Experimental autogenous tooth transplantation in the dog: a comparison between one- and two-stage surgical techniques

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Nethander G, Skoglund A, Kahnberg K-E. Experimental autogenous tooth transplantation in the dog: a comparison between one- and two-stage surgical techniques. Acta Odontol Scand 2003;61:223–229.

Crucial if tooth transplantation is to succeed is preservation of the vitality of the cells of the periodontium and cementum of the tooth graft. Poor contact between the tissues of the recipient site and the root surface when teeth are transplanted to recipient sites prepared immediately prior to transplantation (one-stage technique) could, after transplantation, result in insufficient nutrition to the cells of the root surface and contribute to necrosis of the cells. To improve nutrition, tooth transplantation to recipient beds left to heal for 14 days was performed (two-stage technique). Clinical trials of tooth transplantation by the two-stage technique resulted in a low incidence of tooth graft loss and root resorption. The different results between these two methods, as well as difficulties in evaluating clinical trials, called for an experimental model to be established. In 5 beagle dogs, fully developed autogenous teeth were transplanted using both one-stage and two-stage surgical techniques. The control teeth were transplanted by the one-stage method to recipient beds prepared immediately before transplantation. The test teeth were transplanted using the two-stage method to recipient beds prepared and left to heal for 5 days prior to transplantation. Four pairs of teeth (1 test and 1 control) were transplanted in each dog. One pair of incisors and one pair of premolars were transplanted in the maxilla and in the mandible. Altogether 20 pairs of teeth were included in the study. One pair of teeth fractured during extraction and was therefore excluded from the study. Two pairs of teeth were lost in the first hours after transplantation. Evaluation of the remaining 17 pairs of teeth was made by routine histological examinations after a 6-month period of healing. The blinded examination failed to show a difference between the two surgical methods in terms of frequency of various types of root resorption. The differences between the results after long-term observation of human teeth transplanted by the one- and two-stage tooth transplantation techniques were not found by this experimental model. ☐ *Dogs; experimental; histology; tooth; transplantation*

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If tooth transplantation is to be successful, it is crucial to preserve the vitality of the cells of the remaining periodontal membrane and root cementum of the transplanted tooth (1, 2). Exarticulated teeth, whether caused by accident or of iatrogenic origin, will have a compromised blood supply due to the rupture of the tissues, including the blood vessels. Immediate replantation of exarticulated teeth is known to have a good prognosis (3), while transplanted teeth show a high prevalence of root resorption after transplantation to recipient beds prepared at the same time as transplantation of the tooth (4–9). One explanation for this difference could be that the shape of the recipient bed of a replanted tooth, as opposed to that of a transplanted tooth, may favour a short diffusion distance from the vascular bed to the root surface cells of the affected tooth. The shape of the recipient bed prepared in the bone to accommodate the root of a tooth transplant will, in most cases, not fit as well as the recipient bed of a replanted tooth.

In order to improve the nutrition and preserve the vitality of the cells of the remaining periodontal membrane and the root cementum, teeth were transplanted using a two-stage surgical method. The teeth were transplanted to the regenerated tissues of recipient beds prepared 14 days prior to transplantation. After transplantation by this method, a low prevalence of tooth graft loss and root resorption of the transplanted teeth was evident (10). On examination, the transplanted teeth showed little loss of periodontal attachment and no difference in bleeding when probed compared to non-transplanted control teeth (11).

Although good clinical results have been reported when teeth are transplanted to recipient beds prepared 14 days prior to actual transplantation (12), the ideal time for the transplantation is not known, i.e. whether it should be performed immediately, as in the one-stage method, or later to a recipient bed containing an already organized granulation tissue. The relationship between the tooth graft and the tissues of the recipient bed as well as the characterization of the recipient bed tissue in terms of inflammation, vascularization, etc., remains to be examined. These questions and the evaluation difficulties encountered in the clinical trials called for an experimental model of the tooth transplantation method.

The aim of this study was to examine, in the dog, by an intra-individual paired experimental study, various pat-

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DOI 10.1080/00016350310004098

terns of root resorption of teeth transplanted to a recipient bed in which the tissue was already under regeneration (the two-stage method). To serve as controls, teeth were transplanted to recipient sites prepared immediately prior to transplantation (the one-stage method).

Materials and methods

A total of 5 adult dogs were used in the study. The 2 male and 3 female beagles were purpose-bred and kept at the Experimental Department of the Faculty of Odontology, Lund University, for a period extending from 2 weeks prior to the experimental start and throughout the entire healing period of 6 months. The dogs were fed a soft diet and water *ad libitum*. Prior to surgery, the teeth were cleaned of plaque and calculus until the marginal gingiva was free of clinical evidence of inflammation.

All surgical interventions were carried out under general anesthesia through intravenous administration of a barbiturate solution (0.1 mg/kg b.w.; Pentothal® sodium, Abbot S.A., Brussels, Belgium). After surgery, the dogs were kept under surveillance until fully recovered from the anesthesia, and fed with liquid food and water ad libitum for 5 days. To prevent infection, all animals received an intramuscular injection of 600,000 IU benzyl-penicillin (Penicillin Procaine Vet®, Novo, Copenhagen, Denmark) once a day, starting the day before the first surgery and extending to 7 days after the second operation. Analgesics were not administered in order to avoid interfering with the normal coagulation process of the blood.

As experimental sites, one of the maxillas and the contralateral part of the mandible were randomly selected on each animal. Control transplantations were carried out on the opposite maxilla and mandible.

At the first operation, the 4 experimental recipient beds were prepared in each dog. A total of approximately 3 ml local anesthesia (Xylocain®-Adrenaline; 20 mg/mL + 12.5 μg/mL, Astra, Södertälje, Sweden) was slowly injected into the mucosa (see below). Mucoperiosteal flaps were raised to expose the buccal surface of the alveolar bone at the maxillary third incisor (I_3), at the maxillary third premolar (P_3), at the mandibular first and second incisors (I_1 and I_2), and at the mandibular third premolar (P_3) regions. These teeth (5 in each dog) were extracted and discharged. The 4 experimental recipient cavities were prepared by removing the buccal cortical bone and approximately half of the cancellous bone of the alveolar process with dental burs during extensive saline irrigation. The size and shape of the cavity were made as standardized as possible and approximately 2 mm wider than the size of the donor tooth estimated by radiographic examination. After cleaning the wound by irrigation of saline the flap was repositioned and kept sutured (Dexon®, Davis & Geck, Gosport, Hampshire, UK) for 5 days.

Prior to the second operation, approximately 10 mL venous blood was collected from each dog. The blood was heparinized (Vacutainer®, heparinized, Venoject Terumu, Belgium) and centrifuged at 7000 rpm for 10 min providing 2500 gravity units (Beckman J6). The supernatant, i.e. the plasma, was extracted and used as an autologous storage medium for the tooth transplant, and kept at room temperature during the period between extraction and insertion.

At the second operation, carried out 5 days after the first, both the experimental and the control tooth transplantations were done with the dogs kept under general anesthesia, see above. A total of approximately 3 mL local anesthesia (Xylocain®-Adrenaline) was injected slowly into the mucosa of both the experimental and the control regions in each animal.

At the experimental sites, the soft tissue covering the marginal entrance to the previously prepared recipient beds was excised. This was done to expose the regenerated tissue of the recipient bed and also to prevent epithelium becoming interposed between the root surface of the transplanted tooth and the recipient bed tissues during insertion of the transplant. At the control sites, extractions of the teeth were done and recipient beds were prepared as described above.

At the donor tooth regions, a total of 1.5 mL of local anesthesia (Xylocain®-Adrenaline) was slowly administered. Prior to extraction of the donor teeth, mucoperiosteal flaps were raised to expose the bone. To accomplish an easy removal of the donor tooth, parts of the cortical bone was carefully removed at the mesial and distal aspects, without trauma to the donor tooth, or the previously prepared experimental recipient beds. The tooth graft was then extracted as gently as possible and transferred to the storage medium. After cleaning by saline irrigation, the flaps of these donor regions were repositioned and sutured (Dexon®).

Subsequently the tooth was transplanted (see below) to its respective recipient bed, both experimental and control, and held in place with sutures (Dexon®) to the mucosa for 5 days. Great care was taken to expose both the experimental and the control teeth to the same amount of trauma.

A total of 40 teeth with fully developed roots were included in the trial. Two of the mandibular incisors in one dog fractured during the extraction and were excluded from the experiment. The remaining 38 teeth were transplanted. The maxillary I_1 was transplanted to the mandibular I_1–I_2 region. The mandibular I_3 was transplanted to the maxillary I_3 region. The maxillary P_1 was transplanted to the maxillary P_3 region, and mandibular P_1 to the mandibular P_3 region.

Ten days after the second operation, under general anesthesia, as described above, the root canals of the transplanted teeth were instrumented with H-files and reamers, using a 0.5% buffered sodium-hypo chlorite solution (Dakin's) for irrigation, and subsequently filled with calcium hydroxide (Calasept®, Scania Dental, Sweden) under aseptic conditions. The cavities were sealed using zinkoxide-eugenol followed by phosphate cement. During the entire follow-up period, the trans-

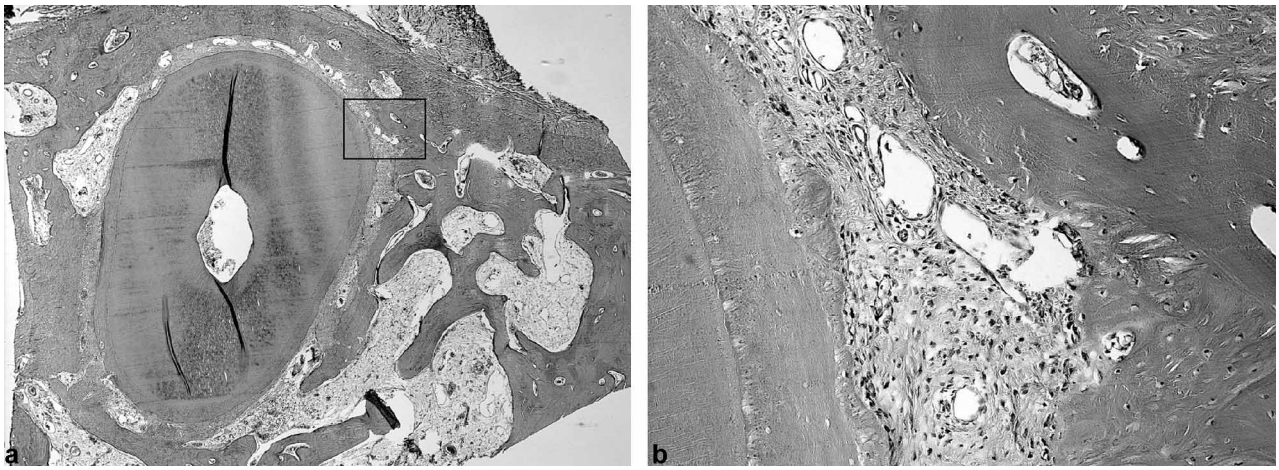

Fig. 1. Root surface conditions categorized as Category 1. The magnified area shows no resorption of the root and no visible inflammatory cells in the vicinity.

planted teeth were treated by cervical application of tetracycline-hydrochloride paste 3 times a week. After a healing period of 6 months, the animals were destroyed by an overdose of Pentothal®. The jaws were removed and fixed in 4% neutral buffered formaldehyde (pH 7.4) and subsequently decalcified in ethylene-diamin-tetra-actetate (EDTA, pH 7.4). Each transplanted tooth with its surrounding tissues was then removed *en bloc*. Finally, the specimens were embedded in paraffin. Sectioning was performed perpendicular to the long axis of the tooth and, at each level, three sections, 5-μm thick, were harvested at regular intervals from the cemento-enamel junction to the apex of the tooth. The mean number of harvested triple sections for incisors was 36 (range 23–45) and for premolars 24 (range 11–29). Finally, the sections were stained with hematoxylin/eosin. Analysis of the respective section was performed by light microscopy (Zeiss, Germany) at 31.25× and 125× magnifications.

The condition of the transplanted tooth and the adjacent tissues was examined using the categories summarized and shown in Figs 1–6. The diagnosis of cementum-like tissue was established when an area was morphologically similar, contained no cells, and stained like the cementum of the transplanted tooth. The diagnosis bone matrix was established when an area was morphologically similar to fibrous bone, stained like the adjacent bone and contained cells.

The material was examined in two ways. First, triple sections of all experimental teeth and their respective control were arranged as pairs and examined blinded in consecutive order starting coronal at the cemento-enamel junction. Pairs were ranked between themselves to

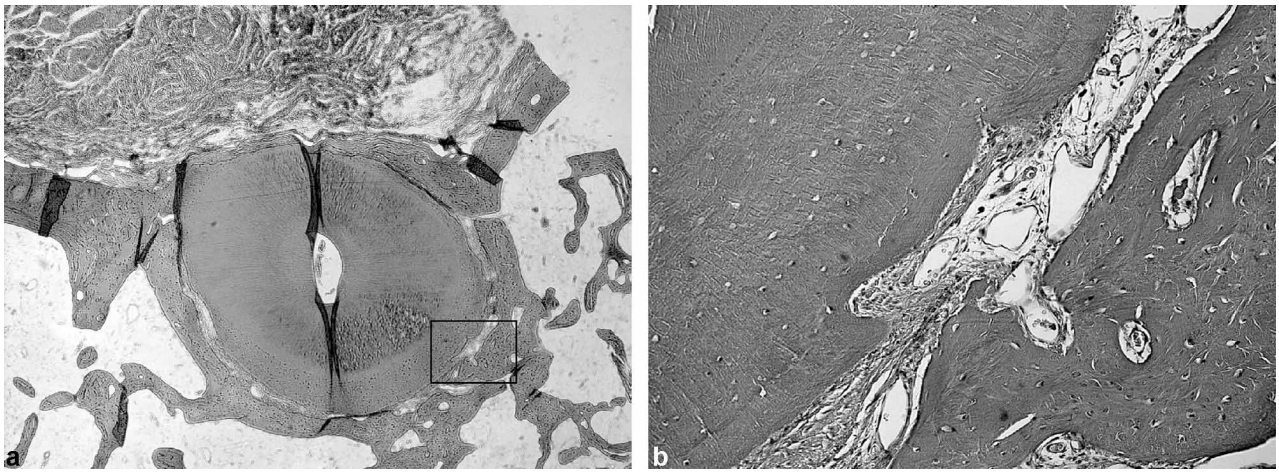

Fig. 2. Root surface conditions categorized as Category 2. The magnified detail shows root resorption only of the cementum with cementum-like tissue on the inner surface of the resorption cavities. No visible inflammatory cells in the vicinity.

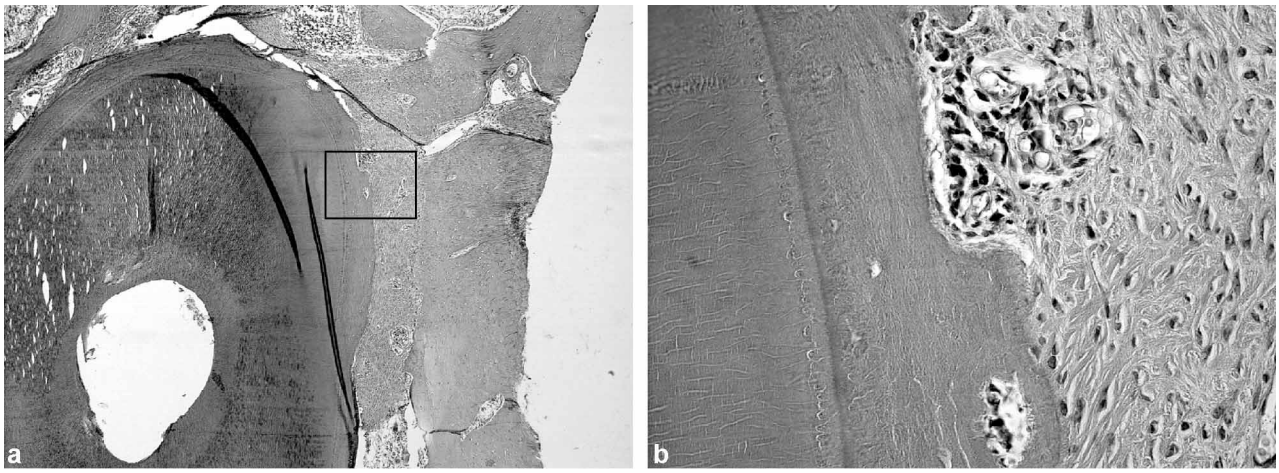

Fig. 3. Root surface conditions categorized as Category 3. The magnified detail shows two areas with presence of root resorption of the cementum without formation of cementum-like tissue and inflammatory cells in the vicinity.

determine the severity of pathological conditions in terms of root resorption, inflammation, soft tissue down-growth, etc. (according to the categories as shown in Figs 1–6, taken all together around the whole periphery of the root surface).

Secondly, in order to examine the extent of the categorized conditions, the root surface and the periodontal tissues were examined at 12 different locations evenly distributed along the entire periphery of the root surface. The locations were defined as the point where the line from the centre of the root canal cavity intersected with the surface of the root. The examination was carried out as a blind study and all triple sections of both the experimental and the control were examined. For each transplanted tooth the total number of examined sites divided the number of sites with a certain condition. The relative prevalence of the defined category was compared with the corresponding relative prevalence of the corresponding control tooth and the difference between the 17 pairs of test and control teeth was tested statistically.

Statistical analysis was performed by use of both the Wilcoxon signed-rank test and Student's *t* test for paired samples using 1% as significance level.

Results

In the first 24 h after the second surgery, 2 pairs of teeth were lost due to fixation failures. After this, no more loss of teeth was recorded and the remaining postoperative period was uneventful. No macroscopic signs of ailments were observed at either test or control sites. After the

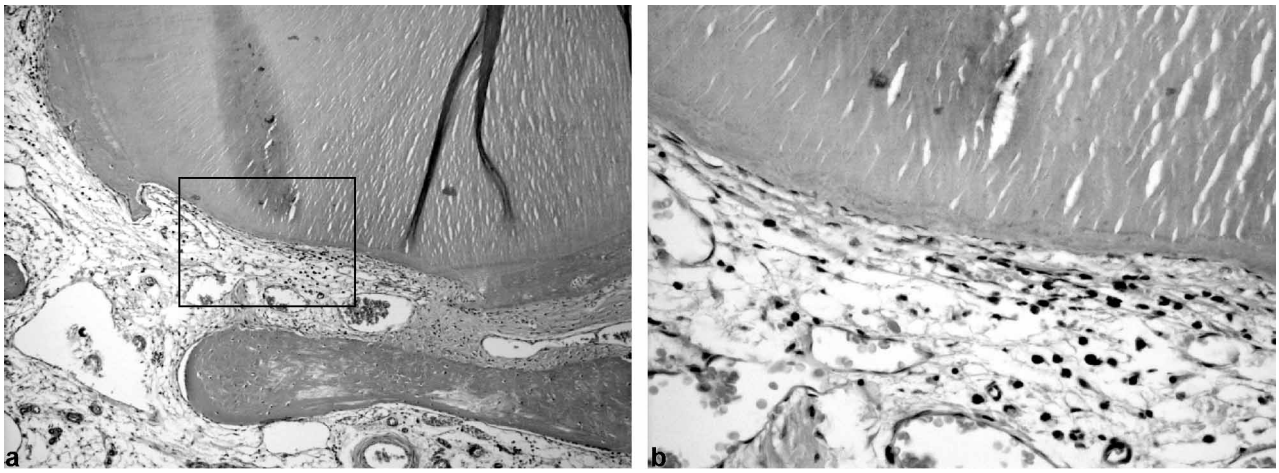

Fig. 4. Root surface conditions categorized as Category 4. The magnified detail shows resorption of the dentine with cementum-like tissue formed on the inner surface of the resorption cavities.

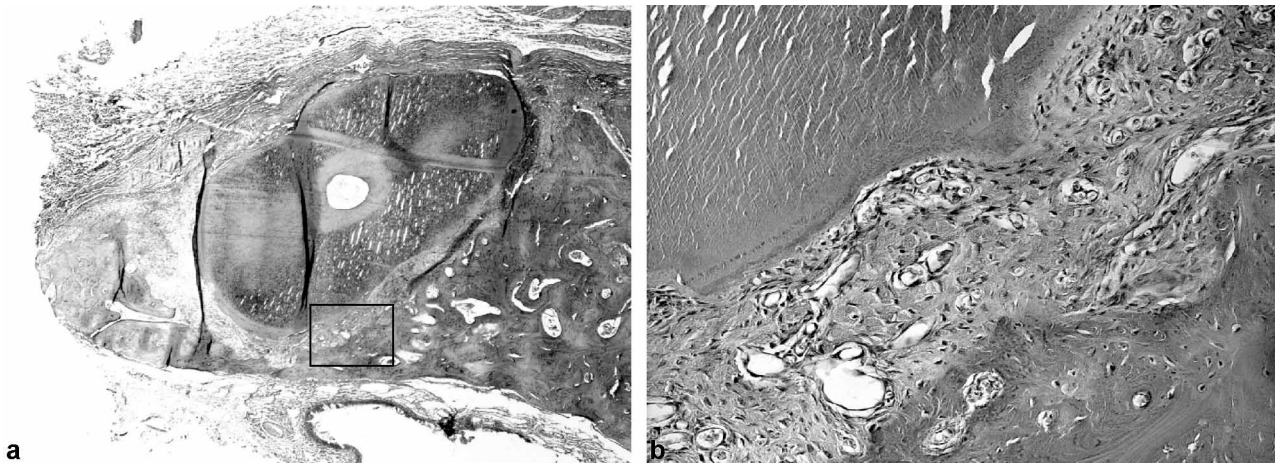

Fig. 5. Root surface conditions categorized as Category 5. The magnified area shows resorption of the dentine with inflammatory cells in the vicinity of the resorption area.

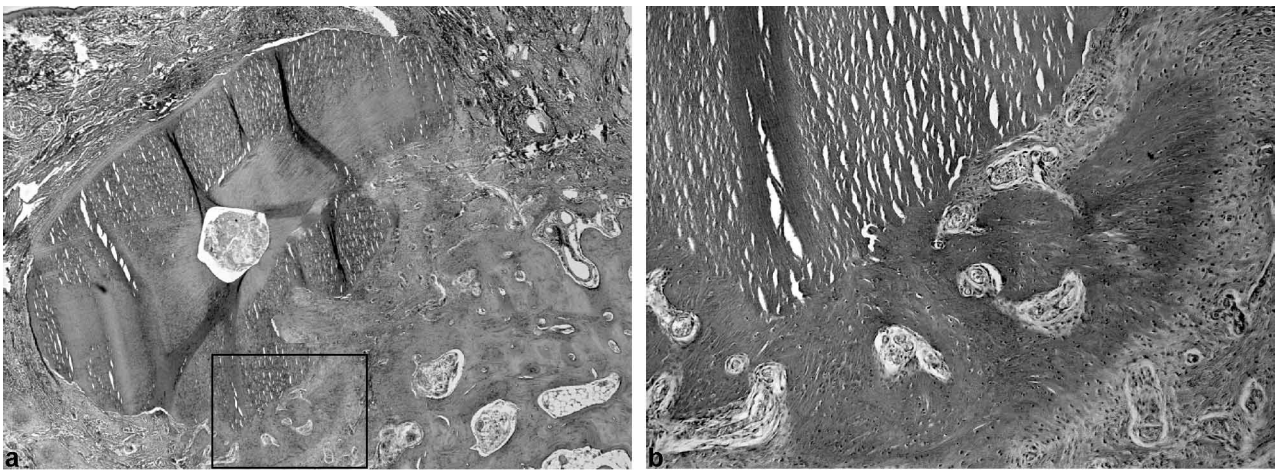

Fig. 6. Root surface conditions categorized as Category 6. Ankylosis. The magnified detail from an area with bone matrix-like tissue in contact with the dentine of the root.

healing period of 6 months, 34 teeth (8 pairs of incisors and 9 pairs of premolars) were harvested and subsequently analysed histologically. Histological examination of the transplanted teeth showed that there was no difference between test and control with regard to the estimated pathology along the whole periphery of the root surface (paired analysis: 56, 91; $n = 17$; $P > 0.05$).

A total of 10,802 sites (5394 control and 5408 test) were examined and the condition of the root surface at each site was categorized according to the criteria.

When comparing the results statistically, no significant differences between test and control were found for any of the categories describing root surface conditions (Table 1).

The material was divided into subgroups of incisors and premolars in order to identify possible differences. Again

Table 1. Results of the histological examination of test and control tooth transplants. The differences between the 17 pairs of test and control teeth were tested using 1% as significant level. Prevalence of root resorption (%)

		Test				Control				
No.	Category	Mean	*s*	Min	Max	Mean	*s*	Min	Max	*t* test
17	1	52.5	22.3	16.3	84.0	51.0	25.9	1.8	91.8	NS
17	2	10.9	14.9	0.0	59.7	7.3	7.7	0.0	26.9	NS
17	3	8.5	6.2	0.8	22.1	7.5	5.2	0.4	17.8	NS
17	4	13.7	10.1	3.2	42.4	14.4	14.4	0.0	60.8	NS
17	5	13.9	19.3	0.0	68.0	18.9	24.5	0.0	78.2	NS
17	6	0.5	1.1	0.0	3.9	0.7	2.2	0.0	8.7	NS
Sum			100.1				99.9			

NS = not significant. *s* = standard deviation.

no significant difference was found between test and control for any of the 6 categories of histological root surface conditions. A further analysis of any difference between test incisors and test premolars showed that there were no significant differences for any of the 6 root surface categories; the material was therefore analysed as consisting of 1 sample of 17 paired test and control tooth transplantations.

To evaluate whether the character of the tissue in the recipient bed had any influence on the result, the whole material was divided into two groups—one consisting of sites situated on the root surface facing the bone, the other containing sites on the root surface facing the soft tissue (gingiva or oral mucosa). When tested, no difference was found between these surfaces of the test teeth in the categorized conditions. For the root surfaces situated towards the bone there was no difference between test and control teeth.

To examine the occurrence of root resorption, frequently present at the marginal root surface, the whole material was again divided into a cervical and an apical part. The cervical part was defined as the coronal third of the sites counted from the cemento-enamel junction. Control teeth showed less resorption of the cervical than the apical cementum. A further subdivision of the cervical and apical material into bone- and non-bone-facing surfaces revealed that, compared to the cervical part, the bone facing apical surface of control teeth had more resorption of the cementum. Test teeth showed no difference in this respect.

Discussion

The two-stage technique, described earlier as a clinical method (10), implies that the recipient bed is prepared surgically prior to transplantation and allowed to heal for 14 days. The period between preparation of the recipient bed and transplantation of the tooth allows in-growth and early maturation of a granulation tissue into the wound. In contrast, in the one-stage technique the recipient bed is prepared and the transplantation is performed at the same surgical intervention. When comparing the overall results of the paired sections of test and control teeth in the present study, no differences were detected between the two transplantation techniques with regard to root resorption.

The teeth were transplanted to recipient beds prepared as grooves into the alveolar bone. Depending on whether the transplantation was performed according to the one-stage method or the two-stage method, one part of the root surface was facing either the repositioned periosteum (1-stage method) or the regenerated tissues of the flap (2-stage method). The contralateral side of the root was facing either the surface of the freshly created bone wound (1-stage method) or the regenerated tissue over 5 days (2-stage method). The major difference in the nutritional conditions due to the contact relationships between the root surface of the test and the control host sites would therefore be expected to be on the side of the root facing the bone. No significant difference was found, however. The reason could be that there was no difference in the contact relationship, and if there was it had no influence on the results.

One explanation for the absence of difference between test and control teeth could be that the experimental recipient bed-healing period of 5 days may have been too short for regeneration and vascularization of the bone wound in the dog compared to the healing period of 14 days used in the clinical 2-stage tooth transplantation trials in man (10). It has been shown that 3 days after extraction the alveolar sockets in dogs were filled with an organizing blood clot and after 7 days the whole alveolus was filled with a young vascular granulation tissue (13). Thus alveolar wounds within the jaws of dogs heal approximately twice as quickly as those in man (14). In the present examination we found no difference for any of the categories between test and control teeth as well as on the surfaces of test teeth situated towards the regenerated tissues of the alveolar bone and the surfaces towards the buccal mucosa. The various conditions of the recipient bed evidently had no influence on the results.

Another explanation could be the fixation method that was used. The transplanted teeth were not rigidly fixed to the surrounding mucosa. Non-rigid fixation of experimentally replanted and transplanted teeth has been shown to result in less incidence of replacement resorption compared to controls with rigid fixation. The results of the present study were in accordance with the results of these experiments (15, 16).

Trauma of the root surface caused root resorption and impaired healing of replanted teeth (18, 19). The force exerted to both the experimental and the control teeth during the extraction far exceeded the force normally used during the two-stage transplantation in clinical practise. This might be another factor that could explain the absence of significant differences in the incidence of root resorption between test and control teeth.

The results of this experimental study, however, do not explain the difference between the results after long-term observation of clinical tooth transplantations by the one-stage (17) and the two-stage transplantation techniques (6).

Conclusion

This experimental study with autogenous free transplantation of teeth to recipient beds in the dog showed no difference in the resorption of the roots compared to teeth transplanted to recipient sites prepared on the same occasion.

Acknowledgements.—This study was supported by grants from the county of Norrbotten.

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Received for publication 5 February 2003
Accepted 19 May 2003