Heliyon 10 (2024) e32873

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Biological alterations associated with the orthodontic treatment with conventional appliances and aligners: A systematic review of clinical and preclinical evidence

Aline Gonçalves [a,b], Quitterie Mathelié-Guinlet [a], Fátima Ramires [a], Francisca Monteiro [b,c], Óscar Carvalho [b,d], Filipe S. Silva [b,d], Albina D. Resende [a,e,f,g], Teresa Pinho [a,*]

[a] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116, Gandra, Portugal
[b] Center for MicroElectroMechanical Systems (CMEMS), University of Minho, Campus Azurém, 4800-058, Guimarães, Portugal
[c] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
[d] LABBELS – Associate Laboratory, Braga, Guimarães, Portugal
[e] Associate Laboratory i4HB-Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, 4585-116 Gandra, Portugal
[f] UCIBIO - Applied Molecular Biosciences Unit, Toxicologic Pathology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS - CESPU), 4585-116 Gandra, Portugal
[g] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal

ARTICLE INFO

Keywords:
Bone formation
Bone resorption
Orthodontic forces
Orthodontic tooth movement
Periodontal ligament
Root resorption

ABSTRACT

Background&objectives: Mechanical forces applied during an orthodontic tooth movement (OTM) propel several biochemical and molecular responses in the periodontal ligament and alveolar bone. Here, we compile the existing clinical and preclinical evidence on these biological changes, aiming to provide a comprehensive discussion on the influence of the mechanical parameters of the OTM in the biological profile of the periodontium.

Material and methods: This systematic integrative review was conducted according to PICOS strategy and PRISMA guidelines. A bibliographic search was performed in three electronic databases (PubMed, Scopus, and Web of Science) to find research articles published until 2023 and written in English. This search resulted in a total of 2279 publications, which were independently assessed by two evaluators using appropriate tools.

Results: Forty-six studies were selected for this review. These revealed that compression, and stretching of the periodontal ligament fibers and cells are observed in the initial phase of the OTM. Specifically, on the tension side, high levels of IL-1β, OPG, and TIMPs are identified. On the compression side, an increase of RANKL, RANK, and MMPs levels predominate.

Conclusion: This paper describes the release profile of common biomarkers according to the orthodontic protocol, suggesting the most appropriate parameters to keep the teeth and their supporting structures healthy. Overall, this manuscript provides a better understanding of the OTM-associated biological phenomena, also highlighting the importance of early evaluation of oral health, and thus it contributes as a fundamental basis for the development of more effective and safe orthodontic treatments with conventional appliances and aligners.

* Corresponding author. UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal.
E-mail address: teresa.pinho@iucs.cespu.pt (T. Pinho).

https://doi.org/10.1016/j.heliyon.2024.e32873
Received 5 July 2023; Received in revised form 6 June 2024; Accepted 11 June 2024
Available online 13 June 2024

1. Introduction

The orthodontic tooth movement (OTM) involves a synergistic sequence of physical phenomena involving the periodontal ligament (PDL), alveolar bone, cement, and gum, that together result in tooth movement through the dentoalveolar complex [1,2]. Under healthy conditions, the applied forces create stress and tension in the PDL and in alveolar bone cells, which respond by modulating the expression and release of biological mediators, such as inflammatory cytokines and growth factors, among others. These mediators stimulate the activation of multiple cells, such as osteoblasts, fibroblasts, and osteocytes, also recruiting and promoting the differentiation of osteoclasts, which results in a balanced cycle of bone tissue, root, and fibers formation and resorption [2–4].

In this sense, orthodontic mechanotransduction promotes different changes in the PDL and surrounding tissues, which are not fully understood yet. Various theories exist proposing an explanation for the mechanisms involved in orthodontic tooth movement, but the pressure-tension theory is the most accepted among the scientific community [2,3,5,6]. This theory supports that chemical signals work as stimuli to cell differentiation, from which two regions result; one compression area, where a decrease in blood flow is observed, and another region of tension, in which the blood flow is maintained or even increased [2,6]. Chemical mediators are released, promoting bone formation on the tension side and bone resorption on the compression side (Fig. 1).

The orthodontic movement comprises three phases: i) an initial phase, usually from 24 h to 48 h after orthodontic application, which is mainly characterized by an immediate and fast movement; ii) a second phase (latency phase), lasting from 20 to 30 days, where a slight or null movement occurs and hyalinization areas increase - no movement occurs until the necrotic tissue is removed; and iii) a final phase (post-latency or movement phase), that lasts until the next activation, in which the speed of movement increases again, gradually or abruptly, corresponding to the greatest tooth movement [2–4,7]. In each of these phases, different biological processes predominate, also reflecting alterations in different biomarkers, such as cytokines and other proteins, in the periodontal space [6,7].

The orthodontic treatment is designed on a case-by-case basis, and therefore its role depends on the classification of each specific congenital or acquired (*e.g.*, traumatic) dental anomaly, as well as on the status of the periodontal tissue and alveolar bone. A thorough evaluation of the oral health of the patient is pivotal to defining the most suitable treatment to be adopted, preventing the risk of damaging the teeth, their roots, and supporting structures, and ensuring the success of the orthodontic treatment [8,9].

Considering the existing literature, a systematic review has become pertinent to provide a synthesized and comprehensive discussion of the current knowledge about this topic. As far as we know, this is the first review evaluating the existing evidence on cellular, molecular, and tissue reactions as a function of the orthodontic treatment protocol specifications. This innovative perspective allowed a direct comparison between the characteristics of an applied mechanical intervention and its effect on the biological activity in cells,

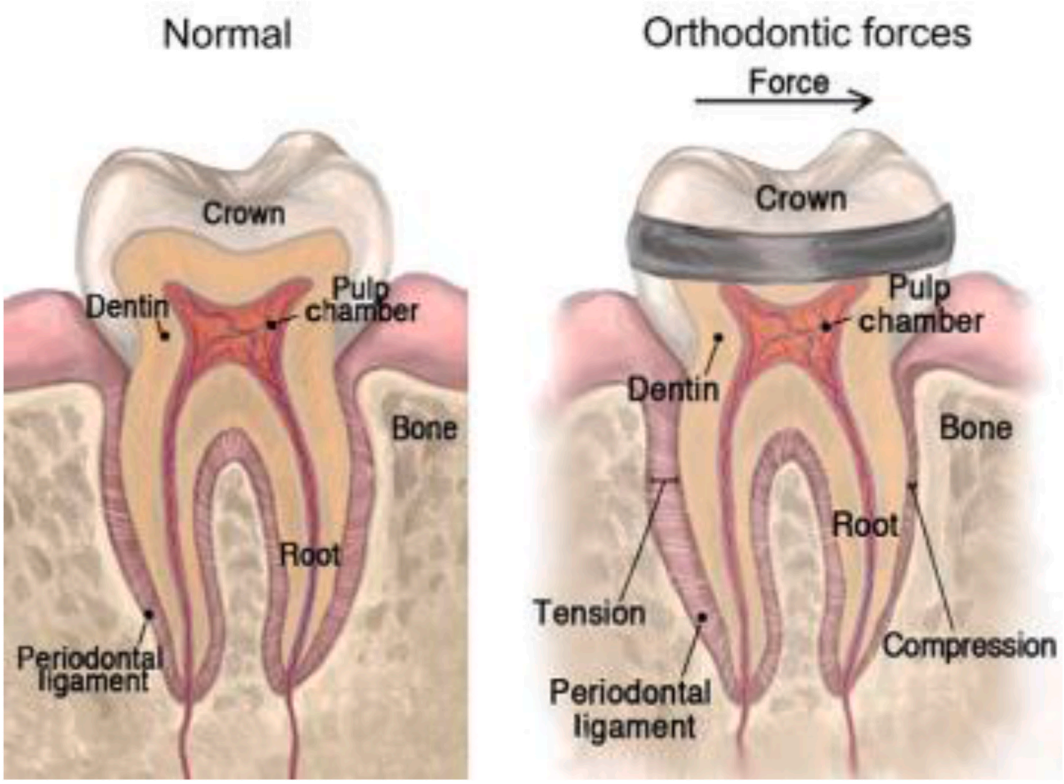

Fig. 1. The effects of mechanical force applied during orthodontic treatment in the periodontal ligament.

animal models, and human patients, combining data from the theoretical and empirical literature. We believe that this systematic integrative review will arouse wide interest in the periodontology community, as it describes a complete pipeline of the phenomena involved in the orthodontic movement. This will certainly contribute as a fundamental basis for future clinical success in orthodontics.

2. Material and methods

2.1. Registration and protocol

This systematic review was elaborated following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 guidelines [10], and recently published systematic reviews with high impact in the orthodontics/dental medicine fields were used as a guide [11]. All the stages of the current review were performed by two clinicians (QMG and AG) and finally revised by a third clinician with several years of expertise in orthodontics (TP), who corroborated the information extracted from the reviewed articles and discussed here.

Also, this systematic review was registered in PROSPERO with the registration number CRD42021251054 (using the previous title: "Biology of the Orthodontic Tooth Movement: a Systematic Review", which was later altered), by April 2021. The registration protocol can be found in PROSPERO database.

2.2. Eligibility criteria

The focused question that this review intends to respond to was defined according to the Population, Intervention, Comparison, Outcomes, and Study design (PICOS) strategy, as presented in Table 1.

Therefore, the following focused questions of this systematic review were defined: i) "What evidence on cellular, biochemical, and metabolic phenomena occurring during the orthodontic movement in cellular, animal, or human models exist in the literature?", and ii) "What mechanical parameters are associated with the most effective orthodontic treatment?". To answer these questions, the eligibility criteria for admission in this systematic review were defined as stated in Table 2.

2.3. Search strategy

A bibliographic survey was conducted in PubMed, Scopus, and Web of Science databases until February 2023. Articles written in English were selected. The keywords and MeSH terms employed in the search strategy are depicted in Table 3. Besides the articles selected from this methodology, a manual search was carried out in books to identify and retrieve articles that were not found in the electronic search.

2.4. Article selection and data collection

An advanced search was performed using the search terms previously exposed. Duplicates were manually removed. The title and abstract of the identified and potentially relevant articles were submitted to a preliminary evaluation, carried out by two authors (AG and FR) independently, to determine whether they met the intended purpose of the study. The potentially eligible studies that met the inclusion criteria were fully analyzed and evaluated for eligibility. Finally, among the full-text selected articles, data were extracted and organized in Table 4 (*in vitro* experiments), Table 5 (animal studies), and Table 6 (clinical trials), including publication data, population under study, intervention parameters, under study, and main biological outcomes. The effect measures of the expression and protein levels of key biomarkers included the mean difference between orthodontically stressed vs no orthodontic treatment groups/models/subjects.

2.5. Quality assessment

Two authors (QMG and AG) independently assessed the quality of the selected articles. For the *in vitro* studies, we adapted the quality assessment strategy proposed by Golbach et al. (2016) [58] and widely used by others [59,60], and a set of criteria was established to evaluate their methodological quality.

The Cochrane Collaboration's tool SYRCLE's (Systematic Review Centre for Laboratory animal Experimentation) risk of bias tool

Table 1
PICOS strategy applied to the current review.

PICOS categories	Applied Criteria
Population	Cell cultures, animals, and humans subjected to orthodontic treatment/stress.
Intervention	Orthodontic treatment.
Comparison	Baseline conditions, control group, and placebo.
Outcomes	Outcomes related to biological alterations in the periodontal space, such as root resorption, bone remodeling, bone formation, and inflammation.
Study design	Randomized trials, cross-sectional studies, prospective and retrospective studies, and preclinical studies (*i.e.*, *in vitro* and animal studies).

Table 2
Eligibility criteria for admission in this review.

Inclusion criteria	Exclusion criteria
●Preclinical and clinical studies that report biological and metabolic alterations induced by orthodontic treatment; ●Studies written in English.	● All types of articles except original research papers (*e.g.*, reviews, meta-analyses, proceeding papers, thesis, dissertations, editorials, commentaries, surveys, unpublished articles, and conference papers/abstracts); ●Studies in which biochemical alterations occurred during orthodontic treatment but that were not caused by the orthodontic forces. ●Articles that do not correctly describe the orthodontic force applied by the conventional/fixed orthodontic appliances. ●Studies that combine the use of orthodontic forces with another stimulus, medicines, or therapeutic agents/compounds that could explicitly impact the periodontium. ●Patients with associated diseases (*e.g.*, periodontal disease, diabetes, among others).

Table 3
Keywords and Mesh terms applied in the electronic search.

Databases	Search strategy
PubMed **Web of Science** **Scopus**	(osteoblast OR bone OR "bone cells" OR "bone tissue" OR "periodontal ligament" OR "periodontal fiber" OR "RANK ligand" OR osteoclast OR osteocyte OR fibroblast OR root) AND ("bone growth" OR "bone remodeling" OR "bone modeling" OR "bone differentiation" OR "bone formation" OR "bone metabolism" OR osseodensification OR osteogenesis OR osteoclastogenesis OR ossification OR "bone resorption" OR "root resorption" OR "apical root resorption") AND (orthodontics OR "orthodontic movement" OR "tooth movement" OR "orthodontic force" OR aligner OR "clear aligners" OR "fixed appliances") AND (biomarker OR "biological marker" OR "biochemical marker" OR cytokine OR "cell response" OR "cells response" OR "cellular response" OR "biological response" OR imaging OR radiographic OR tomography)

for animal intervention studies [61].

The non-randomized clinical trials were evaluated according to the criteria ROBINS-I ("A Cochrane Risk Of Bias Assessment Tool for Non-Randomized Studies") [62]. Both consider the following domains: selection bias, detection bias, bias due to confounding, among others.

3. Results

3.1. Articles selection

From the bibliographic search, a total of 2279 articles resulted. After duplicates removal, 1757 articles remained. Titles and abstracts were screened, and 79 articles were selected for further analysis. These studies were fully read and evaluated individually for eligibility, from which 46 articles were selected and included in this systematic review. No studies were added from other sources. This selection process is illustrated in Fig. 2.

3.2. Study characteristics

Overall, the included studies present high variability concerning the type of study and reported outcomes. Fig. 3a shows the distribution of the type of study; 13 *in vitro* studies using different cell lines (*e.g.*, PDL cells, osteoblasts), 19 animal studies using rodents, and 17 clinical trials conducted in human patients subjected to orthodontic treatment, in a wide age-ranged population. Note that three studies evaluated biological alterations in both *in vitro* and animal models.

The outcomes from the included articles were separated into four subtopics: inflammatory, bone resorption, bone formation, and root resorption. Alternative biological outcomes were also reported. Importantly, multiple studies report more than one type of biological event (Fig. 3b.) and commonly associate them using a cause-effect relationship.

3.3. Results syntheses and individual analysis

Data from the included studies was organized in Table 4 (*in vitro* experiments), Table 5 (animal studies), and Table 6 (clinical trials) according to the year of publication. These tables summarize the findings of the reviewed studies on biological events occurring along tooth orthodontic movement, allowing a direct and comprehensive comparison between studies concerning the applied forces and the reported outcomes. Then, a short overview of the main findings of the revised studies is provided.

The *in vitro* studies confirm a differential expression of the mediators involved in the orthodontic movement in response to distinct mechanical applications.

a. Inflammation

Distinct mechanical protocols have produced the upregulation of different interleukins (IL), such as IL-1β [63], IL-6 [64–66], IL-8

Table 4
In vitro studies.

Publication data	Population under study	Mechanical application parameters	Biological outcomes
Mayahara *et al.* (2012) [12]	Human PDL cells	Intervention: continuous compression of 2.0 g/cm^2 Duration: 24 h	**Bone formation & resorption:** - no significant changes in RANKL expression (RT-PCR); -↑ COX-2 expression (RT-PCR);
Diercke *et al.* (2012) [13]	Primary human cementoblasts from extracted teeth	Intervention: compression delivered by a centrifugal force of 30.3 g/cm^2 Duration: 1, 4, 6 h	**Root resorption:** -↑ RANKL expression after 4 and 6 h of compression (RT-PCR); -↑ RANKL/OPG ratio after 4 and 6 h of compression (RT-PCR); -↑ RANKL protein levels in the cytoplasm of cementoblasts (ICC) -no significant changes in subcellular location nor in expression levels of RANK receptor (RT-PCR); -↓ OPG expression after 1, 4 and 6 h of compression (RT-PCR); -↑ COX-2 expression after 4 and 6 h of compression (RT-PCR);
García-López, Villanueva, and Meikle (2013) [14]	Mouse calvarial osteoblasts and femoral osteoclasts	Intervention: cyclic mechanical deformation with a maximum strain of 0.69 % (delivered to osteoblasts) Duration: 6 s every 90 s for 2–48 h	**Inflammation:** -↑ IL-1β and TNF-α levels from 2 to 24 h, returning to control levels from 24 to 48 h (ELISA); -↑ IL-6 levels over the 48-h time-course (ELISA); **Bone formation & resorption:** -↓ sRANKL levels from 2 to 24 h, returning to control levels from 24 to 48 h (ELISA); -inhibition of osteoclast resorption by culture media over 2–48 h (Pit assay); -no significant changes in OPG levels over 2–24 h, although it increased from 24 to 48 h (ELISA).
Tripuwabhrut *et al.* (2013) [15]	Human osteoblasts derived from alveolar bone	Intervention: continuous compression of 2.0 and 4.0 g/cm^2 Duration: 1, 3 and 7 days	**Inflammation:** -↑ PGE_2 levels after 4-g/cm^2 compression, and tendency to ↑ PGE_2 levels after 2-g/cm^2 compression, after 1-day intervention (ELISA). **Bone formation & resorption:** -↑ RANKL expression after 4-g/cm^2 compression after 1-day intervention (RT-PCR); -↑ ALP and COL I expression in a dose-dependent way after 1-day intervention (RT-PCR); -↓ COL I protein levels after both compression applications after 1-day intervention, ↑ COL I protein levels after 4-g/cm^2 compression after 3-days intervention, and ↑ COL I protein levels after both compression applications after 7-days intervention (ELISA); -no significant changes in OPN and OCN expression after 1-day intervention (RT-PCR); -↓ Runx-2 expression in a force-dependent way after 1-day intervention (RT-PCR); -↓ OPG expression after both compression applications after 1-day intervention (RT-PCR); -↓ OPG protein levels after both compression applications after 1- and 3-days intervention (ELISA); -↑ ALP activity in the culture medium in a force-dependent way after 1-day intervention, and only after 4-g/cm^2 compression after 3- and 7-days interventions (RT-PCR); -↓ ALP activity in the cell lysates after both compression applications after 1- and 7-days interventions, and tendency to ↓ ALP activity after 4-g/cm^2 compression after 3-days intervention (RT-PCR);
Yoshino *et al.* (2014) [16]	Human periodontal ligament cells	Intervention: continuous compression of 4 g/cm^2 Duration: 1, 3, 6, 9, 12, 24 and 48 h	**Inflammation:** -↑ TNF-α levels over the intervention, peaking at 24-h intervention (ELISA); -↑ TNF-α expression over the intervention, peaking after 9-h intervention (RT-PCR); **Root resorption:** -↑ RANKL levels in a time-dependent way (ELISA); -↑ RANKL expression over the intervention, peaking after 9-h intervention (RT-PCR);

(*continued on next page*)

Table 4 (*continued*)

Publication data	Population under study	Mechanical application parameters	Biological outcomes
Proff *et al.* (2014) [17]	Human PDL fibroblast cells	Intervention: static compressive pressure of 2 g/cm^2 Duration: 24 h	**Inflammation:** -↑ IGF-1, IL-8, and PGE_2 gene expression after pressure application (RT-PCR); -↑ PGE_2 levels after pressure application (ELISA); -no significant effects on IL-6 and VEGF expression after pressure application (RT-PCR); **Bone formation & resorption:** -↑ MMP-13 gene expression after pressure application (RT-PCR); -↑ COX-2 gene expression after pressure application (RT-PCR);
Li *et al.* (2015) [18]	Human periodontal ligament cells	Intervention: static compressive pressure of 5, 15 and 25 g/cm^2 Duration: 6, 24 and 72 h, respectively	**Inflammation:** - ↑ PTHrP and IL-11 expression and protein levels after all interventions (RT-PCR and ELISA); - ↑ PGE_2 expression following all forces after 24- and 72-h interventions and following 15- and 25-g/cm^2 compression after 6-h intervention (RT-PCR); **Bone formation & resorption:** -↑ RANKL expression following all forces after 24- and 72-h interventions, and following 15- and 25-g/cm^2 compression after 6-h intervention (RT-PCR); -enhanced osteoclast formation in the presence of osteoclast precursors after all the interventions (TRAP staining); -↓ RANKL protein levels following 15- and 25-g/cm^2 compression after 24- and 72-h interventions, and following 25-g/cm^2 compression after 6-h intervention (ELISA); -heavier compression was associated with greater effects in the biomarkers and in osteoclast formation (TRAP staining); -↑ OPG expression and protein levels following 25-g/cm^2 compression after 24- and 72-h interventions, and following 5- and 15-g/cm^2 compression after 72-h intervention (RT-PCR and ELISA); -↓ OPG expression following 15- and 25-g/cm^2 compression after 6-h intervention, and following 5-g/cm^2 compression after 24-h intervention (RT-PCR); -↓ OPG protein level following 25-g/cm^2 compression after 6-h intervention (ELISA); -↑ COX-2 expression following all forces after 24- and 72-h interventions and following 15- and 25-g/cm^2 compression after 6-h intervention (RT-PCR); **Other biological alterations:** -inhibition of periodontal ligament tissue cells' proliferation following 15- and 25-g/cm^2 compression after all interventions and following 5-g/cm^2 compression after 72-h intervention (MTT assay)
Nettelhoff *et al.* (2016) [19]	Human periodontal ligament fibroblasts and osteoblasts	Intervention: compression of 200 and 400 g/cm^2 Duration: 12 h	**Bone formation & resorption:** -↑ RANKL expression after both pressures in osteoblasts, and only after 200-g/mm^2 compression in PDL fibroblasts (RT-PCR); -↑ RANKL/OPG ratio after both pressures, peaking after 200-g/mm^2 compression, in both cell lines (RT-PCR); -tendency to ↑ MMP-8 protein levels in PDL fibroblasts after both pressures, peaking after 200-g/mm^2 compression (ELISA); -no significant changes in MMP-8 protein levels in osteoblasts (ELISA); -↑ MMP-8/TIMP-1 ratio in PDL fibroblasts in a force-dependent way (ELISA); -no significant changes in MMP-8/TIMP-1 ratio in osteoblasts (ELISA); -↓ osteoblast viability after 400-g/mm^2 compression, in a force-dependent way (MTT); -↑ ALP expression after 400-g/mm^2 compression in osteoblasts in a force-dependent way (RT-PCR);

(*continued on next page*)

Table 4 (*continued*)

Publication data	Population under study	Mechanical application parameters	Biological outcomes
			-tendency to ↑ ALP expression after both pressures in fibroblasts, greater after 200-g/mm^2 compression (RT-PCR); -no significant changes in OCN expression (RT-PCR); -tendency to ↓ OPG expression in both cell lines (RT-PCR); -↑ OPG protein levels in osteoblasts after both pressures, peaking after 200-g/mm^2 compression (ELISA); -tendency to ↓ OPG protein levels in PDL fibroblasts in a force-dependent way (ELISA); -↓ TIMP-1 protein levels in PDL fibroblasts in a force-dependent way (ELISA); -tendency to ↑ TIMP-1 protein levels in osteoblasts after 200-g/mm^2 compression, which ↓ after 400-g/mm^2 compression below control levels (ELISA); **Other biological alterations:** -tendency to ↓ PDL fibroblast viability in a force-dependent way (MTT); -no significant changes in cell apoptosis (TUNEL assay);
Zheng *et al.* (2016) [20]	Human periodontal ligament cells	Intervention: fluid shear stress of 0.006 g/cm^2 Duration: 2, 4, 8, 12 and 24 h	**Inflammation:** -↑ relative FGF-2 expression after all interventions (RT-PCR); -↑ absolute FGF-2 expression after 2- and 4-h interventions (RT-PCR); -↑ relative BMP-2 expression after 4- and 8-h interventions (RT-PCR); -↑ relative TGF-β expression after 8- and 12-h interventions (RT-PCR); -↑ relative VEGF expression from 2- to 8-h interventions in a time-dependent way, slightly ↓ from 8- to 12-h shear stress (RT-PCR); -↓ relative IL-6 expression from 2- to 8-h interventions in a time-dependent way, strongly ↓ from 8- to 12-h shear stress (RT-PCR); **Bone formation & resorption:** -↑ ALP expression after 8-h intervention, returning to control levels after 12-h shear stress (RT-PCR); -↑ OPN expression after 8-h intervention and ↑ OPN protein levels after 12-h shear stress (RT-PCR and ELISA); **Other biological alterations:** -modulation of cells orientation (angle calculation using microscopic images); -inhibition of cell proliferation after 12-h intervention, with ↓ S phase and ↑ G1 phase (Wound healing assay); -no significant changes in cell apoptosis (TUNEL assay); -↑ relative wound area after 12- and 24-h interventions, reflecting inhibition of cell migration (Wound healing assay);
Schröder *et al.* (2018) [21]	Human periodontal ligament fibroblasts	Intervention: compression of 2 g/cm^2 Duration: 24, 48, 72 and 96 h	**Inflammation:** -↑ VEGF-A expression after all interventions, peaking at 24-h compression (RT-PCR); -↑ COX-2 and IL-6 expression after 24- and 48-h compression in a time-dependent way, ↓ from 48 to 96 h (RT-PCR); -no significant changes in FN1 expression (RT-PCR); **Bone formation & resorption:** -↓ MMP-8 expression after 48-h compression, which ↑ from 48 to 72 h (RT-PCR); -↓ RANKL protein levels after 24-h compression, which strongly ↑ after 48- and 72-h compression application, returning to baseline levels after 96-h compression (ELISA); -↑ TRAP-positive cells after all interventions for PDL cocultured with osteoclasts precursor cells, peaking at 72-h compression (TRAP staining);

(*continued on next page*)

Table 4 (*continued*)

Publication data	Population under study	Mechanical application parameters	Biological outcomes
			-↑ P4HA1 expression after 24-, 48- and 72-h compression (RT-PCR); -↑ COL1A2 expression after 24- and 48-h compression (RT-PCR); -tendency to ↓ P4HA1 and COL1A2 expression from 72 to 96 h (RT-PCR); -↑ ALP expression after 24-, 48- and 72-h compression, peaking at 48-h compression and ↓ from 72 to 96 h (RT-PCR); -↓ OPG protein levels after all interventions, peaking after 24-h compression (ELISA);
Schröder *et al.* (2020) [22]	RAW264.7 macrophages	Intervention: -compression of 2 g/cm^2 -static isotropic tensile loading of 16 %. Duration: 2, 4, 24 and 48 h	**Inflammation:** -↑ VEGF-A expression from 2- to 24-h compression in a time-dependent way, returning to control levels after 48-h compression (RT-PCR); -↑ VEGF-A protein levels after 4-h compression (ELISA); -no significant changes in VEGF-A expression nor protein levels after tension applications (RT-PCR and ELISA)); -strong ↑ TNF-α expression after all compression applications, peaking at 48-h compression (RT-PCR); -strong ↑ TNF-α protein levels after 4-h compression (ELISA); -↑ TNF-α expression after 2- and 4-h tension applications (RT-PCR); -↑ TNF-α protein levels after 4-h tension (ELISA); -↑ PGE_2 protein levels after 4-h compression and 4-h tension (ELISA); -strong ↑ IL-6 expression after all compression applications, peaking at 24-h compression (RT-PCR); -↑ IL-6 expression after 4- and 24-h tension, ↓ from 24- to 48-h strength (RT-PCR); -↑ IL-6 protein levels after 4-h compression and 4-h tension (ELISA); **Bone formation & resorption:** -↓ MMP-8 expression after 4-h compression, which strongly ↑ after 24- and 48-h compression (RT-PCR); -↑ MMP-8 expression after 24- and 48-h tension in a time-dependent way (RT-PCR); -↑ MMP-9 expression after 4-, 24- and 48-h compression in a time-dependent way (RT-PCR); -no significant changes in MMP-9 expression after tension applications (RT-PCR); -strong ↑ COX-2 expression after all compression applications (RT-PCR); -↑ COX-2 expression after 2-, 24- and 48-h tension applications (RT-PCR); **Other biological alterations:** -↓ cell number after all compression interventions in a time-dependent way (Coulter Counter); -↓ cell number after 48-h tension and tendency to ↓ after 2- and 24-h tension (Coulter Counter); -↑ cytotoxicity after all compression interventions in a time-dependent way (LDH assay); -no significant changes in cytotoxicity after tension applications (LDH assay);
Lin *et al.* (2022) [23]	Human PDL ligament cells	Intervention: continuous compressive application of 3 and 15 g/cm^2 Duration: 6, 12, 24 and 48 h	**Inflammation:** -↓ TGF- β levels after 3- and 15-g/cm^2 interventions at all periods compared to control (ELISA); -↑ IL-6 levels after 15-g/cm^2 compression delivered for 6, 12 and 24 h compared to control (ELISA); -↑ IL-6 levels after 3-g/cm^2 compression delivered for 12 and 24 h compared to control (ELISA); -↓ IL-6 levels after 3-g/cm^2 compression delivered for 48 h compared to control (ELISA); -↑ IL-6 levels after 15-g/cm^2 compression delivered for 6, 24 and 48 h compared to the 3-g/cm^2 intervention group (ELISA); -↓ TGF- β and Notch1 mRNA expression after 3- and 15-g/cm^2 interventions delivered for 24 h compared to control (RT-PCR); -↑ TGF- β and Notch1 mRNA expression after 3- and 15-g/cm^2 interventions delivered for 48 h compared to control (RT-PCR);

(*continued on next page*)

Table 4 (*continued*)

Publication data	Population under study	Mechanical application parameters	Biological outcomes
			-↑ TGF- β mRNA expression after 15-g/cm^2 intervention delivered for 48 h compared to the 3-g/cm^2 group (RT-PCR); -↑ Notch1 mRNA expression after 15-g/cm^2 intervention delivered for 24 and 48 h compared to the 3-g/cm^2 group (RT-PCR); -↑ HIF-1α mRNA expression after 3- and 15-g/cm^2 interventions delivered for both 24 and 48 h compared to control (RT-PCR); -↑ HIF-1α mRNA expression after 15-g/cm^2 intervention delivered for both 24 and 48 h compared to the 3-g/cm^2 group (RT-PCR); -↑ percentage of Th17 cells after 3- and 15-g/cm^2 interventions compared to control (Flow cytometry); **Other biological alterations:** -↓ cell viability after 15-g compression intervention at all periods (Trypan blue); -no significant changes in cell viability after 3-g compression at all periods (Trypan blue); -↑ cell mortality after 3- and 15-g/cm^2 interventions after 24- and 48-h compression (V-FITC/PI apoptosis kit); -↑ cell mortality a 15-g/cm^2 intervention compared to 3-g/cm^2 intervention after both 24- and 48-h compression (V-FITC/PI apoptosis kit); -no significant differences in apoptosis after 3- and 15-g/cm^2 interventions after 24- and 48-h compression (V-FITC/PI apoptosis kit).

Caption: ABC: alveolar bone crest; ALP: alkaline phosphatase; BMP-2: bone morphogenetic protein 2; CEJ: cement-enamel junction; Col I; collagen type I; COL1A2: collagen type I alpha-2; COX-2: cyclooxygenase-2; CXCL12: C-X-C motif chemokine ligand 12; ELISA: enzyme-linked immunosorbent assay; ICC: immunocytochemistry; IGF-1: insulin-like growth factor 1; IL: interleukin; FGF-2: basic fibroblast growth factor; HIF-1α: hypoxia inducible factor; MMP: matrix metalloproteinase-8; OCN: osteocalcin; OPG: osteoprotegerin; OPN: osteopontin; P4HA1: prolyl-4-hydroxylase-1; PDL: periodontal ligament; PGE2: prostaglandin E2; PTHrP: Parathyroid hormone-related protein; RANKL: receptor activator of nuclear factor kappa-B ligand; RT-PCR: reverse transcription polymerase chain reaction; Runx-2: transcription factor Runx-2; TIMP-1: tissue inhibitors of metalloproteinase-1; TGF-α: transforming growth factor alpha; TNF-α: tumour necrosis factor; TGF- β: transforming growth factor beta; TRAP: tartrate-resistant acid phosphatase; VEGF: vascular endothelial growth factor; VEGF-A: vascular endothelial growth factor A.

Table 5
Animal studies.

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
Kook, Jang, and Lee (2011) [24]	Wistar rats	Intervention: elastic bands between the maxillary 1st and 2nd molars *(inclination movement)* Duration: 3 days	**Bone formation & resorption:** -↑ CD4 and B220 expression, as ↑ TRAP-positive cells, on the compression side, but not on the tension side (IHC, cytometric analysis, TRAP staining); -↑ RANKL expression accompanied by ↑ lymphocytes on the compression side (RT-PCR and confocal analysis).
Nakano *et al.* (2011) [25]	Wistar rats	Intervention: closed-coil springs applying 10- and 50-g force to the upper 1st molar *(inclination movement)* Duration: 10 days Follow-up periods: 0, 3, 7 and 10 days along the intervention	**Bone formation & resorption:** -slightly ↑ RANLK- and M–CSF–positive fibroblasts and osteoblasts at the root surface after 10-g intervention at the 7- and 10-days follow-ups (IHC); -↑ RANLK- and M–CSF–positive odontoclasts and fibroblasts at the root surface after 50-g intervention, at the 7- and 10-days follow-ups (IHC); -no significant differences in RANK and *c-fms*-positive odontoclasts (IHC); -↑ bone resorption lacunae with TRAP-positive osteoclasts and odontoclasts on the surface of the alveolar bone after 50-g intervention, at the 3- and 7-days follow-ups, respectively (H&E staining); -↑ bone resorption lacunae with bigger osteoclasts in the alveolar bone after 10-g intervention at the7-days follow-up (H&E staining); -↑ bone resorption lacunae with osteoclasts in the alveolar bone after 50-g intervention at the 3-days follow-up (H&E staining); -↑ bone resorption lacunae with TRAP-positive osteoclasts on the surface of the alveolar bone after 10-g intervention at the 3- and 7-days follow-ups (TRAP staining, IHC). **Root resorption:** -↑ root resorption lacunae with odontoclasts on the surface of the root after 50-g intervention at the 7-days follow-up (IHC and H&E staining). **Other biological alterations:** -coarse and irregular arrangement of fibers and fibroblasts, ↑ blood capillaries compression and few osteoclasts in bone resorption lacunae in the alveolar bone after 10-g intervention at the 3-days follow-up (H&E staining); -coarse arrangement of fibers and expanded blood capillaries after 50-g intervention at all follow-ups (H&E staining); -no significant changes in tooth movement between the interventions.
Baba *et al.* (2011) [26]	Wistar rats	Intervention: fixed metallic helical springs applying 10-g force to the upper 1st molar *(inclination movement)* Duration: 1, 3 and 6 h	**Inflammation:** -↑ OX6-positive cells throughout the PDL of the compression side after 1 h, ↓ from 3- to 6-h interventions (IHC); -↑ ED1-positive cells in the palatal alveolar crest and interradicular septal root apex of the PDL of the compression side in a time-dependent way (IHC); -no significant changes in OX6- and ED1-positive cells on the tension side (IHC); -no significant changes in IL- 1β nor TNF-α expression in OX6-positive cells in the PDL (real time PCR); -tendency to ↑ IL-1β and TNF-α expression in ED1-positive cells in the PDL (real time PCR); -↓ IL- 1β and TNF-α expression after 1 h, and tendency to ↑ from 1- to 6-h intervention (real time PCR); -↑ HSP27 expression in the PDL after 1 h of intervention, ↓ in a time-dependent way from 1- to 6-h interventions (real time PCR).

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			Other biological alterations: -no significant changes in tooth movement.
Zhou *et al.* (2011)) [27]	Sprague-Dawley rats	Intervention: closed-coil spring applying 100-g force to the right upper 1st molar *(inclination movement)* Duration: 1, 4, 8 and 12 h	**Bone formation & resorption:** -↑ RANKL expression from 1- to 12-h interventions on the compression side, and no significant changes on the tension side (MOD); -↑ RANKL/OPG ratio from 1- to 12-h interventions on the compression side, and ↓ on the tension side (MOD); -↑ osteoclasts from 1- to 8-h interventions, peaking at 4-h intervention and returning to control levels after 12-h intervention (TRAP staining); -↑ OPG expression from 4- to 12-h interventions on the tension side, and no significant changes on the compression side (MOD). **Root resorption:** -↑ root resorption lacunae on the compression side from 4- to 12-h interventions, in a time-dependent way (H&E staining and scanning electron microscopy) -↑ TRAP-positive odontoclasts near the root surface and in the resorption lacunae from 4- to 12-h interventions (TRAP staining);
Kim *et al.* (2012) [28]	Sprague-Dawley rats	Intervention: closed-coil spring applying 100-g mesial force to the upper 1st molar *(inclination movement)* Duration: 3, 7, 10 and 14 days	**Bone formation & resorption:** -osteoclasts lined up in the alveolar bone margin adjacent to the PDL, promoting frontal resorption on the tension side after 3-days intervention (IHC); -↑ new alveolar bone and cementum with osteoclasts and cementoblasts lined up in their margin on the compression side after 10- and 14- days interventions (IHC); -↑ OPN-positive osteocytes, osteoblasts and osteoclasts in the alveolar bone after 3-days intervention, and within the PDL on the tension side, especially near new alveolar bone, after 7- to 14-days interventions (IHC); -↑ osterix-positive cells in the odontoblasts after 3- and 7-days interventions, and in the PDL near the absorbed and new alveolar bone on the tension side after 10- and 14-days interventions, respectively (IHC); -↑ OPN-positive osteocytes and cementocytes in the PDL after 3- and 7-days interventions, and in osteocytes and osteoblasts near the new alveolar bone, cementocytes, cementoblasts and PDL cells on the compression side after 10- and 14-days interventions (IHC); -↑ osterix-positive cells near the alveolar bone after 3- and 7-days interventions, and also on the cementum surface on the compression side after 10- and 14-days interventions (IHC); **Other biological alterations:** -↑ width and stretched fibers in the PDL on the tension side (IHC); -absorption of the crest region of the alveolar bone and ↑ blood capillaries in the area on the tension side after 7-days intervention (IHC); -partial restoration of the alveolar bone and ↑ blood capillaries and coarse fibers in the PDL on the tension side after 10-days intervention (IHC); -normalization of PDL width and alveolar bone height, ↑ cuboidal osteoblasts in the surface of restored alveolar bone and ↑ root resorption on the tooth surface on the tension side after 14-days intervention (IHC); -↑ PCNA-positive cells near the absorbed and new alveolar bone on the tension side after 7-days intervention and after 10- and 14-days interventions, respectively (IHC);

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-↓ width and ↑ condensed cells on the compression side (IHC); -↑ cellular elements, coarse and irregular fiber arrangement, ↑ cementoid, and ↑ resorption lacunae full of osteoclasts on the alveolar bone surface on the compression side after 3- and 7-days interventions (IHC); -↑ PCNA-positive cells on the cementum and alveolar bones surfaces on the compression side after 10- and 14-days interventions (IHC).
Hayashi *et al.* (2012) [29]	Wistar rats	Intervention: closed-coil spring applying 10-g and 50-g forces to the upper 1st molar *(inclination movement)* Duration: 7 days	**Inflammation:** -↑ IL-17, IL-17R and IL-6 protein levels and immunoreactivity in the PDL tissues, mainly after 50-g force (IHC and ELISA); -↑ Th17 cells in the PDL tissue after both interventions, mainly after 50-g force (IHC). **Root resorption:** -↑ root resorption lacunae with multinucleated TRAP-positive odontoclasts in the surface of the root after both interventions, mainly after 50-g force (TRAP staining, IHC). **Movement-related implications:** -equal tooth movement after both forces. **Other biological alterations:** -coarse and irregular arrangement of fibers and fibroblasts, and ↑ blood capillaries compression after both interventions (H&E staining).
Taddei *et al.* (2012a) [30]	C57BL6/J mice	Intervention: coil spring applying 35-g force to the right upper 1st molar *(inclination movement)* Duration: 6 and 12 days Follow-up periods: 12 and 72 h	**Bone formation & resorption:** -↑ TRAP-positive osteoclasts after both interventions (TRAP staining, IHC); -↑ TRAP activity in a time-dependent way on the compression side after both interventions, and ↓ TRAP activity on the tension side after 6-days intervention (TRAP staining, IHC); -↑ RANK and RANKL expression after both interventions, in a time-dependent way (real time PCR); -↑ alveolar bone resorption after 12-h intervention (histological analysis); -↑ OCN expression after 72-h intervention (real time PCR); -↑ OPG and COL-1 expression after both interventions, in a time-dependent way (real time PCR).
Taddei *et al.* (2012b) [31]	C57BL6/J mice	Intervention: open-coil spring applying 10-, 25-, 35- and 50-g force to the right upper 1st molar *(inclination movement)* Duration: 12, 72 h and 6 days	**Inflammation:** -↑ IL-10 after 72-h intervention in both compression and tension sides, mainly on the tension side (real time PCR); -↑ TNF-α expression after 12- and 72-h interventions in both sides, mainly on the compression side (real time PCR); **Bone formation & resorption:** -↑ TRAP activity on the mesial bone surface for all forces after 6-days intervention in a force-dependent way (TRAP staining, IHC); -↑ RANK expression after 12- and 72-h interventions, mainly on the compression side (real time PCR); -↑ RANKL after 12-h intervention both in compression and tension sides, mainly on the compression side, and after 72-h intervention, mainly on the tension side (real time PCR); -↑ MMP-13 expression after 12- and 72-h intervention in both compression and tension sides, although more pronounced after 72-h and on the compression side (real time PCR);

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-↑ Cathepsin K expression on the compression side after 12- and 72-h intervention, in a force-dependent way (real time PCR); -↑ RUNX-2 and OCN expression after 12- and 72-h interventions on the tension side in a force-dependent way (real time PCR); -↑ OPG expression after 12- and 72-h interventions in both compression and tension sides, although more pronounced on the tension side (real time PCR). **Root resorption:** -↑ root resorption after 50-g force after 6-days intervention (histological analysis). **Movement-related implications:** -↑ orthodontic tooth movement after 25- to 50-g forces after 6-days intervention in a force-dependent way (Image J software) −35-force suggested as the optimal force for tooth movement in the used mouse model.
Taddei *et al.* (2013) [32]	C57BL6/J mice	Intervention: coil spring applying 35-g force to the right upper 1st molar *(inclination movement)* Duration: 12, 72 h, 6 and 12 days	**Inflammation:** -↑ IL-10 expression after 72-h intervention, mainly on the tension side (real time PCR); -↑ TNF-α expression after 12- and 72-h interventions both in compression and tension sides (real time PCR); -↑ periostin in the periodontium after 72-h intervention both in compression and tension sides. (real time PCR); **Bone formation & resorption:** -↑ TRAP activity on the compression side after 6- and 12-days interventions (TRAP staining); -↑ alveolar bone resorption after 12-days intervention (histological analysis); -↑ RANK, RANKL, cathepsin K and MMP-13 expression after 12- and 72-h interventions both in compression and tension sides (real time PCR); -↑ RANKL/OPG ratio after 12- and 72-h interventions, peaking at 12-h, mainly on the compression side (real time PCR); -↑ RUNX-2, OPG and OCN in the periodontium after 12- and 72-h interventions both in compression and tension sides. (real time PCR); **Movement-related implications:** -↑ tooth movement and TRAP-positive osteoclasts after 6- and 12-days interventions, in a time-dependent way (TRAP staining).
Yoshino *et al.* (2014) [16]	BALB/c mice	Intervention: closed-coil spring applying 25-g force to the upper left 1st molar *(inclination movement)* Duration: 9 days	**Inflammation:** -↑ TNF-α expression in the PDL tissues. **Bone formation & resorption:** -↑ RANKL expression in the PDL tissues. **Root resorption:** -↑ root resorption lacunae with multinucleated odontoclasts on the surface of the root. **Other biological alterations:** -coarse and irregular arrangement of fibers and compressed blood capillaries.
Alikhani *et al.* (2015) [33]	Sprague-Dawley rats	Intervention: single force application of 3-, 10-, 25-, 50- and 100-g force applied to the upper right 1st molar *(inclination movement)* Follow-up periods: 1, 3, 7, 14 and 28 days after the intervention	**Inflammation:** -↑ chemokines, cytokines and cytokines receptors expression at the 1-day follow-up after all applied forces, generally in a force-dependent way, from 3- to 50-g interventions (RT-PCR);

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-↑ CCL2, CCL5, IL-1 and TNF-α protein levels at the 1-day follow-up after all applied forces in a force-dependent way, from 3- to 25-g interventions (ELISA); -↑ CCL2 and CCL5 protein levels at the 3- and 7-days follow-ups after 10- to 100-g interventions, although most of them ↓ compared to day 1 (ELISA); -↑ IL-1 levels at the 3- and 7-days follow-ups after all interventions, although most ↓ compared to day 1 (ELISA); -↑ TNF-α levels 1 day after 10- to 100-g interventions (ELISA); -no significant changes in TNF-α levels at the 3- and 7-days follow-ups after all interventions (ELISA). **Bone formation & resorption:** -↑ constriction in the area adjacent to the alveolar crest at the 3-days follow-up after all interventions, particularly after 25-, 50- and 100-g forces (histological analysis); -↑ cell-free areas (*i.e.*, hyalinization) after 10- to 100-g interventions in a force-dependent way (histological analysis); -widening and ↑ bone resorption areas at the 7-days follow-up both on the periosteal and endosteal sides, accompanied by ↑ cell-free after 100-g intervention (histological analysis); -widening and ↑ bone resorption areas at the 14-days, but no significant changes in cell-free areas (histological analysis); -↑ RANKL levels after 10- to 100-g interventions at all follow-ups (ELISA); -↑ Cathepsin K-positive cells after all interventions, in a force-dependent way from 10- to 100-g forces, especially next to the alveolar crest and apex area (IHC); -↑ osteoclasts in the endosteal and PDL for heavier and lighter forces, respectively, at the 7-days follow-up (IHC). **Movement-related implications:** -no significant changes in tooth movement among 10- to 100-g forces at the 14-days follow-up, and among 25- to 100-g interventions at the 28-days follow-up (histological analysis)
Li *et al.* (2015) [34]	Rabbits	Intervention: maxillary appliance exerting 80-g force on the right upper molars *(no specified movement)* Duration: 3, 5, 7 and 14 days	**Bone formation & resorption:** -↑ RANKL expression and protein levels in the PDL tissue after all interventions (real time PCR and WB; -↓ OPG expression in the PDL tissue after all interventions in a time-dependent way (real time PCR); -↓ OPG protein levels in the PDL tissue after 7- and 14-days interventions (WB) -↑ RUNX-2 expression and protein levels in the PDL tissue after all interventions (real time PCR and WB).
Seifi *et al.* (2017) [35]	Wistar rats	Intervention: closed-coil springs applying 60-g force to the 1st right upper molar *(inclination movement)* Duration: 21 days	**Inflammation:** -no significant changes in TGF-β1 expression (RT-PCR); **Root resorption:** -↑ root resorption lacunae in the mesial root surfaces with multinucleated giant cells (histologic evaluation).
Matsumoto, Sringkarnboriboon, and Ono (2017) [36]	Sprague-Dawley rats	Intervention: continuous mesio-occlusal 50-g force applied to the 1st left lower molar *(inclination movement)* Duration: 8 and 15 days	**Inflammation:** -↑ IL-1α, IL-1β, TNF-αand PGE_2 expression in the root resorption lacunae after both interventions (IHC); **Root resorption:** -↑ TRAP-positive odontoclasts in the root-resorption lacunae near the alveolar bone on the compression side after both interventions (histochemistry);

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-no significant changes in NSE-positive cells, although some NSE-positive monocytes and macrophages were observed around the periodontal capillaries near the root resorption lacuna and alveolar bone marrow (histochemistry); **Bone formation & resorption:** -↓ COX-1 expression after 8-days intervention, returning to control levels after 15-days intervention (IHC); -↑ COX-2 expression in the root resorption lacunae after both interventions (IHC).
Kaya *et al.* (2020) [37]	Wistar rats	Intervention: coil springs applying 10- and 60-g force to three upper molars *(inclination movement)* Duration: 1, 2, 7, 14, 21, 27, 35 and 42 days	**Bone formation & resorption:** -↑ RANK protein levels 7- and 21-days after 10-g force, and 1- and 21-days after 60-g force on the compression side (IHC); -↑ RANK protein levels 21-days after 60-g force on the tension side (IHC); -↑ RANKL protein levels 21-days after 10-g force on the compression side (IHC); -↑ RANKL protein levels 7-, 21- and 42-days after 60-g force on the tension side (IHC); -↓ spaces on the compression side from 1 day onward (H&E staining); **Root resorption:** -external root resorption and ↑ osteoclastic activity on the compression side 21- and 42-days after 60-g force (H&E staining); -small resorption lacunae at one apical of the root 21-days after 10-g force (H&E staining); -↑ cell apoptosis markers after both forces in both sides, mainly after the heaviest force (H&E staining). **Movement-related implications:** -equal tooth movement after both forces at any follow-up (SC-6 Digital Caliper); -↓ tooth movement rate from days 2–14 after 10-g force, in the first 2 days and from days 21–28 after 60-g force (SC-6 Digital Caliper). **Other biological alterations:** -↑ PDL width on the tension side 21-days after 60-g force (H&E staining).
Marahleh *et al.* (2021) [38]	- C57BL6/J (WT) mice - TNFRs deficient (TNFRsKO) mice	Intervention: closed coil spring applying 10-g force to the maxillary left 1st molar *(inclination movement)* Duration: 6 and 12 days	**Bone formation & resorption:** -↑ RANKL-positive osteocytes after 6- and 12-day treatments in the orthodontically-treated WT mice compared to WT mice control (IHC); -tendency towards ↑ RANKL-positive osteocytes after 6- and 12-day treatments in the orthodontically-treated TNFRsKO mice compared to TNFRsKO mice control, although not significant (IHC); -↑ RANKL-positive osteocytes after 6- and 12-day treatments in the orthodontically-treated WT mice compared to the orthodontically-treated TNFRsKO mice (IHC); -↑ RANKL levels after 6-day intervention compared to 12-day for both mouse models, although not significant (IHC).
Noguchi *et al.* (2022) [39]	- C57BL6/J (WT) mice - TNFRs deficient (TNFRsKO) mice	Intervention: Ni–Ti closed coil spring applying 10-g force to the maxillary left 1st molar *(inclination movement)* Duration: 12 days	**Inflammation:** -↑ VEGF-positive cells and mRNA levels in the alveolar bone surface of the orthodontically-treated WT mice compared to WT mice control (real-time PCR); -↑ VEGF-positive cells and mRNA levels in the alveolar bone surface of the orthodontically-treated TNFRsKO mice compared to TNFRsKO mice control (real-time PCR); (*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-↑ VEGF-positive cells and mRNA levels in the alveolar bone surface of the orthodontically-treated WT mice compared to the orthodontically-treated TNFRsKO mice (real-time PCR).
Wei *et al.* (2022) [40]	Sprague-Dawley rats	Intervention: stainless-steel wire applying 10-g and 50-g force to right maxillary 1st molar *(intrusion movement)* Duration: 0, 2, 6, 24 h, 4, 7 and 14 days	**Bone formation & resorption:** -↓ BV/TV ratio, Tb.N and bone mineral density after 10- and 50-g interventions compared to control, and after 50-g intervention compared to the 10-g group (bone histometric analysis); -↑ Tb.Sp levels after 10- and 50-g interventions compared to control (bone histometric analysis); -no significant changes in Tb.Th between groups for all treatment durations (bone histometric analysis); -↑ $TRAP^{+}$ osteoclasts in the AB side after the 50-g intervention delivered for 24 h, 4, 7 and 14 days compared to all groups (TRAP staining); -no significant differences in TRAP $^{+}$ osteoclasts number in the AB side between 10- and 50-g interventions for all treatment durations (TRAP staining); -↑ SOST mRNA expression in the AB side compared to the CC side after 10-g intervention delivered for 24 h, 4, 7 and 14 days (RT-qPCR); -↑ SOST mRNA expression in the AB side compared to the baseline in the same side after 10-g intervention delivered for 24 h and 4 days, and after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -↑ co-localization of SOST protein with osteocytes after both 10- and 50-g interventions delivered for 4, 7 and 14 days compared to the baseline (IHC); -↑ SOST expression in the AB side compared to the CC side the 50-g intervention delivered for 7 and 14 days compared to the baseline (IHC); -↑ RANKL mRNA expression in the AB side compared to the CC side after 10-g intervention for all treatment durations (RT-qPCR); -↑ RANKL mRNA expression in the AB side compared to the CC side after 50-g intervention delivered for 0, 6, 24 h and 4 days (RT-qPCR); -↑ RANKL mRNA expression in the AB side compared to the baseline in the same side after 10-g intervention delivered for 4 days, and after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -↑ RANKL/OPG mRNA expression ratio in the AB side compared to the CC side after 10-g intervention delivered for 24 h, 4, 7 and 14 days (RT-qPCR); -↑ RANKL/OPG mRNA expression ratio in the AB side compared to the baseline in the same side after 10-g intervention delivered for 4, 7 and 14 days, and after 50-g intervention delivered for 7 and 14 days (RT-qPCR); -↓ OPG mRNA expression in the AB side compared to the baseline in the same side after both 10- and 50- interventions delivered for 7 and 14 days (RT-qPCR). **Root resorption:** -↑ root resorption after the 10-g intervention delivered for 7 and 14 days, and after 50-g intervention delivered for 24 h, 4, 7 and 14 days compared control (micro-CT); -↑ $TRAP^{+}$ osteoclasts after 10- and 50-g interventions delivered for 6, 24 h, 4, 7 and 14 days compared to control, in the CC side groups (TRAP staining); -↓ SOST mRNA expression in the CC side compared to the AB side after 50-g intervention delivered for 4 and 7 days (RT-qPCR); -↓ SOST mRNA expression in the CC side compared to the baseline in the same side after 10-g intervention delivered for 7 and 14 days, and after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); (*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-↓ SOST expression by cementocytes-like cells after the 50-g intervention delivered for 7 and 14 days compared to the baseline (IHC); -SOST labelling in the CC and AB sides did not seem to be differentially affected by force magnitude (IHC); -↑ RANKL mRNA expression in the CC side compared to the baseline in the same side after 10-g intervention delivered for 7 and 14 days, and after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -↓ RANKL/OPG mRNA expression ratio in the CC side compared to the AB side after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -↓ RANKL/OPG mRNA expression ratio in the CC side compared to the baseline in the same side after 10-g intervention delivered for 7 and 14 days, and ↑ ration after 50-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -↑ OPG mRNA expression in the CC side compared to the AB side after 10-g intervention delivered for 4, 7 and 14 days (RT-qPCR); -no significant changes in OPG expression between the AB and CC sides for any treatment duration; -↑ OPG mRNA expression in the CC side compared to the baseline in the same side after 10-g intervention delivered for 4, 7 and 14 days, and ↓ expression after 50-g intervention delivered for 6, 24 h, 4, 7 and 14 day (RT-qPCR). **Movement-related implications:** -↑ tooth movement from days 1–7 after 10-g force, although not significant on day 14 compared to day 7 (micro-CT); -↑ tooth movement significant on day 4 after 50-g force, although not significant on days 7 and 14 compared to days 4 and 7, respectively (micro-CT); -no significant differences in tooth movement between 10- and 50-g groups at all timepoints (micro-CT).
Lin *et al.* (2022) [23]	C57BL6/J mice	Intervention: NiTi coil spring applying 30- and 100-g force between the maxillary incisors and the 1st molar *(inclination movement)* Duration: 7 days	**Inflammation:** -↑ IL-17A-positive area in the bone marrow cavity after 100-g intervention compared to all interventions, more pronounced near the compression side (IHC); -↑ IL-17A and IL-6 levels in alveolar bone and gingiva after 100-g intervention compared to all interventions (IHC and qPCR); -↑ IL-6 relative levels in alveolar bone and gingiva after 30-g intervention compared to control (IHC and qPCR); -↑ HIF-1α relative mRNA levels in alveolar bone and gingiva after 100-g intervention compared to all interventions (qPCR); -↑ Notch1 relative mRNA levels after 30- and 100-g intervention compared to control, and after 100-g intervention compared to 30-g intervention (qPCR); -no significant changes of IL-10, CXCL12 and Foxp3 levels in alveolar bone and gingiva between groups (qPCR). **Root resorption:** -↑ TRAP-positive multinucleated cells in 100-g intervention compared to control (TRAP staining, IHC); -↓ distal root volume of the 1st molar after 100-g intervention compared to control (micro-CT); -evident root resorption on the compression side of the distal root of the 1st molar after the 100-g intervention (micro-CT); -infiltration of TRAP-positive cells into the surface of the absorption root lacunae the 100-g intervention (TRAP staining, IHC).

(*continued on next page*)

Table 5 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			Bone formation & resorption: -significant osteoclast infiltration on the surface of the alveolar bone the 100-g intervention; -↑ osteoclasts and their precursors in the bone marrow cavity the 100-g intervention. **Movement-related implications:** -significantly higher tooth movement after 100-g force intervention than in 30-g force intervention. **Other biological alterations:** -hyalinization change in the periodontal ligament on the compression side of the distal root of the 1st molar the 100-g intervention; -↑ distance between the alveolar bone crest and the cementoenamel junction of the mesial root of the 1st molar after 100-g intervention compared to no force intervention (micro-CT).

Caption: AB: alveolar bone; B220: B cell isoform of 220 kDa; BV/TV: bone volume to tissue volume; CC: cellular cementum; CD4: cluster of differentiation; CCL2: chemokine (C–C motif) ligand 2; CCL5: chemokine (C–C motif) ligand 5; Col I: type I collagen; COX-1: cyclooxygenase-1; COX-2: cyclooxygenase-2; ED1: macrophages/dendritic cells; H&E: hematoxylin and eosin; HSP27: heat shock protein 27; IHC: immunohistochemistry; IL: interleukin; M-CSF: macrophage colony-stimulating factor; micro-CT: micro-computed tomography; MMP-13: matrix metalloproteinase-13; MOD: mean optical density; OCN: osteocalcin; OPG: osteoprotegerin; OPN: osteopontin; OTM: orthodontic tooth movement; OX6: class II antigen; PCNA: proliferating cell nuclear antigen; PCR_ polymerase chain reaction; PDL: periodontal ligament; PGE2: prostaglandin E2; RANK: activator of nuclear factor-kappa; RANKL: receptor activator of nuclear factor kappa-B ligand; Runx-2: transcription factor Runx-2; SOST: sclerotin; Tb.N: trabecular number; Tb.Sp: trabecular separation; Tb.Th: trabecular thickness; TGF-1β: transforming growth factor β1; TNF-α: tumour necrosis factor; TNFRs KO mice: TNF receptor I and II deficient mice; TRAP: tartrate-resistant acid phosphatase; VEGF: vascular endothelial growth factor; WB: western blot; WT mice: wild-type mice.

[17], and IL-11 [67], in different cell lines. Indeed, both tensile loadings [63,64] and 2- to 3-g/cm^2 compressive forces [17,64–66]. Similarly, Yoshino and colleagues (2014) observed that TNF-α levels peaked 24 h after the onset of 4-g/cm^2 compressive forces in human PDL cells [16], and after a tensile strength of 0.69 % in osteoblastic and osteoclastic cultures [14]. In addition, multiple authors detected raised VEGF concentration after compression (2 g/cm^2) in human PDL cells [21,64] and in RAW264.7 macrophages [22], but not after tensile loading [64]. Also, an increase in VEGF expression was observed by Zheng et al. (2016) from 2- to 8-h application of 0.006-g/cm^2 shear waves, slightly decreasing when fluid shear stress was applied up to 12 h [20]. Finally, increased PGE_2 levels following OTM were also associated with the application of compressive pressure in human osteoblasts [68], RAW264.7 macrophages [22], and PDL cells [67,69]. Li et al. (2015) found that PGE2 expression was raised only after the application of compression forces of 5–25 g/cm^2 over 24 and 72 h, but only greater forces (15 and 25 mW/cm^2) were able to modulate PGE2 expression after 6-h compression.

b Bone & root resorption

Concerning bone and root resorption mediators, the most preferred ones among the reviewed articles were the receptor activator of nuclear factor kappa-B ligand (RANKL) and matrix metalloproteinases (MMP). Increased RANKL expression and/or protein levels were observed after 4–6 h [13,16], 12 h [16,19], 24 h [14–16,18], 48 h [21], 72 h [18,21] following the onset of orthodontic application in cells. Although these patterns were mainly observed after compression [65,67,68,70,71], centrifugal pressure applied at 3 g/cm^2 [72] and 0.69 % tensile strain [63] have also augmented RANKL activity. In addition, 2-g/cm^2 compression was shown to be quite effective in upregulating MMPs: two studies reported increased levels of MMP-8 levels after 24, 48, and/or 72 h of in RAW264.7 macrophages [22] and periodontal fibroblasts [21]; MMP-9 levels have also increased after the application of a compressive force for 4, 24 and 48 h in RAW264.7 macrophages [22]; also, increased MMP-13 gene expression was observed after 24-h compression in human PDL fibroblast cells [17]. These results point to a strong contribution of orthodontic-mimicking forces to facilitate extracellular matrix degradation and remodeling processes mediated by MMPs and RANK-L, mainly after 2- to 4-g/cm^2 compression [17,64,65,68,70]. Furthermore, one author observed an increase in tartrate-resistant acid phosphatase (TRAP) expression 72 h after the beginning of the orthodontic stress in PDL fibroblasts [21].

c Bone formation

Regarding bone formation mediators, the analyzed *in vitro* studies monitored osteoprotegerin (OPG), cyclooxygenase-2 (COX-2), and alkaline phosphatase (ALP). Several studies reported a decrease in OPG [13,15,18,21] and ALP [19,21,68,72] expression at distinct time points after a wide range (2–30 g/cm2) of compression forces, in different cell cultures. Also, an acute and transient

Table 6
Clinical studies.

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
Wahab *et al.* (2011) [41]	12 patients (aged 14–25)	Intervention: push coil spring applying 100- and 150-g force to the upper canines *(body movement)* Duration: 6 months Follow-up periods: weekly assessments along the first 6 weeks of intervention, and from this time point until 6 months post retraction	**Root resorption:** -↑ TRAP activity on the tension side after 150-g intervention at the 4-weeks follow-up, returning to baseline levels at the 5- and 6-weeks follow-ups (spectrophotometric assay); -↑ TRAP activity on the compression side after 150-g intervention at the 5-weeks follow-up (spectrophotometric assay); -tendency to ↑ TRAP activity on the tension side after 100-g intervention at the 3-weeks follow-up, returning to baseline levels from 4- to 6-weeks follow-ups (spectrophotometric assay); -tendency to ↑ TRAP activity on the compression side after 100-g intervention at the 6-weeks follow-up (spectrophotometric assay); -no significant changes in root resorption in the canines after both interventions (periapical radiography). Other biological alterations: -linear relationship of cumulative canine movement, which ↑ after 150-g intervention compared with 100-g intervention.
Aras *et al.* (2012) [42]	32 patients (aged 12–18 yo) *(64 extracted upper premolars)*	Intervention: titanium cantilever springs applying buccal tipping with a 150-g force on maxillary premolars *(inclination movement)* Experimental design: EG1: 2 weekly reactivations with intermittent (3-day pause before each reactivation) force.; EG2: 3 weekly reactivations with intermittent force; EG3: 2 weekly reactivations with continuous force; EG4: 3 weekly reactivations with continuous force Duration: 84 days (12 weeks)	**Root resorption:** -tendency towards ↑ root resorption in EG4, although not statistically significant (micro-CT). Movement-related implications: -↑ tooth movement rate after the application of continuous force (EG3 and EG4); -↓ reactivation time induced less root resorption.
Madureira *et al.* (2012) [43]	18 patients (aged 4–40) *(64 extracted premolars)*	Intervention: alloy cantilever with metallic ligature applying 100-g force (gradually reduced) to the premolars *(body movement)* Duration: 3, 15 h, 3, 12 and 21 days (extraction time point)	**Inflammation:** -tendency to ↑ IL-6 levels at the 15-h follow-up (ELISA); -↑ IL-6 and CCL3 levels after 12-days intervention (ELISA); -↑ CCL2 levels after 3- and 12-days interventions (ELISA); -tendency to ↓ IL-6, CCL2 and CCL3 levels from 12- to 21-days interventions (ELISA).
Grant *et al.* (2013) [44]	21 patients (aged 12–20)	Intervention: closed-coil spring applying 100-g force to the upper canines, 3 months after the beginning of orthodontic treatment *(inclination movement)* Duration: 6 weeks Follow-up periods: 4 h, 7 days and 6 weeks along the intervention	**Inflammation:** -↑ IL-1β, IL-8, TNF-α and GM-CSF levels in the canines on the compression and tension sides at all follow-ups (multiplex immunoassays - Luminex multi-analyte technology); -↑ IL-6 levels on the tension side at all follow-ups, although it slightly ↓ at the 7-days follow-up, and also on the compression side at the 4-h and 7-days follow-ups (multiplex immunoassays); -↑ IFN-γ levels in the canines on the tension side at the 4-h follow-up, and tendency to ↑ on the compression side at the 4-h and 7-days follow-ups (multiplex immunoassays); -no significant changes in IL-1β in the 2nd molars both on the compression and tension sides (multiplex immunoassays -↓ GM-CSF levels in the 2nd molars on the tension side at the 7-days and 6-weeks follow-ups, although it slightly ↑ at the 4-h follow-up, and ↑ on the compression side at the 4-h follow-up, then returning to baseline levels (multiplex immunoassays); -↓ IL-8 levels in the 2nd molars on the tension side at all follow-ups in a time-dependent way, and on the compression side at the 7-days and 6-weeks follow-ups, although ↑ at the 4-h follow-up (multiplex immunoassays); -↓ TNF-α and IL-6 levels in the 2nd molars on the tension side at the 7-days and 6-weeks follow-ups, although ↑ at the 4-h follow-up (multiplex immunoassays); -↓ TNF-α levels in the 2nd molars on the compression side at the 7-days follow-up, returning to baseline levels at the 6-

(*continued on next page*)

Table 6 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			weeks follow-up, although it ↑ the 4-h follow-up (multiplex immunoassays); -↑ IL-6 levels in the 2nd molars on the compression side at all follow-ups (multiplex immunoassays); -↑ IFN-γ levels in the 2nd molars on the tension side at the 4-h and 6-weeks follow-ups, and on the compression side at the 4-h follow-up, ↓ below baseline levels at the 7-days follow-up (multiplex immunoassays); Bone formation & resorption: -↑ MMP-9 levels in the canines both on the compression and tension sides at the 7-days and 6-weeks follow-ups (multiplex immunoassays); -↑ MMP-9 levels in the 2nd molars on the compression side at the 4-h follow-up (multiplex immunoassays); -↑ RANKL in the canines and 2nd molars on the compression side at the 6-weeks follow-up, and ↓ RANKL levels in the canines on the tension side at all follow-ups in a time-dependent way (multiplex immunoassays); -tendency to ↑ RANKL levels in the 2nd molars on the tension side at the 7-days and 6-weeks follow-ups (multiplex immunoassays); -↑ RANKL/OPG ratio in the canines and 2nd molars on the compression side at the 6-weeks follow-up (multiplex immunoassays); -↓ OPG levels in the canines on the compression side at the 6-weeks follow-up, although rising at the after 4 h; -no significant changes in OPG levels in the 2nd molars (multiplex immunoassays); -↑ TIMP-1 levels in the canines on the compression side after 6-weeks intervention, and on the tension sides at the 7-days and 6-weeks follow-ups (multiplex immunoassays); -↑ TIMP-2 levels in the canines on the tension side at all follow-ups, and on the compression side after 7 days and 6 weeks (multiplex immunoassays); -↑ TIMP-1 and TIMP-2 levels in the 2nd molars on the compression side at the 4-h follow-up (multiplex immunoassays); Other biological alterations: -↓ distance and speed of movement over the intervention in a time-dependent way, peaking at the 4-h follow-up; -↑ GCF volume both on the compression and tension sides over the intervention.
Madureira *et al.* (2015) [45]	23 patients (aged 10–24) *(68 extracted premolars)*	Intervention: alloy cantilever with metallic ligature applying 100-g force (gradually reduced) to the premolars *(body movement)* Duration: 1, 3, 7, 14, 21 and 28 days (extraction time point)	**Inflammation:** -no significant changes in IL-2, IL-4, IL-10 TNF-α, IFN-γ and IL-17A levels in the PDL nor GCF (flow cytometric immunoassay); -↑ IL-6 levels in the PDL after 1-day intervention, although ↓ in a time-dependent way from day 1–28 (flow cytometric immunoassay); -↑ IL-6 levels in the GCF after 21-days intervention, and tendency to ↑ from 1- to 3-days interventions (flow cytometric immunoassay); -tendency to ↑ IFN-γ levels in the GCF after 1- and 7-days interventions, returning to baseline levels onwards (flow cytometric immunoassay); -tendency to ↑ IL-17A levels from 7- to 21-days in the PDL, and after 3-, 7- and 28-days interventions in the GCF (flow cytometric immunoassay); -positive correlation for IFN-γ levels after 3-days, IL-10 levels after 7-days, IL-17A levels after 14- and 28-days, and TNF-α levels after 28-days interventions between PDL and GCF cytokine concentrations (flow cytometric immunoassay); -negative correlation for IFN-γ levels after 14-days intervention, IL-2 and IL-10 levels after 21-days intervention between PDL and GCF cytokine concentrations (flow cytometric immunoassay); (*continued on next page*)

Table 6 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			Other biological alterations: - ↑ GCF volumes after 7- and 21-days interventions.
Castroflorio *et al.* (2017) [46]	10 patients (aged 19–25.6)	Intervention: 1st aligner applying a distalizing 10-g force to a 2nd molar *(inclination movement)* Duration: 21 days Follow-up periods: 1 h, 7 and 21 days along the intervention	**Inflammation:** -↑ IL-1β levels on the compression side at the 7- and 21-days follow-ups, and on the tension side at the 21-days follow-up, mainly on the compression side (ELISA); -↑ TGF-1β level on the tension side at the 21-days follow-up (ELISA). Bone formation & resorption: -↑ RANKL levels on the tension sides at all follow-ups, and on the compression side at 7- and 21-days follow-ups, peaking at the 21-days follow-up on the compression side (ELISA); -↓ OPG levels both on the compression and tension sides at the 7- and 21-days follow-ups (ELISA); -↑ OPN level on the tension side at the 21-days follow-up (ELISA). Other biological alterations: -no differences in GCF volume at any follow-up (Periotron 8000).
Ahuja *et al.* (2017) [47]	8 patients (aged 13.9–22.9)	Intervention: cantilever spring applying 225-g force to the upper 1st premolars *(body movement)* Duration: 28 days Follow-up periods: 3 h, 1, 3, 7 and 28 days along the intervention	**Inflammation:** -↑ IL-7 and TNF-α levels at all follow-ups in a time-dependent way (multiplex immunoassays); -↑ IL-4 levels in a force-dependent way (multiplex immunoassays); -↓ GM-CSF levels in high-root resorption cases (multiplex immunoassays); -tendency to ↓ IL-1β, IL-4, IL-8 and IFN-γ levels in high-root resorption cases compared to low-root resorption cases (multiplex immunoassays); -no significant changes in IL-7 and TNF-α levels between high- or low-root resorption cases (multiplex immunoassays).
Dudic *et al.* (2017) [48]	29 patients (aged 11.3–43.0 yo) *(57 extracted premolars)*	Intervention: cantilever arm applying 100-g force to upper or lower premolars *(inclination movement)* Duration: 8 weeks	**Root resorption:** -↑ root resorption compared to control (micro-CT); -greater root resorption for mandibular teeth than for maxillary teeth (micro-CT); -significant correlation between tooth displacement and root resorption (micro-CT); Movement-related implications: -↓ tooth displacement in older patient or when an intra- or inter-arch obstacle is present (micro-CT).
Gay *et al.* (2017) [49]	71 patients (aged 18–71 yo) *(1083 teeth)*	Intervention: clear aligners *(no specified movement and force)* Duration: 14 months	**Root resorption:** -↓ root length has been observed on at least one tooth of each patient (radiography); -↓ root length in 41,81 % of the teeth; 3,69 % with severe resorption, 12,18 % with moderate resorption and 25,95 % with slight resorption (radiography); -root resorption is more frequent in the lower lateral and central right incisors, as well as in the upper left first premolar (radiography).
Aman *et al.* (2018) [50]	160 patients (34 ± 16 yo)	Intervention: clear aligners *(no specified movement and force)* Duration: 2.19 ± 0.81 years	**Root resorption:** -minimal root resorption, mainly for the maxillary central incisor (CBCT); -only 2 or 3 patients had severe root resorption (CBCT); -root length reduction was significantly affected by sex, crowding, malocclusion and post-treatment approximation to the cortical plates (CBCT); -race, age, treatment duration, interproximal reduction, previous trauma to the teeth, use of elastics and pre-treatment approximation to the cortical plates do not correlate with orthodontically-induced root resorption (CBCT).

(*continued on next page*)

Table 6 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
Chami *et al.* (2018) [51]	11 patients (mean age: 4.88 yo)	Intervention: clear aligners used in the mandibular arch *(no specified movement and force)* Duration: 21 days Monitoring and follow-up: baseline, 24 h, 7, and 21 days	**Inflammation:** -↓ MIP-1β levels between 24 h and day 21(multiplex immunoassays); -tendency towards ↓ IL-17, MCP-1, IL-1β, IL-7, IL-8, G_CSF, GM-CSF and TNF-α levels over time, from baseline to day 21, although not statistically significant (multiplex immunoassays); -tendency towards ↑ IL-17, MCP-1, MIP-1β and TNF-α at 24 h compared to baseline, although not statistically significant (multiplex immunoassays).
Costello *et al.* (2020) [52]	25 patients (mean age: 31.6 yo) *(994 roots)*	Intervention: clear aligners *(no specified movement and force)* Duration: 73,6 weeks	**Root resorption:** -↓ tooth length for all tooth types, mainly in maxillary central incisors, with anterior teeth showing more root resorption in both arches compared to posterior teeth (CBCT); -no significant differences between angle classification nor crowding status and root resorption, except for the lower canines experiencing mildly and moderately crowded cases (CBCT); -incisors demonstrated the highest frequency of resorption, while canines, premolars and molars exhibiting minimal resorption levels (CBCT); -all tooth types had their greatest level of resorption equal or lower than 0.25 mm (CBCT); -treatment duration and tooth location are not predictors of root resorption; -original tooth length was positively correlated with the level of root resorption.
Akl *et al.* (2021) [53]	20 patients (aged 18–25 yo) *(400 roots)*	Intervention: fixed appliance with infra-zygomatic and palatal miniscrews for intrusion applying 200- and 400-g force to upper premolars and first and second molar *(intrusion movement)* Experimental design: CG: 200-g force applied; EG: 400-g force applied Duration: 6 months	**Root resorption:** -no statistical differences between 200- and 400-g interventions in terms of root resorption, suggesting that force magnitude and root resorption are not correlated (CBCT); -upper 1st premolars are the most affected by root resorption, while the upper 2nd molar is the less affected tooth by root resorption (CBCT).
Ghaleb *et al.* (2021) [54]	8 patients (aged 13–18 yo) *(16 extracted premolars)*	Intervention: transpalatal arch and bracket applying continuous or intermittent 150-g tipping force on maxillary first premolars (*body movement)* Duration: 8 weeks Experimental design: EG1: 150-g continuous force applied; EG2: 150-g intermittent force applied (21 days on, 7 days off)	**Root resorption:** -↑ DPP levels (early root resorption) in the EG1 compared to EG2 after 4 and 8 weeks of treatment (ELISA); -↑ root resorption craters' area and volume in the EG1 after 8 weeks of treatment (CBCT); -correlation between DDP levels and root resorption in both groups. Movement-related implications: -slight ↑ tooth movement rate in patients treated with continuous forces (EG1) after 8 weeks of treatment compared with intermittent force (EG2).
Kloukos *et al.* (2022) [55]	21 patients (aged 17–44 yo)	Intervention: fixed orthodontic appliances brackets and Niti wires applying 80-g force *(no specified movement)* Monitoring and follow-up: baseline, 5 and 14 days after treatment	**Bone formation & resorption:** -no significant differences in CTX and PINP levels in blood serum and GCF at all time points (ELISA); -age and sex do not significantly influence CTX and PINP levels (ELISA); -↑ PINP levels in serum and GCF higher in males than in females (ELISA); -↑ CTX levels in serum and GCF higher in females than in males (ELISA).
Shetty *et al.* (2022) [56]	20 patients (aged 18–30 yo)	Intervention: cantilever spring applying 100- to 150-g force on premolar (*intrusion movement*) Duration: 3 days	**Inflammation:** -tendency towards ↓ IL-6 levels after 3 days of OTM in GCF, although not significant (ELISA); -tendency towards ↑ IL-6 levels after 3 days of OTM in the periodontal ligament, although not significant (ELISA);

(*continued on next page*)

Table 6 (*continued*)

Publication data	Population under study	Mechanical application parameters	Primary biological outcomes
			-no significant differences in IL-6 levels between the control sides assessed through GCF samples and PDL scraping (ELISA); -no significant differences in IL-6 levels between the orthodontically-stimulated sides assessed through GCF samples and PDL scraping (ELISA).
Macrì *et al.* (2023) [57]	28 patients (aged 18–38 yo) *(672 teeth)*	Intervention: clear aligners worn at least 22 h per day *(torque movement)* in maxillary and mandibular arch *(no specified force)* Duration: 25 months	**Root resorption:** -↓ root length for all anterior teeth and upper 1st premolar after clear aligner treatment (CBCT); -lateral incisors presented the greatest root resorption in the upper arch, while this was observed for central incisors in the lower arch (CBCT); -all teeth revealed mild resorption, except for upper lateral incisors which showed moderate resorption (10,79 %) (CBCT).

Caption: CBCT: cone-beam computed tomography; CCL2: chemokine (C–C motif) ligand 2; CCL3: chemokine (C–C motif) ligand 3; CTX: C-terminal telopeptide of type I collagen; DPP: dentin phosphoprotein; ELISA: ELISA: enzyme-linked immunosorbent assay; G-CSF: granulocyte colony-stimulating factor; GCF: gingival crevicular fluid; GM-CSF: granulocyte macrophage colony-stimulating factor; IL: interleukin; INF–Y: interferon-gamma; micro-CT: micro-computed tomography; MIP-1β: macrophage inflammatory protein 1 beta; MMP-9: matrix metalloproteinase-9; MPC-1: mitochondrial pyruvate carrier; OPG: osteoprotegerin; OPN: osteopontin; OTM: orthodontic tooth movement; PDL: periodontal ligament; PINP: N-terminal pro-peptide of type I pro-collagen; RANKL: receptor activator of nuclear factor kappa-B ligand; TGF-1β: transforming growth factor β1; TIMP: tissue inhibitors of metallopeptidase; TNF-α: tumor necrosis factor; TRAP: tartrate-resistant acid phosphatase.

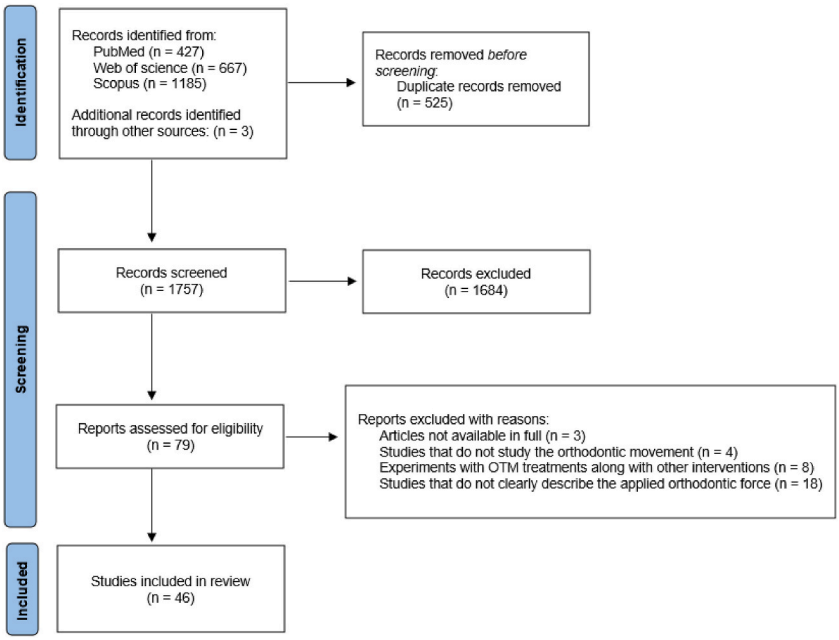

Fig. 2. Flowchart of the study according to PRISMA guidelines.

upregulation of ALP and OPN was observed after 8-h of fluid shear stress, withered 12 h after mechanical stimulation [20].

Furthermore, COX-2 expression increased after the application of 2-g/cm^2 compressive forces for 24 and 48 h in human PDL fibroblasts [21,69] and macrophages [22], as well as after 4 and 6 h of 30-g/cm^2 compression in cementoblast cultures [13]. These results may suggest that heavier compression forces may cause an acute osteogenic response, while lighter forces produce a later COX-2 upregulation.

One study reported a decrease in tissue inhibitor of metalloproteinase-1 (TIMP-1) protein level in PDL fibroblasts after 200- and 400-g/cm^2 compression, in a force-dependent way [19].

d Other biological alterations

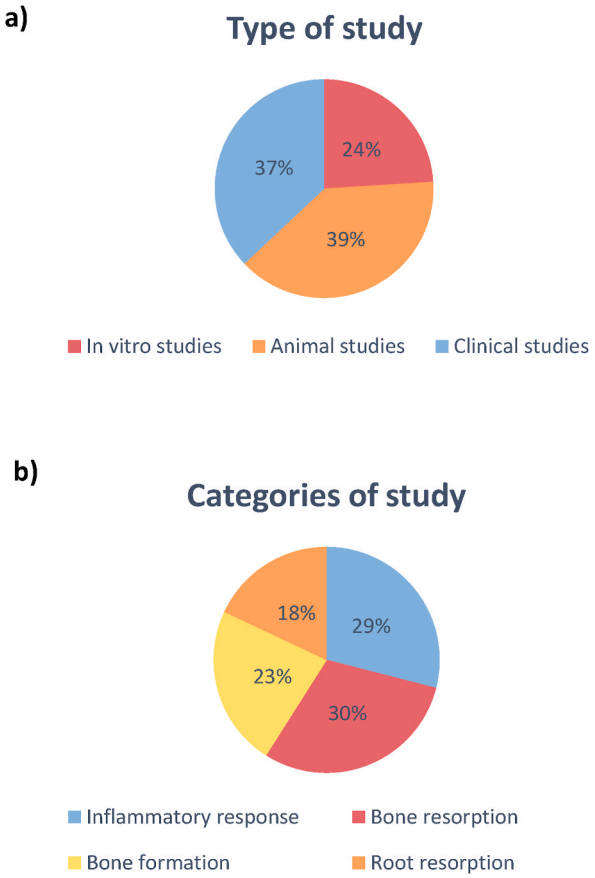

Fig. 3. Studies distribution according to **a)** categories of study; and **b)** the type of study.

The main alternative biological response occurring along the mechanical loading was the inhibition of cell proliferation and viability in human PDL cells after different levels of compression [16,18,19,23] The application of 0.006-g/cm^2 shear stress have also modulated cells orientation, suppressed cell proliferation, and stimulate healing processes after 12 and 24 h in human PDL cells, with signs of apoptosis [20]. Similarly, although the authors observed an increase in PDL fibroblasts mortality after both 3- and 15-g/cm^2 compression (in a dose-dependent way), no significant changes in apoptosis were found.

The animal studies confirm a differential expression of the mediators involved in the orthodontic movement in response to distinct mechanical applications.

a Inflammation

The most mentioned inflammatory mediator (67 % of the included studies) was TNF-α. Several studies reported an increase in cytokine TNF-α levels from 1 to 72 h after orthodontic application, mainly on the compression side, in rodents [16,26,32,33,36]. In addition, multiple authors found an upregulation of different interleukins, including IL-1β and IL-6, in different rat models [23,66,26,29,31,32,36], as well as chemokine ligand 2 (CCL2) and CCL5 protein levels also in rats at 1-, 3-, and 7-days follow-ups [33]. The upregulation of the referred inflammation markers was independent of the type of movement or magnitude of the involved force, since they were associated with a wide range of orthodontic forces (10–100 g) to promote different types of dental movements, although inclination was the most exerted one. In addition, one study has detected an increase of VEGF-positive cells and mRNA expression after 12 days of 10-g OTM in two different mouse models - C57BL6/J (WT) and TNFRs-deficient (TNFRsKO) mice [39]. Importantly, this study showed that the up-regulation of VEGF is increased by the presence of TNF-α during OTM since mice lacking TNF-α receptors exhibit less VEGF-positive cells and gene expression compared to WT mice when both models were subjected to orthodontic forces. In fact, after this publication, the same group, this time led by Marahleh et al. (2021), observed an increase in RANKL-positive osteocytes in the alveolar bone at the compression side of TNFRsKO mice when compared to WT mice using a similar orthodontic intervention [38].

On the other hand, Seifi et al. (2017) did not observe statistically significant differences on the expression of inflammation mediators with or without the application of OTM, even using 60-g force to produce an inclination movement [35].

b. Bone & root resorption

Furthermore, concerning resorption mediators, almost 86 % of the animal experiments assessed the expression and/or protein levels of RANKL and TRAP. Increased RANKL expression and/or protein levels were observed after 4–6 h [16,27], 12 h [16,27,30–32], 24 h [16,30–33,33,34], and several days following the onset of orthodontic application in animal models [32–34,37,38], mainly on the compression side. This upregulation was observed independently of the magnitude of the orthodontic force (10–100 g). Also, upregulation of the RANKL/OPG ratio 12 h after the intervention, especially on the compression side, has been reported in two animal studies after both 35- [32] and 100-g orthodontic forces [27]. In addition, multiple authors observed an increase in TRAP expression 72 h after the beginning of the orthodontic treatment on the compression side of the teeth of Wistar rats [24], as well as from 6 to 12 days in C57BL6/J mice, in a force- and/or time-dependent way (forces up to 50 g) [30–32]. Also, MMP-13 protein levels have risen after 12 and 72 h of force application in C57BL6/J mice after [31,32]. Moreover, multiple authors reported an increase in root resorption lacunae [16,27,29,35,37] and bone resorption [25,30,33], after 10- to 60-g orthodontic forces.

c Bone formation

Regarding bone formation mediators, the most studied one (investigated by 87 % of the animal studies) is osteoprotegerin (OPG). Several studies reported a decrease in OPG expression and/or levels at distinct time points in animal models after both 10- and 50-g intrusion [40] and 80- to 100-g to produce inclination [27,34].

Contrary results were reported by others; different studies observed an upregulation of OPG in a time-dependent way following 10- to 50-g orthodontic forces in C57BL6/J mice [30–32]. Furthermore, the same studies observed an increase in osteocalcin (OCN) expression after 72-h intervention [30,32], in a force-dependent way [31].

d Other biological alterations

Besides the abovementioned alterations, the main alternative biological response occurring along the mechanical loading was an increase of coarse and irregular fibers and blood capillary compression in rodents [16,25,28,29]. Also, it should be noted that three studies observed that heavier forces were not associated with a greater amount of tooth movement when two or more levels of forces (3–100 g) were assessed [29,33,37,40], when producing different types of movements. On the other hand, one study detected a significantly higher OTM under 100-g force compared to 30 g [23].

Clinical studies confirm a differential expression of the mediators involved in the orthodontic movement in response to distinct mechanical applications, in line with the conclusions of preclinical experiments. Despite the wide range of force levels used, similar biological outcomes were often reported among studies.

a. Inflammation

All clinical studies assessing the role of inflammatory mediators during OTM have monitored the expression of either tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), and/or interleukin 6 (IL-6); two studies reported an increase in cytokine TNF-α levels from 1 to 72 h after orthodontic application, more prominent on the compression side [44,47]. Similarly, one clear aligner study observed that TNF-α was increased 24 h and 7 days after intervention [51]. In addition, multiple authors reported an upregulation of IL-1β and/or IL-6 after 1 h to 6 weeks in human gingival crevicular fluid (GCF) [43–45], and after 3 days in periodontal ligament [56]. Moreover, decreased granulocyte-macrophage colony-stimulating factor (GM-CSF) levels have been found in two studies at different time points [47,51]. These results are not in line with Grant et al. (2013) outcomes since they observed an increase in GM- CSF levels on both the compression and tension sides, in a time-dependent way [44].

b Bone & root resorption

Furthermore, concerning bone and root resorption, almost 75 % of the reviewed articles assessed the expression and/or protein levels of RANKL and radiographic findings. In fact, multiple studies mentioned an increase in root resorption and/or a decrease of the root length after OTM applied by conventional appliances [48,53] and clear aligners [49,50,52,53,57], mainly in incisors [49,50,52,57], but also in upper first premolar [53]. One study has detected a higher level of root resorption when OTM was executed under continuous force compared to intermittent force [42]. Moreover, clinical studies have found increased levels of RANKL on the compression side at the 7- and 21-day [46], as well as at the 6-week [44] follow-ups. In addition, Wahab and colleagues (2011) reported that 150-g forces (but not 100-g forces) induced a significant increase in TRAP activity both at the tension and pressure side, although this augment was reversed in the tension side one week after the concentration peak [41]. Also, MMP-9 levels increased after 4-h, 7-day, and 6-week interventions in human patients [44]. Similarly, an upregulation of C-terminal telopeptide of type I collagen (CTX) levels has been noted after 5 and 14 days, in both blood and GCF samples [55].

c. Bone formation

Regarding bone formation mediators, the most studied one was osteoprotegerin (OPG). Two studies reported a decrease in OPG expression and/or levels at distinct time points after 10- [46] and 100-g [44] orthodontic force. TIMP-1 and TIMP-2 upregulation was

also found after 7 days on the tension side and after 6 weeks on both tension and compression sides [44].

d Other biological alterations

Some trials also found an increase in GCF volume after applying 100-g force for 7 days [44,45]. Moreover, two studies also observed that tooth movement tends to be more accelerated under continuous force than under intermittent force [42,54]. Also, one study reported a decrease in OTM rate in older patients or when an intra or inter-arch obstacle was present, as perceived by micro-CT [48].

3.4. Quality assessment data

Among the *in vitro* studies revised here, most of the cases report changes in gene expression and protein release without a previous assessment of the effect of the mechanical stimulus on cell proliferation and/or viability. Besides, four out of 13 studies were not sponsored [12,19,69,24], which might suggest a low risk of sponsorship bias [58].

The most common problem verified when conducting the quality assessment of the animal articles was associated with performance and detection bias, mainly due to the lack of blinding of the investigators regarding the experimental groups [12,16,23,24–27,29,31,33–40]. Similarly, randomization issues were detected, which compromises the quality and verisimilitude of the data collected from such studies.

Finally, 11 out of 17 of the included clinical studies presented a moderate or high risk of bias to missing data [42,48,51], which can lead to incorrect/unrealistic conclusions based on unrevealed data. Complete methodological quality assessment data is provided in Supplementary Tables S1–S3.

4. Discussion

This systematic and integrative review aimed to discuss the biology of orthodontic dental movement, addressing the biological changes that occur in the periodontium during different phases of the orthodontic movement, under different orthodontic protocols, using evidence collected in both preclinical (*i.e.*, *in vitro* and animals) and clinical studies.

Additionally, a comprehensive overview of the effects of different mechanical application parameters on the stressed tissues is also provided. Finally, this review highlights the most common limitations to be considered in future studies in the field and it offers valuable recommendations for filling up the gaps that the literature still presents.

4.1. Histological and biochemical changes

Cellular and tissue reactions begin at the initial phase of movement, immediately after a force application, with the compression and stretching of the fibers and cells of the PDL [44]. This promotes histological changes in the tissues and the release of chemical biomarkers, which modulate bone formation and resorption phenomena. Studies conducted in rats demonstrated that fibers and fibroblasts become thick and irregular, showing compressed blood capillaries on the compression side [16,25,29]. Contrarily, on the tension side, studies found a stretching of the fibers and cells of the PDL, also with expanded blood capillaries [25,28] (Fig. 4).

Concerning cellular alterations, osteoblasts, fibroblasts, and macrophages respond to mechanical stress and induce an inflammatory response, increasing the synthesis and release of different pro-inflammatory enzymes, cytokines, and chemokines [6].

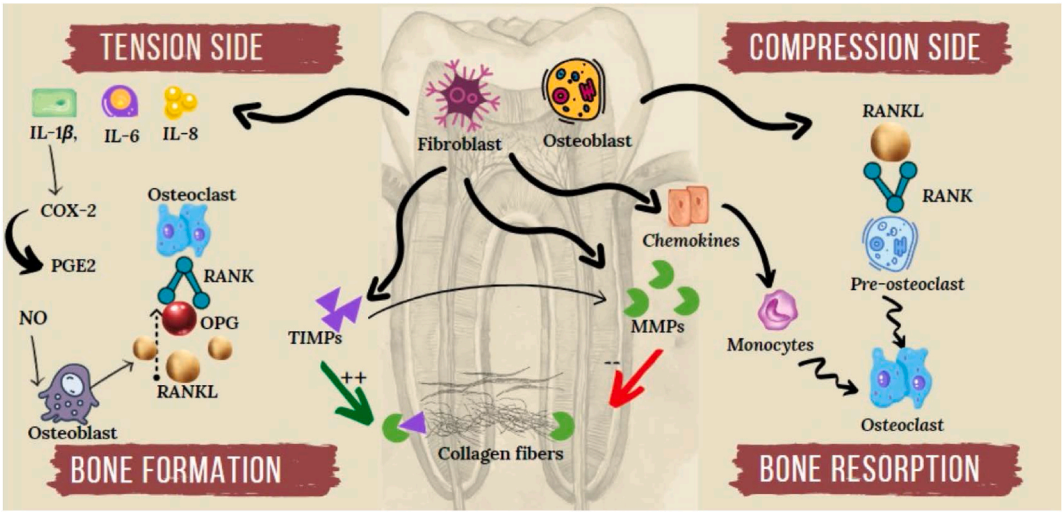

Fig. 4. The main effects produced by the orthodontic force on the tension and compression sides.

Specifically, Schröder et al. (2018) showed that the response of PDL fibroblasts against orthodontic force leads to the initial synthesis of prostaglandins by cyclooxygenase-2 (COX-2), which induces an increased expression of bone resorption mediators (such as RANK-L) and pro-inflammatory cytokines (like IL-1 and TNF- α) [21]. The authors also demonstrated that the expression of genes involved in the remodeling of the extracellular matrix, such as the Collagen Type I Alpha 1 Chain (COL1A2) gene, as well as in angiogenesis phenomena, like VEGF-A, peaked 24 h after the beginning of the orthodontic force application [21]. Another study by Schröder et al. (2020) applying the same mechanical load (*i.e.*, 2 g/cm^2) reported an upregulation of pro-inflammatory COX-2 in macrophages after 2-, 24- and 48-h of tension, as well as IL-6 after the application of 4 and 24 h of a tensile strain. The authors also observed an upregulation of the VEGF-A gene after 2- to 48-h compression, returning to control levels after 48-h intervention, while no significant changes in VEGF-A expression were reported after tension [22]. An increase in cytokines concentration, such as TNF-α and IL-6, was also detected in mouse calvarial osteoblast and femoral osteoclast cultures [14]. Human PDL fibroblast cells also showed an increased expression of IL-8, and PGE_2 (precursors for osteoclastic differentiation) in response to compressive pressure (i.e., 2 g/cm^2) during the first 24 h of stress [69]. Furthermore, Lin and colleagues (2022) reported an upregulation of IL-6 levels and hypoxia-inducible factor (HIF-1α) relative mRNA levels when 3- and 15-g/cm^2 is applied for 24 h in PDL cells, favoring osteoclastogenesis phenomena [23]. In this same study, under the same load and when compared to the no force application group, the levels of other immunoregulatory cytokines, namely the transforming growth factor beta (TGF-β) and the Notch1 protein decreased after compression of 3- and 15 g/cm^2 at 24 h of OTM, while an increase was observed after 48 h [23].

These findings in *in vitro* studies are in line with the results obtained by Taddei et al. (2012) in C57BL6/J mice, who found increased IL-10 and TNF-α levels right after 12-h and 3-day orthodontic treatments [31]. Also, higher levels of VEGF-positive cells and VEGF mRNA were observed along with the application of 10-g force for 12 days in two different mouse strains, mainly for C57BL6-J mice compared to TNF receptor-deficient mice (TNFR-deficient), which suggests that TNF may play a main role during OTM as it modulates VEGF expression, responsible for osteoclast precursors chemotaxis and vascular permeability [39]. The same experimentation, but this time analyzing RANKL-positive osteocytes, has shown a higher level of RANKL expression in WT mice compared to TRNF-deficient mice, suggesting that TNF-α-responsive osteocytes would be important cells for osteoclast formation during OTM [39]. In addition, the increased release of CCL2 and CCL3, which are small proteins of the cytokine family, were proved to attract monocytes which in turn can differentiate into macrophages or osteoclasts [3]. An increase in the levels of these mediators was observed in Sprague-Dawley rats [33], as well as in the GCF of human individuals after force application [43].

In line with preclinical evidence, OTM was proven to modulate the expression and protein release of several inflammatory mediators at an early orthodontic treatment stage, as assessed by analyzing patients' GCF. It should be noted that the GCF is an inflammatory exudate that circulates freely in the gingival sulcus, making it difficult to distinguish between the compression and tension areas, and therefore it should be interpreted as indicative only [43,45,73]. Nevertheless, the revised clinical data indicate that the activation of inflammatory mediators during orthodontic treatment is a very early initial response [6]. From a population of 21 patients, Grant and colleagues (2013) investigated the levels of IL-8, IL-6, IL-1β, and TNF-α, and found increased concentrations of these biomarkers in the canines at 4 h and 42 days after orthodontic appliance, mainly on the compression side [44]. Others observed an upregulation of IL-7 and TNF-α at different time points (*i.e.*, 3 h, 1, 3, 7, and 28 days) in an eight-patient cohort [47], corroborating the previous assumption. In addition, another study conducting an orthodontic treatment with clear aligners reported an increase of macrophage inflammatory protein 1 beta (MIP-1β), an inflammatory chemokine related to the activation and recruitment of monocyte/macrophage cell lines during the initial phase of treatment (between days 7 and 21) [51]. Altogether, the current review has comprehensively collected and analyzed the existing data on OTM-induced histological and biochemical alterations in the periodontal space, highlighting the release of pro-inflammatory cytokines and growth factors during the early stages of the OTM that propel the remodeling of the PDL and alveolar bone.

Importantly, recent evidence has been alerting us to the importance of good oral health to ensure not only the efficacy but also the safety of the orthodontic intervention. The health status of the periodontal tissue is crucial to allow a normal inflammatory process during orthodontic treatment, as well as to prevent further damage in tooth-supporting structures. In patients with periodontal diseases (*e.g.*, periodontitis, gingivitis), the inflammatory pattern of the tissues is aggravated by the increased release of pro-inflammatory agents such as interleukins and metalloproteinases, as well as an overproduction of free radicals, nitric oxide (NO), and endothelial inflammatory biomarkers, commonly observed during the orthodontic intervention [74,75]. Several studies have been describing the risks of applying mechanical loading upon diseased periodontal tissues, demonstrating that mechanical load modulates the inflammatory pattern of periodontal tissues in response to periodontal disease by increasing the expression of several pro-inflammatory mediators and receptors [8,9,76–78]. The application of orthodontic forces in diseased tissues may also potentiate alveolar bone loss and decrease bone apposition [75]. In this sense, we propose that future studies and clinical interventions involving orthodontic treatment should consider a previous evaluation of the patient's oral health in terms of tissue integrity and gingival biofilm burden. Also, the existence of chronic and/or systemic disorders and/or neuromuscular deficits should be addressed by clinicians to enhance usability and comfort during orthodontic treatment. This would allow orthodontists to prevent periodontal damage, bone loss, and extensive root resorption caused by an exacerbated immune host response due to the overproduction of pro-inflammatory cytokines induced by the orthodontic treatment.

4.2. Bone remodeling (formation & resorption)

Osteoclasts are derived from hematopoietic precursors of monocyte/macrophage lineage and regulated by the balance between RANKL, OPG, receptor activator of nuclear factor kappa-B (RANK), and macrophage colony-stimulating factor (M-CSF) [12,19,24,79], and so the increased of osteoclasts formation and the inhibition of their resorption *in vitro* [14,18], and in animal models [24,25,27,28,

33] reflect an increase in bone resorption.

In *in vitro* studies, increased RANKL expression and/or protein levels were observed [13,15,18,19,21]. These data are in line with the results obtained in *in vivo* studies in rats and rabbits, which also detected the upregulation of this biomarker, mainly on the compression side [27,31,34,38,80], as well as in human studies [44]. In addition, OPG levels decreased slightly in response to compressive strength in *in vitro* studies [13,15,18,19,21]. In animal studies, this trend was also observed [34], except for two studies that reported an increase in OPG levels at the 8- and 12-h follow-ups [27,31]. Also, one study conducted in Sprague-Dawley rats detected an upregulation of OPG under 10-g force while a downregulation was observed under 50-g [40], which may indicate an inhibitory effect of high mechanical load concerning OPG expression. Besides, one clinical study found decreased OPG levels at the 24-h follow-up on the compression side [73]. Though some results are slightly contradictory, they suggest that the expression of OPG mediators is more pronounced on the tension side and under light force, although they were also detected on the compression side, while RANKL is more associated with the compression side [73,81].

Levels of COL1 and ALP mRNA, which are markers of early differentiation of osteoblasts, were detected after 1 h of force application in *in vitro* studies [15,19]. Also, it was observed that compressive strength increased the levels of non-collagen proteins such as osteopontin (OPN) and osteocalcin (OCN), as well as transcription factors such as transcription factor Runx-2 [6,19,20]. Animal studies have also found a significant increase in Runx-2, OCN, osterix, and OPN levels on the tension side [28,31]. These results prove that higher concentrations of osteoblast markers and bone formation mediators are more prominent on the tension side compared to the compression side.

Moreover, MMPs (*i.e.*, proteins that degrade the extracellular matrix) were found in *in vitro* [16,20,69,32], animal [31], and human [44] studies, mainly on the compression side. Their activity is balanced by the production of tissue inhibitors of metalloproteinase (TIMPs), which bind to MMPs by inhibiting their proteolytic activity and consequently limiting the degradation of the extracellular matrix [19,20,44]. Higher levels of TIMPs have been detected on the compression sites [44]. Nettelhoff et al. (2016) observed that fibroblasts are mainly responsible for the increase of MMPs and TIMPs, after the application of a compressive force, when compared with osteoblasts, *in vitro* [19].

Besides, three studies saw no differences in tooth movement between the light and heavy force groups at any time in rats [25,37,40]. The only statistically significant outcome detected by the authors was a higher concentration of apoptotic markers, such as caspases 3 and 9, in the heavy force group [37]. These data suggest that the application of higher forces does not increase the activity of bone resorption mediators, and therefore cannot increase the rate of movement. They only expose the tooth to an increased risk of side effects [33,37].

N-terminal pro-peptide of type I pro-collagen (PINP) and CTX, both markers of bone turnover for bone formation and bone resorption, respectively, were discussed in Kloukos and colleagues' (2022) human study. The authors explored their concentration pattern in blood and GCF samples during initial treatment, under 80-g force. No statistically significant alterations have been recorded even when normalized by age and sex of the patients [55].

Although none of the included studies evaluated NO concentration, this also plays an important role in the inflammatory process that occurs in the orthodontic movement. Specifically, NO promotes vascular permeability, allowing monocytes to enter the tissues, which favors bone remodeling [82]. In addition, increased NO reduces the RANKL/OPG ratio, promoting bone formation due to the decreased recruitment of osteoclasts [83]. Therefore, the analysis of NO expression should be considered in future studies. In addition, future clinical studies may consider the assessment of the impact of the OTM on the expression of key biomarkers such as the VEGF, a cytokine involved in angiogenesis (particularly important in the compression side), vascular permeability and tissue neoformation, and ALP, a highly specific biomarker for bone formation.

4.3. Root resorption

One of the most common side effects caused by orthodontic movement is root resorption, which is an inevitable, invariable, and unpredictable pathological consequence [2,84,85]. This consequence could be the result of excessive forces, which causes root cementum resorption, and in more serious scenarios, this resorption could progress to dentine [85]. During orthodontic tooth movement, it is necessary to consider factors such as magnitude, duration, direction, and type of force since they can develop side effects such as root resorption and hyalinization [2].

Studies in rats evaluated the relationship of the force magnitude with the concentration of different root resorption mediators and/or expression; Alikhani et al. (2015) observed a tendency for higher levels of RANKL and chemokines (CCL2 and CCL3) at the 12-h follow-up in rats subjected to a force of 25, 50, and 100 g compared to the groups where 3- and 10-g forces were applied [33]. Other studies in rodents subjected to a force of 50 g had many resorption gaps with odontoclasts near the root surface, compared with the 10-g force group [29]. In line with such results, the authors also observed a marked increase in interleukin 17 (IL-17) and interleukin 17R (IL-17R), which are cytokines that stimulate odontoclast differentiation, in the 50-g force group [25,29]. Hence, multiple evidence exists demonstrating that heavier forces have an increased potential to induce root resorption compared to lighter forces.

Recent studies have found that radiographic analysis is an effective tool for quantifying root resorption. Cone beam computer tomography (CBCT) [50,54,57], micro-computed tomography (micro-CT) [48], and panoramic X-ray [49] have been used by several authors to prove the existence of a significant correlation between OTM and root resorption. In addition, GM-CSF levels were also used to estimate and monitor apical root resorption, although contradictory observations have been reported. Two clinical studies, one using a cantilever spring applying a 225-g force [47] and the other using clear aligners [51], have shown a decrease of these levels at different time points compared to control groups, which indicates greater root resorption in the patients subjected to the orthodontic

treatment. On the other hand, after a 6-week treatment with a closed-coil spring applying 100-g force, a type-dependent increase of GM-CSF levels was observed on both the compression and tension side compared to non-treated patients, suggesting a decrease in root resorption over time.

The severity of orthodontically induced root resorption can be classified into three groups: light, moderate, and severe. Three studies using clear aligners have shown that most teeth that have undergone orthodontic movement suffer from mild resorption after treatment and only a small fraction is affected by severe resorption [49,50,57].

In two studies applying a 150-g tipping force, root resorption was found significantly higher when patients were treated with continuous force than with intermittent force [42,54]. These results might be explained by the fact that during the no-force period, reparative mechanisms can occur spontaneously. Contrary findings have been reported by Akl and colleagues (2021) in their experience carried out on two force groups (200- and 400-g forces), resulting from a lack of correlation between force magnitude and root resorption [53].

Moreover, in addition to mechanical factors, biological cofactors must be taken into consideration when talking about orthodontically induced root resorption. In a clear aligners study, authors have shown that several factors such as sex, crowding, and malocclusion were cofactors that increase root resorption during OTM, while race, age, treatment duration, and previous trauma to the teeth, were not considered significantly influential [50]. However, these results are not in line with those obtained by Costello et al. (2020) for whom angle classification, crowding status, treatment duration, or even tooth location have not been established as predictors of root resorption [52].

In this sense, the possible risks and undesirable side effects of the orthodontic treatment should be carefully addressed by the clinician, to avoid damaging tooth-supporting structures and to achieve a safe and effective movement in the shortest time possible. Depending on the complexity of the malocclusion, oral health, and profile of the patient, the most appropriate treatment must be designed. For instance, Silva and her colleagues (2020) realized that, when esthetics is a decisive factor and crown lengthening is desired without any change in the gingival margin, fibrotomy, and scaling were preferable to conventional orthodontic extrusion alone or with debridement of the open flap before the start of extrusion in dogs [86]. This is a great example to demonstrate that orthodontic treatment is not always the key to solving the malocclusion problem, and the consideration of alternative strategies is required to offer the most appropriate treatment to the patients. Moreover, this also remarks that the orthodontic intervention must be preceded by a complete evaluation of the neuromuscular system (*i.e.*, function and activation pattern), in a way to identify the etiology of the required tooth movement in order to define the best treatment methods and, hence, to maximize the chance of treatment success [74,87,88].

4.4. Phases of the orthodontic movement

Many authors have been reporting that, in the initial phase (*i.e.*, 24–48 h after starting the orthodontic treatment), the RANKL/OPG ratio increases compared to baseline in humans [3,73,81], which is in line with *in vitro* [13,19] and animal [27,32,40] reviewed studies. This result is also consistent with the initial phase described by Burstone in 1962 [89] who supported that, due to hypoxia and ischemia caused by the application of a force, a reduction in RANKL and OPG levels occurs, changing the RANKL/OPG ratio in favor of osteoclastogenesis [2,3,44,81]. After two weeks of orthodontic treatment, this ratio decreases due to the upregulation of OPG compared to RANKL [81], which also occurs in one of the included studies in mice [32]. This represents the second phase, where the movement will only continue when the necrotic or hyalinized tissue is removed [2,3,44,81].

Over longer periods, during the third phase of the orthodontic movement, the RANKL/OPG ratio also increases, which coincides with the increase of the orthodontic movement rate [2,3,44,81]. This aspect was also noted in one clinical study performed in a cohort of 21 patients, six weeks after the beginning of the orthodontic treatment [44].

4.5. Optimal mechanical parameters for orthodontic application

The optimal treatment specifications are related not only to the most effective strategy for tooth alignment but also to the combination of factors that promote less damage to the periodontium and surrounding tissues. As previously said, root resorption is inevitable, but its magnitude can be minored by the application of lighter forces, as long as the desired tooth movement is achieved. Also, excessive forces can occlude the vascularization of the PDL [90]. Several studies have already reported cases in which lighter forces are key for achieving the desired orthodontic result, even when complex movements are required. For instance, Cannavale and colleagues (2013) described a case report in which light forces were crucial to correct a Class I skeletal malocclusion with an ectopic premolar developing in a premolar-molar transposition [88].

In this sense, the provided tables depict the biological outcomes that resulted from different orthodontic interventions, and it is obvious that mechanical application parameters define the produced effects. As expected, the mechanical load is lighter in *in vitro* studies compared with animals and clinical trials, and in animal models compared to human patients. Based on the data provided by the reviewed studies, the preferable range of magnitude of the applied mechanical load in *in vitro* studies is 2–30 g/cm^2 (nine out of 13 cases) [12,13,15,18,21–23,69], although the magnitude of deformation was heterogeneous [19,20]; and 10–60 g/cm^2 to induce molar inclination in animal models (11 out of 19 experiments in rodents) [16,25,26,30–33,35–37,39]. In human patients, the revised studies indicate that a mechanical force of 100 g was able to promote inclination of either canines [44] and premolars [48] with no signs of extensive root resorption; for the intrusion of molars [53] and premolars [53,56], the orthodontic forces vary from 100 to 400 g, with no correlation between the force magnitude and root resorption [53]; finally, the magnitude of the orthodontic force to induce bodily movements of both upper canines [41] and premolars [43,45,54] typically ranges from 100 to 150 g/cm^2, preferably applied in an

intermittent mode to minimize root resorption [54]. These magnitude ranges are similar to the recommendations on the optimal forces for orthodontic movement by Proffit, except for the intrusion movement, since forces of 10–20 g force are indicated [90].

Importantly, studies reporting orthodontic treatments with clear aligners have rarely described the type of movement and the orthodontic force applied (only one out of six studies clearly described the orthodontic protocol with aligners, reporting the use of an initial force of 10 g) [46]. Previous studies investigating the mechanical characteristics of the orthodontic protocol produced by aligners have recognized that these exert variable forces in each tooth, which depend on several factors, including the thermoplastic material properties, thickness of the aligner, width of the aligner edge, direction of tooth movement, designed activation, utilization of attachments, and relative movement to adjacent teeth [91,92], making the orthodontic forces applied by aligners very difficult to quantify. This hampers the comparison of fixed vs removable orthodontic appliances in terms of the most suitable orthodontic forces to promote specific tooth movements for each orthodontic approach.

Based on the biological outcomes reported in the included studies, we suggest that the most suitable parameters to employ in imminent clinical research on the tooth orthodontic movement are: i) force magnitude: 100-g orthodontic force was proved to be preferable to 150-g force to promote body and inclination movements since it causes minor root resorption and tissue damage [42,54]; intrusion movements seem to require heavier orthodontic forces and we recommend the use of 200-g forces, which has been associated with minimal root resorption [53]; ii) periodicity of activation: orthodontic cycles of more than two or three weeks must be avoided, since too long interventions can also promote increased root resorption [28,32,42] or an excessive inflammatory response [47], which suggests that the activation of a new orthodontic cycle should be anticipated; iii) type of force/treatment mode: continuous forces have been established as more likely to induce root resorption than intermittent forces [42,54], possibly because they do not allow cellular response and spontaneous repair.

Overall, although the reviewed protocols show similar orthodontic protocols, it is already demonstrated that small differences in mechanical loading parameters produce extensive effects in the analyzed outcomes, commonly in a time- or force-dependent way. To address this limitation, further studies will be needed to explore mathematical correlations between biomarker concentration/expression and mechanical loading parameters. This can be a useful tool to formulate a precise approach considering the most appropriate parameters to achieve the most effective treatment. Attending to the individual metabolic and anatomical features, the optimal orthodontic treatment specifications should be customized and adjusted along the orthodontic treatment.

4.6. Limitations in the literature

A limitation found in the reviewed studies was the heterogeneity regarding sample size, age, magnitude of force, duration of the intervention, and follow-up periods. These factors were proved to markedly influence the biological outcomes, and so sparse comparable data were collected. Also, the method used to quantify root resorption can be considered as a parameter that can influence the study's results since there are several diagnostic tools with different settings.

Besides, many animal and clinical studies reporting the biological effects of orthodontic tooth movement do not clearly describe the intervention (*i.e.*, applied force, how the force/deformation is applied, duration of the intervention, and other mechanical application parameters). In this sense, many studies were excluded from this systematic review [73,81,82] since the lack of such information hinders the proper comparison between intervention and the respective outcomes. Also, more information about how the deformation and forces are applied to the substrate in *in vitro* studies should be provided. In this sense, future works in the field should fully describe the mechanical stress induced during the orthodontic treatment, allowing a comprehensive analysis of the cause-effect relationship between the applied treatment and the observed outcomes.

5. Conclusions

Orthodontic tooth movement demands a complex response of adaptation of the periodontium, in which multiple mediators play specific roles; on the tension side, an increase in bone formation mediators is observed, whereas bone resorption mediators predominate on the compression side. We have also concluded that the application of heavier forces does not upregulate key biomarkers of inflammation and bone formation, but it increases the risk of root resorption, hyalinization, and exacerbated inflammatory response, so it should be avoided. Therefore, we anticipate that lighter orthodontic force (100-g forces for inclination and body movements of canines, molar, and premolars, and 200-g forces for the intrusion of molars and premolars, although it should be confirmed in future clinical trials), delivered in intermittent mode (*i.e.*, pausing the treatment for some days), possibly with more often cycle activation would represent the most appropriate solution to prevent unnecessary tissue damage.

This paper also highlights the importance of the early identification of dental occlusion problems and the consideration of periodontal diseases, allowing the design of a customized, safe, and more effective treatment. More specifically, the current review reinforces the importance of evaluating the oral health status before the onset of the orthodontic treatment (with both conventional appliances or aligners) to assure the tissues' integrity and the absence of gingival inflammation, maximizing the chance of treatment success. Recommendations for the assessment of the impact of orthodontic treatment on the expression of specific key biomarkers of bone remodeling and vascular permeability (*e.g.*, NO, VEGF, and ALP) in future clinical trials are also provided. We believe this systematic review comprises an important achievement to provide synthesized, clear, and useful information on orthodontic treatments with conventional appliances and aligners to aid clinical practice, contributing as a fundamental basis for clinical success and fostering further developments in the field.

Data availability statement

All data associated with this study is included in the article, supplementary material, and/or referenced in the text.

CRediT authorship contribution statement

Aline Gonçalves: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Quitterie Mathelié-Guinlet:** Formal analysis, Data curation. **Fátima Ramires:** Writing – original draft, Formal analysis, Data curation. **Francisca Monteiro:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation. **Óscar Carvalho:** Writing – review & editing. **Filipe S. Silva:** Writing – review & editing, Funding acquisition. **Albina D. Resende:** Writing – review & editing, Supervision, Methodology. **Teresa Pinho:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Francisca Monteiro reports financial support was provided by Fundação para a Ciência e a Tecnologia. Teresa Pinho reports financial support was provided by UNIPRO.

Acknowledgment

This systematic review was supported by UNIPRO – Oral Pathology and Rehabilitation Research Unit in the scope of the project AlignAgen-GI2-CESPU-2022 and by FCT (Fundação para a Ciência e Tecnologia) through the grant UI/BD/09735/2020 (https://doi.org/10.54499/2020.09375.BD), and in the scope of the projects UIDB/04436/2020 and UIDP/04436/2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32873.

References

[1] Y. Li, L.A. Jacox, S.H. Little, C.-C. Ko, Orthodontic tooth movement: the biology and clinical implications, Kaohsiung J. Med. Sci. 34 (4) (Apr. 2018) 207–214, https://doi.org/10.1016/j.kjms.2018.01.007.
[2] S. Hisham, et al., Cellular and molecular changes in orthodontic tooth movement, Sci. World J. 11 (2011) 1788–1803, https://doi.org/10.1100/2011/761768.
[3] M.A. Asiry, Biological aspects of orthodontic tooth movement: a review of literature, Saudi J. Biol. Sci. 25 (6) (2018) 1027–1032, https://doi.org/10.1016/j.sjbs.2018.03.008.
[4] N.A. Meeran, Cellular response within the periodontal ligament on application of orthodontic forces, J. Indian Soc. Periodontol. 17 (1) (2013) 16–20, https://doi.org/10.4103/0972-124X.107468.
[5] M. Le Gall, J. Sastre, Le déplacement dentaire: bases fondamentales, Int. Orthod. 8 (1) (2010) 1–13, https://doi.org/10.1016/j.ortho.2009.12.001.
[6] H. Huang, L. Ma, S. Kyrkanides, Effects of vascular endothelial growth factor on osteoblasts and osteoclasts, Am. J. Orthod. Dentofacial Orthop. 149 (3) (2016) 366–373, https://doi.org/10.1016/j.ajodo.2015.09.021.
[7] Z. Hakami, H. Kitaura, K. Kimura, M. Ishida, Effect of interleukin-4 on orthodontic tooth movement and associated root resorption, Eur. J. Orthod. 37 (1) (2015) 87–94, https://doi.org/10.1093/ejo/cju016.
[8] M.G. Roscoe, J.B.C. Meira, P.M. Cattaneo, Association of orthodontic force system and root resorption: a systematic review, Am. J. Orthod. Dentofacial Orthop. 147 (5) (May 2015) 610–626, https://doi.org/10.1016/J.AJODO.2014.12.026.
[9] K.M. Clements, A.-M. Bollen, G. Huang, G. King, P. Hujoel, T. Ma, Activation time and material stiffness of sequential removable orthodontic appliances. Part 2: dental improvements, Am. J. Orthod. Dentofacial Orthop. 124 (5) (Nov. 2003) 502–508, https://doi.org/10.1016/S0889-5406(03)00577-8.
[10] D. Moher, et al., Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, Ann. Intern. Med. 151 (4) (2009) 264–269, https://doi.org/10.7326/0003-4819-151-4-200908180-00135.
[11] F.C. de Medeiros, et al., Dental implants in patients with osteoporosis: a systematic review with meta-analysis, Int. J. Oral Maxillofac. Surg. 47 (4) (2018) 480–491, https://doi.org/10.1016/j.ijom.2017.05.021.
[12] K. Mayahara, A. Yamaguchi, H. Takenouchi, T. Kariya, H. Taguchi, N. Shimizu, Osteoblasts stimulate osteoclastogenesis via RANKL expression more strongly than periodontal ligament cells do in response to PGE2, Arch. Oral Biol. 57 (10) (2012) 1377–1384, https://doi.org/10.1016/j.archoralbio.2012.07.009.
[13] K. Diercke, A. Kohl, C.J. Lux, R. Erber, IL-1β und Kompression führen zu einer signifikanten Induktion der RANKL-Expression in primären humanen Zementoblasten, J. Orofac. Orthop. 73 (5) (2012) 397–412, https://doi.org/10.1007/s00056-012-0095-y.
[14] S. García-López, R. Villanueva, M.C. Meikle, Alterations in the synthesis of IL-1β, TNF-α, IL-6, and their downstream targets RANKL and OPG by mouse calvarial osteoblasts in vitro: inhibition of bone resorption by cyclic mechanical strain, Front. Endocrinol. 4 (December 2014) (2013), https://doi.org/10.3389/fendo.2013.00160.
[15] P. Tripuwabhrut, M. Mustafa, C.G. Gjerde, P. Brudvik, K. Mustafa, Effect of compressive force on human osteoblast-like cells and bone remodeling: an in vitro study, Arch. Oral Biol. 58 (7) (2013) 826–836, https://doi.org/10.1016/j.archoralbio.2013.01.004.
[16] T. Yoshino, M. Yamaguchi, M. Shimizu, K. Yamada, K. Kasai, TNF-α aggravates the progression of orthodontically-induced inflammatory root resorption in the presence of RANKL, J. Hard Tissue Biol. 23 (2) (2014) 155–162, https://doi.org/10.2485/jhtb.23.155.
[17] P. Proff, C. Reicheneder, A. Faltermeier, D. Kubein-Meesenburg, P. Römer, Effects of mechanical and bacterial stressors on cytokine and growth-factor expression in periodontal ligament cells, J. Orofac. Orthop. 75 (3) (2014) 191–202, https://doi.org/10.1007/s00056-014-0212-1.
[18] M. Li, J. Yi, Y. Yang, W. Zheng, Y. Li, Z. Zhao, Investigation of optimal orthodontic force at the cellular level through three-dimensionally cultured periodontal ligament cells, Eur. J. Orthod. 38 (4) (2015) 366–372, https://doi.org/10.1093/ejo/cjv052.

[19] L. Nettelhoff, et al., Influence of mechanical compression on human periodontal ligament fibroblasts and osteoblasts, Clin. Oral Invest. 20 (3) (Apr. 2016) 621–629.

[20] L. Zheng, et al., The effects of fluid shear stress on proliferation and osteogenesis of human periodontal ligament cells, J. Biomech. 49 (4) (2016) 572–579, https://doi.org/10.1016/j.jbiomech.2016.01.034.

[21] A. Schröder, K. Bauer, G. Spanier, P. Proff, M. Wolf, C. Kirschneck, Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement, J. Orofac. Orthop. 79 (5) (2018) 337–351, https://doi.org/10.1007/s00056-018-0145-1.

[22] A. Schröder, et al., Effects of compressive and tensile strain on macrophages during simulated orthodontic tooth movement, Mediat. Inflamm. 2020 (2020), https://doi.org/10.1155/2020/2814015.

[23] J. Lin, J. Huang, Z. Zhang, X. Yu, X. Cai, C. Liu, Periodontal ligament cells under mechanical force regulate local immune homeostasis by modulating Th17/Treg cell differentiation, Clin. Oral Invest. 26 (4) (Apr. 2022) 3747–3764, https://doi.org/10.1007/S00784-021-04346-0/METRICS.

[24] S.H. Kook, Y.S. Jang, J.C. Lee, Human periodontal ligament fibroblasts stimulate osteoclastogenesis in response to compression force through TNF-α-mediated activation of CD4+ T cells, J. Cell. Biochem. 112 (10) (2011) 2891–2901, https://doi.org/10.1002/jcb.23205.

[25] Y. Nakano, M. Yamaguchi, S. Fujita, M. Asano, K. Saito, Expressions of RANKL/RANK and M-CSF/c-fms in root resorption lacunae in rat molar by heavy orthodontic force, Eur. J. Orthod. 33 (September 2010) (2011) 335–343, https://doi.org/10.1093/ejo/cjq068.

[26] S. Baba, N. Kuroda, C. Arai, Y. Nakamura, T. Sato, Immunocompetent cells and cytokine expression in the rat periodontal ligament at the initial stage of orthodontic tooth movement, Arch. Oral Biol. 56 (5) (2011) 466–473, https://doi.org/10.1016/j.archoralbio.2010.11.010.

[27] J. Zhou, et al., Expression of osteoprotegerin and receptor activator of nuclear factor κB ligand in root resorption induced by heavy force, rats Die Expression von Osteoprotegerin und des Rezeptor-Akti- vators des Nuklearfaktor-κ-B-Liganden bei der durch starke Kraftei 6 (2011) 457–468, https://doi.org/10.1007/s00056-011-0050-3.

[28] J.Y. Kim, B.I. Kim, S.S. Jue, J.H. Park, J.W. Shin, Localization of osteopontin and osterix in periodontal tissue during orthodontic tooth movement in rats, Angle Orthod. 82 (1) (2012) 107–114, https://doi.org/10.2319/030911-173.1.

[29] N. Hayashi, M. Yamaguchi, R. Nakajima, T. Utsunomiya, H. Yamamoto, K. Kasai, T-helper 17 cells mediate the osteo/odontoclastogenesis induced by excessive orthodontic forces, Oral Dis. 18 (4) (2012) 375–388, https://doi.org/10.1111/j.1601-0825.2011.01886.x.

[30] S. Taddei, et al., Role of CCR2 in orthodontic tooth movement, Am. J. Orthod. Dentofacial Orthop. 141 (2) (2012) 153–160.e1, https://doi.org/10.1016/j.ajodo.2011.07.019.

[31] S.R. de A. Taddei, et al., Experimental model of tooth movement in mice: a standardized protocol for studying bone remodeling under compression and tensile strains, J. Biomech. 45 (16) (Nov. 2012) 2729–2735, https://doi.org/10.1016/j.jbiomech.2012.09.006.

[32] S.R. de A. Taddei, et al., The effect of CCL3 and CCR1 in bone remodeling induced by mechanical loading during orthodontic tooth movement in mice, Bone 52 (1) (2013) 259–267, https://doi.org/10.1016/j.bone.2012.09.036.

[33] M. Alikhani, et al., Saturation of the biological response to orthodontic forces and its effect on the rate of tooth movement, Orthod. Craniofac. Res. 18 (S1) (2015) 8–17, https://doi.org/10.1111/ocr.12090.

[34] B. Li, Y.H. Zhang, L.X. Wang, X. Li, X.D. Zhang, Expression of OPG, RANKL, and RUNX2 in rabbit periodontium under orthodontic force, Genet. Mol. Res. 14 (4) (2015) 19382–19388.

[35] M. Seifi, B. Kazemi, D. Ph, S. Kabiri, Analysis of transforming growth factor- β 1 expression in resorptive lacunae following orthodontic tooth movement in an, Animal Model 19 (2) (2017) 278–282, https://doi.org/10.22074/cellj.2016.4218. Introduction.

[36] Y. Matsumoto, S. Sringkarnboriboon, T. Ono, Proinflammatory mediators related to orthodontically induced periapical root resorption in rat mandibular molars, Eur. J. Orthod. 39 (6) (2017) 686–691, https://doi.org/10.1093/ejo/cjx033.

[37] S. Kaya, M. Çifter, V. Olgaç, H. Íssever, G. Isik, Effects of orthodontic force magnitude on cell apoptosis and RANKL-induced osteoclastogenesis, Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 81 (2) (2020) 100–112, https://doi.org/10.1007/s00056-019-00205-6.

[38] A. Marahleh, et al., Effect of TNF-α on osteocyte RANKL expression during orthodontic tooth movement, J. Dent. Sci. 16 (4) (Oct. 2021) 1191–1197, https://doi.org/10.1016/J.JDS.2021.03.006.

[39] T. Noguchi, et al., Tumor necrosis factor-α enhances the expression of vascular endothelial growth factor in a mouse orthodontic tooth movement model, J. Dent. Sci. 17 (1) (Jan. 2022) 415–420, https://doi.org/10.1016/J.JDS.2021.08.011.

[40] T. Wei, Z. Shan, X. Wen, N. Zhao, G. Shen, Dynamic alternations of RANKL/OPG ratio expressed by cementocytes in response to orthodontic-induced external apical root resorption in a rat model, Mol. Med. Rep. 26 (1) (Jul. 2022) 1–11, https://doi.org/10.3892/MMR.2022.12744/HTML.

[41] R.M. Wahab, et al., Crevicular tartrate resistant acid phosphatase activity and rate of tooth movement under different continuous force applications, Afr J Pharm Pharmacol 5 (20) (2011) 2213–2219, https://doi.org/10.5897/AJPP11.304.

[42] B. Aras, L.L. Cheng, T. Turk, S. Elekdag-Turk, A.S. Jones, M.A. Darendeliler, Physical properties of root cementum: Part 23. Effects of 2 or 3 weekly reactivated continuous or intermittent orthodontic forces on root resorption and tooth movement: a microcomputed tomography study, Am. J. Orthod. Dentofacial Orthop. 141 (2) (Feb. 2012) e29–e37, https://doi.org/10.1016/J.AJODO.2011.07.018.

[43] D. Madureira, et al., Kinetics of interleukin-6 and chemokine ligands 2 and 3 expression of periodontal tissues during orthodontic tooth movement, Am. J. Orthod. Dentofacial Orthop. 142 (4) (2012) 494–500, https://doi.org/10.1016/j.ajodo.2012.05.012.

[44] M. Grant, J. Wilson, P. Rock, I. Chapple, Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement, Eur. J. Orthod. 35 (5) (2013) 644–651, https://doi.org/10.1093/ejo/cjs057.

[45] D.F. Madureira, et al., Cytokine measurements in gingival crevicular fluid and periodontal ligament: are they correlated? Am. J. Orthod. Dentofacial Orthop. 148 (2) (2015) 293–301, https://doi.org/10.1016/j.ajodo.2015.03.030.

[46] T. Castroflorio, E.F. Gamerro, G.P. Caviglia, A. Deregibus, Biochemical markers of bone metabolism during early orthodontic tooth movement with aligners, Angle Orthod. 87 (1) (2017) 74–81, https://doi.org/10.2319/022416-159.1.

[47] R. Ahuja, M. Almuzian, A. Khan, D. Pascovici, O. Dalci, M.A. Darendeliler, A preliminary investigation of short-term cytokine expression in gingival crevicular fluid secondary to high-level orthodontic forces and the associated root resorption: case series analytical study, Prog. Orthod. 18 (1) (2017), https://doi.org/10.1186/s40510-017-0177-x.

[48] A. Dudic, C. Giannopoulou, P. Meda, X. Montet, S. Kiliaridis, Orthodontically induced cervical root resorption in humans is associated with the amount of tooth movement, Eur. J. Orthod. 39 (5) (Oct. 2017) 534–540, https://doi.org/10.1093/EJO/CJW087.

[49] G. Gay, et al., Root resorption during orthodontic treatment with Invisalign®: a radiometric study, Prog. Orthod. 18 (1) (Dec. 2017) 1–6, https://doi.org/10.1186/S40510-017-0166-0/FIGURES/4.

[50] C. Aman, et al., Apical root resorption during orthodontic treatment with clear aligners: a retrospective study using cone-beam computed tomography, Am. J. Orthod. Dentofacial Orthop. 153 (6) (Jun. 2018) 842–851, https://doi.org/10.1016/J.AJODO.2017.10.026.

[51] V. de O. Chami, L. Nunes, J. Capelli Júnior, Expression of cytokines in gingival crevicular fluid associated with tooth movement induced by aligners: a pilot study, Dental Press J Orthod 23 (5) (Sep. 2018) 41–46, https://doi.org/10.1590/2177-6709.23.5.041-046.OAR.

[52] C.J. Costello, B. Kerr, T. Weir, E. Freer, The incidence and severity of root resorption following orthodontic treatment using clear aligners, Australasian Orthodontic Journal 36 (2) (Nov. 2020) 130–137, https://doi.org/10.21307/AOJ-2020-014.

[53] H.E. Akl, A.R. El-Beialy, M.A. El-Ghafour, A.M. Abouelezz, F.A. El Sharaby, Root resorption associated with maxillary buccal segment intrusion using variable force magnitudes:A randomized clinical trial, Angle Orthod. 91 (6) (Nov. 2021) 733–742, https://doi.org/10.2319/012121-62.1.

[54] S. Ghaleb, N. Tamish, W. ElKenany, M. Guindi, The effect of two different types of forces on possible root resorption in relation to dentin phosphoprotein levels: a single-blind, split-mouth, randomized controlled trial, Prog. Orthod. 22 (1) (Dec. 2021) 1–12, https://doi.org/10.1186/S40510-021-00388-Y/TABLES/6.

[55] D. Kloukos, et al., Bone turnover markers in gingival crevicular fluid and blood serum of patients with fixed orthodontic appliances, Eur. J. Orthod. 44 (4) (Aug. 2022) 412–419, https://doi.org/10.1093/EJO/CJAB077.

[56] A.A. Shetty, et al., Evaluation of interleukin 6 levels in gingival crevicular fluid and periodontal ligament on application of orthodontic forces, World 13 (1) (Jan. 2022) 17, https://doi.org/10.5005/JP-JOURNALS-10015-1891.

[57] M. Macrì, S. Medori, G. Varvara, F. Festa, A digital 3D retrospective study evaluating the efficacy of root control during orthodontic treatment with clear aligners, Appl. Sci. 13 (3) (2023) 1540, https://doi.org/10.3390/APP13031540.

[58] L.A. Golbach, et al., Calcium homeostasis and low-frequency magnetic and electric field exposure: a systematic review and meta-analysis of in vitro studies, Environ. Int. 92–93 (March) (Jul. 2016) 695–706, https://doi.org/10.1016/j.envint.2016.01.014.

[59] F. Monteiro, Ó. Carvalho, N. Sousa, F.S. Silva, I. Sotiropoulos, Photobiomodulation and visual stimulation against cognitive decline and Alzheimer's disease pathology: a systematic review, Alzheimer's Dementia: Translational Research & Clinical Interventions (2022) e12249, https://doi.org/10.1002/trc2.12249.

[60] M. Silva, et al., Current perspectives on the biomechanical modelling of the human lower limb: a systematic review, Arch. Comput. Methods Eng. (28) (2021) 601–636, https://doi.org/10.1007/s11831-019-09393-1.

[61] C.R. Hooijmans, M.M. Rovers, R.B.M. De Vries, M. Leenaars, M. Ritskes-Hoitinga, M.W. Langendam, SYRCLE's risk of bias tool forfor animal studies, BMC Med. Res. Methodol. 14 (1) (Mar. 2014) 1–9.

[62] J.A. Sterne, et al., ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions, BMJ 355 (Oct) (2016), https://doi.org/10.1136/bmj.i4919.

[63] S. García-López, R. Villanueva, M.C. Meikle, Alterations in the synthesis of IL-1β, TNF-α, IL-6, and their downstream targets RANKL and OPG by mouse calvarial osteoblasts in vitro: inhibition of bone resorption by cyclic mechanical strain, Front. Endocrinol. 4 (December 2014) (2013), https://doi.org/10.3389/fendo.2013.00160.

[64] A. Schröder, et al., Effects of compressive and tensile strain on macrophages during simulated orthodontic tooth movement, Mediat. Inflamm. 2020 (2020), https://doi.org/10.1155/2020/2814015.

[65] A. Schröder, K. Bauer, G. Spanier, P. Proff, M. Wolf, C. Kirschneck, Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement, J. Orofac. Orthop. 79 (5) (2018) 337–351, https://doi.org/10.1007/s00056-018-0145-1.

[66] J. Lin, J. Huang, Z. Zhang, X. Yu, X. Cai, C. Liu, Periodontal ligament cells under mechanical force regulate local immune homeostasis by modulating Th17/Treg cell differentiation, Clin. Oral Invest. 26 (4) (Apr. 2022) 3747–3764, https://doi.org/10.1007/S00784-021-04346-0/METRICS.

[67] M. Li, J. Yi, Y. Yang, W. Zheng, Y. Li, Z. Zhao, Investigation of optimal orthodontic force at the cellular level through three-dimensionally cultured periodontal ligament cells, Eur. J. Orthod. 38 (4) (2015) 366–372, https://doi.org/10.1093/ejo/cjv052.

[68] P. Tripuwabhrut, M. Mustafa, C.G. Gjerde, P. Brudvik, K. Mustafa, Effect of compressive force on human osteoblast-like cells and bone remodeling: an in vitro study, Arch. Oral Biol. 58 (7) (2013) 826–836, https://doi.org/10.1016/j.archoralbio.2013.01.004.

[69] P. Proff, C. Reicheneder, A. Faltermeier, D. Kubein-Meesenburg, P. Römer, Effects of mechanical and bacterial stressors on cytokine and growth-factor expression in periodontal ligament cells, J. Orofac. Orthop. 75 (3) (2014) 191–202, https://doi.org/10.1007/s00056-014-0212-1.

[70] T. Yoshino, M. Yamaguchi, M. Shimizu, K. Yamada, K. Kasai, TNF-α aggravates the progression of orthodontically-induced inflammatory root resorption in the presence of RANKL, J. Hard Tissue Biol. 23 (2) (2014) 155–162, https://doi.org/10.2485/jhtb.23.155.

[71] L. Nettelhoff, et al., Influence of mechanical compression on human periodontal ligament fibroblasts and osteoblasts, Clin. Oral Invest. 20 (3) (Apr. 2016) 621–629. Accessed: Oct. 16, 2023. [Online]. Available: https://link.springer.com/article/10.1007/s00784-015-1542-0.

[72] K. Diercke, A. Kohl, C.J. Lux, R. Erber, IL-1β and compressive forces lead to a significant induction of RANKL-expression in primary human cementoblasts, J. Orofac. Orthop. 73 (5) (2012) 397–412, https://doi.org/10.1007/s00056-012-0095-y.

[73] G. Barbieri, et al., Biochemical markers of bone metabolism in gingival crevicular fluid during early orthodontic tooth movement, Angle Orthod. 83 (1) (2013) 63–69, https://doi.org/10.2319/022812-168.1.

[74] G. Isola, A. Polizzi, S. Santonocito, A. Alibrandi, R.C. Williams, Periodontitis activates the NLRP3 inflammasome in serum and saliva, J. Periodontol. (2021), https://doi.org/10.1002/JPER.21-0049.

[75] A.V.B. Nogueira, et al., Experimental models of orthodontic tooth movement and their effects on periodontal tissues remodeling, Arch. Oral Biol. 130 (March) (2021), https://doi.org/10.1016/j.archoralbio.2021.105216.

[76] A. Nogueira, R. Molon, M. Nokhbehsaim, J. Deshner, J. Cirelli, Contribution of Biomechanical Forces to Inflammation-Induced Bone Resorption, 2017, https://doi.org/10.1111/jcpe.12636.

[77] B. Rath-deschner, et al., Interaction of periodontitis and orthodontic tooth movement — an in vitro and in vivo study, Clin. Oral Invest. (2021), https://doi.org/10.1007/s00784-021-03988-4.

[78] B. Rath-deschner, et al., CXCL5 , CXCL8 , and CXCL10 regulation by bacteria and mechanical forces in periodontium, Ann. Anat. 234 (2021), https://doi.org/10.1016/j.aanat.2020.151648.

[79] H. Kitaura, K. Kimura, M. Ishida, H. Sugisawa, H. Kohara, Effect of cytokines on osteoclast formation and bone resorption during mechanical force loading of the periodontal membrane, Sci. World J. 2014 (January 2014) (2016) 1–7.

[80] A. Marahleh, et al., TNF-A directly enhances osteocyte RANKL expression and promotes osteoclast formation, Front. Immunol. 10 (December) (2021), https://doi.org/10.3389/fimmu.2019.02925.

[81] G.A. Flórez-Moreno, D.M. Isaza-Guzmán, S.I. Tobón-Arroyave, Time-related changes in salivary levels of the osteotropic factors sRANKL and OPG through orthodontic tooth movement, Am. J. Orthod. Dentofacial Orthop. 143 (1) (2013) 92–100, https://doi.org/10.1016/j.ajodo.2012.08.026.

[82] H. Ford, S. Suri, D. Nilforoushan, M. Manolson, S.G. Gong, Nitric oxide in human gingival crevicular fluid after orthodontic force application, Arch. Oral Biol. 59 (11) (2014) 1211–1216, https://doi.org/10.1016/j.archoralbio.2014.07.015.

[83] X. Fan, et al., Nitric oxide regulates receptor activator of nuclear factor-κb ligand and osteoprotegerin expression in bone marrow stromal cells, Endocrinology 145 (2) (2004) 751–759, https://doi.org/10.1210/en.2003-0726.

[84] A. Hohmann, et al., Correspondences of hydrostatic pressure in periodontal ligament with regions of root resorption: a clinical and a finite element study of the same human teeth, Comput. Methods Progr. Biomed. 93 (2) (2009) 155–161, https://doi.org/10.1016/j.cmpb.2008.09.004.

[85] N. Brezniak, A. Wasserstein, Orthodontically induced inflammatory root resorption. Part I: the basic science aspects, Angle Orthod. 72 (2) (2002) 175–179, https://doi.org/10.1043/0003-3219(2002)072<0175:OIIRRP>2.0.CO;2.

[86] V. Silva, et al., Effects of orthodontic tooth extrusion produced by di ff erent techniques, on the periodontal tissues: a histological study in dogs, Arch. Oral Biol. 116 (May) (2020), https://doi.org/10.1016/j.archoralbio.2020.104768.

[87] M.G. Piancino, G. Isola, A. Merlo, D. Dalessandri, C. Debernardi, P. Bracco, Chewing pattern and muscular activation in open bite patients, J. Electromyogr. Kinesiol. 22 (2012) 273–279, https://doi.org/10.1016/j.jelekin.2011.12.003.

[88] R. Cannavale, G. Matarese, G. Isola, V. Grassia, L. Perillo, Early treatment of an ectopic premolar to prevent molar-premolar transposition, Am. J. Orthod. Dentofacial Orthop. 143 (4) (2013) 559–569, https://doi.org/10.1016/j.ajodo.2012.03.035.

[89] C.J. Burstone, Rationale of the segmented arch, Am. J. Orthod. 48 (11) (Nov. 1962) 805–822, https://doi.org/10.1016/0002-9416(62)90001-5.

[90] W.R. Proffit, H. Fields, Contemporary Orthodontics-E-Book: Contemporary Orthodontics-E-Book, Elsevier Health Sciences, 2012.

[91] Y. Liu, W. Hu, Force changes associated with different intrusion strategies for deep-bite correction by clear aligners, Angle Orthod. 88 (6) (Nov. 2018) 771–778, https://doi.org/10.2319/121717-864.1.

[92] L. Gao, A. Wichelhaus, Forces and moments delivered by the PET-G aligner to a maxillary central incisor for palatal tipping and intrusion, Angle Orthod. 87 (4) (Jul. 2017) 534–541, https://doi.org/10.2319/090216-666.1.