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Original Article

One-stage versus two-stage piezocision-assisted orthodontic tooth movement: A preclinical study based on Nano-CT and RT-PCR analyses

Carole Charavet[a,b,c,d,*], Dorien Van Hede[d,e], Sandy Anania[d,f], Nathalie Maes[g], Adelin Albert[h], France Lambert[d,e]

[a] Université Côte d'Azur, Faculté de Chirurgie-Dentaire, Département d'Orthodontie, Nice, France
[b] CHU de Nice, Pôle Odontologie, UEC d'Orthodontie, Nice, France
[c] Laboratoire Microbiologie orAle Immunité Santé MICORALIS (UPR 7354), Université Côted'Azur, France
[d] Dental Biomaterials Research Unit, University of Liège, Belgium
[e] Department of Periodontology, Oral Surgery and Implant Surgery, University Hospital of Liège, Liège, Belgium
[f] Metastasis Research Laboratory (MRL), GIGA-Cancer, Pathology Institute B23, University of Liège, Liège, Belgium
[g] Biostatistics and Medico-Economic Information Department, University Hospital of Liège, Liège, Belgium
[h] Department of Public Health, University of Liège, Liège, Belgium

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ABSTRACT

Objective: To evaluate the effect of a second-stage piezocision on the biological response.
Materials and methods: 60 rats were randomly allocated to 6 experimental groups of 10 rats. Rats undergoing a one-stage piezocision were sacrified on day 7, 28 and 42 (groups 1–3) while rats undergoing a two-satge piezocision were sacrified on day 42, 63 and 90 (groups 4–6), respectively. The biological response was investigated in 3D at the tissue level using Nano-computed tomography (Nano-CT) and, at the molecular level using the qRT-PCR technique. Bone Volume Fraction (BVF) loss was the primary endpoint.
Results: Similar loss of BVF were observed both after the first and second piezocisions. The change in BVF loss between 7 and 28 days after each piezocision were 25.1 ± 13.0 (SE)% and 11.2 ± 11.6 (SE)% respectively and did not differ from each other (p = 0.43). Changes in BVF loss from 7 to 42 days were also comparable in one-stage and two-stage piezocision (4.9 ± 12.3 (SE) vs. -19.9 ± 13.4 (SE), p = 0.19). At the molecular level, all parameters except Translating Ribosome Affinity Purification (TRAP) protein had identical patterns.
Conclusion: Within the limits of the present study, a second piezocision allowed to re-induce the Regional Acceleratory Phenomenon (RAP) effect. Nevertheless, the relevance of the findings to the clinical effect has not been tested.

1. Introduction

"Acceleration" in orthodontics has known a revival of interest over the last five years [1] . Back in the 60's, bone block corticotomies were initially described to accelerate Orthodontic Treatment (OT) [2]. However, the procedure was rather invasive and therefore poorly applied. The technique was updated in the 2000s by the Wilcko brothers [3–6] and possibly combined with alveolar augmentation. More recently, less invasive approaches have been proposed in order to decrease the post-operative discomfort and several minimally invasive techniques [7] such as piezopuncture [8], micro-osteoperforations [9] and piezocision [10–21] are currently available. Specifically, Charavet et al [11]. and Gibreal et al [22]. demonstrated high level of acceptance and satisfaction following piezocision procedures, involving localized corticotomies without elevating a flap.

Several Randomized Controlled Trials (RCTs) showed a significant decrease of the overall OT time when piezocision was applied compared to a conventional approach [10,23–32] without significant adverse events [33]. However, the decrease in treatment time was found up 4 to 6 months after surgery and the benefit of piezocision was no longer observed during the second part of the treatment [10,23]. Specifically, in cases of minor to moderate overcrowdings, the acceleration of the orthodontic tooth movement occurred during the alignment phase whereas no further effect was observed during the fine-tuning phase [19]. The limited efficacy of piezocision was attributed to the temporary effect of the Regional Acceleratory Phenomenon (RAP) (Frost, 1983), a transient biologic storm found after bone injury. Indeed, RAP is induced by bone injury and characterized by an increase of bone turnover associated with a decrease of bone mineral content [34], hence allowing an acceleration of tooth

* Corresponding author at: Faculté de Chirurgie-Dentaire, Département d'Orthodontie, Université Côte d'Azur, 06300 Nice, France.
E-mail address: c.charavet@gmail.com (C. Charavet).

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displacement [34]. Preclinical studies investigated the biological response at the molecular, cellular and tissue levels following a single stage piezocision-assisted orthodontic tooth movement in a rat model [35,36]. The RAP effect was found to be transient and completely reversible. Furthermore, the intensity of the RAP is corticotomy depth dependent [37]. Therefore, as previously suggested [23], performing a second piezocision might be relevant to prolong/re-induce the RAP biological response and maintain the accelerated tooth movement. To the best of our knowledge, a second stage piezocision was never investigated in animal nor in humans.

The objective of this preclinical study was to observe both at tissue and molecular levels the effect of a second stage piezocision compared to a single stage piezocision.

2. Materials and methods

2.1. Ethics approval

The Institutional Animal Care and Use Ethics Committee of the University of Liege (Liège, Belgium) approved the entire study. The Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines [38] were cautiously respected as well as national and European legislated guidelines.

2.2. Animals and study design

A total of 60 adult male OFA (Oncins France Strain A, Charles Rivers Company) rats (body weight 440−480 g) constituted the study material. The animals were acclimatized in the Animal Housing of the University of Liège (Belgium) for 6 days. They were kept in cages at a temperature of 22.5 ± 0.5 °C, with a 12 h:12 h light-dark cycle and were fed with a specific diet composed of a powdered diet (RM3, TechniLab, The Netherlands) and soft food (diet gel, ClearH20, The Netherlands). Their body weight was carefully monitored before and daily after surgery until they recovered their initial weight.

The 60 animals were randomly allocated to 6 experimental groups of 10 rats. In each group, 5 rats were used for tissue analysis (Nano-CT) and 5 other rats for molecular analysis (RT-PCR). Rats of groups 1−3 underwent a single piezocision at day 0 (baseline), whereas rats of groups 4−6 underwent a two-stage piezocision, specifically they received a first piezocision at day 0 (baseline) followed by a second piezocision at day 35. Rats were sacrified at day 7 (group 1), 28 (group 2), 42 (group 3 and 4), 63 (group 5) and 90 (group 6), respectively. The study design is displayed in Fig. 1.

2.3. Sample size calculation

The sample size calculation was based on the difference between bone volume fraction (BVF) (primary endpoint) features after a single or a two-stage piezocision procedures. The other Nano-CT parameters were regarded as secondary endpoints. With 5 rats in each group (total 5 × 6 = 30 rats), a maximal BVF difference of at least two standard deviations between the two piezocision procedures could be discerned with 80% power at the 5% critical level assuming a standard deviation (SD) of 15% and using a two-sided unpaired Student *t*-test. The sample size was doubled to a total of 60 rats to account for molecular parameters evaluation (5 rats in each group) as seen in Fig. 1.

2.4. Experimental procedure

In each animal, the left hemimaxilla was used as the experimental side. The right hemimaxilla did not receive any treatment and served as negative control to account for intra-individual variability. Rats were under general anesthesia [ketamine (8 mg/kg) and xylazine (5 mg/kg); intraperitoneal administration] during the overall procedures. The orthodontic custom-appliance was placed as previously described by Charavet et al. in Fig. 2 [36]: Transbond Self Etching Primer (3M, CA, USA) and a resin composite (Venus Flow, A2, Kulzer, Hanau, Germany) were employed to etch and bond a rectangular metal mesh (5 X 2 mm) on the two first molars and a metal ring (GAC International LLC) with a hook (3M, CA, USA) on the incisors (the anchorage). A 25 g Sentalloy stainless steel coil spring (GAC International LLC) - according to a previous study [35] - was hooked to the incisors to move mesially the two first molars. Corticotomy (3 mm in length and 1.5 mm in depth) were performed using a Piezotome (Satelec, Acteon group, Merignac, France), as represented by the three black lines in Fig. 2. In all groups, the first piezocision was performed at baseline, the day of the orthodontic appliance placement while in groups 4, 5 and 6 the animals underwent also a second piezocision at day 35. The orthodontic custom-appliance as well as the piezocision osteotomies are presented in Fig. 2.

Adrenergic antagonist (Antisedan, 1 mg/kg), antibiotics (Baytril, 5 mg/kg), painkiller (Temgesic, 0.05 mg/kg) and anti-inflammatory (Rimadyl, 5 mg/kg) were delivered postoperatively. The antibiotics (Baytril, 5 mg/kg) were continued for 7 days after the surgery through the drinking water. Animals were euthanized by an overdose of pentobarbital. Samples were collected, fixed in 4% formaldehyde for one day at 4 °C, and then stored in sterile PBS at 4 °C until the Nano-CT acquisition.

2.5 Data Acquisition

2.5.1. 3D tissue level analysis (Nano-CT)

Subsequently to the fixation process, a Nano-CT system (Phoenix NanoTom M, GE Measurement and Control Solutions, Wunstorf, Germany), was used to scan each specimen. The image acquisition conditions were as follows: voltage at 80 kV, current at 190 μA, applied filter of 0.25 mm, voxel size of 7.5 μm, 1.800 images over 360° scan and used 'fast scan' mode. The Phoenix datos|x CT software was then used to perform the volumetric reconstruction of each sample before being reoriented using the DataViewer software (Bruker micro-CT, Kontich, Belgium). For 3D image visualization, serial regions of interest (ROI) were drawn every 10 pictures using the CTAn software (Bruker micro-CT, Kontich, Belgium) comprising the roots of the first and second maxillary molars. Each Volume of interest (VOI) was obtained by interpolation of all ROIs. The VOI was defined as the volume in between the roots of the first and the second maxillary molars since these served as landmarks. A quantitative evaluation of the mineralized bone and the trabecula thickness, number and separation was calculated using a segmentation procedure based on different ranges of greyscales. Then, the following parameters were assessed for each sample: (i) Bone volume fraction (BVF) defined as the ratio of mineralized tissue (Bone Volume: BV) to the total volume (TV) of the VOI: BVF= BV/TV; (ii) Trabecular Number loss (Tb. N); (iii) Trabecular Thickness (Tb. Th); (iv) Trabecular Separation gain (Tb. Sp).

2.6. Molecular analysis (RT-PCR)

All samples were harvested with their surrounding soft tissue, quickly snipped in small pieces and placed in Eppendorf's to be snap frozen in liquid nitrogen. At the end of the *in vivo* experimentation, the frozen samples were transferred into 1 ml of TRIzol (Life Technologies) containing one Stainless-Steel Bead (Qiagen) and disrupted using a TissueLyzer (Qiagen). An RNA extraction using Direct-Zol (Zymo research) was then performed according to the manufacturer's protocol. The RNA concentration was determined using a NanoDrop. The RevertAid H Minus First Strand cDNA Synthesis Kit (ThermoScientific) was used in order to synthetize cDNA. Finally, a SYBR Green quantitative PCR was performed in duplicate (ABI 7900 HT, Applied Biosystem). The different forward and reverse sequences of

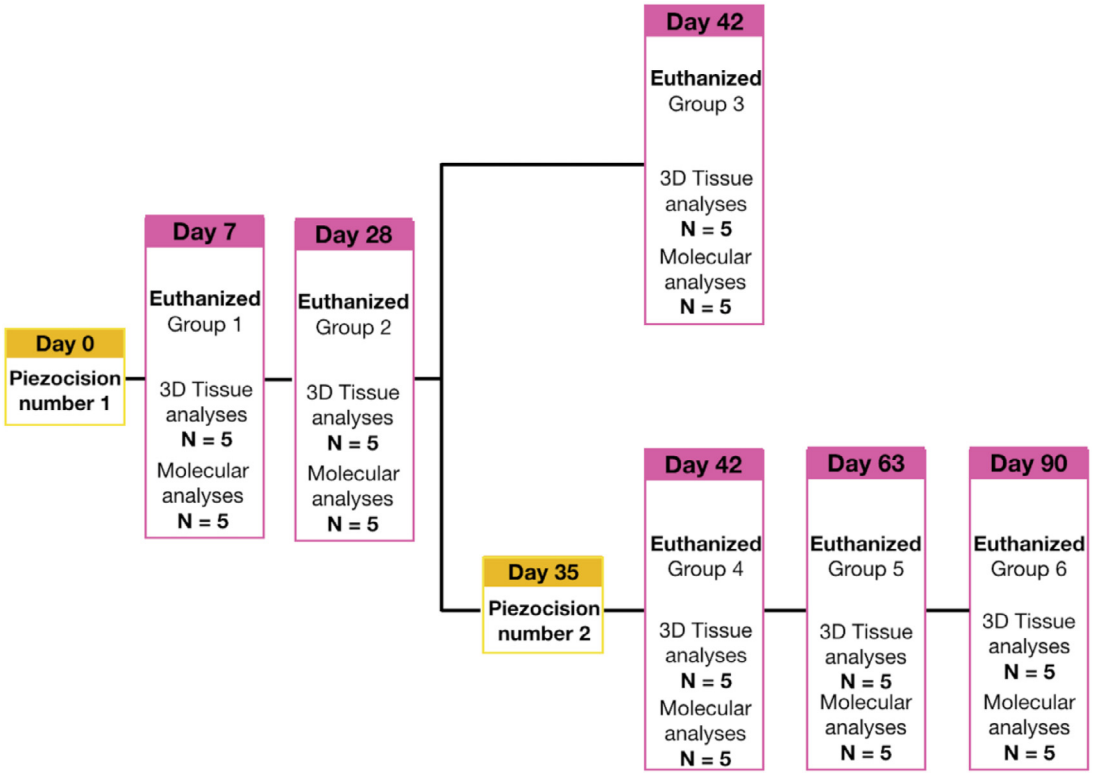

Fig. 1. Study design and numbers of rats assigned to each group for 3D tissue and molecular analyses.

the primers targeting the genes of interest are listed in Table 1. The obtained values were analyzed using the Qbase software (Biogazelle) to calculate the mRNA relative expression levels for target genes normalized to a reference gene (β-actin).

2.7. Statistical analysis

Results were expressed as mean and SD. To eliminate within-individual variability, all measurements of the equipped "experimental" side were corrected by subtracting values of the non-equipped "control" side. To compare the non-linear response (Y) of tissue parameters after one-stage piezocision and two-stage piezocision procedures, a linear model (Model 1) was fitted to the experimental data by multiple regression analysis: $Y = \beta0 + \beta1 \times PZ + (\beta2 \times T2^* + \beta3 \times T3^*) \times (1 - PZ) + [\beta4 \times (T4 - 35)^* + \beta5 \times (T5 - 35)^*] \times PZ$. In this model, PZ=0 (one-stage piezocision) or 1 (two-stage piezocision), T2* is a dummy binary variable equal to 1 if time of measurement is 28 days and 0 otherwise, T3* is a dummy binary variable equal to 1 if time of measurement is 42 days and 0 otherwise, (T4-35)* is a dummy binary variable equal to 1 if time of measurement is 63 days and 0 otherwise, and (T5-35)* is a dummy binary variable equal to 1 if time of measurement is 90 days and 0 otherwise. Note that in this model, times after the second piezocision have been shifted by subtracting 35 days to compare them with those after the first piezocision so that both non-appearing time points T1 and (T3-35) were equal to 7 days and served as reference for other time points. Under Model 1, the null hypothesis $\beta1 = 0$ indicates overall identical effects of one-stage and two-stage piezocision procedures; the null hypothesis $\beta2 = \beta4$ compares the changes in outcome from day 7 to day 28 after each piezocision and the hypothesis $\beta3 = \beta5$ compares the changes in outcome from day 7 to day 42 after each piezocision. For RT-PCR molecular parameters with linear evolutions (BMP2, IL6,

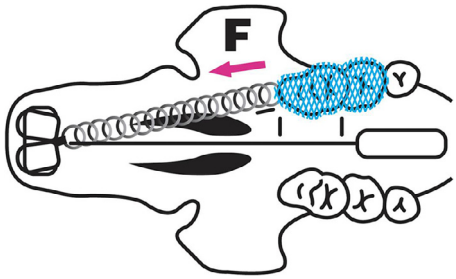

Fig. 2. Occlusal view of the custom-rat orthodontic appliance. Black lines around the first and the second molars represent the flapless osteotomies performed with the piezoelectric device.

Table 1.
Forward and reverse primer sequences (5′–3′) for the reference and the tested genes designed with the Primer 3 online software.

Genes*	Primer sequence 5′–3′
β-Actin Forward	CGT CTT CCC CTC CAT CGT G
β-Actin Reverse	AGG ATG CCT CTC TTG CTC TG
RunX2 Forward	GGC CCT GGT GTT TAA ATG GT
RunX2 Reverse	ACG CCA TAG TCC CTC CTT TT
Rank-L Forward	CCC ATC GGG TTC CCA TAA AGT C
Rank-L Reverse	GCC TGA AGC AAA TGT TGG CGT A
OPG Forward	GTC CCT TGC CCT GAC TAC TCT
OPG Reverse	GAC ATC TTT TGC AAA CCG TGT
BMP-2 Forward	GAA GCC AGG TGT CTC CAA GAG
BMP-2 Reverse	GTG GAT GTC CTT TAC CGT
TRAP Forward	TGC ATG ACG CCA ATG ACA A
TRAP Reverse	GAG GGC ACG GTC AGA GAA C
IL6 Forward	GCC CTT CAG GAA CAG CTA TGA
IL6 Reverse	TGT CAA CAT CAG TCC CAA GA
IL1β Forward	ACT CAT TGT GGC TGT GGA GA
IL1β Reverse	TAG CAG GTC ATC CC
TNFα Forward	CAG CAA CTC CAG AAC ACC CT
TNFα Reverse	GCC AGT GTA TGA GAG GGA CG

* RUNX2 Runt-related transcription factor 2; RANK-L Receptor Activator of nuclear factor Kappa-B Ligand; OPG Osteoprotegerin protein; BMP-2 Bone Morphologic Protein; TRAP Translating Ribosome Affinity Purification; IL Interleukin; TNF Tumor Necrotic Factor.

OPG, TRAP, RANK-L and OPG/RANK-L), a simpler model (Model 2) was fitted to the data, specifically $Y = \beta0 + \beta1$ x PZ + $\beta2$ x T x (1 − PZ) + $\beta3$ x (T-35) x PZ, where PZ = 0 or 1 as before and T is time expressed in days since baseline. The null hypothesis $\beta1=0$ indicates identical overall effect of both piezocision procedures and the null hypothesis $\beta2 = \beta3$ means equal evolution (slopes) after first and second piezocision. Estimates of β regression coefficients were exposed with their standard error (SE). Results were considered significant at the 5% critical level ($p < 0.05$). All calculations were made with SAS version 3.4 (SAS Institute, Cary, NC, USA) and R version 3.2.2.

3. Results

3.1. Animals

All rats remained healthy during the experimentation and, although a reduction of their body weight was recorded the first days following the surgical procedure, they all recovered their initial body weight within 10 days with even a minor increase thereafter. In groups 1, 2 and 6, respectively, one rat was excluded from the tissue analysis due to broken orthodontic appliance. In group 5, one rat was excluded from the molecular analysis for the same reason.

3.2. 3D tissue level outcomes

3.2.1. Loss of bone volume fraction

Fig. 3 displays the loss of BVF (%) over time. Bone demineralization reached a peak 28 days after each piezocision procedure and decreased thereafter. No overall significant difference was observed in BVF loss pattern between first and second piezocision ($\beta1 = 7.8 \pm 12.3\%$; $p = 0.53$); further, the estimates of coefficients $\beta2$ and $\beta4$ which measure the change in BVF loss between 7 and 28 days after each piezocision were 25.1 ± 13.0 (SE)% and 11.2 ± 11.6 (SE)% respectively and did not differ from each other ($p = 0.43$); finally, the estimates of coefficients $\beta3$ and $\beta5$ which measure the change in BVF loss between 7 and 42 days after each piezocision were also comparable (4.9 ± 12.3% vs. −19.9 ± 13.4%, $p = 0.19$).

3.2.2. Trabecular thickness loss

The loss of trabecular thickness after one-stage and two-stage piezocision procedures is displayed in Fig. 4A. By fitting Model 1 to the data, the overall effect of the second piezocision on loss of trabecular thickness was identical to that of the first piezocision ($\beta1 = 4.5 \pm 2.6$, $p = 0.10$); changes between 7 and 28 days after each piezocision were similar ($\beta2 = 0.33 \pm 2.8$ vs. $\beta4 = -0.059 \pm 2.5$, $p = 0.92$) and changes between 7 and 42 days after each piezocision were also comparable ($\beta3 = 2.7 \pm 2.6$ vs. $\beta5 = -2.4 \pm 2.8$, $p = 0.20$).

3.2.3. Trabecular number loss

The decrease of trabecular number in one-stage and two-stage piezocision is portrayed in Fig. 4B. The fitting of Model 1 to the data evidenced no overall difference between one-stag and two-stage piezocision procedures ($\beta1 = -0.0060 \pm 0.0093$, $p = 0.53$) although a slight but not significant increase in trabecular number loss was observed after 21 days compared to 7 days in both groups ($\beta2 = 0.017 \pm 0.0098$, $p = 0.096$ for one-stage piezocision and $\beta4 = 0.015 \pm 0.088$, $p = 0.098$ for two-stage piezocision). The two estimates were not different ($p = 0.89$). The estimates of coefficients $\beta3$ and $\beta5$ which measure the change in trabecular number loss between 7 and 42 days after each piezocision were also similar ($\beta3 = -0.0050 \pm 0.0093$ vs. $\beta5 = -0.014 \pm 0.010$, $p = 0.51$) and not significant.

3.2.4. Trabecular separation gain

By fitting Model 1 to data (Fig. 4C), the evolution of trabecular separation gain was comparable after the first and the second piezocision ($\beta1 = -8.0 \pm 7.7$, $p = 0.31$); changes between 7 and 28 days after each piezocision were similar ($\beta2 = 5.9 \pm 8.1$ vs. $\beta4 = 13.6 \pm 7.2$, $p = 0.49$) and similarly for changes between 7 and 42 days ($\beta3 = -8.3 \pm 8.1$ vs. $\beta5 = -10.9 \pm 8.4$, $p = 0.82$).

3.3. Molecular outcomes (qRT-PCR)

The simpler Model 2 was fitted to data of each qRT-PCR parameter because of their linear evolutions after one-stage piezocision and two-stage piezocision procedures as evidenced in Table 2.

The null hypothesis of overlay of one-stage and two-stage piezocision procedures, namely $\beta1 = 0$ and $\beta2 = \beta3$ respectively, was not rejected for molecular parameters BMP2 ($p = 0.86$ and 0.99, respectively), IL6 ($p = 0.26$ and 0.41, respectively) and RANK-L ($p = 0.91$ and 0.89, respectively), whose concentrations increased after each piezocision, but also for OPG ($p = 0.41$ and 0.71, respectively) whose concentration levels decreased after each piezocision. Concerning the OPG/RANK-L ratio, where concentrations increased after the first piezocision but decreased after the second piezocision, only a tendency for rejecting overlay was noticed ($p = 0.13$ and 0.066, respectively).

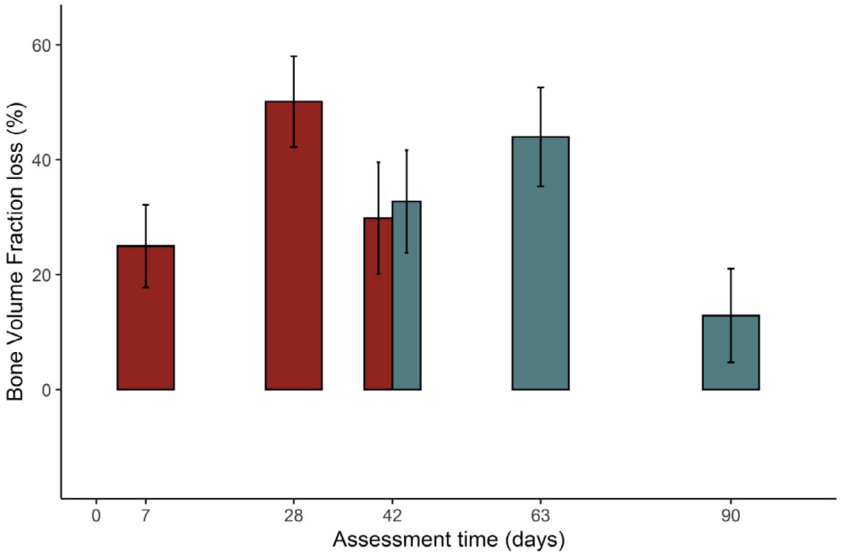

Fig. 3. Bone Volume fraction loss (%) evolution (mean and standard error) after one-stage piezocision groups (red) and after two-stage piezocision groups (blue).

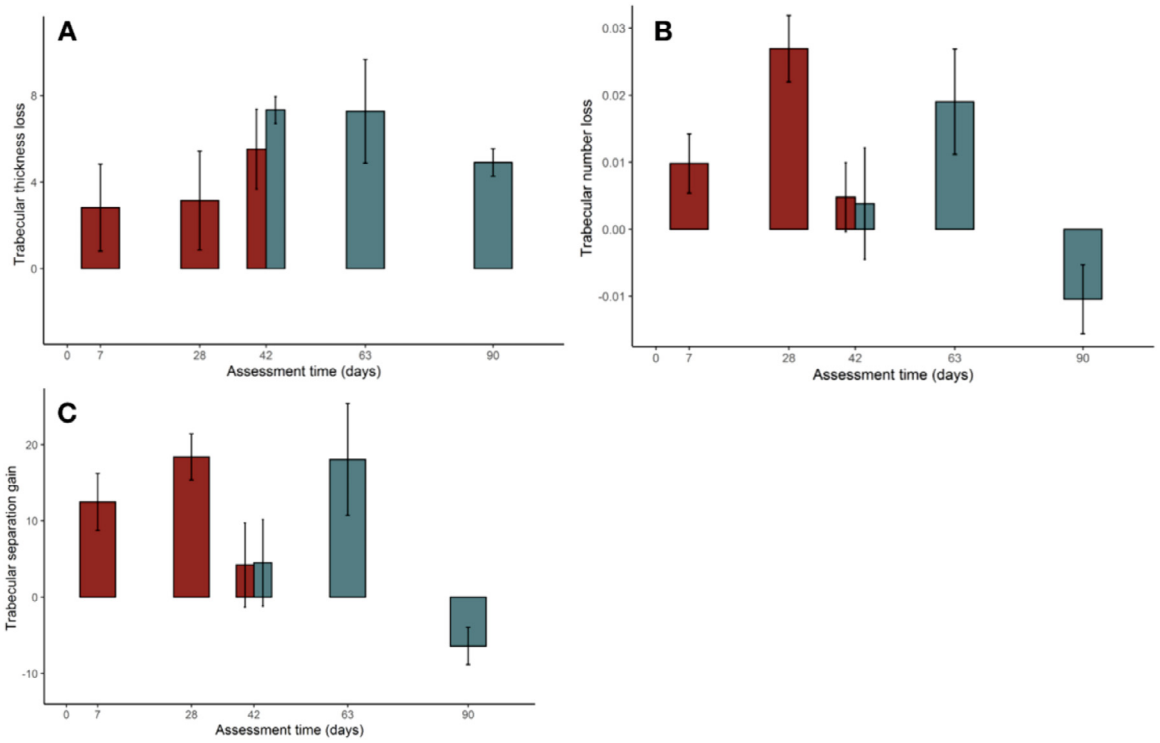

Fig. 4. (A) Trabecular thickness loss, (B) trabecular number loss and (C) trabecular separation gain evolution (mean and standard error) after one-stage piezocision groups (red) and after two-stage piezocision groups (blue).

By contrast, fitting Model 2 to TRAP data evidenced an effect size on concentrations after the second stage piezocision ($\beta 1 = 0.94 \pm 0.44$; $p = 0.045$) but not on slopes (null hypothesis $\beta 2 = \beta 3$, $p = 0.14$) despite a nonsignificant slope in one-stage PZ ($\beta 2$ estimate -0.0086 ± 0.015, $p = 0.57$) and a significant slope in two-stage PZ ($\beta 3$ estimate -0.036 ± 0.0092, $p = 0.0009$).

4. Discussion

This preclinical study aims to explore, for the first time, the effect of a second-stage piezocision on the biological response at the tissue and molecular level. Globally, the biological response appeared similar after first and second stage piezocision, emphasizing for the first time the interest of repeated corticotomies to prolong/re-induce tooth movement acceleration.

Nano-CT technology was used to evaluate bone mineral content, allowing accurate characterization of tissue microarchitecture [39] and quantification of mineralization [40], particularly for small animal models [41].

In this study, the alveolar bone density (BVF) gradually decreased as well as the trabecular thickness and trabecular bone number, after each piezocision procedure until reaching a peak 28 days after each surgery, followed by a progressive remineralization three-dimensional bone structure. These results correlate the data from Dibart et al [35]. investigating the tissue response on a rat model following a single stage of piezocision-assisted orthodontic tooth movement up to 56 days; they found that the bone content decreased significantly

Table 2.
Relative expression of molecular outcomes (mean ± SD) after one-stage and two-stage piezocision procedure.

	Assessment time				
Molecular parameters*	Day 7	Day 28	Day 42	Day 63	Day 90
One-stage piezocision					
BMP2	0.84 ± 0.23	1.0 ± 0.31	1.1 ± 0.32		
IL6	1.4 ± 0.44	1.3 ± 0.94	1.6 ± 0.95		
OPG	1.3 ± 0.34	0.81 ± 0.62	0.97 ± 0.53		
TRAP**	1.2 ± 0.56	0.98 ± 0.14	0.95 ± 0.87		
RANK L	1.1 ± 0.50	1.1 ± 0.12	1.4 ± 0.31		
OPG/RANK L	0.85 ± 0.23	1.3 ± 0.75	1.9 ± 1.2		
Two-stage piezocision					
BMP2			0.89 ± 0.27	1.0 ± 0.37	1.2 ± 0.22
IL6			0.65 ± 0.073	1.5 ± 0.82	1.7 ± 1.1
OPG			1.4 ± 0.62	1.1 ± 0.24	0.77 ± 0.37
TRAP**			2.4 ± 1.1	0.91 ± 0.51	0.61 ± 0.33
RANK L			0.95 ± 0.24	1.2 ± 0.82	1.3 ± 0.83
OPG/RANK L			1.6 ± 0.83	1.8 ± 1.5	0.97 ± 0.91

* BMP-2 Bone Morphologic Protein; IL Interleukin; OPG Osteoprotegerin protein; TRAP Translating Ribosome Affinity Purification protein; RANK-L Receptor Activator of nuclear factor Kappa-B Ligand;.
** significantly different evolutions;.

until day 28 (peak) and were progressively restored to baseline bone faction at day 56. Similarly, Charavet et al [36]. demonstrated a decrease of the bone mineral content a after a single stage piezocision from baseline to day 28 (peak) followed by a progressive remineralization to baseline values at day 42. Tissue level response was also investigated in rat model after corticotomies performed with round burs [42] and a decrease of the bone mineral content was found at day 7, however, it was reestablished to baseline values at day 28. Therefore, the piezocision seems to be more effective to maintain the reduction of the bone density when compared to a surgical trauma induced by a round bur. Additionally, the present study demonstrated for the first time that the decrease of the bone density can be repeated when applying a second osteotomy at day 35, with a very similar pattern.

Additionally, the relative expression of molecular outcomes, which was globally comparable after each piezocision procedure. More specifically, the RANKL and OPG expressions, which both regulate osteoclast differentiation, were comparable after the first and the second piezocision, in accordance with the study of Zhou et al. on bone remodeling in corticotomy-assisted orthodontic tooth movement in rats [43].

Based on the present results both at the tissue and molecular level and the literature cited above, in rat model, the RAP phenomenon seems to start after the first intentional piezocision bone injury reaching a peak after 28 days to progressively decrease, and a second stage piezocision at day 35 allows to maintain the RAP effect to yield a second peak after 63 days to finally disappear as remineralization sets in. Translating this RAP 2-wave pattern to clinical practice would be therefore highly relevant as clinical randomized trial demonstrated that piezocision allowed OTM acceleration for a period of only 4 to 6 months [10]. Thereby, accelerated OTM would be hypothetically effective for up to 1 year. Interestingly, a second-stage bone injury procedure performed in humans by the micro-osteoperforations (MOP) technique, which corresponds to flapless transmucosal bone puncturing, has shown encouraging results. Indeed, Jaiswal et al [44]. recently studied 16 patients requiring bilateral maxillary first premolar extraction for orthodontic treatment, in whom the left and right sides were randomly allocated to one-time MOP and two-time MOP (one month after the first MOP). They found that the rate of maxillary canine retraction was significantly higher in the two-time MOP side after two months and the levels of interleukin (IL-1β) in gingival crevicular fluid was immediately higher in the two-time MOP than the one-time MOP. Further clinical studies would be necessary to fully validate the benefit of a second stage piezocision, as the results of animal study may be difficult to transfer to clinical practice, although Meikle [45] reported that the anabolic and catabolic phases of bone remodeling on rats are analogous to those in humans. Patients reported outcomes measures (PROMs) should also be investigated to evaluate the post-surgical discomfort in balance with patient satisfaction in terms of final results and treatment duration.

One limitation related to the study design should be underlined: to respect the ARRIVE guidelines and reduce the use of laboratory animals, the study design limited the number of experimental groups in the longer time points. Finally, to perform the displacement of the molars, a 25-g Sentalloy stainless steel coil spring alone was employed between the incisors and the first two molars, which did not allow, to achieve predictable and controllable movements over such a long observation period. Therefore, orthodontic tooth movement was not considered and it represents an additional limitation of the present study.

5. Conclusion

The present study demonstrated that repeating a piezocision procedure prolonged/re-induce the RAP effect characterized by the decrease in bone mineral content. The biological phenomenon at the tissue and molecular level were similar after the first and the second piezocision. Therefore, a second stage of piezocision might be relevant to prolong acceleration of the orthodontic tooth movement. Nevertheless, the relevance of our findings to the clinical effect has not been tested. Further clinical studies are needed to confirm this hypothesis.

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Declaration of Competing Interest

None.

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