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Healing Following Implantation of Root with Remaining Periodontal Ligament Cultured *In vitro*

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Abstract

Background: Intentional replantation is extraction of a tooth to do extraoral root canal therapy, curettage of apical lesion when present, and its replacement in its socket. The most common causes of failure in intentionally replanted teeth are external resorption and ankylosis caused by periodontal ligament damage. We hypothesized that if proliferative cells derived from healthy periodontal ligament could cover a damaged site, the success rate of implantation and replantation would increase and thus widen its application. The purpose of this study was to histologically evaluate the influence of tissue culture on healing following implantation of root with remaining periodontal ligament cultured in vitro into a mandibular bone cavity.

Methods: Twenty incisors of 5 beagles were extracted and forty trimmed roots (4.0×3.0 mm) were made using a bur. Periodontal ligament on the root surface of thirty trimmed roots was retained and remaining ten trimmed roots were removed periodontal ligament on the root surface. Thirty trimmed roots with remaining periodontal ligament were divided into three groups according to each culture sequence, namely 0 week (not cultured), 2 and 4 weeks. Following the culture period, the roots were implanted into bone cavities created in the mandible. Four weeks postsurgery, the specimens were prepared for histological analysis.

Results: In the 0w group, ankylosis was observed in three of 10 cases. However, ankylosis did not occur at all in the 2w and 4w groups. However, there were no significant differences in any parameter (normal periodontum, ankylosis, surface resorption, inflammatory resorption) among the three groups.

Conclusion: The mechanical injury that occurred during extraction of the teeth could be responsible for the ankylosis. If the entire root surface is covered with cultured periodontal ligament-derived cells and that could prevent ankylosis, the success rate of intentional replantation and implantation might increase.

Keywords: Periodontal ligament; Transplantation; Tissue culture; Regeneration; Ankylosis; Incisor model; Resorption

Introduction

The periodontal ligament that remains attached to the root surface has been capable of regenerating periodontal ligament after implantation [1,2]. Based on this property, intentional replantation is extraction of a tooth to do extraoral root canal therapy, curettage of apical lesion when present and its replacement in its socket [3-9]. At the moment, the success rate of intentional tooth replantation and implantation based on the healing of periodontal ligament ranged from 50 [10] to 95% [11]. On the other hand, failed cases with root resorption and ankylosis have also been reported [12-14]. Andreasen et al. reported that root resorption and ankylosis occurred when more than 9 mm2 of periodontal ligament was damaged [15]. Clinically, curved and multirooted teeth were at high risk of periodontal damage when the tooth was extracted. In the case of root cracks and periodontally involved teeth, part of the periodontal ligament is destroyed or infected, thus reducing the amount of healthy periodontal ligament. Studies have reported that enamel matrix derivative (EMD) application could increase survival rate in the tooth [16,17], but other studies found no difference in the healing [18-20]. Therefore, EMD application was not exactly effective.

Periodontal healing after transplantation of the teeth with seeding periodontal ligament cells was investigated in some studies [21-23]. However, the cells were collected from other site of periodontal ligament of donor teeth, which must have to be extracted. To solve the problem the supply of periodontal ligament cells, we hypothesized that if proliferative cells derived from healthy periodontal ligament could cover a damaged site using the tissue culture, the success rate of implantation and replantation would increase and thus widen its application. We previously reported that periodontal ligament cells could populate on the planed root surface (≈5 mm) from the remaining periodontal ligament by 4-6 week tissue culture [24]. However, in the periodontal ligament cultured by tissue culture, the number of cells in the deep layers of the remaining periodontal ligament decreased and those in the superficial layer of periodontal ligament increased over time [24,25]. The healing after tissue cultured periodontal ligament was implanted in the bone defect is not well understood. The purpose of this basic study was to examine histologically the effect of tissue culture on the healing of the root surface when the extracted teeth was implanted in a bone cavity after

tissue culture of the periodontal ligament remaining on the extracted teeth.

Materials and Methods

Animals

Twenty maxillary incisors (I3 and I2) of five beagle dogs (1-year-old males; mean weight: 11 kg) were used. This study protocol followed the guidelines for care and use of laboratory animals of Hokkaido University Graduate School of Medicine. All surgical procedures were performed under general anesthesia by an intramuscular injection of Medetomidine Hydrochloride (5 μg/kg, Domitor®; Meiji Seika, Tokyo, Japan) and Ketamine Hydrochloride (2.9 mg/kg, Ketaral 50®; Sankyo, Tokyo, Japan) and local infiltration (xylocaine 2% with 1:80,000 epinephrines, Xylocaine®; DENTSPLY SANKIN, Tokyo, Japan). The dogs received plaque control, consisting of twice-weekly brushing and application of 0.5% chlorhexidine gluconate solution, to establish healthy gingival conditions with the pocket depth of 1-2 mm before the surgical procedure.

Preparation of Teeth

The incisors were carefully extracted with forceps and then immersed in sterile phosphate buffer saline containing antibiotics at the following concentrations: 30 μg/ml Kanamycin Sulfate, 30 μg/ml Amphotericin B (Gibco, Grand Island, NY, USA). The tooth crown and the pulp of the root canal were removed and the remaining root was hemisectioned axially and trimmed to 4.0×3.0 mm using a bur and the periodontal ligament of the root were retained (Figure 1). Total forty trimmed roots were obtained.

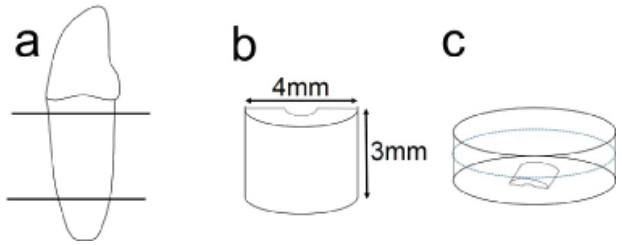

Figure 1: Schematic illustration of methods in the experimental group a) The extracted tooth was sectioned, and the tooth crown and contents of the root canal were removed, b) The remaining root was hemisectioned axially and trimmed to 4.0×3.0 mm, c) The trimmed root was placed in a dish with root-surface sides up and was incubated in α-MEM with 120 μg/ml Kanamycin Sulfate, 3.0 μg/ml Amphotericin B and 15% Fetal Bovine Serum

Twenty trimmed roots were placed in a dish with root-surface sides up and were incubated in α-MEM with 120 μg/ml Kanamycin Sulfate, 3.0 μg/ml Amphotericin B and 15% Fetal Bovine Serum (Gibco, Grand Island, NY, USA) at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air.

Thirty trimmed roots with remaining periodontal ligament were divided into three groups according to each culture sequence, which were 0 week (not cultured) (n=10), 2 (n=10) and 4weeks (n=10).

In the Remove group (n=10), the root surfaces of remaining trimmed roots were carefully planned by curretts in order to remove the periodontal ligament.

Transplantation of Trimmed Roots

Full-thickness flaps were raised at recipient sites in the buccal body of mandible. The sockets of 4.0×3.0 mm were prepared in cortical bone for transplantation of trimmed roots using rotating burs under water irrigation in the recipient sites (Figure 2). The trimmed roots were placed into the sockets and flaps were sutured (Gore-Tex CV-5 Suture, W. L., Gore and Associates Inc., Flagstaff, AZ, USA).

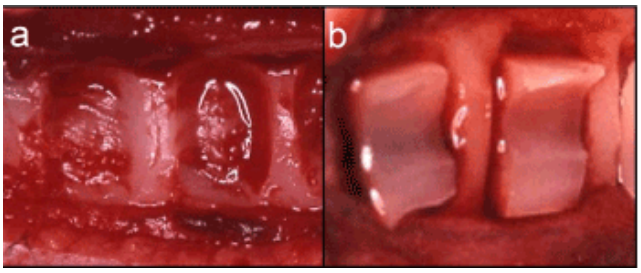

Figure 2: Bone cavity preparation and implantation of the trimmed roots a) Full-thickness flaps were raised at recipient sites and bone cavity was prepared, b) The external surface side of the trimmed roots was implanted toward the bone wall

Twenty trimmed roots of the 0w group and Remove group were prepared and immediately transplanted without tissue culture in the sockets. Sutures were removed 10 days after transplantation.

Histologic processing and histometric analysis

The dogs were sacrificed 4 weeks after transplantation. Block sections were dissected, fixed in 10% buffered formalin, decalcified in Plank-Rychlo solution, trimmed, dehydrated, and embedded in paraffin. Serial sections of 5 μm thickness were prepared in the bucco-lingual plane. Sections were stained with hematoxylin and eosin.

The following measurements were performed on the root surface with remaining periodontal ligament of the teeth by light microscopy (Figure 3): 1) Root length: Length of the root surface; 2) normal periodontium: combined longitudinal heights of the periodontal ligament had a normal histologic appearance. A few inflammatory cells were accepted; 3) surface resorption: combined longitudinal heights of resorption lacunae on the root surface without inflammatory cells in the area; 4) inflammatory resorption: combined longitudinal heights of resorption lacunae on the root surface with inflammatory cells in the area; 5) replacement resorption (ankylosis): combined longitudinal heights of ankylosis. Values were expressed in percentages of root length.

Data analyses

The mean and standard deviation for each measurement were calculated for each tooth from selected sections. Statistical differences were analyzed with the Kruskal-Wallis test using the Scheffe multiple comparison procedure.

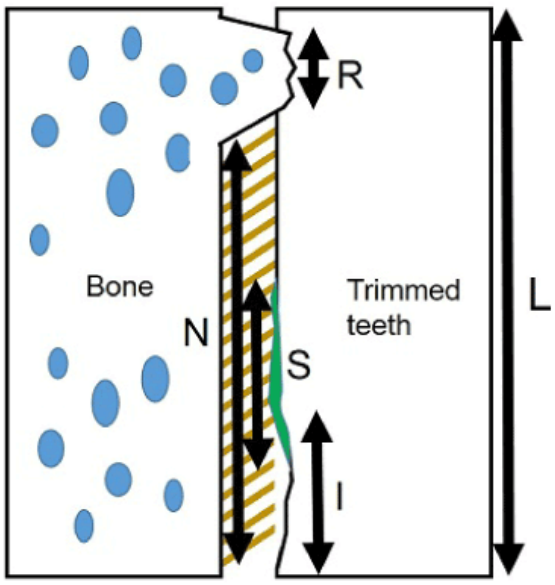

Figure 3: The linear measurements in histometric analysis. L) Root length: length of the root surface; N) normal periodontium: combined longitudinal heights of the periodontal ligament had a normal histologic appearance; R) replacement resorption (ankylosis): combined longitudinal heights of ankylosis; S) surface resorption: combined longitudinal heights of resorption lacunae on the root surface without inflammatory cells in the area; I) inflammatory resorption: combined longitudinal heights of resorption lacunae on the root surface with inflammatory cells in the area

Results

Histological observations

In the 0w group that was implanted immediately after extraction, normal periodontium was observed between the bone wall inside bone cavity and the root surface, and that fiber was oriented perpendicular or parallel to the root surface or in a combination of those (Figure 4). Inflammatory resorption was observed in five of ten roots. Ankylosis was observed in three of ten.

In the 2w group, normal periodontium was observed between the bone wall inside the bone cavity and the root surface as in the case with the 0w group. Inflammatory resorption was observed in one of ten roots. Ankylosis was not observed.

In the 4w group, normal periodontium was observed between the bone wall inside the bone cavity and the root surface as in the 0w and 2w groups. Inflammatory resorption was observed in two of ten roots. Ankylosis was not observed at all.

In the Remove group, normal periodontium was not observed at all. Ankylosis and inflammatory resorption was observed in all specimens.

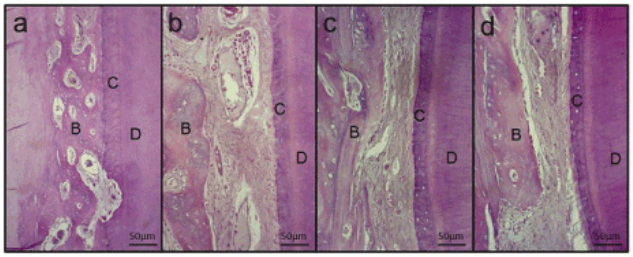

Figure 4: Periodontal healing of root surface after transplantation. a) The remove group that was implanted immediately after extraction and remove of periodontal ligament on root surface (Hematoxylin and Eosin original magnification ×40): The ankylosis and inflammatory resorption was observed on the root surface, b) The 0w group that was implanted immediately after extraction (Hematoxylin and Eosin original magnification ×40): The connective tissue formation was observed between the bone surface of inner wall of bone cavity and root surface, c) The 2w group that was implanted after tissue culture for 2 weeks (Hematoxylin and Eosin original magnification ×40): The connective tissue formation was observed, d) The 4w group that was implanted after tissue culture for 4 weeks (Hematoxylin and Eosin original magnification ×40): The connective tissue formation was observed. D: Dentin, C: Cementum, B: Bone

Parameter	Remove group (n = 10)	0w group (n = 10)	2w group (n = 10)	4w group (n = 10)
Normal periodontum	0.00±0.00	92.70±6.54*	95.20±4.26*	94.52±5.44*
Surface resorption	0.00±0.00	2.95±4.45	4.42±4.28†	4.80±4.73†
Inflammatory resorption	1.48±1.56	2.38±2.94	0.38±1.20	0.68±1.58
Replacement resorption	89.58±28.36	1.97±4.48*	0.00±0.00*	0.00±0.00*

n = number of sites; SD = standard deviation.

* Significantly different from remove group ($p < 0.01$); Kruskal-Wallis test; statistical differences between the four groups.

† Significantly different from remove group ($p < 0.05$); Kruskal-Wallis test; statistical differences between the four groups.

Table1: The healing on the root surface (Group mean ± SD in percentage)

Histometric Observations

Normal periodontum was significantly less in the Remove group compared to the 0w, 2w and 4w groups ($p<0.01$) (Table 1). However, there were no significant differences in normal periodontum among the 0w group (92.70 ± 6.54%), the 2w group (95.20 ± 4.26%) and the 4w group (94.52 ± 5.44%). Ankylosis was significantly greater in the Remove group compared to the 0w, 2w and 4w groups ($p<0.01$). Ankylosis was observed in the 0w group (1.97 ± 4.48%) and was not observed in the 2w and the 4w groups (0.00 ± 0.00%) at all, however,

there were no significant differences in that among the three groups. Surface resorption were observed significantly less in the Remove group compared to the 2w and 4w groups ($p<0.05$).

Discussion

In the periodontal ligament cultured by tissue culture, the number of cells in the deep layers of the remaining periodontal ligament decreased and those in the superficial layer of periodontal ligament increased over time [24]. This study examined the influence of attachment to bone cavity after implantation on the change and proliferation of the periodontal ligament cells. In the 0w group that was implanted immediately after preparation of trimmed root, periodontal ligament-like tissue was observed between the root surface and the bone wall in the bone cavity. The periodontal ligament remaining in the graft used in this study is thought to have induced periodontal ligament formation as in previous studies [19]. The normal periodontum of the 2w and 4w groups was similar to that of the 0w group. Most of the superficial cells of the tissue cultured periodontal ligament were in varying mitotic stages during the culture period [26]. Moreover, McCulloch at al. and Inoue et al. reported that cells that resembled those with high division potential in the central part of the normal periodontal ligament were distributed in the superficial cell layer of the periodontal ligament [27]. These cells could be concerned with periodontal ligament formation after implantation [26,27].

In the 0w group that was implanted immediately after preparation of the trimmed root, ankylosis was observed in three of 10 cases. However, ankylosis did not occur at all in the 2w and 4w groups. The mechanical injury that occurred during extraction of the teeth and preparation of trimmed root could be responsible for the ankylosis [16,28]. We have reported that periodontal ligament cells could populate on the planed root surface (≈5 mm) from the remaining periodontal ligament by 4-6 week tissue culture of extracted teeth [24]. In the culture groups, ankylosis did not occur because the proliferative cells would populate on the lesion site.

Intentional tooth implantation was less risky of infection and drying than implantation of an avulsed tooth. However, the success rate remains from 50% to 95% [10,11]. Most of the causes of failure were ankylosis and root resorption. A clinical study reported that ankylosis would occur because extraction damaged periodontal ligament in daily clinical situation [29]. If the entire root surface is covered with cultured periodontal ligament-derived cells and that could prevent ankylosis, the success rate of intentional replantation and implantation might increase. We have reported that the cell migration from the remaining periodontal ligament on the planed root surface was ≈1mm by 2 week tissue culture. The culture period may be need 2 or more weeks. However, this technique cannot be applied easily in the clinical setting because favorable environmental conditions are necessary to perform tissue culture. Further investigations are required to evaluate the safety of this technique.

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