The Effect of Cultured Autologous Periodontal Ligament Cells on the Healing of Delayed Autotransplanted Dog's Teeth

Yixiang Wang, DDS, Gary Shun-pan Cheung, DDS, PhD,† Xiangliang Xu, DDS,‡ Shijie Zhao, DDS,‡ and Chengfei Zhang, DDS, MD‡§*

Abstract

Introduction: The regeneration of the periodontal structure for avulsed teeth extended dry times has been a goal of dentists. The aim of this study was to investigate a new strategy of delayed replantation for avulsed teeth that were not suitable for immediate replantation. **Methods:** Extracted dog's premolar teeth were maintained in a dry environment for a month after isolation and proliferation of the periodontal ligament (PDL) cells. Then, tooth roots coated with 1×10^6 cultured autologous PDL cells were autotransplanted in artificial sockets created in the mandible. The dogs were sacrificed 60 days after transplantation. Histologic analyses showed that a root-PDL-bone complex was found in all cases of the PDL cell–loaded samples. **Results:** The new PDL-like connective tissue was located between the alveolar bone and the transplanted roots, with fibers inserting into the newborn cementum on one end and alveolar bone on the other. For the control samples, no PDL-like tissue was found, and ankylosis was commonly observed. **Conclusions:** The results indicated that cultured autologous PDL cells assist in the re-establishment of periodontal architecture of autotransplanted teeth that is devoid of viable periodontal cells. *(J Endod 2010;36:264–267)*

Key Words

Delayed autotransplantation, periodontal ligament, periodontal regeneration, replantation

From the *Research Laboratory of Oral and Maxillofacial Surgery; ‡Department of Oral Anatomy and Physiology; §Special Dental Department, Peking University School and Hospital of Stomatology, Beijing, China; and †Area of Endodontics, Faculty of Dentistry, the University of Hong Kong, HKSAR, China.

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Address requests for reprints to Dr Chengfei Zhang, Floor 3A, Prince Philip Dental Hospital, 34 Hospital Road, Saiyingpun, Hong Kong SAR, China. E-mail address: zchengfei@yahoo.com.

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Dentoalveolar traumas are unexpected events and may occur at any time of life, although these seem to be more prevalent in children and adolescents. Avulsion is probably the most severe form of dentoalveolar injuries; the tooth is completely displaced out of its socket, affecting not only the pulp but also the periodontal apparatus including the periodontal ligament (PDL), the alveolar bone, and gingival tissues. The PDL is the layer of connective tissues that connects the cementum (of tooth root) and the alveolar bone. It fixes the tooth in the alveolar socket and attenuates any occlusal loads acting on the tooth (1). All fibers of the PDL are severed in avulsion injury; the survival of the PDL cells on the root surface after injury plays a critical role in the healing of the replanted tooth. Successful replantation is dependent on the re-establishment of the PDL, thus preventing ankylosis or any replacement root resorption (2). Great efforts in shortening the extra-alveolar time and selecting the appropriate storage media have been made to maintain the viability of the remaining PDL cells (3–9). A review of published clinical trials (4, 8, 10–14) indicated variable rates of survival for replanted teeth. A success rate of 4% to 50% has been reported (15). Ankylosis seems to be the most frequent complication associated with the avulsed but replanted teeth, with the ultimate result of replacement resorption and failure of the replanted tooth (16).

The development of tissue engineering and periodontal regeneration sheds some light on the management of avulsed teeth. Several experimental studies have shown that the PDL has a high natural ability of regeneration, contributing positively to the healing process after replantation (17–20). Herr et al (21) reported that fibroblasts originating from both the remaining PDL and alveolar bone compartments are responsible for the repair of the periodontium. Nowadays, it has been confirmed that PDL cells have the characteristics of stem cells that are able to differentiate and form all the components of the periodontium (22–24). The purpose of this study was to examine the effect of delayed autotransplantation in combination with the periodontal tissue engineering using autologous PDL cells on periodontal healing.

Materials and Methods

Reagents

The following reagents were obtained for use in this study: ketamine hydrochloride (Gutian Pharmaceutical, Fujian, China); Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (both from Invitrogen, Carlsbad, CA); and various antibiotics agents (Sigma-Aldrich, Colorado Springs, CO).

Isolation and Proliferation of Dog's PDL Cells

This study was approved by the Institutional Animal Care and Use Committee of the Peking University School of Stomatology. One-year-old mongrel dogs (weight 8-12 kg) were used for this study. The dogs were anesthetized using an intramuscular injection of ketamine hydrochloride (Gutian Pharmaceutical, Fujian, China) at 2 mg/kg body weight before operation. Under a sterile condition, both the left and right third and fourth mandibular premolars were extracted by using an elevator and dental forceps and immediately immersed into an ice-cold phosphate-buffered saline (PBS) solution containing 100 U/mL penicillin and 100 μg/mL streptomycin. The extracted teeth were transferred to the laboratory for PDL cell culture. The wounds were sutured, and the dogs were fed on a soft diet. Before isolation of the PDL cells, the dental pulp and

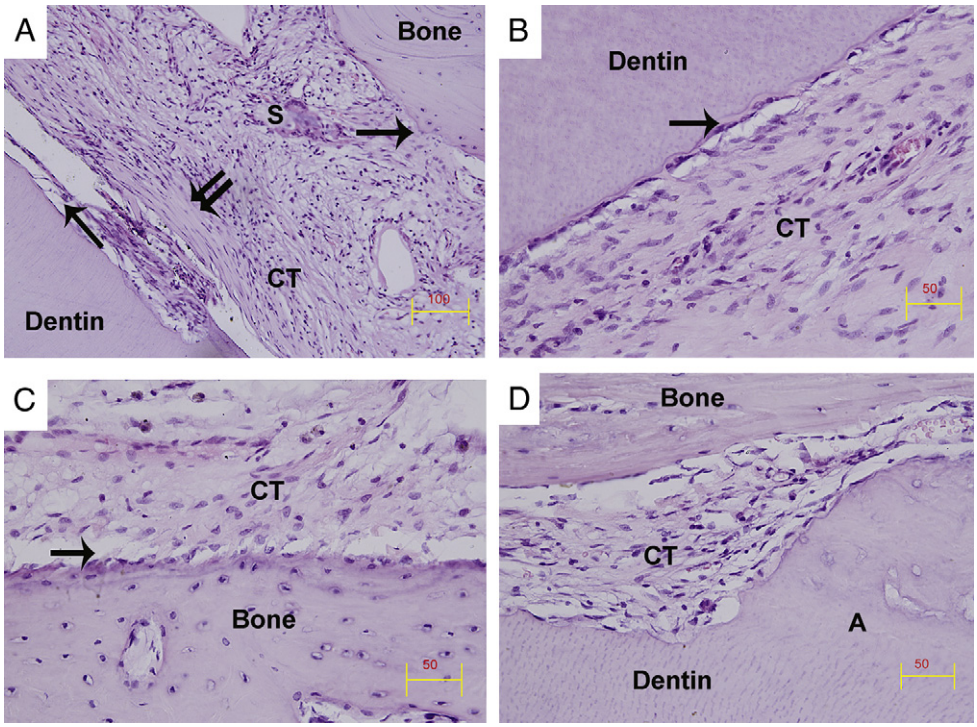

Figure 1. The experimental group (PDL cells—loaded samples) showing fibrous connective tissue in the artificial periodontal space (*A*), with fibers inserting into the newborn cementum on one end (*B*) and alveolar bone on the other (*C*). Ankylosis was also found in some area in this group (*D*). Bone, alveolar bone; S, undegraded scaffold; CT, fibrous connective tissue; A, ankylotic area; single arrows, fibers embedded in the surface of the root surface or the alveolar bone; double arrow, fiber bundle-like structure (hematoxylin and eosin stain, original magnification: ×200 for Fig. 1*A* and ×400 for the others, unit of scale bar: μm).

any gingival tissues near the cervical region of the tooth were completely removed in order to avoid contamination by other tissue types. The PDL cells were harvested by six sequential digestions of PBS containing collagenase (1 mg/mL) and dispase (2 mg/mL). Cells isolated in runs two to six were pooled and cultured until confluence in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin (DMEM complete medium) at 37°C in 5% CO_2 atmosphere. The sixth to eighth passage of PDL cells was used for the periodontal reconstruction. After obtaining the PDL cells, the teeth were kept in a dry environment for 30 days until autotransplantation.

Preparation of Autograft PDL Cells–Alginate Hydrogel Vehicles

Two hours before autotransplantation, root canal treatment of the extracted teeth was completed in the laboratory. The canals were obturated with gutta-percha, and then their external surfaces were disinfected with 70% alcohol before transferring into a physiologic saline solution before the autotransplantation procedure. The cultured PDL cells were digested with 0.25% trypsin containing 0.02% EDTA (Sigma-Aldrich, St Louis, MO); rinsed with PBS once; and then resuspended in 1.2% alginate sodium solution, which is a 4:1 mixture of Co^{60}-treated (irradiation at 5 kGy for 24 hours) and -untreated solution. The cell density was adjusted to 1×10^7 PDL cells/mL. Then, 20 μL of 1 mol/L $CaCl_2$ solution was added to 100 μL of PDL cell suspension to form an alginate hydrogel with 1×10^6 PDL cells.

Autotransplantation of Autologous Tooth Roots

A mucoperiosteal flap was raised from the first to the fourth premolar on each side of the mandible under general anesthesia. Artificial sockets matching the shape of the extracted autologous tooth roots were formed by a low-speed sterile round bur under 0.9% cold physiologic saline irrigation. Two artificial sockets were created in each side. The roots were divided into two groups: the experimental group, autotransplanted roots with alginate hydrogel containing about 1×10^6 PDL cells, and the control group, roots with alginate hydrogel only. Samples in the former group were transplanted into the artificial sockets at the right side of the mandible and then covered with a restorable membrane (Puros Pericardium Membrane; Zimmer Dental, Inc, CA); the wound was closed with 4-0 silk suture. The same procedures were performed for the control group in the contralateral side.

Histologic Analysis

Two months after transplantation, the dogs were sacrificed with an overdose of ketamine hydrochloride. Mandibular block segments containing the autotransplanted roots were resected from the jaw and immersed into 10% neutral buffered formalin for 3 days. The specimens were decalcified in 14% EDTA solution (pH = 7.0) and embedded in paraffin after dehydration in a graded alcohol series. Histologic sections were cut perpendicular to the long axis of the roots at a thickness of 5 μm at 30-μm intervals, stained with hematoxylin and eosin, and examined under an optical microscope (Olympus DP, Tokyo, Japan) for the formation of periodontium-like architecture. Based on a modified version described by Kirakozova et al (25), two blinded examiners assessed the type of periodontal healing in each section. A sample with the presence of a PDL-like structure was scored as favorable healing, whereas a sample without a PDL-like structure but with root resorption or ankylosis in the artificial periodontal space was scored as unfavorable healing.

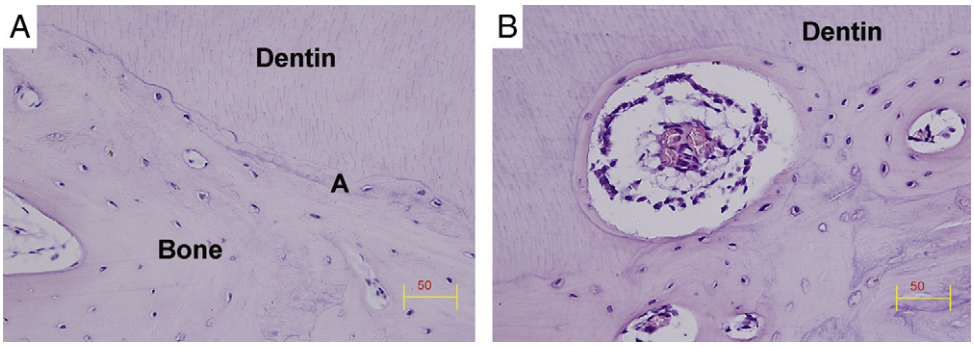

Figure 2. The control group showing areas with ankylosis and the artificial periodontal space filled by newborn bony tissue (A) and the presence of replacement root resorption. B, alveolar bone; A, ankylotic area (hematoxylin and eosin stain, original magnification ×400, unit of scale bar: μm).

Statistical Analysis

The measurements for favorable and unfavorable healing of roots were collected as percentages of the total number of roots in each group. The chi-square test was used to determine whether the periodontal healing pattern was different in the two groups. MedCalc statistical software (MedCalc 9.2.1.0; Frank Schoonjans, Mariakerke, Belgium) was used to analyze all data at $P < 0.05$.

Results

The PDL cells derived from the extracted teeth were successfully isolated and expanded by using the method described previously. After 1 month, the extraction wounds healed and were covered with healthy mucosa. Favorable healing after autotransplantation in the newly created socket was observed in the experimental group. No periradicular infection was found clinically, and the dogs recuperated very well for 2 months postoperatively before they were sacrificed for histologic examination.

In the experimental group, there was clear formation of a root-PDL-bone complex in all histologic sections; the "newborn" PDL-like structure was located between the alveolar bone and the transplanted root (Fig. 1*A*). The root surface was lined with a thin layer of "new" cementum into which Sharpey fibers were attached (Fig. 1*B*). These PDL-like fibers were found extending into the alveolar bone (Fig. 1*C*). The still-intact scaffold (alginate hydrogel) was also found in the PDL-like connective tissue (Fig. 1*A*) and in the restored alveolar bone (Fig. 1*C*). There were some patchy areas of ankylosis in this group of samples (Fig. 1*D*).

The control group showed an absence of PDL-like connective tissue. Ankylosis (Fig. 2*A*) and root resorption (Fig. 2*B*) were virtually noticed in all specimens. Table 1 shows the distribution of the positive roots per groups for the statistical analysis. The experimental group had significantly more favorable healing and less unfavorable healing than the control group ($P < 0.05$).

Discussion

The "reconstruction" of the periodontium after delayed replantation mediated by cultured PDL cells is based on the idea of the periodontal tissue engineering. Because the success rate of the traditional replantation procedure (for teeth with various extra-alveolar time after avulsion) is rather unpredictable, varying from 4% to 50% (15), it is necessary to develop a new strategy for the treatment. Delayed autotransplantation combined with cultured autologous PDL cells, as was shown earlier, is a novel regimen to tackle this clinical problem. Typically, PDL cells will remain viable for a finite period of time on the root surface after the tooth is avulsed; these cells play an important role in the periodontal healing of the replanted transplanted teeth (26). Regeneration of the PDL architecture is effective in preventing ankylosis and replacement resorption of the transplanted tooth roots (26).

In this study, to avoid any influence on the outcome because of any remaining PDL cells surviving on extracted teeth, roots were deliberately stored in a dry environment for 1 month. An artificial bony socket was also created to house the tooth. It is well known that the extraoral time and storage medium are critical for periodontal healing of replanted teeth; storage of less than 1 hour in an appropriate medium is recommended. In the present study, a PDL-like tissue was successfully regenerated by using this new strategy of replantation together with cultured autogenous PDL cells despite the dry storage of the avulsed tooth for an extended period.

Alginate hydrogel was used as the scaffold here because the hydrogel allows even distribution of the PDL cells within the material to fill the space in the artificial sockets. Alginate hydrogel has been used as a scaffold in hard (bone) tissue engineering (27, 28). To control the degradation rate of this scaffold, an irradiated (by Co^{60}) sodium alginate solution was mixed with an untreated solution at the ratio of 4:1. Gradual degradation of the alginate hydrogel left spaces for the newly formed periodontal tissue (although we could not match the rates of hydrogel degradation and periodontal regeneration rate perfectly). In

TABLE 1. Percentage of Positive Roots per Group Used for Statistical Analysis*

	Experimental group percent (total roots)	Control group percent (total roots)	Chi-square test *P* value†
Favorable healing	100 (6)	0 (6)	0.002
Unfavorable healing: ankylosis	0 (6)	100 (6)	0.002
Unfavorable healing: Root resorption	0 (6)	83.3 (6)	0.015

*Three sections per root were evaluated.

†$p < 0.05$ was considered statistically significant.

some specimens of the experimental group, there was only a small amount of undegraded hydrogel sporadically found in the PDL-like connective tissues or the reconstructed alveolar bones, suggesting that the degradation rate of the alginate hydrogel could be controlled further.

Our results showed that the PDL-like structure was found in samples of autotransplantation with PDL cells but not in the control group. Specimens showing a normal appearance of the architecture were found in some areas of the autotransplanted teeth of the experimental group. In contrary, ankylosis and resorption were commonplace for the control group. Because the newly formed PDL-like connective tissues were embedded into cementum on one end and alveolar bone on the other, it is clearly shown that the cultured PDL cells have the potential to regenerate the periodontal tissues.

Although a regenerated PDL-like structure had been found in this study, there were no newly formed fiber bundles with functional orientation of a healthy periodontium. This may be because of the following: (1) the autotransplanted roots were embedded and covered by the mucoperiosteum and thus the root did not receive any mechanical stimuli from occlusion and (2) the mismatch in the rates of alginate hydrogel degradation and periodontal regeneration might affect the organization of PDL fibers in that space.

The new strategy shed some lights on the treatment of avulsed teeth, but more studies need to be performed before patients with avulsed teeth might benefit from this approach. One viable source of PDL cells to establish a PDL cell bank might be the stem cell bank derived from extracting the third molar or the first premolars as necessitated by orthodontic treatment.

In conclusion, this preliminary study has shown the beneficial effects of cultured autologous PDL cells on periodontal healing of avulsed teeth after a delayed autotransplantation in dogs. The results indicated that periodontal ligament cells have the potential to regenerate periodontal tissues in the artificial alveolar socket. Delayed autotransplantation combined with cultured autologous PDL cells might be an alternative management for the avulsed teeth that have left extraorally for an extended period of time. Further studies with a larger sample are necessary.

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