

Effect of mechanical forces on the behavior of osteoblasts: a systematic review of in vitro studies

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Abstract

Mechanical loading can play a critical role in bone modeling/remodeling through osteoblasts, with several factors being involved in this process.

The present study aims to systematically review the effect of mechanical stimulation on human osteoblast cell lineage combined with other variables.

The PubMed and Scopus databases were electronically searched for studies analyzing the effect of compression and tension on human osteoblasts at different differentiation stages. Studies that used carcinogenic osteoblasts were excluded. In addition, studies that did not analyze the osteogenic differentiation or proliferation of cells were excluded. The risk of bias of the studies was evaluated using the modified CONSORT (Consolidated Standards of Reporting Trials) checklist. A total of 20 studies were included. The cells were subjected to tension and compression in 5 and 15 studies, respectively. The application of uniaxial and cyclic strain increased the proliferation of osteoblasts. The same increased pattern could be observed for the osteogenesis of the cells. The impact of the tensile force on the expression of the osteoclastic markers differed based on the loading characteristics. On the other side, the impact of compression on the proliferation of osteoblasts varied according to the magnitude and duration of the force. Besides, different patterns of alternations were observed among the osteogenic markers in response to compression. Meanwhile, compression increased the expression of the osteoclastic markers. It has been shown that the response of the markers related to bone formation or resorption can be altered based on the differentiation stage of the cells, the cell culture system, and the magnitude and duration of the force.

Keywords: proliferation, differentiation, compression, osteoblast, tension

Cite as

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Introduction

Mechanical forces of physiological magnitudes applied to the bone tissue of the human body lead to the preservation and strengthening of the body's bone mass. Weightlessness caused by space flight decreases bone mineralization, while moderate mechanical loading, such as regular exercise, can improve bone tissue mineralization and density.^{1,2} The force produced during occlusion is naturally transmitted to the alveolar bone through the periodontal ligament fibers. It was reported that masticatory hypofunction, followed by a chronic soft diet intake, reduces bone mass.³ In distraction osteogenesis, mechanical strain induces bone formation by osteoblasts.⁴ The clinical success of orthopedic and dental implant osseointegration relies on appropriate mechanical loading factors.⁵

Mechanical loading can cause biological changes in various human cells. It stimulates the osteogenic differentiation in human amniotic epithelial cells and human dental pulp stem cells.^{6,7} Compressive forces induce bone resorption and remodeling in human periodontal ligament cells.^{8,9} External cyclic forces enhance osteogenic differentiation of mesenchymal stem cells (MSC) of the axial skeleton through Notch signaling induction.¹⁰ Extracellular matrix production and tenogenic differentiation of human adipose-derived stem cells are enhanced by receiving an appropriate mechanical loading regimen.¹¹

The osteoblast lineage includes bone-forming and bone-remodeling cells in the human body¹² that play critical physiologic and therapeutic roles by responding to various types of stimuli caused by mechanical forces. These cells are mechanical sensors that can transduce mechanical stimuli into biochemical signals (cell-to-cell communication or the production of paracrine factors).¹³ Therefore, mechanical forces can affect the bone modeling/remodeling process. A wide range of in vitro experiments have investigated gene expression and proliferation changes of osteoblasts under different methods of loading application.¹⁴ Mechanical strain can affect bone formation using bone matrix deformation and, therefore, the fluid shift within the osteocyte's canaliculi.¹⁵

In orthodontic treatments, mechanical forces are used to move the tooth bodily or change its inclination. During typical orthodontic tooth movement, a compression side and a tension side are created by the applied force.¹³ The effect of these two forces on osteoblasts has been investigated in several studies in vivo and in vitro. It has been shown

that there are various factors affecting bone regeneration in the presence or absence of loading application, such as strain parameters (frequency, cycle number, and stimulation duration)⁴ and scaffolds and medium growth factors.¹⁶ Nevertheless, this systematic review aimed to summarize and compare the osteoblast's behavior in response to the application of tension or compression in vitro and examine the factors that can influence these responses.

Methods

Eligibility criteria

Type of participants and interventions

Studies that analyzed the impact of mechanical forces on each type of the human cell osteoblast lineage were included. Articles analyzing the behavior of carcinogenic cells were excluded. Studies that merely assessed the effect of medium mechanical features on the behavior of cells were excluded. Additionally, those that stimulated the cell through non-mechanical forces or forces other than compression or tension were excluded.

Type of outcome measurement

Studies that analyzed the effects of mechanical stimulation on the human osteoblast cell's behavior (proliferation and differentiation) were included. Studies that merely assessed factors other than those mentioned were excluded.

Type of studies

All in vitro studies stimulated human cells of the osteoblast lineage at different stages of differentiation through mechanical force (tension and compression) were included. All animal studies, abstracts, letters, and reviews were excluded.

Information source and search strategy

PubMed and Scopus electronic databases were searched based on the combination of relevant keywords (Table 1). In addition, the reference lists of indicated articles were manually searched to find possible related studies.

Table 1. Key words

Mechanical force (free text)	Behavior (MeSH term)	Behavior (free text)	Cells (MeSH term)	Cells (free text)
tens*	"cell proliferation" [MeSH]	prolif*	"osteoblasts" [MeSH]	osteoblast*
compress*				
stretch	"cell differentiation" [MeSH]	diff*	"osteoclasts" [MeSH]	osteoclast*
"mechanical strain"				

MeSH – Medical Subject Headings.

Study selection and data extraction

Two reviewers performed study selection and data extraction independently. Any disagreement was discussed and resolved by a third independent expert. After removing duplicated studies using the EndNote reference manager (EndNote X9.1), the initial screening of titles and abstracts was done according to the mentioned eligibility criteria. Full texts of potentially eligible studies were reviewed in the next step. The study was designed according to The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Fig. 1).¹⁷

Data items

The study's methods and results were reviewed for data extraction. Extracted data items are as follows: 1) Cell lineage, 2) Type of mechanical stimulation, 3) Mechanical device and loading characteristics, 4) Cell culturing medium, and 5) Cell response to mechanical stimulation (proliferation and differentiation).

Critical appraisal

Assessing the quality of studies was done based on the modified CONSORT checklist,¹⁸ including the following 14 items (Table 2): structured summary (yes/no), scientific back-

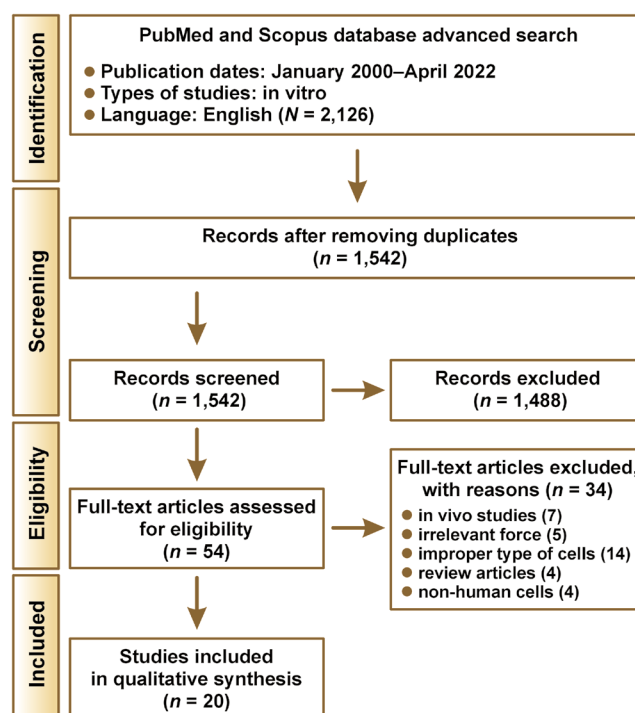


Fig. 1. Flow diagram

ground and explanation of rationale (yes/no), specific objectives and/or hypotheses (yes/no), explained interventions insufficient details (yes/no), defined outcome measurements

Table 2. Modified CONSORT (Consolidated Standards of Reporting Trials) checklist for in vitro studies

Study	Abstract	Introduction		Methods										Results	Discussion	Other	
	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14		
Bhatt et al. ⁴ 2007	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y		
Brezulier et al. ¹³ 2020	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Grimm et al. ¹² 2015	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Ignatius et al. ²⁴ 2005	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Jansen et al. ¹⁹ 2004	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y		
Jansen et al. ²⁰ 2006	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y		
Jansen et al. ²¹ 2010	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Kaspar et al. ²⁶ 2000	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Kaspar et al. ²⁹ 2002	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Kokkinos et al. ⁵ 2009	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	N	Y		
Kreja et al. ²⁵ 2008	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Kusumi et al. ³⁰ 2005	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y		
Rath et al. ²⁸ 2012	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y		
Sanchez et al. ³³ 2012	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y		
Tripuwabhut et al. ³² 2012	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y		
Tripuwabhut et al. ¹⁴ 2013	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y		
Weyts et al. ²³ 2003	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y		
Wozniak et al. ³¹ 2000	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	Y	Y		
Zhang et al. ²⁷ 2019	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y		
Zhu et al. ²² 2008	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y		

1 – structured summary; 2a – background; 2b – objective; 3 – intervention; 4 – outcome; 5 – sample size; 6 – randomized sequence generation; 7 – allocation concealment; 8 – implementation; 9 – blinding; 10 – statistical methods; 11 – outcomes and estimation; 12 – limitations; 13 – funding; 14 – protocol; Y – yes; N – no.

methods (yes/no), sample size determination (yes/no), randomizing sequence generation (yes/no), allocation concealment (yes/no), implementation, usage of proper statistical method (yes/no), expressing the results for each group and the estimated size of the effect and its precision (yes/no), addressing trial limitations (yes/no), addressing sources of potential bias (yes/no), imprecision, and, if relevant, the multiplicity of analyses (yes/no), identifying the sources of funding (yes/no), and availability of the full study protocol (yes/no).

Results

Among 1542 studies, 1488 studies were excluded based on their titles and abstracts (Fig. 1). The full text of the 54 remaining studies was analyzed, and 34 studies were excluded due to the following reasons: 7 studies performed the analysis in in-vivo conditions, five studies applied irrelevant forces such as microgravity and shear stress, 14 studies used improper lineages such as osteosarcoma cells, and four articles used animal osteoblasts. Finally, 20 studies were included for qualitative data synthesis. Among the included studies, tension and compression were applied in 15 and 5 studies, respectively (Table 3).

Cell lineage

Human fetal (SV-HFO)^{19–24} and adult osteoblast from the tibia, femur,^{25,26} calvaria,²⁷ or from subchondral bone pieces²⁸; primary human osteoblasts from Cambrex Bio Science⁴; osteoblast-like cells from tibia/femur/calvaria/ilia²⁹; human bone marrow-derived osteoblasts (HBMDOs) from the femoral diaphysis⁵; and Clonetics normal human osteoblasts (NHOst),³⁰ preosteoblasts, and osteoprogenitors³¹ were used to evaluate the effect of tension. For compression assessment, the included studies used alveolar bone osteoblasts,^{14,32} human fetal osteoblasts,¹³ human tibia osteoblasts,³³ and commercial human osteoblast cell lines.¹²

Force type

The effect of tension on the biological activities of the primary human osteoblast lineage at different stages of differentiation was evaluated in fifteen studies, and five articles analyzed the effect of compression.

Force device

In the included studies, the desired tension was applied by an FX-4000T Flex-cell BioFlex Tension Plus Unit,^{4,19–23,27,31} a 4-point bending device,^{5,26,29} a six-station stimulation apparatus,^{24,25} or other stretching systems.^{28,30} To apply the compressive force, two studies added lead weights to glass wells,^{14,32} one study used a Flex-cell compression system,³³ one study used plastic tube caps,¹³ and one used a centrifuge system.¹²

Medium

Assessing the tension effect, an osteogenic growth medium was used for cell culture in twelve studies. In one of them, 1- α ,25-dihydroxycholecalciferol, vitamin K1, and ascorbic acid were added to cultures that were tested for osteocalcin (OCN) synthesis.³⁴ One study supplemented the osteogenic medium with osteogenic protein-1 (OP-1)²⁶ and another added hams F12.²⁴ Three studies used standard mediums^{5,25,28} supplemented with vitamin D3²⁵ or collagenase II.²⁸ Among the compression-related studies, cells were cultured in a standard medium in two studies^{32,33} and an osteogenic medium in one study.¹² One study added hams F12 to the medium¹³ and one study analyzed the behavior of osteoblasts in both standard and lysates cultures.¹⁴ In addition, one study assessed the effect of clodronate on the behavior of osteoblasts.¹²

Cell response

Tension

Proliferation

The proliferative response of the cells depends on loading characteristics such as frequency and cycle number²⁹ and the differentiation stage of osteoblasts is another factor affecting the cells' proliferation and apoptosis after the force is applied.²³ It was shown that mechanical loading could positively affect the proliferation of osteoblastic precursor cells in a (COL 1) matrix²⁴ and promote the viability of the cells, decreasing the expression of two apoptosis 'executioner' caspases, and increasing proliferation in a time-dependent manner.²⁷ Eight hours of 9% uniaxial strain increased the osteoblasts' proliferation rate by up to three times, more than 3% and 6%.⁴ Also, the application of cyclic stretch (two days for 30 minutes per day with a frequency of 1 Hz and a strain magnitude of 1000 μ strain) increased the proliferation rate by about 10–48%.²⁶ In contrast, it has been reported that the immediate effect of stretch on DNA synthesis (0/5 hours) is not significant. Also, the cells which received the most intense stretching exhibited the lowest proliferation.⁵

Osteogenesis

Mechanical strain can stimulate bone formation using different signals and pathways. Wozniak et al. claimed that avb3-integrin activation is one of the mechanisms. It was shown that strain causes the redistribution of avb3-integrin on the cell's surface.³¹ Extracellular signal-regulated kinase (ERK1/2) signaling is also affected by mechanical loading. The effect of force on ERK1/2 is different based on the osteoblasts' strain characteristics and differentiation stage.¹⁹ Wnt/b-catenin signaling has an inhibitory role in osteoblastic differentiation independent of the

Table 3. Comparison of studies

Study	Cell type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation tests	Proliferation tests	Results
Tripuwabhut et al. ³² 2012	HOB from the alveolar bone, cultured on plates	compression (continuous)	1, 2, 3, and 4 g/cm ² for 1, 3, 24, 48, and 72 h by adding lead weights into glass wells	standard	– RT-PCR – human cytokine group I 2-plex express assay kit	– MTT	– proliferation: 1 h had no ss effect, 3, 4 g/cm ² for 3–48 h decreased – IL-6 and CXCL8 mRNA increased in a force-dependent manner after 24 h (peaked at 4 g) – IL-6 and CXCL8 protein reduced in a force-dependent manner after 24 h (the lowest at 4 g)
Brezulier et al. ¹³ 2020	HFO cultured on 2D and 3D models	compression (continuous)	1 and 4 g/cm ² , using a plastic tube cap	DMEM + Ham's F-12	– RT-PCR – ELISA	– glucose consumption – propidium iodide and Hoechst staining	– proliferation (2D): at 24 h – no ss difference, at 72 h – control = 1 g/cm ² > 4 g/cm ² – proliferation (3D): at 24 h – control = 1 g/cm ² < 4 g/cm ² , at 72 h – no ss difference – ALP (2D, 3D): no ss difference – Col 1 (2D, 3D): no ss difference – OCN (2D): no ss difference; OCN (3D): 4 g/cm ² > control – OPN (3D): no ss difference – Runx2 (2D, 3D): 4 g/cm ² > control – IL-6, IL-8 and OPG (2D, 3D): increased from 24 to 72 h – OPG: no ss difference between 2D and 3D
Grimm et al. ¹² 2015	HOB	compression (continuous)	34.9 g/cm ² by centrifuging	osteoegenic osteogenic + clodronate	– PCR – ELISA – IS	– MTT	– proliferation: no ss difference – RANKL: increased – OPG: decreased – clodronate reduced the effect of force – OPG protein: increased, clodronate + compression decreased
Sanchez et al. ³³ 2012	HOB from the tibia, cultured on plates	compression (cyclic)	1 MPa, 1 Hz for 4 h, using the Flexcell® Compression Plus System	DMEM + 5% FBS+ penicillin + streptomycin + glutamine	– RT-PCR – ELISA – IS	N/A	– IL-6, IL-8, RANKL, FGF-2, MMP-3, MMP-9, and MMP-13: increased – OPG, Col 1 and MMP-2: no ss difference
Tripuwabhut et al. ¹⁴ 2013	HOB from the alveolar bone, cultured on plates	compression (continuous)	2 and 4 g/cm ² for 1 day by adding lead weight into glass wells	standard standard + lysate	– RT-PCR – ALP activity – IS – ELISA	N/A	– OCN and OPN: no ss difference – Col 1: increased – Runx2 and OPG: decreased – RANKL: increased at 4 g/cm ² – ALP: increased – ALP (lysate medium): decreased – PGE2: increased
Bhatt et al. ⁴ 2007	HOB cultured on plastic dishes	stretch (cyclic uniaxial)	3%, 6% and 9%, 1 Hz for 8 h, using the Flexcell strain apparatus	osteogenic	– RT-PCR – IS	– 3H-thymidine	– proliferation: increased (peaked at 9%) – Col 1: increased (peaked at 9%) – BMP-2: increased at 9% – OPN and ON: increased (peaked at 3%) – OCN: increased

Study	Cell type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation tests	Proliferation tests	Results
Kaspar et al. ²⁶ 2000	HOB from the tibia and the femur, cultured on silicon dishes	sinusoidal strain (cyclic)	1,000 μ -strain, 1 Hz over 2 days, for 30 min per day, using a 4-point bending device	osteogenic (for the evaluation of OC synthesis: + 1 α ,25-dihydroxycholecalciferol + vitamin K1 + ascorbic acid)	– ALP activity – ELISA	– Coulter counter	– proliferation: increased – pre-COL: increased – ALP: decreased – OCN: decreased
Kaspar et al. ²⁹ 2002	HOB from the tibia, the femur, the calvaria, and the iliac bone, cultured on silicon dishes	sinusoidal strain (cyclic)	A: 1,000 μ -strain, 1 Hz, 4, 60, 1,800 and 3,600 cycles B: 1,000 μ -strain, 0.1, 1, 10, and 30 Hz, 1,800 cycles C: 1,000 μ -strain, 0.1, 1, 10, and 30 Hz, 30, 300, 3,000, and 9,000 cycles, 5 min all by using a 4-point bending device	osteogenic + penicillin	N/A	– Coulter counter	– proliferation: A: peaked at 1,800 cycles, decreased at 3,600 cycles B: no ss difference between different frequencies > control C: 1 Hz and 300 cycles – the highest, 30 Hz and 9,000 cycles – the unstimulated group, other frequencies and cycle numbers – increased
Zhang et al. ²⁷ 2019	HOB from the calvaria, cultured on a collagen-coated BioFlex® plates	tension (cyclic, equibiaxial)	2%, 0.2 Hz, 5 s, every 60 s for 6, 12 and 24 h, using the Flexcell FX-4000 Strain Unit	osteogenic + antibiotic	– RT-PCR	– MTT	– proliferation: increased after 24 h (higher than at 6 and 12 h), at 6 and 12 h vs. control – no ss difference – CALCR: decreased at 6 h, and increased at 12 and 24 h – CTSK, Col10A1 and CHRD: increased in a time-dependent manner – Col10A1 and CHRD: decreased at 24 h
Kreja et al. ²⁵ 2008	HOB from the tibia or the femur, cultured on silicone dishes	strain	A: continuous, 1% for 30 min, 6 h, 24 h, and 72 h, cell harvesting immediately after stimulation B: continuous, 1% for 30 min, cell harvesting at different time points (0 min, 30 min, 1 h, 3 h, and 5 h) C: continuous, 1% for 6 h, cell harvesting at 3 h and 18 h D: intermittent, 1% on 3 consecutive days (3 \times 30 min, 3 \times 3 h and 3 \times 6 h), cell harvesting immediately after stimulation E: 8%, continuous (30 min) or intermittent on 3 consecutive days (3 \times 3 h), cell harvesting immediately after stimulation	DMEM + FCS + vitamin D	– RT-PCR	N/A	A, B, C, E: – RANKL, OPG, M-CSF, and OCIL: no ss difference D, E: – RANKL: increased by intermittent stimulation 3 \times 3 h and 3 \times 6 h at a magnitude of 1%, and 3 \times 3 h at a magnitude of 8% strain
Rath et al. ²⁸ 2012	HOB from subchondral bone pieces, cultured on BioFlex culture plates coated with collagen type I	tensile strain (continuous)	5% for 4 and 24 h	TCM + FCS+ antibiotic/ antimycotic solution	– RT-PCR	N/A	4 h: – ColA1 and COX-2: increased – BMP-2, BMP-7, OCN, and OPN: no ss difference 24 h: – ColA1, COX-2, BMP-2, BMP-7, OCN, and OPN: no ss difference

Study	Cell type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation tests	Proliferation tests	Results
Kusumi et al. ³⁰ 2005	NHOB	tensile strain (cyclic and continuous)	cyclic: 2%, 7% and 14%, 0.2, 0.25 and 0.3 Hz, 10, 20, 30, or 45 min, and once a day for 4 h for 1, 2 or 3 successive days continuous: 7% for 3 days	OGM	– ELISA – RT-PCR – Western immunoblotting analyses	N/A	cyclic: – OPG: increased – sRANKL and RANKL: decreased continuous: – OPG, sRANKL and RANKL: no ss difference
Ignatius et al. ²⁴ 2005	SV-HFO	tension	1%, 1 Hz, 1,800 cycles, 30 min every day, cell harvesting immediately after loading on days 3, 7, 10, 14, 17, 21, a six-station stimulation apparatus	osteogenic+ Ham's F-12	– RT-PCR	– Coulter counter	– proliferation: increased – Col 1: constant from day 7 to 21, increased at days 3 and 7 – ALP: increased from day 1 to 21 in both groups (control and stimulated), at days 7 and 17 > control – OPN: increased from day 1 to 21 in both groups, at days 3 and 14 > control – OCN: increased from day 1 to 21 in both groups, at days 3 and 21 > control – Cbfa1: increased at days 3 and 7
Weyts et al. ²³ 2003	SV-HFO cultured on collagen-coated plates	stretch (biaxial)	0.4%, 0.9% and 2.5%, 0.5 Hz for 72 h in the presence or absence of osteogenic factors	aMEM without phenol red + FCS + glycerophosphate + dexamethazone	– PICP RIA – DNA quantities – ALP activity – calcium detection kit	– Sysmex cell counter	– proliferation: day 7 – decreased at all magnitudes in the both standard and inducing media; day 14 – increased at all magnitudes in the inducing medium and no ss difference in the standard medium, day 21 – no ss difference – DNA levels: increased from day 7 to 21, at day 21, ss higher in the inducing medium – pro-Col1: decreased from day 7 to 21, no ss difference between the media – ALP: peaked at day 14, ss higher in the inducing medium at days 14 and 21 – calcium accumulation: increased from day 7 to 21 in the inducing medium, ss higher in the inducing medium at day 21
Jansen et al. ¹⁹ 2004	SV-HFO cultured on collagen-coated BioFlex plates	stretch (biaxial)	0.4% for 5, 15 and 60 min on day 7, 14 or 21, using the Flexcell strain apparatus	osteogenic	– Western blot – ALP activity	N/A	ERK1/2 phosphorylation: – duration: a rapid increase with a max at 5–15 min, at 60 min, a decrease toward baseline – day: the strongest at days 14 and 21 – day 21: differentiated cells >> non-differentiated – after day 21: in the presence of osteogenic factors >> in the absence of osteogenic factors
Jansen et al. ²⁰ 2006	SV-HFO cultured on collagen-coated BioFlex plates	stretch (biaxial, cyclic)	0.5 Hz for 15 min on days 7, 14 and 21, using the Flexcell strain apparatus	aMEM without phenol red + HEPES + charcoal-treated FCS + CaCl ₂ + streptomycin + penicillin + dexamethasone + β-glycerophosphate	– ALP activity – DNA level – calcium deposition – alizarin red S – Western blot	N/A	– DNA levels: increase in mineralizing cultures > non-mineralizing cultures – ALP: increased in mineralizing cultures (peaked at day 14), in non-mineralizing cultures, no change – calcium deposition, alizarin red S: at day 7 – no mineralization, at day 14 – the onset of mineralization, at day 21 – full mineralization – MMP-1 and MMP-3: increased – gene expression: the most – MMP-1, MMP-2, MMP-14, and TIMP-2, the least – MMP-8

Study	Cell type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation tests	Proliferation tests	Results
Jansen et al. ²¹ 2010	SV-HFO cultured on collagen-coated BioFlex plates	stretch (biaxial, cyclic)	short term: a single bout, cyclic, 0.4%, 0.5 Hz for 15 min on days 5 and 14 long term: repetitive bouts of 15 min 5 times per day on day 5–21, using the Flexcell strain apparatus	aMEM without phenol red + HEPES + charcoal-treated FCS + CaCl ₂ + streptomycin + penicillin + dexamethasone + β -glycerophosphate	– ALP activity – DNA level – calcium deposition – alizarin red S – Western blot	N/A	– DNA levels: no ss difference – ALP: peak at day 14, stretched – lower at first, higher during the mineralization phase – alizarin red S: peak at day 14, stretched – lower at first, higher during the mineralization phase
Zhu et al. ²² 2008	SV-HFO cultured on collagen-coated Bioflex plates	tension	0.8%, 1.6%, 2.4%, and 3.2%, 1 Hz for 48 h, using the Flexcell strain apparatus	osteogenic + G418	– RT-PCR – ALP activity	N/A	– Col1: enhanced by the increasing strain gradually – ALP: increased at 0.8% and 1.6%, no change at higher magnitudes – OCN: increased at higher magnitudes of strain (2.4% and 3.2%), no change at 0.8% and 1.6%, elongation had no impact – Cbfa1/Runx2 mRNA: increased only at the highest magnitude of strain
Kokkinos et al. ⁵ 2009	HBMDO from the femoral diaphysis, cultured on Ti-6Al-4V	homogeneous strain	500 and 1,000 μ -strain, 0.5 and 1 Hz for 0.5, 1.5, 3, and 6 h, using a four-point bending device	standard	– RT-PCR	– DNA synthesis	– DNA synthesis: at 0.5 h – no effect, at 1,000 μ -strain, 1 Hz – the lowest stimulatory result – Cbfa1 mRNA: peak at 0.5 Hz, 500 μ -strain and 3 h
Wozniak et al. ³¹ 2000	preosteoblasts, osteo-progenitors and osteoblasts from human bone marrow, cultured on collagen/vitronectin-coated supports	strain (cyclic)	70,000 μ -strain, 0.05 Hz for 48 h at 3 cycles/min (10 s on/10 s off)	osteogenic + OP1	– IHC – flow cytometric analysis – Western immunoblotting analysis of cell lysates – alizarin red S	N/A	– aVb3: synthesis – no ss difference, redistribution – increased the number and size of the plaque-like sites of aVb3 expression – OCN: increased – OPN: strain stimulated the secretion of the 168-kDa molecule so that it did not accumulate in the cell – alizarin red S: increased the intensity of mineralized nodules

HOB – human osteoblasts; HFO – human fetal osteoblasts, HBMDO – human bone marrow-derived osteoblasts; NHOB – normal human osteoblasts; DMEM – Dulbecco's Modified Eagle Medium; Ham's F12 – nutrient mixture F-12; FBS – fetal bovine serum; OC – osteoclasts; FCS – fetal calf serum; TCM – tissue culture medium; OGM – osteogenic growth medium; MEM – minimum essential medium; RT-PCR – reverse transcription polymerase chain reaction; ELISA – enzyme-linked immunosorbent assay; IS – immunocytochemical staining; ALP – alkaline phosphatase; PICP RIA – procollagen type I carboxy-terminal peptide radioimmunoassay; IHC – immunohistochemistry; IL – interleukin; CXCL8 – interleukin 8; Col1 – collagen type I; OCN – osteocalcin; OPN – osteopontin; Runx2 – Runt-related transcription factor 2; OPG – osteoprotegerin; RANKL – receptor activator of nuclear factor kappa B ligand; FGF-2 – fibroblast growth factor 2; MMP – matrix metalloproteinase; PGE₂ – prostaglandin E₂; BMP – bone morphogenetic protein; ON – osteonectin; CALCR – calcitonin receptor; CTSK – cathepsin K; Col10A1 – collagen type X alpha 1; CHRD – chordin; M-CSF – macrophage colony-stimulating factor; OCIL – osteoclast inhibitory lectin; ColA1 – collagen alpha 1; COX-2 – cyclooxygenase 2; sRANKL – soluble RANKL; ERK1/2 – extracellular signal-regulated kinase 1/2; aVb3 – integrin alpha V beta 3; ss – statistically significant; N/A – data not available.

ERK pathway. Mechanical loading affects this signaling in a time-dependent manner (initial increase followed by a long period of inhibition after stretch).²¹ There are some ways that osteoblastic differentiation can be monitored, such as the detection of DNA levels (cell density), matrix production (procollagen secretion), maturation (alkaline phosphatase (ALP) activity), and mineralization (calcium levels) for instance.²³ Mechanically strained HBMDOs and SV-HFO/SV40 produced higher intensities of mineralized nodules.^{20,21,23,31} ALP activity was increased in stretched HFOs,^{20–24} but higher strain magnitudes did not affect it.²² Mechanically stimulated osteoblasts showed lower levels of it.²⁶ Biomechanical loading increases the COL 1 expression in human osteoblasts and HFOs in general,^{4,22,26} but the day of culture²⁴ and duration of loading application²⁸ can change its expression results. HFOs,²⁴ preosteoblasts, and osteoprogenitors³¹ showed higher OCN and osteopontin (OPN) expression and secretion levels after mechanical stimulation. It was reported that the mRNA expression of OCN and OPN in osteoblasts and HBMDOs increased, but the peak depended on the strain magnitude.^{4,5,22} Kasper et al. concluded that strain reduces OCN expression in osteoblasts.²⁶ In contrast, Rath et al. reported that it does not affect the expression of OCN and OPN in the same cells.²⁸

Cyclic uniaxial stretch increased bone morphogenetic protein-2 (BMP-2) expression in primary human osteoblasts,⁴ but continuous tensile strain made no significant change in the expression of BMP-2 and BMP-7.²⁸ Core binding factor- α 1 (Cbfa1) is the osteoblast-specific transcription factor through which mechanical loading can affect osteoblast differentiation.²¹ Cbfa1 expression in HBMDOs peaked at 500 $\mu\epsilon$ and decreased at higher magnitudes of mechanical loading (1000 $\mu\epsilon$),²¹ while HFOs exhibited increased expression of it only at the highest magnitude (3.2%).²²

Osteoclastogenesis

Changes in RANKL/RANK/OPG mRNA and protein synthesis are influenced by loading characteristics.²⁵ Differences in the results of changes due to cyclic and continuous force confirm this statement.³⁰ Among the various matrix metalloproteinases (MMPs), MMP-1, and MMP-3 significantly increased under the influence of the applied force.²⁰

Compression

Proliferation

Among three studies^{12,13,32} that assessed osteoblast proliferation, one study mentioned the magnitude and duration-dependent manner of compression.³² In addition, one study¹³ mentioned that osteoblasts had different reactions to compression in 2D and 3D conditions, and

only one study¹² mentioned that compression has no significant effect on the proliferation of osteoblasts.

Osteogenesis

To assess the osteoblastic differentiation/activity, the expression, release, or production of the following factors was analyzed: ALP, COL 1, OCN, OPN, and osteoprotegerin (OPG). It has been mentioned that the alternation in the expression of osteogenic factors differs in 2D and 3D conditions except in the case of runt-related transcription factor-2 (RUNX2), which increased in both conditions.¹³ However, the decrease in the expression of RUNX2 can also be seen.¹⁴ It has been mentioned that the expression of OPG decreased following compression.^{12,33} One study¹⁴ mentioned that the expression of ALP and COL 1 increased, while one study showed lower rates of COL 1 following compression, and another study³³ mentioned no significant difference in the expression of COL 1. In addition, no significant difference in the expression of OCN and OPN was seen.¹⁴

Osteoclastogenesis

The rate of interleukins (IL-6, IL-8), MMP, RANKL, and cytolagenase 2 were analyzed to assess osteoclastogenesis. Four studies^{12,32,33} mentioned that compression increased the rate of osteoclastogenic factors. However, one study³³ mentioned no statistically significant difference in the expression of MMP-2.

Discussion

Osteoblasts face different types of mechanical forces in the human body. The success of treatment procedures such as distraction osteogenesis and orthodontic treatments is related to the reaction of osteoblasts to mechanical forces, specifically tension and compression. Several studies have been done using in-vitro conditions to analyze the behavior of osteoblasts and monitor the expression of specific factors that play a crucial role in bone formation and absorption. The results can be beneficial in anticipating the reaction of osteoblasts to the mechanical forces in the human body.

Cell lineage

Primary osteoblasts have different sensitivities to mechanical strain compared to SV40-immortalized osteoblasts.²⁰ It can be hypothesized that due to the rigidity of the extracellular matrix following mineralization, the effect of mechanical forces on osteoblasts will be reduced.³⁵ In fact, the differentiation stage affects the mechanosensitivity of osteoblasts. For instance, the expression of MMP-3 increased 25-fold after applying tensile force

on the fifth day of cultivation.²⁰ However, it increased by lower amounts (5 folds) when the force was applied on the seventh day of cultivation when the medium was more mineralized.²⁰ In addition, the tensile force did not affect MMP-3 expression when the force was applied on the 21st day of cultivation.²⁰

Force device

The included studies applied tension through different devices. The protocol of mechanical force has a more significant effect on the accuracy of results compared to the specific device that was used. In terms of tensile force, it is essential to make sure that mechanical force is distributed evenly between cells. It is mentioned that this goal can be reached by placing cells in the center of the bio-flex plate accurately.

Force characteristics

Despite the controversies about the exact amount of physiological mechanical strain in the human body, it is claimed that its normal range is between 50–1500 μ s. Below this range is defined as the disuse mode and causes bone weakness. Bone remodeling is started at 1000–1500 μ s and can be continued up to 3000 μ s, which is the start of micro-damage manifestations.³⁶ a high number of included studies applied supraphysiological forces, which may jeopardize the reliability of the results. This may have resulted from not seeing significant differences at lower magnitudes.

Some studies mentioned that prolonging the duration of mechanical force leads to desensitization of cells and reduces the positive/negative effect of mechanical forces on osteoblasts.^{14,37} The duration of force application among studies that applied continuous tension did not exceed several hours.^{28,35,38} Only Kreja et al.³⁹ applied continuous force for 72 hours. However, they did not mention a significant effect of continuous force, while gene expression altered following intermittent force application in the mentioned study.

Cell culture system

The effect of mechanical stimulation can be altered based on the cell culture system.²⁴ To assess the molecular mechanisms concerning the effect of mechanical forces on gene expression and how mechanical forces convert to molecular signals, it is recommended to use 3D conditions rather than conventional 2D environments. In addition, the 3D condition will provide an environment that more resembles in-vivo conditions. It must be considered that before the cultivation of cells under 3D conditions, such as what has been done in Brezulier et al.¹³ and Ignatius et al.,²⁴ it is essential to assess the viability of 3D conditions through cell viability assays such as MTT or BrdU

assays prior to the commencement of the experiment. However, among all included studies, only two studies analyzed the behavior of osteoblasts in 3D conditions.^{13,24} In addition, 2D conditions have some artifacts compared to the in-vivo conditions. For instance, Bhatt et al.⁴⁰ mentioned that in in-vivo conditions, tensile stretch with a frequency of 1 cycle/12 hours is used. However, this frequency had no significant effect on the behavior of osteoblasts using in-vitro conditions.⁴⁰

The composition of the medium can affect the response of stem cells to mechanical forces. For instance, Kreja et al.³⁹ aimed to analyze the osteoclastogenic response of osteoblasts to different magnitudes and frequencies of strain. Considering the positive effect of vitamin D3 on the RANKL expression, it has been added to the medium to induce osteoclastogenesis.³⁹ In addition, FCS, which was added to the medium in this study,³⁹ contains osteoclastogenic cytokines, proteins, and growth factors.

Proliferation and viability

The proliferative response of osteoblasts is one of the primary changes that occur after force application.²⁹ Factors such as culture conditions, mechanical stimulation parameters, and duration of loading application can strongly affect the results,^{5,23,29,34,37,41} but no correlation was found between cellular responsiveness and donor variability of bone cell origin.²⁹ an appropriate number of cycles and strain frequency intensifies proliferation and cell viability^{23,27,29,34} while compressive force generally inhibits cell proliferation³⁷ except in cases in which proliferation had already been down-regulated because of other reasons (by clodronate for example).¹² In comparison with 2D cultures, seeding the cells in 3D cultures resulted in better proliferative responses and adaptation to compressive forces because of better nutrient access or low cell concentration density.^{24,41}

Differentiation

To assess osteogenesis/osteoclastogenesis, the expression and protein production of osteogenic factors such as ALP, OCN, OPN, RUNX2, and COL 1 can be analyzed. Considering the fact that these genes consist of long-term and short-term markers, elongation of cultivation times may provide more accurate results. Therefore, a portion of included studies^{12,41} extended the duration of the experiment by several weeks to obtain more reliable results, while some performed analyses several hours following the stimulation.³⁷ This variety can be justified by considering that the duration of the analysis would be set based on the type of markers that were analyzed.

Cells obtained from different patients may exhibit different behaviors.^{26,39} For instance, Kaspar et al.²⁶ mentioned that even in the control group of their study, which was not subject to mechanical forces, osteoblasts showed different

behaviors. Included studies that derived osteoblasts from human bone did not discuss the common source of osteoblasts in the control or experimental groups.

Alkaline phosphatase

ALP is an early osteogenic differentiation marker. Studies showed that mechanical forces can increase the proliferation in the high ALP activity stage of osteoblasts. For instance, Weyst et al.⁴² mentioned that the ALP activity of HFO will peak 14 days following the mechanical force. In this stage, mechanical strain increases proliferation. However, in the earlier or later phases, stretch decreased or did not alter proliferation, respectively.

Different types of osteoblasts may show contradicting behavior to compression. For instance, the amount of 4 gr/cm² compression has no effect on the ALP expression of HFO while increasing the expression in HOB. In the case of tensile forces, different magnitudes and frequencies of stimulation increased ALP activity in HOB and HFO.^{20,21,35,42} Moreover, among all included studies, the rate of ALP activity peaked after 14 days of force application, which is considered the initiation of mineralization.

PGE2 can induce both osteoblastic and osteoclastic procedures. It has been hypothesized that PGE2 may increase the production of ALP and COL 1.¹⁴ The same increasing pattern following the expression of ALP can confirm the increasing effect of PGE2 on ALP and COL 1.¹⁴

Collagen type I

It makes up 90% of organic materials in the bone matrix²⁰ and is considered an early osteoblast differentiation marker. It is apparent that proliferation and the expression of COL 1 downregulate before the upregulation of osteogenic genes. However, it has been shown that COL 1 enhances osteogenesis.^{43,44} Cells that were cultured on collagen matrixes had higher osteogenic gene expression and expressed the osteogenic factors earlier compared to those cultured on plastic dishes.⁴⁵ In addition, collagen causes uniform mineralization compared to focal mineralization on plastic dishes.⁴⁵

It has been assumed that mechanical stimulation will alter the expression of COL 1 directly. However, Sanchez et al.⁴⁶ mentioned that the production of MMP-3 caused by mechanical stimulation affects the rate of COL 1 expression. They mentioned that 4 hours following the compression, there were no changes in the rate of COL 1. However, MMP-3 increased at this time point. They hypothesized that the rate of COL 1 will increase in the next phase of differentiation.

Osteocalcin

It is the most plentiful non-collagenous protein expressed only by fully differentiated osteoblasts and is

critical for bone metabolism.⁴⁷ The effect of mechanical forces on OCN expression depends on the force's type, magnitude, frequency, and duration. For instance, it has been mentioned that compression has no significant effect on OCN expression. In contrast, tension alters its expression in different patterns. Among all included studies, the expression of OCN decreased when 0.1% strain was applied.^{26,38} However, Ignatius et al.²⁴ mentioned higher expression of OCN following 0.1% strain. Different types of osteoblastic cells were used in the mentioned studies, which may be the reason for conflicting results.

The amount of 0.05%, 2.4%, 3%, 3.2%, and 7% strain increased OCN expression in osteoblasts with no conflicts between studies. Since OCN expression will be recognized in the late stages of the differentiation, performing the analyses at least 48 hours after the stimulation, which was most common among the included studies, may give more accurate results. For instance, Rath et al.²⁸ analyzed the expression after 4 hours and 24 hours of mechanical force and did not mention any significant differences. It does not mean that OCN cannot be recognized at the earlier time points. For instance, Bhatt et al.⁴⁰ analyzed the expression 12 hours after the stimulation and showed higher rates of OCN following the mechanical force.

Cbfa1 is the key to converting mechanical stimulation to osteogenic differentiation. The expression of this marker can be increased following mechanical tension.^{22,38} It can increase MSCs differentiation to osteoblasts and regulate osteoclastic function by binding to osteoblastic acting elements (OSEs), which are located in the promoter region of the osteoblast's specific genes such as OCN, OPN, BSP, and COL I.⁴⁸

Osteopontin

This factor is a late osteoblastic differentiation marker. A 4 gr/cm² compression will decrease the expression of OPN in HFO¹³ while having no significant effect on HOB.¹⁴ The expression of OPN increased following the tensile force in all of the studies, independent of the magnitude, duration, and frequency of the cyclic tensile force.^{24,40,49} However, same as OCN, since it is a late-stage factor, a minimum time is needed to recognize significant differences in the expression of this gene. Rath et al.²⁸ was the only study that mentioned no significant difference in the rate of OPN. This can be because Rath et al.²⁸ was the only study that applied continuous tensile force while others applied cyclic forces.

Runt-related transcription factor 2

Runx2 is a transcription factor playing a crucial role in MSC functionality in forming osteoblasts, osteoclasts, osteocytes, and bone lining cells. Its positive and negative regulation can impact the bone formation process.⁵⁰

Among the included studies, ALP and COL 1 factors did not increase or decrease in the same manner as RUNX2, which suggests that they are produced in a RUNX2-independent manner.^{13,14} However, it has been mentioned that OCN may be expressed through a Runx2-dependent pathway.¹⁴ HOB and HFO showed contradicting results about the effect of compression on RUNX2 expression. In fact, a 4 gr/cm² compressive force increases the expression of RUNX2 in HFO¹³ while decreasing the expression in HOB.¹⁴ On the contrary, with compression, high magnitudes of tensile force can increase the expression of RUNX2.²²

Osteoclastogenesis

Receptor activator of nuclear factor kappa-B ligand

This factor has a significant effect on orthodontic tooth movement by increasing the bone resorption rate. Besides osteoblasts, this factor can be found in odontoclasts, osteocytes, and fibroblasts during tooth movement. The expression of RANKL will increase following compression in both HOB and HFO, independent of the force's magnitude. In addition, continuous tensile forces have no significant effect on the expression of RANKL, while cyclic tensile force decreases RANKL expression^{30,39}. It has been proven that IL-8 can increase the expression of RANKL, which can be confirmed by considering that among included studies, RANKL and IL-8 alter in the same pattern in response to mechanical forces.⁴⁶

Interleukin 6 and interleukin 8

These two markers, which can act in a paracrine and autocrine manner, are considered osteoclastogenic factors. These two markers can be found in the gingival fluid during orthodontic tooth movement. Tripuwabhurt et al.³² mentioned that although compression increased the expression of IL-6, IL-8, and C-X-C motif chemokine ligand 8 (CXCL8), it reduced the rate of IL-6 and CXCL8 protein levels.³² This controversy can be justified by considering the negative feedback control in post-transcriptional procedures.³² The expression of IL-6 and IL-8 increased following the compression in both HOB and HFO, independent of the magnitude and type of compression.^{13,32,33}

Osteoprotegerin

This factor neutralizes the enhancing effect of RANKL on osteoclastogenesis.¹³ Grimm et al.¹² mentioned that compression decreased the expression of OPG. However, the protein levels of OPG increased. This contradiction can be justified by considering the physiological sequences that lead to protein production.¹² Compression increased the expression of OPG in HFO,¹³ while lower

rates of this marker were seen following the compressive force in HOB.^{14,33} In addition, the continuous tensile force had no significant effect on the expression of OPG.^{30,39} However, the rate of OPG increased following the cyclic tensile force.³⁰ It must be mentioned that the effect of tension on HFO was not evaluated.

Limitations

More studies are needed to assess the independent effect of magnitude, duration, and frequency of force on the behavior of osteoblasts.

A portion of the studies did not mention the differentiation stage of osteoblasts at the time of analyses.

Studies with the same osteoblast source for control and experimental groups are needed.

More studies that analyze the effect of mechanical forces in 3D conditions are needed.

More studies that apply mechanical forces in the magnitudes close to in-vivo conditions are needed.

Conclusions

This study aimed to analyze the in-vitro studies that applied tension or compression forces (two significant forces in dentofacial deformity treatments) to osteoblasts from different aspects. It has been shown that the response of markers that are related to bone formation or absorption can be altered based on the differentiation stage of the cells, the cell culture system, and the magnitude and duration of the force. Our results can be useful to compare different in-vitro conditions to physiological conditions to specify what best resembles in-vitro conditions of the human body environment during treatments such as orthodontic tooth movement and distraction osteogenesis.

Ethics approval and consent to participate

Not applicable.

Data availability


All data generated and/or analyzed during this study is included in this published article.


Consent for publication


Not applicable.

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