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Research Paper

Effectiveness of two regeneration materials in autogenous tooth transplantation in larger sockets:an experimental study in beagle dogs ☆

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Abstract

Objective: To evaluate the effectiveness of regenerous tissue and bone substitute in autogenous tooth transplantation in the larger recipient socket. Methods:In 3 Beagle dogs, 18 incisors were transplanted to the recipient sockets, 2 mm wider mesio-distally. The regenerous tissue group, the bone substitute group and the control group contained 7, 7 and 4 teeth respectively. No additional material was used in control group. Clinical and radiographic examinations were done every month and were sacrificed 3 months later. Subsequently, decalcified sections were prepared for routine histological evaluation. Ordinal scores for root surface resorption were analyzed using the Kruskal-Wallis test. Results:All donor teeth survived. A statistically significant difference was found among all three treatment groups(P = 0.0001). The proliferating tissue in space positively affected the periodontal healing without any resorption. Inflammatory resorption of the root surface and formation of new bone were observed in the bone substitute group. Surface resorptions of the roots were found in the control group. Conclusion:Proliferating tissues enhance the regeneration of periodontal tissues in larger recipient sockets and prevent root resorption. Sinbone HT is beneficial for the stabilization of the transplanted teeth in larger sockets.

Key words: autogenous tooth transplantation; regenerous tissue, periodontal regeneration, beagle dogs

INTRODUCTION

Unrestorable teeth are usually extracted and replaced by some prosthesis. In planning the treatment for such cases, usually clinicians have various treatment options including fixed or removable prosthesis, dental implants, and autogenous tooth transplants. However, most of them are limited to factors such as number and periodontal health of remaining tooth in arch, degree of alveolar ridge resorption, certain systemic diseases and treatment cost. Autogenous tooth transplantation could be an alternative way to procure the required treatment in some of these cases should suitable donor teeth available. Autogenous tooth transplantation(a procedure in which a tooth is taken from one socket and transplanted to another socket in same individual)[1] has been described and documented by several clinicians since early 1950s[2-5].According to these authors, successful tooth transplantation depends upon the optimal and uneventful healing of periodontium. Favorable periodontal ligament(PDL) healing depends upon the vitality of remaining PDL cells in the donor root, the shape and size of the recipient socket and the vascularity of the recipient bed. Optimal contact with the recipient site can improve blood supply and nutrition to the periodontal ligament cells and thereby decrease the rate of resorption which is the common cause of transplant failure[1, 6-8]. To improve nutrition and preserve cell activity in these tissues, Nethander *et al.*[9,10] and Akihiko *et al.*[11], suggested that teeth should be transplanted

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together with regenerated tissues which reduce the occurrence of root resorption following transplantation. Hydroxyapatite (HA) and Tricalcium phosphate(TCP) are popular alloplastic bone substitutes because of their handling characteristics and their availability in various sizes[12,13]. Moreover, HA has clearly been identified as a suitable surface for periodontal regeneration and cementogenesis[14]. The initial stability of the transplanted tooth with optimal periodontium healing(essential for successful transplantation) may be provided by these bone substitutes. Hence, the purpose of this study was to evaluate the effectiveness of regenerous tissues, as well as bone substitutes in autogenous tooth transplantation in larger recipient sockets.

MATERIALS AND METHODS

Animal Model

Three(2 male and 1 female) healthy beagle dogs weighing 13 kg were used in this study. Age ranged from 12-15 months. The protocol design and procedures were approved by the Animal Research Center of People's Hospital of Jiangsu Province, Nanjing. All the three dogs were kept under good supervision at the Experimental Department, Animal Research Center, University of Traditional Chinese Medicine, Nanjing, 1 month prior to the surgery and throughout the entire healing period of 3 months.

Prior to the experimental surgeries, a preparatory period of 2-3 weeks permitted the establishment of a clinically healthy gingiva by means of manual scaling and Tinidazole oral rinsing. The dogs were fed a soft, nutritious diet and water *ad libitum*.

Methodology

All the surgical procedures were performed under general anesthesia in sterile conditions. The animals were premedicated with 0.02 mg/kg IM ketamine Hcl (Hengrui Medical Co., Ltd., Jiangsu, China). General anesthesia was induced with 0.03 mg/kg IV Pentobarbital sodium(Sinopharm Chemical Reagent Co. Ltd. Jiangsu) followed by analgesic Fentanyl Citrate injection 0.4 mg/kg IV(Yichang Humanwell Pharmaceutical Co., Ltd. China) and muscle relaxant suxamethonium chloride injection 0.2 mg/kg IV(Shanghai Xundong haipu Pharmaceutical Co., Ltd, Shanghai, China). A prophylactic antibiotic Benzylpenicillin 4,00,000 IU (Shangdong Lukang Medical Co., Ltd, Shangdong, China) was administered. An endotracheal tube(Kendal Curity, Kendall-Gammatron, Sampran, Thailand) was placed and the animals were maintained on isoflurane gas(1%-2%) in 100% oxygen using positive pressure ventilation.

As experimental sites, both sides mandible were selected in each dog. The second and third premolar(P_2, P_3) areas were selected as recipient sites. The lower right quadrant was assigned to the two-stage surgical approach for regenerous tissue group. The lower left quadrant was assigned to the single stage surgical approach for the control and bone substitute group. Both mesial and distal sockets of P_2 and P_3 were randomly selected as an individual recipient bed in each dog.

A total of three groups, 7 teeth in first two groups and 4 teeth in another group were utilized in this experiment.

A) Regenerous Tissue Group:

At the recipient sites, the epithelial covering was excised to expose the regenerous tissues inside the socket bed. Care was taken not to have any epithelial tissue entrapped in between the donor root surface and socket bed tissues. For regenerous tissue group, right maxillary I_1 and right and left maxillary I_2 were randomly selected as donor teeth.

First Surgery:

For the recipient area, gingival incisions were performed mesially from the second premolar and distally to the third premolar teeth. Then, mucoperiosteal flaps were raised. After vertical inter-radicular section performed under saline irrigation, each root was carefully elevated and then gently extracted. An implant machine (Surgi XT 2000, Chang Ming Trading Co., Ltd., Taipei, Taiwan) was used for recipient bed preparation. Each socket was reshaped at a low speed of 1200 rpm with a standard implant drill bur(Ø4.5 mm, 10 mm length) under copious saline irrigation. The shape and size of recipient sockets were made approximately 2 mm wider mesiodistally than the size of the donor teeth, estimated radiographically. The prepared sockets were then irrigated with normal physiological saline to remove any residual debris. Then the flap was repositioned and sutured with non-absorbable black silk suture(3-0, Nanjing Yijia Dental Equipment Co., Ltd.)

Second Surgery:

After one week, the second surgery was performed. At the donor site, following elevation of mucoperiosteal flap, the tooth was carefully extracted and transferred into the normal physiological saline to prevent periodontal ligament from drying. The donor sockets were irrigated with normal saline and flaps repositioned and sutured. The crowns of the teeth were reduced to approximately 2-3 mm coronal to Cemento-Enamel Junction(CEJ) under copious saline irrigation and the cut surfaces were smoothened. Root canal therapy(RCT) was performed for each tooth. The mean extra-oral period for every donor tooth was 20-25 mins.

Endodontically treated donor teeth were then transferred to their respective socket beds and gently pressed with finger to ensure the proper adaptation with regenerous tissues. Then sutured firmly in place with non-absorbable 3-0 black silk suture.

B) Bone Substitute Group:

For bone substitute group, left maxillary I_1, right and left mandibular I_1 were randomly selected as donor teeth. The recipient sites were immediately prepared after extraction of P_2 and P_3. All the other procedures were carried out in a similar fashion to that of regenerous tissue group. Donor teeth were transferred to their recipient sockets and the remaining space was filled with bone substitute(SinboneHT HA60%-TCP40%, particle size 0.25-1.00 mm, Purzer Pharmaceuticals Co. Ltd., Taiwan) and were firmly held in place with suture.

C) Control Group:

For this group, right and left mandibular I_2 were randomly selected as donor teeth. Following extraction and surgical preparation of the recipient sites, all the other procedures were carried out in a similar fashion to that of other group.

A total of 18 teeth with fully developed roots (maxillary and mandibular incisors) were included in this experiment.

Oral hygiene was maintained using mouthwash irrigation for a week after surgery. Antibiotics were continued for 2 days following surgery. Sutures were removed 1 week postsurgery.

Careful clinical examination was done every month for inflammation, infection, tooth mobility or any other pathology. Periapical radiographs were taken at 1 month, 2 month and 3 month after surgery for each dog. After a healing period of 3 months, all dogs were sacrificed by overdose of Pentobarbital.

Histological Processing and Specimen Preparation

Following sacrifice, the mandibles were removed and were fixed in 4% neutral buffered formalin for 24 hours. The tissue blocks containing experimental areas were subsequently decalcified with Plank-Rychlo solution for 10 weeks. The end point of decalcification was radiographically determined. Decalcified specimens were trimmed, dehydrated in ethanol and finally embedded in paraffin. For each specimen, 4 μm sections were serially sectioned with a diamond microtome(Leica RM2265, Leica Microsystems Nussloch GmbH, Nussloch, Germany) along the long axis of the tooth in a mesio-distal direction.

Histological Evaluation

The sections were stained with Hematoxylin & Eosin (H&E) stain and examined under a light microscope (Olympus CX21FS1, Olympus Optical Co., Ltd, Shenzhen, China) equipped with a computerized image system. 5 longitudinal sections were randomly selected from each transplanted tooth and to evaluate any pathological conditions in terms of root resorption, the periodontal tissues were examined at 3 different locations (coronal, middle and apical). Scores were recorded at each location using the following ordinal criteria: 0 = little or no resorption; 1 = resorption up to the cemental layer; 2 = resorption up to the dentinal layer. A total of 270 sites(Regenerous tissue group: 105, Bone substitute group: 105 and Control group: 60) were examined.

Statistical Analysis

All statistical analyses were performed using STATA v9.0(Stata Inc., College Station, TX, USA) statistical software program. The ordinal histological scores from all three groups were compared in terms of root resorption using the *Kruskal-Wallis test*. Further differences among each two groups were analyzed using *Kruskal-Wallis test* with *Bonferroni Adjustment* for multiple comparisons. A P-value < 0.05 was considered statistically significant.

RESULTS

Clinical and Radiographic Observations

All 18 transplanted teeth survived. Clinical healing was uneventful with limited signs of inflammation in all experimental sites following transplantation(Fig. 1). The initial inflammation immediately after surgery was comparable in all three groups. After the second month of surgery, some radiolucencies randomly distributed along the entire length of teeth were seen in some transplanted teeth with bone substitute. The control group showed unclear periodontal space in the areas of tooth transplantation. Precise radiographic diagnoses could not be made because of the inadequate resolution of the images.

Histological Observations

As stated in Table 1, significant difference(P = 0.0001) was found among all three groups in terms of root resorption. In the regenerous tissue group, periodontal healing was observed with adequate width of periodontal ligament in 6 transplanted teeth(Fig. 2). This group showed no significant root resorption in comparison to other groups. The entire root surface was almost completely covered with new cementum, and regenerated periodontal ligament separated the new bone from the cementum. Sharpey's fibers inserted into the cementum were frequently observed. Only one tooth showed some resorption cavities. In Bone Substitute group, numerous proliferating bone cells and fibroblasts were observed(Fig. 3). A varying amount of new immature bone was laid in root-bone interface. Few inflammatory cells with resorption cavities were seen in all 7 transplanted teeth. The resorption was extended up to the dentinal layer invading cementum. In control group, many small resorption cavities were visible in the cemental layer of all transplanted teeth(Fig. 4). This group showed resorption up to the cemental layer which was significantly higher(P = 0.0001) than in other two groups.

Table 1 Number of histological root resorption scores for the three groups of specimens

Score	Control (n = 60 sites)	Regenerous tissue (n = 105 sites)	Bone substitute (n = 105 sites)
0	7	94	20
1	49	11	21
2	4	0	64
Median(25%, 75%)	1 (1, 1)	0(0, 0)	2 (1, 2)
Kruskal-Wallis test	KW statistic	χ^2=136.97, P < 0.0001*	

25%, 75% = percentiles. *Statistically significant difference at less than the 0.01% probability level.

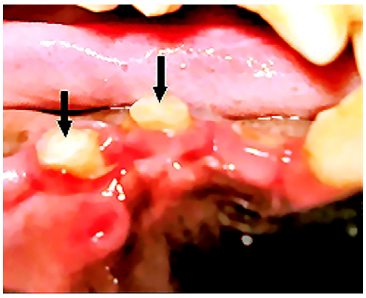
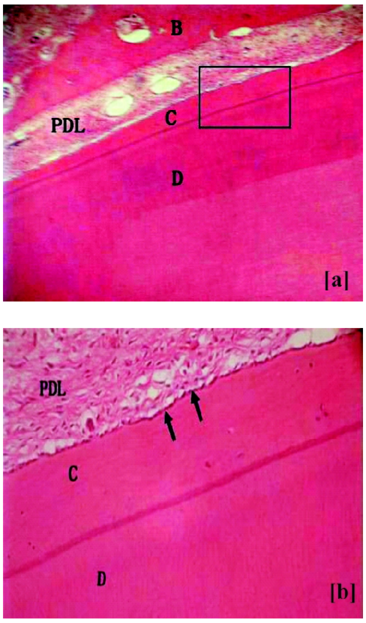
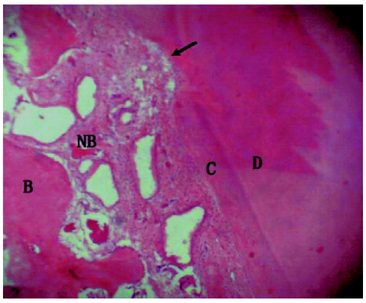


Fig. 1 Healing at 3 months(Arrows showing the soft tissue healing around the transplanted teeth)



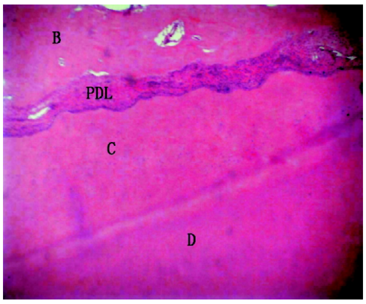
[a]:No root resorptions were observed(H&E stain, × 10). Higher magnification of the framed area in [a], showed functional attachment of periodontal ligament[b], × 40; B: Alveolar Bone, C: Cementum, D: Dentin, PDL: Periodontal Ligament.

Fig. 2 Histologic Photograph of the experimental site of Regenerous Tissue Group



B:Alveolar Bone,C: Cementum, D:Dentin, NB: New Alveolar Bone (HE stain, × 40).

Fig. 3 Histologic Photograph of the experimental site of Bone Substitute Group Inflammatory resorption was observed, arrow represents the resorption cavity.



B:Alveolar Bone,C:Cementum,D:Dentin,PDL:Periodontal Ligament (HE stain, × 10).

Fig. 4 Histologic Photograph of the experimental site of the Control Group Irregular surface of the cementum (surface resorption) was observed.

DISCUSSION

The periodontal ligament has demonstrated a remarkable capacity for repair and regeneration and when up to 1 mm in diameter of the periodontal ligament is lost, through disease or trauma, it is still capable of replenishing itself via differentiation from adjacent cells[1].The provision of a good blood supply is very important in the wound healing following transplantation which is directly influenced by the distance between the donor tooth and the recipient socket. It is the general rule that smaller distances between donor tooth and recipient site provide better wound healing. To our knowledge, however, no report has been issued regarding the optimal distance of the transplanted root surface and the alveolar bone in tooth transplantation. Lee S-J Jung[15], used computer aided rapid prototyping for tooth transplantation where a computer aided resin donor tooth model was made to reproduce the exact shape and size of the donor tooth, thus maintaining the optimal distance between the recipient socket wall and donor root surface. Clinical data from this study showed the positive value of optimal contact between these two surfaces. Nethander[9] first showed that in humans, the regenerous tissue obtained after 14 days of healing period, positively affected the successful outcome of the transplanted teeth in the recipient sockets 2 mm larger than the estimated donor tooth size. Only based on the radiographic examination, the wound healing could not be assessed as a histological examination of human replanted teeth showed that surface resorption occurs in most cases and is not demonstrable radiographically[1]. Moreover, only a 5-year follow up was done. To overcome the difficulties in evaluating clinical trials and make a detailed histological assessment of the effect of regenerous tissue, Nethander[10], performed two-stage surgery for autotransplantation in beagle dogs. The recipient sockets were left to heal for 5 days and the donor teeth were transplanted into the respective sockets containing proliferating connective tissues. No differences were detected between one-stage technique and two-stage technique with regard to root resorption. Hence his study couldn't confirm whether the regenerous tissue had positive effect or no effect at all. In accordance with his study, this experiment was performed on the recipient sockets and left to heal for 7 days. Although the results showed that the 7 days healing period in dogs was appropriate for proper wound healing, for humans, the healing period of 14 days might be essential(as wound healing in dogs is two times faster than in humans[10]). Even though this two stage surgery protocol provides a good capillary bed for the donor tooth(reducing the space in between the donor tooth surface and the recipient socket) its requirement for two-fold surgery remains a problem.

Bioactive material such as hydroxyapatite was found to be compatible with PDL like interfaces[16]. In addition, HA when used with transplantation also showed abundant new bone formation. The space which might have been occupied by the blood clot leading to the necrosis, was subjected to the proliferating bone cells. According to Tsukiboshi M **et al.**[1], there must be sufficient alveolar bone support in all dimensions to allow for stabilization of the transplanted tooth. If the distance between the transplanted tooth and recipient socket becomes larger then the transplanted tooth may become unstable inside the large socket leading to the delayed or improper periodontal healing. In this study, bone substitute filled in this space allowed the transplanted tooth to remain stable. Further stabilization of transplanted teeth was achieved with sutures whereas the reduction of the crown length and smoothening of the cut surface helped to overcome the tongue interference. At the end of the 3-month healing period, all the transplanted teeth were found stable which indicates suturing is entirely feasible. Moreover, the rigid splinting techniques[17] to stabilize the transplanted tooth which results in ankylosis can be avoided.

Bone substitute materials must, of course, be biocompatible, noninfectious, and nonantigenic. Although most are not considered to be osteogenic or osteoinductive, they should at least be osteoconductive and capable of undergoing normal physiologic remodeling to yield functional bone capable of supporting functional osseointegration of dental implants or transplants. Shirakata **et al.**[18], mentioned that faster resorption of the material would be desirable to avoid the risk of any infection of the residual material during periodontal healing. HA/TCP was found to have all above properties, except the resorption rate which requires a long period of observation. The present study showed HA/TCP is evidently a promising material for stabilization of transplanted tooth. However, because of the short 3-month observation period, the study could not reveal the resorption rate of HA/TCP or define the role of HA/TCP on PDL healing. Therefore, further studies are necessary to investigate the exact mechanism of HA/TCP on PDL healing. These bone substitutes along with certain proven techniques such as Guided Tissue Regeneration(GTR) may provide favorable environment for better PDL healing in autogenous tooth transplantation.

In comparison to two stage surgery, one stage surgery was found to result in relatively more resorption and limited or no PDL space. The period of healing might be too short to reveal replacement resorption (ankylosis) as it is generally observed only after 3-4 months of transplantation.

Another important factor to be taken into consideration is the extra-oral time period of the transplanted tooth. Andreasen and colleagues[19,20] observed normal periodontal ligament healing in more than 80% of cases after an extra-oral time of 18 mins. In this regard, the average extra-oral time of 20-25 mins achieved during this study seems to be in a reasonable safe margin. In addition, RCT was performed outside the recipient socket within this limited period of time. All transplanted teeth with RCT done during the transplantation survived showing no adverse effects which indicate RCT during transplantation is a possible way. From the clinical point of view, it would be an effective way to perform RCT during the same occasion of transplantation which may reduce the incidence of inflammatory resorption.

Within the limitations of this study, it can be concluded that regenerous tissues enhance periodontal tissue regeneration in autogenous tooth transplantation to the larger recipient sockets whereas HA/TCP has a beneficial role in the stabilization of the transplanted teeth.

Clinical Implications:

①If nonfunctional teeth such as third molar are available, autotransplantation can be considered the treatment of choice. In some cases, autotransplantation makes placement of a fixed partial denture(instead of a removable one) possible.

② Autotransplantation can be considered for orthodontic patients with congenitally missing teeth or for those with trauma-induced tooth losses by using strategically extracted teeth for orthodontic treatment as donor teeth.

③ If suitable donor teeth are available, autotransplantation can be an alternative to dental implant in some patients in whom dental implants become impossible due to inadequate bone support, implant failure due to rejection and patients in growing stages, i.e. children.

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