REVIEW

In silico design of treatment strategies in wound healing and bone fracture healing

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Wound and bone fracture healing are natural repair processes initiated by trauma. Over the last decade, many mathematical models have been established to investigate the healing processes in silico, in addition to ongoing experimental work. In recent days, the focus of the mathematical models has shifted from simulation of the healing process towards simulation of the impaired healing process and the in silico design of treatment strategies. This review describes the most important causes of failure of the wound and bone fracture healing processes and the experimental models and methods used to investigate and treat these impaired healing cases. Furthermore, the mathematical models that are described address these impaired healing cases and investigate various therapeutic scenarios in silico. Examples are provided to illustrate the potential of these in silico experiments. Finally, limitations of the models and the need for and ability of these models to capture patient specificity and variability are discussed.

Keywords: wound healing; bone regeneration; mathematical modelling; treatment strategies; patient variability

1. Introduction

Skin and skeleton are among the largest organ systems in the human body showing extraordinary abilities for both self-regeneration and repair. However, in some cases, the body is unable to repair the damage and treatment of these

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impaired healing cases amounts to a considerable economic cost. Because of the complex nature of the healing process, a multi-disciplinary approach is necessary to identify the best treatment strategy. This paper focuses on the contribution of mathematical models in the design of such treatment strategies. Mathematical models that look into in silico therapy design are discussed, and examples are given to illustrate their potential. Furthermore, the need for incorporation of patient variability and specificity into the models is discussed, as well as the ability (or disability) of the various types of models to do so.

2. The healing process

Wounds and bone fractures that heal successfully progress through several different temporal yet overlapping phases—haemostasis/inflammation, reparation and remodelling (Thackham et al. 2008). The progression through these phases is mediated by mechanical and biochemical cues present at the trauma site, e.g. the release of many growth factors at different times during the healing process. Figure 1 gives a schematic overview of these different phases.

The immediate response to trauma is aimed at stopping blood from being lost by forming a blood clot within the trauma site. This occurs in the haemostatic phase, which should last only for a few hours. In the inflammatory phase, neutrophils and monocytes (which become macrophages in the wound) degrade necrotic tissue and, in the case of wound healing or open fractures, remove bacteria. This phase typically lasts for a matter of days for both wound and fracture healing.

In the next phase, the reparation phase, different processes occur in different trauma locations. In wound healing, this phase is also known as the proliferative phase during which keratinocytes, fibroblasts and endothelial cells (ECs) migrate into and proliferate within the wound region. The keratinocytes create an epithelial layer that covers the top of the wound, while the fibroblasts start to produce collagen, which is the primary component of the extracellular matrix. The ECs start to form tiny capillaries within the wound region from the existing blood vessels (angiogenesis). In humans, this phase lasts for about two weeks. In fracture healing, the reparative phase (time until clinical union) usually takes six to eight weeks, for relatively uncomplicated fractures. The invasion of inflammatory cells in the callus area is followed by the invasion of fibroblasts, mesenchymal stem cells and ECs (Taguchi et al. 2005), all of them producing and responding to a myriad of growth factors guiding the process. In the first part of the reparative phase, the soft-callus phase, the initially formed granulation tissue is gradually being replaced by fibrous tissue. In this soft-tissue matrix, mesenchymal stem cells will start differentiating, guided by cues from their microenvironment, such as the presence of biological factors (Einhorn 1998) and the perceived mechanical stimuli (Carter et al. 1998). Direct differentiation towards osteoblasts is observed near the cortex, away from the fracture site. These osteoblasts produce woven bone matrix, the hard callus, by a process called intramembranous ossification. Mesenchymal stem cells differentiate into chondrocytes (cartilage forming cells) in the central fracture area, where the soft callus will gradually take on the appearance of cartilage, mechanically stabilizing the fractured zone. The chondrocytes mature towards hypertrophic chondrocytes, initiating biochemical preparations in the
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(i)

(ii)

(iii)

(iv)

(v)

Figure 1. Schematic representation of different phases of wound and fracture healing: (a) the intact situation; (b) trauma; (c) the haemostatic and inflammatory phase; (d) the reparative phase with the soft-callus stage (i) and the hard-callus stage (iii) for fracture healing and the proliferative phase (ii) for wound healing; and (e) the remodelling phase.

cartilage matrix to undergo calcification. This concludes the soft-callus phase of healing. In the second part of the reparative phase, the hard-callus phase, blood vessels invade the calcified cartilage, bringing along osteoblasts. These osteoblasts, receiving enough oxygen and subjected to the proper mechanical stimuli, will produce a hard-callus tissue consisting of mineralized woven bone matrix by a process called endochondral ossification. When the fractured ends are connected by a bony callus, clinical union is reached.

Already during the reparative phase, the final remodelling phase begins. In wound healing, the fibroblasts remodel the extracellular matrix in order to improve the mechanical strength of the tissue. In healing fractures, osteoclasts resorb the unnecessary or poorly placed parts of the regenerate and lamellar bone is formed. This gradually reverts the blood supply to a normal state and restores the bone at the regeneration site to its original shape and strength. Both remodelling processes take place for a prolonged period of time, up to several years in humans.

3. Treatment strategies in wound healing

(a) From trauma to failure

Wounds that do not heal through an orderly and timely process and are unable to produce a repair response with functional integrity are called chronic wounds (Lazarus et al. 1994). Chronic wounds, such as foot and leg ulcers, often remain
in a prolonged inflammatory state due, in part, to an inability to kill the bacteria (Roy et al. 2009). These problematic wounds are often the result of an underlying disease, such as diabetes or venous insufficiency (Mathieu 2002). Furthermore, the estimated cost of treatment for chronic wounds in the United States is a staggering $5–10 billion (Kuehn 2007). Because of the complex nature of the healing process and a lack of guidelines for optimizing treatment (Hunt et al. 2000; Grey et al. 2009), a multi-disciplinary approach is necessary to identify the best treatment strategy for the management of chronic wounds (Kjaer et al. 2005; Harding 2006; Grey et al. 2009).

One reason for the failure of chronic wounds to heal is an insufficient level of oxygen. Wounds are, by their nature, hypoxic, which is defined as a lower partial pressure of oxygen ($pO_2$) compared with the $pO_2$ level of the same tissue under healthy conditions (Sen 2009). The $pO_2$ in a dermal wound ranges from 0 to 10 mmHg at the centre to 60 mmHg at the wound edge, with the $pO_2$ in the arterial blood being about 100 mmHg (Gordillo & Sen 2003). While a hypoxic environment can stimulate an angiogenic response for acute wounds, cells cannot function if their metabolic need for oxygen is not met, as inflammatory cells, fibroblasts and bacteria compete for oxygen in the wound environment (Gordillo & Sen 2009). Fibroblasts, for example, need 30–40 mmHg to properly deposit collagen (Rodriguez et al. 2008). Furthermore, it has been shown that oxygen levels control bacteria killing, epithelialization, angiogenesis, collagen synthesis and matrix deposition (Tompach et al. 1997; Rodriguez et al. 2008; Gordillo & Sen 2009).

(b) From experiments to patients

Methods for improving the healing response of a chronic wound include debridement, topical administration of growth factors, the use of commercially engineered substitutes, wound vacuum-assisted closures (VACs) and the application of hyperbaric and oxygen (Argenta & Morykwas 1997; Morykwas et al. 1997; Mason et al. 1999; Curran & Plosker 2002; Wood 2002; Grey & Harding 2006), each working with varying degrees of success (Steed et al. 1996; Fries et al. 2005). Wound debridement is one of the most common methods for treating a problematic wound. Debridement is the process of removing non-viable or dead tissue from the wound. Such tissue can not only impede the healing process, but also harbour the bacteria that can lead to infection (Grey & Harding 2006). However, the technique is only successful in about 25 per cent of the patients with diabetic foot ulcers (Steed et al. 1996). As a consequence, other techniques are used to treat chronic wounds. One such technique is the topical administration of growth factors like platelet-derived growth factor (PDGF), fibroblast growth factor or transforming growth factor-β1 (TGF-β1). However, clinical trials have produced mixed results and the PDGF is the only growth factor to have been approved for use, as it is the only one shown to be effective through randomized double-blinded studies (Richard et al. 1995; Mi et al. 2007). Another method for treating chronic wounds is through the use of currently available commercially engineered skin substitutes, such as Apligraf and Dermgraft. Apligraf is an artificial skin comprised of both an epidermal and a dermal layer of skin seeded with cells on a scaffold (Curran & Plosker 2002). The treatment protocol is that the artificial skin is added to the wound once
per week for five weeks. Dermagraft is a dermal skin substitute comprised of fibroblasts seeded on a scaffold, with a treatment protocol of one application per week for eight weeks (Marston 2004). Another therapeutic strategy for problematic wounds is the VAC device. The device consists of a porous sponge applied to a wound, covered with an occlusive dressing, with a tube connecting the sponge to a vacuum pump, which applies a suction pressure to the sponge (Vishal Saxena et al. 2004). Several studies have suggested that the VAC reduces infection, improves angiogenesis and is effective in treating both acute and chronic wounds (Argenta & Morykwas 1997; Mason et al. 1999; McCallon et al. 2000; Armstrong et al. 2002).

Yet another strategy for improving the healing response of chronic wounds is through the use of supplemental oxygen, such as through hyperbaric or topical oxygen therapy (Thackham et al. 2008; Gordillo & Sen 2009). Hyperbaric oxygen is a US Food and Drug Administration-approved treatment for chronic and non-healing wounds (Rodriguez et al. 2008). Patients are placed in a chamber where they breathe 100 per cent oxygen at 2–3 atm of pressure for 60–120 min per session. Typically, treatment is given once per day, 5 days a week for two to six weeks, depending upon the severity of the wound (Thackham et al. 2008; Gordillo & Sen 2009). For topical oxygen therapy, typical treatment involves attaching a plastic, inflatable device around the wound region that can deliver 100 per cent oxygen at or slightly above 1 atm. Suggested guidelines for treatment are for 90 min once per day for 4 days, followed by 3 days with no treatment. This process is repeated for several weeks or months until the wound has healed (Gordillo et al. 2008; Rodriguez et al. 2008; Gordillo & Sen 2009).

In comparing the two forms of treatment, hyperbaric oxygen has the advantage of delivering a much higher concentration of oxygen to the wound, with a \( pO_2 \) that can range from 400–1600 mm Hg, when compared with topical oxygen, which delivers 40–80 mm Hg (Gordillo & Sen 2009). The increased pressure (in contrast to normobaric treatment) creates a steeper pressure gradient, which helps the supplemental oxygen reach the cells (Lin et al. 2008). For example, hyperbaric oxygen treatment at 3 atm roughly triples the oxygen diffusion between the capillaries and tissue (Grolman et al. 2001). However, the increased level of oxygen also creates the risk of oxygen toxicity, but the effects can be minimized if the oxygen is breathed intermittently for 60–120 min (Lin et al. 2008). Topical oxygen also has the advantages of using a portable device, being less expensive and delivering oxygen directly to the wound without the dependence of a vascular network (unless hyperbaric oxygen is delivered in a monoplace chamber). However, the studies for topical oxygen on treatment outcomes and underlying mechanisms are more limited than hyperbaric oxygen (Gordillo & Sen 2003). Still, identifying the optimal treatment strategies remains an active area of research (Gordillo et al. 2008) as the clinical success of these treatments varies (Fries et al. 2005).

(c) From models to therapies

One multi-disciplinary approach that can be used to explore different, and perhaps, novel treatment strategies is mathematical modelling (Schugart et al. 2008; Thackham et al. 2008). The first mathematical model of wound
healing dates back to the 1980s and early 1990s. They were developed by Murray and co-workers (Murray et al. 1983, 1988; Sherratt & Murray 1990; Sherratt et al. 1992; Tranquillo & Murray 1992) in the 1980s and early 1990s, and are summarized in Murray (2002, 2003). These models and others developed in the 1990s can be conveniently categorized into one of the four groups—epidermal healing, repair of the dermal extracellular matrix, wound contraction and wound angiogenesis (for a review, see Sherratt & Dallon 2002). Most of these models are deterministic and formulated using differential equations.

A number of recent models were also formulated using differential equations (Vishal Saxena et al. 2004; Mi et al. 2007; Waugh & Sherratt 2007; Schugart et al. 2008; Flegg et al. 2009). These models have been developed to analyse strategies for improved healing, such as wound VACs, commercially engineered skin substitutes and hyperbaric oxygen.

Vishal Saxena et al. (2004) developed a mechanical model to analyse VAC therapy. The mechanical model was solved using a finite-element method, and the simulation results are consistent with the in vitro strain levels shown to promote cell proliferation and illustrate the surface undulations observed in wound cross sections. Their modelling approach may be used to analyse treatment strategies that promote cell proliferation, growth-factor production and wound angiogenesis through the application of micromechanical forces.

Waugh & Sherratt (2007) developed a mathematical model using ordinary differential equations to capture the effects of commercially engineered skin substitutes. Their model is an extension of their previous work (Waugh & Sherratt 2006), and the equations were solved using the MATLAB command ode15s (The Mathworks Inc., Natick, MA, USA). Their work shows that the key component with these treatment protocols is hyaluronan, with healing being similar to that observed in clinical trials. Waugh and Sherratt suggest that the model can be used to explore the effects on interpatient variability or the gradual release of treatment components.

The models of Schugart et al. (2008) and Flegg et al. (2009) use partial differential equations (PDEs) to analyse the effects of hyperbaric oxygen on the promotion of wound angiogenesis. Both models use the ‘snail-trail’ model of angiogenesis (Balding & McElwain 1985), which assumes that EC tips guide EC sprouts into the wound region. The model of Schugart et al. is an extension of the work of Pettet et al. (1996a,b) and the equations were solved using the MATLAB command pdepe (The Mathworks Inc.). Their simulations (shown in figure 2) agree with the experimentally observed results that: (i) the inflammatory response of macrophages peaks 3 days post-wounding (Roy et al. 2009) and (ii) extreme hypoxia and hyperoxia both inhibit wound angiogenesis, but there is optimal level of healing for wound hyperoxia (Hopf et al. 2005). Xue et al. (2009) extend the work of Schugart et al. for an ischaemic cutaneous wound by incorporating a free-boundary approach (i.e. the boundary of the wound changes with respect to time) and solving the system of equations using a finite-difference method. The model of Flegg et al. was solved using a finite-volume method, and their simulation results agree with the clinically relevant observations that: (i) normobaric oxygen therapy cannot be a useful substitute for hyperbaric oxygen, (ii) premature discontinuation of therapy can have negative consequences, and (iii) healing of a chronic wound can
Figure 2. Simulation results from the model of Schugart et al. (2008). (a) Macrophage density peaks at 3 days, which is in good agreement with the work of Roy et al. (2009), for an acute wound. (b) Endothelial cell density for different normalized hypoxic oxygen concentrations, where oxygen concentrations less than 1 are hypoxic. O₂ concentration: dotted line, 0.25; dashed-dotted line, 0.5; dashed line, 0.75; solid line, 1.0. (c) Endothelial cell density for different normalized hyperoxic oxygen concentrations, where oxygen concentrations greater than 1 are hyperoxic, which are measured using a hyperbaric oxygen chamber. O₂ concentration: dotted line, 4.0; dashed-dotted line, 3.0; dashed line, 2.0; solid line, 1.0. The average endothelial cell density increases with oxygen level from 0.5 to 2. When the hypoxic level is below 0.5, the wound is sufficiently hypoxic and vessel growth is stunted. When the hyperoxic level is above 2, the wound becomes extremely hyperoxic and derails tissue repair.
occur using hyperbaric oxygen, even with just a few capillary tips present. With their model, the authors also predict that patients with the following conditions are unlikely to respond to hyperbaric oxygen: (i) poor arterial supply of oxygen, (ii) extreme hypoxia, and (iii) dysfunctional EC response to oxygen. Both Schugart et al. and Flegg et al. suggest that mathematical models can be used to help identify protocols with hyperbaric oxygen that best improve the healing response for problematic wounds. Furthermore, a mathematical model that compares treatment results between hyperbaric and topical oxygen therapies (no models have been employed for topical oxygen therapy) may be useful in providing insights into why topical oxygen significantly increases the expression of vascular endothelial growth factors (VEGFs), but hyperbaric oxygen does not (Gordillo et al. 2008), or analysing the combined effect of using a topical administration of growth factor with supplemental oxygen (Gordillo & Sen 2009).

Vermolen and colleagues have recently focused on computational problems in wound healing for deterministic systems (Vermolen & Adam 2007; Javierre et al. 2009a,b). Computational challenges arise because cell movement is dominated by a chemotactic response to growth factors, which is called an advection-dominating process, as opposed to random motion (Lauffenburger 1983; Stokes & Lauffenburger 1991) (for a review of computational approaches for solving advection-dominating equations in wound healing, see Gerisch & Chaplain (2006) and Thackham et al. (2009)). The work of Vermolen and colleagues analyses changes in wound morphology, as most models assume either planar or axisymmetric wounds (Javier et al. 2009b). Vermolen & Adam (2007) first developed a model for epidermal wound healing with a general two-dimensional shape using the finite-element method. Then, Javierre et al. (2009a) employed a level-set method for a two-dimensional epidermal wound to describe the time evolution of the wound boundary. Javierre et al. (2009b) further extended their work on wound morphology to include mechanical factors. To do so, they extended the model employed by Olsen et al. (1995) by analysing the influence of wound morphology on contraction using the finite-element method.

An alternative approach to deterministic models is to use agent-based models (ABMs). ABMs have the advantage of accounting for the stochastic nature of biological processes. Mi et al. (2007) developed an ABM to analyse different treatment strategies with wound debridement and topical administration of growth factors. They produced the expected results of healing when analysing for different treatment strategies—debridement, release of PDGF, reduction in tumour necrosis factor-α and increase of TGF-β1. Mi et al. suggest that a drug company could use a mathematical model to test a new drug before going through the expensive process of basic science testing, toxicology and clinical trials.

ABMs can also provide a framework for multi-scale models of cells and tissues for wound healing. Smallwood and colleagues use agent-based models for a multi-scale approach to wound healing (Walker et al. 2004a,b, 2006a,b; Sun et al. 2007, 2008). A multi-scale approach has also been developed for deterministic systems by using a hybrid continuous-discrete model (Anderson & Chaplain 1998), such as when studying the flow through a vascular network (McDougall et al. 2002; Chaplain et al. 2006).
4. Treatment strategies in fracture healing

(a) From trauma to failure

Nonunions, hypertrophic or atrophic, and synovial pseudarthrosis are generally differentiated based on radiographic and histological appearance (Rodriguez-Merchan & Forriol 2004). There is no universally accepted definition of nonunion of a fracture (Marsh 1998). Numerous adverse mechanical and biological factors influence the development of nonunion: excess motion, a large interfragmentary gap, loss of blood supply, severe periosteal and soft-tissue trauma (McKibbin 1978; Whiteside 1978; Hulth 1989; Oni et al. 1989; Marsh et al. 1994; Canaã dell & Forriol 1997; An et al. 1999; Park et al. 1999; Landry 2000). General factors such as old age, cachexia and malnutrition, anticoagulants, anti-inflammatory agents, etc., may contribute to the establishment of nonunions, but are not the primary causes (Hulth 1989; Aaron 1996). Of the over six million fractures annually occurring in the USA, 5–10% develop into delayed or nonunions (Praemer et al. 1992, 1999; Einhorn 1995, 1998), costing large amounts of money to the society. Despite the large amount of information existing in the literature, additional research is still required to determine the exact mechanisms that lead to nonunions and, subsequently, the optimal therapeutic strategies for each type of nonunion. Animal experiments looking at new treatment strategies are arduous and lengthy, leaving much room for input from mathematical models.

(b) From experiments to patients

Classical treatment strategies for (hypertrophic) nonunions are aimed at restoring optimal mechanical circumstances to induce healing. Excess motion of the fracture fragments will be reduced using plates, external or intramedullary fixators (Patel et al. 2000; Hsu et al. 2005; Olson & Hahn 2006). Additionally, the application of appropriate dynamic stimulation of the fracture has been shown to enhance the healing process (Goodship et al. 1998; Kenwright & Gardner 1998).

Animal studies indicate a clear improvement of the combination of growth factors and stabilization over stabilization alone (reviewed in Heckman et al. 1991; Heckman 1999; Moucha & Einhorn 2005). Many studies also show that the administration of growth factors at fracture induction is sufficient to induce normal healing in environments that would otherwise lead to nonunion (Wildemann et al. 2004) and extensively reviewed in Dinopoulos & Giannoudis (2007)). Eckardt et al. (2005) demonstrated that, with a semi-rigid fixation in rabbits, both the use of growth factors (VEGFs) and autografts was able and sufficient to restart the healing process in the established tibial nonunions without changing the mechanical environment. Finally, Taguchi et al. (2008) demonstrated that, in a rat nonunion model, administration of growth factors (recombinant bone morphogenetic protein; rhBMP2) was sufficient to recapitulate the healing process without fixation of the mechanically unstable healing environment.

In current clinical practice, the combination of stabilization and biological intervention (whether it is the resection of the nonunion tissue, the use of autografts or the administration of growth factors) is the strategy that is most often followed after a nonunion has been established. Administration of growth factors (bone morphogenetic protein; BMP2) has been shown to lead to similar
results as the use of autografts, yet avoiding the risk for donor site morbidity and pain for the patient (Friedlaender & Horowitz 1992; Johnson & Urist 1998; Cook 1999; Friedlaender et al. 2001; Dimitriou et al. 2005; Giannoudis & Tzioupis 2005; Ronga et al. 2006; Zimmermann et al. 2006; reviews by Southwood et al. (2004) and Dinopoulos & Giannoudis (2007)).

(c) From models to therapies

Because of the complexity of the fracture healing process, mathematical models in the last decade have focused on the reparative phase. The process of bone remodelling does not only take place after fractures, but is a constantly ongoing, life-long process, actively restoring micro-damage and renewing the bone matrix throughout the entire body. The mathematical models of bone remodelling described in Kroll (2000), Pivonka et al. (2008), Ryser et al. (2009) and Adachi et al. (2010) are more focused on these latter applications of the process and are not discussed here.

The first models predicting the process of bone regeneration date from the late 1990s and relate cell fates to the magnitude of mechanical stimuli using simple diagrams (Prendergast et al. 1997; Carter et al. 1998; Claes & Heigele 1999). Since then, continuum models using PDEs and fuzzy logic have been formulated, looking at the influence of either biological stimuli or mechanical stimuli on the regeneration process, or a combination of both. An overview of these models can be found in Geris et al. (2009). Here, we focus on those models that have been applied in order to design optimal and new treatment strategies for specific cases of (impaired) bone regeneration: atrophic nonunions, distraction osteogenesis and overload/underload-induced impaired healing.

Geris et al. (2006, 2008, in press) have developed a mathematical model using PDEs to simulate normal and impaired bone regeneration cases. Atrophic nonunion experiments performed by Brownlow et al. (2001) and Reed et al. (2003) were simulated. Several key features, such as the absence of cartilage and bone and the presence of a well-vascularized fibrous tissue, were captured by the model. A number of treatment strategies were designed, involving the administration of mesenchymal stem cells and/or growth factors at different locations and different times after fracture induction. Application of the most successful treatment strategy, administration of mesenchymal stem cells centrally in the callus area, three weeks after fracture, resulted in the restoration of a normal-healing process, leading to successful healing 16 weeks after fracture (Geris et al. submitted). The mathematical model was further applied to investigate the observed unicortical healing and demonstrated the importance of the central location of cell administration in obtaining bicortical union.

In Peiffer et al. (submitted), the model by Geris et al. (2008) was extended to incorporate a discrete description of the vasculature. A deterministic algorithm for blood-vessel formation was incorporated describing the growth of blood vessels as a result of the movement of leading ECs (tip). Movement of the tip cells was guided by chemotaxis and haptotaxis. Furthermore, tip cells were assumed to move along the fibres present in the matrix. The orientation of the fibres was chosen randomly, thereby generating a stochastic component in the movement of the tip cells (Sun et al. 2005), and new branching and anastomosis algorithms for angiogenesis were implemented. A delayed healing
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Figure 3. (i) Simulated evolution of the tissue fractions and (ii) spatial distribution of blood vessels in the callus for impaired endochondral ossification and under various treatment strategies (Peiffer et al. submitted). (a) Normal healing. (b) Impaired endochondral ossification leading to delayed union. (c) Daily bolus injections from post-fracture week 3 onwards. (d) Injection of a slow release VEGF-carrier on post-fracture week 3. For the blood-vessel structure, only one-quarter of the callus domain is shown (because of assumed symmetry), as schematically represented in (a)ii.

situation, caused by a shortage in the available VEGF in the callus area was simulated (figure 3; Peiffer et al. submitted). Administration of additional VEGF in the callus area by means of daily bolus injections or a slow-release carrier was simulated, showing an enhanced healing response (in terms of both extracellular matrix and blood-vessel formation) for the latter treatment.

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Mathematical models developed by Reina-Romo et al. (2010) and Isaksson et al. (2007) focus on distraction osteogenesis (DO). This is a process in which a controlled displacement of a bone fragment is used to generate new bone in order to bridge critical size defects or lengthen short bones (Ilizarov 1989a; Richards et al. 1998). Within limits, the rate of bone formation during DO has been directly related to the distraction rate (Ilizarov 1989b; Li et al. 1999, 2000) and frequency (Aarnes et al. 2002). Both computational studies investigated the effect of several combinations of distraction rates and frequencies to arrive at a good clinical result in the shortest possible time frame. Furthermore, they looked into the upper and lower limits of the distraction rate leading to nonunion and premature clinical union, respectively.

Controlling the mechanical environment of the callus is a very important aspect of all clinical treatment strategies in bone regeneration. Andreykiv et al. (2007) and Bailón-Plaza & van der Meulen (2003) used their mathematical models of bone regeneration (PDEs) to investigate the effect of mechanically stimulating otherwise (underload-induced) non-healing fractures based on experimental work by Goodship et al. (1998). Andreykiv et al. (2007) investigated the limits of active mechanical loading, leading to optimal healing. Bailón-Plaza & van der Meulen (2003) focused on determining the period during which this active loading should start in order to stimulate normal healing to occur.

Starting from an overload-induced nonunion situation, Geris et al. looked into treatment strategies combining mechanical and biological factors. In the mathematical model, several factors were influenced by mechanical loading. Whereas, most processes that occur during fracture healing report an influence from mechanical loading in vitro, it might be reasonable to assume that not all of these interactions are equally important for the progression of a normal-healing process in vivo. Depending on the dominating biology–mechanics interactions that are implemented, the mechanobioregulatory model by Geris et al. (in press) demonstrates that different treatment strategies are necessary to obtain restoration of normal healing. Figure 4 (unpublished results) shows that when proliferation, osteogenic differentiation, bone-matrix production and endochondral replacement of chondrocytes are the biological processes that are most influenced by mechanical loading, both adequate stabilization of the fracture environment and administration of osteogenic growth factors are necessary to restart normal-healing processes. When mechanical loading mainly influences proliferation, osteogenic differentiation and bone-matrix production, administration of growth factors leads to a bony union three weeks after treatment, with or without removing the overload conditions. In contrast to these results, yet another combination of mechanics and biology (proliferation-dependent on mechanical loading) generated only an adequate healing response upon stabilization of the fracture area, irrespective of the administration of additional growth factors. For each of these simulation results (different treatment modalities required for successful healing), a reported clinical or experimental counterpart can be found. Under comparable conditions (in terms of environmental factors, loading and biology), the outcome of the healing process can be substantially different from one patient to the other. The need for patient-dependent modelling will be discussed in the next section.
5. Prospects

This review focused on models of wound healing and bone fracture healing that tried to design or optimize conditions and treatments leading to successful healing process. The modelling process is not a one-step process, but...
rather an iterative cycle of model definition, experimental validation, model optimization, experimental validation and so on (Kitano 2002). Care must be taken that the model predictions are interpreted in their appropriate context. Invariably, creating a mathematical model of biological phenomena constitutes a simplification of the processes under investigation (as do experimental models). This simplification can be the omission of certain biological or mechanical factors involved, the restriction to a single time or length scale, the implementation of phenomenological descriptions of highly complex processes, etc. Demarcation of the application area for each model is a necessary step in the assessment of its predictive powers.

One major issue with mathematical models is the parameter values that need to be defined and the sparseness of experimental data that is available to do so. Moreover, because of the simplifications that are introduced, some model parameters cannot be validated at all. While it would be better if we could quantitatively measure all parameters, we can still gain insights from a qualitative description/simulation of the physiological process. The significance of the models in this case is that, even though they are a qualitative representation of the physiological process, virtual experiments can still be constructed by exploring different healing responses and can still provide insights into how, for example, a wound might respond to the given treatment (Flegg et al. 2009). However, if the aim of the model is to provide a quantitative prediction of a physiological process, the model parameters need to be quantified. Moreover, if the quality of the data these parameters are derived from or based upon is poor, then so will be the model outcome as coined by the expression ‘garbage in, garbage out (GIGO)’. As the last decade has seen a surge of interest in the translational applications of mathematical modelling in biomedicine (e.g. the National Institute of Health roadmap, http://www.nihroadmap.nih.gov/researchteams/), it is important to design guidelines for the design, development and the use of these models to avoid the GIGO problem. Vodovotz et al. (2007) provide a first proposal for such guidelines based on their experience on modelling of acute illness.

In the 2009 Philosophical Transactions of the Royal Society A theme issue on the virtual physiological human, Geris et al. (2009) extensively discuss issues related to the corroboration and measurement of mechanical aspects of the bone regeneration models that are also mentioned in this review, including issues related to knowledge on mechanical properties of the tissues involved in healing and transduction of and response to mechanical signals. While these particular issues might pose severe limitations on the applicability of the models in clinical practice, they will not be reiterated here and the reader is referred to Geris et al. (2009, in press) for further discussion. To assess the dynamics of the biology incorporated in most models discussed in this review, continuous non-destructive data-collection methods are preferable. Molecular imaging techniques are becoming increasingly refined and constitute a suitable data-collection method, as they can monitor and quantify real-time biology, such as gene expression and cell migration in both space and time (Bar et al. 2003; Mayer-Kuckuk & Boskey 2006).

Despite these advances in data-collection techniques, model corroboration by comparison with animal experimental results is still a long way apart from patient-specific models for clinical use. Computational (image-based) techniques to obtain patient-specific structural models are successfully used in preoperative
planning environments (Clijmans et al. 2008). Mathematical models on the intracellular level (gene/protein networks) have not only led to unexpected insights in, for example, the regulation of cell apoptosis (Fussenegger et al. 2000; Hoffmann et al. 2002; Bentele et al. 2004; Janes et al. 2005; Bagci et al. 2006; Cheong et al. 2006), but have also demonstrated their usefulness in clinical decision-making and the stratification of patients for personalized therapy in cancer (Faratian et al. 2009). For the tissue/organ-level models discussed here, it is still unclear how we can relate patient information from blood or urine samples to local changes in biology (growth factors, cell behaviour) in the different tissues. Despite the current inability of the discussed models to capture patient specificity, they can be (and are being) used to model patient variability. Although this might not provide the clinician with a tool to identify the optimal strategy for a particular patient (in contrast to Capelli et al. (2010) and Perez del Palomar et al. (2010)), it can be used to investigate the disease risk or test the effect of a treatment strategy on an entire population, ‘clinical trials in silico’ (An 2004; Clermont et al. 2004; Kumar et al. 2008; Li et al. 2008). In purely continuum models, such as those of Flegg et al. (2009), Schugart et al. (2008) and Waugh & Sherratt (2007), and the models of fracture healing discussed above, several options exist to investigate the interpatient variability. A first tool is the use of sensitivity analyses. Parameter values describing, for example, proliferation rates, apoptosis rates, growth-factor consumption and production rates are typically different from one patient to the next. Isaksson et al. (2008, 2009) performed extensive multi-parameter sensitivity analyses based on the design of experiments concept to investigate the influence of cell process-related parameter values, as well as extracellular-matrix material properties on the simulated fracture healing process. This highly systematic approach allows to determine the most influential parameters and could contribute the most to interpatient variability.

A second way to investigate interpatient variability in continuum mechanobioregulatory models is by investigating the nature of the biology–mechanics interaction. A genetic component (and therefore an inherent subject-specificity) in the reaction of bone to mechanical loading has been demonstrated (Li et al. 2005; Srivastava et al. 2005; Robling et al. 2007). This genetic influence on bone response has not yet been reported in a bone-regeneration context. However, it seems reasonable to assume that there will be a genetic effect, if only for the fact that some of the cells and processes involved in bone adaptation are also involved in bone regeneration. In (modelling) practice, this boils down to making various biological processes less or more dependent on mechanical loading. The simulations have shown that, for different combinations of mechanics and biology, specific treatment strategies can be applied which will lead to the resumption of the healing process only in that particular case. Simulated treatment strategies involving both biological and mechanical interventions led to resumption of healing in all simulated cases. Therefore, as long as the exact failure mechanism is not known for a specific patient, the current clinical practice of intervening in both mechanics and biology of the bone fracture healing process is at the moment, although not always necessary, the optimal treatment strategy to maximize the chance for success.

Hybrid models combine elements of discrete (cell or agent based) and continuum modelling in one and the same modelling framework. In the models presented by Anderson & Chaplain (1998) and Peiffer et al. (submitted),
blood-vessel formation is described in a discrete way, while the spatio-temporal evolution of cell concentration and extracellular-matrix densities is described by means of PDEs. As the movement of the blood-vessel-tip cells has a stochastic component, running such models various times for the same conditions will lead to slightly different results. As the stochasticity in these models is limited to the movement of the tip cells, the spread of the results is quite small (<3% in Peiffer et al. submitted). Pure agent-based models, such as the one developed by Walker et al. (2004b) and Mi et al. (2007), have the intrinsic ability to account for the stochastic nature of all of the biological processes incorporated in the model. Running consecutive numerical experiments will not only lead to the prediction of an average healing response, but also to the prediction of the variation on this average response, much the same as observed in a patient population. The importance of this ability to assess output variability becomes more pronounced when looking at the model predictions for clinical treatment strategies. Mi et al. (2007) investigated various scenarios with their model that could lead to the formation of non-healing diabetic foot ulcers, based on the hypotheses that have been put forward in the literature. Subsequently, they simulated a routine therapy, being debridement or the physical removal of dead or dying tissue. On average, they observed a good healing response for all debridements, irrespective of the time point they were carried out on. However, taking into account the variability, they observed that only for debridement carried out at the earlier time point, the healing response was significant, corresponding to the current clinical observations. This is an important—and in light of the applicability of models in clinical practice also an essential—advantage of the incorporation of statistical techniques into simulations of treatment strategies. Obviously, since a model is a simplification of reality and does not contain a description of all processes involved as discussed above, some sources of variability remain unaccounted for in the model. Furthermore, as the nature of the statistical behaviour might constantly be changing, even the most precise statistical description might introduce some essential error (Edwards 1960; Massoud et al. 1998).

Given the strong points of the models discussed in this review, as well as their limitations, it seems reasonable to conclude that these models are not ready (yet) to be used by the clinicians as a tool in their daily practice. However, these models can be (and are being) used as a research tool during the experimental research phase. In silico tests can be executed to investigate various hypotheses on the aetiology and treatment of pathologies. Using them as a first screening tool will help to design and plan experimental research in a more intelligent way. While experimental trials remain necessary to validate the in silico predictions, models can help to focus on which experiments need to be run and which do not, potentially leading to significant cost savings.

### 6. Conclusion

In this review, an overview of the wound and bone fracture healing process, the possible causes for failure known to date and the currently used experimental and clinical models and strategies used to treat impaired healing cases are given. Because of the complex nature of the problem at hand, mathematical models can contribute (i) by investigating in silico potential failure mechanisms and
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(ii) by designing new treatment strategies. Examples of potential contributions are shown. An essential aspect of the applicability of these models in clinical practice is their ability to capture patient-specificity and interpatient variability. Depending on the type of model (continuum versus discrete), various options exist to achieve this goal.

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