

# HISTOLOGIC EVALUATION OF AN IMMEDIATELY LOADED TITANIUM IMPLANT RETRIEVED FROM A HUMAN AFTER 6 MONTHS IN FUNCTION

Marco Degidi, MD, DDS  
Antonio Scarano, DDS  
Maurizio Piattelli, MD, DDS  
Adriano Piattelli, MD, DDS

## KEY WORDS

Conical abutment connection  
Crestal bone remodelling  
Dental implants  
Histology  
Immediate loading  
Microgap

*Marco Degidi, MD, DDS, is a visiting professor at the Dental School, University of Chieti, Chieti, Italy, and is in private practice in Bologna, Italy. Antonio Scarano, MD, DDS, is a researcher, Maurizio Piattelli, MD, DDS, is an associate professor, and Adriano Piattelli, MD, DDS, is a professor of oral medicine and pathology at the Dental School, University of Chieti, Chieti, Italy.*

*Address correspondence to Professor Piattelli at Via F. Sciucchi 63, 66100 Chieti, Italy (e-mail: apiattelli@unich.it).*

Clinical and histologic studies have demonstrated that immediate loading can be successfully used in implant dentistry. Many factors are thought to be of importance in obtaining mineralized tissues at the interface. This study describes the implant interface of an immediately loaded implant with a conical implant-abutment connection that had been inserted in the posterior mandible for 6 months. Histology showed that mineralized tissue was present at about  $74\% \pm 6\%$  of the implant interface. No gaps, fibrous tissue, or inflammatory infiltrate were present at the interface. The bone adjacent to the implant was mainly lamellar ( $90\% \pm 4.5\%$ ). Tetracycline was used to label trabecular bone, and labeled bone was found in direct contact with the implant surface. The extensive labelling by tetracycline demonstrated a large quantity of newly formed bone at the implant interface. The distance between the 2 lines marked by tetracycline was  $85 \pm 5$   $\mu$ m. The results of this study show that immediately loaded dental implants can form mineralized tissues at the bone-implant interface.

## INTRODUCTION

An important prerequisite for obtaining mineralized tissues instead of fibrous tissues at the interface of dental implants was an unloaded healing period of 3 to 4 months in the mandible and 6 months in the maxilla.<sup>1,2</sup> Early or immediate loading of dental implants was thought to disturb bone healing and result in fibrous repair.<sup>3-5</sup>

However, several clinical and histologic studies in man and experimental animals have demonstrated that implants that were immediately loaded were clinically successful and developed bone at the implant interface.<sup>6-49</sup>

Immediate loading has the advantages of providing immediate restoration of esthetics and functions,<sup>34</sup> reducing the number of patients visits, reducing the morbidity of a second surgical intervention, and facilitating the

TABLE  
Teeth replaced with implants

Diameter, mm	Length, mm
30 implant Ankylos	4.5 × 11
31 implant Ankylos	5.5 × 11
32 implant Ankylos*	3.5 × 8
26 implant XiVE	3.8 × 18
23 implant XiVE	3.8 × 18
21 implant XiVE	4.5 × 15
20 implant XiVE	3.8 × 15
19 implant XiVE	5.5 × 11
18 implant XiVE	4.5 × 11
17 implant XiVE	3.8 × 8

\* Retrieved implant.

functional rehabilitation of the patient.<sup>37</sup> Earlier implant restoration and function increase patient satisfaction.<sup>32</sup> Very high implant success rates are reported for early and immediately loaded implants. Jaffin et al<sup>27</sup> reported an overall survival rate of 95%, Ganeles et al<sup>25</sup> reported a rate of 99.4%, and Gatti et al<sup>29</sup> reported a rate of 96%. De Bruyn and Collaert<sup>38</sup> reported a 99.3% implant success in early loaded machined implants inserted in completely edentulous mandibles. Collaert and De Bruyn<sup>39</sup> reported a 100% success in early loaded implants with a fixed cross-arch restoration in the mandible. Romeo et al<sup>49</sup> and Gatti and Chiapasco<sup>40</sup> reported a 100% cumulative success rate after 2 years of functional loading in immediately loaded implant-retained mandibular overdentures. Animal experimentation cannot be extrapolated and transferred to a clinical application in humans. There are few human reports on histologic findings for implants placed by a 1-stage approach,<sup>50</sup> and there is a need to investigate the bone-healing processes at the interface,<sup>32</sup> especially concerning the question of which type of bone response is present around immediately loaded implants inserted in poorer quality bone.<sup>51</sup> Clinical stability is insufficient

to evaluate the occurrence of osseointegration, and it has been suggested that an analysis of human biopsies of immediately loaded implants is the best way to ascertain the quality and quantity of the peri-implant hard tissues.<sup>51</sup>

The aim of this study was to evaluate the peri-implant mineralized tissues of an immediately loaded implant, with a conical implant abutment connection placed in the posterior mandible of a man, after a 6-month loading period.

#### MATERIALS AND METHODS

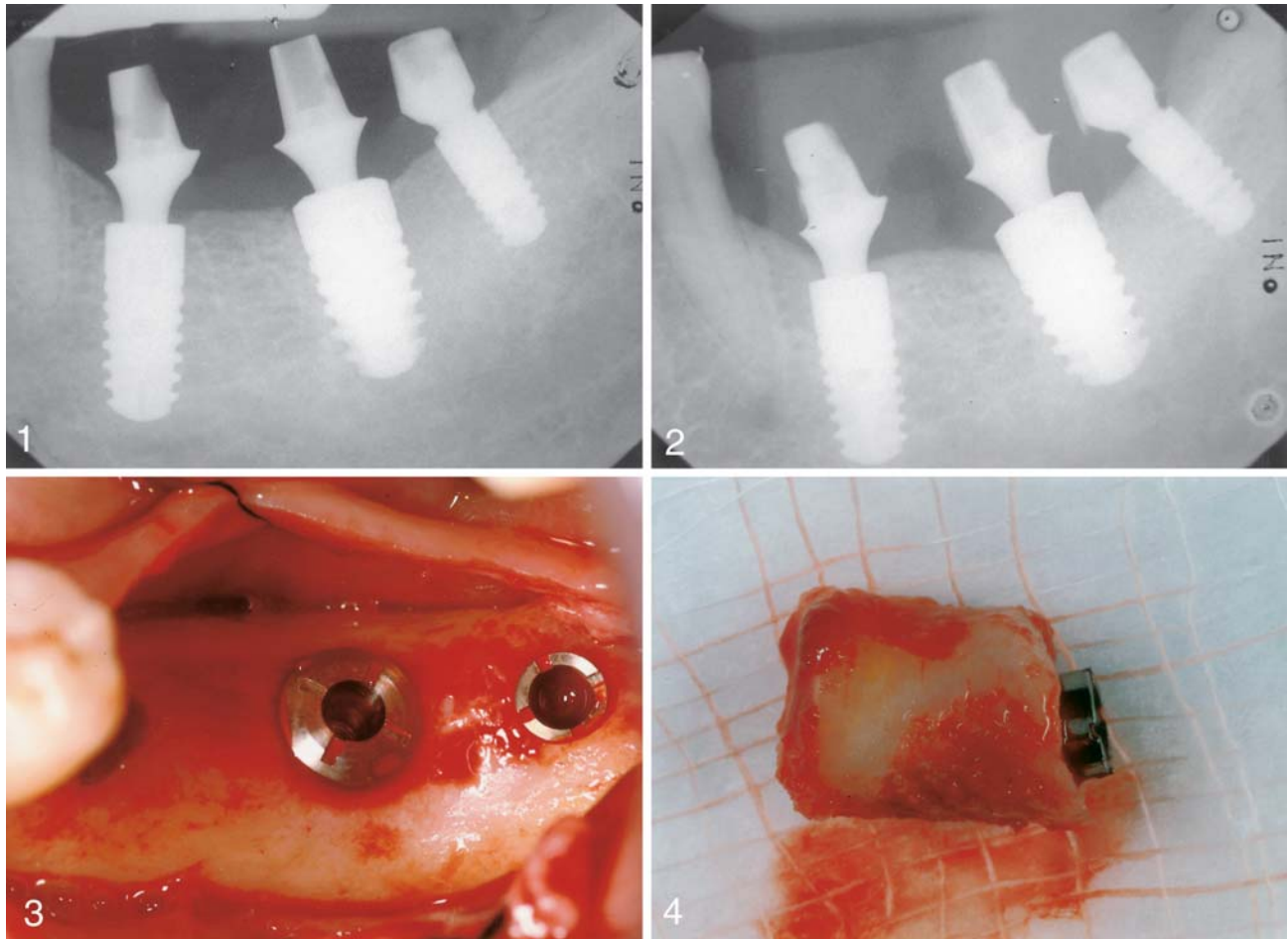
Three Ankylos dental implants (Dentsply, Friadent, Mannheim, Germany) were inserted in the right posterior mandible and 7 XiVE implants (Dentsply) were inserted in the left posterior mandible of a 65-year-old man with a partially edentulous mandible (Table). The patient did not smoke and he gave a written informed consent to the protocol, which was approved by the Ethical Committee of the University of Chieti, Chieti, Italy. All the implants were immediately loaded with a provisional resin restoration the same day of the implant surgery (Figure 1). The patient was given oxytetracycline (500 mg) at 30 and 60 days after the implant insertion. After a 6-month loading period (Figure 2), the most distal mandibular implant was retrieved with a 5.5-mm trephine bur (Figures 3 and 4). This implant had substituted tooth #32 and was a 3.5- × 8-mm Ankylos implant inserted in D3 bone with an insertion torque of 20 Ncm. The implant stability quotient value was 61 at implant insertion and 65 before implant retrieval.

#### Specimen processing

The implant and surrounding tissues were washed in saline

solution and immediately fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.15 M cacodylate buffer at 4°C and pH 7.4. The specimen was processed by the Precise 1 Automated System (Assing, Rome, Italy),<sup>52</sup> dehydrated 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 along its longitudinal axis with a high-precision diamond disc at about 150 μm and was ground down to about 30 μm with a specially designed grinding machine. Three slides were obtained, stained with acid fuchsin and toluidine blue, and examined with a transmitted light Leitz Laborlux microscope (Leitz, Wetzlar, Germany) and a Zeiss fluorescence microscope (Zeiss, Göttingen, Germany). Filters of wavelengths 510 to 560 nm (green filter), 450 to 490 nm (blue filter), 355 to 425 nm (violet filter), and 340 to 380 nm (ultraviolet filter) (Zeiss) were used with the fluorescence microscope. The green fluorescent lines, indicative of tetracycline labelling, were photographed with sensitive photographic film (640T Chrome, Imation Spa, Segrate, Italy).

The histomorphometry was carried out with a light microscope (Laborlux S, Leitz) connected to a high-resolution video camera (3CCD, JVC KY-F55B, Media Cybernetics Inc, Immagini & Computer SnC, Milano, Italy) and interfaced to a monitor and personal computer (Intel Pentium III 1200 MMX, Media Cybernetics Inc, Immagini & Computer SnC, Milano, Italy). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Media Cybernetics Inc, Immagini & Computer SnC, Milano, Italy) and a histome-



FIGURES 1–4. FIGURE 1. Periapical X ray after implant placement. FIGURE 2. Periapical X ray 6 months after immediate loading of the implants. FIGURE 3. Clinical aspect during implant removal. FIGURE 4. Implant removed by trephine.

try software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc, Immagini & Computer Snc, Milano, Italy). The images were analyzed for the percentage of woven bone and lamellar bone, the percentage of bone with a tetracycline label, and the distance between the 2 tetracycline-labeled fluorescent lines. The birefringent organization of bone collagen fibers was determined under polarized light microscopy, in conjunction with the microphotographic image, and was used to distinguish lamellar from woven bone. Only the portion of the implant inserted in bone, with the exclusion of the epithelial and

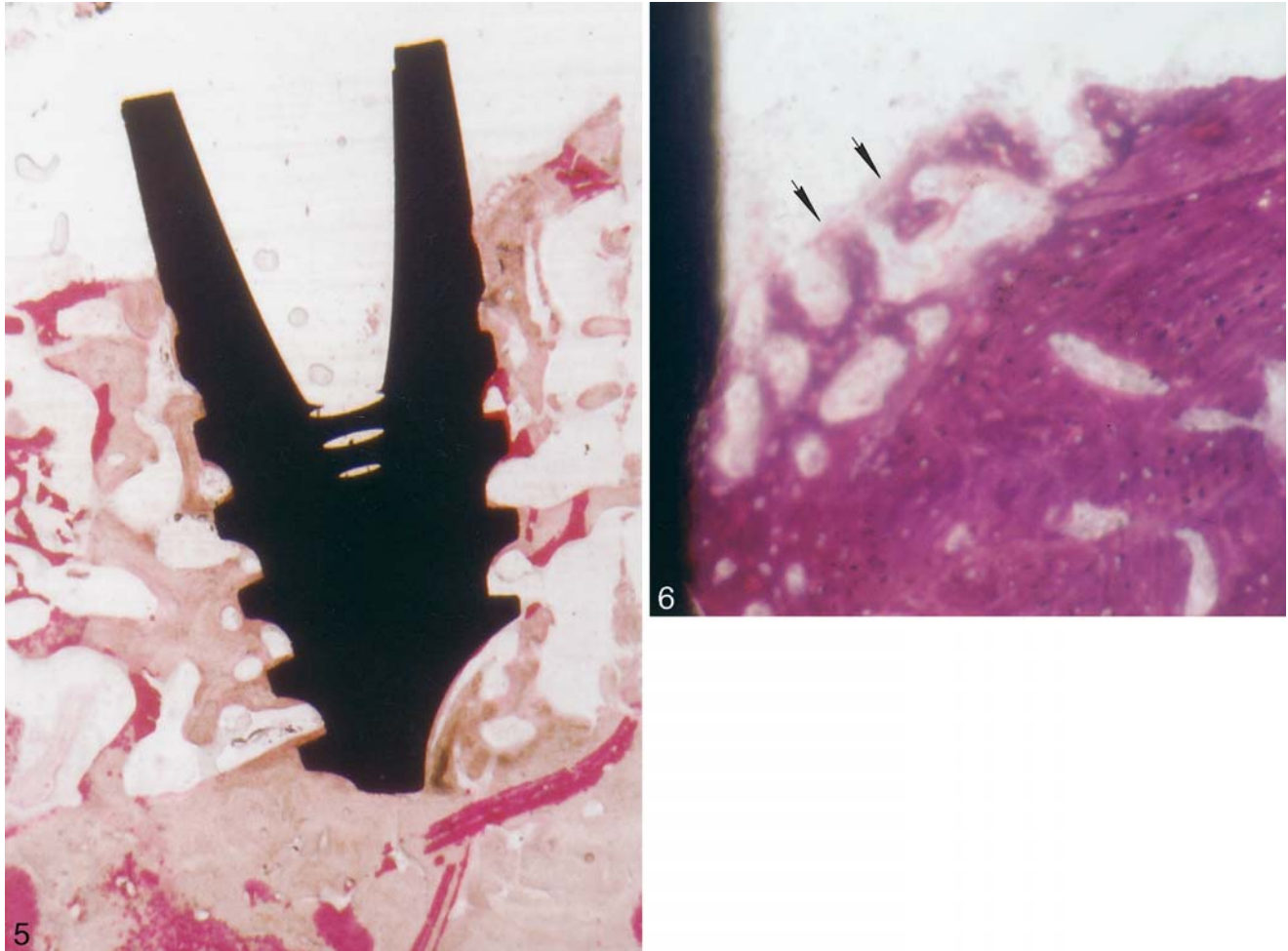
connective supracrestal tissues, was used in the histomorphometrical evaluation.

### RESULTS

Mature mineralized bone was in close contact with the implant surface (Figure 5). Actively secreting osteoblasts were observed in the marrow spaces in only a few portions of the interface. The cortical bone peri-implant trabeculae were thick. This cortical bone tissue was recognized as mature lamellar bone under polarized-light microscopy. At low magnification, it was possible to observe a small quantity of newly formed bone trabeculae at the most coro-

nal portion on 1 side of the implant (Figure 6). At higher magnification, it was possible to see that these trabeculae were surrounded by osteoblasts actively secreting osteoid matrix. No osteoclast activity was present in the coronal portion, and no inflammatory cells were observed near the implant surface. The bone adjacent to the implant surface was mainly lamellar ( $90\% \pm 4.5\%$ ) and the remaining  $9\% \pm 0.21\%$  was woven bone.

The fluorescence was observed in the newly formed bone layer. In many areas, the trabecular bone labeled with the tetracycline was in direct contact with the implant surface (Figure 7).



FIGURES 5–6. FIGURE 5. The implant is surrounded by lamellar bone. This bone is in close contact with the implant surface (acid fuchsin and toluidine blue, original magnification  $\times 12$ ). FIGURE 6. Small newly formed bone trabeculae present in the most coronal portion of the implant (arrows) (acid fuchsin and toluidine blue, original magnification  $\times 40$ ).

The extensive labelling by tetracycline indicated a large quantity of newly formed bone (Figure 8). Tetracycline labeled  $15.2\% \pm 0.8\%$  trabecular bone. The distance between the 2 lines marked by tetracycline was  $85 \pm 5 \mu\text{m}$  (Figure 9). The bone-implant contact percentage was  $74\% \pm 6\%$ .

#### DISCUSSION

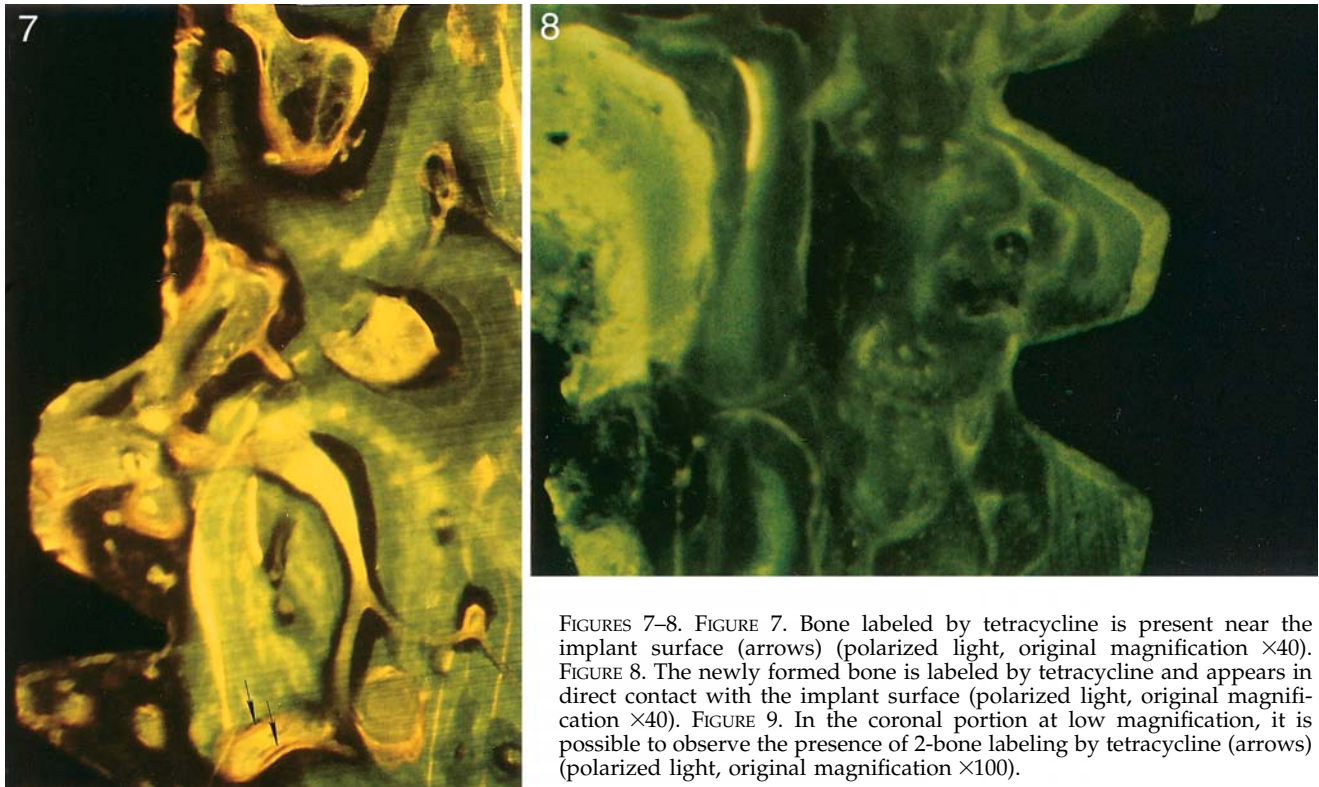
Only a few immediately loaded implants have been retrieved from humans and examined histologically.<sup>17,21–24,28,35,47</sup> Surface characteristics of each implant

type are important in determining the pattern of healing under loading, and histologic evidence of osseointegration is needed for each implant type with a different surface.<sup>28</sup> This documentation can be obtained only by histologic analysis of loaded implants retrieved from human.<sup>23</sup> The special thread design of the Ankylos implant system is characteristic for the progressive different shape of the threads, provided from the coronal to the apical aspects.<sup>33,34</sup> These implants are made from grade II c.p. titanium and have a high-polished collar of 2 mm and a sandblasted surface.<sup>33</sup> More-

over, the Ankylos implant system has an internal seal between the implant and the abutment and does not have a microgap.<sup>33</sup>

Reports indicate that once immediately loaded implants have clinically osseointegrated, they appear to take on the long-term predictability of conventionally healed and loaded implants.<sup>25</sup> The surface characteristics of an implant are extremely important for the osseointegration, especially in the very demanding situations of immediate loading. Several surface treatments (eg, particle blasting, plasma spray coatings, acid etching) have been





FIGURES 7–8. FIGURE 7. Bone labeled by tetracycline is present near the implant surface (arrows) (polarized light, original magnification  $\times 40$ ). FIGURE 8. The newly formed bone is labeled by tetracycline and appears in direct contact with the implant surface (polarized light, original magnification  $\times 40$ ). FIGURE 9. In the coronal portion at low magnification, it is possible to observe the presence of 2-bone labeling by tetracycline (arrows) (polarized light, original magnification  $\times 100$ ).

proposed to improve the implant-surface characteristics and to increase the quantity and quality of bone at the interface with increased interlocking. The surface of the Ankylos implant system is sandblasted with 120- $\mu\text{m}$  aluminum-oxide particles, which may positively influence bone integration under loading conditions because of its high porosity.<sup>33</sup> The histologic data from the present study confirm that immediate loading did not impede osseointegration and that the bone-healing sequence was not disturbed by the stresses transmitted at the interface under these mechanical conditions, even if the implant had been inserted in soft bone (D3). Rocci et al<sup>35</sup> reported very high bone-implant contact (84.2%), with apparent undisturbed healing in implants that had been inserted in bone-quality sites 3 or 4 and that had been biomechanically challenged.<sup>15</sup> The results of this study,

moreover, confirmed those reported by Testori et al.<sup>51</sup> The load-bearing capacity of the implant interface was maintained over replacement of the necrotic bone, and the loading of the implants did not interfere with osseointegration. Using the same implants in an experimental study in monkeys, Romanos et al<sup>33</sup> found no statistically significant differences in the bone-implant contact percentages in the immediate-loading and delayed-loading groups. They concluded that immediately loaded splinted implants inserted in the posterior mandible can osseointegrate with a peri-implant response similar to that of delayed-loaded implants.<sup>33</sup> New bone formation and active remodelling may be observed when the bone is mechanically stimulated.<sup>33</sup> Also, Rocci et al<sup>35</sup> showed that signs of active remodelling were more marked near the implant surface: this remodelling was seen as

formation of secondary osteons or resorption and bone formation at the surface of bone trabeculae.

In this study, the bone labeled by tetracycline was in direct contact with the implant surface in many areas. The splinting of the implant probably decreased the amount of micromotion during the early healing phase, giving the implant a higher tolerance to deleterious micromotion. Rigid splinting and minimal lateral forces may be critical factors for success.<sup>25</sup> Loading within physiologic limits could have stimulated bone formation as a result of the bone adaptation to loading.<sup>35</sup> Also, it has been shown that early daily periods of cyclic micromotion increase the rate of healing.<sup>53</sup>

The presence of newly formed bone in the coronal peri-implant area is striking and could be related to the absence of a micro-gap because of the conical connection. In this way, there is probably no bacterial colonization and leak-

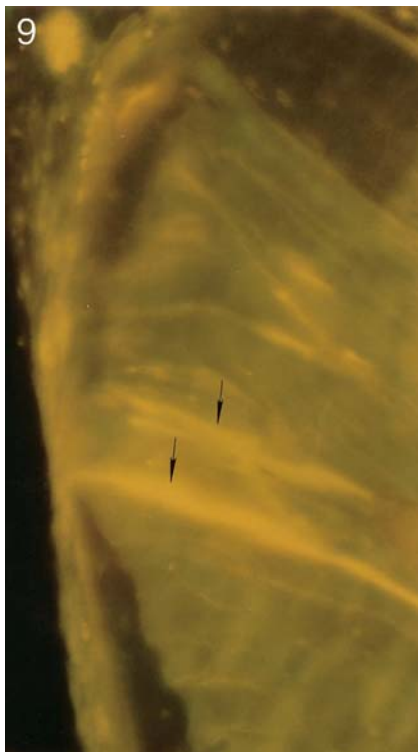


FIGURE 9. In the coronal portion at low magnification, it is possible to observe the presence of 2-bone labeling by tetracycline (arrows) (polarized light, original magnification  $\times 100$ ).

age at the implant-abutment interface; this bacterial leakage has been correlated to peri-implant bone crestal loss.<sup>54-64</sup>

Remodelling is a process of resorption and formation that replaces the preexisting bone and permits the bone adaptation.<sup>65</sup> The remodelling rate or bone turnover is the period of time needed for new bone to substitute the preexisting bone.<sup>65</sup> The bone remodelling rate is also expressed as a percentage or volume of new bone within a specific time period and may be directly related to the strength of the implant interface and the degree of risk for the bone-implant interface.<sup>65</sup> Lamellar bone forms at a rate of 1 to 5 mm each day, whereas woven bone can form at rates of more than 60 mm each day.<sup>65</sup> A higher risk is related to a higher turnover

rate because the bone that is formed at the interface is less mineralized, less organized, and weaker.<sup>65</sup> In the specimen in this study, the distance between the 2 fluorescent lines was 85 mm. This total divided by 30 days produces a rate of new bone formation of 2.83 mm per day, which is well within the above-mentioned limits of lamellar bone formation. The presence of lamellar bone at the interface is, in theory, advantageous for long-term implant survival because it is more mature and resistant to resorption.<sup>65</sup>

### CONCLUSION

The results of this study show that a high percentage of bone contact can be obtained even in immediately loaded implants inserted in low bone quality. Immediate loading does not interfere with bone formation and does not impede osseointegration.

### ACKNOWLEDGMENTS

This work was partially supported by the National Research Council (C.N.R.), Rome, Italy; the Ministry of Education, University, and Research (M.I.U.R.), Rome, Italy; and the Research Association for Dentistry and Dermatology (A.R.O.D.), Chieti, Italy. The help of Friadent (Mannheim, Germany) is gratefully acknowledged.

### REFERENCES

1. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15 year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg.* 1981;10:387-416.
2. Brånemark PI, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand Reconstr Surg.* 1977;11(suppl 16):1-132.
3. Brunski JB. Forces on dental implants and interfacial stress transfer. In: Laney WR, Tolman DE, eds. *Tissue Integration in Oral, Orthopaedic and Maxillofacial Reconstruction.* Chicago, Ill: Quintessence; 1992:108-124.
4. Brunski JB. Influence of biomechanical factor at the bone-biomaterial interface. In: Davies JE, ed. *The Bone-Biomaterial Interface.* Toronto, Ontario, Canada: Toronto University Press; 1991:391-405.
5. Carter DR, Giori NJ. Effect of mechanical stress on tissue differentiation in the bony implant bed. In: Davies JE, ed. *The Bone-Biomaterial Interface.* Toronto, Ontario, Canada: University of Toronto Press; 1991:367-379.
6. Chiapasco M, Gatti C, Rossi E, Haefliger W, Markwalder TH. Implant-retained mandibular overdentures with immediate loading. A retrospective multicenter study on 226 consecutive cases. *Clin Oral Implant Res.* 1997;8:48-57.
7. Tarnow, DP, Emtiaz S, Classi A. Immediate loading of threaded implants at stage 1 surgery in edentulous arches: ten consecutive case reports with 1- to 5-year data. *Int J Oral Maxillofac Implants.* 1997;12:319-324.
8. Schnitman PA, Wöhrle PS, Rubenstein JE, DaSilva JD, Wang NH. Ten-year results for Branemark implants immediately loaded with fixed prostheses at implant placement. *Int J Oral Maxillofac Implants.* 1997;12:495-503.
9. Salama H, Rose LF, Salama M, Betts NJ. Immediate loading of bilaterally splinted titanium root-form implants in fixed prosthodontics. A technique reexamined: two case reports. *Int J Periodont Restor Dent.* 1995;15:345-361.
10. Randow K, Ericsson I, Nilner K, Petersson A, Glantz PO. Immediate functional loading of Brånemark dental implants. An 18-month clinical follow-up study after 24 months. *Clin Oral Implant Res.* 1999;10:8-15.
11. Szmukler-Moncler S, Salama H, Reingewirtz Y, Dubruille JH. Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature. *J Biomed Mater Res.* 1998;43:192-203.
12. Szmukler-Moncler S, Piattelli A, Favero GA, Dubruille JH. Considerations preliminary to the application of early and immediate loading protocols in dental implantology. *Clin Oral Implant Res.* 2000;11:12-25.
13. Chaushu G, Chaushu S, Tzohar A, Dayan D. Immediate loading of single-tooth implants: immediate versus non-immediate implantation. A clinical

- report. *Int J Oral Maxillofac Implants*. 2001;16:267-272.
14. Lazzara RJ, Porter SS, Testori T, Galante J, Zetterquist L. A prospective multicenter study evaluating loading of Osseotite implants two months after placement: one-year results. *J Esthet Dent*. 1998;6:280-289.
15. Misch CE. Non-functional immediate teeth in partially edentulous patients: a pilot study of 10 consecutive cases using the maestro dental implant system. *Compendium*. 1998;19:25-36.
16. Linkow LI, Donath K, Lemons JE. Retrieval analyses of a blade implant after 231 months of clinical function. *Implant Dent*. 1992;1:37-43.
17. Trisi P, Emanuelli M, Quaranta M, Piattelli A. A light microscopy, scanning electron microscopy and laser scanning microscopy analysis of retrieved blade implants after 7 to 20 years of clinical function. *J Periodontol*. 1993;64:374-378.
18. Piattelli A, Ruggeri A, Trisi P, Romasco N, Franchi M. A histologic and histomorphometric study of the bone reactions to non submerged unloaded and loaded single implants in monkeys. *J Oral Implantol*. 1993;19:314-320.
19. Piattelli A, Corigliano M, Scarano A, Quaranta M. Bone reactions to early occlusal loading of two-stage titanium plasma-sprayed implants: a pilot study in monkeys. *Int J Periodont Restor Dent*. 1997;17:163-169.
20. Piattelli A, Corigliano M, Scarano A, Costigliola G, Paolantonio M. Immediate loading of titanium plasma-sprayed implants: a pilot study in monkeys. *J Periodontol*. 1998;69:321-327.
21. Piattelli A, Trisi P, Romasco N, Emanuelli M. Histological analysis of a screw implant retrieved from man: influence of early loading and primary stability. *J Oral Implantol*. 1993;19:303-306.
22. Piattelli A, Paolantonio M, Corigliano M, Scarano A. Immediate loading of titanium plasma-sprayed screw-shaped implants in man: a clinical and histological report of two cases. *J Periodontol*. 1997;68:591-597.
23. Ledermann PD, Schenk R, Buser D. Long-lasting osseointegration of immediately loaded bar-connected TPS screws after 12 years of function: a histologic case report of a 95-year-old patient. *Int J Periodont Restor Dent*. 1999;18:553-556.
24. Piattelli A, Scarano A, Paolantonio M. Immediately loaded screw implant removed for fracture after a 15-year loading period: histological and histochemical analysis. *J Oral Implantol*. 1997;23:75-79.
25. Ganeles J, Rosenberg MM, Holt RL, Reichman LH. Immediate loading of implants with fixed restorations in the completely edentulous mandible: report of 27 patients from a private practice. *Int J Oral Maxillofac Implants*. 2001;16:418-426.
26. Romanos G, Toh CG, Siar CH, et al. Peri-implant bone reactions to immediately loaded implants. An experimental study in monkeys. *J Periodontol*. 2001;72:506-511.
27. Jaffin RA, Kumar A, Berman CL. Immediate loading of implants in partially and fully edentulous jaws: a series of 27 case reports. *J Periodontol*. 2000;71:833-838.
28. Testori T, Szmukler-Moncler S, Francetti L, et al. Immediate loading of Osseotite implants: a case report and histologic analysis after 4 months of occlusal loading. *Int J Periodont Restor Dent*. 2001;21:451-459.
29. Gatti C, Hefliger W, Chiapasco M. Implant-retained mandibular overdentures with immediate loading: a prospective study of ITI implants. *Int J Oral Maxillofac Implants*. 2000;15:383-388.
30. Branemark PI, Engstrand P, Ohrenell LO, et al. Branemark novum: a new treatment concept for rehabilitation of the edentulous mandible. Preliminary results from a prospective clinical follow-up study. *Clin Implant Dent Relat Res*. 1999;1:2-16.
31. Ericsson I, Randow K, Nilner K, Peterson A. Early functional loading of Branemark dental implants: 5-year clinical follow-up study. *Clin Implant Dent Relat Res*. 2000;2:70-77.
32. Vernino AR, Kohles SS, Holt RA, Lee HM, Caudill RF, Kinealy JN. Dual-etched implants loaded after 1- and 2-month healing periods: a histologic comparison in baboons. *Int J Periodont Restor Dent*. 2002;22:399-407.
33. Romanos GE, Toh CG, Siar CH, Swaminathan D, Ong AH. Histological and histomorphometric evaluation of peri-implant bone subjected to immediate loading: an experimental study with macaca fascicularis. *Int J Oral Maxillofac Implants*. 2002;17:44-51.
34. Siar CH, Toh CG, Romanos G, et al. Peri-implant soft tissue integration of immediately loaded implants in the posterior macaque mandible: a histomorphometric study. *J Periodontol*. 2003;74:571-578.
35. Rocci A, Martignoni M, Burgos PM, Gottlow J, Sennerby L. Histology of retrieved immediately and early loaded oxidized implants: light microscopic observations after 5 to 9 months of loading in the posterior mandible. *Clin Implant Dent Relat Res*. 2003;5(suppl):88-98.
36. De Bruyn M, Kisch J, Collaert B, Linden U, Nilner K, Dvarsater L. Fixed mandibular restorations on three early-loaded regular platform Branemark implants. *Clin Implant Dent Relat Res*. 2001;3:176-184.
37. Cooper LF, Rahman A, Moriarty J, Chaffee M, Sacco D. Immediate mandibular rehabilitation with endosseous implants: simultaneous extraction, implant placement, and loading. *Int J Oral Maxillofac Implants*. 2002;17:517-525.
38. De Bruyn H, Collaert B. Early loading of machined-surface Branemark implants in completely edentulous mandibles: healed bone versus fresh extraction sites. *Clin Implant Dent Relat Res*. 2002;4:136-142.
39. Collaert B, De Bruyn H. Early loading of four or five Astra Tech fixtures with a fixed cross-arch restoration in the mandible. *Clin Implant Dent Relat Res*. 2002;4:133-135.
40. Gatti C, Chiapasco M. Immediate loading of Branemark implants: a 24-month follow-up of a comparative prospective pilot study between mandibular overdentures supported by conical transmucosal and standard MKII implants. *Clin Implant Dent Relat Res*. 2002;4:190-199.
41. Lorenzoni M, Pertl C, Zhang K, Wegscheider WA. In patient comparison of immediately loaded and non-loaded implants within 6 months. *Clin Oral Implant Res*. 2003;14:273-279.
42. Wolfinger GJ, Balshi TJ, Rangert B. Immediate functional loading of Branemark system implants in edentulous mandibles: clinical report of the results of developmental and simplified protocols. *Int J Oral Maxillofac Implants*. 2003;18:250-257.
43. Malò P, Rangert B, Nøber M. "All-on-four" immediate-function concept with Branemark system implants for completely edentulous mandibles: a retrospective clinical study. *Clin Implant Dent Relat Res*. 2003;5(suppl):2-9.
44. Vanden Bogaerde L, Pedretti G, Dellacasa P, Mozzati M, Rangert B. Early function of splinted implants in maxillas and posterior mandibles using Branemark system machined-surface implants: an 18-month prospective clinical multicenter study. *Clin Implant Dent Relat Res*. 2003;5(suppl):21-28.
45. Rocci A, Martignoni M, Gottlow J. Immediate loading in the maxilla using flapless surgery, implants placed in predetermined positions, and prefabricated provisional restorations: a retrospective

3-year clinical study. *Clin Implant Dent Relat Res.* 2003;5(suppl):29–36.

46. Malò P, Friberg B, Polizzi G, Gualini F, Vighagen T, Rangert B. Immediate and early function of Branemark system implants placed in the esthetic zone: a 1-year prospective clinical multicenter study. *Clin Implant Dent Relat Res.* 2003;5(suppl):37–46.

47. Degidi M, Petrone G, Iezzi G, Piattelli A. Histologic evaluation of a human immediately loaded titanium implant with a porous anodized surface. *Clin Implant Dent Relat Res.* 2002;4:110–114.

48. Degidi M, Piattelli A. Immediate functional and non functional loading of dental implants: a 2 to 60 months follow-up study of 646 titanium implants. *J Periodontol.* 2003;74:225–241.

49. Romeo E, Chiapasco M, Lazza A, et al. Implant-retained mandibular overdentures with ITI implants. A comparison of 2-year results between delayed and immediate loading. *Clin Oral Implant Res.* 2002;13:495–501.

50. Becker W, Sennerby L, Becker BE, Henry P. Clinical and histologic findings for microimplants placed in one stage and loaded for three months: a case report. *Clin Implant Dent Relat Res.* 2002;4:47–52.

51. Testori T, Szmukler-Moncler S, Francetti L, Del Fabbro M, Trisi P, Weinstein RL. Healing of Osseotite implants under submerged and immediate loading conditions in a single patient: a case report and interface analysis after 2 months. *Int J Periodont Restor Dent.* 2002;22:345–353.

52. Piattelli A, Scarano A, Quaranta M. High-precision, cost-effective system for producing thin sections of oral tissues containing dental implants. *Biomaterials.* 1997;18:577–579.

53. Lacroix D, Prendergast PJ. A mechanoregulation model for tissue differentiation during fracture healing: analysis of gap healing and loading. *J Biomech.* 2002;35:1163–1171.

54. Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Biologic width around titanium implants. A histometric analysis of the implant-to-gingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. *J Periodontol.* 1997;68:186–198.

55. Hermann JS, Buser D, Schenk RK, Higginbottom FL, Cochran DL. Biological width around titanium implants. A physiologically formed and stable dimension over time. *Clin Oral Implant Res.* 2000;11:1–11.

56. Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants: a radiographic evaluation of unloaded non-submerged and submerged implants in the canine mandible. *J Periodontol.* 1997;68:1117–1130.

57. Persson LG, Lekholm U, Leonhardt A, Dahlen G, Lindhe J. Bacterial colonization on internal surfaces of Branemark system implant components. *Clin Oral Implant Res.* 1996;7:90–95.

58. Quirynen M, Bollen CM, Eyssen H, van Steenberghe D. Microbial penetration along the implant components of the Branemark system. An in vitro study. *Clin Oral Implant Res.* 1994;5:239–244.

59. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implants.* 1997;12:527–540.

60. Piattelli A, Scarano A, Paolantonio M, et al. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. *J Periodontol.* 2001;72:1146–1150.

61. Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. *Clin Oral Implant Res.* 1993;4:158–161.

62. Hermann JS, Schofield JD, Schenk RK, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged implants in the canine mandible. *J Periodontol.* 2001;72:1372–1383.

63. Hermann JS, Buser D, Schenk RK, Cochran DL. Crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged and submerged implants in the canine mandible. *J Periodontol.* 2000;71:1412–1424.

64. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibaldi S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. *J Periodontol.* 2003;74:346–352.

65. Misch CE, Bidez MW, Sharawy M. A bioengineered implant for a predetermined bone cellular response to loading forces. A literature review and case reports. *J Periodontol.* 2001;72:1276–1281.