Effect of Piezoelectric Sutural Ostectomies on Accelerated Bone-Borne Sutural Expansion

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Purpose: The present study investigated the effect of piezoelectric sutural ostectomies on accelerated bone-borne sutural expansion.

Materials and Methods: Sixteen male New Zealand white rabbits (20 to 24 weeks old) were randomly divided into 4 experimental groups (n = 4): group 1, conventional rapid sutural expansion; group 2, accelerated sutural expansion; group 3, accelerated sutural expansion with continuous ostectomy; and group 4, accelerated sutural expansion with discontinuous ostectomy. All sutural ostectomies were performed using a piezoelectric instrument (Woodpecker DTE, DS-II, Guangxi, China) before expander application with the rabbits under anesthesia. Modified hyrax expanders were placed across the midsagittal sutures of the rabbits and secured with miniscrew implants located bilaterally in the frontal bone. The hyrax expanders were activated 0.5 mm/day for 12 days (group 1) or with a 2.5-mm initial expansion, followed by 0.5 mm/day for 7 days (groups 2 to 4). After 6 weeks of retention, the bone volume fraction, sutural separation, and new bone formation were evaluated using micro-computed tomography and histomorphometry. Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney U tests and Spearman’s rho correlation (P < .05).

Results: Ranking of the median sutural separation was as follows: group 1, 3.05 mm; group 2, 3.97 mm; group 4, 4.78 mm; and group 3, 5.66 mm. The least and most bone formation were observed in groups 1 (63.63%) and 3 (75.93%), respectively. Spearman’s correlation showed a strong, positive, and significant correlation (r = 0.932; P < .01) between the new sutural bone formation and amount of sutural separation.

Conclusions: Piezoelectric sutural ostectomies increased the rate of sutural separation and promoted new sutural bone formation/osteogenesis. Continuous ostectomy gave better results than discontinuous ostectomy.

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Orthodontic treatment of adult patients with maxillary transverse deficiency using tooth-borne maxillary expansion appliances is often associated with minimal skeletal expansion, the risk of vestibular protrusion or extrusion of posterior teeth, fenestration in bone lamella, an inability to open the palatal suture, and/or treatment relapse. To limit these undesirable effects, bone-borne maxillary expanders were developed by attaching appliances directly to the palatine bone using miniscrew implants. The use of direct forces to expand mature midpalatal sutures can, however, lead to high sutural stresses and variable degrees of pain.

Surgically assisted rapid maxillary expansion procedures (SARME) can facilitate transverse maxillary expansion in mature patients or in adolescents and young adults in whom orthodontic treatment has been ineffective. It was first described by Kole in 1959 and has undergone many modifications since. The areas of bony resistance include the piriform aperture pillars, zygomatic buttresses, pterygoid junctions, and midpalatal suture. The midpalatal suture has been reported to offer the greatest resistance to maxillary expansion. Transcending bony resistance at the midpalatal suture is essential for successful maxillary expansion in adult patients.

Methods of making surgical procedures for SARME simpler, safer, and more predictable have been explored and include the use of piezoelectric surgery. The latter significantly reduces traumatic side effects, operative site bleeding, procedural time, and healing time associated with conventional rotary protocols. Piezoelectric surgery uses high-frequency micro-vibrations for precise cutting of tooth and bone structures without damaging the adjacent soft tissues. In addition to SARME-related procedures, piezoelectric surgery has many other applications, including apicectomy, alveolar bone augmentation, sinus augmentation, and various maxillary and mandibular corticotomies. Currently, no consensus has been reached pertaining to the extent and surgical procedure for SARME. Surgical techniques range from midline (sutural), lateral and/or anterior maxillary osteotomies to full Le Fort I osteotomy from the piriform aperture to the pterygomaxillary fissure, together with a midpalatal split.

Accelerated bone-borne sutural expansion protocols involving large initial appliance activation were recently investigated. Although conventional rapid sutural expansion regimens involve daily activations of 0.25 to 0.5 mm/day, initial expansions of up to 4 mm have been studied. The findings indicated that the protocol involving a 2.5-mm initial expansion was the optimal amount for accelerated sutural expansion. This accelerated sutural protocol could be further enhanced using piezoelectric sutural ostectomy. The objective of the present study was to investigate the effect of piezoelectric sutural ostectomies on accelerated bone-borne sutural expansion using micro-computed tomography (micro-CT) and histomorphometry. Differences between continuous and discontinuous sutural ostectomies were also explored.

**Materials and Methods**

**ANIMAL SELECTION AND PREPARATION**

The University of Malaya Faculty of Medicine institutional animal care and use committee approved the present study (approval no. 2015-16/006/DENTAL/R/ASH), and all animal studies were performed according to the standards specified by the institutional animal care and use committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Sixteen male New Zealand white rabbits aged 20 to 24 weeks were acquired for the study. The midsagittal sutures of the rabbits, which act analogously to midpalatal sutures, were targeted, because they are wide enough for expander application and miniscrew implant placement. The relatively small size of the rabbits permitted whole animal insertion and repeated measurements in a micro-CT scanner. Because the haversian system of rabbits is similar to that of humans, the model also allowed for some extrapolation of the results to clinical care. Adult male rabbits were chosen to reduce the confounding factors arising from growth and hormonal changes. A sample size of 4 rabbits was determined using the resource equation method and previous similar studies. The rabbits (approximate weight 3.0 to 4.0 kg) were procured from a licensed farm, examined, weighed, and observed twice daily for 2 weeks for acclimatization until conducting the experiment. The rabbits were subsequently randomly divided into 4 experimental groups (n = 4): group 1, conventional rapid sutural expansion; group 2, accelerated sutural expansion; group 3, accelerated sutural expansion with continuous ostectomy; and group 4, accelerated sutural expansion with discontinuous ostectomy. Preoperative intramuscular ketamine (30 mg/kg) and xylazine (3 mg/kg) were given to the rabbits. The distraction sites were then anesthetized using 2 mg/kg Marcaine (Abbott Laboratories, Chicago, IL) with 1:200,000 epinephrine. General anesthesia was subsequently accomplished using 1% to 3% isoflurane in a 2:1 oxygen/nitrous oxide mixture administered through special face masks.

**SURGICAL PROCEDURES**

The skin and the periosteum were reflected midway between the anterior and posterior limits of the orbital...
rims exposing the midsagittal suture and the frontal bone of the rabbits. For groups 3 and 4, sutural ostectomies were performed on the midsagittal sutures using a piezoelectric device (Woodpecker DTE, DS-II, Guangxi, China) and diamond surgical tip (model US1; size 4 mm; thickness 0.5 mm). The ostectomies were continuous (20 mm long through the midsagittal suture) and discontinuous (4-mm cuts with 4-mm intermissions over 20 mm) in groups 3 and 4 (Fig 1), respectively. The piezoelectric cuts, which were 1.5 to 2 mm deep, were made on nearly the whole suture with selected portions left intact (inner surface of the suture toward the nose). Pilot holes were drilled using a size 2 round bur at low speed (less than 600 rpm) and with copious saline solution irrigation for all groups. Four Dentos (Daegu, Korea) miniscrew implants (5.0 mm in length and 1.7 mm in diameter) were then manually placed on both sides of the midsagittal suture with a hand-driver to secure the expansion appliance.

EXPANSION PROCEDURES

Customized expansion appliances were fabricated by laser welding four 0.9-mm stainless steel U loops to Hyrax expanders (Leone Spa, Firenze, Italy). The modified Hyrax appliances were secured by engaging the heads of the miniscrew implants (Fig 1). The surgical flaps were subsequently closed, and the rabbits were given postoperative sulfamethoxazole 200 mg and trimethoprim 40 mg (0.4 mL) to prevent infection and meloxicam 0.2 mg/kg to minimize discomfort. The Hyrax expanders were activated 0.5 mm/day for 12 days in group 1 and 2.5 mm initially, followed by 0.5 mm/day for 7 days in groups 2, 3, and 4. The total activation for the Hyrax expanders was standardized at 6 mm for all 4 groups. On completion of the active expansion, the screws of the Hyrax appliances were fixed with light-cured acrylic and left passive. After 6 weeks of retention, sutural separation and new bone formation were assessed using micro-CT. The rabbits were then euthanized with a high dose (90 mg/kg) of phenobarbitone.

MICRO-CT ANALYSIS

Before micro-CT imaging, the rabbits were again anesthetized with ketamine and xylazine. Post-treatment scans of the distraction sites were obtained using high-resolution in vivo X-ray micro-CT imaging (XtremeCT; Scanco Medical, Bassersdorf, Switzerland). Serial CT images were acquired transverse to the midsagittal sutures at 60 kVp and 900 µA. One thousand projections were achieved per rotation with an integration time of 300 ms and a voxel size 41 µm. An aggregate of 160 slices located between the anterior and posterior miniscrew implants was obtained. A region of interest (ROI) 3 mm to the left and right of the midline was identified and applied to all samples (Fig 2A). Three-dimensional (3D) images of the ROI were automatically reconstructed from the 160 slices using the Scanco Micro-CT, version 6.5.3, software (Fig 2C). The grayscale images were smoothed using a gaussian filter with a sigma value of 0.9 and support value of 1. Lower and upper threshold values of 130 to 1000 units were applied to calculate the bone volume fraction (BV/TV), where BV is the mineralized bone volume and TV is the total tissue volume.

After segmentation of bone tissues using these threshold values, a 3D color map of the suture tissue separation was generated. Separation maps of the suture tissue indicate maximum separation values with blue to red colors, indicating increasing degrees of tissue separation (Fig 2B). To calculate the sutural space volume, micro-CT images were exported into Mimics Medical, version 17.0, imaging software (Mimics; Materialise, Leuven, Belgium). The lower and upper thresholds were set for all specimens between 224 to 1249 units to separate soft tissue from bone tissue. Thus, the soft tissue was highlighted on the display in specific colors (yellow) using so-called masks. The mask was cropped to select the ROI and used for all specimens. Region growing (computer-assisted separation of different tissues) and manual deletion of tissues (using a multiple slice editing tool) were performed until only the ROI remained. The software then calculated the sutural soft tissue volume (sutural space volume) by voxel addition and reconstructed a 3D image (Fig 3).

The amount of sutural separation was established using the Radiant Digital Imaging and Communications in Medicine viewer, version 3.4.1. The most anterior and posterior slices for the ROI were first ascertained to locate the bony outline of the sutures. Sutural separation was then calculated using the distance measurement tool (Fig 4) at the point equidistant between the outer surface and inner surface of the frontal bone and using an average of 2 separate readings. The intraexaminer reliability for mapping the ROI and measuring the sutural space volume and sutural separation were assessed by repeating the procedures blinded after 2 weeks. Reliability was evaluated using the Cronbach α test.

HISTOLOGIC ANALYSIS

After sacrificing the rabbits, a standardized area, including the midsagittal region and adjacent bone, was dissected, fixed with 4% paraformaldehyde prepared in phosphate-buffered saline for 48 hours at 4°C, washed scrupulously with running water, and decalciﬁed in 10% ethylenediaminetetraacetic acid (2 Na, pH
FIGURE 1. A, Group 1 immediately after 0.5-mm hyrax activation; B, group 2 immediately after 2.5-mm hyrax activation; (Fig 1 continued on next page.)

FIGURE 1 (cont’d). C, group 3 immediately after continuous ostectomy; and D, group 4 immediately after discontinuous ostectomy. Dotted arrows indicate the location of the miniscrew implants. MHE, modified hyrax expander; MSIs, miniscrew implants (5.0 mm in length, 1.7 mm in diameter); MSS, midsagittal suture; UL, U loop made from 0.9-mm stainless steel wire.

7.4) for 2 weeks, dehydrated with a series of ethanol solutions, and embedded in paraffin. The paraffin blocks were sectioned coronally to 4-μm-thick slices in a microtome at 20°C and mounted on polarized glass slides. For each paraffin block, 3 slides were assigned for histologic examination.

The histologic specimens were stained with hematoxylin and eosin (H&E) and digitized using the Panoramic SCAN digital slide scanner (3DHISTECH, Budapest, Hungary). The histologic images were subsequently assessed using the Panoramic viewer software, version 1.15.3 (3DHISTECH) at 4× magnification. The amount of sutural separation was determined at the upper, middle, and lower parts of the lateral edge of each suture. The position of the 3 segments was defined by the incremental lines separating old from newly formed bone. The distance between the 2 sides of the 3 suture segments was measured using the micrometer measuring tool in the software and averaged for each photomicrograph and rabbit. The expanded suture with the adjacent 2 mm of frontal bone yielded a total of 48 sections, from which 240 microscopic fields (4 peripheral and 1 central) were obtained for the various experimental groups. Quantitative analysis
was performed using the Image-Pro Express software (Media Cybernetics Inc., Bethesda, MD) for Windows. A 48-point grid was overlaid on each microscopic field to measure the amount of newly formed bone, blood capillaries, and nonosteoid tissue in the sutures using the point-counting method. The number of points on the newly formed bone matrix, blood capillaries, and nonosteoid tissue were quantified and statistically analyzed.

Interexaminer reliability for measuring the amount of sutural separation and point-counting method was assessed by 2 blinded examiners. Reliability was again evaluated using the Cronbach \( \alpha \) test.

**STATISTICAL ANALYSIS**

All data were analyzed using the Statistical Package for Social Sciences, version 20.0 (SPSS for Windows, SPSS Inc., Chicago, IL). Normality testing was performed using the Shapiro-Wilk test. Because the data were not normally distributed, nonparametric Kruskal-Wallis and Mann-Whitney \( U \) tests \( (P < .05) \) were used to determine the significant differences in sutural separation, BV/TV, sutural space volume, sutural tissue separation, newly formed bone, and angiogenesis among the experimental groups. Spearman's rho correlations \( (P < .05) \) were also performed to establish the associations between the different variables.

**Results**

The rabbits did not display any obvious signs of systemic illness, distress, or local adverse effects throughout the study. Also, no substantial changes in body weight were observed between the experimental groups during the expansion and retention periods. The bone-borne modified hyrax appliances successfully expanded the midsagittal sutures in all 4 experimental groups. No dislodgement or failure of the miniscrew implants was witnessed. Reliability testing revealed no significant differences in micro-CT analysis between assessment periods \( (\alpha = 0.92) \) or examiners regarding the sutural measurements and point-counting method \( (\alpha = 0.89 \text{ and } \alpha = 0.94, \text{ respectively}) \).

**MICRO-CT ANALYSIS**

The median sutural separation was 2.84, 3.69, 5.54, and 4.91 mm for groups 1 to 4, respectively (Table 1). Paired comparisons showed statistically significant differences in sutural separation between experimental groups. Sutural separation in the ostectomized rabbits (groups 3 and 4) was significantly greater than the groups without ostectomy (groups 2 and 1). Continuous ostectomy (group 3) yielded larger sutural separation than that with discontinuous ostectomy. The sutural separation for group 3 was 50.14% and
95.07% greater than that for groups 2 and 1, respectively.

The median BV/TV, sutural space volume, and sutural tissue separation after 6 weeks of retention are listed in Table 1. With the exception of groups 2 and 4, paired comparisons showed statistically significant differences in the BV/TV between all groups. The sutural space volume and sutural tissue separation in group 3 were significantly lower than those of the other groups. Likewise, the volume and separation for group 2 were significantly lower than those for group 1. Spearman’s correlation showed a strong, positive, and significant association ($r = 0.906; P < .01$) between BV/TV and sutural separation. In contrast, the correlation between BV/TV and sutural space volume was strong, negative, and significant ($r = -0.953; P < .01$).
Biometric investigation of the histologic sections showed median sutural separations of 3.05, 3.97, 5.66, and 4.78 mm for groups 1 to 4, respectively (Table 1). Paired comparisons showed statistically significant differences in biometric sutural expansion between the groups. Groups 3 and 4 showed significantly greater sutural separation than groups 1 and 2. The sutural separation for group 3 was 42.57% and 85.57% greater than that for groups 1 and 2, respectively. The sutural separation for group 3 was significantly larger than that for group 4.

The H&E-stained sections for group 1 showed less osteogenic connective tissues within the bony bridge and bone trabeculae than in the other groups (Fig 5). Furthermore, the expanded sutures were clearly outlined and filled with a variable quantity of irregular woven bone trabeculae with primitive bone marrow and cellular tissues. Bone trabeculae were observed to be discontinuous in the center of the sutural gaps and continuous at the margins for the latter group. The osteoid tissues, which were oriented from the margins to sutural gap centers, appeared to be less dense in group 1 than in the other groups (Fig 6). Microscopic sections for group 2 showed thick bridges and matured bone trabecula with osteoblastic activity and a medullary space that virtually closed the sutural gaps. In addition, dense osteoid tissues with congested blood vessels were observed (Fig 5). Furthermore, areas of bone remodeling and apposition and osteon formation, filled by osteocytes with regular bone disposition, were frequently seen in the sutural gaps in group 2 (Fig 6). Bony islands and bone marrow-containing areas were, however, less obvious in sutural gaps of group 2 compared with group 3.

In group 3 (continuous ostectomy), microphotography of the histlogic section revealed bone regeneration between the distracted segments and areas of new bone formation lined by osteoblast cells (Fig 5), the presence of numerous blood vessels and newly formed osteocytes, and intense osteogenic activity (Fig 6). This tissue was characterized by the presence of...
of trabecular bone with connective tissue on the interim (osteoid tissue) and mature bone tissue at the margin of the defect. The distraction gap was filled with mature and immature bone, together with additional angiogenesis associated with high osteoblastic activity and intense bone deposition. New blood
capillaries with high osteoblastic activity, intense bone deposition, and remodeling featured more prominently in group 3 than in the other groups (Fig 6).

The histologic section of group 4 (discontinuous ostectomy) demonstrated less bone formation than in group 3 but more than that in groups 1 and 2 (Fig 5). It also exhibited less trabeculae and more fibrous callus occupying most areas of the expanded suture compared with group 3 (Fig 6).

Group 3 had significantly more new bone formation and blood capillaries than the other groups. Group 4 also had significantly more blood capillaries than in groups 1 and 2. In addition, a significantly greater percentage of nonosteoid tissue was observed in group 1 than in the other groups. The proportion of nonosteoid tissue in group 3 was significantly lower than that in groups 2 and 4. Spearman's correlation showed a strong, positive, and significant correlation ($r = 0.932; P < .01$) between new sutural bone formation and sutural separation.

Discussion

The present study examined the effect of piezoelectric sutural ostectomies on accelerated bone-borne sutural expansion and compared the differences
between continuous and discontinuous sutural ostectomies. The midpalatal split technique for SARME was originally described in 1938. Converse and Horowitz subsequently proposed the use of both labial and palatal cortical osteotomies, and Pogrel et al suggested a midpalatal cut combined with transection of the lateral support for expansion. Instead of a single midline maxillary osteotomy, other investigators used 2 paramedian palatal osteotomies from the posterior nasal spine to a point just distal to the incisive canal. Timms and Vero advocated 3 surgical stages for maxillary expansion based on the patient’s age. Stage 1 (midpalatal osteotomy) is performed for patients aged 25 years or older, or younger if rapid maxillary expansion had been attempted without success. Stage 2 (midpalatal and lateral osteotomies) is indicated for patients aged 30 years or older, and stage 3 (midpalatal, lateral maxillary, and anterior maxillary osteotomies) for patients aged 40 years or older. The midpalatal split or sutural ostectomy was traditionally performed with burrs, osteotomes, or reciprocating saws between the central incisors with a soft tissue incision. In recent years, the use of minimally invasive piezoelectric ostectomies was advocated to decrease the adverse side effects of conventional instrumentation.

Anchorage for the customized Hyrax expanders was achieved with miniscrew implants. The success rate of the miniscrew implants (100%) was greater than that reported by Liu et al (88%) using a similar rabbit model. The incongruity can be attributed to the use of more and longer miniscrew implants in our study (5 vs 3 mm) and the strength of the laser welded joint between the U loops and Hyrax expanders.
Furthermore, Liu et al.\(^2\) used nickel-titanium open-coil springs that provided limited control over the direction and amount of the forces placed on the miniscrew implants. Moreover, sutural resistance to the expansion in our study was probably lower than that in the study by Liu et al.\(^2\) owing to the sutural ostectomies (groups 3 and 4). Our success rate was comparable to that of Carrillo et al.,\(^3\) who reported 99% success when miniscrew implants were used for orthodontic anchorage in beagle dogs. Garfinkle et al.\(^4\) found that loaded miniscrew implants have a greater success rate than unloaded ones and postulated that the applied forces augment the initial mechanical retention and stimulate osseous adaptation.

Both continuous and discontinuous ostectomies increased the potential for sutural expansion. Micro-CT and histologic analysis confirmed the greater extent of sutural separation in the ostectomized groups. The differences in sutural separation can be attributed in part to the reduced sutural resistance in these experimental groups. Our findings corroborated those of Wright\(^5\) on the frontonasal sutures of adult rabbits. Wright\(^6\) concluded that mature sutures expanded with adjunctive surgery undergo 31% more sutural separation than those without surgery. The quantum of sutural expansion was, however, much greater in our study and could be ascribed to the accelerated sutural expansion protocol comprising 2.5-mm initial Hyrax activation and effective miniscrew implant anchorage.

The greater BV/TV and new sutural bone formation in the ostectomized groups (ie, groups 3 and 4) were associated with more blood capillaries and smaller amount of nonosteoid tissue. The local blood supply affects the pattern of bone-forming process, with bone formation occurring in regions with adequate vascularity and cartilage formation in ischemic areas.\(^7\) The development of blood vessels during the first weeks after surgery might therefore be the crucial factor affecting tissue regeneration. Penetrating blood vessels increase the oxygen partial pressure and pH in the surrounding tissues. Low oxygen partial pressure and pH stimulate osteogenic cells to differentiate into chondroblasts, leading to cartilaginous tissue formation and, consequently, less bone development.\(^8\) Clinical and laboratory studies on the use of piezoelectric instruments for tooth excision and bone surgery reported ease of tooth and bone removal, greater precision, and favorable osseous responses. In addition, patient discomfort appeared to be reduced, resulting in greater acceptance.\(^9\) Furthermore, piezoelectric surgery was found to promote biologic effects on odontoblast-like cells, osteoblasts, and osteogenic cells and might be potentially beneficial for osseointegration.\(^10\) Because sutural bone remodeling is enhanced with piezoelectric ostectomies, more new bone formation in the sutures is anticipated. The increased amount of new sutural bone formation observed explained the negative correlation of BV/TV and sutural space volume.

Ostectomies and subsequent regional acceleratory phenomenon (RAP) have also been shown to increase the cellular response at the site of injury. Sebaoun et al.\(^11\) found that selective alveolar decortications resulted in a threefold increase in osteoclastic activity and bone apposition at the site of injury. Hou et al.\(^12\) demonstrated that expansion alone promoted bone resorption through increased osteoclast activity and activated proliferation of periosteal cells to form new bone and cartilage in rats. Because expansion alone has the capacity to induce remodeling similar to RAP, it might be plausible to increase its effect by causing surgical insult with piezoelectric ostectomies.\(^13\) Continuous ostectomy performed better than discontinuous ostectomy. The smaller BV/TV and new sutural bone formation with discontinuous ostectomy was associated with a lower percentage of blood vessels. The sutural areas that were not ostectomized might have been subjected to large expansion forces that might rupture the blood capillaries, leading to less bone remodeling. In addition, RAP, which normally follows ostectomy, might not occur in these regions, which could also decrease new bone formation.

Piezoelectric surgery is able to enhance the biologic responses of osteoblasts and osteogenic cells and could have potential benefits in osseointegration.\(^14\) These events could explain the lower percentage of BV/TV and new sutural bone formation in the discontinuous piezoelectric ostectomy group (group 3).

Our study had several limitations. First, we used an animal model for sutural expansion; thus, the results should not be directly extrapolated to clinical practice, because the human palate is structurally different and larger in dimension. A longer retention period is also needed to confirm the favorable preliminary data. Further histologic and animal studies with larger sample sizes are needed to evaluate the long-term stability of the newly formed bone. Accelerated bone-borne sutural expansion augmented with continuous piezoelectric ostectomy holds promise as a technique for increasing sutural expansion and reducing the treatment duration in mature patients with transverse maxillary deficiency.

In conclusion, the effect of continuous and discontinuous piezoelectric sutural ostectomies on accelerated bone-borne sutural expansion was investigated. Within the limitations of our study, the following conclusions can be made: 1) piezoelectric ostectomy increases the rate of sutural separation and promotes new sutural bone formation/osteogenesis; and 2) continuous ostectomy offered better outcomes than discontinuous ostectomy.
PIEZOELECTRIC SUTURAL OSTECTOMIES ON ACCELERATED SUTURAL EXPANSION

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