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Original Article

Revisiting peri-implant soft tissue – histopathological study of the peri-implant soft tissue

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Abstract: Peri-implant soft tissues are essential for osseointegration. The peri-implant mucosa may lack vascular supply, and histological observation, even without plaque, shows the presence of inflammatory cells. The objectives of this study were to assess the histopathological changes of the epithelium and connective tissue around the implant. Twenty patients of both genders were studied. Twelve weeks after implant placement, fragments of peri-implant gingival sulcus were harvested and processed for light microscopy. Group I (10): without clinical inflammatory signs (control); Group II (10): with clinical inflammatory signs. Histopathological parameters were analyzed and classified in 3 grades: mild, moderate or severe (grade 1, 2 or 3). Control group showed only slight changes, grade 1. In group II we found edema with moderate to severe cellular and nuclear changes. There are more women than men with all grades of inflammation. All patients with moderate edema are male and all patients with severe edema are female. A significant association ($p=0.007$) exists between these two variables. Significant differences were found when comparing the degree of inflammation with nuclear alterations ($p=0.001$) and the same results when comparing the degree of edema and nuclear changes ($p<0.001$). This study demonstrates that clinical examination can be used, with a small margin of error, to monitor and control the state of the peri-implant mucosa. In clinics the predisposition of female patients to greater degree of edema and inflammation should be accounted for.

Keywords: Implant, inflammation, peri-implant soft tissue, gingiva

Introduction

To Branemark [1] “osseointegration is achieved if the peri-implant mucosa heals rapidly in the marginal region, sealing supporting structures”.

Clinical characteristics of the peri-implant soft tissues should be evaluated for the presence or absence of edema, redness, as well as variations in the rates of plaque and bleeding [2], knowing that the placement of an implant will condition variable pathological changes resulting from the surgical procedure that can be translated into gingival atrophy, and are influenced by other situations, such as sex, age or other conditions of each patient.

The epithelium found in the wound margin around implants is morphologically and phenotypically oral epithelium, resembling the epithelium surrounding adjacent teeth. Based on

these principles, the essential function of the epithelium during the healing process is to cover the exposed connective tissue and establish a barrier that has features common to the junctional epithelium at the level of the groove. On the other hand, the connective tissue itself prevents tissue migration towards the apical epithelium [3]. For Berglundh et al. [4] the reason because epithelium stops its apical migration may be due to the interaction between the soft tissue and the titanium oxide layer of the implant. Thus, the epithelium has the capacity to proliferate around the implant representing a supra-alveolar attachment. Cochran et al. [5] demonstrated the adhesion of epithelial cells and fibroblasts to smooth and rough titanium surfaces. Their results confirmed that the bond between connective tissue and epithelium could prevent migration over the sides of titanium. The existence of similar binding characteristics between the mucosa and the titanium surface [junctional epithelium and connective

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tissue area] was found subsequently by studies evaluating different implant systems [6].

The interaction between the surface of titanium (Ti) and epithelial cells occurs through an anchoring mechanism formed by hemidesmosomes which act as anchor plates that bind epithelial cells to the basal lamina [7]. The mucopolysaccharides of the basal lamina in contact with the dental implant represent the biological barrier that resists the trauma that can occur at this level [8].

The peri-implant mucosa interface at the top of the implant/Ti has the characteristics of cicatricial tissue rich in collagen and with scarce cells [1]. Immunohistochemical extracellular matrix of healthy tissue around the implants is very similar, in what concerns the pattern of distribution of collagens type I, III, IV, VII and distribution of fibronectin and laminin, to gingival tissues. Despite this fact the peri-implant connective tissue contains a larger amount of collagen type V and VI. The presence of type V collagen - collagenase resistant - in the peri-implant connective tissue may function as a mechanical barrier to the invasion by bacteria [9].

The histological observation of the surface of the implant, in animal models and humans, even without plaque, shows the presence of inflammatory cells. In biopsies taken from the interproximal region of implants in clinically healthy subjects and in patients with clear signs of inflammation were found inflammatory infiltrate of soft tissues. The presence of T cells was associated with an effective immune response as a functionally stable condition for long-term clinical success of osseointegrated dental implants [10].

Implants have no local vascular plexus from the periodontal ligament [11], as a result, the apical tissue, the epithelium and peri-implant mucosa may lack vascular supply [12], nevertheless Buser et al. [13] demonstrated that adjacent to the implant surface there are dense fibers, 50 to 100 micra wide. Apparently this inner zone corresponds to a lax connective tissue which includes vascular components [14]. These studies established that the contact area of the tissue, adjacent to implants was similar to that of the complex of tissue around the teeth containing, among other elements,

perpendicular fibers. Thus, some changes in the mucosa were described as repairing mechanisms in an attempt to maintain a stable biological dimension [15].

So, microscopic structure of the gum around the teeth and implants has common characteristics. Both are covered by keratinized epithelium but in the case of implants the epithelium is smaller, so some authors concluded that there is a reaction that occurs between the connective tissue and the surface of titanium oxide for "connective tissue integration" [4].

Gingiva and mucosa of teeth and implants have common features, but differ in the composition of connective tissue, the alignment of the collagen fiber bundles and distribution of vascular structures in the apical compartment of the epithelium bonding.

Objectives

The objectives of this study are to assess the histopathological changes that occur in the epithelium and connective tissue around the implant.

Material and methods

The population of the study was 20 patients, from a private practice, who went to the dentist to replace a missing tooth with an implant (**Table 1**).

The average age was 46.30 ± 13.24 years, with an age range between 25-65 years, six male (30%) and 14 females (70%). Exclusion criteria were: inadequate oral hygiene, smoking habits, chronic systemic disease or relevant parafunctional habits.

In all patients we performed a scaling prior to surgery. The surgical technique, implant placement and collection of gingival tissue were performed by the same surgeon. The implants placed were 35 MkII Branemark System, Nobel Biocare (Goteborg, Sweden), with lengths between 10 and 12 mm and 3.3 mm in diameter. Patients were evaluated at the beginning of the prosthetic phase, i.e. 12 weeks after implant placement when fragments containing about 4x1 mm of gingiva from the peri-implant gingival sulcus were harvested. After macroscopic observation, biopsies were fixed in 10% buff-

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Table 1. Implant placement

Tooth	n
Right superior canine (13)	4
Right superior 2nd premolar (15)	3
Right superior 1st molar (16)	4
Left superior central incisive (21)	1
Left superior canine (23)	2
Left superior 1st molar (26)	6
Total	20

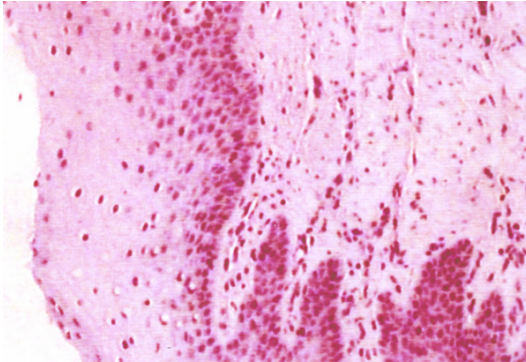


Figure 1. Slight intracellular edema of the epithelial cells (Control g.). (H.E. x200).

ered formalin and processed for light microscopy.

The patients were divided into two groups of 10 patients each: Group I: patients without clinical inflammatory signs (control); Group II: patients with clinical inflammatory signs (patients).

The histopathological parameters analyzed were: inflammation of the chorion, the cellular swelling and nuclear changes and classified into three grades, mild, moderate or severe (Grade 1, 2 or 3).

All patients signed an informed consent document, and were treated according to the principles of the Declaration of Helsinki for medical research involving human subjects, and identifiable human material.

Statistical study

For quantitative variables, we used the Student t test. To compare qualitative variables the chi-square test. Groups of two were compared using Yates correction and Fisher's exact test. It was considered as the minimum level of significance level of $p < 0.05$. Data were processed with SPSS software version 15.0.1.

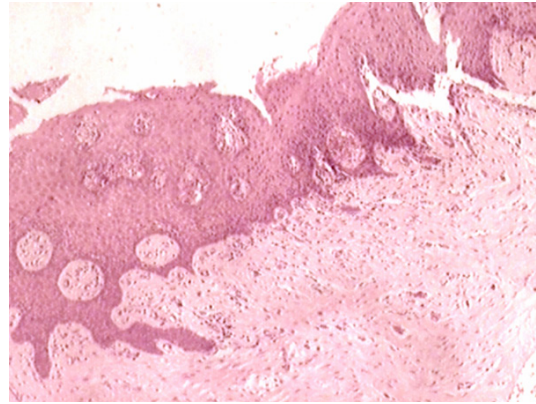


Figure 2. Fibroblasts and collagen fibers, together with some signs of superficial inflammatory infiltrate (Control g.). (H.E. x100).

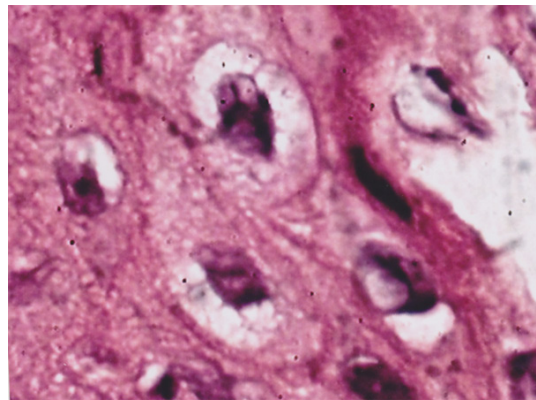


Figure 3. Severe intracellular edema. Cell membrane rupture. Distorted hyperchromatic nuclei (Patient g.). (H.E. x1000).

Results

Group I (control)

Biopsies of this group showed only slight changes, grade 1, especially in the epithelium, such as discrete intracellular edema and a mild inflammation without significant changes. We observed slight changes in epithelial basal layer and the spinous layer presents changes in its architecture and some cells with intracellular edema (**Figure 1**). The chorion has some inflammatory cells, between epidermal ridges and remaining fibroblasts/fibrocytes and collagen fibers (**Figure 2**).

Group II (patients)

In this group there is edema with moderate to severe cellular changes and nuclear changes

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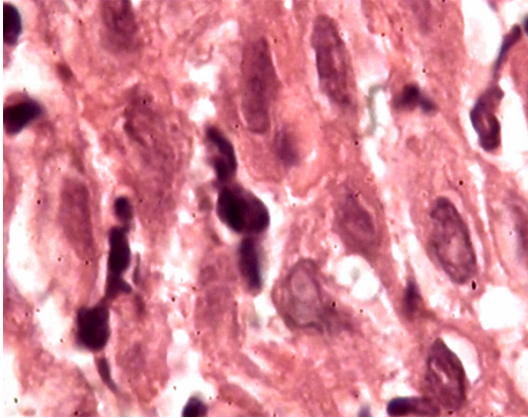


Figure 4. Deeper portion alterations. Marked cellular alterations, including nuclear fragmentation (Patient g.). (H.E. x1000).

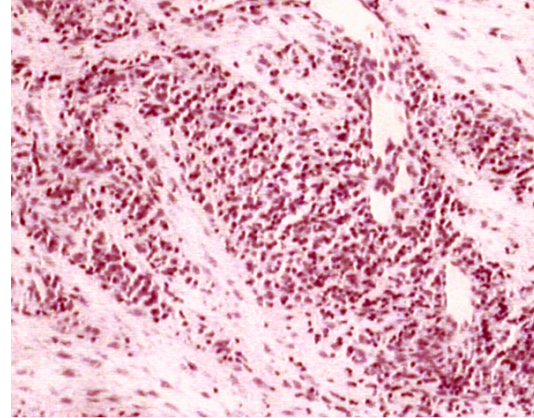


Figure 5. Severe inflammatory infiltrate (mainly lymphocytes), occupying most of the observed chorion (Patient g.). (H.E. x200).

Table 2. Comparison between sex and edema grade

	Edema Grade			Total (n %)
	Slight (n %)	Moderate (n %)	Severe (n %)	
Male	3 (50%)	3 (50%)	0 (0%)	6 (100%)
Female	7 (50%)	0 (0%)	7 (50%)	14 (10%)
Total	10 (50%)	3 (15%)	7 (35%)	20 (100%)

p=0.007.

Table 3. Inflammation grade

	Inflammation Grade			Total (n %)
	Slight (n %)	Moderate (n %)	Severe (n %)	
Control	10 (100%)	0	0	10 (100%)
Patients	1 (10%)	8 (80%)	1 (10%)	10 (10%)
Total	11 (55%)	8 (40%)	1 (5%)	20 (100%)

P<0.001.

with heterogeneous distribution and peripheral chromatin, hyperchromasia, nuclear fragmentation and nuclear distortion (Grade 2 and 3). The intracellular edema is very pronounced leading in some cases to the rupture of the cell membrane. We also observed nuclei with abnormal configurations (**Figures 3 and 4**). The chorion is occupied by a dense inflammatory infiltrate corresponding to a severe degree of inflammation (**Figure 5**).

Statistical correlations

There were no statistically significant differences (p=0.26) between the mean age of patients and the degree of inflammation, although the patients with severe inflammation are younger

than average. There are more women than men in all grades of inflammation in the distribution by sex. All patients with moderate edema are male and all patients with severe edema are female. Significant association (p=0.007) was found between the two variables (**Table 2**).

Group I individuals had mild inflammation, while 90% of the group II had moderate or severe inflammation (p<0.001) (**Table 3**). The degree of edema observed was considered mild in 10 patients (50%), moderate in 3 patients (15%) and severe in 7 patients (35%). All patients (100%) with

severe edema also had severe inflammation (p=0.001) (**Table 4**).

The degree of inflammation was not affected by the length of the implant (p=0.71) or by tooth type (p=0.79).

Concerning nuclear changes no significance was found concerning the mean age of patients (p=0.13), or gender (p=0.11).

Significant differences were found when comparing the degree of inflammation with nuclear alterations (p=0.001) (**Table 5**) and the same results when comparing the degree of edema and nuclear changes, five of seven patients with severe edema have nuclear changes of grade 3 (p<0.001) (**Table 6**).

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Table 4. Comparison between inflammation grade and edema grade

Inflammation Grade	Edema Grade			Total (n %)
	Slight (n %)	Moderate (n %)	Severe (n %)	
Slight	10 (90.9%)	0 (0%)	1 (9.1%)	11 (100%)
Moderate	0 (0%)	3 (37.5%)	5 (62.5%)	8 (100%)
Severe	0 (0%)	0 (0%)	1 (100%)	1 (100%)
Total	10 (50%)	3 (15%)	7 (35%)	20 (100%)

p=0.001.

Table 5. Comparison between inflammation grade and nuclear alterations grade

Inflammation Grade	Nuclear Alterations			Total (n %)
	Slight (n %) grade 1	Moderate (n %) grade 2	Severe (n %) grade 3	
Slight	10 (91.9%)	1 (9.1%)	0 (0%)	11 (100%)
Moderate	0 (0%)	4 (50%)	4 (50%)	8 (100%)
Severe	0 (0%)	0 (0%)	1 (100%)	1 (100%)
Total	10 (50%)	5 (25%)	5 (25%)	20 (100%)

p=0.001.

Table 6. Comparison between edema grade and nuclear alterations grade

Edema Grade	Nuclear Alterations			Total (n %)
	Slight (n %) grade 1	Moderate (n %) grade 2	Severe (n %) grade 3	
Slight	10 (100%)	0 (0%)	0 (0%)	10 (100%)
Moderate	0 (0%)	3 (100%)	0 (0%)	3 (100%)
Severe	0 (0%)	2 (28.6%)	5 (71.4%)	7 (100%)
Total	10 (50%)	5 (25%)	5 (25%)	20 (100%)

P<0.001.

Discussion

The gingival mucosa of natural teeth and around implants have common morphological features but differ in the composition of connective tissue, the orientation of collagen fibers and the vasculature [4, 16-20].

The behavior of the peri-implant soft tissue is crucial and fundamental in the evolution and success of the implant. An inadequate response implies the failure of implant treatment. Some authors such as van Steenberghe [21], apply the same parameters to the teeth and implants, so that their results can establish a clear relationship between implant failure and inflammation of peri-implant soft tissue.

The soft tissue around the implants is very similar in structure and composition to the tissues

surrounding the tooth. Supracrestal tissue surrounding the implant comprises a keratinized gingival epithelium, junctional epithelium and apical connective tissue and the alveolar bone [4].

In the case of teeth, transeptal collagen fibers are inserted into the root surface (in acellular cement), forming bundles of fibers that attach the tooth gingival complex, preventing the apical migration [22].

In implants, connective tissue is present but 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 epithelial [16, 23].

As mentioned above, the soft tissue around the implant is very similar in structure and composition to the tissues surrounding the tooth. In fact, they exhibit a keratinized epithelium and hemidesmosome adhesion as occurs around the teeth, which indicates that there are no differences across the epithelial tissue between them [4].

In relation to connective tissue, the main difference between the implant and the tooth is given by the orientation of the collagen fibers underneath the epithelial attachment. The supracrestal gingival tissue has a perpendicular orientation to the tooth surface while the peri-implant tissues have their collagen fibrils oriented parallel to the implant surface, forming a ring around it. In the case of teeth, there is an insertion of collagen fibers while there is no binding to the implants [4].

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Marginal bone resorption has been linked to the state of the soft tissues around implants. In our study, all patients had a proper oral hygiene. Lindqvist et al. [24] found that patients with good oral hygiene had less bone resorption around the implants. Other authors, such as Rocha dos Santos [25] indicated that hygiene is an important factor in the maintenance of the implants.

The degree of keratinization of the tissue is another factor that has been associated with marginal resorption; however results are contradictory on this point [26]. Bessis [27] indicates that the existence of non-keratinized mucosa is compatible with maintenance of the implant.

The peri-implant soft tissue plays an essential role during wound healing [28]. Epithelial cells located in the periphery of the wound are coded to divide and migrate until the epithelial continuity is completely restored [1].

According to Berglundh et al. [4] healthy soft tissues around teeth and implants should have a pinkish color and be firm. The epithelium is separated from the connective tissue by a basement membrane rich in fibers of type IV collagen and laminin [3].

Our histological observations, described above, are similar to the ones of Listgarten et al. [29] regarding the structure of the peri-implant gingiva.

The real interaction between the epithelium and the implant remains unclear. The present knowledge of this interface has been obtained from in vitro experiments using cultured cells [30, 31]. In our study we observed a basal lamina which acts as anchor plate connecting the epithelial cell to the connective tissue. McKinney et al. [32] also described the basement membrane, demonstrating the presence of laminin, which acts as a molecular adhesive between epithelial cells and the various layers forming the basal lamina. So, the supracrestal compartment plays an important role in maintaining the barrier between the implant and the intraoral environment. In our study, in individuals without clinical signs of inflammation, fibroblasts and collagen fibers were observed in contact with the implant head, maintaining supracrestal integrity.

On the other hand, Lang et al. [1] describe connective tissue apical to the junctional epithelium with more collagen (85% vs. 60%), fewer fibroblasts (5-15% vs. 3.1%), and some blood vessels (6.4% vs. 7.3%) than in regions corresponding to the connective tissue around the teeth. So the supra-alveolar portion of the peri-implant mucosa in the tissue/implant interface has the characteristics of scar tissue, rich in collagen and poor in cells.

In our study, more than half (55%) of the samples had a moderate degree of inflammation, although this was more severe in younger patients, pointing out a possible hyper-reactivity of the tissues.

Berglundh and Donati [33] described a chorionic inflammatory infiltrate dominated by plasma cells and B lymphocytes in patients with no inflammation, which coincides with the observations of our control group. In this group we also observed few macrophages and polymorphonuclear cells [PMNn] as did studies of Nakajima et al. [34], Noda et al. [35], Donati et al. [36] and Kim et al. [37].

As expected, patients with obvious clinical signs of inflammation [pain, bleeding, etc.] had grade 2 and 3 histologic inflammation. This was expected and in accordance with Sanz et al. [38] that reported, in patients with peri-implant inflamed tissues, migration of leukocytes and inflammatory infiltrate composed of mononuclear cells, plasma cells and increased blood vessels occupying approximately 65% of all tissue. Cornelini et al. [39] also described in inflamed tissues: lymphocytes, plasma cells, and some PMNn neutrophils. Gualini and Berglundh [40] observed a chorion with dense inflammatory infiltrate, richer in B lymphocytes than in T lymphocytes, although other authors as Bouillon et al. [41] reported the highest prevalence in the inflammatory infiltrate of T cells, in addition to plasma cells and macrophages.

Half of our samples had a mild degree of cellular edema. When comparing the degree of edema with sex, it was observed that women had higher levels of edema [moderate to severe] showing a significant statistically association ($p=0.007$). This could be due to hormonal reasons related to the gender physiology. Also as expected, individuals from the control group had lower degrees of edema than subjects from the patient group ($p<0.001$).

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In all cases, histopathologically, we observed cellular swelling, reaching a cytoplasmic area/cell ratio of 4:1, observed before rupture of the cell membranes. The nuclear changes were identified as nuclear fragmentation.

Albouy et al. [42, 43], in patients with obvious clinical signs of inflammation, found ulcerated epithelium and dense inflammatory infiltrates. These infiltrates were rich in lymphocytes and plasma cells. The PMNs grouped together in the periodontal pockets and blood vessels in the deeper portion of the connective tissue.

Conclusions

This study clearly demonstrates that clinical examination can be used, with a small margin of error, to monitor and control the state of the peri-implant mucosa, both in healthy subjects and patients. In the clinical management the predisposition of female patients to greater degree of edema and inflammation should be taken into account.

Disclosure of conflict of interest

None.

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