated bone could be observed. **(C, D)** With a ball bur, the fixture shoulders were exposed and the bone peak modeled. **(E, F)** After 4 months with a provisional prosthesis, the gingival tissue was healed and mature enough to start the final implant-supported restoration. **(G, H)** Zirconia abutments were placed to prepare the marginal fit. A zirconia/ceramic bridge was finally connected.

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After 4 weeks a frenectomy was performed (**Figure 2A**). After 8 weeks from tooth extractions, a full thickness flap was raised and four implants (Nobel Biocare, Gothenburg, Sweden; 2 implants 4.3 x 13 and 2 implants 3.5 x 13)**[6]** were placed (**Figures 2B and 2C)**. At the time of implant placement, a bone augmentation using a mixture of 50% of demineralized xenograft (Bio-Oss, Geistlich AG, Wolhusen, Switzerland) and 50% of autogenous bone graft (harvested from ramus) was placed to assure an over-sized bone augmentation. A non-resorbable ePTFE (expanded-polytetrafluorethylene) titanium reinforced membrane (Gore-Tex, WL Gore and Associates, Inc., Newark, USA) was used to cover the grafts. The flap was then coronally shifted and sutured (**Figures 2D to 2F**).

After 6 months, the retroalveolar radiographs confirmed a stable bone and implant integration, and the gingival tissue appeared healed and mature **(Figures 2G to 2I).** A surgical re-entry was then performed. The non-resorbable membrane was removed (**Figures 3A and 3B)**. In order to access to the implant heads, a ball bur was used to eliminate the excess bone and to design a natural bone contour with some inter-implant bone peaks (**Figure 3C)**. Healing caps (6mm long) were then placed and the full thickness flap sutured (**Figure 3D)**. After 10 days, an implant-supported provisional restoration was constructed to model the soft tissue healing and to create esthetic inter-implant papillae. Four months after the provisional phase (**Figures 3E and 3F)**, the final restoration was fabricated and connected. A thick gingival tissue biotype with an esthetic aspect was obtained (**Figures 3G to 3H**). During the 3-year follow-up, the final aspect was stable.

3. Discussion

Achieving an esthetic aspect for implant-supported rehabilitation in the maxillary anterior area is an important requirement to consider a treatment as a success in this region **[7]**. However the treatment is never a “one-shot” treatment, but is always a therapeutic construction associating several surgical and prosthodontic steps.

To ensure a proper treatment outcome, a multi-discipline as well as step-by-step approach is essential **[8]**. In this reported case, all the steps were performed under the guidance of the final restoration template. The therapeutic strategy was ruled by the final objective. This prosthesis-guided multi-staged approach assured not only the esthetic success, but also forced us to follow all the necessary steps to change the gingival tissue biotype from thin to thick. This is an important result, since it is a key to maintain a long-term esthetic success **[9]**.

After tooth extraction/avulsion, most of the bone loss occurs in the first 3 months. The buccal plate resorption is greater (2 times more) than the lingual one **[10]**. Such dramatic changes of the bone profile are probably caused by the loss of periodontal vessels, and the thin and compact bone architecture of the buccal plate. These changes lead to the reduction of the bucco-lingual width, then to the bone height loss **[10]**. The severity of the bone resorption may pose problems for clinicians: it creates an esthetic concern during the design of an implant-supported restoration or a conventional prosthesis; and it makes implant placement challenging due to the lack of adequate bone support. Several techniques have been proposed to try to reduce the post-extractive bone resorption **[10,11]**. In the reported case, the compromised teeth were removed, and post-extraction sockets were filled with collagen sponges and then covered with a full thickness flap to minimize potential bone resorption **[10,11]**. The choice of this collagen sponges is debatable, as many materials are available for this indication and no clear recommendations exist on this matter. The

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advantage of this sponge filling is that it does not disturb the natural process of healing of the alveoli. As the alveolar sockets had their 4 walls, the use of a more compact bone material was not required. The full thickness flap was coronally advanced to assure an adequate soft tissue coverage of the site. This strategy provides some additional benefit for the following surgical step, as more soft tissue was available during the implantation and bone augmentation procedure and during the final step of soft tissue modeling with provisional prosthesis.

The frenum was quite strong and high on the alveolar ridge. Frenectomy was needed and planned before to start the implant and grafting surgery, in order to improve the flap mobility, to ensure a tension-free flap coverage of the grafted area and to reduce the postoperative flap retraction after surgery **[12,13]**. On the longer term, the elimination of the frenum was needed in order to avoid any stress on the peri-implant bone and soft tissues. The frenum insertions can often the source of gingival and bone dehiscences and then implant contaminations, leading to unesthetic aspects and even to peri-implantitis with potential loss of the implants **[14]**.

At the second stage of implant surgery (implant uncovering), it was observed that implants were deeply submerged under the vertically augmented bone. During the re-entry surgery, the excess bone over the implants was eliminated and the bone profile was modeled to create inter-implant bone peaks, to support the future healthy papillae between the implants **[12,15]**. This strategy can help to prevent papillae disappearance and hence it reduces the unesthetic problem known as “black triangle disease” **[12]**. In the following step, the soft tissue was modeled by the pressures of the provisional restoration. Using the ovate pontic concept allows clinicians to mold the soft tissues, and the gingival peri-implant contour can be somehow designed. Su et al. showed that by changing the abutment or crown contour, soft tissue can be molded in a different dimension that fits the needs of a final prosthesis **[3]**.

The change of the tissue biotype (from thin to thick) is another factor that contributes to the good results noted in this case. The thick tissue helps to maintain the soft tissue dimension, allows to manage an esthetic inter-dental triangle, hence ensures the long-term implant esthetic result **[9]**. This change of biotype remains a quite ultimate and difficult objective to reach and control in this kind of treatments. However, this change of biotype is only possible when the environment is globally treated, what implies to reach a natural and functional bone volume and a proper soft tissue reorganization at the end of the treatment.

Finally, this article focused on a general modern philosophy of implant dentistry, and the potential therapeutic options are in fact endless to reach the same final objectives. This is particularly true with the development of new technologies, materials and techniques to simplify and improve the clinical results, for example the use of platelet concentrates **[4,5]** or improved implant design or surfaces **[6]**.

As a conclusion, the use of a multi-discipline and multi-step approach is often the ideal way to a stable esthetic and functional outcome. This approach is now a key philosophy of modern implant dentistry, and should be always kept in mind by all clinicians.

Disclosure of interests

The authors have no conflict of interest to report.

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Research article

Long-term stability of osseointegrated implants in bone regenerated with a collagen membrane in combination with a deproteinized bovine bone graft: 5-year follow-up of 20 implants

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Abstract

Background and objectives. The use of preimplant bone graft is often needed for an adequate implant placement. This clinical study evaluated the 5-year stability of 20 implants placed in bone that had been previously regenerated with a deproteinized bovine bone graft and a collagen membrane.

Materials and Methods. Clinical and radiological data were collected one and 5 years after implant placement.

Results. All implants remained stable throughout the study period with a mean Periotest value of -2.65. X-ray examination showed stable bone crest levels without angular defects and a mean bone loss between the 1st and the 5th year examination of 0.287 mm.

Discussion and Conclusion. The 20 implants were successfully integrated and were maintained in function over a 5-year follow-up period. Based on the clinical and radiological favourable results, we conclude that regenerated bone, formed under a collagen barrier membrane combined with a deproteinized bovine bone graft, responds like pristine bone to implant placement.

Keywords. Biomaterials, bone regeneration, bone grafting, dental implants.

1. Introduction

The use of osseointegrated implants to replace missing teeth is a recommended treatment modality for partially **[1]** and completely edentulous patients **[2]**. As the long-term prognosis of dental implants is adversely affected by inadequate bone volume, successful implant therapy requires adequate bone volume at the potential implant sites. In cases of deficient alveolar ridges, several surgical alternatives are used to increase the alveolar bone volume for implant placement **[3,4]**. One surgical technique uses barrier membranes for guided bone regeneration (GBR), which allows localized jawbone defects to be filled with new bone **[5]**. A well documented GBR surgical procedure is the lateral ridge augmentation technique with a second stage surgical approach in which implants are placed in the newly augmented bone ridge.

Clinical studies showed that autogenous bone graft in combination with a non-resorbable expanded polytetrafluoroethylene (e-PTFE) membrane, is a potential treatment for horizontal ridge augmentation before implant placement **[6,7]**. Frequent complications associated with non-resorbable membranes are soft tissue dehiscences during the healing period **[8,9]** and membrane bacterial contamination **[8]**. In addition, membrane removal during implant placement requires an extensive surgical exposure of the newly formed bone **[10]**.

One major disadvantage of the use of autogenous bone graft is the morbidity associated with the harvesting procedure **[11]**. Due to these disadvantages, the use of a resorbable membrane (causing fewer flap dehiscences) and in combination with bone substitutes (to avoid the morbidity associated with harvesting autogenous grafts) seems to be an effective surgical alternative for lateral ridge augmentation before implant placement **[10,12,13]**.

The aim of this study was to evaluate the 5-year long-term stability of 20 implants placed in a previously augmented ridge, using a collagen membrane in combination with a deproteinized bovine bone graft.

2. Materials and Methods

Twenty non-submerged ITI implants (Straumann AG, Basel, Switzerland)**[14]** were inserted in recipient sites of 10 partially edentulous patients (5 women and 5 men). Four to ten months prior to implant placement, a successful horizontal ridge augmentation was made with a deproteinized bovine bone graft (Bio-Oss, Geistlich AG, Wolhusen, Switzerland) covered by a collagenous membrane (Bio-Gide, Geistlich AG, Wolhusen, Switzerland). Patient, implant-site and implant characteristics are listed in **Table 1**.

Patient number	Gender	Age	Implant site	Implant type	Implant length
1	F	34.8	22	4.1 mm Ø	12 mm
2	H	44.4	21	4.1 mm Ø	12 mm
3	F	25.9	21	3.3 mm Ø	12 mm
4	F	67.3	13	4.1 mm Ø	12 mm
5	F	46.7	24	4.1 mm Ø	12 mm
			25	4.1 mm Ø	12 mm
			26	4.1 mm Ø	12 mm
6	H	60.1	25	4.1 mm Ø	12 mm
			26	4.1 mm Ø	12 mm
			27	4.1 mm Ø	12 mm
7	H	46.2	11	4.1 mm Ø	12 mm
8	F	56.1	25	4.1 mm Ø	12 mm
			26	4.1 mm Ø	12 mm
			15	4.1 mm Ø	12 mm
			16	4.1 mm Ø	12 mm
9	H	31.2	15	4.1 mm Ø	12 mm
			16	4.8 mm Ø	10 mm
			25	4.1 mm Ø	12 mm
			27	4.1 mm Ø	12 mm
10	H	70.1	21	3.3 mm Ø	12 mm

Table 1. Characteristics of patients and implants placed following ridge augmentation using the staged GBR procedure.

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After completion of implant restoration, the patients were monitored in a maintenance program. Over a 5-year period, they were examined annually using the same protocol as for prospective long-term studies of non-submerged ITI implants in pristine bone **[15]**. The following clinical and radiological parameters were evaluated for each implant:

- Suppuration in the peri-implant sulcus (0 = no suppuration, 1 = suppuration).
- Modified plaque index (mPLI) assessed at four aspects around the implants **[16]**. For each implant, one mPLI value was calculated based on the mean of the four obtained values.
- Modified sulcus bleeding index (mSBI) assessed at four aspects around the implants **[16]**. For each implant, one mSBI value was calculated based on the mean of the four obtained values.
- Probing depth (PD) measured at four aspects around the implants. For each implant, one PD value was calculated based on the mean of the four obtained values.
- The distance from the implant shoulder to the mucosal margin (DIM), measured at four aspects around the implants with the same periodontal probe (Hu-Friedy PGF-GFS, Hu-Friedy, Chicago, IL,USA).
- Clinical attachment level (AL) assessed at four aspects around the implants and calculated for each site by adding probing depth and recession depth (AL = PD + DIM).
- Height of keratinized mucosa (KM): the distance between the marginal soft tissue and the mucogingival junction, measured in mm on the vestibular site of each implant with the same periodontal probe.
- Periotest value: the Periotest (Siemens, Bensheim, Germany) method was utilized as previously described **[17]**.
- The distance between the implant shoulder and the first visible bone-implant contact (DIB) was measured at the mesial and distal aspects of each implant, using standardized periapical radiographs with the long-cone paralleling technique and the Rinn System holding device (XCP Instruments, Rinn Corporation, Elgin IL, United States). To evaluate radiological assessment of crestal bone loss around the implants computerized images were used aided by a software system (Digora for Windows, version 2.1 rev. 2, Soredex, Helsinki, Finland). For each implant, one DIB value was evaluated by calculating the average of the mesial and distal values. The 5-year DIB values were compared with the 1-year DIB values to evaluate the crestal bone changes around the implants over the 4-year period between both examinations (DIB5y – 1y).

Based on clinical and radiological findings, each implant was classified as either successful or non successful, using the success criteria followed in previous prospective studies of implants in non-regenerated bone **[15]**:

1. Absence of persistent subjective complaints such as pain, foreign body sensation, and/or dysaesthesia
2. Absence of peri-implant infection with suppuration
3. Absence of implant mobility
4. Absence of continuous radiolucency around the implant

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Statistical analysis of the study results was conducted using the statistical program SPSS 15 (Statistical Package for Social Sciences, SPSS Inc., Chicago). To determine if the quantitative variables followed a normal distribution, the Shapiro-Wilk test was applied. The variables that followed a normal distribution were expressed with the mean ± standard deviation (mean ± SD), while the variables that were not normally distributed were expressed with the median and the aptitude. The comparison of clinical parameters PPD, DIM, AL, KM, Periotest value and DIB between the first (1st year) and the second (5th year) examination was carried out with the t test for paired data with a normal distribution and the Wilcoxon Signed Rank test for variables that were not normally distributed. The significance level chosen in all statistical tests was 95% ($p<0.05$).

3. Results

During the 5-year observation period, none of the 10 patients complained of pain, foreign body sensation or dysaesthesia at implant sites. The peri-implant soft tissues were healthy without signs of infection or suppuration. The clinical parameters at the 1- and 5-year examinations are summarized in **Tables 2 and 3** respectively.

Implant Number	Loc.	Supp.	mPLI	mSBI	PD	DIM	AL	KM	Perio
1	22	0	0	0	2	0	2	5	-5
2	21	0	0	0	2	0	2	5	-7
3	21	0	0	0	2	0	2	5	-5
4	13	0	0	0.5	2	0	2	5	-5
5	24	0	0	0	2	0	2	3	-7
6	25	0	0	0	2.25	0	2.25	3	-8
7	26	0	0	0	2	0	2	3	-6
8	25	0	0	0	3	0	3	3	-2
9	26	0	0	0	3	0	3	3	-3
10	27	0	0	0	3	0	3	3	-3
11	11	0	0	0	2.25	0	2.25	5	2
12	25	0	0	0	2.25	0	2.25	1	-2
13	26	0	0	0	3	0.25	3	1	-2
14	15	0	0	0	2	0	2	3	-1
15	16	0	0	0	2	0	2	3.5	--1
16	15	0	0	0	2.5	0	2.5	5	-5
17	16	0	0	0	2.5	0	2.5	3	-4
18	25	0	0	0	2	0	2	2	-5
19	27	0	0	0	3	0	3	2	-5
20	21	0	0	0	2.75	0	2.75	3	-6
Mean/Median		0	0	0.025	2.25	0.01	2.25	3.55	-3.9
SD		0	0	0	0.42	0.05	0.42	1.14	2.63

Table 2. Clinical parameters at the 1-year examination.
Implant number: consecutive number of implant; Loc.: location of implant according to WHO-classification; Supp: Suppuration; mPLI: modified plaque index; mSBI: modified sulcus bleeding index; PD: probing depth; DIM: distance implant shoulder to the mucosal margin; AL: clinical attachement level; KM: keratinized mucosa; Perio: PerioTest value.

The mean value for the mPLI and mSBI were below 0.5 and did not show any significant differences between the initial and the final examination. The median PD at the 1-year examination was 2.25 mm and 2.5 at the 5-year examination respectively and their difference was statistically significant (p=0.031). DIM values were stable and recorded between 0 mm and 1.5 mm at the 5-year examination. The difference between the 1-year and 5-year median DIM values was not statistically significant (p=0.25). The measurements of DIM values allowed the calculation of the clinical attachment level (AL=PD+DIM). The AL values ranged from 2 mm to 4 mm, resulting in a median value of 2.75 mm at the 5-year examination versus 2.25 mm at the 1-year examination. Their difference was statistically significant (p=0.01). All implants showed ankylotic stability during the 5-year observation period. The median KM value ranged from 3.55 mm at the 1-year examination to 3.05 mm at the 5-year examination. Their difference was statistically significant (p=0.026). The evaluated Periotest values varied from -8 to 3 with a mean value of -3.9 at the 1-year examination and from -7 to 4 with a mean value of -2.65 at the 5-year examination. Their difference was statistically significant (p=0.021).

Implant Number	Loc.	Supp.	MPLI	mSBI	PD	DIM	AL	KM	Perio
1	22	0	0	0	3	0	3	3	-3
2	21	0	0	0	3	0	3	5	-7
3	21	0	0	0	2.25	0	2.25	5	4
4	13	0	0	1	2	1.5	3.5	2	-4
5	24	0	0	0	2.25	0	2.25	3	-5
6	25	0	0	0	2	0.25	2.25	3	-7
7	26	0	0	0	2	0.25	2.25	3	-6
8	25	0	0	0	3	0	3	3	0
9	26	0	0	0	3	0	3	3	-1
10	27	0	0	0	3	0	3	3	-3
11	11	0	0	0	3	0	3	5	6
12	25	0	0	0	2.5	0	2.5	2	-2
13	26	0	0	0	3	0	3	3	-2
14	15	0	0	0	2	0	2	3	1
15	16	0	0	0	2	0	2	3	-1
16	15	0	0	0	2.5	0	2.5	5	-5
17	16	0	0	0	2.5	0	2.5	3	-4
18	25	0	0	0	2	0	2	2	-6
19	27	0	0	0	3	0	3	2	-5
20	21	0	0	0	3	0	3	2	-3
Mean/Median		0	0	0.05	2.5	0.1	2.75	3.05	-2.65
SD		0	0	0	0.44	0.33	0.44	0.99	3.45

Table 3. Clinical parameters at the 5-year examination.
Implant number: consecutive number of implant; Loc.: location of implant according to WHO-classification; Supp: Suppuration; mPLI: modified plaque index; mSBI: modified sulcus bleeding index; PD: probing depth; DIM: distance implant shoulder to the mucosal margin; AL: clinical attachement level; KM: keratinized mucosa; Perio: PerioTest value.

The 5-year periapical radiographs showed normal peri-implant bone structures for all implants, without a continuous peri-implant radiolucency **(Figure 1)**. All implants showed

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stable crestal bone levels and no sign of angular defects. Mean DIB values at the 1- and 5-year examinations were 2.592 mm and 2.897 mm respectively. Direct comparison of the 1st and 5th year examinations showed a mean bone loss of 0.287 mm between both examinations **(Table 4)**.

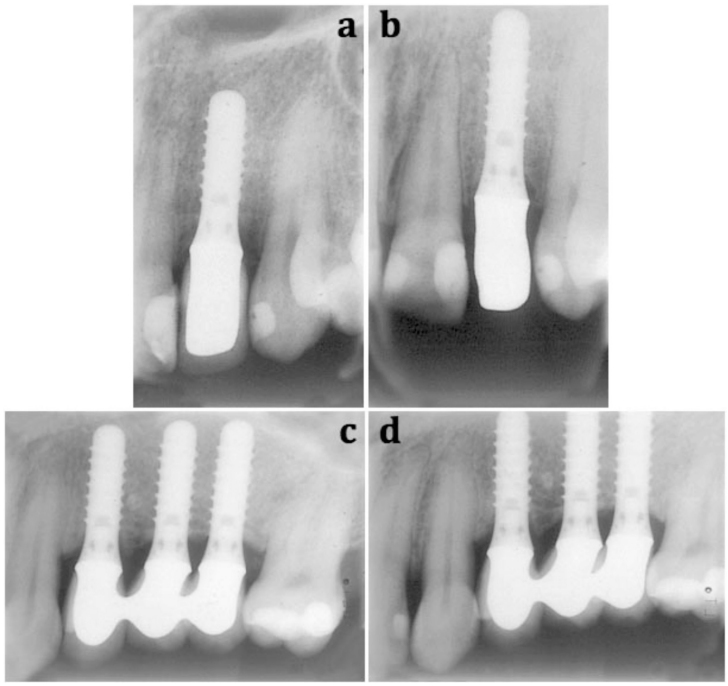

Figure 1. Radiological follow-up. Normal peri-implant bone structures around implants 1-year after implants placement (**a et c**). Stable crestal bone level with no signs of angular defect 5-year after implant placement (**b et d**).

Examination period	Minimum	Maximum	Mean (SD)
Year 1	1.52	4.885	2.592 (0.846)
Year 5	1.73	5.025	2.879 (0.863)
ΔDIB 5y-1y			0.287 (0.282)

Table 4. DIB values of 20 implants (DIB: distance from implant shoulder to first bone to implant contact).

4. Discussion

This clinical study presents clinical and radiological one and 5-year data of 20 implants. These were inserted in bone that had been previously augmented with a deproteinized bovine bone graft (Bio-Oss), combined with a collagen barrier membrane (Bio-Gide). The effectiveness of the combined collagen membrane and a deproteinized bovine bone graft, on horizontal ridge augmentation before implant placement, had been confirmed by other clinical studies **[7,10,13]**.

After the osseointegration of implants, a continuous clinical evaluation is necessary. This allows the detection of early signs of peri-implant disease. The clinical and radiological results obtained are comparable with those of various studies on non-submerged implants placed in pristine, non-regenerated bone **[15,18]**. The mean mPLI values were very low and

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the peri-implant soft tissues were in good health, without signs of infection or suppuration, indicating the patients' excellent oral hygiene. The mSBI values were also low as shown in this study.

The depths of peri-implant recession, five years after implants insertion, were stable and ranged between 0 to 1.5 mm. The median PD and AL values (2.25 and 2.75 respectively at the 5-year examination) were the same or slightly lower than those found in previous studies **[15,18]**. However, controversies exist on the extent to which these parameters are appropriate indicators for a possible pathology of the peri-implant structures **[19]**, since the difference between the used periodontal probes and the exerted pressure certainly influence the results of probing around the implants. Care should be taken when making direct comparisons of PD and clinical AL between different studies as differences when exerting pressure and between various periodontal probes may impact results differently when the implants are examined.

Keratinized mucosa was present on the vestibular site of all implants, as a result of soft tissue manipulation during implant surgery **[13]**. During the 20 implant placements in this study, the initial incision line was moved slightly to the palatal side of the ridge to preserve as much keratinized mucosa as possible on the vestibular side of the future implant restoration **[20]**.

All implants revealed a firm anchorage in the jaw bone during the study period, without presence of mobility, confirmed by the values of Periotest. The mean Periotest value was -2.65 five years after implants insertion and was proportional to the mean Periotest values of previously published studies **[19]**. However, its value as a reliable parameter for implant outcome is unclear. As Periotest values also depend on the implant type, its length, its width, bone quality and length of follow-up time **[17]**, further studies are needed to determine whether changes in Periotest values reveal initial alterations to the original bone to implant interface before other clinical parameters **[16]**. The Periotest values in this study confirmed the absence of implants mobility and their survival through the 5-year follow-up period.

The distance between the implant shoulder and the first visible bone to implant contact was measured on the mesial and distal side of each implant, utilizing standardized periapical radiographs. The mesial and distal radiological bone level of each implant reflects the vestibular and lingual bone levels. The 5-year x-ray examination showed stable crestal bone levels, without the presence of angular defects, with a mean bone loss of 0.287 mm between the two examinations. The mean DIB value of 2.879 mm at the 5-year examination was similar to published radiological data on non-submerged implants in non-regenerated bone **[7,18]**.

According to the clinical and radiological observations, all 20 implants were considered successfully integrated, with functional ankylosis and were effectively maintained in function over a 5-year follow-up period. They did not present persistent subjective complaints such as pain, foreign body sensation dysaesthesia, peri-implant tissue infection, mobility, and continuous radiolucency around the implants **[15]**. The survival and success rates in a 5-year observation period were 100%. These favourable results concurred with results from 5-year studies on ITI implants inserted in non-regenerated bone **[15,18]**. Based on these results we can conclude that regenerated bone, formed underneath collagen membranes, responds like pristine bone to implant placement.

The present study confirms the favourable results of other long-term studies on implants in regenerated bone using the GBR process. In the literature, different success rates

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were obtained depending on the technique and bone material used during the regeneration treatment, for example a GBR procedure with a synthetic hydroxyapatite (HA) spacer under a collagen membrane **[12]**, or various forms of bone regeneration with allograft or collagenated equine xenograft in combination with platelet-rich fibrin autogenous membranes **[3,4]**. The quantity of new bone biomaterials available nowadays on the market is considerable. Each combination of biomaterials and techniques must be evaluated very carefully in order to define the adequate clinical protocol for each combination.

5. Conclusion

Clinical and radiological results of the present study on 20 implants placed in regenerated bone showed that all implants were successfully integrated at the 5-year examination. They met the success criteria and functioned free of complications for patients. The analysis of clinical parameters concurred with the results of studies on implants inserted in non-regenerated bone as well as on implant placed simultaneously with some other GBR techniques. This therapeutic option seems therefore to have a very favourable prognosis. However, many biomaterials and techniques are nowadays available, and this study recalls us the need of adequate investigation and validation of each new therapeutic solution.

Disclosure of interests

The authors have no conflict of interest to report.

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Research article

Anchorage of machined and TPS-coated dental implants of various lengths: An *in vivo* study in the dog maxilla

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Abstract

Background and objectives. The use of short implants is nowadays frequent in daily practice. The objective of this experimental study was to test the correlation between extremely different implant surfaces and the anchorage of short implants.

Materials and Methods. The anchorage of machined-surface and titanium-plasma sprayed (TPS) implants of various lengths was investigated in the dog maxilla. Machined-surface fixtures, 7 and 10 mm long, and TPS implants, 6 and 10 mm long, were reverse-torqued after 3 months of healing.

Results. Failure mode varied with the implant system used. For TPS implants, implant loosening coincided with the peak reverse-torque. The mean was 55.13 and 90.14 Ncm for the 6 mm and 10 mm long implants, respectively; the difference was statistically significant. For machined-surface implants, 2 torque values were measured, a mobilization and peak torque. Mobilization torque for the 7 and 10 mm fixtures was 19.50 and 22.12 Ncm, respectively. Peak torque was 29.63 and 39.25 Ncm, respectively; all differences were not statistically significant. The 6 mm TPS implants were more firmly anchored than the 7 and 10 mm machined-surface fixtures. The torque data measured in the maxilla were significantly lower than the data in the mandible, by half approximately.

Discussion and Conclusion. In this experiment, parameters that influenced implant anchorage were: 1) the jaw bone quality (mandible vs. maxilla), 2) the implant surface and design, 3) implant length for TPS-coated implants. The present data suggest that treatment planning in terms of implant length selection and appropriate healing periods is implant system specific.

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

1. Introduction

Implant therapy, for partially and fully edentulous patients, is widely accepted as a safe and highly reproducible treatment. In the posterior region of the maxilla, where the sinus often limits the use of long implants, the need of complex surgical interventions prior to implant placement has been justified by the old paradigm that longer implants guarantee better success rates **[1]**. This paradigm is largely debated due to the technological evolutions

of the implant systems, as the recent improvements of implant designs and surfaces reduced significantly the influence of the length parameter. However, it remains a significant parameter, particularly for complex treatments using sinus-lift and immediate implantation in the severely resorbed maxilla **[2,3]**.

Machined-surface and Titanium Plasma-Spayed (TPS) implants are almost no more used nowadays, as these 2 technologies are sometimes considered obsolete in dental impant surface science **[4]**. However from a scientific standpoint, these 2 technologies remain very interesting as they represent the 2 extremes of implant surface technologies: the machined-surface was the smoother surface available at the microscale (with no official chemical modifications or engineered nanostructures), what made this implant an important basis of comparison for the development of new surface treatments **[4]**. On the other side, the TPS surface is often considered as the rougher implant surface (at the microscale) that was used in modern implantology, what made this implant an important tool for the research of osseointegration through bone/implant surface biomechanical interlocking **[4]**. These 2 surfaces represent 2 different concepts and approach of osseointegration **[5]**. As they are so extremely different, they are particularly useful in comparative studies to investigate some specific mechanisms.

The machined-surface fixtures and the TPS implant systems have been extensively documented clinically over the years. Users of machined-surface implant systems repeatedly reported that short implants ≤10 mm were at a higher failure risk than longer ones, particularly in the maxilla **[6]**. In contrast, users of the TPS-coated implant system observed similar survival rates for both shorter (≤10 mm) and longer implants, whatever the location **[7]**.

In this study, we investigated the different implant bone anchorage of machined-surface and TPS-coated implants in a dog maxilla model depending on their short or standard lengths. For each implant system, the anchorage of implants of 2 different lengths was evaluated using the removal torque test after 3 months of healing in the dog maxilla, to complete our previous investigations in the mandible **[8]**.

2. Materials and methods

2.1. Implant design and surfaces

Implants selected for the study were commercially available standard implants. Sixteen Brånemark implants of diameter 3.75 mm (Nobelbiocare AG, Göteborg, Sweden) were distributed into eight 7 mm long and eight 10 mm long implants **(Figure 1)**. Sixteen solid screw Straumann implants (Straumann AG, Basel, Switzerland) of diameter 4.1 mm were distributed into eight 6 mm long and eight 10 mm long implants **(Figure 1)**. Surface state of the Brånemark fixtures is machined **(Figure 2a)** whereas surface state of the Straumann implants is roughened by titanium plasma-spraying **(Figure 2b)**.

These surfaces and implant systems were widely tested and characterized in the literature. Following the recently defined classification **[5,9]**, the machined-surface Brånemark fixtures are smooth at the microscale and smooth at the nanoscale. Straumann implants are maximally rough at the microscale and smooth at the nanoscale. Both surface technologies do not display chemical modifications, even if some minor contaminants may sometimes be found. The differences between the 2 surfaces are therefore only their microtopography, as previously explained. Moreover, the 2 implants systems do not have exactly the same screw design, and this bias is discussed further.

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Figure 1. Commercially available Brånemark and Straumann implants used in this study. From left to right, 7 mm Brånemark, 6 mm ITI, 10 mm Brånemark and 10 mm Straumann implants.

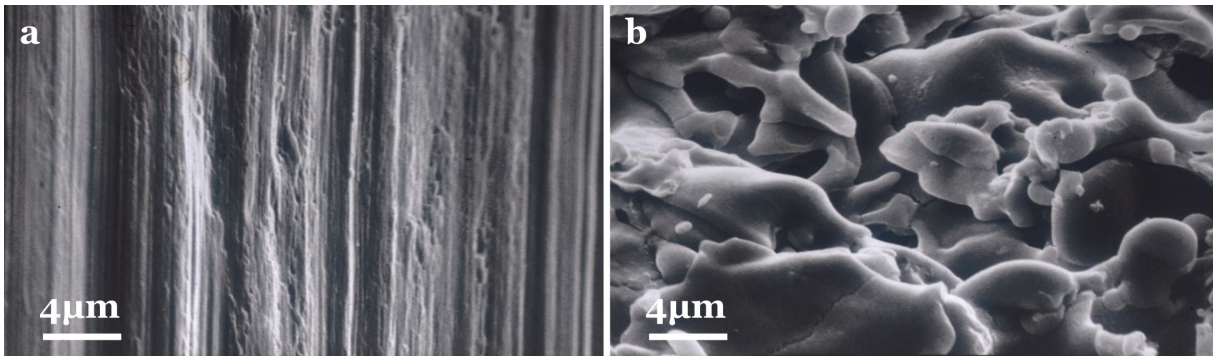

Figure 2. Scanning electron microscopy micrographs of the implants surfaces.
(a) Brånemark fixture, the surface feature corresponds to the machining grooves (x 3000).
(b) Straumann implant, the surface is roughened by titanium plasma-spraying (x 3000).

2.2. Experimental procedure

After protocol approval by the local institutional animal ethics committee, the animal study was conducted in an accredited experimental surgery center (Biomatech-Namsa, Chasse-sur-Rhône, France). Four Anglo-French adult male dogs (14-17 months old), weighing 30-31 kg were selected for this study. This breed can accommodate 10 mm long implants without encroaching the vital structures of the mandibular canal and the maxillary sinus **[8]**, whereas in beagle dogs the available bone height is limited to 6-8 mm. The surgical protocol was described previously **[8]**. Briefly, bilateral extractions of the PM1-PM4 premolars and the M1-M2 molars were performed in the maxilla. After 3 months of healing, 4 Brånemark fixtures (2 x 7 mm long and 2 x 10 mm long) were inserted in one side of the posterior maxilla and 4 Straumann implants (2 x 6 mm long and 2 x 10 mm long) in the other side. Particular care was taken to get the entire implant length in contact with surrounding bone. Bone height was evaluated during the drilling sequence, and when bone height was insufficient to host the entire implant, another site was prepared. For this reason dog 3 hosted 3 implants of 6 mm instead of 2 whilst dog 4 received 3 implants of 10 mm. **Table 1** shows implant distribution in each hemi-maxilla.

Implant placement was performed following the manufacturers' recommendations; Brånemark fixtures were left to heal in a submerged way according to the two-stage surgical procedure **[10]**. Straumann implants were inserted following the one-stage transmucosal

technique **[11]**. During the 3-months healing period, the dogs were left on a soft diet; Straumann implants were professionally cleaned 3 times a week.

	Machined-surface fixtures				TPS implants			
	Distal			Mesial	Mesial			Distal
Dog 1	10	10	7	7	6	6	10	10
Dog 2	10	10	7	7	6	6	6	10
Dog 3	10	10	7	7	6	10	10	10
Dog 4	7	7	10	10	6	10	6	10

Table 1. Implant distribution of the machined-surface Brånemark and TPS-coated Straumann implants.

2.3. Clinical evaluation, radiographic examination and removal torque measurements

Three months after implant placement, the soft tissue condition was evaluated at each maxillary segment. A mid-crestal incision was performed for the Brånemark submerged fixtures, a sulcular incision for the non-submerged Straumann implants. Each posterior maxilla was exposed by reflecting a muco-periostal flap and implant stability was clinically tested. The maxillary bone segment containing the implants was resected, radiographed and then secured in a bench-vise. The cover screws were carefully removed and a customized device (Straumann AG, Basel, Switzerland) was screwed on the implants to allow application of the reverse-torque. Within half-an-hour after bone resection, implant anchorage was assessed with a HSIOS HD 100 portable digital torque-meter (Intechnik, Adliswil, Switzerland). After resection of the last bone segment, the dogs were sacrificed with a lethal dose of Dolethal® (Laboratoire Vetoquinol, Paris, France).

2.4. Statistical analysis

The reverse-torque values were statistically evaluated with a 1-way analysis of variance (ANOVA) taking the implant as the analyzed unit. The Student-Neumann-Keuls method was used for pairwise comparisons. Differences were considered significant at $p<0.05$.

3. Results

3.1. Soft tissue condition and implant stability

All the Brånemark fixtures remained submerged without mucosal ulceration; the Straumann implants remained uncovered with the soft tissues in good condition. All implants were clinically stable without peri-implant radiolucency on the radiographs.

3.2. Straumann TPS implants removal torque measurements

During maxilla resection of the first dog, the distal bony wall of the most distal implant was torn-off accidentally, excluding this implant from analysis **(Table 2)**. During removal torque application, implants held firmly in the bone until loosening; the peak torque value was reached without early signs of discernible mobilization. A steep decrease in

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removal torque value followed **(Figure 3)**. For the 10 mm long implants, the mean reverse-torque value was 90.14 ± 14.60 Ncm; it was 55.13 ± 23.94 Ncm for the 6 mm long implants **(Table 2)**. Increasing implant length by 4 mm (66.7%) enhanced significantly implant anchorage by 63.5% **(Table 3)**.

	TPS implants		**Machined fixtures**			
	peak torque		mobilization torque		peak torque	
	6 mm	**10 mm**	**7 mm**	**10 mm**	**7 mm**	**10 mm**
Dog 1	92	82	34	12	42	30
	50	-	10	18	26	30
Dog 2	60	74	16	16	46	36
	34	93	26	19	27	30
Dog 3	12	101	26	18	33	35
	67	98	11	24	25	45
Dog 4	59	111	9	37	12	53
	67	72	24	33	26	55
Mean	**55.13**	**90.14**	**19.5**	**22.12**	**29.63**	**39.25**
SD	± 23.94	± 14.60	± 9.26	± 8.68	± 10.68	± 10.39

Table 2. Removal torque measured for the Straumann TPS-coated implants and the Brånemark machined-surface fixtures. For the machined-surface fixtures, 2 sets of torque values are displayed, the mobilization and peak torque values. Average torque and standard deviation are given.

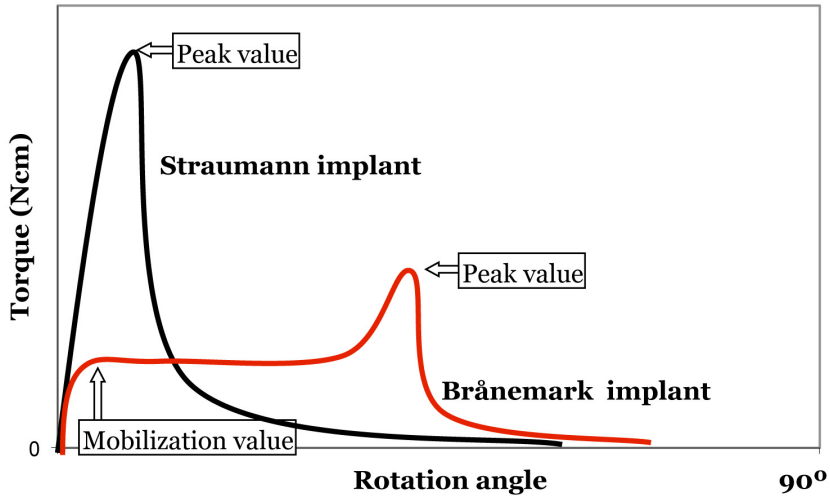

Figure 3. Schematic representation of the loosening modes of the 10 mm long Straumann and Brånemark implants. Note the mobilization torque level for the Brånemark implants, the plateau attained during the rotation phase, the peak-value and the steep decrease of the reverse-torque value.

3.3. Brånemark machined-surface fixtures removal torque measurements

In contrast to TPS implants, implant loosening of the machine-surfaced implants was progressive as shown in **Figure 3**. Implants were immobile until a certain torque was reached. Once mobilized, implants slightly rotated; while rotating, increase in torque

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resistance was minimal **(Figure 3)**. After a certain rotation angle, a peak torque value was reached; it was followed by a steep decrease. The reverse-torque at initial mobilization was recorded as the mobilization reverse-torque value; the higher reverse-torque was recorded as the peak torque value. Both torque values are given in **Table 2**.

The mean peak torque to loosen the 10 mm long implants was 39.25 ± 10.39 Ncm; the mean mobilization torque was 22.12 ± 8.68 Ncm. The mean peak torque required to unscrew the 7 mm long implants was 29.63 ± 10.68 Ncm; the corresponding mobilization value was 19.50 ± 9.26 Ncm. Increasing fixture length by 3 mm (43%) enhanced the peak anchorage by 32%, the mobilization torque increased by 13%.

Peak torque values were compared between implant systems **(Table 3b)**. The 6 mm Straumann implants were better anchored than the 7 mm Brånemark fixtures (+86%). The 10 mm Straumann implants were more firmly anchored than the equivalent Brånemark fixtures (+130%). When considering the mobilization torque for the Brånemark implants, the difference in anchorage between the 6 mm Straumann and the 7 mm Brånemark implants was +183%. The anchorage difference between the 10 mm implants of both implant systems was +307% **(Table 3a)**.

(a)	**Straumann implants** peak torque	**Brånemark fixtures** mobilization torque	
	6 mm	**7 mm**	**10 mm**
Straumann 6 mm peak torque	-	2.83 (S)	2.49 (S)
Straumann 10 mm peak torque	1.64 (S)	4.62 (S)	4.07 (S)
Brånemark 10 mm mobilization torque	-	1.13 (NS)	-

(b)	**Straumann implants** peak torque	**Brånemark fixtures** peak torque	
	6 mm	**7 mm**	**10 mm**
Straumann 6 mm peak torque	-	1.86 (S)	1.4 (S)
Straumann 10 mm peak torque	1.64 (S)	3.04 (S)	2.3 (S)
Brånemark 10 mm peak torque	-	1.32 (NS)	-

Table 3. Torque ratios and multiple pairwise comparisons according to implant length and implant system. Divisor is on the horizontal scale. **(a)** The mobilization values for the Brånemark fixtures were considered. **(b)** The peak values for the Brånemark fixtures were considered. S = statistically significant difference, NS = not statistically significant difference.

The reverse-torque values of the 2 implant groups were statistically different ($p<0.001$). A multiple pairwise comparison was performed with the Student-Neumann-Keuls method. The mobilization and peak torque values of the Brånemark implants were examined in consecutive order. When mobilization torques were examined, the means were statistically different for all implant groups, except for the 7 mm and 10 mm Brånemark implant groups **(Table 3a)**. When peak torque values were examined, the 7 mm and 10 mm Brånemark implant groups, as well as the 6 mm Straumann and the 10 mm Brånemark implant groups, were not statistically different **(Table 3b)**.

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4. Discussion

4.1. Two different anchorage/loosening modes

This study confirmed the existence of 2 distinct loosening modes in the maxilla, as previously reported in the mandible **[8]**. For TPS implants, loosening occurred at the same time as the peak reverse-torque, followed by a steep decrease in reverse-torque. This loosening mode has been associated with the rupture of a micro-mechanical bound at the implant interface. Scanning electron microscopy (SEM) and histology of the implant interface, confirmed that the TPS-coated surface displayed attached bone, and that bone fragments were found at distance from the interface **[8]**. For the machined-surface implants, a progressive loosening with 2 distinct torque values was repeatedly observed. SEM observation of the implant interface showed that the fracture line remained at the interface, no bone was found attached to the machined surface **[8]**.

These 2 patterns of loosening modes reveal 2 different forms of osseointegration. They highlight that the extreme roughness of the TPS implants promotes a very strong bone/implant biomechanical interlocking, while the machined-surface implants promote a simple surface ankylosis with limited interlocking. This difference reveals 2 different concepts of osseointegration that somehow still exist nowadays: some implant systems are promoting biomechanical interlocking while others are searching a more biochemical interlocking. However, nowadays many implant systems try to combine the 2 concepts to reach the osseointegration (for example moderate microroughness and Calcium Phosphate impregnation)**[12]**, and the 2 extremes represented by machined-surface and TPS were mostly abandoned **[9]**.

4.2. Factors influencing the anchorage

It may not be possible to identify the factors responsible for the differences in anchorage observed for these implants due to confounding differences between the 2 implant systems such as differences in design (distinct thread shape and pitch 0.6 vs. 1.25 mm), diameter (3.75 mm vs. 4.1 mm) and surface state (machined vs. TPS-coated). However, the analysis of the literature may allow us to support the surface as the main explanation of our results.

Carr et al. **[13]** compared the removal peak torque of machined-surface implants and TPS-coated implants of similar design and length, placed in the posterior maxilla of baboons. They found that TPS-coated implants were better anchored by a factor x2.2 near to the x2.3 factor measured in the present study **(Table 3a)** for Straumann and Brånemark implants of the same length. Differences in anchorage between the Straumann and the Brånemark implants may be better explained by differences in surface state (machined vs. TPS), rather than by differences in implant design (thread shape, pitch, and diameter). Noteworthy, the 10 mm long Brånemark implant has an apical hole but the 7 mm (Brånemark) implant does not have this feature. As the loosening pattern and torque values for both implant groups were similar, this suggests that the apical hole has no relevant retentive function.

Nowadays, machined-surface implants were abandoned due to their too weak biomechanical interlocking. TPS were also abandoned for various reasons that are not so clearly documented, but were mostly related to a too strong microroughness that was related with some risks of peri-implantitis **[14]**. Modern implants are mostly using an intermediate microroughness, sometimes in combination with various forms of chemical modifications **[5,9]**.

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4.3. Implant system and clinical recommendations

This study also requires to remember the evolutions of our practice with the evolution of technologies. When these surfaces were marketed, conflicting clinical recommendations have been made by Brånemark and Straumann users. For Brånemark implants, bicortical anchorage has been recommended **[10,15]**. Short implants have been considered at higher failure risk and placement of the longest possible implants privileged to take advantage of the available bone height **[15]**. In the posterior region, replacement of one implant per missing root (support value, SV = 1) has been encouraged to decrease the loading risk factor **[16]**. Long healing periods of 3-4 months in the mandible and 6-8 months in the maxilla have been mandatory **[10]**.

Unlike Brånemark implants, bicortical anchorage has not been suggested for TPS-coated Straumann implants and the 12 mm long implant is typically the longest implant inserted **[11]**. Shorter Straumann implants are not considered at higher failure risk and placement of fewer implants than the number of replaced roots (SV < 1) has been suggested **[17]**. Healing periods of 3-4 months have been recommended in both the mandible and the maxilla **[11]**.

These recommendations were based on the experience of clinicians and are supported by the current results. Nowadays, the number of new implant systems is considerable and most companies are not large enough to develop proper validated clinical recommendations. This study recalls us that differences in surface treatment promote differences in bone anchorage – particularly for short implants in the maxilla – and justify different clinical approaches. It is important to have adapted recommendations for the use of each implant system.

4.4. Implant anchorage and bone quality

The present experimental protocol was designed to obtain anchorage data from the mandible and the maxilla of the same animals. As mandible and maxilla differ in their bone structure, an aim was to observe how implant anchorage was affected by bone quality. Mandibular implants were better anchored than those inserted in the maxilla. For all implant surfaces and all implant lengths, the reverse-torque values in the mandible were roughly twice (1.74-2.13) the maxilla **(Table 4)**. In all groups, the differences in anchorage were significant when tested with the Student-t test for independent groups. Noteworthy, the TPS-coated screws inserted in the maxilla achieved at least the same anchorage as the Brånemark fixtures inserted in the mandible **(Table 4)**.

	Straumann implants peak torque		**Brånemark fixtures**			
			mobilization torque		peak torque	
	6 mm	**10 mm**	**7 mm**	**10 mm**	**7 mm**	**10 mm**
Mandible	104.88	192.25	36.67	38.57	61.88	69.13
Maxilla	55.13	90.14	19.5	22.12	29.63	39.25
Mandible/Maxilla ratio	1.90	2.13	1.88	1.74	2.09	1.76
Statistical significance	p=0.001	p=0.0001	p=0.0004	p=0.02	p<0.0001	p<0.0001

Table 4. Removal torque values of the mandibular and maxillary implants. The mandibular/maxillary torque ratio approximated 2 for all implant groups; it was statistically significant for all groups.

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The differences in anchorage between the 2 jaws might justify the recommendation for distinct healing times in the mandible and in the maxilla; indeed for Brånemark fixtures, it was advised at least 3 months of healing in the mandible and 6 months in the maxilla **[10]**. No such difference was advocated for Straumann implants since 3-4 months of healing was recommended for both jaws **[11]**. Hence, if 3-4 months of healing is appropriate in the maxilla for TPS-coated implants, a shorter healing period in the mandible may not jeopardize the integration prognosis for TPS-coated implants. Therefore, in the mandible, the 3-month healing period recommended for TPS-coated implants **[11]** could be viewed as a therapeutic reserve, as previously suggested **[8]**. The TPS-coated implants could conceivably be loaded as early as 6 weeks, like the SLA (sandblasted with large grit and acid attacked) implants, since similar torque data after 4, 8 and 12 weeks have been reported for TPS and SLA implants in mini-pigs **[18]**.

The differences in anchorage, due to bone quality and site (mandible or maxilla), corroborate the common knowledge to adjust healing times to bone quality. Thus, implants inserted in type IV bone might require a longer healing time than implants inserted in type I or II bone.

Finally, it is important to keep in mind that the implant design and bone osteotomy are also important factors, combined with the surface treatment of the implants. It can be expected that the right combination of these various elements can allow us to improve and accelerate the anchorage of new generations of implants, whatever the bone quality **[19,20]**.

5. Conclusion

In conclusion, distinct failure modes and different levels of anchorage were measured for machined-surface and TPS-coated implants. The present data suggest that the differences in anchorage are more likely due to differences in surface than to differences in implant design. This study illustrates the importance of the implant system characteristics for the adequate clinical use of short implants in the maxilla, and the need for proper recommendations depending on each system on the market.

Disclosure of interests

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