Original Article

The Effect of Restoration Type on Osseointegration of Dental Implants: A Histological Analysis in Dogs

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ABSTRACT

Background and Objective: **The concept of immediate occlusal loading with dental implants offers several advantages. In this research, the effect of type of restoration on Osseointegration rate of dental implants was histologically evaluated.**

Materials and Methods: **Three months prior to implantation, the lower premolar teeth of 15 dogs were extracted. Then, 3 to 4 Maestro™ implants were placed in the healed extraction sites for each dog (n=48). Dividing the dogs into 3 groups, the implants were either loaded 48 hours later, with metallic or prefabricated acrylic crowns, or were left unloaded until the time of sacrifice. Three months after implant insertion, the animals were sacrificed and the samples were investigated to define the Osseo integration rate, lamellar and woven bone percentage and local inflammation of the regenerated bone.**

Results: **No significant difference in the observed criteria was observed among the three groups ($p>0.05$); however, the unloaded group had the highest osseointegration rate and the group with metallic crown loaded had the least. The prosthesis type had no significant effect on the implant success rate ($p>0.05$). Lamellar and woven bone percentage of regenerated bone also did not differ in the three groups ($p>0.05$). One implant from each group failed in this study.**

Conclusion: **Compared to unloaded implants, bone regeneration seems to have similar histological characteristics around immediately loaded dental implants and if properly carried out, these two kinds of restoration materials do not seem to affect the osseointegration and the bone regeneration process.**

Key words: Dental implants, Osseo integration, Bone regeneration, Dental restoration

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Introduction

It has been shown that immediate loading of dental implants did not cause untoward effects on the formation of mineralized tissue at the interface, producing, on the contrary, a higher bone-implant percentage than in submerged implants, and thus, immediate loading can be a possible alternative procedure in implant dentistry (1). It has also been reported that with immediately loaded implants, patients resumed function quickly and that the masticatory function was uniformly judged to be superior to pretreatment time (2). Immediate loading shortens the total rehabilitation time, with an increased patient satisfaction and the avoidance of delays in the final rehabilitation. Primary stability is a key factor in the success of immediately loaded implants; because a high degree of primary stability helps the resistance against micromotions. Micromotion is the movement of implant surface against the surrounding bone tissue, during functional loading (3).

Long-term maintenance and success of osseointegrated implants involves continued remodeling activity at the periphery of the implant to avoid bone fatigue fracture (4) and to replace bone that may have sustained micro-fractures as a result of cyclic loading (5). Woven bone is produced in response to extraordinary loading conditions and provides a rapid, almost immediate increase in the sectional geometry of bone. The amount of new, less-mineralized bone at the interface, as well as the type of bone (woven or lamellar), influence the strength of the interface (6). The micro-strain environment may affect the turnover rate of bone adjacent to an implant during prosthetic loading (7). The rate of bone turnover in the regional environment of an implant has a great clinical importance for the long-term maintenance of dental implants (7).

Several histologic studies on humans and experimental animals have found that kind of restoration material did not impede osseointegration and did not produce untoward effects on bone formation in a peri-implant location (1;8;9). Also, clinical studies have shown very high percentages of success for immediately loaded implants in different clinical situations (10). Factors such as primary stability and immediate immobilization (splinting) of dental implants (10), adequate bone density and absence of overloading the dental implants have been reported to influence osseointegration and the prognosis of immediate loading (11).

The aim of the present study was to histologically determine the effect of kind of restoration material on the osseointegration rate of Maestro™ dental implants (Biohorizons® implant systems Inc., Birmingham, USA) and its effect on bone regeneration and bone remodeling process in dogs.

Materials and Methods

Fifteen dogs were utilized in this prospective study. The protocol of this study was approved by the ethics committee of School of Dentistry, Shahed University, Tehran, Iran and the National Animal Care Society, Tehran, Iran. A period of 10 days was decided to standardize the diet and environmental conditions of the dogs (12).

An IM injection of ketamin hydrochloride (5 mg/kg) and diazepam (1 mg/kg) were used to sedate the animals prior to operation. The oral cavity of the animals was then thoroughly rinsed with a 1:1 mixture of povodine iodine 10% and chlorhexidine solution. Then, with a local injection of lidocaine 2% with 1:100000 epinephrine, the area around lower premolars was locally anesthetized. Following complete anesthesia of the tissues, the lower premolar teeth were atraumatically extracted. This was performed to standardize the comparisons. The technique consisted of separating the roots with a high speed bur in presence of intense water spray and extracting the separated roots with forceps. Twelve sockets were omitted from the study due to bone or root fractures. Three months later after confirming proper bone regeneration with radiography, forty-eight 4.0×9.0 mm Maestro™ dental implants were placed in the site of the regenerated bone (3 to 4 implants for each dog) using one-stage non-submerged technique. The primary stability of the implants was checked after implant insertion. The implants were then divided into 3 groups (n=16 for each). The first and second groups were loaded with prefabricated metallic and acrylic crowns (8 implants for each type of crown in each group) 48 hours after implantation respectively. The third group was left unloaded (did not use crown) until the day of sacrifice. Following operation, animals were taken care of according to the protocol of Tehran Veterinary School with a special soft diet and supporting medicaments. The reason for prescribing a soft diet for the dogs was to inhibit overloading of the implants.

Three months after primary surgery, the animals were sacrificed using vital perfusion with formalin 10% and the implants and surrounding tissues were retrieved using cutting disks. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Iran Acryl, Semnan, Iran). After the polymerization was complete, the resin blocks were cut along the longitudinal axis of the implants, preparing a cross-section of bone-implant interface using Accutom-50 cut-off machine (Struers, Copenhagen, Denmark). The representative section for each implant was then analyzed with a stereomicroscope (Olympus SZX_ILLB200, Olympus Optical Co. Ltd., Japan) and photographed with a high-resolution video camera (JVC TK_C1380E, Japan) that was linked to a monitor and a personal computer (Intel Pentium IV 2.0 GHz). The images were captured with the aid of a histometry software with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics, Milano, Italy). These images were used to calculate the osseointegration rate of implant surface. These measurements were later confirmed with the ones performed using the captured slides of transmitted light microscope to see if grinding and further processing of specimens had a significant effect on calculation of the osseointegration rate of implants. The specimens were then processed to obtain thin ground sections with a diameter of 30 µm using the same cut-off machine. The midsagital section was then stained with acid fuchsin and toluidine blue and was observed by an optical microsope (Nikon ECLIPSE E400, Japan) and photographed with another digital camera (Nikon Fujix HC_300Zi, Japan) that was connected to the same personal computer. After defining the osseointegration rate with the aid of the histometry software program, in order to define the type of regenerated bone and evaluate the amount of inflammation, the samples were decalcified with 10% formic acid and the implants were taken out. The sections were then stained with H&E and the bone tissue was observed under the same optical microscope and under a transmitted light microscope (Nikon E400, Epidsa, Tokyo, Japan) and images were captured with the same image-capturing facilities (Figures 1). The amount of inflammation present in the region was graded by the pathologist based on a scale of 0 to 3, where 0 stands for no inflammatory cells, and 3 stands for severe inflammation in all regions. The amount of lamellar and woven bone in the distance within 2 mm of implant surface was also calculated under transmitted light microscope by the aid of the histometry software.

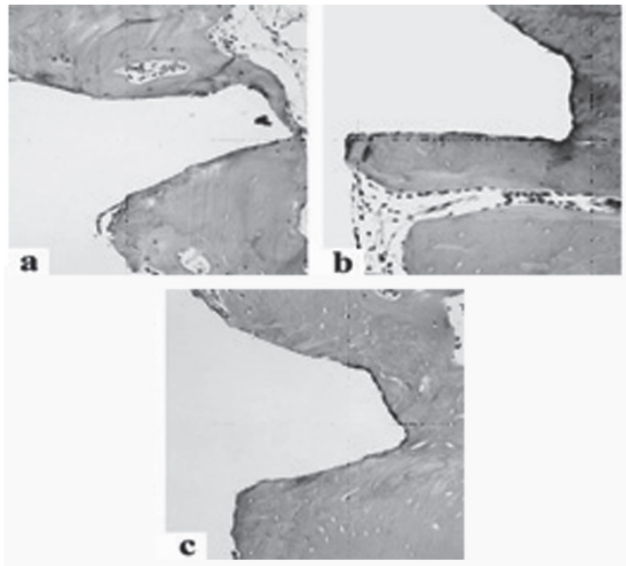

Figure 1: Histologic view of bone surrounding the implant. Bone tissue seems to be well generated and organized along the implant surface in all of the three groups. Few inflammatory cells could be detected along the contact surface. a) implants loaded 48 hours after surgery; b) implants loaded 1 week after surgery; c) unloaded implants (H&E ×100)

Statistical analysis was carried out using analysis of variance test (One way ANOVA). All the values were expressed as means ± SD. Statistically significant differences were set at $p<0.05$.

Results

Osseointegration of dental implants was evident in the three groups and newly formed bone and bone trabeculae were found in contact with the implant surface (Figures 1 and Table 1). Some portions of the implant surface were in direct contact with woven bone and some other areas were covered with lamellar bone. The orientation of lamellar bone varied in different regions and it was either perpendicular or parallel to the implant surface (Figures 3 and 4). Uncalcified osteoid matrix and bone marrow tissue were also found in contact with implant surface. The distribution of these areas did not seem to differ between the three groups. No inflammatory cells were found close to implant surface in none of the three groups.

Table 1: Osseointegration rate and bone generation composition around loaded and unloaded dental implants

Index	Unloaded group		Loaded groups			
			1 week		48 hours	
	Mean	SD	Mean	SD	Mean	SD
Osseointegration rate (%)	51.5	3.2	50.6	1.8	49.5	3.1
Lamellar bone formation at 2 mm (%)	60.4	1.9	60.1	1.8	59.9	1.8
Woven bone formation at 2 mm (%)	31.7	2.2	31.7	2.6	31.4	2.2

The three groups also showed no significant difference in the osseointegration rate of implants ($p>0.05$). The formation of lamellar and woven bone in the area within 2 millimeters of implant surface also did not seem to differ in the three groups ($p>0.05$). Prosthesis type did not have a significant effect on either the osseointegration rate or the type of bone generated in the area ($p>0.05$). No difference was also observed in the local inflammation of the three groups and the level of inflammation remained low in all of the observed samples. One implant from each group failed in this study. None of the remaining implants showed a level of mobility until the time of sacrifice.

No significant difference was discovered between the osseointegration rate calculated by the histometry software using images of stereomicroscope vs. transmitted light microscope.

Discussion

Mechanical load plays an important role in the development, maintenance and adaptation of the skeleton (12). Wolf's law demonstrates the connection between mechanical events such as stress or strain and biological events such as bone remodeling, bone formation and resorption (13). Bone adaptation is dependent upon strain magnitute, duration, frequency, history, type (compression, tension, or shear) and distribution (14). Finite element analysis studies have shown that the distribution of loud on substructure is deferent between deferent materials (14).

It has been reported that immediate loading may have the potential to increase the density of the alveolar bone around endosseous implants (15). It has also been reported that remodeling is evident and appeared to be more active near the implant surfaces (16) and peri-implant mineralized bone areas show a higher density within the threads of immediately loaded implants and also, new bone formation and active remodeling may be observed when the bone is mechanically stimulated (17).

Based on an animal study, Van Oosterwyck et al (9) have reported that excessively high dynamic implant loading can produce a pathologic overloading of bone, determining a higher level of marginal bone loss or, sometimes, a loss of osseointegration. Engquist et al (10) has also perceived overloading as the major cause for loss of immediately loaded implants; though these results are in disagreement with Heitz-Mayfield et al (13) who reported that in the presence of peri-implant mucosal health, a period of 8 months of excessive occlusal load, did not result in loss of osseointegration of dental implants and marginal bone loss. Therefore, restoration material can create deferent load and quality and loading within physiologic limits can be speculated to stimulate bone adaptation to loading, while this could explain that the loss of one implant from each group in this study could be a result of over loading, as oral hygiene was carefully maintained for the animals and primary stability was checked.

The results of this study confirm that the early loading of implants does not seem to compromise osseointegration and bone formation around the implants. These results are in agreement with those reported by Zubery et al that showed success rate does not differ between loaded and unloaded implants in dogs (17). The results of this study also confirm those of Degidi et al (18) that reported bone remodeling rate does not differ between different type of restoration of dental implants and kind of material had not interfered with lamellar or woven bone formation.

Conclusion

Comparing to unloaded implants, bone regeneration seems to have similar histologic characteristics around two kinds of restoration material used in this study in loaded dental implants and if properly carried out, the kind of restoration does not seem to affect osseointegration of implants and the type of bone generated in the area. The authors suggest further research on the effect restoration kind and type of dental material used instead of super structure on the pattern of bone regeneration and trabecular orientation and the bone remodeling process.

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