

Autotransplantation or replantation of cryopreserved teeth: a case series and literature review

Michiko Yoshizawa¹, Takahiro Koyama², Naoya Izumi¹, Kanae Niimi¹, Yukiko Ono¹, Hisao Ajima², Akinori Funayama¹, Toshihiko Mikami¹, Tadaharu Kobayashi¹, Kazuhiro Ono³, Ritsuo Takagi², Chikara Saito¹

¹Division of Reconstructive Surgery for Oral and Maxillofacial Region, Department of Tissue Regeneration and Reconstruction, Course for Oral Life Science, Niigata University Graduate School of Medical and Dental Sciences;

²Division of Oral and Maxillofacial Surgery, Department of Oral Health Science, Course for Oral Life Science, Niigata University Graduate School of Medical and Dental Sciences;

³Division of Oral Science for Health Promotion, Department of Oral Health and Welfare, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Abstract – Background: The aim of this report was to evaluate the outcome of autotransplantation or replantation of cryopreserved teeth clinically and radiographically. Donor teeth were slowly frozen in a controlled-rate freezer using 5% dimethylsulfoxide (DMSO) and 6% hydroxyethyl starch (HES) as protectants. Seven cryopreserved teeth, with duration of storage ranging from 4 to 36 months, were autotransplanted or replanted at Niigata University Medical and Dental Hospital. Endodontic treatment involving root canal debridement followed by interim root canal filling with calcium hydroxide was started 3 weeks after the operation and continued with replacement of the calcium hydroxide filling at 2-week to 3-month intervals. Three transplants showed periodontal regeneration clinically and radiographically, whereas replacement root resorption was observed in the remaining transplants. From the results, it can be concluded that cryopreserved tooth autotransplantation has potential for clinical use; however, the risk of replacement root resorption remains.

Key words: autotransplantation; cryopreservation; replantation; tooth

Correspondence to: Michiko Yoshizawa, Division of Reconstructive Surgery for Oral and Maxillofacial Region, Department of Tissue Regeneration and Reconstruction, Course for Oral Life Science, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-Dori, Chuo-ku, Niigata 951-8514, Japan
Tel.: +81 25 227 2878
Fax: +81 25 223 6516
e-mail: yoshi@dent.niigata-u.ac.jp

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Tooth autotransplantation is a potential treatment option for recovering the occlusal function of lost teeth, as the transplanted tooth can function as a normal tooth following successful transplantation (1). Since 1994, we have performed more than 50 immediate autotransplantations of teeth with complete root formation annually at Niigata University Medical and Dental Hospital, with a survival rate of nearly 90% (2, 3). Nevertheless, the limited indications of this procedure represent a major disadvantage—both a healthy donor tooth and a healthy recipient site must be available simultaneously. We have been conducting labora-

tory and clinical investigations on the cryopreservation of donor teeth to expand the indications for tooth autotransplantation. Previous studies showed that teeth cryopreserved for a maximum of 4 weeks showed nearly the same level of periodontal regeneration as that observed with immediately transplanted teeth, although the healing with cryopreserved teeth proceeded more slowly in rats (4, 5). In the present study, we evaluated the outcome of autotransplantation of cryopreserved teeth clinically and radiographically. Herein, we present a case series with a review of the literature.

Materials and methods

The protocol of this study was approved by the Institutional Review Board of Niigata University Dental School in compliance with the Helsinki Declaration. All patients provided informed consent before undergoing treatment.

Patients

This study included seven patients (three men and four women) in whom cryopreserved teeth with complete root formation were autotransplanted at Niigata University Medical and Dental Hospital. The age of patients at the time of tooth cryopreservation ranged from 16 to 44 years with a mean age 30.67 years (Table 1).

Cryopreservation

Donor teeth were carefully extracted, and the labial, mesial, lingual or palatal, and distal surfaces of the donor tooth were sketched and photographed, and the condition of the periodontal ligament was recorded after removal. Donor teeth were immersed in autogenous plasma containing 5% dimethylsulfoxide (DMSO) and 6% hydroxyethyl starch (HES; Kyokutouseiyaku Co., Tokyo, Japan) in cryotubes for 5–10 min at 4°C. The cryotubes were slowly frozen at a rate of $-1^{\circ}\text{C min}^{-1}$ from room temperature to the freezing point (-7°C), followed by limited super cooling (-40°C) for 5 min to eliminate latent heat in a programmed freezer (Taiyo-Toyo Sanso Co., Tokyo, Japan). The cryotubes were then cooled at a rate of $-0.5^{\circ}\text{C min}^{-1}$ to -80°C and transferred to a freezer maintained at -152°C (Sanyo Co., Tokyo, Japan) (4, 5).

Transplantation procedures

The transplantation procedure was based on that used for immediate tooth transplantation (1, 3). Mucoperiosteal flaps at the recipient site were made after local anesthesia. The recipient socket was prepared with a bone trephine bar (GC Co., Tokyo, Japan) and surgical round bar before thawing the cryopreserved donor tooth, based on the size and shape of the cryopreserved donor tooth with reference to its sketches and photographs before cryopreservation. The donor tooth in the cryotube was thawed in a warm water bath at 37°C , and the tooth was rinsed with physiological saline. The

match between the recipient socket and the donor tooth was checked. Prior to transplantation, the donor tooth was treated with an enamel matrix derivative, Straumann® Emdogain (Seikagaku Co., Tokyo, Japan). The tooth was transplanted to the recipient socket, and the flaps were sutured with 4-0 silk. All transplanted teeth were stabilized with orthodontic wire and resin or 4-0 silk sutures. The sutures were removed after 7 days, and the wire splint was removed 3 weeks postoperatively.

Endodontic treatment

Endodontic treatment involving debridement of the root canal system followed by interim root canal filling with calcium hydroxide was started 3 weeks after the operation and continued with replacement of the calcium hydroxide filling at 2-week to 3-month intervals. All transplanted teeth were filled with thermoplasticized gutta-percha when no signs of failure were seen clinically and radiographically (6).

Postoperative examination and evaluation of prognosis

The patients were evaluated by clinical and radiographic examination after 1, 2, and 3 weeks and at 2, 3, and 4 to 5, 6, 9, and 12 months. Thereafter, the patients were followed up at intervals of 6–12 months. At each visit, all cryopreserved transplants were evaluated clinically and radiographically. The cases were classified into two groups: good cases, which exhibited periodontal regeneration clinically and radiographically, and other cases, which presented abnormal findings (i.e., failure of initial healing, root resorption, periodontal inflammation, and delay in bone regeneration) (3). Root resorption was diagnosed based on clinical findings and dental radiographs and classified into two types: inflammatory resorption and replacement resorption as described previously (7). Inflammatory resorption was defined as periradicular radiolucency, and replacement resorption was defined as a lack of clinical mobility, high percussion sound, and radiographic confirmation of the disappearance of the periodontal space.

Results

The cryopreserved transplanted teeth comprised 3 first premolars extracted for orthodontic treatment, 3 third molars that were impacted or dislocated, and one

Table 1. Characteristics of cryopreserved teeth autotransplantation or replantation

Case	Age (years)	Sex	Donor teeth	Duration of cryopreservation (months)	Recipient sites	Postoperative course	Observation period (months)
1	24	M	44	24	47	Good	88
2	16	M	33	8	33	Good	13
3	44	F	48	4	47	Good	13
4	21	F	38	5	37	Replacement root resorption	25
5	44	M	18	5	17	Replacement root resorption	12
6	35	F	34	36	11	Replacement root resorption	45
7	35	F	44	36	12	Replacement root resorption	45

canine located in the fracture line of the jaw. The mean duration of cryopreservation was 13.7 months, ranging from 4 to 36 months. All cryopreserved third molars were transplanted to the position of second or first molar, while 3 first premolars were transplanted to the position of incisor or second molar. A cryopreserved canine removed during fracture of the mandible was transplanted to the same position after healing of the jaw fracture. Three transplants were judged to be good cases, whereas replacement root resorption was observed in the remaining four transplants, although it had not progressed (Figs 1 and 2).

Discussion

Occasionally, we encounter situations in which tooth transplantation or replantation cannot be carried out immediately after the recipient tooth is removed because the tissue in the recipient area has not healed adequately or the teeth have been totally dislocated due to severe damage of the alveolar bone during an injury, and so forth. The indication of tooth transplantation can be expanded by cryopreservation of donor teeth, which allows storage of donor teeth for extended periods of time. Teeth extracted for orthodontic reasons or impacted third molars are excellent candidates for cryopreservation and future transplantation (8). In the first clinical case report, a first premolar cryopreserved for 18 months was autotransplanted in connection with orthodontic treatment, and it showed clinically and radiographically normal periodontal healing with no signs of root resorption or marginal bone loss (9, 10). Paulsen et al. (11) also performed auto-

transplantation and orthodontic treatment, together with cryopreservation, in connection with complicated trauma in the anterior region of an 8-year-old girl. In the present study, however, 4 of the 7 cryopreserved teeth showed replacement root resorption. Replacement root resorption was reported to occur after transplantation due to lack of cementum or loss of precementum and cementoblasts resulting from root surface injury (12–15). Drying of the root surface is also a cause of replacement root resorption (15).

Ice injury is the most serious problem that tissue faced during cryopreservation. Ice injury to living cells is caused by a change in osmotic pressure due to increased solute concentrations during freezing and ice crystal formation (16). Schwartz et al. (17, 18) reported that slow and controlled-rate freezing reduces the ice injury. Periodontal regeneration similar to that of immediately transplanted teeth was observed after transplantation of slowly frozen teeth. They also used cryoprotectants, such as 5% DMSO, which protect the cells from extra- and intracellular ice crystal damage and from the osmotic shock during freezing and thawing, to reduce the ice formation. Politis et al. (19) reported that after cryopreservation, periodontal ligament and epithelial rests of Malassez were not damaged micrographically when subjected to slow and controlled-rate freezing.

Previously, we had cryopreserved teeth based on the procedure suggested by Schwartz et al. and histologically examined the periodontal regeneration process after transplantation of the cryopreserved teeth subcutaneously in rats (4, 5). The automatically slow and controlled-rate freezing in a medium containing 5%

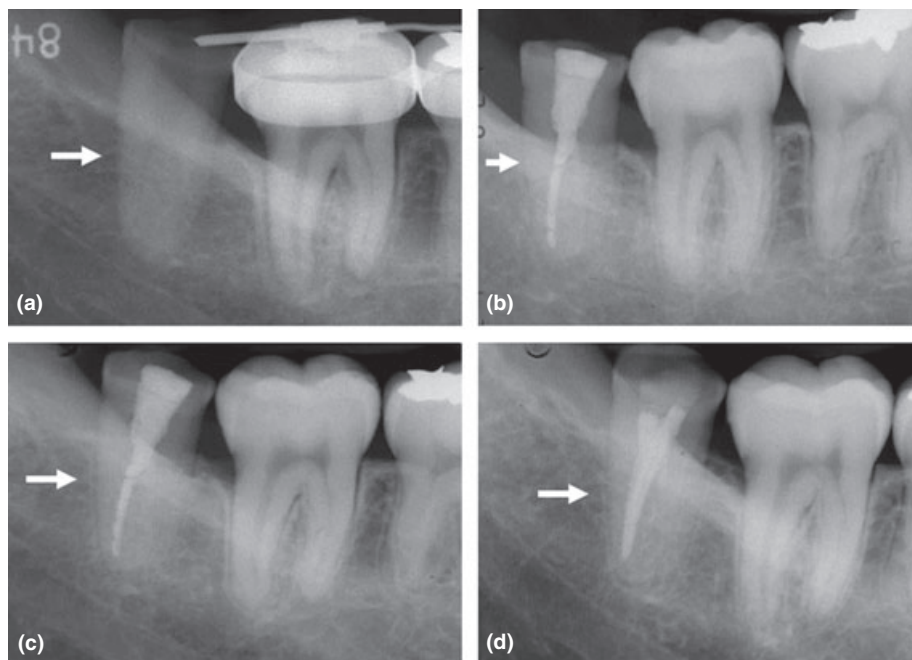


Fig. 1. Case 1. A successful case of cryopreserved tooth autotransplantation: a 24-year-old man whose lower right first premolar was extracted for orthodontic treatment, cryopreserved for 24 months, and then autotransplanted to the position of the lower right second molar, which was congenitally missing. Periapical radiographs taken at: (a) 3 weeks; (b) 6 months; (c) 16 months; (d) 24 months after autotransplantation. The continuous periodontal space and lamina dura can be observed at 16 months. No root resorption was observed up to 24 months (arrows; cryopreserved transplanted tooth).

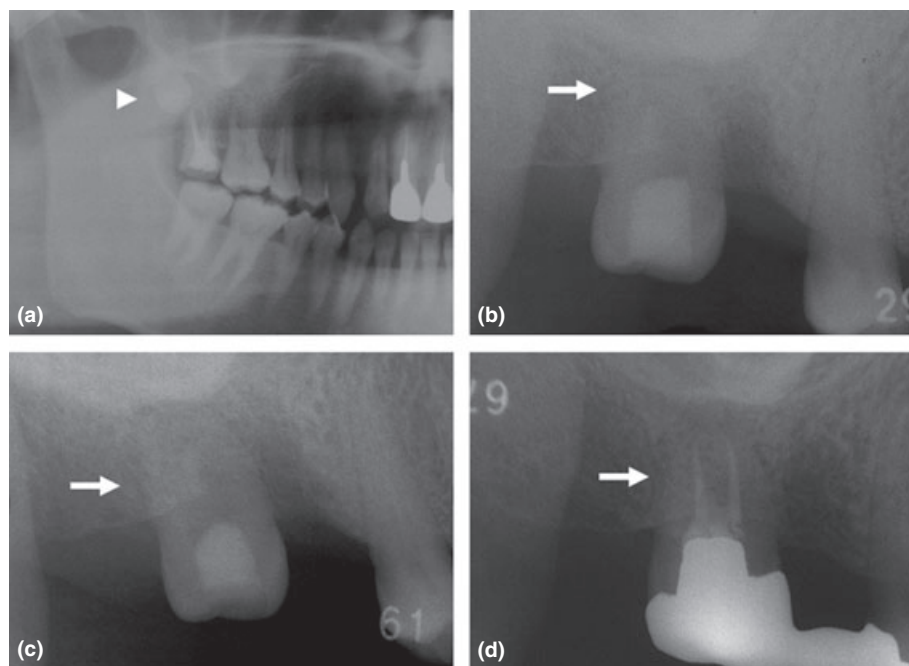


Fig. 2. Case 2. Replacement root resorption seen after cryopreserved tooth autotransplantation: a 44-year-old man whose upper right first and second molars were removed due to tooth fracture. At the same time, the third molar (arrowhead) was removed, cryopreserved for 5 months, and then transplanted to the position of the lost upper right second molar. (a) Preoperative panoramic radiograph (arrowhead; donor tooth). Periapical radiographs taken at: (b) 3 weeks; (c) 2 months; (d) 12 months after autotransplantation. The continuous periodontal space and lamina dura were not observed up to 12 months (arrows; cryopreserved transplanted tooth).

DMSO and 6% HES, which improved the survival rate of cryopreserved blood stem cells, resulted in regeneration of normal periodontal tissue with no evidence of root resorption and abnormal morphological changes. However, the alkaline phosphatase (ALP) activity was weak in the periodontal ligament of molars cryopreserved overnight 2 weeks after transplantation. Moreover, the acellular cementum had a rougher surface, and cementoblasts were seen less frequently 1 week after transplantation in both molars cryopreserved overnight and for 4 weeks. Although the periodontal tissues of both molars cryopreserved overnight and for 4 weeks regenerated similarly to those of immediately transplanted teeth 3 or 4 weeks after transplantation, the delay in periodontal regeneration after transplantation of cryopreserved teeth might have caused replacement root resorption in our clinical cases. The delay in periodontal regeneration may be influenced by the procedure of cryopreservation and thawing including the equilibration of cryoprotectants as well as the storage duration. The mean duration of cryopreservation, 13.7 months (ranging from 4 to 36 months), in the present study was longer than that in our previous study. The teeth stored for 24 months showed good periodontal healing, whereas the teeth stored for 5 months exhibited replacement root resorption. Considering these observations, the procedures of cryopreservation and thawing including the equilibration of cryoprotectants may have to be improved to prevent replacement root resorption. Slow equilibration of cryoprotectants before cryopreservation and thawing,

which was shown by Schwartz et al. (18) to enhance normal periodontal healing, might be important for preventing replacement root resorption because it was not used in our study. Moreover, other cryopreservation procedures, such as slow freezing with a magnetic field to prevent ice formation without a high concentration of cryoprotectants, have been reported recently (20, 21). Further experimental studies exploring the procedures for cryopreservation and thawing to promote periodontal regeneration and the limitations of long-term storage are required. In addition, it is possible that cryopreservation of teeth for long-term storage leads to enamel cracks due to ice crystallization (22). Cracks in enamel and dentin cause inflammation with consecutive root resorption and bone loss. Although there were no findings of cracks in cryopreserved donor teeth or inflammatory root resorption after transplantation in our seven cases, long-term observation is needed after transplantation of cryopreserved teeth.

In conclusion, cryopreserved tooth autotransplantation has a potential for clinical use; however, a risk of replacement root resorption remains. Further studies are needed to promote the regeneration of the periodontium after cryopreserved tooth autotransplantation.

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Conflict of interest statement

There is no financial interest to disclose on this study.

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