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Research Article

A Peri-Implant Soft Tissue Biopsy Technique to Analyze the Peri-Implant Tissue Sealing: A Non Invasive Approach for Human Histologies

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Abstract

Aim: To describe a minimally invasive technique for peri-implant soft tissue biopsy.

Materials and Methods: Peri-implant mucosa was removed by gentle dissection still attached to the titanium pre-fabricated abutment. The biopsy was prepared for light microscopy according to the ground sectioning technique. The section was grounded to 30 - 50 µm thickness using a micro-grinding unit.

Results: The histological findings revealed the presence of a thick and dense multiple bundles of collagen fibers near and parallel to the abutment surface.

Conclusion: The proposed protocol can allow a precise histological evaluation of the sample. Furthermore it might be of help for researchers interested in studying a human model rather than an animal one without removing the osteointegrated implant thus avoiding to raise any ethical concern.

Keywords: *Peri-Implant Soft Tissue Sealing; Mucosa; Peri-Implant Mucosa; Peri-Implant Bone Loss; Peri-Implantitis; Mucositis; Mucosa Thickness*

Clinical Relevance

Scientific Rationale for Study: Allow to perform peri-implant soft tissue analysis with a minimally invasive approach.

Principal Findings: Thick collagen fibers near and parallel to the abutment surface.

Practical Implications: The minimally invasive biopsy technique described in this manuscript would be of help for future research aiming to describe the peri-implant mucosa, soft tissue sealing and long-term stability of the peri-implant tissues.

Introduction

In implantology, peri-implant tissue health and soft-tissue aesthetics is essential for long term biological, restorative and aesthetic success. However the need for keratinized mucosa and its role in the maintenance of peri-implant health and soft-tissue integration remains a debated issue. According to some authors [1] the absence of adequate keratinized mucosa in endosseous dental implants, especially in posterior implants, can be associated with higher plaque accumulation and gingival inflammation but not with more annual bone loss. Data from other published studies revealed conflicting results with regard to the influence of keratinized mucosa on plaque score and soft-tissue inflammation. Most studies showed that the amount of soft-tissue recession was significantly increased at implant sites with narrow keratinized mucosa [2,3], but the amount of keratinized mucosa had little effect on deepening of peri-implant pockets [4]. A band of keratinized mucosa could not be absolutely necessary for the maintenance of peri-implant tissue, whereas lack of adequate keratinized mucosa around the implant might impede proper oral hygiene performance and compromise the aesthetic results. The majority of the studies [1,5] failed to find an association between keratinized mucosa width and peri-implant probing depth; however, the study by Zigdon and Machtei [6] showed that implants with wider mucosal band presented with higher mean probing depth than those with narrower band of keratinized mucosa (3.1 mm vs. 2.7 mm). They considered that shallower probing depth at implants sites with narrow keratinized mucosa might be related to soft-tissue recession. Therefore, less pocket formation may be more common in areas with less keratinized mucosa.

Based on the lack of evidence in the literature on the role of the peri-implant mucosa it was felt to develop a non-invasive protocol to study the implant mucosa in a state of either health or disease but without removing the osteointegrated implant.

When it comes to the surgical protocol for soft tissue biospy of the peri-implant mucosa, the published articles suggested the simultaneous removal of the endosseous implant and the above mucosa in order to allow the soft tissue to be maintained firm and attached to the underlying bone for a correct histological evaluation [7,8]. By the use of the described technique it is possible to avoid the disruption of the fragile mucosa for histological analysis but at the same time it is not applicable in humans without raising an ethical concern for the invasiveness of the procedure. Accordingly this type of protocol can be rarely applied to humans and almost all published data are then reporting the histological findings based on animal models.

The purpose of this study was to propose a new and non-invasive surgical protocol for peri-implant soft tissue biopsy.

Materials and Methods

A 64 year-old-female patient presented to the clinic for the treatment of the full maxilla due to a failing fixed partial denture. The medical history revealed hypertension and the use of cardioaspirin for prevention of cardiovascular disease. The patient signed a written informed consent form before starting the treatment. An immediate full maxilla reinforced provisional was delivered the same day the old prosthesis were atraumatically sectioned. Endodontic re-treatments were then performed and endosseous dental implants (Nobel Replace CC Groovy Nobel Biocare AB, Zurich, Switzerland) were inserted in the maxillary posterior sextants simultaneously with bilateral sinus elevation and GBR as well as osseous resective surgery for the treatment of a “gummy smile”.

After allowing a proper healing time of 4 months a second stage surgery was executed to uncover the implants and perform a soft tissue manipulation. At the same time of uncovery and after assessing the osteointegration of the dental implants a definitive titanium pre-fabricated abutment (Esthetic Abutment Conical Connection NP 3.5 Nobel Biocare AB, Zurich, Switzerland) was positioned and the flap was then sutured back around the abutment (5.0 PGA C3 Hu-Friedy Mfg. Co., LLC Chicago, IL USA). Suture were removed after 7 days and no medications were prescribed since the surgery performed was minimally invasive and the patient did not show any discomfort.

After allowing the tissue to heal and mature for 3 months (Figure 1) the patient accepted, by signing a second informed consent, to have the mucosa covering the abutment removed to proceed to the prosthetic phase.

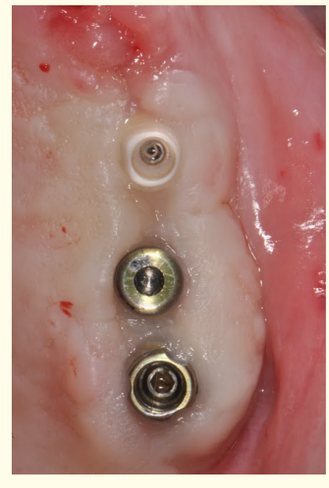
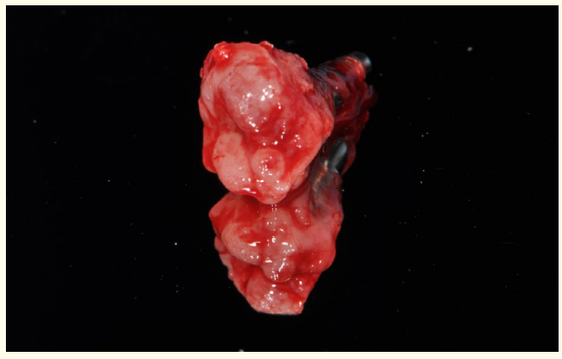


Figure 1: *Occlusal view before soft tissue biopsy.*

A circular incision was executed coronally and a thickness of about 1.5 mm of mucosa was left intact around the abutment. The inner screw of the abutment was removed and by gentle dissection from the base of the implant platform the mucosa was separated without disrupting it (Figure 2-4). A titanium healing abutment was then inserted into the implant platform (Figure 5).



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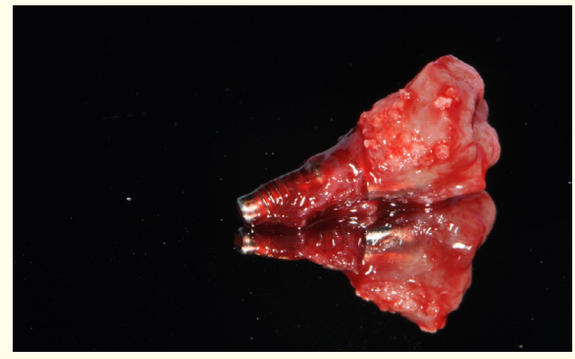

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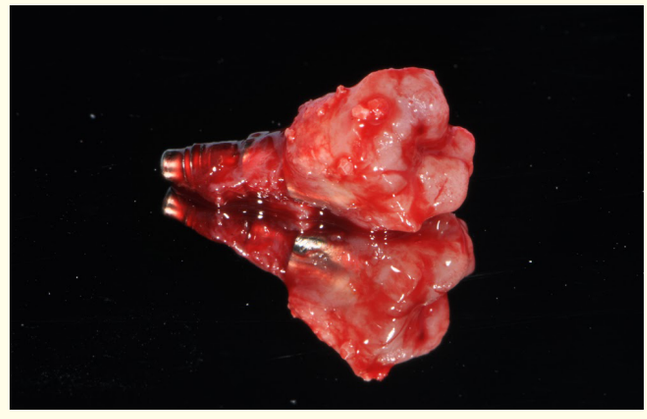

(4)

Figure 2, 3 and 4: *The abutment is removed. The mucosa is maintained firmly attached to the titanium surface of the pre-fabricated esthetic abutment*

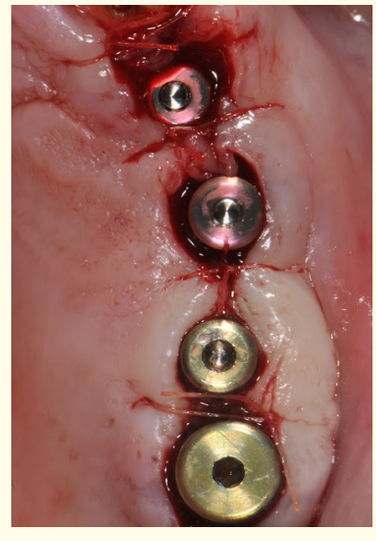

Figure 5: *Suturing and healing abutment positioning after mucosa biopsy.*

Histotechnical Preparation

The biopsy was prepared for light microscopy according to the ground sectioning technique. In short, the specimen was dehydrated in a graded series of ethanol: 60%, 80%, 96% and absolute ethanol each 48 hrs. Then the specimen was infiltrated with a graded series of ethanol and Technovit 7200 VLC (Kulzer, Hanau, Germany) embedding resin over a period of at least 12 days at standard temperature and pressure while constantly shaking. This was finished with the sample being placed in 3 consecutive containers of 100% Technovit 7200 VLC (Kulzer, Hanau, Germany) each for 24 hrs at standard temperature and pressure and constant shaking. Following dehydration and infiltration the specimen was light polymerized in Technovit 7200 VLC for 10 hrs using a light polymerization unit (Exakt, Norderstedt, Germany) allowing water-cooling. The temperature during polymerization never exceeded 40°C. In the following the polymerized block was sectioned using a band-saw unit (Exakt, Norderstedt, Germany) equipped with a diamond-coated band. The section was grounded to 30 - 50 µm thickness using an Exakt micro-grinding unit. The section then was polished stepwise using Struers diamond pastes. Finally, the section was stained with Sanderson's RBS stain and counter stained with acid fuchsin (Dorn and Hart, Villa Park, USA). The sections were evaluated with a Leica 205A stereomicroscope and a Leica 6000 DM light microscope (Leica Microsystems, Glattbrugg, Switzerland).

Results

Peri-implant mucosa is composed of 2 compartments: a marginal junctional epithelium and a zone of connective tissue attachment. Both structures consist mainly of collagen.

The histological findings revealed the presence of a thick multiple layers of collagen fibers firmly attached to the abutment. Two sections were produced, one through the center of the abutment (section 1) and the second located more in the periphery of the abutment (section 2).

An overview of section 1 showed connective tissue completely covering the coronal part of the abutment. Three different parts could be distinguished. Two laterally placed (labeled in Figure 6 as 1) and a middle part (labeled in Figure 6 as 2). Part 1 revealed in the upper part connective tissue infiltrated with leucocytes and in the lower part dense collagen fibers oriented parallel to the abutment surface (Figure 7). Part 1 was bordered by a keratinized oral epithelium. Part 2, interposed between parts 1 and 3, revealed a highly inflamed connective tissue (Figure 8). Out from the oral epithelium of part 3, a newly formed epithelium is interposed between parts 2 and 3 (Figure 9). Part 3 showed similar elements as part 1 but in addition graft particles embedded in connective tissue (Figure 10).

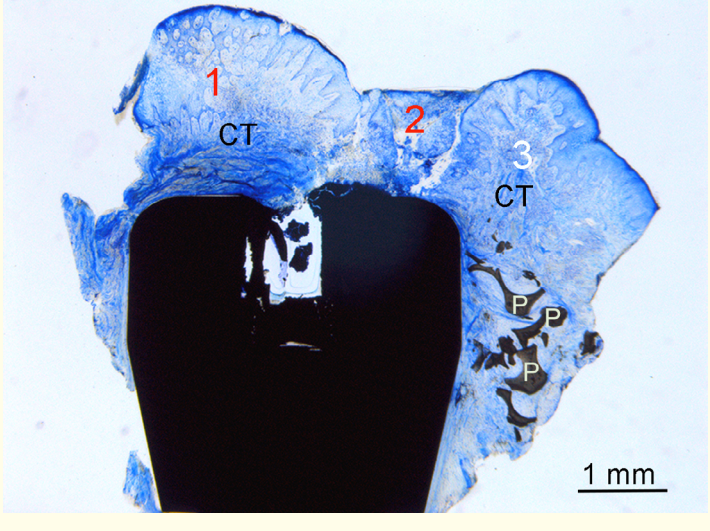

Figure 6: *Overview light microscopic micrograph of section 1 showing parts 1-3., being part 1 and 3 lateral and part 2 central. Note the presence of graft particles (P) surrounded by connective tissue (CT) in part 3.*

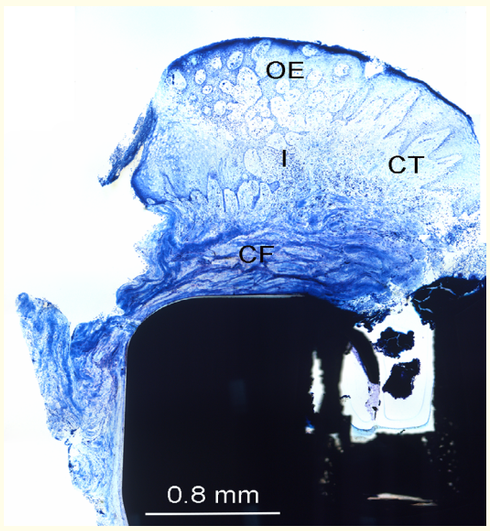

Figure 7: *Light microscopic micrograph of part 1 characterized by slightly inflammed (I) connective tissue (CT), the presence of dense collagen fibers near the abutment surface and a keratinized oral epithelium (OE)..*

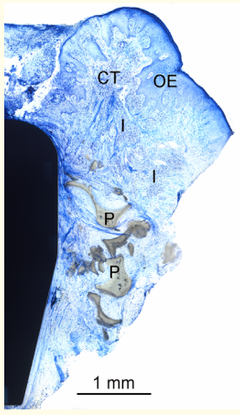

Figure 8: *Light microscopic micrograph showing part 02 next to part 3. The Figure shows the presence of an inflammatory infiltrate (IF) in part 3, oral epithelium (OE), connective tissue (CT), and graft particles (P).*

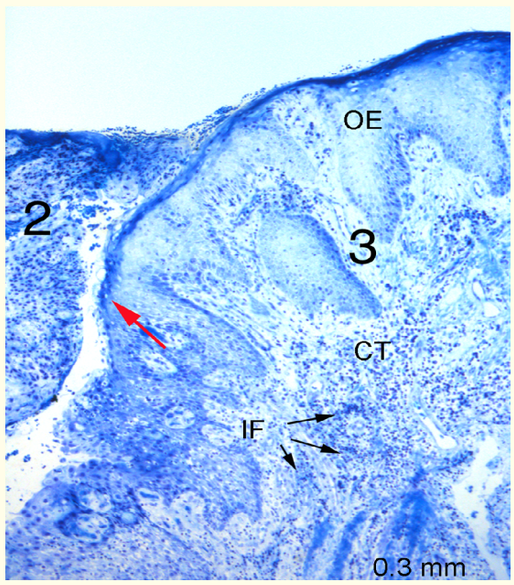

Figure 9: *Higher magnification of the border between part 2 and 3 showing the presence of an epithelium (red arrow). Note the presence of an inflammatory infiltrate (IF) in part 3 and in particular in part 2, ora epithelium (OE) and connective tissue (CT).*

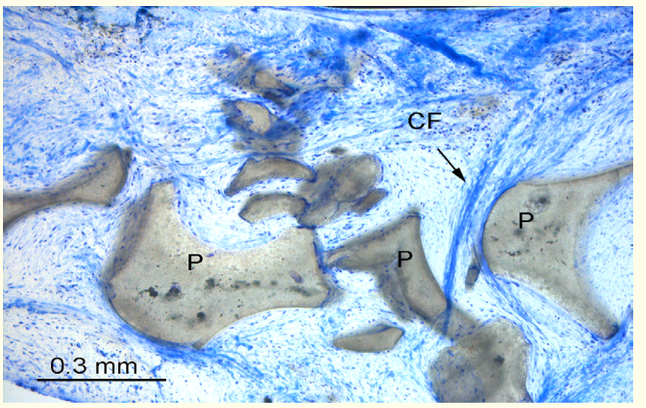

Figure 10: *Micrograph of part 3 revealing the presence of graft particles (P) embedded in connective tissue (CT).*

Section 2 also showed connective tissue with a keratinized epithelium covering the abutment and the presence of graft particles (Figure 10). Part 2 as observed in section 1 was no longer present but a missing keratinized layer was still visible (Figure 11). Again dense bundles of collagen fibers were present near and parallel to the abutment surface (Figure 12).

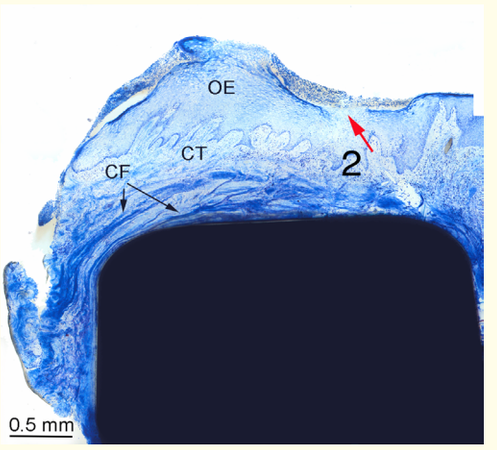

Figure 11: *Note the absence of a keratinized layer in the middle (red arrow). Oral epithelium (OE) is facing the oral cavity, a thick layer of connective tissue (CT) is between the oral epithelium (OE) and multiple layers of collagen fibers (CF) running parallel to the titanium abutment.*

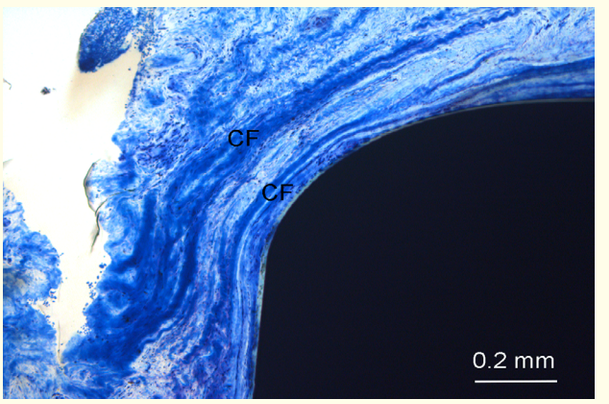

Figure 12: *Collagen fibers (CF) near and parallel to the abutment surface.*

Discussion

It is well known that connective tissue is present in the implant mucosa but it is not inserted directly on the surface. In the peri-implant tissues there is a higher proportion of collagen and fibroblasts arranged parallel to the surface of the implant inserted directly into the bone to form a collar that gives consistency and tonicity to the mucosa. This interaction between the tissue and the titanium implant surface is essential, as in teeth, to inhibit the apical migration of the junctional epithelium thus preventing bone loss. Achieving optimal gingival esthetics around anterior implants is a challenging procedure, and maintaining it over time can be an equally demanding task. Despite the high success rates achieve with osseointegrated implants the peri-implant mucosal response is not clearly understood. There are conflicting results in the current literature with regard to the significance of keratinized mucosa in peri-implant health. Several studies indicated that a band of keratinized mucosa is not important for the maintenance of peri-implant tissue. Other studies supported the preservation or the reconstruction of keratinized mucosa in order to maintain the soft tissue stability around dental implants. The novel surgical approach that is proposed clearly demonstrates that this could be of help when a detailed study of the mucosa is needed on patients. The histological analysis of the peri-implant soft tissue collar might represent the link between osteointegration and stability of the peri-implant mucosa.

Conclusions

Various factors are crucial for predictable long-term peri-implant tissue stability, including the biologic width; the papilla height and the mucosal soft-tissue level; the amounts of soft-tissue volume and keratinized tissue; and the biotype of the mucosa.

The biotype of the mucosa is congenitally set, whereas many other parameters can, to some extent, be influenced by the treatment itself.

Although it is authors' opinion that a minimal thickness of the soft tissue collar is needed for the mucosa to be stable overtime, the evidence related to the effect of keratinized and thickness of the mucosa on the changes of attachment or bone levels is limited, and conclusions could not be drawn at present. The proposed protocol might be of help for researcher to study a human model rather than an animal one without removing the osteointegrated implant.

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Disclosure of any Conflicts of Interests if Applicable

The authors reported no conflict of interest.

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