Review

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

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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 body[12] 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.

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 remov-

Table 1. Key Words

Mechanical Force (free text)	Behavior (Mesh Term)	Behavior (Free Text)	Cells (Mesh Term)	Cells (free text)
Tens*	"Cell Prolifera-tion"[Mesh]	Prolif*	"Osteoblasts"[Mesh]	Osteoblast*
Compress*	"Cell Differentia-tion"[Mesh]	Diff*	"Osteoclasts"[Mesh]	Osteoclast*

ing 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 background and explanation of rationale (yes/no), specific objectives and/or hypotheses (yes/no), explained interventions insufficient details (yes/no), defined outcome measurements methods (yes/no), sample size determina-

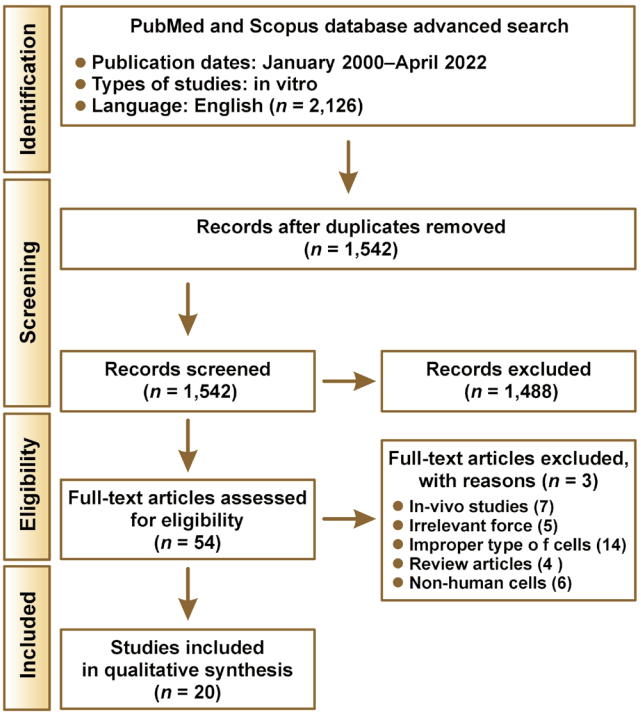

Fig. 1. Flow diagram

Table 2. Modified CONSORT checklist for in vitro studies

Auther/year	Abstract	Introduction		Method								Result	Discussion	Other	
	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14
Bhatt et al (2007) (7)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y
Brezulier et al (2020) (46)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Grimm et al (2015) (20)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Ignatius et al (2005) (35)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Jansen et al (2004) (30)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Jansen et al (2006) (31)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Jansen et al (2010) (71)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kaspar et al (2000) (43)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kaspar et al (2002) (40)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kokkinos et al (2009) (8)	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	N	Y
Kreja et al (2008) (36)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kusumi et al (2005) (41)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Rath et al (2012) (39)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Sanchez et al (2012) (45)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Tripuwabhrut et al (2012) (47)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Tripuwabhrut et al (2013) (23)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Weyts et al (2003) (34)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y
Wozniak et al (2000) (42)	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	Y	Y
Zhang et al (2018) (38)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Zhu et al (2008) (33)	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 method, 11: outcomes and estimation, 12: limitations, 13: funding, 14: protocol, Y:yes, N:no

tion (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 pieces[28]; primary human osteoblasts from Cambrex Bio Science[4]; osteoblast-like cells from tibia/femur/calvaria/ilia[29]; human bone marrow-derived osteoblasts (HBMDOs) from the femoral diaphysis[5]; and Clonetics normal human osteoblasts (NHOst),[30] preosteoblasts, and osteoprogenitors[31] 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-alpha,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 D3[25] 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 medium[13] 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 number[29] 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) matrix[24] 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 μstrain) 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	Prolifera-tion Tests	Result
Tripuwabhrut et al. (2012) (47)	HOB from alveolar bone cultured on plates	Compression (continues)	1.0, 2.0, 3.0, and 4.0 g/ cm2 for 1, 3, 24, 48, and 72 h by adding lead weights into glass wells	Standard	- RT-PCR - human cytokine group I 2plex express assay kit	- MTT	- Proliferation: 1h had no ss effect, 3, 4 g/cm2 for 3–48 h de-creased - IL6 and CXCL8 MRNA increased in force depend-ent manner after 24h (peaked at 4g) - IL6 and CXCL8 protein reduced in force dependent man-ner after 24h (lowest at 4g)
Brezulier1 et al (2020) (46)	HFO cultured on 2D and 3D model	Compression (continues)	1, 4 g/cm2 using plas-tic tube cap	DMEM + hams F12	- RT-PCR - ELIZA	- Glucose consump-tion - Propidium iodide and Hoechst staining	- Proliferation (2D): 24h: no ss difference; 72h: control = 1g/ cm2 > 4g/cm2 - Proliferation (3D): 24h: con-trol=1g/cm<4g/cm; 72h: no ss difference - ALP (2D, 3D): no ss differ-ence - COL1 (2D, 3D): no ss difference - OCN: 2D: no ss diff; 3D: 4g/ cm2 > control - OPN (3D): no ss diff - RUNX2 (2D, 3D): 4g/cm2 > control - IL-6,8, OPG (2D,3D): in-creased from 24 to 72, - OPG: no ss difference between 2D and 3D
Grimm et al (2015) (20)	HOB	Compression (continues)	34.9 g/cm2 by centri-fuge	Osteogenic - Osteogenic + clodronate	- PCR - ELI-SA - IS	- MTT	- Proliferation: No ss differ-ence - RANKL: In-creased - OPG: Decreased - Clodronate re-duced the effect of force - OPG protein: in-creased, clodro-nate + compres-sion decreased
Sanchez et al (2012) (45)	HOB from tibia cul-tured on plate	Compression (cyclic)	1 MPa, 1 Hz for 4h by Flexercell Compres-sion Plus system	DMEM + 5% FBS + penicil-lin + streptomycin + glutamine	RT-PCR - ELI-SA - IS	N/A	- IL-6, IL-8, CC-2, RANKL, FGF-2, MMP-3, MMP-9, MMP-13: Increased - OPG, Col-1, MMP-2: No ss diff
Tripuwabhrut (2013) (23)	HOB from alveolar bone cultured on plate	Compression (continues)	2 and 4.0 g/cm2 for 1d by adding lead weight to glass	Standard - Standard + lysate	- RT-PCR - ALP activity - IS - ELISA	NM	- OCN and OPN: No ss diff - Col 1: Increased - Runx2, OPG: Decreased - RANKL: In-creased at 4g/ cm2 - ALP: Increased - ALP (lysate me-dium): De-creased - PGE-2: Increased
Bhatt et al. (2007) (7)	HOB cultured on plas-tic dishes	Stretch (cy-clic uniaxial)	3%, 6%, 9% 1 Hz for 8 h by Flexcell strain apparatus	Osteogenic	- RT-PCR - IS	H thymi-dine	- Proliferation: Increased peaked at 9% - Col 1: Increased peaked at 9% - BMP-2: Increased at 9% - OPN, ON: Increased peaked at 3% - OCN: Increased

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Kaspar et al. (2000) (43)	HOB from tibia and femur cultured on sili-con dishes	Sinusoidal strain (cyclic)	1000 µstrain 1 Hz over two days for 30 min per day by 4-point bending device	Osteogenic (For OC synthesis eval-uation: + 1alpha,25-di hydroxy chole calciferol + vita-min K1+ ascorbic acid)	- ALP activity - ELISA	Coulter Counter	- Proliferation: Increased - pre COL: In-creased - ALP: Decreased - OCN: Decreased
Kaspar et al. (2002) (40)	HOB from tibia, femur, calvaria and iliac cul-tured on silicon dishes	Sinusoidal strain (cyclic)	-A: 1000µstrain 1Hz 4, 60, 1800 and 3600 cycles -B: 1000µstrain 0.1, 1, 10, 30Hz 1800 cycles -C: 1000 µstrain 0.1, 1, 10, 30Hz 30, 300, 3000 and 9000 cycles 5 min All by 4-point bending device	Osteogenic + penicillin	N/A	Coulter Counter	- Prolifera-tion: -A: peaked at 1800 cycles 3600 cycles: Decreased -B: no ss difference between differ-ent frequencies > control -C: 1Hz and 300 cycle= high-est 30Hz and 9000 cycles= unstimulated group Other frequencies and cycle numbers= Increased
Zhang et al (2019) (38)	HOB from calvaria cultured on collagen coated Bioflex plate	Tension (cy-clic, equibiaxi-al)	2% 0.2 HZ 5 s, every 60 s for 6, 12, and 24 h by a Flexer-cell FX-4000 Strain Unit	Osteogenic + antibiotic	- RT-PCR	-MTT	- Proliferation: Increased after 24 h (higher than 6,12h) 6h, 12h vs control : No ss diff - CALCR: decreased at 6h and increased at 12,24h - CTSK, COL10A1, CHRD: in-creased in a time dependent man-ner - COL10A1, CHRD: de-creased at 24h
Kreja et al. (2008) (36)	HOB from the tibia or femur cultured on sili-cone dishes	Strain	-A: continuous 1% for 30 min, 6 h, 24 h, 72 h cell harvest-ing 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 (3×30 min, 3×3 h, and 3×6 h) cell harvesting immediate-ly after stimulation -E: 8% continuous (30 min) or intermit-tent on 3 consecutive days (3×3 h) cell harvesting immediate-ly after stimulation	DMEM + FCS + vitamin D	- RT-PCR	N/A	- A, B, C, E: - RANKL, OPG, M-CSF, OCIL: No ss diff - D, E: - RANKL: Increased by intermittent stim-ulation 3×3 h and 3×6 h at a magni-tude of 1% and 3×3 at a magni-tude of 8% strain

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Rath et al (2012) (39)	HOB from subchondral bone pieces cultured on BioFlex® culture plates coated with col-lagen type I	tensile strain (continuous)	5% for 4 and 24 hours	TCM+ FCS+ anti-biotic/antimycotic solution	- RT-PCR	N/A	-4h: -COLA1, COX2: In-creased - BMP2, BMP7, OCN, OPN: No ss diff -24h: -COLA1, COX2, BMP2, BMP7, OCN, OPN: No ss diff
Kusumi et al (2005) (41)	NHOBs	Tensile strain (cyclic and continuous)	Cyclic: 2%, 7%, 14% 0.2, 0.25, 0.3 Hz 10, 20, 30, or 45min, and once a day for 4h for 1, 2, or 3 successive days Continuous: 7% for 3 days	OGM	-enzyme-linked immunosorbent assay -RT -PCR -Western immunoblotting analyses	N/A	- Cyclic: -OPG: In-creased - sRANKL, RANKL: De-creased - Continuous: -OPG, sRANKL, RANKL: No ss diff
Ignatius et al (2005) (35)	SV-HFO	tension	1% 1 HZ 1800 cycles 30min every day Cell harvesting: immediately after loading on days 3, 7, 10, 14, 17, 21 six-station stimula-tion apparatus	Osteogenic+ Hams F12	-RT-PCR	- Coulter Counter	- Proliferation: Increased -COL I: constant from day7 to 21, increased at 3, 7 - ALP: in-creased from day1 to 21 in both groups (control and stimulated), Day 7,17>control -OPN: : increased from day1 to 21 in both groups Day 3, 14>control -OCN: : increased from day1 to 21 in both groups Day 3, 21>control -cbfa1: increased at day 3,7
Weyts et al (2003) (34)	SV-HFO cultured on collagen coated plates	Stretch (bi-axial)	0.4, 0.9,2.5% 0.5 HZ For 72h in the presence or absence of osteogenic factors	aMEM without phenol red + FCS + glycerophos-phate+ dexametha-zone	-PICP RIA -DNA quantities -ALP activity -calcium detection kit	- Sysmex cell coun-ter	- Prolifera-tion: -day 7: decreased at all magnitudes in both standard and inducing medium -day14: increased at all magnitudes in inducing me-dium and no diff in standard me-dium -day21: No ss diff - DNA levels: increased from 7 to 21d, at 21d ss higher in induc-ing medium -pro COLI: de-creased from 7d to 21d, no ss diff between medi-ums - ALP: peaked at 14d, ss higher in induc-ing medium at 14,21d -calcium accu-mulation: in-creased from 7d to 21d in induc-ing medium, ss higher in induc-ing medium at 21d
Jansen et al. (2004) (30)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial)	0.4% for 5, 15, 60 min on day 7, 14, or 21 by Flexercell strain apparatus	Osteogenic	-Western blot - ALP activity	N/A	- ERK1/2 phos-phorylation: -duration: rapid increase with a max between 5-15 min 60 min: decrease toward baseline -day: strongest at day 14,21 -Day 21: differentiated cells>>non-differentiating -After day 21: in the presence of osteogenic fac-tors>>in the ab-sence of osteo-genic factors

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Jansen et al (2006) (72)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial, cyclic)	0.5 Hz for 15 min on days 7, 14, 21 by Flexercell strain appa-ratus	aMEM without phenol red + HEPES+ charcoal treat-ed fetal calf se-rum + CaCl2 + streptomycin + penicillin + dexa-methasone + b-glycerophosphate	-ALP activity -DNA level -Calci-um Deposition -Alizarin Red S -Western blot		- DNA levels: increase in mineralizing cultures > non-mineralizing cultures -ALP: - miner-alizing cultures: increase, peaked at day 14. - nonmineralizing cultures: no change -Calcium Deposi-tion, Alizarin Red S: Day 7: no mineraliza-tion, day 14: onset of mineral-ization, day 21: full mineraliza-tion -MMP-1, MMP-3: in-crease -gene expres-sion: most= MMP-1, -2, -14, TIMP-2. Least= MMP-8
Jansen et al (2010) (71)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial, cyclic)	-short term: single bout cy-clic 0.4% 0.5 Hz for 15 min on days 5,14 -long term: repetitive bouts of 15 min for five times per day, day 5-21 by Flexercell strain apparatus	aMEM without phenol red+ HEPES+ charcoal treat-ed fetal calf se-rum+ CaCl2+ streptomycin+ penicillin+ dexa-methasone+ b-glycerophosphate	-ALP activity -DNA level -Calci-um Deposition -Alizarin Red S -Western blot	N/A	- DNA levels: No ss diff -ALP: peak at day14 ,stretched: lower at first, higher during the min-eralization phase -Alizarin Red S: peak at day14 ,stretched: lower at first, higher during the mineralization phase
Zhu et al (2008) (33)	SV-HFO cultured on collagen coated Bioflex plates	tension	0.8%, 1.6%, 2.4%, 3.2% 1 HZ for 48h by Flexercell strain apparatus	Osteogenic + G418	-RT-PCR -ALP activity	N/A	- COLI: en-hanced by the increasing strain gradually - ALP: increased at 0.8% and 1.6%, no change at higher magni-tudes. - OCN: increased at higher magni-tudes of strain (2.4% and 3.2%).no change at 0.8% and 1.6%. elongation had no effects - Cbfa1/Runx2 mRNA: in-creased only at the highest mag-nitude of strain
Kokkinos et al (2009) (8)	HBMDO from the femoral diaphysis cul-tured on Ti-6Al-4V	Homogeneous strain	500, 1000 µstrain 0.5, 1 Hz for 0.5 h, 1.5 h, 3 h, 6 h by four point bending device	standard	-RT-PCR	- DNA synthesis	- DNA synthesis: - 0.5 h: no effect - 1000 µstrain, 1 Hz: lowest stimulato-ry result - Cbfa1 mRNA: peak =0.5 Hz, 500 µstrain, 3 h
Wozniak et al (2000) (42)	preosteoblasts, oste-oprogeni-tors, osteoblasts from human bone marrow cultured on colla-gen/ vitronectin-coated supports	Strain (cyclic)	70,000 µstrain 0.05 Hz For 48h at 3 cycles/minute (10s on/10 s off)	Osteogenic + OP1	-IHC -flow cytometric analysis -Western analysis -immuno-blotting of cell lysates -Alizarin red-S	N/A	-avb3: - syn-thesis: no diff - redistribution: increased the number and size of the plaquelike sites of avb3 expression -OCN: : in-creased -OPN: strain stimulate secretion of the 168-kDa mole-cule such that it does not accumu-late in the cell -Alizarin Red S: increased the intensity of min-eralized nod-ules

HOB: human osteoblasts, HFO: human fetal osteoblasts, HBMDO: human bone marrow derived osteoblasts, NHOBs: normal human osteoblasts, OGM: osteogenic growth medium, TCM: tissue culture medium, Ss: statistically significant

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 culture[24] and duration of loading application[28] can change its expression results. HFOs,[24] preosteoblasts, and osteoprogenitors[31] 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-α1 (Cbfa1) is the osteoblast-specific transcription factor through which mechanical loading can affect osteoblast differentiation.[21] Cbfa1 expression in HBMDOs peaked at 500 με and decreased at higher magnitudes of mechanical loading (1000 με),[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 study[13] mentioned that osteoblasts had different reactions to compression in 2D and 3D conditions, and only one study[12] 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 study[14] mentioned that the expression of ALP and COL 1 increased, while one study showed lower rates of COL 1 following compression, and another study[33] 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 cytocolagenase 2 were analyzed to assess osteoclastogenesis. Four studies[12,32,33] mentioned that compression increased the rate of osteoclastogenic factors. However, one study[33] 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 μs. Below this range is defined as the disuse mode and causes bone weakness. Bone remodeling is started at 1000–1500 μs and can be continued up to 3000 μs, 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 proliferation[37] 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/cm^2 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 matrix[20] 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/cm^2 compression will decrease the expression of OPN in HFO[13] 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/cm^2 compressive force increases the expression of RUNX2 in HFO[13] 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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