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Short Communication

Cell migration capability of vascular endothelial growth factor into the root apex of a root canal model *in vivo*

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Abstract: Once a tooth develops deep caries and the dental pulp tissue is irreversibly infected, the infected dental pulp tissue should be removed, and filling material should be placed in the root canal. Endodontically treated teeth are prone to root fracture or periapical periodontitis; however, dental pulp tissue has the potential to prevent root fracture or periapical periodontitis. Therefore, dental pulp regeneration after pulpectomy may help prolong tooth life. In this study, a new method of dental pulp regeneration was developed. Vascular endothelial growth factor-adsorbed collagen gel was injected into the root canal of a prepared root canal model, placed into the dorsum of a rat, and cultured for 3 weeks. After retrieving the implant, histological analysis was performed. It was found that rat somatic cells were recruited into the root apex of the transplanted root canal model. These findings suggest a new potential technique for engineering dental pulp tissue.

Keywords: vascular endothelial growth factor; dental pulp; cell homing.

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Introduction

Once caries reaches the dental pulp tissue and irreversible pulpitis develops, the infected dental pulp tissue should be removed, and root canal treatment should be performed. However, endodontically treated teeth are prone to cracking and infection at the root apex, and salivary microleakage can be a problem (1). If we can regenerate dental pulp tissue after pulpectomy, teeth with regenerated dental pulp will maintain the ability to regrow dentin, defend against exogenous stimuli, and perform immunological functions. This technique will also prevent the need for tooth extraction due to root fraction or periapical periodontitis.

The preclinical safety, feasibility, and efficacy of dental pulp regeneration using the transplantation of dental pulp stem cells (DPSCs) have been established (2). However, while the application of dental pulp regeneration has been thoroughly examined, this method requires the extraction of teeth and having blood drawn to obtain autologous DPSCs and serum. In addition, there are financial expenses for the expansion of DPSCs in a cell-processing center, the transportation of DPSCs to each hospital, and standard operating procedures to reduce the risk of a cell-derived immunoreaction.

It has been reported that somatic stem cells and progenitor cells recruit cells to an injured area, regenerating injured tissue in a process known as cell homing. Stem cells and progenitor cells have this cell homing ability, and the cells around an injured area release growth factor to recruit stem cells and progenitor cells (3). Several researchers have attempted to induce the regeneration of tissue or organs, like bone or teeth, using

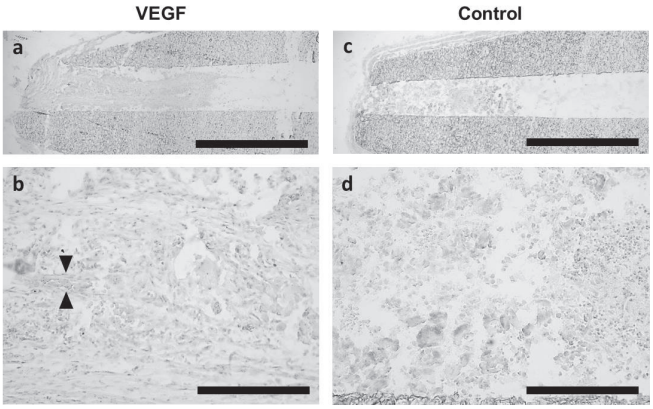

Fig. 1 VEGF-recruited rat somatic cells into the root apex of the root canal model. A large number of rat somatic cells and blood vessel-like tissue were observed in the root apex of the root canal model injected with the VEGF-adsorbed collagen gel at three weeks after transplantation (a, b). Fewer cells and no blood vessel-like tissue were observed in the root apex of the root canal model injected with VEGF-free collagen gel (c, d). Blood vessel-like structure tissue (arrowheads). Scale bars: (a, c) = 1 mm; (b, d) = 125 μm.

this mechanism (4,5).

Vascular endothelial growth factor (VEGF) is a glycoprotein that promotes the proliferation and differentiation of vascular endothelial cells to bind with VEGF receptors (VEGFRs) on vascular endothelial cells, inducing vasculogenesis and angiogenesis (6). In angiogenesis, smooth muscle cells and pericytes are recruited to stabilize differentiation, proliferation, and survival of endothelial cells to maintain blood vessels. Pericytes are mural cells surrounding the endothelial cells of capillaries throughout the body and may be differentiated into myoblasts, fibroblasts, osteoblasts, chondrocytes, neurospheres, adipocytes, and mesenchymal stem cells (7).

In the present study, the goal was to establish a safer, less expensive, and easier method of dental pulp regeneration using only VEGF and scaffold to recruit stem cells and progenitor cells into the root canal.

Materials and Methods

Endodontic treatment in a root canal model

An access opening was made through the lingual crown of the root canal model (Nissin, Kyoto, Japan) into the pulp chamber, and the root canal preparation was done with hand reamers (0.02 taper, size from #15 to #60). After root canal preparation, the root canal model was washed with 6% sodium hypochlorite solution and placed in 6% sodium hypochlorite solution for at least 24 h. VEGF at a dose of 10 ng/mL (VEGF-A165; Sigma, St. Louis, MO, USA) adhered to a collagen gel solution (Nitta Gelatin, Osaka, Japan) (8). VEGF-adsorbed collagen gel solution was carefully injected into the root canal of the prepared root canal model with a 30-G needle and incubated at 37°C for 10 min. GC Fuji LINING (GC, Tokyo, Japan) was used for sealing and cured with PenCure (Morita Mfg., Suita, Japan) for 20 s. Additionally, VEGF-free collagen gel solution was injected into another root canal model and incubated as a control.

Implantation

Prior to the study, all experiments were reviewed and approved by the Animal Care and Use Committee of Nagasaki University Graduate School of Biomedical Sciences (Permit Number: 150309-1-2). Six-week-old male rats (Texam, Nagasaki, Japan) were anesthetized with a combination of medetomidine-midazolam-fentanyl. A linear incision was made, and a subcutaneous pocket was created in the dorsum. Treated root canal models with VEGF-adsorbed or VEGF-free collagen gel were subcutaneously implanted for 3 weeks.

Histological analyses

The implanted rats were fixed, and the implants were then retrieved and placed in 10% buffered formalin. They were mounted in optimal cutting temperature compound and frozen with liquid nitrogen. Sections were made with a cryostat (CM1950; Leica, Wetzlar, Germany), then thawed for 20 s and placed into 100% ethanol and dyed with hematoxylin and eosin (HE) staining.

Results

To evaluate the chemotaxis of rat somatic cells, VEGF-adsorbed collagen gel was injected into the root canal of a prepared root canal model and placed in the dorsum of 6-week-old rats. Three weeks after placement, the

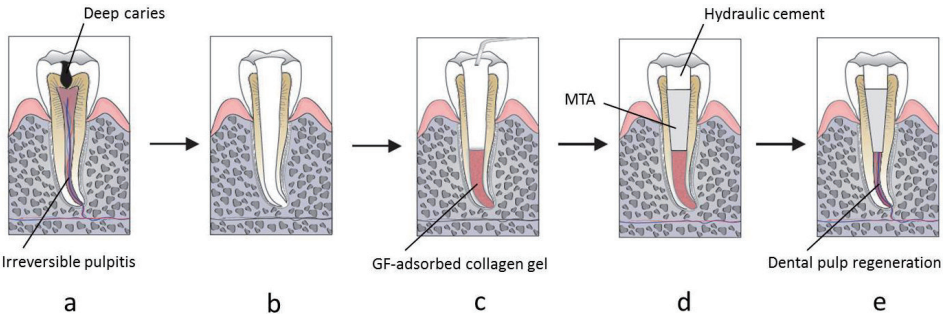

Fig. 2 Schematic illustration of a new method of root canal filling. Once dental pulp tissue is irreversibly infected and should be removed (a). After root canal preparation, the root canal is washed with 17% ethylenediaminetetraacetic acid solution and 6% sodium hypochlorite solution and dried completely (b). A growth factor-adsorbed scaffold is carefully injected into the apical third of the root canal with a 30-G needle (c) and sealed with MTA. Hydraulic cement is temporarily filled on the MTA (d). After the treatment, growth factor recruits somatic cells from the periapex, and new dental pulp tissue is constructed (e).

implant was retrieved and dyed with HE staining. A large number of rat somatic cells and blood vessel-like tissue were observed in the root apex of the root canal model injected with the VEGF-adsorbed collagen gel (Fig. 1a, b). Furthermore, fewer cells and no blood vessel-like tissue were observed in the root apex of the control root canal model that was injected with VEGF-free collagen gel (Fig. 1c, d).

Discussion

In this study, a large number of rat somatic cells are observed in the root apex of the root canal model injected with VEGF-adsorbed collagen gel. It is unclear if this occurs because VEGF induces angiogenesis, recruiting multipotent pericytes in the root apex of the root canal model. In future studies, we hope to characterize these cells with immune staining.

Kim et al. showed that cells were recruited into the root canal by growth factors (8). That was the first study to regenerate dental pulp tissue with a growth factor. However, cells were recruited by not only the root apex but also the access hole because the tooth was not sealed at the access hole. We need to recruit somatic cells only by the root apex. In the current study, rat somatic cells are recruited only from the periapex into the root apex of our root canal model injected with VEGF-adsorbed collagen without any cell transplantation.

We believe that the regeneration of dental pulp tissue in the apical third of the root canal is sufficient. The success rate of pulpectomy is high, but secondary caries or inappropriate treatment can easily cause periapical periodontitis. When microleakage occurs, the regenerated dental pulp tissue at the root apex may respond to the penetration of bacteria, and endodontic retreatment may be required to prevent periapical periodontitis. Dental pulp tissue regenerated in the apical third may also reduce the risk of root fracture in the apex, which is often difficult to find and treat, even with a microscope.

Once the dental pulp tissue is irreversibly infected, it should be removed (Fig. 2a). After root canal preparation, the root canal is washed with 17% ethylenediaminetetraacetic acid solution and 6% sodium hypochlorite solution and dried completely (Fig. 2b). A growth factor-adsorbed scaffold is then carefully injected into the apical third of the root canal with a 30-G needle (Fig. 2c) and sealed with mineral trioxide aggregate (MTA). Hydraulic cement is temporarily filled on the hardened MTA (Fig. 2d). After the treatment, growth factor will recruit somatic cells from the periapex, and new dental pulp tissue is constructed (Fig. 2e).

As there are root canal branches in the apical 3 mm of the root canal, cells should be recruited >3 mm from the apical foramen for apical barrier formation. In this study, cells were recruited ≤3 mm from the apical foramen. This necessitated a prolonged culture period to achieve optimal results. A translucent root canal model used in the present study, allowing microscopic monitoring of whether the prepared root canal wall is clean and smooth and whether the collagen gel has been injected correctly without any air bubbles. In this way, we can compare the number of cells recruited into the root canal model and evaluate the appropriate root canal preparation, including the apical preparation size and taper and the appropriate use of a scaffold and growth factors.

In conclusion, 3 weeks after root preparation, irrigation, injection with VEGF-adsorbed collagen, and sealing, rat somatic cells are recruited from the periapex into the root apex of our root canal model without any cell transplantation. While this study has several issues to address, this technique may represent a safe, reasonable,

and simple new method of dental pulp regeneration that is easily applied in the clinical setting.

Acknowledgments

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Conflict of interest

None declared.

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