Mechanics Research Communications 42 (2012) 22–31

Contents lists available at SciVerse ScienceDirect

Mechanics Research Communications

journal homepage: www.elsevier.com/locate/mechrescom

Recent advances in mechanobiological modeling of bone regeneration

Hanna Isaksson[a,b,*]

[a] Division of Solid Mechanics and Division of Orthopedics, Lund University, Lund, Sweden
[b] Department of Applied Physics, University of Eastern Finland, Kuopio, Finland

ARTICLE INFO

Article history:
Received 20 August 2011
Available online 22 November 2011

Keywords:
Skeletal regeneration
Tissue differentiation
Tissue growth
Finite element
Mechano-regulation

ABSTRACT

Skeletal regeneration and bone fracture repair involves complex cellular and molecular events that result in new bone formation. Many of the critical steps during bone healing are dependent on the local mechanical environment in the healing tissue. Computational models are used together with mechano-regulation algorithms to predict the influence of mechanical stimuli on the tissue differentiation process during bone healing.

This paper reviews the field of computational mechanobiology with focus on bone healing. The history of mechanoregulatory modeling is described, as well as the recent advances and current problems. Most recent advances have been focusing on integrating the mechano-regulatory algorithms with more sophisticated description of the cellular and molecular events. Achieving suitable validation for the models is the most significant challenge. Thus far, focus has been on corroborating mechanoregulatory models by comparing existing models with well characterized experimental data, identify shortcomings and further develop improved computational models of bone healing. Ultimately, these models can be used to help unraveling the basic principles of cell and tissue differentiation, optimization of implant design, and potentially to investigate treatments of non-union and other pathologies.

1. Introduction

Bone regeneration and fracture healing is so common in life that it is easy to overlook how astonishing it is as a biomechanical phenomenon. In contrast to other adult biological tissues, which heal with the production of scar tissue, bone heals with bone. The repair includes complex and multifactorial processes of cellular and molecular events that results in new bone formation. New bone is formed and continuously remodeled until its mechanical properties are restored and the original site of injury can hardly be recognized. Most commonly, the bone heals sequentially by tissue differentiation, where several intermediate tissues are formed that stabilizes the fracture and finally results in bony bridging of the fracture. The principles of bone fracture healing are similar to other bone forming and regenerative processes, e.g. long bone growth during fetal development, limb lengthening (distraction osteogenesis), bone ingrowth (osseointegration) on implants, and bone tissue engineering.

Impaired healing has been associated with a variety of factors, including the mechanical and the biological environments. It is well recognized that mechanical stimulation can induce fracture healing or alter its biological pathway (Claes et al., 1997, 1998; Goodship and Kenwright, 1985). However, the mechanisms by which mechanical stimuli are transferred into a biological response remain partly unknown. A better understanding of these processes would enable the development of more accurate and rational strategies for fracture treatment and would open up unlimited fields of research in other disciplines of regenerative medicine.

Mechanobiology describes the mechanisms by which mechanical loads regulates biological processes through signals to cells (in contrast to biomechanics which is the study of the mechanical behavior of biological systems) (van der Meulen and Huiskes, 2002). When the mechanisms of mechanically regulated tissue formation are better understood, then physiological conditions and pharmacological agents may be developed to promote better and faster bone tissue formation. Computer modeling is having a profound effect on the field of mechanobiology (Prendergast, 1997). The relationship between global mechanical loads and the local stresses and strains that influence the tissue formation can be calculated using computational models. In fact, many biological processes, including bone repair, are so complex that physical experimentation is often either too time consuming, too expensive, or impossible. As a result, mathematical models that simulate the complex systems are more extensively used. In mechanobiology, computational models have been developed and used together with *in vivo* and *in vitro* experiments to quantitatively determine the rules that govern the effects of mechanical loading on cells and tissue differentiation, growth,

* Corresponding author at: Division of Solid Mechanics, Lund University, Box 118, 221 00 Lund, Sweden. Tel.: +46 46 22 24423.
E-mail address: hanna.isaksson@solid.lth.se

0093-6413/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.mechrescom.2011.11.006

adaptation and maintenance of bone (van der Meulen and Huiskes, 2002).

Mechanical perturbations are applied to model geometry, and the local mechanical environment is calculated using the finite element method (FEM). The biological aspects of the computations are based on different premises for local mechanical variables stimulating certain cellular activities, for example cell division (proliferation), or changes in bone structure (bone remodeling). Computational models are gradually becoming more sophisticated with increasing computational power and mechanobiological knowledge. Both experimental and computational studies are critical to advance our knowledge in mechanobiology. Integration of the two fields is important, since models can help interpret experiments and experiments can provide relationships and observations for model development.

This article will summarize the mechano-regulatory algorithms available in the literature, with focus on studies that have used these algorithms in combination with FEM to study bone repair and the recent advances in the field. Some of the related problems are identified, e.g. how to sufficiently validate the necessary assumptions. Finally, the future potential of corroborated mechanobiological models is discussed, including how mechanical modeling can be used to improve our understanding of basic biology during bone regeneration and for developing clinical treatment protocols for fracture healing, or in tissue engineering. Given the extensive amount of work in this area it is not possible to describe all literature in detail. Therefore, the focus is to describe the background and to highlight some of the recent advances and future directions that may be important in bone regeneration.

2. Bone fracture repair

Bone fractures when its strain limit is exceeded, most commonly by physical trauma. The fracture results in a series of tissue responses that remove tissue debris, re-establish the vascular supply, and produce new skeletal matrix (Einhorn, 1995). Once a fracture has healed and undergone remodeling, the structure will have returned to the pre-injury state.

Bone healing generally occurs through either primary or secondary healing. Primary fracture healing (also known as direct healing, or intramembranous bone formation), involves direct cortical remodeling without any external tissue (callus) formation (Perren, 1979). It occurs only under small displacements, with either a small gap or direct contact of the fractured compact bone ends. It is a slow process that can take months to years until healing is complete. In contrast to primary healing, secondary healing occurs in the presence of some interfragmentary movement between the fractured bone ends, and is the process by which most fractures heal naturally. It involves a sequential tissue differentiation processes by which the bone fragments are first stabilized by an external callus (Fig. 1) (Perren and Rahn, 1980; Perren and Claes, 2000). Recovery of bone strength is generally more rapid than in primary healing. The callus stabilizes the fracture by enlarging its cross sectional area and increasing its stiffness through tissue differentiation. The interfragmentary movement decreases with healing time, as the callus stiffens. Finally, the hard callus bridges the bony fragments and reduces the interfragmentary movement to such a low level that bone formation can occur in the gap. The process of bone repair by secondary healing is divided into three overlapping stages; inflammation, repair (formation of soft and hard callus tissue), and remodeling (resorption of the callus) (Fig. 1) (Frost, 1989a,b). The mechanical environment primarily plays a crucial role in the reparative phase of healing, which will be the focus of this review.

Shortly, during inflammation, mesenchymal stem cells migrate towards the fracture region to form a loose granulation tissue (Gerstenfeld et al., 2003; Postacchini et al., 1995). The cells divide (proliferate), to later differentiate and change their cell phenotype to tissue specific cells that can generate fibrous tissue, cartilage or bone, respectively (Einhorn, 1998). Intramembranous bone formation also occurs in secondary repair, although at some distance from the fracture gap (Fig. 1). This rapid formation of woven bone begins several millimeters away from the fracture gap (Einhorn, 1998). Concurrently, callus formation through endochondral ossification occurs at and around the fracture gap. The soft callus consists of fibrous and/or cartilaginous connective tissues, which have developed from the mesenchymal tissue. The amount of cartilage that is formed is dependent on the amount of mechanical stimulation (Claes et al., 1997; Epari et al., 2006a). The formation of cartilage usually begins at the cortical bone ends and expands radially. Eventually the cartilage calcifies, which allows the ingrowth of bone. The bone formation occurs step by step towards the fracture plane. Once bony bridging of the callus has occurred and reunited the fracture ends, the processes of bone remodeling and resorption dominates the activities in the callus.

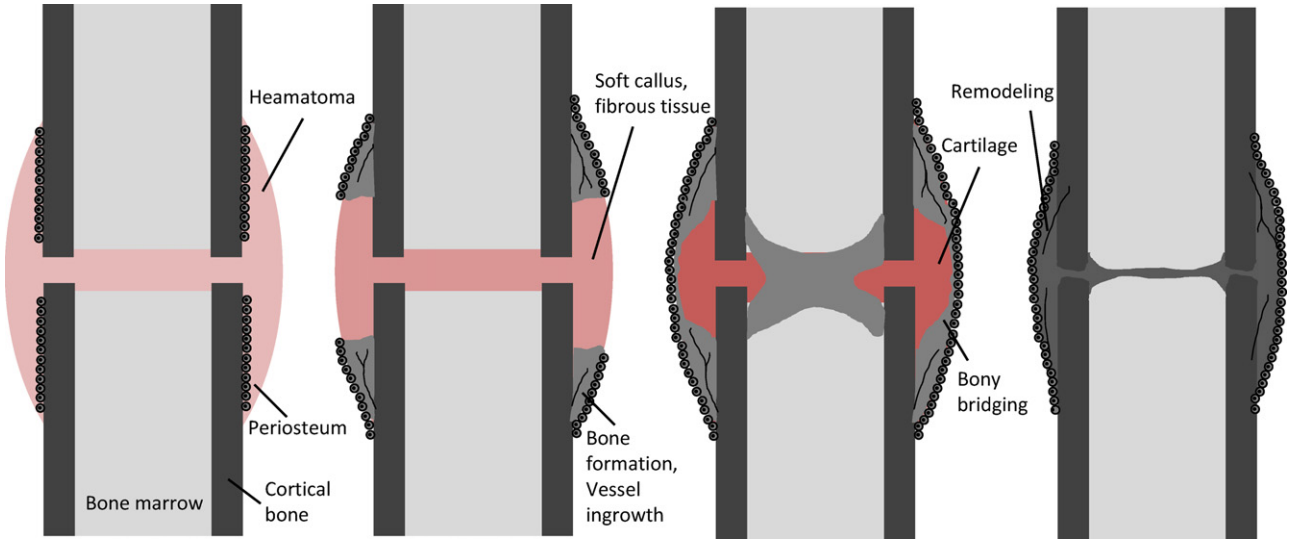

Fig. 1. Secondary bone fracture healing occurs through a sequential tissue differentiation process, from an initial heamatoma (blood cloth), through stages of soft and hard callus formation, external bony bridging and finally bone remodeling.

The less organized woven bone is gradually replaced by more highly organized and stiff lamellar bone, and remodeling of the newly formed bone tissue and of the fracture ends restores the original shape and lamellar structure of the bone (Einhorn, 1998).

2.1. The relationship between mechanical stimulation and bone healing

Mechanical stimulation can induce fracture healing or alter its biological pathway (Claes et al., 1997, 1998; Goodship and Kenwright, 1985). The most dominant mechanical factors identified are the fracture geometry (pattern and gap size) and the magnitude, direction and history of the interfragmentary motion between the bone ends. These factors determine the local strain field in the callus. The distribution of local strain in the healing tissue is believed to provide the mechanobiological signal for regulation of the fracture repair process that stimulates cellular reactions. Small gaps are beneficial for a fast and successful healing process, while larger gaps result in delayed healing, with decreased size in the external callus and reduced bone formation in the fracture gap (Claes et al., 1997). The amount of interfragmentary movement is dictated by external load and fixation stability. A stiff fixator limits the stimulation of callus formation, while flexible fixation enhances callus formation. Unstable fixation can lead to excessive motion and result in non-union (Claes et al., 1995; Epari et al., 2006b; Kenwright and Goodship, 1989). The direction of the interfragmentary movement influences the healing process. Moderate axial interfragmentary movement is widely accepted to enhance fracture repair by stimulating formation of periosteal callus and increasing the rate of healing (Kenwright et al., 1991). Shear movements, however, have resulted in contradicting results. Experimental studies have shown that shear movements at the fracture site result in healing with decreased external callus formation, delayed bone formation in the fracture gap, and inferior mechanical stability, compared to healing with axial movement (Augat et al., 2003). However, other experimental investigations have demonstrated superior healing under shear, compared to axial motion (Bishop et al., 2006; Park et al., 1998). Hence, the effect of shear, appears to be highly sensitive to timing, magnitude, and/or gap size (Augat et al., 2005). More details are available in a recent review of *in vivo* experimental models that have been used to investigate the effect of mechanical loading during bone healing (Epari et al., 2011). Despite extensive experimental knowledge, it is still uncertain how these global mechanical stimuli translates into pressure, fluid flow or shear at the tissue and cell level. That translation can be investigated with computational tools.

Additionally, numerous other factors are highly important for successful bone healing. For example, sufficient soft tissue coverage is crucial to restore the vascular supply and provide the cells with oxygen and nutrients. Moreover, the biochemical milieu including many growth factors affects the healing. However, those are beyond the scope of this article, and for more information the reader is referred to the following review articles (Aspenberg, 2005; Garrison et al., 2010; Keramaris et al., 2008; Nauth et al., 2010).

3. Computational bone mechanobiology

Computational modeling using FEA has significantly improved the methodology of the design process in biomedical applications (Prendergast, 1997). Computational mechanobiology attempts to determine the quantitative rules that govern the effects of mechanical loading on tissue differentiation, growth, adaptation and maintenance (van der Meulen and Huiskes, 2002). By utilizing FE models together with models describing biological activities, these processes are simulated adaptively (Fig. 2).

The modeling is based on the premise that local mechanical variables stimulate cell expression to regulate tissue composition, density or structure. Modeling considerations include force application at the boundary, force transmission through the tissue matrix, mechanosensation and transduction by cells, and transformation of extracellular matrix characteristics (Fig. 2). These parts are combined and represented by variables, parameters and mathematical relationships in a FE model. Some of these variables are known, or can be measured (e.g. morphology, mechanical tissue properties, external loading characteristics), whereas others have to be estimated. Some of the most common proposed mechano-regulatory algorithms that have been used to study tissue differentiation and bone healing are described below.

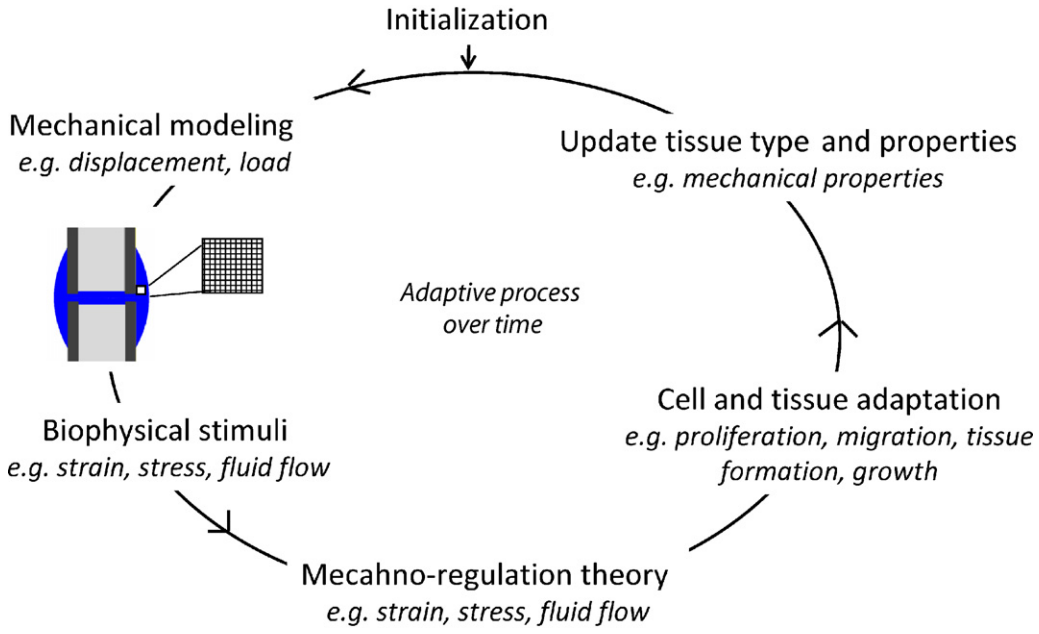

Fig. 2. An adaptive mechanobiological modeling scheme of bone healing.

4. Mechanoregulatory algorithms

4.1. Early theories

In 1960, Pauwels proposed the first rigorous theoretical framework by which the effects of mechanical forces on tissue differentiation pathways occur through mechanical deformation of the tissues (Pauwels, 1960). He suggested that tissues were suited to sustain distinct mechanical stressing. Fibrous tissue forms in regions of tension and cartilaginous tissue are suited to support hydrostatic pressure. Hence, he identified strain and pressure as two distinct stimuli that would stimulate fibrous tissue and cartilage, respectively. Primary bone formation requires a stable, low-strain mechanical environment and endochondral bone formation will proceed only after the soft tissues have stabilized the environment sufficiently to create this low strain environment (Pauwels, 1960) (Fig. 3a). The fundamental concept was that in the case of a healing fracture, it is impossible for direct bone formation to bridge an unstable gap without being destroyed. Therefore the purpose of the intermediate tissues is to stabilize and stiffen the fracture callus and to create a mechanically undisturbed environment where bone can form. Pauwels' theory was based on clinical observation and logic, but he did not have the means of measuring or calculating the tissue strains or stresses in detail.

Perren and Cordey (1980) proposed that tissue differentiation is controlled by the resilience of the callus tissues to strain. Their idea was that a tissue that ruptures or fails at a certain strain level cannot be formed in a region experiencing strains greater than this (Fig. 3b). The interfragmentary strain is determined by taking the longitudinal fracture-gap movement and dividing it by the size of the gap. As a tissue in the fracture gap stiffens, the interfragmentary strain is reduced allowing healing by progressive tissue-differentiation from the initial granulation tissue, to fibrous tissue, cartilaginous tissue and finally bone. However, the hypothesis only considered axial strains and the important strain contributions from radial and circumferential strains were not accounted for.

4.2. Single phase finite element models

Based on the ideas of Pauwels, Carter et al. (1988) proposed a model in which local stress or strain history explained tissue differentiation over time. Later, they proposed a more generalized mechano-transduction model (Carter et al., 1998) (Fig. 3c). When the tissue is subjected to high tensile strains (above the tension line) fibrous tissue is produced. Production of cartilaginous tissue is predicted to occur under high pressure, i.e. to the left of the pressure line, since this tissue can support and resist hydrostatic pressure. When the hydrostatic pressure is low, i.e. to the right of this line, formation of bone occurs. No specific threshold values were specified for tension or pressure lines. The studies by Carter et al. were the first to employ FEA to explore relationships between local stress/strain levels and differentiated tissue types. They modeled the tissue in the callus as a single solid (linear elastic) phase. They investigated the predictions of the model for a developing joint, endochondral ossification during fracture healing, and healing around orthopedic implants (Carter et al., 1988, 1998; Giori et al., 1995). Carter's studies stressed that a good blood supply is necessary for bone formation, while a compromised blood supply favors cartilaginous tissue formation. Carter's mechanobiological model has also been used to study oblique fractures (Blenman et al., 1989), pseudoarthrosis formation (Loboa et al., 2001), asymmetric fractures (Gardner et al., 2004) and distraction osteogenesis (Morgan et al., 2006). However, none of the studies predicted tissue differentiation adaptively over time.

Claes and associates performed an interdisciplinary study comparing data from animal experiments, FEA and cell cultures to assess the influence of gap size and interfragmentary strain on bone healing (Claes et al., 1997, 1998). Based on histological observations, Claes and Heigele (1999) formulated a mechano-regulation algorithm, similar to that of Carter. For the first time, they quantified thresholds for when the various tissues were to form (Fig. 3d). The FEA used to determine the thresholds, was a solid hyperelastic analysis, performed at a few specific time points during fracture healing. By comparing the mathematical analysis of stress and strain with histology they could attribute intramembranous bone formations to local strains of less than 5% and hydrostatic pressure between ± 0.15 MPa. Compressive hydrostatic pressures greater than -0.15 MPa and strains smaller than 15% appeared to stimulate endochondral ossification, with all other conditions corresponding to areas of fibrous tissue or fibrocartilage. Their theory was based on observations that bone formation occurs mainly near calcified surfaces. This algorithm has also been combined with other rules of bone healing, using an iterative FE model controlled by 'fuzzy logic' (Ament and Hofer, 2000) to investigate trabecular bone fracture healing (Shefelbine et al., 2005).

4.3. Biphasic and adaptive finite element models

In a biphasic analysis of a tissue differentiation experiment around an orthopedic implant, it was found that the stresses on the tissues are generated both by the tissue matrix and by the drag forces from interstitial fluid flow (Huiskes et al., 1997; Prendergast et al., 1997). This indicated the need for biphasic models. Prendergast et al. (1997) introduced a model of tissue differentiation based on a biphasic poroelastic FE model of the tissues, and proposed two biophysical stimuli: shear (deviatoric) strain in the solid phase and fluid velocity in the interstitial fluid phase as the mechano-transduction variables. High magnitudes of either, favors fibrous tissue, and only when both stimuli are low enough, can bone formation occur (Fig. 3e).

Lacroix et al. applied this algorithm to investigate tissue differentiation during fracture healing based on an 2D axisymmetric FE model (Lacroix and Prendergast, 2002; Lacroix et al., 2002). Their adaptive poroelastic model was able to simulate direct periosteal bone formation, endochondral ossification in the external callus, stabilization when bridging of the external callus occurs, and resorption of the external callus (Lacroix and Prendergast, 2002). The model was able to predict slower healing with increasing gap size and increased connective tissue production with increased interfragmentary strain. These studies introduced the first biological representations by prescribing stem cell concentrations initially at the external boundaries and using a diffusive mechanism to collectively simulate migration, proliferation and differentiation of cells. This model has later been used for successful predictions of tissue differentiation in a rabbit bone chamber (Geris et al., 2004), and during osteochondral defect healing (Kelly and Prendergast, 2005).

4.4. Comparison of biophysical stimuli

Although different in theory, the mechano-regulation algorithms described above were shown to be able to predict normal bone healing reasonably well. Geris et al. (2003) compared the ability of the algorithms by Claes and Heigele (1999) and Prendergast et al. (1997) to predict bone formation inside a rabbit bone chamber. They introduced both algorithms in one geometrical model, but used different material descriptions for each algorithm. They found that the fluid flow was important for the predicted differentiation patterns in the bone chamber. However, they were not able to separate the models in terms of their validity (Geris et al., 2003).

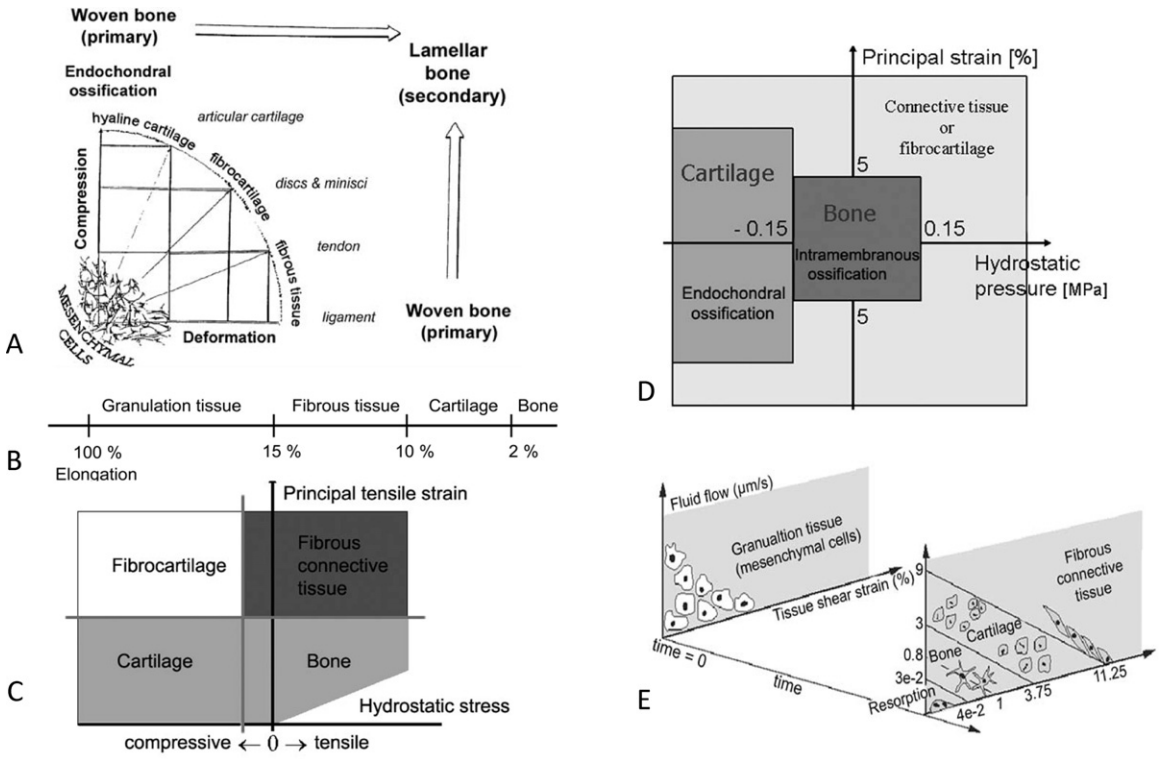

Fig. 3. Mechano-regulatory algorithms. (A) Pauwels scheme for differentiation of mesenchymal cells into musculoskeletal tissues, depending on the combination of volumetric and deviatoric deformation (Pauwels, 1960). (B) Perren and Cordey's idea based on how much elongation each tissue type can tolerate (Perren and Cordey, 1980). (C) Mechanobiological model based on tensile strain and hydrostatic pressure as proposed by Carter et al. (1998). (D) The fracture healing model proposed by Claes and Heigele (1999), including threshold values for when each tissue type will form. (E) The tissue differentiation scheme proposed by Prendergast et al. (1997) based on the magnitudes of fluid velocity and tissue shear strain.

Figures A–D are adapted based on Pauwels (1960), Perren and Cordey (1980), Carter et al. (1998), Claes and Heigele (1999), figure E is reprinted from Lacroix and Prendergast (2002).

Isaksson et al. (2006b) compared the previously described and new potential mechano-regulation algorithms' abilities to predict the general tissue distributions in normal fracture healing under cyclic axial load. All algorithms were implemented into the same versatile computational FEA model, which allowed direct comparison between the algorithms. Several algorithms, based on different biophysical stimuli, were equally well able to predict normal fracture healing processes (Isaksson et al., 2006b). To corroborate the algorithms further, they also compared the predictions with extensive *in vivo* experimental bone healing data under two distinctly different mechanical conditions: axial compression or torsional rotation (Isaksson et al., 2006a). None of the established algorithms properly predicted the spatial and temporal tissue distributions observed experimentally under all loading modes and time points. However, the algorithm based on deviatoric strain and fluid flow (Prendergast et al., 1997), predicted the experimental results the best (Isaksson et al., 2006a).

4.5. Models of callus growth

It is known that during the tissue differentiation process the callus not only changes in stiffness and cell density, but also it tends to change shape. In all studies described above, tissue volumetric growth was neglected. Isaksson et al. (2007) included volumetric growth into an adaptive FE model of distraction osteogenesis (limb lengthening) using the algorithm by Prendergast et al. (1997). By including volumetric growth of individual tissue types, it was shown to correctly predict experimentally observed spatial and temporal tissue distributions during distraction osteogenesis, as well as known perturbations due to changes in distraction rate and frequency (Isaksson et al., 2007). Volumetric growth was modeled based on the matrix production rates of each tissue type. Matrix production were simulated based on the biphasic swelling model (Wilson et al., 2005), by applying a swelling pressure to the element and considering the subsequent volume expansion as being an increase in matrix. The tissue was allowed to swell for 24 h and were then assumed stress free and used as input for the next increment. The relaxation behavior of the tissue during the 24 h corresponded well with that measured experimentally. However, the evolution of the predicted reaction forces over time were not corroborated by experimental data (Isaksson et al., 2007).

Garcia-Aznar et al. (2007) developed a continuum mathematical model that simulated the process of tissue regulation and callus volumetric growth during fracture healing adaptively. The model attempted to mimic events such as stem cell migration, proliferation, differentiation and cell death of all the cell types, as well production and degradation of the different tissues involved. They also included criteria for tissue damage, calcification and remodeling. They chose the second invariant of the deviatoric strain tensor as the stimulus guiding the tissue differentiation process. The volumetric growth was based on the amount of tissue production and modeled using a separate FE model based on thermal expansion. Even though the predicted callus geometries in their growth model were not completely physiological at the boundaries, it was able to predict increased callus size for increased interfragmentary movements (Fig. 4) (Garcia-Aznar et al., 2007), as well as realistic variations when gap size and fixator stiffness were varied (Gomez-Benito et al., 2005, 2006). This model was improved by Reina-Romo

et al. (2009) with respect to how it accounts for load history and used to simulate distraction osteogenesis. This study also assumed complete stress relaxation after each increment. However, in a second study, Reina-Romo et al. (2010) presented a macroscopic growth mixture formulation and showed that by accounting for the pre-traction stresses that are generated during distraction osteogenesis, both variations in distraction rate and the evolution of the resulting reaction forces over time can be predicted.

4.6. Accounting for biological factors

With the exception of the models described under tissue growth, the studies above included a highly limited description of cellular mechanisms. The model by Lacroix and Prendergast (2002) used a diffusion mechanism to collectively simulate migration, proliferation and differentiation of cells. Based on that scheme, it was identified that the healing speed was most sensitive to cell diffusion rate. Since real cell activity and tissue production rates were not modeled, the "model time" had low physical meaning (Isaksson et al., 2006b). There are several cell and tissue types involved during bone healing, and they all have varying rates for each activity that is modeled, e.g. migration, proliferation or rate of matrix production. Therefore, recent developments in this area have focused on describing the different cellular activities occurring during bone healing in more detail.

4.6.1. Mathematical models based on biochemical factors

Bailon-Plaza and van der Meulen (2001) developed a mathematical framework to study the effects of growth factors during fracture healing. They used finite difference methods to simulate sequential tissue regulation and cellular events, studying the evolution of tissue specific cells in the callus. In their model, cell differentiation was controlled by the presence of two growth factors (instead of mechanical stimulation, as the models described above). The rate of change of cell density, matrix density and growth factor concentrations, as well as matrix synthesis and degradation and growth factor diffusion, were included into their model.

4.6.2. Cell-phenotype specific activities

Isaksson et al. (2008a) took a step towards a more mechanistic modeling of cellular activity in bone healing. The formulation included mechanical modulation of cell phenotype and skeletal tissue-type specific activities and rates, by describing the temporal and spatial distributions of fibrous tissue, cartilage and bone, as regulated by four cell types, mesenchymal stem cells, fibroblast, chondrocytes and osteoblasts. At each time point and location, each cell type can migrate, proliferate, differentiate and/or apoptose, depending on their mechanical stimulation and the activity of other cell types in the environment. They can also produce tissue, or stimulate tissue removal. This model was shown to correctly predict the normal fracture healing processes (Fig. 5), as well as delayed and non-union due to excessive loading, and also the effects of some specific biological perturbations and pathological situations. For example, alterations due to periosteal stripping or impaired cartilage remodeling (endochondral ossification) compared well with experimental observations (Isaksson et al., 2008a). The model requires extensive parametric data as input, which was gathered, as far as possible, from literature. Since many of the parameter magnitudes are not well established, a factorial analysis was conducted using 'design of experiments' methods and Taguchi orthogonal arrays (Isaksson et al., 2008b). A few cellular parameters were thereby identified as key factors in the process of bone healing. These were related to bone formation, and cartilage production and degradation, which corresponded to those processes that have been suggested to be crucial biological steps in bone healing. Bone healing was found to be sensitive to parameters related to fibrous tissue and cartilage formation. These parameters had optimum values, indicating that some amounts of soft tissue production are beneficial, but too little or too much may be detrimental to the healing process (Isaksson et al., 2008b). However, in these studies, all cell activities were modeled on an element basis and anisotropy in the cell movement was not accounted for.

4.6.3. Stochastic cell modeling

Pérez and Prendergast (2007) developed a new model for cell dispersal in the callus. A 'random walk' model was included to represent cell migration both with and without a preferred direction, which implies anisotropic proliferation and migration of cells. The

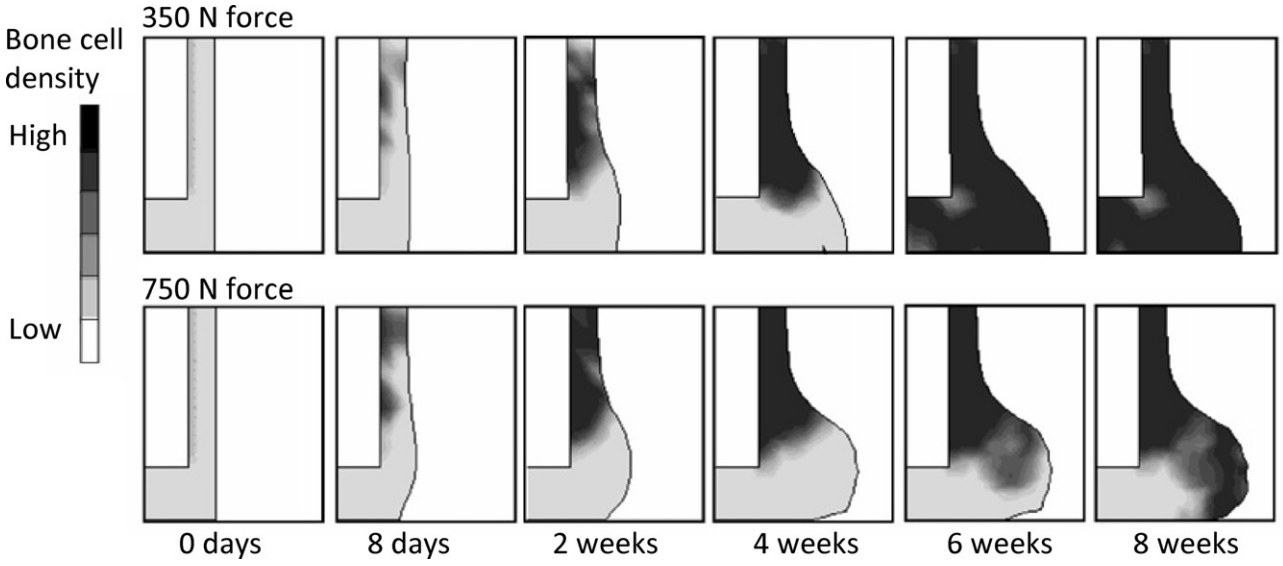

Fig. 4. Simulated bone healing based on the model by Garcia-Aznar et al. (2007) including volumetric growth. Increased load lead to delayed bone healing and somewhat larger callus growth.

Part of the figure is reprinted from Garcia-Aznar et al. (2007).

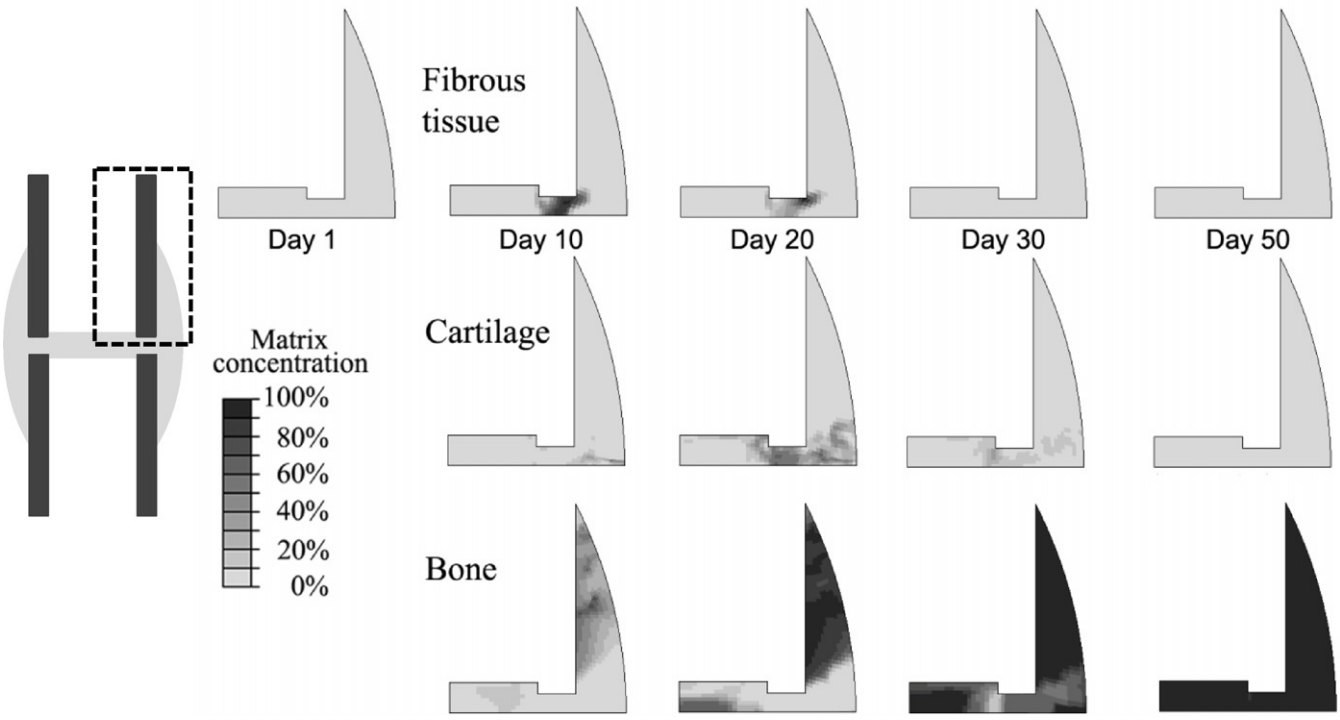

Fig. 5. Simulated bone healing based on the model by Isaksson et al. (2008a) with more mechanistic cell description, showing spatial and temporal tissue densities of fibrous tissue, cartilage and bone.

Reprinted from Isaksson et al. (2008a).
© 2008 with permission from Elsevier.

cell mechanisms were modeled as an internal lattice inside each element, which accounts more for the differences between the tissue and cell levels. The study simulated an implant–bone interface in 2D, using the stochastic cell model and the mechano-regulatory model by Prendergast et al. (1997). The predictions of both models are similar, although the 'random walk' model was able to predict a more irregular tissue distribution than the diffusion model. Due to the stochastic nature of the model, each simulation gave slightly different results. In a simulation of experimental data from a bone chamber experiment, qualitative agreement with histological data was found (Khayyeri et al., 2009). However, despite some variability between the simulations, the full variability found between specimens in the experiment could not be predicted by the mechanoregulation algorithm until differences in mechano-sensitivity between different individuals were modeled as differences in cell activity rates (Khayyeri et al., 2011). A similar model was also used in a 3D computational simulation of bone healing in a human tibia with more realistic loading (Byrne et al., 2011). The main phases of healing, including the resorption phase was predicted with qualitative agreement with known clinical outcomes (Fig. 6).

4.6.4. Vascularization of the tissue

Thus far, the mechanical environment was the only regulator of cell activity. However, sufficient blood supply is required to provide the cells with nutrients and oxygen. Hence, angiogenesis (in-growth of new blood vessels) is a key factor in bone healing. Low oxygen environment promotes cartilage formation, whereas bone can only form in high oxygen environments (Keramaris et al., 2008). Geris et al. (2006, 2008) further developed the model by Bailon-Plaza and van der Meulen (2001), to also account for angiogenesis through the regulation of a growth factor, and compared the results with experimental data of normal fracture healing. The diffusion of oxygen is limited to a few hundred micrometers from capillaries, and therefore the morphology of the new vascular network plays a critical role in bone healing. Checa and Prendergast (2009) further developed the stochastic cell model by Pérez and Prendergast (2007), to account for angiogenesis. They simulated tissue differentiation in a bone/implant gap under shear loading, and found that their model could predict capillary networks similar to those found in experimental studies. This also resulted in more 'heterogenous' patterns of tissue differentiation. The model accounted for the mechanical influence and showed that higher loads caused a slower vascular development and delayed bone tissue formation. It has also been used to evaluate the effect of cell seeding and mechanical loading on scaffolds, demonstrating the possibilities for these models in tissue engineering approaches (Checa and Prendergast, 2010). Moreover, this model was used to investigate the inter-species differences that exist in bone repair, where small animals show faster healing compared to larger animals (Checa et al., 2011). Histological data from rat and sheep bone healing was compared to computer simulations, and they concluded that geometrical (size) differences between the species alone cannot explain the distinctions observed experimentally during secondary bone healing between sheep and rat. However, the study could not conclude weather these differences are due to differences in cell behavior, material properties, or the mechano-sensitivity (Checa et al., 2011).

5. Problems and potentials

The FEM is an incredibly powerful tool that has allowed scientists and engineers to accurately predict mechanical responses in biological tissues and simulate complex processes such as bone healing adaptively. Moreover, modern software has removed many of the time-consuming tasks involved in creating the models, and also opened up the use to more investigators. However, the ease of use also greatly increases the potential for misuse of the method and prediction of inaccurate results (Jacobs and Kelly, 2011). It is

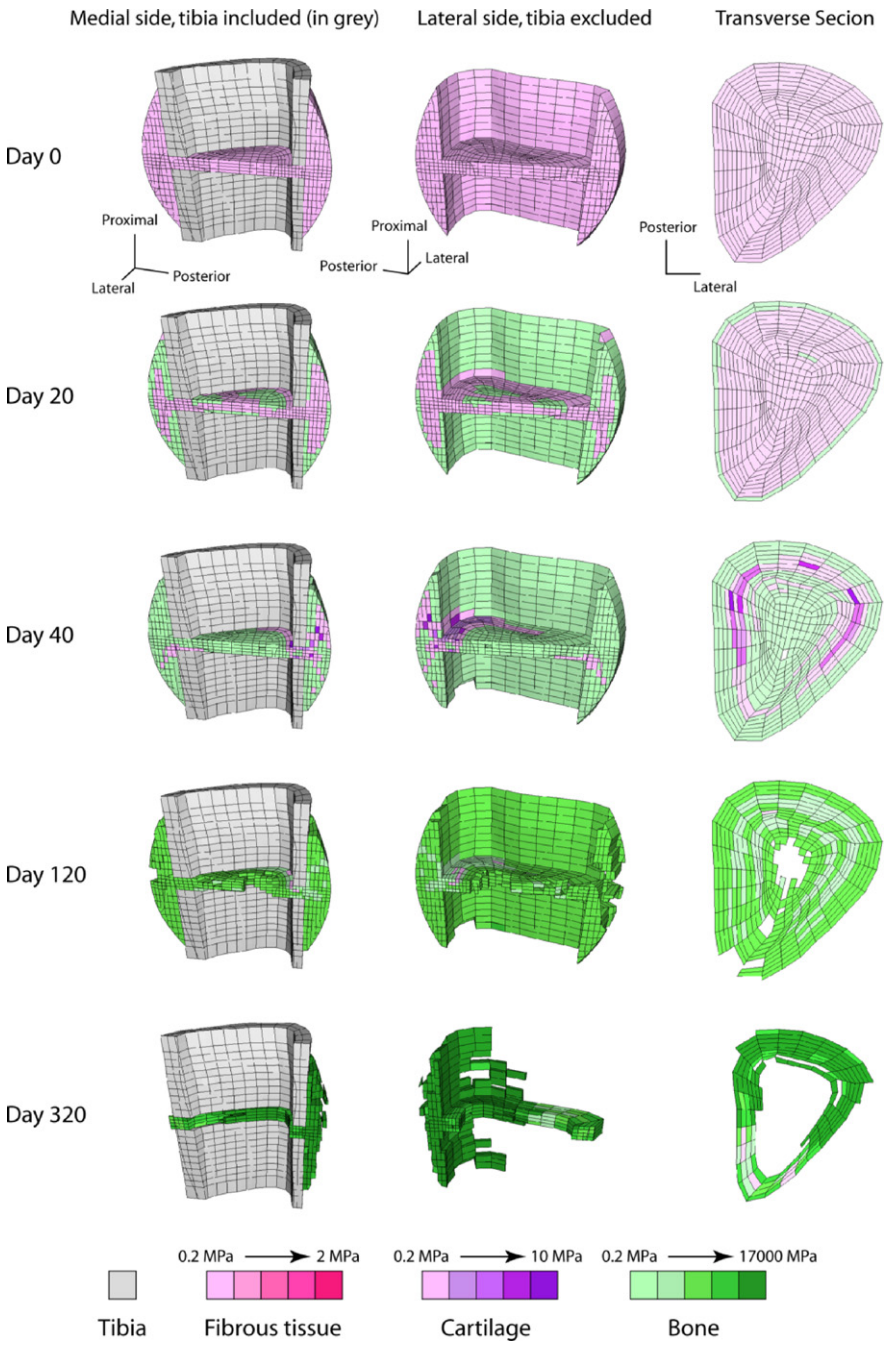

Fig. 6. Simulated bone healing based on the stochastic cell modeling by Pérez and Prendergast (2007), which was used by Byrne et al. (2011) to model 3D bone healing.

important to remember that a computational model is not better than its worse assumption. As described above, the mechano-biological models are rapidly becoming more complex, and each new mechanical or biological process that is included in the model requires many more assumptions to be made. The key problem in mechanobiological modeling is validation; To 'what extent does the assumptions and parameters in the model reflect reality' (Jacobs and Kelly, 2011).

5.1. Validation

Thus far, validation in this area has focused on comparing the simulation results with experimental data. Ideally, the experimental data should be obtained by the same investigator team (Isaksson et al., 2006a, 2007, 2009a; Khayyeri et al., 2009, 2011). However, since that is not always available, it is also common to corroborate parts of the model against experimental data obtained by other laboratories (Isaksson et al., 2008a). The danger then is that it is not always possible to know all the details in the experimental setups, and for example tissue mechanical properties or boundary conditions may differ greatly between experimental setups. With the increasing level of complexity, however, we are often faced with situations where we are not able to determine parameter values as accurately as we would like. For example, cell migration rates determined *in vitro* are used as estimations for *in vivo* rates, or experimental data from different species are

used and scaled. In those situations it is necessary to conduct a parametric analysis or a sensitivity analysis. For example, design of experiments or factorial analysis have been used to assess the importance of the assumptions made with respect to the cell activity rates (Isaksson et al., 2008b), the mechanical properties of the tissues (Isaksson et al., 2009b), and the assumptions regarding angiogenesis (Checa and Prendergast, 2009). If the model is not sensitive to the parameters that are less well-known, then more confidence can be gained in the simulation outcome. However, if the simulation strongly depends on a parameter for which experimental data is not available, then the specific simulation may not be of much value (Jacobs and Kelly, 2011). Parametric studies have identified the cellular parameters related to endochondral bone formation (Isaksson et al., 2008b) and three material properties (permeability of granulation tissue, Young's modulus of cartilage and permeability of immature bone) as particularly important. Hopefully, this will lead to more experimental studies aimed to quantify these parameters.

5.2. Future potential

Although there are important limitations, especially regarding validation of the involved assumptions, mechano-biological modeling has lead to important advances in the field. Corroborated mechanobiological models can be used to improve our understanding of basic biology during bone regeneration, and to identify areas that need further investigation. Hence, validated models can be useful when designing new experiments, and together theoretical models and animal experiments can lead to new research questions and advances in mechanobiology. One of the most important future applications of mechanobiology is for the development of new clinical therapies, for example in bone fracture healing, distraction osteogenesis or osteoporosis. Geris et al. (2010a,b). evaluated their models' ability to predict certain pathological cases of fracture healing, and took a first step to attempts to test therapeutic strategies by injecting mesenchymal stem cells and growth factors that promotes bone formation at different locations and time points. Another area of application is the improvement of implant design. With orthopedic implants such as prostheses, cells migrate up the implant surface and begin to synthesis matrix. However, if the micromotion is too high bone will not form to stabilize the implant-instead a soft tissue layer will form (Prendergast, 2006). An important future domain of applicability is in bone tissue engineering and regenerative medicine (Boccaccio et al., 2011). Appropriate biophysical stimuli are needed in bone scaffolds, in addition to nutrients and sufficient oxygen supply, to favor appropriate tissue differentiation (Prendergast et al., 2009). This has been the focus of many computational studies recently by e.g. investigating scaffold mechanical properties, porosity and cell seeding etc., and was recently reviewed by Boccaccio et al. (2011). Moreover, one of the key challenges for the future, is to determine the capabilities and limitations of mechano-biological modeling when it comes to real hypothesis testing type of investigation in biology, compared to descriptive research such as showing associations between mechanical and biological parameters or behavior (Jacobs and Kelly, 2011). It appears rather clear that engineers have many reasons to be interested in computational modeling of tissue differentiation and bone regeneration.

5.3. Conclusion

Many biological processes, including bone healing, are so complex that for certain research questions physical experimentation is either too time consuming, too expensive, or impossible. As a result, computational models are used more extensively. Mechano-regulatory theories have been developed and shown able to explain how the mechanical environment influences tissue differentiation, growth, maintenance, remodeling and degeneration. Over the last two decades, the computational models employed for studies of bone healing have progressed from single phase linear elastic models which were evaluated at only one time point (Carter et al., 1998) via hyperelastic (Claes and Heigele, 1999) to poroelastic material descriptions implemented in models that adaptively predict tissue distributions over time (Prendergast et al., 1997). Poroelastic material description is especially important when describing the soft tissues involved in the early stages of healing, and has become the standard material description. Unfortunately, some of the material properties of these soft tissues are not yet well characterized (Isaksson et al., 2009b). Recently, the focus has shifted from further development of the mechanical analyses, towards implementing more biological aspects, including effects of different cell types, growth factors and directed cell movement and in-growth of blood vessels. The models are becoming more multifaceted as the knowledge about the complex biological processes during bone healing increases. We are also on the verge of simulating patient specific data, or including genetic or inter-specimen variability into the models. Despite the remaining challenge to achieve sufficient validation, mechanobiology is a field where mechanical modeling can contribute significantly to our understanding of basic physiology and pathology and outline future areas of research.

Conflict of interest

The author has no conflicts of interest.

Acknowledgements

Financial support from the European Commission (FRACQUAL - 293434) and the Swedish Agency for Innovation Systems.

References

Ament, C., Hofer, E.P., 2000. A fuzzy logic model of fracture healing. J. Biomech. 33, 961–968.
Aspenberg, P., 2005. Drugs and fracture repair. 76, 741–748.
Augat, P., Burger, J., Schorlemmer, S., Henke, T., Peraus, M., Claes, L., 2003. Shear movement at the fracture site delays healing in a diaphyseal fracture model. J. Orthop. Res. 21, 1011–1017.
Augat, P., Simon, U., Liedert, A., Claes, L., 2005. Mechanics and mechano-biology of fracture healing in normal and osteoporotic bone. Osteoporos. Int. 16, S36–S43.
Bailon-Plaza, A., van der Meulen, M.C., 2001. A mathematical framework to study the effects of growth factor influences on fracture healing. J. Theor. Biol. 212, 191–209.
Bishop, N.E., van Rhijn, M., Tami, I., Corveleign, R., Schneider, E., Ito, K., 2006. Shear does not necessarily inhibit bone healing. Clin. Orthop. Relat. Res. 443, 307–314.
Blenman, P.R., Carter, D.R., Beaupre, G.S., 1989. Role of mechanical loading in the progressive ossification of a fracture callus. J. Orthop. Res. 7, 398–407.
Boccaccio, A., Ballini, A., Pappalettere, C., Tullo, D., Cantore, S., Desiate, A., 2011. Finite element method (FEM), mechanobiology and biomimetic scaffolds in bone tissue engineering. Int. J. Biol. Sci. 7, 112–132.
Byrne, D.P., Lacroix, D., Prendergast, P.J., 2011. Simulation of fracture healing in the tibia: mechanoregulation of cell activity using a lattice modeling approach. J. Orthop. Res. 29, 1496–1503.
Carter, D.R., Blenman, P.R., Beaupre, G.S., 1988. Correlations between mechanical stress history and tissue differentiation in initial fracture healing. J. Orthop. Res. 6, 736–748.
Carter, D.R., Beaupre, G.S., Giori, N.J., Helms, J.A., 1998. Mechanobiology of skeletal regeneration. Clin. Orthop. 355S, S41–S55.
Checa, S., Prendergast, P.J., 2009. A mechanobiological model for tissue differentiation that includes angiogenesis: a lattice-based modeling approach. Ann. Biomed. Eng. 37, 129–145.
Checa, S., Prendergast, P.J., 2010. Effect of cell seeding and mechanical loading on vascularization and tissue formation inside a scaffold: a mechano-biological model using a lattice approach to simulate cell activity. J. Biomech. 43, 961–968.
Checa, S., Prendergast, P.J., Duda, G.N., 2011. Inter-species investigation of the mechano-regulation of bone healing: comparison of secondary bone healing in sheep and rat. J. Biomech. 44, 1237–1245.
Claes, L., Augat, P., Suger, G., Wilke, H.J., 1997. Influence of size and stability of the osteotomy gap on the success of fracture healing. J. Orthop. Res. 15, 577–584.

Claes, L.E., Wilke, H.J., Augat, P., Rubenacker, S., Margevicius, K.J., 1995. Effect of dynamization on gap healing of diaphyseal fractures under external fixation. Clin. Biomech. 10, 227–234.
Claes, L.E., Heigele, C.A., Neidlinger-Wilke, C., Kaspar, D., Seidl, W., Margevicius, K.J., Augat, P., 1998. Effects of mechanical factors on the fracture healing process. Clin. Orthop., S132–S147.
Claes, L.E., Heigele, C.A., 1999. Magnitudes of local stress and strain along bony surfaces predict the course and type of fracture healing. J. Biomech. 32, 255–266.
Einhorn, T.A., 1995. Enhancement of fracture-healing. J. Bone Joint Surg. Am. 77, 940–956.
Einhorn, T.A., 1998. The cell and molecular biology of fracture healing. Clin. Orthop., S7–S21.
Epari, D., Duda, G., Thompson, M., 2011. Mechanobiology of bone healing and regeneration: in vivo models. Proc. Inst. Mech. Eng. H 224, 1543–1553.
Epari, D.R., Schell, H., Bail, H.J., Duda, G.N., 2006a. Instability prolongs the chondral phase during bone healing in sheep. Bone 38, 864–870.
Epari, D.R., Taylor, W.R., Heller, M.O., Duda, G.N., 2006b. Mechanical conditions in the initial phase of bone healing. Clin. Biomech 21, 646–655.
Frost, H.M., 1989a. The biology of fracture healing. An overview for clinicians. Part I. Clin. Orthop., 283–293.
Frost, H.M., 1989b. The biology of fracture healing. An overview for clinicians. Part II. Clin. Orthop., 294–309.
Garcia-Aznar, J.M., Kuiper, J.H., Gomez-Benito, M.J., Doblare, M., Richardson, J.B., 2007. Computational simulation of fracture healing: influence of interfragmentary movement on the callus growth. J. Biomech. 40, 1467–1476.
Gardner, T.N., Mishra, S., Marks, L., 2004. The role of osteogenic index, octahedral shear stress and dilatational stress in the ossification of a fracture callus. Med. Eng Phys. 26, 493–501.
Garrison, K., Shemilt, I., Donell, S., Ryder, J., Mugford, M., Harvey, I., Song, F.V.A., 2010. Bone morphogenetic protein (BMP) for fracture healing in adults. Cochrane Database Syst. Rev. 16, CD0069.50.
Geris, L., Van Oosterwyck, H., Vander, S.J., Duyck, J., Naert, I., 2003. Assessment of mechanobiological models for the numerical simulation of tissue differentiation around immediately loaded implants. Comput. Methods Biomech. Biomed. Engin. 6, 277–288.
Geris, L., Andreykiv, A., Oosterwyck, H.V., Sloten, J.V., Keulen Fv, F.F., Duyck, J., Naert, I., 2004. Numerical simulation of tissue differentiation around loaded titanium implants in a bone chamber. J. Biomech. 37, 763–769.
Geris, L., Gerisch, A., Maes, C., Carmeliet, G., Weiner, R., Vander Sloten, J., Van Oosterwyck, H., 2006. Mathematical modeling of fracture healing in mice: comparison between experimental data and numerical simulation results. Med. Biol. Eng. Comput. 44, 280–289.
Geris, L., Gerisch, A.J.V., Weiner, S., Oosterwyck, R.H.V., 2008. Angiogenesis in bone fracture healing: a bioregulatory model. J. Theor. Biol. 251, 137–158.
Geris, L., Reed, A.A., Vander Sloten, J., Simpson, A.H., Van Oosterwyck, H., 2010a. Occurrence and treatment of bone atrophic non-unions investigated by an integrative approach. PLoS Comput. Biol. 6, e1000915.
Geris, L., Sloten, J.V., Van Oosterwyck, H., 2010b. Connecting biology and mechanics in fracture healing: an integrated mathematical modeling framework for the study of nonunions. Biomech. Model. Mechanobiol. 9, 713–724.
Gerstenfeld, L.C., Cullinane, D.M., Barnes, G.L., Graves, D.T., Einhorn, T.A., 2003. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J. Cell Biochem. 88, 873–884.
Giori, N., Ryd, L., Carter, D., 1995. Mechanical influences on tissue differentiation at bone-cement interfaces. J. Arthroplasty 10, 514–522.
Gomez-Benito, M.J., Garcia-Aznar, J.M., Kuiper, J.H., Doblare, M., 2005. Influence of fracture gap size on the pattern of long bone healing: a computational study. J. Theor. Biol. 235, 105–119.
Gomez-Benito, M.J., Garcia-Aznar, J.M., Kuiper, J.H., Doblare, M., 2006. A 3D computational simulation of fracture callus formation: influence of the stiffness of the external fixator. J. Biomech. Eng. 128, 290–299.
Goodship, A.E., Kenwright, J., 1985. The influence of induced micromovement upon the healing of experimental tibial fractures. J. Bone Joint Surg. Br. 67, 650–655.
Huiskes, R., van Driel, W.D., Prendergast, P.J., Soballe, K., 1997. A biomechanical regulatory model for periprosthetic fibrous-tissue differentiation. Mater. Med. 8, 785–788.
Isaksson, H., Donkelaar, C.C., Huiskes, R., Ito, K., 2006a. Corroboration of mechanoregulatory algorithms for tissue differentiation during fracture healing: comparison with in vivo results. J. Orthop. Res. 24, 898–907.
Isaksson, H., Wilson, W., van Donkelaar, C.C., Huiskes, R., Ito, K., 2006b. Comparison of biophysical stimuli for mechano-regulation of tissue differentiation during fracture healing. J. Biomech. 39, 1507–1516.
Isaksson, H., Comas, O., Donkelaar, C.C., Mediavilla, J., Huiskes, R., Ito, K., 2007. Bone regeneration during distraction osteogenesis: mechano-regulation by shear strain and fluid velocity. J. Biomech. 40, 2002–2011.
Isaksson, H., van Donkelaar, C.C., Huiskes, R., Ito, K., 2008a. A mechano-regulatory bone-healing model incorporating cell-phenotype specific activity. J. Theor. Biol. 252, 230–246.
Isaksson, H., van Donkelaar, C.C., Huiskes, R., Yao, J., Ito, K., 2008b. Determining the most important cellular characteristics for fracture healing using design of experiments methods. J. Theor. Biol. 255, 26–39.
Isaksson, H., Gröngröft, I., Wilson, W., van Donkelaar, C.C., van Rietbergen, B., Tami, A., Huiskes, R., Ito, K., 2009a. Remodeling of fracture callus in mice is consistent with mechanical loading and bone remodeling theory. J. Orthop. Res. 27, 664–672.
Isaksson, H., van Donkelaar, C.C., Ito, K., 2009b. Sensitivity of tissue differentiation and bone healing predictions to tissue properties. J. Biomech. 42, 555–564.
Jacobs, C.R., Kelly, D.J., 2011. Cell mechanics: the role of simulation. In: Fernandes, P.R., Bártolo, P.J. (Eds.), Advances on Modeling in Tissue Engineering, 20, pp. 1–14.
Kelly, D.J., Prendergast, P.J., 2005. Mechano-regulation of stem cell differentiation and tissue regeneration in osteochondral defects. J. Biomech.
Kenwright, J., Goodship, A.E., 1989. Controlled mechanical stimulation in the treatment of tibial fractures. Clin. Orthop., 36–47.
Kenwright, J., Richardson, J.B., Cunningham, J.L., White, S.H., Goodship, A.E., Adams, M.A., Magnussen, P.A., Newman, J.H., 1991. Axial movement and tibial fractures. A controlled randomised trial of treatment. J. Bone Joint Surg. Br. 73, 654–659.
Keramaris, N., Calori, G., Nikolaou, V., Schemitsch, E., Giannoudis, P., 2008. Fracture vascularity and bone healing: a systematic review of the role of VEGF. Injury 39, S45–S57.
Khayyeri, H., Checa, S., Tägil, M., Prendergast, P.J., 2009. Corroboration of mechanobiological simulations of tissue differentiation in an in vivo bone chamber using a lattice-modeling approach. J. Orthop. Res. 27, 1659–1666.
Khayyeri, H., Checa, S., Tägil, M., Aspenberg, P., Prendergast, P.J., 2011. Variability observed in mechano-regulated in vivo tissue differentiation can be explained by variation in cell mechano-sensitivity. J. Biomech. 44, 1051–1058.
Lacroix, D., Prendergast, P.J., 2002. A mechano-regulation model for tissue differentiation during fracture healing: analysis of gap size and loading. J. Biomech. 35, 1163–1171.
Lacroix, D., Prendergast, P.J., Li, G., Marsh, D., 2002. Biomechanical model to simulate tissue differentiation and bone regeneration: application to fracture healing. Med. Biol. Eng. Comput. 40, 14–21.
Loboa, E.G., Beaupre, G.S., Carter, D.R., 2001. Mechanobiology of initial pseudarthrosis formation with oblique fractures. J. Orthop. Res. 19, 1067–1072.
Morgan, E.F., Longaker, M.T., Carter, D.R., 2006. Relationships between tissue dilatation and differentiation in distraction osteogenesis. Matrix Biol. 25, 94–103.
Nauth, A., Giannoudis, P., Einhorn, T., Hankenson, K., Friedlaender, G., Li, R., Schemitsch, E., 2010. Growth factors: beyond bone morphogenetic proteins. J. Orthop. Trauma 24, 543–546.
Park, S.H., O'Connor, K., McKellop, H., Sarmiento, A., 1998. The influence of active shear or compressive motion on fracture-healing. J. Bone Joint Surg. Am. 80, 868–878.
Pauwels, F., 1960. A new theory on the influence of mechanical stimuli on the differentiation of supporting tissue. The tenth contribution to the functional anatomy and causal morphology of the supporting structure. Z. Anat. Entwicklungsgesch. 121, 478–515.
Pérez, M., Prendergast, P.J., 2007. Random-walk models of cell dispersal included in mechanobiological simulations of tissue differentiation. J. Biomech. 40, 2244–2253.
Perren, S.M., 1979. Physical and biological aspects of fracture healing with special reference to internal fixation. Clin. Orthop., 175–196.
Perren, S.M., Cordey, J., 1980. The concept of interfragmentary strain. In: Uhthoff, H.K. (Ed.), Current Concepts of Internal Fixation of Fractures. Springer-Verlag, Heidelberg, pp. 63–77.
Perren, S.M., Rahn, B.A., 1980. Biomechanics of fracture healing. Can. J. Surg. 23, 228–232.
Perren, S.M., Claes, L., 2000. Biology and biomechanics in fracture management. In: Ruedi, T.P., Murphy, W.M. (Eds.), AO Principles of Fracture Fanagement. Thieme, Stuttgart, pp. 7–32.
Postacchini, F., Gumina, S., Perugia, D., De Martino, C., 1995. Early fracture callus in the diaphysis of human long bones. Histologic and ultrastructural study. Clin. Orthop. Relat Res., 218–228.
Prendergast, P.J., 1997. Finite element models in tissue mechanics and orthopaedic implant design. Clin. Biomech. 12, 343–366.
Prendergast, P.J., Huiskes, R., Soballe, K., 1997. ESB Research Award 1996. Biophysical stimuli on cells during tissue differentiation at implant interfaces. J. Biomech. 30, 539–548.
Prendergast, P.J., 2006. Prosthesis fixation for orthopaedics. In: Webster, J.E. (Ed.), Encyclopaedia of Medical Devices and Instrumentation. Wiley, New Jersey, pp. 192–198.
Prendergast, P.J., Checa, S., Lacroix, D., 2009. Computational models of tissue differentiation. In: De, S.E.A. (Ed.), Computational Modelling in Biomechanics. Springer Science, NY, pp. 353–372.
Reina-Romo, E., Gómez-Benito, M., García-Aznar, J., Domínguez, J., Doblaré, M., 2009. Modeling distraction osteogenesis: analysis of the distraction rate. Biomech. Model. Mechanobiol. 8, 323–335.
Reina-Romo, E., Gómez-Benito, M., García-Aznar, J., Domínguez, J., Doblaré, M., 2010. Growth mixture model of distraction osteogenesis: effect of pre-traction stresses. Biomech. Model. Mechanobiol. 9, 103–115.
Shefelbine, S.J., Augat, P., Claes, L., Simon, U., 2005. Trabecular bone fracture healing simulation with finite element analysis and fuzzy logic. J. Biomech. 38, 2440–2450.
van der Meulen, M.C., Huiskes, R., 2002. Why mechanobiology? A survey article. J. Biomech. 35, 401–414.
Wilson, W., van Donkelaar, C.C., van Rietbergen, B., Huiskes, R., 2005. A fibril-reinforced poroviscoelastic swelling model for articular cartilage. J. Biomech. 38, 1195–1204.