Histologic Analysis of the Oral Mucosa Lining Osseointegrated Implant Cover Screws: A Study in Humans

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Osseointegrated dental implants are inserted into the alveolar ridge, and for them to function as tooth replacements, the surrounding tissues need to adapt to them. Just as with teeth, dental implants traverse the oral mucosa and have access to the contaminated environment of the oral cavity. Therefore, periodontal and peri-implant tissues are important for establishing a protective barrier. The aim of the present study was to perform a histologic analysis of the mucosa surrounding osseointegrated implant cover screws. For this study, 17 mucosal specimens were obtained from 12 patients during the second surgical session for implant exposure to the oral environment. After histologic preparation, specimens were sectioned perpendicularly to the mucosal surface to a thickness of about 3 μm, stained with 1% toluidine blue, and examined under light microscopy. All specimens showed a keratinized, stratified, squamous epithelium with well-defined strata. In the lamina propria, unorganized dense connective tissue was noted in the reticular layer, and in 4 samples, a chronic inflammatory infiltrate was seen in this region. The papillary layer presented tall connective papillae consisting of loose connective tissue. The results of this study confirm the hypothesis that the mucosa that conceals osseointegrated implant cover screws has the same morphologic characteristics as the alveolar masticatory mucosa. Furthermore, clinical conditions of normality in peri-implant tissues may not coincide with situations of histologic normality.

Key Words: periodontology, soft tissue–implant interactions, clinical research

INTRODUCTION

The formation of normal dentition is determined by a complex development process, with all the end components constituted by cells and their products, resulting in the formation of dental tissues. Periodontal tissues include the periodontal...
ligament, alveolar bone, cement, and gingival tissues. The main function of these is to unite the tooth to the maxilla and provide biological sealing between the contaminated oral cavity environment and the internal aseptic environment.¹

Osseointegrated dental implants have become common because of their high success rate and represent a predictable rehabilitation option for cases of tooth loss. They were developed by Branemark in the early 1960s, and their clinical function depends on their direct anchorage in bone.²

The first trials with oral implants concentrated on the clinically useful attempt to anchor implants to bone. The successful efforts of Branemark and colleagues in obtaining a predictable osseointegration did not, however, focus with the same intensity on the study of the mucosa-implant interface.³,⁴

Experiments in animals have shown tissue responses to implants primarily in terms of bone formation and the remodeling process. Most of these studies were conducted in animals such as monkeys and dogs; others were done in guinea pigs and rats. Using the rat experimental model, the tissue responses to implantation, particularly the processes of epithelial regeneration and formation of bone around the implants, could be reported chronologically as observed by optic microscopy.⁵ Nevertheless, few significant data consider the mucosa-implant interface, especially in humans. The few existing studies practically report what was found in samples of animal experiments.³

Gould et al⁶ confirmed in vivo (electron microscopy, in a single individual) their previous discoveries in vitro that a layer of epithelial cells was inserted into the titanium surface. This experiment showed several layers of epithelial cells adjacent to the implant surfaces, with an intercellular union achieved via desmosomes. At the epithelium-implant interface, clear evidence of hemidesmosomes, as well as material that had the appearance of basal lamina, could be seen.³

Using light and electron microscopy in healthy oral mucosa samples that surrounded titanium (Branemark-type) and sapphire crystal implants (from a single surgical session) in 20 individuals, Arvidson et al⁷ revealed that the external epithelium adjacent to the implant fold was stratified, keratinized (similar to masticatory mucosa), and continuous with sulcal epithelium (non-keratinized), and this was continuous with junctional epithelium. The apical portion of the junctional epithelium, consisting of only a few cellular layers, ended approximately 1–2 mm from the bone, and under electron microscopy, these cells adjacent to the implants exhibited condensed structures similar to hemidesmosomes. The supraperiosteal connective tissue was characterized by a 3-dimensional network of collagen fibers running in different directions.

Using titanium implants in a monkey maxilla model, which were restored 1 month after placement surgery and were maintained under occlusal loads for 14 months, Ruggeri et al⁸ for the first time suggested the term “circular ligament,” representing collagen fibers originating subepithelially and on the alveolar bone crest, which migrated to form a dense connective tissue bundle around the “neck” of implants not submerged by a load.

Piatelli et al⁹ confirmed the presence of collagen fibers running in a circular pattern around 3 implants placed in a human. The circular ligament, however, was confined to the connective tissue layer immediately coronal relative to the alveolar bone, whereas apically to the junctional epithelium, the connective tissue contained fibers running predominantly parallel to the implant surface. The authors furthermore reported that the peri-implant connective tissue seemed to be important in prevent-
ing apical growth of the epithelium and in guaranteeing the longitudinal success of the osseointegrated implants.10

Analyzing the apical-coronal dimensions of the biological distance components of nonsubmersed implants, both loaded and unloaded, Cochran et al4 found the values of 1.36, 1.01, and 1.05 mm for peri-implant connective tissue in the periods of 3 months from cicatrization without load, and 3 and 12 months from cicatrization with functional loads, respectively. The discreet narrowing was attributed to the long-term maturation of the peri-implant mucosa connective tissue as a result of function (load). Data from this study suggest that a biological width with a stable dimension, as is seen around natural teeth, exists around titanium implants of a single surgical session (both with and without load).

Current data indicate that the presence of a layer of distinct connective tissue is expected adjacent to oral implants. The layer, ranging from 0.75–2.2 mm in thickness, is interposed between the junctional epithelium and the alveolar bone. The connective tissue adjacent to the implant surface is probably avascular, and immediately coronal relative to the alveolar bone, it contains collagen fibers running in a circular pattern around the implant. It is suggested that this internal layer represents postoperative scar tissue, and that vascularization of the peri-implant mucosa appears to be derived almost exclusively from periosteal blood vessels.3

The clinical significance of the peri-implant tissue dimensions and structure in maintaining the crest bone and osseointegration of implants is not yet clear,3 nor is it clear whether this dense connective “scar” tissue, with a probable barrier function,9 forms immediately after implants are placed, in 2 surgical sessions, or only when they are exposed to the oral medium, whether or not functionally loaded.

Thus, the structural and functional analysis of the mucosa that comes into contact with the osseointegrated implants in an area, which, from the time of its exposure to the oral cavity will be occupied by components such as the sulcus, epithelium, and peri-implant connective tissues essential to the osseointegration process,6,9,11 is of fundamental importance in elucidating the formation process of these structures.

**PROPOSITION**

The purpose of this project is to perform a histologic analysis of the mucosa that lines the covering screw of osseointegrated implants of 2 surgical sessions.

**MATERIALS AND METHODS**

After approval was obtained from the Research Ethics Committee of Federal University of Uberlândia (Report 006/04, CEP registration #135/03), 17 samples were collected of oral mucosa lining the covering screws of Branemark-type implants that had healed clinically in isolation from the oral cavity (Figures 1 and 2); these were obtained from 12 individuals between the ages of 20 and 55 years. To obtain the samples, a circular punch-type scalpel (Branemark system) approximately 5 mm in diameter was used (Figure 3).

According to preestablished inclusion criteria for research, patients were selected (1) who had no systemic abnormalities with implants with a minimum length of 10 mm and a diameter of 3.75 mm, and (2) who, at the time of the second surgical session, did not present clinically identifiable conditions of inflammation in the area to be operated, or a previous history of an inflammatory reaction in this area. To indicate the circular scalpel, a strip of remaining inserted gingiva approximately 4 mm wide had to be present (Figure 3).
The samples (Figures 4 and 5) measured around 5 mm in diameter (extent of the epithelium) and between 3 and 4 mm in thickness (epithelium-connective direction). Approximate measurements were made with a periodontal probe. After the samples were processed, semiserial cuts approximately 3 μm thick were made perpendicular to the mucosal surface; these were stained with 1% toluidine blue, mounted on glass slides, and analyzed under a light microscope.

**Results**

In all the mucosa samples, the epithelium observed was stratified, squamous, kerati-
nized, or parakeratinized, with well-defined strata. In the basal layer, which consisted of cubic cells, some mastocytes could be observed (Figure 6).

In the spinous layer, the thickest stratum, polyhedral cells were noted, presenting cytoplasmic intercellular projections (Figure 7). The granulose stratum, which consisted of about 3 cellular layers, presented flattened cells with basophilic granules in the cytoplasm (Figure 7). The corneal stratum, which was composed of layers of dead cells,
was parakeratinized (Figure 8) in some samples and orthokeratinized in others (Figure 7).

In all the mucosa samples, a predominance of dense unorganized connective tissue was observed, as is characteristic of the masticatory mucosas. The papillary layer of the lamina propria (Figure 8) displayed tall connective papillae made up of loose connective tissue with many fibroblasts amid the delicate web of collagen fibers. In the reticular layer, the dense unorganized connective tissue, amid thick collagen fiber bundles, displayed resident connective cells such as fibroblasts and mastocytes (Figure 9).

In some samples, the presence of metachromatic material was detected in the reticular layer (Figure 10). In 4 samples, a chronic inflammatory infiltrate was observed in this region (Figure 11).

**DISCUSSION**

The first experiment with oral implants focused on a clinically useful attempt to anchor the implants to the bone, generating consensual data in the world literature as to their mechanism and predictability. However, the successful efforts of various authors to elucidate the osseointegration process did not focus with the same intensity on the study of the mucosa-implant interface.

The periodontal mucosa, as well as the mucosa-implant interface, is an extremely important region for the integrity of an individual as a whole, because in no other part of the body is there such a complex relationship (mucosa “vs” teeth or implants) separating the organism’s internal environment from a contaminated region such as the oral cavity. Therefore, some authors justify the clinical success of implants by the clinical and histologic characteristics of the junctional epithelium and of the adjacent conjunctive tissue, which effectively promote the marginal sealing of this area.

Furthermore, connective tissue seems be important in preventing apical growth of the epithelium and in guaranteeing the longitudinal success of osseointegrated implants.

In the literature, descriptions of the junctional epithelium report the presence of a basal lamina and hemidesmosomes promoting the adhesion of this tissue to the implant surface, in a manner similar to that of periodontal tissues.

The peri-implant connective tissue presents structural differences when compared with periodontal connective insertion tissue, and it consists of dense connective tissue, probably avascular in the supraosseous portions adjacent to the implant, with organized circumferential collagen fibers, suggesting that this internal layer represents postsurgical scar tissue. Some studies suggest that the structure of the peri-implant connective tissue is probably related to the absence of progenitor cement cells that eventually may guarantee direct insertion of collagen fibers into the implant.

A major part of the information on epithelial and connective tissues (lamina propria) that come into contact with the osseointegrated implants, arises from in vivo studies conducted in experimental animals such as dogs, monkeys, and rats. These studies represent a larger volume of scientific work compared with experimental studies in humans.

In this study, in the epithelial tissue that covered the samples, the following strata were observed: basal, spinous, granulose, and corneal. They were well defined and morphologically similar to the normal masticatory mucosa, as is the adjacent connective tissue that in the papillary portion presented loose connective tissue with many fibroblasts amid the delicate web of collagen fibers and in the reticular layer displayed a dense unorganized connective.
tissue. These data suggest that the “circular ligament” proposed by Ruggeri et al., containing fibers organized in a circular pattern around the supraosseous portion of the implant, probably forms after the implant starts to work, after a second operative session, or after accidental exposure of the implant cover cap to the oral cavity.

The presence of chronic inflammatory infiltrate in the adjacent connective tissue observed in 4 samples suggests that in some cases, communication may occur between the implants and the oral cavity, although this may not be observed on routine clinical examination. The covering cap, which may become contaminated at the time of surgical insertion of the implant or during the period awaiting osseointegration, thus may serve as a niche for bacterial deposits.

Surgical exposure of the osseointegrated implant cover cap may be cogitated to facilitate oral hygiene procedures when implants programmed to remain protected by the oral mucosa eventually communicate with the oral cavity.

The data obtained in this experiment are consistent with those of Arvidson et al. in terms of the description of the masticatory mucosa, although the region analyzed by these authors was adjacent to functioning implants. It is not possible to make structural comparisons between the present study and investigations of Gould et al. and Piattelli et al., because the implants observed in those studies were exposed to the oral cavity.

Peri-implant tissues are fundamental for the predictability of osseointegrated implants and for maintaining the health of the individual as a whole. However, the tolerance to implants exerted by these tissues, their behavior in situations of aggression (mainly by bacterial plaque), possible changes related to age, and their real physiologic function must be explained, as much is lacking for a complete understanding of this excellent treatment modality.

**CONCLUSIONS**

The mucosa that lines osseointegrated implant covering screws presents the same morphologic characteristics as are noted in the masticatory mucosa that covers the alveolar ridge.

Conditions of clinical normality detected in peri-implant tissues on routine clinical examination may not coincide with situations of normality when microscopically analyzed.

**ABBREVIATIONS**

BL: basal layer  
CF: collagen fibers  
CL: corneal layer  
CP: connective papillae  
ECI: epithelium-connective interface  
ER: epithelial ridges  
F: fibroblasts  
GL: granulous layer  
M: mastocytes  
m: microcirculation  
SL: spinous layer

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