Review

Mechanobiology of indirect bone fracture healing under conditions of relative stability: a narrative review for the practicing clinician

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Abstract. *Background and aim:* Mechanical influence on secondary fracture healing remains an incompletely understood phenomenon. This is of special importance in biological osteosynthesis, where sility is sacrificed for the sake of an optimal biological fracture environment. Under condition of relative stability, a wide range of biomechanical conditions can be achieved. Mechanobiology, which studies mechanical influences on biological systems has become a large, interdisciplinary field. The aim of this article is to present a comprehensive synthesis of the literature for the practicing clinician, with insights relevant to their practice of fracture care. *Methods:* The MEDLINE online database (Pubmed) was searched in September 2021 for relevant articles. *Results:* The search provided 816 results, which were scanned by the first author by the title and abstract. With relevance to the research topic, 59 articles were chosen and read in detail. Another 70 articles were added by screening the references of relevant articles. A total of 129 articles were read and analysed *Conclusions:* Mechanical environment plays a crucial role in the fracture healing process. The definition of an optimal mechanical environment still evades us, due to the complexity of the problem. Computational models could replicate the complex mechanical environment of bone healing in humans but require detailed knowledge of mechano-transduction and material properties of healing tissues. The literature reminds us of the importance of adequate stiffness of constructs used under conditions of relative stability. Hopefully, further research in this field will result in not only empirical but more accurate and evidence-based assessments of osteosynthesis fixations. (www.actabiomedica.it)

Key words: Mechanobiology, Bone fracture, Secondary Bone Healing, Relative Stability, Osteosynthesis, Orthopaedic Trauma Surgery.

Introduction

The bone fracture healing process presents an astonishing integration of engineering and biology. Bone is one of the rare tissues which heals without a scar, with full regeneration of form and function – *restitutio ad integrum*. (1) To fully appreciate this phenomenon basic knowledge from the field of mechanics is necessary. The term *stability* is a qualitative measure used to describe the amount of movement in the fracture gap under physiologic loading. The same movement can be quantified through the calculation of the Young's elastic module of a tissue or construct, representing its *stiffness*. The term *strength* in this context usually represents the *ultimate tensile strength*, which defines the maximum stress (load) a material or construct can withstand before failure. A similar term often used in fracture care is *rigidity*, which describes the mechanical behaviour (*stiffness*, *strength*) of an implant (plate, intramedullary nail, etc) (2).

The science of bone fracture treatment has seen a rapid development since the second part of the 20th century. Especially through the pioneering work and research of the "Arbeitsgemeinschaft für Osteosynthesefragen", *eng. Association for the Study of Internal Fixation* (*AO group)* a paradigm shift has taken place, with the primary goal of treatment being not the healed fracture, but a *functional* bone, limb and patient (3). In-vivo studies on bone healing showed that under the conditions of anatomic reduction (no fracture gap) and *absolute stability* (no interfragmentary movement) healing occurred through osteonal remodeling, without the formation of callus, allowing early pain-free movement of the limb (Figure 1).

However, due to the detrimental effects of direct fracture exposure, reduction techniques and implant disturbance on fracture vascularity, emphasis has been given to osteosynthesis techniques which put the respect for the biological fracture environment in the forefront (4). These techniques rely upon indirect, functional reduction techniques that do not disturb the bone healing unit. Moreover, methods of fracture stabilization which induce mechanical conditions of *relative stability* are increasingly being used. The latter allow elastic, reversible movement between the main bone fragments to occur under physiological loading. The respect for biology, however, comes at the price of mechanical stability. These techniques induce secondary or indirect fracture healing with callus formation (Figure 2).

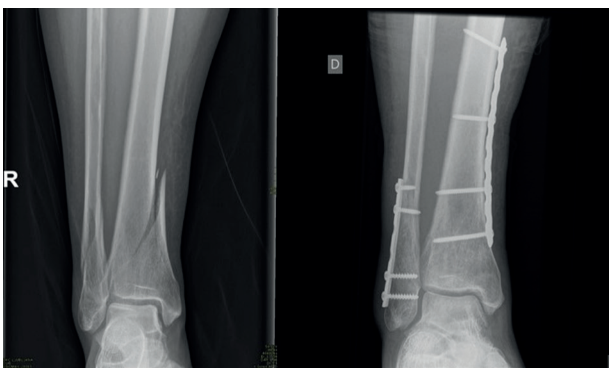

Figure 1. Distal tibia fracture treated with lag screws and antiglide plate (absolute stability).

In contrast to absolute stability, which represents a single mechanical environment without interfragmentary movement, relative stability encompasses a broad spectrum of mechanical environments from the theoretical infinite instability of two independent bone fragments suspended in midair on one end, to the rigid osteosynthesis which induces a mechanical environment close to those with absolute stability on the other. Within this bandwidth, secondary fracture healing can occur in a vast proportion of mechanical environments, which is why fractures in nature are able to heal so well, despite nonanatomic reduction and interfragmentary movement (IFM) (Figure 3).

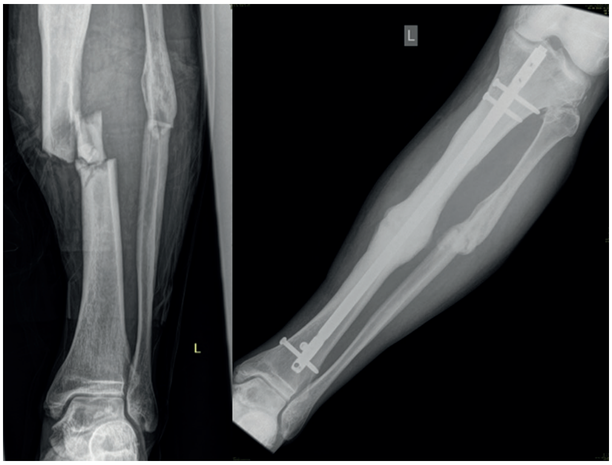

Figure 2. Tibia shaft fracture treated with a locking intramedullary nail (relative stability).

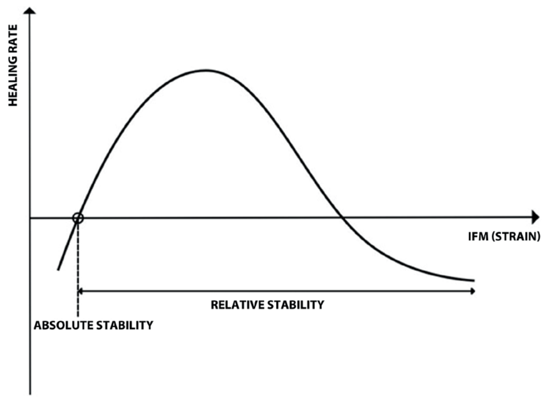

Figure 3. Bone healing under relative stablity: Based on "A unified theory of bone healing and nonunion", Elliott et al, Bone Joint J, 2016.

Secondary fracture healing has been described in detail from a qualitative perspective, however a precise quantitative understanding of the mechanisms by which loading influences the mechanical environment of fracture healing is still lacking (5). At both ends of the spectrum of relative stability, fracture healing disturbances occur due to either insufficient or excessive IFM. Without the definition of an optimal mechanical environment and in absence of clinical tools to assist surgeons in predicting the exact biomechanical features of an osteosynthesis, 5-10% of treated fractures still show a disturbance in the healing process (6). A detailed understanding of the process required research to move to the tissue and cellular levels of bone healing. The complexity of the problem appropriately grows, with a plethora of interdependent biological and mechanical variables that alter the outcome (7). Hence, *mechanobiology*, the science which explains the influence of mechanical conditions on biological processes, has moved out of the boundaries of medicine and presents an interdisciplinary field combining clinical experience with knowledge from bioengineering, molecular biology and imaging technologies (8). With better understanding of the basic mechano-transduction pathways, computational models could be developed to tailor individual fracture treatment plans, minimizing the chance of healing disturbances and, if possible, enhance the healing process.

The aim of the present review is to provide a comprehensive synthesis of the research done in this field. We wish to provide the insights from experimental and computational research to the practicing clinician and to shed light on areas in need of future research.

Methods

The MEDLINE online database (Pubmed) was searched in September 2021 for relevant articles with the following search terms: ("mechanical environment" OR "mechan*" OR "Stress, Mechanical"[Mesh] OR "Mechanotransduction, Cellular"[Mesh] OR "Weight-Bearing"[Mesh]) AND ("Fractures, Bone"[Mesh] OR "fracture site") AND ("Fracture Healing"[Mesh] OR "healing process" OR "healing environment" OR "healing progress") AND ("Information Theory"[Mesh] OR "Models, Theoretical"[Mesh] OR "Models, Biological"[Mesh] OR "Models, Structural"[Mesh] OR "model*" OR "paradigm*")

The search was performed with additional filters: "full text", "Journal Article", "German", "English".

The search provided 816 results, which were scanned by the first author by the title and abstract. With relevance to the research topic, 59 articles were chosen and read in detail. Another 70 articles were added by screening the references of relevant articles. A total of 129 articles were read and analysed.

Secondary Fracture Healing

The purpose of secondary bone healing is to stabilize the main fracture line, where IFM is greatest. This is achieved through the development of the fracture callus, a complex healing unit. The process can be arbitrarily divided into four distinct phases. After the formation of the fracture hematoma, a strong inflammation response is initiated, resulting in formation of mechanically weak granulation tissue. The reparative phase can be split into soft and hard callus formation. Soft callus is characterized by the presence of all types of non-mineralized tissues such as fibrous tissue, cartilage, and osteoid matrix. Soft callus gradually evolves in hard callus when mineralized cartilage and bone (woven) appear. When the callus is fully bridged with woven bone, the fourth phase of remodeling re-establishes the original anatomy and mechanical characteristics (lamellar bone) depending on the physiological loading regime.

The process is not linear as phases overlap with each other in space and time. Within the same time frame from initial injury, different tissue patterns can be found in specific locations of the healing unit. The callus is a dynamic, heterogenous structure, responding to the ever changing mechanical and biological environments. Despite a myriad of conditions to which a fracture can be exposed, certain common patterns of healing could be observed in histological analysis of bone healing (9,10). We can divide the fracture healing zone into four parts with distinct mechanical and biological characteristics:

- cortical fracture ends
- endosteal callus
- periosteal callus
- fracture gap

Initially, the greatest amount of movement seems to occur within the fracture gap, where connective tissue predominantly forms. On periosteal and to a lesser degree endosteal surfaces remote from the fracture site, bone starts to form early within the healing process through intramembranous ossification, due to low strains and an abundance of progenitor cells (11).

Areas within the callus at the level of the fracture gap, exposed to intermediate strains, tend to differentiate into cartilage. With escalating degrees of IFM or construct instability, the callus replies with proliferation of repair tissues and an increase in volume, where the *stiffness* increases with third power of its radius (12). Once the callus is bridged, cartilage mineralizes and chondrocyte hypertrophy leads to their programmed cell death (apoptosis). Blood vessels from the periosteal and surrounding tissues infiltrate the callus and enable endochondral ossification to proceed from the periphery towards the fracture gap. The cuff of woven bone stabilizes the original fracture gap, where fibrocartilage is converted to bone. With reestablishment of bone continuity, loads are now shifted back through the original cortex. The callus is thus shielded from stress and undergoes remodeling.

Mechano-transduction

There is therefore an ability of the Mesenchymal Stem Cells (MSC) in the healing zone to sense changes in their mechanical environment. Exactly through which mechanisms this mechano-transduction occurs is still unknown (13).

In the second half of the 20. century, Pauwels observed that reparative cells under tension tend to develop a fibrous phenotype, while hydrostatic pressure (compression) induces differentiation down a chondrogenic lineage (causative histogenesis) (14). He postulated there is no stimulus that would itself stimulate bone formation and new bone is formed on already established repair tissues through endochondral ossification.

In 1980 Perren introduced the "Interfragmentary Strain Theory", where he observed that healing tissue can only form between its induction and tolerance thresholds specific for that tissue type (15). The term strain implies tissue deformation under load and can be defined as change in length divided by the original length, which is in this case the length of the fracture gap (Figure 4).

As good as this model is for a qualitative description, it represents an oversimplification with limited use in experimental and clinical studies, since the interfragmentary strain (IFS) is not uniformly distributed over the fracture zone and the callus. Moreover, the material characteristics of tissues measured *in-vitro* may well be different in the *in-vivo* setting during different stages of healing and loading directions.

Based on the causal histogenesis theory, Claes and Heigele (16) and Carter (17) developed a mechanobiological model, quantifying the boundaries for tissue differentiation under strain and hydrostatic pressure (Figure 5).

Fundamentally, their work still incorporates Pauwels' dogma that not only magnitude, but also the direction of strain and stress (compression) are important.

The letter was challenged by Lacroix and Prendergast (18) who developed a bi-phasic material properties model, as opposed to previous models which

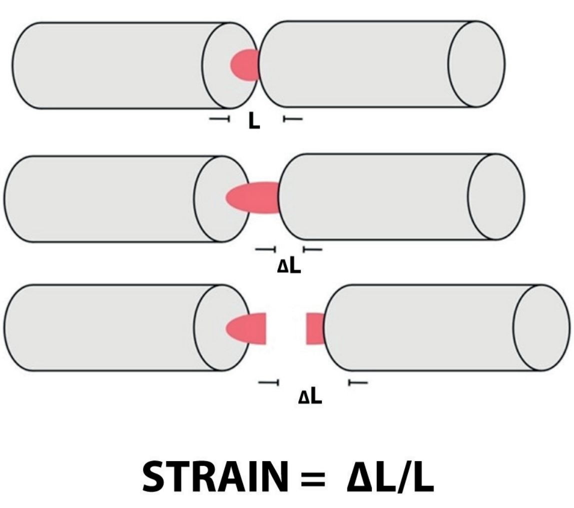

Figure 4. Interfragmentary Strain Theory (2).

assumed healing tissues to be linear elastic. As a measure of deviatoric deformation in the solid phase, cell shape deformation or strain was taken. For the volumetric changes in the fluid phase, fluid flow velocity was calculated. The model predicts that cells respond only to different magnitudes of shape and volume changes, while the direction does not seem to be of importance in the differentiation pathway.

Epari (19) compared the predictive value of the three established Finite Element Analysis (FEA) models validating them against an experimental study with histological analysis of fracture healing. For comparison, a fourth model with only deviatoric strain as mechanical stimulus was established. The study showed that none of the models reached a predictive accuracy which would warrant their use in clinical practice.

Qualitatively all models gave similar results, while quantitative differences in predicting tissue formation were observed. Similarities between the models were predictable, since all measure invariants of cell deformation, deviatoric strain and dilatational strain (hydrostatic pressure, fluid flow velocity) were observed, although in different ways. Surprisingly, the model with strain measures only was equally good at predicting bone healing. The study was, however, limited to the initial phase of healing, while volumetric and fluid flow changes might be important in the later stages with solidification of the healing tissues. The quantitative differences between the models most probably arise from the different values of material properties and thresholds for tissue differentiation. The study from Isaksson (5) found that the Lacroix/Prendergast model was the only one able to effectively predict healing under torsional loading.

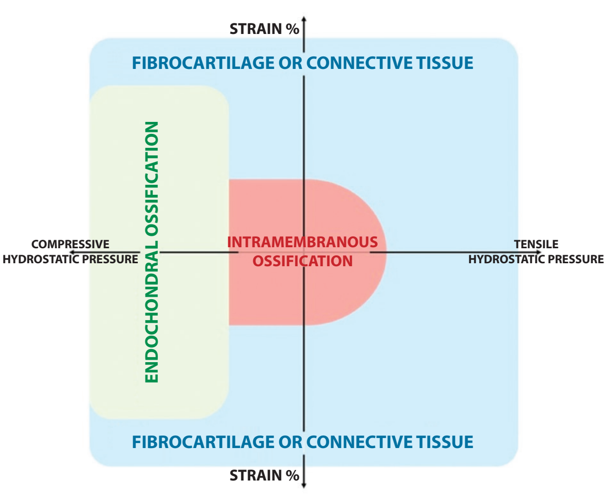

Figure 5. Mechano-biological model based on strain and hydrostatic pressure (16).

To sum up, it seems that cells can sense their mechanical environment through changes in shape and volume. Although certain mechano-transduction pathways have been found to regulate intracellular pathways and gene expression (20), the exact mechanisms are still unknown. Different computational models have been successfully used to predict bone healing in simplified mechanical conditions. Deviatoric strain or change in cell shape seems to have the greatest influence, at least in the initial stage of healing. However, based on the current state of the art, extrapolations to bone healing in the clinical setting cannot be made yet.

Osteosynthesis and fracture mechanical environment

The mechanical environment of a fracture is on a gross scale determined by loading and the degree of fracture stabilization. Loading is induced by the forces/moments of gravity and through muscular activity (8). In the clinical setting, loading conditions are complex and might occur through combinations of translational and rotational movements (Table 1).

Not only the direction and magnitude, but also the rate, duration, and number of cycles of loading must be considered. Thus, the exact conditions vary greatly between anatomic regions and are mostly unknown. On the other hand, the stability (*stiffness*) of a construct depends upon the non-linear sum of the intrinsic stability of a fracture and the type of the chosen osteosynthesis (OS) (Table 2).

Loading of a construct produces IFM which is converted into heterogenous IFS along the healing unit and in the fracture gap. MSC sense the changes in the mechanical environment and mount an adequate

Table 1. Loading movements in 6 degrees of freedom.

Translational movements	Rotational movements
Axial translation	Internal-external rotation
Antero-posterior translation	Ante-recurvatum
Medio-lateral translation	Varus-Valgus

Table 2. Factors determining loading and construct stability (fracture + OS).

Loading	Fracture stability	Osteosynthesis stability
Anatomic area	Bone/segment	Implant type/material
Gravity	Fracture geometry	Implant dimensions
Muscular activity	Contact surface	Implant geometry
Rehabilitation protocol	Reduction	Implant application
Pain induced shielding	Comminution	Trans-fixation method

response thorough proliferation and tissue differentiation. As the callus grows and matures, it constantly alters the biomechanical environment, with the process either ending with fracture union, delayed healing or non-union.

Another independent crucial parameter is the dimension of the fracture gap. According to the IFS theory a larger gap would produce a more favourite mechanical environment, lowering the strains in interfragmentary tissues. However, clinical experience and experimental studies show that the biological potential of the bone healing unit to bridge a gap is limited and worst results were achieved with large fracture gaps (inadequate reduction) and interfragmentary movements (construct instability) (18,21,22).

Conditions of relative stability in fracture care are mostly induced with the use of locking intramedullary nails, external fixators and plates used in the bridging mode (internal fixator principle). Every implant produces a unique mechanical environment, which can be altered also by changing its size, geometry, material, working length, mode of application and transfixation method (conventional screws, locking screws, bolts,). Implants are anisotropic and therefore do not produce equal stiffness under loads from different directions. This has been of special interest in research of the impact from translational shear on fracture healing. It's role as a detrimental movement has long been a matter of controversy. Some traditional studies (23-26) concluded that shear intrinsically delays/prevents fracture healing by stimulating fibrous tissue formation. Later studies (27-29) explained that those results most probably stem from the anisotropic behaviour of implants used, where the same loading produced a much greater strain in the translational direction than in the axial. Moreover, these studies showed adequate healing when intermediate strain magnitudes in both axial and shear directions when compared.

Appreciating the complexity of the problem and the number of variables which are not known or cannot be measured directly in the clinical environment, it becomes clear why research has been limited to experimental animal studies. However, these studies are very heterogenic regarding animal species (sheep, rat, rabbits), bones (femur, tibia, metatarsal), loading conditions (axial, shear, torsion, combinations), methods of fixation (unilateral fixators, ring fixators, intramedullary nails, plates), construct stiffness, fracture gap sizes, mechanical stimulus (force, rate, duration, number of cycles), length of observation/study and outcome measures. These limitations preclude a unified quantification of secondary bone healing. Nonetheless, animal studies have given many qualitative insights into how mechanical modulation of the fracture environment affects the healing process. They have shown that mechanical loading is not only able to prevent delayed healing, but also acceleration in the process itself can be achieved (30).

The optimal mechanical environment that would allow this in a clinical setting remains elusive, due to the limitations mentioned above and to the dynamic nature of the process, which leads us to believe the optimal values differ within the developing healing stages. Moreover, they led into questioning the traditional way we treat fractures, allowing ever increasing weight-bearing and unrestricted movement as the healing progresses. The controversial theory of dynamization, which has been for years incorporated into fracture treatment, has been challenged by the opposite "reverse dynamization". Both are presented in the following section.

Dynamization and reverse dynamization

Dynamization has been a vaguely define term in orthopaedic trauma surgery. It encompasses two mechanically quite distinct techniques:

1. destabilization of a construct, leading to decreased *stiffness* and thus increased IFM at constant physiologic loading (external fixator modulation)
2. allowing compression at the fracture site, thus obliterating the fracture gap, and increasing *stiffness* due to interfragmentary contact. At constant physiological loading, lower IFM would be expected (removal of static locking in intramedullary nail fixation).

In animal experimental studies of mechanobiology, the external fixator was the most frequent choice of fixation. Non-interference with the fracture environment, modulation and measurement options were the major arguments in its favor (31). When researching the effect of dynamization, the first definition applied. Studies have shown that although some degree of IFM is needed to stimulate the healing process, further dynamization delayed healing, by inducing additional instability (32). In an attempt to reduce the additional IFM, additional callus mostly constituted of fibrocartilage forms. If the gap can be bridged, more time is needed for endochondral ossification to replace large volumes of cartilage (33,34). Statistically faster and more complete bony bridging was seen under stable fixation. (32,35,36)

Not all bone healing stages seem to be equally sensitive to changes in the mechanical environment. (8) The initial stages of healing (the first two weeks) seem to be of particular importance, since MSC determine their differentiation fate based on mechanical conditions in this period. Also, the strong biological response in the inflammation phase seems to override the mechanical stimulation, as no differences in tissue distribution were found in this phase despite different loading regimes (37-39). On the other hand, the periosteal gap increasingly narrows as bridging nears completion in the later phases. Moreover, tissues of the hard callus have very low strain tolerance. Inducing additional instability in these phases might produce a high IFS environment preventing formation of a bridge over the two halves of the healing front. Additional fibrous and cartilage tissue needs to form to abolish the extra movement, thus delaying healing. If unsuccessful, a hypertrophic non-union develops with later formation of a pseudo-arthrosis.

A similar situation might be attributed to allowing the patients to progressively load and exercise the injured limb when seeing progression of callus formation on X-rays. However, it has been proven that just through muscle activation similar loads and IFM can be achieved as with weight-bearing (40).

These observations led to the development of the theory of "reversed dynamization". Here, high strain tolerance of the tissues in the initial stages is exploited to produce effective mechanical stimulation of the healing process. In the latter stages, stiffness is increased and/or loading decreased, thus enabling the previously produced large callus to quickly mineralize and ossify. Some studies have shown that faster union with higher rates of bone stiffness and strength can be achieved (41-43). The majority of these animal studies were performed under simplified mechanical conditions, with IFM being applied in one or two directions. Loading regimes in animals are hard to control and continuously measure. Under such conditions IFS measurements are not accurate enough to make any quantitative parallels with the complex bone healing environment in humans.

Finite Element Analysis (FEA) models

At this point, the number of mechanical variables stemming from loading conditions, fracture geometry, fracture gap size, type of osteosynthesis and tissue material properties that need to be controlled to set up an experimental environment to accurately study bone healing can be appreciated. Moreover, the bi-phasic nature of tissues and biological processes such as neo-angiogenesis, MSC concentration, migration, proliferation rate and incompletely understood tissue differentiation algorithms and thresholds need to be accounted for. With the limitations of human and animal experimental studies, computational models such as the *FEA* were introduced from the field of engineering (44). They allow for simulations of different mechanical and biological conditions. However, they still need verification against an experimental model and histological analysis to confirm the validity and accuracy of their predictions.

In the FEA, a computer model of the desired bone and fixation method are made (33). Apart from geometry, material properties of cortical bone and construct need to be defined. Further, a fracture is simulated, usually through a transverse osteotomy, and a fracture gap width defined. Next boundary conditions of the healing unit or callus are determined. To simulate the initial phase of bone healing, material properties of granulation tissues are inserted. Next, the loading regime is defined in its magnitude, direction, rate and number of cycles. The above-mentioned biologic factors are incorporated through the "fuzzy logic" algorithm (45). Application of a mechano-transduction model (see section "Mechano-transduction") defines the rules and thresholds of sequential tissue differentiation. The simulation is repeated as an iterative process until bone bridging or non-union occurs.

So far, simplified models of FEA have been developed, which can effectively predict bone healing under limited mechanical conditions (46). With better understanding of mechano-transduction and further development of the FEA technique, complex biomechanical environments could be simulated accurately. Optimal mechanical conditions for specific fractures could thus be determined, allowing the surgeon to plan a tailored osteosynthesis with a reliable outcome.

Clinical Implications

Mechanobiology has yet to develop to the point where clear, quantitative recommendations regarding the mechanical fracture environment can be made. However, based on results from the current literature, adequate stability or stiffness must be established. The four cornerstones of the AO principles (3) have withstood the test of time and are to be always respected:

1. Fracture reduction and reestablishment of anatomical relationships
2. Stable fixation as required by the "personality" of the fracture
3. Preservation of the blood supply to soft tissues and bone
4. Early and safe mobilization of the limb and patient

As construct instability leads to delayed healing, we present some methods to increase stiffness with the use of following implants (47):

Monolateral External fixator

- Increase the number and diameter of Schanz screws
- Increase the distance between the Schanz screws
- Pre-tension Schanz screws before locking
- Decrease the bar to bone distance
- Increase the number of connecting bars
- Apply the fixator in multiple planes

Locked Intramedullary Nailing

- Reaming - insertion of a larger diameter nail
- greater contact area between bone and nail -decreased working length
- Increase the number of locking screws
- Insert locking screws in multiple planes
- Use ASLS – Angular Stable Locking System

Plate in bridging mode

Application of bridging plates, especially with rigid angular stable systems, requires special attention. Due to the position of the plate remote from the bone axis, bending moments arise. This leads to much greater IFM on the opposite side of the fracture than under the plate due to the lever arm effect (48). This asymmetrical mechanical environment is hard to optimize. Stiff implants with multiple locking screws and a short working length would thus supress the mechanical stimulation needed for healing, especially in the area under the plate. Thus, the following general recommendations are in order:

- Increase plate length
- Increase working length of the plate
- 0.5 ratio of filled to empty screw holes - CAVE: metaphyseal fractures with short segments, osteoporotic bone!
- Use of locking screws according to the internal fixation principle

Except for the external fixator, the only variable a clinician can modulate in the postoperative setting is the rehabilitation protocol. As joint mobilization is imperative for prevention of the "fracture disease" and an amount of IFM is desired for stimulation of the healing process, excessive IFM, especially in the later stages, seems to be detrimental and delays bony bridging. The traditional method of ever-increasing weight bearing and load exercises with the progression of healing has thus been put under question by the reverse dynamization theory. The clinical problem is of course multifaceted, for example in geriatric population where immediate full weight bearing and early mobilization are of paramount importance. Based on the reviewed literature, however, the authors cannot give recommendations regarding optimal loading protocols. We believe every case of osteosynthesis presents a unique situation and must be tailored accordingly by the treating surgeon, who knows best what kind of mechanical environment has been established.

Conclusions

Mechanobiology has become a broad interdisciplinary area of research where many important facets of secondary fracture healing still need clarification. Detailed knowledge about mechano-transduction, material properties of healing tissues and an accurate differentiation algorithm are prerequisites for the establishment of computational models, which could replicate the complex mechanical environment of bone healing in humans. Also, further refinements of experimental studies, against which such FEA models could be validated, is warranted. So far, we have learned that the mechanical environment plays a crucial role in the fracture healing process. As we move away from rigid fixations for the sake of a better biological environment, the literature remind us of the importance of adequate stiffness of constructs used under conditions of relative stability. The definition of an optimal mechanical environment still evades us, due to the complexity of the problem. Orthopaedic trauma surgeons are at the heart of coordinating this process by choosing appropriate fixation methods and postoperative loading protocols. Hopefully, further research in this field will result in not only empirical but more accurate and evidence-based assessments of established osteosynthesis fixations.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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