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Effects of orthodontic load on the periodontium of autogenously transplanted teeth in beagle dogs*

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Abstract: Objective: To observe the periodontal healing of autogenously transplanted teeth loaded orthodontically after autotransplantation in Beagle dogs. Methods: Forty-eight teeth were autogenously transplanted, 24 of which were loaded postoperatively with orthodontic force at different time points and for different durations. Periodontal healing was evaluated by probing pocket depth (PPD), the expression of relevant proteins, and histomorphometric analyses. Results: The dental pockets of loaded and non-loaded teeth were both much deeper after the first postoperative week than before transplantation (P<0.05). Later, the PPD, which was measured after postoperative weeks 1, 3, 5, 9 and 13, gradually became shallow. The expressions of alkaline phosphatase (ALP) and basic fibroblast growth factor (bFGF) were higher in loaded teeth than in non-loaded teeth (P<0.05), and in groups subjected to two weeks duration of loading than in other groups at the same load time point (P<0.05). For the same load duration, the expressions of ALP and bFGF in teeth loaded after postoperative week 4 were higher than those of other treatments (P<0.05). According to histomorphometric analyses, an orthodontic force on transplanted teeth applied after postoperative weeks 4 or 8 for two weeks duration should be favorable for periodontal healing. Conclusions: It is advisable to apply an appropriate magnitude of force on autotransplanted teeth, such as orthodontic force, at appropriate time points and for a suitable duration, to achieve the optimal clinical prognosis following autogenous tooth transplantation. These results may serve as a basis for subsequent studies in humans so as to make clinical improvements.

Key words: Autogenous tooth transplantation, Periodontium healing, Orthodontic load
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1 Introduction

Autogenous tooth transplantation (ATT) is an alternative method for preserving teeth which involves extracting intact teeth surgically to replace missing or non-functional teeth in the same individual. Fong (1953) reported the first successful case in which an immature mandibular third molar was extracted to replace a lost first molar. Studies of ATT then were followed in various forms, such as clinical observation and laboratory experiments. Tooth survival rates ranged from 74% to 100% (Andreasen *et al.*, 1990a; 1990b; Czochrowska *et al.*, 2002; Tsukiboshi, 2002; Jonsson and Sigurdsson, 2004; Mensink and van Merkesteyn, 2010). However, there were still some unsatisfactory complications, such as root resorption or ankylosis (Andreasen, 1980a; 1980b; 1981a; 1981b; 1981c; Andreasen and Kristerson, 1981; Schwartz *et al.*, 1985; Andreasen *et al.*, 1990a; 1990b; 1990c; 1990d; 1995; Terheyden *et al.*, 1995). The success of autotransplantation involved favorable healing of the periodontium. This depended upon

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numerous factors, such as the developmental stage and the form of the donor tooth, the exposure time in air, the storage conditions after removal, the attachment of the periodontal ligament to the extracted tooth, the site and shape of the recipient socket, the method and duration of tooth fixture after transplantation, and the endodontic treatment time of the transplanted tooth (Blomlof *et al.*, 1983; Andreasen *et al.*, 1990b; Schwartz *et al.*, 2002; Bauss *et al.*, 2008). Increasingly, research shows that the application of mechanical stimuli to the donor teeth is good for healing of the periodontium after autotransplantation. Mine *et al.* (2005) noted that proper occlusal force facilitates the periodontal healing of transplanted teeth. Furthermore, they found that it is necessary to ligature the autotransplanted teeth and the adjacent teeth together after surgery to stabilize the transplanted teeth in the socket. Nevertheless, it is still unclear how the periodontium reacts to mechanical stimuli at different initial time points and for different durations. Thus, further scientific research is needed. This study was designed to determine the appropriate time points of application and the duration of orthodontic loads upon autotransplanted teeth by assessment of periodontal health status, the expression of relevant proteins and histomorphometric analyses.

2 Materials and methods

2.1 Animals

Twelve 12-month-old male beagle dogs (10–12 kg) were supplied by the Experimental Animal Center of Sichuan University, China. All dogs had intact dentition with healthy periodontal tissue. They were fed on time (twice daily) with a quantitative diet in semi-liquid form and given fresh water ad libitum. The protocols of animal research were approved by the Bioethics Committee of Sichuan University, China.

2.2 Experimental animal model

According to different initial time points of application of orthodontic load (after postoperative weeks 2, 4, 8, and 12), 12 dogs were divided randomly into four groups (three in each group) marked A, B, C, and D, respectively. In the same way, on the basis of different load durations (1, 2, and 4 weeks), three dogs in each group were randomly numbered 1, 2, and 4, respectively. Bicuspids from the same position on each side of the mouth were chosen and exchanged as the donor teeth contralaterally. Those on the left were designated as the loaded group (T_1) and those on the right as the non-loaded group (T_0).

2.3 Periodontal examination

A periodontal probe (scale, 1 mm; diameter of pointed end, 0.5 mm) was used to measure the probing pocket depth (PPD) before surgery. Six sites (mesial, medial, and distal sites in the buccal and lingual sides of each tooth examined) were measured using the probe parallel to the long axis of the tooth. The average of the six values was recorded as the PPD. The same method was performed to measure the PPD of the teeth after postoperative weeks 1, 3, 5, 9, and 13. The PPD data were analyzed using a matched *t*-test with statistic software SPSS 18.0.

2.4 Surgical procedures

To ensure the best fitting socket to facilitate the embedding of the transplanted teeth, the corresponding contralateral bicuspids were selected for transplantation. All surgical procedures were performed under general anesthesia by intravenous injection using 0.02 g/ml ketamine and 0.03 g/ml pentobarbital sodium (1 mg/kg body weight), and local anesthesia using 0.4–0.6 ml lidocaine (0.002 g/ml; all purchased from North China Pharmaceutical Group Corp., China). After anesthesia, the periodontal flap was elevated and the bicuspids extracted bilaterally. The extracted teeth were conserved in sterile saline for less than 30 min. According to the size and form of the root of the donor teeth, a surgical drilling unit (Satelec, Bordeaux, France) with irrigation of saline was used to recondition the recipient tooth sockets so as to make an approximate 1 mm gap between the tooth socket wall and the donor tooth. The transplanted teeth were fixed by 8-ligation and orthodontic bonder to the neighboring teeth. The dogs received an intramuscular injection of penicillin (400 000 IU/kg; North China Pharmaceutical Group Corp., Shijiazhuang, China) and the surgical area was washed with chlorhexidine mouthwash for three postoperative days. One week later, root canal therapy was carried out on the transplanted teeth, and the fixtures were removed simultaneously.

2.5 Orthodontic loading

The transplanted bicuspids on the left were loaded at 2, 4, 8 and 12 weeks after surgery. For stabilization of the load installation, grooves (depth 0.2 mm) were prepared with a low-speed handpiece on the neck of the replanted and anchored teeth. Ni-Ti closed-coil springs (Aosu Medical Devicement Group Corp., Hangzhou, China) were applied. The force applied was measured using an orthodontic dynamometer (Minnesota Mining and Manufacturing, St. Paul, USA) to ensure that the magnitude of the load was always 85 g (Fig. 1). The springs were fixed with ligature wire (diameter 0.2 mm) between them (Fig. 2). The duration of application of the load was 1, 2 or 4 weeks according to the experimental model. The animals were sacrificed after the orthodontic load was removed, and then the teeth and their surrounding periodontal tissues were removed as specimens.

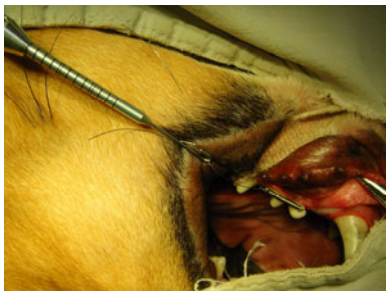

Fig. 1 Spring force measured by orthodontic dynamometer (the load magnitude is 85 g)

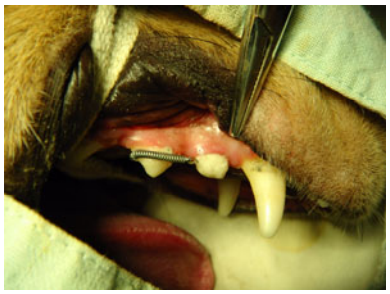

Fig. 2 Ni-Ti closed-coil spring fixed with ligature wire between the replanted and anchorage teeth

2.6 Analysis of expression of alkaline phosphatase (ALP) and basic fibroblast growth factor (bFGF)

The specimens were frozen in liquid nitrogen until RNA extraction. Total RNA was extracted to examine the mRNA expressions of ALP and bFGF using RNAiso™ Plus (TaKaRa Bio Inc., Japan) according to the manufacturer's protocol. RNA was reverse transcribed to synthesize first-strand cDNA with an ExScript RT Reagent kit (TaKaRa Bio Inc., Japan). The real-time quantitative polymerase chain reaction (PCR) was performed using SYBR® PrimeScript™ RT-PCR Kit II (TaKaRa Bio Inc.), running on an Applied Biosystem7300 Real-Time PCR System (PerkinElmer, USA). A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer was used as an internal control. The primer sequences used are shown in Table 1.

Table 1 Primer sequences for real-time PCR

Gene	Primer sequence
ALP	F: 5′-CCCTCTCCAAGACATACAACACC-3′
	R: 5′-GTGACCTCGTTTCCCTGAGTC-3′
bFGF	F: 5′-CGTTGTGTCCATCAAAGGAG-3′
	R: 5′-GTGCCACATACCAACTGGAGTA-3′
GAPDH	F: 5′-GTGATGCTGGTGCTGAGTATGT-3′
	R: 5′-AGAAGGAGCAGAGATGATGACC-3′

F: forward; R: reverse

The expressions of ALP and bFGF were evaluated by ΔC_T. The values of ΔC_T were analyzed by analysis of variance and *t*-tests. The results were considered statistically significant at $P<0.05$.

2.7 Histomorphometric evaluation

The fixed specimens were decalcified in 10% ethylinediaminetetraacetic acid before dehydration and embedding in paraffin. They were sectioned into 5-μm slices, which were stained with Masson stain.

After microscopic observation, all slices were photographed. The resulting images were processed by computer software (Image-Pro Plus 4.5 and Adobe Photoshop 7.0) to calculate the root resorption rate (%) according to (Goldie and King, 1984)

$$\text{Root resorption rate}=\frac{\text{Root resorption area}}{\text{Root total area}}\times 100\%.$$

The root resorption rate data were analyzed using a 3×4 factorial experimental design model (Table 2), using matched *t*-tests, variance analysis, and the least significant difference (LSD) test with statistical software SPSS 18.0. The results were considered statistically significant at $P<0.05$.

Table 2 3×4 factorial experimental design model

Duration	Postoperative time point			
	Week 2	Week 4	Week 8	Week 12
1 week	X_1Y_1	X_2Y_1	X_3Y_1	X_4Y_1
2 weeks	X_1Y_2	X_2Y_2	X_3Y_2	X_4Y_2
4 weeks	X_1Y_3	X_2Y_3	X_3Y_3	X_4Y_3

X: postoperative time point to load on the autotransplanted teeth; *Y*: duration of load on the autotransplanted teeth

3 Results

3.1 PPD examination

Neither loading nor non-loading of replanted teeth produced mobility or dislocation. However, the dental pockets following either treatment were much deeper after postoperative week 1 than pre-transplantation ($P<0.05$) (Fig. 3a). As time went on, the PPD gradually became shallow, as shown by measurements after postoperative weeks 1, 3, 5, 9, and 13 (Fig. 3b). There were no significance differences between the PPDs of loaded and non-loaded teeth at corresponding time points ($P>0.05$).

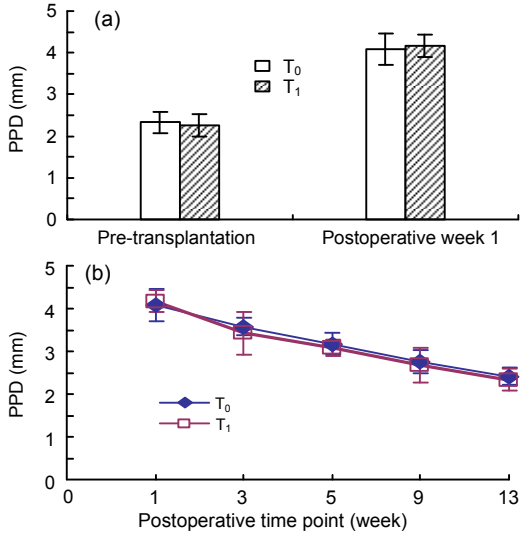

Fig. 3 PPDs of autotransplanted teeth in non-loaded group (T_0) and loaded group (T_1)
(a) Pre-transplantation and after postoperative week 1; (b) After postoperative weeks 1, 3, 5, 9 and 13. Data are expressed as mean±standard deviation (SD), n=6

3.2 mRNA expression levels of ALP and bFGF

ALP is an enzyme secreted by osteoblasts during the cell differentiation phase, and its mRNA expression level is consistent with the process of bone formation. bFGF is widely distributed in fibroblasts, vascular endothelial cells, osteoblasts, undifferentiated mesenchymal cells, and extracellular matrix of the periodontal tissue (Gao *et al.*, 1996).

The mRNA expression level of bFGF is associated with vascular changes, proliferation and differentiation of fibroblasts and osteoblasts in the process of tooth movement. Thus, the effect of the orthodontic load on the periodontium of autotransplanted teeth can be evaluated by the expression levels of ALP and bFGF.

The mRNA expression levels of ALP and bFGF showed consistency (Fig. 4). On the whole, the expressions of both ALP and bFGF were higher in the T_1 than in the T_0 groups ($P<0.05$). The expressions of the two target proteins in groups with two weeks duration of loading were higher than those of other groups at the same load time point ($P<0.05$). Furthermore, with the same load duration, the expressions of ALP and bFGF in teeth loaded at postoperative week 4, were higher than those of other groups ($P<0.05$).

Masson-stained slices showed different degrees of periodontium remodeling at various time points and after different durations of loading (Fig. 5).

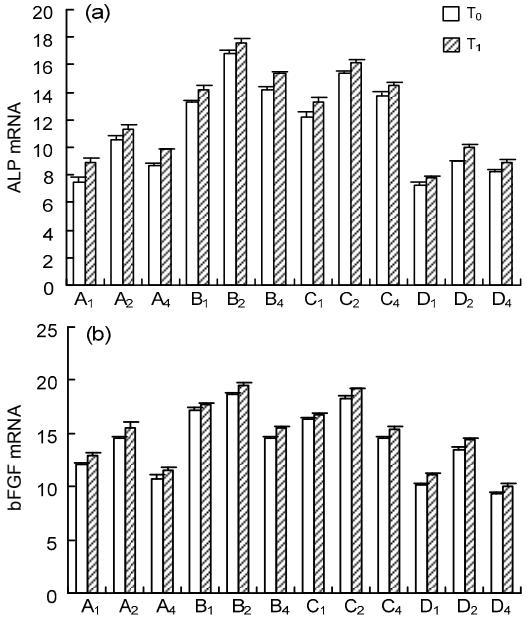

Fig. 4 mRNA expression levels of ALP (a) and bFGF (b) of autotransplanted teeth in non-loaded group (T_0) and loaded group (T_1)
Data are expressed as mean±SD, n=3

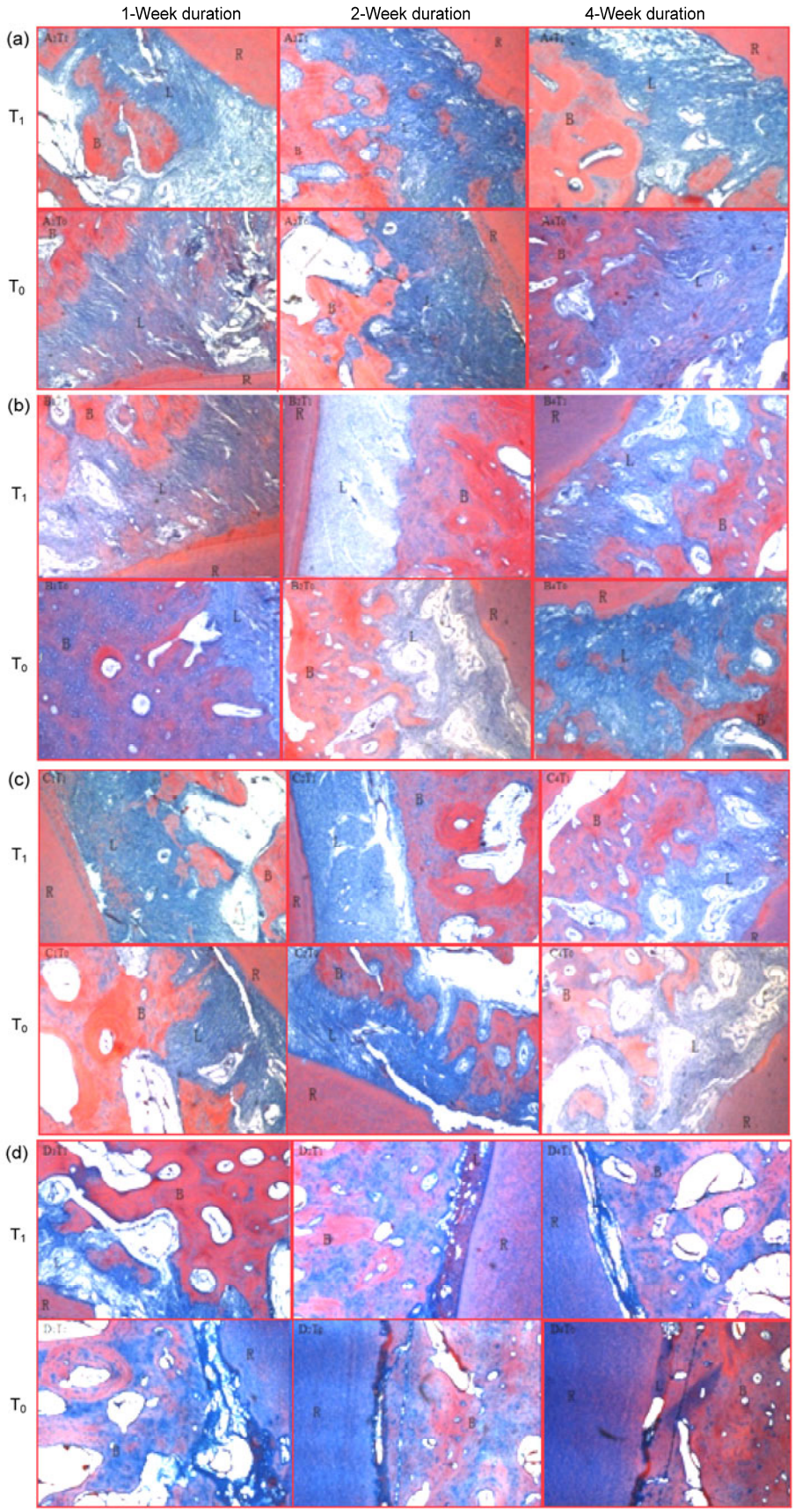

Fig. 5 Masson-stained images of autotransplanated teeth after postoperative week 2 (a), week 4 (b), week 8 (c), and week 12 (d) with different durations of loading treatments

In (a), 1-week durations in T_0 and T_1 groups are named as A_1T_0 and A_1T_1, respectively. In (b), 1-, 2-, and 4-week durations in T_1 group are named as B_1T_1, B_2T_1, and B_4T_1, respectively. The same rule for naming is applied for other images in (a)–(d). T_1: loaded group; T_0: non-loaded group; B: bone; R: root; L: periodontal ligament. Magnification 40×

The arrangement of the periodontal ligament fibers of loaded teeth was more organized than that of non-loaded teeth. Compared with non-loaded groups, fewer absorptive lacunae could be seen on the surface of root and alveolar bone in loaded groups. More favorable periodontal restoration features can be seen in B_2T_1 (Fig. 5b) and C_2T_1 (Fig. 5c), including the width of periodontal ligaments, fiber arrangement, root resorption, and the state of alveolar bone. The periodontal ligaments were thin in DT_1 and DT_0 (Fig. 5d), and some parts even had disappeared (D_2T_0 and D_4T_0), implying a tendency towards ankylosis.

In autotransplanted teeth, the mean root resorption rate was (7.6±0.2)% in non-loaded groups and (7.5±0.2)% in loaded groups (Table 3). There was a significant difference between T_0 and T_1.

According to analysis of variance of the 3×4 factorial experimental design model, there were no statistically significant differences among the *X* or *Y* groups. However, the interaction between the two factors ($X\times Y$) was significant (Table 4).

On the basis of the LSD test, there was a statistically significant difference between X_2Y_2 and the other groups (Table 5).

Table 3 Measurement of the root resorption rate of teeth in non-loaded group (T_0) and loaded group (T_1) (n=24)

Group	Root resorption rate (%)	t	P
T_0	7.6±0.2	5.326	<0.05
T_1	7.5±0.2		

Table 4 Analysis of variance of the 3×4 factorial experimental design model

Factor	F	P
X	1.112	>0.05
Y	0.705	>0.05
$X\times Y$	4.864	<0.05

Table 5 Least significant difference (LSD) of the 3×4 factorial experimental design model

Factor	LSD	P
Y_2-Y_1	5.82	<0.05
Y_3-Y_2	6.31	<0.05
Y_3-Y_1	1.46	>0.05
X_4-X_1	0.89	>0.05
X_4-X_2	7.65	<0.05
X_4-X_3	2.34	>0.05
X_3-X_2	9.21	<0.05
X_3-X_1	1.78	<0.05
X_2-X_1	6.42	<0.05

4 Discussion

ATT offers a biological and feasible treatment for replacing missing teeth. The general indications for autotransplantation include teeth lost due to trauma, caries or periodontal diseases, the need for re-seating of impacted or ectopic teeth in their normal positions, and agenesis of teeth (Tanaka *et al.*, 1998; Muramoto *et al.*, 2000; Hayashi *et al.*, 2001; Kaneko *et al.*, 2001; Eui-Seok *et al.*, 2002; Kallu *et al.*, 2005). In recent years, the survival rate of teeth following autotransplantion has increased by means of strategies such as infection control, improvements in surgical procedures, and splinted biting buffering, which contribute to periodontal healing. However, the common complications, such as root resorption or ankylosis, still cause concern for dentists. Some studies have shown that mesenchyma-originated cells, such as fibroblasts, osteoblasts, osteoclasts, odontoblasts, and odontoclasts, take part in the modeling and remodeling of periodontal tissues, which require proper mechanical stimuli. For this reason, it is essential to optimize the application and the duration of the stimuli applied to autotransplanted teeth. The aim of this study was to investigate the optimal conditions for loading autotransplanted teeth.

The PPDs of teeth before and after surgery were measured to evaluate their periodontal status in different operative periods. Considering the confounding factors resulting from various postoperative load conditions, the periodontal examination time points for pre- and post-operation comparisons were defined as pre-operation and postoperative week 1. We found deeper pockets in teeth after postoperative week 1 but the depth became shallower over time in both loaded and non-loaded teeth. Thus, it appears that ATT surgery might result in deeper pockets after short postoperative periods. Among the possible reasons, the oral hygiene of beagle dogs should be considered, as it was not easy to control, was not the same as in humans, and hindered the dissipation of inflammation after surgery. Following loading of the appropriate orthodontic force, the periodontal healing of the replanted teeth showed no unfavorable signs.

The remodeling of alveolar bone is a complex physiological process, which is regulated by a variety of enzymes and bioactive factors (Uematsu *et al.*, 1996; Ren *et al.*, 2002), including ALP,

bonemorphogenic protein (BMP), transforming growth factor-p (TGF-p), insulin-like growth factor-I (IGF-I), fibroblast growth factors (FGFs), platelet-derived growth factor (pDGF), tumor necrosis factor (TNF), epidermal growth factors (EGFs), and growth hormone (GH). ALP is an enzyme that is closely associated with the formation of mineralized tissues, and its activity is considered an important index of osteogenesis (Batra *et al.*, 2006). bFGF can induce the proliferation of periodontal tissue (Takayama *et al.*, 1997) and promote the secretion of extracellular matrix by fibroblasts and osteoblasts, and angiogenesis (Matsuda *et al.*, 1992). Accordingly, the mRNA expression levels of ALP and bFGF can to some extent reflect the effect of orthodontic loads on the periodontium of autotransplanted teeth. The expressions of both ALP and bFGF were higher in orthodontically loaded teeth than in unloaded teeth. This demonstrated that orthodontic load was helpful to periodontal healing of autotransplanted teeth. At the same time points of orthodontic loading, ALP and bFGF were highly expressed in the groups with two-week duration of loading. With the same duration of loading, the highest expression of the two factors was in groups loaded after postoperative week 4.

According to the histomorphometric analyses of the periodontia of autotransplanted teeth, the root resorption rate of loaded teeth was less than that of unloaded teeth. This shows that orthodontic load could contribute to the improvement of root resorption and reconstruction of the periodontium. Moreover, the 3×4 factorial experimental design model showed that different degrees of root resorption occurred at various time points and after different durations of orthodontic load. Also, the timing and duration of the load showed an interaction with the root resorption rate. Accordingly, the optimal junction point should be investigated. On the basis of this study, the root resorption rate was the lowest among the autotransplanted teeth, which were loaded after postoperative week 4 for 2-week duration. This treatment was very beneficial for the reconstruction of periodontal tissue in autotransplanted teeth. However, the Masson-stained sections revealed a favorable state of periodontal restoration in teeth after postoperative week 4 and week 8 with 2-week duration. Thus, the appropriate time points and duration of load should be considered.

It has been reported that the healing speed of the wound following tooth extraction in dogs is about twice that of humans (Hubsch and Hansen, 1969). Therefore, the data from this research might serve as useful basis for subsequent clinical studies in humans, and may help dentists to choose a suitable time and duration for orthodontic loading of autotransplanted teeth during the surgical process. Further study is needed to achieve the optimal prognosis for the preservation of teeth through autogenous transplantation.

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Compliance with ethics guidelines

Lu LU, Hui-fang SUN, Han XUE, Jing GUO, and Yang-xi CHEN declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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