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A rat model for orthodontic translational expansive tooth movement

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Structured Abstract

Objectives – To present the development of an experimental model in rats for translational expansive tooth movement.

Setting and Sample – Section of Periodontology at Department of Dentistry Aarhus University. Twenty male Wistar rats in two pilot experimental settings plus seven animals without any intervention serving as controls.

Material and Methods – The second molar (group P1) or the second and third molar (group P2) in the maxillae of the animals were moved buccally using transpalatal β -titanium springs. In the group P2, two spring types (high force and low force) and two preangulations (0° passive or 30° torsion moment) were tested. The amount and type of tooth movement achieved and the resulting skeletal effect were assessed on microCT images, histological analysis was performed on few selected specimens.

Results – Expansive translational root movement amounting half a tooth width was achieved. Comparison of the amount of tooth movement at the right and left side of the maxilla showed that the expansion was rather symmetrical in the P2 group. Skeletal widening of the maxilla contributed in the P2 group to approximately one-third of the total root movement, whereas two-thirds were dental movement.

Conclusion – With the model used in the P2 group, further research on translational expansive tooth movement and its effect on the periodontium can be pursued. In models for orthodontic expansion, it is strongly recommended to separately evaluate skeletal and dental effects.

Key words: bone dehiscence; orthodontics; periodontium; recession; tooth movement

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Introduction

Orthodontic arch expansion is often associated with clinically important issues like alveolar bone loss, development of gingival recession, and lack of treatment stability. Although orthodontic expansion has been associated with development of bone dehiscences and gingival recessions (1, 2), most retrospective clinical studies fail to observe any clinically relevant recession development after expansion. In these studies, the expansion was evaluated by measuring changes in arch length between crowns of incisors and molars (3, 4) or in tooth inclination (5). However, the change in position of the root to the alveolar envelope (6) may be of greater importance in regard with dehiscence and recession development.

Adverse effects with relatively low incidence are difficult to investigate in prospective human trials. An alternative would be to use an animal model. The available experiments in rodents are either on mesio-distal tooth movements, for example toward edentulous regions or extraction sockets (7–18) or on small amounts of facial inclination (19, 20). These models do not involve translational movement beyond the skeletal envelope.

There is no small-animal model for expansive translational tooth movement with well-defined forces and the possibility for evaluation of the amount of root movement (position change), type of movement (inclination change), and skeletal widening (maxillary expansion by sutural bone apposition). Such a model would be relevant to examine the incidence and severity of bone dehiscences and gingival recessions by expansive orthodontic tooth movement, to investigate relevant factors in the development of bone dehiscences and gingival recessions, and to minimize those adverse effects during and after orthodontic treatment. Thus, the aim of the present report is to present the development of an experimental model in rats for palatal translational expansive tooth movement and to evaluate the type (rotation vs. translation), the

distance, and the dental or skeletal components of the achieved movement.

Material & methods

Animals and anesthesia protocol

Twenty-seven, 3-month old, male, Hannover–Wistar rats (WH-M) (TaconicA/S, Ry, Denmark) with a body weight of 364 ± 12 g were used in this pilot experiment. The animals were kept in pairs in transparent plastic cages, and were maintained under standardized conditions (12 h light/12 h dark; temperature 19–23°C); standard rodent chow and water were provided *ad libitum*. The appliances were placed under general anesthesia by subcutaneous injection of 1 ml/kg of dilution with sterile saline, Atropin 1 mg/ml, and Immobilon® vet. and reversed by the same amount of dilution with sterile saline and Revivon® vet. as described by Fisker et al. (21). The study was approved by the Danish Inspectorate for Animal Experiments (2010/561-1849).

Orthodontic appliances

A modified biomechanical approach as described by Rebellato (22) was used to achieve translational facial tooth movement with torque control by expansive force and to minimize facial inclination of the teeth.

Two types of springs ($0.016'' \times 0.022''$ β -titanium 0.5 helix and $0.016'' \times 0.022''$ β -titanium ends welded on round $0.016''$ β -titanium 1.5 helix) were manually bent according to a template, and the force deactivation curve (insertion at 9 mm, activation to 5 mm, 0.2 mm stepwise deactivation to 9 mm) in every appliance was measured using strain gauge technology with a custom-built designed force system identification (FSI) apparatus (23). The HF springs were initially activated to deliver 50 ± 8 cN per side. By a projected tooth movement of approximately 2 mm on each side of the jaw (i.e., one tooth width) the force would gradually decrease to 28 ± 6 cN; similarly, the LF spring would gradually decrease from 25 ± 4 cN to 15 ± 3 cN per side.

Force comparability

Force comparability was based on comparison of surface areas of human and rat first molars. Cylinder-shaped roots with dimensions as described by Wheeler (24) were assumed for humans (average length/neck diameter: 12 mm/4 mm, 12 mm/4 mm, and 13 mm/5 mm, for the mesio-buccal, disto-buccal, and palatal root, respectively), while right circular cone-shaped roots were assumed for rats. Root length and diameter at half root length were measured on multiplanar reconstructions along the pulp of each root.

Experimental groups

Control (C)

Seven animals without any intervention (i.e., no appliances) served as controls.

Pilot 1 (P1)

In 10 animals, only the second molars (M2) were bilaterally moved with a transpalatal spring of the HF type. A transpalatal bar extending to the mid-buccal surfaces of M1 and M3 on both sides was placed to retain tooth position and prevent skeletal

widening of the maxilla due to opening of the mid-palatal suture. The appliances were conventionally bonded with resin directly on the teeth (Fig 1A–D).

Pilot 2 (P2)

In 10 animals, both the second (M2) and third molars (M3) were bilaterally moved with a transpalatal spring: a HF-spring (i.e., per tooth, 25 ± 4 cN decreasing during deactivation to 14 ± 3 cN) was used in five animals, while in the rest, LF-springs (i.e., per tooth, 13 ± 2 cN decreasing during deactivation to 8 ± 2 cN) were placed. In three animals in each force group, the springs were passively glued exerting a facially directed force to both sides of the jaw (P2 0°). In two animals in each force group, the springs were inserted in tubes glued with an angulation of 30° aiming to apply an uprighting torque moment in addition to the facially directed force, with the intention to prevent facial inclination of the molars (P2 30°). A transpalatal bar extending to the mid-buccal surface of M1 was placed to retain tooth position and prevent skeletal widening of the maxilla due to opening of the mid-palatal suture (Fig. 1E–L).

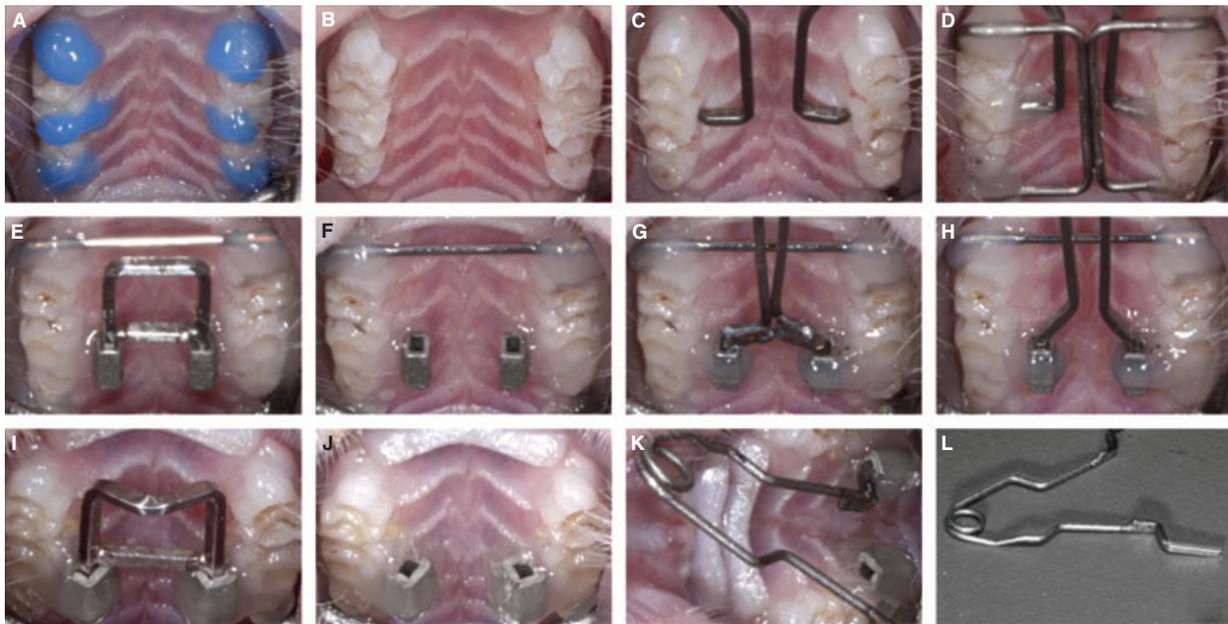


Fig. 1. The appliance was either directly bonded to M2 in P1 group (A–D) or inserted and bonded to 0.018" × 0.025" sandblasted tubes on M2 and M3 in P2 group (G–H). To achieve a torque moment as well with the round LF-springs, a piece of .016" × 0.022" β -titanium wire was laser-soldered to the round wire (L). The tubes were bonded with a positioning guide (E,I) either passively 0° (E,F) or with a torque moment of 30° (I,J). A transpalatal bar was used with the intention to prevent opening of the mid-palatal suture and minimize skeletal effect (D and F–H).

The appliances were fixed with resin (Transbond XT™ Light Cure Adhesive, 3M, Rüslikon, Switzerland) using a total etch, total bond technique. The animals were sacrificed at various time points, corresponding to various amounts of expansion by clinical inspection, but never exceeding one tooth width.

Histology and microCT

The animals were sacrificed with CO₂, and the lower cranial skeleton including the maxillae was dissected free and fixed in 70% alcohol. Some of the specimens (three of group P2) were divided into two pieces along the mid-palatal suture; one of the resulting hemimaxillae of each of those three P2 animals was then dehydrated in ascending concentrations of ethanol series and embedded in methylmethacrylate. Undecalcified vertical or horizontal sections of ~500 microns in thickness were cut using a low-speed diamond saw with coolant (Varicut® VC-50; Leco, Munich, Germany). After mounting the sections onto acrylic glass slabs, they were ground and polished to a final thickness of about 100 μm (Knuth-Rotor-3; Struers, Rodovre/Copenhagen, Denmark). The sections were stained with toluidine blue and basic fuchsin. Digital photography was performed using a ProgRes® C5 digital camera (Jenoptik Laser; Optik Systeme GmbH, Jena, Germany) connected to a Zeiss Axioplan microscope (Carl Zeiss, Göttingen, Germany). The specimens from groups P1 and P2 were then digitized with a microCT scanner (microCT-40; SCANCO Medical AG, Brüttsellen, Switzerland) in a cylindrical sample holder (30 or 36 μm, 70 kVp, 113 μA, integration time 200 ms, average data 2×). The maxillae of all specimens in the control group were aligned, split in two halves, embedded, and scanned at 12 μm (maximal diameter of the sample holder 10 mm) to increase resolution and maintain the possibility to evaluate facial roots on both sides by non-decalcified histology.

Morphometrical evaluation of amount of facial tooth movement, type of movement, and skeletal width

All measurements were gathered on multiplanar reconstructed slices of microCT data. For mea-

suring, the data set of each specimen was first oriented by setting a coordinate system using a multiplanar reconstruction algorithm of Osirix v.3.9.4 64-bit (Pixmeo Sàrl, Geneva, Switzerland). The hard tissue based reference structures for the alignment were the mid-palatal suture for the sagittal plane and the furcations for the transverse plane. Morphometrical evaluation was performed on one frontal slice per tooth and side. Slices through the center of the disto-facial root of the first molar (M1D), the mesio-facial root of the second molar (M2M), and the mesio-facial root of the third molar (M3M) were selected.

The vertical bisector of the rectangle, with corners set at the cemento-enamel junction (CEJ) on the buccal and palatal side of the tooth and at the root tips (RT) of the buccal and palatal roots, was assumed to represent tooth axis. The root center point (RC) was constructed by the bisection of the two angle bisectors of the same rectangle. The amount of tooth movement was assessed by measuring and comparing distances from RC to the caudal corner of the mid-palatal suture (MPS) between the control and the experimental groups (Fig. 2A).

The type of tooth movement was assessed by measuring and comparing tooth inclinations defined as the angle between the tooth axis and a line between RC and MPS (RC-MPS) between the control and the experimental groups (Fig. 2A). Symmetry of tooth movement in groups P1 and P2 was evaluated by comparing right-to-left side RC-MPS distances.

To estimate any possible skeletal effect of the appliance (i.e., widening of the maxilla with bone apposition at the mid-palatal suture), the distance from the center of the palatal opening of the major palatal nerve (NPM) to MPS was measured on one additional frontal slice containing the NPM canal (Fig. 2B).

Statistical Methods

The right- and left-side measurements were averaged, except for three animals of group P2 contributing with only one hemimaxilla, where the other half was used for histological processing.

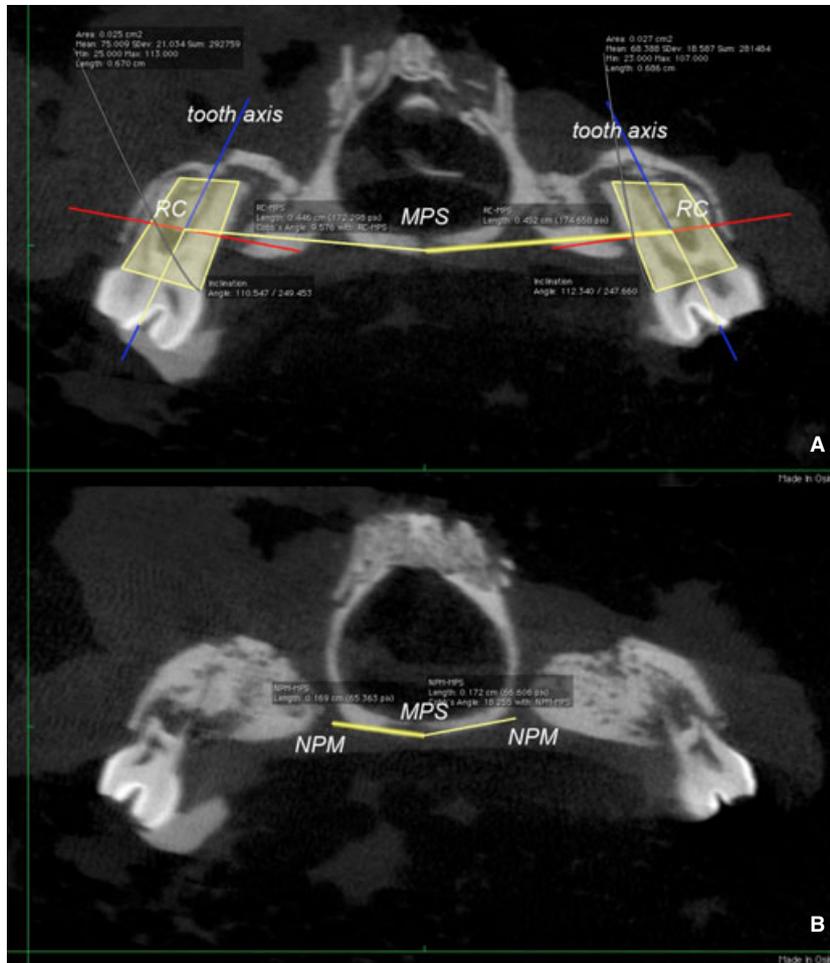


Fig. 2. Geometrical constructions for measuring after multiplanar reconstruction of microCT. Slices from the animal with biggest skeletal effect due to lost transpalatal retention bar. (A) The vertical bisector of the rectangle with corners set at the cemento-enamel junction (CEJ) on the buccal and palatal side of the tooth and at the tips of the buccal, and palatal roots (RT) was assumed to represent root axis. The root center point (RC) was constructed by the bisection of the two angle bisectors of the same rectangle. The amount of tooth movement was assessed by measuring the distance from the caudal corner of the mid-palatal suture (MPS) to RC. The type of tooth movement was assessed by measuring tooth inclination, defined as the angle between the tooth axis and a line between RC and MPS. (B) The distance from MPS to the opening of the major palatal nerve (NPM) was used to estimate skeletal effect.

Nonparametric comparisons were performed with Kruskal–Wallis test with Gaussian approximation, followed by Dunn’s post hoc test. The level of significance was set at $p < 0.05$. The GraphPad Prism v5.00 for Macintosh (GraphPad Software, San Diego, CA, USA) was used.

Results

The appliances did not seem to have any apparent effect on the chewing function of the animals, as indicated by the gradual normal increase of body weight throughout the course of the experiment. The three hemimaxillae pro-

cessed for histological evaluation showed fenestration or dehiscence of the facial cortical bone, thinning of the gingiva, and bone apposition on the cortical bone surface adjacent to the facially displaced roots (Fig. 3). Root resorption at the pressure zone and bone apposition at the tension zone were observed. Vertical histological cuts of rat maxillae in contrast to horizontal slices have the advantage of including the mid-palatal suture and the buccal root with periodontal tissues on the same slice for each root prominence. Therefore, it is possible on vertical sections to evaluate the position and inclination of the tooth simultaneously to the assessment of the periodontal tissues.

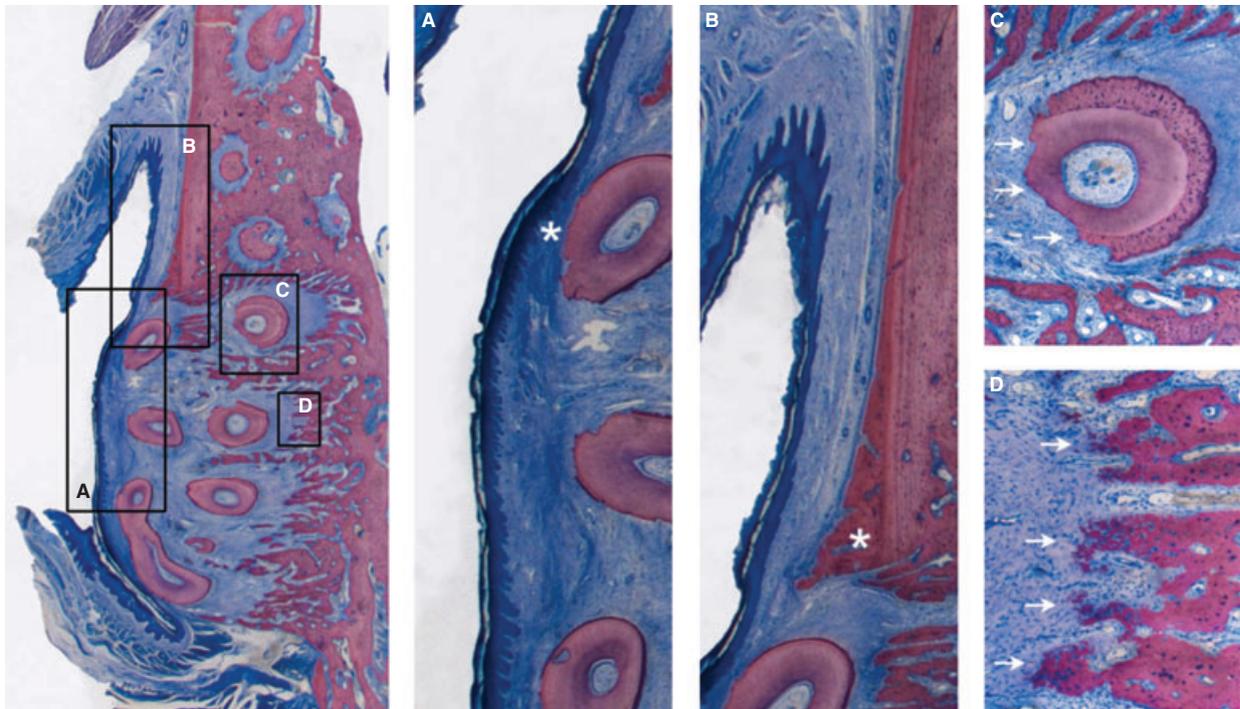


Fig. 3. Micrograph of a specimen from the P2 group (high force, 0 degrees) with translational facial tooth movement of M2 and M3. Complete resorption of the facial cortical bone and thinning of the gingiva are observed (A, asterisk). Recessions are not present at this root level. Triangular-shaped bone apposition on the cortical bone surface lateral to the root (B, asterisk). Root resorption (arrows) at the pressure zone (C) and bone apposition (arrows) at the tension zone (D) can be observed.

A reconstructed image – based on microCT data – of a representative specimen from each group is presented in Fig. 4. The results of the morphometric evaluation are presented in Figs 5 and 6.

Control group

Average root length and root diameter of the first molar are 2.79 ± 0.12 mm/ 0.81 ± 0.05 mm for the centro-buccal root, 1.7 ± 0.11 mm/ 0.42 ± 0.09 mm for the mesial root, 2.25 ± 0.11 mm/ 0.58 ± 0.06 mm for the disto-buccal root, 2.55 ± 0.18 mm/ 0.67 ± 0.04 mm for the centro-palatal root, and 2.38 ± 0.16 mm/ 0.59 ± 0.03 mm for the disto-palatal root. A ratio of 11.56 results by dividing the mean total root surface of human (536.4 mm^2) and rat (46.4 mm^2) first molars.

P1 group

In five of 10 animals, the spring was lost and tooth movement of M2 was not consistently achieved; these animals were considered as fail-

ures and were excluded from any further analysis. In the remaining five animals, the appliances were in place for 23–87 days, resulting in a facial movement and inclination of M2 was achieved (appliances in place for 23–87 days). The difference of the medians for RC-MPS between P1 and C group was $335 \mu\text{m}$ ($p > 0.05$). The difference of the medians in inclination of M2 between P1 and C was 22.1° ($p < 0.05$); Dunn's multiple comparison test, while a non-significant difference of the medians of $50 \mu\text{m}$ in terms of skeletal width (NPM – MPS) was observed (Fig. 6). Right-to-left-side differences in regard to RC-MPS showed tendency for higher variance in the P1 compared with the C group. Loss of rigid connection between the spring and the tooth in some of the animals resulted in a lack of torque expression and uncontrolled facial inclination (Fig. 7).

P2 group

Three ($1 \times \text{LF } 0^\circ$, $1 \times \text{HF } 0^\circ$, and $1 \times \text{LF } 30^\circ$) of 10 animals in the P2 group lost the appliance; these

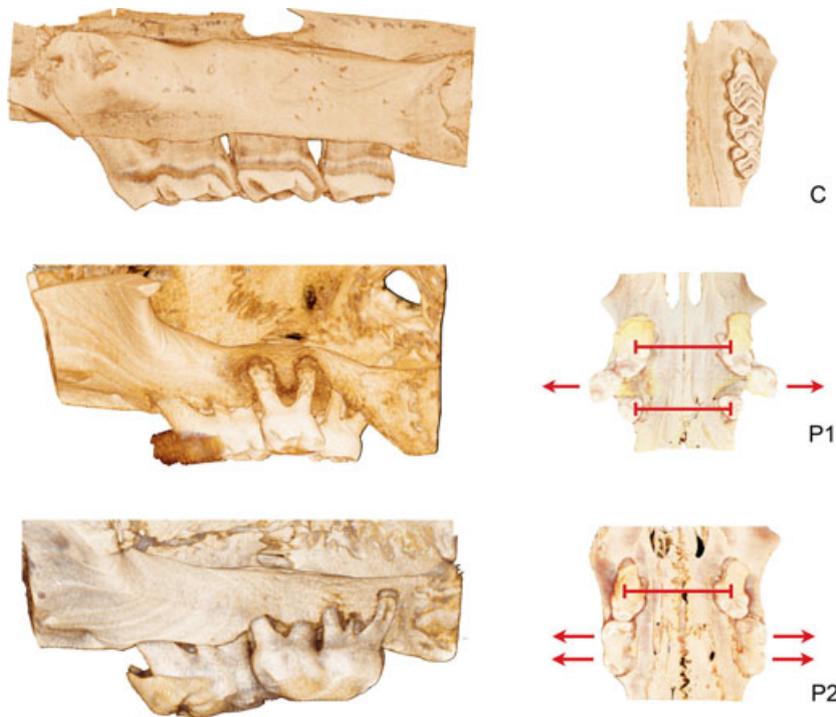


Fig. 4. A reconstructed image – based on microCT data – of a specimen from each group (C, P1, and P2).

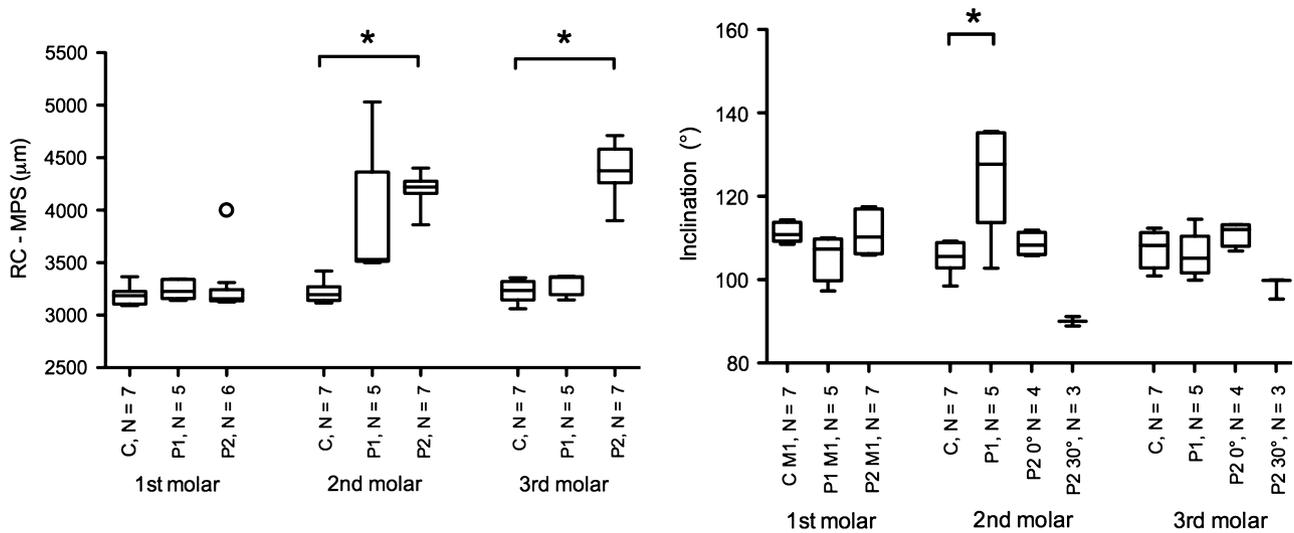


Fig. 5. Box-plots of distances from the root center to the mid-palatal suture RC-MPS (A) and of tooth inclination (B) in groups C, P1, and P2. Significant differences are marked with an asterisk. The measurement of tooth position at M1 for one animal (circle) in the P2 group subgroup low force 0° was excluded due to loss of the retention appliance. The P1 group shows significant facial inclination of M2. The P2 group shows most translational tooth movement and no clinical significant change in inclination. The P2 subgroup 30° shows a non-significant tendency of facial root torque.

animals were considered as failures and were excluded from further analysis. In the remaining seven animals, a facial movement and inclination of M2 and M3 was achieved (appliances in place for 34–73 days). In one of these seven animals, the retention appliance was lost, but with the spring still functional in place; thus, data

from this animal were not included in the analysis of skeletal effects and analysis of M1 (indicated with a circle in Fig. 5). Statistically significant differences were found for RC-MPS of M2 and M3 compared with M2 and M3 of the control group, respectively, (Kruskal–Wallis test $p < 0.01$; Dunn’s multiple comparison test

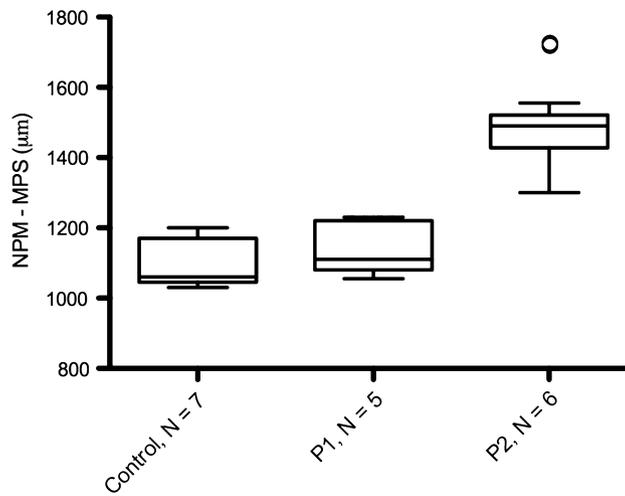


Fig. 6. Box-plot of the distances from the opening of the major palatine nerve (NPM) to the mid-palatal suture (MPS) in groups C, P1, and P2. A significant skeletal effect of slightly more than one-third of the tooth movement was detected for the P2 group despite the retention bar at M1. The retention bar between M1 and M3 prevented skeletal side effects in the P1 group. One animal (circle) in the P2 group was excluded because the retention appliance was broken.



Fig. 7. Transverse cut at second molar (M2) of a rat from the discontinued P1 group. M2 rotated over a hypomochlion at the limbus alveolaris, while the position of the root was nearly unchanged. An uprighting moment did not develop because a rigid connection between wire and tooth was lost while the spring was still active and in place. It is questionable, if such an inclination would be stable without retention appliance. Artifacts are present in the central lower part of the picture caused by the metal appliance during microCT scan with a resolution of 36 µm.

significant for both M2 and M3). The root positions of M2 and M3 were on median 1025 µm and 1140 µm more facially compared with the control group. The range of differences between right- and left-side root positions in P2 groups was 140 µm at M2 and 120 µm at M3 (N = 4, M2: max 20 µm min -120 µm, M3 min -40 µm max 60 µm).

No statistical differences were found for inclinations of group P2 compared with the control group at corresponding sites (Dunn's multiple comparison test $p > 0.05$). A tendency to buccal root torque was seen in group P2, subgroup 30° for inclination. The medians P2 subgroup 30° compared with P2 subgroup 0° differed by -18.3° for M2 and by -12.2° for M3. The skeletal effect of the expansive springs was significant

for the P2 group (Dunn's multiple comparison test $p < 0.05$). The medians of NPM - MPS between P2 and C differed by 430 µm. After subtracting this skeletal widening of the maxilla from the difference in position of the moved teeth, a net dental movement of 595 µm for M2 and 610 µm for M3 results. Thus, the skeletal effect (widening of the maxilla) in contrast to pure dental movement accounted on median for 41 and 38% of total position change at M2 and M3, respectively.

Discussion

Currently, it is unclear whether arch expansion by translational movement leads always to bone

dehiscence or gingival recession development and also which parameters (e.g., force magnitude, duration of force application, distance of movement beyond the skeletal envelope etc.) may be of importance in these processes. Such issues are difficult to address in randomized controlled clinical studies and therefore preclinical *in vivo* research seems warranted. Compared to large animals, use of small animals (i.e., rodents) is generally preferable due to economical and ethical concerns. To the best of our knowledge, most animal studies in rodents related to orthodontics involved mesio-distal tooth movements, for example toward edentulous regions or extraction sockets (7–18), while only a few regarded facial inclination (19, 20). No rodent study up to now involved translational movement beyond the skeletal envelope and the present report is the first to describe an experimental *in vivo* model in rats for translational expansive tooth movement.

In the present study, use of transpalatal β -titanium springs placed in directly bonded tubes on both M2 and M3 (P2, subgroup 0°) was the most effective in creating expansive buccal translational root movement of about 595 μm for M2 and 610 μm for M3. M3 moved slightly more buccally comparing with M2, apparently due to the circular opening of the spring. Nevertheless, comparison of the tooth movement distances in the right and left side of the maxilla showed that a rather symmetrical expansion was achieved. In the present study, the time of sacrifice varied considerably among animals, but the amount of variation in tooth movement distance was rather low, indicating that there is a variation among animals regarding the speed of tooth movement despite the standardized orthodontic appliances, similarly to what is observed in humans. Although some buccal root torque has probably developed during tooth movement also in this group, application of buccal root torque (P2, subgroup 30°) was not required to avoid facial inclination for the two spring types used in this model. Pressure-tension areas in the periodontium along the root of an orthodontically moved tooth are expected to vary with the type of movement; therefore, the amount and type of

root movement are the most relevant features when assessing the effects of tooth movement on the periodontium. Evaluation of only tooth crown position could be misleading about the true amount and type of tooth movement and pressure-tension areas along the root. Thus, measuring root position and inclination is more preferable than only assessing tooth crown position, as it was done in previous studies (19, 20).

The biomechanical approach with torque control used in the present study is different from previously described biomechanical approaches (19, 20), where a non-rigid connection between spring and tooth was used. Indeed, the movement with the biomechanical approaches described in those studies, resembles the facial inclination of the teeth observed in the P1 group in the present study, which was the result of loss of torque control due to frequent composite breakage at the spring-tooth interface but with a still functional spring. Thus, due to the above shortcomings and the high drop out in the P1 group observed herein, direct bonding of the spring to the tooth seems not the appropriate approach, if translational tooth movement is desired.

In the present study, comparability of the force levels with those in humans was attempted using the root surface area of first molars, although it is recognized that species differences in anatomy and physiology are complex and using simple size ratios is an oversimplification; our calculations for force comparability differ from those in a previous investigation (25). Similarly to the study of Ren et al. (25), it may be concluded that a further reduction in force levels below 13 cN per tooth (equals 156 cN for humans under the assumption of a comparability factor to humans of 12) could be more appropriate for future experiments with this method.

In a previous study, in non-human primates, involving buccal tooth movement of maxillary anterior teeth, considerable unintentional tooth extrusion was observed in addition to the expansive tooth movement (26). In the present study, vertical side effects of the appliances in the P1 and P2 groups did not seem to occur, as no

obvious vertical differences to the control teeth were observed neither by clinical nor by microCT inspection. In our model, the applied expansive forces may also exert skeletal effects (e.g., palatal expansion), which in turn may influence the true amount of tooth movement in relation to the alveolar envelope. Corbrige et al. (27) have recently shown skeletal widening of the palate as well as tooth movement through the alveolar process with decreased facial bone thickness by expansion with a quadhelix appliance in children. The increased distance from the mid-palatal suture to the major palatine nerve foramen in the P2 group, compared with the C group, indeed indicates a partly skeletal effect of the appliance. Obviously, the use of a single transpalatal bar placed on M1 for dental anchorage was not enough to completely prevent such skeletal effects, that is, relative to the fixed M1, the two hemimaxillae moved buccally, and new bone had formed at the mid-palatal suture. This might be considered as a shortcoming of the model; however, the effect of skeletal expansion was considered and attempted estimated herein. The skeletal effect observed in P2 might have diminished tooth movement relative to the alveolar envelope by slightly more than one-third. In contrast, the use of two interconnected connected transpalatal bars at M1 and M3 in P1 might have efficiently prevented any significant skeletal effects of the appliance.

In the present model, evaluation of the effect of expansive tooth movement on bone and gingiva is obviously only possible through histological evaluation after sacrifice. When evaluation of the effect of expansive tooth movement only on the hard tissues would be needed, use of *in vivo*-microCT could be a future approach as long as artifacts by metal are controlled, and the resolution is sufficient. Finally, it should be kept in mind that the present study consisted of pilot experiments, to test feasibility of translational

expansion up to one tooth width, using the least possible number of animals.

Conclusion

A model to move rat molars in a symmetrical buccal translational way with different magnitudes of orthodontic forces was established. Further research on the effect of translational expansive tooth movement on the periodontium seems possible with this model.

Clinical relevance

Dental arch expansion is an orthodontic treatment option to resolve crowding or to compensate skeletal discrepancies. Adverse effects (i.e., bone dehiscence and gingival recession) have been associated with arch expansion, but little is known about which factors are of importance. The purpose of these pilot studies was to establish an animal model for expansive translational tooth movement for future research on possible factors of importance in the development of bone dehiscences and gingival recessions, with the aim to prevent or minimize these adverse effects.

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