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RESEARCH

Digital subtraction radiography evaluation of longitudinal bone density changes around immediate loading implants: a pilot study

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Objective: The aim of this study was to assess longitudinal quantitative changes in bone density around different implant loading protocols and implant surfaces measured by digital subtraction radiography (DSR).
Methods: 12 patients received bilateral homologous standard and TiUnite® (Nobel Biocare, Kloten, Switzerland) single-tooth implants under 2 implant—loading protocols: immediate loading (8 patients, 16 implants, 12 maxillary) and conventional loading (4 patients, 8 implants, 4 maxillary). Standardized periapical radiographs were taken immediately after implant placement (baseline image) and at the 3-month, 6-month and 12-month follow ups. Radiographic images were digitized and submitted to digital subtraction using the DSR system® (Electro Medical System, Nyon, Switzerland), resulting in three subtracted images. Quantitative analysis of bone density was performed using Image Tool® software (University of Texas Health Science Centre, San Antonio, TX) to assess pixel value changes in five areas around the implants (crestal, subcrestal, medial third, apical–lateral and apical).
Results: Repeated-measures analysis of variance showed that grey levels were significantly influenced by follow-up time and implant-loading protocol. A linear increase in grey levels was found for immediate loading (IML) implants and a significant decrease in grey levels was observed in the 12-month follow up for conventional loading implants. No effect of implant surface treatment was observed.
Conclusion: In conclusion, IML protocol induced mineral bone gain around single-tooth implants after the first year under function for cases with favourable bone conditions.
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Introduction

A favourable bone healing process around implants is a major contributing factor for successful treatment with dental implants using either conventional or immediate loading (IML) protocols. Clinical studies have shown that the IML protocol is a suitable alternative to conventional two-stage implants based on the prevalence of post-operative negative clinical outcomes such as osseointegration failure, implant mobility, pain and discomfort.[1,2] Similar findings were found in animal experiments[3–6] and human histological studies.[7,8]

A systematic review of clinical studies concluded that it is possible to successfully load dental implants immediately or soon after their placement in selected patients, although failure rates tend to be greater when compared with conventional loaded implants.[9] Other radiographic parameters such as marginal bone level changes may not differ in different loading protocols,[9] but implant design and surface, implant–abutment configuration, implant position and surgical technique are determinants in marginal bone level preservation.[10–12]

Evidence suggests that high primary stability, which is essential for IML protocols,[9] is dependent on several factors including bone density and quality, implant shape, design and surface characteristics, and surgical technique.[13] An increased bone density around the implants ensures greater resistance to occlusal forces in

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the long-term follow up, especially for IML implants. However, quantitative assessment of changes in mineral bone density around implants is scarce and not properly reported in the literature.

Histological and histomorphometric animal studies have shown that IML implants become osseointegrated with a hard-tissue peri-implant response similar to conventional loaded implants, increasing the ossification process of the alveolar bone around endosseous implants.[14,15] Similarly, previous studies using digital subtraction radiography (DSR) found a continuous increase in peri-implant bone density over time following implantation.[16,17] However, these studies do not report reliable quantitative measurements and comparison between different loading protocols is not provided. The aim of this study was to assess longitudinal quantitative changes in bone density around different implant loading protocols and implant surfaces, measured by DSR.

Materials and methods

Sample

12 adult partial edentulous patients with bilateral homologous single-tooth spaces were selected to participate in the study. 8 patients were female and 4 were male, with an age range of 24–61 years (mean, 37.3 years; standard deviation, 8.8 years). Each patient had either single bilateral edentulous spaces located in the maxilla (lateral incisor, canine or premolar region) or single bilateral edentulous spaces located in the mandible (molar region), opposing natural teeth or a tooth-borne fixed partial denture. Screening panoramic radiographs showed sufficient bone dimensions for installation of a 3.75 × 13 mm implant at each site. Clinical examination revealed healthy gingival tissues and the absence of relevant systemic diseases such as diabetes; none of the patients were smokers and all were free of parafunctional habits which could influence osseointegration. All implant sites were classified as bone type II (implant bone site with a thick layer of compact bone surrounding a core of dense trabecular bone) or bone type III (implant bone site with a thin layer of compact bone surrounding a core of dense trabecular bone) according to Lekholm and Zarb's[18] classification, which considers panoramic and periapical radiographs and surgeon's hand-felt perception of drilling resistance during bone preparation for implant placement.

The research protocol was approved by the local ethics committee. All patients were fully informed about their participation and signed an informed consent form.

Implant treatment protocol

Patients received bilateral 13 mm- tall implants: on one side, an oxidized surface implant [MK III TiUnite® (cylindrical, external hexagon connection); Nobel Biocare, Kloten, Switzerland], and on the other side, a machined surface standard implant (Brånemark system implant®, turned, cylindrical, external hexagon connection; Nobel Biocare). The location of both types of implants was randomly assigned for the right and left sides. 16 implants were placed in the maxilla (8 TiUnite and 8 standard) and 8 implants were placed in the mandible (4 TiUnite and 4 standard). Implant placement was performed by the same implantologist and according to the manufacturer's recommended protocol. All surgical procedures were performed without intercurrences and post-operative follow up was favourable for all patients.

Resonance frequency measurement was used to evaluate implant stability immediately after placement (Osstell ISQ®; Integration Diagnostics, Gothenburg, Sweden). Immediate loading of implants was performed when implant stability quotient values were higher than 60 and there was a minimum final insertion torque of 30 N cm. Such conditions were satisfied for 8 patients (16 implants—12 in the maxilla and 4 in the mandible) and therefore they were assigned to the IML protocol procedures. The remaining four patients (eight implants—four in the maxilla and four in the mandible) did not comply with these requirements and were assigned to the conventional two-stage protocol.

For IML implants (8 patients, 16 implants), provisional crowns were cemented following implant placement and adjustment of occlusal and proximal contacts in centric occlusion and eccentric excursion. For the remaining implants (four patients, eight implants), rehabilitation was performed after a 6 month healing period and second stage surgery.

Radiograph acquisition and subtraction radiograph protocol

Each of the 24 implants was radiographed immediately after surgery and at the 3 month, 6 month and 12 month follow ups. An X-ray unit [Heliodent 70 (70 kVp, 7 mA) was used at 30 cm focus receptor distance with an exposure time of 0.32 s for the molar region and 0.25 s for the premolar, canine and lateral incisor regions. Radiographic film holders and vinyl polysiloxane-based material were used for bite recording. Vertical angulation of the X-ray beam was also recorded for each implant. The radiographic films [IP-21 Insight Single X- ay Film ("F" speed); Eastman Kodak Company, Rochester, NY] were developed by an X-ray film processor (Peri-Pro III; Air Techniques, lnc., Hicksville, NY) containing fresh developer and fixer solutions. Film holders were disinfected and identified before storage. 4 patients did not return for one (n = 3) or two (n = 1) radiographic exam recalls; three of these patients were from the IML protocol group.

All periapical radiographs (n = 86) were digitized in greyscale mode with a 400-dpi resolution using the scanner included in the DSR® system (Electro Medical

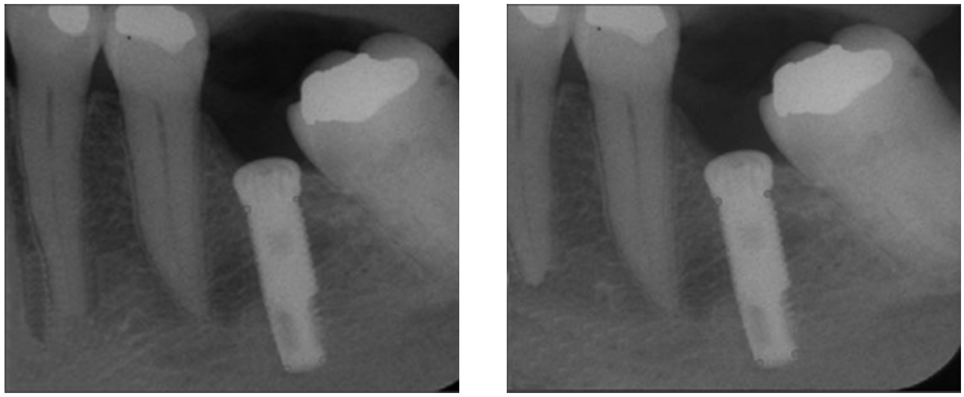

Figure 1 (a) Baseline and (b) follow-up images with the four reproducible anatomical landmarks used for alignment of the images

System, Nyon, Switzerland) and stored as 8-bit tagged image file format (TIFF) images.

The DSR® system was also used for the subtraction radiography procedure. The baseline radiographs (obtained immediately after surgery) and the post-operative images were aligned by selecting four common reference points recorded on the implant surface for the alignment of pairs of radiographs (Figure 1). Following the selection of an area of interest on the images, the follow-up images were subtracted from the baseline image with the software compensating for any subtle differences in geometric projection and film contrast correction between the pairs of images. Changes were depicted as a darkened area for mineral bone loss, a neutral grey for no change and a brightened area for increased mineral bone gain.

For each implant, three subtraction radiographs (SRs) were obtained: SR-3, SR-6 and SR-12. The SR-3 image was obtained automatically by the software by subtracting the baseline and the 3-month follow up images (images were aligned and superimposed to create a subtracted image saved in TIFF format). In the same way, the SR-6 image was obtained by subtraction of the pair baseline and 6 month follow-up images and the SR-12 image was obtained by subtraction of baseline and 12 month follow-up images. A total of 62 SR images were obtained and saved as TIFF files (Figure 2).

Bone density assessment

Image Tool® software (University of Texas Health Science Centre, San Antonio, TX) was used for peri-implant bone density measurements on SR images. The histogram tool was used for measuring the mean pixel grey value of a selected region, ranging from 0 to 255 on the greyscale.

For each implant, 9 10×10 pixel regions of interest (ROIs) were selected on peri-implant bone. ROIs were distributed at the five levels around the implant: crestal, subcrestal, mid-implant, apical–lateral and at the apex (Figure 3). The ROIs were positioned two pixels away from the implant surface to avoid making contact with the metallic surface of the implant image. Nine mean pixel values were obtained for each implant on each SR image. In order to ensure the reliability of the method, an identical 10×10 pixel ROI was selected on the implant area, which was considered as an internal control. These additional measurements were made using the same SR images, focussing on areas without brightness intensity changes.

Greyscale values ranged from 0 (black pixel) to 255 (white pixel). In the ROIs of the peri-implant area, pixel

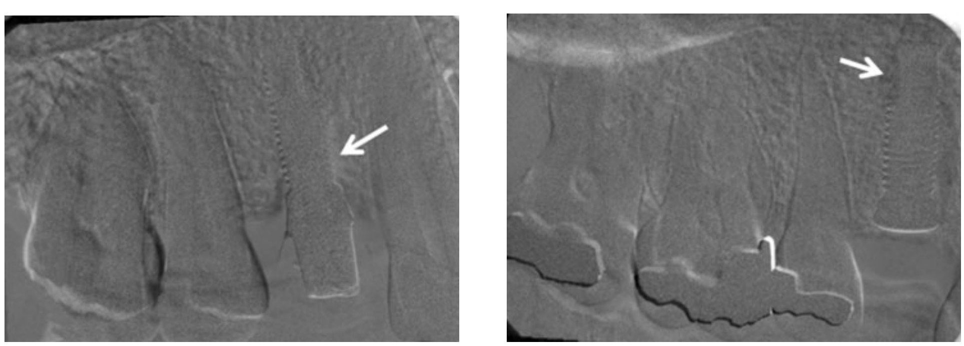

Figure 2 Subtraction radiograph images: (a) the arrow indicates an area of increased grey levels; (b) the arrow indicates an area of decreased grey levels

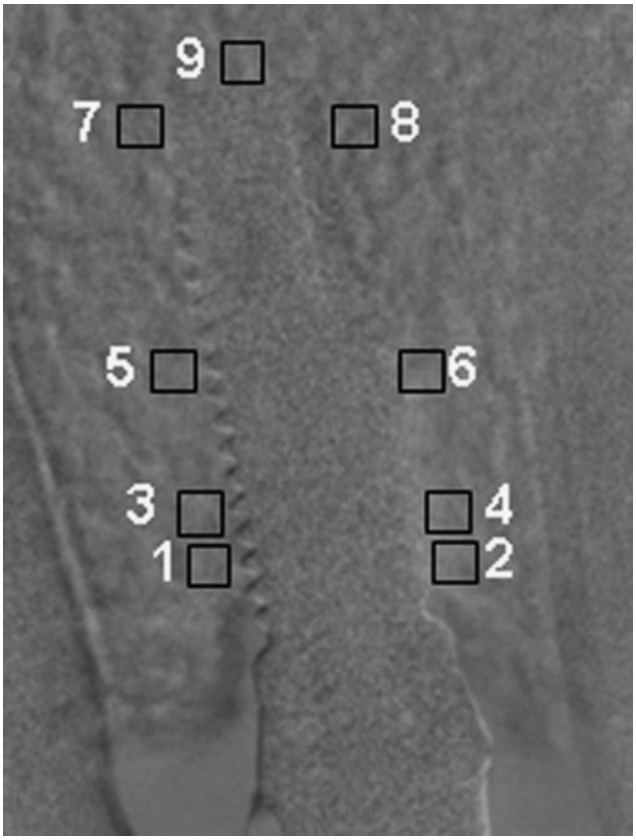

Figure 3 To measure peri-implant bone density, the operator selected 9 10 × 10 pixel regions of interest around the implant at five levels: crestal (1,2); subcrestal immediately below the crestal (3,4); mid-implant (5,6); apical–lateral area (7,8); and apex region (9)

values around 128 indicate unchanged bone density. Pixel values above 128 indicate mineral gain (increased bone density) while pixel values below 128 represent mineral loss (decreased bone density). In control areas, pixel grey values were around 128.

Peri-implant grey level measurements were performed by the same radiologist using a trackball-driven cursor on 8 × magnified images on a monitor. Mesial and distal mean pixel values at four levels next to the implant (crestal, subcrestal, mid-implant and apical–lateral) were averaged for each level, except at the apex region, where only one mean value was obtained.

Data analysis

Descriptive analysis of pixel grey values was tabulated according to ROI area, loading protocol and implant surface, expressed as mean and SD. Repeated-measures analysis of variance (ANOVA) was used to test the effect of follow-up time, implant-loading protocol and implant surface on the bone density around implants. Hypothesis testing included within-subjects contrast at the 3 month, 6 month and 12 month follow up, according to the implant surface, and between-subjects contrast of different patients concerning implant-loading protocols. Statistical significance was set at $p < 0.05$. SPSS 17.0 software (IBM Corporation, Armonk, NY) was used for data analysis.

Results

Table 1 summarises the descriptive values of grey levels for all levels of the independent variables (follow-up time, ROI area, loading protocol and implant surface). The effects of these variables and their interactions are expressed as *p*-values of the repeated-measures ANOVA in Table 2. The results showed that follow-up time and interaction with loading protocol had significant linear effects on grey levels in most ROI areas ($p < 0.05$). No effect of implant surface during the follow-up periods was observed in any of the five ROI areas. The grey value of the unchanged ROI area was a homogeneous background of 128 pixel grey values with slight deviation (± one grey level), irrespective of the tissue structure (bone, teeth or implant).

A detailed descriptive analysis of pixel grey values is presented in Table 3 for both conventional and IML implants. An overall visualization of pixel grey values in Figure 4 shows a significant decrease in bone density at the 12 month follow up for conventional loading implants and a linear increase in bone density for IML implants.

Table 1 Descriptive measures of pixel grey values (means and standard deviations)

		3 months	*6 months*	*12 months*
ROI area (n = 24)	Crestal	130.0 (5.8)	132.3 (9.0)	130.3 (11.3)
	Subcrestal	129.9 (9.1)	131.3 (7.6)	128.9 (12.8)
	Mediolateral	126.8 (5.2)	124.4 (9.0)	122.3 (11.1)
	Apical–lateral	124.9 (10.7)	126.0 (5.1)	125.9 (9.0)
	Apical	128.3 (13.6)	130.3 (11.7)	130.9 (12.1)
Loading protocol	Conventional (n = 8)	128.9 (2.3)	127.5 (3.0)	120.6 (9.8)
	Immediate (n = 16)	127.3 (6.9)	129.6 (4.9)	130.5 (6.7)
Implant surface	Standard (n = 12)	128.4 (3.4)	128.1 (3.8)	126.7 (5.1)
	TiUnite® (Nobel Biocare, Kloten, Switzerland) (n = 12)	127.1 (7.8)	129.6 (4.9)	128.9 (11.4)
Overall (n = 24)	—	127.8 (5.8)	128.9 (4.3)	127.8 (8.7)

ROI, region of interest; n, number of implants.

Table 2 Results of repeated-measures analysis of variance (*p*-values) for tests of within-subjects contrasts, according to follow-up time, implant surface, implant loading protocol and factor interactions

	ROI area				
Factors	*Crestal*	*Subcrestal*	*Medial third*	*Apical–lateral*	*Apical*
Follow-up time	0.032*	0.058	0.006*	0.048*	0.624
Follow-up time × implant-loading protocol	0.018*	0.027*	0.003*	0.046*	0.662
Follow-up time × implant surface	0.331	0.714	0.570	0.904	0.749

ROI, region of interest.
* statistically significant effect.

Table 3 Measurements of pixel grey values for ROI areas according to implant-loading protocol (mean and standard error)

ROI area	*Implant loading protocol*	*3 months*	*6 months*	*12 months*
Crestal	Conventional	132.8 (1.64)	131.3 (2.14)	122.9 (5.16)
	Immediate loading	128.7 (1.72)	132.9 (2.80)	133.5 (2.50)
Subcrestal	Conventional	134.2 (1.48)	130.5 (1.26)	123.3 (5.28)
	Immediate loading	127.9 (2.83)	131.8 (2.48)	131.4 (3.33)
Mediolateral	Conventional	126.7 (1.81)	125.1 (1.57)	115.5 (2.78)
	Immediate loading	126.9 (1.75)	124.0 (2.92	125.0 (2.95)
Apical–lateral	Conventional	124.8 (2.04)	124.4 (1.50)	118.4 (4.24)
	Immediate loading	124.9 (3.51)	126.8 (1.47)	128.8 (1.67)
Apical	Conventional	125.2 (3.32)	125.4 (3.76)	121.4 (5.70)
	Immediate loading	129.6 (4.74)	133.2 (3.46)	134.6 (2.77)

ROI, region of interest.

Discussion

Our study showed that the SR method revealed different bone healing progression in the follow-up period for IML implants. A slight linear increase in pixel grey values was observed for IML implants and a significant decrease was observed for conventional loading implants 12 months after placement. Previous studies concluded that the IML protocol stimulates bone neoformation in response to occlusal load,[19] increases bone-implant contact percentage and decreases the risk of the presence of fibrous connective tissue at the interface.[20,21] Differences between conventional and IML protocols were evidenced by a clinical pilot study carried out by Barone et al[19] using volumetric CT scanning for densitometric assessment. Higher measures of bone mineralization in the IML group were observed 6 months after surgery.

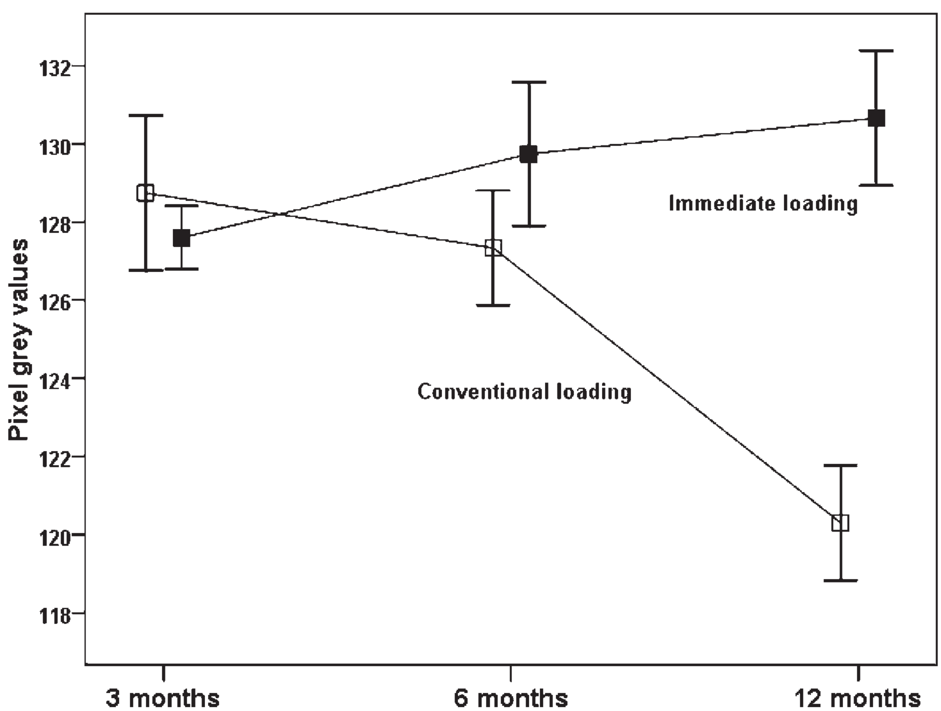

Figure 4 Follow-up mean and standard error of pixel grey values according to implant loading protocol

Previous studies reported that SR was considered a reliable method for quantitative longitudinal assessment of bone changes at implant sites.[17,22] Jeffcoat and Reddy[22] had related that quantitative SR could facilitate the study of dental implants in the future because few surrogate outcomes had been developed to replace histology for the determination of osseointegration. More recently, in a clinical study of implants in the posterior maxilla, Appleton et al[16] used the SR method and evidenced less crestal bone loss in progressively loaded implants compared with conventional loaded implants after a 12 month follow-up period. This study did not observe statistically significant differences in grey levels in the selected ROIs around implants (crestal, subcrestal, mid-implant, apical–lateral and implant apex) but suggested a strong trend towards gaining more density over time.[16] In our study, using the same ROI areas in SR images, we observed a significant effect of follow-up time, mainly influenced by implant-loading protocol, in all ROI areas except the apical area. Wakoh et al[17] also used the SR technique, successfully detecting peaks of bone change around dental implants.

It is hypothesized that in the conventional loading implants, the lower bone–implant contact probably hinders load distribution, resulting in lower peri-implant bone density after delayed loading between the 6 month and 12 month follow ups. At this stage, it is supposed that a new inflammatory process takes place and remodelling may suppress bone neoformation.

No difference in grey levels was observed between standard and TiUnite implants. However, it must be emphasized that low-quality bone sites were not included in this study and IML was restricted to implants with high primary stability. In adverse conditions, better osteoconductive properties of TiUnite implants have the potential for promoting a high level of implant osseointegration,[23] especially in type IV bone in the posterior maxilla.[24] Additionally, our study sample included only single-tooth crowns and the results may not be applicable to extensive rehabilitations.

For unchanged areas, pixel grey values were around 128. The grey values for such sites can vary slightly owing to the influence of many factors in the subtraction technique including difficulties in standardization of image acquisition, film developing and image digitization.

Our study provides additional radiographic evidence for the clinician in the decision-making process using the SR technique, but it must be emphasized that the SR protocol requires a standardized radiographic technique. Another important technical factor is the acquisition of a homogeneous background and gamma correction to reduce potential interferences and allow the detection of subtle changes in the image background. In our study, reproducible measurements were obtained using registration of the horizontal and vertical coordinates of the image to locate any addressable point on the computer display screen, *i.e.* together, the x and y coordinates located any specific ROI position on the screen, allowing reliable identification of bright or dark areas and indication of mineral gain or loss. In addition, quantitative assessments are highly dependent on the operator's ability and skill to obtain reproducible measurements and reliable interpretation of bone density changes around implants.

It must be emphasized that all implants included in this study were successfully osseointegrated and had no negative clinical outcome, since selection of cases excluded patients with poor bone conditions or systemic diseases that disturb the bone healing process. Surgical and prosthodontic procedures were performed under controlled conditions. Subtraction analysis using pixel grey values in cases with pathological changes, which are commonly observed in clinical settings, may be useful to identify minor or incipient pathological bone changes, but changes must be interpreted with caution when correlating quantitative bone changes with clinical and radiographic parameters.

Future studies with larger study samples under controlled conditions are needed to reaffirm our findings to allow them to be applicable in other clinical human settings. In addition, future SR studies need to explore the influence of bone density on the primary implant stability and variations according to site-specific regions of the maxilla and mandible, as demonstrated previously in CT clinical studies.[25–28]

In conclusion, this study showed that the IML protocol induced mineral bone gain around single-tooth implants after the first year under function for cases with favourable bone conditions and a decrease in bone density around conventional loading implants. In addition, no effect of implant surface was observed on longitudinal assessment of the pixel grey values around the implants.

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