

Dental Implants: Early Versus Standard Two-Stage Loading (Animal Study)

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Direct bone formation on the implant surface is a treatment goal in implant dentistry. It was always thought that a healing period of 3 months for the lower jaw and 5–6 months for the upper jaw was required for osseointegration to occur. Recent studies, however, show that with the early loading protocol, osseointegration is possible as well. The goal of this study was to evaluate clinical, histologic, and histomorphometric parameters of implants with early loading protocols and implants that did not undergo the early loading protocol. In this experimental study, the first to the fourth premolar teeth were extracted from the lower jaws of 3 dogs. After a healing period of 3 months, 12 BioHorizons internal implants 4.5 × 10.5 mm were placed in the mandible of the dogs. The implant stability quotient (ISQ) was recorded. After 3 weeks, half of the implants were exposed, and after recording ISQ, polycarbonate crowns were placed on them and occlusion was adjusted so that there was no contact in centric occlusion and no lateral movement with the opposing teeth. After 3 months, the ISQs were recorded for all the implants. Bleeding upon probing and pocket depths were measured for the early loading implants. All the implants were removed using a trephine bur, and cross-sections of 150 μm were prepared, from which the bone implant contact (BIC) and the type of bone around them were obtained. Statistical analysis was conducted with independent *t* test, paired *t* test, and repeated analysis of variance. The BIC for the early loaded group was 46.17% ± 12.89%, and for the unloaded group was 44.4% ± 10.45%. This difference was not statistically significant (*P* = .811). The ISQ for the implants in the early loaded group (before they were removed) was 71 ± 7.35 and that of the unloaded group was 66.75 ± 11.86. These differences were statistically insignificant. With regard to the result of this study, and the fact that the 2 groups showed no statistically significant differences in a number of major aspects, such as BIC and ISQ, it seems possible to load implants with an earlier than usual protocol with no adverse effects on implant success. It is necessary, however, to follow certain protocols for this type of loading.

Key Words: dental implants, early loading, dog, bone to implant contact, histomorphometric study

INTRODUCTION

Dental implants are increasingly used to replace missing teeth. Direct contact between implant surfaces and the bone is a treatment goal in implant dentistry.¹

The prerequisite for the 2-stage surgical protocol described by Branemark et al² to achieve osseointegration is to place the implant below the osseous crest (subcrestally), cover the implants with soft tissue for 3–6 months, and keep pressure off the

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implants for this period. After this protocol, a second surgery to uncover the implants and apply prosthetic abutments is required. Many completely or partially edentulous patients have been treated using this protocol.²⁻⁴ In any situation, it was believed that a healing period of at least 3 months in the lower arch and 5–6 months in the upper arch was required for osseointegration to occur.^{2,3,5} The rationale for this waiting period and delayed loading is that early loading of implants can cause formation of fibrous tissue around the implants instead of direct contact with the bone.^{5,6} Recent studies, however, show that although immediate or early loading can induce the formation of fibrous tissue around dental implants, it is not necessarily responsible for formation of fibrous tissue around them.⁷

The logic of immediate and early loading is to reduce the formation not only of fibrous tissue but also of woven bone and to induce formation of mature lamellar bone to resist occlusal forces.^{1,8} It should also be noted that early loading is done when the bone is at its weakest point after surgery (3–5 weeks).¹

There are different criteria for evaluating the health of dental implants, such as survival rate, crestal bone loss, probing depth, bleeding index, and mobility.⁹⁻¹³ Osseointegration, which is direct contact of the bone with the implant body, can provide prosthetic support and transmit occlusal forces to the bone.¹⁴ According to Branemark's definition, osseointegration is the direct contact of the bone with the implant, as can be observed under a magnifying optical microscope on at least some point of the implant body.² It is important to note that osseointegration refers to the direct contact between the implant and the bone, which can be verified under a light microscope. However, 100% osseointegration never occurs around the implant surface.

At the light microscopy level, successful cases have shown 30%–95% osseointegration.¹⁵ Success in osseointegration depends on 4 important basic factors: (1) biocompatible implants, which means the materials used in the implants should not stimulate the patients' immune system; (2) close contact between the bone and the implant; (3) use of a surgical technique with the most minimal mechanical and heat traumas caused by the drill; and (4) minimized force on implants after their

placement. It was thought that a period of 3–6 months was necessary for complete healing before implant loading; however, recent studies show that integration occurred in implants loaded early integrated and those loaded later and that early loading induces osseointegration and even prevents bone loss.¹⁶

In this study, we used the following definitions for loading time (as defined in a congress held at Barcelona, Spain, in May 2002¹⁷): immediate loading, the prosthesis is delivered on the same day of implant surgery; early loading, the prosthesis is delivered after implant surgery in a separate appointment that is earlier than the standard protocol (3–6 months); delayed loading, the prosthesis is delivered in a separate appointment after a healing period of 3–6 months; occlusal loading, the crown or the bridge is in contact with the opposing teeth in centric occlusion; nonocclusal loading, the crown or the bridge is not in contact with the opposing teeth. Ghanavati et al¹⁸ demonstrated that loading time had no effect on the presence of inflammatory cells, spread of the lamellar and woven bone, and bone-implant contact (BIC). Sagara et al¹⁹ conducted a study on 3 groups of implants (group 1 = one stage on soft diet; group 2 = one stage on hard diet; group 3 = two stages). In general, new bone formation was seen on the surfaces of all the implants in the 3 groups, and no fibrous tissue was seen in any of the 3 groups. The amount of BIC was compared between the 3 groups, and the highest was obtained in group 3 (57.4% ± 15.6%), which was significantly different from that in group 2 (35.5% ± 11.7%) and group 1 (40.1% ± 19.6%); however, the differences in BIC between groups 1 and 2 were not significant. In a study by Rocci et al,²⁰ the histomorphometric measurements in 5 patients with 2 implants immediately loaded showed BIC of 92.9%, whereas implants with early loading showed BIC of 81.4%. The authors concluded that both groups of early loading and immediate loading did osseointegrate normally. In addition, other studies showed survival rates of 93.4%, 94.1%, 96.2%, 96%, 97.7%, 99.1%, and 100% for early loading.²¹⁻³¹

MATERIAL AND METHODS

This study was an experimental animal study. The inclusion criterion required the dog to be 1 to 4

years old. Each subject's lower first to fourth premolars were extracted, and the canines and first molars were retained. Each group consisted of 6 implants (unloaded and early loaded implants).

After we described the study protocol and obtained approval from the ethics committee of the Esfahan Dental School, we selected 3 dogs for the study. The dogs were examined by a veterinarian to check health status and whether they met the study criterion. Each dog was anesthetized with intravenous ketamine hydrochloride (5 mg/kg), followed by inhaled nitrous oxide. This procedure was used at every step in which anesthesia was necessary.

The posterior teeth in the lower jaw from the first to the fourth premolars were extracted. These teeth had divergent roots; hence, they were separated from the furcation area using a high-speed hand-piece and then taken out separately using forceps to minimize trauma during the extraction. Periodical radiographs were taken after extraction to ensure that no root tips were left behind.

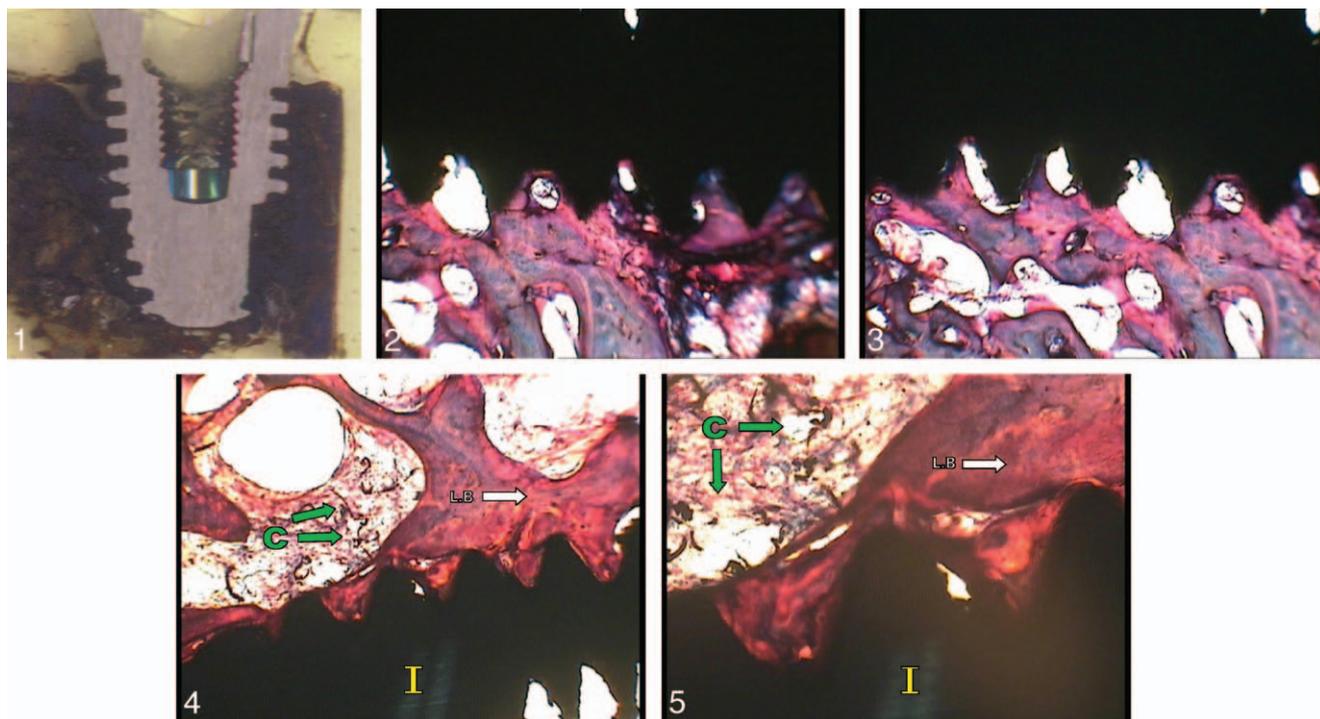
After 3 months, the dogs were anesthetized. Periapical radiographs were taken to confirm complete healing. The flap was reflected using a crestal incision, and the site was prepared with a starter drill for implant placement. Drill depths of 2×10.5 mm and 2.5×10.5 mm were made. The final preparation was made with 3.0- and 3.2-mm width-increasing drills. In each lower jaw, 4 internal implants (Biohorizons Implant Systems, Birmingham, Ala) with a 4.0-mm diameter and a 10.5-mm length were placed. These lengths and measurements were based on those used in the study by Schnitman et al³² The implants were placed with a Novag micromotor (Micro Dispenser, Novag AG, Goldach, Switzerland) and rotated at 800 rpm according to the manufacturer's recommendation. A torque of 50 Ncm was recorded by using a digital torque wrench to establish good primary stability and to ensure that all of the implant threads were in the bone and only their high polished surfaces were above the bone.

We ensured that all of the implants had the proper initial stability by this way.^{33,34} Upon removal of the abutments and with the aid of a proper smart peg (type 27) and Osstell (Integration Diagnostics, Savedalen, Sweden), the implant stability quotient (ISQ) of each implant was recorded, the cover screws were placed, and all of the

implants were submerged. The final positions of the implants were verified using periapical radiographs. Three weeks after the implants were placed, the dogs were anesthetized, half of the implants were randomly exposed, and the abutments were placed and tightened up to 30 Ncm using a ratchet and a torque wrench. The polycarbonate crowns (Williams Dental Service, Täby, Sweden) were relined using an acrylic resin trim (Trim II, Harry J. Bosworth Co, Skokie, Ill) and cemented onto the abutments using zinc phosphate cement (De Trey Zinc Cement AD, International Ltd, Wey Bridge, UK). Occlusion was adjusted to prevent contact in centric occlusion or lateral movement.

Three months after the implant placement, with the dogs on a plaque control program and soft diet in the first 4 weeks and a regular diet thereafter, the last step was started. As in the previous steps, the dogs were anesthetized. The implants with polycarbonate crowns were measured for probing mesiobuccal, mesiolingual, distolingual, and distobuccal depths (in millimeters). The bleeding upon probing was recorded. At the end of the procedure, the provisional crowns and the abutments were removed. The implants were removed along with the surrounding bone using a trephine bur with a 10-mm diameter. It is important to note that by using this method it was not necessary to kill the dogs, which is a strong point of this study. To prepare the samples for the next steps, the following procedures were done:

1. The samples were first fixed and dehydrated.
2. The samples were placed in blocks containing cold-cure acrylic resin with the aid of a surveyor (Meliodent, Heraeus Kulzer, Berkshire, UK).
3. Cross-sections of all the samples were prepared by means of a grand section using a cross-section instrument (Accutom-50, Struers, Copenhagen, Denmark).
4. Cross-sections were cut from the longitudinal axis of the implants at a thickness of 150 μ m (Figure 1).
5. The samples were stained (Masson's Trichrom Staining, AR 173, Dako, Glostrup, Denmark) and placed under a microscope. The samples stained for LAM (Figure 1) were studied under a light microscope with a digital lens of $\times 40$ magnification. The BIC and the percentages of the different types of bone were recorded (and repeated 2 times). For verification purposes, BIC was again



FIGURES 1–5. **FIGURE 1.** Low magnification: it is possible to see that the coronal portion of the implant is covered by compact, mature, lamellar bone original magnification $\times 1$). **FIGURE 2.** Bone trabeculae formation around the loaded implant (bone implant contact [BIC]) (Masson’s trichrome; $\times 40$). **FIGURE 3.** Bone trabeculae formation around the control implant (BIC) (Masson’s trichrome; $\times 40$). **FIGURE 4.** Quality of bone and connective tissue around the loaded implant (Masson’s trichrome; $\times 400$); C = Connective tissue (green arrow), L.B. = lamellar none (white arrow); I = implant. **FIGURE 5.** At higher magnification, it is possible to see the types of bone (lamellar and woven) and connective tissues available at the interface. (Masson’s trichrome; $\times 100$); C = connective tissue (green arrow); L.B. = lamellar bone (white arrow); I = implant.

read with the help of images of the cross-sections of the samples and Adobe Photoshop 7.0 (San Jose, Calif). The statistical analysis was carried out using the SPSS 11.5 software (SPSS, Chicago, Ill), an independent *t* test, repeated measures analysis of variance, and a paired *t* test.

RESULTS

The different parameters were evaluated in the 2 different loading systems: the early loading versus unloading protocol. These parameters included BIC, ISQ, probing depth, bleeding upon probing, and histologic findings of bone type 2 mm around the implants.

Bone-implant contact

In the early loaded group, the BIC was within the range of 37%–70%, with a mean of $46.17\% \pm 12.89\%$; for the unloaded group, BIC was within the range of 35%–60%, with a mean of $44.40\% \pm$

10.45%. One of the samples in the unloaded group ($n = 5$) was lost during the preparation. The results of the independent statistical testing showed no difference in mean BIC between the early loaded and the unloaded groups ($P = .611$; Figures 2 and 3; Table 1).

Implant stability quotient

The ISQs obtained from the Ostell devices right after implant placement (ISQ1), 3 weeks after placement (ISQ2), and 3 months after placement (ISQ3) were recorded. Table 2 shows that the ISQ1 values for the early loaded group were within in the range of 56–73, with a mean of 62.5 ± 6.53 , whereas those of the unloaded group were within 51–78, with a mean of 62.17 ± 10.59 .

For the early loaded group, the ISQ2 values measured in the third week were within the range of 54–60, with a mean of 57.67 ± 2.88 . The ISQ3 values for the early loaded group were within the range of 62–78, with a mean of 71 ± 7.35 , whereas

TABLE 1
Percent bone implant contact values in 2 groups

Group	N	Mean	SD	SE	95% CI for Mean		Minimum	Maximum	Significance Between Groups
					Lower Bound	Upper Bound			
Early	6	46.17	12.89	5.26	32.64	59.69	37	70	.811
Unloaded	5	44.40	10.45	4.68	31.42	57.38	35	60	

those for the unloaded group were in the range of 51–80, with a mean of 66.75 ± 11.86 .

To evaluate the effect of early loading on the ISQ value, the ISQ value before implant removal was obtained for the early loaded and unloaded groups, for which statistical analysis showed no significant difference between the two groups ($P = .473$). We obtained a statistically significant difference between the ISQ1, ISQ2, and ISQ3 values in the early loaded group ($P = .009$). The paired t test result showed a statistically significant difference between the ISQ2 and ISQ3 values ($P = .014$). However, there was no statistically significant difference between the ISQ1 and ISQ2 values ($P = .191$) or between the ISQ1 and ISQ3 values ($P = .059$). In the unloaded group, there was no statistically significant difference between the ISQ obtained on the day of implant placement and that obtained before implant removal ($P = .581$) according to the results of the paired t test.

Probing depth

Probing depth was measured at 4 points: MB, DB, ML, and DL for early loaded implants. This variable was not possible to measure for unloaded implants because they remained submerged until the day they were removed. The data obtained are recorded in Table 3.

Bleeding upon probing

The bleeding status of each group was documented. In the early loaded group, upon probing, 3 samples (50%) had bleeding, whereas the other 3 samples (50%) had none. This variable was not measurable for the unloaded implants because they stayed submerged until the day they were removed.

Type of bone 2 mm around the implants

In the 2-mm implant vicinity, which consisted of woven and lamellar bones, some connective and inflammatory tissues could be seen. It should be noted that one of the unloaded samples ($n = 5$) was lost during the preparation. Woven bone formed 33.2%–40.0% (mean \pm SD = $36.22\% \pm 2.63\%$) of the bones surrounding the early loading implants and 32.3%–40.2% ($35.80\% \pm 2.97\%$) of the bones surrounding the unloaded implants (Figures 4 and 5). Lamellar bone formed 52.2%–60.5% ($56.75\% \pm 3.32\%$) of the bones around the early loading implants and 53%–61% ($57.8\% \pm 3.11\%$) of the bones around the unloaded implants (Table 4; Figures 4 and 5). Connective and inflammatory tissues of the early loaded implants were $7.03\% \pm 0.9\%$ and for the unloaded implants were $6.8\% \pm 3.41\%$. To determine the effect of early loading on the type and the amount of bone around the implants, comparison of the early

TABLE 2
Statistical data on the implant stability quotient in different time intervals in unloaded and early loaded implants

Time	Group	N	Mean	SD	SE	95% CI for Mean		Minimum	Maximum	Significance Between Groups
						Lower Bound	Upper Bound			
Day of insertion	Early	6	62.50	6.53	2.66	55.65	69.35	56.00	73.00	.949
	Unloaded	6	62.17	10.59	4.32	51.05	73.28	51.00	78.00	
3 weeks later	Early	6	57.67	2.88	1.17	54.65	60.68	54.00	60.00	-----
3 months later	Early	6	71.00	7.35	3.00	63.29	78.71	62.00	78.00	.473
	Unloaded	6	66.75	11.86	4.84	54.31	79.19	51.00	80.00	

TABLE 3
Probing pocket depth at 4 points of early loaded and unloaded implants

Location	Mean	SD	SE	95% CI for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
PPDMB	2.17	0.41	0.17	1.74	2.60	2	3
PPDML	2.33	0.82	0.33	1.48	3.19	1	3
PPDDB	2.00	0	0	2.00	2.00	2	2
PPDDL	2.17	0.75	0.31	1.38	2.96	1	3

*PPDMB indicates probing pocket depth mesio-buccal; PPDML, probing pocket depth mesio-lingual; PPDB, probing pocket depth disto-buccal; PPDDL, probing pocket depth disto-lingual.

loaded and unloaded groups was made using an independent *t* test (Table 4).

In lamellar and woven bone content of the bones surrounding the implants, there was no significant difference between the 2 groups (*P* = .605), as well as in connective and inflammatory tissue composition (*P* = .582).

DISCUSSION

This study was conducted to evaluate the clinical, histologic, and histomorphometric parameters of Biohorizons dental implants using the early loading protocol. The parameters, that is, BIC, insertion torque, pocket depth at 4 points, and type of bone in the 2-mm vicinity of the implants, were evaluated and used as criteria for success in early loading of implants as opposed to unloaded implants. Early loading can provide patients with a shorter treatment time and, therefore more comfort.

A total of 12 Biohorizons implants with a 10.5-mm length and a 4-mm diameter were placed in the lower jaws of 3 dogs; 6 of the implants were not loaded at all, and 6 were loaded 3 weeks after the placement of the implants with polycarbonate crowns. A review of the literature on implant

loading indicates the important role of mechanical forces on bone loss prevention. Wolf's law describes the correlation between mechanical events, such as stress and strain, and biological events, such as bone remodeling formation or resorption.³⁵⁻³⁷ Bone contact depends on strain value and type (compressive, tensile, or shear), time, and frequency.³⁸ In an animal study by Ghanavati et al,¹⁸ the BIC in the unloaded group was 51.5% ± 3.2%, whereas that in the early loaded group was 50.6% ± 1.8%. Although the value is a bit higher in the former group, the difference is not statistically significant. In a study by Sagara et al,¹⁹ of 3 groups of implants studied, the BIC was 57.4% ± 15.6% in the unloaded group, where implants remained submerged during the healing period; 40.1 ± 19.6% in the group where implants were exposed to the oral cavity without loading; and 35.5 ± 11.7% in the third group with loading. These differences were not statistically significant.

In a study by Rocci et al,²⁰ the BIC was 92.9% for the immediate loading group and 81.4% for the early loading group; however, the differences were not statistically significant. The results of our study also indicate a difference between the 2 groups; that is, the BIC in the early loaded group was higher

TABLE 4
Composition of bone in the 2 mm around implants

Type of Tissue	Group	N	Mean	SD	SE	95% CI for Mean		Minimum	Maximum	Significance Between Groups
						Lower Bound	Upper Bound			
Woven bone (%)	Early	6	36.22	2.63	1.07	33.46	38.98	33.20	40.00	.811
	Unloaded	5	35.80	2.97	1.33	32.11	39.49	32.30	40.20	
Lamellar bone (%)	Early	6	56.75	3.32	1.36	53.26	60.24	52.20	60.50	.605
	Unloaded	5	57.80	3.11	1.39	53.93	61.67	53.00	61.00	
Inflammatory and connective tissue (%)	Early	6	7.03	0.90	0.37	6.08	7.98	6.00	8.00	.582
	Unloaded	5	6.40	2.55	1.53	2.56	11.04	4.50	12.70	

than that of the unloaded group ($46.17\% \pm 12.89\%$ versus $44.4 \pm 10.45\%$, respectively). However, this difference was not statistically significant ($P = .811$). The results of this study are consistent with the results of the study by Ghanavati et al¹⁸ and Sagara et al.¹⁹ However, in this study, the BIC was $46.17\% \pm 12.89\%$, whereas in a study by Rocci et al,²⁰ it was 81.4% . This finding may be due to the differences between the 2 studies.

In the study by Rocci et al,²⁰ machined-surface implants were used and the loading time was 5–9 months. In this study, however, the surfaces of the implants were treated with resorbable blast media and the loading time was 3 months. In addition, the thread designs of the implants were different in the 2 studies. All of these factors could be the cause of the differences between the 2 studies. Therefore, it seems that whether or not loading was performed during the first 3 months has no effect on BIC.

Another aim of this study was to determine the stability of the implants by resonance frequency analysis. In a study by Olsson et al,²¹ implants were placed in the upper jaws of 10 patients and an ISQ of 60.1 was obtained. In our study, the implants were placed in the lower jaws of the dogs and an ISQ of 62.5 ± 6.53 was obtained for the early loading group and 62.17 ± 10.59 for the unloaded group. The values obtained in these 2 studies are similar. The ISQ value obtained in our study was slightly higher than that obtained in the other study, where the implants were placed in the upper jaws of humans; the difference may be due to the differences between dog and human bones. In addition, in our study, the implants were placed in the lower jaws of the dogs, where bone is denser.

An interesting point is that it has been reported in earlier studies that implant stability is at its weakest 3 weeks after implant placement.³³ In other words, the implant-bone surface is at its weakest after 3–5 weeks of implant placement.³⁴ The results of the current study also support this finding, as the ISQ in the early loaded group right after implantation (ISQ1) was 62.5 ± 6.53 , and that after 3 weeks, when the implants were loaded (ISQ2), was 57.67 ± 2.88 . Before the implants were removed after 3 months, ISQ3 increased to 71.0 ± 7.35 . Therefore, ISQ2 was the lowest of the 3 measured ISQ values. These results confirm the findings of earlier studies. The difference between the ISQ2 and the ISQ3 values was statistically significant ($P = .14$); however,

the difference between the ISQ1 and ISQ3 values was not significant ($P = .059$). The differences might have been significant if our sample size had been larger. Our ISQ value before implant removal in the early loaded group was greater than that in the unloaded group (71 ± 7.35 versus 66.75 ± 11.86), which shows higher stability for the early loaded implants than for the unloaded implants. However, this difference was not statistically significant ($P = .473$).

The survival rates of the dental implants using different loading protocols are discussed in various journal articles. Most of these discuss the survival rates of early loaded and unloaded implants within a specific time frame. In a study by Ghanavati et al,¹⁸ the overall survival rate of implants in the early and unloaded groups was 93.7%. Furthermore, in a study by Olsson et al,²¹ a survival rate of 93.4% was recorded for the early loaded implant after 1 year. De Smet et al²² reported a 94.1% success rate. In the study by Randow et al,²³ a 100% success rate was obtained. In a study by Cooper et al,²⁴ the success rate was 96.2%. In the study by Vanden Bogaerde et al,²⁵ a 96% success rate was obtained, whereas in the study of Testori et al,²⁶ a 97.7% success rate was obtained. Rocuzzo et al²⁷ reported a 100% success rate in their study, whereas Cochran et al²⁸ reported 99.1% (all in the early loaded group). Payne et al,²⁹ Roynesdal et al,³⁰ and Misch and Degidi³¹ reported a 100% success rate for all of the implants. Similarly, in this study, a survival rate of 100% was obtained in the unloaded and loaded groups. Therefore, none of the implant placements failed whether the load was placed early or during the healing phase. Lamellar and woven bone distribution (separately) between the loaded and unloaded groups showed no significant difference in our study; these results are similar to those of obtained by Ghanavati et al.¹⁸

Based on the aforementioned results, it can be concluded that loading time has no effect on the type of bone formed. As mentioned previously, the goal of early loading is not only to reduce fibrous tissue formation but also to induce lamellar bone formation to resist occlusal forces.^{1,8} The results of this study showed no significant difference in bone type in the 2 different loading systems, although in the early loaded group, the percentage of lamellar bone ($56.75\% \pm 3.32\%$) was higher than that of woven bone ($36.22\% \pm 2.63\%$).

In the study by Ghanavati et al,¹⁸ the degree of

inflammation around the dental implants was assessed by a pathologist on a scale of 0 to 3 (0 = no inflammation and 3 = severe inflammation). Inflammatory cells were not observed in any of the groups. In this study, the inflammatory and connective tissues (or nonbony tissues in general) comprised $7.03\% \pm 9\%$ of the surrounding area of the dental implants in the early loading group and $6.40\% \pm 2.55\%$ of that in the unloaded group; however, the differences are not statistically significant ($P = .582$). Therefore, the loading time had no effect on the degree of inflammation or the connective tissue formation.

CONCLUSION

In view of the present discussion, it can be concluded that there was no statistically significant difference between the unloaded and loaded groups in extent of BIC at the light microscope level. No implant failures were seen in either group. Contrary to the belief that for osseointegration to occur, a time interval of >3 months is required, loading can be done earlier with no adverse effect on osseointegration or implant survival rate.

ABBREVIATIONS

BIC: bone implant contact
 DB: distobuccal
 DL: distolingual
 ISQ: implant stability quotient
 MB: mesiobuccal
 ML: mesiolingual

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