ORIGINAL ARTICLE

SEM AND IN VIVO STUDY OF ADHESION CHARACTERISTICS OF TITANIUM AND POLYTETRAFLUROETHYLENE SURFACES HEALING SCREWS

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Received: Mar 24, 2024; Accepted: May 12, 2024; Published: Jun 25, 2024

Abstract

Background: The long-term maintenance of implants and the prostheses they support depends on many factors. Specifically, the patient's biotype is important where the presence of a significant thickness of keratinized gingiva should ensure the presence of a real biological seal, whose formation is also influenced by the healing screw. The healing screws normally used are made of titanium; however, their cost induces the dentist to use them for improper single use. The possibility of using materials other than titanium such as polytetrafluoroethylene (PTFE) for the construction of healing screws can therefore be advantageous.

Objectives: The aim of this experimental clinical study is to test new PTFE healing screws by exploiting their chemical-physical characteristics and undoubted economic advantages.

Materials and methods: 10 patients were taken into consideration and 2 implants were inserted in each. In each patient in the second phase, a titanium healing screw and a PTFE healing screw were inserted. After 7 days, they were removed to allow analysis under an electron microscope. Results: SEM analysis of the screws showed that the PTFE surface has the ability to accumulate much less organic material than the titanium control screws. Furthermore, PTFE healing screws have been shown to reduce the level of inflammation induced by reuse compared to traditional healing screws.

Conclusions: the reduced cost of the PTFE screw, together with the intrinsic characteristics of the material, represent promising characteristics, which can make their use advantageous in clinical practice.

Keywords: implants; biological width; PTFE healing screw; titanium healing screw.

Introduction

The concept of osseointegration, whose foundations were born during the 1960s thanks to Brånemark's studies,1,2 is a prerogative for implant success.3 This phenomenon is influenced by a series of factors on which research is continuing to focus, examining the various aspects in depth in order to find new strategies that make some phases no longer dependent on the human factor.
The various facets of osseointegration can be analyzed according to molecular and micro- and macrostructural aspects. Cellular physiological mechanisms are at the basis of this phenomenon\textsuperscript{4-7} which can also be influenced by the geometric and surface characteristics of the implant;\textsuperscript{8,10} by the bone quality of the implant site;\textsuperscript{1,12} by the surgical technique used\textsuperscript{13-16} and by factors attributable to the patient, such as post-surgery healing phenomena, any comorbidities or the ability to scrupulously follow the indications in the post-operative period.\textsuperscript{17}

Implantology uses two different techniques: the submerged technique, also called biphasic, and the non-submerged technique, also called monophasic.

Using the submerged technique, two different operating phases are distinguished. The first sees the positioning of an implant inside the bone for its total length, up to the head of the implant, and the whole thing will be covered by mucosa. Subsequently the bone, not loaded and with an implant which is therefore supposed to be free of mobilization, will heal. It is once again underlined that primary stability plays a primary role in osseointegration. There is a very interesting study that show how it could be obtain a better and fast osteointegration using the magnetic static fields.\textsuperscript{18-20} In these studies, the magnetic fields can improve applied ion the screw of the implant can improve the differentiation of the osteoblast and increase the matrix around the implants. The once healing has occurred, we move on to the second phase characterized, however, by the surgical exposure of the implant to proceed with the incorporation of the abutments in order to optimize prosthetic rehabilitation.\textsuperscript{18,21} In another studies Author show that it could be right the use of photobiomodulation for improve the bone healing.\textsuperscript{22-23} However, the maintenance of the bone around of the implant seems to depend by the position of the implant respect to the biological width.\textsuperscript{24} The advantages of this technique are well documented by over twenty years of clinical success, while the main disadvantages lie in the need for two surgical stages and in the long intervals of time during which the patient is forced to wear removable prostheses.

The non-submerged technique consists in positioning the implant with its exposure in the oral cavity. Specifically, we will have a coronal portion of the fixture which, emerging on the surface, creates a transmucosal neck starting from the intraosseous portion of the implant. This undoubtedly turns out to be an enormous advantage, when compared to biphasic, both for greater comfort for the patient, who does not have to undergo surgery again, and for the clinician.\textsuperscript{25} Other benefits of this technique lie in the possibility of positioning the implant platform above the plane of the bone crest; in this way the micro-gap present in the implant-pillar connection is no longer placed below the bone plane, but rather above it.\textsuperscript{26}

The disadvantages of this technique, however, consist in the fact that there will be no guarantee of a bone seal on the implant, therefore bacterial colonization will be more likely responsible for a probable implant failure. Scrupulous hygiene will therefore be necessary to avoid the formation of gum pockets and bone loss. Another disadvantage is the greater transmucosal profile that occurs with monophasic implants (which sometimes protrude through the mucosa into the oral cavity for 3-5 mm), which however represents a risk for the fixture to be traumatized during the healing period. In fact, the higher the healing screw, the greater the risk of micromovement of the future abutment;\textsuperscript{27} this can strongly influence the osseointegration process, and consequently the success, of both transmucosal and submerged implants subjected to immediate loading.

However, the authors have demonstrated that there are no substantial differences in follow-ups between the submerged and non-submerged systems.\textsuperscript{28}

The most recent clinical recommendations see the monophasic approach as preferable, in accordance with the advantages mentioned above. Despite this, it is assumed that the biphasic strategy may be preferable if there is no certainty of the primary stability that the implant can achieve or one wishes to resort to guided bone regeneration (GBR).\textsuperscript{29}

The long-term maintenance of the implants and the prosthesis they support depends on many factors, including the phenotype of the patient's mucosal tissue,\textsuperscript{30} the material with which the prosthetic abutment is constructed,\textsuperscript{31} the duration of the second phase, i.e. the number of times we are forced to unscrew and re-tighten the healing screws, the crestal or subcrestal position of the implant,\textsuperscript{28} the correct function of the prosthesis.\textsuperscript{32}

As regards the patient's mucosa, the presence of a significant thickness of keratinized gingiva should ensure the presence of a real biological seal described in 1998 by Kawahara and Hashimoto.\textsuperscript{33-35}
biological seal represented by the peri-implant soft tissues is essential for an adequate osseointegration process and it is necessary that there is no bacterial penetration through it. To have an adequate seal, the junctional epithelium must adhere to the implant surface in the absence of plaque, since it is only thanks to this condition that the epithelial cells are able to synthesize the proteoglycans necessary for the attachment.

Numerous studies have evaluated the adhesion capacity of the junctional epithelium to the titanium of the implant and the concept of how epithelial cells, both in culture and in vivo, have the ability to form a hemidesmosomal basal lamina system with the surface of titanium, creating an adhesion surface.42-46

The biological width of the peri-implant soft tissues was extensively described in a study carried out by Berglundh in 199647 which highlights how a certain minimum biological width of the peri-implant mucosa is always required and that bone resorption can take place to allow the formation of an adequate soft tissue attachment. Studies carried out by Kawahara et al48 made it possible to identify three areas of the seal: the plaque area located in the most superficial area of the implant fissure which represents the growth site of the bacteria and corresponds to the areas of the sulcular epithelium; the bare area where epithelial adhesion is normally determined by a glue-like mucous layer that covers the implant surface and, finally, the deeper epithelial attachment area, where we will find responsible epithelial cells in single file in the implant/dental interface of adhesion via hemidesmosomes. However, it is still not clear today whether this area in which we have the biological seal described by Kawahara is susceptible to increase or reduction through maneuvers adopted by the dentist. In particular, we know that hemidesmosomes, however, prefer titanium and reject gold, as described by Abrahamson and others in 199849 and this poses a mandatory condition for choosing the abutment material if we want to obtain the hemidesmosomal seal: only titanium and no gold.

Furthermore, this band of hemidesmosomes will be able to adhere to the titanium only if it is devoid of the keratin layer that normally forms during the maneuvers of unscrewing and re-tightening the healing screws; this second condition requires us to reduce the prosthetic steps to a minimum, avoid contaminated healing screws that trigger epithelial and therefore keratin inflammatory reactions and ultimately forces us to peel the transmucosal tunnel well described by Abrahamson’s group in 1998.50 The stability of the prosthetic abutment and the accuracy is very important for the absolutely reducing of bacteria colonization.51-55

These preliminary considerations make us understand the absolute importance and delicacy of the step in which the healing screws are involved. The healing screws normally used are made of titanium and should be disposable; however, clinical practice teaches that the dentist is accustomed to sterilizing the screws used at the end of the work, without, however, proceeding with their decontamination first. The residues and irregularities that inevitably form trigger premature inflammatory processes and a harmful production of keratin which effectively undermines the possibility of the formation of the biological seal. Precisely this accumulation of plaque triggers inflammation of the soft tissues of the small transmucosal passage, inevitably determining a response of the epithelial cells which results in the production of keratin on the surface. This layer thus formed effectively prevents the hemidesmosomes from coming into contact with the titanium of the healing screw first and of the abutment afterwards, thus resulting in the deepening of the Kawahara plaque area (similar to sulcular epithelium) and the notable reduction of the area active in the biological seal, i.e. the area of cells in single file and devoid of keratin.

The cost of titanium healing screws actually induces the dentist to use them for non-improper single use, as the original philosophy would dictate. Reuse should include an absolute removal of debris accumulated during their use in the mouth, decontamination and subsequent re-sterilization. Steps without which a new use could be the cause of a possible trigger of inflammatory reactions.

The possibility therefore of using material other than titanium for the construction of healing screws can reduce the problems of possible desired inflammatory reactions. A material widely used in dentistry and characterized by not triggering inflammatory processes is tetrafluoroethylene (PTFE). Vascular prostheses, heart valves,47 vascular stents, sutures for general dental surgery are currently produced with this material.48 For this reason it was possible to create healing screws in PTFE as a material

already used in the past, widely certified as the basis of many products already on the market and CE marked.

The aim of this clinical study is to compare, under a scanning electron microscope (SEM), the intrinsic adhesion characteristics of 2 different types of healing screws: a traditional type in titanium and a new type in molded polytetrafluoroethylene.

Materials and Methods

In this study, 10 male patients with ages ranging between 36 and 67 years were taken into consideration.

2 implants were inserted in each patient for a total of 20 Biomet 3i implants with a diameter of 4 millimeters and variable length.

In each patient in the second phase, a titanium healing screw supplied by the same company and a PTFE healing screw produced by Super-charged Production s.r.l. were inserted of the WHITE type.

After 7 days, assessments of the degree of healing of the soft tissues were performed on each patient using the method VAS to highlight the different clinical behavior, and the differentiated measurement of the bleeding index was then performed. Subsequently, the titanium healing screws and the WHITE screws were removed and replaced with new ones, to allow scanning electron microscope (SEM) analysis of the surfaces. Finally, a small biopsy was performed to evaluate the expression of CD20 and CD3 to evaluate the level of inflammation induced 15 days after 3 loosening and retightening.

Through the use of SEM, a morphological investigation was conducted at different magnifications of the surfaces of the titanium and PTFE screws.

Results

1. **SEM analysis before use in the mouth**

   The surfaces of the healing screws (Figures 1A and 1B) after coating with gold powder, showed their original texture, free of any organic marial. The PTFE screw (Figures 2A and 2B) shows a surface microstructure characterized by parallel reliefs of materials (called nodes) connected by perpendicular fibers (called fibrils).

   The surface of the titanium screw shows a smooth texture. The differences found are due to the different nature of the material and the different manufacturing procedures.

2. **SEM analysis after use in the mouth**

   Following their use in the oral cavity and sterilization, the screws were analyzed again under SEM. As can be seen from Figures 3A and 3b, particularly in the higher magnification image (Figure 3C), the surface of the titanium healing screw shows residues of organic material. The nature of the material was confirmed by a spectroscopic analysis, which identified a distribution of emission spectra typical of organic material ("amorphous curve", images not shown). The morphological analysis of the PTFE screw did not show significant increases in material deposited on the surface, as confirmed by the spectroscopic analysis.

   Contrary to the texture characteristics, the PTFE screw, with a lower quantity of organic material, confirms the excellent physical-chemical qualities of the material in terms of anti-adhesion. This shows that repeated clinical use of a titanium screw still presents adherent residues which make it qualitatively unsuitable for the use for which it was intended. The
PTFE screw, ten times cheaper, with excellent chemical-physical properties, which has the same ease of use as the titanium screw, is also preferable from an aesthetic point of view.

**Figure 1A.** Titanium screw BEFORE use, the surface appears to be free of any organic material (23x)

**Figure 1B.** Titanium screw BEFORE use, the surface appears to be free of organic material (92x)

**Figure 2A.** PTFE screw BEFORE use, the surface is free of organic material (23x)

**Figure 2B.** PTFE screw BEFORE the use, the surface is free of organic material (92x)

**Figure 3A.** Titanium screw AFTER use, the surface appears to be moderately contaminated with organic material (23x)

**Figure 3B.** Titanium screw AFTER use, visible organic material (92x)

**Figure 3C.** Titanium screw AFTER use, organic material visible at high magnification (200x)

**Figure 4A.** PTFE screw AFTER use, the surface is not contaminated by organic material (23x)

**Figure 4B.** PTFE screw AFTER use, the surface is not contaminated by organic material (92x)
Discussion

SEM analysis of the screws showed that the PTFE surface has the ability to accumulate much less organic material than the titanium control screws. It was also observed by immunohistochemical analysis that CD3 and CD20 proteins, the levels of which correlate with the presence of an inflammatory infiltrate, varied in the biopsy samples. It is possible to believe that a lower adhesion of organic residues on the surface of the WHITE screws corresponds to a smaller inflammatory infiltrate in the peri-implant oral mucosa; the lower inflammatory response of the peri-implant tissues would determine a reduced production of keratin in the transmucosal route. It is interesting to underline that to obtain the biological seal useful for protecting the implant abutment system and capable of keeping the inflammatory infiltrate away, it is necessary for a hemidesmosomal attachment to form towards the titanium of the abutment. But this seal inevitably does not form when the epithelium reacts to the inflammatory stimuli triggered by possible residues present on the healing screws with a keratotic reaction which in fact makes the exposure and consequent attack of the integrins of the hemidesmosomes unlikely. The White screws showed good behavior in terms of soft tissue color (Vas scale) which may suggest a poor or absent inflammatory infiltrate.

It can therefore be concluded that the use of WHITE healing screws compared to traditional healing screws has shown that it can reduce the level of inflammation induced by the reuse of titanium screws. Although the use of titanium screws must be disposable, daily clinical practice often does not respect this principle, which is fundamental for obtaining the biological seal and protection of the implant-abutment system, essential for the long-term maintenance of the artefacts implant-prosthetics. A further advantage of the WHITE healing screws is represented by the possibility of being able to print this type of healing screws with the shapes most compliant with prosthetic trends in an extremely economical way. In fact, the reduced cost of the screw WHITE, together with the intrinsic characteristics of the material they are made of, PTFE, represent promising characteristics, which can make their use advantageous in clinical practice.

Conclusions

It can therefore be concluded that the use of WHITE healing screws compared to traditional healing screws has shown that it can reduce the level of inflammation induced by the reuse of titanium screws.

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Declarations

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the University ethics committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Source of funding

The work was not funded.

Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used Conceptualization, F.B. and L.M.; methodology, S.G.; software, A.M.M.; validation, F.S., and F.B.;
formal analysis, A.M.; investigation, S.G.; resources; data curation, C.B.; writing—original draft preparation, FB.; writing—review and editing, LM.; visualization, C.B.; supervision, F.S. All authors have read and agreed to the published version of the manuscript.

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Journal Bulletin of Stomatology and Maxillofacial Surgery, Vol. 20 № 2

SEM և IN VIVO ՊՈԼՈՄԵՐԸՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ
ՆԵՐԱՐԱՄԱՆՈՒՄ ԼՆԴՐԸՆԵՐԻ ԿԱՐodoxyում

Լուսիա, Նիկոլա, ՈւՄԱՐԵՆՅԵՐԻ ՈՒՍՈՒՄԻ ՀԱՄԱՐ

ՄԱԿԵՐԵՍՆԵՐԻ ՄԱԿԵՐԵՍԸ:

1. Թույլ տալու մակերեսից: պտուտակները
2. այլ մակերեսներից: պտուտակները

ՄԱԿԵՐԵՍԸ:

Նժամակավորություն: Բամբինին, Պելեցչաու, Բելֆիորետտի, Սերենա, Բարբերի, Լ'Աքվիլայի, Կլինիկական

Այս հանրամասն մակերեսը պտուտակ է, որոնք կարող են այս մակերեսից ուսումնասիրության համար: Նյութեր այս մակերեսից կարող են օգտագործվել հիվանդների վրա որպես համար բաժին, երկարատև սպասարկումը

Այդ մակերեսները ուսումնասիրվել են բացի պտուտակային պատմության, որը կարող է դեմքի այլ մակերեսներից կամ այլ տեխնոլոգիաների համար։ Գործով պտուտակների այս մակերեսից կարող է դեմքի այլ մակերեսներից կամ այլ տեխնոլոգիաների համար։