Aus der Poliklinik für Kieferorthopädie

der Ludwig-Maximilians-Universität München

Vorstand: Prof. Dr. med. dent. Andrea Wichelhaus

INVESTIGATION OF INTER- AND INTRACELLULAR COMMUNICATION DURING SIMULATED ORTHODONTIC TOOTH MOVEMENT WITH THE "WEIGHT APPROACH BASED" *IN VITRO* MODEL

Dissertation
zum Erwerb des Doctor of Philosophy (Ph.D.)
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

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Date of oral defence:	07.04.2020

Dedicated to my family for endless love, support and encouragement.

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List of Abbreviations

COX2	Cyclooxygenase2
HB-GAM	Heparin binding growth associated molecule
hOBs	human alveolar osteoblasts
hPDFs	Human periodontal ligament fibroblasts
OPG	Osteoprotegerin
OTM	Orthodontic tooth movement
P2RX7	Purinergic Receptor P2X 7
PGE2	Prostaglandin E2
PLGA	Polylactic-co-glycolic acid
PLLA	hydrophilically modified poly-L-lactide matrix
PPI	Protein-protein interaction
PTGS2	Prostaglandin-Endoperoxide Synthase 2
RANKL	Receptor activator of nuclear factor kappa B ligand
RoB	Risk of bias
RUNX2	Runt-related transcription factor 2
STARCARD	STAndard Reporting requirements in CARies Diagnostic Studies
TNFRSF11B	Tumor necrosis factor receptor superfamily member 11B
TNFSF11	Tumor necrosis factor ligand superfamily member 11
TNFα	Tumour necrosis factor alpha
WAB	Weight approach based

INTRODUCTORY SUMMARY

Name of the project: Use of weight approach based *in vitro* models to investigate inter- and intracellular communication during simulated orthodontic tooth movement

The following project was done at the Department of Orthodontics and Dentofacial Orthopedics, University Hospital, Ludwig-Maximilians-Universität München, under the supervision of Dr. Uwe Baumert and Prof. Dr. Andrea Wichelhaus. It is considered as a main topic of the PhD thesis and contains two ***published*** *studies used for fulfilling the requirements for Ph.D. program completement.*

Orthodontic tooth movement (OTM) is based on the initiation of bone remodelling upon orthodontic force application (Wichelhaus 2017). Histologically, the events in tooth supporting tissues and surrounding alveolar bone during OTM have been well described (Davidovitch 1991). However, knowledge about its molecular background remains fragmented (Davidovitch and Krishnan 2015).

OTM represents a complicated process, guided by many molecular events, which are spatially and temporary coordinated by different cell types, signalling factors and networks (Wichelhaus 2017). The complex morphological structure of the clinical situation and corresponding *in vivo* models makes it impossible, to answer questions like: how individual cell types sense the force; how they convert mechanical stimuli into molecular signals, and how this signals further contribute to bone remodelling. As such, many *in vitro* models have been introduced to systematically breakdown and analyse individual processes involved in OTM by focusing on specific cell types and types of force (Baumert et al. 2004; Yang et al.

2015). One of these *in vitro* models is the so called “weight approach based” (WAB) *in vitro* loading model (Yang et al. 2015). This model is used to investigate molecular events on the compression side of the tooth during OTM applying the static unilateral compressive force on the cells, which is one of the dominant forces in the treatment with fixed mobile appliances (Kanzaki et al. 2002; Yang et al. 2015).

Briefly, cells are precultured in cell culture dishes as 2D or 3D cultures and then subjected to static compressive force by placing a weight directly over them (Yang et al. 2015). This is mostly achieved, by placing a glass cylinder filled with lead granules on top of a glass disc directly onto the cells. The force level is adjusted with the lead granules within the glass cylinder.

So far, numerous studies using WAB *in vitro* loading model have been published. They provide valuable information on the response of different cell types to static compressive force (Yang et al. 2015).

PUBLICATION 1. IN VITRO WEIGHT-LOADED CELL MODELS FOR UNDERSTANDING MECHANO-DEPENDENT MOLECULAR PATHWAYS INVOLVED IN ORTHODONTIC TOOTH MOVEMENT: A SYSTEMATIC REVIEW

In order to get a clear overview of the so far published knowledge and to identify existing gaps, primary aim of this study was, to identify all articles using WAB *in vitro* loading model in the field of orthodontics. Special attention was given on details of cell culture, force duration & magnitude and findings on molecular events related to OTM. Studies using 2D and 3D WAB setups were assessed separately. Out of 2,284 initially identified studies applying the 2D WAB setup, 56 studies were considered as relevant for the systematic review. The 3D setup was

identified in 1,042 studies, and 15 studies matched inclusion criteria for the systematic review (Janjic et al. 2018).

2D WAB setup: Most of the studies using the 2D WAB setup used a force magnitude of 2 g/cm^2 (Janjic et al. 2018). This force magnitude is considered to induce a peak in the production of cytokines and expression of mRNAs coding for osteoclastogenic molecules (Kang et al. 2013; Kanzaki et al. 2002; Kim et al. 2013). Force was applied usually for up to 24 h (Janjic et al. 2018). Independently of the cell type used, gene expression analysis showed an increased expression of proinflammatory mediators and osteoclastogenesis stimulating factors (Janjic et al. 2018), which is in line with *in vivo* findings (Vansant et al. 2018). Human periodontal ligament fibroblasts (hPDFs) were the cell type that was examined mostly (Janjic et al. 2018). Main attention was given to the following genes and metabolites: *RANKL (TNFSF11), OPG (TNFRSF11B), COX2 (PTGS2)* and PGE_2. Additionally, force application never exceeded 72 h (Janjic et al. 2018). Clinically, the first week of OTM is the period, in which significant changes on histological level were described (Reitan 1960). Therefore, 72 h of force application might be too short to elucidate all important molecular events on the compression side of the tooth during OTM. Another observation of this review was, that not enough attention is dedicated to cell proliferation and cell viability monitoring, which can be considered as a bias introducing issue, especially in studies with longer duration of force application (Janjic et al. 2018).

3D WAB setup: Among the studies with 3D WAB setup, the application of three different types of scaffolds have been described so far: collagen gel scaffolds, polylactic-co-glycolic acid (PLGA) scaffolds or those made from a hydrophilically modified poly-L-lactide (PLLA) matrix (Janjic et al. 2018). Hydrophilically modified PLLA scaffolds are especially suitable for long-term force application, even up to 14 days (Liao et al. 2016). Otherwise, the duration of other

studies ranged between 0.5 to 72 h. The force magnitude ranged between 5 to 35 g/cm^2 in studies using PGLA and hydrophilically modified PLLA scaffolds and between 0.5 to 9.5 g/cm^2 in ones using collagen scaffolds. Mostly investigated cell type were hPDFs. According to our results, studies using 3D WAB setup showed obvious differences in molecular findings. We attribute these contradictory results to high methodological differences between the studies. Even though promising, WAB studies applying a 3D setup are still not well established as those using a 2D setup. In order to make results of this studies reliable and comparable to *in vivo* situation, it is necessary to establish proper scaffolds for use in combination with WAB models and define suitable force magnitudes for each of them (Janjic et al. 2018).

The second part of the review focused on 2D WAB studies with hPDFs and human alveolar osteoblasts (hOBs) and bone derived cells lines. Information collected from these studies was used to generate list of all so far examined genes, separately for each cell type. Based on this data, STRING analysis was performed (STRING database 10.5, URL: https://string-db.org/) (Szklarczyk et al. 2017), protein-protein interaction (PPI) networks were generated and genes with the highest number of interactions were identified. Additionally, STRING analysis of both sets of genes was used to identify KEGG pathways and select the ones relevant to OTM. Identified pathways in this review can be considered as a useful source for discovering the new genes important for OTM and should be considered in future conducted studies using WAB loading model (Janjic et al. 2018).

PUBLICATION 2. EFFECT OF THE STATIC COMPRESSIVE FORCE ON IN VITRO CULTURED PDL FIBROBLASTS: MONITORING OF THE VIABILITY AND GENE EXPRESSION OVER SIX DAYS

In a previously described systematic review we identified all studies related to the field of orthodontics using the 2D WAB *in vitro* loading model to apply static compressive force on hPDFs (Janjic et al. 2018). This review identified the future need for:

- longer lasting studies with WAB *in vitro* loading model, in order to broaden the understanding of molecular events on the compression side of tooth and hPDFs' role in OTM.
- additional attention to monitor cell proliferation and viability during force application.

Therefore, the aim of second study in this project was to prolong the use of the WAB model to 6 days. Static force of 2 g/cm^2 was used to compress the hPDFs and monitor its effect on inflammatory genes and mediators (*COX2, IL6, TNFα*, PGE_2), genes involved in the bone remodelling (*RUNX2, P2RX7*) and mechanosensing genes (*cFOS, HB-GAM*) on a daily basis. To exclude possible negative influence of prolonged WAB loading model application on cells, on each day of the experiment cell proliferation and cell viability were assessed using the Alamar Blue® assay and the Live/Dead viability/cytotoxicity Kit, respectively (Janjic Rankovic et al. 2019).

Inflammation contributes significantly to bone resorption and osteoclastogenesis on the compressive side during OTM. In line with this, this study described increased gene expression of inflammatory genes *COX2, IL6, TNFα. COX 2* and *IL6* showed temporary upregulation, while TNFα remained upregulated until day six. In addition to increased *COX2* gene expression, increased concentrations of PGE_2 were measured in the cell culture supernatant.

Concentrations of secreted TNFα remained under the detection limit. *RUNX2* and *P2RX7* on the other hand showed temporarily downregulations at certain timepoints of experiment. This was consistent with previous reports, since these genes have been previously recognized as the contributors to osteogenesis (Vansant et al. 2018). The mechanosensing gene *cFOS* was upregulated during the whole experiment, while *HB-GAM* mostly remained unchanged (Janjic Rankovic et al. 2019).

As far as we know, this is the only study that used WAB *in vitro* loading model for a period of 6 days applying static compressive force on hPDFs. Published studies using the WAB loading model on hPDFs examined molecular events within the first 96h of static force application (Janjic et al. 2018; Schröder et al. 2018). Up to this period of time, our findings are mostly in line with the published literature (Kang et al. 2010; Kanzaki et al. 2002; Mayahara et al. 2007; Schröder et al. 2018). However, no comparable data from *in vitro* studies for longer periods of force application exist. The results of this study suggest, that the molecular events are still high after 6 days of the force application, introducing the need of further studies that will, not only confirm our results, but also broaden the knowledge on molecular events after longer terms of force application.

AUTHOR`S-CONTRIBUTIONS TO BOTH PUBLICATIONS

Publication 1. Participated in the development of the study design and research question. Defined the inclusion and exclusion criteria for the systematic review. Designed and tested the search strategy based on predefined research question and eligibility criteria. Conducted the search in PubMed electronic database and screened all identified records on the basis of title and abstract. Obtained and checked full text of all potentially relevant records in order to identify and included studies matching eligibility criteria. Tried to identify additional studies by crosschecking the reference lists of already included studies. Extracted data from the studies in the predefined tables. Crated gene lists and performed the STRING analysis in the "hOB and bone derived cells lines" group. Wrote the main draft of the manuscript and revised it according to co-authors comments together with senior author. Created illustrations and tables.

Publication 2. Formulated the research question based on the gaps in knowledge identified in the Publication 1. Planned the experiment and methodology design together with the senior author. Tested and established the WAB set up, based on the descriptions from previously published literature. Conducted the experiment and performed the cell viability and cell proliferation assessments, ELISA assays and RT-qPCR analysis on the collected samples. Collected and organised raw data from the assays and prepared them for the further statistical analysis. Participated in figure creation and graphical presentation of the results. Wrote the main draft of the manuscript and finalized it together with the senior author.

CONFIRMATION OF CO-AUTHORS

By signing, the following co-authors confirm that:

- the extent of their contributions (content-related and volume) in the publications submitted, and
- their agreement to the submission of the publications.

	Name of co-author	Extend of contribution (content-related and volume)
1.	Docheva, Denitsa	Publication 1: Contributed to the study design and data analysis. Contributed to manuscript revision. Publication 2: Contributed to the study design. Revised the manuscript. Agreed to the submission of both publications.
2.	Trickovic Janjic, Olivera	Publication 1: Participated in drafting the manuscript and helped revising the paper. Agreed to the submission of publication 1.
3.	Wichelhaus, Andrea	Publication 1: Participated in drafting the manuscript and helped revising the paper. Publication 2: Revised the manuscript. Agreed to the submission of both publications.
4.	Baumert, Uwe	Publication 1: Conceived the idea of the study and participated in the development of the study design, acquisition of data, analysis and interpretation of data, supervised manuscript writing and wrote parts of the manuscript. Publication 2: Participated in study design and supervised the experiments. Did the statistical analysis part of the data presentation. Supervised writing of the manuscript and wrote parts of the manuscript. Agreed to the submission of both publications.

PUBLICATION 1

In Vitro Weight-Loaded Cell Models for Understanding Mechanodependent Molecular Pathways Involved in Orthodontic Tooth Movement: A Systematic Review

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Stem Cells International. 2018 Jul 31; 2018:3208285.

doi: 10.1155/2018/3208285.

Hindawi
Stem Cells International
Volume 2018, Article ID 3208285, 17 pages
https://doi.org/10.1155/2018/3208285

Review Article

In Vitro Weight-Loaded Cell Models for Understanding Mechanodependent Molecular Pathways Involved in Orthodontic Tooth Movement: A Systematic Review

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Academic Editor: Andrea Ballini

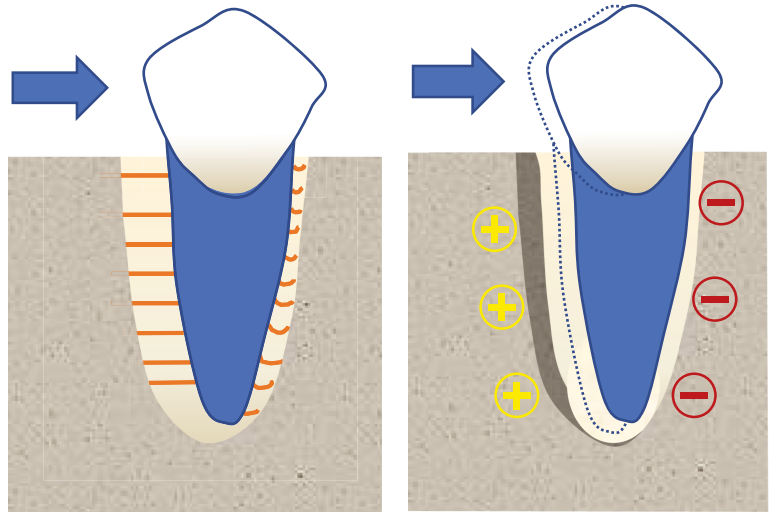
Cells from the mesenchymal lineage in the dental area, including but not limited to PDL fibroblasts, osteoblasts, and dental stem cells, are exposed to mechanical stress in physiological (e.g., chewing) and nonphysiological/therapeutic (e.g., orthodontic tooth movement) situations. Close and complex interaction of these different cell types results in the physiological and nonphysiological adaptation of these tissues to mechanical stress. Currently, different *in vitro* loading models are used to investigate the effect of different types of mechanical loading on the stress adaptation of these cell types. We performed a systematic review according to the PRISMA guidelines to identify all studies in the field of dentistry with focus on mechanobiology using *in vitro* loading models applying uniaxial static compressive force. Only studies reporting on cells from the mesenchymal lineage were considered for inclusion. The results are summarized regarding gene expression in relation to force duration and magnitude, and the most significant signaling pathways they take part in are identified using protein-protein interaction networks.

1. Introduction

The aim of orthodontics is to move an abnormally positioned tooth through the application of a continuous force on its surface. This force stimulates bone remodelling in the surrounding tissue, namely, the periodontal ligament (PDL) and the alveolar bone, resulting in the bone removal in the direction of the tooth movement and bone apposition in the opposite direction (Figure 1). Thus, the underlying mechanism of orthodontic tooth movement (OTM) is the stimulation of bone remodelling by the application of an orthodontic force [1].

Histologically, the effects of orthodontic force on the tooth and its surrounding tissues are now well understood and the underlying stages in OTM are identified [2]. Human periodontal ligament cells (hPDLCs) and human osteoblasts (hOBs) are recognized as the cell types originating from the mesenchymal lineage, which play the most dominant role during OTM. Unlike hOBs, which represent well a characterized cell type, hPDLCs represent a mixed population of mostly fibroblast-like cells [3]. Among them, mesenchymal stem cells are of special importance as the source of progenitors responsible for the regeneration and remodulation of not only PDL itself but also alveolar bone [4].

In order to better understand morphological changes during OTM, it is important to elucidate molecular and cellular signaling mechanisms between and within these cell types. The complex *in vivo* structure of the tissues involved



(a) (b)

Figure 1: Bone remodelling during orthodontic tooth movement. (a) Initial displacement of the tooth due to stretching of the fibres within the PDL on the tension side and compression on the opposite with the application of the orthodontic force. (b) Bone apposition on the tension side and resorption on the compression side as the result of the long-term force application.

makes it impossible to investigate force sensing and cellular communication of individual cells. Therefore, *in vitro* models using cells isolated from the PDL or from alveolar bone were established and different types of forces mimicking those found during OTM were applied [5]. These *in vitro* models are used to answer open questions including but not limited to how cells sense force, how they convert mechanical stress into molecular signals, and how these molecular signals influence the specific response of these cells to that specific force.

On the basis of the most commonly used approaches to apply mechanical stress on cells, present *in vitro* loading models can be classified into those using substrate deformation-based approaches, hydrostatic pressure approach, centrifugation approach, fluid flow approach, vibration approach, and weight approach [6]. Also, there has been increasing interest in moving from conventional monolayer, two-dimensional (2D) *in vitro* loading models to three-dimensional (3D) *in vitro* loading models.

Weight-based *in vitro* loading models have been successfully used over several years to investigate the effect of static, compressive, unidirectional force on the cells. In models using 2D cell cultures, cells are precultured in cell culture dishes (e.g., 6-well plates). After reaching the desired confluency, the cells are subjected to weight-based compression. In most cases, a glass slide is laid on top of the cell monolayer. Then, a weight is applied by positioning a glass cylinder filled with lead granules on top of this slide. The glass slide is used to secure even distribution of the force [7]. Increasing or reducing the number of granules in the glass cylinder adjusts the level of compressive force (Figure 2(a)). The same type of force is applied by slight modifications of this model: some authors used a stack of glass slides of different heights (e.g., [8]) or glass discs of different thicknesses (e.g., [9]) replacing the glass cylinder filled with lead granules. This *in vitro* loading model can also be used to apply static compressive force on 3D cell cultures. In this case, the same principle is used, except that the cells are embedded in a 3D matrix that is then compressed in the described manner (Figure 2(b)). Yang et al. [6] coined the term "weight approach"-based (WAB) for this *in vitro* model. To refer to this specific setup, we will also use WAB throughout this publication.

The primary aim of this review was to identify all articles related to the field of orthodontics using either a 2D or 3D WAB *in vitro* loading model and provide an overview of the details of their use: the most commonly used loading durations, force magnitudes, and scaffolds and their findings regarding gene expression and substance secretion in relation to force application. The secondary objective was to discover most commonly examined genes and to identify important pathways in OTM that most of the identified genes from these studies are involved in, focusing especially on hPDLCs.

2. Materials and Methods

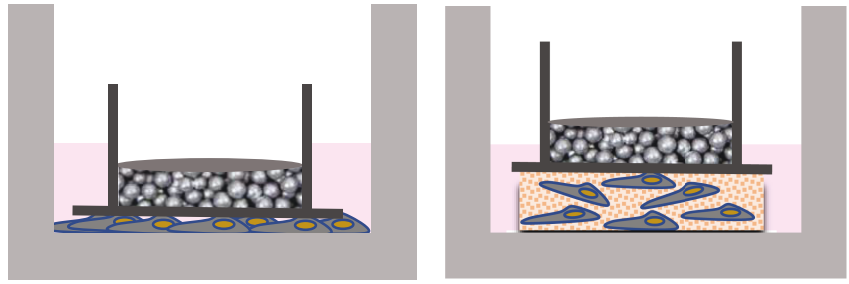
To conduct this review, the "Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols" (PRISMA-P) 2015 statement was consulted [10].

2.1. Defining the Eligibility Criteria. Inclusion criteria were as follows:

(i) Studies in the field of dentistry that examined the effect of mechanical stress on tooth surrounding tissues

(ii) Application of the 2D or 3D WAB *in vitro* loading model…

(iii) …on hPDLCs, hOBs, or all bone-like cell types/lines of human or animal origin



(a) (b)

Figure 2: Schematic illustration of the static 2D (a) and 3D (b) *in vitro* loading model based on the weight approach applied in the literature (details are found in the text).

(iv) Only studies written in English language, identified on the PubMed database until 01.12.2017, were taken into consideration

2.2. Literature Search and Study Selection Process. Separate search strategies were created for studies using either the 2D or the 3D *in vitro* setup for mechanical cell loading (Supplement 1). Searches were performed in the PubMed database following these predefined search strategies.

After identification of relevant studies in the PubMed database, the downloaded records from each search were imported into the bibliographic software EndNote X8 (Clarivate Analytics, Philadelphia, Pennsylvania, USA). All records were examined by two reviewers independently (MJ and UB), according to predefined inclusion and exclusion criteria (see above): first by title, then by abstract. If the abstract was not available, the full text of the report was obtained. Records that were obviously irrelevant were excluded, and the full texts of all remaining records were acquired. After the full-text assessment, the final list of included articles was generated. Any disagreements during this process were dissolved through discussion with another review author (DD) until reaching a consensus. The articles that did not meet all inclusion criteria after full-text assessment were excluded from further examination. Additional relevant studies were further identified through forward and backward reference chaining and hand-search of specific journals. Study quality assessment of the included studies was not performed, since the goal of this article was to provide an overview of all findings in the field only.

2.3. Data Extraction. The following information was extracted from each study obtained in full length: author, journal, year of publication, and used cell type. Force magnitude and duration, examined genes or substances, gene expression, or substance secretion details were recorded only if their response was directly connected to mechanical force stimulus. Gene symbols were used in the tables whenever possible. In case the identity or variant of a gene was doubtful or not clear primer sequences were examined using Primer-BLAST (URL: https://www.ncbi.nlm.nih.gov/tools/primer-blast/) [11]. If Western blot, ELISA, or inhibition experiments were reported, we tried to verify the antibodies and/or inhibitor specificity to determine the exact protein species (variant). Additionally, the method used for evaluation of the gene/substance expression was recorded. Data regarding the used scaffolds were collected for studies applying 3D WAB *in vitro* setups.

The following tables were prepared to summarize the findings: (1) studies applying the 2D WAB *in vitro* loading model on human primary cells from the orofacial region (i.e., hPDLCs, hOBs, and human oral bone marrow cells), (2) studies applying the 2D WAB *in vitro* loading model on human and nonhuman cells and cell lines not included in the first table, and (3) studies applying the 3D WAB *in vitro* loading model on human and nonhuman cells and cell lines.

2.4. STRING Analysis. The examined genes and metabolites using the 2D approach were summarized in two separate lists: one for hPDLFs and one for hOBs and other human bone-derived cell lines. Protein-protein interaction (PPI) networks were generated for both lists separately using the STRING database (10.5, URL: https://string-db.org/) [12]. From within STRING, the KEGG database [13] was queried to identify the main pathways involved. Only pathways with a false discovery rate below 1.00*E*−05 were considered.

3. Results

3.1. Study Selection Process. Figure 3 summarises the results of both 2D and 3D searches using a flow chart according to PRISMA. Separate searches were conducted for the studies applying either the 2D or 3D (Supplement 1) WAB *in vitro* loading models.

The search formula applied to identify 2D WAB *in vitro* loading studies is shown in Supplement 1. Altogether, 2284 abstracts were identified in the PubMed database (Figure 3).

Additionally, 7 articles were identified through forward and backward reference chaining and hand-search of specific journals. After reading the titles and abstracts of all identified studies, we excluded 2184. The remaining 107 articles were then checked by full-text reading. Fifty-six of them meet our inclusion criteria and were included for further analysis. The remaining did not meet the inclusion criteria. Reasons for their exclusion are listed in Supplement 1.

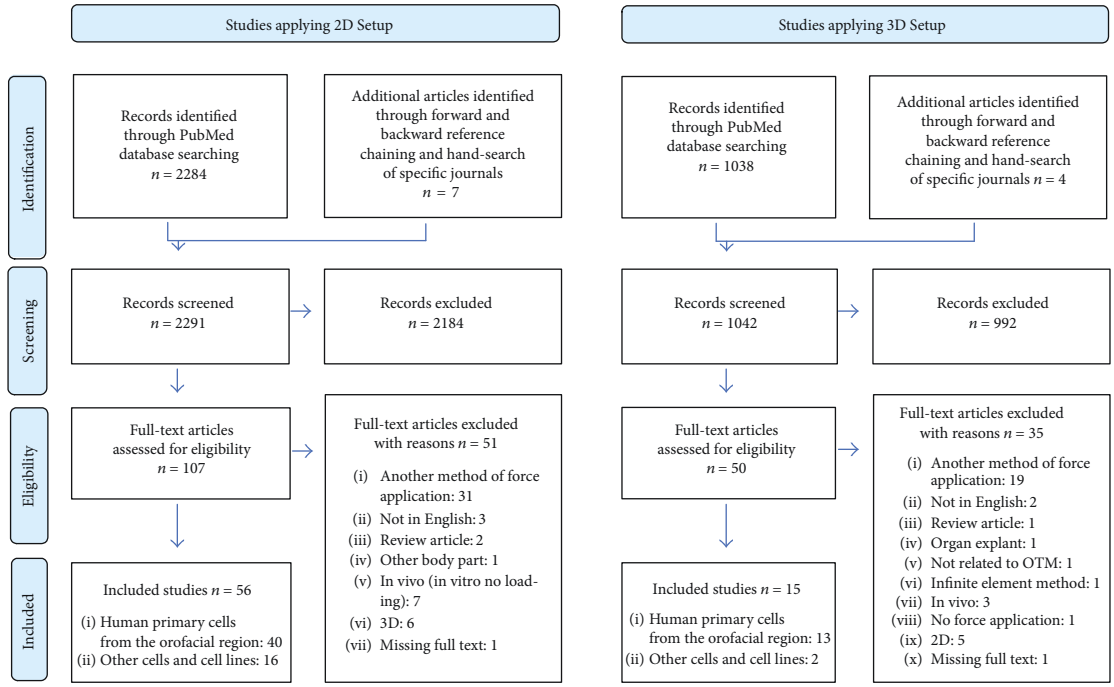


Figure 3: PRISMA flow diagram of the review process.

The search formula applied to identify 3D WAB *in vitro* loading studies is shown in Supplement 1. We identified a total of 1038 articles in PubMed (Figure 3). Additional 4 articles were discovered through forward and backward reference chaining and hand-search of specific journals. After initial screening, we excluded 992 articles and proceeded with full-text reading of the 50 articles. Finally, 17 of them meet our inclusion criteria. The remaining articles were excluded from further analysis. Reasons for their exclusion are summarized in Supplement 1.

All studies fulfilling the inclusion criteria were organised into three different supplementary tables: Supplement 2 summarises 2D WAB *in vitro* loading studies using human primary cells from the orofacial region. In Supplement 3, the two-dimensional WAB *in vitro* loading studies using human nonorofacial-derived cells and animal cells and cell lines are found. Supplement 4 summarises the 3D WAB *in vitro* loading studies.

3.2. Force Durations and Force Magnitudes Used in the Studies

3.2.1. 2D WAB In Vitro Loading Model. In these studies, compression forces ranging from 0.25 g/cm^2 to 5 g/cm^2 were applied on cells in 2D culture. The most commonly used compressive force was 2 g/cm^2, irrespectively which cell type was used in the study. In most of the studies, the force was applied for 24 h (Supplements 2 and 3).

3.2.2. 3D WAB In Vitro Loading Model. Force duration and magnitude depended on the scaffold used (Supplement 4). In most of the studies, scaffolds made from collagen gel and the polylactic-co-glycolic acid (PLGA) were applied. One of the studies [14] used a hydrophilically modified poly-L-lactide (PLLA) matrix. Collagen gel scaffolds were used with force magnitudes varying between 0.5 g/cm^2 and 9.5 g/cm^2; the most commonly used force was 6 g/cm^2. Force was applied for 0.5 to 72 h. Most commonly used force application periods were 12 and 24 h. Force levels between 5 and 35 g/cm^2 were applied to cells embedded in PLGA scaffolds. The most commonly applied force was 25 g/cm^2. The duration of force application was from 3 to 72 h. The study using the hydrophilically modulated PLLA matrix [14] applied force magnitudes from 5 to 35 g/cm^2. The duration of force application varied between one day and 14 days.

3.3. Cell Types Used in the Studies

3.3.1. 2D WAB In Vitro Loading Model. Forty of these studies used human primary cells isolated from the tooth surrounding tissues (Supplement 2): hPDLCs, hOBs, and human orofacial bone marrow-derived cells (hOBMC). The remaining studies used other cells and cell lines from human and animal sources: MG63, RAW264.7, ST-2, Saos-2, OCCM-30, MC3T3-E1, C2C12, U2OS, rat-derived PDLCs, or bone marrow-derived osteoblasts and the cementoblast cell line HCEM-SV40 (Supplement 3).

3.3.2. 3D WAB In Vitro Loading Model. hPDLCs and human gingival fibroblasts were used in 13 studies (Supplement 4). The remaining two studies used cell types and lines from the nonoral region or nonhuman origin (Supplement 4): the murine cell line MC3T3-E1 and murine osteoblasts.

Taken together, the most commonly used cells were hPDLCs. They were used in total 51 studies (2D: 38; 3D: 13) (Supplements 2 and 4). According to the isolation

method applied, we distinguished between the following sources: "explant method" [15, 16] (2D: 18; 3D: 4), "enzyme digestion method" [4] (2D: 9; 3D: 6), commercial sources (2D: 3; 3D: 1), or "no detailed information of isolation available" (2D: 8; 3D: 2).

3.4. Genes and Substances Examined in the Studies. A complete overview of genes and metabolites examined in 2D and 3D WAB studies and details of their expression can be found in Supplements 2 and 3 (2D) and Supplement 4 (3D).

In this review, special attention was paid to hPDLCs as the most examined cell type among studies and their prominent role in OTM. The most examined genes and metabolites in relation to hPDLCs were TNF superfamily member 11 (TNFSF11), TNF receptor superfamily member 11B (TNFRSF11B), prostaglandin-endoperoxide synthase 2 (PTGS2), and prostaglandin E_2 (PGE_2). In Table 1, details regarding their expression/secretion, including the information at which time points or force magnitudes the highest/lowest value was reached, is summarized.

3.5. STRING Analysis and KEGG Pathways

3.5.1. Construction of Protein-Protein Interaction (PPI) Network. In order to elucidate the molecular mechanisms of OTM and the role of the hPDLCs and bone cells in this process, we used STRING to construct PPI networks. Two separate gene lists were compiled from those studies using hPDLCs ("hPDLC list"; data from Supplement 3) and from those using hOBs or human bone-cells and cell lines ("hOB list"; data from Supplements 2 and 3). The hPDLC list contained 48 different genes (Figure 4(a)) and the hOB list 51 different genes (Figure 4(b)).

Two separate PPI networks were obtained, based on the interactions with a high level of confidence (>0.700) (Figure 4). Nodes in the networks represent the proteins produced by a single protein-coding gene locus; edges represent protein-protein interaction. Based on the colour of the edge, eight different interactions based on "gene neighbourhood," "gene fusion," "cooccurrence," "coexpression," "experiments," "databases," and "text mining" can be differentiated [12]. The top 10 nodes with the highest degree of connections from each of the two gene lists are also shown in Figure 4. PPI enrichment p values for each constructed network were calculated in STRING. These show that both PPI networks had significantly more interactions than expected and that the nodes are not random (PP enrichment p value $< 1.0E{-}16$).

3.5.2. Identification of KEGG Pathways. According to our STRING analysis, KEGG pathways relevant for OTM for each set of genes are listed in Table 2.

4. Discussion

In vivo bone remodelling during OTM represents a complex biological process, triggered by mechanical stimuli. OTM involves numerous events, spatially and temporary orchestrated and coordinated by different cell types, signaling factors, and networks [1]. Systematic breakdown and analysis of individual components of this complex process is the key for understanding its molecular background and a possible way to accelerate and improve it. Therefore, a variety of *in vitro* mechanical loading models have been established [5, 6]. The *in vitro* loading model based on the weight approach has been considered as the most appropriate loading model for the stimulation of the orthodontic force on the compressive site [6].

4.1. Characteristics of 2D and 3D WAB In Vitro Loading Models

4.1.1. Conventional 2D WAB. In vitro *loading model*, initially described by Kanai et al. [7], has been used for more than two decades for studying the compression-induced osteoclastogenesis and is still considered as the gold standard. It represents a simple and effective method for application of static compressive, unidirectional force to a cell monolayer.

The advantages of WAB *in vitro* loading model are the following:

(i) It reduces the need for animal studies, which are costly and time consuming.

(ii) It enables the analysis of specific cell types independently or in cocultures with other cells of interest.

(iii) Human primary cells can be used for better approximation to clinical situation.

From our point of view, the main disadvantage is its missing impact of the natural surrounding environment. There has been an increasing interest in the development of the 3D cell culture WAB *in vitro* loading model during the last years, in order to approximate the *in vitro* situation to the *in vivo* situation.

4.1.2. 3D WAB In Vitro Loading Model. During the last years, more studies have been using cells incorporated into biological scaffolds instead of monolayer cultures. This is due to the demand of mimicking an extracellular matrix, which is beneficial for cell behaviour, instead of growing cells on artificial plastic cell culture surface [46]. According to our data, three types of scaffolds have been used so far in combination with the 3D WAB *in vitro* loading model. The first identified studies used collagen I scaffolds [26, 47, 48]. Although the collagen gels are still widely used for this purpose, there is the increasing interest in the development of scaffolds composed of synthetic polymers. In 2011, Li et al. [33] introduced the PLGA scaffolds that had a higher stiffness in comparison to collagen gels and an elastic modulus very close to that of human PDL. The only disadvantage was that cells growing in PLGA displayed a disordered grow pattern that differs from the one in natural PDL [33]. Liao et al. [14] went one step further and introduced a hydrophilically modified PLLA matrix. This matrix displayed several advantages: higher nutrient and oxygen permeability and a better cell attachment, making it more suitable for long-term force application [14].

4.2. Force Magnitude Used in the Studies. According to Schwarz [49], optimal orthodontic force (OOF) in clinical

Table 1: Top four examined genes or substances in studies applying 2D or 3D *in vitro* WAB loading model on hPDLCs. For each gene or substance, cell culture type and literature reference are given. Additionally, examined force durations and magnitudes are summarized. Force effect on gene expression/substance secretion was evaluated (increase/decrease/no change). In all cases, the most prominent changes (increase/decrease) or "no change" are noted. For each change of expression/secretion, the corresponding maximum (increase/decrease) of force duration and magnitudes are additionally provided.

Gene symbol or metabolite	Cell culture	Reference	Examined force applied		Gene expression			Substance secretion		
			Duration (h)	Magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)
PGE_2	2D	Benjakul et al. in press [17]	48	1.5	na			Increase (qPCR: GAPDH)	48	1.5
		Jin et al. 2015 [18]	0; 0.5; 3; 6; 12	2.0	na			Increase (ELISA)	12	2.0
		Kang et al. 2010 [19]	0.5; 2; 6; 24; 48	2.0	na			Increase (ELISA)	48	2.0
		Kanzaki et al. 2002 [20]	0.5; 1.5; 6; 24; 48 (+ELISA: 60)	0.5; 1.0; 2.0; 3.0; 4.0 (ELISA: 2.0)	na			Increase (ELISA)	60	2.0
		Kirschneck et al. 2015 [21]	24	2.0	na			Not explicitly stated (ELISA)		
		Liu et al. 2006 [22]	48	2.0	na			Increase (ELISA)	48	2.0
		Mayahara et al. 2007 [23]	3; 6; 12; 24; 48	2	na			Increase (ELISA)	48	2
		Premaraj et al. 2013 [24]	0.5; 1; 3; 6	5.0	na			Increase (ELISA)	1	5.0
		Proff et al. 2014 [9]	24	2	na			Increase (ELISA)	24	2
		Römer et al. 2013 [25]	24	2	na			Increase (ELISA)	24	2
	3D (Coll. gel)	de Araujo et al. 2007 [26]	3; 12; 24; 48; 72	6.0				Increase (EIA)	72	6.0
	3D (PLGA)	Li et al. 2016 [27]	6; 24; 72	5.0; 15.0; 25.0	na			Increase (ELISA)	24	15.0…25.0
		Yi et al. 2016 [28]	24	25.0				Increase (ELISA)	24	25.0
PTGS2	2D	Jin et al. 2015 [18]	0; 0.5; 3; 6; 12	2.0	Increase (qPCR: GAPDH)	12	2.0			
		Kang et al. 2010 [19]	0.5; 2; 6; 24; 48	2.0	Increase (qPCR: GAPDH)	48				
		Kanzaki et al. 2002 [20]	0.5; 1.5; 6; 24; 48	0.5; 1.0; 2.0; 3.0; 4.0	Increase (sqPCR: ACTNB)	6	2.0			
		Kirschneck et al. 2015 [21]	24	2.0	Increase (qPCR: POL2RA)	24	2.0			
		Liu et al. 2006 [22]	48	2.0	Increase (sqPCR: ACTNB)	48	2.0			
		Mayahara et al. 2007 [23]	3; 6; 12; 24; 48	2	Increase (qPCR: GAPDH)	48	2			
		Mayahara et al. 2010 [29]	3; 6; 12; 24; 48	2.0	Increase (qPCR: GAPDH)	48	2			
		Premaraj et al. 2013 [24]	6	0.2; 2.2; 5.0	nd			Increase (WB)	6	5.0
		Proff et al. 2014 [9]	24	2	Increase (qPCR: POL2RA)	24	2	Increase (WB)	24	2
		Römer et al. 2013 [25]	24	2	Increase (qPCR: POL2RA)	24	2			
		Wongkhantee et al. 2007 [30]	24	0; 1.25; 2.5	Increase (sqPCR: GAPDH)	24	2.5			
	3D (Coll. gel)	de Araujo et al. 2007 [26]	1; 3; 6; 12; 24; 48; 72	3.6; 6.0; 7.1; 9.5	Increase (sqPCR: GAPDH)	6	7.1			

Table 1: Continued.

Gene symbol or metabolite	Cell culture	Reference	Examined force applied		Gene expression			Substance secretion		
			Duration (h)	Magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)
	3D (PLGA)	Li et al. 2016 [31]	6; 24; 72	25.0	Increase (qPCR: GAPDH)	6	25.0			
		Li et al. 2013 [32]	6; 24; 72	25.0	Increase (qPCR: GAPDH)	6	25.0			
		Li et al. 2016 [27]	6; 24; 72	5.0; 15.0; 25.0	Increase (qPCR: GAPDH)	6	25.0			
		Li et al. 2011 [33]	6	5; 15; 25; 35	Increase (qPCR: GAPDH)	6	35.0			
		Yi et al. 2016 [28]	24	25.0	Increase (qPCR: GAPDH)	24	25.0	Increase (WB)	24	25.0
TNFRSF11B	2D	Benjakul et al. in press [17]	48	1.5	No change (qPCR: GAPDH)			No change		
		Jin et al. 2015 [18]	0; 0.5; 3; 6; 12	2.0	No change (qPCR: GAPDH)					
		Kanzaki et al. 2002 [20]	0.5; 1.5; 6; 24; 48	0.5; 1.0; 2.0; 3.0; 4.0	No change (sqPCR: ACTNB)					
		Kim et al. 2013 [8]	0.5; 2; 6; 24; 48	2.0	Transitory downregulated. (qPCR: GAPDH)	6	2.0	Transitory downregulation (ELISA)	6	2.0
		Kirschneck et al. 2015 [21]	24	2.0	No change (qPCR: POL2RA)					
		Lee et al. 2015 [34]	0; 2; 4; 8; 24; 48	2.5	No change (qPCR: ACTNB)					
		Liu et al. 2017 [35]	6; 12; 24	0.5; 1.0; 1.5	nd			Decrease (WB)	n. g.	1.5
		Luckprom et al. 2011 [36]	2; 4	2.5	No change (sqPCR: GAPDH)					
		Mitsuhashi et al. 2011 [37]	1; 3; 6; 9; 12; 24	4.0	No change (qPCR: ACTNB)					
		Nakajima et al. 2008 [38]	0; 1; 3; 6; 9; 12; 24	0.5; 1.0; 2.0; 3.0; 4.0	nd			Increase (ELISA)	24	0.5
		Nishijima et al. 2006 [39]	48	0; 0.5; 1.0; 2.0; 3.0	nd			Decrease (ELISA)	48	2.0
		Römer et al. 2013 [25]	24	2	No change (qPCR: RNA-polymerase-2-polypeptide A)					
		Yamada et al. 2013 [40]	12	4.0	Decrease (qPCR: GAPDH)	12	4.0	Decrease (ELISA)	12	4.0
		Yamaguchi et al. 2006 [41]	0; 3; 6; 9; 12; 24; 48	0.5; 1.0; 2.0; 3.0	n. d.			Decrease (ELISA)	12…48	2.0
	3D (Coll. gel)	Kaku et al. 2016 [42]	12; 24	0.5; 1.0; 2.0	Increase (qPCR: GAPDH)	12	1.0			
	3D (PLLA modif.)	Liao et al. 2016 [14]	1 d; 3 d; 7 d; 14 d	5.0; 15.0; 25.0; 35.0	No change (qPCR: GAPDH)					

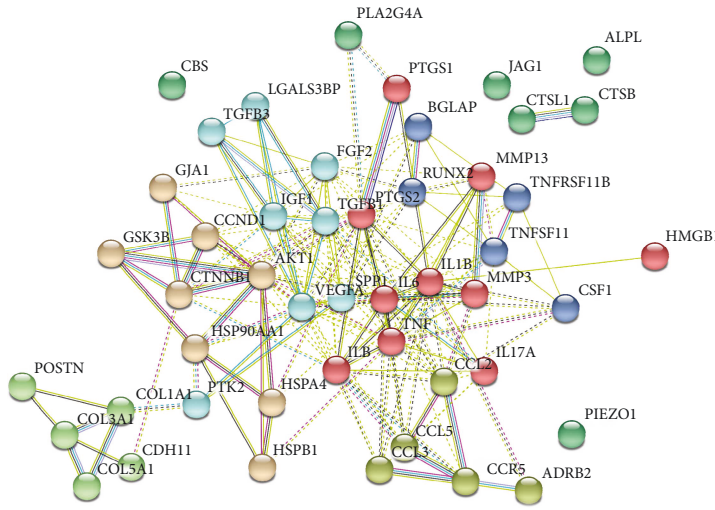
Table 1: Continued.

Gene symbol or metabolite	Cell culture	Reference	Examined force applied		Gene expression		Substance secretion		
			Duration (h)	Magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)
	3D (PLGA)	Jianru et al. 2015 [43]	3; 6; 12 (WB: 12)	25.0	Decrease followed by increase (qPCR: GAPDH)	3 (decrease) 12 (increase)	25.0	Increase (WB)	12	25.0
		Li et al. 2016 [31]	6; 24; 72	25.0	Decrease followed by Increase (qPCR: GAPDH)	6 (decrease) 72 (increase)	25.0			
		Li et al. 2016 [27]	6; 24; 72	5.0; 15.0; 25.0	Decrease followed by increase (qPCR: GAPDH)	6 (decrease) 72 (increase)	15.0 (decrease) 25.0 (increase)	Decrease followed by Increase (qPCR: GAPDH)	6 (decrease) 72 (increase)	25.0 (decrease) 25.0 (increase)
		Li et al. 2011 [33]	6; 24; 72	25	Decrease followed by increase (qPCR: GAPDH)	6 (decrease) 72 (increase)	25.0			
		Yi et al. 2016 [28]	24	25.0	Decrease (qPCR: GAPDH)	24	25.0	No change (WB)		
TNFSF11	2D	Benjakul et al. in press [17]	48	1.5	Increase (qPCR: GAPDH)	48	1.5	Increase (qPCR: GAPDH)	48	1.5
		Jin et al. 2015 [18]	0; 0.5; 3; 6; 12	2.0	Increase (qPCR: GAPDH)	12	2.0			
		Kang et al. 2013 [44]	2; 48	2.0	Increase (qPCR: GAPDH)	48	2.0			
		Kanzaki et al. 2002 [20]	0.5; 1.5; 6; 24; 48	0.5; 1.0; 2.0; 3.0; 4.0	Increase (sqPCR: ACTNB)	48	2.0	Increase (WB): 40-kDa+ 55-kDa	48	2.0
		Kikuta et al. 2015 [45]	1; 3; 6; 9; 12; 24 (+ELISA: 48)	4.0	Increase (qPCR: GAPDH)	12	4.0	Increase (ELISA)	24	4.0
		Kim et al. 2013 [8]	0.5; 2; 6; 24; 48	2.0 ++	Increase (qPCR: GAPDH)	24	2.0	Increase (ELISA)	48	2.0
		Kirschneck et al. 2015 [21]	24	2.0	Increase (qPCR: POL2RA)	24	2.0			
		Lee et al. 2015 [34]	0; 2; 4; 8; 24; 48	2.5	Increase (qPCR: ACTNB)	24	2.5			
		Liu et al. 2017 [35]	6, 12, 24	0.5; 1.0; 1.5	nd			Increase (WB: GAPDH)	ng	1.5
		Liu et al. 2006 [22]	48	2.0	Increase (sqPCR: ACTNB)	48	2.0			
		Luckprom et al. 2011 [36]	2; 4	2.5	Increase (sqPCR: GAPDH)	2	2.5	Increase (WB)	4	2.5
		Mitsuhashi et al. 2011 [37]	1; 3; 6; 9; 12; 24	4.0	Temporary increase (qPCR: ACTNB)	6…9	4.0			
		Nakajima et al. 2008 [38]	0; 1; 3; 6; 9; 12; 24	0.5; 1.0; 2.0; 3.0; 4.0	nd			Increase (ELISA)	24	4.0
		Nishijima et al. 2006 [39]	48	0; 0.5; 1.0; 2.0; 3.0	nd			Increase (ELISA)	12…48	2.0
		Römer et al. 2013 [25]	24	2	Increase (qPCR: RNA-polymerase-2-polypeptide A)	24	2			

Table 1: Continued.

Gene symbol or metabolite	Cell culture	Reference	Examined force applied		Gene expression			Substance secretion		
			Duration (h)	Magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)	Increase/decrease/no change	Change in relation to force duration (h)	Change in relation to force magnitude (g/cm^2)
		Wongkhantee et al. 2007 [30]	24	0; 1.25; 2.5	Increase (sqPCR: GAPDH)	24	2.5	Increase (WB; ACTNB)	24	2.5
		Yamada et al. 2013 [40]	12	4.0	Increase (qPCR: GAPDH)	12	4.0	Increase (ELISA)	12	4.0
		Yamaguchi et al. 2006 [41]	0; 3; 6; 9; 12; 24; 48	0.5; 1.0; 2.0; 3.0	nd			Increase (ELISA): sRANKL Increase (WB)	12…48 12	2.0 2.0
	3D (Coll. gel)	Kang et al. 2013 [44]	2; 48	2.0	Increase (qPCR: GAPDH)	2	2.0			
	3D (PLLA modif.)	Liao et al. 2016 [14]	1 d; 3 d; 7 d; 14 d	5.0; 15.0; 25.0; 35.0	Increase (qPCR: GAPDH)	Day 14	35.0			
		Jianru et al. 2015 [43]	3; 6; 12 (WB: 12)	25.0	Increase (qPCR: GAPDH)	6	25.0	Increase (WB)	12	25.0
		Li et al. 2016 [31]	6; 24; 72	25.0	Increase (qPCR: GAPDH)	6	25.0			
		Li et al. 2016 [27]	6; 24; 72	5.0; 15.0; 25.0	Increase (qPCR: GAPDH)	6	25.0	Decrease (ELISA)	72	25.0
	3D (PLGA)	Li et al. 2011 [33]	6; 24; 72	5; 15; 25; 35	Increase (qPCR: GAPDH) Increase followed by no change (qPCR: GAPDH)	6 6 (increase) 72 (no change)	25…35.0 25 25			
		Yi et al. 2016 [28]	24	25.0	Increase (qPCR: GAPDH)	24	25.0	Increase (WB)	24	25.0

2D: two-dimensional cell culture; 3D (Coll. gel): three-dimensional cell culture, collagen gel; 3D (PLGA): three-dimensional cell culture using PLGA scaffolds; 3D (PLLA modif.): three-dimensional cell culture, hydrophilically modified PLLA scaffolds; qPCR: quantitative polymerase chain reaction (e.g., real-time PCR); sqPCR: semiquantitative polymerase chain reaction, followed by reference gene used; nr: not reported; na: not applicable; ELISA: enzyme-linked immune absorbent assay; WB: Western blot; IF: immunofluorescence; FLM: fluorescence microscopy; EIA: enzyme immunoassay.

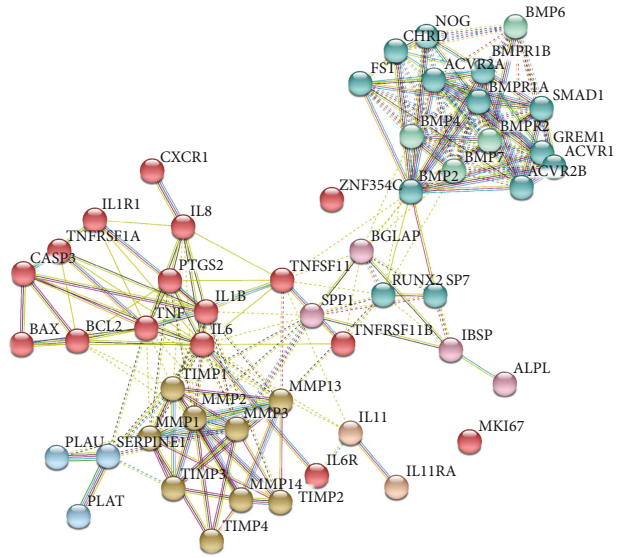


hPDLC list:

ADRB2, AKT1, ALPL, BGLAP, CBS, CCL2, CCL3, CCL5, CCND1, CCR5, CDH11, COL1A1, COL3A1, COL5A1, CSF1, CTNNB1, CTSB, CTSL, CXCL8, FGF2, GJA1, GSK3B, HMGB1, HSP90AA1, HSPA4, HSPB1, IGF1, IL17A, IL1B, IL6, JAG1, LGALS3BP, MMP13, MMP3, PIEZO1, PLA2G4A, POSTN, PTGS1, PTGS2, PTK2, RUNX2, SPP1, TGFB1, TGFB3, TNF, TNFRSF11B, TNFSF11, VEGFA

Gene	Number of interactions
VEGFA	24
IL6	23
IL1B	21
TNF	18
AKT1	18
TGFB1	18
CXCL8 (IL8)	17
IGF1	17
FGF2	16
PTGS2	15

(a)



hOB list:

ACVR1, ACVR2A, ACVR2B, ALPL, BAX, BCL2, BGLAP, BMP2, BMP4, BMP6, BMP7, BMPR1A, BMPR1B, BMPR2, Casp3, CHRD, CXCR1, FST, GREM1, IBSP, IL11, IL11RA, IL1B, IL1R1, IL6, IL6R, IL8, MKI67, MMP1, MMP13, MMP14, MMP2, MMP3, NOG, PLAT, PLAU, PTGS2, RUNX2, SERPINE1, SMAD1, SP7, SPP1, TIMP1, TIMP2, TIMP3, TIMP4, TNF, TNFRSF11B, TNFRSF1A, TNFSF11, ZNF354C

Gene	Number of interactions
IL6	22
BMP2	19
TNF	16
BMP4	15
BMP7	15
IL1B	15
MMP2	15
BMPR1A	14
BMPR1B	14
BMPR2	14

(b)

Figure 4: Protein-protein interaction networks for the (a) "hPDLC list" and the (b) "hOB list". The gene lists are shown in the lower left part of each subfigure. Those genes with the highest number of interactions ("top 10") are given in tables in the lower right part of each subfigure.

Table 2: KEGG pathways relevant for OTM with false discovery rates below $1.00E-05$ derived from STRING analysis using the set of examined genes from human periodontal ligament cells ("hPDLC list"; top panel) and human bone and bone-related cells and cell lines ("hOB list"; bottom panel). "X", gene involved in that specific pathway.

(a)

KEGG ID	4060	4668	4510	4620	4370	4062	4380	4010	4064
KEGG name	Cytokine-cytokine receptor interaction	TNF signaling pathway	Focal adhesion	Toll-like receptor signaling pathway	VEGF signaling pathway	Chemokine signaling pathway	Osteoclast differentiation	MAPK signaling pathway	NF-kappa B signaling pathway
False discovery rate	2.62*E*–15	2.06*E*–12	3.90*E*–11	2.04*E*–09	9.47*E*–08	1.33*E*–07	2.29*E*–07	1.42*E*–06	1.86*E*–05
ADRB2									
AKT1		X	X	X	X	X	X	X	
ALPL									
BGLAP									
CBS									
CCL2	X	X				X			
CCL3	X			X		X			
CCL5	X	X		X		X			
CCND1			X						
CCR5	X					X			
CDH11									
COL1A1			X						
COL3A1			X						
COL5A1			X						
CSF1	X	X					X		
CTNNB1			X						
CTSB									
CTSL									
CXCL8 (= IL8)	X			X		X			X
FGF2								X	
GJA1									
GSK3b			X			X			
HMGB1									
HSP90AA1									
HSPA4									
HSPB1					X			X	
IGF1			X						
IL17A	X								
IL1B	X	X		X			X	X	X
IL6	X	X		X					
JAG1		X							
LGALS3BP									
MMP13									
MMP3		X							
PIEZO1									
PLA2G4A					X			X	
POSTN									
PTGS1									
PTGS2		X			X				X

Table 2: Continued.

KEGG ID	4060	4668	4510	4620	4370	4062	4380	4010	4064
KEGG name	Cytokine-cytokine receptor interaction	TNF signaling pathway	Focal adhesion	Toll-like receptor signaling pathway	VEGF signaling pathway	Chemokine signaling pathway	Osteoclast differentiation	MAPK signaling pathway	NF-kappa B signaling pathway
False discovery rate	2.62*E*–15	2.06*E*–12	3.90*E*–11	2.04*E*–09	9.47*E*–08	1.33*E*–07	2.29*E*–07	1.42*E*–06	1.86*E*–05
PTK2			X		X	X			
RUNX2									
SPP1			X	X					
TGFB1	X							X	
TGFB3	X						X	X	
TNF	X	X		X			X	X	X
TNFRSF11B	X						X		
TNFSF11	X						X		X
VEGFA	X		X		X				

(b)

KEGG ID	4350	4060	4064	4390	4668	4210	4380	4620	4066
KEGG name	TGF-beta signaling pathway	Cytokine-cytokine receptor interaction	NF-kappa B signaling pathway	Hippo signaling pathway	TNF signaling pathway	Apoptosis	Osteoclast differentiation	Toll-like receptor signaling pathway	HIF-1 signaling pathway
False discovery rate	8.33*E*–23	2.37*E*–21	8.32*E*–11	5.07*E*–09	1.01*E*–08	6.26*E*–08	1.02*E*–05	6.79*E*–05	7.16*E*–05
ACVR1	X	X							
ACVR2A	X	X							
ACVR2B	X	X							
ALPL									
BAX						X			
BCL2			X			X			X
BGLAP									
BMP2	X	X		X					
BMP4	X			X					
BMP6	X			X					
BMP7	X	X		X					
BMPR1A	X	X		X					
BMPR1B	X	X		X					
BMPR2	X	X		X					
Casp3					X	X			
CHRD	X								
CXCR1		X							
FST	X								
GREM1									
IBSP									
IL11		X							
IL11RA									
IL1b		X	X		X	X	X	X	
IL1r1		X	X		X	X			

Table 2: Continued.

KEGG ID	4350	4060	4064	4390	4668	4210	4380	4620	4066
KEGG name	TGF-beta signaling pathway	Cytokine-cytokine receptor interaction	NF-kappa B signaling pathway	Hippo signaling pathway	TNF signaling pathway	Apoptosis	Osteoclast differentiation	Toll-like receptor signaling pathway	HIF-1 signaling pathway
False discovery rate	8.33*E*–23	2.37*E*–21	8.32*E*–11	5.07*E*–09	1.01*E*–08	6.26*E*–08	1.02*E*–05	6.79*E*–05	7.16*E*–05
IL6		X					X	X	X
IL6R		X							X
IL8		X	X				X	X	
MKI67									
MMP1									
MMP13									
MMP14									
MMP2									
MMP3									
NOG	X								
PLAT									
PLAU			X						
PTGS2			X						
RUNX2									
SERPINE1				X					X
SMAD1	X			X					
SP7									
SPP1							X	X	
TIMP1									X
TIMP2									
TIMP3									
TIMP4									
TNF	X	X	X		X	X	X	X	
TNFRSF11B		X							
TNFRSF1A		X	X		X	X			
TNFSF11		X	X						
ZNF354C									

orthodontics should be equal to capillary blood vessel pressure (≈25 g/cm^2) [49]. On a tissue level, OOF should enable the desired clinical outcome without causing the unwanted side effects, for example, root resorption. On the cellular level, it should evoke best biologic cellular response without inhibiting the cell proliferation significantly [27]. Optimal orthodontic force *in vitro* varies between different models. Estimation of OOF for each *in vitro* model is of crucial importance for their successful application in OTM simulation [20, 33].

In 2D cell culture WAB *in vitro* loading models, applied forces varied between 0.2 and 5.0 g/cm^2. Our data suggest that 2.0 g/cm^2 was the most commonly used force magnitude in the studies so far. According to Kanzaki et al. [20], this force magnitude proved to induce the best cellular response. Few studies reported a decrease in cell viability in a force-dependent manner, especially with the application of 4 g/cm^2 force [20, 37, 50, 51].

In studies applying the 3D WAB *in vitro* loading models, the force magnitude used was chosen depending on the stiffness of the scaffold. Studies using collagen gel scaffolds most commonly applied 6 g/cm^2 force onto their *in vitro* models. According to Araujo et al. [47], this force was corresponding to the therapeutic orthodontic force, giving the best cellular response. For PLGA scaffolds, the force magnitude showing the best performance was 25 g/cm^2 (range: 5–35 g/cm^2). The same range of forces were applied in the study of Liao et al. [14] using a hydrophilically modified PLLA scaffold matrix. This range also corresponds to the one used in clinical settings, which indicates that these scaffolds are closest to the mechanical properties of *in vivo* PDL [14, 33]. This qualifies them also as a suitable model for investigation of light and

heavy forces, which are considered as a cause of orthodontic treatment failure.

4.3. Duration of the Force Application. The length of the force application in the studies rarely exceeded 72 h. In most of the cases, force was applied up to 24 and 48 h. Considering the fact that the first 10 days are of crucial importance for OTM ([52], p. 303), the duration of force application in most of the conducted studies is insufficient to fully understand the molecular background of OTM. Additionally, we would like to point out that only a few studies observed cell viability during the experiment. Most of them confirmed a reduction of cell viability, not only due to the force level but also depending on time [19, 50, 51]. We assume that one of the limitations, especially in the 2D WAB *in vitro* models, is compromised nutrient and oxygen supply in the pressure area. To overcome especially the time limitation of previous models, Liao et al. [14] introduced the hydrophilically modified PLLA matrix as a new scaffold for 3D cultures. They have shown that this scaffold can be used for up to 14 days without affecting cell viability, claiming that it provides good perfusion of the nutrients and oxygen over longer periods of time [14]. Establishing an *in vitro* model suitable for long-term force application (up to or more than 10 days) is beneficial for progress in this research field.

4.4. Role of PDL and hPDLCs in OTM. Due to lack of PDL, ankylosed teeth and implants cannot undergo OTM, which depict best PDL's key role in transmitting the mechanical stimulus and initiating the process of bone remodelling [1, 53]. Beside its mechanotransduction properties, it also contributes to tissue homoeostasis and repair, mostly due to the presence of mesenchymal stem cells which are an important part in the normal hPDLC population [4]. This portion of hPDLCs is known to be present in a higher extent in hPDLCs isolated with the "enzyme digestion method" [54], commonly used among the studies in this review, especially in the 3D group.

4.5. Most Examined Genes in the Studies That Used hPDLCs. To explain the contribution of hPDLCs in OTM on the molecular level, we summarised all data regarding the most commonly examined genes and substances in this cell type (Table 1). These were *TNFSF11*, *PTGS2*, and PGE_2, known as osteoclastogenesis inducers, and *TNFRSF11B*, known as an osteoclastogenesis inhibitor.

TNFSF11 (also known as "RANKL") [55] plays a crucial role in bone resorption on the compression side during OTM, inducing the osteoclast formation. *TNFSF11* showed an increased gene expression in all studies that used the 2D WAB *in vitro* loading model (Table 1). In most of the studies using this model, *TNFSF11* gene expression, as well as protein secretion, was positively correlated with both force duration and magnitude reaching the maximum expression level after 12–24 hours of force application. Studies using the 3D WAB *in vitro* loading model also reported an increase in the TNFSF11 secretion, most of them after 6 hours of force application (Table 1). In cells grown in PLGA scaffolds, a positive correlation between force magnitude and gene expression but a negative correlation between force duration and gene expression was noticed.

TNFRSF11B, also referred to as osteoprotegerin (OPG), is TNFSF11's antagonist that inhibits osteoclastogenesis [55]. Most of the studies applying the 2D WAB *in vitro* loading model reported no observed change in gene expression ($n = 8$), with exception of two studies that reported downregulation [40] or transitory downregulation [8] (Table 1). Considering protein secretion, results were contradictory. Most studies, however, reported a decrease in protein secretion or did not report any change. Results from studies using 3D WAB *in vitro* loading were also contrary, depending on the scaffold used. In a study using collagen gel scaffolds, an increase in *TNFRSF11B* gene expression was observed [26]. In all studies applying PLGA scaffolds, a decrease in TNFRSF11B secretion was positively correlated with force magnitude and negatively correlated with force duration [27, 28, 31, 33, 43]. With one exception [28], a comparison of *TNFSF11* and *TNFRSF11B* gene expression in the aforementioned studies showed that a rapid down/regulation of *TNFRSF11B* appears parallel to a rapid upregulation of *TNFSF11* in 3D WAB *in vitro* loading. Since both genes represent antagonists in bone turnover regulation, this was explained as a good representation of the cyclic changes in the bone metabolism on the compression side during OTM [31, 33]. It was also suggested that downregulation of *TNFSF11* in later stages might have something to do with other inducers for prolonged osteoclastogenesis promotion [33].

Gene expression of *PTGS2* was increased upon force application in both 2D and 3D studies. In most of the 2D WAB studies, *PTGS2* showed a positive correlation between the duration of the experiment and gene expression (Table 1). In those studies, using the 3D WAB *in vitro* loading model, *PTGS2* seemed to be negatively correlated with force duration and positively correlated with force magnitude. On the other hand, PTGS2 protein quantity was shown to be in positive correlation with both duration and force magnitude using Western blotting (Table 1). Since PTGS2 is involved in prostaglandin E_2 metabolism, an upregulation of *PTGS2* gene expression (maximum at 24 to 48 h after force application) is correlated with an upregulation of PGE_2 secretion (maximum at 48 h after force application) in all studies (Table 1).

Taken together, there seems to be some inconsistency between studies using the 2D and the 3D WAB *in vitro* loading model. The results within the 2D WAB group of studies are quite similar and comparable. However, a noticeable higher heterogeneity among those studies using the 3D WAB *in vitro* loading model is recognizable. This heterogeneity can be related to the type of scaffolds used.

4.6. STRING PPI Analysis. We performed STRING PPI analysis for two selected sets of genes ("hPDLC list" and "hOB list"). PPI enrichment p values obtained from both PPI networks (Figure 4) had significantly more interactions than expected. This implicates that the genes examined in the studies were not chosen randomly. From our point of view, this is not surprising, since most of the

studies were selecting "the genes of interest" for their analysis, all previously known or suspected to be involved in bone metabolism. Just a few of the studies performed microarray analysis in order to identify all genes responding to force application [26, 32, 44, 48].

In addition, KEGG pathways relevant for OTM, identified for each set of genes in STRING analysis (Table 2), can be useful source for discovering new genes that might influence OTM.

5. Conclusions

In summary, the WAB *in vitro* loading model represents a simple and very efficient way to investigate molecular events during OTM. The purpose of this review was to provide an overview of all used forms of the WAB *in vitro* loading model (2D and 3D in combination with different scaffolds), present all current findings, and point out at certain questions for their further improvement.

3D WAB *in vitro* loading models have shown to be promising for use in future research by bringing a more real environment in *in vitro* setups. However, unlike well-established 2D models that provide comparable results, 3D models show inconsistency in results. Obviously, there is a need for further improvement in order to establish standardised *in vitro* models that will provide comparable results. Also, there is a need to elucidate molecular events during longer periods of force application. Therefore, the future goal is to establish both 2D and 3D loading models that will allow us to conduct long-term investigations. The study of Liao et al. [14] is a good example for this, and there should be more research in that direction.

Abbreviations

2D: Two-dimensional
3D: Three-dimensional
ATP: Adenosine triphosphate
cAMP: Cyclic adenosine monophosphate
ECM: Extracellular matrix
ELISA: Enzyme-linked immunosorbent assay
H_2S: Hydrogen sulfide
hOBMCs: Human oral bone marrow cells
hOBs: Human osteoblasts
hPDLCs: Human periodontal ligament cells
KEGG: Kyoto encyclopedia of genes and genomes
NO: Nitric oxide
OOF: Optimal orthodontic force
OPG: Osteoprotegerin
OTM: Orthodontic tooth movement
PDL: Periodontal ligament
PGE_2: Prostaglandin E_2
PLGA: Polylactic-co-glycolic acid
PLLA: Poly-L-lactide acid
PPI: Protein-protein interaction
PTGS2: Prostaglandin-endoperoxide synthase 2
RANKL: Receptor activator of nuclear factor kappa-*B* ligand
ROS: Reactive oxygen species
STRING: Search tool for the retrieval of interacting genes/proteins
TNF: Tumor necrosis factor
TNFRSF11B: TNF receptor superfamily member 11b
TNFSF11: TNF superfamily member 11
WAB: Weight approach based.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgments

Mila Janjic received a study grant from BAYHOST (Bayerisches Hochschulzentrum für Mittel-, Ost- und Südosteuropa, Regensburg, Germany) and from the Fund for Young Talents of the Republic of Serbia (Government of the Republic of Serbia, Ministry of Youth and Sports, Belgrade, Serbia).

Supplementary Materials

Supplementary 1. Search strategy designed for the studies applying the *in vitro* loading model based on a weight approach on cells in 2D or 3D cell culture and lists the excluded studies after full-text reading with reasons.

Supplementary 2. Studies applying the 2D weight approach on human primary cells from the orofacial region, that is, human periodontal ligament cells (hPDLC), human oral bone marrow cells (hOBMC), and human alveolar bone osteoblasts (hOB). For each gene or metabolite force magnitude and force duration, the change in gene expression or substance secretion (increase, decrease, and no change) and the techniques applied are given.

Supplementary 3. Studies applying the 2D weight approach on human and nonhuman cells and cell lines not included in Supplement 2 (i.e., human primary cells from the orofacial region). For each gene or metabolite force magnitude and force duration, the change in gene expression or substance secretion (increase, decrease, and no change) and the techniques applied are given.

Supplementary 4. Studies applying the 3D weight approach on human and nonhuman cells and cell lines. For each gene or metabolite force magnitude and force duration, the change in gene expression or substance secretion (increase, decrease, and no change) and the techniques applied are given.

References

[1] A. Wichelhaus, *Orthodontic Therapy - Fundamental Treatment Concepts*, Georg Thieme, New York, 2017.

[2] Z. Davidovitch and V. Krishnan, "Biological basis of orthodontic tooth movement: an historical perspective," in *Biological Mechanisms of Tooth Movement, V. Krishnan and Z. Davidovitch Eds*, pp. 3–14, Wiley, Chichester, West Sussex, UK, 2015.

[3] Z. Davidovitch, "Tooth movement," *Critical Reviews in Oral Biology and Medicine*, vol. 2, no. 4, pp. 411–450, 1991.

[4] B. M. Seo, M. Miura, S. Gronthos et al., "Investigation of multipotent postnatal stem cells from human periodontal ligament," *The Lancet*, vol. 364, no. 9429, pp. 149–155, 2004.

[5] U. Baumert, I. Golan, B. Becker et al., "Pressure simulation of orthodontic force in osteoblasts: a pilot study," *Orthodontics and Craniofacial Research*, vol. 7, no. 1, pp. 3–9, 2004.

[6] L. Yang, Y. Yang, S. Wang, Y. Li, and Z. Zhao, "*In vitro* mechanical loading models for periodontal ligament cells: from two-dimensional to three-dimensional models," *Archives of Oral Biology*, vol. 60, no. 3, pp. 416–424, 2015.

[7] K. Kanai, H. Nohara, and K. Hanada, "Initial effects of continuously applied compressive stress to human periodontal ligament fibroblasts," *The Journal of Japan Orthodontic Society*, vol. 51, pp. 153–163, 1992.

[8] S. J. Kim, K. H. Park, Y. G. Park, S. W. Lee, and Y. G. Kang, "Compressive stress induced the up-regulation of M-CSF, RANKL, TNF-α expression and the down-regulation of OPG expression in PDL cells via the integrin-FAK pathway," *Archives of Oral Biology*, vol. 58, no. 6, pp. 707–716, 2013.

[9] P. Proff, C. Reicheneder, A. Faltermeier, D. Kubein-Meesenburg, and P. Römer, "Effects of mechanical and bacterial stressors on cytokine and growth-factor expression in periodontal ligament cells," *Journal of Orofacial Orthopedics / Fortschritte der Kieferorthopädie*, vol. 75, no. 3, pp. 191–202, 2014.

[10] D. Moher, PRISMA-P Group, L. Shamseer et al., "Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement," *Systematic Reviews*, vol. 4, no. 1, 2015.

[11] J. Ye, G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, and T. L. Madden, "Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction," *BMC Bioinformatics*, vol. 13, no. 1, p. 134, 2012.

[12] D. Szklarczyk, J. H. Morris, H. Cook et al., "The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible," *Nucleic Acids Research*, vol. 45, no. D1, pp. D362–D368, 2016.

[13] J. Du, Z. Yuan, Z. Ma, J. Song, X. Xie, and Y. Chen, "KEGG-PATH: Kyoto encyclopedia of genes and genomes-based pathway analysis using a path analysis model," *Molecular BioSystems*, vol. 10, no. 9, pp. 2441–2447, 2014.

[14] W. Liao, M. Okada, K. Inami, Y. Hashimoto, and N. Matsumoto, "Cell survival and gene expression under compressive stress in a three-dimensional in vitro human periodontal ligament-like tissue model," *Cytotechnology*, vol. 68, no. 2, pp. 249–260, 2016.

[15] B. Ragnarsson, G. Carr, and J. C. Daniel, "Basic biological sciences isolation and growth of human periodontal ligament cells in vitro," *Journal of Dental Research*, vol. 64, no. 8, pp. 1026–1030, 1985.

[16] M. J. Somerman, S. Y. Archer, G. R. Imm, and R. A. Foster, "A comparative study of human periodontal ligament cells and gingival fibroblasts *in vitro*," *Journal of Dental Research*, vol. 67, no. 1, pp. 66–70, 1988.

[17] S. Benjakul, S. Jitpukdeebodintra, and C. Leethanakul, "Effects of low magnitude high frequency mechanical vibration combined with compressive force on human periodontal ligament cells *in vitro*," *European Journal of Orthodontics*, In press.

[18] Y. Jin, J. Li, Y. Wang et al., "Functional role of mechanosensitive ion channel Piezo1 in human periodontal ligament cells," *The Angle Orthodontist*, vol. 85, no. 1, pp. 87–94, 2015.

[19] Y. G. Kang, J. H. Nam, K. H. Kim, and K. S. Lee, "FAK pathway regulates PGE2 production in compressed periodontal ligament cells," *Journal of Dental Research*, vol. 89, no. 12, pp. 1444–1449, 2010.

[20] H. Kanzaki, M. Chiba, Y. Shimizu, and H. Mitani, "Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor κB ligand up-regulation via prostaglandin E2 synthesis," *Journal of Bone and Mineral Research*, vol. 17, no. 2, pp. 210–220, 2002.

[21] C. Kirschneck, P. Proff, M. Maurer, C. Reicheneder, and P. Römer, "Orthodontic forces add to nicotine-induced loss of periodontal bone : An in vivo and in vitro study," *Journal of Orofacial Orthopedics*, vol. 76, no. 3, pp. 195–212, 2015.

[22] L. Liu, K. Igarashi, H. Kanzaki, M. Chiba, H. Shinoda, and H. Mitani, "Clodronate inhibits PGE2 production in compressed periodontal ligament cells," *Journal of Dental Research*, vol. 85, no. 8, pp. 757–760, 2006.

[23] K. Mayahara, Y. Kobayashi, K. Takimoto, N. Suzuki, N. Mitsui, and N. Shimizu, "Aging stimulates cyclooxygenase-2 expression and prostaglandin E_2 production in human periodontal ligament cells after the application of compressive force," *Journal of Periodontal Research*, vol. 42, no. 1, pp. 8–14, 2007.

[24] S. Premaraj, I. Souza, and T. Premaraj, "Focal adhesion kinase mediates β-catenin signaling in periodontal ligament cells," *Biochemical and Biophysical Research Communications*, vol. 439, no. 4, pp. 487–492, 2013.

[25] P. Römer, J. Köstler, V. Koretsi, and P. Proff, "Endotoxins potentiate COX-2 and RANKL expression in compressed PDL cells," *Clinical Oral Investigations*, vol. 17, no. 9, pp. 2041–2048, 2013.

[26] R. M. S. de Araujo, Y. Oba, and K. Moriyama, "Identification of genes related to mechanical stress in human periodontal ligament cells using microarray analysis," *Journal of Periodontal Research*, vol. 42, no. 1, pp. 15–22, 2007.

[27] M. Li, J. Yi, Y. Yang, W. Zheng, Y. Li, and Z. Zhao, "Investigation of optimal orthodontic force at the cellular level through three-dimensionally cultured periodontal ligament cells," *The European Journal of Orthodontics*, vol. 38, no. 4, pp. 366–372, 2016.

[28] J. Yi, B. Yan, M. Li et al., "Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression," *Archives of Oral Biology*, vol. 64, pp. 51–60, 2016.

[29] K. Mayahara, A. Yamaguchi, M. Sakaguchi, Y. Igarashi, and N. Shimizu, "Effect of Ga-Al-As laser irradiation on *COX-2* and *cPLA$_2$-α* expression in compressed human periodontal ligament cells," *Lasers in Surgery and Medicine*, vol. 42, no. 6, pp. 489–493, 2010.

[30] S. Wongkhantee, T. Yongchaitrakul, and P. Pavasant, "Mechanical stress induces osteopontin expression in human periodontal ligament cells through rho kinase," *Journal of Periodontology*, vol. 78, no. 6, pp. 1113–1119, 2007.

[31] M. Le Li, J. Yi, Y. Yang et al., "Compression and hypoxia play independent roles while having combinative effects in the

osteoclastogenesis induced by periodontal ligament cells," *The Angle Orthodontist*, vol. 86, no. 1, pp. 66–73, 2016.

[32] Y. Li, M. Li, L. Tan et al., "Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression," *Archives of Oral Biology*, vol. 58, no. 5, pp. 511–522, 2013.

[33] Y. Li, W. Zheng, J. S. Liu et al., "Expression of osteoclastogenesis inducers in a tissue model of periodontal ligament under compression," *Journal of Dental Research*, vol. 90, no. 1, pp. 115–120, 2010.

[34] S. Y. Lee, H. I. Yoo, and S. H. Kim, "CCR5-CCL axis in PDL during orthodontic biophysical force application," *Journal of Dental Research*, vol. 94, no. 12, pp. 1715–1723, 2015.

[35] F. Liu, F. Wen, D. He et al., "Force-induced H_2S by PDLSCs modifies osteoclastic activity during tooth movement," *Journal of Dental Research*, vol. 96, no. 6, pp. 694–702, 2017.

[36] P. Luckprom, K. Kanjanamekanant, and P. Pavasant, "Role of connexin43 hemichannels in mechanical stress-induced ATP release in human periodontal ligament cells," *Journal of Periodontal Research*, vol. 46, no. 5, pp. 607–615, 2011.

[37] M. Mitsuhashi, M. Yamaguchi, T. Kojima, R. Nakajima, and K. Kasai, "Effects of HSP70 on the compression force-induced TNF-α and RANKL expression in human periodontal ligament cells," *Inflammation Research*, vol. 60, no. 2, pp. 187–194, 2011.

[38] R. Nakajima, M. Yamaguchi, T. Kojima, M. Takano, and K. Kasai, "Effects of compression force on fibroblast growth factor-2 and receptor activator of nuclear factor kappa B ligand production by periodontal ligament cells *in vitro*," *Journal of Periodontal Research*, vol. 43, no. 2, pp. 168–173, 2008.

[39] Y. Nishijima, M. Yamaguchi, T. Kojima, N. Aihara, R. Nakajima, and K. Kasai, "Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells *in vitro*," *Orthodontics and Craniofacial Research*, vol. 9, no. 2, pp. 63–70, 2006.

[40] K. Yamada, M. Yamaguchi, M. Asano, S. Fujita, R. Kobayashi, and K. Kasai, "Th17-cells in atopic dermatitis stimulate orthodontic root resorption," *Oral Diseases*, vol. 19, no. 7, pp. 683–693, 2013.

[41] M. Yamaguchi, N. Aihara, T. Kojima, and K. Kasai, "RANKL increase in compressed periodontal ligament cells from root resorption," *Journal of Dental Research*, vol. 85, no. 8, pp. 751–756, 2006.

[42] M. Kaku, J. M. Rosales Rocabado, M. Kitami et al., "Mechanical loading stimulates expression of collagen cross-linking associated enzymes in periodontal ligament," *Journal of Cellular Physiology*, vol. 231, no. 4, pp. 926–933, 2016.

[43] Y. I. Jianru, L. I. MeiLe, Y. YANG, W. ZHENG, L. I. Yu, and Z. ZHAO, "Static compression regulates OPG expression in periodontal ligament cells via the CAMK II pathway," *Journal of Applied Oral Science*, vol. 23, no. 6, pp. 549–554, 2015.

[44] K. L. Kang, S. W. Lee, Y. S. Ahn, S. H. Kim, and Y. G. Kang, "Bioinformatic analysis of responsive genes in two-dimension and three-dimension cultured human periodontal ligament cells subjected to compressive stress," *Journal of Periodontal Research*, vol. 48, no. 1, pp. 87–97, 2013.

[45] J. Kikuta, M. Yamaguchi, M. Shimizu, T. Yoshino, and K. Kasai, "Notch signaling induces root resorption via RANKL and IL-6 from hPDL cells," *Journal of Dental Research*, vol. 94, no. 1, pp. 140–147, 2014.

[46] M. Weinreb and C. E. Nemcovsky, "*In vitro* models for evaluation of periodontal wound healing/regeneration," *Periodontology*, vol. 68, no. 1, pp. 41–54, 2015.

[47] R. M. Santos de Araujo, Y. Oba, and K. Moriyama, "Role of regulator of G-protein signaling 2 (RGS2) in periodontal ligament cells under mechanical stress," *Cell Biochemistry and Function*, vol. 25, no. 6, pp. 753–758, 2007.

[48] Y. H. Lee, D. S. Nahm, Y. K. Jung et al., "Differential gene expression of periodontal ligament cells after loading of static compressive force," *Journal of Periodontology*, vol. 78, no. 3, pp. 446–452, 2007.

[49] A. M. Schwarz, "Tissue changes incidental to orthodontic tooth movement," *International Journal of Orthodontia, Oral Surgery and Radiography*, vol. 18, no. 4, pp. 331–352, 1932.

[50] K. Kanjanamekanant, P. Luckprom, and P. Pavasant, "Mechanical stress-induced interleukin-1beta expression through adenosine triphosphate/P2X7 receptor activation in human periodontal ligament cells," *Journal of Periodontal Research*, vol. 48, no. 2, pp. 169–176, 2013.

[51] P. Tripuwabhrut, K. Mustafa, P. Brudvik, and M. Mustafa, "Initial responses of osteoblasts derived from human alveolar bone to various compressive forces," *European Journal of Oral Sciences*, vol. 120, no. 4, pp. 311–318, 2012.

[52] W. R. Proffit and H. W. Fields Jr., *Contemporary Orthodontics*, Mosby, St. Louis, 2000.

[53] G. J. King, S. D. Keeling, and T. J. Wronski, "Histomorphometric study of alveolar bone turnover in orthodontic tooth movement," *Bone*, vol. 12, no. 6, pp. 401–409, 1991.

[54] K. Tanaka, K. Iwasaki, K. E. Feghali, M. Komaki, I. Ishikawa, and Y. Izumi, "Comparison of characteristics of periodontal ligament cells obtained from outgrowth and enzyme-digested culture methods," *Archives of Oral Biology*, vol. 56, no. 4, pp. 380–388, 2011.

[55] M. Yamaguchi, "RANK/RANKL/OPG during orthodontic tooth movement," *Orthodontics & Craniofacial Research*, vol. 12, no. 2, pp. 113–119, 2009.

Supplement 1

Janjic et al., *In Vitro* Weight Loaded Cell Models for Understanding Mechano-dependent Molecular Pathways Involved in Orthodontic Tooth Movement: A Systematic Review

Table 1. Search strategy designed for the studies applying the *in vitro* loading model based on a weight approach on cells in 2D cell culture.

FIELD		FORCE		TSSUE/ CELLS
orthodont* OR orthodontic tooth movement OR orthodontic forces	AND	mechanical stress OR compress* force OR continuous* compress* force OR compressive loading OR loading OR compress* OR mechanical force OR compressive loading OR static compressive loading OR mechanical stress	AND	bone OR periodontal ligament OR periodontal ligament cells OR periodontal ligament fibroblast OR PDL OR RAW OR hPDLCs OR osteoclast* OR osteoblast* OR Saos-2 OR bone remodelling OR PBMCs

Final look of the prepared entry for the PubMed database:
(orthodont* OR orthodontic tooth movement OR orthodontic forces) AND (mechanical stress OR compress* force OR continuous* compress* force OR compressive loading OR loading OR compress* OR mechanical force OR compressive loading OR static compressive loading OR mechanical stress) AND (bone OR periodontal ligament OR periodontal ligament cells OR periodontal ligament fibroblast OR PDL OR RAW OR hPDLCs OR osteoclast* OR osteoblast* OR Saos-2 OR bone remodelling OR PBMCs)

Table 2. List of excluded studies after full text reading with reasons – 2D studies.

Reason for exclusion (N)	Study
Another method of force application (31)	Basdra et al. (1997) [1]; Chien et al. (2006) [2]; Chien et al. (2009) [3]; Diercke et al. (2012) [4]; Diercke et al. (2012) [5]; Grimm et al. (2015) [6]; Guo et al. (2015) [7]; Hou et al. (2014) [8]; Imamura et al. (1990) [9]; Ito et al. (2014) [10]; Jacobs et al. (2013) [11]; Konermann et al. (2016) [12]; Korb et al. (2016) [13]; Li et al. (2009) [14]; Li et al. (2013) [15]; Liu et al. (2017) [16]; Liu, et al. (2009) [17]; Maeda et al. (2007) [18]; Maeda et al. (2015) [19]; Morikawa et al. (2016) [20]; Nakao et al. (2007) [21]; Sen et al. (2015) [22]; Shu et al. (2017) [23]; Wang et al. (2015) [24]; Wolf et al. (2016) [25]; Wu et al. (2015) [26]; Xu et al. (2014) [27]; Xu et al. (2015) [28]; Yang et al. (2010) [29]; Zhang et al. (2013) [30]; Zhang et al. (2016) [31]
Not in English (3)	Huang et al. (2006) [32]; Jiang et al. (2006) [33]; Xu et al. (2008) [34]
Review article (2)	Takano-Yamamoto et al. (2017) [35]; Yamaguchi et al. (2005) [36]
Other body part (1)	Ichimiya et al. (2007) [37]
Missing full text (1)	Ikeda et al. (2016) [38]
In vivo (7)	Cobo et al. (2016) [39]; Gluhak-Heinrich et al. (2006) [40]; Hayashi et al. (2012) [41]; Madureira et al. (2012) [42]; Nakano et al. (2015) [43]; Wolf et al. (2013) [44]; Xu et al. (2017) [45]
3D (6)	de Araujo et al. (2007) [46]; de Araujo et al. (2014) [47]; Li et al. (2016a) [48]; Li et al. (2016b) [49]; Liao et al. (2016) [50]; Yi et al. (2016) [51]

Table 3. Search strategy designed for studies applying the *in vitro* loading model based on a weight approach on 3D cell culture.

FIELD		FORCE		TISSUE/CELLS		3D MODEL
orthodontic force OR periodont* OR orthodontic tooth movement OR tooth movement OR OTM OR orthodont* OR orthodontic force	AND	mechanical stress OR mechan* stress OR compressive force OR static compressive force OR mechanical loading OR mechanical stress OR static compressive force OR static compress* OR static force OR loading OR compress* OR compressive loading OR pressure OR continuous compressive force OR continuous compress* OR Static Compress* OR mechanical force OR compressive stress	AND	periodontal ligament cells OR periodont* OR periodontal ligament OR PDL OR PDL cells OR periodontal ligament fibroblasts OR periodontal ligament cells OR osteoblast* OR osteoclast* OR alveolar bone OR bone resorption OR PDL tissue OR human gingival fibroblasts OR periodontal tissue	AND	three-dimensional culture system OR collagen OR collagen gel* OR three-dimensional model OR 3D OR 3D loading model OR 3-D model OR in vitro model OR 3-D in vitro model OR Gels OR 3-D culturing OR 3D culturing OR poly lactic-co-glycolic acid scaffolds OR PLGA scaffolds OR Scaffolds OR PLGA OR PDL tissue model OR Three-Dimensional Cultured OR three-dimensional gels OR periodontal ligament tissue model OR tissue model OR *in vitro* tissue model* OR porous poly scaffold OR periodontal ligament like tissue model

Final look of the prepared entry for the PubMed database:

(orthodontic force OR periodont* OR orthodontic tooth movement OR tooth movement OR OTM OR orthodont* OR orthodontic force) AND (mechanical stress OR mechan* stress OR compressive force OR static compressive force OR mechanical loading OR mechanical stress OR static compressive force OR static compress* OR static force OR loading OR compress* OR compressive loading OR pressure OR continuous compressive force OR continuous compress* OR Static Compress* OR mechanical force OR compressive stress) AND (periodontal ligament cells OR periodont* OR periodontal ligament OR PDL OR PDL cells OR periodontal ligament fibroblasts OR periodontal ligament cells OR osteoblast* OR osteoclast* OR alveolar bone OR bone resorption OR PDL tissue OR human gingival fibroblasts OR periodontal tissue) AND (three-dimensional culture system OR collagen OR collagen gel* OR three-dimensional model OR 3D OR 3D loading model OR 3-D model OR in vitro model OR 3-D in vitro model OR Gels OR 3-D culturing OR 3D culturing OR poly lactic-co-glycolic acid scaffolds OR PLGA scaffolds OR Scaffolds OR PLGA OR PDL tissue model OR Three-Dimensional Cultured OR three-dimensional gels OR periodontal ligament tissue model OR tissue model OR in vitro tissue model* OR porous poly scaffold OR periodontal ligament like tissue model)

Table 4. List of excluded studies after full text reading with reasons – 3D studies.

Reason for exclusion (N)	**Study**
Another method of force application (17)	Berendsen et al. (2009) [52]; Chang et al. (2008) [53]; Chang et al. (2015) [54]; Deschner et al. (2012) [55]; Diercke et al. (2011) [56]; Gharibi et al. (2013) [57]; Guo et al. (2015) [7]; Hou et al. (2014) [8]; Huang et al. (2009) [58]; Jacobs et al. (2013) [11]; Oortgiesen et al. (2012) [59]; Saminathan et al. (2013) [60]; Saminathan et al. (2015) [61]; Wolf et al. (2016) [25]; Wu et al. (2015) [26]; Xu et al. (2017) [62]; Yang et al. (2010) [29]; Zhang et al. (2013) [30]; Zhao et al. (2008) [63]
Not in English (2)	An et al. (2009) [64]; Huang et al. (2006) [32]
Organ explant (1)	Duncan et al. (1984) [65]
Not related to OTM (1)	Tabeian et al. (2017) [66]
Infinite element method (1)	Xin et al. (2002) [67]
Review article (1)	Wang et al. (2016) [68]
In vivo (3)	Gluhak-Heinrich et al. (2006) [40]; Moura et al. (2014) [69]; Zhao et al. (2008) [70]
Missing full text (1)	Zhang et al. (2016) [71]
No force application (1)	Cobo et al. (2016) [39]
2D (5)	Chen et al. (2015) [72]; Feng et al. (2017) [73]; Liu et al. (2017) [74]; Tripuwabhrut et al. (2013) [75]; Wolf et al. (2014) [76]

References

1. E. K. Basdra, "Biological reactions to orthodontic tooth movement," *Journal of Orofacial Orthopedics,* vol. 58, no. 1, pp. 2-15, 1997.
2. C. H. Chien, S. Otsuki, S. A. Chowdhury et al., "Enhancement of cytotoxic activity of sodium fluoride against human periodontal ligament fibroblasts by water pressure," *In Vivo,* vol. 20, no. 6b, pp. 849-56, 2006.
3. C. H. Chien, H. Sakagami, M. Kouhara et al., "Effect of simulated orthodontic forces on fluoride-induced cytotoxicity in MC3T3-E1 osteoblast-like cells," *In Vivo,* vol. 23, no. 2, pp. 259-65, 2009.
4. K. Diercke, A. Kohl, C. J. Lux et al., "IL-1β and compressive forces lead to a significant induction of RANKL-expression in primary human cementoblasts," *Journal of Orofacial Orthopedics,* vol. 73, no. 5, pp. 397-412, 2012.
5. K. Diercke, A. Konig, A. Kohl et al., "Human primary cementoblasts respond to combined IL-1β stimulation and compression with an impaired BSP and CEMP-1 expression," *European Journal of Cell Biology,* vol. 91, no. 5, pp. 402-12, 2012.
6. S. Grimm, C. Walter, A. Pabst et al., "Effect of compressive loading and incubation with clodronate on the RANKL/OPG system of human osteoblasts," *Journal of Orofacial Orthopedics,* vol. 76, no. 6, pp. 531-42, 2015.
7. T. Guo, L. Zhang, A. Konermann et al., "Manganese superoxide dismutase is required to maintain osteoclast differentiation and function under static force," *Scientific Reports,* vol. 5, pp. 8016, 2015.
8. J. Hou, Y. Chen, X. Meng et al., "Compressive force regulates ephrinB2 and EphB4 in osteoblasts and osteoclasts contributing to alveolar bone resorption during experimental tooth movement," *Korean Journal of Orthodontics,* vol. 44, no. 6, pp. 320-9, 2014.
9. K. Imamura, H. Ozawa, T. Hiraide et al., "Continuously applied compressive pressure induces bone resorption by a mechanism involving prostaglandin E_2 synthesis," *Journal of Cellular Physiology,* vol. 144, no. 2, pp. 222-8, 1990.
10. M. Ito, T. Arakawa, M. Okayama et al., "Gravity loading induces adenosine triphosphate release and phosphorylation of extracellular signal-regulated kinases in human periodontal ligament cells," *J Investig Clin Dent,* vol. 5, no. 4, pp. 266-74, 2014.
11. C. Jacobs, S. Grimm, T. Ziebart et al., "Osteogenic differentiation of periodontal fibroblasts is dependent on the strength of mechanical strain," *Archives of Oral Biology,* vol. 58, no. 7, pp. 896-904, 2013.
12. A. Konermann, A. Kantarci, S. Wilbert et al., "Verification of γ-Amino-Butyric Acid (GABA) signaling system components in periodontal ligament cells in vivo and in vitro," *Cellular and Molecular Neurobiology,* vol. 36, no. 8, pp. 1353-1363, 2016.
13. K. Korb, E. Katsikogianni, S. Zingler et al., "Inhibition of AXUD1 attenuates compression-dependent apoptosis of cementoblasts," *Clinical Oral Investigations,* vol. 20, no. 9, pp. 2333-2341, 2016.
14. J. Li, L. Jiang, G. Liao et al., "Centrifugal forces within usually-used magnitude elicited a transitory and reversible change in proliferation and gene expression of osteoblastic cells UMR-106," *Molecular Biology Reports,* vol. 36, no. 2, pp. 299-305, 2009.
15. F. F. Li, F. L. Chen, H. Wang et al., "Proteomics based detection of differentially expressed proteins in human osteoblasts subjected to mechanical stress," *Biochemistry and Cell Biology,* vol. 91, no. 2, pp. 109-15, 2013.
16. J. Liu, Q. Li, S. Liu et al., "Periodontal ligament stem cells in the periodontitis microenvironment are sensitive to static mechanical strain," *Stem Cells Int,* vol. 2017, pp. 1380851, 2017.
17. J. Liu, Z. Zhao, J. Li et al., "Hydrostatic pressures promote initial osteodifferentiation with ERK1/2 not p38 MAPK signaling involved," *Journal of Cellular Biochemistry,* vol. 107, no. 2, pp. 224-32, 2009.
18. A. Maeda, K. Soejima, K. Bandow et al., "Force-induced IL-8 from periodontal ligament cells requires IL-1β," *Journal of Dental Research,* vol. 86, no. 7, pp. 629-34, 2007.
19. A. Maeda, K. Bandow, J. Kusuyama et al., "Induction of CXCL2 and CCL2 by pressure force requires IL-1β-MyD88 axis in osteoblasts," *Bone,* vol. 74, pp. 76-82, 2015.
20. T. Morikawa, K. Matsuzaka, K. Nakajima et al., "Dental pulp cells promote the expression of receptor activator of nuclear factor-κB ligand, prostaglandin E_2 and substance P in

mechanically stressed periodontal ligament cells," *Archives of Oral Biology,* vol. 70, pp. 158-164, 2016.
21. K. Nakao, T. Goto, K. K. Gunjigake et al., "Intermittent force induces high RANKL expression in human periodontal ligament cells," *Journal of Dental Research,* vol. 86, no. 7, pp. 623-8, 2007.
22. S. Sen, K. Diercke, S. Zingler et al., "Compression induces Ephrin-A2 in PDL fibroblasts via c-fos," *Journal of Dental Research,* vol. 94, no. 3, pp. 464-72, 2015.
23. R. Shu, D. Bai, T. Sheu et al., "Sclerostin promotes bone remodeling in the process of tooth movement," *PLoS One,* vol. 12, no. 1, pp. e0167312, 2017.
24. H. Wang, R. Wang, Z. Wang et al., "ClC-3 chloride channel functions as a mechanically sensitive channel in osteoblasts," *Biochemistry and Cell Biology,* vol. 93, no. 6, pp. 558-65, 2015.
25. M. Wolf, S. Lossdörfer, P. Römer et al., "Short-term heat pre-treatment modulates the release of HMGB1 and pro-inflammatory cytokines in hPDL cells following mechanical loading and affects monocyte behavior," *Clinical Oral Investigations,* vol. 20, no. 5, pp. 923-31, 2016.
26. J. Wu, M. Song, T. Li et al., "The Rho-mDia1 signaling pathway is required for cyclic strain-induced cytoskeletal rearrangement of human periodontal ligament cells," *Experimental Cell Research,* vol. 337, no. 1, pp. 28-36, 2015.
27. H. Xu, X. Han, Y. Meng et al., "Favorable effect of myofibroblasts on collagen synthesis and osteocalcin production in the periodontal ligament," *American Journal of Orthodontics and Dentofacial Orthopedics,* vol. 145, no. 4, pp. 469-79, 2014.
28. H. Xu, D. Bai, L. B. Ruest et al., "Expression analysis of a-smooth muscle actin and tenascin-C in the periodontal ligament under orthodontic loading or *in vitro* culture," *Int J Oral Sci,* vol. 7, no. 4, pp. 232-41, 2015.
29. Y. Yang, Y. Yang, X. Li et al., "Functional analysis of core binding factor a1 and its relationship with related genes expressed by human periodontal ligament cells exposed to mechanical stress," *European Journal of Orthodontics,* vol. 32, no. 6, pp. 698-705, 2010.
30. P. Zhang, Y. Wu, Q. Dai et al., "p38-MAPK signaling pathway is not involved in osteogenic differentiation during early response of mesenchymal stem cells to continuous mechanical strain," *Molecular and Cellular Biochemistry,* vol. 378, no. 1-2, pp. 19-28, 2013.
31. L. Zhang, W. Liu, J. Zhao et al., "Mechanical stress regulates osteogenic differentiation and RANKL/OPG ratio in periodontal ligament stem cells by the Wnt/β-catenin pathway," *Biochimica et Biophysica Acta,* vol. 1860, no. 10, pp. 2211-9, 2016.
32. S. G. Huang, J. X. Zhang, P. Y. Xiong et al., "[Effect of continuously compressive pressure on the expression of RANKL mRNA in human periodontal ligament cells in vitro]," *Zhong Nan Da Xue Xue Bao Yi Xue Ban,* vol. 31, no. 4, pp. 518-22, 2006.
33. L. Y. Jiang, Z. H. Zhao and J. Wang, "[Effects of mechanical tensile stress on the expression of ICAM-1 mRNA in osteoblasts differentiated from rBMSCs]," *Sichuan Da Xue Xue Bao Yi Xue Ban,* vol. 37, no. 3, pp. 438-41, 2006.
34. H. Y. Xu, H. Zhou and G. Z. Rao, "[The effect of continuously compressive press on the shape of cells and expressions of MMP-9,TRAP in human osteoclasts]," *Shanghai Kou Qiang Yi Xue,* vol. 17, no. 3, pp. 285-8, 2008.
35. T. Takano-Yamamoto, T. Fukunaga and N. Takeshita, "Gene expression analysis of CCN protein in bone under mechanical stress," *Methods in Molecular Biology,* vol. 1489, pp. 283-308, 2017.
36. M. Yamaguchi and K. Kasai, "Inflammation in periodontal tissues in response to mechanical forces," *Archivum Immunologiae et Therapiae Experimentalis,* vol. 53, no. 5, pp. 388-98, 2005.
37. H. Ichimiya, T. Takahashi, W. Ariyoshi et al., "Compressive mechanical stress promotes osteoclast formation through RANKL expression on synovial cells," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics,* vol. 103, no. 3, pp. 334-41, 2007.
38. M. Ikeda, Y. Yoshimura, T. Kikuiri et al., "Release from optimal compressive force suppresses osteoclast differentiation," *Mol Med Rep,* vol. 14, no. 5, pp. 4699-4705, 2016.
39. T. Cobo, C. G. Viloria, L. Solares et al., "Role of periostin in adhesion and migration of bone remodeling cells," *PLoS One,* vol. 11, no. 1, pp. e0147837, 2016.
40. J. Gluhak-Heinrich, S. Gu, D. Pavlin et al., "Mechanical loading stimulates expression of connexin 43 in alveolar bone cells in the tooth movement model," *Cell Commun Adhes,* vol. 13, no. 1-2, pp. 115-25, 2006.

41. N. Hayashi, M. Yamaguchi, R. Nakajima et al., "T-helper 17 cells mediate the osteo/odontoclastogenesis induced by excessive orthodontic forces," *Oral Diseases,* vol. 18, no. 4, pp. 375-88, 2012.
42. D. F. Madureira, A. Taddei Sde, M. H. Abreu et al., "Kinetics of interleukin-6 and chemokine ligands 2 and 3 expression of periodontal tissues during orthodontic tooth movement," *American Journal of Orthodontics and Dentofacial Orthopedics,* vol. 142, no. 4, pp. 494-500, 2012.
43. Y. Nakano, M. Yamaguchi, M. Shimizu et al., "Interleukin-17 is involved in orthodontically induced inflammatory root resorption in dental pulp cells," *American Journal of Orthodontics and Dentofacial Orthopedics,* vol. 148, no. 2, pp. 302-9, 2015.
44. M. Wolf, S. Lossdörfer, N. Abuduwali et al., "Potential role of high mobility group box protein 1 and intermittent PTH (1-34) in periodontal tissue repair following orthodontic tooth movement in rats," *Clinical Oral Investigations,* vol. 17, no. 3, pp. 989-97, 2013.
45. H. Xu, Y. He, J. Q. Feng et al., "Wnt3α and transforming growth factor-β induce myofibroblast differentiation from periodontal ligament cells via different pathways," *Experimental Cell Research,* vol. 353, no. 2, pp. 55-62, 2017.
46. R. M. Santos de Araujo, Y. Oba and K. Moriyama, "Role of regulator of G-protein signaling 2 (RGS2) in periodontal ligament cells under mechanical stress," *Cell Biochemistry and Function,* vol. 25, no. 6, pp. 753-8, 2007.
47. R. M. Santos de Araujo, Y. Oba, S. Kuroda et al., "RhoE regulates actin cytoskeleton organization in human periodontal ligament cells under mechanical stress," *Archives of Oral Biology,* vol. 59, no. 2, pp. 187-92, 2014.
48. M. Li, J. Yi, Y. Yang et al., "Investigation of optimal orthodontic force at the cellular level through three-dimensionally cultured periodontal ligament cells," *European Journal of Orthodontics,* vol. 38, no. 4, pp. 366-72, 2016.
49. M. L. Li, J. Yi, Y. Yang et al., "Compression and hypoxia play independent roles while having combinative effects in the osteoclastogenesis induced by periodontal ligament cells," *Angle Orthodontist,* vol. 86, no. 1, pp. 66-73, 2016.
50. W. Liao, M. Okada, K. Inami et al., "Cell survival and gene expression under compressive stress in a three-dimensional in vitro human periodontal ligament-like tissue model," *Cytotechnology,* vol. 68, no. 2, pp. 249-60, 2016.
51. J. Yi, B. Yan, M. Li et al., "Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression," *Archives of Oral Biology,* vol. 64, pp. 51-60, 2016.
52. A. D. Berendsen, T. H. Smit, X. F. Walboomers et al., "Three-dimensional loading model for periodontal ligament regeneration in vitro," *Tissue Eng Part C Methods,* vol. 15, no. 4, pp. 561-70, 2009.
53. H. H. Chang, C. B. Wu, Y. J. Chen et al., "MMP-3 response to compressive forces in vitro and in vivo," *Journal of Dental Research,* vol. 87, no. 7, pp. 692-6, 2008.
54. M. Chang, H. Lin, M. Luo et al., "Integrated miRNA and mRNA expression profiling of tension force-induced bone formation in periodontal ligament cells," *In Vitro Cellular and Developmental Biology. Animal,* vol. 51, no. 8, pp. 797-807, 2015.
55. B. Deschner, B. Rath, A. Jager et al., "Gene analysis of signal transduction factors and transcription factors in periodontal ligament cells following application of dynamic strain," *Journal of Orofacial Orthopedics,* vol. 73, no. 6, pp. 486-95, 497, 2012.
56. K. Diercke, S. Sen, A. Kohl et al., "Compression-dependent up-regulation of ephrin-A2 in PDL fibroblasts attenuates osteogenesis," *Journal of Dental Research,* vol. 90, no. 9, pp. 1108-15, 2011.
57. B. Gharibi, G. Cama, M. Capurro et al., "Gene expression responses to mechanical stimulation of mesenchymal stem cells seeded on calcium phosphate cement," *Tissue Engineering Part A,* vol. 19, no. 21-22, pp. 2426-38, 2013.
58. L. Huang, Y. Meng, A. Ren et al., "Response of cementoblast-like cells to mechanical tensile or compressive stress at physiological levels in vitro," *Molecular Biology Reports,* vol. 36, no. 7, pp. 1741-8, 2009.
59. D. A. Oortgiesen, N. Yu, A. L. Bronckers et al., "A three-dimensional cell culture model to study the mechano-biological behavior in periodontal ligament regeneration," *Tissue Eng Part C Methods,* vol. 18, no. 2, pp. 81-9, 2012.

60. A. Saminathan, K. J. Vinoth, H. H. Low et al., "Engineering three-dimensional constructs of the periodontal ligament in hyaluronan-gelatin hydrogel films and a mechanically active environment," *Journal of Periodontal Research,* vol. 48, no. 6, pp. 790-801, 2013.
61. A. Saminathan, G. Sriram, J. K. Vinoth et al., "Engineering the periodontal ligament in hyaluronan-gelatin-type I collagen constructs: upregulation of apoptosis and alterations in gene expression by cyclic compressive strain," *Tissue Engineering Part A,* vol. 21, no. 3-4, pp. 518-29, 2015.
62. H. Y. Xu, E. M. Nie, G. Deng et al., "Periostin is essential for periodontal ligament remodeling during orthodontic treatment," *Mol Med Rep,* vol. 15, no. 4, pp. 1800-1806, 2017.
63. Y. Zhao, C. Wang, S. Li et al., "Expression of Osterix in mechanical stress-induced osteogenic differentiation of periodontal ligament cells in vitro," *European Journal of Oral Sciences,* vol. 116, no. 3, pp. 199-206, 2008.
64. Y. Y. An, H. Zhou, Y. S. Ruan et al., "[Mass chromatographic analysis on different protein expression of human periodontal ligament cell under static pressure]," *Shanghai Kou Qiang Yi Xue,* vol. 18, no. 1, pp. 56-60, 2009.
65. G. W. Duncan, E. H. Yen, E. T. Pritchard et al., "Collagen and prostaglandin synthesis in force-stressed periodontal ligament *in vitro*," *Journal of Dental Research,* vol. 63, no. 5, pp. 665-9, 1984.
66. H. Tabeian, A. D. Bakker, B. F. Betti et al., "Cyclic Tensile Strain Reduces TNF-alpha Induced Expression of MMP-13 by Condylar Temporomandibular Joint Cells," *Journal of Cellular Physiology,* vol. 232, no. 6, pp. 1287-1294, 2017.
67. H. Xin, X. Ma, L. Ying et al., "The application of infinite element method to endodontic endosseous implant stress analysis," *Zhonghua Kou Qiang Yi Xue Za Zhi,* vol. 37, no. 3, pp. 183-6, 2002.
68. T. Wang, G. Li, J. Chen et al., "Three-dimensional stress In vitro promotes the proliferation and differentiation of periodontal ligament stem cells implanted by bioactive glass," *Cell Mol Biol (Noisy-le-grand),* vol. 62, no. 10, pp. 62-7, 2016.
69. A. P. Moura, S. R. Taddei, C. M. Queiroz-Junior et al., "The relevance of leukotrienes for bone resorption induced by mechanical loading," *Bone,* vol. 69, pp. 133-8, 2014.
70. Z. Zhao, Y. Fan, D. Bai et al., "The adaptive response of periodontal ligament to orthodontic force loading - a combined biomechanical and biological study," *Clin Biomech (Bristol, Avon),* vol. 23 Suppl 1, pp. S59-66, 2008.
71. X. Zhang, W. G. Guo, H. Cui et al., "In vitro and in vivo enhancement of osteogenic capacity in a synthetic BMP-2 derived peptide-coated mineralized collagen composite," *J Tissue Eng Regen Med,* vol. 10, no. 2, pp. 99-107, 2016.
72. Y. Chen, A. Mohammed, M. Oubaidin et al., "Cyclic stretch and compression forces alter microRNA-29 expression of human periodontal ligament cells," *Gene,* vol. 566, no. 1, pp. 13-7, 2015.
73. L. Feng, Y. Zhang, X. Kou et al., "Cadherin-11 modulates cell morphology and collagen synthesis in periodontal ligament cells under mechanical stress," *Angle Orthodontist,* vol. 87, no. 2, pp. 193-199, 2017.
74. F. Liu, F. Wen, D. He et al., "Force-induced H_2S by PDLSCs modifies osteoclastic activity during tooth movement," *Journal of Dental Research,* vol. 96, no. 6, pp. 694-702, 2017.
75. P. Tripuwabhrut, M. Mustafa, C. G. Gjerde et al., "Effect of compressive force on human osteoblast-like cells and bone remodelling: an *in vitro* study," *Archives of Oral Biology,* vol. 58, no. 7, pp. 826-36, 2013.
76. M. Wolf, S. Lossdörfer, K. Küpper et al., "Regulation of high mobility group box protein 1 expression following mechanical loading by orthodontic forces *in vitro* and *in vivo*," *European Journal of Orthodontics,* vol. 36, no. 6, pp. 624-31, 2014.

Supplement 2. Studies applying the 2D weight approach on human primary cells from the orofacial region, i.e. human periodontal ligament cells (hPDLC), human oral bone marrow cells (hOBMC), and human alveolar bone osteoblasts (hOB). For each gene or metabolite force magnitude and force duration, the change in gene expression or substance secretion (increase, decrease, no change), and the techniques for analysis applied are given.

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm^2]	Duration [h]		
Asano et al. 2011 [1]	hPDLC (exp)	*CXCL8*	1.0; 2.0; 3.0; 4.0	0; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		CCL2	1.0; 2.0; 3.0; 4.0	0; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
Benjakul et al. in press [2]	hPDLC (exp?)	PGE_2	1.5	48	n. a.	Increase (ELISA)
		TNFSF11	1.5	48	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNFRSF11B	1.5	48	No change (qPCR: GAPDH)	No change (ELISA)
		RUNX2	1.5	48	Decrease (qPCR: GAPDH)	n. r.
Cao et al. 2014 [3]	hPDLC (dig)	*ADRB2*	1.5 0.5; 1.0; 1.5; 2.0	0; 2; 4; 6; 8; 12 6	n. r	Increase (WB)
	hOBMC	*ADRB2*	1.5 0.5; 1.0; 1.5; 2.0	0; 2; 4; 6; 8; 12 6	n. r	No change (WB)
Chae et al. 2011 [4]	hPDLC ($$)	ROS	3.0	4	n. a.	Increase (FLM)
		IL1B	3.0	24	Increase (qPCR: GAPDH)	Increase (ELISA)
		CXCL8	3.0	24	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL6	3.0	24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNF	3.0	24	Increase (qPCR: GAPDH)	Increase (ELISA)
Chen et al. 2015 [5]	hPDLC (exp)	*COL1A1*	2.0	24	Decrease (qPCR: ACTB)	n. r.
		COL3A1	2.0	24	Decrease (qPCR: ACTB)	n. r.
		COL5A1	2.0	24	No change (qPCR: ACTB)	n. r.
		microRNAs	2.0	24	Increase (qPCR: U6snRNA)	n. r.
Feng et al. 2017 [6]	hPDLC (dig)	*CDH11*	0.5; 1.0; 1.5; 2.0 1.0	24 4; 8; 12; 24	Decrease (qPCR: GAPDH)	Decrease (WB)
		COL1A1	0.5; 1.0; 1.5; 2.0 1.0	24 4; 8; 12; 24	Decrease (qPCR: GAPDH)	Decrease (WB)
		CTNNB1	0.5; 1.0; 1.5; 2.0 1.0	24 4; 8; 12; 24	n. r.	Decrease (WB)
Feng et al. 2016 [7]	hPDLC (dig)	*COL1A1*	1.0	24	Decrease (qPCR: GAPDH)	n. r.
		TGFB1	1.0	24	Decrease (qPCR: GAPDH)	n. r.
		TGFB3	1.0	24	Decrease (qPCR: GAPDH)	n. r.

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm²]	Duration [h]		
		TGF-β (*antibody specificity not identifiable)	1.0	24	n. a.	Decrease (WB)
He et al. 2015 [8]	hPDLC (dig)	Effect on macro-phages in co-culture	1	24	n. r.	n. r.
Jin et al. 2015 [9]	hPDLC (dig)	*PTGS2*	2.0	0.5; 3; 6; 12	Increase (qPCR: GAPDH)	n. r.
		NFKB (*antibody specificity not identifiable)	2.0	3	n. r.	Increased nuclear translocation (WB)
		TNFRSF11B	2.0	0.5; 3; 6; 12	No change (qPCR: GAPDH)	n. r.
		PGE_2	2.0	12	n. a.	Increase (ELISA)
		PIEZO1	2.0	0.5; 3; 6; 12 WB: 3	Increase (qPCR: GAPDH)	Increase (WB)
		TNFSF11	2.0	0.5; 3; 6; 12	Increase (qPCR: GAPDH)	n. r.
Kang et al. 2013 [10]	hPDLC (dig)	*IL1B*	2.0	2; 48	No change (qPCR: GAPDH)	n. r.
		TNF	2.0	2; 48	No change (qPCR: GAPDH)	n. r.
		TNFSF11	2.0	2; 48	Increase (qPCR: GAPDH)	n. r.
		MMP3	2.0	2; 48	Decrease (qPCR: GAPDH)	n. r.
		MMP13	2.0	2; 48	Increase (qPCR: GAPDH)	n. r.
Kang et al. 2010 [11]	hPDLC (?)	*PTGS2*	2.0	0.5; 2; 6; 24; 48	Increase (qPCR: GAPDH)	n. r.
		PTK2	2.0	0.5; 2; 6; 24; 48	n. r.	p-FAK: Increase (WB) overall FAK: no change (WB)
		PGE_2	2.0	0.5; 2; 6; 24; 48	n. a.	Increase (ELISA)
Kanjanamekanant et al. 2013 [12]	hPDLC (?)	*IL1B*	1.0; 1.5; 2.0; 2.5	1, 3, 5	Increase (sqPCR: GAPDH)	Increase (ELISA)
Kanjanamekanant et al. 2014 [13]	hPDLC (?)	*IL1B*	0; 0.5; 1.0; 1.5; 2.0; 2.5	3	n. r.	ELISA
		ATP	2.0	3	n. a.	ELISA
Kanzaki et al. 2002 [14]	hPDLC (exp)	*PTGS1*	0.5; 1.0; 2.0; 3.0; 4.0[+]	0.5; 1.5; 6; 24; 48	No change (sqPCR: ACTNB)	n. r.
		PTGS2	0.5; 1.0; 2.0; 3.0; 4.0[+]	0.5; 1.5; 6; 24; 48	Increase (sqPCR: ACTNB)	n. r.
		TNFRSF11B	0.5; 1.0; 2.0; 3.0; 4.0[+]	0.5; 1.5; 6; 24; 48	No change (sqPCR: ACTNB)	n. r.
		PGE_2	2.0	0.5; 1.5; 6; 24; 48,60	n. a.	Increase (ELISA)
		TNFSF11	0.5; 1.0; 2.0; 3.0; 4.0 WB: 2.0	0.5; 1.5; 6; 24; 48 WB: 48, 96	Increase (sqPCR: ACTNB)	Increase (WB): 40-kDa + 55-kDa
Kikuta et al. 2015 [15]	hPDLC (exp)	*IL6*	4.0	1; 3; 6; 9; 12; 24 ELISA: 1; 3; 6; 9; 12; 24; 48	Increase (qPCR: GAPDH)	Increase (ELISA)
		JAG1	4.0	1; 3; 6; 9; 12; 24 (	Increase (qPCR: GAPDH)	Increase (ELISA)

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm²]	Duration [h]		
		TNFSF11	4.0	1; 3; 6; 9; 12; 24 ELISA: 1; 3; 6; 9; 12; 24; 48	Increase (qPCR: GAPDH)	Increase (ELISA)
Kim et al. 2013 [16]	hPDLC (dig)	*PTK2*	2.0	0.5; 2; 6; 24; 48	n. r.	p-FAK/FAK-ratio: Increase (WB)
		CSF1	2.0	0.5; 2; 6; 24; 48	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNFRSF11B	2.0	0.5; 2; 6; 24; 48	Transitory downregulated. (qPCR: GAPDH)	Transitory downregulation (ELISA)
		TNFSF11	2.0	0.5; 2; 6; 24; 48	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNF	2.0	0.5; 2; 6; 24; 48	Increase (qPCR: GAPDH)	Increase (ELISA)
Kirschneck et al. 2015 [17]	hPDLC (exp)	*PTGS2*	2.0	24	Increase (qPCR: POL2RA)	n. r.
		IL6	2.0	24	Increase (qPCR: POL2RA)	Not explicitly stated (ELISA)
		TNFRSF11B	2.0	24	No change (qPCR: POL2RA)	n. r.
		PGE_2	2.0	24	n. a.	Not explicitly stated (ELISA)
		TNFSF11	2.0	24	Increase (qPCR: POL2RA)	n. r.
Kunii et al. 2013 [18]	hPDLC (exp)	*IL6*	1.0; 2.0; 3.0; 4.0	3; 6; 9; 12; 24 ELISA: 3; 6; 9; 12; 24; 48; 72	Increase (qPCR: GAPDH)	Increase (ELISA)
Lee et al. 2015 [19]	hPDLC (?)	*CCL3*	2.5	2; 4; 8; 24; 48 WB: 24; 48; 72; 96	Increase (qPCR: ACTNB)	Increase (WB)
		CCL5	2.5	2; 4; 8; 24; 48 WB: 24; 48; 72; 96	Increase (qPCR: ACTNB)	Increase (WB)
		CCR5	2.5	2; 4; 8; 24; 48 WB: 24; 48; 72; 96	Increase (qPCR: ACTNB)	Increase (WB)
		ALPL	2.5	24	Increase (qPCR: ACTNB)	n. r.
		RUNX2	2.5	24	No change (qPCR: ACTNB)	n. r.
		BGLAP	2.5	24	No change (qPCR: ACTNB)	n. r.
		TNFSF11	2.5	24	Increase (qPCR: ACTNB)	n. r.
		TNFRSF11B	2.5	24	No change (qPCR: ACTNB)	n. r.
		POSTN	2.5	24	Increase (qPCR: ACTNB)	n. r.
		IL12 *(*forward and reverse primers are identical. Primer Blast- no results)*	2.5	24	No change (qPCR: ACTNB)	n. r.
		COL1A1	2.5	2; 4; 8; 24; 48	Increase (qPCR: ACTNB)	n. r.
Liu et al. 2017 [20]	hPDLC (dig)	*CBS*	0.5; 1.0; 1.5	6; 12; 24	n. r.	Increase (WB)
		H_2S	0.5; 1.0; 1.5	6; 12; 24	n. a.	Increase

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm²]	Duration [h]		
		CCL2	0.5; 1.0; 1.5	6; 12; 24	n. r.	Increase (WB)
		TNFSF11	0.5; 1.0; 1.5	6; 12; 24	n. r.	Increase (WB)
		TNFRSF11B	0.5; 1.0; 1.5	6; 12; 24	n. r.	Decrease (WB)
Liu et al. 2006 [21]	hPDLC (?)	*PTGS2*	2.0	48	Increase (sqPCR: ACTNB)	n. r.
		IL1B	2.0	48	n. r.	No change (ELISA)
		PGE_2	2.0	48	n. a.	Increase (ELISA)
		TNFSF11	2.0	48	Increase (sqPCR: ACTNB)	n. r.
		NO	2.0	48	n. a.	Increase (HPLC-Griess)
Luckprom et al. 2011 [22]	hPDLC (?)	*TNFRSF11B*	2.5	2; 4	No change (sqPCR: GAPDH)	n. r.
		TNFSF11	2.5	2; 4	Increase (sqPCR: GAPDH)	Increase (WB)
		ATP	2.5	2; 4	n. a.	Increase (WB)
		GJA1	2.5	2; 4	n. r. (sqPCR: GAPDH)	n. r.
Mayahara et al. 2007 [23]	hPDLC (exp)	*PTGS2*	2	3; 6; 12; 24; 48	Increase (qPCR: GAPDH)	n. r.
		PGE_2	2	3; 6; 12; 24; 48	n. a.	Increase (ELISA)
Mayahara et al. 2010 [24]	hPDLC (exp)	*PTGS2*	2.0	3; 6; 12; 24; 48	Increase (qPCR: GAPDH)	n. r.
		PLA2G4A	2.0	3; 6; 12; 24; 48	Increase (qPCR: GAPDH)	n. r.
Mitsuhashi et al. 2011 [25]	hPDLC (exp)	*HSPB1*	4.0	1; 3; 6; 9; 12; 24	No change (qPCR: ACTNB)	n. r.
		HSPA4	1.0; 2.0; 4.0	1; 3; 6; 9; 12; 24	Increase (qPCR: ACTNB)	Increase (ELISA; WB)
		HSP90AA1	4.0	1; 3; 6; 9; 12; 24	Increase (qPCR: ACTNB)	n. r.
		TNFRSF11B	4.0	1; 3; 6; 9; 12; 24	No change (qPCR: ACTNB)	n. r.
		TNFSF11	4.0	1; 3; 6; 9; 12; 24	Temporary increase (qPCR: ACTNB)	n. r.
		TNF	4.0	1; 3; 6; 9; 12; 24	Temporary increase (qPCR: ACTNB)	n. r.
Nakajima et al. 2008 [26]	hPDLC (exp)	*FGF2*	0.5; 1.0; 2.0; 3.0; 4.0	1; 3; 6; 9; 12; 24	Increase (sqPCR: ACTNA)	Increase (ELISA)
		TNFRSF11B	0.5; 1.0; 2.0; 3.0; 4.0	1; 3; 6; 9; 12; 24	n. r.	Increase (ELISA)
		TNFSF11	0.5; 1.0; 2.0; 3.0; 4.0	1; 3; 6; 9; 12; 24	n. r.	Increase (ELISA)
Nishijima et al. 2006 [27]	hPDLC (exp)	*TNFSF11*	0.5; 1.0; 2.0; 3.0	48	n. r.	Increase (ELISA)
		TNFRSF11B	0.5; 1.0; 2.0; 3.0	48	n. r.	Decrease (ELISA)
Premaraj et al. 2011 [28]	hPDLC ($$)	*AKT1*	0.2; 2.2; 5.0	6	n. r.	WB: Increase p-Akt
		GSK3b	0.2; 2.2; 5.0	6	n. r.	WB: Increase in p-GSK-3β

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm²]	Duration [h]		
		CTNNB1	0.2; 2.2; 5.0	6	n. r.	WB: Increase in nuclear dephos-β-catenin
Premaraj et al. 2013 [29]	hPDLC ($$)	*AKT1*	5.0	6	n. r.	Increase in dephos-Akt (WB)
		PTGS2	0.2; 2.2; 5.0	6	n. r.	Increase (WB)
		CCND1	0.2; 2.2; 5.0	6	n. r.	Increase (WB)
		PTK2	0.2; 2.2; 5.0	0.5; 1; 3; 6	n. r.	Increase in p-FAK (WB)
		PGE_2	5.0	0.5; 1; 3; 6	n. a.	Increase (ELISA)
		CTNNB1	5.0	6	n. r.	Increase in dephos-β-catenin
		NO	5.0	0.2; 0.5; 1; 2	n. a.	Increase (Griess Reagent System)
Proff et al. 2014 [30]	hPDLC (exp)	*PTGS2*	2	24	Increase (qPCR: POLR2A)	Increase (WB)
		IGF1	2	24	Increase (qPCR: POLR2A)	n. r.
		IL6	2	24	No change (qPCR: POLR2A)	n. r.
		CXCL8	2	24	Increase (qPCR: POLR2A)	Decrease (WB, ELISA)
		MMP13	2	24	Increase (qPCR: POLR2A)	n. r.
		VEGFA	2	24	No change (qPCR: POLR2A)	n. r.
		PGE_2	2	24	n. a.	Increase (ELISA)
Römer et al. 2013 [31]	hPDLC (exp)	*PTGS2*	2	24	Increase (qPCR: POLR2A)	n. r.
		TNFRSF11B	2	24	No change (qPCR: POLR2A)	n. r.
		PGE_2	2	24	n. a.	Increase (ELISA)
		TNFSF11	2	24	Increase (qPCR: POLR2A)	n. r.
Tripuwabhrut et al. 2013 [32]	hOB	*COL1*	2.0; 4.0	24 ELISA: 24; 72; 7d	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNFSF11	2.0; 4.0	24; IF: +72	Increase (qPCR: GAPDH)	IF; not detectable with ELISA
		TNFRSF11B	2.0; 4.0	24; ELISA: 24;72	Decrease (qPCR: GAPDH)	Decrease (ELISA)
		PGE_2	2.0; 4.0	24	n. a.	Increase (ELISA)
		SPP1	2.0; 4.0	24	No change (qPCR: GAPDH)	n. r.
		BGLAP	2.0; 4.0	24	No change (qPCR: GAPDH)	n. r.
		RUNX2	2.0; 4.0	24	Decrease (qPCR: GAPDH)	n. r.
		ALPL	2.0; 4.0	24; Activity: 24; 72; 7d	Increase (qPCR: GAPDH)	Activity: Increase extracellular Activity: decrease intracellular
Tripuwabhrut et al. 2012 [33]	hOB	*MKI67*	2.0; 4.0	24	Decrease (qPCR: GAPDH)	n. r.

Reference	Cell type[a]	Gene/ metabolite symbol	Examined force applied		Gene expression [b, c] (Increase/ decrease/ no change)	Substance secretion [c,d] (Increase/ decrease/ no change)
			Magnitude [g/cm^2]	Duration [h]		
		BAX	2.0; 4.0	24	No change (qPCR: GAPDH)	n. r.
		BCL2	2.0; 4.0	24	No change (qPCR: GAPDH)	n. r.
		IL6	2.0; 4.0	24	Increase (qPCR: GAPDH)	Decrease (ELISA)
		CXCL8	2.0; 4.0	24	Increase (qPCR: GAPDH)	Decrease (ELISA)
Wolf et al. 2014 [34]	hPDLC (?)	*HMGB1*	4.0	8	n. r.	Increase (ELISA)
Wolf et al. 2013 [35]	hPDLC (?)	*HMGB1*	4.0	24	n. r.	Translocation to cytoplasm (IF) Increase (ELISA)
Wongkhantee et al. 2007 [36]	hPDLC (exp)	*PTGS2*	1.25; 2.5	24	Increase (sqPCR: GAPDH)	n. r.
		SPP1	0.5; 0.75; 1.0; 1.25;2.5	1; 4; 8; 24; 48	Increase (sqPCR: GAPDH)	Increase (WB)
		TNFSF11	1.25; 2.5	24	Increase (sqPCR: GAPDH)	Increase (WB)
Yamada et al. 2013 [37]	hPDLC (exp)	*IL6*	4.0	12	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL17A	4.0	12	n. r.	No change (ELISA)
		TNFRSF11B	4.0	12	Decrease (qPCR: GAPDH)	Decrease (ELISA)
		TNFSF11	4.0	12	Increase (qPCR: GAPDH)	Increase (ELISA)
Yamaguchi et al. 2004 [38]	hPDLC (exp)	*CTSB*	0.5; 1.0; 2.0; 3.0	3; 6; 9; 12; 24	Increase (sqPCR: GAPDH)	Increase (ELISA)
		CTSL	0.5; 1.0; 2.0; 3.0	3; 6; 9; 12; 24	Increase (sqPCR: GAPDH)	Increase (ELISA)
Yamaguchi et al. 2006 [39]	hPDLC (exp)	*TNFRSF11B*	0.5; 1.0; 2.0; 3.0	3; 6; 9; 12; 24; 48	n. r.	Decrease (ELISA)
		TNFSF11	0.5; 1.0; 2.0; 3.0	3; 6; 9; 12; 24; 48	n. r.	Increase (ELISA): sRANKL Increase (WB)
Zhang et al. 2017 [40]	hPDLC (dig)	*LGALS3BP*	0.5; 1.0; 1.5; 2.0;	24	n. r.	Increase (ELISA):

[a] hPDLC (exp) – hPDLC isolated with explant method; hPDLC (dig) – hPDLC isolated with digestion method, hPDLC (?) – hPDLC, isolation method not given; hPDLC ($$) – hPDLC from commercial sources; hOB – human osteoblasts; hOBMC – human oral bone marrow cells

[b] qPCR – quantitative PCR (e.g. real time PCR); sqPCR – semi-quantitative PCR; followed by reference gene used

[c] n. r. – not reported; n. a. – not applicable

[d] ELISA – Enzyme linked immune absorbent assay; WB – western blot; IF – immunofluorescence; FLM, fluorescence microscopy; HPLC-Griess – High Pressure Liquid Chromatography, Griess detection method; p-FAK/FAK – phosphorylated and non-phosphorylated focal adhesion kinase (FAK); kDa – kilo Dalton; pAkt – phosphorylated protein kinase B; p-GSK-3β – phosphorylated glycogen synthase kinase-3-beta; dephos-β-catenin – dephosphorylated β-catenin; sRANKL – soluble RANKL

References

1. M. Asano, M. Yamaguchi, R. Nakajima et al., "IL-8 and MCP-1 induced by excessive orthodontic force mediates odontoclastogenesis in periodontal tissues," *Oral Diseases,* vol. 17, no. 5, pp. 489-98, 2011.
2. S. Benjakul, S. Jitpukdeebodintra and C. Leethanakul, "Effects of low magnitude high frequency mechanical vibration combined with compressive force on human periodontal ligament cells *in vitro*," *European Journal of Orthodontics,* in press.
3. H. Cao, X. Kou, R. Yang et al., "Force-induced Adrb2 in periodontal ligament cells promotes tooth movement," *Journal of Dental Research,* vol. 93, no. 11, pp. 1163-9, 2014.
4. H. S. Chae, H. J. Park, H. R. Hwang et al., "The effect of antioxidants on the production of pro-inflammatory cytokines and orthodontic tooth movement," *Molecules and Cells,* vol. 32, no. 2, pp. 189-96, 2011.
5. Y. Chen, A. Mohammed, M. Oubaidin et al., "Cyclic stretch and compression forces alter microRNA-29 expression of human periodontal ligament cells," *Gene,* vol. 566, no. 1, pp. 13-7, 2015.
6. L. Feng, Y. Zhang, X. Kou et al., "Cadherin-11 modulates cell morphology and collagen synthesis in periodontal ligament cells under mechanical stress," *Angle Orthodontist,* vol. 87, no. 2, pp. 193-199, 2017.
7. L. Feng, R. Yang, D. Liu et al., "PDL progenitor-mediated PDL recovery contributes to orthodontic relapse," *Journal of Dental Research,* vol. 95, no. 9, pp. 1049-56, 2016.
8. D. He, X. Kou, R. Yang et al., "M1-like macrophage polarization promotes orthodontic tooth movement," *Journal of Dental Research,* vol. 94, no. 9, pp. 1286-94, 2015.
9. Y. Jin, J. Li, Y. Wang et al., "Functional role of mechanosensitive ion channel Piezo1 in human periodontal ligament cells," *Angle Orthodontist,* vol. 85, no. 1, pp. 87-94, 2015.
10. K. L. Kang, S. W. Lee, Y. S. Ahn et al., "Bioinformatic analysis of responsive genes in two-dimension and three-dimension cultured human periodontal ligament cells subjected to compressive stress," *Journal of Periodontal Research,* vol. 48, no. 1, pp. 87-97, 2013.
11. Y. G. Kang, J. H. Nam, K. H. Kim et al., "FAK pathway regulates PGE_2 production in compressed periodontal ligament cells," *Journal of Dental Research,* vol. 89, no. 12, pp. 1444-9, 2010.
12. K. Kanjanamekanant, P. Luckprom and P. Pavasant, "Mechanical stress-induced interleukin-1beta expression through adenosine triphosphate/P2X7 receptor activation in human periodontal ligament cells," *Journal of Periodontal Research,* vol. 48, no. 2, pp. 169-76, 2013.
13. K. Kanjanamekanant, P. Luckprom and P. Pavasant, "P2X7 receptor-Pannexin1 interaction mediates stress-induced interleukin-1 beta expression in human periodontal ligament cells," *Journal of Periodontal Research,* vol. 49, no. 5, pp. 595-602, 2014.
14. H. Kanzaki, M. Chiba, Y. Shimizu et al., "Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis," *Journal of Bone and Mineral Research,* vol. 17, no. 2, pp. 210-20, 2002.
15. J. Kikuta, M. Yamaguchi, M. Shimizu et al., "Notch signaling induces root resorption via RANKL and IL-6 from hPDL cells," *Journal of Dental Research,* vol. 94, no. 1, pp. 140-7, 2015.
16. S. J. Kim, K. H. Park, Y. G. Park et al., "Compressive stress induced the up-regulation of M-CSF, RANKL, TNF-a expression and the down-regulation of OPG expression in PDL cells via the integrin-FAK pathway," *Archives of Oral Biology,* vol. 58, no. 6, pp. 707-16, 2013.
17. C. Kirschneck, P. Proff, M. Maurer et al., "Orthodontic forces add to nicotine-induced loss of periodontal bone : An in vivo and in vitro study," *Journal of Orofacial Orthopedics,* vol. 76, no. 3, pp. 195-212, 2015.
18. R. Kunii, M. Yamaguchi, Y. Tanimoto et al., "Role of interleukin-6 in orthodontically induced inflammatory root resorption in humans," *Korean Journal of Orthodontics,* vol. 43, no. 6, pp. 294-301, 2013.
19. S. Y. Lee, H. I. Yoo and S. H. Kim, "CCR5-CCL axis in PDL during orthodontic biophysical force application," *Journal of Dental Research,* vol. 94, no. 12, pp. 1715-23, 2015.

20. F. Liu, F. Wen, D. He et al., "Force-induced H_2S by PDLSCs modifies osteoclastic activity during tooth movement," *Journal of Dental Research,* vol. 96, no. 6, pp. 694-702, 2017.
21. L. Liu, K. Igarashi, H. Kanzaki et al., "Clodronate inhibits PGE_2 production in compressed periodontal ligament cells," *Journal of Dental Research,* vol. 85, no. 8, pp. 757-60, 2006.
22. P. Luckprom, K. Kanjanamekanant and P. Pavasant, "Role of connexin43 hemichannels in mechanical stress-induced ATP release in human periodontal ligament cells," *Journal of Periodontal Research,* vol. 46, no. 5, pp. 607-15, 2011.
23. K. Mayahara, Y. Kobayashi, K. Takimoto et al., "Aging stimulates cyclooxygenase-2 expression and prostaglandin E_2 production in human periodontal ligament cells after the application of compressive force," *Journal of Periodontal Research,* vol. 42, no. 1, pp. 8-14, 2007.
24. K. Mayahara, A. Yamaguchi, M. Sakaguchi et al., "Effect of Ga-Al-As laser irradiation on *COX-2* and *$cPLA_2$-a* expression in compressed human periodontal ligament cells," *Lasers in Surgery and Medicine,* vol. 42, no. 6, pp. 489-93, 2010.
25. M. Mitsuhashi, M. Yamaguchi, T. Kojima et al., "Effects of HSP70 on the compression force-induced TNF-a and RANKL expression in human periodontal ligament cells," *Inflammation Research,* vol. 60, no. 2, pp. 187-94, 2011.
26. R. Nakajima, M. Yamaguchi, T. Kojima et al., "Effects of compression force on fibroblast growth factor-2 and receptor activator of nuclear factor kappa B ligand production by periodontal ligament cells *in vitro*," *Journal of Periodontal Research,* vol. 43, no. 2, pp. 168-73, 2008.
27. Y. Nishijima, M. Yamaguchi, T. Kojima et al., "Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells *in vitro*," *Orthodontics and Craniofacial Research,* vol. 9, no. 2, pp. 63-70, 2006.
28. S. Premaraj, I. Souza and T. Premaraj, "Mechanical loading activates β-catenin signaling in periodontal ligament cells," *Angle Orthodontist,* vol. 81, no. 4, pp. 592-9, 2011.
29. S. Premaraj, I. Souza and T. Premaraj, "Focal adhesion kinase mediates β-catenin signaling in periodontal ligament cells," *Biochemical and Biophysical Research Communications,* vol. 439, no. 4, pp. 487-92, 2013.
30. P. Proff, C. Reicheneder, A. Faltermeier et al., "Effects of mechanical and bacterial stressors on cytokine and growth-factor expression in periodontal ligament cells," *Journal of Orofacial Orthopedics,* vol. 75, no. 3, pp. 191-202, 2014.
31. P. Römer, J. Köstler, V. Koretsi et al., "Endotoxins potentiate COX-2 and RANKL expression in compressed PDL cells," *Clinical Oral Investigations,* vol. 17, no. 9, pp. 2041-8, 2013.
32. P. Tripuwabhrut, M. Mustafa, C. G. Gjerde et al., "Effect of compressive force on human osteoblast-like cells and bone remodelling: an *in vitro* study," *Archives of Oral Biology,* vol. 58, no. 7, pp. 826-36, 2013.
33. P. Tripuwabhrut, K. Mustafa, P. Brudvik et al., "Initial responses of osteoblasts derived from human alveolar bone to various compressive forces," *European Journal of Oral Sciences,* vol. 120, no. 4, pp. 311-8, 2012.
34. M. Wolf, S. Lossdörfer, K. Küpper et al., "Regulation of high mobility group box protein 1 expression following mechanical loading by orthodontic forces *in vitro* and *in vivo*," *European Journal of Orthodontics,* vol. 36, no. 6, pp. 624-31, 2014.
35. M. Wolf, S. Lossdörfer, R. Craveiro et al., "Regulation of macrophage migration and activity by high-mobility group box 1 protein released from periodontal ligament cells during orthodontically induced periodontal repair: an in vitro and in vivo experimental study," *Journal of Orofacial Orthopedics,* vol. 74, no. 5, pp. 420-34, 2013.
36. S. Wongkhantee, T. Yongchaitrakul and P. Pavasant, "Mechanical stress induces osteopontin expression in human periodontal ligament cells through rho kinase," *Journal of Periodontology,* vol. 78, no. 6, pp. 1113-9, 2007.
37. K. Yamada, M. Yamaguchi, M. Asano et al., "Th17-cells in atopic dermatitis stimulate orthodontic root resorption," *Oral Diseases,* vol. 19, no. 7, pp. 683-93, 2013.

38. M. Yamaguchi, Y. Ozawa, A. Nogimura et al., "Cathepsins B and L increased during response of periodontal ligament cells to mechanical stress in vitro," *Connective Tissue Research,* vol. 45, no. 3, pp. 181-9, 2004.
39. M. Yamaguchi, N. Aihara, T. Kojima et al., "RANKL increase in compressed periodontal ligament cells from root resorption," *Journal of Dental Research,* vol. 85, no. 8, pp. 751-6, 2006.
40. Y. Zhang, X. Kou, N. Jiang et al., "Effect of intraoral mechanical stress application on the expression of a force-responsive prognostic marker associated with system disease progression," *Journal of Dentistry,* vol. 57, pp. 57-65, 2017.

Supplement 3. Studies applying the 2D weight approach on human and non-human cells and cell lines not included in Table 1. For each gene or metabolite force magnitude and force duration, the change in gene expression or substance secretion (increase, decrease, no change), and the techniques for analysis applied are given.

Reference	Cell type (species) [a]	Gene symbol or metabolite	Examined force applied		Gene expression [c,d] (Increase/ decrease/ no change)	Substance secretion [d,e] (Increase/ decrease/ no change)
			Magnitude [g/cm^2][b]	Duration [h]		
Goga et al. 2006 [1]	MG63 (*H. s.*)	*CASP3*	2.0; 4.0 N/cm^2	12; 24	n. r.	Increase
Hayakawa et al. 2015 [2]	RAW264.7 (*M. m.*)	*Nfatc1*	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Tnfsf11	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Tnfrsf11a	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Ctsk	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Clcn7	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Mmp9	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Tcirg1	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Dcstamp	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Ocstamp	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Itgav	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Itgb3	0.114; 0.215; 0.301; 0.387; 0.53^{++}	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
Hoshina et al. 2004 [3]	Bone marrow derived osteoblasts (*R. n.*)	*Spp1*	0.9	12; 24; 72	No change (qPCR: GAPDH)	n. r.
		Bglap	0.9	12; 24; 72	Decrease (qPCR: GAPDH)	n. r.
		Alpl	0.9	12; 24; 72	n. r.	No change (activity)
Inubushi et al. 2014 [4]	ST-2 (*M. m.*)	*Tnf*	0.5	2; 24	Decrease (qPCR: 18S)	n. r.
		Ptgs2	0.5	2; 24	Increase (qPCR: 18S)	n. r.
		Tnfsf11	0.5	2; 24	Increase (qPCR: 18S)	n. r.
		Tnfrsf11b	0.5	2; 24	Decrease (qPCR: 18S)	n. r.
Koyama et al. 2008 [5]	Saos-2 (*H. s.*)	*IL1B*	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL6	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)

Reference	Cell type (species) [a]	Gene symbol or metabolite	Examined force applied		Gene expression [c,d] (Increase/ decrease/ no change)	Substance secretion [d,e] (Increase/ decrease/ no change)
			Magnitude [g/cm^2] [b]	Duration [h]		
		IL8 (CXCL8)	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	No change (qPCR: GAPDH)	No change (ELISA)
		IL11	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNF	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL1R1	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24;	Increase (qPCR: GAPDH)	n. r.
		IL6R	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24;	Increase (qPCR: GAPDH)	n. r.
		CXCR1	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24;	Increase (qPCR: GAPDH)	n. r.
		IL11RA	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24;	No change (qPCR: GAPDH)	n. r.
		TNFRSF1A	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24;	No change (qPCR: GAPDH)	n. r.
Matsunaga et al. 2016 [6]	Cementoblast cell line (HCEM-SV40) (*H.s.*)	*RUNX2*	0.25 gf/cm^2	12	Decrease$^+$ (qPCR: GAPDH)	n. r.
		ALPL	0.25 gf/cm^2	12	Decrease$^+$ (qPCR: GAPDH)	n. r.
		WNT5A	0.25 gf/cm^2	12	Decrease$^+$ (qPCR: GAPDH)	n. r.
		SPON1	0.25 gf/cm^2	12	Decrease$^+$ (qPCR: GAPDH)	n. r.
Mitsui et al. 2005 [7]	Saos-2 (*H.s.*)	*PTGS2*	1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		IBSP	0.5, 1.0, 2.0; 3.0 WB: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (WB)
		SPP1	1.0	1; 3; 6; 9; 12; 24	Increase followed by decrease (qPCR: GAPDH)	Increase (WB)
		PGE_2	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24	n. a.	Increase (ELISA)
Mitsui et al. 2006 [8]	Saos-2 (*H.s.*)	*MMP1*	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		MMP2	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		MMP3	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		MMP13	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		MMP14	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TIMP1	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TIMP2	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TIMP3	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		TIMP4	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		PLAT	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		PLAU	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		SERPINE1	0.5, 1.0, 2.0; 3.0; ELISA: 1.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	Increase (ELISA)
Mitsui et al. 2006 [9]	Saos-2 (*H.s.*)	*BMP2*	0.5, 1.0, 2.0; 3.0	1; 3; 6; 9; 12; 24; WB: 2	Increase (qPCR: GAPDH)	Increase (WB)
		BMP4	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	Increase (WB)
		BMP6	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	Increase (WB)
		BMP7	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	Increase (WB)

Reference	Cell type (species) [a]	Gene symbol or metabolite	Examined force applied		Gene expression [c,d] (Increase/ decrease/ no change)	Substance secretion [d,e] (Increase/ decrease/ no change)
			Magnitude [g/cm^2] [b]	Duration [h]		
		BMPR1A	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		BMPR1B	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		ACVR1	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		BMPR2	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		ACVR2A	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		ACVR2B	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		CHRD	0.5, 1.0, 2.0, or 3.0	9	Decrease followed by increase (qPCR: GAPDH)	n. r.
		GREM1	0.5, 1.0, 2.0, or 3.0	9	Decrease followed by increase (qPCR: GAPDH)	Decrease followed by increase (WB)
		FST	0.5, 1.0, 2.0, or 3.0	9	Decrease followed by no change (qPCR: GAPDH)	Decrease followed by no change (WB)
		NOG	0.5, 1.0, 2.0, or 3.0	9	Decrease followed by increase (qPCR: GAPDH)	Decrease followed by increase (WB)
		RUNX2	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		SP7	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		ZNF354C	0.5, 1.0, 2.0, or 3.0	9	Increase (qPCR: GAPDH)	n. r.
		SMAD1	1.0	9	n. d.	Increase p-Smad1 (WB)
Rego et al. 2011 [10]	OCCM-30 (*M.m.*)	*Bmp2*	0.2 kPa	12	Increase (qPCR: GAPDH)	n. r.
		Bglap2	0.2 kPa	12	Increase (qPCR: GAPDH)	n. r.
		Ptgs2	0.2 kPa	1; 3; 6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Tnfsf11	0.2 kPa	12; 24	Increase (qPCR: GAPDH)	n. r.
		Tnfrsf11b	0.2 kPa	12; 24	No change (qPCR: GAPDH)	n. r.
		PGE_2	0.2 kPa	6; 12;24	n. a.	Increase
		Ptger1	0.2 kPa	1	No change (qPCR: GAPDH)	n. r.
		Ptger2	0.2 kPa	1	No change (qPCR: GAPDH)	n. r.
		Ptger3	0.2 kPa	1	No change (qPCR: GAPDH)	n. r.
		Ptger4	0.2 kPa	1	No change (qPCR: GAPDH)	n. r.
Sanuki et al. 2010 [11]	MC3T3-E1 (*M.m.*)	*Ptgs2*	1.0; 3.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Csf1	1.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		Tnfsf11	1.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Increase (qPCR: GAPDH)	Increase (ELISA)
		Tnfrsf11b	1.0; 3.0	1; 3; 6; 9; 12; 24; ELISA: 24	Decrease (qPCR: GAPDH)	Decrease (ELISA)
		PGE_2	1.0; 3.0	24	n. a.	Increase (ELISA)
Takahashi et al. 2003 [12]	PDLC (*R. n.*)	*Mmp8*	0.1; 0.2; 0.3 kPa	72	Decrease (sqPCR: GAPDH)	n. r.
		Mmp13	0.1; 0.2; 0.3 kPa	72	Decrease (sqPCR: GAPDH)	n. r.

Reference	Cell type (species) [a]	Gene symbol or metabolite	Examined force applied		Gene expression [c,d] (Increase/ decrease/ no change)	Substance secretion [d,e] (Increase/ decrease/ no change)
			Magnitude [g/cm^2][b]	Duration [h]		
Yanagisawa et al. 2007 [13]	C2C12 (*M.m.*)	*Runx2*	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Msx2	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Dlx5	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Sp7	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Zfp354c	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Sox5	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Sox9	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Myod1	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		Pparg	0.25; 0.5, 1.0, 2.0	1; 3; 6; 9; 12; 24 (WB: 24)	Increase (qPCR: GAPDH)	Increase (WB)
		p38-MAPK *(*Antibody specificity not specified)*	0.5	WB/ELISA: 5, 10, 20, 30, 60 min	n. r.	Increased p-P38 MAPK (WB) Increased p-P38 MAPK (ELISA)
Zhang et al. 2010 [14]	MC3T3-E1 (*M.m.*)	*Il17a*	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17b	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17d	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17c	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il25	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17f	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17ra	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17rb	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17rd	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17rc	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il17re	1.0; 2.0	1; 3; 6; 9; 12; 24	Increase (qPCR: GAPDH)	n. r.
		Il1a	1.0; 2.0	2	n. r.	Increase (ELISA)
		Il6	1.0; 2.0	2	n. r.	Increase (ELISA)
Zhang et al. 2017 [15]	OCCM-30 (*M.m.*)	*Piezo1*	2.0	0; 3; 6; 9; 12; 24;	Decrease (qPCR: GAPDH)	Decrease (WB)
		Tnfrsf11b	2.0	0; 3; 6; 9; 12; 24;	Decrease (qPCR: GAPDH)	n. r.
		Spp1	2.0	0; 3; 6; 9; 12; 24;	Decrease (qPCR: GAPDH)	n. r.
		Bglap	2.0	0; 3; 6; 9; 12; 24;	Decrease (qPCR: GAPDH)	n. r.
		Hacd1	2.0	0; 3; 6; 9; 12; 24;	Decrease (qPCR: GAPDH)	n. r.
Zhou et al. 2013 [16]	U2OS (*H.s.*)	*RUNX2*	1.0	1; 4; 8; 12; 24	Decrease (qPCR: GAPDH)	n. r.
		BGLAP	1.0	1; 4; 8; 12; 24	Decrease (qPCR: GAPDH)	n. r.
		ALPL	1.0	1; 4; 8; 12; 24	Decrease (qPCR: GAPDH)	n. r.

Reference	Cell type (species) [a]	Gene symbol or metabolite	Examined force applied		Gene expression [c,d] (Increase/ decrease/ no change)	Substance secretion [d,e] (Increase/ decrease/ no change)
			Magnitude [g/cm^2][b]	Duration [h]		
		IBSP	1.0	1; 4; 8; 12; 24	Increase (qPCR: GAPDH)	n. r.
		IL1B	1.0	1; 4; 8; 12; 24	Increase (qPCR: GAPDH)	n. r.
		IL6	1.0	1; 4; 8; 12; 24	Increase (qPCR: GAPDH)	n. r.
		PTGS2	1.0	1; 4; 8; 12; 24	Increase (qPCR: GAPDH)	n. r.

[a] Origin of cells: *H. s. – H. sapiens*; *M. m. – M. musculus*; *R. n. – Rattus norvegicus*
[b] [+]Analysis was done 7, 14 or 21 days after WAB; [++] Calculated according to information given in the respective study
[c] qPCR – quantitative PCR (e.g. real time PCR); sqPCR – semi-quantitative PCR; followed by reference gene used
[d] n. r. – not reported; n. a. – not applicable
[e] ELISA – Enzyme linked immune absorbent assay; WB – western blot; IF – immunofluorescence; p-Smad1 – phosphorylated Smad1 ("similar to mothers against decapentaplegic 1"); p-P38 MAPK – phosphorylated P38 mitogen-activated protein kinases

References:

1. Y. Goga, M. Chiba, Y. Shimizu et al., "Compressive force induces osteoblast apoptosis *via* caspase-8," *Journal of Dental Research,* vol. 85, no. 3, pp. 240-4, 2006.
2. T. Hayakawa, Y. Yoshimura, T. Kikuiri et al., "Optimal compressive force accelerates osteoclastogenesis in RAW264.7 cells," *Mol Med Rep,* vol. 12, no. 4, pp. 5879-85, 2015.
3. S. Hoshina, K. Matsuzaka, Y. Motoyoshi et al., "Osteoblast-like cell behavior of rat bone marrow under continuous compressive force *in vitro*," *Biomedical Research,* vol. 25, no. 3, pp. 109-117, 2004.
4. T. Inubushi, A. Kawazoe, M. Miyauchi et al., "Lactoferrin inhibits infection-related osteoclastogenesis without interrupting compressive force-related osteoclastogenesis," *Archives of Oral Biology,* vol. 59, no. 2, pp. 226-32, 2014.
5. Y. Koyama, N. Mitsui, N. Suzuki et al., "Effect of compressive force on the expression of inflammatory cytokines and their receptors in osteoblastic Saos-2 cells," *Archives of Oral Biology,* vol. 53, no. 5, pp. 488-96, 2008.
6. K. Matsunaga, C. Ito, K. Nakakogawa et al., "Response to light compressive force in human cementoblasts *in vitro*," *Biomedical Research,* vol. 37, no. 5, pp. 293-298, 2016.
7. N. Mitsui, N. Suzuki, M. Maeno et al., "Optimal compressive force induces bone formation via increasing bone sialoprotein and prostaglandin E_2 production appropriately," *Life Sciences,* vol. 77, no. 25, pp. 3168-82, 2005.
8. N. Mitsui, N. Suzuki, Y. Koyama et al., "Effect of compressive force on the expression of MMPs, PAs, and their inhibitors in osteoblastic Saos-2 cells," *Life Sciences,* vol. 79, no. 6, pp. 575-83, 2006.
9. N. Mitsui, N. Suzuki, M. Maeno et al., "Optimal compressive force induces bone formation via increasing bone morphogenetic proteins production and decreasing their antagonists production by Saos-2 cells," *Life Sciences,* vol. 78, no. 23, pp. 2697-706, 2006.
10. E. B. Rego, T. Inubushi, A. Kawazoe et al., "Effect of PGE_2 induced by compressive and tensile stresses on cementoblast differentiation *in vitro*," *Archives of Oral Biology,* vol. 56, no. 11, pp. 1238-46, 2011.
11. R. Sanuki, C. Shionome, A. Kuwabara et al., "Compressive force induces osteoclast differentiation via prostaglandin E_2 production in MC3T3-E1 cells," *Connective Tissue Research,* vol. 51, no. 2, pp. 150-8, 2010.
12. I. Takahashi, M. Nishimura, K. Onodera et al., "Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats," *Journal of Dental Research,* vol. 82, no. 8, pp. 646-51, 2003.
13. M. Yanagisawa, N. Suzuki, N. Mitsui et al., "Effects of compressive force on the differentiation of pluripotent mesenchymal cells," *Life Sciences,* vol. 81, no. 5, pp. 405-12, 2007.

14. F. Zhang, C. L. Wang, Y. Koyama et al., "Compressive force stimulates the gene expression of IL-17s and their receptors in MC3T3-E1 cells," *Connective Tissue Research,* vol. 51, no. 5, pp. 359-69, 2010.
15. Y. Y. Zhang, Y. P. Huang, H. X. Zhao et al., "Cementogenesis is inhibited under a mechanical static compressive force via *Piezo1*," *Angle Orthodontist,* vol. 87, no. 4, pp. 618-624, 2017.
16. S. Zhou, J. Zhang, H. Zheng et al., "Inhibition of mechanical stress-induced NF-κB promotes bone formation," *Oral Diseases,* vol. 19, no. 1, pp. 59-64, 2013.

Supplement 4. Studies applying the 3D weight approach on human and non-human cells and cell lines. For each gene or metabolite force magnitude and force duration are given, the change in gene expression or substance secretion (increase, decrease, no change), and the techniques for analysis applied are given.

Reference	Cell type [a]	Gene symbol or metabolite	Scaffold [b]	Examined force applied		Gene expression [d,e] (Increase/ decrease/ no change)	Substance secretion [e,f] (Increase/ decrease/ no change)
				Magnitude [g/cm^2][c]	Duration [h]		
Santos de Araujo et al. 2014 [1]	hPDLC (exp)	F-actin (Triton X-100 insoluble fraction)	Collagen gel	6.0 (IF/WB: 3.0)	48	n. r.	Decrease (WB) Reversible inhibition of stress fibre formation (IF)
		RND3	Collagen gel	6.0 (IF/WB: 3.0)	12; 24	n. r.	Increase (WB)
		RHOA	Collagen gel	6.0 (IF/WB: 3.0)	12; 24	n. r.	No change (WB)
		RGS2	Collagen gel	6.0 (IF/WB: 3.0)	12; 24	n. r.	Increase (WB)
Santos de Araujo et al. 2007 [2]	hPLDF (?)	*RGS2*	Collagen gel	6.0	qPCR: 6; 12; 24 sqPCR: 6 WB: 12; 24; 48	Increase (sqPCR & qPCR: GAPDH)	Increase (WB)
		$cAMP_i$	Collagen gel	6.0	1; 3; 6; 12	n. r.	Increase (EIA)
de Araujo et al. 2007 [3]	hPDLC (exp)	PGE_2	Collagen gel	6.0	3; 12; 24; 48; 72	n. r.	Increase (EIA)
		PTGS2	Collagen gel	3.6; 6.0; 7.1; 9.5	1; 3; 6; 12; 24; 48; 72	Increase (sqPCR: GAPDH)	n. r.
		HSPA5	Collagen gel	6.0	6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		IL6	Collagen gel	6.0	6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		RND3	Collagen gel	6.0	6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		IL1B	Collagen gel	6.0	6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		RCAN1	Collagen gel	6.0	6; 12; 24	Increase (qPCR: GAPDH)	n. r.
		INP4A	Collagen gel	6.0	6; 12; 24	Decrease (qPCR: GAPDH)	n. r.
Kaku et al. 2016 [4]	hPDLC (dig)	*TNFRSF11B*	Collagen gel	0.5; 1.0; 2.0	12;24	Increase (qPCR: GAPDH)	n. r.
		COL1A2	Collagen gel	0.5; 1.0; 2.0	12;24	No change (qPCR: GAPDH)	n. r.
		LOX	Collagen gel	0.5; 1.0; 2.0	12;24	Increase (qPCR: GAPDH)	n. r.
		PLOD1	Collagen gel	0.5; 1.0; 2.0	12;24	No change (qPCR: GAPDH)	n. r.
		PLOD2	Collagen gel	0.5; 1.0; 2.0	12;24	Increase (qPCR: GAPDH)	n. r.
		PLOD3	Collagen gel	0.5; 1.0; 2.0	12;24	No change (qPCR: GAPDH)	n. r.
Kaneuji et al. 2011 [5]	MC3T3-E1 (*M. m.)*	*TNFRSF11B*	Collagen gel	7.5; ELISA: 2.5; 7.5	24; ELISA: 48	Increase (sqPCR: β-Actin)	Increase (ELISA)
Kang et al. 2013 [6]	hPDLC (dig)	*IL1B*	Collagen gel	2.0	2;48	Increase (qPCR: GAPDH)	n. r.
		TNF	Collagen gel	2.0	2;48	Increase (qPCR: GAPDH)	n. r.
		TNFSF11	Collagen gel	2.0	2;48	Increase (qPCR: GAPDH)	n. r.
		MMP3	Collagen gel	2.0	2;48	Increase (qPCR: GAPDH)	n. r.

Reference	Cell type [a]	Gene symbol or metabolite	Scaffold [b]	Examined force applied		Gene expression [d,e] (Increase/ decrease/ no change)	Substance secretion [e,f] (Increase/ decrease/ no change)
				Magnitude [g/cm^2][c]	Duration [h]		
		MMP13	Collagen gel	2.0	2;48	Increase (qPCR: GAPDH)	n. r.
Lee et al. 2007 [7]	hPDLC (?)	*ALPP*	Collagen gel	1.76	2; 12 ELISA: 72	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL6	Collagen gel	1.76	2; 12 ELISA: 72	Increase (qPCR: GAPDH)	Decrease (ELISA)
		CXCL8	Collagen gel	1.76	2; 12 ELISA: 72	Increase (qPCR: GAPDH)	No change (ELISA)
Li et al. 2016 [8]	hPDLC (dig)	*TNFSF11*	PLGA	25.0	6;24;72	Increase (qPCR: GAPDH)	n. r.
		TNFRSF11B	PLGA	25.0	6;24;72	Decrease (6h) followed by Increase (24,72h) (qPCR: GAPDH)	n. r.
		PTGS2	PLGA	25.0	6;24;72	Increase (qPCR: GAPDH)	n. r.
		IL1B	PLGA	25.0	6;24;72	Increase (qPCR: GAPDH)	n. r.
		HIF1A	PLGA	25.0	6;24;72	No change (qPCR: GAPDH)	n. r.
		VEGFA	PLGA	25.0	6;24;72	Increase (qPCR: GAPDH)	n. r.
Li et al. 2013 [9]	hPDLC (dig)	*CCL20*	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		STC1	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		IL1RN	PLGA	25.0	6; 24; 72	Increase (6; 24h) followed by decrease(72h) (qPCR: GAPDH)	n. r.
		NOG	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		FGF7	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		FOS	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		MAP3K8	PLGA	25.0	6; 24; 72	Decrease (6h) followed by increase (24; 72h) (qPCR: GAPDH)	n. r.
		JUN	PLGA	25.0	6; 24; 72	Decrease (6h) followed by increase (24; 72h) (qPCR: GAPDH)	n. r.
		CDK1	PLGA	25.0	6; 24; 72	Decrease (qPCR: GAPDH)	n. r.
		CCNA2	PLGA	25.0	6; 24; 72	Decrease (qPCR: GAPDH)	n. r.
		KIF11	PLGA	25.0	6; 24; 72	Decrease (qPCR: GAPDH)	n. r.
		KIF23	PLGA	25.0	6; 24; 72	Decrease (qPCR: GAPDH)	n. r.
		CYR61	PLGA	25.0	6; 24; 72	Decrease (6h) followed by increase (24; 72h) (qPCR: GAPDH)	n. r.
		COX1	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		PTGS2	PLGA	25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
Li et al. 2016 [10]	hPDLC (dig)	*TNFSF11*	PLGA	5.0; 15.0; 25.0	6; 24; 72	Increase (qPCR: GAPDH)	Decrease (ELISA)

Reference	Cell type [a]	Gene symbol or metabolite	Scaffold [b]	Examined force applied		Gene expression [d,e] (Increase/ decrease/ no change)	Substance secretion [e,f] (Increase/ decrease/ no change)
				Magnitude [g/cm^2][c]	Duration [h]		
		PTGS2	PLGA	5.0; 15.0; 25.0	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		PTHLH	PLGA	5.0; 15.0; 25.0	6; 24; 72	Increase (qPCR: GAPDH)	Increase (ELISA)
		IL11	PLGA	5.0; 15.0; 25.0	6; 24; 72	Increase (qPCR: GAPDH)	Increase (ELISA)
		TNFRSF11B	PLGA	5.0; 15.0; 25.0	6; 24; 72	Increase (qPCR: GAPDH)	Decrease followed by Increase (ELISA)
		PGE_2	PLGA	5.0; 15.0; 25.0	6; 24; 72	n. a.	Increase (ELISA)
Li et al. 2011 [11]	hPDLC (exp)	*TNFSF11*	PLGA	5; 15; 25; 35	6; 24; 72	Increase (6; 24h) followed by decrease (72h) (qPCR: GAPDH)	n. r.
		PTGS2	PLGA	5; 15; 25; 35	6	Increase (qPCR: GAPDH)	n. r.
		TNFRSF11B	PLGA	25	6; 24; 72	Decrease (6h) followed by increase (24; 72h) (qPCR: GAPDH)	n. r.
		IL1B	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		CXCL8	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		IL11	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		FGF2	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		PTHLH	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		RUNX2	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		BMP2	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		POSTN	PLGA	25	6; 24; 72	Decrease (qPCR: GAPDH)	n. r.
	hGF (exp)	*CXCL8*	PLGA	25	6; 24; 72	Increase (qPCR: GAPDH)	n. r.
		TNFSF11	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		TNFRSF11B	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		PTHLH	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		IL11	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
		FGF2	PLGA	25	6; 24; 72	No change (qPCR: GAPDH)	n. r.
Liao et al. 2016 [12]	hPDLC ($$)	*TNFSF11*	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	Increase (qPCR: GAPDH)	n. r.
		BMP2	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	Increase (qPCR: GAPDH)	n. r.
		ASPN	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	Increase (qPCR: GAPDH)	n. r.
		ALPP	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	Increase (qPCR: GAPDH)	n. r.
		TNFRSF11B	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	No change (qPCR: GAPDH)	n. r.
		COL1A1	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	Decrease (qPCR: GAPDH)	n. r.
		FGF2	PLLA modif.	5.0; 15.0; 25.0; 35.0	1d; 3d; 7d; 14d	No change (qPCR: GAPDH)	n. r.
Shen et al. 2017 [13]	MC3T3-E1/SC14	*Runx2*	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Increase (WB)

Reference	Cell type [a]	Gene symbol or metabolite	Scaffold [b]	Examined force applied		Gene expression [d,e] (Increase/ decrease/ no change)	Substance secretion [e,f] (Increase/ decrease/ no change)
				Magnitude [g/cm^2][c]	Duration [h]		
		Alp	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Increase (WB); Increase (Activity)
		Ocn	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Increase (WB)
		Rankl	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Not detectable (ELISA)
		Opg	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Increase (ELISA)
	mOB	*Runx2*	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	n. r.
		Alp	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	Increase (Activity)
		Ocn	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	n. r.
		Rankl	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	n. r.
		Opg	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	n. r.
		Ocn	Collagen gel	0; 1; 2; 3; 4; 5	24	Increase (qPCR: Bactn)	n. r.
Jianru et al. 2015 [14]	hPDLC (dig)	*TNFSF11*	PLGA	25.0	3; 6; 12 (WB: 12)	Increase (qPCR: GAPDH)	Increase (WB)
		TNFRSF11B	PLGA	25.0	3; 6; 12 (WB: 12)	Decrease (3h) followed by increase (6,12h) (qPCR: GAPDH)	Increase (WB)
		NFATC2	PLGA	25.0	3; 6; 12	Increase (qPCR: GAPDH)	n. r.
Yi et al. 2016 [15]	hPDLC (exp)	*TNFSF11*	PLGA	25.0	24	Increase (qPCR: GAPDH)	Increase (WB)
		TNFRSF11B	PLGA	25.0	24	Decrease (qPCR: GAPDH)	No change (WB)
		PTHLH	PLGA	25.0	24	Increase (qPCR: GAPDH)	n. r.
		PTGS2	PLGA	25.0	24	Increase (qPCR: GAPDH)	Increase (WB)
		CXCL8	PLGA	25.0	24	Increase (qPCR: GAPDH)	n. r.
		IL11	PLGA	25.0	24	Increase (qPCR: GAPDH)	n. r.
		PGE_2	PLGA	25.0	24	n. a.	Increase (ELISA)

[a] hPDLC (exp) – hPDLC, isolated with explant method; hPDLC (dig) – hPDLC, isolated with digestion method, hPDLC (?) – hPDLC, isolation method not given; hPDLC ($$) – hPDLC from commercial sources; hOB – human osteoblasts; hOBMC – human oral bone marrow cells; hGF – human gingival fibroblasts
Origin of non-human cells: *M. m.* – *M. musculus*
[b] PLGA – Poly lactic-co-glycolic acid; PLLA modif. – Hydrophilically modified poly-L-lactide acid matrix
[c] IF – immunofluorescence; WB – western blot; ELISA - Enzyme linked immune absorbent assay;
[d] qPCR – quantitative polymerase chain reaction (e.g. real time PCR); sqPCR – semi-quantitative polymerase chain reaction; followed by reference gene used
[e] n. r. – not reported; n. a. – not applicable
[f] ELISA – Enzyme linked immune absorbent assay; WB – western blot; IF – immunofluorescence

References

1. R. M. Santos de Araujo, Y. Oba, S. Kuroda et al., "RhoE regulates actin cytoskeleton organization in human periodontal ligament cells under mechanical stress," *Archives of Oral Biology,* vol. 59, no. 2, pp. 187-92, 2014.
2. R. M. Santos de Araujo, Y. Oba and K. Moriyama, "Role of regulator of G-protein signaling 2 (RGS2) in periodontal ligament cells under mechanical stress," *Cell Biochemistry and Function,* vol. 25, no. 6, pp. 753-8, 2007.
3. R. M. de Araujo, Y. Oba and K. Moriyama, "Identification of genes related to mechanical stress in human periodontal ligament cells using microarray analysis," *Journal of Periodontal Research,* vol. 42, no. 1, pp. 15-22, 2007.
4. M. Kaku, J. M. Rosales Rocabado, M. Kitami et al., "Mechanical loading stimulates expression of collagen cross-linking associated enzymes in periodontal ligament," *Journal of Cellular Physiology,* vol. 231, no. 4, pp. 926-33, 2016.
5. T. Kaneuji, W. Ariyoshi, T. Okinaga et al., "Mechanisms involved in regulation of osteoclastic differentiation by mechanical stress-loaded osteoblasts," *Biochemical and Biophysical Research Communications,* vol. 408, no. 1, pp. 103-9, 2011.
6. K. L. Kang, S. W. Lee, Y. S. Ahn et al., "Bioinformatic analysis of responsive genes in two-dimension and three-dimension cultured human periodontal ligament cells subjected to compressive stress," *Journal of Periodontal Research,* vol. 48, no. 1, pp. 87-97, 2013.
7. Y. H. Lee, D. S. Nahm, Y. K. Jung et al., "Differential gene expression of periodontal ligament cells after loading of static compressive force," *Journal of Periodontology,* vol. 78, no. 3, pp. 446-52, 2007.
8. M. L. Li, J. Yi, Y. Yang et al., "Compression and hypoxia play independent roles while having combinative effects in the osteoclastogenesis induced by periodontal ligament cells," *Angle Orthodontist,* vol. 86, no. 1, pp. 66-73, 2016.
9. Y. Li, M. Li, L. Tan et al., "Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression," *Archives of Oral Biology,* vol. 58, no. 5, pp. 511-22, 2013.
10. M. Li, J. Yi, Y. Yang et al., "Investigation of optimal orthodontic force at the cellular level through three-dimensionally cultured periodontal ligament cells," *European Journal of Orthodontics,* vol. 38, no. 4, pp. 366-72, 2016.
11. Y. Li, W. Zheng, J. S. Liu et al., "Expression of osteoclastogenesis inducers in a tissue model of periodontal ligament under compression," *Journal of Dental Research,* vol. 90, no. 1, pp. 115-20, 2011.
12. W. Liao, M. Okada, K. Inami et al., "Cell survival and gene expression under compressive stress in a three-dimensional in vitro human periodontal ligament-like tissue model," *Cytotechnology,* vol. 68, no. 2, pp. 249-60, 2016.
13. X. Q. Shen, Y. M. Geng, P. Liu et al., "Magnitude-dependent response of osteoblasts regulated by compressive stress," *Scientific Reports,* vol. 7, pp. 44925, 2017.
14. Y. I. Jianru, L. I. MeiLe, Y. Yang et al., "Static compression regulates OPG expression in periodontal ligament cells via the CAMK II pathway," *J Appl Oral Sci,* vol. 23, no. 6, pp. 549-54, 2015.
15. J. Yi, B. Yan, M. Li et al., "Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression," *Archives of Oral Biology,* vol. 64, pp. 51-60, 2016.

PUBLICATION 2

Effect of the static compressive force on in vitro cultured PDL fibroblasts: monitoring of the viability and gene expression over six days

Mila Janjic Rankovic, Denitsa Docheva, Andrea Wichelhaus and Uwe Baumert

Clinical Oral Investigation [Epub ahead of print, 15-11-2019]

doi: 10.1007/s00784-019-03113-6

ADDITIONAL CONTRIBUTIONS

Name of the project: Systematic exploration/analysis of the caries diagnostic studies

*This project consists of four studies**.** The development of the studies is still in progress. Therefore, they are considered as **unpublished** work, included as **"additional contributions"** in the cumulative dissertation. The project is coordinated by Prof. Dr. Jan Kühnisch from the Department of Operative Dentistry and Periodontology, University Hospital, Ludwig-Maximilians-Universität München.*

Two systematic reviews with meta-analyses on caries diagnostic studies were conducted to identify and summarize so far published knowledge in this field. Reviews were done separately for studies performed on proximal and occlusal tooth surfaces. Analysing all identified studies in detail, it became obvious that the methodology of many caries diagnostic studies is heterogeneous and therefore, the comparability of their findings is limited. Aiming to eliminate these problems, a scientific network compiled of experts in the field and young scientist was formed with the aim to establish and recommend standards for future studies in a form of checklist adapted for specific needs in caries diagnostic trials: STAndard Reporting requirements in CARies Diagnostic Studies (STARD checklist). Also, a tool to assess internal validity of caries diagnostic studies was developed (tailor-made risk of bias (RoB) -analysis), and has been additionally applied in 2 meta-analyses.

From October 2017 – March 2019, three 2-day workshops were conducted to discuss, evaluate, agree and publish the findings from the literature in a form of two systematic reviews and to develop consensus recommendations.

Mila Janjić Ranković will be the first author in Systematic review with meta-analyses on caries diagnostic studies performed on proximal surfaces. Further, as the co-author in systematic review with meta-analyses on caries diagnostic studies performed on occlusal surfaces, she contributed by performing in parallel all steps concerning the literature search, identification of the studies, data extraction and RoB evaluation. As the result of the overall work two consensus papers on the methodology of caries detection and diagnostic studies are planned. The work group aim at finishing the work on the project papers until the end of the 2019.

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REFERENCES

Baumert U, Golan I, Becker B, Hrala BP, Redlich M, Roos HA, Palmon A, Reichenberg E, Mussig D (2004). Pressure simulation of orthodontic force in osteoblasts: a pilot study. Orthod Craniofac Res; 7(1):3-9.

Davidovitch Z (1991). Tooth movement. Crit Rev Oral Biol Med; 2(4):411-50.

Davidovitch Z, Krishnan V (2015). Biological basis of orthodontic tooth movement. Biological Mechanisms of Tooth Movement.]: Wiley.

Janjic M, Docheva D, Trickovic Janjic O, Wichelhaus A, Baumert U (2018). In Vitro Weight-Loaded Cell Models for Understanding Mechanodependent Molecular Pathways Involved in Orthodontic Tooth Movement: A Systematic Review. Stem Cells Int; 2018:3208285.

Janjic Rankovic M, Docheva D, Wichelhaus A, Baumert U (2019). Effect of the static force on in vitro cultured PDL fibroblasts: monitoring of the viability and gene expression over six days. Clin Oral Investig; DOI: 10.1007/s00784-019-03113-6.

Kang KL, Lee SW, Ahn YS, Kim SH, Kang YG (2013). Bioinformatic analysis of responsive genes in two-dimension and three-dimension cultured human periodontal ligament cells subjected to compressive stress. J Periodontal Res; 48(1):87-97.

Kang YG, Nam JH, Kim KH, Lee KS (2010). FAK pathway regulates PGE_2 production in compressed periodontal ligament cells. J Dent Res; 89(12):1444-9.

Kanzaki H, Chiba M, Shimizu Y, Mitani H (2002). Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis. J Bone Miner Res; 17(2):210-20.

Kim SJ, Park KH, Park YG, Lee SW, Kang YG (2013). Compressive stress induced the up-regulation of M-CSF, RANKL, TNF-a expression and the down-regulation of OPG expression in PDL cells via the integrin-FAK pathway. Arch Oral Biol; 58(6):707-16.

Liao W, Okada M, Inami K, Hashimoto Y, Matsumoto N (2016). Cell survival and gene expression under compressive stress in a three-dimensional in vitro human periodontal ligament-like tissue model. Cytotechnology; 68(2):249-60.

Mayahara K, Kobayashi Y, Takimoto K, Suzuki N, Mitsui N, Shimizu N (2007). Aging stimulates cyclooxygenase-2 expression and prostaglandin E_2 production in human periodontal ligament cells after the application of compressive force. J Periodontal Res; 42(1):8-14.

Reitan K (1960). Tissue behavior during orthodontic tooth movement. Am J Orthod; 46(12):881-900.

Schröder A, Bauer K, Spanier G, Proff P, Wolf M, Kirschneck C (2018). Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement. J Orofac Orthop; 79(5):337-351.

Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C (2017). The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res; 45(D1):D362-d368.

Vansant L, Cadenas De Llano-Perula M, Verdonck A, Willems G (2018). Expression of biological mediators during orthodontic tooth movement: A systematic review. Arch Oral Biol; 95:170-186.

Wichelhaus A (2017). Orthodontic Therapy - Fundamental Treatment Concepts. New York: Georg Thieme.

Yang L, Yang Y, Wang S, Li Y, Zhao Z (2015). *In vitro* mechanical loading models for periodontal ligament cells: from two-dimensional to three-dimensional models. Arch Oral Biol; 60(3):416-24.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all who contributed to the development of my PhD thesis:

Prof. Wichelhaus who has been a truly dedicated and supportive mentor over previous years. I appreciate her conscientious guidance and encouragement to accomplish this assignment. It has been truly an honour and a privilege working as a part of her team

Dr. Baumert who introduced me to the topic of this thesis and shared his passion for research with me. I am extremely thankful for all valuable guidance and fruitful discussions during our daily coffee meetings. He cared so much about my work, and always responded to my questions and queries promptly.

Prof. Kühnisch, who I am also hugely appreciative for great support, advice and assistance. I would like to thank him for teaching me everything about systematic reviews and for the great discussions during PhD seminars.

Prof. Docheva for great ideas, advice and invaluable support.

Ms. Schreindorfer, Ms. Müller and **Dr. Shi** for teaching me new techniques in the lab and always being there for me.

Ms. Hackl and **Dr. Diegelmann** for being there to answer my questions and help me.

I gratefully acknowledge the funding I received from **BAYHOST** (Bayerisches Hochschulzentrum für Mittel-, Ost- und Südosteuropa, Regensburg, Germany) and from the **Fund for Young Talents of the Republic of Serbia** (Government of the Republic of Serbia, Ministry of Youth and Sports, Belgrade, Serbia).

My thanks and appreciations also go to my **colleagues from the PhD program**. I remember all nice discussions and invaluable moments during our studies.

At the end I would like to express the special gratitude to my **beloved husband Djordje** and **my parents** for supporting my dreams and ambitions and being always by my side.

LIST OF PUBLICATIONS

Journal publications

Janjic Rankovic M, Docheva D, Wichelhaus A, Baumert U. Effect of the static compressive force on in vitro cultured PDL fibroblasts: monitoring of the viability and gene expression over six days. Clin Oral Investig. 2019; DOI: 10.1007/s00784-019-03113-6.

Janjic M, Docheva D, Trickovic Janjic O, Wichelhaus A, Baumert U. In vitro weight-loaded cell models for understanding mechanodependent molecular pathways involved in orthodontic tooth movement: a systematic review. Stem Cells Int. 2018; 2018: 3208285.

Petrovic M, Kesic L, Obradovic R, Savic Z, Mihailovic D, Obradovic I, Avdic-Saracevic M, Janjic-Trickovic O, **Janjic M**. Comparative analysis of smoking influence on periodontal tissue in subjects with periodontal disease. Mater Sociomed. 2013; 25(3):196-8.

Presentations at Congresses

Janjic M, Wichelhaus A, Baumert U. Comparison of Two Controls Used in WAB In Vitro Experiments. CED-IADR/NOF Oral Health Research Congress. 19.-21.09.2019, Madrid, Spain.

Janjic M, Wichelhaus A, Baumert U. In vitro loading of PDL cells: viability and gene expression over 6 days. The Göttingen Spirit Summer School: Symposium on mineralization and biometric concepts in dental research. 18.-19.09.2018, Göttingen, Germany.

Janjic M, Docheva D, Wichelhaus A, Baumert U. In vitro Models for Studying Bone Remodeling in Orthodontics: a Systematic Review. 25th Annual Meeting of the European Orthopaedic Research Society. 13.-15.09.2017, Munich, Germany.

Tričković Janjić O, Nikolić M, **Janjić M**, Stojkovic B. Significance of Sealant Retention Related To Caries [Poster]. 22. Balkan Stomatological Society Congress. 4.-6.05.2017, Thessaloniki, Greece.

Janjic M, Pancic D. Presence of the osteoclasts in mandible of the human embryo from ninth to twelfth week of gestation. 6th International Pirogov Scientific Medical Conference of Students and Young Scientists. 24.03.2011, Moscow, Russia.

Janjic M, Kostic M. Hromogranin A-Immunoreactivity in hypophysis and pharyngeal hypophysis of the human embryo. 5th International Pirogov Scientific Medical Conference of Students and Young Scientists. 18.03.2010, Moscow, Russia.

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