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Histological evaluation of periodontal regeneration in autogenous tooth transplantation in the dog: a comparison between one and two-stage surgical techniques, a pilot study

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Abstract – *Objective*: To compare the periodontal regeneration, associated with autogenous tooth transplantation in dogs, using either one- or two-stage surgical techniques. *Methods*: The study group 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 group 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. Clinical examinations were done every week and the animals were euthanized 9 weeks later. Subsequently, decalcified sections were prepared for routine and imunohistochemical histological evaluation. Periodontal healing was evaluated by undertaking histomorphometric analysis and analyzed using the Mann–Whitney test ($P = 0.05$). *Results*: All the transplanted teeth in both groups survived. No statistically significant difference was found in the complete healing between the treatment groups ($P = 0.053$). There was no difference between the occurrence of inflammatory root resorption ($P = 0.135$) and replacement root resorption ($P = 0.081$). *Conclusions*: This study demonstrated that there was no difference between the two surgical techniques in terms of preventing root resorption 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 (6–8). Immediate replantation of exarticulated teeth is known to have a good prognosis, while transplanted teeth to recipient beds prepared at the same time, show a high prevalence of root resorption (4). To improve nutrition and preserve cell activity in these tissues Nethander et al. (6) and Katayama et al. (7) suggested that teeth should be transplanted to the sockets with regenerative tissues, which may reduce the root resorption following transplantation.

The aim of this study was to examine in dogs, by an experimental study, various patterns of root resorption in teeth transplanted to a recipient bed, in which the tissue was under regeneration during 7 days (the two-stage method). To serve as control, teeth were transplanted to recipient beds prepared immediately before transplantation (the one-stage method).

Materials and methods

Animal preparation

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, and maintained in the EZN during the experimental periodand were observed daily by the doctor and technicians of the EZN.

Six non-carious and periodontally sound mature incisors and six non-carious and periodontally sound mature premolars from three male Beagle (age 5 months;

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

Experimental procedures

All selected teeth, incisors (n = 6) and premolars (n = 6) 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.

Treatment groups

The teeth were extracted as atraumatically as possible. The roots and sockets were gently rinsed with 5 ml each of normal saline solution immediately before the following treatment protocols:

Group A (n = 6): The teeth are transplanted to the recipient beds prepared immediately before.

Group B (n = 6): The teeth are transplanted to a recipient bed in which healing tissue was under regeneration during 7 days.

Group C (n = 3): Negative control. No treatment was performed on the teeth.

After transplantation the teeth are splinted with vycril 3/0 (9, 10). 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 buprenorfine once daily.

Specimen processing

The animals were euthanized at 9 weeks after transplantation. The dogs were deeply anesthetized 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% paraformoldehyde in phosphate buffer (pH 7.4). Jaw blocks containing the transplanted teeth were ressected and fixed in the same fixative, decalcified in 50% formic acid and 20% sodium citrate and embedded in paraffin. The paraffin embedded blocks were subsequently sectioned longitudinally in a buco-lingual direction at a thickness of 5 μm, corresponding to the root canal. The section that was technically the best was stained with hematoxylin and eosin (11). The other sections stained for immunohistochemistry for keratin MNF116 (12).

Histomorphometric evaluation

Histomorphometric 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 40× magnification. The histology of the periodontium was classified by using the classification as follows: percentage of complete healing; percentage of superficial root resorption; percentage of inflammatory root resorption; percentage of replacement root resorption.

Statistical analysis

The percentage of each histologic classification for each root and each treatment group was calculated. The differences among the groups were statistically compared by using the Mann–Whitney test. The significance level was set at 5%.

Results

The dogs tolerated the operative procedures well, and their behavior did not change. No teeth were lost and the number of transplanted teeth was 12. The results of the histomorphometric analysis for each treatment group are presented in Table 1. The periodontal width observed was adequate in both groups (Fig. 1). In teeth transplanted to the recipient beds prepared immediately before (group A), the mean occurrence of complete healing was (81%), compared with group B, 94% (teeth transplanted to a recipient bed in which the tissue was under regeneration during 7 days). But there was no significant difference (P = 0.050). The mean occurrence of replacement resorption in group A (9%), compared with group B (3%), was no significantly different (P = 0.202). The mean occurrence of superficial root resorption was high in group A (8%), compared with group B (3%), but there was no significant difference (P = 0.084). In roots with inflammatory root resorption, numerous odontoclasts were observed (Fig. 2). The mean occurrence of inflammatory root resorption was

Table 1. Periodontal healing pattern for different groups and statistical comparison by using the Mann–Whitney test (P = 0.05)

	Groups	
Classification	A (n = 6)	B (n = 6)
Complete healing (P = 0.050)	81 ± 13	94 ± 6
Inflammatory resorption (P = 0.021),	11 ± 6	3 ± 4
Replacement resorption (P = 0.202)	9 ± 10	3 ± 4
Superficial resorption (P = 0.084)	8 ± 4	3 ± 5

Values are given as mean (%) ±SD.
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.

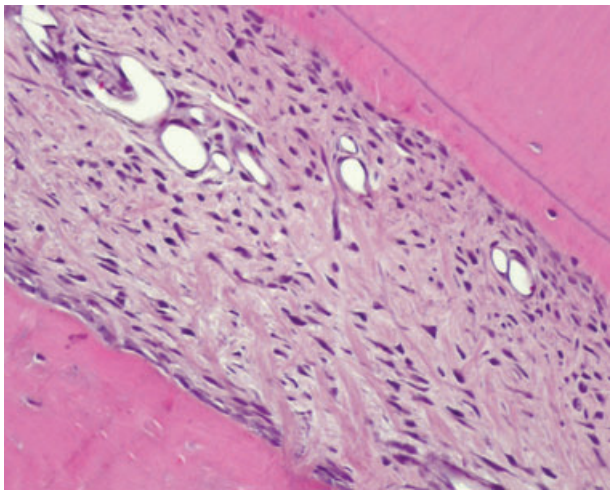

Fig. 1. Histological photograph of the experimental root surface with complete healing (Dog 1, teeth 21 – Group A). (H&E ×200).

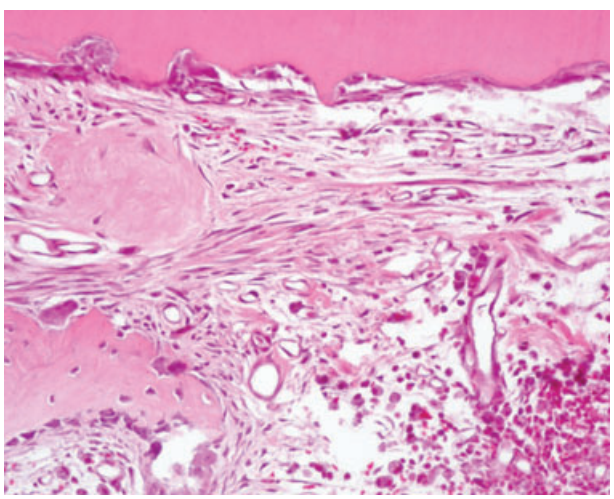

Fig. 2. Histological photograph of the experimental root surface with root resorption (Dog 3, teeth 31 – Group A). (H&E ×200).

higher with technique A (11%), than with technique B (3%), with significant difference ($P = 0.021$). In the histological observations at 9 weeks after transplantation, the space of the periodontal ligament was reestablished and we can see by MNF116 the epithelial cells of Malassez (Fig. 3).

Discussion

Studies on the complications of root resorption after transplantation teeth have been based on models in monkeys, rats and dogs (5, 6, 13, 14). Transplantation to a recipient bed in which the tissue was under regeneration described by Nethander et al. (6) implies that the recipient bed is prepared surgically prior to transplantation and allowed to heal for 5 days. In this study, we

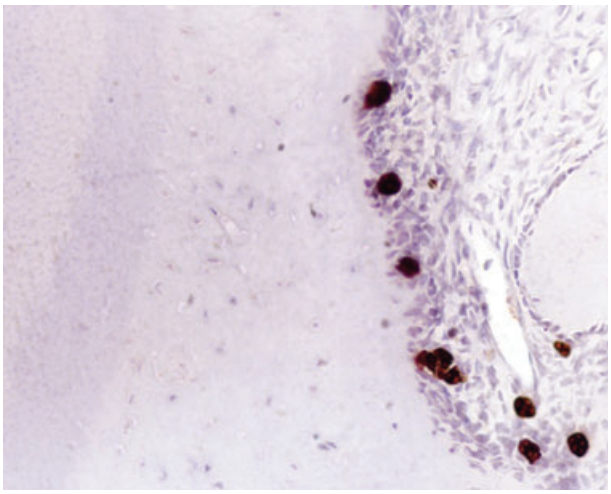

Fig. 3. Histological photograph of the experimental root surface cells of Malassez (arrow) (Dog 3, teeth 31 – Group A). (MNF116 ×200).

used transplant teeth in dogs, after 7 days of regeneration. 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. In the one stage technique, the recipient bed is prepared and the transplantation is performed in the same surgical intervention.

The mean occurrence of replacement resorption was high in teeth transplanted to the recipient beds prepared immediately before (9%), compared with teeth transplanted to a recipient bed in which the tissue was under regeneration during 7 days (3%), with no significant difference ($P = 0.202$). All teeth presented the occurrence of superficial root resorption with 8% in group A, compared with 3% in group B, but there was no significant difference ($P = 0.084$). The mean occurrence of inflammatory resorption was high in teeth transplanted to the recipient beds prepared immediately before (11%), compared with teeth transplanted to a recipient bed in which the tissue was under regeneration (3%), with significant difference ($P = 0.021$).

The complete healing is superior in the two-stage technique (94%), compared with one-stage (81%), but there was no significant difference ($P = 0.050$). This may be explained by the fact that in technique B, the alveolus was filled with young vascular granulation tissues and had a good nutritional condition due to the contact relationships between the root surface and this tissue (15).

Katayama et al. (7) demonstrated in dogs that transplanted proliferating tissues promoted reconstruction of a normal periodontium in injured teeth. The effect is probably due to the expression of fibroblast growth factor (FGF) that is a potent mitogen for mesenchymal cells, including bone and PDL cells, and also stimulate osteogenic expression of stromal bone marrow cells.

They demonstrated that the mRNA expression of FGF and alkaline phosphatase (ALP) increased in proliferating tissue. They concluded that this tissue may promote the regeneration of PDL tissue and prevent ankilosis and root resorption following the transplantation of teeth.

Conclusion

This experimental pilot study indicated that there were no differences in the wound healing process between one-stage and two-stage techniques of transplantation and that periodontal ligament tissue contains osteoprogenitor cells that have the ability to participate in alveolar bone regeneration.

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