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# Comparison of peri-implant soft tissues in submerged versus transmucosal healing: A split mouth prospective immunohistochemical study

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ABSTRACT

*Objective:* The present split-mouth prospective study involves an immunohistochemical evaluation of peri-implant soft tissue healing after the osseointegration period, comparing submerged and transmucosal approaches using two-piece implant systems. The null hypothesis was that both surgical procedures elicit a similar immune response of the peri-implant soft tissues.

*Design:* Thirty-one healthy patients were included in this study, in which two implants were placed in the right and left maxillary pre-molar regions. A total of 62 dental implants were analyzed, establishing a control side with 31 submerged implants, and a study side with 31 exposed implants bearing healing abutments. After a three-month healing period, a soft tissue biopsy was collected and prepared for immunohistochemical analysis of the proportions of different lymphocyte subpopulations.

*Results:* The comparative analysis between the submerged and transmucosal approaches failed to identify statistically significant differences in CD19+ B cells, CD4+ T cells, CD8+ T cells, CD25+ T cells or γd T cells. However, significant differences in NK lymphocytes ($p = 0.012$) were recorded with the submerged surgical procedure.

*Conclusions:* Peri-implant soft tissue immune response with submerged or transmucosal healing protocols demonstrated comparable outcomes after the osseointegration period. There is sufficient evidence that the null hypothesis of no difference cannot be rejected. To the best of our knowledge, this is the first study of its kind. Further research is therefore needed to further clarify the role of these lymphocyte subpopulations in peri-implant soft tissues.

## 1. Introduction

A number of different techniques have been used in recent years for dental implant placement. While some commercial brands (Straumann Dental Implant System, Waldenburg, Switzerland) historically indicated placement using a one-stage technique, others (Nobel Biocare AB, Gothenburg, Sweden) required a two-stage technique (Astrand et al., 2002). The original two-stage surgical concept developed by Brånemark et al. (1977) comprised a first stage in which the dental implant was inserted in the bone, followed after an osteointegration period by second stage placement of the transmucosal abutment. In contrast, in the one-stage surgical procedure the implant and transmucosal abutment are both positioned in the same step, leaving the abutment exposed within the mouth throughout the implant osteointegration process.

The advantages of the transmucosal surgical procedure include the need for only one surgical step, resulting in greater convenience for the patient, and increased cost-effectiveness compared with the submerged technique, which requires second-step surgery to expose the implant neck. However, the latter technique ensures implant osteointegration in an environment protected from the oral cavity, thanks to the overlying mucosa. Despite all the factors that appear to influence bone remodeling and the peri-implant soft tissues, such as mucosal thickness, the presence of bacterial biofilm, compression of the healing tissues by an interim prosthesis, etc., the exact biological response to these factors remains unclear. In this regard, also remains unclear whether the transmucosal approach constitutes a risk factor for peri-implant disease compared to submerged healing. A meta-analysis published by Faot

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et al. (2015) found peri-implant crevicular fluid to contain inflammatory mediators such as IL-1β and TNF-α, that can be used as additional criteria for a more robust diagnosis of peri-implant infection. A significant difference has been observed between the concentration of cytokines (IL-1β, IL-6, IL-10 and TNF-α) at sites with peri-implantitis compared to healthy peri-implant tissue (Ata-Ali, Flichy-Fernández et al., 2015). As reported by Renvert, Widén, and Persson (2017), it seems logical to find lower levels of key proinflammatory cytokines and bacteria at implants with a clinically stable treatment outcome.

From the pathogenic perspective, the peri-implant soft tissues suffer aggression from both the bacterial presence in the oral cavity and the implant itself, which induces an inflammatory reaction and tissue damage through the stimulation of humoral and cellular immune responses, with the activation of macrophages, polymorphonuclear cells, T lymphocytes and plasma cells. These proinflammatory effects could result in destruction of the peri-implant tissues. Olmedo et al. (2012), in a study of 153 biopsies of the supra-implant oral mucosa of submerged dental implants, found that 41% exhibited metal particles, and the immunohistochemical study confirmed the presence of T lymphocytes associated with the metal particles. To the best of our knowledge, no studies have investigated all the lymphocyte subpopulations in peri-implant soft tissue healing. Knowledge of the lymphocyte populations in healthy peri-implant soft tissues with a view to evaluating differences around implants placed in one or two surgical steps could help solve doubts as to which surgical approach is best.

The primary null hypothesis of this study is that there is no significant difference in peri-implant soft tissue response between the two surgical techniques following the implant osteointegration period. The present split-mouth prospective study involves an immunohistochemical analysis of the proportions of CD19+ B cells, CD4+ T cells, CD8+ T cells, CD25+ T cells, NK cells and γδ T cells in the peri-implant soft tissues, comparing the submerged and transmucosal surgical approaches.

## 2. Material and methods

### 2.1. Patients

Thirty-one consecutive patients were included in this split-mouth prospective study in the Department of Oral Medicine and Surgery (Madrid Complutense University, Madrid, Spain). The patients were required to have a sufficient amount of vertical and horizontal bone in the recipient sites to allow implant placement without the need for bone regeneration techniques. They were also required to present sufficient keratinized gingival tissue (at least 2 mm) (Negri et al., 2016), and to meet appropriate standards of oral hygiene (plaque and gingival indices < 15%), since the study sought to evaluate healthy peri-implant mucosa, and no signs of peri-implant tissue disease were therefore allowable (Ata-Ali, Ata-Ali, & Bagan, 2015). All the patients were required to present healthy periodontal conditions of the neighboring teeth. Patients with severe diabetes were excluded, as were those with bleeding disorders, serious systemic diseases, a history of radiotherapy in the head or neck region, treatment with bisphosphonates, active infectious diseases such as hepatitis, HIV infection or tuberculosis, or treatment for chronic conditions (involving the administration of phenytoin, cyclosporin or calcium channel blockers). Smokers and pregnant or breast-feeding women were also excluded.

Before the start of the study, the patients received full information about the study design, and written consent was obtained. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of San Carlos Clinic Hospital (Madrid, Spain)(Ref.: 13/449E).

### 2.2. Surgical technique

All surgical treatments were performed under local anesthesia. Phibo® treatment surface Avantblast (TSA) implants (Phibo Dental Solutions, Sentmenat, Barcelona, Spain) were used in the patients treated with two one-piece implants in the left and right maxillary premolar regions. No early functional loading was made. Implant treatment was performed by a single clinician, following standard protocols for implant placement with supracrestal incision, mucoperiosteal flap release, site drilling with a pilot drill and subsequent drills up to the implant diameter. A total of 62 implants were studied, and all of them were 4.5 mm in diameter and 13 mm in length. Thirty-one implants were placed with the one-stage technique (on the left side) and 31 with two-stage technique (on the right side):

- **One-stage technique** (transmucosal protocol): Following transmucosal implant placement, the healing abutments classically used during second stage surgery were placed. As a result, the cover screw was not used, and the peri-implant mucosa was sutured around the abutment.
- **Two-stage technique** (submerged protocol): Following implant insertion in the bone, the cover screw was placed and the mucoperiosteal flap was adapted for primary closure. Second stage surgery was performed 12 weeks later, after the osteointegration period. Once the implant was exposed in the mouth, a healing abutment was placed and the mucosa was adapted around it.

Both the cover screws and the healing abutments unwire tightened to a torque of 15 Ncm. Postoperatively, amoxicillin-clavulanate (Augmentine® 875/125 mg, GlaxoSmithkline, Madrid, Spain) was administered every 8 h for one week, and ibuprofen 600 mg (Normon, Madrid, Spain) was prescribed as needed. A 0.2% chlorhexidine gluconate solution was used twice a day for 7 days until the sutures were removed.

After a three-month observation period (when no complications occurred), a radiological control was performed, including periapical radiographs. All the dental implants were clinically stable, and all were osseointegrated, with no surgical and/or postsurgical complications.

### 2.3. Sample collection

Infiltration anesthesia was applied at the top of the upper vestibule, complemented by infiltration in the palatal region. In cases with total mucosal coverage, samples were collected making a circular incision at the top of the implant, while for implants fitted with healing abutments an incision was made in order to carefully remove a half-moon shaped fragment of the marginal mucosa surrounding the healing cap limited to the vestibular side and penetrating the soft tissue down to the bone. The soft tissue portion was then prepared for immunohistochemical study. As soon as they had been collected, the samples were placed in sterile containers with physiological saline and sent to the Department of Immunology (Ramón y Cajal Hospital, Madrid, Spain) within a maximum of two hours. The samples carried an identifying code (single blinding), as a result of which the person analyzing them was unaware of which group they belonged to.

### 2.4. Sample processing

#### 2.4.1. Sample disaggregation

Before performing flow cytometry, cell individualization was carried out by disaggregating the tissue samples. The biopsies were incubated for 60 min under constant agitation in a 5 ml volume of Roswell Park Memorial Institute (RPMI) medium, supplemented with 10% fetal calf serum (FCS) with 1 mg/ml collagenase and 40 μg/ml deoxyribonuclease (DNase) agar. The addition of collagenase boosted sample disaggregation, leaving cells in suspension in the medium. The released cells were then centrifuged and washed twice in phosphate buffer solution (PBS) to prepare them for processing.

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