

Original Article

Development of Oral and Extra-Oral Endosseous Craniofacial Implants by Using a Mesh Structure for Connective Tissue Attachment

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Connective tissue attachment to a mesh structure incorporated on the surface of oral implants and extra-oral endosseous craniofacial implants (EOECI) was investigated. Two types of implants were prepared: TI and TI-Mesh. TI was composed of an upper and a lower component, both comprised of a titanium cylinder, which could be connected using a titanium screw. The composition of the TI-Mesh was similar, but the lower cylinder had a lateral groove that was covered with a titanium mesh. In animal experiments performed using rat calvaria, the lower component was first implanted and was left submerged for 3 weeks, then the upper component was mounted percutaneously. After an additional 2 weeks, each implant and the surrounding tissues were harvested and evaluated. Histological observations revealed collagen fibers originating from surrounding hypodermal tissues anchored to the mesh structures of the TI-Mesh whereas no such collagen fibers were observed around TI. Significantly greater values of the attachment strength, the thickness of the dermal tissue, the thickness of hypodermal tissue, and the attachment lengths were observed in TI-Mesh than those of TI. Thus connective tissue attachment with collagen fibers anchored to the mesh was achieved by incorporating mesh structures into the percutaneously placed implants.

Key words: Soft-tissue implant interactions, tissue-implant interface, connective tissue attachment, animal experiments, mesh

Introduction

Oral implants and extra-oral endosseous craniofacial implants (EOECI) are commonly used in clinical practice. They are based on well-established technology of osseointegration¹⁻⁴ and enable firm bonding of the implants to the bone tissues. However, the bonding of implants to soft tissue has not yet been thoroughly investigated.

At the interface between soft tissue and an implant, the collagen fibers in the peri-implant tissues cannot anchor to the implant, and run parallel to the implant surface.^{5,8} With regard to natural teeth and the gingiva, however, soft tissue attachment is established, whereby the dento-gingival fibers from subepithelial connective tissue run into the cementum of the root's cervix and firmly attach the gingiva to the tooth. Accordingly, the bond between oral implants and soft tissue is inferior compared to that of natural teeth and surrounding soft tissue owing to a lack of connective tissue attachment, which leads to inferior marginal sealing and then to poor resistance against infection.⁹⁻¹¹ Further, in the case of EOECI, the lack of soft tissue attachment leads to the far more serious clinical problem of downgrowth.¹²⁻¹⁵

Hence, improvement of the strength of attachment between the implant and soft tissue is important in order to overcome these problems. Many methods of improving the attachment of soft fibrous tissues to artificial materials have been investigated. Among these methods, surface treatment processes such as coating with hydroxyapatite¹⁶ or laminin-derived peptide,¹⁷ micromachining of grooves,¹⁸ and laser ablation (Laser-Lok)¹⁹ have been reported to increase cell adhesion to

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the surface of materials. However, attachment of the extracellular matrix to the surface of materials is another issue that remains to be investigated.

We developed a mesh structure for spontaneous anchoring of collagen fibers originating from adjacent connective tissues to the implant. Previous animal experiments have shown that a mesh with a spacing of approximately 200 μm was favorable for soft tissue attachment and for increasing attachment strength.²⁰ However, the efficacy of the titanium implant with a titanium mesh structure has not been confirmed in EOECI or oral implants models, in which the implant is placed onto the bone percutaneously or permucosally. In this study, implants with a mesh structure were percutaneously anchored to rat calvaria, and the effectiveness of the mesh structure was examined under conditions approximating those of clinically used implants.

Materials and Methods

Experimental implants

The materials used for making experimental implants were CP titanium rods and CP titanium meshes (Grade 1, machined surface; Nilaco, Tokyo, Japan). Scanning electron microscopic (SEM) images of these materials are shown in Fig. 1. The dimensions of the titanium mesh were determined using a measuring microscope (VH-8000; Keyence, Osaka, Japan); the fiber diameter and mesh spacing were found to be 120 μm and 213 μm , respectively.

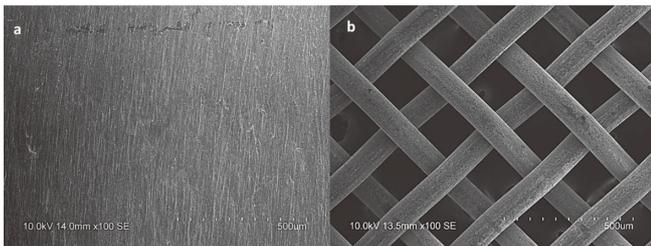


Figure 1. Scanning electron microscopy (SEM) observations. (a) Lateral surface of the titanium rod used for TI and TI-Mesh implants. (b) Titanium mesh used for covering the groove of TI-Mesh implants.

Two types of experimental implants were prepared in this study, which we called TI and TI-Mesh. Photographs of these experimental implants are shown in Fig. 2, and their dimensions are presented in Fig. 3 in a schema representing implantation. The TI implant was composed of upper and lower components. The lower component was cylindrical, with a diameter of

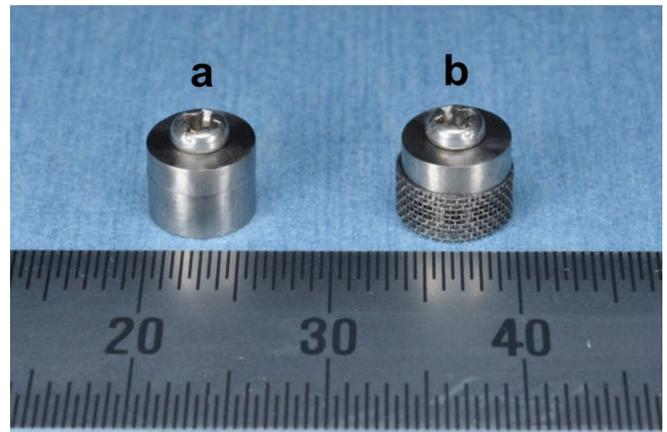


Figure 2. Experimental implants. The upper components were mounted onto the lower components with a titanium screw. (a) TI implant. (b) TI-Mesh implant incorporating a titanium mesh covering a groove on the lateral surface.

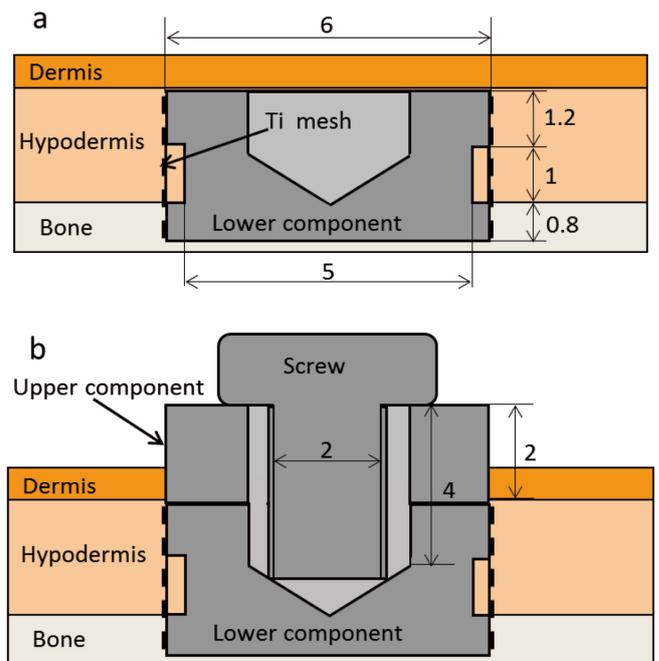


Figure 3. A schema of implantation depicting the specimen's dimensions.

(a) First surgery. Implantation of the lower component alone. The implant was fixed to the bone and left submerged for 3 weeks. (b) Second surgery. The upper component was mounted onto the lower component using a titanium screw. After mounting, the upper portion of the connected implant was left exposed for the following 2 weeks.

6 mm and a height of 3 mm, and it had a female M2 screw hole on the upper surface. This screw hole was used for mounting the upper component at the second surgery and for the connection of the jig at the mechanical testing stage, as described later. The upper

component was also cylindrical, with outer and inner diameters of 6 mm and 2 mm respectively and a height of 2 mm. The TI-Mesh implant was similarly composed of 2 components, and the upper component was similar to that of the TI implant. The lower component had the same dimensions as that of the TI, but it had a lateral groove of 1-mm width and 0.5-mm depth together with the titanium mesh covering the whole lateral surface; thus the lower component of TI-Mesh had the outer diameter of 6.24 mm. Bonding of the mesh to the titanium cylinder was achieved with the use of dental adhesive (Super-Bond C&B; Sun medical, Shiga, Japan). Under a stereomicroscope (SMZ1000; Nikon, Tokyo, Japan), the mesh was adhered to the lateral surfaces above and below the groove with paying particular attention not to allow the adhesive to overflow into the groove. After hardening, the protruded adhesive over the mesh was carefully removed with the use of dental silicone point abrasives. These experimental implants were degreased with acetone for 10 sec, rinsed with 70% ethanol and then with distilled water, dried at room temperature, sterilized with ethylene oxide gas, and used for subsequent experiments.

Animal Experiments

Twenty 15-week-old male Sprague-Dawley rats (body weight: 500–600 g) were used in this study. The experiment was conducted in compliance with the guidelines issued by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and the study protocol was approved by the committee (Approval No. 0070176).

First, animals underwent implantation of the lower component of 1 of the 2 types of implants. Before surgery, the animal was initially anesthetized using isoflurane (Isoflu; Abbott Lab., Abbott, IL, USA) and then placed under general anesthesia using medetomidine (0.5 mL/kg; Domitor; Pfizer AH, Exton, PA, USA) and sodium pentobarbital (0.5 mL/kg; Somnopentyl, Schering-Plough AH, Omaha, NE, USA). Enrofloxacin (5 mg/kg; Baytril; Bayer AG, Leverkusen, Germany), an antimicrobial agent, was also administered intramuscularly to prevent infection. Then the animal's head was shaved and disinfected with iodine solution, and a straight incision was made extending from the nasal bone to the midsagittal crest. A periosteal flap was elevated to expose the calvaria, in which a hole of 6-mm diameter and 0.8-mm depth was created using round and fissure dental burs under infusion with physiological saline. After hemostasis, the bone surface was washed with saline and air-dried. Then the

dental adhesive was applied to the bottom surface of the implant, and the implant was placed into the hole as shown in Fig. 4. The protruded adhesive was carefully removed with a probe. Immediately after hardening of the adhesive, hypodermal and dermal layers were closed using 6-0 nylon sutures. After surgery, atipamezole hydrochloride (10 mL/kg; Antisedan; Pfizer), a medetomidine antagonist, was administered intramuscularly to reduce the recovery time from anesthesia.

The implant was left submerged for 3 weeks, and then the animal was subjected to a second surgery. The soft tissue above the implanted lower component was removed using a 6-mm-diameter soft tissue punch to expose the top surface of the lower component, and the upper component was mounted onto it with a titanium screw as shown in Fig. 4. The upper portion of the connected implant was left exposed for 2 weeks.

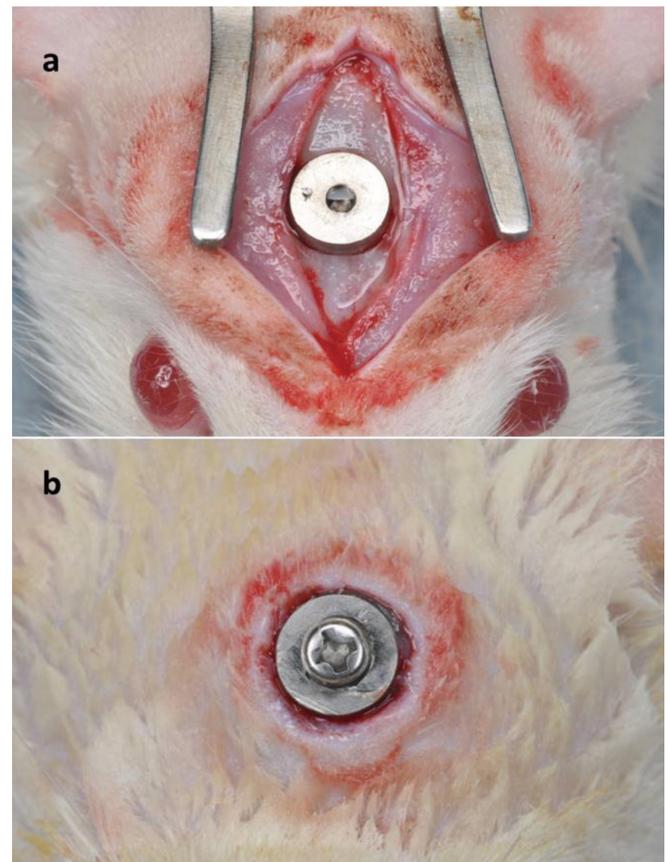


Figure 4. Intraoperative photographs.

(a) First surgery. The lower component was inserted into the hole prepared on the calvaria and fixed via the application of a dental adhesive. (b) Second surgery. The upper component was mounted onto the lower component using a titanium screw. The upper portion of the connected implant was left exposed.

Harvesting of samples

At the end of the implantation period, the implant was retrieved along with the surrounding bone and skin tissues, and mechanical tests and histological observations were performed. Of the total 20 animals, 10 were used for mechanical testing (5 each for TI and TI-Mesh implants) and the remaining 10 for histological observation.

Mechanical testing

The harvested implant with the surrounding tissues was immediately frozen at -24°C . Within a week, preparation of the sample for mechanical testing was performed without thawing, in which the bone tissues adhering to the implants (the bone tissues beneath or lateral to the implants) were carefully removed under the stereomicroscope using a dental round bur. Since the bone tissues were of 0.1 - 0.2 mm thickness, it took no more than several seconds to remove them. Then, the samples were soaked in physiological saline for 30 min and thawed. The attachment strengths of TI and TI-Mesh implants to the surrounding soft tissues were measured by pull-out tests, in which a testing machine (Autograph AG-X; Shimadzu, Kyoto, Japan) was used as illustrated in Fig. 5. The soft tissues and bone were sandwiched between aluminum plates with a center hole of 8 mm diameter and fixed to the lower crosshead of the testing machine. The upper component of the implant was removed and the lower component was connected with a screw to a universal joint under the load cell fixed on the upper crosshead. The mechanical test was performed at a crosshead speed of 2 mm/min and the data of load and displacement were recorded via a computer. The area of the mesh facing the tissues was adopted as the nominal area and calculated to be 19.6 mm^2 (1 mm [the height of the effective mesh] \times $3.14 \times 6.24\text{ mm}$ [the diameter of the implant]); the peak load measured was divided by this nominal mesh area, and the resulting value was determined as the attachment strength of TI-Mesh implant. The nominal area of TI implant at the time of implantation was similarly calculated to be 56.5 mm^2 (3 mm [the height of the titanium component] \times $3.14 \times 6.0\text{ mm}$ [the diameter of the implant]); however, considerable downgrowth of skin tissues was observed in the experiments and usage of this nominal area was found to yield too small estimation for the strength. Observing that the attachment length of connective tissues to TI implant turned to be ca. 1 mm, the nominal area of TI implant at the time of harvest was assumed to be 18.8 mm^2 (1 mm [the attachment length] \times $3.14 \times 6.0\text{ mm}$

[the diameter of the implant]) and the attachment strength was evaluated. It should also be noted that the statistical analysis demonstrated the same results on estimated strengths independent of the utilized nominal areas.

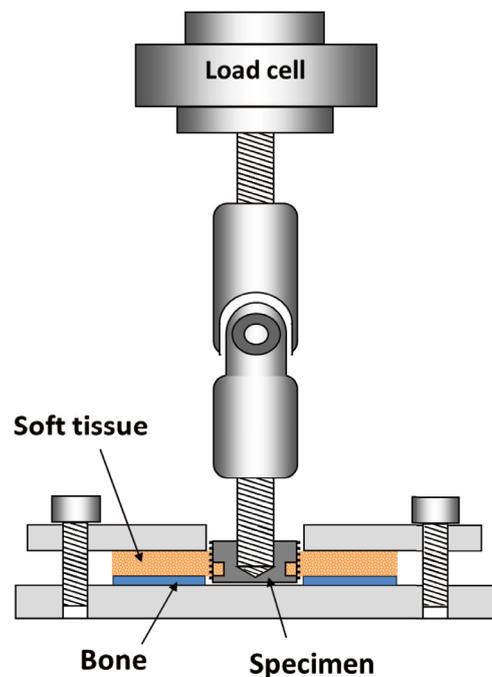


Figure 5. Schema of the mechanical testing of harvested samples.

Histological observation

For histological observation, the experimental implants and their surrounding tissues were fixed in 10% formalin immediately after harvest. They were then dehydrated in a graded series of ethanol dilutions, defatted with xylene, and embedded in methylmethacrylate resin (Osteoresin; Wako, Osaka, Japan). Thin sections of approximately $50\text{-}\mu\text{m}$ thickness were prepared using a diamond disc microtome (SP1600; Leica Microsystems, Bensheim, Germany); these sections were stained with toluidine blue and observed under a light microscope (Eclipse 50i; Nikon) equipped with a digital camera (Digital Sight DS-SM5; Nikon). The collagen fibers in these sections were also observed under polarized light.

Quantitative morphological evaluation of the histological sections was performed. The areas of the adjacent tissues of 1.5-mm width²¹ around the implant were specified in the histological section, and the average thickness of dermal and hypodermal tissues were determined after dividing the measured area by the width 1.5 mm. Attachment length was estimated as

the thickness of soft connective tissue (dermal tissue plus hypodermal tissue) facing the implant surface.

Statistical analysis

The attachment strength of the implant to the soft tissue, the thickness of the dermal tissue and hypodermal tissue in the surrounding soft tissue, and the attachment length of the soft tissue to the implant were statistically compared between TI and TI-Mesh implants using the non-parametric Mann-Whitney *U* test. All statistical analyses were performed using the “R” software (version 2.13.0; <http://www.r-project.org/>). Differences were considered statistically significant if $p < 0.05$.

Results

Macroscopic observation

During the experimental period, the condition of the body, in general, and the tissue around the implant were observed every day and no serious complications such as infection were observed. At the time of the harvest, significant downgrowth was observed in all the rats with TI implants, whereas none of the rats with TI-Mesh implants showed downgrowth.

Attachment strengths

The attachment strengths of both implants are shown in Fig. 6. The median (quartile range) of attachment strength for TI-Mesh implant was 268 (194 – 317) kPa, while that for TI implants 12 (12 – 24) kPa. The

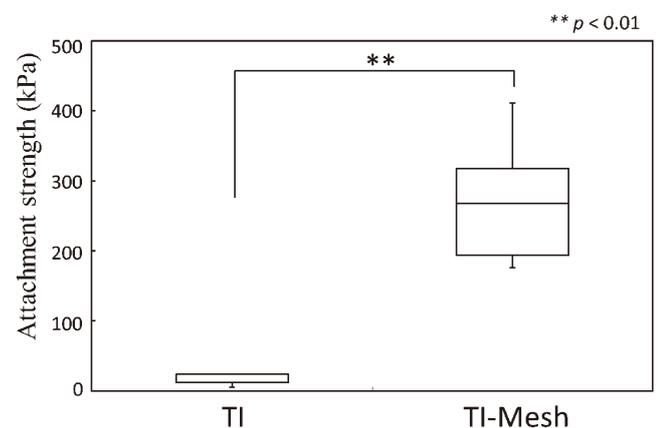


Figure 6. Boxplot of Attachment strength of the implants to soft connective tissues.

The bottom and top of the box are the first and third quartiles, the band inside the box is the median, and the ends of the whiskers are the minimum and maximum of all of the data. The attachment strength of TI-Mesh implants was significantly greater than that of TI implants ($p < 0.01$).

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Histological observation

Typical histological sections stained with toluidine blue are shown in Fig. 7. Significant downgrowth was observed in conjunction with the TI implants, whereas none was evident with the TI-Mesh implants. Collagen fibers from the connective tissues surrounding TI-Mesh implants entered into the groove as shown in Fig. 8, as observed under polarized light. Collagen fibers penetrated into the groove through the mesh and were oriented perpendicular to the implant, and thus

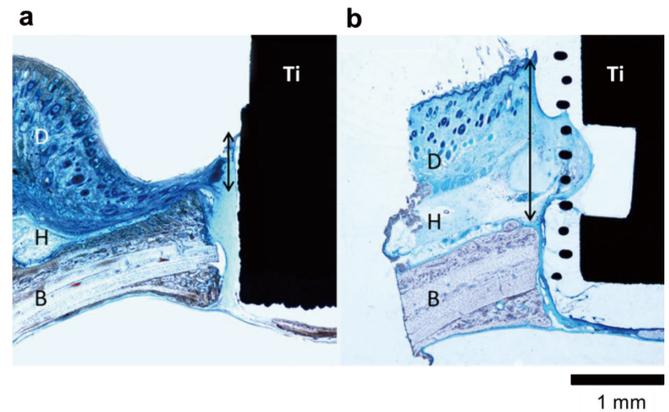


Figure 7. Light microscopy observations (toluidine blue stain). (a) TI implant. (b) TI-Mesh implant. D: dermis, H: hypodermis, B: bone, Ti: titanium implant. The arrows in (a) and (b) indicate the length of soft connective tissue attachment. Cross sections of the titanium mesh showed circular black spots (b).

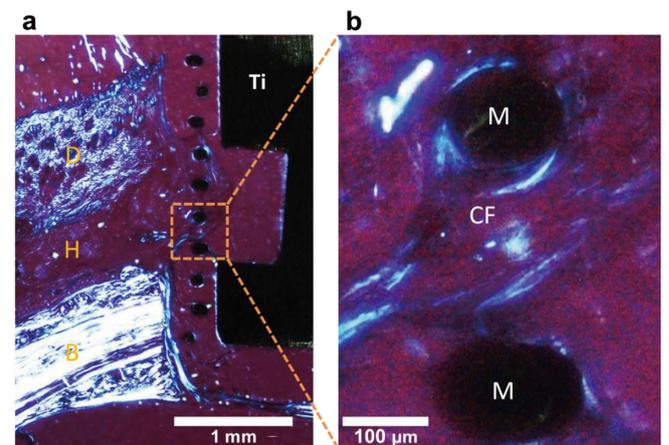


Figure 8. The TI-Mesh implant shown in Fig. 6, as observed under polarized light.

A magnified view of the box in (a) is shown in (b). Collagen fibers were penetrating into the groove through the mesh and were oriented perpendicular to the implant, and anchoring of the connective tissues to the titanium mesh was confirmed. D: dermis, H: hypodermis, B: bone, Ti: titanium implant, M: Ti mesh, CF: collagen fibers.

anchoring of the connective tissues to the titanium mesh was achieved.

The results of quantitative morphological evaluation of the histological sections are shown in Fig. 9. The

thicknesses of dermal and hypodermal tissues were 0.81 (0.58 – 1.09) mm and 0.78 (0.59 – 1.02) mm for TI-Mesh implants, and 0.34 (0.25 – 0.58) mm and 0.071 (0.0 – 0.35) mm for TI implants respectively. The attachment lengths were 1.74 (1.60 – 2.18) mm for TI-Mesh implants and 0.95 (0.77 – 1.20) mm for TI implants. Significant differences were confirmed between each pair of corresponding values derived from TI-Mesh and TI implants ($p < 0.01$).

Discussion

Peri-implant downgrowth is one of the major clinical concerns relating to the long-term survival of implants.^{22,23} A probable cause of it is that the collagen fibers from the surrounding soft tissues are aligned parallel to the material's surface and are not anchored to the implant.^{7,19,24} Therefore, it was expected that if anchoring of collagen fibers from the surrounding soft tissues to the surface of the implants was induced, then enhanced attachment of fibrous connective tissue to the implants could be achieved. A study investigating the anchoring of collagen fibers to implants via the use of a mesh structure on the implant's surface showed that a mesh with approximately 200- μ m spacing was favorable.²⁰ However, whether or not this mesh structure also effectively prevented peri-implant downgrowth was not investigated. Hence, in this study, downgrowth was examined with regard to titanium implants that included a mesh structure and placed percutaneously.

In this study, an experimental model was developed in which the specimen was implanted onto the rat calvaria and the efficacy of the implant with mesh structures was evaluated with regard to soft tissue attachment and downgrowth. This kind of model has not been reported elsewhere. The most technically difficult aspect of this model was fixation of the implant to the calvaria. Since this bone was as thin as 1 mm, the conventional technique of osseointegration¹⁻⁴ was found to be difficult to implement. Hence, a dental adhesive, which had been proven to be biocompatible and usable for bonding titanium implants to living bone tissues,²⁵ was employed, and it yielded successful fixation. We believe this model is useful to investigate the efficacy of connective tissue attachment to prevent the epithelial downgrowth.¹⁹ However, the fact that osseointegration was not achieved in this model was a potential limitation of this study.

Regarding the experimental period, Paquay *et al.*²⁶ reported that acute inflammation due to surgery

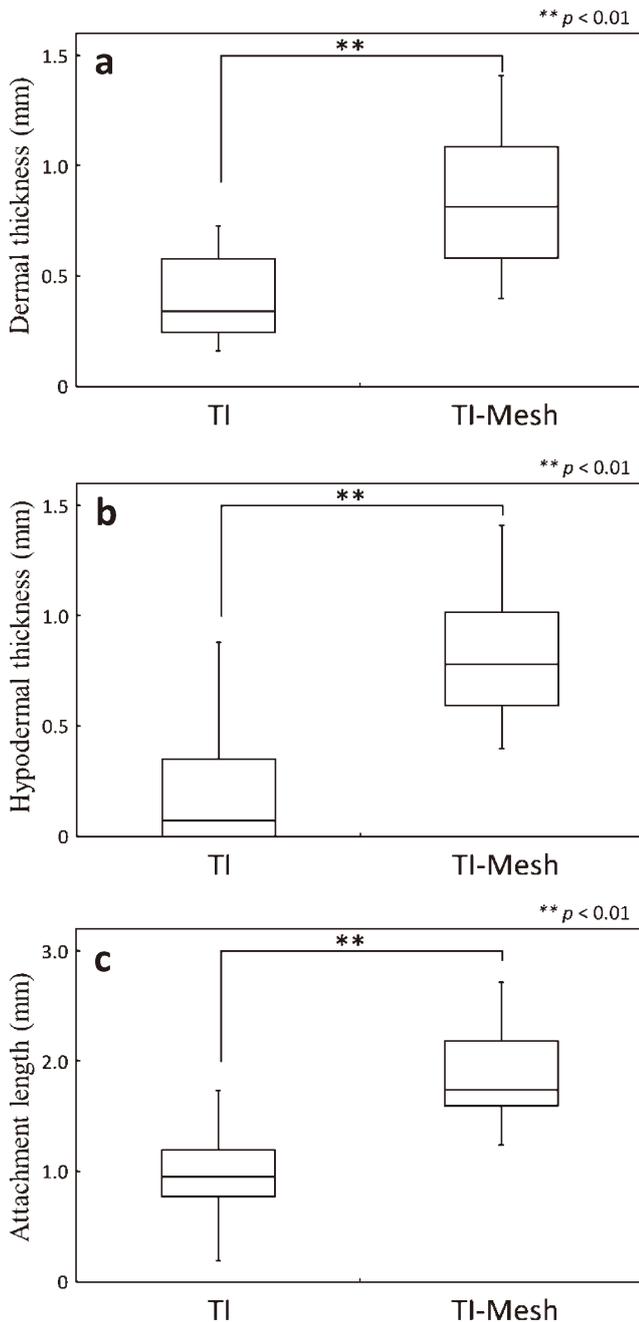


Figure 9. Boxplot of (a) average thickness of dermal tissue, (b) average thickness of hypodermal tissue, and (c) attachment length.

The bottom and top of the box are the first and third quartiles, the band inside the box is the median, and the ends of the whiskers are the minimum and maximum of all of the data. Significant differences were confirmed for each of these values ($p < 0.01$).

continued for 2 weeks and the necessary healing period was approximately 3 weeks, in their investigation into experimental percutaneous devices fabricated with a sintered titanium fiber mesh. Therefore, in this study, the lower components were left submerged for 3 weeks to allow the interface between the implant and the hypodermal tissue to mature. Thereafter, the upper components were mounted onto the lower components in order to investigate downgrowth. A pilot experiment showed that all of the TI implants had dislodged approximately 3 weeks after the mounting of the upper component. Hence, the experimental period after the mounting of the upper component was determined to be 2 weeks.

The retrieved implants were evaluated with regard to the attachment strength of soft tissue to the implant, the orientation of the collagen fibers, and the quantitative morphological parameters of the soft tissues. The space between the TI-Mesh implant and the aluminum plate was smaller than that around TI implant. This yielded the decrease in maximum bending displacement of the specimen. However, such displacement was inherently small since the bending deformation of the bone tissue in the specimen was small. On the other hand, the failure of the specimen was caused by the attachment failure of soft connective tissues after large deformation. Hence the above mentioned difference in the spaces was considered to have relatively small impact on the experimental results. The length of downgrowth may be defined as the sinking length of the epithelial tissue from the position of initial healing after the surgery; however, such a position could not be determined from the histological sections obtained from the samples in which downgrowth had already occurred. Therefore, the attachment length of the soft tissues to the implant surface was measured instead. Greater attachment length is considered to correspond with less downgrowth. Furthermore, since it is difficult to strictly identify the location on the implant where epithelium stopped, such histomorphological quantities closely related to the attachment length as the thickness of dermal tissue and hypodermal tissues were also measured.

The mechanical attachment strength between TI-Mesh implants and soft connective tissue was greater than that of TI implants. These results are consistent with the results of our previous studies.^{20,27} Thus, it was verified that the mechanical attachment strength was increased by using implants with the surface mesh structure, even if it was placed onto the bone percutaneously.

Significant differences were found with regard to all

the quantitative variables evaluated in the quantitative morphological analyses ($p < 0.01$). The mesh structure was considered to prevent the downgrowth; however, it is possible to speculate that the difference in the surfaces (titanium and the dental adhesive) was responsible for the above mentioned differences. Actually we performed a pilot study in which one-piece implants (the implants which were not separated to the upper and lower components) were prepared and implanted into the rat calvaria percutaneously. The downgrowth was confirmed in either case of the Ti surface (one-piece TI implant) or the dental adhesive surface (one-piece TI-Mesh implant). In the case of the one-piece TI-Mesh implant, the downgrowth took place before the anchoring of collagen fibers to the mesh and disabled the functionality of the mesh structure completely. Hence we concluded that the mesh structure effectively prevented the occurrence of downgrowth whereas the dental adhesive surface did not.

While no studies on the downgrowth of the soft tissues surrounding implants have been reported elsewhere to date, the circumference of the implant in the present study approximates that of EOECI. The occurrence of downgrowth around our control TI implants is consistent with the findings of clinical cases of EOECI.¹²⁻¹⁴ On the other hand, such downgrowth was not observed in the experimental group implanted with TI-Mesh. Downgrowth is far more frequently observed in the skin tissue around EOECI than in the masticatory mucosa that surrounds an ordinary oral implant. This difference may stem from differences between skin tissue and masticatory mucosa. Skin tissue is relatively soft and covering the soft hypodermal tissue and muscle layer, thereby it shows high mobility. In contrast, the masticatory mucosa is relatively hard and it is located directly on hard bone tissue; thus it has less mobility.^{28,29} In order to reduce the amount of downgrowth around EOECI, procedures in which the peri-implant soft tissue is thinned or the split thickness skin is grafted (STSG technique) to limit tissue movement are recommended.³⁰⁻³³ In our results, increased attachment strength achieved via the anchoring of collagen fibers to the mesh structure was considered to have effectively decreased mobility and prevented downgrowth. Goldman²⁴ and Nevins¹⁹ have also asserted that connective tissue attachment of the collagen fibers is the most important factor in preventing downgrowth. In this study, the efficacy of the mesh structure for preventing downgrowth around the implant was proven quantitatively, at least in a rat

model of EOECI.

In fact, this experiment could be regarded as an approximating model of EOECI, in which clinicians experience many serious problems stem from the lack of adhesion of skin to implant, low stiffness of peri-implant tissue, excessive thickness of peri-implant tissue, and mobility of peri-implant skin.¹⁵ The present results suggested that an implant with a mesh structure may help overcome these adverse events. However, further investigations concerning the long-term stability of the attachment and implantation studies relating to the oral environment are necessary with regard to clinical applications.

Conclusions

This study showed that connective tissue attachment via collagen fibers was achieved by incorporating a mesh structure onto the experimental implant, even if the implant was placed on the bone percutaneously. This attachment enabled the dermal and hypodermal tissue to remain healthy and prevented down-growth. Implants with a mesh structure may be favorably applied in clinical cases of EOECI and oral implantation.

Acknowledgements

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