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Osseointegration of dental implants in *Macaca fascicularis*

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Abstract. Osseointegration is an important factor in determining the success of a dental implant. It can be assessed from the osseointegration that occurs between the implant and the bone. The implant stability is determined by the osseous support at the implant-bone interface, which is commonly evaluated by histomorphometric analysis. This study aimed to evaluate whether the osseointegration level measured by a Low Resonance Frequency Analyzer (LRFA) gave results as good as those obtained by histomorphometric examination. Six male *Macaca fascicularis* were used in this study. In each animal, two types of loading were performed: immediate and delayed loading. Clinical examination and LRFA measurement were performed to determine osseointegration at the first and second weeks and at the first, second, third, and fourth months. After four months, histomorphometric examination was performed. The relationship between the histomorphometric examination and LRFA measurement was compared using the Pearson correlation coefficient. There was no significant difference in the osseointegration between immediate loading and delayed loading ($p > 0.05$) The bone-implant contact percentage in the first group did not differ significantly from that in the second group. Statistical analysis showed that there was a strong correlation between LRFA measurement and histomorphometric examination. Osseointegration could be evaluated through LRFA measurement as well as through histomorphometric examination.

1. Introduction

The success of an implant is assessed from the osseointegration between the implant and the bone. A detailed evaluation using radiographic photos and light and electron microscopy examinations is needed to examine osseointegration. These examinations reveal the growth of connective tissue around the implant. This connective tissue may cause clinical mobility and further failure of the dental implant [1-4]. Osseointegration is the tissue integration that occurs at the interface area between a bone and a functional and loaded implant [3]. Osseointegration is the only way to increase the success of the tissue integration of a root-formed implant. If the direct development of bone apposition in the interface area is disturbed, the percentage of fibrous tissue grown will increase progressively, and consequently, the implant will have to be removed. Osseointegration could also occur successfully in a blade/plate-formed implant with high percentage of bone apposition around the root-formed implant [1]. The biomechanical condition around an implant plays an important role in determining the quality and composition of the new interface. For example, a study showed that if an implant is stable in the bone at the time of placement, then osseointegration will occur successfully at its interface [5].

Palmer et al. noted that osseointegration is basically a type of integration between the bone and the implant surface. It is not an absolute phenomenon; however, the proportion of the entire bone implant surface can be measured histologically. The highest contact level is found in cortical bone compared to

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cancellous bone. High-density cortex and trabeculae-type bone have higher contact degree with an implant. The contact degree between the bone and the implant increases with time [6]. The surface of a functioning implant during recovery shows three types of tissue integration: osseointegration, osseopreservation, and periosteal integration. All three have been proved to be safe and effective. All endosteal implant surfaces have similar types of tissues irrespective of how tissue integration has developed. They contain vascular and neural tissues; the prominent tissues are cortical and cancellous bone, bone marrow, and collagen fiber. The percentage of each tissue type and their surface distribution on the implant differs depending on the type of endosteal tissue integration. A dental implant practitioner should understand the biomechanics of tissue integration, which play an important role in the diagnosis and therapy plan, as well as the restoration and long-term prognosis [1,6,7].

Lawrence et al. noted that implant stability is the characteristic needed to achieve osseointegration [8,4]. At the time of implant surgery, for achieving good initial stability, excellent alveolar bone quality, proper implant design, and good surgical technique are required. An implant engrafted in bone with optimum density will have higher stability compared to one engrafted in cancellous bone [9-11]. Lawrence et al. reviewed various methods for assessing the stability of an engrafted implant from the time of insertion to the development of complete osseointegration. These methods include the percussion test, radiograph, periotest, dynamic modal test, reverse torque, resonance frequency analysis, impulse test, Implatest, and Implatest FFT signature analysis. Implatest is the newest method for noninvasively assessing the development of osseointegration at the time of implant placement; it can also be used for long-term assessment during the development of osseointegration [11].

Marina et al. found that the osseointegration quality could be evaluated by using an ultrasonic device. Ultrasonic transmission depends directly on the acoustic differences between neighboring materials. The higher the acoustic transmission of a material, the higher is its transmission intensity. By using an ultrasonic device, the ultrasonic transmission across the implant surface can be detected from the wave dilatation to monitor the osseointegration of an inserted implant [12]. Histomorphometry can also be used for examination; it is a standardized and accurate method for assessing osseointegration in dental implants. A histomorphometric examination can be performed by measuring the amount of bone-implant contact (BIC). The greater the BIC, the more successful is the osseointegration.

2. Materials and Methods

In this study, six 6-year-old *Macaca fascicularis* weighing 4.5–6 kg were used. The study procedures were approved by the Ethics Committee of the PSSP. Each subject received five dental implants (Imtek IMD USA) of 7-mm length and 2.1-mm diameter on the mandibular jaw. The implants were placed into the mandibula in place of teeth that were extracted 1 month earlier. All subjects were quarantined for 6 weeks, and they were free of systemic or local diseases. There were five different treatment groups. Group 1: immediate implant loading on the same day as implant placement, with normal contact restoration. Group 2: immediate implant loading on the same day as implant placement, with light contact restoration. Group 3: implant loading 14 days after implant placement, with normal contact restoration. Group 4: implant loading 14 days after implant placement, with light contact restoration. Group 5: delayed implant loading as a control group. The subjects were evaluated clinically and radiographically immediately after implant placement and after 7 days, 14 days, 1 month, and 2 months.

The titanium implants were placed in the subjects' jaws two months after tooth extraction. Before implantation, the subjects were intravenously anesthetized with ketamine (15 mg/kg). After sedation, which took ~5 min, general anesthesia was intravenously administered with a 1.8-ml Propofol bolus (4 mg/kg). Then, the implant was inserted without opening the lid (flapless). To determine the location of the implant placement is by creating a sign using the probe. The specimens were sectioned longitudinally along the major axis. The core specimens were fixed with 10% buffered formalin. Each core was decalcified with 5% formic acid and embedded in paraffin. A series of 4–6 μm thick longitudinal sections were cut through the central area of the core specimens by using a microtome.

Three slides were obtained for each specimen. The slide was stained with hematoxylin and eosin (H&E). Histomorphometric analysis was performed by measuring the BIC and calculating the percentage. A projection microscope (Olympus) with a digital image capture device was used for morphometric measurements (with 40x magnification).

3. Results and Discussion

3.1 Results

Histomorphometric measurements could be performed in only five out of six existing teeth. Table 1 shows the descriptive histomorphometric results according to the five treatment groups.

Table 1. Histomorphometric scores of five treatment groups

Histomorphometric	Treatment				
	ILNC1	ILNC14	ILLC1	ILLC14	Control
N	5	5	5	5	5
Minimum	0.1975	0.3698	0.5029	0.6108	0.6400
Maximum	0.5653	0.5813	0.6733	0.8040	0.8302
Median	0.3690	0.5318	0.5168	0.7980	0.8045
Mean	0.3759	0.5067	0.5631	0.7423	0.7566
SD	0.1326	0.0803	0.0755	0.0859	0.0848

Distribution of research groups:
ILNC1: Immediate Loading Implant, Normal Contact, Day 1
ILNC14: Immediate Loading Implant, Normal Contact, Day 14
ILLC1: Immediate Loading Implant, Light Contact, Day 1
ILLC14: Immediate loading implants, Light Contact, Day 14

The histomorphometric scores showed wide variations across the four groups. ILNC1 showed the most variation. ILLC14 and the control group had the highest mean score, and ILNC1 had the lowest mean score. In the five treatment groups, the histomorphometric score follows a normal distribution ($p > 0.05$).

Table 2. Correlation between histomorphometry and LRFA

Group	r	p-value
ILCN1	0.81	0.093
ILCN14	0.84	0.037
ILLC1	0.95	0.014
ILLC14	0.89	0.041
Control	0.89	0.041

Statistical analysis using Pearson's correlation test
$p > 0.005$ indicates no statistically significant difference

Pearson's correlation test was used to analyze the relationship between histomorphometry and LRFA. A strong correlation was found between histomorphometry and LRFA in the ILLC1, ILLC14, and control groups ($p < 0.05$).

3.2 Discussion

Histomorphometric examination is a standardized and accurate method for assessing the osseointegration of dental implants. A histomorphometric examination can be performed by measuring the amount of BIC. The greater the BIC percentage, the more successful is the osseointegration [13-

15]. In this study, histomorphometric examination was performed as a benchmark for other methods to evaluate osseointegration, such as LRFA. The histomorphometric examination results showed that the score for each test parameter and different controls was statistically significant. The lowest and highest scores were seen for normal occlusal contact on the first day and the control group, respectively. A difference was seen between normal and light occlusal contact with good intervention performed immediately on the first day and occlusal contacts performed after 14 days.

Previous research noted that osseointegration should not be defined as the occurrence of 100% bone apposition [5,16-18]. BIC of only 20% at a microscopic level that is distributed at the interface area can also be considered as osseointegration. The average BIC reaches ~35.6%. In this study, almost all histomorphometric examinations showed BIC above 20%; only ILLC1 showed a score of 19.17%. Therefore, based on the assumption, nearly all groups showed adequate histomorphometric scores, although with different percentages.

4. Conclusion

Osseointegration is crucial to the success of dental implant treatments. Osseointegration can be evaluated through histomorphometric measurements, which are considered the most accurate method for this purpose. There is a positive correlation between histomorphometry and LRFA. LRFA is more suitable because it is noninvasive and can be performed clinically.

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