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Does removal of the original pulp tissue before autotransplantation influence ingrowth of new tissue in the pulp chamber?

Wim G.M. Laureys[1], Luc R. Dermaut[1], Claude A. Cuvelier[2], Guy A.M. De Pauw[1]

Departments of [1]Orthodontics and [2]Anatomic Pathology, University Ghent, Ghent, Belgium

Correspondence to: Laureys Wim, Department of Orthodontics, University Ghent, University Hospital – P8, De Pintelaan 185, 9000 Ghent, Belgium
Tel.: +32 9 332 40 06
Fax: +32 9 332 38 51
e-mail: wim.laureys@ugent.be

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Abstract – In an attempt to extend the indication area for autotransplantation of vital teeth, two possibilities can be proposed: (i) The enlargement of the apical foramen, with the aim to facilitate revascularization and ingrowth of new tissue. The ingrowth of tissue will eliminate the need for endodontic treatment when mature teeth are transplanted and (ii) the cryopreservation of teeth in case they cannot be transplanted immediately to the receptor site. Teeth with an ideal stage of root formation can be cryopreserved to perform transplantation later. Although pulpcell cultures survive crypreservation *in vitro*, the pulp tissue cannot survive the cryopreservation procedures when it is kept inside the pulpchamber. Therefore, the pulp tissue has to be removed before cryopreservation. It has been demonstrated that revascularization and ingrowth of new tissue can occur in an empty pulp chamber (1). The aim of this study was to find out if revascularization and ingrowth of new pulp tissue is influenced by removal of the original pulp tissue before autotransplantation. Twenty nine single-rooted teeth from three adult beagle dogs were transplanted after resection of the root tip. One group of teeth ($n = 14$) had the pulp tissue removed before transplantation. The other group ($n = 15$) had the original pulp left *in situ*. The transplanted teeth were histologically analysed 90 days post-transplantation. In the group with the tissue left *in situ*, 12 teeth (80%) showed a pulp chamber totally filled or at least 1/3 to 2/3 filled with viable tissue. In the group with the pulp tissue removed, 11 teeth (79%) had no or little vital tissue in the pulp chamber. The necrotic masses that develop in the original pulp tissue immediately after transplantation are a possible stimulating factor in the repair process of the pulp. As a conclusion, it can be stated that in case of autotransplantation of teeth, it is advisable to leave the pulp tissue *in situ* to stimulate the revascularization and ingrowth of new tissue after transplantation.

Autotransplantation of immature teeth has become an evidence-based procedure with a success rate of almost 98% if teeth are transplanted in ideal circumstances (2–6). The best long-term results are achieved with a root formation of two-thirds completed, atraumatic extraction and transplantation to a suitable acceptor site in a minimum of time. However, these different conditions restrict the indication area for autotransplantation and are very much age related.

In an attempt to enlarge the indication area for autotransplantation, cryopreservation offers new possibilities. Cryopreservation can be indicated when, at the ideal moment of time for transplantation, the recipient site is too small to accept the tooth to be transplanted and orthodontic treatment is needed to gain the necessary space. A cryopreserved tooth will have a vital periodontal ligament, but unfortunately does not have a vital pulp. Pulp tissue has a very complex structure and the penetration of cryoprotective agents throughout the small apical foramen of fully erupted teeth into the pulp chamber is very poor. In the literature, most studies on cryopreservation described transplantation of permanent teeth with a closed apex, facilitating successful endodontic treatment after transplantation (7–9).

It is known from a series of studies by Skoglund et al (10–13) that, as a rule, the original pulp of autotransplanted immature teeth and of mature apicoectomized teeth becomes necrotic after transplantation. Repair occurs through the ingrowth of well-vascularized cell-rich connective tissue. Fewer cells and blood vessels are found in new pulp tissue and the new tissue after obliteration resembles bone or cementum in major parts of the pulp cavity.

It has been demonstrated in two earlier studies (1, 14) that teeth can revascularize after autotransplantation if the original pulp tissue is removed at the moment of extraction. This procedure offers the possibility to store teeth in a tooth bank (cryopreservation) and to autotransplant those in a later stage expecting the pulp chamber will be filled with new vital tissue.

However, in the previously mentioned studies it was found that sometimes revascularization and ingrowth of new tissue does not occur. So far, it is unknown whether revascularization and ingrowth of new tissue in the pulp chamber is influenced by the removal of the original pulp tissue.

Therefore, the aim of this study was to find out if revascularization and ingrowth of new pulp tissue is influenced by the removal of the original pulp tissue before autotransplantation.

Material and methods

The experimental material consisted of 30 single-rooted mature teeth (18 incisors and 12 first premolars) from three adult beagle dogs (Fig. 1). A positive advice for the use of beagle dogs in this experimental study was given by the Ethical Committee for animal research of the University of Ghent (ECP 02/27).

Unfortunately, one incisor was lost during the surgical procedure because of a root fracture finally resulting in a sample of 29 teeth.

Figure 1 shows the schematic view of the used teeth in the dog's dentition.

The teeth were extracted and apicoectomized with a wire cutter to enlarge the apical foramen, holding the teeth by the crown with extraction forceps (1). One group of teeth had (n = 14) the entire pulp tissue removed from the apical side with the use of a nerve broche. In the other group (n = 15), the pulp tissue was left *in situ*. In the group with the pulp removed, the apical diameter varied between 0.53 and 1.52 mm. Whereas 78.5% of the teeth had an apical diameter smaller than 1 mm. In the group with the pulp left *in situ* the apical foramen varied between 0.31 and 1.09 mm, 93.2% had a diameter smaller than 1 mm.

All teeth were immediately transplanted to their original site (replantation) and some positioned in infra-occlusion because of the smaller root length after apicoectomy. No enlargement of the alveolus was carried out to prevent extra surgical manipulation. A total of 29 teeth were atraumatically replanted. No pressure was exercised on the teeth during replantation. Only the lower incisors needed splinting by means of a flexible wire (twistflex 0.014 inch) during the experimental period.

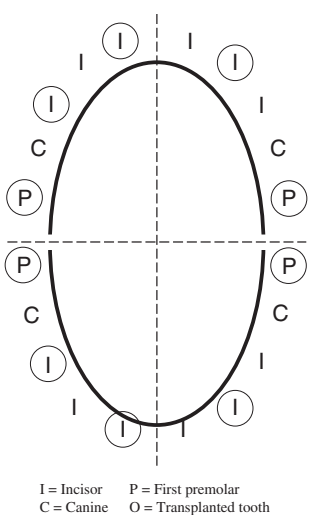

Fig. 1. Schematic view of the used teeth in the dog's dentition.

The observation period for all teeth was 90 days post-transplantation. The dogs were sacrificed, the jaws excised and fixed in 4% neutral buffered formaldehyde. After demineralization (25% EDTA), histological paraffin sections were made and stained with haematoxyline-eosine.The serial sections (5 μm) were made as much as possible parallel to the long axes. Because of the curved roots, it was seldom possible for the whole length of the pulp chamber of the same tooth to be in one section. The consecutive central serial sections of the pulp chamber were used.

The sections were examined under light microscope. A classification was made in the amount of vital tissue filling the pulp chamber 90 days post-transplantation: (i) more than 2/3 of the pulp chamber filled, (ii) between 1/3 and 2/3 of the pulp chamber filled, (iii) $<1/3$ of the pulp chamber filled (apical area only) (iv) no ingrowth.

The periodontal health of the transplanted teeth was not measured in this study.

Results

The amount of vital tissue in the pulp chamber 90 days after transplantation is illustrated in Fig. 2.

Figure 2 amount of vital tissue in the pulp chamber 90 days post-transplantation.

1. Seven of 29 teeth (24%) showed a pulp chamber, totally filled with new tissue. In only one tooth of these seven, the pulp tissue was removed before transplantation. In the other six teeth the pulp tissue was not removed, only the apex was widened (apicoectomy).

The pulp chamber in these teeth with the original pulp left *in situ* showed a more or less thick layer of tertiary dentine, which was aligned with odontoblasts. (Fig. 3a,b)

On the contrary, the pulp chamber of the tooth with the original pulp removed, showed only a small layer of tertiary dentine, which was not fully aligned with a layer of odontoblasts (Fig. 4, coronal part) (Fig. 5, apical part).

Revascularization and ingrowth of new tissue was observed in the entire pulp chamber, but the apical half of the pulp chamber showed more blood vessels

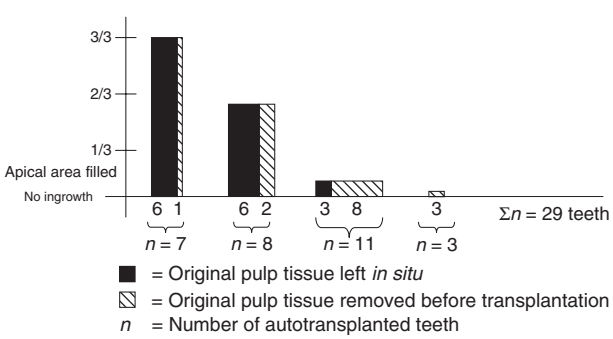

Fig. 2. Amount of vital tissue in pulp chamber 90 days post-transplantation.

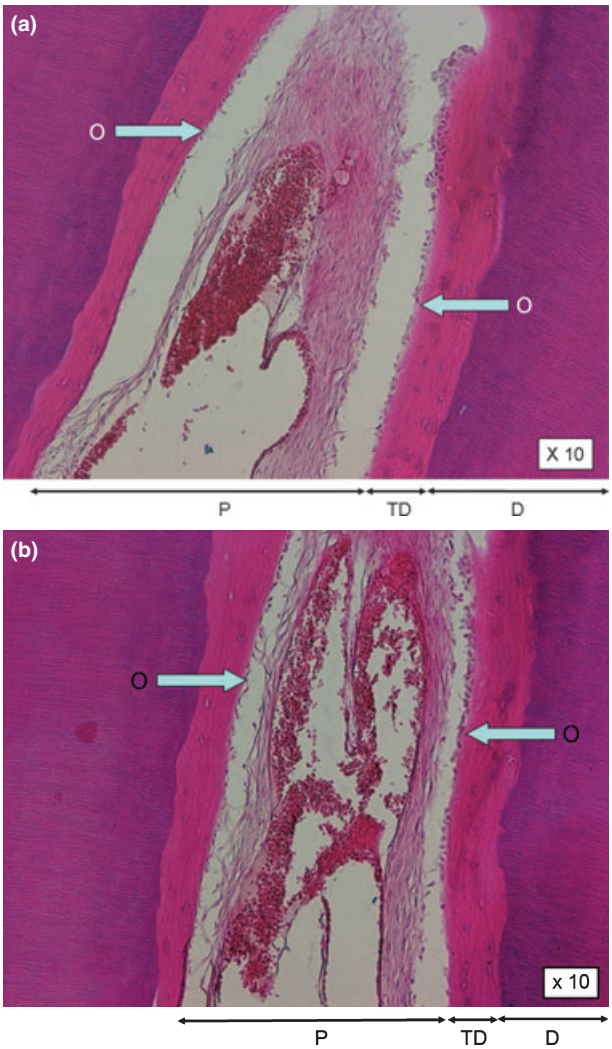

Fig. 3. (a,b) Example of teeth with pulp left *in situ*, thick layer of tertiary dentine and odontoblasts. P, pulp chamber; TD, tertiary dentine; D, dentine; O, layer of odontoblasts.

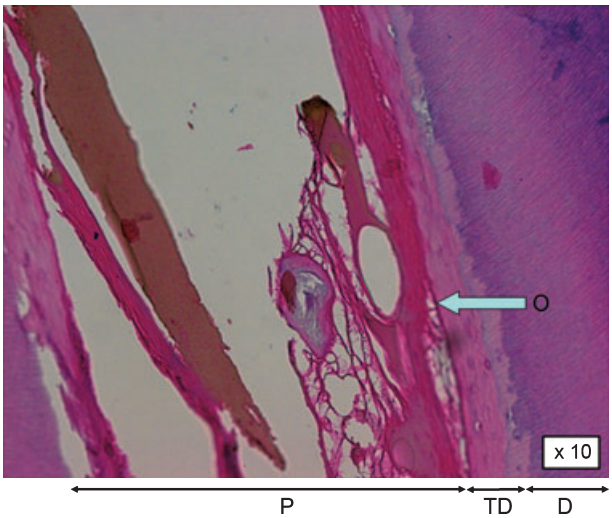

Fig. 4. Example of teeth with original pulp removed before transplantation. P, pulp chamber; TD, tertiary dentine; D, dentine.

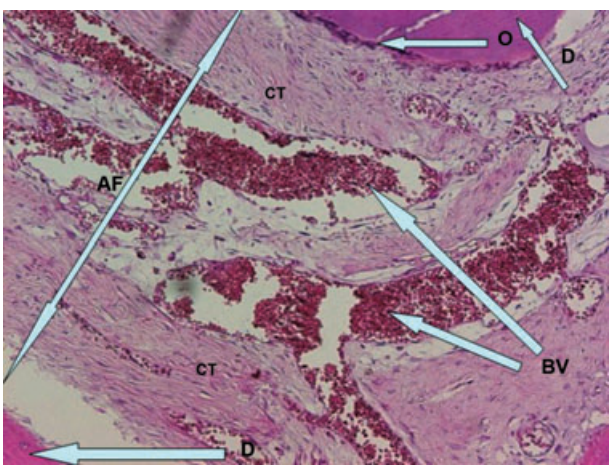

Fig. 5. New well-stained connective tissue, in apical part of pulpchamber, 90 days post-transplantation. D, dentine; O, odontoblast-like cells; CT, connective tissue; BV, bloodvessels with erythrocytes; AF, apical foramen.

filled with erythrocytes and more well-stained cells. The tissue at the coronal half of the pulp chamber was more scar-like tissue.

Figures 4 and 5 show the more coronal part and the apical part of the new tissue in the pulp chamber of the tooth that had its original pulp tissue removed before transplantation.

2. Eight of 29 teeth (28%) showed 1/3 to 2/3 refill of the pulp chamber with vital tissue. Six of these eight teeth had the original pulp tissue left *in situ*; two of the eight teeth had the original pulp tissue removed.

3. Eleven of 29 teeth (38%) showed only the start of revascularization and had vital pulp tissue only at the apical foramen. Less than 1/3 of the pulp chamber was filled. Eight of these 11 teeth had the original pulp tissue removed (Fig 6a,b).

4. Three of 29 teeth (10%) showed no revascularization at all; these three teeth had the original pulp removed before transplantation. No vital tissue was seen in the pulp chamber.

No significant difference was seen between maxillary and mandibullar teeth and also between incisors and premolars.

Discussion

According to Skoglund et al. (10–13), the revascularization and ingrowth of new tissue in the pulp chamber after transplantation or replantation of teeth with an open apex is characterized by three stages if the original pulp tissue is left *in situ*:

Stage 1: Necrosis of the original pulp tissue. Cell activity in the pulp chamber drops because blood flow in the pulp is stopped.

Stage 2: Ingrowth of new tissue in the first 10–30 days after transplantation. The cell activity in the pulp

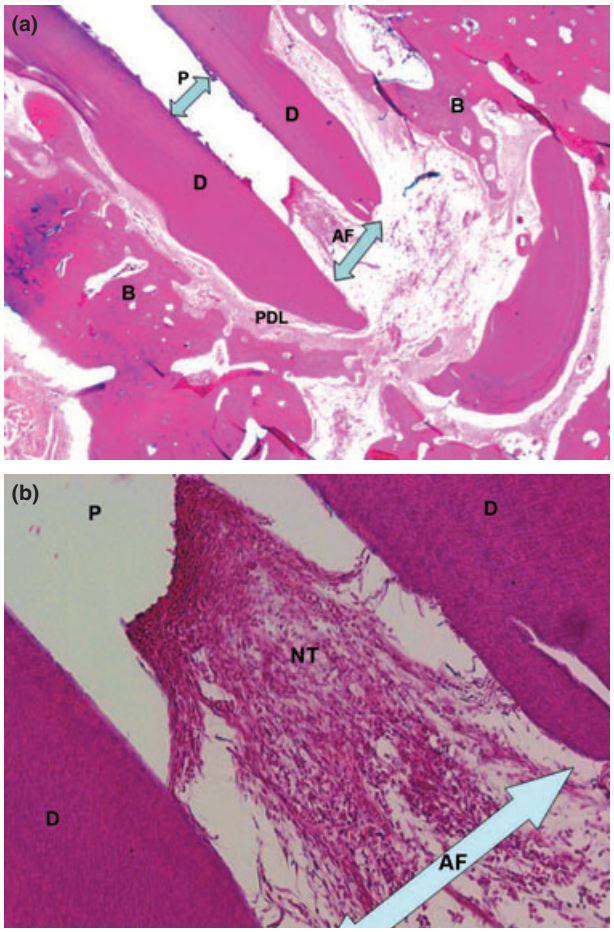

Fig. 6. (a) (×2.5) Start of revascularization in empty pulpchamber, 90 days post-transplantation. (b) (×10): Start of revascularization in empty pulpchamber, 90 days post-transplantation. D, dentine; P, pulpchamber (empty); B, bone; PDL, periodontal ligament; AF, apical foramen; NT, new tissue starting ingrowth.

chamber rises, starting from the apical side and growing to the top of the pulp chamber. The old, original tissue is replaced by new cell-rich connective tissue.

Stage 3: 180 days post-transplantation the cell activity in the pulp chamber drops again and the obliteration of the pulp chamber and formation of a more scar-like tissue starts.

The same stages of revascularization and ingrowth of new tissue (stage 2 and 3) are demonstrated in transplanted teeth when the original pulp tissue is removed before transplantation.

It was shown that revascularization of the empty pulp chamber after removal of the original pulp tissue, starts in the first 10 days post-transplantation. Eighteen days post-transplantation blood vessels can be demonstrated in the coronal part of the pulp chamber (1). In another study, it was demonstrated that 75% of all teeth with an open apex and empty pulp chamber prior to transplantation, had the pulp chamber totally filled with new cell-rich connective tissue 30 days post-transplantation (14).

However, in this study today, the amount of vital pulp tissue after ingrowth in the empty pulp chamber is less than that in the previously mentioned studies (1, 14). A possible explanation could be that in accordance with the 3rd stage of revascularization, as proposed by Skoglund et al. (10–13), the vitality is dropping 90 days post-transplantation. The viable new pulp tissue present at 30 days post-transplantation (14) or 40 days post-transplantation (1) changes into scar tissue and obliteration starts.

In this study, the group with the original pulp tissue left *in situ* comprised 12 of 15 teeth (80%) with the pulp chamber totally filled or for at least 1/3 to 2/3 with viable tissue 90 days post-transplantation. According to the studies of Skoglund et al. (10–13), the pulp chamber was possibly filled with more viable new tissue 30 days post-transplantation. However, 90 days post-transplantation, the vitality has dropped and scar formation was observed.

So in this study the moment of observation (90 days post-transplantation) in both the 'empty' as the '*in situ*' group is less favourable for the amount of vital tissue present in the pulp chamber.

In the group with the pulp tissue removed before transplantation, 11 of 14 teeth (79%) had no or little vital tissue in the pulp chamber 90 days post-transplantation. Only two teeth had the pulp chamber filled for 1/3 to 2/3 and one tooth had the pulp chamber totally filled with vital tissue.

Compared with the group where the pulp tissue was left *in situ*, it seems that the revascularization and ingrowth of new tissue is more difficult if the original pulp tissue is removed before transplantation.

Immediately after autotransplantation of immature teeth the original pulp tissue becomes necrotic (10–13). These necrotic masses in the original pulp tissue are a possible stimulating factor in the repair process of the pulp. Vojinovic and Vojinovic (15) showed that retromigration of cells from the periodontium into the apical pulp in immature teeth is possible. In certain conditions, these periodontal precursor cells can differentiate into odontoblasts. It was demonstrated that necrotic foci in the pulp chamber have a coordinating effect on this cellular activity.

As it is not possible to cryopreserve pulp tissue, teeth of a tooth bank need to undergo an endodontic treatment after transplantation (7, 8, 16). To avoid this endodontic treatment afterwards, it has been demonstrated that making the pulp chamber empty before cryopreservation offers new possibilities in revascularization and ingrowth of new pulp tissue (1).

If cryopreservation before transplantation is not a treatment option, the original pulp tissue of immature teeth and mature apicoectomized teeth can better be left *in situ* before transplantation to let it necrotize after transplantation, facilitating revascularization and ingrowth of new tissue.

A large number of variables, such as size of the apical foramen, splinting of the transplanted tooth, atraumatic infra-occlusion at the moment of transplantation, influences the revascularization and ingrowth of new tissue in the pulp chamber after transplantation.

The effect of these variables was not examined because these variables were equally divided in the '*in situ*' group as in the 'pulp removal' group.

Conclusion

Revascularization and ingrowth of new pulp-like tissue after autotransplantation of teeth with an empty pulp chamber and an open apex was possible although in general the amount of ingrowth was lesser compared with the teeth with the original pulp tissue left *in situ* at the moment of transplantation.

A few months after transplantation, the cell activity in the pulp drops, scar tissue is formed and obliteration starts.

In case of autotransplantion of teeth, it is advisable to leave the tissue *in situ* before transplantation to stimulate the revascularization and ingrowth of new pulp tissue.

Future research has to find out whether it is possible to cryopreserve teeth with an open apex, with the pulp tissue left *in situ*, and transplant them successfully afterwards without endodontic treatment.

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