Light-Microscopic Evaluation of the Dimensions of Peri-Implant Mucosa Around Immediately Loaded and Submerged Titanium Implants in Monkeys

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Background: Immediate loading of dental implants is a successful treatment concept. The importance of healthy peri-implant soft tissues for the long-term success of dental implants has been widely recognized. The aim of this study was to evaluate the peri-implant soft tissues around immediately loaded and submerged implants in monkeys.

Methods: A total of 48 implants were inserted in six *Macaca fascicularis* monkeys. For 24 implants (test implants), the prosthetic abutments were inserted immediately, and a custom-made metal superstructure was cemented after 3 days; the other 24 implants were left unloaded (control implants). Block sections of bone segments containing the implants were retrieved 9 months after surgical placement. A histomorphometric measurement of sulcular epithelium (SE), junctional epithelium (JE), and connective tissue (CT) contact percentage of the soft tissues around test and control implants was carried out.

Results: In some specimens, the peri-implant epithelium was very similar to a pocket epithelium, whereas in others it was possible to observe an SE and a long junctional-like epithelium with a moderate amount of inflammatory cells. The supracrestal peri-implant CT was dense and organized in collagen fibrous bundles in an annular pattern around the implant. No statistically significant differences were present in the dimensions of SE, JE, and CT in test and control implants ($P > 0.05$).

Conclusion: Immediate loading did not produce changes in the dimensions of the peri-implant soft tissues. *J Periodontol 2008;79:1697-1703.*

KEY WORDS

Animal studies; dental implants; histology.

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The importance of healthy peri-implant soft tissues for the long-term success of dental implants has been recognized.[1,2] In animal studies,[3,4] it was observed that peri-implant soft tissues are composed of epithelium, with a mean vertical height of 2 mm, supported by an underlying high-collagen, cell-poor connective tissue (CT), with a mean height of 1 mm; these tissues show a great similarity to dentogingival tissues surrounding natural teeth. The coronal surface of immediately and delayed-loaded implants consisted of a keratinized stratified squamous epithelium supported by fibrous CT with minimal inflammation.[3] Human biopsies of healthy keratinized mucosa surrounding implants showed a CT rich in inflammatory cells and poor in collagenous components.[5,6] In humans, the CT barrier around loaded implant abutments had a normal keratinized mucosa continuing in a thin sulcular epithelium, with the CT having a high density of cells around the implant with a small number of blood vessels.[5]

One major factor that could influence the success of an implant is the possibility that oral epithelium attaches to the implant in the same manner as with a tooth, sealing off the oral environment from bone.[7,8] It was noted that even if there is a moderate loss of the peri-implant

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bone, this loss remains stable if the soft tissues are firm and intact.[8] The epithelial attachment on teeth derives from the fusion of the reduced enamel epithelium with the oral epithelium.[9-11] The peri-implant mucosa seems capable of attachment to the titanium abutment surface,[12] as in the natural dentition the junctional epithelium (JE) can attach to the implant surface via a basal lamina and hemidesmosomes.[13] An electron microscopic study[8] demonstrated the in vivo presence of hemidesmosomes and basal lamina at the epithelium–titanium interface. However, the importance of this finding should not be overstressed because the presence of hemidesmosomes is regularly encountered in epithelial cell cultures of many biocompatible materials.[12] It is not clear why JE around the implant should be kept from proliferating apically to the region where bone is in contact with the implant surface.[13] However, such epithelial proliferation or migration is limited,[12,13] particularly in the absence of inflammation. It is possible that the formation of a mature collagen seal at the bone level prevents this migration.[12] Relatively limited data are available about the peri-implant supracrestal soft tissues because of the technical difficulties with preparing histologic sections combining soft tissue, bone, and implants.[14,15] Moreover, the structure and dimensions of peri-implant soft tissues in immediately loaded dental implants has been investigated only rarely.[3,16,17] Therefore, it is important to evaluate the response of the soft tissues around immediately loaded implants.[2]

The aim of this study was to evaluate the characteristics and the dimensions of the peri-implant mucosa surrounding immediately loaded and submerged implants in monkeys.

MATERIALS AND METHODS

The protocol was approved by the Ethical Committee of Maimonides University, Buenos Aires, Argentina. The implant surgery technique and peri-implant bone reactions around these immediately loaded and submerged implants‡ were reported in a previously published study.[18] Briefly, the titanium implants were approved for human use and have been on the market in Italy for ~20 years; the implants are screw-shaped with a diameter of 4 mm and a length of 10 mm. The plasma spray covered 7.5 mm of the body length of the screw, whereas the neck was 2.5 mm and was made of smooth titanium. The body of the implant consisted of three threads, each with a pitch of 1.5 mm, whereas five threads were present in the apical portion. Six 8-year-old *Macaca fascicularis* monkeys, weighing ~8 to 10 kg, were used in the present study. The surgical procedure was performed under intramuscular anesthesia with ketamine hydrochloride (20 mg/kg), atropine sulfate (0.05 mg/kg), and chlorpromazine hydrochloride (1 mg/kg). The two premolars and the first molars of the maxilla and mandible were extracted, and the sites were allowed to heal for 4 months before implant placement. A total of 48 implants were inserted: 24 in the mandible and 24 in the maxilla. A mucoperiosteal flap was elevated, and bone artificial sockets were prepared with a 3.99-mm bur used on a specially designed electric machine operated at 100 revolutions per minute under generous saline irrigation to minimize the bone damage due to overheating. The sockets were treated with a manual 4.00-mm cutting instrument, and threading of bone was done with a custom-made instrument. The implants were inserted with torque values ≥35 Ncm. Implant stability was evaluated clinically and was good in all cases.

The implant abutted 1 mm over the crestal bone with the entire plasma-sprayed surface covered.

For 24 implants (12 in the maxilla and 12 in the mandible; test implants), titanium standard straight abutments for cement retention were inserted immediately, and a direct impression was made with vinylsiloxane material in standard trays. Because it was not possible to deliver any prosthesis at the time of implant placement, a custom-made metal superstructure was fabricated and cemented after 3 days. The implants were splinted in a bridge in centric occlusion.

The animals were fed a solid standard diet. Twenty-four implants (12 in the maxilla and 12 in the mandible) were left unloaded (control implants). Healing screws were immediately inserted in the control implants, and the soft tissues healed in such a way that the implants were transgingival. Healing screws were 1 to 2 mm higher than masticatory mucosa and were not subjected to occlusal loads. Sutures were removed after 1 week. A three-times-per-week plaque-control regimen was introduced, consisting of toothbrushing and topical application of 1% chlorhexidine gluconate gel, on test and control implants. Examinations, including implant mobility, plaque index,[19] and bleeding on probing (BOP), were carried out once per month. This regimen continued until implant retrieval. In all six animals, block sections of the bone segments containing the implants were retrieved 9 months after surgical placement (Fig. 1), and the bone defects were treated with guided bone regeneration using bioabsorbable membranes.

Processing of Specimens

The implants and the surrounding tissues were stored immediately in 10% buffered formalin and processed to obtain thin ground sections. The specimens were dehydrated in a series of graded alcohols and embedded in a glycolmethacrylate resin.§ Longitudinal

‡ P.H.I., Legnano, Italy.
§ Technovit 7200 VLC, Kulzer, Wehrheim, Germany.

Figure 1.
Block sections of the bone segments containing the implants were retrieved 9 months after surgical placement. At low-power magnification, bone (B) is visible around a large portion of the implant interface. (Basic fuchsin-toluidine blue; original magnification ×6.) Bar = 500 μm.

cut sections of 150 μm along the major axis of the implants were prepared using a cutting system.‖

Three slides were obtained and stained with basic fuchsin and toluidine blue. A double staining with von Kossa and acid fuchsin was done to evaluate the degree of bone mineralization. Each slide, after polishing, was immersed in $AgNO_3$ for 30 minutes and exposed to sunlight. Then, the slides were washed under tap water, dried, immersed in basic fuchsin for 5 minutes, washed, and mounted.

Histomorphometry

Measurement of sulcular epithelium (SE), JE, and CT was carried out using a light microscope¶ connected to a high-resolution video camera# and interfaced to a computer system.** This optical system was associated with a digitizing pad†† and a histometry software package with image-capturing capabilities.‡‡

Statistical Analysis

Statistical analysis was performed, and the mean value for each soft tissue was calculated. The unpaired *t* test was used, and *P* values ≤0.05 were considered statistically significant.

RESULTS

Clinical Observations

During the observation period, no implant was lost, and no clinical attachment loss was observed around any implant. No implant showed measurable mobility, and the implant surfaces were free from visible plaque. Although a careful plaque-control regimen was performed during the entire study, consisting of toothbrushing and topical application of 1% chlorhexidine gluconate gel over test and control implants, slight peri-implant inflammation was detected, and positive BOP values were often observed.

The light-microscopic results of the peri-implant soft tissues were similar around test and control implants and are reported together.

Maxilla

Light-microscopy observations. The peri-implant soft tissues were in close contact with the implant neck. These tissues were lined by a keratinized squamous epithelium. In the superior part, the cellular layers were numerous and similar to those seen in normal free gingiva. The more apical part of the peri-implant epithelium showed different features, probably as a result of plaque accumulation. In some specimens, the peri-implant epithelium was very similar to a pocket epithelium, with inflammatory cells in the epithelial tissue and CT; in others, it was possible to observe an SE and a long JE-like epithelium in which a moderate amount of inflammatory cells was present and included two to four flattened epithelial cell layers interconnected by several desmosomes and with hemidesmosomes facing the implant surface. The oral SE was hyperplastic, and ulcerations were present in some areas. SE was observed at the interface with the abutment, while JE was present at the interface with the implant. The epithelium was never attached to the abutment.

The plane of the histologic section made it possible to identify the implant surface in contact with epithelial cells and CT (Figs. 2 and 3).

Artifactual separation of the adjacent CT from the implant was a frequent observation, and this separation tended to leave minimal amounts of material on the implant surface. Most cells in the CT appeared to be fibroblasts and inflammatory cells, and these were embedded within a delicate fiber network. These collagen fibers were oriented obliquely near the implants, whereas they were perpendicular to the implant surface at a distance from the implant.

‖ Precise 1 Automated System, Assing, Rome, Italy.
¶ Laborlux S, Leitz, Wetzlar, Germany.
# 3CCD, JVC KY-F55B, JVC, Yokohama, Japan.
** Intel Pentium III 1200 MMX, Intel, Santa Clara, CA.
†† Matrix Vision, Oppenweiler, Germany.
‡‡ Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD; Immagini & Computer, Milan, Italy.

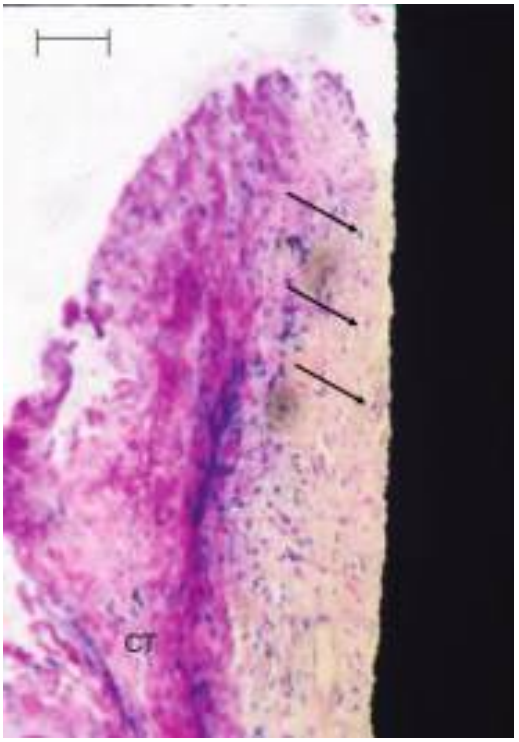

Figure 2.
Overview of ground section showing peri-implant tissues covered with keratinizing oral epithelium. Epithelial cells (arrows) and CT are present at the interface with the implant surface. The peri-implant soft tissues are in close contact with the implant neck. (Basic fuchsin-toluidine blue; original magnification ×40.) Bar = 200 μm.

Histomorphometric observations. In test implants, the mean SE was 1.3 ± 0.2 mm, the mean JE was 2.1 ± 0.3 mm, and the mean CT was 1.9 ± 0.4 mm. In control implants, the mean SE was 1.1 ± 0.3 mm, the mean JE was 1.9 ± 0.2 mm, and the mean CT was 1.7 ± 0.3 mm.

Mandible

Light-microscopy observations. An inflammatory infiltrate was present within the peri-implant mucosa. The oral SE was hyperplastic with elongated rete pegs and areas of ulceration. There were some polymorphonuclear leukocytes throughout this epithelium. The implant surface adjacent to this region had isolated clumps of subgingival plaque. At the interface with the abutment only SE was observed, while JE was shown interfacing the implant surface. The JE was non-ulcerated and composed of a layer that was three to five cells thick. This epithelium extended apically to various degrees, depending on the position of the serial sections. The CT approximating this superficial portion of the pocket had an inflammatory infiltrate composed primarily of plasma cells with some polymorphonuclear leukocytes, lymphocytes, and occasional macrophages. There were many small blood vessels in the area immediately below the epithelium. Supracrestal peri-implant CT was dense and organized in fibrous bundles in an annular pattern around the implant (Fig. 4).

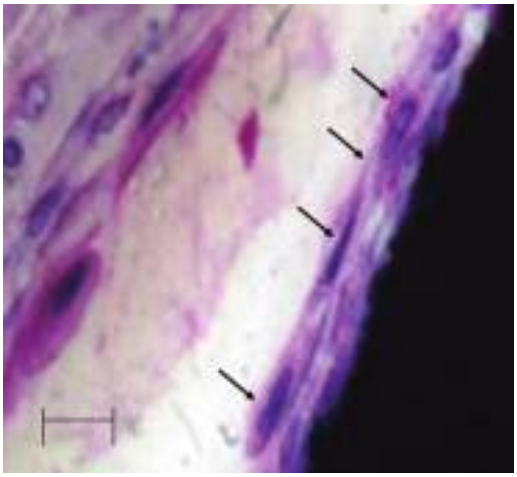

Figure 3.
JE (arrows) is interposed between CT and alveolar bone crest. The epithelial cells adhere to the implant surface. (Basic fuchsin-toluidine blue; original magnification ×100.) Bar = 10 μm.

Figure 4.
Longitudinal section through CT around implant viewed in polarized light. Note the presence of collagen fibers oriented parallel to the implant surface near the metal surface (arrows). A dense tissue organized in fibrous bundles formed an annular pattern around the implant. (Basic fuchsin-toluidine blue; original magnification ×40.) Bar = 200 μm.

Histomorphometric observations. In test implants, the mean SE was 1.2 ± 0.2 mm, the mean JE was 1.2 ± 0.2 mm, and the mean CT attachment was 1.5 ± 0.3 mm. In control implants, the mean SE was 1.1 ± 0.2 mm, the mean JE was 1.0 ± 0.2 mm, and the mean CT attachment was 1.3 ± 0.4 mm.

Statistical Analysis

No statistically significant differences were present in the SE, JE, and CT dimensions in test and control specimens ($P > 0.05$).

DISCUSSION

Implants properly installed and loaded can work successfully for ≥20 years as abutments for fixed restorations or as fixed supports for removable prosthetic restorations.[13] In the area of the mandible between the mental foramina, a >95% 5-year success rate can be expected.[20] The long-term success of oral implants is dependent on a direct bone–implant contact in a sufficient portion of the implant to achieve "osseointegration."[21] Conversely, a good integration of the supracrestal soft tissues with the implant surface seems to be highly desirable.[22] The present study evaluated, histologically and histomorphometrically, the soft tissue characteristics around immediately loaded implants placed in a one-stage surgical procedure compared to those around unloaded submerged implants. CT attachment and JE provide important information about the location of the apical extension of JE cells, crestal bone height, and the extent of bone–implant contact.[4] No differences were observed in the mean values of SE, JE, and CT attachment between test and control groups. These results are comparable to previous studies[4,7,23] that did not find differences in soft tissues around submerged and nonsubmerged, loaded and unloaded implant systems. In the present study, collagen fibers were distributed in a circular or oblique way near the implants, whereas they were perpendicular to the implant surface at a distance from it. These observations agree with those of other human and animal studies[1,24-26] but disagree with other investigators who found fibers running more or less parallel to the implant surface in animals[16,22,27] and humans.[7,28,29]

In animal studies,[30,31] peri-implant soft tissues responded to a prolonged period of plaque accumulation with the development of an inflammatory lesion.

In the present study, despite a strict plaque-control regimen, slight clinical peri-implant inflammation was often detected. The histologic data confirmed this observation, an inflammatory infiltrate was often present within the peri-implant mucosa, and the implant surface adjacent to this region had isolated clumps of subgingival plaque. These observations are in accordance with those of another study,[4] whereas other research did not show obvious signs of inflammation.[32]

A complete understanding of the biology of peri-implant tissues is lacking, and the underlying mechanisms of attachment and the factors that influence the integrity of this biologic seal are not well understood.[1,4] Experimental work in vivo and in vitro showed the presence of JE and a fibrous CT.[8,33,34] Histologic data from animal studies and human biopsies indicated a limited or absent inflammatory infiltrate of the CT around titanium abutments,[12,35,36] and a normal mucosa was frequently present around the implants;[13,37,38] moreover, biopsies from mucosa around stable implants contained only a very small inflammatory cell infiltrate, located in a narrow zone beneath the inner implant epithelium.[39,40] Some investigators[41] believed that the implant sulcus can remain healthy indefinitely. This led workers[32] to examine the extent to which epithelial cells and fibroblasts adhere to a number of substrates in vivo and in vitro. Other investigators[12,20] stated that the findings of peri-implant mucositis and peri-implant pockets were not associated with accelerated marginal bone loss and were not a factor in implant success. The marginal soft tissues adjacent to the implant consisted of a collagenous stroma covered with a keratinized epithelium.[24] The JE, arranged like a collar around the implants, consisted of layers of non-keratinized flattened squamous cells.[24] The outer implant epithelium, facing the oral cavity, resembled the masticatory oral mucosa.[24]

Some investigators[4] observed many peri-implant collagen fibers, mainly aligned parallel to the implant surface. However, the titanium/CT interface lacked a mechanical attachment of inserting collagen fibers.[4] The CT integration zone had a low density of blood vessels and a large number of fibroblasts.[4] Close CT adaptation through a thin, avascular, and collagen fiber-rich, scar-like tissue was documented in human and animal models.[30,41,42] There is considerable evidence that marginal soft tissue integration plays a fundamental role in establishing an effective seal between the oral environment and the endosseous part of a titanium implant.[43]

CONCLUSIONS

Our findings agree with other experimental studies[4,17] that demonstrated that immediate loading did not negatively affect soft tissue integration.

The animal model used in this study shows a rapid healing process, and a similar study with an earlier histologic examination (i.e., 3 months) should probably be carried out to evaluate if differences exist in the soft tissues around immediately and delayed-loaded implants at earlier time periods.

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