Dental Traumatology

Dental Traumatology 2012; **28**: 97–100; doi: 10.1111/j.1600-9657.2011.01040.x

Evaluation of dentin formed in autogenous tooth transplantation in the dog: a comparison between one- and two-stage surgical techniques

Manuel Marques Ferreira[1], Maria Filomena Rabaça Botelho[2], Lina Carvalho[3], Maria Reis Silva[3], Barbara Oliveiros[2], Eunice Virgínia Palmeirão Carrilho[1]

[1]Department of Dentistry, University School of Medicine; [2]Department of Biophysics and Biomathematics, University School of Medicine; [3]Department of Pathologic Anatomy, University School of Medicine, Coimbra, Portugal

Correspondence to: Manuel Marques Ferreira, Departamento de Medicina Dentária, Hospitais da Universidade de Coimbra, Rua Bissaya Barreto- Bloco de Celas, 3000 Coimbra, Portugal
Tel.: +239484183
Fax: +351239834616
e-mail: m.mferreira@netcabo.pt

Accepted 6 May, 2011

Abstract – This study was designed to compare the thickness of dentin formed associated with autogenous tooth transplantation in dogs, using either one- or two-stage surgical techniques. The study consisted of three Beagles, older than 5 months, in which six incisors and six premolars were transplanted to mechanically prepared recipient sockets. One group was transplanted using a one-stage method to recipient beds prepared immediately before transplantation. The second groups of teeth were transplanted using a two-stage method in which the recipient beds were prepared and left to heal for 7 days before transplantation. Dogs were injected with xylenol orange, calcein and oxytetracycline at 2 days before, 3 and 9 weeks after transplantation, respectively, for vital staining. Clinical examinations were carried out every week, and the animals were euthanized 9 weeks later. The jaws were resected, fixed in formaldehyde and embedded in resin. Undemineralized sections were cut and examined by fluorescent microscopy. The thickness of dentin formed in the third week after transplantation and 9 weeks was evaluated by undertaking histomorphometric analysis and analysed using the Mann–Whitney *U* test ($P = 0.05$). All the transplanted teeth in both groups survived, and the dentin was formed. No statistically significant difference was found in the thickness of dentin formed in the third week and formed in the third to ninth week between the treatment groups ($P = 0.999$ and $P = 0.998$, respectively). This study demonstrated that there was no difference between the two surgical techniques in terms of the thickness of dentin formed in transplanted teeth.

Autogenous tooth transplantation could be an alternative way to restore the arch if there is a suitable donor tooth available (1, 2). Successful tooth transplantation depends upon the optimal and uneventful healing of the periodontium (3, 4). It depends upon the vitality of remaining periodontal ligament cells in the donor root, the shape and the site of the recipient socket and the vascularity of the recipient bed (5–8). Immature teeth are more useful for transplantation than fully mature teeth (4). To improve nutrition and preserve cell activity in these tissues, Nethander et al. (6), Katayama et al. (7) and Ferreira et al. (9) suggested that teeth should be transplanted to the sockets with regenerative tissues, which may reduce the root resorption and the circulation and innervations recover the original pulp tissue, and dentin development continues after transplantation of immature teeth.

The aim of this study was to examine, by an experimental study in dogs, the disturbance in formation and calcification of dentin in teeth transplanted to a recipient bed, prepared immediately before transplantation (the one-stage method) or in which the tissue was under regeneration during 7 days (the two-stage method).

Material and methods

Before the start of the experiment, application was made to the Direcção de Serviços de Meios de Defesa da Saúde, Bem-estar e Alimentação Animal da Direcção Geral de Veterinária (Portugal). The animal experimental procedures were performed in Estação Zootécnica Nacional (EZN) – Instituto de Tecnologia Biomédica, Santarém, Portugal, in accordance with the International Guiding Principles for Animal Research. The animals were acquired from Universidade de Córdova, Espanha, maintained in the EZN during the experimental period and were observed daily by the doctor and technicians of the EZN.

Twelve non-carious and periodontally sound incisors and twelve non-carious and periodontally sound premolars from three male Beagles (age 5 months; body weight

11.73 ± 1.13 kg) were selected. All the experimental procedures were performed with the animals under general anaesthesia, using preanaesthetic sedation with 0.05 mg kg^{-1} of body weight of acepromazine (Calmivet®; Vetoquinol, Lure, France) administered intramuscularly and anaesthetic induction with 10 mg kg^{-1} of body weight of thiopental (Pentotal®; Queluz de Baixo, Portugal) administered intravenously. After intubation, the anaesthesia was maintained with a mixture of oxygen and 1–2% of isoflurane (Isoflo; Veterinaria Esteve, Barcelona, Spain). Throughout the duration of general anaesthesia, the dogs received normal saline solution intravenously.

All selected teeth, incisors (n = 12) and premolars (n = 12) were extracted as atraumatically as possible, under aseptic conditions. The alveolus at each site was enlarged with a bur Ø 3.5 to receive the incisors and Ø 4.2 to receive the premolars (Strauman, Basel, Switzerland), under copious irrigation with normal saline solution.

The roots and sockets were gently rinsed with 5 ml each of normal saline solution immediately before the following treatment protocols.

Group A (n = 12) – The teeth are transplanted to the recipient beds prepared immediately before.

Group B (n = 12) – The teeth are transplanted to a recipient bed in which healing tissue was under regeneration during 7 days.

Group C (n = 6) – Control group. No treatment was performed on the teeth.

After transplantation, the teeth are splinted with vycril 3/0 (9–11). The animals were given standard food softened with hot water. Immediately after transplantation and for five subsequent days, the animals were given enrofloxacine (5 mg kg^{-1} of body weight), to prevent postoperative infection. In addition, for possible postsurgical pain, each animal was given 0.01 mg kg^{-1} of body weight of buprenorphine once daily.

For vital staining with fluorescent dyes, all dogs were injected subcutaneously with xylenol orange (90 mg kg^{-1}) 2 days before transplantation, calcein (5 mg per 7 kg) 21 days after transplantation and oxytetracycline 62 days after transplantation. The animals were euthanized 63 days after transplantation. The dogs were deeply anaesthetized with an overdose of intravenous pentobarbital at 100 mg kg^{-1} of body weight. After the dissecation of the carotid vein, perfusion was performed with 40 ml of 4% paraformaldehyde in phosphate buffer (pH 7.4). Jaw blocks containing the transplanted teeth were resected and fixed in the same fixative. Following fixation, the specimens were dehydrated in graduated ethyl alcohol solutions from 60% to 100% with continuous agitation. Specimens were embedded in resin (Technovit 7200 VLC; Kulzer, Exact Advanced Technologies, Nordestedt, Germany) without any decalcification. The ground sections were obtained by cutting the teeth longitudinally with a high speed saw, water cooled (Exact System, Hamburg, Germany). The sections were mounted on acrylic slides and ground to approximately 100 microns. One 100 micron section in the middle of teeth was selected to represent each tooth.

Morphometry evaluation

A total of 30 sections with 100 μm thickness representing 30 teeth were observed under fluorescent microscope Nikon Eclipse 80i and the thickness of dentin was measured for the transplanted and control teeth. Images were input into a computer using a digital device camera Nikon DXm 1200C (NIKON INSTRUMENTS, Badhoevedorp, The Netherlands), with fixed magnification ×100. To visualize the fluorochromes in the newly formed dentin, chroma filters (Chroma Technology Corp., Bellows Falls, VT, USA) were used, G2A for the xylenol orange (excitation 510–560 nm), B2A for calcein (excitation 450–490 nm) and UV2A for the oxytetracycline (excitation 330–380 nm). Morphometric analysis was performed with the aid of the software ImageJ 1.30 (Image Processing and Analysis in Java; National Institute of Mental Health, Bethesda, MD, USA) at 100× magnifications. The width of dentin was measured in 6 vertical lines in every section and the mean value calculated. The thickness of the new dentin formed within the first 3 weeks was designated D1, and the thickness of dentin formed within 3–9 weeks was designated D2.

For statistical analysis, the differences among the groups were statistically compared using the Mann–Whitney U test. The significance level was set at 5%. All statistical analysis was performed using SPSS version 1.4 statistical software (SPSS, Japan Inc., Tokyo, Japan).

The dogs tolerated the operative procedures well, and their behaviour did not change. No teeth were lost, and the number of transplanted teeth was 24. In the morphometric evaluation, tree lines were seen in both control and transplanted teeth (Figs 1 and 2). Results of the analysis of weekly changes in dentin thickness are summarized in Fig. 3.

In teeth transplanted to the recipient beds prepared immediately before (group A), the mean thickness of dentin D1 was 35.5 ± 25.3, with no significant difference from the control teeth (P = 0.067), and D2 was 55.15 ± 36.3, with significant difference from the control teeth (P = 0.042; Table 1).

In teeth transplanted to a recipient bed in which the tissue was under regeneration during 7 days, the mean

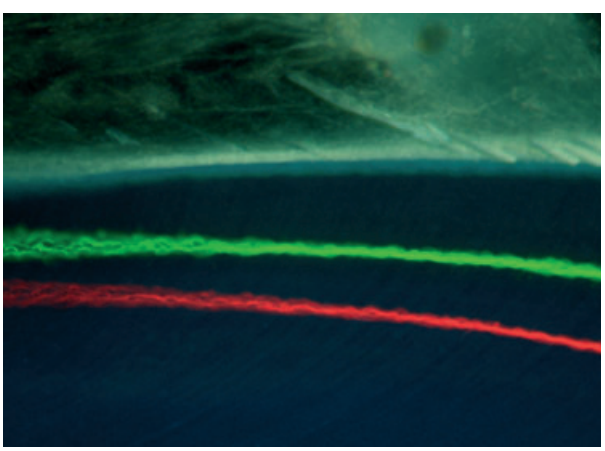

Fig. 1. Control teeth 9 weeks after transplantation labelled with xylenol O range (XO), calcein (C) and oxytetracycline (OXT) histomorphometric analysis (×100).

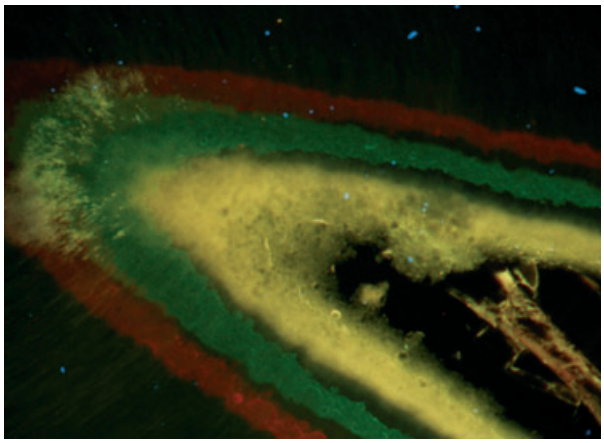

Fig. 2. Transplanted teeth 9 weeks after transplantation labelled with xylenol orange, calcein and oxytetracycline histomorphometric analysis (×100).

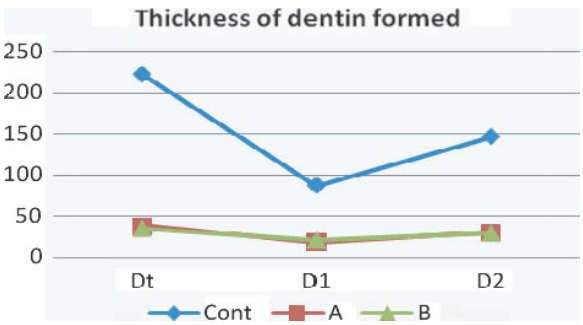

Fig. 3. The mean values of thickness of dentin formed in the control group (Cont) were compared with the transplanted teeth. A = one-stage group; B = two-stage group.

Table 1. The mean values of thickness of dentin formed every week in the control teeth and the experimental transplanted groups

Groups	D1(% ±SD)	D2(% ±SD)
Group C (n = 6)	87.2 ± 54.2	146.6 ± 85.5
Group A (n = 12)	35.5 ± 25.3	55.15 ± 36.3
Group B (n = 12)	41.6 ± 18.1	60.9 ± 24.3

Group A- transplanted teeth to the recipient beds prepared immediately before.
Group B- transplanted teeth to a recipient bed in which the tissue was under regeneration.
Group C- control group, teeth not transplanted.

thickness of dentin D1 was 41.6 ± 18.1 and D2 was 60.9 ± 24.3, and the Mann–Whitney U test revealed a significant difference between the control teeth $P = 0.047$ and $P = 0.027$ (Table 1).

Discussion

Studies of tooth transplantation have been based on models in monkeys, rats and dogs (5, 6, 9, 12, 13). It is suggested that pulp necrosis is the main cause of periapical inflammation and inflammatory root resorption. Previous studies of transplantation of mature teeth reported that the pulp necrotized and that calcified tissue did not form. In contrast, in transplanted immature teeth, the odontoblast and dental pulp tissue were alive after transplantation. When revascularization is delayed, original pulp tissue is necrotized and bone-like tissue then forms in the pulp. During a prolonged extra-alveolar period, the pulp suffers and necrotizes. This indicates that increased handling of the graft in attempts to adapt the tooth to the socket represents a risk of contamination with bacteria and damages the architecture and function of the pulp and periodontal ligament. To prevent this, we made the transplantation to a recipient bed in which the tissue was under regeneration as described by Nethander et al. (6), where the recipient bed is prepared surgically prior to transplantation and allowed to heal for 5 days. This allows in-growth and early maturation of granulation tissues into the wound, into which the transplanted teeth come in direct contact, improving the revascularization. In this study, we used transplanted teeth in dogs, after 7 days of regeneration (the two-stage technique) or performed in the same surgical intervention (the one-stage technique).

The periodontal ligament has demonstrated a remarkable capacity for repair and regeneration. All transplanted teeth demonstrated a similar progression of regeneration. In addition, the osteoblast-like cells lining the newly formed alveolar bone suggest that osteogenic cells in periodontal ligament might proliferate and differentiate into osteoblast-like cells.

The fluorescent labelling demonstrated the development of dentin formed. The new dentin formed was bonded by the old dentin confirmed by the labelling experiment with xylenol orange. In the control teeth, the amount of dentin formed gradually decreased, which suggests that the dentin in the physiological conditions is very actively formed in the first postnatal week. Fluorescent labelling also demonstrated development of dentin in transplanted teeth. The D2/D1 ratio for transplanted teeth in the one-stage was 1:6 and is the same ratio that is found in the control teeth. The D2/D1 ratio for transplanted teeth in the two-stage was 1:4, a lower ratio than in the control teeth.

This experimental study demonstrated that tooth transplantation makes a disturbance in the pulp tissue and formation rate of dentin. There were no differences in the wound healing process between one-stage and two-stage techniques in transplanted teeth.

Autotransplantation can be an alternative to dental implant in some patients in whom dental implants become impossible because of inadequate bone support and patients in growing stages (1–4, 12, 14). This study may improve understanding of both the biology and clinical application of tooth transplantation. We think that tooth autotransplantation is still a very useful method for replacing missing teeth, provided that the extraoral time and other factors are well controlled. The results confirmed that in tooth transplantation, the formation and calcification of dentin continues, although some disturbance in the pulp tissue occurs. This finding may improve understanding of pulp biology and the

requirement to control pulp revascularization, as a very important step to make the root canal treatment to prevent the apical periodontitis.

References

1. Clokie CML, Yau DM, Chano L. Autogenous tooth transplantation: an alternative to dental implant placement? J Can Dent Assoc 2001;67:92–6.
2. Northway WM, Konigsberg S. Autogenic tooth transplantation. The "state of the art". Am J Orthod 1980;77:146–62.
3. Lee SJ, JungI Y, Choi SY, Kum KY. Clinical application of computer aided rapid prototyping for tooth transplantation. Dent Traumatol 2001;17:1114–9.
4. Andreasen JO, Paulsen HU, Yu Z, Bayer T, Schwartz O. A long-term study of 370 autotransplanted premolars. Part II. Tooth survival and pulp healing subsequent to transplantation. Eur J Orthod 1990;12:14–24.
5. Bosco AF, Neto MS, Nagata MJH, Pedrini D, Sundefeld MLMM. Avaliação dos tecidos periodontais de dentes com rizogénese completa autotransplantados para alvéolos recém criados ou em fase inicial de cicatrização. Estudo histológico em macacos. Rev Odontol UNESP S Paulo 2000;29:1–23.
6. Nethander G, Skoglund A. Experimental autogenous tooth transplantation in the dog a comparison between one and two-stage surgical techniques. Acta Odontol Scand 2003;61:223–9.
7. Katayama A, Ota M, Sugito H, Shibukawa Y, Yamada S. Effect of proliferating tissue on transplanted teeth in dogs. Oral Surg Oral Med Oral Pathol 2006;101:e110–8.
8. Lindskog S, Pierce AM, Blomlöf L, Hammarström L. The role of necrotic periodontal membrane in cementum resorption and ankylosis. Endod Dent Traumatol 1985;1:96–101.
9. Ferreira M, Botelho M, Lina C, Oliveiros B, Carrilho E. Histological evaluation of periodontal regeneration in autogenous tooth transplantation in the dog: a comparison between one and two-stage surgical techniques, a pilot study. Dent Traumatol 2010;26:76–9.
10. Bauss O, Schilke R, Fenske C, Engelke W, Kiliaridis S. Autotransplantation of immature third molars: influence of different splinting methods and fixation periods. Dent Traumatol 2002;18:322–8.
11. Nasjelti C, Castelli W, Caffese R. The effect of different splinting times on replantation of teeth in monkeys. Oral Surg Oral Med Oral Pathol 1982;53:557–66.
12. Pedroso JA, Neto MS, Okamoto T, Nunes NA. Transplante dental homógeno para alvéolos imediatamente após a exodontia ou em fase inicial de cicatrização – análise histológica em ratos. Rev Odontol UNESP S Paulo 1993;22:85–95.
13. Skoglund A, Tronstad L, Wallenius K. A microangiographic of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg Oral Med Oral Pathol 1978;45:17–23.
14. Tsukiboshi M. History and future prospects. In: Tsukiboshi M, editors. Autotransplantation of Teeth, 2ª edn. Tokyo, Japan: Quintessence Publishing Co, Inc; 2001. p. 170–1.