



The role of angiogenesis and pulpal healing in tooth replantation and allograft transplantation

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ABSTRACT

Tooth transplantation is one of the treatment options for extracted teeth that can be considered before dental implantation. Although the success rate of tooth transplantation is lower than that of implantation, tooth replantation and transplantation have the great advantage of using natural teeth. Tooth replantation might be considered a promising option in some cases. In present study, the expression patterns of revascularization and pulpal healing, which are the most important for the pulp viability, were analyzed after tooth replantation and allograft in mice. The inflammatory response and root dentin resorption were observed and not different between replantation and allograft in initiation of healing process. However, bonelike tissue formation, pulp revascularization and pulp healing were faster in replantation. The difference of healing patterns between tooth replantation and allograft found in present study will be helpful to select the treatment option and to understand healing mechanism.

1. Introduction

Tooth transplantation is a viable treatment option for edentulous spaces resulting from avulsed teeth. There are two types of treatments in tooth transplantation depending on who the donor is. One is the allograft, where a tooth is transferred from one individual to a different individual [1]. The other is auto-transplantation or replantation. Auto-transplantation is the process of transplanting the tooth from one site into another in the same individual [2], while replantation involves replanting the tooth with a vital or nonvital pulp in the same alveolar socket [1]. Replantation is usually executed in cases of avulsed teeth following trauma. On the other hand, allo- or auto-transplantation is performed at the extraction site of the missed tooth because of various reasons, including caries and periodontitis [1].

The reported success rate for auto-transplantation and replantation varies depending on age (root apex closure), tooth type, and the cause of avulsion [3]. However except in special cases, it is lower than around 96%, which is widely known for the recent success rate of dental

implants and keep increasing [4]. The most important factors in the success of tooth replantation or transplantation are blood vessel recruitment and innervation into the pulp. Teeth are richly supported by blood vessels and peripheral nerves [5,6]. In order to carry out its life-long functions, innervation of peripheral sensory nerve is essential for mediating sensory functions as well as blood flow, supply oxygen and nutrients, and dispose of waste products [7]. Nevertheless, the transplanted tooth pulp tissue needs to be connected to the general blood supply for initial tissue survival [8].

There are two ways to supply blood to the pulp tissue. One is anastomosis between preexisting vessels of transplanted pulp tissue and ingrowing blood vessels through apical foramina. The other is revascularization of the pulp tissue with the ingrowth of newly formed vessels. It is known that anastomosis rarely occurs, and most of the blood supply comes from revascularization with ingrowth of newly formed vessels as observed in a previous study with dogs [9]. In successful cases after transplantation, pulpal regeneration, re-innervation, and revascularization have to be shown simultaneously [10,11]. Ideally, pulp tissue

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functionality should be conserved after replantation or transplantation, which implies the presence of a functional vascular network [8].

Another criterion that can determine the success of a transplantation is maintaining the vitality of the pulp. Tooth injuries, such as in cavity preparation and tooth replantation/transplantation cause degeneration of the odontoblast layer in the dental pulp [11–14]. Pulpal responses to tooth replantation can be divided into at least two types of healing patterns; dentin and/or bonelike tissue formation in the pulp tissue [15, 16]. The biological properties of the dentin-pulp complex are still unknown, especially with respect to its ability to form hard tissue [11,17]. As such, it is necessary to observe odontoblast differentiation along with the pulpal healing pattern in tooth replantation or transplantation. The intermediate filament, nestin, can be used as a specific marker for odontoblasts of adult teeth [18,19].

Although several studies have been conducted on replantation or allograft in mice and rats, a study focusing on the revascularization and pulp regeneration spatio-temporally in both operations has not been conducted yet. In the present study, angiogenic tendency and pulp regeneration were analyzed by verifying the expression of vascular endothelial growth factor (VEGF), von Willebrand Factor (vWF) as angiogenic factors and nestin as pulp regeneration respectively at 3, 5, and 7 days after replantation or allograft. The present study shows the initial healing process in tooth replantation and allograft.

2. Materials and methods

All experiments were performed according to the guidelines of the Yonsei University Health System, Intramural Animal Care and Use Committee (YUHS-IACUC). YUHS-IACUC complies with the Guide for the care and use of laboratory animals (National Research Council, USA). And YUHS-IACUC has passed AAALAC International accreditation program since 2003.

2.1. Animals

Hsd:ICR(CD-1®) 3-week-old male mice were purchased from KOA-TECH (Pyeongtaek, Korea). All mice were housed in a temperature-controlled room (22 °C) under artificial illumination (lights on from 05:00 to 17:00) and 55% relative humidity and had ad libitum access to food and water. PN 3W-stage mice were used in this study. Mice were randomly assigned three heads per experimental group (18 mice in total).

2.2. Tooth replantation and allograft

Tooth replantation and allograft are described in the schematic diagram in Fig. S1. The upper right first molar (M1) was extracted with a pair of modified dental tweezers and then immediately repositioned into the original socket. For allograft, donor M1 was extracted from the littermate of the recipient. The extracted teeth were immersed in physiological saline before replantation and allograft. Within 3 min after immersed, the extracted teeth were replanted or transplanted to the sockets and pressed for 2 min with gauze. The upper right second molar (M2) and third molar (M3) of the same animal were used as controls. Three mice were used for each experimental group.

2.3. Immunohistochemistry

Samples were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), decalcified in 10% EDTA (pH 7.4, BE021, Biosolution Co., Ltd., Korea) for 4 weeks at 4 °C and then embedded in paraffin using standard procedures. Sections (5- μ m thickness) of the specimens were incubated in 10 mM citrate buffer (pH 6.0) at 60 °C overnight or proteinase K (10 μ g/mL, AM2546, Thermo Fisher Scientific, USA) for 20 min at 37 °C. The specimens were incubated with anti-VEGF (SC-152, Santa Cruz Biotechnology, Inc., USA; dilution 1:100), anti-vWF (vWF;

AB7356, Merck, Darmstadt, Germany; dilution 1:150), and anti-Nestin (MAB353, Merck, Darmstadt, Germany; dilution 1:200) antibodies at 4 °C overnight. The specimens were incubated with rabbit anti-goat Alexa Fluor 555 (A21431, Invitrogen, OR, USA; dilution 1:200), goat anti-rabbit Alexa Fluor 555 (A21428, Invitrogen, OR, USA; dilution 1:200) or goat anti-mouse Alexa Fluor 555 (ab150114, Abcam, UK; dilution 1:200). The sections were examined using a confocal laser microscope (DMi8, Leica, Germany).

2.4. Image analysis

Root thickness and inflammatory region ratio were measured at the Hematoxylin and Eosin (HE) stained section images. HE stained images were selected from two mesio-distally sectioned serial slides containing both mesial root canal and distal root canal for each mouse. Three thickness measurements were obtained within 250 μ m from the apex of each M1 distal root using image-analysis software (imageJ, ver. 1.38e, NIH, USA). Therefore, the number of measured populations is 18 per experimental group (3 mice \times 2 slides \times 3 measures).

2.4.1. Root thickness measurement

The thickness value included cementum and root dentin. If the apex foramen was obliterated, the thickness was measured with full thickness of root from mesial wall to distal wall, then half of the value was obtained (Fig. 1E arrowheads). Reference thicknesses were measured at M2 of the slides used for measuring M1 thicknesses of replantation and allograft measurement, and slides that were difficult to measure whole thickness of M2 according to the sectioned direction were excluded as outliers.

2.4.2. Inflammatory region measurement

The inflammatory region ratio was measured and calculated as the area of inflammation to the entire periodontal ligament (PDL) space area of M1 using same software (Fig. S2). The PDL space area was collected from the HE images with polygon selection tool. Upper margin of the cervix was set to the line from the Cemento-Enamel-Junction (CEJ) to the alveolar crest. The pulp region in the root canal is not included. In order to measure inflammatory mononucleated cell and granulocyte infiltration, the color information of inflammatory cells was obtained from each HE images, and color threshold tool of the software was used for inflammatory region. The ranges of threshold values were set as follow; In 8bit HSB scale, hue low limit: 172–178, high limit: 224–229, saturation low limit: 104–112, high limit: 250–255, brightness: 0–255 (full range). The number of measured populations is 6 per experimental group. The reference of the inflammatory region ratio was measured in the PDL space of M2.

2.4.3. Immunohistochemistry quantification

For quantification of immunohistochemistry data, same software was used. ROI was set within the pulp region. The signal intensity was measured as the ratio of the pixel area occupied by the signal above the threshold similar to inflammatory region ratio measurement. Threshold was set for excluding non-specific background signal. The number of measured populations is 3 per experimental group. In the same slide, one threshold value was applied, and this value was set to exclude the non-specific background signal of M2 pulp. The threshold values were presented as follow; In Red stacks of 8bit RGB scale, for vWF, low limit: 100–140, high limit: 255, for VEGF, low limit: 58–64, high limit: 255, for nestin, low limit: 71–94, high limit: 255.

2.4.4. Statistical analysis

All the quantitative data were expressed as the mean \pm SD. Statistical analysis was performed by two-way analysis of Variance (ANOVA) with Tukey's multiple comparisons test between different time points or Sidak's multiple comparisons test between operation groups, $p < 0.05$ was considered significant.

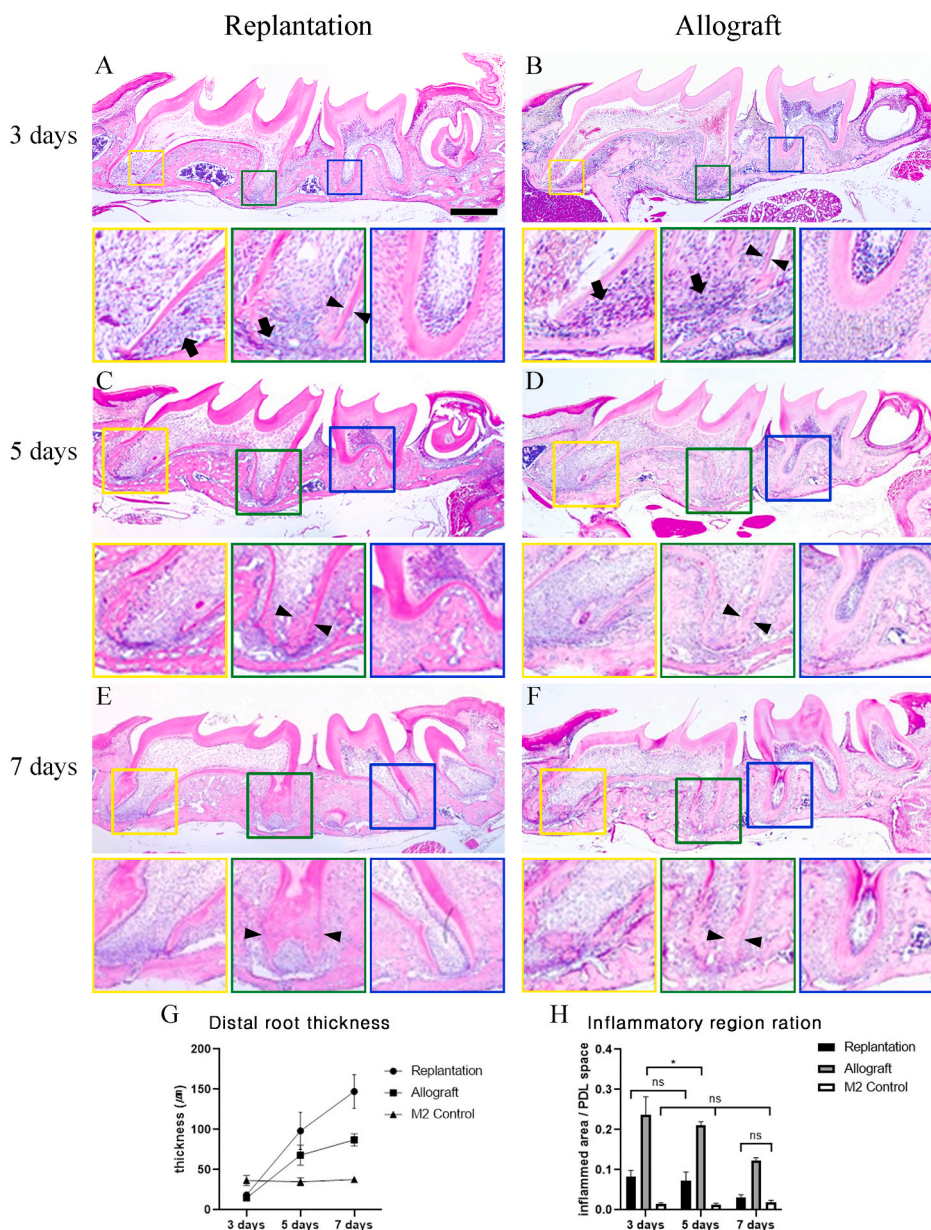


Fig. 1. Comparison of histology between molars after tooth replantation and allograft.

(A) Three days after tooth replantation, the dentin becomes thin and immunocompetent cells are observed around the apex of the pulp. (B) In the tooth allograft, inflammation becomes more severe than in replantation. (C) Five days after tooth replantation, inflammatory cells remain and bone-like tissue is observed in the pulp apex. (D) In the tooth allograft, the dentin becomes thin in the furcation zone and the bone-like tissue is observed similarly to replantation. (E) Seven days after tooth replantation, most inflammatory cells disappear and the bone-like tissue became thicker than it was after 5 days. (F) In allograft, inflammatory cells remain and the bone-like tissue is thickened. (G) Distal root thickness of M1. All the thickness changes of replantation and allograft along time factor showed significantly increased ($p < 0.0001$). (H) Inflammatory region ratio. The p -values of all comparison for which significance is not indicated are < 0.0001 . Scale bar; 500 μm . *, $p < 0.05$; ns, not significant.

3. Results

3.1. Inflammatory response and bone-like tissue formation after tooth replantation and allograft

To analyze histological changes between tooth replantation and allograft, hematoxylin and eosin (HE) staining was performed. Three days after tooth replantation, the root dentin of M1 was resorbed and became thinner than that of M2, which was not affected by the operation (Fig. 1A arrowheads). This resorption was observed in the dentin surrounded by the periodontal ligament (PDL) space, including the furcation zone. The inflammatory region, which consisted of macrophages mononucleated cells and immunocompetent cells, was observed around the apex of the pulp (Fig. 1A arrow). In allografts, more inflammatory cells were recruited than in replantation (Fig. 1B arrow) and the root dentin also became thin (Fig. 1B arrowheads). Root dentin resorption did not differ significantly between the replantation and allograft groups. Five days after replantation, inflammatory cells remained, and bone-like tissue was observed in the mesial and distopalatal apical

foramen (Fig. 1C). In the allografts, bone-like tissue formation was also observed at the distopalatal root apex of M1, similar to replantation (Fig. 1D arrowheads). However, the bone-like tissue thickness of the allograft was lower than that of replantation. Seven days after replantation, most inflammatory cells disappeared and the distal root apical foramen was closed (obliterated) by newly formed bone-like tissue (Fig. 1E). In the allografts, inflammatory cells remained and the bone-like tissue became thick (Fig. 1F). Root thicknesses of distal roots were increased significantly at seven days compared to three or five days in both operations (Fig. 1G). In comparison between operation groups, the thickness of the replants was higher than that of the allografts significantly ($p < 0.0001$). For the reference, M2 mesial root thickness was not changed at all. Inflammatory region ratio was reduced at seven days compared to three or five days in both operations (Fig. 1H). It was also found to be significant in comparison according to the type of operation ($p < 0.0001$). The inflammatory region ratio of M2 PDL space were below 2.00% regardless of time factor. Especially, the inflammation of replanted tooth PDL was relieved at seven days. The thickness and inflammatory region ratio two-way ANOVA data were presented in

Table S1. Taken together, both tooth replantation and allograft cause inflammation in the root pulp and PDL space around the apex. Overall, the process of healing is more advanced in replantation than in the allograft.

3.2. Angiogenesis marker expression after replantation and allograft

To investigate angiogenic capacity between tooth replantation and allograft, the expression of vWF and VEGF as angiogenesis markers was examined. vWF is another marker of angiogenesis. Three days after tooth replantation, vWF expression was observed in the pulp chamber and horn (Fig. 2A arrowheads). vWF expression was strongly observed, specifically in the distal part of the pulp cavity (Fig. 2B). In the case of the allografts, vWF was strongly expressed on the distal side of the pulp (Fig. 2C arrowheads), although its expression was observed intensively at the blood vessels in the pulp of M2 and M3 (Fig. 2D). Five days after replantation, vWF was rarely expressed in the pulp canal and pulp horn (Fig. 2E, F arrowheads). In the allografts, vWF was strongly expressed in the pulp horns and roof of the pulp cavity (Fig. 2G, H arrowheads). Seven days after replantation, vWF was expressed sparsely in the pulp horn and floor (Fig. 2I, J). In the allografts, vWF expression was rarely observed (Fig. 2K, L). Taken together, vWF was expressed in the pulp chamber 3 days after transplantation and then decreased gradually (Fig. 2M).

At 3 days after tooth replantation, VEGF expression was observed at the pulp cavity and apex of M1 (Fig. S3A arrowheads), whereas VEGF was expressed weakly throughout the pulp of M2 and M3 (Fig. S3B). In the case of the tooth allograft, VEGF was strongly expressed in the pulp chamber and distal canal compared to M2 and M3 (Fig. S3C arrowheads, D). Five days after replantation, VEGF was strongly expressed overall in the pulp chamber including pulp horns (Fig. S3E, F). In the allografts, VEGF expression was shifted to the pulp horn (Fig. S3G, H). Seven days

after replantation, VEGF was expressed uniformly in the replanted tooth, similarly to M2 and M3 (Fig. S3I, J). In the allografts, the VEGF signal remained in the pulp horn (Fig. S3K, L) similar to 5 days after the allograft. Thus, VEGF expression in allografts continues to be longer than in replantation (Fig. S3M).

3.3. Pulpal healing with odontoblast differentiation

To investigate pulpal healing after tooth replantation and allograft, nestin expression was examined. Three days after tooth replantation, nestin was expressed intensively in the pulp apex of M1 (Fig. 3A arrowheads), whereas its expression was localized at the odontoblast layer of M2 and M3 (Fig. 3B, D). In the allografts, nestin was expressed in the pulp floor and distal canal (Fig. 3C arrowhead). In both replantation and allograft, nestin expression in odontoblasts of the coronal side and pulp floor, which may have existed previously, was decreased at three days after operation. Five days after tooth replantation, nestin expression was observed throughout the pulp strongly (Fig. 3E, F arrowheads). In the allografts, nestin expression in dental pulp was similar to that in replantation (Fig. 3G, H arrowheads). Seven days after replantation, nestin was expressed at the odontoblast layer, similarly to M2 and M3 (Fig. 3I, J). In the allografts, nestin expression remained all over the pulp, although it decreased in the pulp region excluding the odontoblast layer (Fig. 3K, L arrowheads). Thus, odontoblastic differentiation started from the apex to the chamber of the pulp. This response of the pulp became normal within 7 days in replantation, whereas it was delayed in the allograft (Fig. 3M).

4. Discussion

This is the first report of the initial healing process comparing tooth replantation and allograft in mice. An experimental mouse model for

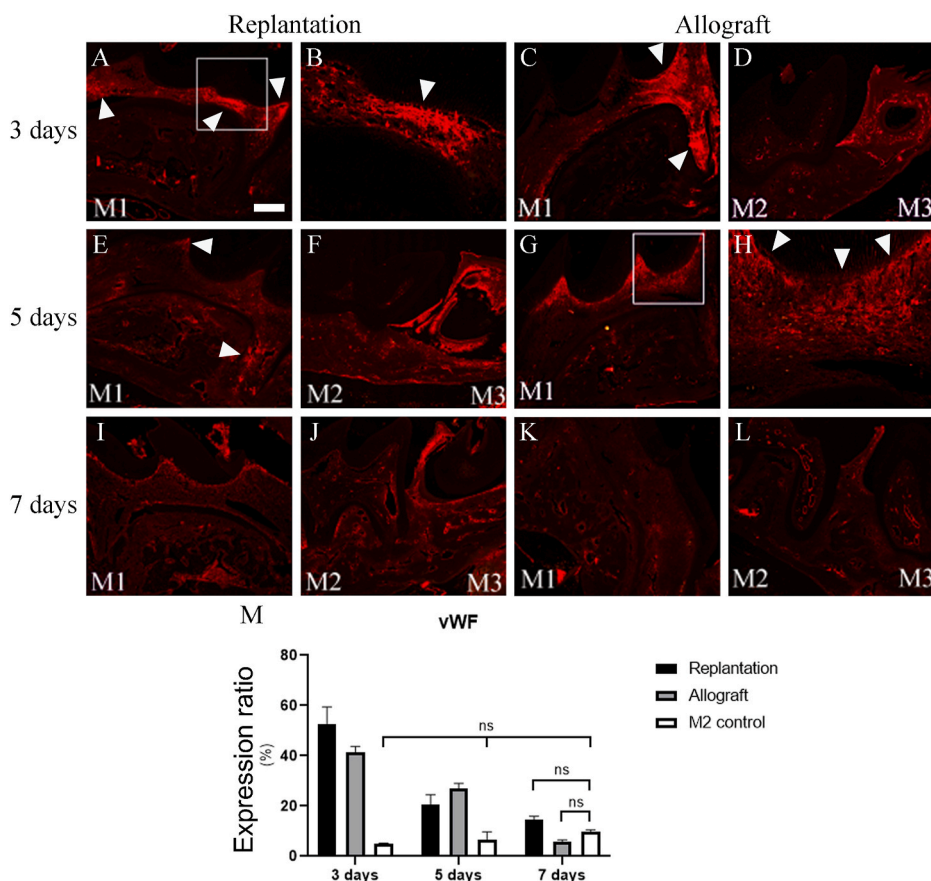


Fig. 2. vWF expression patterns between tooth replantation and allograft.

(A) Three days after replantation, vWF expression is observed in the pulp cavity and horn (arrowheads). (B) High magnification of panel A. vWF expression is strongly expressed specifically in the distal region of the pulp cavity. (C, D) In the case of the tooth allograft, vWF is expressed in the distal pulpal region (arrowheads), which is intensively expressed in blood vessels of the pulp of M2 and M3. (E, F) Five days after replantation, vWF is rarely expressed around the apex and pulp horn (arrowheads). (G, H) In the allograft, vWF is strongly expressed in the pulp horn (arrowheads). (I, J) Seven days after replantation, vWF is expressed sparsely in the pulp horn and floor. (K, L) In the tooth allograft, vWF is expressed in blood vessels of the pulp, similar to M2 and M3, although its expression is rarely observed. (M) Quantified expression ratio of vWF. All the comparison which are not indicated to ns are different significantly ($p < 0.05$). Scale bars; 100 μ m; ns, not significant.

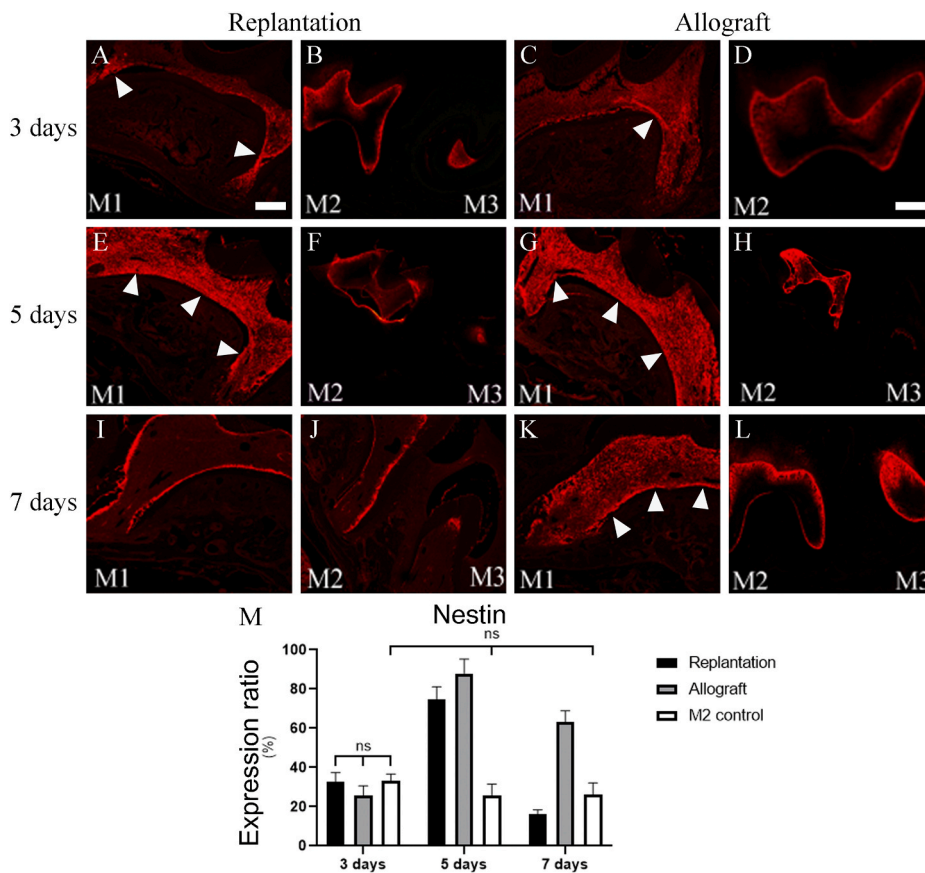


Fig. 3. Comparison of pulpal healing between tooth replantation and allograft.

(A, B) Three days after tooth replantation, nestin is expressed intensively in the pulp apex of M1 (arrowheads), whereas its expression is localized at the odontoblast layer of M2 and M3. (C, D) In the allograft, nestin is expressed in the pulp floor and root canal (arrowhead). (E, F) Five days after replantation, nestin expression is observed throughout the pulp and strongly in the pulp floor (arrowheads). (G, H) In the allografts, nestin is expressed in dental pulp similarly to replantation (arrowheads). (I, J) Seven days after replantation, nestin is expressed in the odontoblast layer, similarly to M2 and M3. (K, L) In the allografts, nestin expression remained overall in the pulp. (M) Quantified expression ratio of nestin. All the comparison which are not indicated to ns are different significantly ($p < 0.05$). Scale bars; A-C, E-L, 100 μ m; D, 50 μ m; ns, not significant.

tooth replantation was established in 2007 [20]. Mouse tooth allograft was first reported in 2009 [21]. According to previous researches, pulpal healing responses can be divided into at least two types: tertiary dentin and bonelike tissue formation [10,11,15,20,22]. In this study, bonelike tissue formation was observed in all experimental groups. Both tooth replantation and allograft cause the inflammatory response around the root apex and the root dentin resorption in the initial stage of healing. Following inflammation, bonelike tissue is formed and thickened, and pulp canal obliteration proceeds during the healing process. In replantation, this healing process is faster than allograft and obliteration is observed in the distal root of the pulp apex 7 days after replantation after operation. This phenomenon in replantation is faster than the previously known healing process period of 14 days [20] and may be caused by immediate transplantation after tooth extraction.

Revascularization is an essential process for successful tooth transplantation. The anastomosis between preexisting vessels of transplanted pulp tissue and ingrowing blood vessels rarely occurs [8]. In both the replantation and allograft of the present study, evidence of anastomosis was not observed. From our results, angiogenesis markers were detected from the root canal through the pulp floor to the roof of the pulp chamber and horn sequentially in both operations. However, there was a time difference between the two operations. vWF showed the highest expression at day 3 in replantation, while the highest level at day 5 in allograft and became similar to that of M2 at day 7. Although VEGF expression of M1 became similar to M2 and M3 at 7 days after replantation, the expression pattern at day 5 of M1 is maintained until day 7 in the allograft. Based on these results, it can be inferred that the revascularization process is faster in replantation than in allograft.

Nestin is mainly expressed in the early stages of nervous tissue and muscle development [23]. However, nestin is expressed in both epithelial and mesenchymal components of developing rodent teeth, and its expression progressively becomes restricted to the differentiation

of odontoblasts [19]. In both replantation and allograft, nestin signal of mature odontoblasts was decreased at initial healing process. In replantation, the expression of nestin was started at the pulp canal and continuously distributed throughout the pulp. The result that nestin expression was detected only on the odontoblast layer, similar to M2 and M3 at 7 days after replantation, inferred that the odontoblast differentiation of the healing process was completed at that time. However, in allografts, nestin expression was strongest at 5 days after the operation. The expression decreased slightly but continuously remained overall in the pulp at 7 days after the allograft. This suggests that pulp regeneration in the tooth allograft was delayed compared to that in the replantation.

In Fig. 4, angiogenesis and pulp healing patterns are presented as the healing responses of two types of tooth transplantation: replantation and allograft. The present study showed the overall healing process in tooth transplantation and allograft. Despite a number of studies on tooth transplantation in various species, including humans, accurate information on the healing pattern is still stacked in a veil. An exact understanding of the properties of the dentin-pulp complex is needed for future regenerative treatment of the dental pulp.

5. Conclusion

Tooth transplantation comprises inevitable host responses, including inflammation. For survival of the transplanted tooth, revascularization and pulp regeneration are the essential healing processes. The inflammatory response and tooth resorption were similar between the replantation and allograft in the initiation of healing. However, following healing processes such as bonelike tissue formation, pulp revascularization, and pulpal healing were faster in replantation. In replantation, the expression of the marker reached the maximum value at day 5, and then it appeared similar to the expression pattern of normal

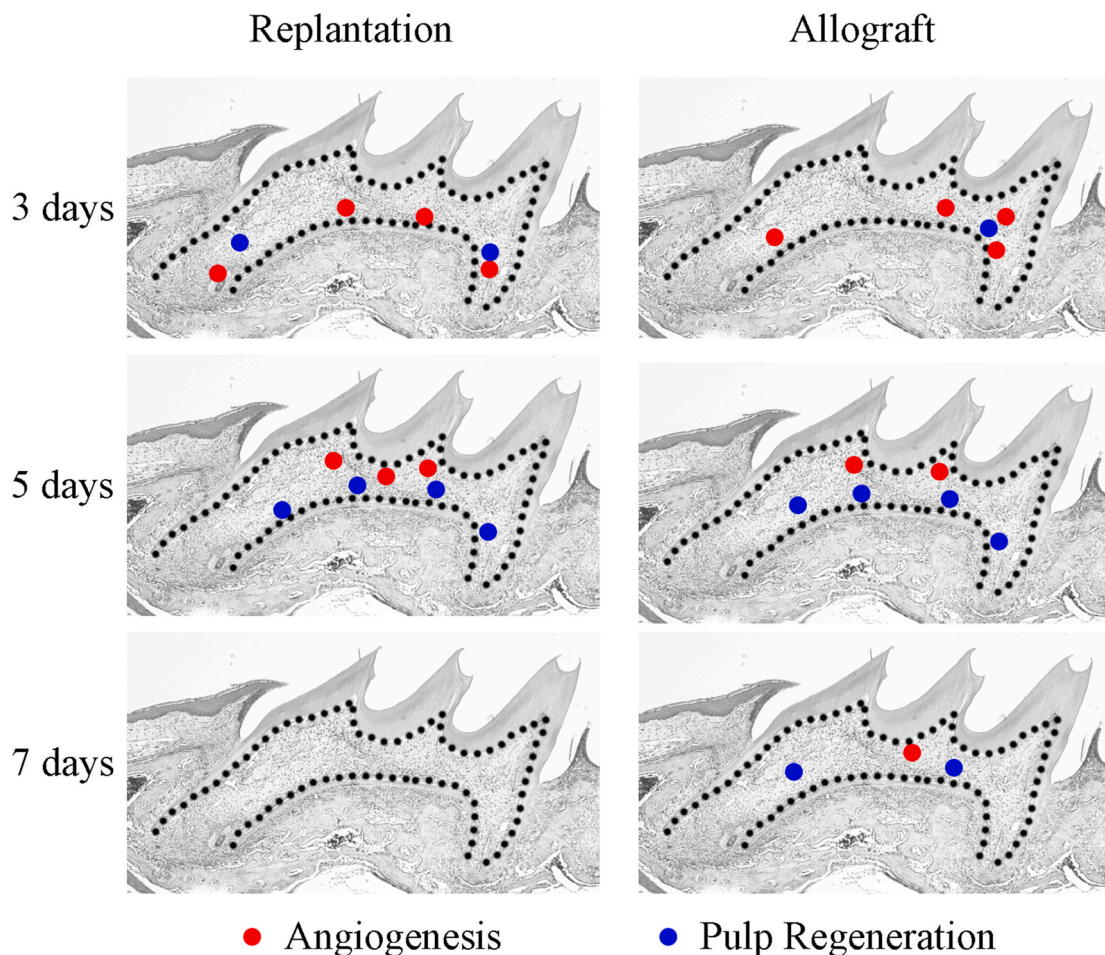


Fig. 4. Angiogenesis and pulp healing patterns between tooth replantation and allograft.

Three days after tooth replantation, angiogenesis markers are expressed in the pulp chamber and around the pulp canal. Nestin is strongly expressed in the root canal. After 5 days, the expression of angiogenesis markers is shifted to the pulp chamber, especially the pulp horn. Nestin expression is observed overall in the pulp, strongly at the pulp floor. After 7 days, angiogenesis markers are expressed uniformly in the replanted tooth. Nestin expression is observed in the odontoblast layer. In tooth the allografts, angiogenesis markers and nestin expression was generally retarded compared to that in replantation. After 3 days, angiogenesis markers are expressed in the pulp chamber, especially around the distal pulpal region. Nestin is expressed at the pulp floor and canal. After 5 days, VEGF and vWF expressions are shifted to the pulp chamber and horn. Nestin is expressed in the pulp overall. After 7 days, angiogenesis markers are rarely expressed in the pulp horn. Nestin is expressed throughout the pulp.

teeth, whereas the allograft still showed excessive expression after 5 days. Specifically, the 5 days after surgery seems to be the critical time-point of retardation of the healing in mouse molar. This study will yield useful fundamental data and guidelines for future research to understand the healing process of tooth transplantation.

CRediT authorship contribution statement

Dong-Joon Lee: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Seung-Jun Lee:** Writing - original draft. **Min-Jung Lee:** Investigation, Formal analysis. **Eun-Jung Kim:** Validation, Funding acquisition. **Hayato Ohshima:** Conceptualization, Methodology, Investigation. **Han-Sung Jung:** Conceptualization, Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2021.100945>.

References

- [1] J.R. Natiella, J.E. Armitage, G.W. Greene, The replantation and transplantation of teeth. A review, *Oral Surg. Oral Med. Oral Pathol.* 29 (1970) 397–419.
- [2] D. Ong, Y. Itskovich, G. Dance, Autotransplantation: a viable treatment option for adolescent patients with significantly compromised teeth, *Aust. Dent. J.* 61 (2016) 396–407.
- [3] D.R. Steiner, Avulsed maxillary central incisors: the case for replantation, *Am. J. Orthod. Dentofacial* 142 (2012), 8+.

- [4] M.S. Howe, W. Keys, D. Richards, Long-term (10-year) dental implant survival: a systematic review and sensitivity meta-analysis, *J. Dent.* 84 (2019) 9–21.
- [5] O. Shadad, R. Chaulagain, K. Luukko, P. Kettunen, Establishment of tooth blood supply and innervation is developmentally regulated and takes place through differential patterning processes, *J. Anat.* 234 (2019) 465–479.
- [6] N. Vongsavan, B. Matthews, The vascularity of dental pulp in cats, *J. Dent. Res.* 71 (1992) 1913–1915.
- [7] L. Olgart, Neural control of pulpal blood flow, *Crit. Rev. Oral Biol. Med.* 7 (1996) 159–171.
- [8] C. Rombouts, T. Giraud, C. Jeanneau, I. About, Pulp vascularization during tooth development, regeneration, and therapy, *J. Dent. Res.* 96 (2017) 137–144.
- [9] I. Claus, W. Laureys, R. Cornelissen, L.R. Dermaut, Histologic analysis of pulpal revascularization of autotransplanted immature teeth after removal of the original pulp tissue, *Am. J. Orthod. Dentofacial Orthop.* 125 (2004) 93–99.
- [10] A. Shimizu, K. Nakakura-Ohshima, T. Noda, T. Maeda, H. Ohshima, Responses of immunocompetent cells in the dental pulp to replantation during the regeneration process in rat molars, *Cell Tissue Res.* 302 (2000) 221–233.
- [11] H. Tsukamoto-Tanaka, M. Ikegame, R. Takagi, H. Harada, H. Ohshima, Histochemical and immunocytochemical study of hard tissue formation in dental pulp during the healing process in rat molars after tooth replantation, *Cell Tissue Res.* 325 (2006) 219–229.
- [12] K. Nakakura-Ohshima, J. Watanabe, S. Kenmotsu, H. Ohshima, Possible role of immunocompetent cells and the expression of heat shock protein-25 in the process of pulpal regeneration after tooth injury in rat molars, *J. Electron. Microsc.* 52 (2003) 581–591.
- [13] H. Ohshima, K. Nakakura-Ohshima, K. Takeuchi, M. Hoshino, Y. Takano, T. Maeda, Pulpal regeneration after cavity preparation, with special reference to close spatio-relationships between odontoblasts and immunocompetent cells, *Microsc. Res. Tech.* 60 (2003) 483–490.
- [14] R. Ogawa, C. Saito, H.S. Jung, H. Ohshima, Capacity of dental pulp differentiation after tooth transplantation, *Cell Tissue Res.* 326 (2006) 715–724.
- [15] H. Ohshima, K. Nakakura-Ohshima, H. Yamamoto, T. Maeda, Alteration in the expression of heat shock protein (Hsp) 25-immunoreactivity in the dental pulp of rat molars following tooth replantation, *Arch. Histol. Cytol.* 64 (2001) 425–437.
- [16] A. Quispe-Salcedo, H. Ida-Yonemochi, H. Ohshima, Effects of a triple antibiotic solution on pulpal dynamics after intentionally delayed tooth replantation in mice, *J. Endod.* 40 (2014) 1566–1572.
- [17] N. Kawashima, T. Okiji, Odontoblasts: specialized hard-tissue-forming cells in the dentin-pulp complex, *Congenital. Anom.* 56 (2016) 144–153.
- [18] I. About, D. Laurent-Maquin, U. Lendahl, T.A. Mitsiadis, Nestin expression in embryonic and adult human teeth under normal and pathological conditions, *Am. J. Pathol.* 157 (2000) 287–295.
- [19] C. Terling, A. Rass, T.A. Mitsiadis, K. Fried, U. Lendahl, J. Wroblewski, Expression of the intermediate filament nestin during rodent tooth development, *Int. J. Dev. Biol.* 39 (1995) 947–956.
- [20] T. Hasegawa, H. Suzuki, H. Yoshie, H. Ohshima, Influence of extended operation time and of occlusal force on determination of pulpal healing pattern in replanted mouse molars, *Cell Tissue Res.* 329 (2007) 259–272.
- [21] H. Unno, H. Suzuki, K. Nakakura-Ohshima, H.S. Jung, H. Ohshima, Pulpal regeneration following allogenic tooth transplantation into mouse maxilla, *Anat. Rec.* 292 (2009) 570–579.
- [22] I. Kvinnsland, K.J. Heyeraas, M.R. Byers, Regeneration of calcitonin gene-related peptide immunoreactive nerves in replanted rat molars and their supporting tissues, *Arch. Oral Biol.* 36 (1991) 815–826.
- [23] U. Lendahl, L.B. Zimmerman, R.D. McKay, CNS stem cells express a new class of intermediate filament protein, *Cell* 60 (1990) 585–595.