

Review

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Mechanobiology of bone remodeling and fracture healing in the aged organism

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Abstract: Bone can adapt to changing load demands by mechanically regulated bone remodeling. Osteocytes, osteoblasts, and mesenchymal stem cells are mechanosensitive and respond to mechanical signals through the activation of specific molecular signaling pathways. The process of bone regeneration after fracture is similarly and highly regulated by the biomechanical environment at the fracture site. Depending on the tissue strains, mesenchymal cells differentiate into fibroblasts, chondrocytes, or osteoblasts, determining the course and the success of healing. In the aged organism, mechanotransduction in both intact and fractured bones may be altered due to changed hormone levels and expression of growth factors and other signaling molecules. It is proposed that altered mechanotransduction may contribute to disturbed healing in aged patients. This review explains the basic principles of mechanotransduction in the bone and the fracture callus and summarizes the current knowledge on aging-induced changes in mechanobiology. Furthermore, the methods for external biomechanical stimulation of intact and fractured bones are discussed with respect to a possible application in the elderly patient.

Keywords: bone; mechanostimulation; regeneration; ultrasound; vibration.

Abbreviations

BMP, bone morphogenetic protein; CSF, colony-stimulating factor; COX-2, cyclooxygenase-2; ER, estrogen receptor; IGF-I, insulin-like growth factor-I; LIPUS, low-intensity pulsed ultrasound; LMHFV, low-magnitude, high-frequency vibration; MSCs, mesenchymal stem

cells; OPG, osteoprotegerin; PGE₂, prostaglandin E₂; RANKL, receptor activator of nuclear factor- κ B (NF- κ B) ligand; TGF- β , transforming growth factor- β ; vitamin D3, 1,25-dihydroxycholecalciferol.

Mechanobiology of bone tissue

Bone mass is maintained during life via constant bone formation and resorption, a process termed as bone remodeling. Bone-forming osteoblasts synthesize collagen and regulate extracellular matrix mineralization, whereas bone-resorbing osteoclasts maintain bone degradation by acidifying and solubilizing the bone mineral. The most numerous cells in bone tissue, the osteocytes, form a close communication network with their neighbor osteocytes and the other bone cells through gap junctions. Osteocytes are involved in regulating osteoblast and osteoclast activity and survival.

At the endocrine and molecular levels, several factors can influence the bone remodeling process. Hormones, including estrogen, insulin, cortisol, epinephrine, parathyroid hormone, and 1,25-dihydroxycholecalciferol (vitamin D3), regulate the activity of bone cells and control the balance between resorption and formation. Many growth factors and signaling pathways can exert osteoanabolic or osteocatabolic functions. For example, the process of osteoclast formation is regulated by colony-stimulating factor (CSF), several interleukins, parathyroid hormone, calcitonin, and vitamin D3 as well as the ratio of receptor activator of nuclear factor- κ B (NF- κ B) ligand (RANKL) to osteoprotegerin (OPG), both factors secreted by osteoblasts. Signaling pathways involved in osteoblast recruitment and maturation include transforming growth factor- β (TGF- β), bone morphogenetic proteins (BMPs), insulin-like growth factor-I (IGF-I), and the Wnt/ β -catenin signaling pathway [1].

In 1892, the anatomist and surgeon Julius Wolff postulated that bone remodeling is not only influenced by biological factors but is also under tight biomechanical control for a more efficient adaptation to changing load situations [2]. In 1987, Harold Frost extended Wolff's theory and demonstrated the dependence of bone

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formation on the quality and frequency of the mechanical stimulus. Frost postulated that different biomechanical loading ranges provoked either bone formation or resorption (the “mechanostat theory”), which was also shown by others [3–6]. The cellular and molecular mechanisms involved are not yet fully understood. One well-established theory suggests that osteocytes are the main mechanosensory cells in the bone [7–9]. Osteocytes may act as a sensor of local bone stresses, which arise from bending and compressive forces during walking. Tissue deformation induces interstitial bone fluid flow and osteocytes are able to sense the flow-induced shear stress on the surface of their cell bodies. Ion channels and integrin receptors are critical for the transduction of the mechanical signals into biochemical signals inside the cells. The process of converting external mechanical forces into a biochemical response is termed as cellular mechanotransduction [10]. Experimental studies demonstrated the involvement of numerous molecular pathways and mediators in mechanotransduction [11–13]. One main osteocytic mediator for load-induced bone formation is prostaglandin E₂ (PGE₂), which is secreted after the mechanically induced expression of cyclooxygenase-2 (COX-2) [10, 14]. Furthermore, mechanically stimulated osteocytes react by increasing their OPG/RANKL ratio, thus interacting with osteoclasts. Additionally, the osteocytic expression of sclerostin can be influenced by mechanical load [15]. Sclerostin is a regulator of osteoblastic bone formation. When sclerostin binds to low-density lipoprotein receptor-related proteins 5 and 6 on the cell membrane of osteoblasts, it inhibits canonical Wnt/ β -catenin signaling and reduces osteoblastic bone formation. Therefore, sclerostin acts as a coupling factor between osteocytes and osteoblasts.

In recent years, growing evidence has suggested that also other cells involved in bone metabolism, for example, bone-lining cells, osteoblasts, and mesenchymal stem cells (MSCs), may be mechanosensitive [16]. The *in vitro* mechanical stimulation of these cells led to increased osteogenic differentiation and matrix mineralization [17–19]. Therefore, the adaptation of bone to mechanical load involved several interacting cell types, signaling molecules, and pathways.

Disturbed mechanobiology in the aged organism

The process of mechanotransduction in the bone can be disturbed by several pathological conditions, such as during postmenopausal osteoporosis and in the aged

organism. Postmenopausal osteoporosis is characterized by the loss of ovary-derived estrogen, leading to a high bone turnover, an imbalance of bone homeostasis toward increased bone resorption, and a subsequent bone loss. The estrogen receptor (ER) signaling pathway is also important for the transmission of mechanical signals. In his “mechanostat theory” [4], Frost postulated that estrogen might decrease the mechanical threshold for bone formation and can sensitize the bone to mechanical stimuli. This was confirmed in several experimental studies [20], and it was shown that mechanotransduction is altered in osteoblasts from estrogen-deficient, osteoporotic patients [21]. However, the influence of estrogen on mechanically regulated bone formation appears to be strongly dependent on the timing of estrogen administration [22]. Moreover, the expression ratio of the two ERs, ER α and ER β , appears to be essential, with ER α probably increasing mechanosensitivity and ER β decreasing it [23]. The expression of both receptors is largely regulated by estrogen [24].

Similarly, in the aged organism, bone mass is gradually lost. In contrast to postmenopausal osteoporosis, the mechanism is not a high bone turnover but rather a low bone turnover. The reasons might be the reduction of the proliferation and differentiation capacity of stem cells, decreased physical activity, increased inflammatory cytokine levels, and reduced expression of several osteoanabolic mediators, including sex hormones, IGF-I, and molecules of the Wnt/ β -catenin signaling pathway [25–28].

Because many such mediators are also involved in mechanotransduction, it has been recently proposed that the mechanosensitivity of bone cells *per se* may decrease during aging, which can contribute to senile bone loss. However, the influence of age on the mechanotransduction processes in the bone remains controversial. There are experimental studies showing increased [29, 30], decreased [31–33], and unaffected [34–36] mechanoresponsiveness of bone tissue during aging, which depends on the applied stimulus and the determined outcome parameters. In clinical studies, both an anabolic response to physical exercise and no change have been reported in older humans in comparison to young control groups [37, 38]. Nevertheless, clinical studies have reported the effectiveness of external mechanostimulation on bone formation in aged osteoporotic patients. In particular, the so-called “low-magnitude, high-frequency whole-body vibration” (LMHFV) was shown to improve bone mass in aged postmenopausal women [39–42]. Therefore, even if the threshold value at which bone reacts to mechanical loading may be altered in the aged subject,

mechanostimulation may still represent a therapeutic option to reduce aging-induced bone loss.

Mechanobiology of fracture healing

The biomechanical environment is not only critical for bone homeostasis but also during fracture healing. The rigid fixation of long-bone fractures resulting in minimal interfragmentary movements induces direct intramembranous bone healing, whereas flexible fixation with higher interfragmentary movements results in callus healing with endochondral bone formation [43, 44]. Too flexible fixation can result in nonunions with hypertrophic fibrous tissue near the fracture gap. Similarly, too low biomechanical stimulation is detrimental for bone healing. The underlying mechanism of biomechanical influence on fracture healing is described in Pauwels' theory of "causal histogenesis" [45]. He postulated the profound influence of the mechanical environment on tissue differentiation. In more detail, Claes et al. demonstrated in 1998 that, if there are high stresses at the fracture area, mesenchymal cells are likely to form fibrous tissue, whereas osseous tissue is generated under low stress conditions. At intermediate stresses, mesenchymal cells will differentiate into chondrocytes and initiate cartilaginous callus formation, which initially bridges the fracture gap [46–48]. Several molecular factors are influenced by the mechanical environment during bone regeneration. In the inflammatory phase of fracture healing, cytokines, including chemokine C-X-C motif ligand 3, von Willebrand factor, macrophage-CSF, and tumor necrosis factor- α , are altered depending on the biomechanical environment at the fracture site [49–51]. During the endochondral ossification process, signaling pathways and molecules involved in chondrocyte maturation, including Indian hedgehog and collagen 2, are demonstrated to be decreased in the fracture calli of stabilized fractures compared to nonstabilized fractures [52]. Additionally, the expression of several components of the BMP signaling cascade, including BMP-2, Noggin, p-Smad, and BMP receptor-1A, is strongly influenced by the mechanical environment. Yu et al. [53] proposed that biomechanical stimuli might activate the osteoanabolic BMP signaling pathway, thereby influencing the cell-fate decision during the regeneration process. Lienau et al. [51] demonstrated that important osteoblastic mediators, including BMPs, IGF-I, OPG, and TGF- β , are reduced in animals with delayed fracture healing due to rotational instability. A serum analysis of fracture patients demonstrated increased levels of TGF- β and IGF-I in patients with flexible osteosynthesis [54]. Other experimental studies

showed a differential expression of angiogenic factors, including vascular endothelial growth factor and cysteine-rich angiogenic inducer 61, depending on the biomechanical environment at the fracture site [49, 50]. Genome-wide expression arrays comparing activated or repressed genes during nonstimulated and biomechanically stimulated fracture healing showed a differential expression of more than 100 genes, mainly associated with chondrocytic/osteoblastic differentiation, cell adhesion, or cell signaling pathways [55]. Therefore, the biomechanical environment critically influences cell-fate decision and thus fracture healing.

Fracture healing and mechanostimulation in the aged organism

Both clinical and experimental studies indicated that fracture healing is disturbed in the aged organism [56–62]. Experimental studies demonstrated a reduced osteogenic capacity of MSCs [63], disturbed cartilaginous and bony callus maturation [61, 64], decreased callus vascularization [60], and lower expression of osteoanabolic signaling molecules [63, 65]. Additionally, aging-induced changes in the inflammatory and oxidative stress response may be one reason for disturbed bone healing [66]. Confirming this, Xing et al. [67] demonstrated that the rejuvenation of inflammatory cells increased bone and callus formation in aged mice. Therefore, cells from both hematopoietic and mesenchymal lineages appear to be involved in aging-induced delayed healing.

Because it was also shown that the expression of the mechanosensitive gene COX-2 is markedly reduced in the fracture callus of aged animals [68], the question arises as to whether aging can also disturb the mechanobiological control of fracture healing and whether the "optimal" biomechanical conditions for successful bone healing are the same in young and aged patients. There are only a few studies investigating the effect of aging on the mechanobiology of fracture healing. It was demonstrated in aged rats that mechanical optimization of fracture fixation failed to improve healing [69]. Young control animals displayed a significantly larger callus volume and stiffness after semi-rigid fixation compared to rigid fixation. However, in aged animals, there were no differences between the two fixation groups. Likewise, Mehta et al. [70] demonstrated that changing the biomechanical environment did not alter bony callus formation, callus microstructure, or mineralization in aged animals unlike in young animals. In another study, the authors showed a different mechanoresponsiveness

of several genes, including TGF- β , MMP-9, and MMP-13, in aged compared to young rats and a reduced ability of aged MSCs to sense and adapt to mechanical stimuli [49]. Together, these studies suggested that reduced mechanotransduction in the aged organism may indeed contribute to disturbed bone regeneration. In contrast, a recent study demonstrated that changing the biomechanical environment at the fracture site did influence bone healing in aged rats [71]. High interfragmentary movements led to increased callus size with greater amounts of cartilaginous tissue. However, the late phase of fracture healing was not influenced by fracture fixation stability. In summary, the mechanobiology of fracture healing in the aged organism requires further investigation.

A further possibility, in addition to fracture fixation to influence bone healing mechanically, is the application of external biomechanical stimuli. In the literature, many different methods are described to influence the healing process [72–74]. One promising approach is the application of low-intensity pulsed ultrasound (LIPUS). Treatment with LIPUS during callus formation was generally demonstrated to accelerate the healing process in both clinical and experimental studies [73, 75–80]. Importantly, LIPUS also augmented fracture repair in aged patients [77] and animals [81, 82]. Molecular analyses showed increased neovascularization and bone formation in the fracture callus of aged individuals [82]. Interestingly, LIPUS treatment reduced the healing time in aged wild-type mice but not in COX-2 knockout mice, indicating a critical role of this mechanoresponsive gene and its downstream mediator PGE₂. Confirming this, injections with a PGE₂ receptor agonist restored the positive effects of LIPUS on fracture healing [83]. Therefore, reduced COX-2 and PGE₂ expression in the fracture callus of aged subjects may be critical for the healing process, whereas their expression can be increased by external mechanical stimulation to accelerate the regeneration. Another non-invasive biomechanical treatment to counteract delayed fracture healing is the application of whole-body LMHFV. *In vitro* experiments using preosteoblastic and MSCs demonstrated an increased osteogenic response after vibration therapy [17, 84, 85]. However, *in vivo* studies investigating the effects of LMHFV on fracture healing produced conflicting results, which appeared to be due to different animal models. Vibration therapy accelerated bone regeneration in estrogen-deficient, osteoporotic animals [86–90], whereas no or even negative effects were shown in estrogen-competent animals [90, 91]. Therefore, the success of LMHFV during fracture healing appears to be profoundly influenced by the estrogen level. Notably, aged estrogen-deficient mice also displayed improved

fracture healing after LMHFV [92]. Therefore, this method could be suitable to accelerate fracture healing in aged and postmenopausal patients. However, further studies are required to evaluate the safety and efficacy of LMHFV during bone healing in clinical practice.

In conclusion, mechanotransduction on the tissue, cellular, and molecular levels is strongly influenced by aging. The mechanoresponsiveness of both intact and fractured bones may differ between young and aged subjects. Particularly during the process of fracture healing, which is under tight biomechanical control, external mechanostimulation is able to influence the healing process even in the aged organism. Therefore, therapies such as LIPUS and LMHFV might have the potential to counteract delayed bone regeneration in the elderly. However, further studies and randomized clinical trials are needed to prove the effects of biomechanical stimulation on fracture healing in the aged patient.

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Author Contributions

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Supplemental Material: The article (DOI: 10.1515/iss-2016-0021) offers reviewer assessments as supplementary material.

Reviewer Assessment

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Melanie Haffner-Luntzer*, Astrid Liedert and Anita Ignatius

Mechanobiology of bone remodeling and fracture healing in the aged organism

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Reviewers' Comments to Original Submission

Reviewer 1: anonymous

Oct 06, 2016

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	10
Custom Review Question(s)	Response
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	2
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	2
Does the introduction present the problem clearly?	N/A
Are the results/conclusions justified?	2
How comprehensive and up-to-date is the subject matter presented?	2
How adequate is the data presentation?	N/A
Are units and terminology used correctly?	N/A
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	2
Does the reader get new insights from the article?	1 - Low/No
Please rate the practical significance.	2
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	N/A
Please rate the appropriateness of the references.	2
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	2
Are you willing to review the revision of this manuscript?	Yes

Comments to Author:

This manuscript represents a compact summary on some important aspects of mechanobiology on bone remodeling and fracture healing. It also provides a short glance on the effects of aging on these two processes. The manuscript is well written and comprehensive. However, it often remains superficial not really providing any additional insight or improved understanding of any of the processes in mechanobiology in association with aging. Most of the content is long and well known and well accepted in the bone and fracture community. For readers not familiar with the topic, the manuscript might provide a first glance on the subject.

In summary, the manuscript provides a compact overview on how bone remodeling and fracture healing are affected by mechanics without providing any new insights or raising any new thoughts. It thus might be a nice read for readers not familiar with bone, fracture and orthopaedics.

Some minor issues could be addressed in order to improve the manuscript:

Page 2 - 5 only discusses healthy bone and thus misses the point of the review article on fracture healing. Actually, the topic of this review article is mainly covered on page 7 and 8, not much more than one page. So it would be suggested to modify the title not only limiting the topic to fractured but also to healthy bone.

LIPUS and LMHFV are only two of various measures to externally affect fracture healing. Some older methods like electric or magnetic fields have demonstrated promising results in pre-clinical studies. Shock wave treatment is still be applied to accelerate healing and/or treat delayed unions. Some more recent methods like ultraviolet light stimulation demonstrated some potential. None of these have been included in the review. Moreover, most of the recent clinical trials on ultrasound and some on vibration therapy have not been included. The use of ultrasound in distraction osteogenesis has been ignored.

In the conclusion it is stated that there is strong influence of aging on mechanotransduction. This statement can be challenged as it is mainly based on pre-clinical and in vitro models of artificial aging and or artificially induced metabolic mechanisms. How strong the individual effects are in naturally occurring human aging still has to be demonstrated. Finally, there is no good reason to highlight LIPUS and LMHFV in light of the lack of their effect on healing in some more recent clinical trials.

Reviewer 2: anonymous

Oct 01, 2016

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	100
Custom Review Question(s)	Response
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	5 - High/Yes
How comprehensive and up-to-date is the subject matter presented?	5 - High/Yes
How adequate is the data presentation?	5 - High/Yes
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	5 - High/Yes
Does the reader get new insights from the article?	5 - High/Yes
Please rate the practical significance.	5 - High/Yes
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	N/A
Please rate the appropriateness of the references.	5 - High/Yes
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	5 - High/Yes
Are you willing to review the revision of this manuscript?	Yes
Comments to Author:	
This is an excellent review that adequately covers the topic.	

Authors' Response to Reviewer Comments

Oct 13, 2016

Reply to the reviewer's comments:

Reviewer 1

This manuscript represents a compact summary on some important aspects of mechanobiology on bone remodeling and fracture healing. It also provides a short glance on the effects of aging on these two processes. The manuscript is well written and comprehensive. However, it often remains superficial not really providing any additional insight or improved understanding of any of the processes in mechanobiology in association with aging. Most of the content is long and well known and well accepted in the bone and fracture community. For readers not familiar with the topic, the manuscript might provide a first glance on the subject. In summary, the manuscript provides a compact overview on how bone remodeling and fracture healing are affected by mechanics without providing any new insights or raising any new thoughts. It thus might be a nice read for readers not familiar with bone, fracture and orthopaedics. Some minor issues could be addressed in order to improve the manuscript:

Page 2 - 5 only discusses healthy bone and thus misses the point of the review article on fracture healing. Actually, the topic of this review article is mainly covered on page 7 and 8, not much more than one page. So it would be suggested to modify the title not only limiting the topic to fractured but also to healthy bone.

Answer: Thank you for this suggestion, we changed the title to "Mechanobiology of bone remodeling and fracture healing in the aged organism"

Reviewer 1: LIPUS and LMHFV are only two of various measures to externally affect fracture healing. Some older methods like electric or magnetic fields have demonstrated promising results in pre-clinical studies. Shock wave treatment is still be applied to accelerate healing and/or treat delayed unions. Some more recent methods like ultraviolet light stimulation demonstrated some potential. None of these have been included in the review. Moreover, most of the recent clinical trials on ultrasound and some on vibration therapy have not been included. The use of ultrasound in distraction osteogenesis has been ignored.

Answer: The reviewer is right, we did not include all methods for external biomechanical stimulation in our review. We just aimed to give some examples because this was not the main topic of the review. We included the statement: "In the literature, many different methods are described to influence the healing process [72-74]. One promising approach is the application of low-intensity pulsed ultrasound (LIPUS)..." in our manuscript.

Concerning the reviewer's statement about recent clinical trials, we additionally included the most recent studies about application of LIPUS during fracture healing. However, we did not include studies about the treatment of apparent Non-Unions with LIPUS, since the biological and mechanobiological properties of Non-Union fracture tissue may differ from fresh callus tissue, but this was not the topic of our review.

Concerning LMHFV, we did not find other recent clinical trials in the literature.

Reviewer 1: In the conclusion it is stated that there is strong influence of aging on mechanotransduction. This statement can be challenged as it is mainly based on pre-clinical and in vitro models of artificial aging and or artificially induced metabolic mechanisms. How strong the individual effects are in naturally occurring human aging still has to be demonstrated. Finally, there is no good reason to highlight LIPUS and LMHFV in light of the lack of their effect on healing in some more recent clinical trials.

Answer: Thank you for this suggestion, we added the statement: "However, further studies and randomized clinical trials are needed to prove the effects of biomechanical stimulation on fracture healing in the aged patient." to our conclusion.

Reviewer 2

This is an excellent review that adequately covers the topic.

Answer: Thank you for this positive comment.

Reviewers' Comments to Revision

Reviewer 1: anonymous

Oct 14, 2016

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	15
Custom Review Question(s)	Response
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	N/A
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	N/A
Are units and terminology used correctly?	N/A
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	5 - High/Yes
Does the reader get new insights from the article?	1 - Low/No
Please rate the practical significance.	1 - Low/No
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	N/A
Please rate the appropriateness of the references.	5 - High/Yes
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	2
Are you willing to review the revision of this manuscript?	Yes