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# The use of gene therapy vs. corticotomy surgery in accelerating orthodontic tooth movement

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## Structured Abstract

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**Objective** – Alveolar corticotomy surgery is an adjunctive therapy for reducing orthodontic treatment duration. The activation pathways of bone resorption involved in the tooth movement (TM) process are directly linked to the receptor activator of the nuclear factor- $\kappa$ B ligand (RANKL). Whether similar molecular pathways through RANKL, are shared by the acceleratory TM process (corticotomy-induced or not), sustained acceleration would therefore be expected with transgenic overexpression of this factor. We hypothesize that maintaining transgenic overexpression of RANKL will accelerate TM under force over time rather than at the beginning of therapy only; this contrasts with the corticotomy procedure.

**Material and Methods** – We transfected the pcDNA3.1(+)-mRANKL transgene *in vitro* into NIH3T3 cells, then evaluated by PCR, Western blot and *ex vivo* resorption assay. Quantification of RANKL immunofluorescence, fluorescence-based tartrate-resistant acid phosphatase+ (TRAP+) osteoclast counts and histological analyses of the bone resorption area were performed and clinically correlated after a 32-day *in vivo* experiment comparing corticotomy and gene therapy.

**Results** – *In vitro* experiments resulted in increased level of RANKL protein (46%,  $p < 0.05$ ) and greater mineral resorption (39%,  $p < 0.05$ ) compared to the controls. *In vivo* results showed increased RANKL immunoexpression for both corticotomy (twofold) and transfection groups (threefold) after 10 days. After 32 days, a similar result was obtained for the transfected group but not for the surgery group. These data correlate with the clinical effect of decelerating TM in the surgery group.

**Conclusions** – Selective gene therapy with RANKL has been experimentally tested as an alternative method to corticotomy surgery, showing higher effectiveness than surgical methods used for acceleratory purposes in orthodontics.

**Key words:** corticotomy surgery; experimental therapy; gene therapy; orthodontics; tooth movement

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## Introduction

Alveolar corticotomy surgery is an adjunctive therapy for reducing the orthodontic treatment period by nearly a half (1, 2). However, the short accelerated movement period and the morbidity rates from this kind of surgery both demand that alternative therapies be sought (3, 4). The tooth movement (TM) process is a biological event directly dependent on bone

resorption and apposition. The biomolecular pathways of osteoclast activation are closely linked to the ratio between the receptor activator of the nuclear factor- $\kappa$ B (RANKL) and the osteoprotegerin (OPG) (5). As a result, an elevated level of RANKL with respect to OPG is associated with greater osteoclast differentiation, survival and activation rates, remodeling the bone further. This strong evidence is supported by several studies in various pathological conditions such as osteoporosis, periodontal disease and osteosarcoma (6–8). Overexpression of the membrane-bound or soluble RANKL protein levels would be related, therefore, to higher catabolic osteoclast activity, as illustrated in the OPG<sup>-/-</sup> knockout model (9, 10).

It has been shown empirically that corticotomy-assisted malocclusion therapy shortens TM phases, because of the increased rate of bone remodelling under the so-called regional acceleratory phenomenon (RAP) (11, 12). Taking this into consideration, several authors have recently supported alveolar corticotomy or, in global terms, surgically facilitated orthodontic therapy (SFOT), as an adjunctive therapy for shortening malocclusion treatment time (2, 13, 14). Considering all the data, we hypothesize that the sustained overexpression of RANKL will not only selectively activate osteoclast and increase osteoclastogenesis and bone resorption but also lead to the acceleration of TM under force over time rather than at the beginning of therapy only; this contrasts with the corticotomy procedure. To test our hypothesis, we first performed the *in vitro* transfection of the RANKL gene. Once demonstrated *in vitro*, we compared corticotomy- or gene therapy-assisted orthodontics by an *in vivo* study of Wistar rats under experimental force combined with alveolar corticotomy or genetic RANKL transfection.

## Materials and methods

### *In vitro* experimental design

Cloning of the RANKL gene, construction of the RANKL plasmid and *in vitro* transfection assay

The MC3T3-E1 cell line (DMSZ, Brunswick, Germany) was subjected to total RNA extraction with TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions. Total RNA was then reverse-transcribed into cDNA and subjected to PCR amplification (Promega, Sunnyvale, CA, USA). Two specific primers were designed using mouse RANKL

cDNA plus restriction site, primer *Xho*I site + RANKL upstream (5'GGGCTCGAGATGCCTGAGGCCAGCCA-TTTGC3') and downstream primer (5'CCAGTCTAGACGAAATGAGTCTCAGTCTATGTCC3') + *Xba*I site. Each cycle consisted of a heat denaturation step at 94°C for 1 min, an annealing step at 50°C for 45 s and an extension step at 72°C for 60 s. DNA was then purified following manufacturer's protocol (Quiagen, Germantown, MD, USA). The plasmid vector pcDNA3.1(+) and the RANKL cDNA containing the restriction site-tagged RANKL sequence were digested with *Xho*I and *Xba*I enzymes (New England BioLabs, Ipswich, MA, USA). The insert was ligated into the linearized plasmid expression vector pcDNA3.1(+) using the T4 DNA ligase kit (Invitrogen, Carlsbad, CA, USA). The ligation product was cloned into competent *Escherichia coli* (One shot<sup>®</sup> Top10; Invitrogen), checked by PCR and finally sequenced.

The RANKL expression vector (pcDNA-mRANKL) was driven by a cytomegalovirus promoter: the sequence encoding the functional protein for soluble mouse RANKL (AF019048, 606–1086 bp). The plasmid endotoxin level was considered endotoxin-free when the resulting concentration was 0.1 EU/ $\mu$ g plasmid. The RANKL transgene was transfected (Lipofectamine<sup>™</sup> 2000; Invitrogen) in the NIH3T3 cell line (DSMZ, Brunswick, Germany). Total RNA, total protein and medium (Total protein extraction kit; Millipore, Billerica, MA, USA) were collected 72 h after transfection. RANKL gene and protein expression were subsequently analysed using PCR and Western blot with anti-RANKL antibody (sc-7628; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), respectively. Spectrophotometric measurements were obtained using Quantity One<sup>®</sup> 1-D (Bio-Rad, Hercules, CA, USA).<sup>1</sup>

<sup>1</sup>The Western blot test, in this experiment, is used to quantify the protein produced by the transfected cells and is used here to detect increases in RANKL protein production following transfection. The test is based on protein charges that allow them to be recognized in the membrane by molecular weight and detection of antibodies anti-RANKL. The electrophoresis method is used to separate different DNA fragments according to molecular length. Based on DNA charge, different fragments are observed in gel after an electrical current is applied. Larger fragments move more slowly and lag behind the smaller fragments as they migrate through the gel. Plasmids, generally speaking, are commercial bacteria-derived DNA fragments with a well-defined, sequenced form, prepared to incorporate an extra DNA fragment (in this case, a RANKL insert). They have a promoter region, necessary to start off protein synthesis from the DNA fragment. Vectors are biomolecular systems, prepared to incorporate a DNA fragment (plasmid) and transfer it into a cell using their membrane fusing properties or infection potential, depending on their viral or non-viral nature.

*Ex vivo* functional mineral resorption assay

The functional integrity of total RANKL protein was tested by mineral resorption assay (Osteologic®; BD Biosciences, Erembodegem, Belgium). Rat bone marrow cells were collected (15), seeded on mineral slice discs and cultured in D-MEM 10% FBS, supplemented with 1,25-(OH)2D3 ( $1 \times 10^{-8}$ M), with or without 50% of the collected culture media. The medium was changed every day for 10 days to maintain a pH that was not proresorptive. The resorption pit areas were traced from randomly selected fields and measured using IMAGE J 1.36 software (NIH, Bethesda, MD, USA).

***In vivo* experimental design**

## Experimental animals

Seventy-two 7-week-old Wistar rats were divided into five groups for a 32-day split-mouth study (Fig. 1A). All animal procedures were carried out under general anaesthesia and having obtained ethical permission from the US Ethics Committee.

## Animal welfare evaluation and bone mineral density measurements

Animal growth was monitored and compared every 2 days. Additionally, bone mineral density was recorded to determine the consequences on systemic bone metabolism using the X-ray bone densitometer, Hologic II-QDR-TM-1000.

## Tooth movement appliance and measurements

In brief, the experimental force appliance consisted of a pre-formed I-shaped stainless steel structure, bonded onto the first and third molars serving as anchorage units. This method enabled a correct split-mouth measurement to a standard point on the stainless steel bar (Fig. 1B, C). TM was measured using a minicalliper on the second molars using a medial force vector toward the palatal mid-line, delivered by a Sentalloy close-coil spring, activated to 10 cN force (GAC, New York, NY, USA). The measurements between hemimaxillae were thus perfectly comparable.

***In vivo* gene RANKL transfection**

For *in vivo* transfection, we used a hemagglutinating virus of Japan envelope vector (GenomONE®; Ishihara sangyo kaisha Ltd., Osaka, Japan), according to the manufacturer's instructions. (16) A 4 µl solution containing pcDNAmRANKL combined with 1:900 000

adrenaline as vasoconstrictor was administered in the palate, 0.5 mm medial to the second molar of the animals in G3 and G5, every 6 days; the control side received just the non-functional plasmid and same concentration of vasoconstrictor (Fig. 1A). Optimal solution administration (quantity and time interval) was determined immunohistochemically from the previous pilot experiments (data not shown).

**Corticotomy surgery procedure**

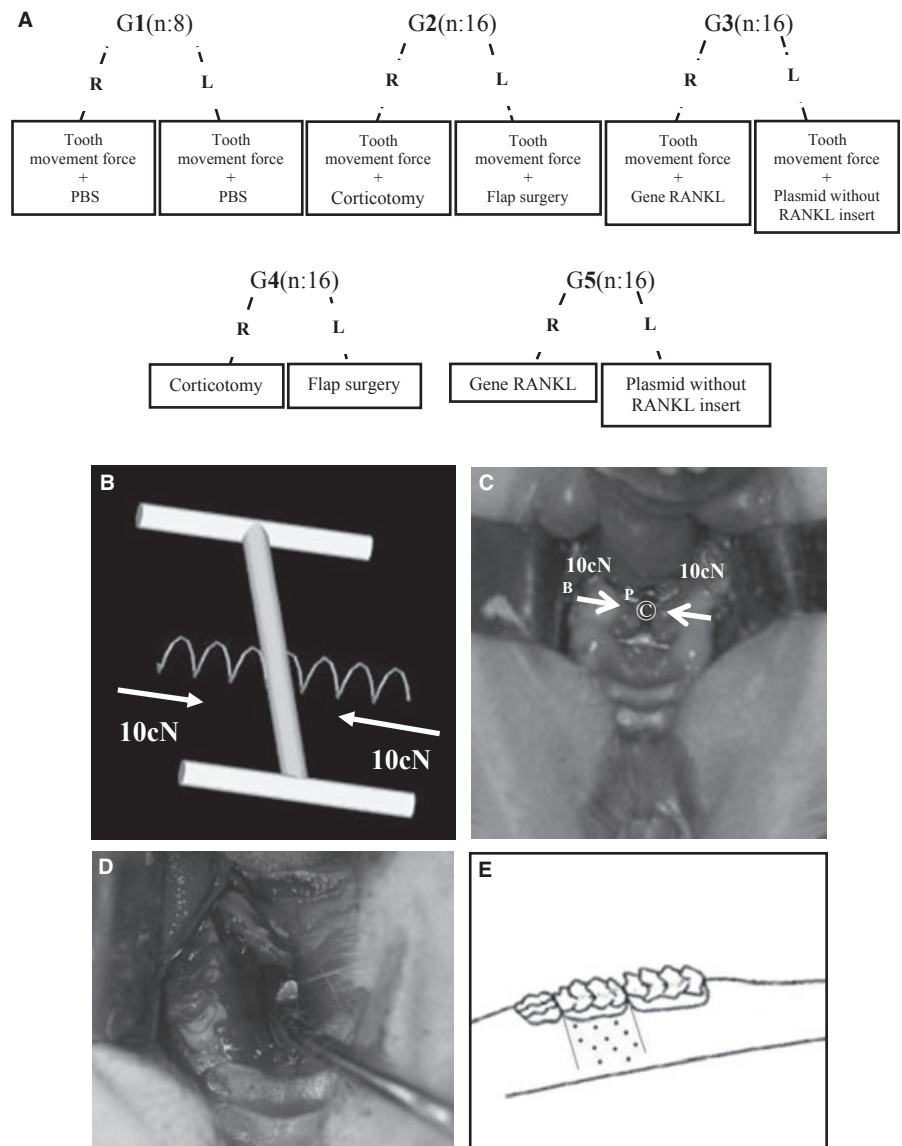
All surgery was performed under a dissecting microscope, using microsurgical tables, microdrills and microscalpels. Surgical design consisted of making antero-posterior and apical-occlusal cortical cuts under saline irrigation, around the right maxillary second molar, with a further ten cortical miniholes, 0.3 mm in diameter and 0.3 mm deep, in the palatal plate (Fig. 1D, E). On the control side, only flap surgery was reproduced. Post-operative management consisted of antibiotics and analgesics for 3 days.

**Histological examinations**

The upper jaws were surgically removed and fixed overnight, decalcified with 10% EDTA in 0.01 M PBS for 4–6 weeks at 4°C, dehydrated and embedded in paraffin. Periodontal tissue from the palatal area of the upper second molars was examined with haematoxylin/eosin staining in serial cross-sections 4 µm thick. The histomorphological resorption area was evaluated in 20 serial cross-sections per group using IMAGE J 1.36 software. Intramethod error was  $73.465 \mu\text{m}^2$  when a single researcher in blind tests analysed 40 randomly selected samples.

**Immunofluorescence analysis for RANKL and tartrate-resistant acid phosphatase (TRAP)**

Fluorescence-based RANKL staining was performed using a goat polyclonal anti-RANKL antibody (Santa Cruz Biotechnology). For immunofluorescence detection of RANKL, the sections were incubated with the FITC-conjugated anti-goat IgG, washed, mounted in PBS-glycerol and observed under a fluorescence microscope. Fluorescence-based TRAP staining was performed using ELF97 (Invitrogen) as a phosphatase substrate (17, 18).



**Fig. 1.** *In vivo* experimental design: (A) Total experimental sample and group distribution. Group 1 (G1) served as external control, under palatal force and with a 4  $\mu$ l PBS injection to both hemi-maxillae. Group 2 (G2), under palatal force and with corticotomy to the right hemi-maxilla; on the left side, simple flap surgery. Group 3 (G3), under palatal force and with a 4  $\mu$ l receptor activator of the nuclear factor- $\kappa$ B ligand (RANKL) gene transfection to the right hemi-maxilla; in the left hemi-maxilla, administration of a non-functional plasmid solution. Group 4 (G4), with no applied orthodontic force and flap surgery to the right hemi-maxilla; corticotomy to the left hemi-maxilla. Group 5 (G5), with no applied orthodontic force and RANKL gene to the right hemi-maxilla; injection of a non-functional plasmid solution to the left hemi-maxilla. L, left hemi-maxilla, R, right hemi-maxilla; (B) Experimental appliance design. Movement was performed in a palatal direction (arrows) to second molars; (C) Experimental tooth movement (TM) appliance bonded on the first and third molars. TM force vectors description; P, palatal; b, bucal; ©, mid-line point; (D) Surgical area used for the corticotomy. (E) Outline of the type of corticotomy performed on the animals.

## Statistics

Data were analysed for statistical differences using the Kruskal–Wallis test, followed by a Bonferroni-type multiple comparison (Tukey type). Differences of  $p < 0.05$  were considered significant.

## Results

### *In vitro* assay results

Plasmid vector pcDNA3.1(+)-mRANKL construction, transfection and RANKL protein *in vitro* evaluation

pcDNA3.1(+)-mRANKL was successfully constructed and transfected *in vitro* (Fig. 2A). As expected, membrane-bound RANKL and soluble protein overexpres-

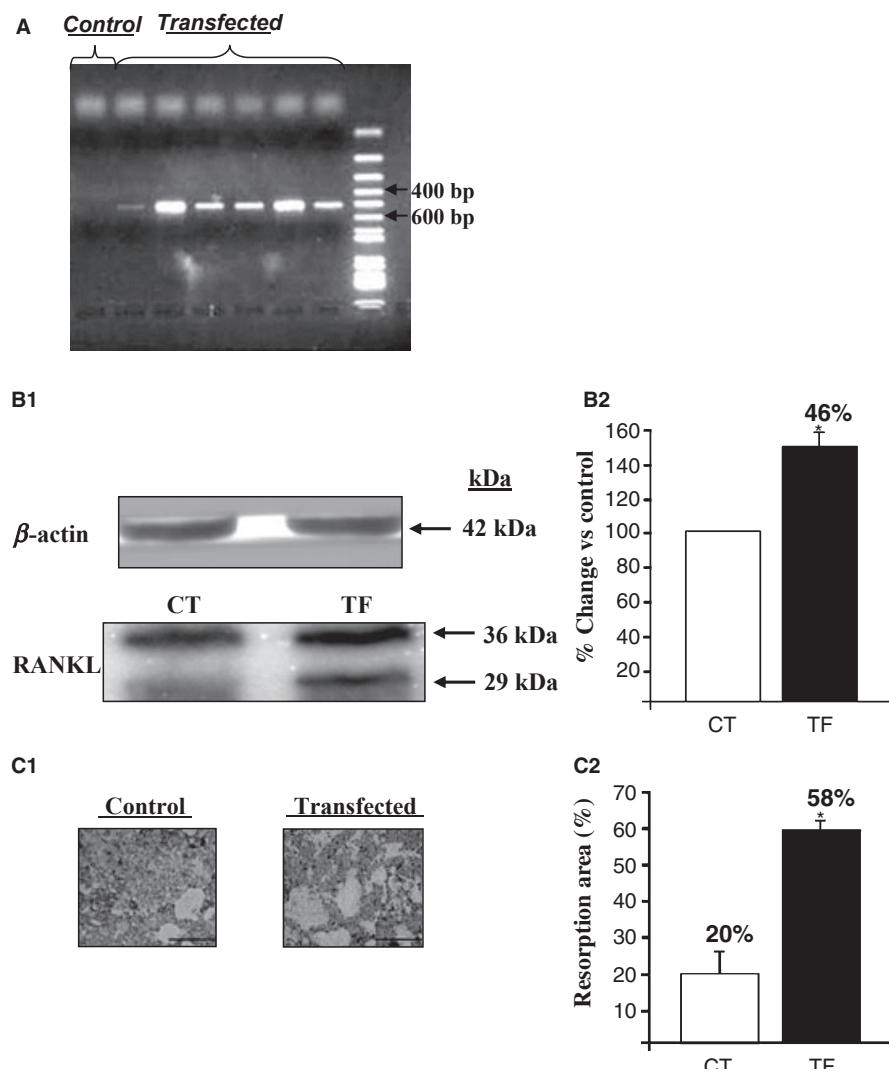
sion were detected by Western blot on transfected NIH3T3 cells (Fig. 2B1). Furthermore, Western blot spectrophotometric measurements showed a 46% ( $p < 0.05$ ) higher RANKL expression in transfected cells compared to the controls (Fig. 2B2).

### *Ex vivo* experimental results

#### Overexpressed RANKL protein activity

The functional integrity of overexpressed RANKL proteins was demonstrated by a 10-day mineral resorption assay (Fig. 2C1). This overexpression triggered a substantial increase in osteoclastic resorptive activity. As representative of ten mineral resorption assays, a higher bone resorption rate of 37.9% ( $p < 0.05$ ) compared to the controls was observed (Fig. 2C2).





**Fig. 2.** *In vitro* and *ex vivo* experimental results: (A) Positive receptor activator of the nuclear factor- $\kappa$ B ligand (RANKL) expression in transfected NIH3T3 cells, as shown by PCR; (B1) Expression of RANKL protein demonstrated by Western blot assay from cell culture production. Soluble and membrane-bound form (approximately 29 and 36 kDa), respectively; (B2) Bar chart of spectrophotometric measurements obtained with  $\beta$ -actin as loading control.  $*p < 0.05$ ; (C1) Comparative resorption assay; (C2) Bar chart of resorption area for the control and transfected groups. The resorption pit area of the transfected group makes up 57.8% of the total area measured; compare this with the 19.9% observed for the control group  $\times 40$ . Bar = 100  $\mu$ m. CT, control; TF, transfected.  $*p < 0.05$ .

### ***In vivo* experimental results**

#### **Animal welfare**

Induced bone resorption, whether through corticotomy or genetic transfer, did not affect the animal growth or bone mineral density with respect to the control groups (Data not shown).

*In vivo* gene transfection and corticotomy initially enhanced RANKL protein expression. After 10 days of the *in vivo* experiments, the corticotomy surgical procedure and local RANKL gene transfection resulted in increased RANKL expression when compared with the control groups, as shown by immunofluorescence (Figs 3A–E.1–4 and 4F). However, after 32 days, while protein levels in the transfection group were similar, there was a substantial decrease in the group undergoing surgery (Fig. 3G). It can be seen that TM in the surgery group progressively decreased

over the period of the experiment (Fig. 4A, B). A parallel reduction in RANKL levels was reported on the tenth day and on the day 32 for this group (Fig. 3B.1, 2). Significantly, the relative intensity of RANKL immunofluorescence observed in the different groups had reduced on the final day of observation in the experimental period, except for the transfected group (Fig. 3G).

#### ***In vivo* RANKL gene transfection increased osteoclastogenesis**

As shown in Fig. 5A–D, the transfected animals showed the highest TRAP+ osteoclast count per measured area after 10 days, when compared with those obtained from the surgery and control groups (Fig. 5E). This fact is a significant reflection of the integrity of the functional activity of the overexpressed RANKL protein *in vivo*. As shown in the merged microphotograph

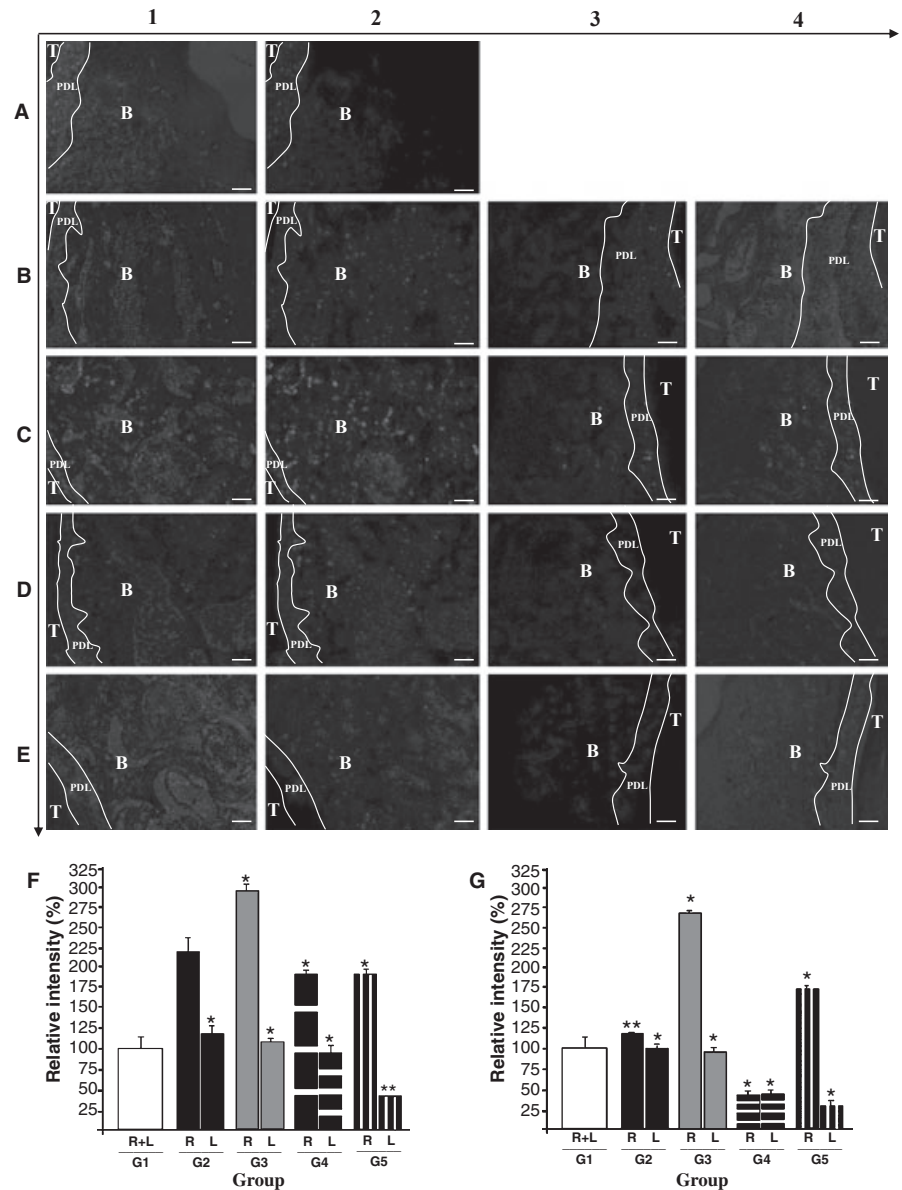


Fig. 3. Receptor activator of the nuclear factor- $\kappa$ B ligand (RANKL) expression detection via immunofluorescence for groups 1–5 (A–E, respectively), both hemi-maxillae at the mid-point of the observational period (day 10). (A–E.1) Merged immunofluorescence microphotographs from right hemi-maxillae when combined with serial H–E slides. (A–E.2) Right hemi-maxillae; (A–E.3) Left hemi-maxillae. (A–E.4) Merged immunofluorescence microphotographs left hemi-maxillae  $\times 40$ . Bar = 50  $\mu$ m. T, tooth; PDL, periodontal ligament; B, alveolar bone; (F) Relative fluorescence intensity for RANKL expression in groups 1–5 both hemi-maxillae, compared to external control (G1), after 10 days; (G) Relative fluorescence intensity for RANKL expression in groups 1–5 both hemi-maxillae, compared to G1 after 32 days. Note that measurements were performed on the last transfection on day 30. R, right hemi-maxilla; L, left hemi-maxilla. \* $p < 0.05$ ; \*\* $p < 0.01$ .

detail (Fig. 5D), most TRAP<sup>+</sup> osteoclast cells presented more than the two nuclei per cell characteristic of this type of cell. Interestingly, the time-dependent osteoclast formation observed in the surgery group increases significantly in parallel with the lower RANKL levels detected (Figs 3G and 5E). Note that the number of TRAP<sup>+</sup> osteoclast cells has remained relatively constant for all the different groups, except the surgery groups (Fig. 5E, F).

#### Local *in vivo* RANKL gene transfection and corticotomy affects TM

Both groups under TM force combined with genetic transfection or corticotomy resulted in a statistically significant ( $p < 0.05$ ) increase in final TM compared to

the internal and external control groups (Fig. 4A, B). The transfection group presented the highest final TM rate, with 41.27% and 23.61% higher average final TM rates than the external control group and the corticotomy group (G2 corticotomy), respectively (Fig. 4B). Group G2 corticotomy showed a 21.63% ( $p < 0.05$ ) higher final TM rate than the external control group (G1). Significantly, plasmid vector transfection with no RANKL insert and under TM force (G3, non-functional vector) did not significantly increase TM with respect to the external control group. Similarly, the group G2 flap surgery did not experience any statistically significant TM increase from that of the G1 (Figs 4A, B and 6A–E1).

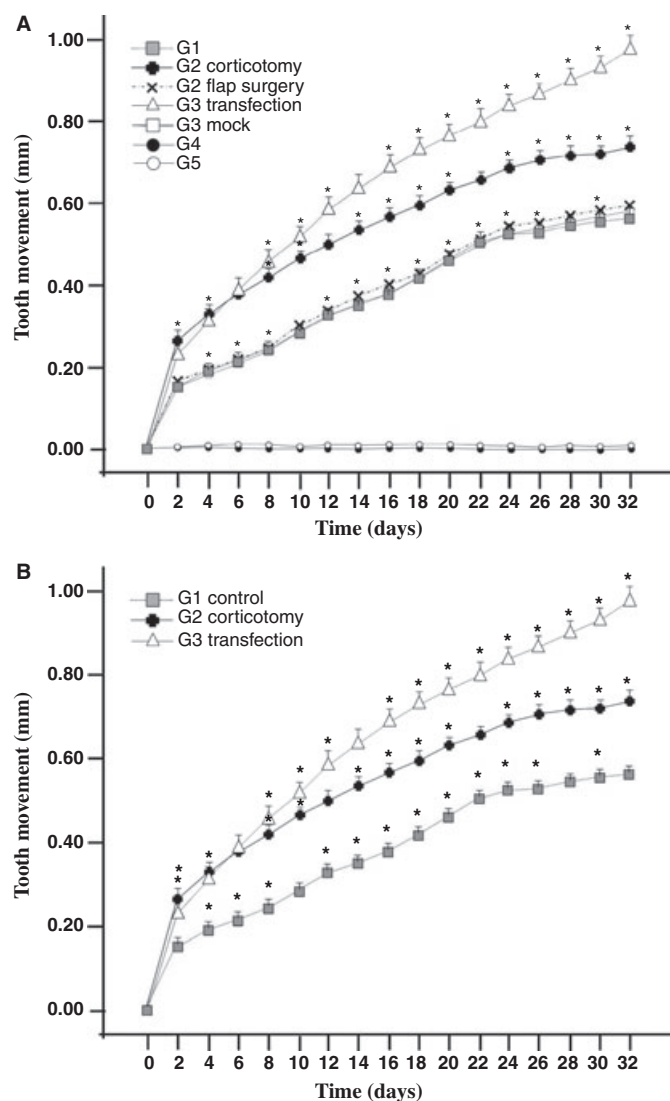


Fig. 4. (A) Clinical tooth movement (TM) data registered on the hemi-maxillae of the five groups. To simplify, the hemi-maxillae of groups 1, 4 and 5 were summarized as 1 per group, because almost identical clinical results were obtained for each of the respective hemi-maxillae in the group. (B) Statistical comparison of TM measurements registered for the groups the highest final TM rate – G2 corticotomy and G3 transfection – and the external control group (G1). The measurement error in a blind test was 0.01 mm when a single researcher measured 40 samples. \* $p < 0.05$ .

#### Effects of RANKL gene transfection on alveolar bone resorption

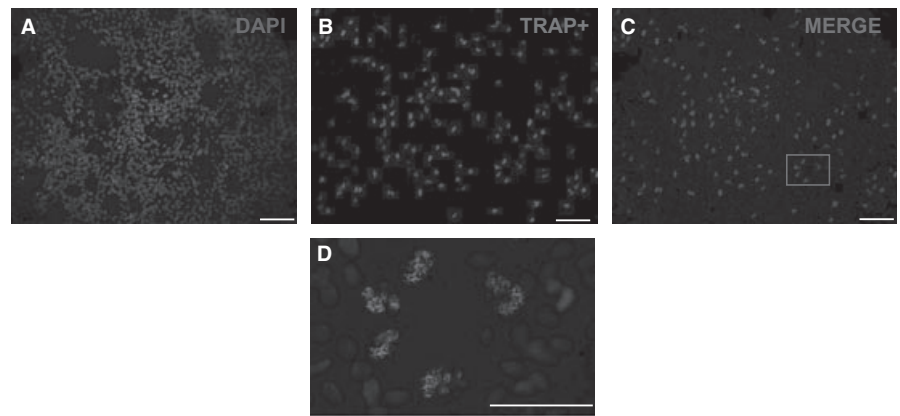
As expected, histological records obtained from groups under TM force with corticotomy (Fig. 6B.2) or RANKL transfection (Fig. 6C.2) showed a larger bone resorption area than the control groups (Fig. 6 A.2,3, B.3, C.3, D.2,3, E.2,3, F). Significantly, when the same random area was analysed histologically (Fig. 6.G), markedly induced bone resorption was observed throughout the experimental period in the RANKL transfection group without TM force (Fig. 6E.2), although there were no

consequences for TM (Fig. 4A). However, plasmid vector transfection with no insert (Fig. 6C.3) did not significantly increase mineral loss when compared to the G1 (Fig. 6A.2,3) as a consequence of TM force. Interestingly, the transfection group under orthodontic force maintained a higher bone resorption rate than the corticotomy group under force throughout the experiment.

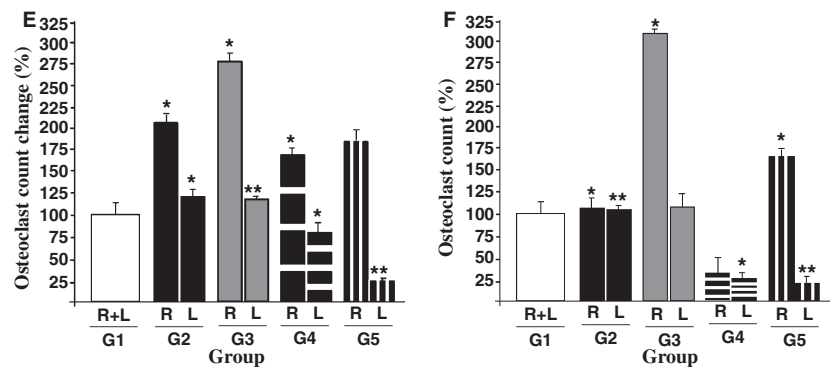
## Discussion

In this study, induced bone resorption through the RANKL activation pathway was evaluated to determine future clinical alternatives for reducing the malocclusion treatment time. Previous studies have determined that there is an increase in RANKL over OPG, its physiological antagonist, in the alveolar bone, owing to inflammatory pathologies, such as periodontitis (8) or even periapical lesions (19). Furthermore, positive imbalances of RANKL, also known as OPGL, ODF, TNFSF11 or TRANCE in the scientific literature, have been previously defined and located in the bone resorption area in the direction of TM (20). In agreement with the same findings, substantial decreases in the rate of TM have been attributed to local increases in OPG over RANKL in the bone resorption area in the direction of TM (21). Overall, the present research, both *in vitro* and *in vivo*, not only demonstrates an increase in bone resorption, but also that there is a direct impact on the TM mechanisms. Continued bone resorption on the TM side owing to local RANKL gene transfection shows that the RANKL molecule is at least one key acceleratory factor, as Kazanki et al. have also demonstrated with great elegance (22).

At the same time, alveolar corticotomy has been proposed as a adjunctive therapy for an increasing TM rate (2, 13, 14), and although other authors (23) maintain that alveolar corticotomy does not bring about a significant increase in the TM rate, the present findings would seem to contradict this. This could be due, at least in part, to the experimental TM appliance and the magnitude of force used for the rat model in these studies, about 100 g per molar – equivalent to over 5000 g per molar in humans – and which would be considered a heavy force for evaluating experimental rat TM as other authors have reported (24). Nevertheless, in our experiments, we did not use



**Fig. 5.** ELF97-based fluorescent tartrate-resistant acid phosphatase (TRAP) staining. (A–C) Microphotographs A to C showed representatives images obtained for TRAP+ osteoclast-like cells, visualized at day 10 of the experiment in G3. (A) Nuclei visualization; (B) TRAP+ cells; (C) Merged microphotographs; (D) Merged image detail obtained from red squared area of panel C. Most TRAP+ osteoclast-like cells presented more than the two nuclei per cell characteristic of this type of cell. ELF97-based fluorescent TRAP staining (green). Nuclei visualized by DAPI (blue)  $\times 40$ . Bar = 50  $\mu\text{m}$ ; (E) Results of osteoclast count for groups 1–5, both hemi-maxillae compared to controls at day 10; (F) Results of osteoclast count for groups 1–5, treated hemi-maxillae compared to control hemi-maxillae, at day 32. R, right hemi-maxilla; L, left hemi-maxilla. \* $p < 0.05$ ; \*\* $p < 0.01$ .



so-called 'light forces' – that is, <10 g per rat molar – for the corticotomy and transfection groups, because of evidence, reported by many authors, of the need for a heavier force in corticotomy as adjunctive therapy to induce orthodontic TM (2, 14). In addition, most of the previous TM studies took the upper first molars in a mesial direction as their experimental unit (25, 26). However, the physiological distal drift of the rat molars might be interfering with the objective results extrapolated from those experiments (24). To avoid misinterpreting the results, we adopted a new experimental TM model, with an I-shaped appliance that generates a constant magnitude and direction force in the mid-palatal direction, minimizing experimental error.

Numerous reports have described the pharmacological acceleration of TM (27–29). However, these drugs are quickly flushed away through the bloodstream, thus proving useless, moreover, when systemic administration is used. As a mechanism, local gene transfer facilitates prolonged yet always time-limited protein expression, regardless of the blood circulation, (16) particularly when a vasoconstrictor adjunct has been used. In this study, transfer to the periodontal tissue was carried out with a mixed non-viral system (16) to

take advantage of the fusing properties of the viral membrane and, at the same time, to avoid viral recombination, neoplastic transformation, high toxicity or genome incorporation as a consequence of the viral transfer method (30). In our *in vivo* experiments, no inflammatory or rejection responses were observed clinically during the observational period; in addition, the TM rate increased linearly during the experimental period. Nonetheless, it is possible that an immune response may occur because of the repeated injections to the viral membrane system, even when a non-viral method is used. Thus, a longer experimental period could lead to neutralization of RANKL protein production.

The aims of this study were to compare corticotomy surgery and RANKL gene transfer to the periodontal tissue as two methods that might substantially reduce the treatment time for malocclusion through induced osteoclastogenesis and bone resorption with no apparent systemic bone imbalance. Despite the fact that the corticotomy group under TM force (G2 corticotomy) showed a greater initial TM increase, it experienced a gradual decrease in TM. This clinical observation correlates with the decrease in the RANKL levels detected and the lower TRAP+ cell count (Fig. 5E,



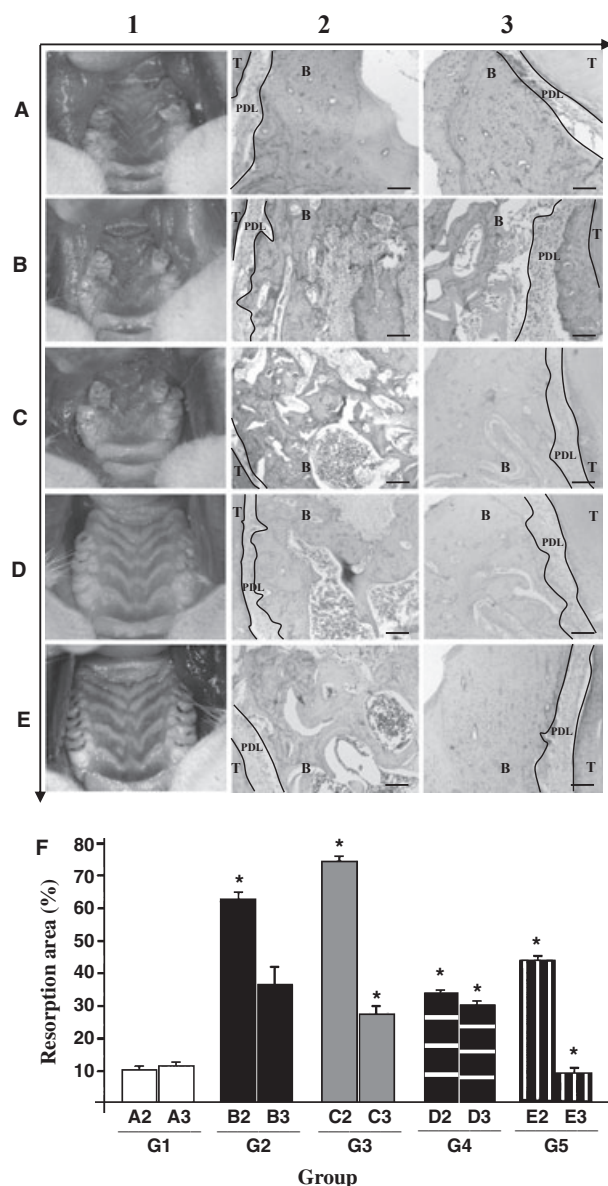


Fig. 6. Clinical intra-oral and histological results of the *in vivo* experiments. (A-E.1) Representative clinical intra-oral results for group 1-5; (A-E.2) Representative histological microphotographs for groups 1-5, right hemi-maxillae; (A-E.3) Representative histological microphotographs for groups 1-5, left hemi-maxillae. H-E  $\times 40$ . Bar = 50  $\mu$ m. T, tooth; PDL, periodontal ligament; B, alveolar bone; (F) Graph of results of *in vivo* bone resorption area.  $*p < 0.05$ . (G) Transversal section of a rat molar. The squared area represents the randomly selected area for standardized histological examination of the palatal area. P, palatal side; B, buccal side.

F). Thus, the observed effect might be caused by a compensatory homeostatic effect, as occurs with any other surgical procedure in conjunction with the RAP (11). This fact seriously limits the possibilities of prolonged TM acceleration over time without fresh surgery. In actual practice, osteopenic state (RAP) using SFOT is perpetuated by generating internal strain gradients through weekly or bi-weekly adjustments of the appliance that leads to an effective acceleratory purpose. Catabolic and anabolic phenomena have previously been referred to as causing this acceleration (31). In our experiments, by day 32, induced RANKL overexpression increased TM to 41.27% ( $p < 0.05$ ) compared to the control group. Furthermore, TM accel-

eration was observed with a correspondingly sustained increase in RANKL levels and a significant increase in active osteoclast cells.

The search for selective acceleration of TM rather than more rudimentary and aggressive surgery with its derived risks and morbidity should be stated for the orthodontist. The orthodontist should progress towards finding a more accurate method for accelerating TM.

## Conclusions

Despite the limitations of the study, on the basis of our results, gene therapy has been tested as an alter-

native method to corticotomy, showing higher efficacy than standard surgical methods used for acceleratory purposes in orthodontics. Nevertheless, further evidence is needed – focusing on toxicity or reducing secondary effects – to determine its future clinical applicability.

## Clinical relevance

Corticotomy-assisted orthodontics is increasingly used for accelerating TM and shortening orthodontic treatment time. We investigated the biological molecular basis of TM acceleration, based on the

biological mechanisms underlying corticotomy-assisted orthodontics, and, more specifically, we compared corticotomy surgery and RANKL gene transfer to the periodontal tissue as two methods that might substantially reduce orthodontic treatment time. This kind of biological study will benefit future clinical orthodontics, which, like other biomedical disciplines, has to adapt to advances in biological applications to optimize clinical results and efficiency of treatment.

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## References

- Wilcko MT, Wilcko WM, Pulver JJ, Bissada NF, Bouquot JE. Accelerated osteogenic orthodontics technique: a 1-stage surgically facilitated rapid orthodontic technique with alveolar augmentation. *J Oral Maxillofac Surg* 2009;67:2149–59.
- Iino S, Sakoda S, Ito G, Nishimori T, Ikeda T, Miyawaki S. Acceleration of orthodontic tooth movement by alveolar corticotomy in the dog. *Am J Orthod Dentofac Orthop* 2007;131:448.e1–8.
- Schwartzman V, Schwartzman R. Corticotomy. *Clin Orthop Relat Res* 1992;280:37–47.
- Andrle J, Subrtová I, Subrt I. [Complications of corticotomy]. *Cesk Stomatol* 1970;70:193–6.
- Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan C R, Burgess T et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;93:165–76.
- Mori K, Le Goff B, Berreur M, Riet A, Moreau A, Blanchard F et al. Human osteosarcoma cells express functional receptor activator of nuclear factor-kappa B. *J Pathol* 2007;211:555–62.
- Romas E. Clinical applications of RANKL ligand inhibition. *J Intern Med* 2009;39:110–6.
- Han X, Lin X, Seliger AR, Eastcott J, Kawai T, Taubman MA. Expression of receptor activator of nuclear factor-kappaB ligand by B cells in response to oral bacteria. *Oral Microbiol Immunol* 2009;24:190–6.
- Oshiro T, Shiotani A, Shibasaki Y, Sasaki T. Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegerin-deficient mice. *Anat Rec* 2002;266:218–25.
- Blair JM, Zhou H, Seibel MJ, Dunstan CR. Mechanisms of disease: roles of OPG, RANKL and RANK in the pathophysiology of skeletal metastasis. *Nat Clin Pract Oncol* 2006;3:41–9.
- Frost HM. The regional accelerated phenomenon. *Orthop Clin North Am* 1981;114:372–82.
- Burstone CJ. The biophysics of bone remodelling during orthodontics-optimal force considerations. In: Norton LA, Burstone CJ, editors. *The Biology of Tooth Movement*. Boca Raton: CRC Press; 1989. pp. 321–34.
- Wilcko WM, Wilcko T, Bouquot JE, Ferguson DJ. Rapid orthodontics with alveolar reshaping: two case reports of decrowding. *Int J Periodontics Restorative Dent* 2001;21:9–19.
- Kim SJ, Park YG, Kang SG. Effects of corticision on parodontal remodeling in orthodontic tooth movement. *Angle Orthod* 2009;79:284–91.
- Scheven BA, Visser JW, Nijweide PJ. In vitro osteoclast generation from different bone marrow fractions, including a highly enriched haematopoietic stem cell population. *Nature* 1986;321:79–81.
- Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N et al. Hemagglutinating virus of Japan (HJV) envelope vector as a versatile gene delivery system. *Mol Ther* 2002;6:219–26.
- Filgueira L. Fluorescence-based staining for tartrate-resistant acid phosphatase (TRAP) in osteoclasts. *J Histochem Cytochem* 2004;52:411–4.
- Matsushita T, Chan YY, Kawanami A, Balmes G, Landreth GE, Murakami S. Extracellular signal-regulated kinase 1 (ERK1) and ERK2 play essential roles in osteoblast differentiation and in supporting osteoclastogenesis. *Mol Cell Biol* 2009;29:5843–57.
- Fukada SY, Silva TA, Saconato IF, Garlet GP, Ávila-Campos MJ, Silva JS et al. iNOS-derived nitric oxide modulates infection-stimulated bone loss. *J Dent Res* 2008;87:1155–9.
- Ogasawara T, Yoshimine Y, Kiyoshima T, Kobayashi I, Matsuo K, Akamine A et al. In situ expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. *J Periodontol Res* 2004;39:42–9.
- Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H. Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. *J Dent Res* 2004;83:920–5.
- Kanzaki H, Chiba M, Arai K, Takahashi I, Haruyama N, Nishimura M et al. Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. *Gene Ther* 2006;13:678–85.
- Lee W, Karapetyan G, Moats R, Yamashita DD, Moon HB, Ferguson DJ et al. Corticotomy-/osteotomy-assisted tooth movement microCTs differ. *J Dent Res* 2008;87:861–7.
- Ren Y, Maltha JC, Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement – a critical review and a proposed solution. *Eur J Orthod* 2004;26:483–90.
- Kawarizadeh A, Bourauel C, Götz W, Jäger A. Early responses of periodontal ligament cells to mechanical stimulus in vivo. *J Dent Res* 2005;84:902–6.

26. Xie R, Kuijpers-Jagtman AM, Maltha JC. Osteoclast differentiation during experimental tooth movement by a short-term force application: an immunohistochemical study in rats. *Acta Odontol Scand* 2008;66:314–20.
27. Yamasaki K, Shibata Y, Fukuhara T. The effect of prostaglandins on experimental tooth movement in monkeys (*Macaca fuscata*). *J Dent Res* 1982;61:1444–6.
28. Kalia S, Melsen B, Verna C. Tissue reaction to orthodontic tooth movement in acute and chronic corticosteroid treatment. *Orthod Craniofac Res* 2004;7:26–34.
29. Chung CJ, Baik HS, Soma K. Bone formation and tooth movement are synergistically enhanced by administration of EP4 agonist. *Am J Orthod Dentofac Orthop* 2007;132:427.e13–20.
30. St George JA. Gene therapy progress and prospects: adenoviral vectors. *Gene Ther* 2003;10:1135–41.
31. Sebaoun JD, Kantarci A, Turner JW, Carvalho RS, Van Dyke TE, Ferguson DJ. Modeling of trabecular bone and lamina dura following selective alveolar decortication in rats. *J Periodontol* 2008;79:1679–88.

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