Regenerative endodontic procedures with minced pulp tissue graft in mature permanent teeth : An exploratory clinical study with a focused *in vitro* study

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Regenerative endodontic procedures with minced pulp tissue graft in mature permanent teeth : An exploratory clinical study with a focused *in vitro* study

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A Dissertation Submitted
to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy in Dental Science

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December 2024

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The Graduate School
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December 2024

감사의 글

2017년, 보존과 전공의로서 대학원 과정을 시작했던 순간이 엊그제 같은데, 어느덧 2024년 12월, 이 과정을 마무리하는 시점에 서게 되었습니다. 돌이켜보니, 그 시간들은 짧지 않았지만, 한편으로는 순식간에 흘러간 듯합니다. 여러 측면에서 제 부족함을 깊이 느낄 수 있었던 시간이었지만, 많은 분들의 도움 덕분에 지금의 제가 있을 수 있었다는 것을 깨달은, 참으로 감사한 시간이기도 했습니다.

우선, 치과대학 학생 시절부터 대학원 학위 과정에 이르기까지 항상 이끌어 주시고 아낌없는 지도를 베풀어 주신 김의성 교수님께 진심으로 감사드립니다. 교수님의 귀한 가르침과 따뜻한 격려가 있었기에 제가 치과의사로 성장하고, 보존과 전문의를 거쳐 대학원 과정을 무사히 마칠 수 있었습니다. 저의 논문 심사를 위해 소중한 시간을 내주시고 아낌없는 조언과 귀중한 충고를 해 주신 김선일 교수님, 김도현 교수님, 이석준 교수님, 송윤정 교수님께도 깊은 감사를 드립니다. 교수님들께서 보내주신 애정 어린 관심과 지도가 있었기에 제 학위논문의 완성도를 높이고 성공적으로 마무리할 수 있었습니다. 아울러, 전공의와 대학원 과정을 거치는 동안 저에게 관심과 격려를 아낌없이 보내주신 노병덕 교수님, 박성호 교수님, 정일영 교수님, 박정원 교수님, 신수정 교수님, 신유석 교수님께도 감사드립니다. 실험부터 논문 작성까지 항상 도움과 응원을 아끼지 않아 주신 연구실 식구들, 그리고 치과대학 및 보존과 동기들, 선후배님들께도 진심으로 감사의 마음을 전합니다.

마지막으로, 저의 오랜 학업 기간 동안 아낌없는 믿음과 사랑으로 항상 곁에서 큰 힘이 되어 주신 아버지, 어머니, 장인어른, 장모님께 깊은 감사와 사랑의 마음을 전합니다. 또한, 언제나 곁에서 사랑과 응원을 보내주며 제 삶의 가장 큰 원동력이 되어 준, 제가 세상에서 가장 사랑하는 아내 재희와 딸 지유에게도 무한한 감사와 사랑을 보냅니다.

2024년 12월 김 욱 성

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Abstract

Regenerative endodontic procedures with minced pulp tissue graft in mature permanent teeth : An exploratory clinical study with a focused *in vitro* study

Regenerative endodontic procedures (REPs) using cell-based approaches have emerged as novel treatment modalities. The minced pulp tissue graft was proposed as a simplified, cell-based REP that can bypass challenges associated with cell culture processes, demonstrating promising results in a laboratory study. However, there remains a significant gap in clinical applications, and foundational research on this procedure is still limited. This study is divided into two parts. Part I aims to present the treatment procedure and outcomes through an exploratory clinical study on REPs using minced pulp tissue grafts, focusing on identifying the tentative factors influencing pulpal tissue regeneration within this protocol. In Part II, using a reverse translational approach, the study investigates these identified tentative factors, specifically those that can be further validated at the *in vitro* level.

Part I - Healthy patients requiring non-surgical root canal treatment were enrolled. MP tissue obtained from the third molar was grafted into the instrumented, disinfected, and blood-filled root canal. Patients were evaluated clinically and radiographically. Follow-ups for the six enrolled cases ranged from 19 to 42 months. Radiographically, all the teeth showed favorable outcomes. In two teeth, intracanal calcification was observed in the apical third; however, there was no recovery in the sensibility tests. One tooth exhibited intracanal calcification in the apical third and showed recovery in the sensibility tests. Considering these outcomes and clinical variables, the size of the apical foramen and the composition of the transplanted pulp tissue were identified as tentative influencing factors.

Since it is evident that a larger apical foramen creates a more favorable environment for regeneration, reverse translational research was planned to validate the impact of the composition of the transplanted pulp tissue. Therefore, the objective of Part II was specified

to investigate the differences in VEGF and NGF secretion between the coronal and radicular minced pulp of mature permanent teeth at the tissue level, as these cytokines are crucial for promoting angiogenesis and neurogenesis, which are key processes in REPs.

Part II - Human dental pulp tissues were obtained from healthy adult mature permanent third molars with closed apices. The tissues were separated into coronal and radicular portions, minced, and incubated for 3 days. VEGF and NGF levels in the supernatants were then measured using ELISA. Pulp tissues were obtained from five patients, with ages ranging from 21 to 37 years. The total weight of the pulp tissue ranged between 6 and 20 mg. The weight ratio of coronal pulp to radicular pulp varied, with an average ratio of approximately 3.5:1. There was no difference in VEGF expression per unit weight between coronal and radicular pulp. NGF production was either confirmed to be present in trace amounts or not detected at all in both the coronal and radicular regions.

When performing simplified cell-based regenerative endodontic procedures using minced pulp tissue grafts, a larger apical size results in more favorable outcomes, and the overall quantity of pulp tissue is likely more important than the ratio of coronal to radicular pulp. To promote more efficient pulp-dentin complex regeneration, it may be worth considering the addition of growth factors and/or scaffolds to the minced pulp tissue.

Keywords: clinical study; minced pulp; transplantation; cell- and tissue-based therapy; regenerative medicine; reverse translational research; coronal pulp; radicular pulp; vascular endothelial growth factor (VEGF); nerve growth factor (NGF)

This dissertation includes material from the article titled ‘Regenerative Endodontic Procedures With Minced Pulp Tissue Graft in Mature Permanent Teeth: A Clinical Study’ published in the *Journal of Endodontics*, *51*(1), 43–53. (Kim et al., 2025). Specifically, parts of the Introduction, Part I, and Discussion sections draw from this article. The material is included here with the permission of the publisher.

I. Introduction

The term 'regenerative endodontic procedures' refers to the use of biologically based procedures designed to restore and repair damaged structures, such as dentin, root structures, and cells of the pulp-dentin complex (Murray et al., 2007). Iwaya et al. were the pioneers in applying the technique of revascularization in a clinical context to address sinus tract and apical periodontitis in immature permanent teeth (Iwaya et al., 2001). Their approach was based on research conducted on the revascularization of immature dog teeth that had been autotransplanted and reimplanted (Skoglund & Tronstad, 1981; Skoglund et al., 1978), as well as the use of a combination of antibiotics for disinfecting root canals (Hoshino et al., 1996; Sato et al., 1996). The therapy successfully resolved apical periodontitis and associated clinical symptoms and signs, while also promoting the thickening of the canal walls and the closure of the apex in immature permanent teeth. The revascularization protocol was introduced by Banchs and Trope (Banchs & Trope, 2004) based on previous studies involving revascularization of reimplanted teeth (Kling et al., 1986), disinfection of root canals (Hoshino et al., 1996), and formation of blood clots in the canal area (Nygarrd-Östby & Hjortdal, 1971). Furthermore, mineral trioxide aggregate (MTA) was used as an intracanal barrier due to its superior sealing ability and biocompatibility (Parirokh & Torabinejad, 2010; Torabinejad & Parirokh, 2010). This strategy has been broadly accepted by numerous studies in the literature and the *Clinical Considerations for a Regenerative Procedure* by the American Association of Endodontists (AAE, 2021). In addition to eliminating clinical symptoms and indicators of apical periodontitis, this treatment also promoted the closure of immature permanent teeth with apical periodontitis and the thickening of the canal walls. Therefore, in the case of immature permanent teeth with necrotic pulps, regenerative endodontics was proposed as an alternative treatment instead of the traditional apexification method (Hargreaves et al., 2008).

Conventional endodontic therapy remains the primary method of conservative treatment for irreversible pulpitis or pulp necrosis in adult teeth. Undoubtedly, this strategy

has an exceptionally high rate of success. According to a systematic study conducted by Ng et al., success rates range from 86% to 93% over a follow-up period of two to ten years (Ng et al., 2010). However, the treated tooth experiences a loss of vitality, making it more prone to fractures and reinfections (He et al., 2017). Therefore, it is crucial to broaden the application and scope of regenerative endodontic procedures to include the mature permanent teeth of adults, given the challenges and limitations of conventional treatments.

Because of their age, adult patients must be treated with certain considerations. Initially, the probability of revascularization is diminished in adult patients. Over time, degenerative changes in arteries and nerves reduce the formation of new blood vessels throughout the body on a systemic basis (Lähteenvuo & Rosenzweig, 2012). Within the pulp-dentin complex, aging can lead to a reduction in its blood supply (Morse, 1991), as the pulp chamber becomes smaller due to the growth of dentin. Additionally, calcifications become more common (Iezzi et al., 2019), and the narrowing of the root tip occurs gradually due to the deposition of dentin and cementum. Another aspect to examine is the alterations in the functionality and capacity of stem cells. The regenerative capacity of multipotent stem cells diminishes over time (Brohlin et al., 2012; Chambers et al., 2007). This phenomenon may be attributed to the decline in the differentiation ability of MSCs over time, rather than the quantity of MSCs that reach the root canal systems of adults, as this quantity remains constant regardless of age (Chrepa et al., 2015). An additional aspect to take into account is the reduced size of the apical constriction in fully developed teeth (He et al., 2017).

In contrast to the objective of regenerative endodontics, which aims to develop tissue resembling the pulp-dentin complex, the histologic examination of tissue formed using current clinical protocols yielded structures more akin to cementum and bone. These tissues were observed within the root canal of immature permanent teeth with necrotic pulps and apical periodontitis (Lei et al., 2015; Nosrat et al., 2012; Paryani & Kim, 2013; Shimizu et al., 2012; Torabinejad & Faras, 2012). This suggests that in addition to stem cells from the apical papilla, which may aid in the regeneration of the pulp-dentine complex, other types of stem cells capable of producing mineralized tissue like cementum and bone were also transported into the root canal following the production of periapical bleeding. The results of the current cell homing method in regenerative endodontic procedures might be seen as more of a repair process rather than true regeneration. It has been suggested that cell homing approach is a guided pulp repair process (Diogenes et al., 2016). In addition, research has demonstrated that intracanal calcification associated with regenerative endodontic procedures with cell homing approach is a frequent phenomenon (Song et al., 2017). While revascularization-related intracanal calcification may not hinder the healing

process of apical periodontitis, certain instances can progress to the full obliteration of root canals and hinder the normal functioning of dental pulp tissues (Song et al., 2017).

Focusing on authentic pulp-dentin regeneration, cell-based strategies alongside tissue engineering have gained attention (Lin et al., 2021). This approach requires a cell source to be delivered into the host for tissue to regenerate to its original or near-original state. Along with the cell source, growth factors and a physical scaffold that promote cell growth and differentiation are key elements of regenerative endodontics, merging the principles of tissue engineering and regenerative medicine (M. Nakashima & A. Akamine, 2005). One notable example of a cell-based approach is the study by Nakashima et al., which demonstrated the safety of mobilized dental pulp stem cell (MDPSC) transplantation in pulpectomized teeth with irreversible pulpitis, as well as the efficacy of regenerative therapy using MDPSCs combined with G-CSF for pulp/dentin regeneration (Nakashima et al., 2017). Additionally, a subsequent report detailing two cases (Nakashima et al., 2022) illustrated the applicability of this method for pulp regenerative cell therapy in multirooted molars. Furthermore, they reported a case applying this treatment to mature teeth with apical periodontitis in a later study (Nakashima & Tanaka, 2024). The case report by Meza et al. detailed a successful outcome of regenerative autologous cellular therapy, employing mesenchymal stem cells derived from inflamed dental pulp and leukocyte platelet-rich fibrin (L-PRF), in a mature tooth (Meza et al., 2019). Gomez-Sosa et al. presented a case demonstrating the application of allogenic bone marrow mesenchymal stem cells in regenerative endodontic treatment for a mature nonvital tooth (Gomez-Sosa et al., 2022). Brizuela et al. provided clinical evidence on the safety and efficacy of the endodontic use of allogenic umbilical cord mesenchymal stem cells encapsulated in a plasma-derived biomaterial through a randomized controlled clinical trial (Brizuela et al., 2020). Moreover, they reported the outcomes of two cases diagnosed with pulp necrosis and apical periodontitis in mature teeth treated using this method, with a follow-up period of 5 years (Brizuela et al., 2024).

Despite advances, autologous stem cell transplantation remains experimental in clinical regenerative endodontics, primarily due to complexities such as challenges in stem cell isolation and expansion, the requirement for good manufacturing practice (GMP) facilities, the establishment of stem cell banks, government regulatory issues, the need for clinicians with specialized skills, training for chair-side assistants, and the associated higher costs (Huang et al., 2013).

To overcome the drawbacks of cell-based procedures, Liang et al. (Liang et al., 2018) proposed pulp tissue grafting for REPs. Direct pulp tissue transplantation bypasses *in vitro*

cell expansion of dental pulp stem cells (DPSCs), making it feasible in day-to-day endodontic practice. The study demonstrated that human minced pulp tissue (MP) yielded mesenchymal stem cells (MSCs) and retained the odontogenic and osteogenic differentiation capacities of pulpal MSCs (Liang et al., 2018).

Conventional forward translational research, or benchtop-to-bedside research, follows a linear path from lab studies to clinical trials but is often hindered by high failure rates despite substantial investment in scientific advancements. In contrast, reverse translational research, or bedside-to-benchtop research, starts with real patient outcomes in clinical settings and investigates the molecular mechanisms underlying these observations. This cyclical approach integrates patient data into each research iteration, allowing for continuous refinement and potential improvements in treatment strategies and therapeutic development. (Musyuni et al., 2023; Pezzulo & Levin, 2016; Shakhnovich, 2018).

Although the laboratory study on the characteristics of minced pulp tissue has demonstrated promising potential for pulp tissue regeneration (Liang et al., 2018), there remains a significant gap in clinical applications, and foundational research on this procedure is still limited. Nevertheless, as the MP graft uses autologous tissue and can be integrated into the existing regenerative endodontic procedure with minimal modifications, anticipated side effects are likely negligible. With cautious optimism based on these findings, an exploratory clinical study using a forward translational approach was planned, along with a reverse translational *in vitro* study to explore conditions that may impact the therapeutic efficacy of this procedure.

Therefore, this study is structured with two key objectives, divided into Part I and Part II. Part I aims to present the treatment procedure and outcomes through an exploratory clinical trial on the regenerative endodontic procedure using minced pulp tissue graft, focusing on identifying the tentative factors influencing pulpal tissue regeneration within this protocol. In Part II, using a reverse translational approach, the study investigates the identified tentative factors, specifically those that can be further validated at the *in vitro* level.

Part I

II. Material and Methods

1. Patient selection

Patients were recruited from the Department of Conservative Dentistry at Yonsei University Dental Hospital, Seoul, Korea. This clinical study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol and project were evaluated and approved by the Yonsei University College of Dentistry Institutional Review Board (Approved No.13-0069). The treatment options were discussed with all patients and signed informed consent forms explaining the aim of the study and potential complications were obtained.

The inclusion criteria were as follows: patients aged 19–35 years with no sex predilection, who had a tooth requiring non-surgical root canal treatment or retreatment with a periodontal pocket depth < 3 mm. Furthermore, patients needed to possess a third molar that was prone to extraction without odontosection and without a carious cavity involving the pulp. Patients were excluded if they had systemic conditions that made routine dental treatment difficult or if they were taking medications that could interfere with healing or hemostasis.

2. Clinical procedure

The clinical procedures were illustrated schematically in Figure 1.

First appointment

Teeth requiring root canal treatment were anesthetized with 2% lidocaine (with 1:100,000 epinephrine), isolated with a rubber dam, and any caries or defective restorations were removed. Missing walls were reconstructed using composite resin if needed. Access to the pulp chamber was opened, and the working length was determined using an electronic apex locator (Root ZX; Morita, Tokyo, Japan), as confirmed by periapical radiography. Canal enlargement was performed using rotary files with 2.6% sodium hypochlorite as an irrigant. Apical gauging was performed using a hand file and was documented. Irrigant was activated using an ultrasonic device (Endosonic Blue; Maruchi, Wonju, South Korea), followed by the application of calcium hydroxide as an intracanal medicament, and the tooth was temporized using temporary filling material (Caviton; GC, Tokyo, Japan).

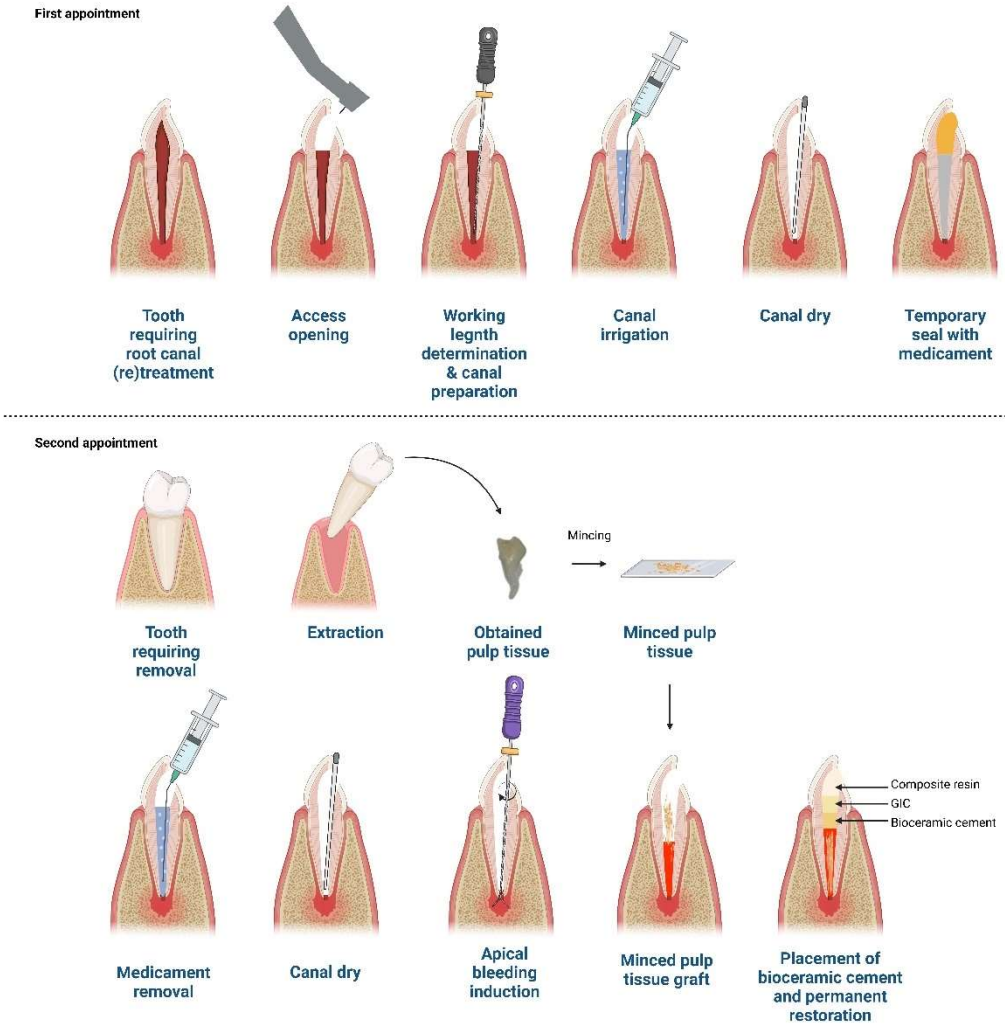

Figure 1 Schematic diagram of the clinical procedures

Second appointment

Patients were then recalled 1-4 weeks after the initial treatment. In cases with persistent infection, additional canal disinfection procedures were performed. Otherwise, pulp transplantation was initiated.

The third molar planned for extraction was anesthetized using 2% lidocaine (with 1:100,000 epinephrine), extracted, promptly rinsed, and stored in sterile saline. The tooth prepared for the root canal treatment was anesthetized with 3% mepivacaine, isolated with a rubber dam, and the temporary filling material was removed. Apical patency was confirmed using a #10 K-file, and the canal was irrigated with 2.6% NaOCl (20 mL/canal for 5 min) and then thoroughly rinsed with sterile saline. The dentin was conditioned with 17% EDTA (20 mL) for gentle irrigation, followed by rinsing with sterile saline. The canal was dried with sterile paper points and apical bleeding was induced using a pre-curved K-file extending 2 mm beyond the apical foramen, achieving sufficient bleeding at the mid-level of the canal, which was verified using a microscope. A deep notch was carefully created in the extracted tooth using a high-speed diamond bur so as not to damage the pulp and then split using a crown spreader to collect the pulp. The pulp tissue was minced on a sterile glass plate into fine pieces (approximately 0.5 mm in length) using a surgical blade, ensuring it remained moist with saline throughout the mincing process. The MP was transplanted into the canal, topped with hydraulic calcium silicate cement (RetroMTA; BioMTA, Seoul, Korea), and allowed to set for 5 minutes before the cavity was restored with glass ionomer cement (Fuji II LC; GC, Tokyo, Japan) and packable composite resin. After treatment, the patient was prescribed 200-mg ibuprofen every 8 hours for 7 days and 500-mg amoxicillin for the same period.

Patients were informed that in cases of unsuccessful transplantation, the transplanted pulp would be removed, and conventional endodontic treatment would be performed. They adhered to the clinical/radiographic follow-up periods of 1, 2, 3, 6, and 12 months, followed by every 6 months, if available.

3. Clinical and radiographic evaluation

After the procedure, patients adhered to clinical and radiographic follow-up periods at 1, 2, 3, 6, and 12 months, followed by every 6 months, if available. At each visit before and after the procedure, patient discomfort was assessed, and clinical examinations, including mobility, percussion, and sensibility tests (ice, heat, EPT), were performed. Periapical radiographs were taken before the procedure and at each follow-up visit, while CBCT scans were conducted before the procedure and then every six months until the second year after the procedure.

Based on the American Association of Endodontists' *Clinical Considerations for a Regenerative Procedure* (AAE, 2021), which was adapted for mature permanent teeth

(Garrido-Parada et al., 2022), the primary goal was the elimination of symptoms and evidence of bony healing. The secondary goal was to achieve a recovery of positive response to vitality testing.

III. Results

Six patients without systemic diseases who required root canal treatment for a tooth and third molar extraction simultaneously were enrolled (Figure 2A, B, 3A, B, 4A, B, C, 5A, B, 6A, B, C, 7A, B, C, D). The patient details are shown in Table 1. There was no gender-based restriction in the inclusion criteria; however, all the enrolled patients were men, aged 23.3 ± 2.4 years (range, 20–27 years).

Table 1 Patients' details

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)	27	20	23	24	24	22
Gender	Male	Male	Male	Male	Male	Male
Host tooth No.	#45	#12	#24	#15	#22	#14
Donor tooth No.	#28	#18	#18	#28	#18	#48
Pre-operative clinical findings						
Percussion pain	+	+	+	-	+	-
Apical lesion	-	+	+	-	+	+
Pulpal diagnosis	IP	PN	PN	IP	PT	PN
Periapical diagnosis	SAP	CAA	CAA	NA	SAP	CAA

CAA, chronic apical abscess; IP, irreversible pulpitis; NA, normal apical tissue; PN, pulp necrosis; PT, previously treated; SAP, symptomatic apical periodontitis.

Patient 1

When the third molar was extracted to obtain the pulp tissue, caries penetrating the dentin were observed (Fig. 2C). This may have influenced the condition of the dental pulp, which presented a mildly inflamed appearance. Consequently, the harvested pulp tissue was segmented into three sections. The most coronal section was discarded, while the remaining two sections, one containing both coronal and radicular portions and the other from the radicular portion, were utilized for transplantation (Fig. 2D). Owing to methodological oversight in the early stages of the study, the pulp tissue was not finely minced but divided into larger sections. Furthermore, the insufficient induction of apical bleeding was compensated by introducing fresh blood from the extraction site.

Postoperative (Fig. 2E) follow-up up to 1 year and 7 months revealed a sustained symptom-free condition. Periapical radiolucency was not observed radiographically, and slight calcification in the apical third of the root canal was confirmed. (Fig. 2F, G). However, throughout the follow-up period, the tooth consistently showed negative responses to sensibility tests.

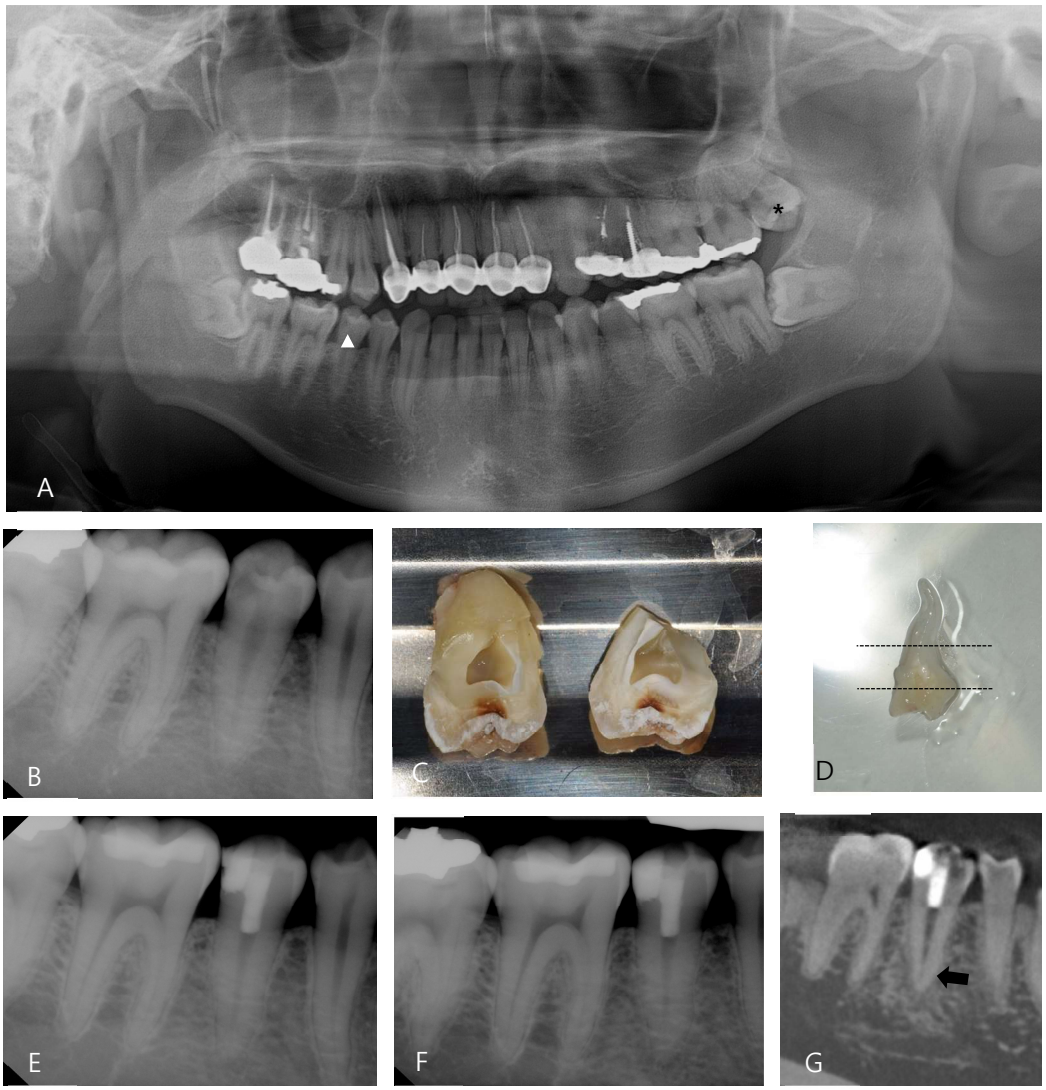

Figure 2 Treatment overview of mandibular right second premolar in patient 1
(A) Panoramic view of patient 1 with donor tooth #28 marked with asterisks and recipient tooth #45 marked with an arrow. (B) Preoperative radiograph of tooth #45. (C) Sectioned donor tooth #18. (D) Dental pulp was collected from the sectioned tooth #28. In patient 1, the pulp was cut into three pieces, as marked with a dashed line; the coronal third was discarded, and the remaining two pieces were used for transplantation. (E) Postoperative radiograph of tooth #45 after procedure. (F) Follow-up periapical radiograph taken 1 year 7 months after procedure. (G) CBCT taken 1 year and 7 months after the procedure shows canal calcification in the apical third of the root canal marked with an arrow.

Patient 2

The harvested pulp tissue consisted of the coronal pulp from the pulp chamber and the radicular pulp from the palatal root (Fig. 3C). Owing to insufficient apical bleeding induction, fresh blood collected from the extraction socket was transplanted along with the MP (Fig. 3D).

After pulp transplantation (Fig. 3E), the patient missed follow-up visits and did not maintain contact. However, at 42 months post-operation, the patient presented to the dental hospital with discomfort in another area. A panoramic radiograph taken at this visit revealed a significant reduction in the periapical lesions without canal calcification (Fig. 3F).

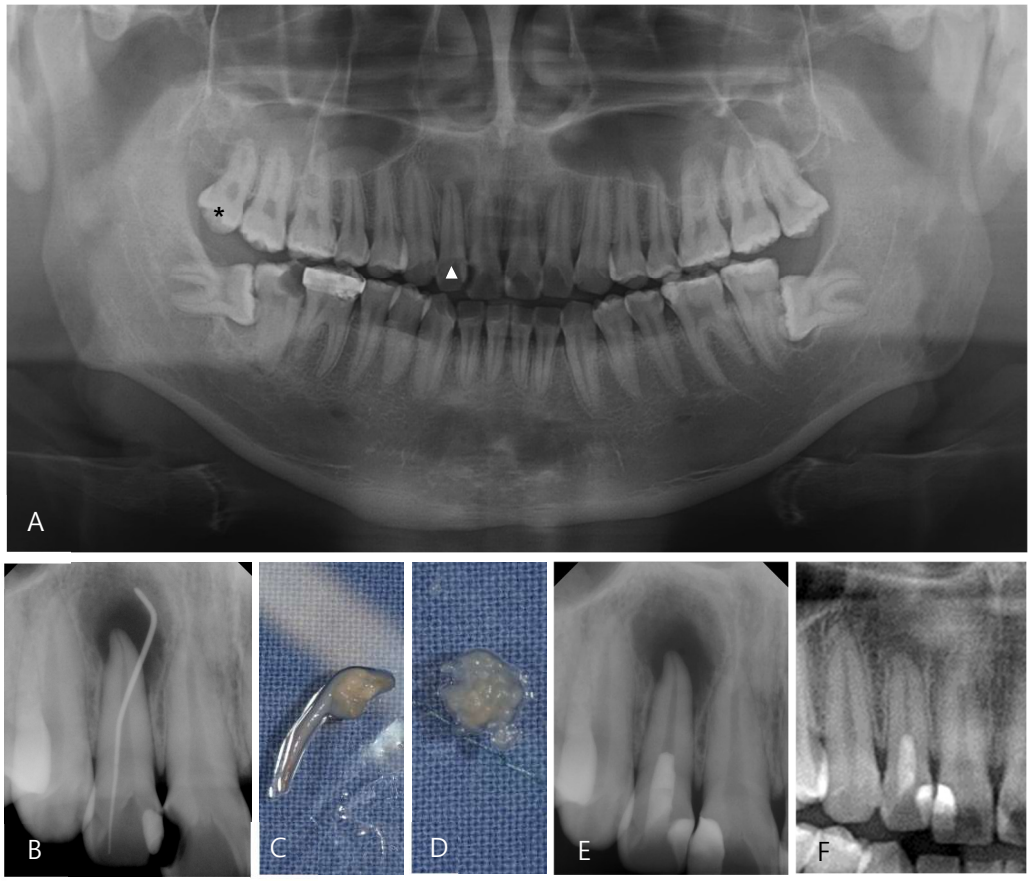

Figure 3 Treatment overview of maxillary right lateral incisor in patient 2

(A) Panoramic view of patient 2 with donor tooth #18 marked with asterisks and recipient tooth #12 marked with an arrow. (B) Preoperative radiograph of tooth #12 with periapical lesion and gutta-percha tracing. (C) Obtained pulp tissue. (D) Minced pulp of tooth #18 ready for transplantation. (E) Postoperative radiograph of tooth #12 after procedure. (F) Follow-up panoramic radiograph taken 3 years and 6 months after procedure showing decreased size of periapical lesion.

Patient 3

The pulp obtained by splitting the tooth primarily originated from the coronal region (Fig. 4D). The pulp tissue was minced (Fig. 4E), sufficient apical bleeding was induced, the MP was transplanted into the canals (Fig. 4F), and the cavity was sealed (Fig. 4G, H).

The patient exhibited no remarkable signs or symptoms during the 24-month postoperative period. The apical lesion resolved, with noticeable canal calcification in the apical one-third area (Fig. 4I, J). The tooth consistently tested negative on sensibility tests at all appointments.

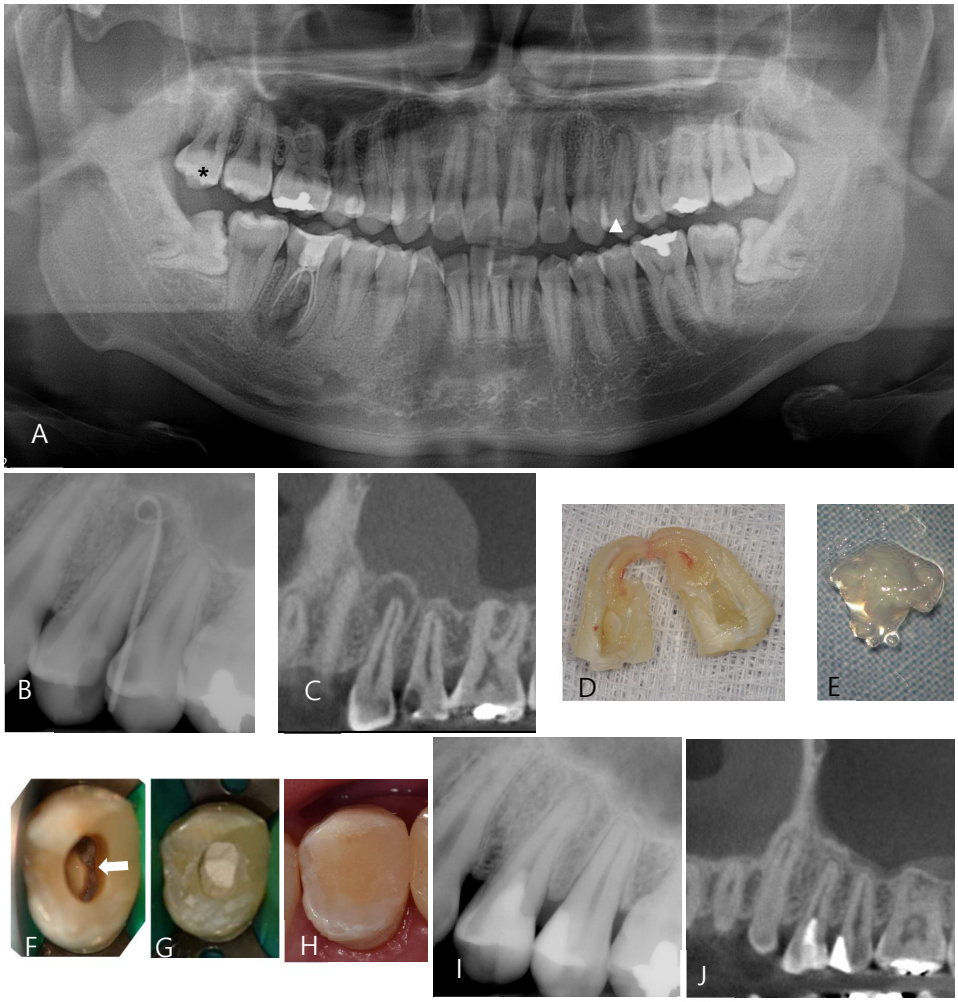

Figure 4 Treatment overview of maxillary left first premolar in patient 3

(A) Panoramic view of patient 3 with donor tooth #18 marked with asterisks and recipient tooth #24 marked with an arrow. (B) Preoperative radiograph of tooth #24 with periapical lesion and GP tracing. (C) Preoperative CBCT radiograph showing periapical lesion on tooth #24 and mucosal thickening of the maxillary sinus. (D) Sectioned photograph of tooth #18. (E) Minced pulp of tooth #18 ready for transplantation. (F) Photograph of minced pulp placed (arrow) inside the root canal. (G) RetroMTA was placed after minced pulp transplantation. (H) Postoperative clinical photograph of recipient tooth. (I) Periapical radiograph taken 2 years after the procedure shows complete healing of the periapical lesion (J) CBCT taken 2 years after the procedure showing complete healing of periapical lesion and mucosal thickening. Canal calcification is visible in the apical third of tooth #24.

Patient 4

Most of the pulp tissue obtained from the donor tooth was from coronal tissue (Fig. 5C). Minor amounts of radicular pulp tissue were procured. The MP (Fig.5D) was transplanted in the presence of blood from the induced apical bleeding (Fig. 5E, F).

Up to 42 months postoperatively, the patient reported no subjective discomfort. No remarkable abnormalities were observed during clinical examinations. Radiographically, the tooth remained stable without any discernible periapical lesions or evidence of root canal narrowing (Fig. 5G). Sensibility tests showed no recovery throughout the follow-up.

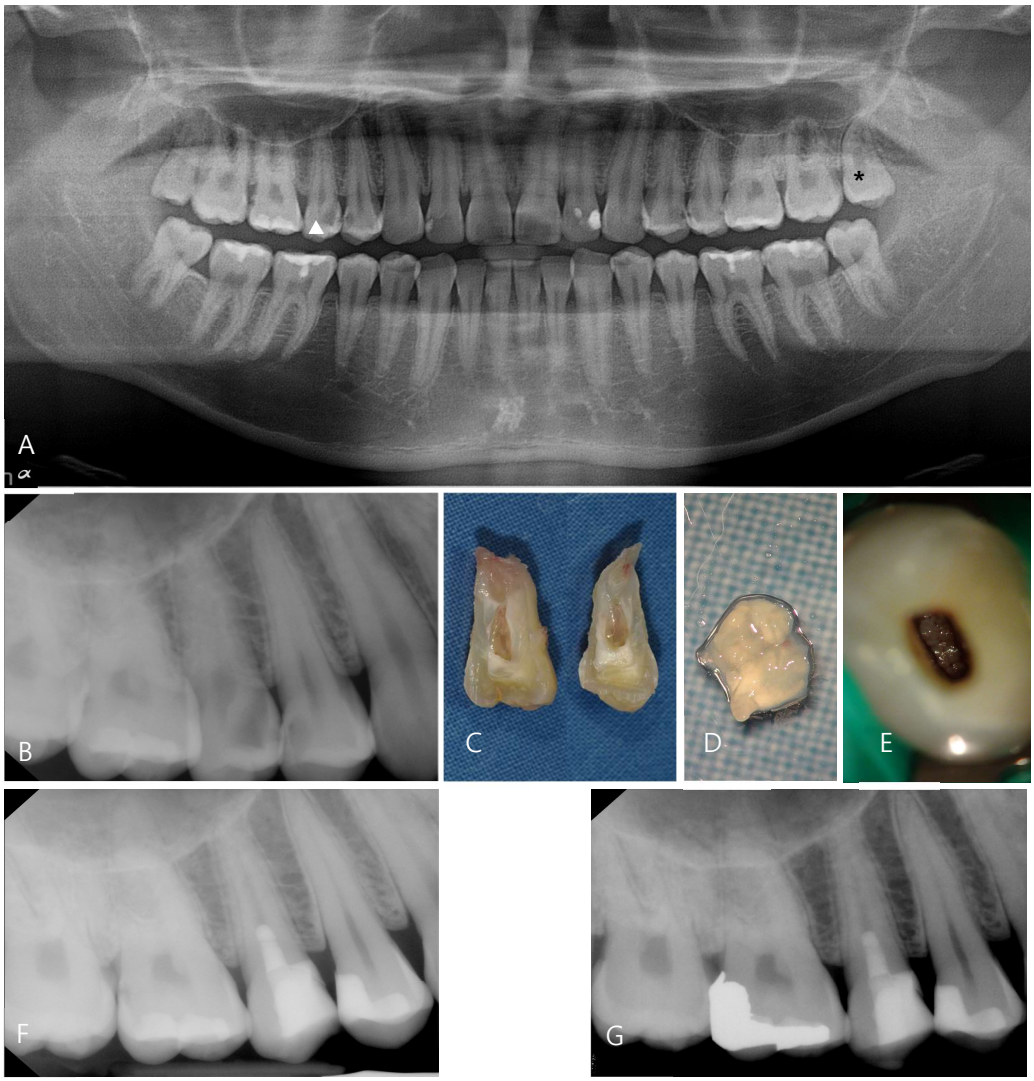

Figure 5 Treatment overview of maxillary right second premolar in patient 4

(A) Panoramic view of patient 4 with donor tooth #28 marked with asterisks and recipient tooth #15 marked with an arrow. (B) Preoperative radiograph of tooth #15. (C) Clinical photograph sectioned tooth #28. (D) Obtained pulp tissue from sectioned tooth #28. (E) Photograph of tooth #15 after minced pulp transplantation. (F) Postoperative radiograph. (G) Follow-up periapical radiograph taken 3 years 6 months after procedure.

Patient 5

Most pulp tissue originated from the pulp chamber of the donor tooth (Fig. 6D, E). The recipient tooth had previously undergone a root canal treatment; therefore, the filling material within the root canal was thoroughly removed. The absence of residual filling material was confirmed radiographically and under an operating dental microscope. Sufficient induction of apical bleeding was achieved, and the MP was transplanted (Fig. 6F).

Over 40 months, no adverse clinical observations were noted. Radiographically, there was a continual decrease in the periapical lesion (not fully resolved), with no intracanal calcification detected (Fig. 6G, H, I). Sensibility tests consistently showed no response.

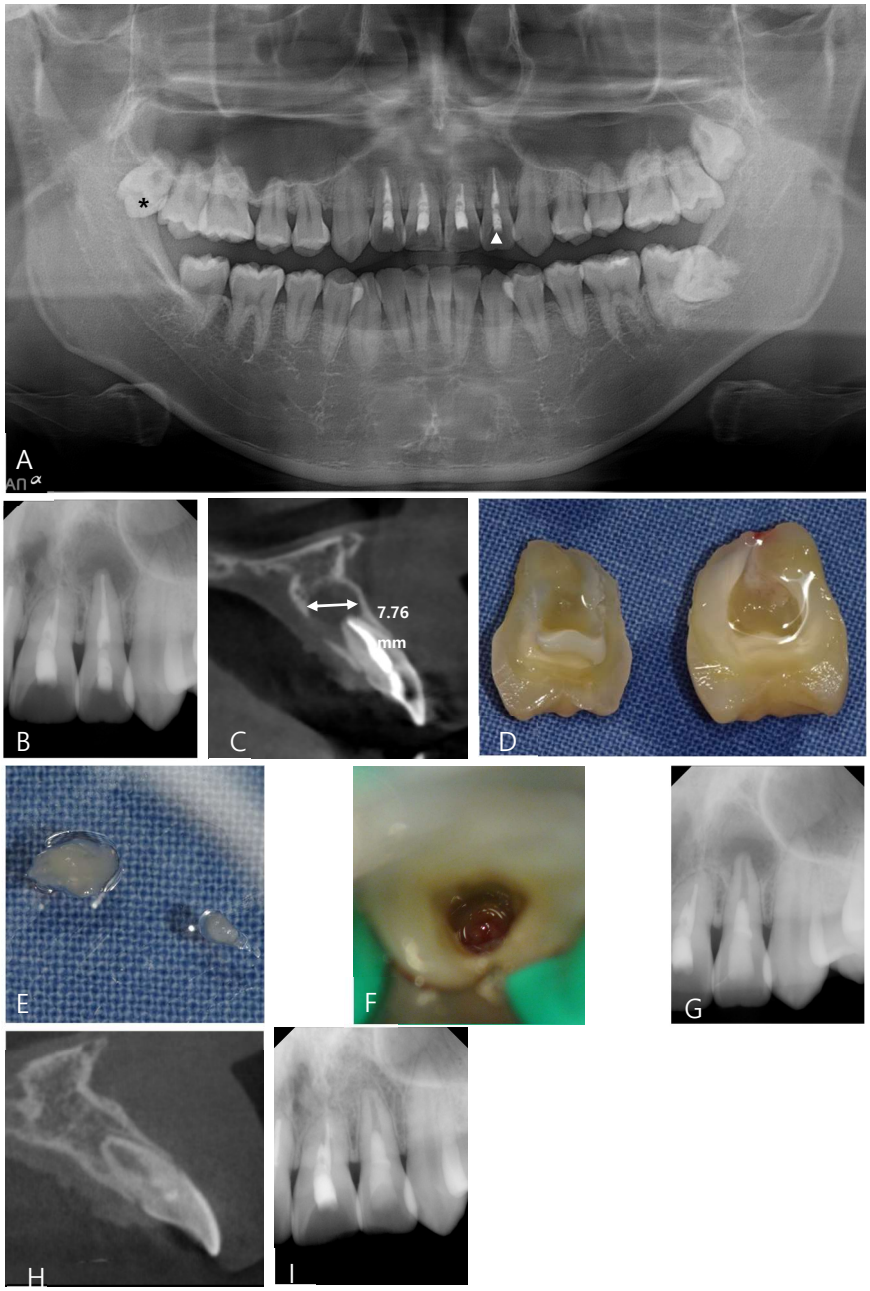

Figure 6 Treatment overview of maxillary left lateral incisor in patient 5

(A) Panoramic view of patient 5 with donor tooth #18 marked with asterisks and recipient

tooth #22 marked with an arrow. (B) Preoperative radiograph of tooth #22 with periapical lesion. (C) Preoperative CBCT of tooth #22 with a periapical lesion of size 7.76 mm and canal filling material. (D) Sectioned tooth #18. (E) Obtained pulp tissue. (F) Photograph of tooth #22 after transplantation of minced pulp. (G) Postoperative radiograph of tooth #22 after the procedure. (H) CBCT image of tooth #22 at 2 years 9 months follow-up. (I) Periapical view of tooth #22 taken 3 years 4 months following the procedure showing the reduced size of the periapical lesion.

Patient 6

The obtained pulp tissue comprised one piece connecting the distal root pulp to the coronal pulp and another piece from the mesial root pulp, providing adequate tissue (Fig. 7E). Both pieces were finely minced, mixed (Fig. 7F), and introduced into the prepared canals (Fig. 7G, H, I).

The patient remained symptom-free during the 1- and 2-month follow-ups, with no abnormalities observed during clinical examinations. By the 3rd month, stinging pain on consuming hot foods was reported, showing positive responses to hot tests and percussion. By the 4-month follow-up, pain intensity decreased, but frequency increased, with no response to percussion or heat tests, and reduced apical lesion on radiographs. At the 6- and 12-month follow-ups, no discomfort was reported, and clinical tests, including percussion, bite, and sensibility tests were negative. Radiographs showed a decreasing trend in the apical lesion. By the 18th month, the ice and hot tests were negative, whereas the electric pulp test (EPT) was positive, with no discomfort reported. Moreover, the apical lesion disappeared and intracanal calcification in the apical one-third area was observed (Fig. 7J). At the 40th month, positive responses to both EPT and ice tests, indicated nerve reinnervation, with sustained resolution of periapical lesion radiographically. The intracanal calcification remained confined to the apical third (Fig. 7K).

No systemic side effects were observed in any patient during the follow-up period. Radiographic outcomes, including the presence and changes in apical lesions and intracanal calcifications, as well as the variables encountered during treatment, are described in Table 2.

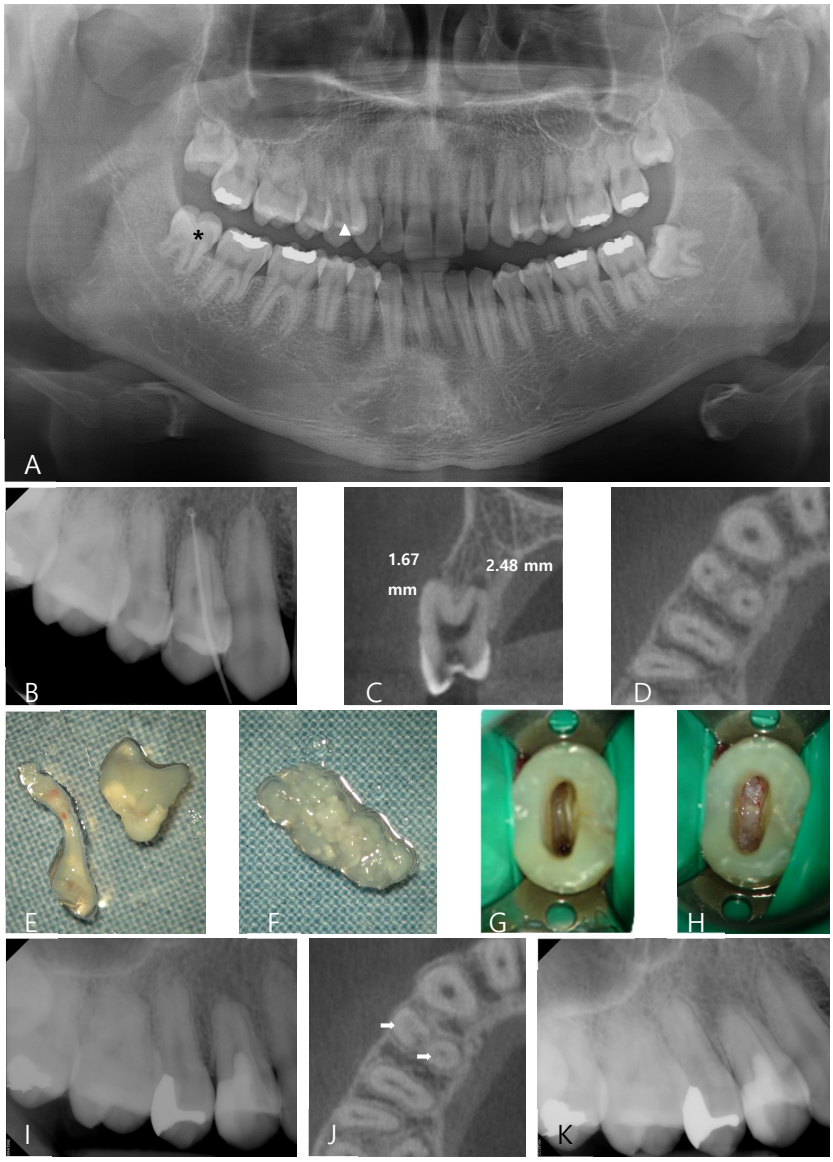

Figure 7 Treatment overview of maxillary right first premolar in patient 6

(A) Panoramic view of patient 6 with donor tooth #48 marked with asterisks and recipient tooth #14 marked with an arrow. (B) Preoperative radiograph of tooth #14 with GP tracing. (C) CBCT taken preoperatively shows buccal cortical bone fenestration with a periapical lesion measuring 1.67 mm and a lesion on the palatal aspect measuring approximately 2.48 mm. (D) Axial view of preoperative CBCT (E) Dental pulp collected from sectioned tooth

#48 (F) Minced pulp tissue (G), (H) Photograph of tooth #14 after canal preparation and subsequent minced pulp transplantation, respectively. (I) Postoperative periapical radiograph of tooth #14 after the procedure. (J) Axial view of CBCT at 1 year 6 months. Canal calcification is observed in the apical third of both buccal and palatal canals (arrows). (K) Follow-up periapical radiograph taken 3 years and 4 months after the procedure.

Table 2 Operative outcomes and variables during treatment

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Apical lesion Pre-operative Post-operative	 Absent Absent	 Present Decreased	 Present Resolved	 Absent Absent	 Present Decreased	 Present Resolved
Apical bleeding induction	Insufficient	Insufficient	Sufficient	Sufficient	Sufficient	Sufficient
Apical preparation size	#55	#35	#50	#40	#40	#60(buccal) #80(palatal)
Composition of acquired pulp tissue	Coronal and radicular pulp but not minced	Coronal and radicular pulp	Mainly coronal pulp	Mainly coronal pulp	Mainly coronal pulp	Coronal and radicular pulp
Last follow-up (month)	19	42	24	42	40	40
Recovery to sensibility test	-	Not available	-	-	-	+
Post-operative Intracanal calcification	Present at apical 1/3	Absent	Present at apical 1/3	Absent	Absent	Present at apical 1/3

Part II

In Part I of this study, various variables were observed under clinical conditions. It was evident that a larger apical foramen resulted in a more favorable environment for regeneration, aligning with the observed outcomes. Additionally, clinical outcomes suggested another significant variable: the quantity and composition of pulp tissue from extracted third molars, especially variations in the ratio of coronal to radicular pulp. Favorable regeneration, including re-innervation and apical remodeling of the root canal- specifically, canal calcification at the apical area- was observed when the quantity of pulp tissue was substantial and the ratio of coronal to radicular origins was balanced.

Angiogenesis/vasculogenesis and neurogenesis are critical for pulp regeneration (Nakashima et al., 2009), as pulp vasculature supplies nutrition and oxygen, serves as a conduit for metabolic waste transport, and regulates inflammation. Additionally, the pulp nerve fibers contribute to angiogenesis, facilitate the extravasation of immune cells to control inflammation, support pulp tissue maintenance, and enhance pulp defense mechanisms. The close association between pulp innervation and vasculature is essential for maintaining pulp homeostasis (Misako Nakashima & Akifumi Akamine, 2005).

Angiogenesis involves the growth of new vessels from the pre-existing capillaries and is fundamental to normal development and healing (Folkman & Shing, 1992). The endothelial cells lining the pulpal vasculature play a major role in the angiogenic process. When injured, the release of cytokines stimulates an inflammatory response that leads to the recruitment of progenitor cells to the injury site, aiding in the healing process (Mathieu et al., 2005). Vascular endothelial growth factor (VEGF), a potent pro-angiogenic cytokine secreted by many cell types, is a key regulator of both physiological and pathological angiogenesis. VEGF acts on the vasculature by inducing the proliferation, differentiation, and migration of vascular endothelial cells. It has been identified in dental pulp cells and the dentine matrix, in which, under pathological conditions, it can be released to stimulate angiogenesis (Artese et al., 2002; Tran-Hung et al., 2008; Zhang et al., 2011).

Nerve growth factor (NGF) is the firstly discovered and best characterized neurotrophic factor, known to play a critical protective role in the development and survival of sympathetic, sensory and forebrain cholinergic neurons. NGF promotes neuritis outgrowth both *in vivo* and *in vitro* and aids nerve cell recovery after ischemic, surgical or chemical injuries (Aloe et al., 2015). Also, NGF signaling in dental pulp is linked to the differentiation of odontoblasts, the synthesis of dentin matrix, and the attraction of neurons. This indicates that NGF has a vital role in the regenerative processes of damaged and

diseased human teeth (Mitsiadis et al., 2017).

To validate the findings from Part I of this study regarding the impact of pulp tissue composition by anatomic origin (coronal/radicular) on pulpal regeneration, an *in vitro* study was planned. Therefore, the objective of Part II was specifically defined to investigate the differences in VEGF and NGF secretion between the coronal and radicular minced pulp of mature permanent teeth at the tissue level.

IV. Materials and Methods

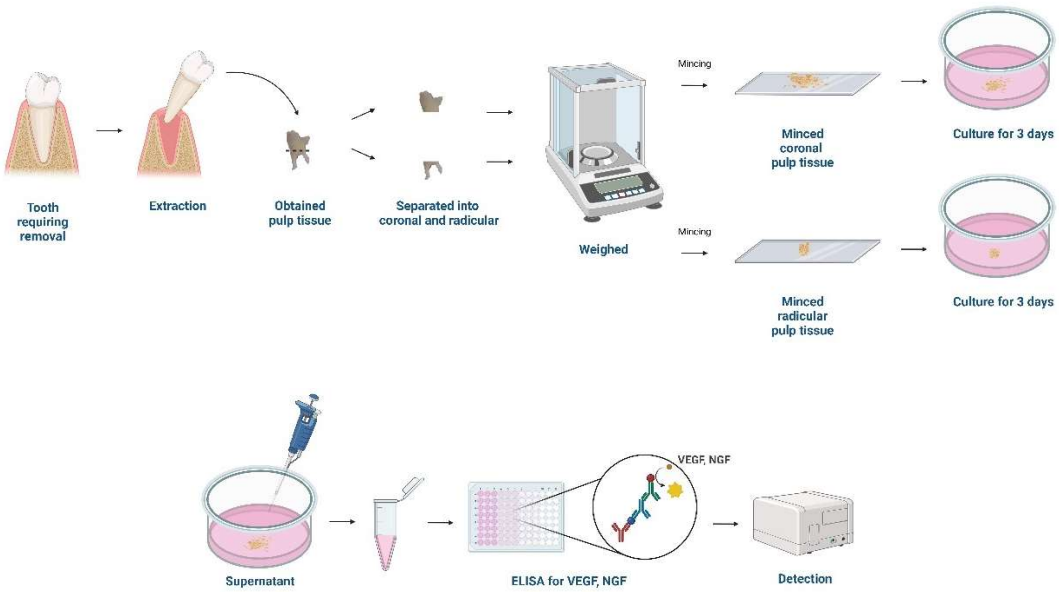

Figure 8 Schematic workflow of the *in vitro* study

The experimental procedures for the in vitro study are summarized in the schematic workflow in Figure 8.

1. Preparation of pulp tissues

Human dental pulp tissues were obtained from freshly extracted third molars with closed apices, which were free of pulp-involving caries, unrestored, and did not require odontosection. These third molars were selected from different healthy patients at Yonsei University Dental Hospital, Seoul, Korea. Patients under medication or pregnant women were excluded from the study. Informed consent was obtained from each patient before extraction, and the study was approved by the Institutional Review Board of Yonsei University Dental Hospital (Institutional Review Board number: 13-0069).

All teeth were stored in sterile saline within sterile Falcon tubes on ice prior to being split to obtain pulp tissue. The root surface of each tooth was scraped with a blade to remove the attached periodontal ligament (PDL), which could contaminate the pulp sample. A deep notch was carefully created in each extracted tooth using a high-speed diamond bur to avoid damaging the pulp, and then the tooth was split using a crown spreader to collect the pulp tissue. This process was performed with sterilized instruments in an aseptic environment. Collected pulp tissues were stored in sterile saline, in sterile Falcon tubes on ice, immediately transferred to the laboratory, and processed under a hood.

The pulp tissue was gently washed twice with Dulbecco's modified eagle's medium (Gibco, Waltham, MA, USA) supplemented with 3% penicillin-streptomycin (Gibco). The tissues were separated by their anatomical origins, coronal and radicular, based on the cemento-enamel junction, and each sample was weighed. After weighing, the tissues were minced into fine pieces (approximately 0.5 mm in length) using a sterilized surgical blade. The minced tissues were then seeded into complete medium (Dulbecco's modified eagle's medium supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum [Gibco]) and incubated in a humidified atmosphere of 5% CO_2 at 37°C for three days to obtain culture supernatants.

2. Enzyme-linked immunosorbent assay

Concentrations of VEGF and NGF in the culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions. Absorbance of the plates was read at 450 nm using a VersaMax Multiplate Reader spectrophotometer. ELISA data were obtained from four independent experiments, each performed in triplicate.

3. Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. For data analysis, the t-test was used for normal distributions, while the Mann-Whitney U test was applied to non-normal distributions. A p-value of less than .05 was considered statistically significant in all tests. Statistical analyses were performed using IBM SPSS Statistics for Windows (version 29.0; IBM Corp., Armonk, NY, USA).

V. Results

1. Patient demographics and composition of obtained pulp tissues

Pulp tissues were obtained from five patients, with ages ranging from 21 to 37 years, and a mean age of 27.2. The gender distribution of the patients included two females and three males. Pulp tissues were exclusively collected from maxillary third molars, as mandibular third molars often required odontosection during extraction. The total weight of the pulp tissue obtained from each extracted tooth ranged from 6 to 20 mg. The weight ratio of coronal pulp to radicular pulp varied significantly, with an average ratio of approximately 3.5:1. This ratio ranged from 2:1 to 10.6:1, and the difference in quantity between coronal and radicular pulp tissues was statistically significant (Table 3).

2. VEGF and NGF production of minced pulp tissues

The ELISA test was conducted to measure the levels of angiogenic cytokine (VEGF) and neurotrophic cytokine (NGF) (Table 4). It was found that VEGF production was significantly higher in coronal pulp tissue compared to radicular pulp tissue (Figure 8A). However, when adjusted for tissue weight differences, there was no statistically significant difference in VEGF production per unit weight between coronal and radicular pulp. Additionally, the variability in VEGF production per unit weight was greater in the apical region (Figure 8B). NGF production was detected in only one of the five tissue samples, and even in that case, only in trace amounts. In the remaining four samples, NGF production was not detected.

Table 3 Patient information and weight of obtained pulp tissues

Sample	Age	Sex	Tooth	Weight of coronal pulp (mg)	Weight of radicular pulp (mg)	Total weight of pulp tissue (mg)	Weight ratio (coronal pulp / radicular pulp)
1	23	M	#28	12.7	1.2	13.9	10.6
2	27	M	#18	8.9	4.2	13.1	2.1
3	37	F	#18	4.4	1.6	6.0	2.8
4	28	M	#18	9.2	4.7	13.9	2
5	21	F	#18	14.6	5.6	20.2	2.6

Table 4 VEGF and NGF levels in coronal and radicular minced pulp tissues

Sample	VEGF (pg/ml)		VEGF per unit weight (pg/ml per mg)		NGF (pg/ml)	
	Coronal	Radicular	Coronal	Radicular	Coronal	Radicular
1	41.35	9.07	3.26	7.56	3.23	0.63
2	63.52	47.22	7.14	11.24	-	-
3	79.51	53.71	18.07	33.57	-	-
4	96.75	6.28	10.52	1.34	-	-
5	312.34	11.96	21.39	2.14	-	-

-; not detected

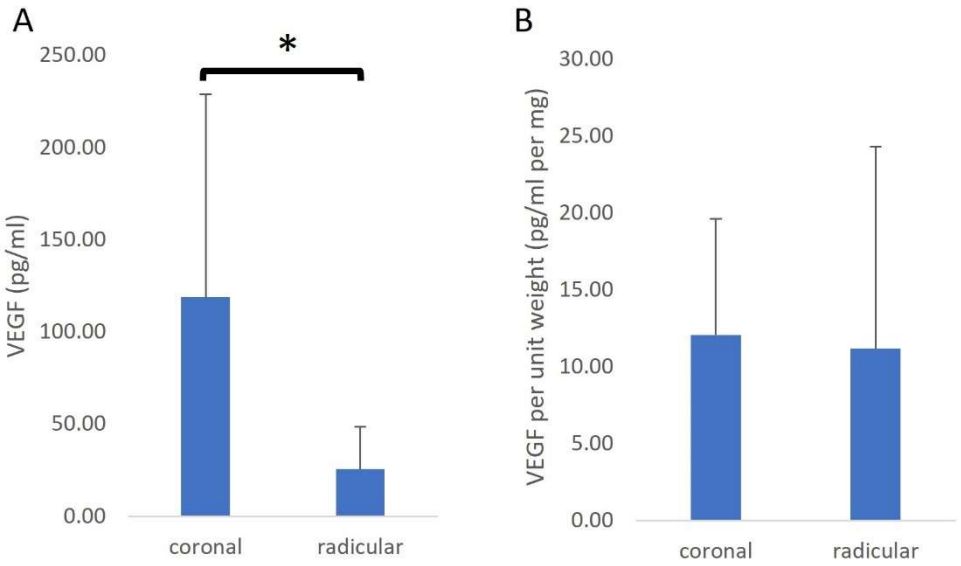

Figure 9 VEGF levels in coronal and radicular minced pulp tissues

(A) Total VEGF production from radicular and coronal areas. (B) VEGF production per unit weight by region. (Data are presented as the mean ± SD. * $p < .05$)

VI. Discussion

Recent clinical trials and case reports on cell-based REPs for adult teeth have used autologous DPSCs (Meza et al., 2019; Nakashima et al., 2022; Nakashima et al., 2017; Nakashima & Tanaka, 2024), as well as allogenic MSCs derived from the umbilical cord (Brizuela et al., 2024; Brizuela et al., 2020) or bone marrow (Gomez-Sosa et al., 2022). However, these methodologies require GMP facilities and cell expansion, presenting challenges for their integration into routine clinical practice (Huang et al., 2013). Considering these challenges, the clinical section (Part I) of this study implemented an MP graft model (Liang et al., 2018), a cell-based approach that is cost-effective, avoids transplant rejection concerns, and does not complicate the clinical setting. To the best of our knowledge, this is the first clinical investigation of autologous MP grafting in teeth.

Follow-ups from 19 to 42 months confirm that the primary goal, the elimination of symptoms and evidence of bony healing, was achieved in all cases. Considering the inclusion of teeth with a variety of endodontic diagnostic conditions, MP grafting appears

to be a viable treatment method for adult teeth requiring endodontic therapy.

In Part I of this study, the regeneration and condition of the pulp tissue were indirectly assessed through radiographic examination and sensibility tests. Radiographically, apical remodeling, observed as calcification and narrowing of the canal at the apex, suggests that regeneration of pulp-like tissue with odontogenic/osteogenic activity occurs, particularly in the apical region, which is indicative of matrix production (Gomez-Sosa et al., 2022). Additionally, the recovery of responses to thermal stimuli (hot and cold tests) and to electrical tests can be considered as evidence of the presence of neural-like cells within the pulp cavity and reinnervation, suggesting the regeneration of a more organized pulp-dentin complex (AAE, 2021). It is challenging to exclude the possibility that the observed regeneration is attributable to the activity of stem cells from the periapical area (periodontal ligament or periapical bone) (Chrepa et al., 2015), which may have migrated via apical bleeding induction. Considering that cells from the apical papilla are the primary source of REPs in immature teeth and are absent in mature adult teeth, and noting the absence of complete canal obliteration (Song et al., 2017) despite the canal space being filled with blood during the transplantation of MP, it is plausible to suggest that the regeneration of pulp-like tissue could be the result of either the survival and differentiation of MSCs derived from the grafted MP at the apical part or the interaction between the grafted MP and stem cells from the periapical area.

A tooth with apical foramen sizes of 0.6 mm (buccal) and 0.8 mm (palatal) demonstrated the most organized regeneration of the pulp-dentin complex, exhibiting both recovery in sensibility tests and apical remodeling (patient 6). In teeth with apical foramen sizes of 0.5-0.55 mm, only apical remodeling was observed, suggesting a less mature form of regeneration (patient 1,3). Meanwhile, teeth with foramen sizes ranging from 0.35 to 0.4 mm showed neither apical remodeling nor sensibility recovery, indicating that the transplanted MP may not have survived or been adequately differentiated (patient 2,4,5). This pattern indicates a correlation between the size of the apical foramen and treatment outcomes, with larger foramen sizes linked to a more favorable form of regeneration. This relationship can be elucidated by considering two aspects: oxygen diffusion is only effective within 0.1–0.2 mm from a blood supply source (Krogh, 1919a, 1919b), and a consistent observation across numerous studies is the massive death of transplanted cells, often attributed to oxidative stress and nutrient depletion (Degano et al., 2008; Logeart-Avramoglou et al., 2010). Therefore, ensuring adequate blood supply and promoting angiogenesis through a larger apical foramen are crucial for the success of MP grafts as these factors directly influence cell survival and overall tissue regeneration.

Intracanal calcification related with CF-RET is a common occurrence, with a prevalence of 62.1% (Song et al., 2017). While root canal calcification is not considered as a disease, an excessive amount of calcification can hinder the vitality and function of revascularized tissues. This can also affect any future endodontic therapy that may be necessary (Nosrat et al., 2012; Shah et al., 2008; Song et al., 2017). Furthermore, the complete obliteration of the root canal space prevents the restoration of pulpal function by revascularization. Regarding the type of calcification in the root canal area, research involving large animals and histologic case reports suggest the production of ectopic bone, cementum, and fibrotic tissue, which closely resemble dystrophic calcification (Lei et al., 2015; Martin et al., 2013; Shimizu et al., 2012; Thibodeau et al., 2007; Wang et al., 2010). Therefore, total obliteration of the root canal can be considered an undesirable outcome. In our study of six cases, there was no instance of complete root canal obliteration during follow-up periods ranging from 19 to 42 months. When examining other cell-based approach studies (Brizuela et al., 2024; Brizuela et al., 2020; Gomez-Sosa et al., 2022; Meza et al., 2019; Nakashima et al., 2022; Nakashima et al., 2017; Nakashima & Tanaka, 2024), there were no reported cases of entire canal obliteration either. The absence of total obliteration within the root canal might be explained through several scenario. Firstly, this might be due to the result of ideal regeneration of pulp tissue through the transplanted tissue. Secondly, the transplanted tissue could have acted as a physical barrier. Lastly, in the coronal portion where blood supply might be less efficient, massive cell death of the transplanted tissue could have occurred and the byproducts of this cell death could have served as chemical inhibitors, preventing excessive odontogenic-/osteogenic differentiation of stem cells present in the canal.

Upon closer examination of the progression of patient 6, which exhibited re-innervation, initial positive response to hot test, suggestive of C-fiber (Bender, 2000) regeneration, was noted at three months following the minced pulp tissue graft. This response to hot test disappeared in subsequent evaluations at four, six, and twelve months. At the eighteen-month follow-up, responses to the Electric Pulp Test (EPT) emerged, and by 40 months, positive responses to both the ice test and EPT were observed, indicating A-delta fiber (Ingle & Bakland, 2002) regeneration. This sequence intriguingly mirrors the exact reverse of the progression typically seen in clinical scenarios, transitioning from normal pulp through pulpitis to pulp necrosis, as demonstrated by sensibility test results. Therefore, in this case, it can be assumed that the transplanted minced pulp tissue was successfully engrafted, providing minced pulp-derived mesenchymal stem cells, differentiating into pulp-like tissue, and progressively remodeling into more organized tissue. Given the challenge in precisely determining the recovery point of cold test

responsiveness between 18 to 40 months, these findings highlight the imperative for an extended follow-up period exceeding 18 months when employing this therapeutic modality.

When examining reinnervation in cell-based approaches, two clinical studies reported reinnervation rates of 80% (Nakashima et al., 2017) and 50% (Brizuela et al., 2020), respectively. Additionally, numerous case reports have documented reinnervation (Brizuela et al., 2024; Gomez-Sosa et al., 2022; Meza et al., 2019; Nakashima et al., 2022; Nakashima & Tanaka, 2024). However, in our treated patients, only one patient showed sensibility recovery, indicating a lower rate compared to these studies, underscoring the secondary goal of REPs in mature teeth (Garrido-Parada et al., 2022). In studies achieving sensibility response recovery with transplanted expanded DPSCs, the apical size was between 0.45-0.55 mm (Nakashima et al., 2022; Nakashima et al., 2017; Nakashima & Tanaka, 2024). Similarly, a case report involving cultured and transplanted DPSCs, where sensibility test response was restored, recorded an apical foramen size of 0.35 mm (Meza et al., 2019). In contrast, the patient in our study, where reinnervation occurred, had a larger apical size. This observation is consistent with the findings of Liang et al. that minced pulp-derived MSCs replicate more rapidly than DPSCs during the initial phase and have a shorter replicative lifespan (Liang et al., 2018). These results suggest that a larger apical foramen is needed for MP transplantation, which likely requires a more robust blood supply and rapid angiogenesis for success than DPSC transplantation, as reflected in our observed outcomes.

Another distinction between our study and others regarding the cell-based approach is the presence of additional growth factors and/or scaffolds, such as granulocyte colony-stimulating factor (Nakashima et al., 2022; Nakashima et al., 2017; Nakashima & Tanaka, 2024), platelet-poor plasma (Brizuela et al., 2024; Brizuela et al., 2020), leukocyte platelet-rich fibrin (Meza et al., 2019), and pre-clotted platelet-rich plasma (Gomez-Sosa et al., 2022). In our cases, the transplantation environment for MP relied solely on endogenous elements derived from EDTA irrigation or induced apical bleeding supplemented by fresh blood from the extraction socket when necessary. This microenvironment may have been insufficient for transplanted MP. Additionally, in Part II of this study, NGF was either detected at trace levels or not detected at all using the ELISA method, indicating an extremely low quantity of NGF present in the pulp tissue obtained from split mature permanent teeth. Although various neurotrophic factors contribute to neurogenesis, NGF is a well-established, critical factor for this process (Mitsiadis & Pagella, 2021). The presence of NGF in such low quantities in minced pulp tissue suggests that simply applying minced pulp in regenerative endodontic procedures may be insufficient. Therefore, incorporating additional growth factors and/or scaffolds might enhance the efficacy of minced pulp tissue

grafts in REPs, thereby increasing the potential for successful neurogenesis.

Anatomically, the dental pulp is divided into coronal and radicular parts. Although their histological characteristics are similar, their developmental mechanisms differ (Jernvall & Thesleff, 2000; Yamashiro et al., 2003). The crown forms during the early embryonic stage, whereas root formation occurs in the late embryonic and postnatal stages (Huang & Chai, 2012), indicating potential differences in genetic traits and developmental phenotypes between the crown and root. Excluding patients whose pulp tissue was not finely minced and those for whom sensibility tests were not feasible due to noncompliance, significant differences in the composition of the transplanted pulp tissue were identified. The most successful case involved the transplantation of nearly equal amounts of coronal and radicular pulp. Several studies have explored the differences between the apical complex and coronal pulp in immature permanent teeth (Joo et al., 2018; Kim et al., 2016; Park et al., 2020), as well as comparative analyses between the pulp tissue of immature and mature permanent teeth (Gomez-Sosa et al., 2021; Gomez-Sosa et al., 2019). However, in the context of mature permanent teeth, there is a lack of research on whether differences exist in the properties of coronal and radicular pulps, particularly in terms of angiogenesis, neurogenic activity, stemness, and hard tissue-forming capacities. Consequently, Part II of this study aims to address this gap by specifically investigating these properties at the *in vitro* level.

In Part II of this study, mature permanent teeth (third molars) from adults were split to obtain pulp tissue. Sufficient quantities of coronal pulp were generally accessible, whereas radicular pulp was often obtained in much smaller amounts. The ratio of coronal to radicular pulp varied significantly, ranging from approximately 2 to 10 times less radicular pulp. This substantial variation is likely due to morphological variability in the apical region, a recognized characteristic of mature teeth (Gao et al., 2016). Since this study only used third molars, this variability appears to have been even more pronounced. The difficulty in obtaining consistent tissue samples likely contributes to the scarcity of studies that specifically compare the characteristics of coronal and radicular pulp.

A study investigating the vascularity and VEGF/VEGFR2 expression in different regions (coronal, middle, and apical) of pulp tissue from mature permanent teeth using immunohistochemistry methods found that the pulps exhibited significantly greater VEGF expression in the coronal region than in the middle region. In addition, there was very little pulp tissue present for examination in the apical region of mature teeth, and samples were variable. Some specimens contained few blood vessels, whereas others showed numerous small immunopositive vessels (Friedlander et al., 2018). In the above study, the coronal

region was defined as the dentine–pulp tissue above the cementoenamel junction, whereas the apical region included 2–3 mm of apical root (dentine and mature pulp tissues). The middle region was defined as the area between the coronal and apical zones. Therefore, the middle and apical regions combined correspond to the radicular pulp in our study. This interpretation explains our finding that VEGF expression per unit weight was similar between the coronal and radicular pulp.

Additionally, while the aforementioned study utilized unerupted and impacted human third molar teeth, our study used erupted third molars, which may account for some differences in the findings. The large ranges observed in the study are consistent with our findings, suggesting that VEGF production in pulp tissue varies significantly among individuals. These individual differences could be influenced by various factors, with external forces applied to the teeth, such as occlusal forces or orthodontic forces, appearing to be one of those contributing factors (Caviedes-Bucheli et al., 2021).

When examining the expression of NGF in mature permanent teeth using immunohistochemistry, it was found that the most intense NGF labeling was detected in the odontoblastic processes, while weaker staining was observed in the odontoblastic bodies. Notably, NGF staining was generally absent in dental pulp fibroblasts, although sporadic NGF immunoreactivity was observed in some of these cells (Mitsiadis et al., 2017). This suggests that during the procedure of splitting the tooth to obtain pulp tissue, the odontogenic processes within the dentinal tubules may have been left within the split tooth rather than being included with the obtained pulp tissue. Additionally, the amount of NGF inherently present and secreted by the pulp tissue itself may be so low that, when diluted in the culture medium, it becomes difficult to detect or is only present in trace amounts.

In the Part I of this study, it was anticipated that the difference in the ratio of coronal to radicular pulp tissue obtained from the donor tooth when grafting minced pulp tissue might influence pulp tissue regeneration. However, based on the *in vitro* results showing no difference in VEGF production per unit weight between coronal and radicular pulp, it appears that the ratio does not significantly impact angiogenesis. Nevertheless, considering that the total VEGF production was greater in the coronal pulp, it suggests that the overall quantity of pulp tissue, regardless of its anatomical location, may be more critical for successful regeneration.

As this exploratory clinical study (Part I) included only six patients, the small sample size is a notable limitation. Additionally, the treatment protocol relied on the availability of a suitable donor tooth for pulp tissue, which limits its broader applicability. The inability to perform histological evaluations of the internal condition of the root canal after the

procedure, due to ethical constraints, also presents a challenge. Despite these limitations, the clinical follow-up provided valuable insights into the use of MP grafts in regenerative endodontic procedures for mature permanent teeth.

Part II of the study also has limitations. The small sample size (N=5) limited the ability to draw definitive conclusions, and the analysis focused solely on VEGF and NGF as representative cytokines. Future investigations will require a larger number of samples and repeated experiments. Additionally, future studies should broaden their scope to include other angiogenic and neurogenic/neurotrophic factors. Further research is also necessary to optimize conditions that enhance the regenerative potential of minced pulp tissue.

VII. Conclusions

The use of MP grafts in REPs for mature permanent teeth in adults has been explored. This exploratory clinical study suggests that MP grafts represent a feasible and simplified cell-based approach, potentially establishing a new paradigm in REPs. When performing REPs using MP grafts, a larger apical size yields more favorable outcomes; furthermore, the overall quantity of pulp tissue is likely more significant than the ratio of coronal to radicular pulp. To promote more efficient pulp-dentin complex regeneration, adding growth factors (e.g., NGF) and/or scaffolds to the minced pulp tissue may be beneficial. Further laboratory studies and randomized clinical trials involving a larger patient cohort are necessary to optimize and assess the efficacy of this simplified cell-based regenerative endodontic procedures.

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Abstract in Korean

성숙 영구치에서 미세 절단 치수 조직 이식을 통한 재생 근관 치료: 탐색적 임상 연구와 역전이 시험관 내 연구

세포 기반 접근법을 이용한 재생 근관 치료는 새로운 치료 방법으로 떠오르고 있다. 한 실험실 연구에서 미세 절단 치수 조직의 특성에 대한 유망한 결과를 바탕으로, 미세 절단 치수 조직 이식을 통한 간소화된 세포 기반의 재생 근관 치료 프로토콜이 제안되었다. 이 치료 프로토콜은 세포 배양 과정과 관련되어 발생하는 어려움들을 우회할 수 있는 방법으로 보이나, 해당 실험실 연구와 임상적 적용까지는 큰 간격이 존재하며, 이와 관련한 기초 연구 또한 제한적이다. 본 연구는 두 부분으로 구성되어 있다. 제 1 부는 성숙 영구치에서 미세 절단 치수 조직을 이용한 재생 근관 치료의 치료 과정과 결과를 탐색적 임상 연구를 통해 제시하며, 본 치료 프로토콜을 통한 치수 조직 재생에 영향을 미치는 잠정적 요인들을 식별하는 데 초점을 맞춘다. 제 2 부에서는 역전이 연구(reverse translational research) 접근법을 이용하여 이러한 잠정적 요인들에 대해 시험관 내 연구 수준에서 검증이 가능한 요인에 대해 탐구하는 것을 목표로 하였다.

제 1 부 – 비외과적 근관 치료를 필요로 하는 건강한 환자들을 대상으로 하였다. 제 3 대구치에서 얻은 미세 절단 치수 조직을 근관 성형, 세척, 소독이 완료된 후 혈액으로 채워진 근관내에 이식하였다. 환자들은 임상 및 방사선학적으로 평가되었다. 등록된 6 명의 환자에 대한 추적 관찰은 19 개월에서 42 개월까지 이루어졌다. 방사선학적으로, 모든 치아에서 양호한 결과가 확인되었다. 6 개의 치아 중 2 개는 근관 내 석회화 및 치수생활력 검사에 대한 반응 회복이 나타나지 않았고, 1 개 치아는 근관 석회화는 확인되지 않았으며 치수생활력 검사가 불가능하였다. 2 개의 치아는 근관 내 석회화가 근관의 근단부 3 분의 1 에서 관찰되었지만, 치수생활력 검사에서 검사에서 회복이 나타나지 않았다. 1 개의 치아는 근관의 근단부 3 분의 1 에서 석회화가 나타났으며, 치수생활력 검사에서 양성반응 회복이 확인되었다. 이러한 결과와 임상 변수들을 고려했을 때, 근단공의 크기와 이식된 치수 조직의 구성(근관부 치수, 근단부 치수)을 잠재적으로 결과에 영향을 미치는 요인으로 판단하였다.

근단공의 크기가 클수록 치수-상아질 복합체 재생에 유리한 환경을 조성할 수 있다는 것은 명백하였기 때문에, 이식된 분쇄 치수 조직의 기원에 따른 구성 차이(치관부 치수, 치근부 치수)의 영향을 검증하기 위해 역전이 연구를 계획하였다. 따라서 본 연구의 제 2 부의 목표는 성숙 영구치 미세 절단 치수조직의 치관부와 치근부 기원에 따른 혈관 내피

성장 인자(VEGF)와 신경 성장 인자(NGF)의 – 재생 근관 치료 과정에서 중요한 혈관형성과 신경형성 촉진에 중요한 역할을 하는 것으로 알려진 – 분비 차이를 조사하는 것으로 구체화하였다.

제 2 부 – 건강한 성인의 성숙한 영구 제 3 대구치에서 치수 조직을 얻었다. 얻어진 조직을 치관부와 치근부로 나누고 잘게 조각낸 후, 3 일 동안 배양하여 배양 상등액을 얻었다. 상등액에서 혈관 내피 성장 인자와 신경 성장 인자의 수준을 효소면역측정법(ELISA)를 사용하여 측정하였다. 치수 조직은 21 세에서 37 세 사이의 5 명의 환자에게서 얻었다. 각 발치된 치아에서 얻은 치수 조직의 총 무게는 6mg 에서 20mg 사이였다. 치관부 치수와 치근부 치수의 무게 비율은 평균 약 3.5:1 이었다. 단위 무게당 혈관 내피 성장 인자의 발현은 치관부와 치근부 치수 사이에 차이가 없었다. 얻어진 치수 조직에서 신경 성장 인자의 발현은 극미량으로 확인되었거나 치관부와 치근부 모두에서 검출되지 않았다.

미세 절단 치수 조직 이식을 이용한 간소화된 세포기반 접근의 재생 근관 치료를 수행할 때, 더 큰 근단공의 크기는 보다 양호한 결과를 나타냈다. 또한, 이식하는 치수 조직의 치관부와 치근부 기원에 따른 비율보다는 치수 조직의 양이 더 중요한 것으로 보인다. 보다 효율적인 치수–상아질 복합체 재생을 촉진하기 위해, 미세 절단 치수 조직에 성장 인자 및/또는 스캐폴드를 추가하는 것을 고려해 볼 만하다.

핵심되는 말: 임상 연구; 분쇄 치수 조직; 조직 이식; 세포/조직 기반 치료; 재생 의학; 역전이 연구; 치관부 치수; 치근부 치수; 혈관 내피 성장 인자; 신경 성장 인자