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Does osseointegration occur around nonprimary stabilized titanium implants? A histomorphometric analysis

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ABSTRACT

Aim The aim of this study was to analyze the levels of bone-implant contact (BIC) and bone area between the threads (BABT) of primary and nonprimary stabilized titanium implants histologically in rats.

Materials and methods Fourteen Sprague-Dawley rats were categorized as two groups: primary (PS) and nonprimary stabilization (NPS) groups. Totally, 14 titanium implants with machined surfaces were integrated into the tibial bones of the rats with and without primary stabilization in the PS and NPS groups, respectively. After 12 weeks of healing, the rats were sacrificed, block sections were acquired for histological analysis.

Results The BIC ratios (%) in the PS and NPS groups were 53.23±5.65% and 40.79±5.59%, respectively and was significantly higher in the PS group as compared with that in the NPS group (P<0.05). The mean BABT ratios (%) in the PS and NPS groups were 55.71±7.32% and 37.14±2.67% respectively. The BABT ratio (%) was significantly higher in the PS group than in the NPS group (P<0.05).

Conclusion The results suggest that osseointegration may occur despite the absence of primary stability at the end of a 12-week healing period but primary stabilization at the time of installation appears to be critical in achieving maximum BIC and BABT.

KEYWORDS: Dental implant, Histomorphometric analysis, Osseointegration, Primary stabilization

INTRODUCTION

Titanium dental implants have been used frequently for many years as an option for the rehabilitation of partial and total edentulism (1). The success and survival rates of dental implants are directly related to the quantity and quality of the bone around the implants. Primary stabilization, which refers to the initial rigid placement of a dental implant during surgery, is necessary for a successful osseointegration (2). Osseointegration, as defined by the percentage of bone-implant contact (BIC), refers to the balance between the bone and implant, and is required for long-term functional success. Rigid placement depends on the bone quality in which the implant is inserted, the groove design of the implant, and the surgical technique. However, it is very difficult to achieve tight implant placement, especially when the bone quality and quantity are insufficient (3). In such cases, the stability of the implant can be compromised.
During osseointegration, bone formation occurs in two different types: new bone is formed as a result of direct contact with the implant surface (contact osteogenesis), or in nontightly fitting implants new bone formation occurs from neighboring bone tissue (distance osteogenesis). When there is a gap between the bone and the implant surface, a blood clot fills this area and forms a matrix. This matrix is eventually displaced by woven bone. This kind of healing in the peri-implant bone tissue is called endoosseos-intrabony healing (4, 5).
According to a number of studies in the literature, when the distance between the peri-implant bone and the surface of the implant is greater than 1 mm, bone-implant fusion may not be fully achieved (4, 6-8). In these in vivo studies, the authors reported that bone-implant contact (BIC) at the coronal level was dependent on the amount of space between the surface of the titanium implant and the adjacent bone surface. In another in vivo study, similar healing was demonstrated in implants with a distance of more than 1 mm and 2 mm between the implant and the bone (9).

Since the concept of immediate loading has been introduced, the importance of primary stability has increased. However, the amount of mechanical attachment needed to achieve optimal bone healing around implants remains unclear. In addition, there is no clear consensus on how rigid mechanical engagement influences osseointegration. Excessive pressure on bone may cause hyalinization of the bone around an implant during the early healing period, and this may cause a prolonged healing time for osseointegration (10). Little is known about the effects of rigid primary stability on achieving successful and satisfactory osseointegration or the outcomes of inserting implants without mechanical engagement using oversized drilling when they are unloaded and submerged. Thus, the aim of this study was to histologically examine the levels of bone-implant contact (BIC) and bone area between the threads (BABT) of rigid and loosely placed titanium implants that could move vertically and rotationally, without primary stability after a long-term healing period.

MATERIALS AND METHODS

Animals and experimental design

All experimental and surgical protocols in this study were conducted at the Fırat University Experimental Research Center, Elazıg, Turkiye, and complied with the ARRIVE Guidelines for Animal Research by the National Centre for the Replacement Refinement & Reduction (11). Ethical consent for this study was obtained from local ethics committee for animal experiments at Fırat University (2017/193). The experimental research center of Fırat University provided the animals used in this study, and the management of the animals was controlled by the Institutional Board for the Care and Use of Laboratory Animals. In this study, 14 female Sprague-Dawley rats (weight: 280–320 g) aged 0.5–1 years were used. The rats were housed in temperature-controlled cages in a 12 h:12 h light–dark cycle, with free access to food and water during the study period.

The rats were divided randomly into a nonprimary stabilization (NPS) group and a primary stabilization (PS) group. In the NPS group, titanium implants were placed surgically, and primary stabilization was not achieved during the integration of the implants, thereby allowing the implants to move vertically and rotationally in their sockets. A three-dimensional gap (0.5 mm in width) was left between the implant surface and the bony walls of the implant bed. In the PS group, the titanium implants were inserted surgically, and primary stabilization was achieved during the integration of the implants. Thus, the implants did not move vertically or rotationally in their sockets.

Surgical procedures

The surgical procedures were performed under general anesthesia. General anesthesia was administered by an intramuscular injection of 40 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine. All the surgical procedures were carried out under sterile conditions. Following general anesthesia, the rats' right tibial skins were shaved and irrigated with povidone iodine. After general anesthesia was achieved, a linear incision (1.5–2 cm long) was made on the skin of the tibial crest. After the skin incision, a periosteal elevator was used to reach the metaphyseal part of the tibial bone. Implant sockets were created using appropriate drills progressively under sterile serum physiological perfusion. After the bone sockets had been prepared, titanium implants with machined surfaces (4.5 mm length and 2.5 mm diameter) were inserted. Following surgical implantation of the implants, the subcutaneous tissues and skin were repositioned in their original position in layers. Then, they were sutured with 4-0 polyglactin absorbable sutures. After the surgical procedure, all the animals received an analgesic (0.1 mg/kg tramadol hydrochloride) and antibiotics (50 mg/kg, cefazolin sodium) which were administered intramuscularly for 3 days. In total, 14 machine-surfaced titanium implants were placed into the corticocancelleous part of the right tibial bones. The same researcher performed all the surgical procedures.

Histologic procedures and analysis

At 12 weeks, all the rats were sacrificed and the implants were explanted, with the bone tissue surrounding the implants. The samples were kept in a 10% formaldehyde solution for 7 days. Following fixation, the samples were embedded in 2-hydroxyetylmetacrylate resin to cut easily the undecalcified bone and titanium using an Exakt® microtome (Leica Biosystems, Nussloch, Germany). For the histological analysis, the implants with surrounding bone tissue were ground using an Exakt® grinder (Leica, Germany). Sections that were 50 µm in thickness were prepared for light microscope analysis (Olympus, Tokyo, Japan), and these sections were stained with toluidine blue. All the procedures were performed at the research laboratory of the Faculty of Dentistry at the University of Erciyes, Kayseri, Turkiye. The histological analyses were performed using a light microscope available at the Department of Medical Microbiology, Faculty of Medicine, Fırat University, Elazıg, Turkiye by a single examiner blunt to the study groups. The BIC ratio was determined for each section as a proportion of the total implant surface length in direct contact with the bone (1). The percentage of the ratio of the total bone-containing area to the total thread area was calculated as the percentage of BABT for each implant (12).

Statistical analysis

For the statistical analysis, SPPS software was used (USA). The data were analyzed, and means and standard deviations (%) were calculated. The Student's *T* test was used for the analysis of the data, and a P value of < 0.05 was accepted sufficient for statistical significance.

RESULTS

The histological BIC (%) and BABT (%) data for the NPS and PS groups are shown in Table 1 and Table 2. The mean BIC (%) ratios in the NPS and PS groups were 40.79 ± 5.59% and 53.23 ± 5.65%, respectively. There was a statistically significant difference in the BIC (%) ratios of the two groups (P<0,05). The BIC (%) ratio in the PS group was higher than that in the NPS group (Table 1). With respect to the mean BABT (%) ratios, there was a statistically significant difference between the two groups (P<0,05). The BABT (%) of the PS group was significantly higher than that of the NPS group, being 55.71±7.32 and 37.14±2.67 respectively (Table 2). Figure 1A and B histologically illustrate the results for each experimental group.

Group	N	BIC (%) Mean±SD	P*
N_PS	7	40.79±5.59 α	0.001
PS	7	53.23±5.65	

Values are presented as mean±standard deviation.
*Student T test was applied for statistical analysis.*P<0.05, α Statistically significantlly different compared with the PS- group. N_PS: Non_primary stabilization, PS: Primary stabilization.*

TABLE 1 Bone implant contact (BIC) ratio (%) of the groups

Group	N	BABT (%) Mean±SD	P*
N_PS	7	37,14±2,67 α	0.000
PS	7	55,71±7,32	

Values are presented as mean±standard deviation.
**Student T test. P<0,05, α Statistically significantlly different compared with the PS- group. N_PS: Non_primary stabilization, PS: Primary stabilization,*

TABLE 2 Bone area between the threads (BABT) (%) of the groups

DISCUSSION

In this study, the BIC and BABT of titanium implants installed with (PS) or without primary stabilization (NPS) on the tibiae of rats were evaluated histologically in an experimental implant model. Primary stability is defined as the mechanical connection between the dental implant and the bone without biological integration. Poor primary stability is accepted as one of the most important causes of implant failures (13). The achievement of a high success rate for implants hinges on primary stability, which serves as a fundamental prerequisite for successful osseointegration and the differentiation of bone cells (14). In preclinical studies, the degree of osseointegration is commonly evaluated histologically, and the BIC and BABT are calculated from histological sections and have a high level of evidence (1-4, 15, 16). It has been reported that histomorphometric examination is a reliable approach to investigate implant osseointegration, allowing for the determination of BIC, new bone formation, and bone quality around the integrated dental implants (17). According to a previous study, recent developments in surface treatments improved the BIC, even in the presence of low-quality bone and early loading (18). Titanium is the most successful of many implant materials in terms of its biocompatibility and mechanical properties. In the present study, machined surfaced titanium implants were used.

Micromobility at the implant-bone interface can ruin the healing process of the bone, causing fibrous encapsulation (19, 20). Although loosely placed implants may become fixed by partial bone growth during the first 12 weeks after placement, these result in only irregular direct contact between the implant and bone (6). On the other hand, if initial stabilization is sufficient, fixation with appositional lamels of bone along the implant surface occurs (6). Marcu et al. (21) reported that the contact surface at the bone-implant interface increased and bone with high resistance proliferated as an adaptive reaction to restore the resistance of the area, weakened following the trauma caused by the insertion of the implant.

Ivanoff et al. (22) reported high BIC values of rotation-

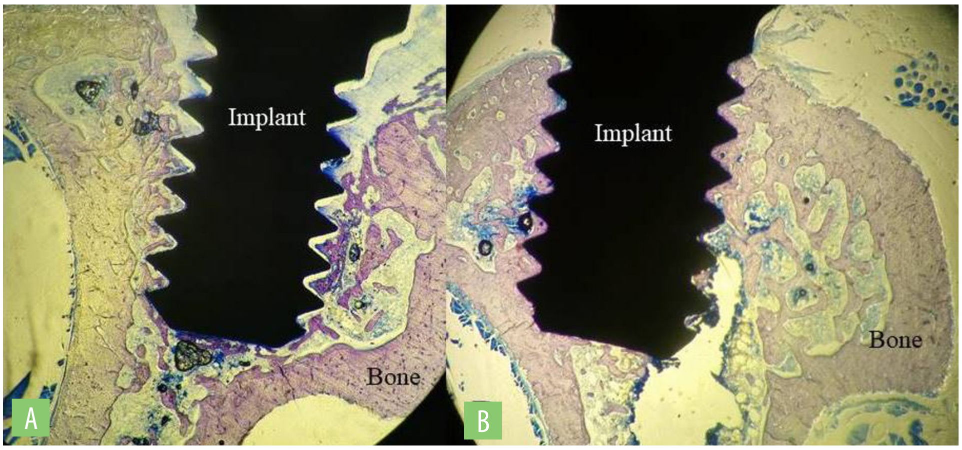

FIG. 1A and B
Non-decalcified histologic images of the Non_Primary Stabilized and Primary Stabilized group implants. (A) Non_Primary stabilized (B) Primary stabilized implants (4X). (X: 10 Times Magnification).

ally mobile implants as long as they were not totally mobile. They concluded that initial rotational mobility unless total mobility, irrespective of whether the implant was in cortical or trabecular bone, was not the sole cause of decreased integration of unloaded implants. Jung et al. (15) demonstrated that acid-etched, sandblasted, and hydroxyapatite-coated titanium implants inserted into sockets prepared with an oversized drill achieved the same level of osseointegration as immobile implants with primary stability after both 4 and 8 weeks of healing. A study that evaluated implants with rotational and vertical movement using resonance frequency analysis suggested that osseointegration in the absence of primary stability was not statistically different as compared with that of a control group at the end of a 12-week healing period (2). In contrast, in a study on dog jaws, in which implants were placed in small and large defect sites, Sivolella et al. (4) reported that large and narrow distances around the implants significantly decreased the osseointegration level when compared with a control group. Carlsson et al.(6) compared osseointegration of implants with a distance of 0.35 and 0.85 mm around the implant surfaces, with the implants placed in sockets with the same diameter. After 6 and 12 weeks of healing, they concluded that osseointegration was achieved in a control group-primary stabilized implants, but not in the other groups (6). In another study, according to histological analysis, osseointegration was not observed in any implants installed without primary stability (20). The authors suggested that the lack of osseointegration in the test group-nonprimary stabilized implants, was the result of implant instability facilitating the formation of fibrous connective tissue between the implant surface and the newly formed bone.

In the present study, the mean BIC (%) ratio and BABT (%) ratio for the total implant surfaces in the PS group was statistically significantly higher than that in the NPS group. This finding is in accordance with that of other studies, which reported that primary stability was critical for osseointegration (4, 6, 20). In research on dogs, Jung et al. (3) concluded that the level of osseointegration of implants in a control group was not statistically different after 4- and 8-week healing periods. In their study, although the mean amount of BIC remained unchanged in the control group, it increased in the experimental group-nonprimary stabilized implants with time. When they examined the pattern of osseointegration, they observed no change after 8 weeks as compared with that after 4 weeks. However, they found changes in the newly formed bone, with well-organized lamellar bone substituting the woven bone adjacent to the implants. In a 12-week study on dog jaws, Sivolella et al. (4) demonstrated that the gap between the bone and the implant was filled with intense connective tissue, which would later be replaced by bone.

In the present study, the bony contact and bone area between the threads observed at the loosely placed-NPS implants was significantly lower than that in the rigid implants, with a ratio of 40.79% and 37,14% respectively. This contact may have arisen as a consequence of proliferation from neighboring bone tissue (i.e., distance osteogenesis) (23). The surface of the implant may have acted as a body promoting osseointegration. On the other hand, connective tissue at the supracrestal region may have emigrated between the bone and the implant surface, thereby disturbing the osseointegration process, as no membranes were used to seal the top of the implants. However, this connective tissue that adhered to the implant surface may be mineralized over time and integrated into the implant surface. The results of a previous study showed that the percentage of BIC at all the nonprimary stabilized implants was high and similar to that at primary stabilized implants after 4 months, regardless of whether membranes were used (24). The difference between this study and the present one may be due to the surface characteristics of the implants. Machined surfaced titanium implants were used in the present study, whereas sandblasted and acid-etched (SLA-surfaced) implants were employed in the other study. As reported previously, the use of SLA-surfaced implants may affect the amount of BIC acquired during healing (25). Surface modifications are accepted as effective in increasing bone healing and improving the grade of osseointegration (26, 27). However, in the present study, the implants without primary stability showed some degree of osseointegration.

Schenk et al. (28) proposed that bone bridging during the closure of a defect was related to the extent of the gap and that woven bone filled the gap in defects < 1 mm. Other studies suggested that bone healing was disturbed when a gap of 0.5 to 1 mm wide was present between the implant and the bone after implant placement (6, 8, 29). Thus, in the NPS group, the implants were placed in sockets, with gaps of 0.5 mm between the implants and the bone bed. Although the amount of new bone in touch with the implant is related to the initial size of the marginal gap, the generation of a coagulum in the defect, its retention, and replacement with a transient matrix are crucial in the resolution of the defect.

This study has some limitations. The long-term success and survival rate of the implants in this study could not be evaluated. More comparative studies with larger sample sizes are needed to increase scientific and statistical power.

CONCLUSION

Within the limits of this study, we can conclude that osseointegration may be obtained in the absence of primary stability at the end of a 12-week healing period, even when using machined surfaced titanium implants. The initial bone contact at the time of implant installation appears to be critical in achieving maximum BIC and BABT following healing. Further investigation is indicated because of the clinical importance of these results for long-term implant stability.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contribution

VET: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Validation, Visualization, Writing-original draft.
AS: Formal analysis, Project administration, Supervision, Writing-review and editing.
SD: Conceptualization, Methodology, Project administration, Supervision, Writing-review and editing.
AB: Methodology, Project administration, Supervision, Writing-review and editing.

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