Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effects of different types of palatal lateral excisions on growth and development of maxilla and dental arch

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Received Dec. 23, 2007; revision accepted June 2, 2008

Abstract: Objective: This study aimed to explore the effects of different types of palatal lateral excisions on the growth and development of the maxilla and dental arch, and to investigate the underlying mechanisms. Methods: A total of 112 3-week-old Sprague-Dawley (SD) male rats were randomly divided into a control and 3 experimental groups: the mucoperiosteal denudation group, the mucosal flap excision group, and the periosteum excision group. In the experimental groups, bilateral mucoperiosteal, mucosal flap and periosteum were excised respectively in the lateral one half of the palate. Four rats in each group were randomly chosen for sacrifice every two weeks. The maxilla was dissected following the excision. The widths of the maxilla and dental arch were measured and the histological phenomena were investigated at different phases. At the same time, 12 animals in each group were sequentially injected with calcein every two weeks. Three animals in each group, whose fluorescent labeling was used, were sacrificed for investigating bone formation at Week 8 following injection. Results: (1) Each experimental group presented the constriction of the maxilla and dental arch. The upper first molars in the experimental groups inclined medially. The mucoperiosteal denudation group showed the largest degree of effect followed by the periosteum excision group. The indices of the mucosal flap excision group, which retained the structures of the periosteum layer, had the most approximate values to the control group; (2) Different histological changes among the experimental groups were detected. The fibers penetrated into the palatal bone as Sharpey's fibers in the mucoperiosteal denudation group. The pattern of bone deposition was the bundle type. Sharpey's fibers were not found in the mucosal flap and periosteum excision groups and the depositions of palatal bone were the lamellar type as those in the control group; (3) The rates of bone deposition in the experimental groups decreased compared with the control group. The rates in different phases were the most approximate values to those of the control group in the mucosal flap excision group, which has the same structure of periosteum as the control group. Conclusion: There were different effects on the growth and development of the maxilla and dental arch in different types of palatal lateral excisions. Periosteum is important for bone formation and deposition pattern. The prevention of Sharpey's fibers forming and attaching to the palatine can effectively avert the following malformation.

Key words: Lateral excision, Maxilla growth, Dental arch growth, Periosteum, Calcein, Sharpey's fibers
doi:10.1631/jzus.B0720015 **Document code:** A **CLC number:** R78

INTRODUCTION

Cleft palate is one of the most common birth defects worldwide. Palatoplasty has been considered the primary means of treatment. Nowadays, surgery is considered one of the major factors causing disturbances in the facial growth of cleft palate patients. This is especially true in the mucoperiosteal denudation of bone followed by wound contraction and formation of scar tissues on the palatal lateral denuded bone area (Kremenak and Searls, 1971; Wijdeveld *et al*., 1991; Ishikawa *et al*., 1998; Pigott *et al*., 2002; Shetye, 2004; Weinzweig *et al*., 2006).

Inhibition of face and occlusion growth appeared after surgery. The possible mechanisms of facial deformation are intrinsic developmental defi-

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ciencies, functional distortions, scar tissues disturbance, surgical techniques, blood flow alteration, sequence treatment schedule, and so on. There have been many investigations of the adverse effects on the maxilla and dental arch growth (Graber, 1949; Lynch and Peil, 1966; Searls and Biggs, 1974; Wijdeveld *et al*., 1987).

In the studies of Wijdeveld *et al*.(1989; 1991), palatal surgery according to von Langenbeck technique was simulated in beagle dogs and resulted in inhibition of the growth and development of dento-alveolar structures. They attributed this result to: (1) the initial wounds construction, (2) the firm attachment of scar tissues to the palatine by means of Sharpey's fibers, (3) no elastic fibers demonstrated in the healing tissue, and (4) periodontal fibers being fanned out into the scar tissues.

Prevention of this inhibition of the dento-alveolar growth might be accomplished by the separation of scar tissues and palatine, by covering the areas of palatal bone denudation with membranes or by modifying the techniques of palatal repair. In previous studies (In de Braekt *et al*., 1995; Shi *et al*., 2003; Ophof *et al*., 2004; 2008), biocompatible, biodegradable membranes or mucosa from autogenic cheek, palate and tissue-engineering oral mucosa membranes were used to prevent the development of Sharpey's fibers. It remains to be elucidated whether the growth inhibition could be prevented by membrane covering.

Another way to prevent scar tissue attachment is by modifying the surgical techniques, mainly including the mucosal flap technique (Perko, 1974) and the partially flap technique (Leenstra *et al*., 1995a; 1995b). These two techniques preserve the periosteum in the palatal lateral excisions area, avoid the denudation of bone, and prevent Sharpey's fibers attachment (Leenstra *et al*., 1995a). Comparing the mucoperiosteal technique with the partially split flap technique in cleft palate patients, researchers found that the partially split flap technique can prevent construction of the dental arch efficiently (Leenstra *et al*., 1996; Noguchi *et al*., 2003), and that the speech evaluation, dental arch shape and velopharyngeal function were more advantageous (Ito *et al*., 2006; Vander Poorten *et al*., 2006).

The aim of this study was to evaluate the histological development of the maxilla and dental arch, wound healing and bone deposition in Sprague-Dawley (SD) rats. The evaluation was performed after simulated lateral excisions of palatal repair according to the von Langenbeck technique, the partially split flap technique or the mucosa covering with autogenic palate. The results were assessed in order to provide references for surgery design as well as to improve the management of cleft palate.

MATERIALS AND METHODS

Animals and surgical procedures

Rats are suitable experimental animals for studies of the maxillary growth influenced by palatal surgery and the subsequent healing process. The crest-time of maxilla and dental arch growths in rats is 23~75 d after birth. In this phase, the experimental intervention effect can be expressed preferably (Searls and Biggs, 1974). So we used a total of 112 purebred 3-week-old SD male rats in this study (Experimental Animal Center, Sichuan University, China). Throughout the experiments, the principles of laboratory animal care (National Research Council of China; 1996) were followed. The weight of rats was (50±5) g. The animals were randomly divided into a control group (n=28) and three experimental groups: the mucoperiosteal denudation group (n=28), the mucosal flap excision group (n=28), and the periosteum excision group (n=28). The experimental groups were anesthetized with muscular injections of 7 mg/kg sodium pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, IL, USA). Operative incisions began at the posterior margin of the anterior palatine foramen and ended on the level of distal surface of the maxillary second molar (Fig.1). The lateral incisions of palatal repair according to von Langenbeck technique, the partially split flap technique and the autogenic palatal mucosa covering technique were simulated respectively in three experimental groups. The bilateral halves of palatal mucoperiosteum were excised in the mucoperiosteal denudation group. The bilateral halves of the palatal mucosal flap were excised in the mucosal flap excision group, even though the periosteum of the lateral areas was preserved. The bilateral halves of the palatal mucosal flap were elevated, and the periosteum under the wound surface was excised in the periosteum excision group.

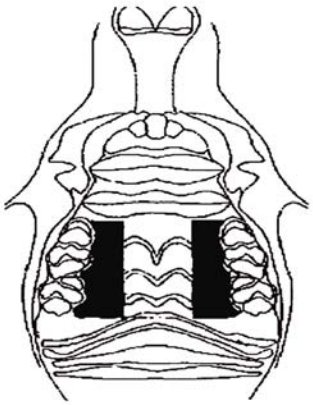


Fig.1 Diagram of the rat palate. Hatched areas are the soft tissue excision areas. Operative incision began at posterior margin of anterior palatine foramen and ended on the level of distal surface of the maxillary second molar. Bilateral halves of palatal mucoperiosteum, mucosal flap and periosteum were excised in three experimental groups

Growth and development of the maxilla and dental arch

At Weeks 2, 4, 6 and 8 after operation, 3 rats in each group were sacrificed with muscular injection of 7 mg/kg sodium pentobarbital (Nembutal®). The maxilla was dissected and embedded in methyl methacrylate resin after being dehydrated in a graded series of ethanol. Sections of the coronal plane of maxilla were made between the maxillary first molar mesiolingual cusps perpendicular to the occlusal plane by hard tissue cutting machine (LEICA AP1600, Germany) at 100 μm. The sections were fixed on microscope slides, observed under stereomicroscope, and photographed by digital camera.

The outlines of the upper first molars and palates were traced from photographs at 26.5× amplification. The experiments were performed according to the method of Kim *et al*.(2002), defining *A* and *A′*, *B* and *B′* points (Fig.2). Distances between *A* and *A′* points (*AA′*), *B* and *B′* points (*BB′*), representing the maxilla width and dental arch width respectively, were measured three times by a sliding caliper to obtain the mean values. *BB′* value minus *AA′* value was defined as *CC′*, representing the degree of tooth obliquity. The smaller *CC′* value was, the more serious the first molar introversion was. Experimental results were analyzed by statistical software package SPSS 11.5. One-way analysis of variance (ANOVA) was used to analyze the same indices of the four groups from the same period. Least significant difference (*LSD*) was used to analyze the differences between indices for pairs of groups.

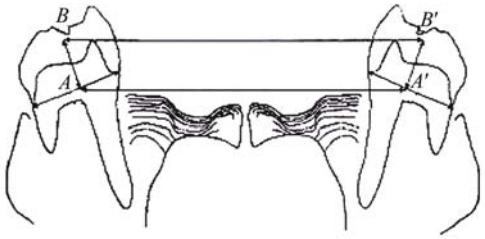


Fig.2 Diagram of the measuring points. The middle points of enamelcement junction between buccal and lingual surfaces of the maxillary first molar assumed as the rotation center of teeth were defined as *A* and *A′* points. The distance between *A* and *A′* points represented the width of maxilla (*AA′*). On the vertical lines to the above connecting lines, there are 30 mm points higher defined as *B* and *B′* points, and the distance between *B* and *B′* points represented the width of dental arch (*BB′*). *BB′* value minus *AA′* value was defined as *CC′*, representing the degree of tooth obliquity. The smaller *CC′* value was, the more serious the first molar introversion was

Histological processing

One rat in each group was anesthetized by muscular injection of 7 mg/kg sodium pentobarbital (Nembutal®) at Weeks 2, 4, 6 and 8 after operation. The maxillae were dissected and immersed in 10% (w/v) neutral formaldehyde as a fixative. Then the blocks were decalcified in 20% (w/v) formic acid and 5% (w/v) sodium citrate for 7 to 10 d, dehydrated and embedded in paraffin. Serial coronal sections of 5 μm were prepared between the maxillary first molar. Dimethyl benzene was used for deparaffinage. The sections were stained with hematoxylin and eosin (H & E). Five sections from each block were stained with trichrome staining (in toluidine for 10 min at 25 °C, 1% (w/v) orange G and saturated picric acid with volume ratio of 1 to 9 for 2 min, and ponceau red for 10 min).

Fluorescent labeling

We used the vital fluorescent bone marker calcein in order to characterize bone growth, which was widely used in the fluorescent analysis to determine many metal ions and as an indicator for titration analysis (Diehl and Ellingboe, 1956; Wallach *et al*., 1959; Jiménez *et al*., 1988).

Twelve rats in each group had 20 mg/kg calcein (Sigma, US) injected into the dorsal subcutaneous area every two weeks. Three labeled rats in each group were sacrificed at Week 8 after fluorescent injection. Hard tissue sections were prepared. Using ultraviolet light exposure at 380 nm, the section was observed by fluorescence microscope (Nikon, UFX-II) and graphed by digital camera. The right borders of section photos were measured at 40× amplification. Distance between two labeled lines was measured three times to obtain the mean value. The rate of bone deposition was estimated using distance divided by time interval. The data were analyzed with statistical software SPSS 11.5. One-way ANOVA was used to analyze the rate of bone deposition in the four groups from the same period. The *LSD* was used to analyze the differences between indices for pairs of groups.

RESULTS

Chronological changes in the maxilla width, dental arch width, and the degree of the upper first molar introversion

Fig.3 shows the chronological changes of the maxilla width, dental arch width, and the degree of the upper first molar introversion. The widths of maxilla and dental arch increased gradually in the control group. Maxillary and alveolar bones grew and developed down and in a lateral orientation. The same growth tendency appeared in the experimental groups, but at a slower rate. The upper first molar was introverted. The widths of the maxilla and dental arch were smaller than those in the control group, and the degrees of the upper first molar introversion were larger than those in the control group, especially in the mucoperiosteal denudation group. The indices of the mucosal flap excision group had the closest approximate values to those of the control group.

One-way ANOVA showed that there was statistical significance in three indices among all the groups at four periods ($P<0.01$). *LSD* revealed no significantly statistical differences between the control group and the mucosal flap excision group in three indices at Week 8 ($P=1.000$, 0.101, 0.141). There were no significantly statistical differences of the *CC′* values between the mucoperiosteal denudation group and the periosteum excision group at Week 2 ($P=0.516$) and Week 8 ($P=0.438$), and between the mucosal flap excision group and the periosteum excision group at Week 4 ($P=0.623$) (Tables 1 and 2).

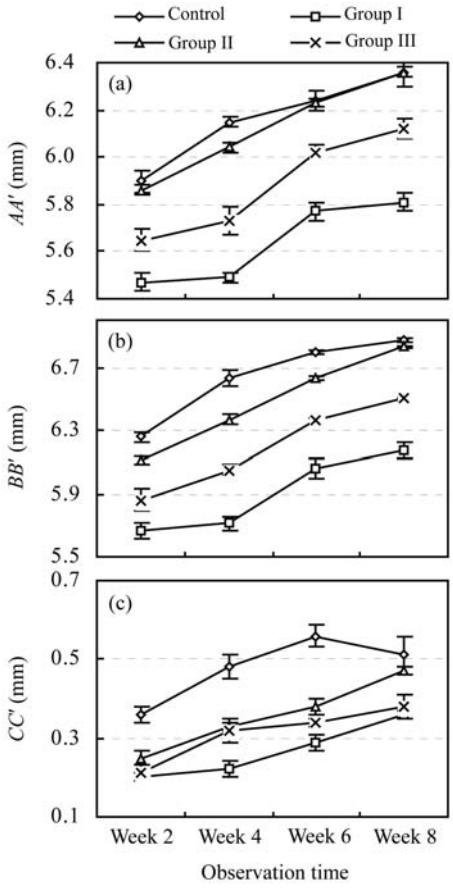


Fig.3 Chronological changes of maxilla width (*AA′*) (a), dental arch width (*BB′*) (b), and the degree of the upper first molar introversion (*CC′*) (c) in the four groups that were measured on different phases

Group I: Mucoperiosteal denudation group; Group II: Mucosal flap excision group; Group III: Periosteum excision group

Histological findings

The palatal bone was covered with parakeratotic stratified squamous epithelium with many villi protruding into the underlying connective tissues in the sections of the control group. Just below the epithelium, the connective tissue layer consisted mainly of a 3D network of coarse collagen fibers. Blood vessels aligned sagitally in the submucous layers. The major palatine artery and branches of the palatal nerves were found at the lateral aspect close to the bone. The periodontal fibers were fanned out into the gingival and the deeper layers of the palatal connective tissues.

Table 1 The maxilla width (*AA′*), the dental arch width (*BB′*) and the degree of the upper first molar introversion (*CC′*) on the different phases in the four groups

Phase	Group	*AA′* (mm)	*BB′* (mm)	*CC′* (mm)
Week 2	Control	5.90±0.05	6.26±0.03	0.36±0.02
	Group I	5.47±0.04	5.67±0.05	0.20±0.01
	Group II	5.86±0.05	6.11±0.03	0.25±0.02
	Group III	5.65±0.05	5.86±0.07	0.21±0.02
Week 4	Control	6.15±0.02	6.63±0.05	0.48±0.03
	Group I	5.49±0.02	5.71±0.04	0.22±0.02
	Group II	6.04±0.02	6.37±0.03	0.33±0.01
	Group III	5.73±0.06	6.05±0.03	0.32±0.03
Week 6	Control	6.24±0.04	6.80±0.01	0.56±0.03
	Group I	5.77±0.04	6.06±0.06	0.29±0.02
	Group II	6.23±0.03	6.63±0.01	0.38±0.02
	Group III	6.02±0.03	6.36±0.02	0.34±0.01
Week 8	Control	6.36±0.06	6.87±0.01	0.51±0.05
	Group I	5.81±0.04	6.17±0.05	0.36±0.01
	Group II	6.36±0.02	6.83±0.01	0.47±0.01
	Group III	6.12±0.04	6.50±0.01	0.38±0.03

All data expressed as mean±*SD*. Group I: Mucoperiosteal denudation group; Group II: Mucosal flap excision group; Group III: Periosteum excision group

Table 2 Results of multiple-comparison tests (*LSD*) for pairs of groups between indices of rats

Phase	Group	*AA′*	*BB′*	*CC′*
Week 2	C vs I	†	†	†
	C vs II	ns	†	†
	C vs III	†	†	†
	I vs II	†	†	†
	I vs III	†	†	ns
	II vs III	†	†	*
	$P^{\#}$	0.000	0.000	0.000
Week 4	C vs I	†	†	†
	C vs II	†	†	†
	C vs III	†	†	†
	I vs II	†	†	†
	I vs III	†	†	†
	II vs III	†	†	ns
	P	0.000	0.000	0.000
Week 6	C vs I	†	†	†
	C vs II	ns	†	†
	C vs III	†	†	†
	I vs II	†	†	†
	I vs III	†	†	*
	II vs III	†	†	*
	P	0.000	0.000	0.000
Week 8	C vs I	†	†	†
	C vs II	ns	ns	ns
	C vs III	†	†	†
	I vs II	†	†	†
	I vs III	†	†	ns
	II vs III	†	†	†
	P	0.000	0.000	0.001

$^{*}P<0.05$; $^{\dagger}P<0.01$; $^{ns}P>0.05$; $^{\#}P$: Significance between groups. C: Control group; I: Mucoperiosteal denudation group; II: Mucosal flap excision group; III: Periosteum excision group

The periosteal part of the mucoperiosteum consisted of a thin layer with some osteoblasts. The palatal bone was of the lamellar type (Fig.4a, see Page 644).

Two weeks after operation, scar tissues were found in the lateral operation areas in the mucoperiosteal denudation group. The epithelium seemed to be somewhat thinner and lacked villi. The connective tissues became thinner, and collagen fibers showed hyperplasia and were arranged disorderly. Slight inflammatory cell infiltration was found. Few inactive osteoblasts appeared on the bone surface. Four weeks after operation, collagenous fibers appeared hyperplastic and thickening, and were arranged horizontally-oriented. The periodontal fibers were fanned out into the scar tissues and connected with the collagenous fibers. The structure of the periosteum disappeared. In the operation areas many thick collagen fibers penetrated into the palatal bone as Sharpey's fibers. Eight weeks after operation, scar tissues were constituted by a great quantity of horizontally-oriented thick collagen fibers. Sharpey's fibers created a firm attachment of the scar tissues to the bone (Fig.4b). The periodontal ligament showed a conjunction between the mucoperiosteal connective tissues and the alveolar bone (Fig.5a). The palatal bone was of the bundle type, with trabecular deposition and osteoid formation (Fig.4b).

Two weeks after operation, the epithelium consisted of parakeratotic stratified squamous epithelium with obvious villi in the mucosal flap excision group. Large blood vessels appeared hyperaemic in the thick connective tissues. Collagenous fibers thickened and became hyperplastic. Inflammatory cells infiltration could be found (Fig.4c). Four weeks after operation, a layer of scar tissues extended from the surface of the former wound areas to the deeper layer of the mucoperiosteum (Fig.5c). The boundary of scar tissues was rather vague. Increasing fibroblast and hyperplastic collagenous fibers were found in the layer of the connective tissues. There were no Sharpey's fibers. The periodontal ligament showed less attachment to the mucoperiosteal connective tissues (Fig.5b). The layer of the periosteum was thick and rich in cells. The palatal bone was of lamellar type (Fig.5c), similar to the control group. Six to eight weeks after operation, fibroblast reduced and collagenous fibers thickened increasingly. The layer of the periosteum became thinner, but small amounts of bone deposition still could be found.

Two weeks after the operation, the epithelium closely resembled those of the control group animals

except for granular layer presenting slight proliferation in the periosteum excision group. Small amounts of inflammatory cells infiltrated in the operation area. Proliferative and indiscriminate osteoplast could be found in the border of the operation area. Four weeks after operation, collagenous fibers thickened and became horizontally-oriented. Scar tissues were connected with the periodontal fibers (Fig.5e). A thin layer of regenerated periosteum appeared in the operation area (Fig.4d). The Sharpey's fibers could not be found. Six to eight weeks after operation, the reticular collagen fibers located in maturated scar tissues, and the palatal bone was of lamellar type with osteoid formation (Figs.4e and 5e).

Fluorescent labeled appearance

Regularly arranged fluorescence labeling lines corresponding to the injection time were clearly observed from the side of the nose to the side of the palatine in the control group at Week 8 after injection (Fig.6a). The marker lines became narrower near the side of the palatine. Strong fluorescence labeling was found on the median suture of palatine, corresponding to the last time of mark (Fig.6b).

Two weeks after injection, the structure of palatine essentially remained normal in the mucoperiosteal denudation group. Massive irregular fluorescent labeling markers could be observed adjoining the side of the nose. A strong fluorescence labeling band was seen on the median palatine suture with irregular arrangement. Flexed marker line aligned on the side of the palatine. At Week 8 after injection, the labeling lines became regularly arranged, corresponding to the injection time, and curved to the side of the palate. The nearer the fluorescence labeling to the side of the palatine was, the narrower the distance between one marker line and the other (Fig.6c). The attenuated fluorescence labeling band was present on the median palatine suture.

The bone lamella of the hard palate remained normal anatomy and structure in the mucosal flap excision group. The labeling lines showed wrinklier compared with the control group. At Week 8 after injection, the regular fluorescence labeling line corresponding to the injection time was observed from the side of the nose to the side of the palatine. The distance between two labeling lines became smaller near the side of the palatine (Fig.6d). Strong labeling band corresponding to the last time of injection appeared on the median suture of the palatine.

Two weeks after marking, the fluorescence labeling lines on the side of the nose were dispersed and irregular in the periosteum excision group. At Week 4 after injection, the labeling lines corresponding to the injection time began to appear. Six to eight weeks after operation, relatively regular fluorescence labeling lines were distributed from the side of the nose to the side of the palatine. Similar to the control group, the distance between the two marker lines became smaller gradually (Fig.6e), and the strong fluorescence labeling band arose on the median suture of the palatine.

One-way ANOVA showed that there was statistical significance of the rates of bone deposition among all the groups at four periods ($P<0.01$). The data of *LSD* analysis showed that there was statistical significance of the indices between all pairs of groups ($P<0.01$), except the rate of bone deposition between the mucoperiosteal denudation group and the periosteum excision group at Weeks 6~8 ($P=0.382$) (Tables 3 and 4).

Table 3 The rate of palatine bone deposition on the different phases in the four groups (n=3)

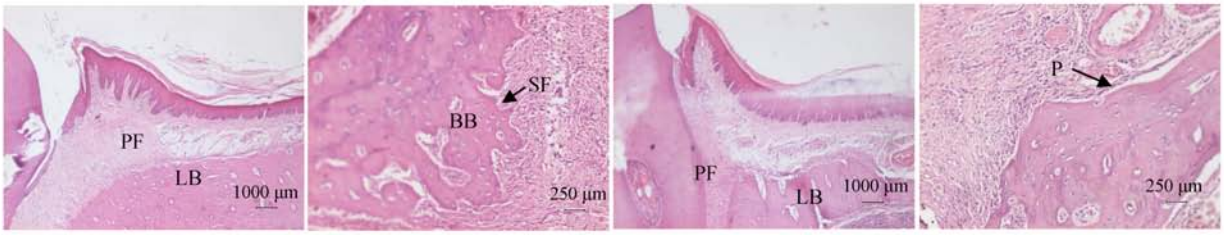
Time	Rate of palatine bone deposition (mm/d)			
	Control group	Group I	Group II	Group III
Weeks 0~2	0.0160±0.0002	0.0110±0.0001	0.0140±0.0002	0.0130±0.0001
Weeks 2~4	0.0140±0.0003	0.0090±0.0002	0.0120±0.0001	0.0110±0.0002
Weeks 4~6	0.0100±0.0002	0.0050±0.0002	0.0080±0.0001	0.0060±0.0002
Weeks 6~8	0.0100±0.0001	0.0050±0.0001	0.0080±0.0002	0.0049±0.0001

Data expressed as mean±*SD*. Group I: Mucoperiosteal denudation group; Group II: Mucosal flap excision group; Group III: Periosteum excision group

Table 4 Results of multiple-comparison tests (LSD) for pairs of groups between indices of rats

Time	C vs I	C vs II	C vs III	I vs II	I vs III	II vs III	One-way ANOVA (P)$^{\#}$
Weeks 0~2	†	†	†	†	†	†	0.000
Weeks 2~4	†	†	†	†	†	†	0.000
Weeks 4~6	†	†	†	†	†	†	0.000
Weeks 6~8	†	†	†	†	ns	†	0.000

†$P<0.01$; $^{ns}P>0.05$; $^{\#}P$: Significance between groups. C: Control group; I: Mucoperiosteal denudation group; II: Mucosal flap excision group; III: Periosteum excision group



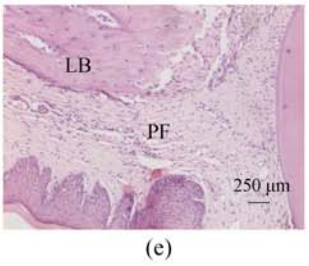
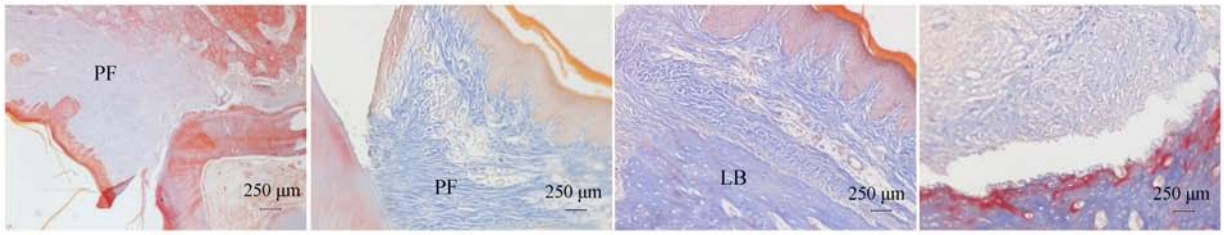


Fig.4 Frontal section of the rat palate (H & E staining)

(a) Control group (Week 8). The periodontal fibers were fanned out into the gingiva and the deeper layers of the palatal connective tissues. Sharpey's fibers were not found. The palatal bone was of the lamellar type; (b) Mucoperiosteal denudation group (Week 8). Sharpey's fibers created a firm attachment of the scar tissues to the bone. The palatal bone was of the bundle type; (c) Mucosal flap excision group (Week 8). The periodontal ligament showed less attachment to the mucoperiosteal connective tissue. The palatal bone was of lamellar type; (d) Periosteum excision group (Week 4). A thin layer of regenerated periosteum appeared in the operation area; (e) Periosteum excision group (Week 8). Scar tissues were connected with the periodontal fibers. The surface of the palatine was flat. PF: Periodonal fibers; LB: Lamellar type bone; SF: Sharpey's fibers; BB: Bundle type bone; P: Periosteum



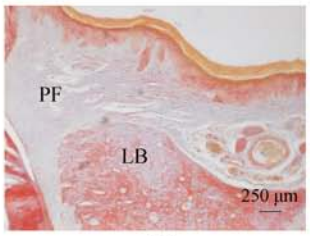
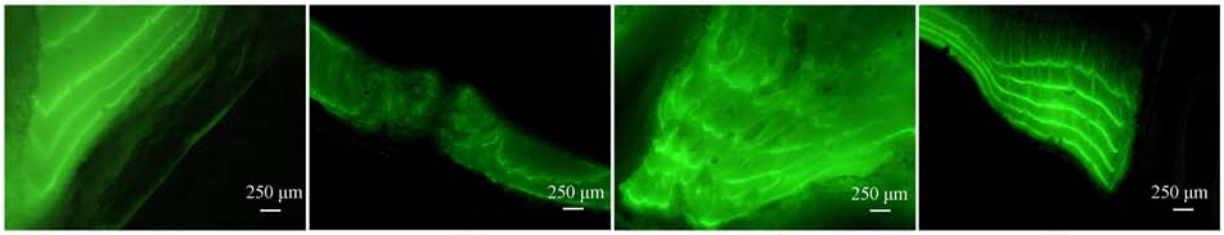


Fig.5 Frontal section of the rat palate (trichrome staining)

(a) Mucoperiosteal denudation group (Week 8). The periodontal fibers were fanned out into the scar tissues and connected with the collagenous fibers; (b) Mucosal flap excision group (Week 8). The periodontal fibers were not connected with the scar tissues; (c) Mucosal flap excision group (Week 8). The palatal bone was of lamellar type. Sharpey's fibers were not found; (d) Periosteum excision group (Week 0). Periosteum was excised completely; (e) Periosteum excision group (Week 8). Scar tissues were connected with the periodontal fibers. The palatal bone was of lamellar type. PF: Periodonal fibers; LB: Lamellar type bone



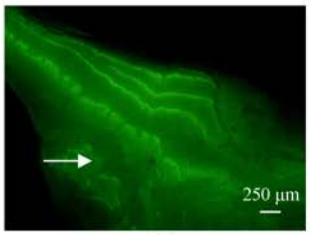


Fig.6 Frontal section of the rat palate (fluorescence microscope image; calcein)

(a) Control group. Fluorescence labeling lines were arranged from the side of the nose to the side of the palatine regularly; (b) Control group. Irregular fluorescence labeling band was found on the median suture of palatine, corresponding to the last time of mark; (c) Mucoperiosteal denudation group. Massive irregular fluorescent labeling markers could be observed adjoining the side of the nose. Flexed marker line aligned on the side of the palatine; (d) Mucosal flap excision group. The regular fluorescence labeling lines corresponding to the injection time (Weeks 0, 2 , 4 , 6 , 8) were observed from the side of the nose to the side of the palatine. The labeling lines showed wrinklier; (e) Periosteum excision group. Arrow shows the fluorescence labeling lines of the 0~2 weeks, which were dispersed and irregular. The fluorescence labeling lines of 4~8 weeks were relatively regularly distributed from the side of the nose to the side of the palatine

DISCUSSION

Earlier studies concerning with the influence of palatoplastic surgery on facial growth and development had used non-bony cleft animal models. The closure of a human palatal cleft is generally achieved by soft tissues alone rather than by osseous surgeries. The wound in the palatal lateral excision area was caused by the medial translocation of soft tissues to close the defect. Furthermore, surgical creation of the palatal bony defect has to undergo the healing process of bone. Our study did not include the artificial creation of bony clefts because of possible complications from extensive trauma.

Our previous study (Meng *et al*., 2007), concerning the roles of different areas of palatine bone denudation on the growth and development of the maxilla and dental arch, showed that surgical effect could be expressed efficiently in one-half of the palate soft tissues operated.

Calcein, a familiar fluorescent reagent, could specifically label calcium salt released into newly formed bone matrix and is capable of rapidly penetrating into the skeletal structures (Suzuki, 1986; Ducy *et al*., 2000). The labeling can be detected under the short wavelength ultraviolet light (Lanyon *et al*., 1982; Burr *et al*., 1989; Lee *et al*., 2000; O′Brien *et al*., 2003). It is very stable once it is incorporated, and free calcein is rapidly cleared from the circulation. Furthermore, its application needs 10~14 d, which is appropriate to our observation time. In addition, calcein could be sensitive to determine the bone deposition despite the lowest application dose (Pautke *et al*., 2007) thus making it a useful vital marker to sites of new bone deposition in our study.

Many methods can be used to prevent the inhibition of maxilla and dental arch growth after palatal repair. In this study, we simulated the lateral excisions of palatal repair according to the partially split flap technique and the method of mucosa origin from autogenic covering on rats to obviate growth inhibition of the palatine, and compared the effects with those in lateral excisions of the von Langenbeck technique, which verified the effect on the growth and development of the maxilla and dental arch.

Different types of palatal lateral excisions may have different effects on the growth and development of the maxilla and dental arch. The mucosal flap excision group, which retained the structures of periosteum layer, showed the weakest effect, and the indices had the most approximate values to those of the control group.

The histological findings in the mucoperiosteum excision group are in agreement with the findings of Wijdeveld *et al*.(1991). The composition of healed tissues was different from normal mucoperiosteum. The scar tissues contained the gross collagen fibers but lacked the elastic fibers of normal palatal mucosa. The firm attachment of the scar tissues to the palatal bone was due to the Sharpey's fibers. The collagen fibers in the wound were also connected with teeth and periodontal ligament. The distribution of tensile forces along the whole palatine was changed. At the same time, local tensile forces might act in medial direction on the teeth. Furthermore, palatal bone was classified into membrane bone. The integrity of the periosteum and the quantity of the osteoblasts directly refer to bony matrix formation and mineral matter deposition. Periosteum was not present in this group. The palatal bone took on the bundle type deposition in histological appearance. Fluorescent labeling appeared blurred and flexed labeling lines, which might be the manifestation of this bundle type deposition. The formation of bundle bone is the result of the wound healing in areas of denuded bone. Bundle bone can be characterized as immature bone and often appears in postnatal life and in places where bone repair takes place (Ham and Cormack, 1979), as is the case in denuded bony areas. In addition, the rates of bone deposition at all four phases were decreased. All of these factors induced the growth inhibition of the hard palate.

Scar tissue areas showed lower perfusion values, reflecting a lower vascular density in the scar, according to Chu *et al*.(2000)'s study on rat palates. In the mucosal flap excision group, the inhibitive growth rate in early period may be due to the operation damage to the partial blood vessels and nerves and the primary construction of scar tissues. The attachment of Sharpey's fibers and connecting collagen fibers with periodontal ligament were not found. The method in this study retained the structures of the periosteum layer and the primary nourished vascellum. The method prevented the attachment of Sharpey's fibers and decreased the contractual degree after full development of scar. Thus local contraction

forces were blocked and the degree of maxilla and dental arch growth inhibition was reduced. Beside the same structure of periosteum, the histological features of the tissues after surgery were the most approximate to those in the control group. Fluorescent labeling showed that the marker lines aligned regularly. They reflected the lamellar type palatal bones formation in these areas. Bone trabecula and osteoid formed along the periosteum. Furthermore, the bone deposition rates at four phases were most approximate to those of the control group. Therefore, the growth rates of maxilla were most approximate to those of the control group in different periods.

The excision of periostea might disturb the maxilla "organic center" and affect the growth and development of maxilla in the periosteum excision group. Compared with the mucosal flap excision group, the same histological features of the tissues were found except that the collagen fibers were connected with periodontal ligament. This method also can prevent the formation of Sharpey's fibers and block local contraction forces. Thus the degree of maxilla and dental arch growth inhibition was reduced. The conjunction of fibers and the periodontal ligament, injured periosteum and the more serious damage of blood vessels and nerves in this group might explain the maxilla and dental arch widths difference in the mucosal flap excision group and the periosteum excision group. Fluorescent labeling showed that the alignment of labeling lines changed from disorder to regular 4 weeks after operation, which might reflect the bundle bone formation switched to the lamellar type deposition. Furthermore, the bone deposition rates of the four phases were slower than those in the mucosal flap excision group. All of these reasons could testify that the method in the periosteum excision group, which simulated the technique of mucosa origin from autogenic covering, could prevent the inhibition of maxilla and dental arch growth after surgery. However, the effect was weaker than that by the method in the mucosal flap excision group which simulated the partially split flap technique.

Above all, we could demonstrate that the remain of the periosteum in palatal lateral area is important to the growth and development of maxilla and dental arch after surgery. Urist and Mclean (1952)'s and Zheng *et al*.(2006)'s work showed that the osteogenic role of periosteum played an important part in membrane bone formation. The growth and development of the palatine perform a pattern of intramembranous ossification and new bony tissue forms along the periosteum layer. Furthermore, intact periosteum can prevent hematoma, development and attachment of Sharpey's fibers. Therefore, the method of retaining periosteum could reduce the degree of maxilla and dental arch growth inhibition following surgery.

CONCLUSION

There were different effects on the growth and development of the maxilla and dental arch in different types of palatal lateral excisions. Periosteum is important for bone formation and the pattern of deposition. The prevention of Sharpey's fibers forming and attaching to the palatine can effectively avert the following malformation.

References

Burr, D.B., Schaffler, M.B., Yang, K.H., Wu, D.D., Lukoschek, M., Kandzari, D., Sivaneri, N., Blaha, J.D., Radin, E.L., 1989. The effects of altered strain environments on bone tissue kinetics. *Bone*, **10**(3):215-221. [doi:10.1016/8756-3282(89)90056-2]

Chu, S., Ishikawa, H., Kim, T., Yoshida, S., 2000. Analysis of scar tissue distribution on rat palates: a laser Doppler flowmetric study. *Cleft Palate Craniofac. J.*, **37**(5): 488-496. [doi:10.1597/1545-1569(2000)037<0488:AOSTDO>2.0.CO;2]

Diehl, H., Ellingboe, J.L., 1956. Indicator for titration of calcium in presence of magnesium using disodium dihydrogen ethylenediamine tetraacetate. *Anal. Chem.*, **28**(5): 882-884. [doi:10.1021/ac60113a030]

Ducy, P., Schinke, T., Karsenty, G., 2000. The osteoblast: a sophisticated fibroblast under central surveillance. *Science*, **289**(5484):1501-1504. [doi:10.1126/science.289.5484.1501]

Graber, T.M., 1949. A ceghalometric analysis of the developmental pattern and facial morphology in cleft palate. *Angle Orthod.*, **19**(2):91-100.

Ham, A.W., Cormack, D.H. (Eds.), 1979. Bone and Bones. *In*: Histology. JB Lippincott, Philadelphia, p.387-388.

In de Braekt, M.M.H., Maltha, J.C., Kuijpers-Jagtman, A.M., 1995. Poly-(L-lactic) acid membranes in palatal surgery in beagle dogs: clinical and histologic evaluation. *Cleft Palate Craniofac. J.*, **32**(4):290-298. [doi:10.1597/1545-1569(1995)032<0290:PLLAMI>2.3.CO;2]

Ishikawa, H., Nakamura, S., Misaki, K., Kudoh, M., Fukuda, H., Yoshida, S., 1998. Scar tissue distribution on palates

and its relation to maxillary dental arch form. *Cleft Palate Craniofac. J.*, **35**(4):313-319. [doi:10.1597/1545-1569(1998)035<0313:STDOPA>2.3.CO;2]

Ito, S., Noguchi, M., Suda, Y., Yamaguchi, A., Kohama, G., Yamamoto, E., 2006. Speech evaluation and dental arch shape following pushback palatoplasty in cleft palate patients: supraperiosteal flap technique versus mucoperiosteal flap technique. *J. Craniomaxillofac. Surg.*, **34**(3): 135-143.

Jiménez, M.D.A., Gil, I.S., Corvillo, M.A.P., 1988. Determination of calciumand magnesium in milk by complexometric titration using protein precipitation and complexation with Palladiazo or other indicators. *Analyst*, **113**(4):633-635. [doi:10.1039/an9881300633]

Kim, T., Ishikawa, H., Chu, S., Handa, A., Iida, J., Yoshida, S., 2002. Constriction of the maxillary dental arch by mucoperiosteal denudation of the palate. *Cleft Palate Craniofac. J.*, **39**(4):425-431. [doi:10.1597/1545-1569(2002)039<0425:COTMDA>2.0.CO;2]

Kremenak, C.R.Jr., Searls, J.C., 1971. Experimental manipulation of midfacial growth: a synthesis of five years of research at the Iowa Maxillofacial Growth Laboratory. *J. Dent. Res.*, **50**(6):1488-1491.

Lanyon, L.E., Goodship, A.E., Pye, C.J., MacFie, J.H., 1982. Mechanically adaptive bone remodelling. *J. Biomech.*, **15**(3):141-154. [doi:10.1016/0021-9290(82)90246-9]

Lee, T.C., Arthur, T.L., Gibson, L.J., Hayes, W.C., 2000. Sequential labelling of microdamage in bone using chelating agents. *J. Orthop. Res.*, **18**(2):322-325. [doi:10.1002/jor.1100180222]

Leenstra, T.S., Kuijpers-Jagtman, A.M., Maltha, J.C., Freihofer, H.P., 1995a. Palatal surgery without denudation of bone favours dento-alveolar development in dogs. *Int. J. Oral Maxillofac. Surg.*, **24** (6):440-444. [doi:10.1016/S0901-5027(05)80474-6]

Leenstra, T.S., Maltha, J.C., Kuijpers-Jagtman, A.M., Spauwen, P.H., 1995b. Wound healing in beagle dogs after palatal repair without denudation of bone. *Cleft Palate Craniofac. J.*, **32**(5):363-369. [doi:10.1597/1545-1569(1995)032<0363:WHIBDA>2.3.CO;2]

Leenstra, T.S., Kohama, G.I., Kuijpers-Jagtman, A.M., Freihofer, H.P., 1996. Supraperiosteal flap technique versus mucoperiosteal flap technique in cleft palate surgery. *Cleft Palate Craniofac. J.*, **33** (6):501-506. [doi:10.1597/1545-1569(1996)033<0501:SFTVMF>2.3.CO;2]

Lynch, J.B., Peil, R., 1966. Retarded maxillary growth in experimental cleft palates mechanical binding of scar tissue in puppies. *Am. Surg.*, **32**(7):507-511.

Meng, T., Shi, B., Huang, X., Zheng, Q., Wang, Y., Wu, M., Lu, Y., Li, S., 2007. Roles of different areas of palatine bone denudation on growth and development of the maxilla and dental arch: an experimental study. *J. Craniofac. Surg.*, **18**(2):391-398. [doi:10.1097/01.scs.0000235109.15860.f7]

Noguchi, M., Suda, Y., Ito, S., Kohama, G., 2003. Dento-alveolar development in unilateral cleft lip, alveolus and palate. *J. Craniomaxillofac. Surg.*, **31**(3): 137-141.

O′Brien, F.J., Taylor, D., Lee, T.C., 2003. Microcrack accumulation at different intervals during fatigue testing of compact bone. *J. Biomech.*, **36**(7):973-980. [doi:10.1016/S0021-9290(03)00066-6]

Ophof, R., Maltha, J.C., von den Hoff, J.W., Kuijpers-Jagtman, A.M., 2004. Histologic evaluation of skin-derived and collagen-based substrates implanted in palatal wounds. *Wound Repair Regen.*, **12**(5):528-538. [doi:10.1111/j.1067-1927.2004.012505.x]

Ophof, R., Maltha, J.C., Kuijpers-Jagtman, A.M., von den Hoff, J.W., 2008. Implantation of tissue-engineered mucosal substitutes in the dog palate. *Eur. J. Orthod.*, **30**(1):1-9. [doi:10.1093/ejo/cjm082]

Pautke, C., Tischer, T., Vogt, S., Haczek, C., Deppe, H., Neff, A., Horch, H.H., Schieker, M., Kolk, A., 2007. New advances in fluorochrome sequential labelling of teeth using seven different fluorochromes and spectral image analysis. *J. Anat.*, **210**(1):117-121. [doi:10.1111/j.1469-7580.2006.00660.x]

Perko, M.A., 1974. Primary closure of the cleft palate using a palatal mucosal flap: an attempt to prevent growth impairment. *J. Maxillofac. Surg.*, **2**(1):40-43. [doi:10.1016/S0301-0503(74)80012-3]

Pigott, R.W., Albery, E.H., Hathorn, I.S., Atack, N.E., Williams, A., Harland, K., Orlando, A., Falder, S., Coghlan, B., 2002. A comparison of three methods of repairing the hard palate. *Cleft Palate Craniofac. J.*, **39**(4):383-391. [doi:10.1597/1545-1569(2002)039<0383:ACOTMO>2.0.CO;2]

Searls, J.C., Biggs, D.R., 1974. Surgically induced maxillary growth inhibition in rats. *Cleft Palate J.*, **11**:1-16.

Shetye, P.R., 2004. Facial growth of adults with unoperated clefts. *Clinics in Plastic Surgery*, **31**(2):361-371. [doi:10.1016/S0094-1298(03)00137-8]

Shi, B., Song, Q.G., Huang, X., 2003. Maxillary growth following tissue engineered oral mucosal implantation on mucoperiosteal denuded palate process in rats. *West China J. Stomatology*, **21**(1):255 (in Chinese).

Suzuki, S., 1986. Histomorphometric study on growing condyle of rat. *Bull. Tokyo. Med. Dent. Univ.*, **33**(1): 23-34.

Urist, M.R., Mclean, F.C., 1952. Osteogenic potency and new bone formation by induction in transplants to the anterior chamber of eye. *J. Bone Joint Surg.*, **34A**:443-476.

Vander Poorten, V., Ostyn, F., van Kerckhoven, W., Wellens, W., Breuls, M., Verdonck, A., Vergalle, C., Schoenaers, J.H., 2006. The Leuven staged supraperiosteal retropositioning repair: long-term velopharyngeal function in non-syndromic cleft palate. *B-ENT.*, **2**(Suppl. 4):35-43.

Wallach, D.F.H., Surgenor, D.M., Soderberg, J., Delano, E., 1959. Preparation and properties of 3,6-dihydroxy-2,4-bis-[*N,N′*-(carboxymethyl)-aminomethyl] fluoran. Utilization for the ultramicrodetermination of calcium. *Anal. Chem.*, **31**(3):456-460. [doi:10.1021/ac60147a041]

Weinzweig, J., Panter, K.E., Seki, J., Pantaloni, M., Spangenberger, A., Harper, J.S., 2006. The fetal cleft palate: IV. Midfacial growth and bony palatal development following in utero and neonatal repair of the congenital caprine model. *Plast Reconstr. Surg.*, **118**(1): 81-93. [doi:10.1097/01.prs.0000221062.93132.91]

Wijdeveld, M.G., Grupping, E.M., Kuijpers-Jagtman, A.M., Maltha, J.C., 1987. Wound healing of the palatal mucoperiosteum in beagle dogs after surgery at different ages. *J. Cranio-Maxillofac. Surg.*, **15**(2):51-57. [doi:10.1016/S1010-5182(87)80018-5]

Wijdeveld, M.G., Grupping, E.M., Kuijpers-Jagtman, A.M., Maltha, J.C., 1989. Maxillary arch dimensions after palatal surgery at different ages on beagle dogs. *J. Dent Res.*, **68**(6):1105-1109.

Wijdeveld, M.G., Maltha, J.C., Grupping, E., de Jonge, J., Kuijpers-Jagtman, A.M., 1991. A histological study of tissue response to simulated cleft palate surgery at different ages in beagle dogs. *Arch. Oral Biol.*, **36**(11): 837-843. [doi:10.1016/0003-9969(91)90033-Q]

Zheng, Y.X., Ringe, J., Liang, Z., Loch, A., Chen, L., Sittinger, M., 2006. Osteogenic potential of human periosteum-derived progenitor cells in PLGA scaffold using allogeneic serum. *J. Zhejiang Univ. Sci. B*, **7**(10):817-824. [doi:10.1631/jzus.2006.B0817]