**Table S1**. List of the reviewed in vitro PDL mechanical loading studies. The reviewed studies investigating the influence of mechanical loading on PDL cells or constructs are listed reporting the applied mechanical stimulus, the loading method, the cell type, the culture method, the stimulation protocol, and the main results.

| Stimulus | Loading method | Cells | Culture method | Stimulation protocol | Results | Study |
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| Compression | Weight | hPDL cells; human gingival fibroblasts | 3D culture on PLGA scaffold | 5, 15, 25, or 35 g/cm² for 6 h<br>25 g/cm² for 6, 24, or 72 h | Upregulation of mRNA expression of PTHrP, IL-11, IL-8, and FGF-2 (potential osteoclastogenesis inducers) in hPDL cells by force ≥ 25 g/cm²<br>Up-regulation of osteoclastogenic genes in hPDL cells at 6 h<br>Increase of osteoclastogenesis inhibitor genes and reduction of hPDL cell proliferation at 24-72 h | Li et al., 2011 and 2013 [126, 127] |
| | | hPDL cells | 2D culture vs 3D collagen gel | 2.0 g/cm² for 2 or 48 h | After 2/48 h, changes in the expression levels of 191/280 and 553/519 genes in 2D and 3D cultured cells, respectively<br>Higher number of activated MAPK and FAK pathways in 3D than in 2D culture | Kang et al., 2013 [128] |
| | | hPDLSCs | - | 1 g/cm² for 24 h | Alteration of cell morphology<br>Reduction of collagen expression<br>Recovery after force withdrawal | Feng et al., 2016 [129] |
| | | rat primary PDLSCs | - | 0-1.5 g/cm² for 12 h | Greater clonogenicity and proliferation<br>Reduced differentiation ability<br>Improved macrophage migration, osteoclast differentiation, and proinflammatory factor expression<br>Upregulation of TRPV4 regulating osteoclast differentiation by affecting the RANKL/OPG system via ERK signalling | Jin et al., 2020 [130] |
| | | hPDLFs | - | 2 g/cm² for 24, 48, and 72 h | Change of morphology (disorganization of actin filaments)<br>Inhibition of proliferation and reduction of viability, followed by a recovery and adaptation after 48 h | Brockhaus et al., 2021 [131] |

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| | hPDLFs | - | 2 g/cm² for 6 h + periodontal pathogens | Inflammatory response supported by GDF15 | Stemmler et al., 2021 [132] |
| | hPDLSCs | - | 0.5-2.5 g/cm² for 12 h | Increase of the autophagy protein LC3, inducing M1 macrophage polarization via the inhibition of the AKT signaling pathway | Jiang et al., 2021 [133] |
| Hydrostatic pressure | hPDL cells | - | 1 or 6 MPa, 10 or 60 min | Increase of RANKL and OPG mRNA expression | Yamamoto et al., 2006 [135] |
| | hPDLFs | - | 150 psi (~1 MPa), 0.1 Hz, 3 h/day for 2 days | Increase of integrins, collagens, and metalloproteinase expression | Wenger et al., 2011 [136] |
| | hPDLSCs | - | 100 kPa, for 1, 6 or 12 h | Increase of RUNX2 and Sp7 genes expression after 1 and 6 h and of ALP release after 1 h<br>Decrease in RUNX2 and Sp7 genes expression after 12 h<br>No changes in ALP release after 12 h | Zhang et al., 2016 [134] |
| | hPDL cells | - | 50 - 150 kPa, 0.1 Hz, 1 h/day for 5 days | No changes in osteogenic markers expression (at 50 kPa)<br>Increase of osteogenic markers expression (at 90 kPa)<br>Decrease of osteogenic markers expression (at 150 kPa) | Jia et al., 2020 [138] |
| Compression device | hPDL cells | 3D culture on matrix of hyaluronan, gelatin, and COLL-1 | 340.6 g/cm², for 1 s every 60 s, for 6, 12, and 24 h | Increase of cell death (12 and 14 h)<br>Increase of ECM genes expression, MMP-1, and TIMP-1 protein levels (24 h)<br>No changes in RANKL, OPG, and FGF-2 expression | Saminathan et al., 2015 [139] |

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| | | hPDLFs | - | 5 and 10% for 12 h | Decrease of cell viability at 10% of compression<br>Increase of ALP gene expression and RANKL/OPG ratio at 5% of compression | Nettelhoff et al., 2016 [140] |
| **Stretch** | Vacuum | hPDLFs | 3D culture on 80-100 µm thick collagen film | 12%, 0.2 Hz, for 6 h/day, for 24 h, 7, 14 or 21 days | Increase of MMP-1 and TIMP-1 protein levels in the supernatants and of COL3A1, MMP3, TIMP1, and OPG gene expression<br>ECM remodelling | Saminathan et al., 2013 [141] |
| | | hPDLSCs | - | 12%, 0.1 Hz for 6, 12 or 24 h | Up-regulation of RUNX2, ALP, and OCN mRNA expression (after 24 h) | Shen et al., 2014 [143] |
| | | hPDLSCs | - | 10%, 1 Hz, for 6, 12, 24 or 48 h | Cell alignment in the strain direction<br>Time-dependent increase of miR-21 expression and ALP activity until 48 h | Wei et al., 2015 [144] |
| | | hPDL cells | - | 12%, 0.1 Hz, for 24 h | Increase of COL1A1, COL3A1 and COL5A1 gene expression<br>ECM remodelling | Chen et al., 2015 [142] |
| | | Pathological and healthy hPDLSCs | - | 6, 8, 10, 12 or 14%, for 12 h | Increase of cell proliferation and osteogenic capacity until 8% for pathological cells and until 12% for healthy cells | Liu et al., 2017 [148] |
| | | hPDLSCs | - | 10%, 1 Hz, for 12 h | Cell alignment in the strain direction<br>Up-regulation of RUNX2, OCN and Sp7 expression | Wang et al., 2019 [145] |
| | | hPDLSCs | - | 10%, 0.1 Hz, for 24 or 72 h | Increase of ALP activity<br>Upregulation of OPN, RUNX2, COLL-1, ALP, OCN and OSX expression | Meng et al., 2022 [147] |

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| | | hPDLSCs | - | 10%, 0.5 Hz, for 1, 3, 6, 12, 24 or 36 h | Generation of ROS<br>Nuclear accumulation of Nrf2<br>Upregulation of OPN, RUNX2, COLL-1 and ALP expression and ALP activity after 3h until 36 h | Xi et al., 2021 [146] |
| | | hPDL cells | - | 2.5, 5 and 10%, for 24 h | Upregulation of CASA complex component gene expression | Salim et al., 2022 [149] |
| | Substrate pulling | hPDL cells | - | 5%, 60 s/returns, resting time = 29 s, for up to 7 days | Decrease of fibrin forming and FACIT collagens mRNA expression (at 48 h)<br>Increase of non-fibril forming collagens (at 48 h)<br>Increase of COLL-1, 4 and 12 from day 2 to day 7 | Nemoto et al., 2010 [153] |
| | | rat PDLFs | 3D culture on 200 μm thick collagen film | 8%, 1 Hz for 8 h/day for 1, 3 or 5 days | Cell alignment perpendicular to the stretching direction (at day 5)<br>Increase of RUNX2 and COLL-1 gene expressions<br>No changes in C-fos and Cox-2 gene expressions | Oortgiensen et al., 2012 [154] |
| | | hPDLFs | - | 8%, 1 Hz, for 15, 30, 60, 180 min or 16 h (static vs cyclic strain) | Cell alignment perpendicular to the stretching direction under cyclic strain (at 16 h)<br>Activation of ERK, p38 and JNK signalling pathways by cyclic strain (at 15 min), the activation returned to the basal level at 180 min<br>Increase of C-fos expression after 15 min of cyclic and static strain (higher expression in cyclic) | Papadopoulou et al., 2017 [155] |

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| | | hPDLSCs | 3D culture on collagen membrane | 20%, for 5 days | No changes in cell viability<br>Increase of the bioactivity of the PDLSC-derived exosomes | Yu et al., 2021 [156] |
| | Substrate inflation | hPDL cells | - | 1, 10 and 20%, 0.1 Hz for 30 min, 1 and 24 h | Cell alignment perpendicular to the stretching direction (at 24 h)<br>Increase of Cx43 gene expression (after 24 h at 10 and 20%) | Xu et al., 2012 [158] |
| | Substrate bending | hPDLSCs | - | 3000 μstrain, 0.5 Hz, for 3, 6, 12, or 24 h | Increase of Satb2, RUNX2 and OSX mRNA expression and protein levels | Tang et al., 2012 [158] |
| Shear stress | Tangential fluid flow | hPDL cells | - | 12 dyn/cm$^2$ for 2 h | Promotion of osteogenic differentiation<br>Increase of ALP activity, expressed osteogenic genes and mineralized nodule formation | Tang et al., 2014 [160] |
| | | hPDL cells | - | 6 dyn/cm$^2$ for 2, 4, 8, 12 h | Induction of osteogenic differentiation (>8 h)<br>Cell alignment along flow direction (2-8 h)<br>Inhibition of proliferation and migration (12 h) | Zheng et al., 2016 [161] |
| | | Immortalized hPDL cells | 3D culture on microfibrous scaffold of COLL-1 | 6 dyn/cm$^2$ for 1, 4 h | Increase of cell spreading, adhesion, and viability | Lin et al., 2021 [164] |
| | | hPDL cells | - | 1, 3, 6, or 9 dyn/cm$^2$ up to 4 h | Promotion of cell proliferation (1-6 dyn/cm$^2$)<br>Inhibition of cell proliferation (9 dyn/cm$^2$)<br>Maintenance of cell viability<br>Increase of F-actin filament density | Shi et al., 2022 [162] |

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| | | | | | Promotion of actin cap expansion, nuclei compression, and increased nuclear pore size<br>Activation of ERK, p38, and JNK<br>Time dependent regulation of AMOT and YAP localization | |
| | Sliding plate | hPDL cells | 3D culture on COLL-1 gel | Static shear strain resulting in 1.36 ± 0.53 Pa for 24 h | Alignment of collagen fibers and cells along principal strain vector | Kim et al., 2011 [163] |

Acronyms: Alkaline phosphatase (ALP); Angiomotin (AMOT); Basic fibroblast growth factor (FGF-2); Bone morphogenetic protein 2 (BMP-2); Chaperone-assisted selective autophagy (CASA); c-Jun N-terminal kinases (JNK); Collagen type I, alpha-1 (COL1A1); Collagen type III, alpha-1 (COL3A1); Collagen type V, alpha-1 (COL5A1); Connexin 43 (Cx43); Cyclooxygenase-2 (Cox-2); Extracellular matrix (ECM); Extracellular signal-regulated kinase (ERK); Fibril-associated with interrupted triple helices (FACIT); Focal adhesion kinase (FAK); Growth differentiation factor 15 (GDF15); human periodontal ligament (hPDL); human periodontal ligament fibroblasts (hPDLFs); Human periodontal ligament stem cells (hPDLSCs); Interleukin 11 (IL-11); Interleukin 8 (IL-8); Matrix metalloproteinase-1 (MMP-1); Matrix metalloproteinase-3 (MMP-3); MicroRNA-21 (miR-21); Microtubule-associated protein 1A/1B-light chain 3 (LC3); Mitogen-activated protein kinase (MAPK); Nuclear factor erythroid-2-related factor-2 (Nrf2); Osteocalcin (OCN); Osteoprotegerin (OPG); Osterix (OSX); p38 mitogen-activated protein kinases (p38); Parathyroid hormone-related protein (PTHrP); Reactive oxygen species (ROS); Receptor activator of nuclear factor kappa-B ligand (RANKL); Runt-related transcription factor 2 (RUNX2); Serine/threonine-specific protein kinases (AKT); Sp7 transcription factor 7 (Sp7); Special AT-rich sequence-binding protein 2 (Satb2); Tissue inhibitor of metalloproteinases-1 (TIMP-1); Transient Receptor Potential Cation Channel Subfamily V Member 4 (TRPV4); Yes-associated protein (YAP).