

Periodontal Ligament Formation Around Titanium Implants Using Cultured Periodontal Ligament Cells: A Pilot Study

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The aim of this study was to investigate whether a new periodontal ligament attachment will form on titanium implants when they are implanted with cultured periodontal ligament cells. Periodontal ligament cells obtained from the teeth of 3 dogs were cultured and attached to the surface of titanium implants. The implants with the cultured autologous periodontal ligament cells were placed in the mandibles of the dogs. After 3 months of healing, histologic examination revealed that, on some implant surfaces, a layer of cementum-like tissue with inserting collagen fibers had been achieved. These results demonstrated that cultured periodontal ligament cells can form tissue resembling a true periodontal ligament around implants. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:193-196)

Key words: cell culture, implants, periodontal ligament, titanium implants

Different kinds of interfaces between implants and host tissues have been recognized. Currently, osseointegrated implants are generally agreed to be the most acceptable implants because of their high long-term clinical survival rate.^{1,2} However, problems still exist with these implants. Since they lack a periodontal ligament, any inflammation around them may cause more serious bone loss than does inflammation around teeth with a periodontal ligament.³ In addition, these implants are ankylosed and do not have the same mobility as natural teeth with a periodontal ligament. To compensate for this difference, in one system a mobile element has been designed for placement in the implant or its suprastructure.^{4,5} But if an implant with a periodontal ligament could be developed, these problems might be resolved.

Buser et al⁶ and Warrar et al⁷ demonstrated that a cementum layer on the implant surface with inserting collagen fibers had been achieved around implants, which were placed in contact with the

periodontal ligament of the retained natural roots. They concluded that dental implants with a true periodontal ligament could be developed. This study aimed to investigate whether a new attachment with a periodontal ligament would form on titanium implants when implanted with cultured periodontal ligament cells.

MATERIALS AND METHODS

Three mongrel dogs, each weighing more than 17 kg, were used in this study. Animal selection and management, surgical protocol, and preparation followed routines approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The mandibular first and second premolars of each dog were extracted bilaterally, creating edentulous regions. All surgical procedures were performed under intravenous sodium pentobarbital anesthesia (Entobar, Hanlim Pharmaceuticals Co, Seoul, Korea) in combination with a local anesthetic agent (lidocaine and 1:80,000 epinephrine). Approximately 3 months after tooth extraction, culturing of periodontal ligament cells was performed. To obtain the periodontal ligament cells, the first maxillary premolar was removed from each dog. The periodontal ligament tissues, removed from the midroot surface of the tooth, were minced, and portions were placed on a surface of the implant in a 25 cm² culture

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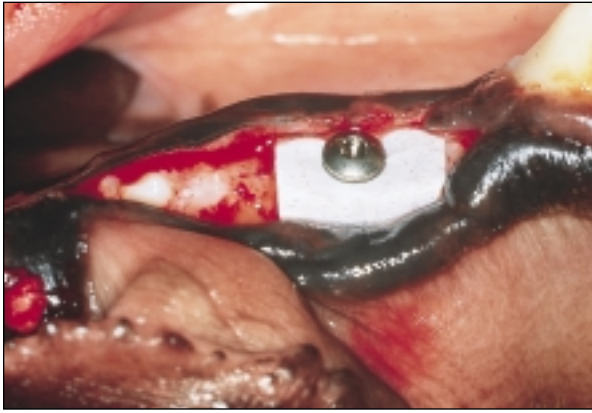


Fig 1 The implant is covered with a Gore-Tex membrane.

flask. The implant was an 8-mm-long, commercially pure titanium screw-type implant with a diameter of 4.1 mm (Institute Straumann AG, Waldenburg, Switzerland). Dulbecco's Modified Eagles Medium (DMEM, Gibco BRL, Grand Island, NY), complemented with 20% fetal calf serum, 1% antibiotic-antimycotic, and 1% glutamine, was used. All cultures were maintained throughout the experiment at 37°C in a humidified 5% CO₂/95% air atmosphere.

When the periodontal ligament cells that grew out of the explants covered the implant surface, the explants were detached from the implant. After complete covering of the implant surface with the periodontal ligament cells was confirmed microscopically (after 4 to 5 weeks), each implant with the cells was then placed in the edentulous regions of the dog from which the periodontal ligament cells had been obtained. Each dog received 2 implants with cultured autologous periodontal ligament cells in the edentulous regions. The edentulous region was opened by a crestal incision. The mucoperiosteal flap was raised and the alveolar ridge was prepared for placement of the implant. A low-speed drill was used for hole preparation. The final implant site was prepared with a trephine bur (outer diameter 4.8 mm) to create a void space between the implant and the surrounding bone, so that the cultured periodontal ligament cells attached to the implant surface were not severed at the time of placement. The implant was placed into the hole and covered by a Gore-Tex membrane (3i/W. L. Gore, West Palm Beach, FL) to prevent gingival connective tissue from growing into the wound around the implant (Fig 1). The membrane was secured by means of a cover screw. The raised mucoperiosteal flaps were then sutured in a manner that ensured that the implant was submerged. The dogs were placed on a normal hard-pellet diet, and oral hygiene protocol was not carried out for the duration of the study.

After 3 months of healing, the implants and their surrounding bone were removed under the same conditions of anesthesia. The specimens were then rinsed in saline, fixed in 10% buffered formalin, dehydrated in alcohol, and embedded in methyl-methacrylate resin. The implants were cut midaxially in a buccolingual plane into 200- μ m-thick sections using the cutting-grinding technique, and they were subsequently ground and polished to a final thickness of approximately 40 μ m. The specimens were stained with villanueva bone stain.⁸ Evaluations were performed using a light microscope.

RESULTS

After 3 months, uneventful healing was seen around 4 of 6 implants. Two implants had to be removed because of peri-implant infection, extensive membrane exposure, and severe mobility. Thus, histologic examination for the 2 failed implants was not performed. The other implants were stable, and the specimens for them showed new connective tissue formation on the implants. There were no healed areas with direct bone-to-implant contact. On some implant surfaces, a thin layer of cementum-like tissue was observed, but the cementum-like tissue was not observed in the opposing bone. The cementum-like tissue was observed on 37% of the evaluated implant surfaces. Between the cementum-like tissue on the implants and the alveolar bone, a periodontal ligament-like tissue was seen. Collagen fibers in the periodontal ligament-like tissue were embedded in the alveolar bone on one side and in the cementum-like tissue of the implants on the other side. The collagen fibers were oriented perpendicular to the implant surface (Figs 2 and 3). However, there were some areas where collagen fibers in the connective tissue layer were arranged parallel to the implant surface, and no layer of cementum-like tissue had formed (Fig 4).

DISCUSSION

There have been several investigations that provide evidence of periodontal ligament formation around dental implants. Nyman et al⁹ demonstrated that periodontal ligament cells possess the ability to re-establish a connective tissue attachment. With their concept, pilot studies have been designed to form new connective tissue attachments on implant materials by repopulation of periodontal ligament cells on implant materials.^{6,7,10,11} These studies showed cementum deposition, with collagen fibers inserted on the implant materials. A new perspective in

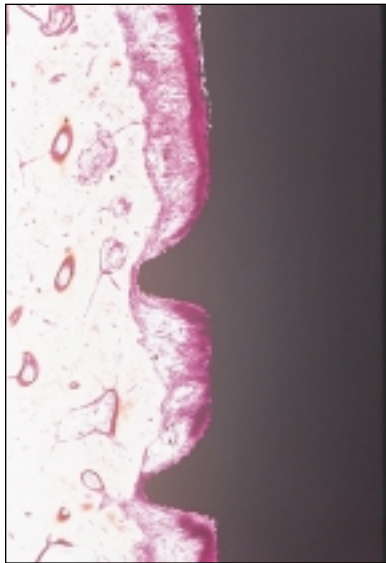


Fig 2 Titanium dental implant 3 months after its placement with cultured autologous periodontal ligament cells. A periodontal ligament-like tissue is seen surrounding the implant (original magnification $\times 40$).

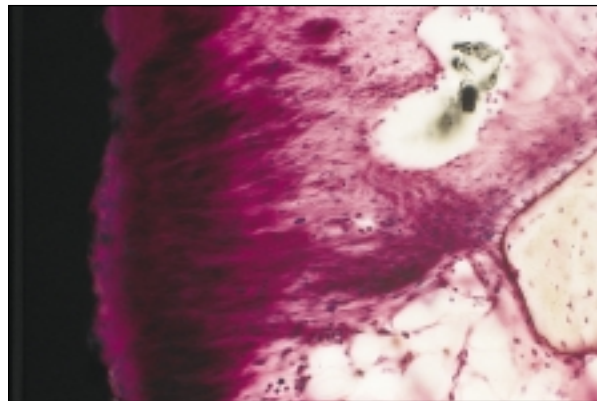


Fig 3 High magnification of the implant surface, where a cementum-like tissue layer was present. Connective tissue fibers, oriented perpendicular to the implant surface run across the periodontal ligament space and insert in the opposing bone (original magnification $\times 200$).

implant dentistry can be opened if such an event occurs through the culture of periodontal ligament cells on dental implants. To the authors' knowledge, there are no published reports on whether cultured periodontal ligament cells can create a new periodontal ligament around dental implants.

The present study showed new connective tissue formation on commercially pure titanium dental implants that had been implanted with cultured periodontal ligament cells. The finding of cementum-like tissue on the implant surface, with collagen fibers inserting perpendicular to the implants, suggests that cultured periodontal ligament cells can produce a tissue resembling a true periodontal ligament on the implant surface. Several authors have demonstrated that cultured periodontal ligament cells maintain their properties to re-establish a connective tissue attachment.¹²⁻¹⁵ Boyko et al¹⁶ showed that cultured periodontal ligament cells could create a new periodontal ligament when reimplanted with a demineralized root. Van Dijk et al¹⁷ demonstrated that cell seeding of cultured periodontal ligament cells could produce a new connective tissue attachment on a planed root surface.

An interesting observation in the present study was that cultured periodontal ligament cells deposited new cementum-like tissue in the areas where collagen fibers were arranged perpendicular to the implant surface, while the cementum-like tissue was not formed in the areas where collagen fibers

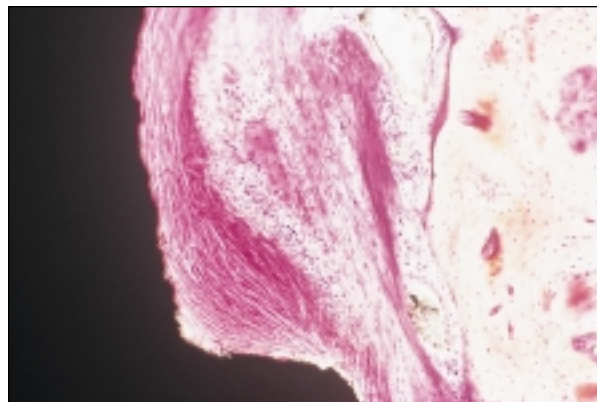


Fig 4 Titanium dental implant 3 months after placement of the implant with cultured autologous periodontal ligament cells. Collagen fibers are arranged parallel to the implant surface, and no layer of cementum-like tissue was found (original magnification $\times 100$).

were arranged parallel to the implant surface. These findings suggest that the formation of cementum-like tissue is in close relationship to collagen fibers inserting perpendicular to the implant surface. The insertion is presumably a result of the fact that they form a biologically active and positive attachment to the implant. Therefore, these findings would imply that the behavior of the cells on the implant surfaces plays an important role in the formation of the cementum-like tissue. It would be interesting to compare the formation of cementum-like tissue on

bioactive materials such as bioglass and hydroxyapatite with its formation on bioinert materials such as commercially pure titanium.

In the present study, cementum-like tissue was observed on some implant surfaces and not on others. One interpretation of this observation is that in the areas where no cementum-like tissue was observed, cementoblasts might not have been present in the periodontal ligament tissue, whereas in the areas where cementum-like tissue was formed, cementoblasts of the periodontal ligament tissue could serve as a source for cells that could then participate in the formation of the cementum-like tissue. Despite the interest in cementoblast culture, thus far there is no established method for harvesting and culturing cementoblasts. To obtain a complete layer of cementum on the implant surface, more studies are certainly needed to harvest and culture a cementoblast population.

It has been pointed out that soft tissue healing following surgical procedures on animals is, in general, less predictable than in human patients because of the limited feasibility of employing postoperative measures to manage inflammation and to protect the surgical site.¹⁸ As a result, the complications of soft tissue dehiscence and subsequent membrane exposure were reported to be much higher in animals than in human subjects.¹⁸ In this study, 2 of the 6 implants exhibited peri-implant infection, membrane exposure, and severe mobility. An explanation for the mucosal complications might be that the mechanical interference of the hard diet and poor oral hygiene contributed to peri-implant infection. This may also be related to the dead space created around the implants so that the cultured periodontal ligament cells would not be severed at the time of implant placement. Nevertheless, two-thirds of the implants remained intact, indicating that it is possible to achieve a new attachment using the experimental design of the present study.

CONCLUSION

From the results of the present study, it can be concluded that a periodontal ligament-like tissue attachment can form around dental implants when they are implanted with cultured periodontal ligament cells. Application of cultured periodontal ligament cells to the implant surface may open a new perspective in implant dentistry.

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