Effects and mechanisms of mechanical stress on secondary fracture healing

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1. ABSTRACT

Fracture healing is an extremely complex biological reconstruction process, affected by age, hormones, blood supply, degree of injury, mechanical load and many other factors, among which, mechanical stimuli is one of the most important. Bone tissue has a good adaptability to mechanical stress, as biomechanical studies have shown that the local mechanical environment of the fracture site plays a regulatory role in the process of fracture healing (1,2). The present article reviews the mechanical mechanisms and effects of mechanical stress on fracture healing.

2. MODES OF FRACTURE HEALING

Biomechanical studies on bone have shown that the mechanical environment of fracture sites determines if fracture healing will be by direct or indirect processes. Direct healing occurs under highly rigid conditions that require the fracture sites to be closely connected, directly reconstructed, and no callus detected by X-ray imaging. Indirect healing occurs under less rigid conditions, including cell differentiation and proliferation at the fracture sites to connect calluses, then the bone matrix mineralizes and is deposited to form woven and lamellar bone tissues to achieve healing. Indirect healing is the

major type of fracture healing and allows subtle movements at the fracture sites to form the connection callus. This process depends largely on stress and tolerance of bone to stress, as too much stress in proportion to bone tolerance can lead to large shifts in fracture sites, resulting in delayed union, non-union, and pseudarthrosis. Therefore, the local stress environment of fracture sites determines the type of fracture healing.

3. THE EFFECTS OF MECHANICAL STRESS ON FRACTURE HEALING

3.1. Duration of mechanical stress

The choice of stress duration is important for bone healing, as studies have shown that the original callus and hematoma tissue are very sensitive to stressful stimuli during the initial stages of fracture healing and stressful stimuli can promote inflammatory responses at the fracture site and release a variety of inflammatory mediators and activate osteoblasts (3). During the initial stage of fracture healing, stress caused by the axial load can promote osteoblasts, cartilage cells, and fibroblasts to form bone, which is beneficial to fracture healing. Takeda et al. (4) reported the effects of mechanical stimulation on tibial fracture in rats during the early stage of bone fracture. One group received stimulation 2 days after fracture-inducing surgery and at postoperative day 14, the amount of callus in the peripheral and central regions was greater in the stimulation group than the control group. Lately, rigid external stimulation that withstood increased callus formation has identified a contribution of mechanical stimulation in the initial period of fracture healing (5). However, in the process of bone healing, repair tissue at the fracture sites gradually becomes rigid, which results in the reduction of strain tolerance. At this time, too much stress can easily damage newly established vascular tissue and hinder the fracture healing process, resulting in delayed union or non-union of the fracture (6).

3.2. Direction of mechanical stress

During fracture healing, the differentiation and proliferation of mesenchymal cells are closely related with the direction of mechanical stress. Compressive stress and shear stress with low intensity can promote the formation of intramembranous ossification and the tensile strength can induce cartilage formation, while high intensity shear stress leads to the formation of fibrous tissue and fibrocartilage and prevents their further differentiation to woven bone and lamellar bone (7). The combination of shear stress and hydrostatic pressure can promote fibrocartilage formation, while the combined shear stress and periodic axial compressive stress promote cartilage formation and endochondral ossification resulting in abundance of the outer callus (8). Most specialists believe that shear stress prevents mesenchymal cell differentiation into cartilage and endochondral ossification, inhibits vascular regeneration of the area around the fracture, and promotes generation of fibrous connective tissue, thus resulting in delayed union, non-union, or pseudarthrosis (9). However, the healing rate of oblique fractures is faster than that of transverse fractures. In addition to the contact area of the fracture site, the role of shear stress is unknown.

Schell et al. (10) used external fixation to treat oblique fractures and found that while the shear interfragmentary movement was > 4 mm, the fracture healed quickly. In fact, neither axial compressive stress nor shear stress has specific parameters to promote fracture healing and the specific biomechanical mechanism remains unclear.

3.3. Intensity and magnitude of mechanical stress

The required stress intensity varies in different phases of fracture healing. In the early phase of healing, the repair tissue at the fracture sites has relatively low stiffness and withstands external force poorly, thus requires lower stress levels. With the increase of stiffness in repair tissue, the load tolerance is increased, as well as the stress level. Only when the stress level of the fracture site and the stiffness of repair tissue are balanced can the tissue achieve good differentiation and healing. Otherwise, the stress is greater than the tissue tolerance, thereby impeding callus formation and inhibiting absorption of necrotic cells at the fracture site, leading to bone atrophy. Conversely, if stress stimulation is not sufficient to cause tissue strain, it is difficult to generate cell differentiation and proliferation, which may lead to delayed union or nonunion of bone. Claes et al. (11) applied goat tibial osteotomy to establish a finite elemental analysis model in three groups based on osteotomy gap size of 1, 2, or 6 mm, respectively. In each group, the fracture site received a minimum fracture strain of 6% and maximum strain of 31%, then results were obtained through finite elemental analysis, which showed that the formation of intramembranous ossification was induced with strain < 5% in the fracture area and hydrostatic pressure < 0.15 MPa, the formation of endochondral ossification was promoted when strain was < 15% in the fracture area and hydrostatic pressure > 0.15 MPa, while under other conditions, connective tissue and fibrous cartilage tissue were formed. Although many studies included relevant parameters of stress on fracture sites using animal models, it is difficult to obtain an ideal model because of the large individual differences and different degrees of fracture. Thus, further research is needed to establish specific parameters relevant to the magnitude of strain during different stages of fracture healing and animal studies will undoubtedly present significance guidance to clinical work.

3.4. Frequency of mechanical stress

Rubin et al. (12) studied the effects of various stress frequencies on bone healing in a turkey ulna model and found that after applying low-amplitude micro-dynamic stress, high frequency (20 Hz) promoted fracture healing more than low frequency (1 Hz). Goodship et al. (13) evaluated the ability of low-magnitude, high-frequency mechanical signals to influence fracture healing by applying a frequency of 30 Hz to a sheep osteotomy model and found that it promoted callus formation and mineralization, and increased callus stiffness. As a living material, stress stimuli and frequency range applied on bone tissue should be considered. The stress stimuli of high frequency (> 30 Hz) may cause osteoporosis and bone resorption, while the stress of low frequency (0.5~3 Hz) can not only promote fracture healing, but also prevent osteoporosis, as low-frequency stress is closer to

physiological frequency of normal walking and load-bearing.

3.5. Mode of mechanical stress

Bone cells are capable of sensing and responding to mechanical forces, but mechanosensitivity declines soon after stimulus initiation, as under continued stimulation, bone is desensitized to mechanical stimuli. Many studies have shown that cyclical or intermittent stress stimulus is more sensitive and osteoblastic compared with continual stress stimulation, thereby promoting fracture healing (14). Intermittent stress stimulus is more effective to stimulate cell activity in bone tissue than continuous stress stimulation. Reportedly, a significant abundance in the amount and vitality of bone cells that express glucose-6-sulfuric acid-cystine and increased intake of ^{3}H-uracil nucleoside are associated with the intensity of stress stimulation. In a study regarding sensing mechanisms of stress load on the mouse tibia, Robling et al. (15) found that bone cells were sensitive to short-term intermittent stress and long-term stress bone induces saturation of cell sensitivity to mechanical signals. An intermittent time of 4—8 h was best for osteogenesis.

4. MECHANICAL STRESS MECHANISMS ON FRACTURE HEALING

The process of fracture healing and bone regeneration is affected by blood supply to fracture sites, the stress environment, and concentrations of hormones, growth factors, and many other factors, among which, mechanical stress stimulus was the most important. However, questions remain, such as: Why does mechanical stimulus promote fracture healing? How are bone cells sensitive to stress stimulation? and What is the transmission mechanism of mechanical signals that convert to biochemical signaling? The development of in vitro cell culture technology and molecular cell biology will present further research opportunities regarding these issues.

4.1. Microdamage and microcirculation

Primary bone injury can initiate normal fracture repair, in which living bone around the necrotic tissue proliferate and differentiate into new tissues and then connect together, in a process referred to as the original callus reaction. This initial reaction occurs regardless of the changes to the internal or external environment; however, its development is limited, unless under favorable conditions. Mechanical stimulation causes repeated microdamage at the fracture sites, prolongs the original callus reaction and inflammatory response, promotes the release of many inflammatory mediators and growth factors, and initiates the bone healing process, thereby inducing the proliferation and differentiation of mesenchymal cells into osteoblasts and chondrocytes. The blood supply of the peripheral fracture area is very important to fracture healing. However, local blood vessels suffer varying degrees of damage; therefore, capillary regeneration and vascularization of surrounding tissues is particularly important. Many studies have shown that during the initial phase of fracture healing, the capillary beds are very sensitive to mechanical stress stimulation.

Appropriate stress stimuli promote capillary growth and tissue vascularization, which transport nutrients for fracture repair and improve local oxygen concentration (16). With capillary growth, more mesenchymal stem cells proliferate and differentiate into osteoblasts under stress and aerobic conditions and vascular endothelial cells can also differentiate into osteoblasts and participate in osteogenesis, thus promoting fracture healing (17).

4.2. Mechanical signal transmission mechanism in bone cells

Bone is a dynamic viscoelastic tissue and very sensitive to mechanical stress stimulation, as bone cells can sense strain at the tissue level directly or indirectly. The mechanical receptors of bone cells include integrin on the bone cell membrane and cytoskeleton, and the three-dimensional mesh structure of the bone lacuna. After osteoblasts and osteoclasts experience stress stimulation, a mechanical signal transmission process in the bone cells is initiated, which can be divided into four steps: mechanical signal coupling, biochemical coupling, signal transmission, and effector cell response. The mechanical signals received by bone cells are transformed into biochemical signals, which induce cellular biochemical effects and promote cell proliferation and differentiation, as well as the secretion and metabolism of growth factors, hormones, active ossification substances, and so on (18, 19). Integrins are a family of membrane receptors and the adhesion molecules of this family mainly mediate intracellular and extracellular matrix adhesion. The cytoskeleton is an organelle, which connects to membrane lipoprotein molecules and is the molecular basis of cell movement, cell morphology, and transmembrane signal transduction. As the main substance that connects intracellular and extracellular signaling, integrin can cause cytoskeleton rearrangement, change microfilament protein conformation, and induce the chromosome to change cell functions. Strain on bone tissue causes the liquid flow of intercellular fluid to the bone lacuna system, which is a receptor sensitive to shear stress and hydrostatic forces of intercellular fluid. Further, it transports biochemical information through gap junctions and is a biochemical signal coupling structure in bone cells. After integrin, the cytoskeleton complex and bone lacunar-bone tube system receive stress stimulation signals through specific conduction channels, such as protein kinase C (PKC), focal adhesion kinase, mitogen activated protein kinase (MAPK), and Ca^{2+} channels, etc. Then, the mechanical information is transferred to the endonuclear matrix to phosphorylate extracellular signal conditioning kinase 2, the c-jun enzyme, p38, and MAPK, then causes intracellular biochemical effects to control transcription expression of endonuclear COX-2, c-fos, and c-jun (21), which promote the synthesis and secretion of growth factor associated with osteoblast activity, such as insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and bone morphogenetic protein (BMP).

4.3. Synthesis and secretion of cellular growth factors and hormones

Bone tissue can sense stressful stimuli and promote responses. In vivo experiments have shown that axial stress
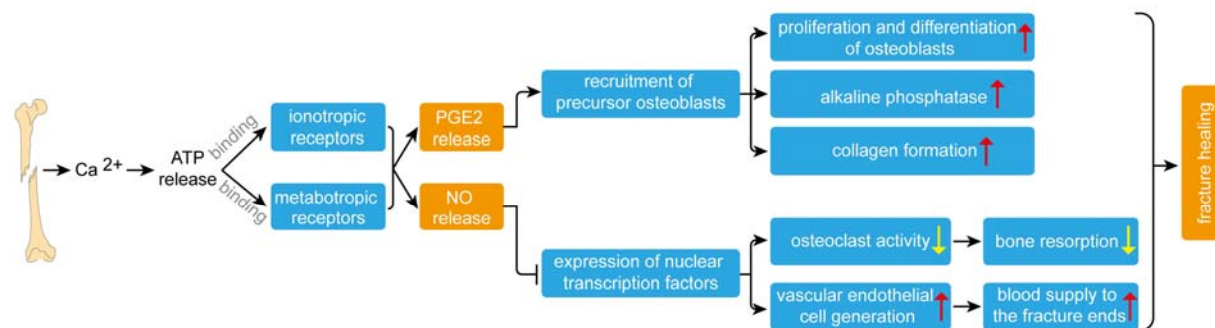
Figure 1. PGE2 and NO in the fracture healing. After bone cells are exposed to fluid sheer stress, the internal flow of extracellular Ca^{2+} can promote adenosine-5'-triphosphate (ATP) release. PGE2 and NO are released followed by ATP binding to ionotropic and metabotropic receptors. Through recruitment of precursor osteoblasts, PGE2 promotes the proliferation and differentiation of osteoblasts and increases alkaline phosphatase activity and collagen formation. Through blocking the expression of nuclear transcription factors, NO inhibits osteoclast activity to reduce bone resorption, and promotes vascular endothelial cell generation to increase blood supply to the fracture ends; therefore, it plays an important role in regulation of bone anabolism, thus promoting fracture healing.

and fluid shear stress in the physiological range releases prostaglandin (PGE2) and nitric oxide (NO) and induces generation of cell growth factors, including BMP, IGF, TGF, VEGF, and platelet-derived growth factor (PDGF) (22). Since there is an abundance of lacuna in bone tissue, bone cells are very sensitive to fluid shear stress. After bone cells are exposed to fluid sheer stress, the internal flow of extracellular Ca^{2+} can promote adenosine-5'-triphosphate (ATP) release. PGE2 and NO are released followed by ATP binding to ionotropic (i.e., purinoceptor 1) and metabotropic receptors (i.e., purinoceptor 14). Through recruitment of precursor osteoblasts, PGE2 promotes the proliferation and differentiation of osteoblasts and increases alkaline phosphatase activity and collagen formation. Through blocking the expression of nuclear transcription factors (i.e., receptor activator of nuclear factor-kappaB ligand), NO inhibits osteoclast activity to reduce bone resorption, and promotes vascular endothelial cell generation to increase blood supply to the fracture ends; therefore, it plays an important role in regulation of bone anabolism, thus promoting fracture healing (23) (figure 1). In addition, after exposure to stress, bone cells produce a series of secondary messenger molecules, such as Ca^{2+}, cathelicidin antimicrobial peptide precursor, and PKC, among others. The secondary messengers transport biochemical signals into the nucleus through a conduction pathway, then regulate the transcription and expression of genes, thereby guiding the synthesis and secretion of cellular growth factors, such as BMP, IGF, TGF, and VEGF. Among these, BMP, TGF, and IGF promote differentiation of mesenchymal cells and precursor osteoblasts into osteoblasts, osteoclasts, and chondrocytes, VEGF plays an important role in the vascularization of fracture sites, and PGF and PDGF promote mitosis in mesenchymal cells, osteoblasts, and chondrocytes to influence fracture healing.

5. CONCLUSION

Fracture healing can be enhanced by load bearing and specific components of the mechanical environment, such as action time, strength, and frequency, can augment or accelerate the process. Although the mechanisms remain unclear, we can clarify these parameters using animal experimental models. In clinical applications, it is very difficult to establish optimal stress parameters for different phases of bone fracture healing because of the individual differences in levels of fracture damage and locations. Further, it is difficult to avoid the limitations in clinical work using only animal experimental results. In further studies, research should be directed at elucidating the intercellular transmission of mechanical signals and the molecular mechanisms to improve the fracture healing rate, shorten treatment duration, and avoid delayed union, nonunion, and osteoporosis.

7. REFERENCES

1. Schuller M, Weninger P, Tschegg E, Jamek M, Redl H and Stanzl-Tschegg S: Micromotion at the fracture site after tibial nailing with four unreamed small-diameter nails--a biomechanical study using a distal tibia fracture model. *J Trauma* 66,1391-1397 (2009)

2. Mark H, Nilsson A, Nannmark U and Rydevik B: Effects of fracture fixation stability on ossification in healing fractures. *Clin Orthop Relat Res* 419,245-250 (2004)

3. Zhang P, Malacinski GM and Yokota H: Joint loading modality: its application to bone formation and fracture healing. *Br J Sports Med* 42,556-560 (2008)

4. Takeda T, Narita T and Ito H: Experimental study on the effect of mechanical stimulation on the early stage of fracture healing. *J Nippon Med Sch* 71,252-262 (2004)

5. Weis JA, Miga MI, Granero-Molto F and Spagnoli A: A finite element inverse analysis to assess functional improvement during the fracture healing process. *J Biomech* 43,557-562 (2010)

6. Ulstrup AK: Biomechanical concepts of fracture healing in weight-bearing long bones. *Acta Orthop Belg* 74,291-302 (2008)

7. Smith-Adaline EA, Volkman SK, Ignelzi MA, Jr., Slade J, Platte S and Goldstein SA: Mechanical environment alters tissue formation patterns during fracture repair. *J Orthop Res* 22,1079-1085 (2004)

8. Sanz-Herrera JA, Doblare M and Garcia-Aznar JM: Scaffold microarchitecture determines internal bone directional growth structure: a numerical study. *J Biomech* 43,2480-2486 (2010)

9. Augat P, Burger J, Schorlemmer S, Henke T, Peraus M and Claes L: Shear movement at the fracture site delays healing in a diaphyseal fracture model. *J Orthop Res* 21,1011-1017 (2003)

10. Schell H, Epari DR, Kassi JP, Bragulla H, Bail HJ and Duda GN: The course of bone healing is influenced by the initial shear fixation stability. *J Orthop Res* 23,1022-1028 (2005)

11. Claes L, Eckert-Hubner K and Augat P: The effect of mechanical stability on local vascularization and tissue differentiation in callus healing. *J Orthop Res* 20,1099-1105 (2002)

12. Rubin J, Rubin C and Jacobs CR: Molecular pathways mediating mechanical signaling in bone. *Gene* 367,1-16 (2006)

13. Goodship AE, Lawes TJ and Rubin CT: Low-magnitude high-frequency mechanical signals accelerate and augment endochondral bone repair: preliminary evidence of efficacy. *J Orthop Res* 27,922-930 (2009)

14. Gardner MJ, van der Meulen MC, Demetrakopoulos D, Wright TM, Myers ER and Bostrom MP: In vivo cyclic axial compression affects bone healing in the mouse tibia. *J Orthop Res* 24,1679-1686 (2006)

15. Robling AG, Burr DB and Turner CH: Recovery periods restore mechanosensitivity to dynamically loaded bone. *J Exp Biol* 204,3389-3399 (2001)

16. Rentz JA, Chapman B, Alvarez PJ and Schnoor JL: Stimulation of hybrid poplar growth in petroleum-contaminated soils through oxygen addition and soil nutrient amendments. *Int J Phytoremediation* 5,57-72 (2003)

17. Lin H, Aubin CE, Parent S and Villemure I: Recent advances in mechanobiological modeling of bone regeneration. *Mech Res Commun* 42,22-31 (2012)

18. Morgan EF, Gleason RE, Hayward LN, Leong PL and Palomares KT: Mechanotransduction and fracture repair. *J Bone Joint Surg Am* 90 (Suppl 1) ,25-30 (2008)

19. Shapiro F: Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. *Eur Cell Mater* 15,53-76 (2008)

20. Liedert A, Kaspar D, Blakytny R, Claes L and Ignatius A: Signal transduction pathways involved in mechanotransduction in bone cells. *Biochem Biophys Res Commun* 349,1-5 (2006)

21. Robling AG, Castillo AB and Turner CH: Biomechanical and molecular regulation of bone remodeling. *Annu Rev Biomed Eng* 8,455-498 (2006)

22. Cui L, Ma YF, Yao W. Cancellous bone of aged rats maintains its capacity to respond vigorously to the anabolic effects of prostaglandin E2 by modeling-dependent bone gain. *J Bone Miner Metab* 19,29-37 (2001)

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