Histologic Analysis of an Immediately Loaded Implant Retrieved After 2 Months

Human biopsy of immediately loaded implants is the most important way to determine the occurrence of osseointegration. Implants inserted in sites with poor bone quality have been associated with lower success rates. The aim of this study is to document the early healing processes in a man around an immediately loaded implant retrieved after a 2-month loading period. An implant was inserted in the mandible of a 32-year-old patient and was loaded into a non-functional loading mode with a fixed provisional prosthesis the same day of the implant surgery. After 2 months, because the patient had difficulty accepting the implant, the implant was retrieved with a 5-mm trephine drill. Before retrieval, the implant appeared to be clinically osseointegrated, and no mobility was present. The pre-existing bone quality was type D4. The implant was surrounded by newly formed bone lamellae with a width of 200 to 400 μm. In many areas it was possible to observe osteoblasts producing osteoid matrix directly on the implant surface. Bone-to-implant contact percentage was 71% ± 3.2%. Even in a poor bone site and after a healing period of only 2 months, we observed a high bone-to-implant contact percentage. We can confirm that immediately loaded implants placed in soft spongy bone after a 2-month healing period can present mineralized tissue at the interface.

Introduction

Fibrous tissue has been described at the bone-implant interface in early or immediately loaded implants. In the past decade, a high clinical and radiographic level of osseointegration has been reported in early and immediately loaded implants. Moreover, high implant survival rates for these implants can be found in the literature. It has been reported that immediate loading does not lead to disruption of the implant-bone interface. The success of functionally stable osteosynthesis systems shows that osteosynthesis screws can be immediately subjected to loading without a loosening of the screw-bone interface.

One of the most important changes in implant dentistry has
been the increased acceptance of immediate loading as a clinical alternative, in carefully selected cases, with a predictable outcome and with high survival rates.\textsuperscript{42} However, a cautious approach is needed when transferring to humans the histologic results obtained in animal experimentation.\textsuperscript{43} Human biopsy of immediately loaded implants is certainly the most important way to determine the occurrence of osseointegration.\textsuperscript{40} The histologic reports of early or immediately loaded implants in humans reported in the literature are few.\textsuperscript{16,17,22–25,28,36,44,47,57,72,73}

Primary implant stability and bone quality have been recognized as key features for the success in immediately loaded implants.\textsuperscript{43} Implants inserted in sites with poor bone quality have been associated with lower success rates.\textsuperscript{74} This is likely related to the lower bone content and the lower bone-to-implant contact percentages because of the cancellous structure of these sites.\textsuperscript{74} Also, the implant surface characteristics have been demonstrated to be important for the bone apposition at the interface, with a faster bone formation at rougher surfaces.\textsuperscript{40} FRIADENT plus surface (DENTSPLY Friadent, Mannheim, Germany) is obtained with a novel grit-blasting and acid-etching technique, and it shows a regular micro-roughness with pores in the micrometer dimension overlaying a macroroughness structure caused by the grit blasing.\textsuperscript{75} This results in a porous topography with an hierarchical surface structure.\textsuperscript{76} The spatial parameters have shown a first macroscopic level of roughness of the dimensions of 100 µm and then a second level of grooves in the dimensions of about 12 to 75 µm, each of which embraces an arrangement of smaller, round groups with diameter of about 1 to 5 µm.\textsuperscript{76} Recently, we have documented the presence of mineralized tissues at the interface of immediately loaded implants retrieved after a loading period of 6 or 10 months.\textsuperscript{36,47,72,73} In this study, we attempted to document the healing processes at the interface after a shorter loading period of 2 months.\textsuperscript{40}

The aim of this study was a histologic and histomorphometric analysis in a man of the bone-titanium interface in an immediately loaded titanium implant with a novel surface that was retrieved after a 2-month loading period.

\section*{Materials and Methods}

A 32-year-old male patient presented with a partial edentulism in the right mandible. His medical history was unremarkable and he did not smoke. A 3.8 × 8 XiVE plus implant (DENTSPLY Friadent) was inserted into the posterior mandibular jaw region and was put into a nonfunctional loading mode with a fixed provisional prosthesis the same day of the implant surgery.\textsuperscript{49} The implant stability quotient (ISQ) at implant insertion was 71. The insertion torque was 7.7 N/cm. The last drill was D3.8. (DENTSPLY-Friadent, Mannheim, Germany).

After some weeks, the patient returned to say that he was uncomfortable with the implant and asked that it be removed. He was referred to a psychologist, but after a few consultations he still requested implant removal. It was then decided to follow his wish. Before retrieval, the implant was radiographically and clinically osseointegrated (Figure 1). After 2 months, the implant and surrounding tissues were then retrieved with a 5-mm trephine drill (Straumann, Waldenburg, Sweden) (Figure 2).

\section*{Processing of specimens}

The implant and the surrounding tissues were stored immediately in 10% buffered formalin and processed to obtain thin ground sections with the Precise I Automated System (Assing, Rome, Italy).\textsuperscript{77} The specimen was dehydration in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimen was sectioned longitudinally along the major axis of the implant with a high-precision diamond disc at about 150 µm and was ground down to about 30 µm. Three slides were obtained, and each was stained with basic fuchsin and toluidine blue. A double staining with von Kossa and acid fuchsin was performed to evaluate the degree of bone mineralization. After polishing, one slide was immersed in silver nitrate for 30 minutes and exposed to sunlight. The slides were then washed under tap water, dried, immersed in basic fuchsin for 5 minutes, and then washed and mounted.

\section*{Histomorphometry}

Histomorphometry of bone-to-implant contact percentage was carried out with a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (SCCD, JVC KY-F55B; JVC, Yokohama, Japan) and interfaced to a monitor and personal computer (Intel Pentium III 1200 MMX; Intel, Santa Clara, Calif). This optical system was associated with a digitizing pad (Matrix Vision GmbH; Matrix Vision, Oppenweiler, Germany) and a histometry software.
package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc, Immagini & Computer Snc, Milano, Italy).

**RESULTS**

The implant was clinically osseointegrated and stable, and no mobility was present. Radiographically, before the retrieval, a slight vertical crestal bone loss was noted (Figure 1).

The implant was surrounded by newly formed bone (Figure 3). At the coronal level, no infrabony pocket, Howship lacunae, or osteoclasts were present (Figure 4). The first bone-to-implant contact was found at about 1 mm from the shoulder of the implant. The pre-existing bone quality was type D4. No apical epithelial migration was found. No inflammatory infiltrate was present around the implant. No gaps or dense fibrous connective tissue were found at the bone-metal interface (Figure 5).

At the coronal level, the newly formed bone was woven and not lamellar bone and completely surrounded the pre-existing cortical bone. A rim of osteoblasts lined the marrow spaces and were producing osteoid matrix. Osteoblasts were also found in direct contact with the implant surface and were producing osteoid matrix directly on the metal surface. Only in a few areas in the coronal portion was it possible to see that this bone was going through an active remodeling process with the presence of some well-structured Haversian canals. The quality of the bone around the apical portion of the implant was poor.

Newly formed, strongly stained bone lamellae with a width of 200 to 400 μm were present around the implant. Far from the implant surface, only a few newly formed thin bone trabeculae were present. No Haversian canals were present in the bone located at the apical portion of the implant. In many fields, it was possible at the interface to observe osteoblasts producing osteoid matrix directly on the implant surface (Figure 6). Few marrow spaces were observed directly on the implant surface. In the apical portion of the implant, osteoblasts and newly formed bone were present, and no osteoclasts were present (Figure 7). Histomorphometric evaluation showed that the bone-to-implant contact percentage was 71% ± 3.2%.

**DISCUSSION**

Because the use of abbreviated healing periods is increasing, it is necessary to balance their benefits (eg, shorter time to completion) with the long-term success rates reported with delayed loading protocols because no quantitative data exist for the early healing processes in humans. In this field, the histologic evidence that it is possible to observe the formation of mineralized tissue at the interface even with shorter healing periods will help support the concept of early loading.

Immediate loading can reduce the treatment period because the soft tissues heal simultaneously with the hard tissues according to the contours of the provisional restoration. Immediate loading has esthetic, psychological, and functional advantages in eliminating second-stage surgery and in reducing patient discomfort and additional costs of the procedure. Most of the cases of immediately loaded implants reported in the literature were in the mandible. Posterior areas of the jaws have been avoided because of scarce bone quality, higher forces produced during mastication, and
FIGURES 3–7. Figure 3. The implant was surrounded by lamellar and woven bone. The bone was in close contact with the implant surface. At the coronal level, no infrabony pocket, Howship lacunae, or osteoclasts were present (acid fuchsin and toluidine blue, original magnification ×12). Figure 4. Higher magnification of the coronal portion of the bone-implant interface. No infrabony pockets or osteoclasts were present (acid fuchsin and toluidine blue, original magnification ×100). Figure 5. Lamellar and woven bone were observed in direct contact with the implant surface; no gaps or connective tissue were present at the bone-implant interface. The first bone-to-implant contact was found at about 1 mm from the shoulder of the implant. The pre-existing bone quality was type D4. No apical epithelial migration was found. No inflammatory infiltrate was present around the implant (acid
presumed higher implant failure rate. Most implant failures have been described in sites where the bone density was low at the start of the procedure. However, studies need to be conducted in soft-bone areas to evaluate if implant surfaces play an important role in immediate loading success and the predictability of immediate loading procedures in this type of anatomic location.

It has been documented that the occurrence of mineralized tissue at the interface is mainly related to the biomechanical stability of the implant and to the amount of micromotion. A threshold of tolerated micromotion has been identified somewhere between 50 and 100 microns. Poor implant stability produces a distortional strain with fibrous tissue formation at the interface. Bone submitted to cyclic micromovements elicits a more physiologic pattern of tissue regeneration than bone that is not stimulated mechanically. From a scientific point of view, the tissue integration of dental implants can be considered successful only if the bone-implant interface is maintained in the long term and if mineralized tissue is present and remains at the interface with the implants.

There is an absence of information about how much bone is needed at the interface of loaded dental implants, even though a recent figure of 50% has been proposed. Trisi et al found a 47.81% bone-to-implant contact percentage in the maxilla in dual-etched implants retrieved after a 2-month period. Testori et al reported a 64.2% bone-to-implant contact percentage in the mandible after a 2-month period. The density of bone at the implant site has been suggested to be important in the amount of bone at the interface; clinically, host-bone density plays an important role in determining the predictability of immediate loading success. In our case, even in a poor bone site, we have observed a high bone-to-implant contact percentage. The type of mineralized tissue at the interface with dental implants in spongous bone is not as satisfying as the extremely dense appearance observed around implants inserted in cortical lamellar bone. Our histologic results are similar to those reported by Testori et al, who found that in the spongous area the implant surface was covered by an almost continuous thin shell of newly formed bone. This particular arrangement corresponds to the characteristics of contact osteogenesis. The clinical efficacy of these thin isolated bone trabeculae to resist loading forces may perhaps be questioned, but the clinical evidence in our case points to a successful osseointegration process because the implant was clinically stable before retrieval. Moreover, Rohrer et al have documented that the trabeculae in the cancellous bone area were generally oriented around the implant in a supporting strut-like buttressing pattern.

The good clinical and histologic results of the present study may also relate to the use of an implant with a rough surface. This surface has been demonstrated to have a hierarchical surface structure because a blasting and etching processes produced a wettable surface. This unique wettability characteristic has been hypothesized to lead to an advanced adhesion of noncollagenous proteins such as sialoprotein and osteopontin, which are the preconditions of contact osteogenesis. Moreover, higher amounts of fibronectin adsorbed on the FRIADENT plus surface may improve host responses such as osteoblast adhesion. The presence of the sharp edges found under scanning electron microscopy on the FRIADENT plus surface could help the adhesion of cells by presenting a differential in the chemistry of the implant surface. The proteins adsorbing to the edge could take up a different shape from those adsorbed to a continuously flat surface. Furthermore, this surface has been reported to have a higher percentage of tetracycline marked bone.

In conclusion, in our study the implant had successfully osseointegrated from a clinical and radiologic point of view and was stable at retrieval time. Mineralized tissue was found covering the implant surface. The good clinical and histologic results of the present study may also relate to the use of an implant with a rough surface. This surface has been demonstrated to have a hierarchical surface structure because a blasting and etching processes produced a wettable surface. This unique wettability characteristic has been hypothesized to lead to an advanced adhesion of noncollagenous proteins such as sialoprotein and osteopontin, which are the preconditions of contact osteogenesis.
a large portion of the implant surface with no foreign body or inflammatory reactions visible, remodeling was present in an area around the implant, and we confirm that implants placed in soft spongy bone and immediately loaded after a 2-month healing period can present a high percentage of mineralized tissue at the interface. Primary stability plays an important role in the implant success, and it may be the fundamental prerequisite for immediate loading more so than is the anatomic location. Micro-motion has been reported to positively influence the healing of bone fractures, and optimal healing is not achieved in total absence of micromotion. Immediate and early loading of dental implants can then function as a mechanical stimuli for osteoblasts.

Immediate loading can therefore be considered an innovative and attractive treatment that can be successfully used today in implantology. Despite that retrieved human specimens are only rarely reported in the literature because of ethical reasons, they play a relevant role in helping understand the biological aspects of the bone response at the interface.

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