# Effect of Remaining Periodontal Ligament on the Healing-up of the Implant Placement

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Abstract: Tooth-shaped titanium implants were placed into a tooth socket with adhering periodontal ligament after extraction to examine the regeneration of periodontal tissues from remaining periodontal ligament cells. The extraction forces of the implants were measured with a materials testing machine. The mechanical strength of the peri-implant ligament increased markedly from 14 (25% of control) to 28 (68% of control) days. The implants and surrounding tissues were observed morphologically. The result showed that cementum-like hard tissues were formed on the surface of the titanium implants and there were many collagen fiber bundles between the cementum-like tissues and alveolar bone at 21 and 28 days. These findings suggest that placement of an implant into a socket with periodontal ligament leads to formation of new cementum-like hard tissues with functionally-oriented collagen bundles and development of adequate mechanical strength.

Key words: cementum tissue, periodontal ligament, tensile test, titanium dental implant, undecalcified specimen

## Introduction

Osseointegration is considered the most acceptable interface connection around dental implants. However, the osseointegrated implants cannot successfully accomplish sensory, nutritive, and homeostatic functions as the normal periodontal ligament (PDL) does.

In this study, we examined the extraction forces of implants by mechanical testing, and assessed the support of new attachment with peri-implant fibrous ligament, and morphologically observed the healing process of the newly-formed attachment tissue around titanium implants inserted in sockets with adhering PDL after tooth extraction in the rat model.

## Materials & Methods

***Design of tooth-shaped titanium implants:***

One tooth was selected as a standard model in eleven extracted left maxillary first molars (Fig. 1a). From a mold of this model, 35 tooth-shaped titanium implants (Fig. 1b) were manufactured.

***Procedure of placing implants:***

Male Wistar rats, aged 41~43 days, were used. Under anesthesia, the left maxillary first molars were extracted. Tooth-shaped implants were then placed into the tooth sockets (Fig. 1c). After the experimental period, the implanted maxillae were collected.

***Tensile test:***

Thirty Wistar rats were divided into implant (n=20) and control (n=10) groups. In the implant group, tooth-shaped implants were placed as described above, and these animals were killed at 14 (n=7) and 28 (n=7) days after implantation. In the control group, 10 rats were killed at 0 day. A load-deformation curve was obtained by extracting each implant from its socket using a materials testing machine at a speed of 5 mm/min. From each load-deformation curve, the maximum load was recorded and considered as extraction force. The differences in the mean extraction forces between any two groups were examined by the Scheffé method.

***Undecalcified sections:***

Twenty-six Wistar rats were divided into implant (n=15), and control (n=8) groups. At 7, 14, 21, and 28 days after implantation, the rats were perfused with 4% parafolmaldehyde solution. Each maxilla containing the implant was embedded in methyl methacrylate. Longitudinal mesio-distal sections were cut parallel to the long axis. The sections were ground down to about 100 ìm, surface-stained with basic fuchsin and methylene blue, and observed with a light microscope under ordinary or polarized light. After that, three sliced specimens were selected from the implant group and surface-stained with alizarin red.

## Results

***Tensile test:***

The load-deformation curves (Fig. 2a) for the PDL in the control group (n=9) were non-linear and roughly sigmoid in shape. The shapes of the load-deformation curves for peri-implant tissues were similar to those for PDL. However, the levels of loads and deformations of the curves for the peri-implant tissue at 14 days (n=7) were less than those for the PDL. The levels for the peri-implant tissue increased considerably at 28 days (n=7).

The mean value of the maximum loads (Fig. 2b) for the peri-implant tissue at 14 days was 75% less than that for the PDL ($p<0.05$). The mean value at 28 days increased markedly (2.7 times) compared to that at 14 days ($p<0.05$). The mean value at 28 days was 32% less than that of natural teeth, although there was no significant difference between the two values.

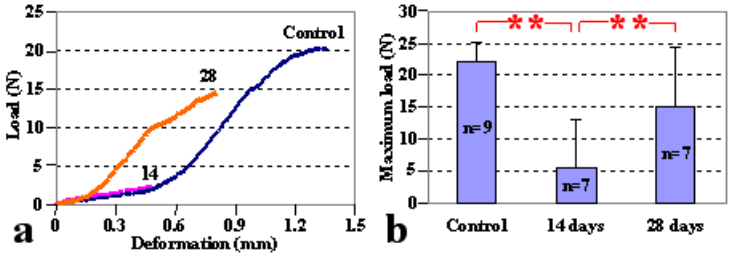
***Morphologic observations***

***Light and polarized light microscopic images:***

At 7 and 14 days after implantation, many round cells were observed adjacent to the implant surface in the peri-implant tissue

Fig.1


Fig.2




(Fig. 3a), as in natural teeth. At 14 days, methylene-blue stained cementum-like tissues were observed close to the implant surface in various regions. The methylene blue-stained the cementum-like tissues were stainable with alizarin red indicating that calcium was deposited in these structures. At 21 and 28 days, methylene-blue stained hard tissue structures were frequently seen in direct contact with the implant surface, as in Fig. 3b.

Polarized light microscopy showed that in the apical peri-implant tissues, birefringent collagen fiber bundles were oriented obliquely and vertically from the implant surface to the alveolar bone at 21 and 28 days. Near the alveolar crest, birefringent fibers extending from the bone were oriented almost perpendicular to the implant surface, however their orientation close to the implant surface was indistinct. In the cervical region, supracrestal birefringent fiber bundles were running from the implant surface to the lamina propia of the gingiva similar to normal periodontal tissues at 7, 14, 21 (Fig. 3c), and 28 days.

## Discussion

The characteristic of the present study is that after tooth-shaped implants were inserted into molar tooth sockets with adhering PDL tissues, the process of the regeneration of the support function of newly-formed attachment with functionally-oriented fiber bundles around the implants was evaluated by biomechanical and histological observations.

Our tensile test data quantitatively demonstrated that the mechanical strength of the peri-implant tissue recovered considerably from 14 to 28 days and that the peri-implant tissues at 28 days exhibited load-deformation curves similar to those for the normal PDL. Thus, the newly-formed peri-implant tissue may develop adequate mechanical strength four weeks after insertion of tooth-shaped titanium implants in the rat model.

At the initial stage, many cementoblast-like cells were found on the implant surfaces through thin amorphous layers (Fig. 3a). A similar layer has also been observed with cementoblast-like cells in repaired cementum on resorbed dentin or enamel [1]. The cementoblast-like cells and amorphous layer seen here might play a role in the initial attachment of collagen fibers.

In the later stages, methylene blue and alizarin red-stained, calcified structures were found near the implant surface through amorphous layers. The calcified structures had direct contact to the implant surface at 28 days (Fig. 3b). The calcified matrix exhibited many extensions into invaginations of the sandblasted implant surfaces. Calcified structures might possibly be involved in the formation of new attachment apparatus around the implants.

In the supra-alveolar regions of osseointegrated implants, most collagen fiber bundles in the peri-implant tissue were oriented parallel and circular to the implant surface. In the present study, functional orientation of supporting fiber bundles was found in supracrestal, transseptal, cervical, middle and apical regions. These results suggest that supporting connective tissues containing cementum-like structures around the implants were regenerated from the PDL tissues which remained in the sockets after tooth extraction. This can be closely related to the increased mechanical strength of the peri-implant tissues at 28 days (Fig. 2).

So far, there have been various studies on effects of coating materials including hydroxyapatite or collagen, occlusal loads, prolonged healing time, application of cultured PDL cells [2], and variety of growth factors [3] to implant surfaces on the functional formation of cementum and supporting fibers in the peri-implant tissues or the PDL tissues. These factors may be important for development of dental implants with functional periodontal tissues. To examine these factors in our rat model would be an interesting future project.

## References

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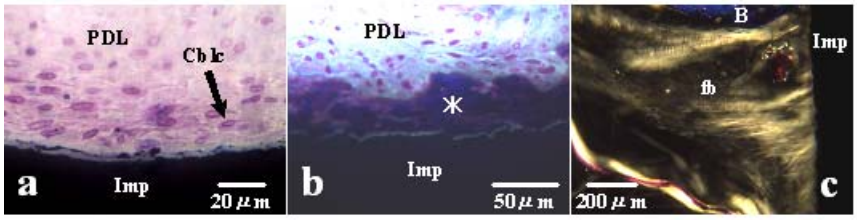
## Legends for figures

Fig. 1. (a) An extracted left maxillary first molar. (b) A tooth-shaped implant. (c) The implant was placed in the socket immediately after extraction of the left maxillary first molar.

Fig.2. (a) Typical examples of load-deformation curves for the

Fig.3




peri-implant tissue or normal periodontal ligament obtained by extracting tooth-shaped implants (14 and 28 days after implantation) or a left maxillary first molar from their sockets. (b) Extraction forces of the control teeth and implants are shown as mean + 1 SD. ** $p<0.05$ (Scheffé method).

Fig. 3. (a) Light micrograph of the mesial side of the mesial root of an implant (Imp) near the alveolar crest at 7 days. Note cementoblast-like cells (Cblc) reside adjacent to thin amorphous layer deposited on the implant surface. (b) Light micrograph of the middle region of the distal root of an implant at 28 days. Methylene-blue stained, calcified matrix structures (asterisk) were seen on the implant surface. (c) Polarized-light micrograph of the cervical region of the mesial root of an implant at 21 days. Birefringent fiber bundles (fb) were running perpendicularly from the implant surface to the lamina propia . B, alveolar bone; PDL, periodontal ligament