Submitted: 08 November, 2022 Accepted: 05 January, 2023 Published: 03 May, 2024 DOI:10.22514/jocpd.2024.071

Journal of Clinical Pediatric Dentistry

CASE REPORT

Deciduous pulp tissue implantation into the root canal of mandibular incisor resulted in pulp revascularization: a case report with a 5-year follow-up

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Abstract

To explore a new method to implant deciduous tooth pulp into the canal of young permanent teeth with necrotic pulps and apical periodontitis for the regenerative endodontic treatment of tooth no: 41 in a 7-year-old male. Briefly, 1.5% Sodium Hypochlorite (NaOCl) irrigation and calcium hydroxide-iodoform paste were used as root canal disinfectant at the first visit. After 2 weeks, the intracanal medication was removed, and the root canal was slowly rinsed with 17% Ethylene Diamine Tetraacetic Acid (EDTA), followed by flushing with 20 mL saline and then drying with paper points. Tooth no: 72 was extracted, and its pulp was extracted and subsequently implanted into the disinfected root canal along with induced apical bleeding. Calcium hydroxide iodoform paste was gently placed over the bleeding clot, and after forming a mineral trioxide aggregate (MTA) coronal barrier, the accessed cavities were restored using Z350 resin composite. The root developments were evaluated *via* radiographic imaging at 6 months, 1 year and 5 years after treatment. Imaging and clinical analysis showed closure of the apical foramen, thickening of the root canal wall, and satisfactory root length growth. Autologous transplantation might be useful to regenerate dental pulp in necrotic young permanent teeth.

Keywords

Autologous transplantation; Deciduous tooth; Pulp; Regeneration

1. Introduction

Research on regenerative endodontic procedures (REPs) for treatment of young permanent teeth with necrotic pulps and apical periodontitis has become a hot topic [1]. The major advantages of REPs are that they allow further development of the root of young permanent teeth and enhance root strength by hard tissue deposits over traditional methods [2]. Several studies on REPs have shown that the newly formed tissues contain multiple types of dentin pulp-like structures and periodontal membrane-like, cementum-like and periapical bone-like tissues [1–3], which all are indicative of repair rather than regeneration.

The physiological development of tooth root is dependent on the presence of part of the surviving dental pulp, apical papilla (SCAPs) and epithelial root sheath (ERS) [2–4]. If the pulp is completely necrotic, the SCAPs and ERS become damaged and the root development depends on the stem cells in the periodontal membrane, cementum and alveolar bone, thereby complicating the treatment of such infected tooth [4]. Under these circumstances, regenerating pulp tissues is the ideal way to improve revascularization. Stem cells, growth factors and scaffolds are the three components that are critical to the success of tissue engineering in these cases [2]. Deciduous pulp meets this requirement for tissue engineering components because of the existence of undifferentiated stem cells, growth factors and the natural extracellular microenvironment [5].

Research on the potential of pulp regeneration *via* autologous transplantation of deciduous pulp into immature necrotic permanent teeth showed tooth development in necrotic young permanent teeth [6, 7]. In this report, we present a case of a patient whose pulp tissue from tooth no: 72 was implanted into tooth no: 41 which contained necrotic pulps and apical periodontitis; and provide discussions on the potential of pulp tissue regeneration *via* autologous transplantation.

2. Subject and methods

2.1 Subject

The patient was a seven-year-old male with a history of a crown fracture one month earlier, for which his local dentist performed root canal therapy. The patient experienced severe pain and visited the dentist one day before presenting at our clinic.

No significant abnormal findings were noted in the extraoral examination. Intra-oral examination showed that tooth no: 41 had a crown fracture (Fig. 1). Sensibility tests, including Cold

Test and Electric Pulp Test (EPT), were performed, and the responses to these tests were negative, although the patient did show sensitivity to percussion. The mobility of tooth no: 41 was II, and periodontal probing depths were 2 mm. Radiographic examination showed that the apical foramen of tooth no: 41 was trumpet-shaped with a thin root canal wall, incomplete filling of gutta-percha and a broken needle (Fig. 2A–B). The needle was located 2 mm inside the middle of the root canal (Fig. 2C–D). The root development of tooth no: 41 was in stage III according to the Cvek classification, following which the patient was diagnosed with pulp necrosis and acute apical periodontitis of tooth no: 41.

Considering the root development was incomplete, the treatment plan was to attempt pulp regeneration with the pulp tissue from exfoliated deciduous teeth as the biocompatible scaffold. Before the treatment, all potential risks and the possible outcomes of the treatment were comprehensively discussed with the parent, and a full consent was obtained.

FIGURE 1. The arrow shows the crown fracture at tooth no: 41.

2.2 Methods

At the first appointment, tooth no: 41 was separated with a rubber dam after local anesthesia with 2% lidocaine (Sinopharm Group Rongsheng Pharmaceutical Co., Ltd, city, China) and access was acquired with water-cooled high-speed #3 diamond burs. The working length (WL) was determined radiographically (Fig. 3). The DG16 endodontic explorer was used to detect the root canal entrance. The gutta-percha was removed using a 15# hand K-file under a dental microscope, and the broken needle was removed by copious irrigation with a large amount of sterile saline using an Ultrasonic activator (p5xs, Acteon, Bordeaux, France). Then, the root canals were gently irrigated with 20 mL of freshly prepared 1.5% NaOCl using closed-end and side-vented needles positioned about 1 mm below the apex. Following copious irrigation with 20 mL sterile saline, the root canals were dried with sterile paper points. Calcium hydroxide iodoform paste (VETAPEX, Morita, Japan) was embedded into the root canal using a Lentulo Spiral filler. The canal was temporarily sealed with 3–4 mm glass ionomer cement (Jiangsu, China), and ibuprofen was prescribed to the patient for 3 days.

During the second appointment 2 weeks later, the tooth was free of clinical symptoms. The patient rinsed his mouth with 3% Gargle hydrogen mouthwash (Likang Disinfectant Hi-Tch Co., Ltd, Shanghai, China) for 30 seconds. Then, his mouth and neighboring areas were disinfected using 3% Betadine (Likang Disinfectant Hi-Tch Co., Ltd, Shanghai, China). Tooth no: 72 and no: 41 were then scrubbed with 1% Betadine (Likang Disinfectant Hi-Tch Co., Ltd, Shanghai, China) for 2 minutes, following local anesthesia with 2% lidocaine without epinephrine (Sinopharm Group Rongsheng Pharmaceutical Co., Ltd, China).

A thick rubber dam sheet was placed with punched holes exposing the tooth after disinfected by scrubbing with 3% Betadine (Fig. 4). The isolated crown surfaces were clean up using 3% Betadine. The access cavity was opened. The calcium hydroxide iodoform paste was removed by copious irrigation

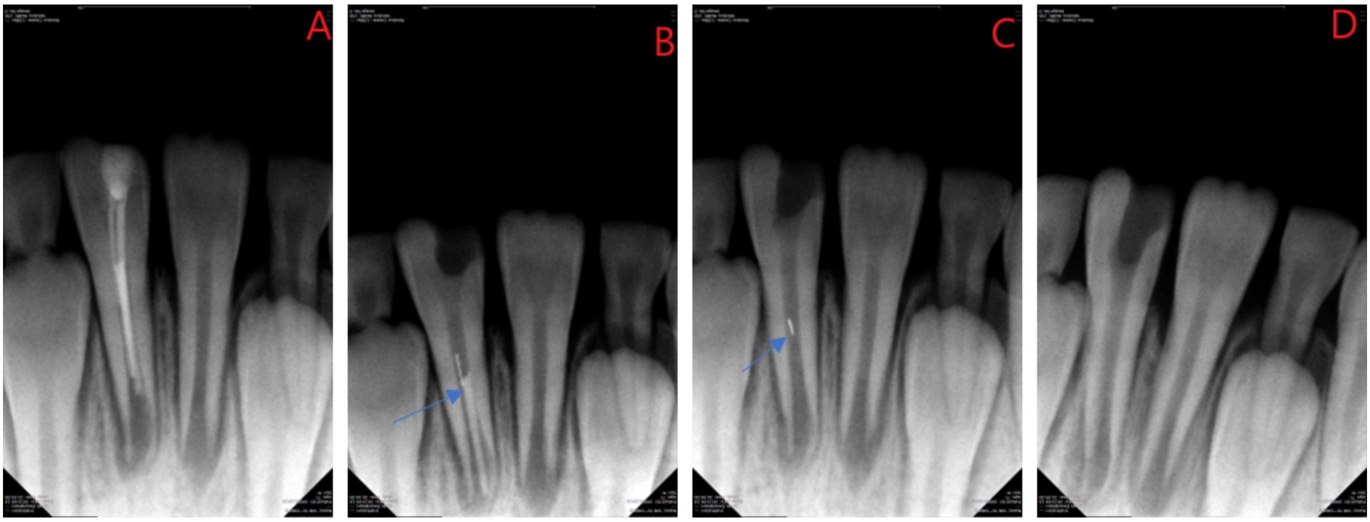

FIGURE 2. Radiographic examination showing that the apical foramen of tooth no: 41 was trumpet-shaped with thin root canal wall. The arrows demonstrate incomplete filling of gutta-percha and (A,B) and a broken needle located inside the middle of the root canal (C,D).

with 1.5% NaOCl first and followed by saline as described above with an Ultrasonic activator (SATELEC, France); the root canals were dried with sterile paper points and gently irrigated again with 20 mL of 17% EDTA, followed by a final flush with 20 mL saline, dried with paper points.

FIGURE 3. Radiographic determination of the working length of tooth no: 41.

FIGURE 4. A thick rubber dam sheet.

For the autotransplantation procedure, as the mandibular incisor was the smallest permanent tooth in the mouth, and the size and shape of the mandibular lateral incisor were close to that of the incisor, it was decided to extract tooth no: 72, whose working length was determined by measuring with a ruler. The crown of tooth no: 72 was removed by creating a deep circumferential notch at CEJ using a high-speed, water-cooled diamond disk (Fig. 5), followed by the separation of the crown from the root by applying gentle digital pressure. The pulp tissue was then quickly removed using a pre-sterilized excavator with minimal trauma. Then, bleeding was created by over-instrumenting at 2 mm past the apical foramen with disinfected 15# K-file (MANI, Japan), and the bleeding was stopped below the cementoenamel junction (CEJ). The whole pulp was quickly released from the canal using a sterile tweezer. Immediately, the pulp tissue was gently laid down into the root canal of tooth no: 41 with disinfected gutta-percha cones marking the working length according to tooth no: 72. The transplanted deciduous pulp was allowed to remain in contact with blood for 1 minute without interruption, vision magnification was used during the process.

FIGURE 5. The tooth no: 72 was extracted and the arrow shows the separated pulp tissue.

Calcium hydroxide iodoform paste (Vetapex, Morita, Japan) was placed over the blood clot, following MTA coronal barrier, and temporarily sealed with glass ionomer cement (GC, Japan). Part of the glass ionomer cement one week later, and was sealed with Z350 resin (3M, USA). After treatment, the patient was prescribed amoxicillin (Sinochem Group Zhongnuo Pharmaceutical Co., Ltd., city, China) for 2 days and was advised on oral hygiene and trauma prevention.

Radiographic examinations were carried out 6 months, 1 year and 5 years after surgery to visualize the apical foramen, width of root walls and root length.

The patient was examined at the indicated time interval after the treatment and reported no clinical symptoms, with negative response to cold test and EPT at all times of the recall period. Additionally, increased root length was observed at the 6-month follow-up (Fig. 6); thickened wall thickness and growth of root length was measured at 1-year (Fig. 7) and 5-year (Fig. 8) after treatment; complete closure was observed at the 5-year follow-up (Fig. 7), and internal mineralization in the apical third (Fig. 7) at the 1-year follow-up, which remained on the 5-year follow-up (Fig. 8). The root development stage of tooth no. 41, according to the Cvek classification, was V at the 5-year follow-up.

3. Discussion

The pulp tissues of the autologous tooth have been used in single-rooted mature premolars and single-rooted immature permanent incisors and proven as a feasible biologic scaffold for regenerative endodontic treatment [7, 8]. Consistently, the results presented here further suggest that autologous pulp transplantation can achieve clinical outcomes and radiographic results that are similar to those using traditional revascularization techniques [7–9]. These include periapical inflammation healing, continued development of the root canal, augmentation of root length, and reduction of apical diameter.

FIGURE 6. Growth of root length observed 6 months after follow-up.

FIGURE 7. The thickening of root canal wall and the growth of root length observed after 1 year of treatment. The arrows demonstrate internal mineralization in the apical third.

FIGURE 8. Thickening of root canal wall and growth of root length observed and complete closure observed at the 5-year follow-up. The arrow demonstrates maintenance of internal mineralization in the apical third.

It is known that relative to the adult human teeth, the deciduous tooth pulp contains a lot of deciduous pulp stem cells with a higher rate of proliferation [5]. The deciduous pulp also contains multiple growth factors, including fibroblast growth factor-2, transforming growth factor-b, vascular endothelial growth factor, and platelet-derived growth factor [10, 11]. It was hypothesized that multiple cytokines and dentin matrix proteins are released from the wall of the root canal following root canal irrigation [12]; and the blood clots in the root canal can also release vascular endothelial growth factor, epidermal growth factor and other growth factors [13]. These cytokines, matrix proteins and growth factors together promote the homing of stem cells and tissue regeneration [14].

It is worth mentioning that radiographic imaging showed internal mineralization at the 1-year follow-up. The potential of new repair tissues to promote continued root development is limited by the extent of damage on Hertwig's epithelial root sheath and apical papilla induced by trauma or apical periodontitis [15], which can lead to unpredictability as the commonly reported problem of progressive root canal obliteration [16]. Deciduous tooth pulp has a very low tendency for calcification [17]. It is therefore a likely result of new vital tissue regeneration from the SCAP and/or autotransplanted deciduous pulp stem cells. However, it is also worth noting that a reasonably large pulp volume that can provide sufficient human exfoliated deciduous teeth (SHED) is important as it can differentiate into pulp-like tissue over time. Insufficient SHED might cause progressive calcific tissue deposits with induced apical bleeding, providing an acute influx of surrounding periodontal tissues with aggressive mineralizing properties [18].

Ischemia renders tissue transplantation challenging [19]. The dental pulp is enclosed in a mineralized shell and has a limited blood supply, so stimulating bleeding is essential for the autologous transplantation of deciduous tooth pulp to reduce damage caused by excessive ischemia in the initial stage of transplantation. In this present study, the bleeding was initiated by instrumentation beyond the apex, not by the apical separation of the primary pulp tissue, which was insufficient for blood supply (Fig. 4). In addition, the ability of SHED to establish vascularization is usually more robust than that of dental pulp stem cells [10, 20]. Thus, in the presence of an open apical foramen [21], SHED may help establish vasculature that will ensure the proper nutrient supply for tissue regeneration over time.

In our case series, the cold test and EPT were negative at any recall period. It is well understood that for regenerative endodontic treatment, positive response to vitality tests is a desirable clinician-based outcome [22]. However, lack of positive response in some cases could be a result of lagging in nerve regeneration. In such cases, Doppler ultrasound evaluation is more appropriate to be conducted to demonstrate revascularization and pulp vitality [23].

Infection control is critical to treatment [24]. The American Association of Endodontists (AAE) recommends 1–1.5% sodium hypochlorite (NaOCl) as the flushing solution [25], which is lower in concentration, thus, less toxic [26]. Therefore, 1.5% NaOCl was used, followed by rinsing with plenty of sterile saline to minimize potential damages to stem cells. Considering that the antibiotic paste at the clinically advocated concentrations could impact the survivability of SCAPs [27], we used calcium hydroxide iodoform paste in the root canal because it has bactericidal effects within the confines of the root canal. These properties are thought to be directly related to its potential of hydrogen (pH) (pH 9–12), as it can inhibit the growth of bacteria and neutralize acidic products in the area of inflammation [28], while iodoform can slowly release free iodine to reduce the exudation of the wound, keep the wound dry and do not have irritational effects on the tissue. In addition, iodoform also has anti-inflammatory and astringent effects [29].

It is important to seal the crown of teeth after transplantation. The general rule is to cover the MTA after hemostasis [25]. However, in our case, in an effort to prevent the discoloration by MTA [30], it was covered directly with calcium hydroxide iodoform, followed by MTA and dental morphology was restored with Z350 resins (3M, USA) after removing part of the glass ionomer cement.

Considering that it may not be possible to perform preoperative CBCT in this type of case, postgraduate residents and inexperienced general dentists should promptly refer to a trained specialist for better treatment guidance.

Although case reports provide helpful insights into rare cases and novel techniques used in clinical practice, they possess a low impact in determining the efficacy of a given treatment method. The case reported in this study also had limitations, which include a risk of over-emphasis on the specifics, possible over-interpretation of information based purely on a limited number of reported patients, insufficient evidence to fully establish a true cause-effect relationship.

4. Conclusion

This case report provide evidence that deciduous tooth pulp implantation can yield favorable clinical and radiographic outcomes but further studies await to validate these findings and to provide better clinical guidelines for treatment of such cases.

AVAILABILITY OF DATA AND MATERIALS

Some or all data, models generated or used during the study are available in a repository (Chinese Clinical Trial Registry) or online (https://www.chictr.org.cn/showproj.aspx?proj=16988) in accordance with funder data retention policies.

AUTHOR CONTRIBUTIONS

XYT and YH—designed the research study and performed the research. XYT—wrote the manuscript. XYT, YH, JJJX, GTS and LCL—participate in the discussion. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The treatment plan was approved by the Ethics Committee and the Institutional Biosafety Committee of the Affiliated Stomatological Hospital of Nanchang University (Jiangxi, P. R. China) (2016002) and the study was registered at the China clinical trial registration center (registration number: CHICTR-BNC-16009977). Inform consent was obtained from the patient and his parents.

ACKNOWLEDGMENT

The authors would like to thank Zezhang T. Wen at the LSUHSC School of Dentistry Department of Comprehensive Dentistry and Biomaterials, New Orleans, LA, USA for his final polish during the preparation of the manuscript.

FUNDING

The Project was funded by Science and Technology Department of Jiangxi Province (20171BBG70042).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Xiaoying Tang, Jiangjinjun Xu, Guangtai Song, Licheng Lai, Yan Huang. Deciduous pulp tissue implantation into the root canal of mandibular incisor resulted in pulp revascularization: a case report with a 5-year follow-up. Journal of Clinical Pediatric Dentistry. 2024; 48(3): 171-176. doi: 10.22514/jocpd.2024.071.