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REVIEW

Role of mathematical modeling in bone fracture healing

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Bone fracture healing is a complex physiological process commonly described by a four-phase model consisting of an inflammatory phase, two repair phases with soft callus formation followed by hard callus formation, and a remodeling phase, or more recently by an anabolic/catabolic model. Data from humans and animal models have demonstrated crucial environmental conditions for optimal fracture healing, including the mechanical environment, blood supply and availability of mesenchymal stem cells. Fracture healing spans multiple length and time scales, making it difficult to know precisely which factors and/or phases to manipulate in order to obtain optimal fracture-repair outcomes. Deformations resulting from physiological loading or fracture fixation at the organ scale are sensed at the cellular scale by cells inside the fracture callus. These deformations together with autocrine and paracrine signals determine cellular differentiation, proliferation and migration. The local repair activities lead to new bone formation and stabilization of the fracture. Although experimental data are available at different spatial and temporal scales, it is not clear how these data can be linked to provide a holistic view of fracture healing. Mathematical modeling is a powerful tool to quantify conceptual models and to establish the missing links between experimental data obtained at different scales. The objective of this review is to introduce mathematical modeling to readers who are not familiar with this methodology and to demonstrate that once validated, such models can be used for hypothesis testing and to assist in clinical treatment as will be shown for the example of atrophic nonunions.

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Introduction

Bone fractures are among the most frequent injuries of the musculoskeletal system. For instance, 15.3 million fractures occur annually[1] in the United States, resulting in 14 million visits to emergency rooms or other health-care facilities.[2] Although most fractures heal without difficulty due to bone's self-repair capacity, a considerable amount of delayed healing and nonunions develop with often unknown causes. Of the estimated 6 million bone fractures in the United States each year, ~5–10 percent will develop slow or delayed union or incomplete healing often referred to as nonunion.[3,4] The reasons for delayed healing and nonunions are often associated with complicated, multisegmental factures, open fractures, infection, insufficient fracture immobilization (fixation), inadequate blood supply and chronic disease states (including diabetes, renal failure, and metabolic bone disease). Treating fractures accounts for nearly half of the $56 billion annual expenditure for trauma.[5] Costs related to fracture repair are expected to rise in the future, given the increase of the aging population in first world countries and the associated increase of osteoporotic patients. Hence, prevention and effective treatment of fractures are essential both for patients' well being and to reduce the financial burden to health-care systems.

From a clinical perspective it is essential to identify respective outcomes of fracture repair and provide suggestions for optimal treatment and/or changes in treatment for individual patients. As has been summarized in Geris *et al.*,[6] there is currently no generally accepted definition of nonunion of a fracture.[7] Differentiation between delayed unions and nonunions is sometimes difficult. A nonunion is generally defined as the cessation of all reparative processes of healing without bony union. As all regulatory processes applying to delayed unions usually occur to a more severe degree in nonunions, the differentiation between delayed and nonunion is often based on radiographic criteria and time. In humans, failure to show any

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progressive change in the radiographic appearance for at least 3 months after the period of time during which normal fracture union would be thought to have occurred, is an evidence of nonunion.[7]

Clinical determinants. The different phases in fracture healing are strongly influenced by physiological and pharmacological factors in the prevailing environment that determine the state of the fracture and the progress of repair. Key features include the nature, location and extent of injury, the biomechanical forces (for example, physiological loading and/or fracture fixation), infection and/or disease, adjunctive drug therapies, nutrition, as well as underlying genetic conditions.[4,8] Classical treatments for nonunions are aimed at restoring the biomechanical integrity of the fracture site, that is, reducing excessive motion of the fracture fragments by using external and/or intramedullary fixators, as excessive movement inhibits repair. In contrast, although fully rigid fixators have been shown to delay fracture repair, certain patterns of dynamic loading have been shown to enhance fracture healing.

The use of growth factors for enhancing fracture healing is a relatively new treatment strategy. However, the challenge for administrating biochemical regulatory factors is developing a delivery system that provides a suitable concentration at the right site of the fracture callus at the optimal time point, which essentially mimics the growth factor concentration pattern observed in normal healing subjects. Despite the large amount of experimental data, additional research is required to determine the exact mechanisms of nonunion and to design the optimum therapeutic response.

Potential roles of quantitative mathematical modeling in fracture repair. As will be highlighted in this review paper mathematical modeling could have an important role in the design of growth factor delivery, optimizing fixation in terms of rigidity in different regions of the fracture callus, timing for providing stem cells and optimizing their differentiation status.

This review first summarizes the most commonly used conceptual models in bone fracture healing including cellular, biochemical and biomechanical regulatory factors (Section 'The Biology of Bone Fracture Healing'). These conceptual models are useful qualitative tools for hypothesis formulation. Subsequently, it will be shown how such qualitative conceptual models can be 'translated' into quantitative mathematical models, which can then be used for integration of spatial and temporal experimental data, hypothesis testing and making predictions on fracture outcomes (Section 'Mathematical Models of Bone Fracture Healing'). This review will help readers who are not familiar with mathematical modeling to become acquainted with the general concept underlying this methodology. Finally, several mathematical modeling approaches applied to different spatial scales will be discussed, including continuous and discrete modeling approaches. Using the model developed by Geris *et al.*[9] as a generic example, it will be shown how computational modeling can be used to improve current understanding of development and treatment of atrophic nonunions. Finally we will outline the challenges in fracture repair studies (Section 'Outlook and Conclusions').

The Biology of Bone Fracture Healing

It is beyond the scope of the current review article to summarize all biological and biomechanical aspects of fracture healing. For recent excellent reviews focusing on fracture biology, the reader is referred to Claes *et al.*,[8] Schindeler *et al.*[10] and Marsell *et al*.[11] Thus, only the most important regulatory mechanisms are discussed below with special emphasis on currently utilized conceptual models of bone fracture healing and how this information can be used to develop mathematical models (Section 'Mathematical Models of Bone Fracture Healing').

Conceptual models of bone fracture healing. The biology of fracture healing is a complex biological process that involves participation of many cell types, a large number of biochemical and biomechanical regulatory factors, and expression of several thousand genes.[4] Fracture healing follows a well-defined sequence of four phases starting with an inflammation phase, followed by two repair phases consisting of soft callus formation and hard callus formation, and completed via a remodeling phase.[8,10] These different phases partially overlap (**Figure 1a**). As has been summarized by Claes *et al.,*[8] this sequence of events has been observed in many animal models being best described in rats. Although the fracture-healing process is similar in larger animals and humans, temporal events occur over a longer time course which could be partly due to the larger spatial area that needs to be bridged.

Inflammation phase. Following bone trauma, a hematoma is formed, consisting of cells from both the peripheral and intramedullary blood supply, as well as bone marrow cells.[12,13] The hematoma is characterized by hypoxia and low pH and acts as a temporary scaffold for the active invasion of inflammatory cells. A large number of proinflammatory cytokines, including TNF-α and interleukin (IL)1β, and growth factors, including members of the transforming growth factor-β superfamily (bone morphogenetic protein (BMP)-2, BMP-4, BMP-6), are released early in the inflammatory phase.[4,13] In addition, angiogenic factors (angiopoetin-1, vascular endothelial growth factor are released due to the hypoxic conditions caused by blood vessel rupture. These factors recruit inflammatory cells and promote angiogenesis. The acute inflammatory response peaks within the first 24 h (that is, IL-6 and IL-1β) and is complete about 7 days after fracture in rats. A transient inflammatory response appears necessary for optimal healing. Anti-inflammatory treatments inhibit healing if given in the first few days following fracture.[14] Mice deficient in TNF-α show impaired fracture healing,[15] and transient exposure to TNF-α has been shown to promote osteoblastic differentiation by mesenchymal stem cells (MSCs).[16]

Repair phases (soft and hard callus). The repair phase requires the deposition of new extracellular matrix with the initial requirement that the bone fracture be stabilized (**Figure 1a**). The recruitment of MSCs and the regulation of their differentiation into chondrocytes and osteoblasts is an essential requirement to provide sufficient cells to produce the required level of matrix formation. Stem cells are largely recruited from local tissues within bone marrow, blood vessels, periosteum, adjacent muscle tissue or from the circulation.[10] Recruitment of sufficient stem cells is a rate-limiting step in bone fracture healing and can

be problematic when the stem cell population is depleted, as occurs with ageing[17] and in diseases such as diabetes.[18] The initial extracellular matrix formed during fracture repair is soft cartilage deposited by chondrocytes. At this time the fracture site is almost completely avascular, and is thus significantly hypoxic. It may well be that the level of hypoxia is a determinant of initial chondrocyte differentiation by MSCs.[19]

In a pattern resembling endochondral bone formation,[20] the chondrocytes then undergo hypertrophy and the cartilage mineralizes. The mineralized cartilage is resorbed by osteoclasts and replaced with woven bone by osteoblasts, thus forming a hard callus that provides rigidity to the fracture site. Woven bone, though mechanically inferior to mature lamellar bone, can be rapidly deposited and mineralized and provide mechanical stability to the fractured bone by providing a large scaffold surrounding the fracture. Vascular invasion accompanies the replacement of the cartilage with woven bone, resulting in increased vascular supply relative to normal bone.[8]

Remodeling phase. The final stage of fracture repair is the replacement of woven bone that bridges the fracture gap by lamellar bone. Woven bone formed in the cortical fracture gap is remodeled into lamellar bone due to formation of secondary osteons. This stage is biochemically controlled by IL-1, TNF-α and BMP-2, with expression of these factors increasing during this phase, as opposed to most of the inflammatory cytokines which are largely reduced. Vascularization during the remodeling phase is reduced to prefracture levels[12] (**Figure 1b**). Periosteal and medullary calluses are being resorbed by osteoclasts that leads to a reshaping of diaphyseal bone, which takes about 5–8 weeks in rats and can take years in humans.

As has been pointed out in Pivonka and Komarova[21] biomedical research, including bone fracture healing, is most commonly 'hypothesis-driven'. Derivation of the hypothesis requires the complexity of real phenomena to be reduced, resulting in development of a conceptual model describing the biological process, which encompasses some logical relationship between the perceived key elements of the whole. The experimental outcomes agree or disagree with the particular stated hypothesis, allowing for the refinement of a conceptual model. In this way, a large collection of individual observations on different aspects of bone fracture healing is generated. Essentially, these observations can be viewed as single pieces of a large puzzle, for example, the systems behavior in bone fracture healing. However, without having the overall picture in mind (that is, the conceptual model), these individual pieces cannot be put together in a systematic way.

In fracture healing the so-called four-phase conceptual model (described above) is most commonly employed and is based on observed histological sections both in animals and humans (**Figure 1a**). As has been pointed out by Schindeler *et al.,*[10] a potential drawback of this four-phase model is that it does not consider the often significant temporal overlap between the different phases, but rather provides a static picture. A useful extension of this four-phase model has been made by Claes *et al.*[8] by including the temporal sequence of healing events, the biomechanical environment (that is, the interfragmentary movement (IFM) at the fracture site), the blood flow (that is, availability of oxygen and nutrients at the fracture site) and the evolution of tissue volume fractions (that is, formation of soft tissue, cartilaginous tissue and bone) into the model (**Figure 1b**). It is important to note that this extended four-phase model now contains time as an explicit variable and can be used for formulation of a certain hypothesis such as: 'What happens if one interferes with the IFM and/or blood flow at a certain time point?'

Another conceptual model of bone fracture healing has been proposed by Little and co-workers[10,22] (**Figure 1c**), in the following, referred to as the anabolic/catabolic model. This model provides an alternative explanation for the underlying mechanisms regulating fracture healing and is based on the balance between anabolic (that is, forming) and catabolic (that is, resorbing) responses, which apply both to hard callus remodeling and soft callus remodeling. In this model, many of the cell types that are recruited to the fracture site at the early stage of fracture healing and the associated expression of growth factors and cytokines are viewed as a general tissue response to injury and are thus nonspecific to bone. On the basis of these observations, Little *et al.* have termed these processes nonspecific anabolism and nonspecific catabolism.[10,22] On the other hand, processes related specifically to bone such as cartilage and bone modeling and remodeling responses are referred to as specific anabolism and specific catabolism (**Figure 1c**). Similar to the extended four-phase model, the anabolic/catabolic model includes a continuous time course, which allows formulation of hypothesis regarding interference with the different nonspecific and specific responses. It is speculated that the speed of fracture healing may be determined by the nonspecific processes (that is, recruitment of cells, revascularization and so on), whereas the strength of repair relates to the mechanically driven balance between bone-specific anabolism and bone-specific catabolism.

On the basis of the discussions above, it is clear that currently employed conceptual models of bone fracture healing start to depart from the classical 'static' view of the four-phase model and incorporate events that are both temporally and spatially determined. Important regulatory mechanisms such as IFM, blood flow and different cell populations participating in fracture repair are now viewed as temporal changing quantities. One of the challenges in presenting conceptual models as a 'temporal superposition' of individual graphs (as has been done in the above models) is that the complexity increases with the number of individual regulatory mechanisms. For example, in the extended four-phase model three graphs (**Figure 1b**, that is, IFM, blood flow and tissue volume fractions) need to be 'mentally' superposed to estimate effects of various regulatory mechanisms on fracture healing.

Another shortcoming of these models is that they only look at the temporal changes of a particular regulatory quantity without considering a spatial region. For example, plotting the tissue volume fractions or cellular distributions implicitly assumes that these quantities are averaged over a certain domain (or volume) of the fracture callus. Observing particularly different cell distributions within the fracture callus, it is clear that different regions of the fracture callus are occupied by different cell types. As such, an (spatial) averaging over the entire fracture callus would lead to a loss of this important information. In addition, biological questions related to the migratory behavior of different cell types into the fracture site require knowledge of the spatial distribution of cells and biochemical regulatory

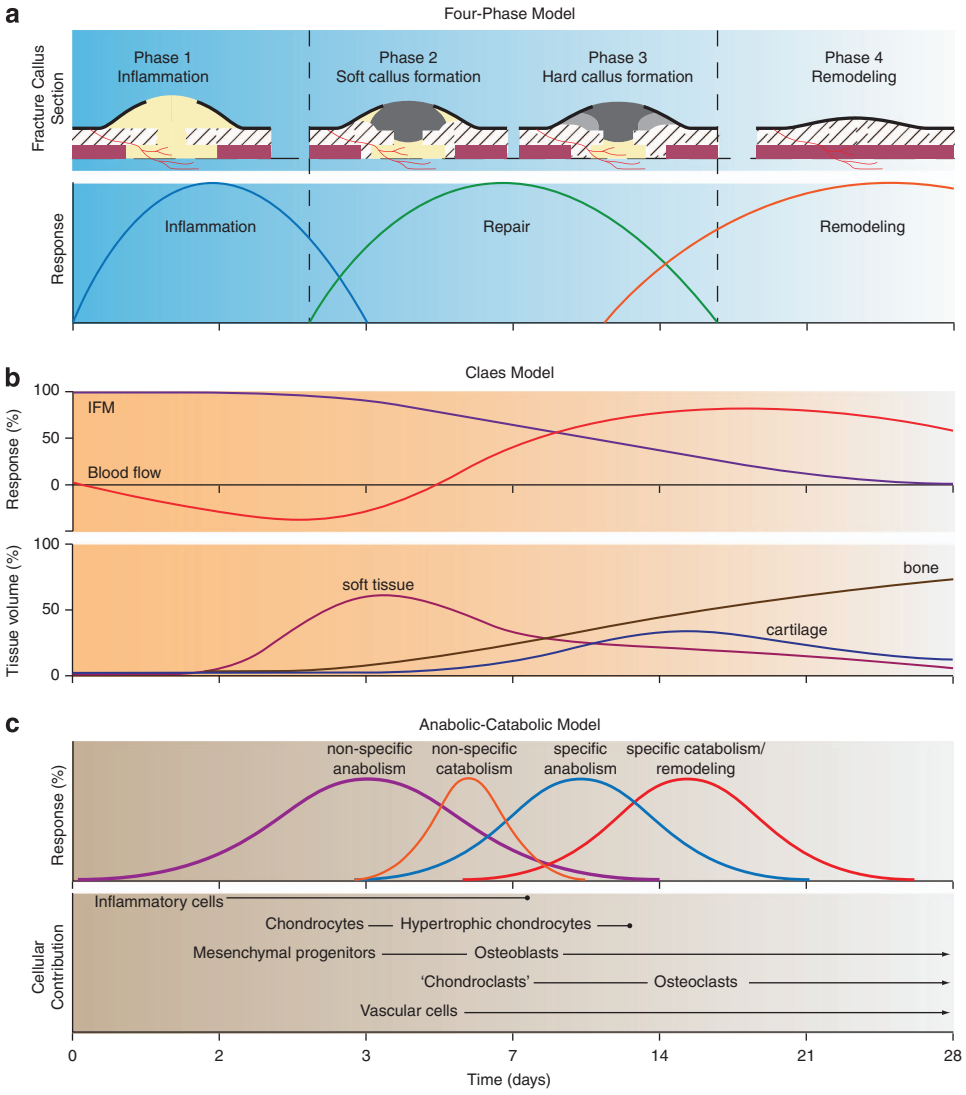

Figure 1 Conceptual models of bone fracture healing: (**a**) four-phase model based on histology providing a static picture and dynamic overlap of phases (yellow = granulation tissue; dark gray, fibrous tissue, light gray, cartilaginous tissue, dashed, bone); (**b**) extended four-phase model according to Claes *et al.*[8] superposing IFM, blood flow and tissue volume fractions; (**c**) anabolic/catabolic model according to Little and co-workers.[10,22]

factors. The concentration gradients of regulatory factors are involved in cell recruitment, stimulation of growth and/or cell differentiation. As one might imagine, incorporation of such spatial components into current temporal models may prove difficult due to the qualitative nature of these models. In contrast, we will show that the development of quantitative mathematical models is a rigorous way to incorporate different cell types and regulatory mechanisms taking into account both spatial and temporal distributions (Section 'Mathematical Models of Bone Fracture Healing'). In the following, we provide a brief outlook of currently developed experimental systems, which have the potential to assess dynamic cell distribution and migratory behaviors in bone fracture healing.

Emerging experimental systems to investigate both spatial and temporal events. Experimental systems for studying bone fracture repair at the tissue level have typically involved animal studies in mice, rats, rabbits, sheep or dogs where bone defects or bone fractures are induced and bone repair assessed by sequential X-ray tracking bone bridging of defects or by sequential euthanasia of animals with histological assessment of cell and tissue changes. These static assessments are limited in their ability to characterize temporal and spatial relationships between repair processes and the role of the surrounding tissues in regulating events and providing a source of stem cells.

Recent advances in multimodality molecular imaging allows noninvasive monitoring of specific biological processes within living subjects and is complimentary to anatomical imaging.[23] Although positron emission tomography provides great sensitivity, it lacks spatial resolution and is associated with radiation dosage and radionuclide toxicity. Similarly, optical imaging has great sensitivity and temporal resolution, but lacks spatial resolution and sufficient penetration depth. By combining molecular with anatomical imaging both spatial and temporal aspects of bone fracture healing can be assessed.

Rowe and co-workers have developed an elegant strategy for assessing changes at the cellular level during bone repair phases. In this experimental system, transgenic mice have been

developed that express fluorescent proteins specific to the degree of osteoprogenitor differentiation (from multi-potential mesenchymal precursor cells, through to functional chondrocytes and osteoblasts and to mature osteocytes). These cells can then be identified in frozen histological specimens to evaluate, at the cellular level, progression of fracture repair and its dependence on cell recruitment and differentiation. Moreover, these tissues can be harvested, cells isolated and sorted using fluorescence-activated cell sorting, and the specific populations assessed for gene expression of growth factors and other cell regulators to determine paracrine and autocrine regulatory functions. These ongoing studies continue to provide data with which to define and calibrate mathematical models of bone fracture repair.[24–27]

Mathematical Models of Bone Fracture Healing

Owing to the complexity of bone fracture healing and the limitations of experimental technologies, purely experimental approaches are unable to fully describe the underlying biochemical and biomechanical mechanisms leading to fracture healing. As has been described in several review papers mathematical modeling provides a powerful tool to formalize the conceptual model underlying the hypothesis, which allows for simulation of complex biological mechanisms *in silico*.[6,21,28] Such models are particularly useful in situations: (i) when simultaneous multiple events make it difficult to predict intuitively the behavior of the system, (ii) when the time/length scales of various events under investigation are significantly different and (iii) when the system exhibits clearly nonlinear (nonobvious) behavior. Hence, *in-silico* models can be regarded as additional (technical) tools, apart from the commonly employed *in-vitro* and *in-vivo* models, for hypothesis testing and to interpret the obtained experimental data and/or extrapolate from or between different data.

Computational models to investigate different aspects of bone fracture healing may be distinguished based on the particular regulatory mechanisms that have been considered (for example, purely biomechanical models, biochemical models and coupled models)[6] or based on the particular scale that they have been applied (for example, cellular-, tissue- and organ scale).[29] In the following, we will summarize some of the currently developed models of bone fracture healing using the latter classification.

Cellular-scale and tissue-scale models. Mathematical models formulated on the cellular scale describe the interactions of different cell types and biochemical regulatory factors (**Figure 2**). These models are commonly referred to as cell-population models and describe the temporal evolution of cell densities and concentration of regulatory factors. These models often neglect spatial influences, and quantities such as cell density and concentration of regulatory factors can therefore be thought of as spatial averages. Commonly employed temporal models in bone remodeling applications are based on the models of Komarova *et al.* and Pivonka *et al.*[30–32]

The most commonly employed mathematical models describing soft-callus and hard-callus formation on the tissue scale are continuous spatio-temporal models based on partial differential equations. In this approach different cell populations (for example, osteoblasts, chondrocytes, and fibroblasts) and regulatory factors are described in a continuous way (**Figure 2b and c**).

A model describing the reparative phase of secondary fracture healing has been developed by Bailón-Plaza and van der Meulen.[33] In this study, the effects of growth factors during both intramembranous and endochondral ossification has been investigated. The model includes the densities of MSCs, chondrocytes and osteoblasts and the concentration of osteogenic and chondrogenic growth factors together with the density of a combined fibrous/cartilaginous extracellular matrix and a bone matrix. These cell densities change due to cell migration, proliferation, differentiation, endochondral replacement and cell removal (**Figure 2c**). Changes in matrix density are a result of synthesis and resorption. Most of the processes modeled are mediated by osteogenic and chondrogenic growth factors, which are produced by the respective cells.

Geris *et al.*[9] further extended this model to include key aspects of healing such as angiogenesis and directed cell migration. Over the past 5 years several additional modifications of this model have been made.[34,35] An example of the Geris *et al.* model together with a detailed description of the model features is given below for the application of atrophic nonunions in a rat model.

An alternative approach to the continuous description of cell populations is the application of a discrete approach where each cell is modeled individually. These so-called agent-based approaches facilitate incorporation of cell cycle, cell proliferation and other complex cell interactions. However, they are computationally very intensive because of the large number of cells (or 'agents) involved. Traditionally, these type of models have emerged from the tumor mathematical modeling literature based on both tumor growth and therapeutic interventions.[36,37]

Buenzli *et al.*[38] have utilized this approach investigating the interaction of osteoclasts in the formation of the resorption cavity in osteonal remodeling. Although early models of blood vessel formation are based on a continuous description,[9] more recent models take into account the discrete nature of blood vessels in the fracture callus.[35] Only recently, Pérez and Prendergast[39] proposed a discrete approach to cell movement based on a 'random-walk' of cells within a lattice to model proliferation and cell migration. This approach facilitates modeling of simultaneous dispersal of different cell phenotypes, the explicit modeling of cell proliferation and apoptosis.

For simulations of heterogeneous cell populations such as the different osteoblast precursor populations residing in the fracture callus (as indicated in Subsection 'Emerging experimental systems to investigate both spatial and temporal events') application of a discrete rather than continuous approach might be more appropriate. However, the increase in model resolution as obtained with agent-based models comes at the cost of estimation of a larger number of model parameters (see Section 'Outlook and Conclusions', for further discussions).

Organ-scale models. Organ-scale models primarily focus on the effect of mechanical stimuli on bone fracture healing.[40,41] These types of models are generally formulated as continuum models based on the theory of (poro)elasticity using finite element analysis to compute the mechanical stimuli experienced in different regions of the fracture callus (**Figure 2a**). To adapt the tissue material properties (determined by the different

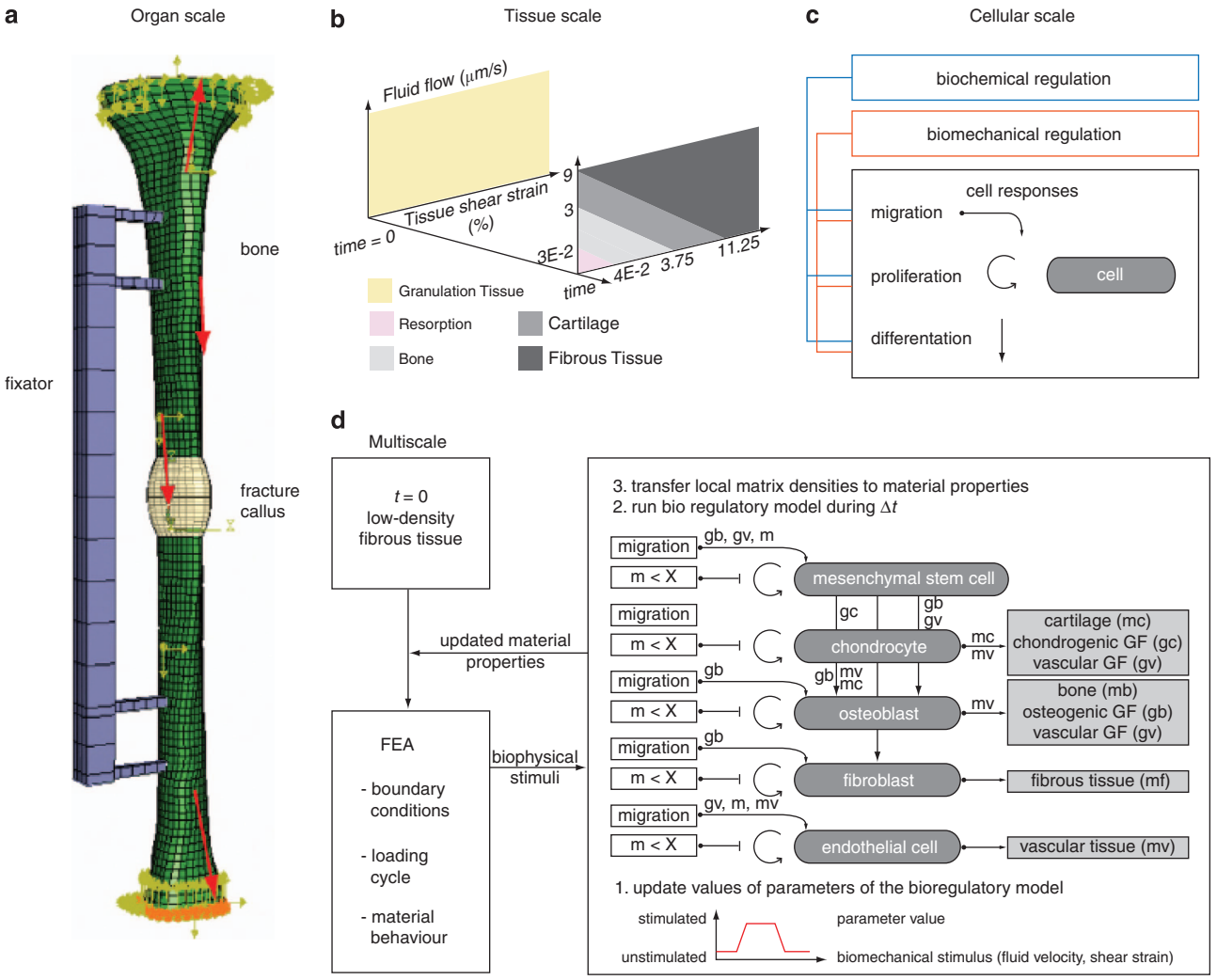

Figure 2 Mathematical models of bone fracture healing: (**a**) Organ-scale model of human tibia including external fixator device using finite element analysis (FEA) modified from Bryne *et al.*;[41] (**b**) tissue-scale model of the biomechanical stimulus based on fluid flow and tissue shear strain according to Prendergast *et al.*[50] to direct cell response; (**c**) cell-scale model including biochemical and biomechanical regulation of cell migration, proliferation and differentiation; (**d**) multiscale model (coupling organ, tissue and cell scale) including biochemical and biomechanical regulation (adapted from Geris *et al.*[6]).

material volume fractions and distributions across the fracture callus) these models are coupled with mechanoregulatory models of cell behavior (**Figure 2b**).

As has been pointed out in the previous section, biomechanical control of the IFM is essential for optimal fracture healing. Mechanical loading leads to tissue deformation, which can induce direct stretching of cells residing in the fracture callus and/or fluid flow.[42,43] It is now well accepted that mesenchymal cells subjected to mechanical loading (via direct stretching and/or pulsatile fluid shear stress) may change their differentiation pathway. Pauwels[44] was the first to formulate a conceptual model of this mechanoregulatory behavior, where cell elongation has been assumed to result in formation of fibrous connective tissue, whereas hydrostatic pressure leads to formation of cartilaginous tissue. In the late 70s Perren and co-workers developed the concept of interfragmentary strain (IFS) (Note that the commonly used terminology interfragmentary deformation is equivalent to the IFS via the strain–deformation relationship), which hypothesized that certain tissue cannot be formed in regions that experience strains larger than a defined failure strain.[45] The IFS conceptual model provides a practical method to evaluate different fracture treatment strategies, but is not applicable for bone healing in general, as it disregards the structural and mechanical (that is, spatial) heterogeneities of the fracture callus (see also comments made on IFM in Section 'The Biology of Bone Fracture Healing'). Further extensions of these early conceptual models have been made first by Carter *et al.*[46,47] using the principal tensile strain and the hydrostatic pressure as mechanoregulatory quantities and later by Claes and Heigele[48] who suggested strain and hydrostatic pressure to regulate cell differentiation (see Geris *et al.*[6] for a summary of these conceptual models and regulatory mechanisms). Isaksson *et al.*[49] compared the different mechanoregulatory mechanisms proposed by Carter *et al.*,[47] Claes and Heigele[48] and Prendergast *et al.*,[50] and found that combined biophysical stimuli of shear strain and fluid velocity was closest to experimental results (**Figure 2b**). Healing under torsional loading conditions could only be predicted if a combined mechanical stimuli of shear strain and fluid velocity was taken into account.

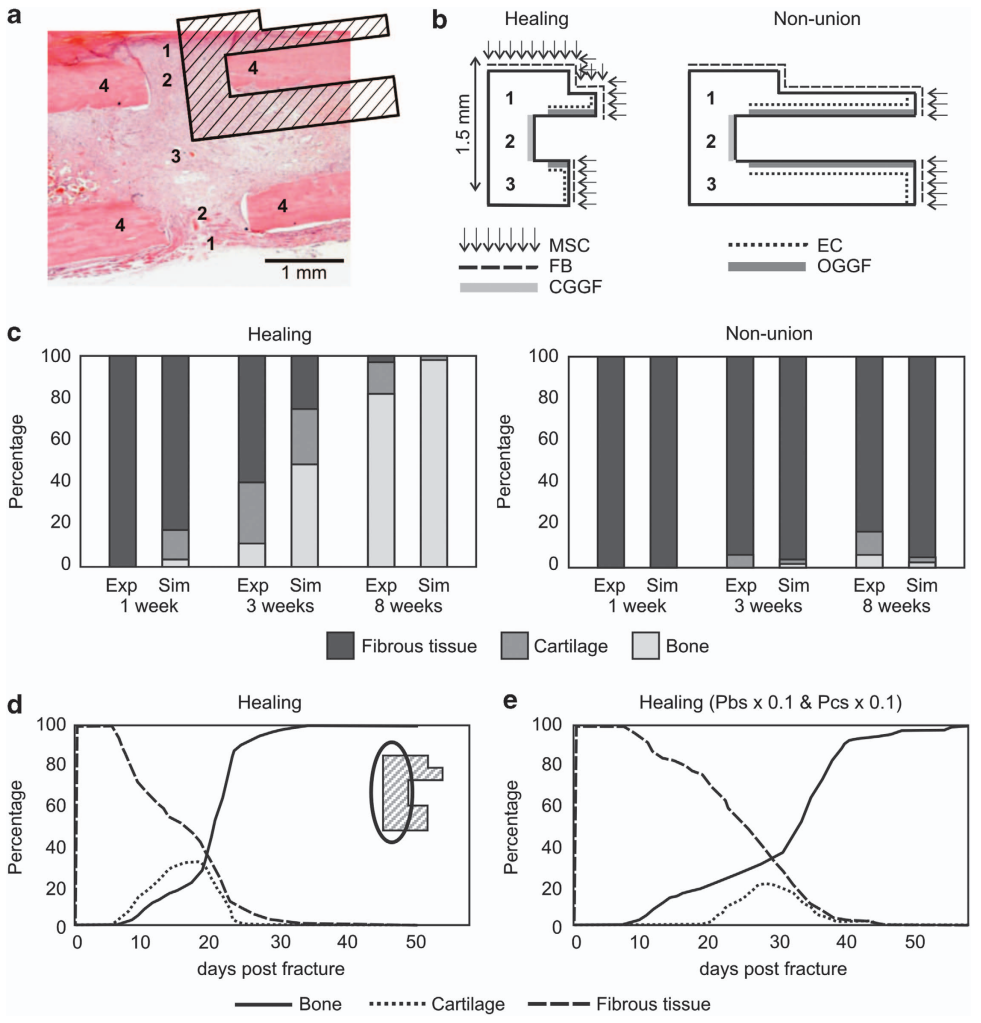

Figure 3 Mathematical model of the regeneration process in healing and nonunion groups: (**a**) different domains of the simulations (1, periosteal callus, 2, intercortical gap, 3, endosteal callus, 4, cortical bone); (**b**) boundary conditions for healing and nonunion model (FB, fibroblast, EC, endothelial cell, CGGF, chondrogenic growth factor, OGGF, osteogenic growth factor); (**c**) comparison of experimentally measured (Exp) and numerically calculated (Sim) tissue constituents present within the interfragmentary gap of healing and nonunion groups; (**d**) temporal evolution of the numerically calculated tissue fraction for the healing group (spatially averaged over the interfragmentary region—see insert); (**e**) temporal evolution of the numerically calculated tissue fraction for the healing group with 10-fold reduced cartilage and bone production rates leading to better correspondence between experimental and simulation results (adapted from Geris *et al.*[57]).

This approach has been successfully applied to investigate tissue differentiation patterns in many areas of bone healing, including osseointegration around implants,[51] osteochondral defect healing,[52] regeneration of osteotomized mandible[53] and tissue differentiation inside bone chambers.[54]

Another application of such organ-scale models is related to identifying optimal fracture fixation and designing particular stabilization devices depending on the type of fracture and the severity of trauma[55,56] (**Figure 2a**). Furthermore, a complete model of bone fracture healing at the organ scale also requires incorporation of bone remodeling and adaptation. Currently, most models of bone fracture healing are primarily concerned with investigation of soft-callus formation and hard-callus formation phases. For a review on bone remodeling at the organ scale we refer to Webster *et al.*[29]

Numerical simulation of atrophic nonunion: Geris *et al.* model. Among the many proposed models using a continuum description we will now focus on the model presented by Geris *et al.*,[9,57] and show how this type of model can be used to address different hypothesis around the occurrence and treatment of bone atrophic nonunions. Such nonunions present a class of nonhealing fractures that display only limited external callus formation. On the basis of experimental studies it was hypothesized that in order to obtain successful healing outcomes blood vessels, growth factors and proliferative precursor cells all need to be present simultaneously in the callus. Using an integrative approach coupling *in-vivo* and *in-silico* models Geris *et al.* systematically investigate these different aspects. Furthermore, the *in-silico* model is used to investigate and design different treatment strategies including cell transplantation for atrophic nonunions.

This model includes key aspects of bone fracture healing such as angiogenesis, cell differentiation and cell migration (**Figure 2d**). We note that further extensions of this model have been made including biomechanical regulation (of cell

proliferation rates) due to mechanical loading[34] and a more accurate (that is discrete) description of the network of blood vessels invading the fracture site using a hybrid model.[35] Although it is currently not feasible to model all different cell types and biochemical regulatory factors involved in fracture healing a commonly made assumption in mathematical modeling (as well as in the development of conceptual models) is to 'lump' different cell types and various regulatory factors into generic groups. As outlined in the previous section osteblasts residing in the fracture callus are a heterogeneous population of osteoblastic cells at various stages of differentiation. In the model of Geris *et al.,* the terminology osteoblast density and chondrocyte density is used to describe an average (that is, a mixture) of osteoblastic cells and chondrocytes. Similar chondrogenic growth factors, osteogenic growth factors and vascular growth factors represent a mixture of various growth factors belonging to the respective class of growth factors.

Experimental system. **Figure 3a** shows the application of the Geris *et al.* model to simulate fracture healing in a rat model of atrophic nonunion. The conceptual model without biomechanical regulation is shown in **Figure 2d**. In this model an osteotomy was performed. The fibula was fractured manually using the three-point bending test and a 1 mm gap introduced at the site of osteotomy. To achieve an atrophic nonunion the periosteum was stripped and the marrow canal was reamed in this animal model.

Initial and boundary conditions. The geometrical domain for the model has been chosen based on the experimental set up assuming symmetry of the fracture site (**Figure 3b**). As with any computational model, prescribing suitable initial and boundary conditions is essential. For the atrophic nonunion case the domain was extended at the distal end (away from the fracture site) over a distance corresponding to the length, where the periosteum has been stripped and the marrow canal reamed in the experiments (**Figure 3b**).

The model developed by Geris *et al.* was first applied for simulation of different fracture healing scenarios. It predicted that for cases where either the periosteum was stripped or the marrow canal was reamed, complete fracture healing is possible with delayed bone formation in those parts of the callus site where the MSC source was removed. Only when both modifications were combined the occurrence of a nonunion was predicted (**Figure 3c**), suggesting that the lack of stem cells due to the applied experimental procedures affects other process downstream in the healing cascade, including blood vessel formation and growth factor production. Comparison of experimental data and simulation results showed good agreement of healing progression (**Figure 3c**). For the nonunion group, the mathematical model predicted formation of small amounts of cartilage and bone by postosteotomy week (POW) 8. For the healing group, both direct bone formation (close to the undamaged cortical bone) and cartilage formation (central part of the callus) were predicted to form by POW 3 (**Figure 3d**). In these simulations the cartilage tissue was replaced by bone via endochondral ossification by week 8. The ossification process could be delayed by reducing the cartilage and/or bone matrix formation rate in the model (**Figure 3e**).

After the model has been tested for different healing scenarios it was then applied to investigate various treatment strategies. Administration of MSCs at 3 weeks postosteotomy, that is, the time when vascularity within the interfragmentary gap was sufficient to keep the injected cells alive has been hypothesized as a successful treatment for atrophic nonunions. The model suggested that, after injection of the cell transplant at POW 3 in the center of the callus region, the amount of bone gradually increases whereas the amount of fibrous tissue decreases up to POW 16. Formation of a small amount of cartilage was also predicted with endochondral ossification still in progress at POW 16 (**Figure 4a$_{I}$, a$_{II}$**). Interestingly, the amount of soft tissue present at POW 16 depended strongly on the exact location of injection of the cell transplant with excentral injection leading to unicortical bridging (**Figure 4b$_{I}$, b$_{II}$**). Another simulation addressed a commonly employed technique of growth factor and/or cell administration, close to but outside of the callus. However, the simulation results showed that such a treatment strategy would result in the formation of a layer of bone closest to the cell source, which would prevent other cells from migrating into the center of the callus (**Figure 4c**).

Outlook and Conclusions

As has been pointed out in this review one of the still open questions in fracture biology (and tissue engineering in general) is related to the transduction of mechanical signals from the tissue level down to the cell or even to the intracellular level. It is currently not clear to which extend mechanical quantities such as strain, strain rate and/or fluid flow are driving cellular responses such as ligand production and/or cell differentiation, proliferation and migration. From the review of Bonewald and Johnson[42] it is clear that osteocytes are the major mechanosensing cells in bone that respond both to changes in the biochemical and biomechanical environment via expression of receptor activator of NFkB ligand, sclerostin and other regulatory factors. It is likely that cells in a regenerating tissue might respond in a similar way to mechanical stimuli, although the mechanical environment, that is, magnitude of strain, strain rate and so on may be quite different. Methods to quantify these different mechanoregulatory mechanisms remain to be determined.

To quantify different mechanical stimuli such as strain, strain rate and fluid velocity *in-vivo* including anatomical realistic geometry, muscle loading, effect of fixator device and realistic tissue properties of the fracture callus are essential. Several multiscale computational models have been developed to address this problem (**Figure 2**). However, these type of models require a large amount of experimental data related to the model parameters, which reflect tissue, cell and regulatory factor properties. Both *in-vitro* and/or *in-vivo* experiments can be designed to estimate these model properties.

Although conceptual models are commonly utilized in bone fracture research, mathematical models have only recently been applied in this field. In view of the ever increasing amount of experimental data available and the growing sophistication of conceptual models used for experimental design and hypothesis testing, mathematical models are novel tools for augmenting experimental analysis, providing new information about potential regulatory mechanisms, and suggesting new hypotheses that allow for a deeper understanding of underlying

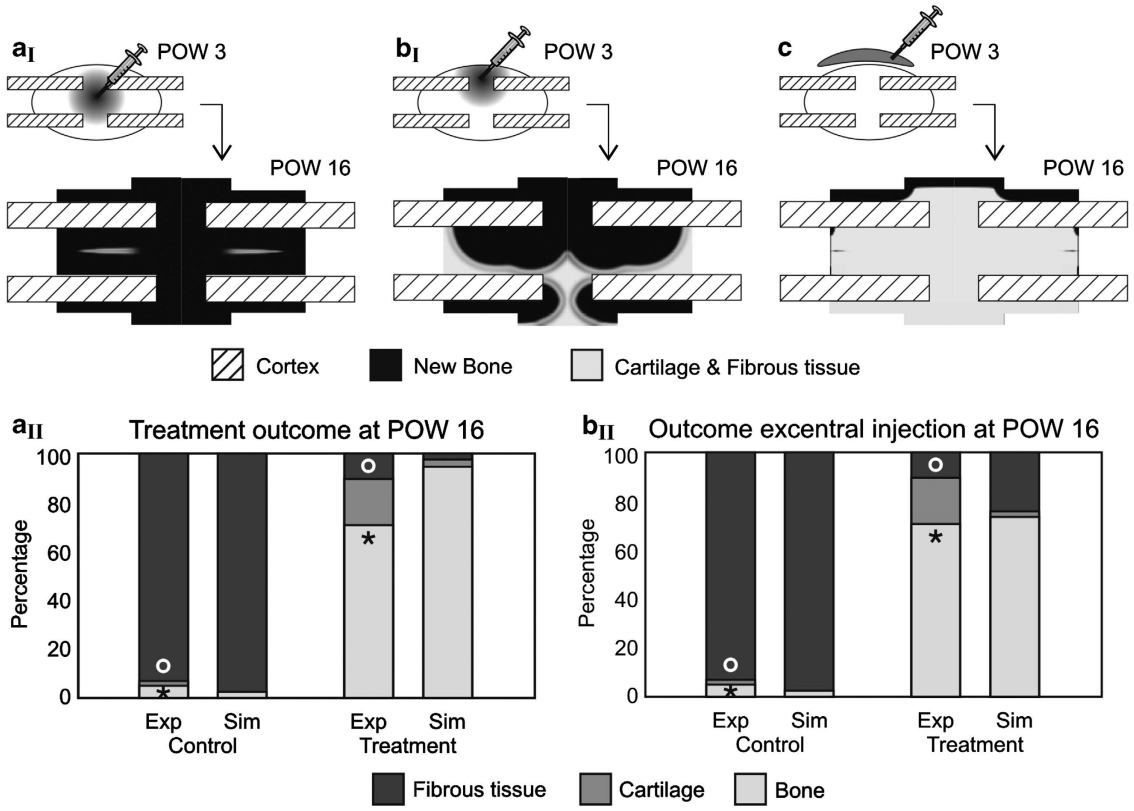

Figure 4 Effect of MSC transplantation on atrophic nonunion: ($\mathbf{a_I}$) simulation results for the treatment with the cell transplant injected at the center of the callus; ($\mathbf{a_{II}}$) comparison of experimentally measured (Exp) and numerically calculated (Sim) tissue constituents present within the interfragmentary gap of control (carrier solution injected) and treatment (MSC transplant) groups ($^*P < 0.005$, Student's *t*-test); ($\mathbf{b_I}$) Simulation results for the treatment with cell transplant injected externally in the callus; ($\mathbf{b_{II}}$) comparison of experimentally measured (Exp) and numerically calculated (Sim) tissue constituents present within the interfragmentary gap of control (carrier solution injected) and treatment (MSC transplant) groups ($^*P < 0.005$, Student's *t*-test); (**c**) simulation results for the treatment with the cell transplant injected outside the callus (adapted from Geris *et al.*[57]).

complexities. In particular, such models can be used as clinical tools to investigate the etiology and treatment of fractures as has been shown here on the example of atrophic nonunions. We believe that utilizing such a combined *in silico–in vivo* approach will help to optimize experimental and clinical studies on fracture healing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. AAOS. Half of all musculoskeletal injuries occur in home. *AAOS Now* 2009; available at http://www.aaos.org/news/aaosnow/apr09/clinical9.asp.
2. Nolte PA, van der Krans A, Patka P, Janssen IMC, Ryaby JP, Albers GHR. Low-intensity pulsed ultrasound in the treatment of nonunions. *J Trauma-Injury Infec Critic Care* 2001;**51**: 693–703.
3. Einhorn TA. Enhancement of fracture-healing. *J Bone Joint Surg Am* 1995;**77**:940–956.
4. Einhorn TA. The cell and molecular biology of fracture healing. *Clin Orthop Relat Res* 1998;**355**:S7–S21.
5. American College of Surgeons Committe on Trauma. National Trauma Data Bank Report. Chicago: American College of Surgeons, 2002.
6. Geris L, Vander Sloten J, Van Oosterwyck H. *In silico* biology of bone modelling and remodelling: regeneration. *Philoss Transact A math Phys Eng Sci* 2009;**367**:2031–2053.
7. Heppenstall RB. Fracture Treatment and Healing. Philadelphia: Saunders, 1980.
8. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. *Nat Rev Rheumatol* 2012;**8**:133–143.
9. Geris L, Gerisch A, Sloten JV, Rd Weiner, Oosterwyck HV. Angiogenesis in bone fracture healing: A bioregulatory model. *J Theor Biol* 2008;**251**:137–158.
10. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: the cellular picture. *Sem Cell Dev Biol* 2008;**19**:459–466.
11. Marsell R, Einhorn TA. The biology of fracture healing. *Injury* 2011;**42**:551–555.
12. Kon T, Cho T-J, Aizawa T, Yamazaki M, Nooh N, Graves D *et al.* Expression of osteoprotegerin, receptor activator of NF-κB ligand (osteoprotegerin ligand) andrelated proinflammatory cytokines during fracture healing. *J Bone Miner Res* 2001;**16**:1004–1014.
13. Cho T-J, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor β superfamily during murine fracture healing. *J Bone Miner Res* 2002;**17**:513–520.
14. Barry S. Non-steroidal anti-inflammatory drugs inhibit bone healing. *Rev Veterin Comparat Orthopaed Traumatol* 2010;**23**:385–392.
15. Gerstenfeld LC, Cho TJ, Kon T, Aizawa T, Tsay A, Fitch J *et al.* Impaired fracture healing in the absence of TNF-α signaling: the role of TNF-α in endochondral cartilage resorption. *J Bone Miner Res* 2003;**18**:1584–1592.
16. Lu Z, Wang G, Dunstan CR, Zreiqat H. Short-term exposure to tumor necrosis factor-alpha enables human osteoblasts to direct adipose tissue-derived mesenchymal stem cells into osteogenic differentiation. *Stem Cells Dev* 2012;**21**:2420–2429.
17. Lepperdinger G, Brunauer R, Gassner R, Jamnig A, Kloss F, Laschober GT. Changes of the functional capacity of mesenchymal stem cells due to aging or age-associated disease—implications for clinical applications and donor recruitment. *Transfus Med Hemother* 2008;**35**:299–305.
18. Manavalan JS, Cremers S, Dempster DW, Zhou H, Dworakowski E, Kode A *et al.* Circulating osteogenic precursor cells in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012;**97**:3240–3250.
19. Hirao M, Tamai N, Tsumaki N, Yoshikawa H, Myoui A. Oxygen tension regulates chondrocyte differentiation and function during endochondral ossification. *J Biol Chem* 2006;**281**:31079–31092.

20. Shapiro F. Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. *Eur Cell Mater* 2008;**15**:53–76.
21. Pivonka P, Komarova SV. Mathematical modeling in bone biology: from intracellular signaling to tissue mechanics. *Bone* 2010;**47**:181–189.
22. Little DG, Ramachandran M, Schindeler A. The anabolic and catabolic responses in bone repair. *J Bone Joint Surg* 2007;**89-B**:425–433.
23. Lambers FM, Kuhn G, Müller R. Advances in multimodality molecular imaging of bone structure and function. *BoneKEy Reports* **1**, Article Number: 37 (2012); doi:10.1038/bonekey.2012.28.
24. Kalajzic I, Staal A, Yang W-P, Wu Y, Johnson SE, Feyen JHM *et al.* Expression profile of osteoblast lineage at defined stages of differentiation. *J Biol Chem* 2005;**280**:24618–24626.
25. Kalajzic I, Kalajzic Z, Kaliterna M, Gronowicz G, Clark SH, Lichtler AC *et al.* Use of type I collagen green fluorescent protein transgenes to identify subpopulations of cells at different stages of the osteoblast lineage. *J Bone Miner Res* 2002;**17**:15–25.
26. Ushiku C, Adams DJ, Jiang X, Wang L, Rowe DW. Long bone fracture repair in mice harboring GFP reporters for cells within the osteoblastic lineage. *J Orthop Res* 2010;**28**:1338–1347.
27. Grcevic D, Pejda S, Matthews BG, Repic D, Wang L, Li H *et al. In vivo* fate mapping identifies mesenchymal progenitor cells. *Stem Cells* 2012;**30**:187–196.
28. Trüssel A, Müller R, Webster D. Toward mechanical systems biology in bone. *Ann Biomed Eng* 2012;**40**:2475–2487.
29. Webster D, Müller R. *In silico* models of bone remodeling from macro to nano—from organ to cell. *Wiley Interdisciplinary Rev Syst Biol Med* 2011;**3**:241–251.
30. Komarova SV, Smith RJ, Dixon SJ, Sims SM, Wahl LM. Mathematical model predicts a critical role for osteoclast autocrine regulation in the control of bone remodeling. *Bone* 2003;**33**:206–215.
31. Pivonka P, Zimak J, Smith DW, Gardiner BS, Dunstan CR, Sims NA *et al.* Model structure and control of bone remodeling: a theoretical study. *Bone* 2008;**43**:249–263.
32. Pivonka P, Zimak J, Smith DW, Gardiner BS, Dunstan CR, Sims NA *et al.* Theoretical investigation of the role of the RANK-RANKL-OPG system in bone remodeling. *J Theor Biol* 2009;**262**:306–316.
33. Bailón-Plaza A, Van Der Meulen MCH. A mathematical framework to study the effects of growth factor influences on fracture healing. *J Theor Biol* 2001;**212**:191–209.
34. Geris L, Sloten J, Oosterwyck H. Connecting biology and mechanics in fracture healing: an integrated mathematical modeling framework for the study of nonunions. *Biomech Model Mechanobiol* 2010;**9**:713–724.
35. Peiffer V, Gerisch A, Vandepitte D, Van Oosterwyck H, Geris L. A hybrid bioregulatory model of angiogenesis during bone fracture healing. *Biomech Model Mechanobiol* 2011;**10**:383–395.
36. Anderson ARA, Chaplain MAJ. Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bull Math Biol* 1998;**60**:857–900.
37. Anderson ARA, Quaranta V. Integrative mathematical oncology. *Nat Rev Cancer* 2008;**8**:227–234.
38. Buenzli PR, Jeon J, Pivonka P, Smith DW, Cummings PT. Investigation of bone resorption within a cortical basic multicellular unit using a lattice-based computational model. *Bone* 2012;**50**:378–389.
39. Pérez MA, Prendergast PJ. Random-walk models of cell dispersal included in mechanobiological simulations of tissue differentiation. *J Biomech* 2007;**40**:2244–2253.
40. Bailón-Plaza A, van der Meulen MCH. Beneficial effects of moderate, early loading and adverse effects of delayed or excessive loading on bone healing. *J Biomech* 2003;**36**:1069–1077.
41. Byrne DP, Lacroix D, Prendergast PJ. Simulation of fracture healing in the tibia: mechanoregulation of cell activity using a lattice modeling approach. *J Orthop Res* 2011;**29**:1496–1503.
42. Bonewald LF, Johnson ML. Osteocytes mechanosensing and Wnt signaling. *Bone* 2008;**42**:606–615.
43. Bonewald LF. The amazing osteocyte. *J Bone Miner Res* 2011;**26**:229–238.
44. Pauwels F. Eine neue theorie über den einfluß mechanischer reize auf die differenzierung der stützgewebe. *Z Anat Entwicklungsgeschichte* 1960;**121**:478–515.
45. Perren SM. Physical and biological aspects of fracture healing with special reference to internal fixation. *Clin Orthop Relat Res* 1979;**138**:175–196.
46. Carter DR. Mechanical loading history and skeletal biology. *J Biomech* 1987;**20**:1095–1109.
47. Carter DR, Blenman PR, Beaupré GS. Correlations between mechanical stress history and tissue differentiation in initial fracture healing. *J Orthop Res* 1988;**6**:736–748.
48. Claes LE, Heigele CA. Magnitudes of local stress and strain along bony surfaces predict the course and type of fracture healing. *J Biomech* 1999;**32**:255–266.
49. Isaksson H, van Donkelaar CC, Huiskes R, Ito K. Corroboration of mechanoregulatory algorithms for tissue differentiation during fracture healing: comparison with *in vivo* results. *J Orthop Res* 2006;**24**:898–907.
50. Prendergast PJ, Huiskes R, Søballe K. Biophysical stimuli on cells during tissue differentiation at implant interfaces. *J Biomech* 1997;**30**:539–548.
51. Andreykiv A, Prendergast PJ, van Keulen F, Swieszkowski W, Rozing PM. Bone ingrowth simulation for a concept glenoid component design. *J Biomech* 2005;**38**:1023–1033.
52. Kelly DJ, Prendergast PJ. Prediction of the optimal mechanical properties for a scaffold used in osteochondral defect repair. *Tissue Eng* 2006;**12**:2509–2519.
53. Boccaccio A, Prendergast P, Pappalettere C, Kelly D. Tissue differentiation and bone regeneration in an osteotomized mandible: a computational analysis of the latency period. *Med Biol Eng Comput* 2008;**46**:283–298.
54. Khayyeri H, Checa S, Tägil M, Prendergast PJ. Corroboration of mechanobiological simulations of tissue differentiation in an *in vivo* bone chamber using a lattice-modeling approach. *J Orthop Res* 2009;**27**:1659–1666.
55. Mihalko WM, Beaudoin AJ, Cardea JA, Krause WR. Finite-element modelling of femoral shaft fracture fixation techniques post total hip arthroplasty. *J Biomech* 1992;**25**:469–476.
56. Chao EY, Kasman RA, An KN. Rigidity and stress analyses of external fracture fixation devices: a theoretical approach. *J Biomech* 1982;**15**:971–983.
57. Geris L, Reed AAC, Vander Sloten J, Simpson AHRW, Van Oosterwyck H. Occurrence and treatment of bone atrophic non-unions investigated by an integrative approach. *PLoS Comput Biol* 2010;**6**:e1000915.