Bone Healing at Functionally Loaded and UnloadedScrew-Shaped Implants Supporting Single Crowns: A Histomorphometric Study in Humans

Daichi Yonezawa, DDS[1]/Adriano Piattelli, MD, DDS[2]/Riccardo Favero, DDS[3]/ Mauro Ferri, DDS[4]/Giovanna Iezzi, PhD, DDS[5]/Daniele Botticelli, MD, PhD[6]

of Applied Prosthodontics, Graduate School of Biomedical Sciences, Nagasaki University;Research Affiliate,
ical, Oral and BiotechnologicalSciences, University of Chieti-Pescara, Chieti, Italy.
emy, Rimini, Italy.
, Departamento de Ciencia dela Salud, Corporación Universitaria Rafael Núñez, Cartagenade Indias, Bolívar,
of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy.
ademy, Rimini, Italy.

Correspondence to: Dr Giovanna Iezzi, Department of MedicalOral and Biotechnological Sciences, University of Chieti- Pescara, Via dei Vestini 31, 66100 Chieti, Italy.
Fax: +39-0871-3554333. Email: gio.iezzi@unich.it

Abstract

Purpose: *To evaluate histologically and histomorphometrically the effect of a delayed load on healing at implants with a moderately rough surface.*

Materials and Methods: *Two solid titanium screw-shaped devices, 5 mm long and 3.5 mm in diameter, were inserted in the distal segments of the alveolar ridgeof 16 volunteer patients in a nonsubmerged fashion. After 2 months, one implant was loaded, while the other was left unloaded. After 2 months, the two implants were collected from 10 patients using a sonic instrument, and ground sections were prepared from the biopsy specimens. Histomorphometric analyseswere performed.*

Results: *After 4 months of healing, biopsy specimens from 10 patients were available for analyses (n = 10). The total bone-to-implant contact percentage was 86.8% ± 6.5% and 84.6% ± 3.7% forloaded and unloaded implants, respectively. New bone was represented by 85.5% ± 6.7% and 83.4% ± 3.9% at the loaded and unloaded sites, respectively. A very small amount of old parent bone was found. The density of the mineralized bone was 76.8% ± 8.3% for the loaded sites and 74.1% ± 10.5% for the unloaded sites. The percentages of new and old bone densities were 69.0% ± 8.3% and 7.8% ± 3.9% at the loadedsites, and 65.9% ± 10.3% and 8.2% ± 4.5% at the unloaded sites, respectively. No statistically significantdifferences were disclosed.* ***Conclusion:*** *Applying a delayed load to implants supporting single crowns did not yield statistically significant differences, and only a tendency of higher osseointegration and bonedensity was observed at loaded sites compared with the unloaded sites.* Int J Oral Maxillofac Implants 2018;33:181–187. doi: 10.11607/jomi.5928

Keywords: *delayed load, dental implants, histometry, human study, morphometry, prosthetic reconstruction*

Various experimental studies have shown that load, both applied immediately and in a delayed fash- ion, has increased bone-to-implant contact1–4 as well as bone density around implants.1,4,5 In an experiment in dogs, the sequential healing at immediately loaded implants placed in extractionsockets and in healed sites was studied.[6] Biopsy speci- mens were collected after 1 week, 2 weeks, 1 month,and 3 months after implant placement and immediateloading. New bone in close contact with the implant surface was found already after 1 week with percent-ages of 7% to 8% at both groups. The amount of new bone increased over time, reaching 68.4% at the healed sites and 61.8% at the extraction socket sitesafter 3 months of healing. Old bone was observed atpercentages of 30.0% and 20.6% at the healed and ex-traction socket sites, respectively. These percentages decreased over time, but parent bone was still foundin contact with the implant surface; however, it was atvery low percentages, 4.2% and 2.9% at the healed andextraction socket sites, respectively. A recent study in humans reported data on 17 im-plants loaded from 4 to 20 years.[7] The implants wereretrieved for different reasons, and a histomorphomet- ric analysis was performed. All implants were integrat- ed into the surrounding hard tissues, with percentagesof bone contact ranging from 32% to 85%. Despite the large amount of literature available onhistometric analyses on retrieved implants[7–13] and re-ports on comparison between loaded and unloadedimplants,[14,15] there is still a lack of confirmation in hu- mans of the outcomes seen in animal experiments.

Hence, the aim of the present study was to evalu-ate histomorphometrically the effect of delayed load on healing at implants with a moderately rough surface.

MATERIALS AND METHODS

This study followed the Declaration of Helsinki on medical protocol and ethics. The study protocol was submitted to and approved by the Ethical Committeeof the Corporación Universitaria Rafael Nuñez, Carta-gena de Indias (Colombia). The procedures and thepossible complications were carefully explained, andinformed consent was collected from each patient. The approval was provided after the presentation of literature related to the topic of the investigation aswell as that related to the safety of the procedures for harvesting of biopsy specimens. A sample size calcu-lation was performed based on animal results,[4,6] con-sidering the higher variability that may be found inhumans compared with animals. All surgeries, patientrecruitment, and follow-up were performed at the Cor-poración Universitaria Rafael Nuñez, Cartagena de In-dias (Colombia).

Patient Recruitment

Sixteen healthy volunteers presenting no contraindi-cations for oral surgery procedures and in need of im-plants for partial oral rehabilitation were planned to berecruited for the study. The volunteer subjects receiveddental treatment free of charge.

The patients had to fulfill the following inclusion criteria: *(1)* ≥ 18 years of age; *(2)* good general health; and *(3)* not pregnant. Moreover, the patients had to have an edentulous zone in the distal segments of thearches, devoid of previous bone augmentation proce-dures and sufficient bone volume for the placement ofat least two implants. The presence of the antagonist teeth in the opposite arch to allow functional load was required both at test and control sites.

Implants

Solid titanium screw-shaped implants were manufac-tured (Sweden & Martina). The implants were 5 mm long, including a polished neck of 0.8 mm, with a core diameter of 2.9 mm, with threads of 0.3 mm ofdepth, reaching a total diameter of 3.5 mm (Fig 1). Theimplants were produced with internal threads andgrooves to make the fixation of healing screws and abutments possible. All the implants were prepared with a ZirTi surface (Sweden & Martina). For more in-formation on the surface, see Caneva et al.[16]

Randomization

Each patient received two implants, one to be loadedafter 2 months from placement and one to be left un-loaded. A researcher (D.B.), neither involved in the se- lection of the patients nor in the surgical and prosthetictreatment, performed the randomization of the siteselectronically (randomization.com). Consequently, the surgeries were carried out by a clinician (M.F.) who wasunaware of which implants would have been loaded orleft unloaded. Sealed opaque envelopes were openedat the time of prosthesis delivery.

Clinical Procedures

After local anesthesia was provided, full-thickness flapswere raised and the recipient sites slightly marked with a lanceolate bur. Subsequently, osteotomies were prepared, reaching a depth of 5 mm first with a drillof 2.0 mm in diameter, followed by a drill of 2.8 mmin diameter. The cortical region was further widenedwith a drill of 3.0 mm in diameter. The implants were placed with the coronal margin flush to the bony crest. A healing abutment was placed on each device, and silk single sutures were provided to secure the flaps around the healing abutment; nonsubmerged healingwas allowed. The sutures were removed after 7 days,and the patients were included in a maintenance fol-low-up during the study. After 2 months, the healing abutment was removedat the sites to be loaded, and a custom-made abutment for prosthesis reconstruction was applied. Impressions were taken, and a cemented single crown made ofresin was secured to the implants (Fig 2). Only vertical contact was allowed at the loaded site. The occlusion was checked at the time of loading, after 1 month, andat the time of biopsies. The control site was left unload-ed, and the abutment was maintained in situ, protrud- ing in the oral cavity approximately 1 mm.

After 2 months, the implants were collected. Thecrowns were removed, and incisions of the mucosawere performed around the neck of the implants, try- ing to leave as small of a portion of soft tissue as pos-sible attached to the coronal region of the implants.The mucosa was then carefully detached from the im- plants and from the surrounding bone. A micro-sawof 0.15 to 0.25 mm of width (SFS 102, Komet Dental Gebr. Brasseler) was mounted on a sonic instrument (Sonosurgery, TeKne Dental) and used to cut the biop-sy specimens.[17] The bone incisions were performed insuch a way to maintain the bone at the buccal aspect. Vertical cuts were made parallel to the long axis of theimplants, as close as possible to it, at the mesial anddistal aspects. A further cut was performed buccally, ina buccolingual direction, reaching the apical extension of the implants. Lingually, a small vertical incision was made coronally, and the biopsy specimen was luxatedtoward the buccal side with a small elevator (Fig 3).

Histologic Preparation of the BiopsySpecimens

The biopsy specimens containing the implants were fixed in 10% buffered formalin immediately after the retrieval. The specimens were first dehydrated in alcohol and then included in a glycol-methacrylate resin (Technovit 7200 VLC; Kulzer). Subsequently, they were polymerized and sectioned following the long axis of the implants using a diamond disk to obtain slides of approximately 150 µm of width. The samples were then ground down to approximately30 µm of width and stained using acid fuchsin andtoluidine blue.

Histologic Examination

In a Nikon microscope (Eclipse Ci, Nikon Corporation), connected to a computer with a video camera (DS-Fi2, Nikon Corporation), the most coronal contact ofthe bone to the surface of the device (B) and the api-cal extension of the bone (A) were identified. Software(NIS-element D, Nikon Corporation) was used to evalu- ate at a magnification of ×200 the amount of newly formed bone, old parent bone, and soft tissues (mar-row, Haversian canals, basic multicellular units (BMUs) in contact with the implant surface included betweenB and A. The mean total mineralized bone was alsocalculated as the sum between new and old bone foreach implant. Moreover, a region of interest (ROI) was also defined as that enclosed between B and A, and from the coreof the implants to a distance of approximately 0.4 mm from it. Newly formed bone, old parent bone, and soft tissue densities were also evaluated within the ROI us- ing a point counting procedure[18] with a lattice withsquares of 50 microns superposed over the tissues at amagnification of ×200. Percentages of the various tis- sues were calculated, including the total mineralized bone tissues, composed of new and old bone.

Data Analysis

Mean values ± standard deviations (SD) were calcu-lated for new bone, old parent bone, and soft tissues(marrow, Haversian canals, BMUs). Moreover, 25th, 50th (median), and 75th percentiles were added in the tables.The Wilcoxon test for dependent variables was used foranalyses, setting the level of significance to $P = .05$. Ex-ploratively, a correlation between the total mineralizedbone and total bone density was also calculated.

RESULTS

Clinical and Histologic Outcomes

During the 4-month healing period, five patients pre-sented symptoms of a viral infection, diagnosed asChikungunya, that suddenly arose in the region. Thesepatients could not comply with the follow-up protocoland were excluded from the study. The implants were removed with a counter-torque when the patients recovered from the disease. Moreover, one biopsy specimen was damaged during collection, and the pa-tient was excluded. Biopsy specimens were safely collected from 10 pa- tients, so that n = 10 for loaded and unloaded sites wasreached for analyses.

At both sites, new bone was found in contact withthe implant surface, reaching mean percentages of 85.5% ± 6.7% and 83.4% ± 3.9% at the loaded and unloaded sites, respectively (Table 1). Small percent-ages (1.1% to 1.2%) were still found in contact withthe implant surfaces at both loaded and unloadedsites. The total mineralized bone was 86.8% ± 6.5% and 84.6% ± 3.7% for loaded and unloaded implants with no statistically significant differences.

The area around the implants was mainly occupiedby newly formed lamellar bone (Figs 4 and 5). Old bonewas still visible in various areas, presenting new bonein close contact to it. At both sites, the new bone pre- sented various cement lines lining areas with different stages of bone maturation. Secondary osteons were noted, as well as areas of resorption, denoting active remodeling processes.

Mineral bone density within the ROI was repre- sented by newly formed bone at a percentage of 69.0% ± 8.3% and 65.9% ± 10.3% at the loaded andunloaded sites, respectively, while old bone was 7.8% ± 3.9% and 8.2% ± 4.5%, respectively (Table 2). The total bone density was 76.8% ± 8.3% for the load-ed sites and 74.1% ± 10.5% for the unloaded sites,without statistically significant differences.

The correlation between the total mineralized bone and total mineralized bone density was $r = 0.3$ and$r = –0.05$ for loaded and unloaded sites, respectively.

DISCUSSION

The aim of the present study was to describe the effectof load on osseointegration. High osseointegrationand bone density were observed both at the loaded and unloaded sites. Although the differences were not significant, a tendency of higher osseointegration andbone density was detected at the loaded compared with the unloaded sites.

Similar histologic assessments were also performedin another recent human study,[19] where 13 subjects were enrolled and received two implants each, one immediately loaded and the other left unloaded. Af-ter 1 and 3 months, in six patients, the osteotomies were prepared with drills, while in the remaining seven patients, osteotomies were used to finalize the preparation. No statistically significant differences were observed between loaded and unloaded sites. Inanother human study,[14] four implants were placed infour patients: two implants were immediately loaded, while two were left submerged. One implant in eachgroup was retrieved with a trephine after 4 weeks and the other after 8 weeks. Bone-to-implant contact was found at higher percentages at the loaded comparedwith the unloaded sites. Indeed, after 4 weeks of heal- ing, bone-to-implant contact was 65.6% at the loadedand 54.7% at the unloaded sites. After 8 weeks, the bone-to-implant contact percentage was 76.2% and 62.3% at the loaded and unloaded sites, respectively. Several other studies on the effect of load on heal-ing have also been performed in animals.[1–6] In an ex-periment in dogs, a lateral static load was applied tothe implants for 3 months, while the controls were left unloaded.[1] Greater bone-to-implant contact as well ashigher bone density were found at the loaded implantscompared with controls. In another experiment in dogs,two implants were placed in each side of the mandible.[4]The recipient sites were prepared to obtain a differenttorque at the two implants, one with approximately 30Ncm and the other with > 70 Ncm, on both sides of themandible. However, only the implants at one side of themandible were immediately loaded, while those at theopposite side were left unloaded. Both bone-to-implantcontact and bone density were found to be higher atthe loaded compared with the unloaded sites. More-over, these parameters were higher at the 30 Ncm sitescompared with the > 70 Ncm sites. In another dog study,implants were placed immediately after tooth extrac-tion and immediately loaded, while the controls wereleft unloaded.[3] Higher bone-to-implant contact was re- ported at the loaded compared with the unloaded sitesafter both 30 and 90 days of healing.

In the present study, only a tendency to higher bone-to-implant contact and bone density was foundat the loaded compared with the unloaded sites. It has to be considered that five patients with devices already placed presented symptoms from an unex-pected endemic infection that made it impossible forthem to comply with the timing required in the proto-col of the study. The implants had to be retrieved with a counter-torque procedure as soon as the patientsrecovered from the disease. These patients had to beexcluded from the study. Moreover, one biopsy speci- men was severely damaged during collection, so six patients were lost in total. These events have obviouslyreduced considerably the power of the present study.

The biopsy specimens in the present study were col-lected by means of a sonic instrument on which a thinsaw was mounted. The instrument was used in close vicinity to the implants to save most of the hard tissue at the donor sites so that only the buccal aspect wasincluded in the biopsy specimens. This method was se-lected based on the reports of an in vitro study.[17] In thatstudy, 4-mm-long and 2.4-mm-diameter screw-shape implants were placed in bovine fresh ribs. Three dif-ferent methods for biopsy specimen collection were adopted, two using trephines and one using a similarsaw used in the present experiment and mounted ona sonic instrument. The trephines were used concen-trically or eccentrically, respectively. It was shown thatthe sonic instrument needed more time to collect bi-opsy specimens. However, it yielded a higher qualityof the specimens retrieved, including the interface be- tween the implant surface and the bone, and a lowervolume of the defects at the donor sites compared withthe trephines.

In the present study, implants were mainly surround-ed by newly formed lamellar bone. However, old bonewas still visible in various areas, and still in contact withthe implant surface in small percentages. It has to beconsidered that, after placement, the implant surface isincontactonlywitholdparentbonethatguaranteestheprimary stability. New bone formation occurred rapidly in the areas not in contact with the mineralized bone,such as in marrow regions or within spaces betweenthe recipient sites and the implants.[20,21] In the zonesin contact with the parent bone, instead, the old bonehas to be first resorbed, leaving space to the new bone,before the occurrence of new bone apposition; the dy-namics of this process are influenced by several factors.In a review[22] of sequential studies on the early phasesof osseointegration, it was shown that the fastest rate ofosseointegration was in rabbits followed by dogs, andthe slowest rate was seen in humans. Moreover, it was shown thatosseointegration was fasterinthe spongiosacompared with the cortical bone and faster at moder-ately rough surfaces compared with turned surfaces.

The high percentage of bone-to-implant contact found in the present study (> 83%) may be related notonly to the quality of the surface used, but may havealso been affected by the method used to select thearea to be analyzed and by the quality of the region in which the implants were placed, mainly composedof cortical bone. Indeed, a moderately rough surfacewas used, and very low or no correlation was foundbetween the bone in contact with the implant surface and the adjacent bone density. This is in agreement with the results reported in experiments in dogs inwhich the healing at moderately rough surfaces was compared with that at turned surfaces. Higher bone-to-implant contact but lower bone density were foundat the rough compared with the turned surfaces.[20,23]This, in turn, means that low correlation may be ex-pected at the moderately rough surfaces.

CONCLUSIONS

Applying a delayed load to implants supporting singlecrowns did not yield statistically significant differences but only a tendency of higher osseointegration andbone density at loaded compared with unloaded sites.

ACKNOWLEDGMENTS

This study has been supported by grants from Sweden & Mar- tina, Due Carrare, Padua, Italy; by ARDEC Academy, AriminumOdontologica Srl, Rimini, Italy; and by the Ministry of Educa- tion, University and Research (M.I.U.R.), Rome, Italy. Tecnoss Srl, Giaveno, Turin, Italy provided free of charge all biomaterials needed for bone regeneration at the donor sites. All the authors declare no conflict of interest with the material presented.

REFERENCES

1. Gotfredsen K, Berglundh T, Lindhe J. Bone reactions adjacent to titanium implants subjected to static load. A study in the dog (I). Clin Oral Implants Res 2001;12:1–8.

2. Berglundh T, Abrahamsson I, Lindhe J. Bone reactions to long- standing functional load at implants: An experimental study in dogs. J Clin Periodontol 2005;32:925—932.
3. Calvo-Guirado JL, Aguilar-Salvatierra A, Gomez-Moreno G, Guardia J, Delgado-Ruiz RA, Mate-Sanchez de Val JE. Histological, radio- logical and histomorphometric evaluation of immediate vs. non- immediate loading of a zirconia implant with surface treatment in a dog model. Clin Oral Implants Res 2014;25:826—830.
4. Rea M, Botticelli D, Ricci S, Soldini C, González GG, Lang NP. Influ- ence of immediate loading on healing of implants installed with different insertion torques—An experimental study in dogs. ClinOral Implants Res 2015;26:90—95.
5. Romanos GE, Toh CG, Siar CH, Wicht H, Yacoob H, Nentwig GH. Bone-implant interface around titanium implants under different loading conditions: A histomorphometrical analysis in the Macacafascicularis monkey. J Periodontol 2003;74:1483—1490.
6. Mainetti T, Lang NP, Bengazi F, Sbricoli L, Soto Cantero L, Botticelli D. Immediate loading of implants installed in a healed alveolar bony ridge or immediately after tooth extraction: An experimental study in dogs. Clin Oral Implants Res 2015;26:435—441.
7. Iezzi G, Piattelli A, Mangano C, et al. Periimplant bone response in human-retrieved, clinically stable, successful, and functioning dental implants after a long-term loading period: A report of 17 cases from 4 to 20 years. Implant Dent 2016;25:380—386.
8. Iezzi G, Fiera E, Scarano A, Pecora G, Piattelli A. Histologic evalu- ation of a provisional implant retrieved from man 7 months after placement in a sinus augmented with calcium sulphate: A case report. J Oral Implantol 2007;33:89—95.
9. Iezzi G, Degidi M, Piattelli A, Shibli JA, Perrotti V. A histological and histomorphometrical evaluation of retrieved human implantswith a wettable, highly hydrophilic, hierarchically microstructured surface: A retrospective analysis of 14 implants. Implant Dent 2013;22:138—142.
10. Iezzi G, Degidi M, Shibli JA, Vantaggiato G, Piattelli A, Perrotti V. Bone response to dental implants after a 3- to 10-year loading period: A histologic and histomorphometric report of four cases. Int J Periodontics Restorative Dent 2013;33:755—761
11. Mangano C, Perrotti V, Raspanti M, et al. Human dental implants with a sandblasted, acid-etched surface retrieved after 5 and 10 years: A light and scanning electron microscopy evaluation of twocases. Int J Oral Maxillofac Implants 2013;28:917—920.
12. Iezzi G, Piattelli A, Mangano C, et al. Peri-implant bone tissues around retrieved human implants after time periods longer than 5 years: A retrospective histologic and histomorphometric evalua- tion of 8 cases. Odontology 2014;102:116—121.
13. Mangano C, Piattelli A, Mortellaro C, Mangano F, Perrotti V, Iezzi G. Evaluation of peri-implant bone response in implants retrieved for fracture after more than 20 years of loading: A case series. J Oral Implantol 2015;41:414—418.
14. Degidi M, Piattelli A, Shibli JA, Perrotti V, Iezzi G. Bone formation around immediately loaded and submerged dental implants witha modified sandblasted and acid-etched surface after 4 and 8 weeks: A human histologic and histomorphometric analysis. Int J Oral Maxillofac Implants 2009;24:896—901.
15. Degidi M, Piattelli A, Shibli JA, Perrotti V, Iezzi G. Early bone forma- tion around immediately restored implants with and without occlusal contact: A human histologic and histomorphometric eval- uation. Case report. Int J Oral Maxillofac Implants 2009;24:734—739.
16. Caneva M, Lang NP, Calvo Guirado JL, Spriano S, Iezzi G, Botticelli D. Bone healing at bicortically installed implants with different surface configurations. An experimental study in rabbits. Clin Oral Implants Res 2015;26:293—299.
17. Ferri M, Lang NP, Angarita Alfonso EE, Bedoya Quintero ID, Burgos EM, Botticelli D. Use of sonic instruments for implant biopsy retrieval. Clin Oral Implants Res 2015;26:1237—1243.
18. Schroeder HE, Münzel-Pedrazzoli S. Correlated morphometric and biochemical analysis of gingival tissue. Morphometric model,tissue sampling and test of stereologic procedures. J Microsc 1973;99:301—329.
19. Donati M, Botticelli D, La Scala V, Tomasi C, Berglundh T. Effect of immediate functional loading on osseointegration of implants used for single tooth replacement. A human histological study. Clin Oral Implants Res 2013;24:738—745.
20. Abrahamsson I, Berglundh T, Linder E, Lang NP, Lindhe J. Early bone formation adjacent to rough and turned endosseous implantsurfaces. An experimental study in the dog. Clin Oral Implants Res 2004;15:381—392.
21. Rossi F, Lang NP, De Santis E, Morelli F, Favero G, Botticelli D. Bone-healing pattern at the surface of titanium implants: An experimen- tal study in the dog. Clin Oral Implants Res 2014;25:124—131.
22. Botticelli D, Lang NP. Dynamics of osseointegration in various human and animal models—A comparative analysis. Clin OralImplants Res 2017;28:742—748.
23. Caroprese M, Lang NP, Baffone GM, Ricci S, Caneva M, Botticelli D. Histomorphometric analysis of bone healing at implants with turned or rough surfaces: An experimental study in the dog. J Oral Sci Rehabil 2016;2:74—79.

FIGURES

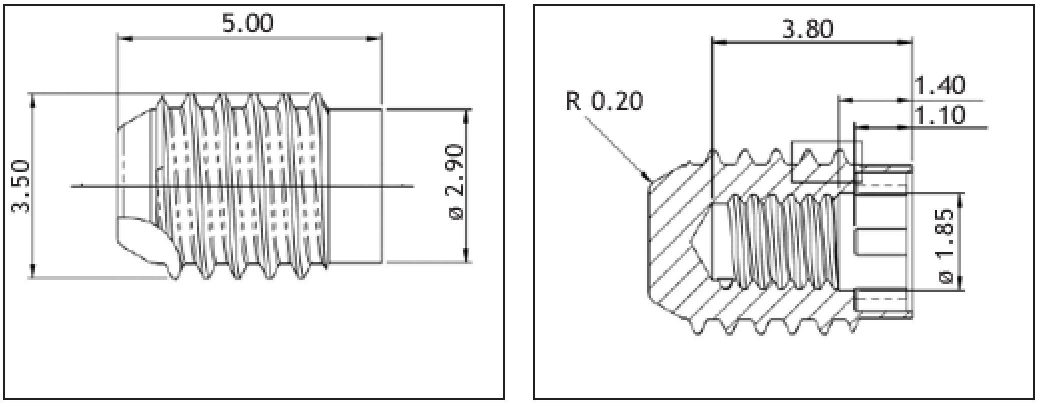

Fig 1 Technical features and measuresof the device

Fig 2 Clinical view of a single crown in resin applied to a device placed in the dis·tal segment of the mandible

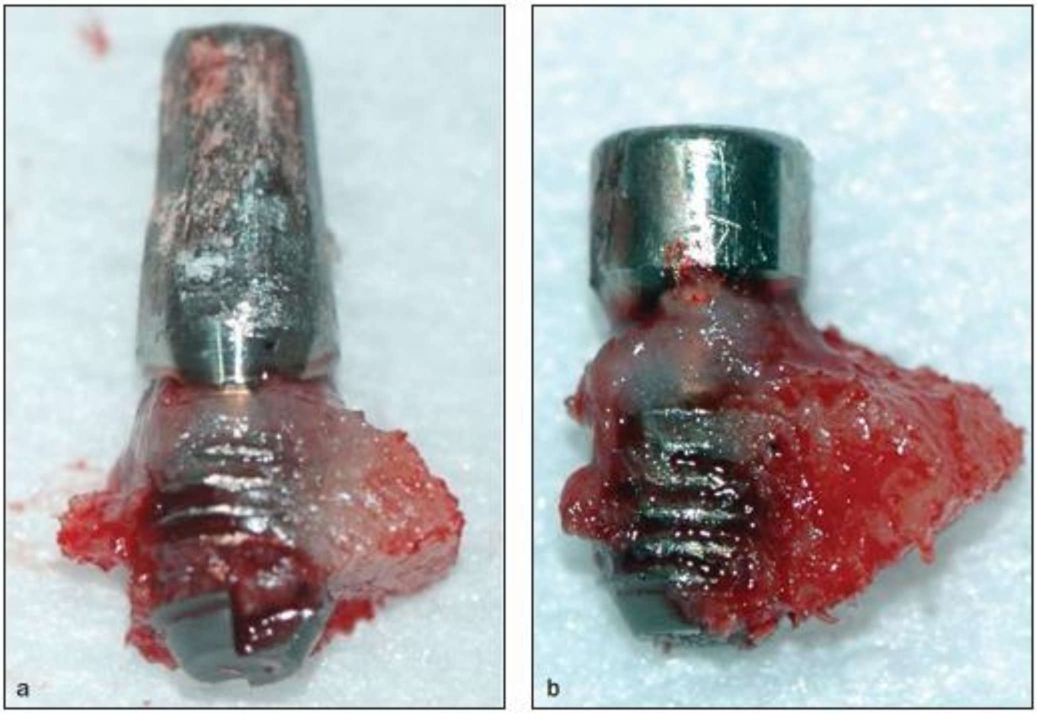

Fig 3 Biopsy specimens of the devices at *(a)* loaded and *(b)* unloaded sites.

Table 1 Tissues in Contact with the Implant Surface in Percentage (n = 10)

		New bone	Old bone	Total mineralized bone	Soft tissues
Loaded (%)	Mean (SD)	85.5 (6.7)	1.3 (1.2)	86.8 (6.5)	13.2 (6.5)
	Median (25th; 75th)	85.9 (82.4; 90.1)	1.2 (0.6; 1.6)	87.1 (82.8; 91.4)	12.9 (8.6; 17.2)
Unloaded (%)	Mean (SD)	83.4 (3.9)	1.2 (0.9)	84.6 (3.7)	15.4 (3.7)
	Median (25th; 75th)	82.7 (82.1; 83.7)	1.3 (0.7; 1.9)	84.0 (83.0; 85.0)	16.0 (15.0; 17.0)

Mean values (SD) and medians (25th and 75th percentiles).
$P > .05$; no statistically significant differences were seen.

Table 1 Tissues in Contact with the Implant Surface in Percentage (n = 10)

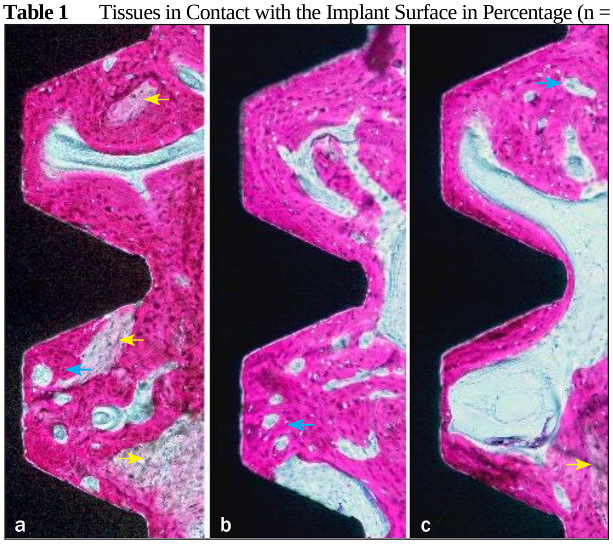

Fig 4 Ground sections illustrating the result of healing at the loaded sites after 4 months from placement and 2 months of loading. Islands of old bone were still visible *(light purple col- or; yellow arrows)* surrounded by vast areas occupied by newly formed bone *(dark purple color)*. Bone remodeling processes were also found *(examples indicated by light blue arrows)*. Mi- crophotographs originally grabbed at ×100 magnification. Acidfuchsin and toluidine blue stain

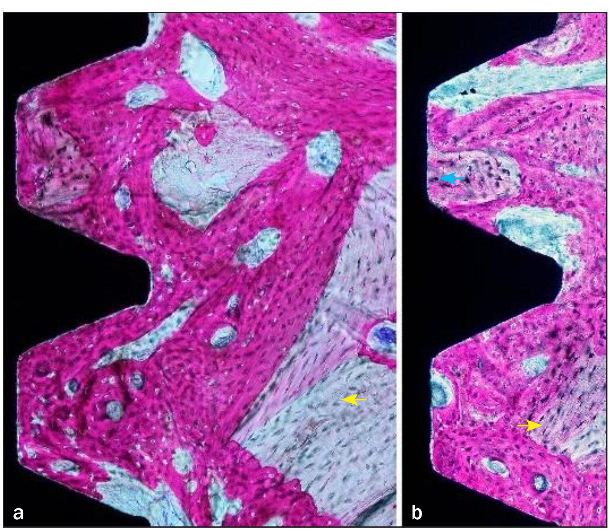

Fig 5 Ground sections illustrating the result of healing at the unloaded sites (control) after 4 months from placement. Newlyformed bone *(dark purple color)* was separating the implant sur-face from the old bone *(light purple color; examples indicated by yellow arrows)*. Only small portions of old bone were found in direct contact with the implant surface *(light blue arrow)*. Mi- crophotographs originally grabbed at ×100 magnification. Acidfuchsin and toluidine blue stain.

Table 2 Tissue Components Around the Surface in Percentage (n = 10)

		New bone	Old bone	Total bone density	Soft tissues
Loaded (%)	Mean (SD)	69.0 (8.3)	7.8 (3.9)	76.8 (8.3)	23.2 (8.3)
	Median (25th; 75th)	66.6 (66.0; 70.9)	6.2 (5.8; 9.9)	79.3 (72.2; 80.8)	20.7 (19.2; 27.8)
Unloaded (%)	Mean (SD)	65.9 (10.3)	8.2 (4.5)	74.1 (10.5)	25.9 (10.5)
	Median (25th; 75th)	67.0 (62.7; 71.2)	8.0 (5.4; 9.1)	75.1 (69.1; 81.9)	24.9 (18.1; 30.9)

Mean values (SD) and medians (25th and 75th percentiles).
$P > .05$; no statistically significant differences were seen.

Table 2 Tissue Components Around the Surface in Percentage (n = 10)