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Computational modelling of bone regeneration using a three-dimensional lattice approach

A thesis submitted to the University of Dublin in partial fulfilment of the
requirements for the degree of

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Trinity College Dublin

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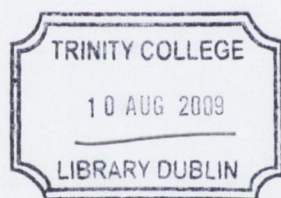
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Thesis

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Publications and presentations arising from this study

Byrne, D.P., Lacroix, D., Kelly, D.J., Prendergast, P.J., 2008. Modelling cell dispersal in the fracture callus, In: Proceedings of the 16th Conference of the European Society of Biomechanics, Lucerne, Switzerland. *Journal of Biomechanics* 41:S464.

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Checa, S., Byrne, D.P., Lacroix, D., Kelly, D.J., Prendergast, P.J., 2008. Comparing diffusion and random-walk methods to model cell dispersal in mechano-regulation algorithms, In: 8th *International Symposium on Computer Methods in Biomechanics and Biomedical Engineering*, Porto, Portugal.

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Byrne, D.P., Kelly, D.J., Prendergast, P.J., 2007. Simulations of Bone Regeneration as a function of Dissolution rate in a regular structured scaffold, In: *Proceedings of the 13th Annual conference of the section of Bioengineering of the Royal Academy of Medicine in Ireland*, Fermanagh, p 75.

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Byrne, D.P., Kelly, D.J., Prendergast, P.J., 2006. Effect of Porosity on Bone Regeneration in Regular structured scaffolds, In: *Proceedings of the 12th Annual conference of the section of Bioengineering of the Royal Academy of Medicine in Ireland*, Galway, p 82.

Abstract

Mechano-regulatory theories are indispensable for developing and understanding how mechanical forces modulate morphological and structural fitness of skeletal tissues. These theories have been incorporated into computational simulations of tissue regeneration and provide considerable predictive power, especially in bone tissue engineering. The primary objective of this thesis was to develop a method to include cellular processes in three-dimensional mechano-regulation computational models – namely proliferation, migration, cellular apoptosis and differentiation. In the first part of this work, tissue-differentiation and bone regeneration is simulated in a regular structured scaffold to investigate varying scaffold parameters on bone formation; such as porosity, Young's modulus and dissolution rate – and this is done under low, high and ramp loading conditions. The simulations predicted that all three design variables have a critical effect on the amount of bone regeneration. In general, it was found that scaffolds conducive to osteogenesis in the initial stages of healing allow for the development of a system that will be able to withstand the applied forces as time progresses. It was therefore concluded that scaffolds must be optimised to suit site-specific loading requirements. In the second part of this work, a fracture healing model of bone regeneration was investigated. The main phases observed during healing were predicted, and the temporal changes in interfragmentary strain and bending stiffness were corroborated by comparison to experimental data and clinical results. For the first time bone healing was simulated beyond the reparative phase by modelling the transition of woven bone into lamellar bone. Bone healing was also found to be sensitive to the permeability values of woven bone.

In summary, this work has further established the potential of mechanobiological computational models in developing our knowledge of cell and tissue differentiation processes during bone healing and can be used to assist optimisation of implant design and investigation of fracture treatments.

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Nomenclature

Roman letters

<i>E</i>	Young's modulus (MPa)
<i>K</i>	Permeability (m^4/Ns)
<i>S</i>	Stimulus

Greek letters

σ	maximum principle stress
γ	octahedral shear strain
v	interstitial fluid flow

Acronyms

ECM	Extracellular matrix
MSC	Mesenchymal stem cell
GAG	Glycosaminoglycan
PLA	Polyactic acid
HA	Hydroxyapatite
β -TCP	Tri-calcium phosphate
RP	Rapid prototype
SFF	Solid free form
FCC	Face cubic centred
BMU	Basic multicellular unit
IFS	Interfragmentary strain
2D	Two-dimensional
3D	Three-dimensional

Chapter 1

Introduction

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1.1 Background

Bone exhibits a lifelong capacity to reform after injury, and it is continually being remodelled to maintain healthy tissue. However the ability of bone to perform its structural functions often becomes impaired due to disease or trauma. This sets about a complex physiological process of bone healing which, in optimal conditions, can reconstitute the bone almost identically to its original shape and function. Even slight perturbations in bone regeneration can have profound consequences, such as non-unions of fractures or extended bone defects following trauma or cancer resection. The main cause for unsuccessful bone healing is the rapid formation of soft connective tissue which may disturb or totally prevent the bone regeneration process. In such circumstances surgical intervention is required to help re-establish the structural integrity of the injured bone.

The mechanical environment is generally accepted as having a major influence on bone regeneration (McKibbin, 1978). Mechanical stimulation can induce bone regeneration or alter its biological pathway. Controlled micromotion at the site of a fracture has been shown to enhance the healing process (Goodship and Kenwright, 1985); whereas excessive movement may cause the opposite effect (Einhorn, 1995). As yet the optimal parameters have not been established. Therefore current challenges involve determining the appropriate mechanical stimulants to promote bone regeneration. Towards this effort, studies analysing the interaction between mechanical signals and biological processes in cells and tissues has been proposed. According to Roesler (1987) such studies date back to the work of Wilhelm Roux in the late 19th century, who suggested that cells react to a mechanical stimulus by locally establishing the appropriate structures. Nowadays such research is often called “mechanobiology”. Understanding the relationship between mechanics and biology could help develop new methods for the stimulation of bone regeneration by mechanical means, and enhance current methods of treatment.

1.2 Computational mechanobiology

Mechanobiology has been defined as “how mechanical forces modulate morphological and structural fitness of the skeletal tissues – bone, cartilage, ligament and tendon” (van der Meulen and Huiskes, 2002). The main aim of mechanobiology

is to determine how loads create mechanical stimuli within tissues, how the cells sense these stimuli and emit signals that are translated into the cascade of biochemical reactions that stimulate cell expression and cell or tissue differentiation (van der Meulen and Huiskes, 2002). The potential of experimental science to answer questions about-mechano-regulation has been limited by the complexity of biochemical factors governing cell/matrix interactions (Lacroix, 2000). In this regard numerical models are invaluable to view complex systems, and can be used to improve the understanding of biological and biomedical problems using a variety of mathematical frameworks. In recent years, computational models have been developed and used together with *in vivo* and *in vitro* experiments to gain an insight to the possible relationships between mechanics and biology.

Theories on the relationship between mechanics and biology were originally proposed in relation to fracture healing. These theories later evolved into 'algorithms'; a finite set of rules that gives a sequence of operations for solving a specific problem. Mechano-regulation algorithms have been proposed to encapsulate the rules that govern the effects of mechanical loading on cells and tissues. In general these algorithms use combinations of strain invariants, hydrostatic pressure or fluid velocity as biophysical stimuli to predict cell differentiation. Computational studies or 'simulations' to predict tissue regeneration combine finite element modelling, (to compute the mechanical stimuli in the tissues), and mechano-regulation algorithms (to adapt the tissue material properties in iterative computational schemes), see Figure 1-1. In particular researchers have considered predictive methods for differentiation, growth and adaptation and maintenance of tissues. As yet, however, we may question the predictive power of these methods.

To date numerous aspects of tissue regeneration have been simulated using these techniques, especially in respect to bone; such as fracture healing, distraction osteogenesis, bone remodelling and adaptation, tissue differentiation at bone-implant interfaces, and bone ingrowth into porous coated implants and scaffolds. Osteochondral defect repair and cardiovascular tissue repair have also been simulated. To reach their full potential these models must closely mimic the clinical situation and require corroboration against experimental data. One key aspect in these simulations is the modelling of the cellular processes. It is clear that the mechanical environment regulates tissue differentiation; however it is only the cells

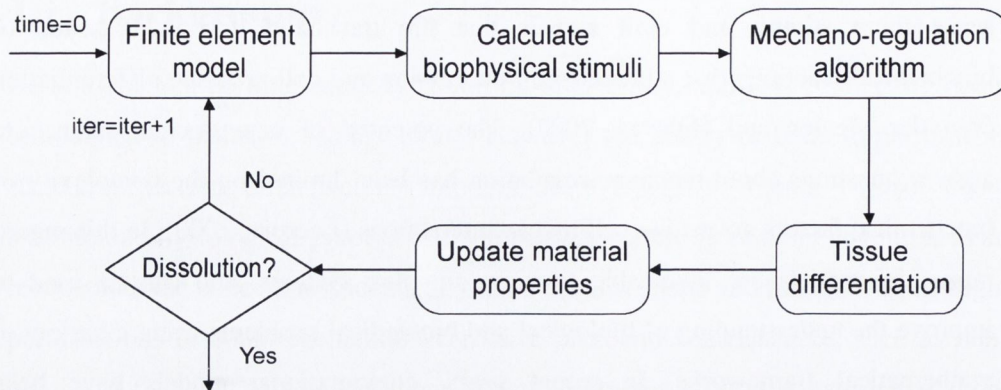


Figure 1-1: Schematic representation of a basic computational simulation, employing a mechano-regulation algorithm

that have the sensing and signalling machinery to respond to the local mechanical forces. Cell migration, proliferation and apoptosis are also believed to be modulated by the mechanical environment; therefore it is important to account for these processes in the computational simulations.

Lacroix *et al.* (2002(a)) introduced a diffusion equation to account for combined migration, proliferation and differentiation of cells. This initial study revealed that stem cell access to the regenerating region has a considerable effect on the healing pattern and rate of healing. Until recently many simulations used diffusion to model cellular activity within the regenerating tissue (Geris *et al.*, 2004; Andreykiv *et al.*, 2005; Boccaccio *et al.*, 2008). However, this approach implicitly assumes that cells attempt to achieve a homogenous population density within the area of analysis. Several authors have since attempted to manipulate/modify the diffusion equation using partial differential equations to more accurately define cellular activity and include apoptosis (Kelly and Prendergast, 2005; Kelly and Prendergast, 2006; Andreykiv *et al.*, 2007; Isaksson, 2007). In these studies, diffusion remains the central method of modelling cellular processes; however despite its convenience to model, diffusion is not the mechanism of cell dispersal; instead cells disperse by crawling or proliferation or are transported in a moving fluid. The outline of these cell-based mechano-regulation simulations is illustrated in Figure 1-2.

In an attempt to better replicate cellular processes Pérez and Prendergast proposed a 'random-walk' model to mimic cell proliferation and migration (Pérez

and Prendergast, 2007). Based on the stochastic nature of cell dispersal, this two-dimensional study also included anisotropic and directed movements. This mechanistic approach allows for the simultaneous dispersal of several cell populations, the explicit modelling of cell proliferation and, more importantly, the possibility of implementing experimentally motivated cell-based rules. Clearly solutions of this kind may not be attainable to any known set of partial differential equation.

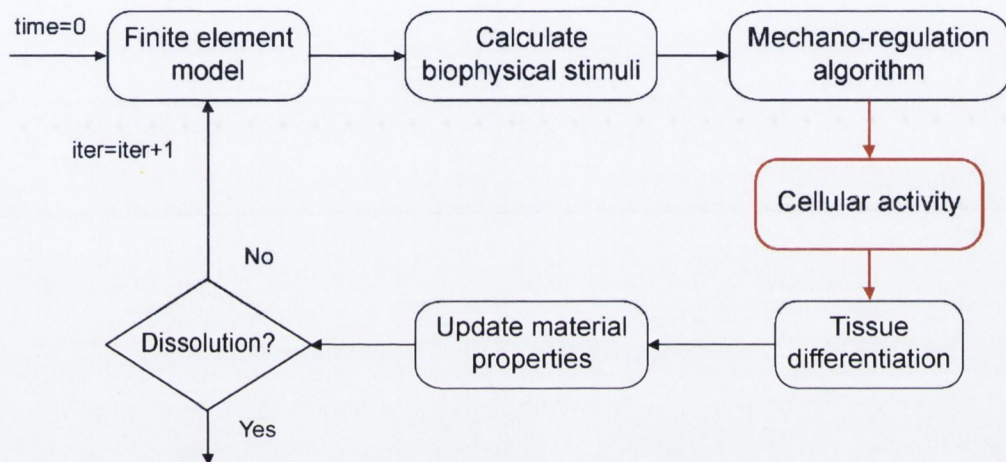


Figure 1-2: Flow chart of a mechano-regulation simulation including cellular activity

1.3 Objective of the thesis

Mechano-regulatory models are indispensable for developing and understanding how mechanical forces modulate morphological and structural fitness of skeletal tissues, but they have not yet been developed to the degree that allows their application to practical problems in bioengineering, or in clinical applications. With sufficient validation they could be used in pre-clinical testing to predict tissue regeneration in a variety of clinical settings, such as defect healing or tissue regeneration around implants. Ideally these simulations should be able to accurately predict biological responses without undue manipulation, or ‘tuning’, of the model parameters; thus only the initial geometry and material properties would be required to simulate the time-course of tissue regeneration in any given clinical application.

The aim of this work is to develop a three-dimensional lattice-modelling approach to account for cellular processes in tissue differentiation, and predict bone regeneration in two separate studies; (i) within a regular structured scaffold and (ii) in a model of fracture healing. If the use of lattice modelling could be corroborated, it could lead to a new systematic approach for computational simulations in both fracture healing and tissue engineering. Therefore it is the author's thesis that simulations of bone regeneration are best achieved using a lattice in which cell activity is modelled, because it can account for processes at a cellular level.

Chapter 2

Literature review

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2.1 Bone structure and composition

Bone tissue is a specialised form of mineralised connective tissue, composed of several cavities ranging in size and shape, containing blood vessels and bone marrow. Depending on the size, shape and distribution of these cavities, bone tissue can be categorised into cortical/compact and cancellous/trabecular bone, see Figure 2-1. The differences between these tissue types are both structural and functional although both have the same matrix composition. Cortical bone is a dense, solid mass with only microscopic channels. Approximately 80% of the skeletal mass in the adult human skeleton is cortical bone, which forms the outer wall of all bones and is largely responsible for the supportive and protective function of the skeleton (Jee, 2001). The bulk of cortical bone is in the shafts of long bones of the appendicular skeleton. The remaining 20% of the bone mass is composed of cancellous bone, which is highly porous and is found in the inner parts of flat bones, the end of long bones and in the cuboidal bones (e.g., vertebrae) (Yaszemski *et al.*, 1996). Generally cancellous bone is located where bones are heavily stressed or where multiaxial stress states exist. The bone matrix is in the form of plates, or struts called trabeculae. The orientation of the trabeculae is variable: in most locations they are anisotropic, but according to Wolff's Law (1892), the architecture of the cancellous bone is continuously adapted throughout life in such a manner that the trabeculae are oriented along the major stress lines, so that it is optimised to support the loads it bears.

Cortical and cancellous bone can be further divided into woven/primary and lamellar/secondary bone. Woven bone is a quickly formed, poorly organised tissue in which collagen fibres are randomly arranged (Martin *et al.*, 1998). This is a provisional material that is eventually resorbed and replaced by lamellar bone through the process of remodelling (Jee, 2001). Lamellar bone is normally less active and consists of an anisotropic matrix of mineral crystals (hydroxyapatite), and densely packed collagen fibres around a central canal (the Haversian canal) containing blood or sometimes nerves. The Haversian system or osteon consists of concentric layers, or lamellae, of compact bone tissue, surrounding osteocytes and canaliculi, this system is separated from other osteons with a cement line lining the

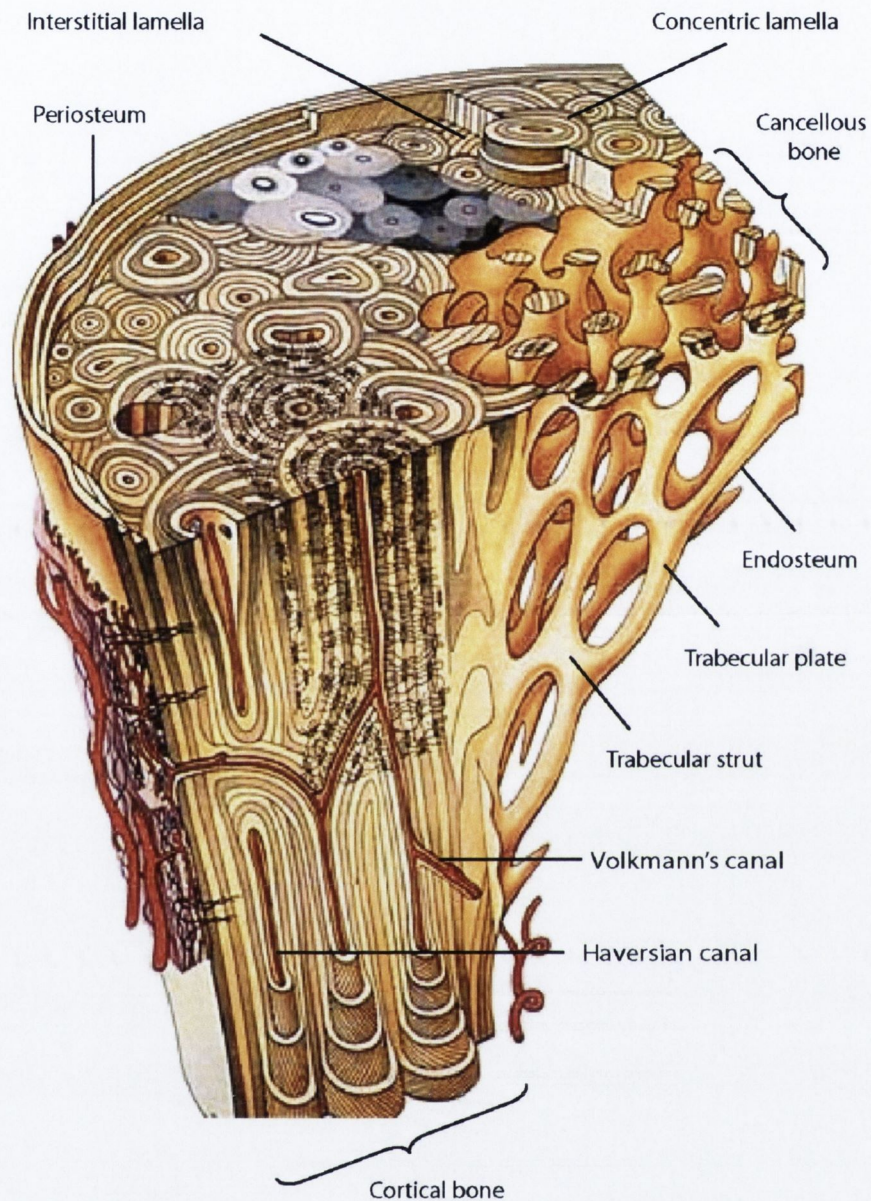


Figure 2-1: Schematic illustration of cortical and cancellous bone tissue in a cross section of a long bone diaphysis (Gray, 1973)

outside of the osteon. The Haversian canals have a network of transversely oriented canals, called Volkmann's canals, which connect the periosteal, and endosteal surfaces and the bone marrow enabling regulation of cell and bone metabolism.

The nonmineralised spaces within bone contain marrow, a tissue composed of blood vessels, nerves, and various types of cells (Martin *et al.*, 1998). The chief function of the marrow is to generate the principal cells present in blood. The

endosteum lines the marrow cavity. This layer covers the trabeculae of cancellous bone and lines the inner surfaces of the central canals. It is also active during bone growth, repair and remodelling. The superficial layer of cortical bone is covered by the periosteum. The periosteum isolates the bone from surrounding tissue, and provides a route for circulatory and nervous supply; it also actively participates in bone growth and repair (Martini and Nath, 2008).

There are four primary cell types within the extracellular matrix of bone tissue, namely osteoprogenitor cells, osteoblasts, osteoclasts, and osteocytes, see Figure 2-2. Together these four cell types are responsible for the delicate balance of bone remodelling and repair, that exists in healthy bone tissue. Osteoprogenitor cells are a small group of mesenchymal cells that are located within the endosteum, and their primary function is to maintain the osteoblast population by producing daughter cells that differentiate into osteoblasts (Glesson, 2006). Osteoblasts are bone forming cells that synthesise and secrete the unmineralised bone matrix (osteoid), or ground substances of bone. These cells play a crucial role in the mineralisation process by elevating local calcium phosphate levels and promoting the deposition of calcium salts within the organic matrix. Once an osteoblast is surrounded by osteoid, the matrix mineralises and they differentiate further to become osteocytes, which reside in enclosures known as lacunae (a pocket sandwiched between layers of matrix).

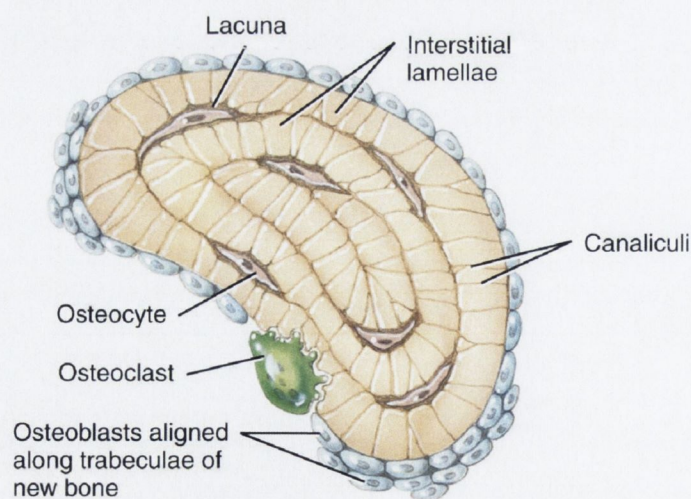


Figure 2-2: Schematic of a trabecular strut cross-section, illustrating bone lacunae and their corresponding canalicular structure (Martini and Nath, 2008)

Although the cells are embedded in these lacunae they are still connected to one another via canaliculi, which allow for the transfer of nutrients, hormones, wastes and mechanical and electrical stimuli. Osteoclasts are large multinucleated cells which are responsible for the removal of bone matrix. They secrete acids and proteolytic enzymes which dissolve bone matrix in a process known as osteolysis or bone resorption. The balance between the bone forming cells (osteoblasts) and the bone resorbing cells (osteoclasts) are very important in maintaining the mechanical integrity of bone.

2.2 Bone formation, growth and remodelling

Bone is formed by two distinct ossification processes, intramembranous and endochondral. Intramembranous ossification involves the formation of woven bone directly from condensed mesenchymal tissue without a cartilaginous framework, while endochondral ossification involves a cartilaginous intermediate formed from mesenchymal tissue, which is later ossified to form new bone (Yaszemski *et al.*, 1996).

Intramembranous ossification forms most of the flat bones (skull, mandible and maxilla) and also contributes to the growth of short bones and the thickening of long bones. In this process, mesenchymal stem cells proliferate and condense around a profuse capillary network, becoming osteoblasts. These osteoblasts begin to secrete osteoid matrix containing collagenous tissue, proteoglycans and other growth factors (Glesson, 2006). As the osteoid matrix becomes calcified some osteoblasts are trapped inside bony pockets and differentiate into osteocytes. During this process, blood vessels are entrapped supplying the embedded osteocytes with nutrients. Thus a network of bone is formed, the meshes of which contain blood vessels, and a delicate connective tissue crowded with osteoblasts. The ossification process proceeds by an overall thickening of the trabeculae through the addition of fresh layers of bone formed by the osteoblasts on their surfaces (Gray, 1973). At this point the mineralising tissue can either remain as an interconnected trabecular network or become the more mineralised cortical tissue through subsequent remodelling around trapped blood vessels. Finally, as the network of trabeculae is established, the primitive mesenchymal tissue among these trabecular branches differentiates into the myeloid (hemopoietic) tissue of the bone marrow (Martin and Seeman, 2003). Those

regions of the mesenchymal tissues that remain uncalcified differentiate into the periosteum and endosteum of developing intramembranous bone.

Most of the long and short bones of the body develop by endochondral ossification. In the first step of this process mesenchymal cells differentiate into cartilage cells (chondrocytes), resulting in a structural matrix for bone development. Growth of the cartilage model occurs mainly by perichondral apposition and interstitial chondrocytic mitosis to form an elongated dumb-bell shape mass of cartilage, consisting of a shaft (diaphysis) and future articular portions (epiphysis) surround by perichondrium (Martin and Seeman, 2003). Over time the cartilage matrix begins to disintegrate and the area becomes vascularised thus developing osteogenic potential. In a study of mechanical forces on embryonic morphogenesis, Nowlan *et al.* (2008) hypothesised that stresses due to muscle contractions play an important role in cartilage and bone growth and differentiation. They found that in the stages just prior to mineralisation, cycles of stimulus (stress, strain and fluid velocity) occur in the cartilaginous tissues as a promoter for ossification, where the cyclic nature of the stimulus is due to muscle contractions. The stresses tended to focus on the mid-shaft region where osteogenesis begins, indicating that stresses induced by muscle contractions may provoke mineralisation.

Following this, osteoblasts penetrate the cartilage and replace it with cancellous bone, forming a primary ossification centre. Ossification continues from this centre toward the ends of the bones. After cancellous bone is formed in the diaphysis, osteoclasts break down the newly formed bone to open up the medullary cavity. The next stage consists of the prolongation of the developing bone through the growth of the epiphyses. Ossification of the epiphyses is similar to that in the diaphysis; except that the cancellous bone is retained instead of being broken down to form the medullary cavity. A region of hyaline cartilage remains over the surface of the epiphysis as the articular cartilage and another area of cartilage remains between the epiphysis and diaphysis. This is the epiphyseal plate or growth region.

The adult skeleton is continuously adapting and renewing its structure throughout life. The development of bone is achieved by growth, modelling and remodelling. Growth and modelling are the two dominant processes that are present in normal growing individuals. The combination of these two processes is often referred to as adaptation. Growth increases the length and diameter of long bones (both internally and externally). This baseline architecture is modified by the

modelling process, which sculpts the bone's size, shape, and curvature to optimally sustain the mechanical loads typically borne by that bone. Modelling adjusts bone architecture and mass via modelling drifts, which add bone to the surfaces and remove (resorb) it from others. This increases or decreases the cross-sectional area of bone. In the normal developing skeleton, growth and modelling result in the production of organized parallel sheets of primary lamellar bone. Once skeletal maturity is reached, modelling is reduced to a low level compared to that which occurred during development.

The renewal process of bone is accomplished through bone remodelling. This process is carried out by a group of bone cells in what is known as a Basic Multicellular Unit (BMU). A BMU consists of osteoclasts and osteoblasts working in a coupled action of bone resorption and deposition (Frost, 1986). The organisation of the BMUs in cortical and cancellous bone differs in regards to structure. In cortical bone the BMU forms a cylindrical canal. Osteoclasts dig a circular tunnel (cutting cone) at front of the channel, dissolving the existing material. The rear of the tunnel is made up of osteoblasts (closing cone) which lay down new bone matrix. The remodelling process in cancellous bone generally takes place on the surface of the trabeculae. The cancellous BMU can be regarded as half a cortical BMU, digging a trench rather than a tunnel, see Figure 2-3. It has been estimated that approximately one million BMUs can be operating at any given moment within an average skeleton, resulting in a total skeletal renewal occurring, on average, every two years in children and every seven to ten years in adults.

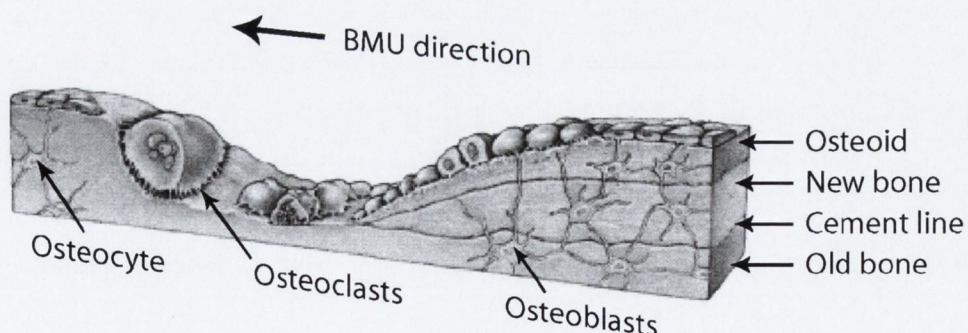


Figure 2-3: Schematic of Bone Modelling Unit (BMU) on the surface of a trabecular strut.

Adapted from Jee, 2001

2.3 Bone regeneration

Bone is a unique tissue that has the dynamic potential to completely repair itself after damage, without any scar-tissue formation – no other tissue displays this attribute. Similar to bone development, the regeneration process is accomplished by the proliferation and differentiation of pluripotent mesenchymal stem cells. Several factors influence the healing process, such as genetic, cellular and biochemical factors, age, the type of fracture, interfragmentary motion and fracture geometry (Goodship *et al.*, 1993; Guilak *et al.*, 2003). Another key issue which has become an area of much interest is the mechanical loading, which determines the biophysical microenvironment local to the differentiating cells. In order to increase our understanding of how the mechanical environment influences the biological response it is important to look at how the bone regeneration process takes place. Fracture healing is the most common condition in which bone regenerates to its original state and function, and will be discussed below. There are also specific clinical settings in which large segments of bone must be resected to treat tumours, disease or other congenital defects. Thus, although most bony injuries heal without problems, there are several conditions under which enhancement of the repair process would be of great benefit to ensure the rapid restoration of skeletal function. It is in these settings that tissue-engineering of bone holds great potential.

2.3.1 Bone repair

Bone fractures are relatively common and usually occur when an accidental overload substantially exceeds the normal range of loading to which the bone has adapted during its growth and development (Martin *et al.*, 1998). Bone fracture repair occurs either by primary fracture healing, where the fracture gap ossifies via intramembranous bone formation without an external callus, or by secondary fracture healing, where a multistage process of tissue regeneration stabilises the bone with an external callus and repairs the fracture via endochondral ossification (Prendergast and van der Meulen, 2001). The majority of fractures heal by secondary healing in a sequence of three overlapping biological phases - the inflammatory, reparative and remodelling phases; see Figure 2-4. The recovery time for a fracture depends primarily on its severity and anatomical site.

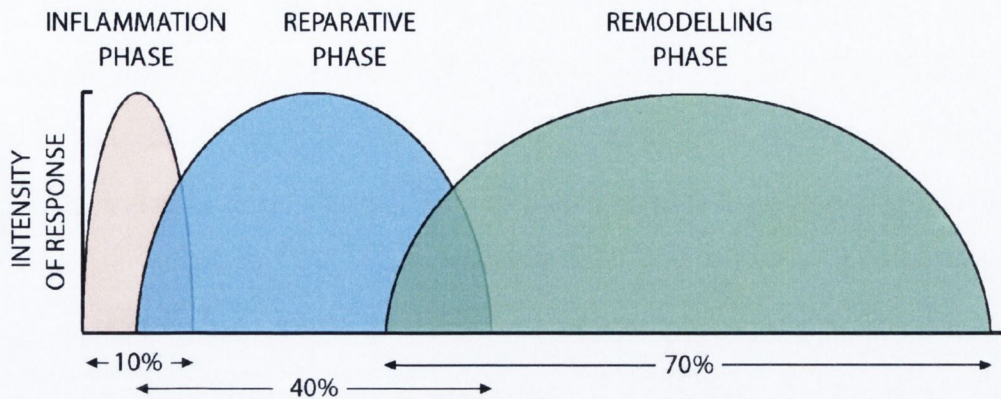


Figure 2-4: An approximation of the relative amounts of time devoted to the inflammation, reparative and remodelling phases in bone healing process. Adapted from Cruess and Dumont, 1975

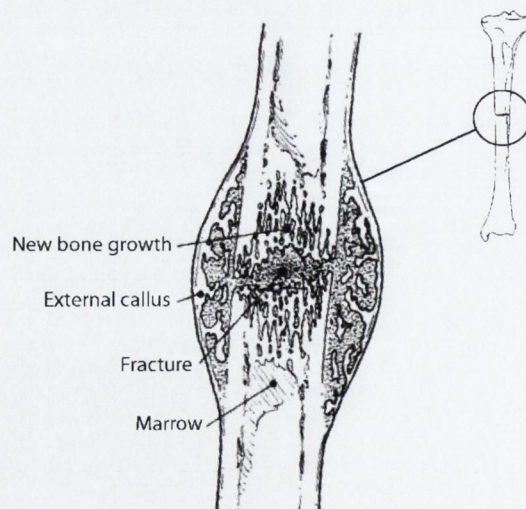
2.3.1.1 Inflammation phase

In the inflammation phase, haematoma and haemorrhage formation results from the disruption of the periosteal and endosteal blood vessels at the site of injury. The disruption of the vasculature leads to necrosis around the trauma site, and the presence of so much necrotic material elicits an immediate and intense acute inflammatory response. The haematoma releases a large number of signalling molecules, including inflammatory cytokines and growth factors, which appear to regulate the initiation of fracture healing and the associated cellular response (Frost, 1986; Einhorn, 1998). Macrophages, leukocytes and other inflammatory cells invade the area. Pluripotent progenitor cells, called mesenchymal stem cells (see section 2.5), also migrate towards the fracture site. Mesenchymal stem cells originate from the periosteum, endosteum, bone marrow and possibly the vasculature of the muscle-tissue surrounding the haematoma (Postacchini *et al.*, 1995; Einhorn, 1998; Gerstenfeld *et al.*, 2003). Proliferation of these cells along with inflammatory cells leads to a mass of granulation tissue.

2.3.1.2 Reparative phase

A key stage in skeletal regeneration is the differentiation of pluripotential mesenchymal cells of the early granulation tissue into cells that form cartilage, fibrocartilage, fibrous tissue or bone (Carter and Beaupré, 2001). This differentiation occurs under strong mechanobiological regulation which will be discussed in section

2.6. Differentiation of mesenchymal stem cells into chondrocytes and osteocytes leads to the generation of the reparative callus. An external hard (periosteal) callus forms along the periphery of the fracture site, while an internal (medullary) callus forms in the centre; see Figure 2-5. Intramembranous ossification occurs at the periphery, beneath the damaged periosteum. This rapidly formed woven bone creates a mineralised hard callus (Prendergast and van der Meulen, 2001). Cells in the granulation tissue continue to generate chondrocytes and hyaline cartilage. This forms the fracture callus in which the fracture gap is eventually bridged by hyaline cartilage and woven bone. While this stage will restore the bone to some of its original strength, it is only a temporary fix. The next stage of the reparative process involves replacing the hyaline cartilage and woven bone with lamellar bone via endochondral ossification. Mineralisation of the cartilage involves a mechanism similar to long bone growth at the growth plate (Einhorn, 1998). The formation of endochondral bone is dependent on the existence of blood capillaries, which originate from the periosteal callus. Angiogenesis occurs subsequently to osteochondral ossification, leading to erosion of mineralised cartilage and the formation of new lamellar bone on the surface as trabecular bone. Eventually all of the original lamellar bone will be replaced by trabecular bone, restoring most of the bone's original strength. When this occurs bony union has been achieved, completing the reparative phase.



*Figure 2-5: Schematic of reparative stage of fracture healing. Adapted from
www.hughston.com*

2.3.1.3 Remodelling phase

Once bony union has been achieved the broken bone is approximately as strong as the intact bone, but it often has greater mass than the original bone, and is therefore less mechanically efficient (Martin *et al.*, 1998). Wolff (1892) recognised that the architecture of the skeletal system corresponds to the mechanical need of the system, therefore remodelling about the fracture takes place for a prolonged period of time until the original contour and internal structure of the bone has been restored. Via the remodelling process the medullary and periosteal calluses are removed, and the remaining woven bone or calcified cartilage is replaced by secondary lamellar bone (cortical or trabecular, as the site indicates).

2.3.1.4 Fracture fixation

In order for bone healing to occur the regenerating tissue at the fracture site needs mechanical stimulation. The mechanical forces transmitted to the callus bring about strain, interstitial fluid flow and fluid pressure, release biochemical mediators, and provide greater vascular response (Lacroix, 2000). Excessive motion in highly unstable fractures does not allow soft-tissue healing and periosteal revascularisation, while total inhibition of motion also prevents adequate healing. The loading at the site of fracture is, in turn, determined by the method of fixation and the physical activity of the patient. Depending on the severity of fracture fixation methods either involve nonrigid immobilisation using plaster casts, or rigid fracture immobilisation with surgical implants such as intramedullary nails, screws and plates or external fixators (Wraight and Scammell, 2006). The majority of fractures are stabilised with plaster casts; however complex fractures, fractures through joint surfaces and fractures with extensive soft-tissue damage require rigid fixation. In chapter 5 tissue regeneration in a fractured tibia with an external fixator is simulated using a mechano-regulation algorithm.

External fixators tend to be used in more severe fractures, especially open fractures with associated lacerations and soft-tissue damage. An external fixator is a system that allows the stabilisation of fragments away from the open wound with the aid of percutaneous screws or wires that are connected to one or more bars on the outside of the skin, see Figure 2-6(a). The rigidity of this fixation is mainly determined by the stiffness of the fixator construct and the quality of the connection between the screws and the bone. The stiffness is described by the interfragmentary

movement occurring under external loads. As the callus stiffens the fixator allows a greater proportion of the load to pass via the bone, see Figure 2-6(b). The load at the external fixator decreases, which leads to decreasing deformation of the fixator frame. During postoperative periods the fixator can be often adjusted to improve skeletal alignment or to apply compressive or distractive forces. Richardson *et al.* (1994) monitored the bending stiffness of the tibia as healing occurs, see Figure 2-7. They concluded that a bending stiffness of 15 Nm/degree indicates successful healing.

Occasionally the healing process encounters complications and a fracture fails to heal, resulting in a non-union. A fracture that does not heal in the expected length of time but which is still progressing is called a delayed union (Martin *et al.*, 1998). Non-unions happen when the bone lacks adequate blood supply and stability. Factors that can increase the risk of non-union include: smoking, old age, severe anaemia, diabetes, and infections.

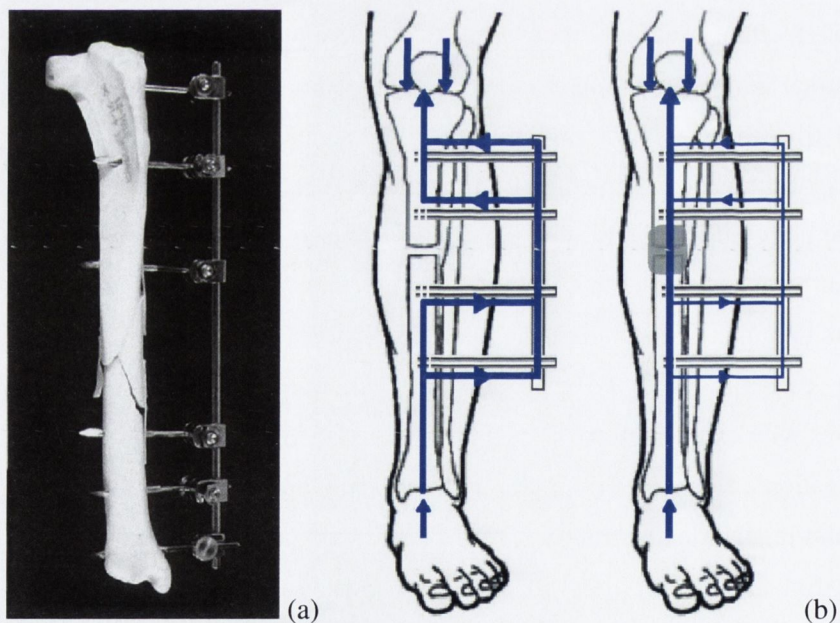


Figure 2-6: (a) External fixator used to increase the stability of the fractured tibia and (b) the load sharing between external fixator and repair tissue in the fracture at the beginning (left) and with loadable callus formation at a later stage of healing (Mow and Huiskes, 2005)

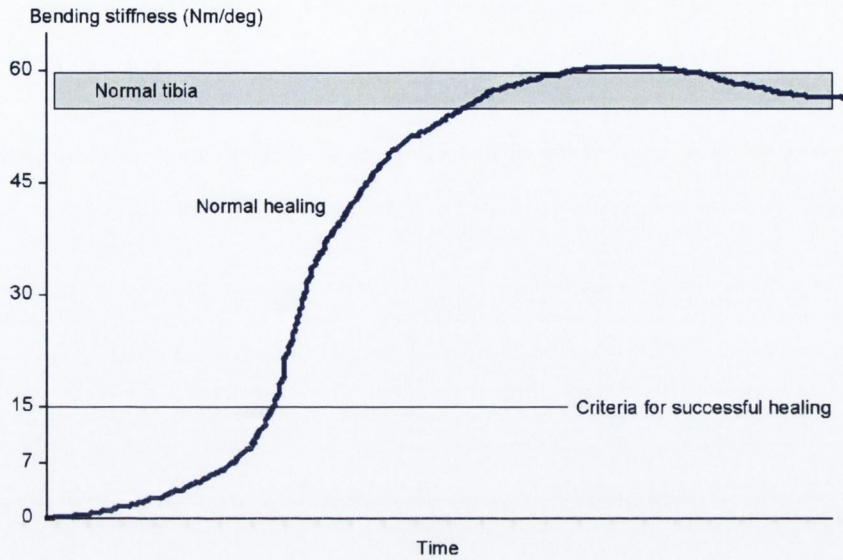


Figure 2-7: Graph illustrating the increase of bending stiffness as healing occurs. Adapted from Richardson et al. (1994)

2.3.2 Bone scaffolds

As mentioned above, bone has a dynamic potential to regenerate itself after damage; however the repair of large bone defects resulting from resection or trauma or non-union fractures still requires the implantation of bone grafts or an engineered scaffold. Natural bone grafts possessing excellent osteoconduction and mechanical stability have been used extensively in clinical settings. Depending on the relationship between the donor and the recipient, natural bone grafts are categorized into autografts, allografts and xenografts. With autografting the donor and the recipient are the same individual - a fraction of the tissue or organ is harvested from an uninjured site and grafted at the non-functional site (Medawar, 1944), see Figure 2-8. The donor tissue from allografts and xenografts are harvested from other humans or other species, respectively. Autografts are considered the gold standard for bone implantation, and have the advantage of immunocompatibility over allografts and xenografts. However problems such as donor site morbidity, risk of infection and the availability of bone tissue of the correct size and shape limit the use of autografts in orthopaedic applications.

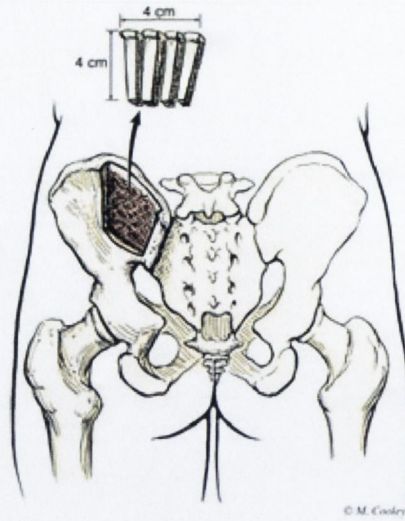


Figure 2-8: Autograft – where bone is harvested from the iliac crest to be placed at the defective site

Approximately one-million surgical cases of bone-grafting procedures are performed annually (Greenwald *et al.*, 2001), and the demand for bone grafts will continue to rise over the next decade as the population ages. One possible remedy for the shortage of bone grafts is a functional tissue-engineered bone graft. Both *in vivo* and *in vitro* tissue engineering approaches are employed for bone-tissue engineering using pluripotent mesenchymal stem cells (MSCs, see Section 2.5). There are currently many three-dimensional scaffolds available to induce the formation of bone from the surrounding tissue or act as a carrier template for implanted cells (Burg *et al.*, 2000). Various biomaterials have been evaluated for their potential use as scaffolds in bone tissue engineering. The ideal biomaterial should be osteoconductive, osteoinductive, biocompatible, and biodegradable (Kwan *et al.*, 2007). Osteoconductivity refers to the ability of the graft to support the attachment of bone forming cells, and allow new cell migration and vessel formation. The osteoinductive quality of scaffolds describes their ability to induce nondifferentiated stem cells or progenitor cells to differentiate along an osteogenic lineage. The scaffold must also be biocompatible so as not to elicit an immunological or clinically detectable foreign body reaction (Hutmacher, 2001). The three main types of biomaterials which have been successfully investigated for use as scaffolds include natural polymers, synthetic polymers, and ceramics. In general natural polymers,

such as collagen and glycosaminoglycan (GAG), contain bioactive domains favourable for biological activities involved in tissue regeneration, whereas synthetic polymers, such as polylactic acid (PLA), feature controllable material properties that can approximate the physical properties of native tissue (Chen *et al.*, 2007). Ceramics have also been widely used, due to their high biocompatibility and osteogenic induction of MSCs; see Figure 2-9(a). Calcium phosphate ceramics, such as hydroxyapatite (HA) and tri-calcium phosphate (β -TCP) closely mimic the structural and chemical characteristics of the mineral component of bone (Chang *et al.*, 2000; Chu *et al.*, 2002), and therefore efficaciously support osteogenic differentiation.

In addition to the biomaterial properties, scaffolds must also conform to many biological and structural requirements. Scaffold properties such as pore size, interconnectivity, micro and macro -porosity, and surface characteristics have been shown to influence the rate and degree at which bone formation occurs. Porosity facilitates the migration and proliferation of precursor cells, allows for the mass transfer of nutrients and oxygen within the construct, and also provides an available volume for new matrix deposition (Jones *et al.*, 2006). It is thus favourable to have a high degree of porosity to allow full ingrowth of new tissue. The porosity in conjunction with the pore size and interconnectivity should also be sufficient to prevent pore occlusion and peripheral tissue formation (Buckley, 2007). Higher porosities have been reported to result in greater bone ingrowth *in vivo* but the consequential lowering of mechanical stiffness and strength sets an upper functional limit for porosity (Karageorgiou and Kaplan, 2005). Thus it would seem that the porosity of the scaffold must lie within a critical range – small enough to maintain

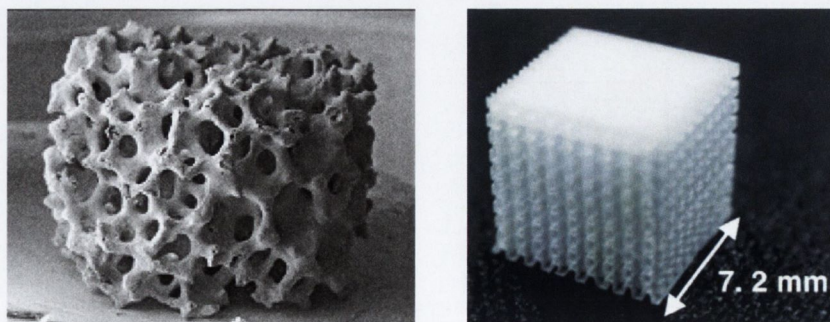


Figure 2-9: (a) Calcium phosphate scaffold and (b) regular structured scaffold fabricated through solid free-form fabrication (SFF)

the mechanical integrity of the scaffold and large enough to provide optimal bioactivity (O'Brien *et al.*, 2004). The mechanical properties of the scaffold construct should be similar to the host tissue; so that it can be mechanically functional (i.e. bear load). Another parameter that must also be controlled is the rate of scaffold degradation. Ideally the rate of degradation should be proportional to the rate of tissue growth, in order to maintain the mechanical function of the bone-scaffold system, see Figure 2-10. However, if the rate of bone ingrowth is exceeded by the rate of resorption, then the implant is likely to fail (Hutmacher, 2000). The optimal mechanical properties should promote appropriate mechanical stimuli through the scaffold to promote bone regeneration. In chapter 3 computer simulations are used to maximise bone regeneration in a regular structured scaffold by appropriate selection of porosity, Young's modulus and dissolution rate under high or low loading conditions.

The ability to control the scaffold architecture, through design and fabrication, has a significant effect in promoting MSC-based bone formation. Success in the stimulation of bone has been shown with scaffolds with random architecture and porosity similar to the native tissue. Depending on the material type several manufacturing techniques have been developed to fabricate scaffolds. These include solvent-casting, gas foaming, melt moulding, fibre bonding, phase

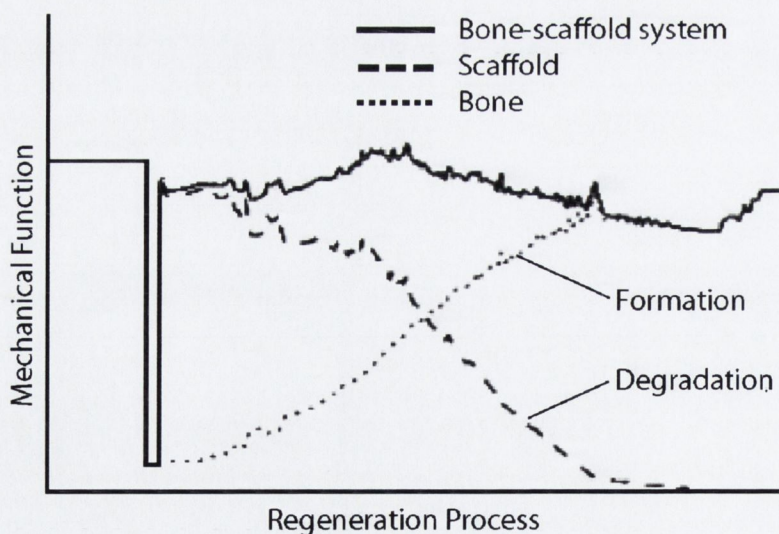


Figure 2-10: Schematic drawing of change in tissue mechanical function in bone tissue regeneration process using biodegradable scaffold. Adapted from Adachi *et al* (2006)

separation, emulsion freeze drying, solution casting and freeze drying. These traditional methods, however, have largely been unsuccessful in controlling the internal architecture to a high degree of accuracy or homogeneity. This inconsistency will result in grossly different mechanical properties throughout the scaffold. If the scaffold is subjected to mechanical loading, the most porous region, which generally coincides with the mechanically weakest region, will fail prematurely and cause implant failure (Liebschner and Wettergreen, 2003). In order to counteract these problems bioprinting a biomedical type of rapid prototyping, and solid free-form fabrication (SFF) technologies are currently being used by investigators to manufacture tissue engineering scaffolds (Hollister *et al.*, 2000; Hutmacher, 2000; Chu *et al.*, 2002). These techniques create the scaffolds by adding material as opposed to the traditional techniques that remove material to create the final part, therefore the architecture can now be laid out with regular, repeated structures, composed of almost any biomaterial that is desired (Taboas *et al.*, 2003), see Figure 2-9(b). Controlling the characteristics of pores, such as geometry, connectivity and porosity in this manner can give lead to the design of a scaffold that more closely emulates the properties of bone and may successfully heal critical size bone defects.

2.4 Stem cells and tissue differentiation

Mesenchymal stem cells (MSCs) are nonhematopoietic progenitor cells found in adult tissues. They are characterised by their extensive proliferative ability in an uncommitted state and hold the potential to differentiate along various lineages of mesenchymal origin in response to appropriate stimuli (Chen *et al.*, 2007). Bone marrow is the best known source for MSCs. In addition, MSCs have been identified in a number of other tissues such as adipose, periosteum, trabecular bone, synovium, skeletal muscle and deciduous teeth (Barry and Murphy, 2004). It is thought that the stem cells in these locations lie dormant in a non-proliferating state until they are needed to participate in local repair and regeneration. MSCs have been shown to differentiate into various mesenchymal lineages, including cellular phenotypes representative of the musculoskeletal tissues, such as osteocytes (bone), chondrocytes (cartilage), myoblasts (muscle), fibroblasts (tendon) and adipocytes (fat). The developmental hierarchy is illustrated in Figure 2-11. Quiescent MSCs become mobilised during repair and remodelling through regulation by external

chemical and physical signals that control their activation, proliferation, migration, differentiation and survival i.e. their fate (Kearney, 2008). MSC fate decisions are controlled by both intrinsic regulators and the extra-cellular environment (Rabbany *et al.*, 2003).

Due to their pluripotent properties, MSCs are an ideal cell source for tissue engineering applications. Procuring stem cells and controlling their activities, as well as developing transplantation technology of stem cells and stem cell derived cellular products, will be fundamental to tissue engineering and to the development of cellular therapies (Palsson and Bhatia, 2003). A range of surface markers can be used to experimentally identify MSCs; however other cell types may also express these

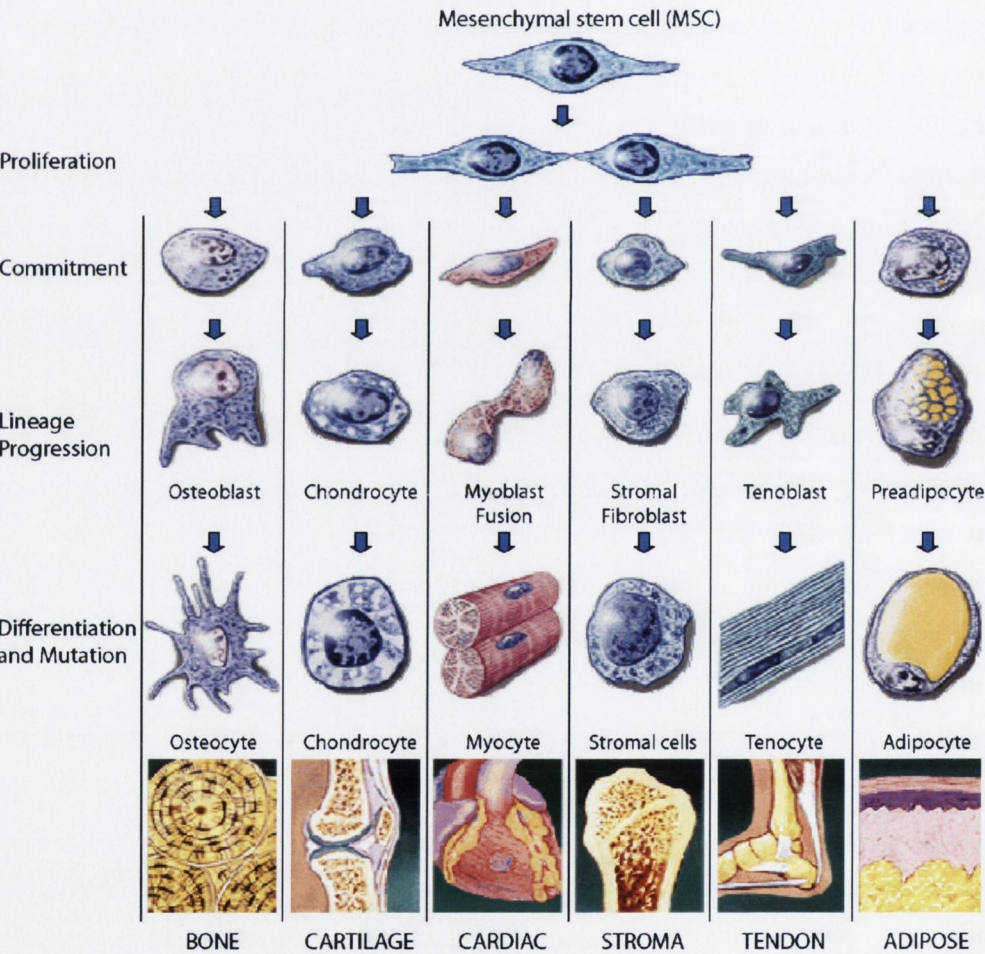


Figure 2-11: Illustration of the multilineage potential of MSCs which can result in the formation of tissues such as bone, cartilage and muscle when signalled with appropriate stimuli. Adapted from Caplan and Bruder (2001)

proteins (McMahon, 2007), therefore it remains a challenge to isolate MSCs specifically from a mixed cell population. MSCs can be used in conjunction with tissue-engineered bone scaffolds to promote and accelerate osteogenesis. To date MSCs have been used in tissue engineering of a number of musculoskeletal tissues, including cartilage, bone, osteochondral constructs, ligament and tendon with varying degrees of success (Chen *et al.*, 2007).

2.5 Mechanobiology

Mechanobiology is the study of interactions between mechanical stimuli and biologic processes at the cellular, tissue, and organ level. Mechanical loading can influence cell proliferation, differentiation and metabolism, and as such plays a crucial role in the growth, adaptation, regeneration and engineering of living tissues. At the centre of mechanobiology is the cellular process of mechanotransduction, or the way cells sense and respond to mechanical forces. Of particular interest to this work, is how the osteogenic pathway, shown in the leftmost column of Figure 2-11, is regulated by mechanical forces within the tissue.

The concept that cellular processes can be regulated by mechanical loading dates back to the late 1800s when Roux introduced his theory of functional adaptation. According to Roesler (1987), Roux proposed that fibrous connective tissue forms under tension, osseous tissue forms under compression and cartilaginous tissue under shear forces (Roux, 1881). Much of the present-day understanding of the regulative effect of mechanical forces on tissue differentiation however comes from Friedrich Pauwels (1980). Pauwels analysed the mechanical environment within a healing fracture callus and hypothesised that two invariants of the stress tensor, the octahedral shear stress (which causes a change in cell shape), and hydrostatic stress (which causes a change in cell volume), guided the cell differentiation pathway. A schematic representation of the concept is illustrated in Figure 2-12.

Pauwels proposed that granulation tissue containing mesenchymal cells would differentiate into cartilage under high levels of hydrostatic stress and low levels of octahedral shear stress; whereas differentiation into fibrous tissue (ligamentogenesis) would result from high levels of octahedral shear stress and low levels of hydrostatic stress. No specific stimulus for bone formation was proposed.

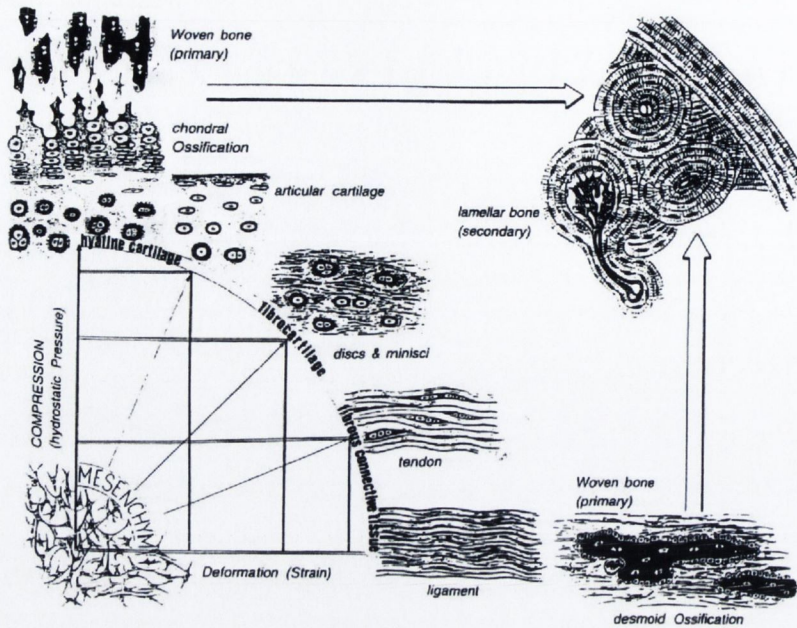


Figure 2-12: A schematic representation of the hypothesised influence of mechanical stimuli on tissue differentiation proposed by Pauwels. Bone regeneration only occurs after stabilisation of the mechanical environment by the formation of soft tissue. Figure taken from Weinans and Prendergast (1996)

Instead he concluded that bone formation occurs once cartilage or connective tissue provided a framework rigid enough for ossification to occur. Pauwels tested his hypotheses using experimental tools: photoelasticity measurements of strain in laboratory models of bone, in addition to the use of “strength of materials” theory (Mow and Huiskes, 2005). His ideas were limited by the fact that he was unable to accurately measure the stresses and strains acting on the tissues in the fracture callus. Towards this effort computational analysis holds great promise in enhancing our understanding of mechano-regulation in skeletal tissues.

2.6 Computational mechanobiology models

Since Pauwels’ time, computational modelling has emerged as an alternative approach to the classical methods of scientific investigation (i.e. experimental and theoretical). One such tool which is widely used in biomechanics is the finite element method. This computer method provides the capability for analysis of any given point inside a structure of arbitrary geometry, and material complexity; and as such can be employed to determine stresses and strains in complex biological

systems. Along with modern histological and biochemical techniques this has led to the development of a number of new theories of mechano-regulated tissue differentiation (Kelly, 2003). These theories attempt to discover how tissue differentiation is regulated by its mechanical environment. Most include a volumetric deformation (change in size) and deviatoric deformation components (change in shape) similar to Pauwels, see Figure 2-13; however no one theory has yet become widely accepted. This section describes some of the mechanoregulatory theories and algorithms that have been proposed to predict and/or simulate tissue formation.

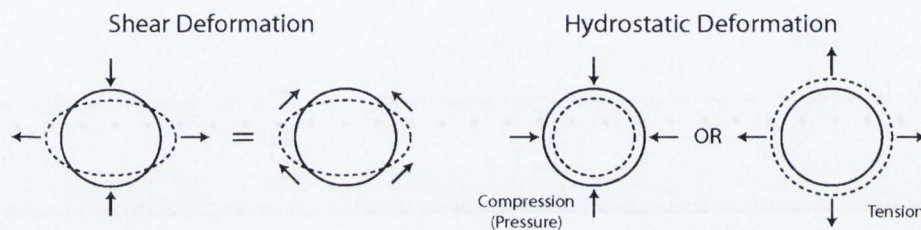


Figure 2-13: Schematic representation of the deformation caused by hydrostatic stress and octahedral shear (or distortional) stress, from Carter et al. (1998)

2.6.1 Interfragmentary strain theory

Based on a qualitative analysis of fracture healing Perren and Cordey proposed that the fracture gap can only be filled with a tissue capable of sustaining interfragmentary strains without rupture (Perren, 1979; Perren and Cordey, 1980). The interfragmentary strain (IFS) is defined as the interfragmentary movement divided by the initial fracture gap size. The strain tolerance varies between tissues due to differences in strength and rigidity. Initially the fracture gap contains granulation tissue, which has the greatest strain tolerance. As healing progresses the regenerating tissue stiffens, causing a reduction in interfragmentary strain. Consequently this creates an environment where stiffer, stronger tissues can form, see Figure 2-14. Thus, healing occurs by a progressive differentiation from the initial granulation tissue to fibrous tissue, cartilage and finally bone. This theory is limited by its simplistic view of fracture healing, as it implies only one tissue is present in the fracture callus at any time, and does not consider the three-dimensional complexity of the callus or multiaxial stress states (Prendergast and van der Meulen, 2001).

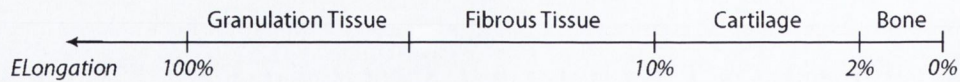


Figure 2-14: Strain tolerance of repair tissues. A tissue cannot exist in an environment where the interfragmentary strain exceeds the strain tolerance of the extracellular matrix of the tissue (Perren and Cordey, 1980)

2.6.2 Single phase models

2.6.2.1 Carter's mechanobiology hypothesis

Based on the framework of Pauwels, Carter *et al.* expanded the concepts relating tissue differentiation to mechanical loading. They proposed that local stress or strain history influences tissue differentiation over time (Carter *et al.*, 1988). These ideas were later developed further and a more general mechano-regulation theory was proposed, see Figure 2-15. In this refined model octahedral shear stress was replaced with distortional strain (octahedral shear strain) based on the belief that biological events at the tissue level are often related to changes in cell shape and local matrix formation (Giori *et al.*, 1993). They postulated that:

- Compressive hydrostatic stress history guides the formation of cartilaginous matrix constituents
- Tensile strain history guides connective tissue cells in their production and turnover of fibrous matrix constituents
- Fibrocartilage is formed when a tissue loading history consists of a combination of high levels of hydrostatic compressive stress and high levels of tensile strain
- Direct bone formation is permitted, in regions exposed to neither significant compressive hydrostatic stress nor significant tensile strain, provided there is an adequate blood supply
- Pre-osseous tissue can be diverted down a chondrogenic pathway in regions of low oxygen tension

Carter *et al.* (1988) were the first to introduce finite element models to determine the effects of mechanical loading on differentiating tissue. Applying their original tissue differentiation theory (based on a combination of octahedral shear stress and hydrostatic stress), in a two-dimensional linear elastic FE model of a fracture callus,

they were able to predict realistic tissue patterns consistent with biological observations (Carter *et al.*, 1988; Blenman *et al.*, 1989). Later, their refined mechano-regulation theory was employed to determine tissue differentiation patterns around implants (Giori *et al.*, 1995), during fracture healing (Carter *et al.*, 1998; Carter and Beaupré, 2001; Gardner *et al.*, 2004), oblique fracture healing (Loboa *et al.*, 2001) and osteochondral defect healing (Carter and Beaupré, 2001). Unlike Pauwels they recognised the influence of vascular perfusion and proposed that low oxygen tension diverts cells down the cartilaginous pathway. The mechanobiological phase diagram in Figure 2-15 presents a basic framework for understanding the initial directions of skeletal tissue differentiation; however no specific values of stress/strain levels were proposed. This semi-quantitative nature of the calculations renders the theory subjective, and hence difficult to falsify.

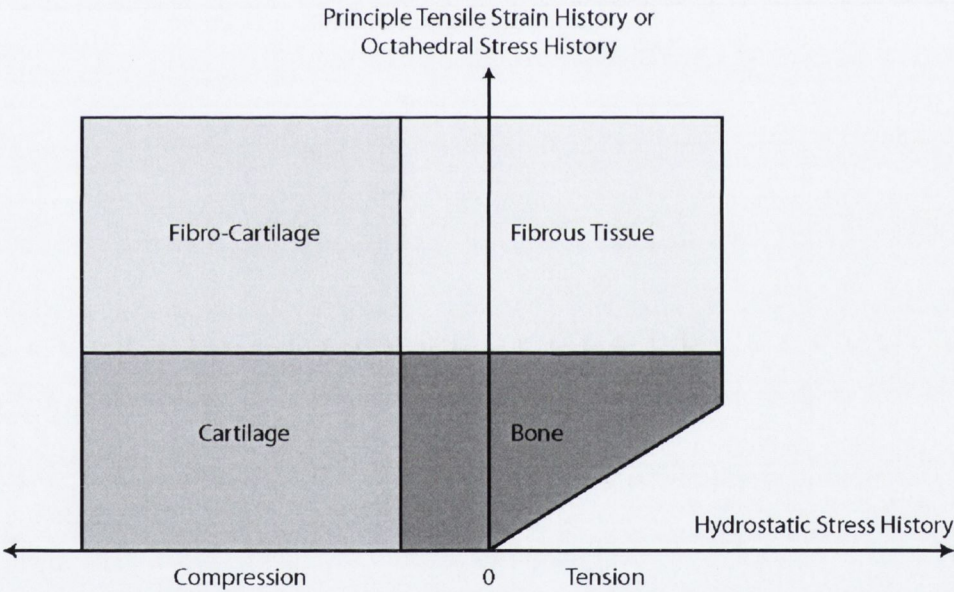


Figure 2-15: Schematic representation of the role hydrostatic stress history and maximum principle tensile strain history on the differentiation of pluripotent mesenchymal tissue in a well-vascularised environment (Carter *et al.*, 1998)

2.6.2.2 Claes and Heigele’s fracture healing model

The mechanoregulation theory of Claes and Heigele was initially presented in quantitative terms, and although the resulting concept is similar to that of Carter *et al.*, they based their mechano-regulation theory on the observation that bone

formation occurs mainly near calcified surfaces and that both intramembranous and endochondral ossification exist in fracture healing. Depending on local strain and hydrostatic pressure different cellular reactions and tissue differentiation processes were predicted to occur (Claes *et al.*, 1998; Claes and Heigele, 1999). They postulated that intramembranous bone formation occurs in regions with very small tissue strains (less than 5%) and hydrostatic pressures (below -0.15 MPa), see Figure 2-16. Endochondral ossification would take place in regions with greater magnitudes of strain and compressive hydrostatic pressure (greater than 15% and -0.15 MPa), while tissue strains above approximately 15% would lead to fibro-cartilage and connective tissue preventing bone healing (Claes *et al.*, 1998; Claes and Heigele, 1999). Using an axisymmetric FE model of a fractured ovine tibia, Claes and Heigele calculated the stresses and strains within the callus. Hyperelastic material properties were used to model the connective tissues while linear elastic properties were used for other tissues. Comparing the computer model of fracture healing with

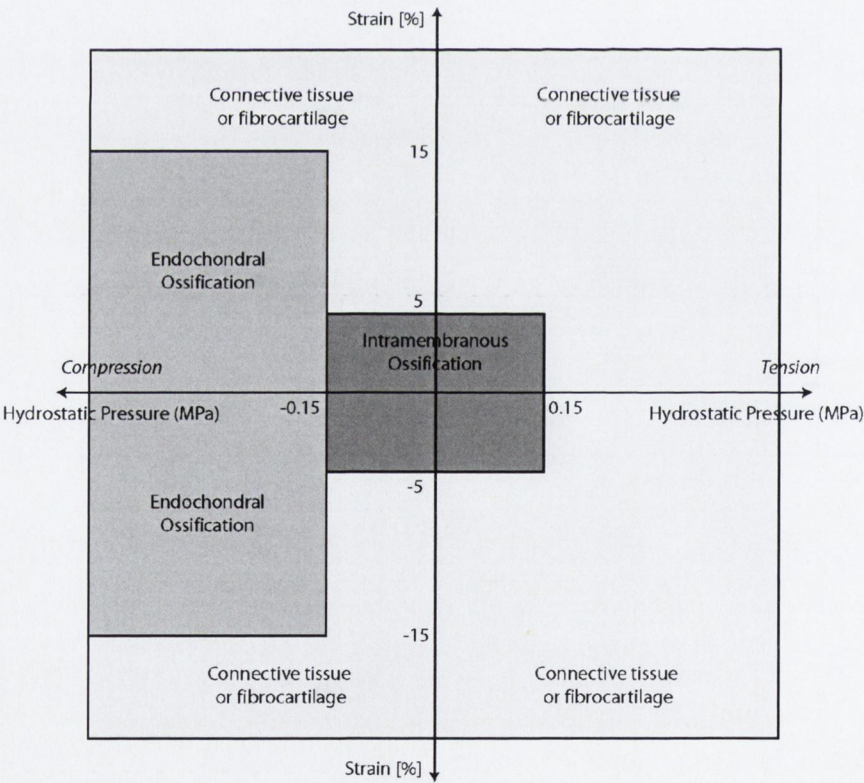


Figure 2-16: The mechano-regulation concept regulated by hydrostatic pressure and strain as proposed by Claes and Heigele (1999)

histological findings from *in vivo* experiments, they were able to demonstrate that the quantitative formulation did indeed properly predict tissue differentiation events in the callus at three states of healing. Like Carter *et al.*, these models are limited in the sense that the tissue types were predicted directly from the stress patterns computed at one moment in time, and therefore the theory could not be tested to see if it could *simulate* the progression of tissue differentiation over time.

2.6.3 Biphasic models

Soft tissues of the body, such as skin, cartilage or myocardium, are composed of large amounts of fluid in a solid matrix of collagens and proteins, (Prendergast *et al.*, 1997). Experimental studies of these tissues have shown that they are viscoelastic – that is, their stress/strain response is time dependent. This behaviour is attributed, in part, to the fact that fluid must flow within the solid matrix if deformation is to occur. The viscoelastic properties of the tissues are governed by its permeability, which is a measure of the ease with which fluid can flow through the pores, and it is inversely proportional to the frictional drag exerted by the fluid flowing through the material. Modelling the biphasic nature of soft tissues has been related to the study of consolidation – the settlement of soils under load (Biot, 1940). Since the principle equation for poroelastic theory used in many finite element packages are based on this theory, a review of Biot's general theory is outlined below.

According to the poroelasticity theory, tissues can be modelled as a mixture of solid and fluid constituents present at each material point. Because of this continuity, the sum of the volume fractions has to equal one and the apparent total density of the mixture, ρ , can be formulated as follows:

$$\rho = (1 - n)\rho^s + n\rho^f \quad (2.1)$$

where n is the porosity (equal to the fluid volume fraction), and ρ^s and ρ^f are the apparent density of the solid and fluid phases respectively. The porosity, n , can be expressed in terms of n_R , the porosity in the reference configuration as follows

$$n = 1 - \frac{1 - n_R}{J} \quad (2.2)$$

where J is the ratio of the current volume, dV , to the reference volume, dV_R , or also the determinant of the deformation gradient with respect to the reference configuration

$$J = \frac{dV}{dV_R} = \det \nabla \mathbf{x} \quad (2.3)$$

where ∇ is the gradient operator and $\mathbf{x}$ the deformation vector. The total stress σ , (positive in tension) acting on the total area of the solid and the pores is separated into inter-granular stress (solid phase stress) σ' , and pore pressure p , (positive in compression) corresponding to the fluid stress when the fluid is inviscid

$$\sigma = \sigma' - p\mathbf{I} \quad (2.4)$$

where $\mathbf{I}$ is the identity matrix. Assuming that the gravity field, the convective terms and the relative acceleration between solid and fluid phases are neglected, the momentum conservation leads to

$$\text{div}(\sigma) = \rho \ddot{\mathbf{u}}_s \quad (2.5)$$

where $\ddot{\mathbf{u}}_s$ is the acceleration of the solid. Using equation 2.4, this equation reads

$$\text{div}(\sigma') - \nabla I p = \rho \ddot{\mathbf{u}}_s \quad (2.6)$$

If we assume the material to be isotropic, and if the infinitesimal strain displacement relationship is assumed, we can write a constitutive law as follows

$$\sigma' = \lambda e \mathbf{I} + 2\mu \varepsilon \quad (2.7)$$

where ε denotes strain tensor, e denotes the dilatational strain, λ and μ are the Lamé constants related to the Young's modulus and Poisson's ratio. Finite strain can be accounted for by using a total or updated Lagrange formulation.

There are two possible ways in which the volume of the tissue can change. The first is expelled fluid from the tissue. This produces a relative fluid velocity according to Darcy's law

$$\dot{\mathbf{u}}_f = -\frac{k}{\nu} \nabla p \quad (2.8)$$

where $\dot{\mathbf{u}}_f$ is the relative fluid velocity vector with respect to the solid velocity, k is the permeability vector and ν is the kinematic viscosity. The second way in which the volume of the tissue can change is by compression of the solid and fluid phases themselves. If we assume that the compression modulus of the porous solid is much smaller than the intrinsic compression modulus of the non-porous solid, the general mass conservation law leads to

$$\text{div}(\dot{\mathbf{u}}_s) + \text{div}(\dot{\mathbf{u}}_f) + \frac{\dot{p}}{Q} = 0 \quad (2.9)$$

where $\dot{u}_s$ is the velocity vector of the solid and $\dot{u}_f$ is the relative fluid velocity vector with respect to the solid velocity, and Q is the Biot material parameter related to the compressibility of the fluid and solid by

$$\frac{1}{Q} = \frac{n}{K_f} + \frac{1-n}{K_s} \quad (2.10)$$

where K_f and K_s are the intrinsic compression modulus of the fluid and solid phases respectively. Thus, six parameters (Young's modulus, permeability, Poisson's ratio, porosity, and solid and fluid compression moduli) need to be known to completely define a poroelastic compressible isotropic model.

The basic FE assumption is the interpolation of coordinates, displacements and pore pressure potentials for each element from the values in nodes. Based on the previous equations, a FE space discretisation is derived via the standard Galerkin procedure. The space discretisation is evaluated at several time steps using an Euler backward time integration.

The biphasic theory proposed by Mow *et al.*, (1980) was applied to cartilage to represent more accurately the nature and behaviour of tissues. They present the biphasic theory as a development of the theory of mixtures. In the biphasic theory, the material is considered to be a continuum mixture of a deformable solid phase and a fluid phase. Simon (1992) showed that the biphasic and poroelastic theories, when applied to biomechanic soft tissues, are effectively equivalent (Prendergast *et al.*, 1997).

2.6.3.1 Prendergast and colleagues

Phase 1

Prendergast and Huiskes (1995) and Prendergast *et al.* (1997), created a poroelastic finite element model of a bone-implant interface to analyse the mechanical environment on differentiating cells. They found that the biophysical stimuli experienced by the regenerating tissue at the implant interface are not only generated by the tissue matrix, but also to a large extent by the drag forces from the interstitial flow. Based on this study, a new mechano-regulation theory was developed taking into consideration that connective tissues are poroelastic and comprise both fluid and solid. They proposed a mechano-regulatory pathway composed of two biophysical stimuli; octahedral strain of the solid phase and interstitial fluid velocity relative to

the solid, see Figure 2-17. The *in vivo* experiments from (Søballe, 1993), where a micromotion device was implanted into the condyles of dogs; provided Prendergast *et al.*, with a means to quantify their regulatory model and demonstrate that it was consistent with observed temporal changes in implant motion. In a separate study, Prendergast and Huiskes (1996) showed that a linear-elastic finite element model of the bone-implant interface was unable to predict ossification patterns similar to those using the biphasic representation.

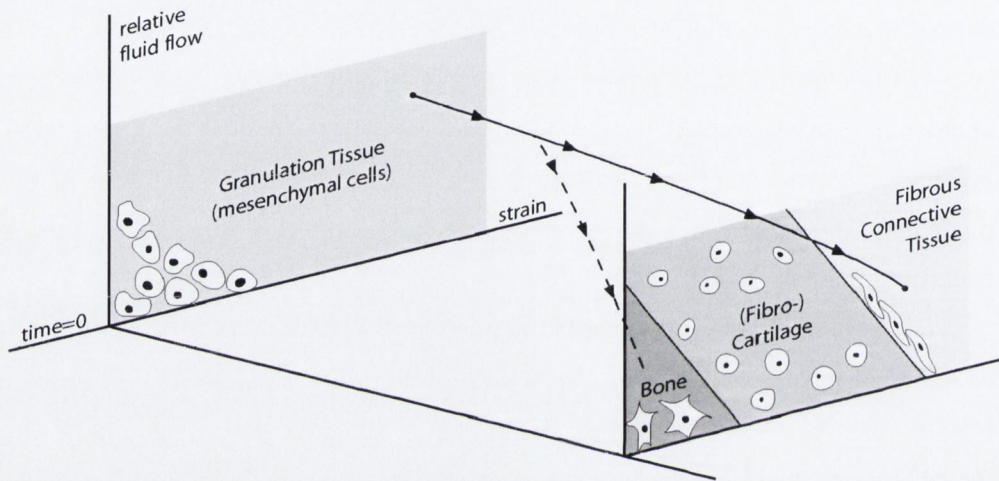


Figure 2-17: Mechano-regulation pathway controlled by tissue shear strain and interstitial fluid flow (Prendergast *et al.*, 1997)

Using this theory for tissue differentiation, a mechano-regulatory *algorithm* was developed where the predicted phenotype depends on the combined value of maximal distortional strain, γ , and relative fluid velocity, v (Huiskes *et al.*, 1997; Driel *et al.*, 1998). The stimulus value, S , determined from the equation:

$$S = \frac{\gamma}{a} + \frac{v}{b} \quad (2.11)$$

where $a = 0.0375$ and $b = 3\mu\text{ms}^{-1}$, dictates the regulatory pathway of three tissue types. High stimulus levels ($S > 3$) promote the differentiation of mesenchymal cells into fibroblasts, intermediate levels ($1 < S < 3$) stimulate the differentiation into chondrocytes, and low levels of these stimuli ($S < 1$) promote the differentiation into osteoblasts. Van Driel *et al.* (1998), and Huiskes *et al.* (1997), employed this algorithm in an iterative FE model to *simulate* tissue differentiation around an implant. In doing so, they were the first to simulate the progression of tissue

differentiation over time, and paved the way for other researchers to predict the time course of tissues using similar methods.

Interestingly Isaksson *et al.* (2006) compared the mechano-regulation theories proposed by Carter *et al.* (1998), Claes and Heigele (1999) and Prendergast *et al.* (1997), in a fracture healing study and found that the concept based on strain and fluid velocity as stimuli corroborated best with experimental results. Thus, this biphasic mechano-regulation algorithm has been included in many simulations to model tissue regeneration in a variety of clinical settings. In the next phase of this work, the focus has shifted towards incorporating a more accurate description of cellular processes. The work carried out in Trinity College Dublin is discussed below.

Phase 2

Lacroix *et al.* (2002(a)) introduced the presence of cells/bioactive factors in the decision process of tissue differentiation. They hypothesised that cell migration, proliferation and apoptosis are likely to be modulated by the mechanical environment and therefore must be modelled in the computational simulations. Progenitor cell migration was described as a diffusive process, in a fracture healing study. This approach implicitly assumes that cells attempt to achieve a homogeneous population density within the fracture callus. It is dictated by the equation:

$$D\nabla^2 n = \frac{dn}{dt} \quad (2.12)$$

where n is the cell density and the constant D is the diffusion co-efficient. Applying the mechano-regulation theory proposed by Prendergast *et al.* (1997), in an iterative two-dimensional finite element model, Lacroix *et al.* (2002(a)) were able to simulate periosteal bone formation and endochondral ossification in the external callus similar to those observed. Using this approach, fracture healing was simulated with different gap sizes and loading magnitudes (Lacroix and Prendergast, 2002(b)). To account for the underactivity of bone areas of low local strain, a resorption field was added for very low stimuli values, ($S < 0.53$), see Figure 2-18.

Several features of fracture healing were accurately predicted, such as intramembranous bone formation far from the fracture site and endochondral ossification in the external callus, stabilisation when bridging of the external callus occurs, and resorption of the external callus. These studies showed that stem cell

access to the regenerating region has a considerable effect on the healing pattern and the healing rate; thus highlighting the importance of including cellular dispersal in tissue differentiation simulations.

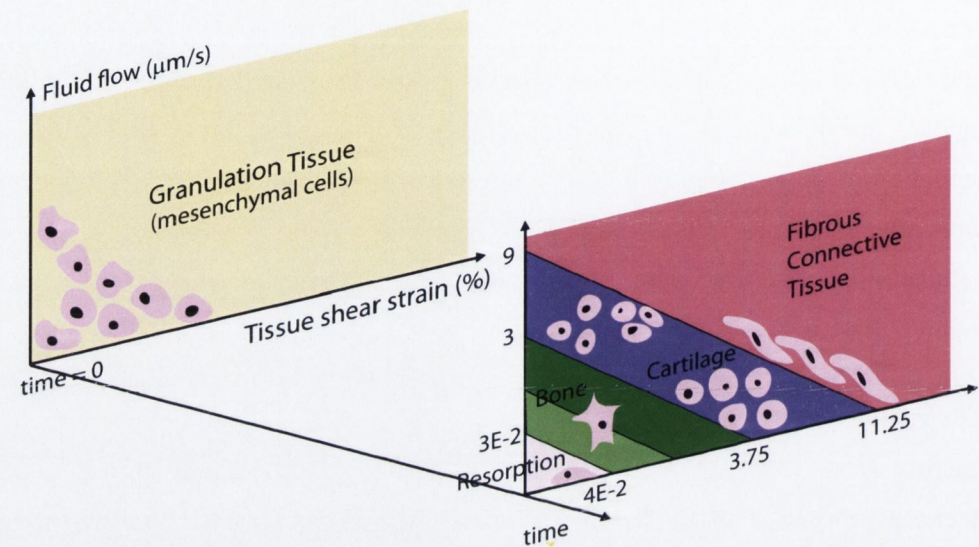


Figure 2-18: Mechano-regulation concept including bone resorption. When the mechanical stimuli become low, the cells are understrained and the osteoclasts start resorbing bone (Lacroix, 2000; Lacroix and Prendergast, 2002(b))

In order to analyse what is occurring physiologically many of these fracture healing studies were simplified into two-dimensions, and assumed a cylindrical bone with an axial load (axisymmetrical analysis). It is questionable whether these representations adequately capture the structural characteristics of a fracture site, and consequently, whether the mechanical stimuli can be accurately determined. In an attempt to amend these limitations Lacroix and Prendergast (2002(c)) performed a three-dimensional healing simulation based on the real geometry of a fractured tibia with an external fixator. Tissue differentiation and bone regeneration was used to simulate the progress of healing under two different loading magnitudes. Healing was shown to be successful under the lower load and unsuccessful under the higher load. The associated limitations/assumptions made in this study are addressed in a fracture study in Chapter 5.

Similar methods were employed by Andreykiv *et al.* (2005), to simulate osseointegration (bone ingrowth) in a glenoid bone implant in two-dimensional study. This study illustrated the positive effects of stiff glenoid components; and

components that provide a uniform load distribution, which result in a reduction of the peak interface micromotions. In another fracture healing study; Andreykiv *et al.*, combined the biphasic mechano-regulation algorithm with a system of partial differential equations to model cell migration, and proliferation, differentiation and resorption of tissues. Unlike the models of Lacroix and Prendergast cell proliferation was modelled explicitly, tissues were modelled separate from cells and tissue production rates were not equal for every tissue. The model was able to predict the tissue differentiation patterns as observed in the animal studies by Claes (1995) and Claes and Heigele (1999).

Phase 3

In Lacroix' model of fracture healing the diffusion coefficient was set to give a steady state cell concentration through the fracture callus at the end of a 16 week healing period. Cell dispersal was therefore assumed to be independent of the tissue differentiation process. Following an extensive review of the literature on mechano-regulated mitosis, Kelly and Prendergast (2003, 2005) discovered that relatively high magnitudes of strain were seen to increase cellular proliferation, while very high magnitudes of stress or strain resulted in cell death. In a study of osteochondral defect repair, they assumed a quadratic relationship between cell proliferation/death and octahedral shear strain, which is illustrated in Figure 2-19. The mechanical environment was therefore used to regulate MSC dispersal, proliferation, differentiation and apoptosis, using the following equation:

$$\frac{dn^i}{dt} = D^i \nabla^2 n^i + P^i(S) n^i - K^i(S) n^i \quad (2.13)$$

where n^i denotes the number of cells of a particular cell phenotype i , D^i is the diffusion coefficient for cell phenotype i , $P^i(S)$ is a proliferation rate and $K^i(S)$ is a cell death rate (either necrosis or apoptosis) for cell phenotype i as a function of the stimulus S .

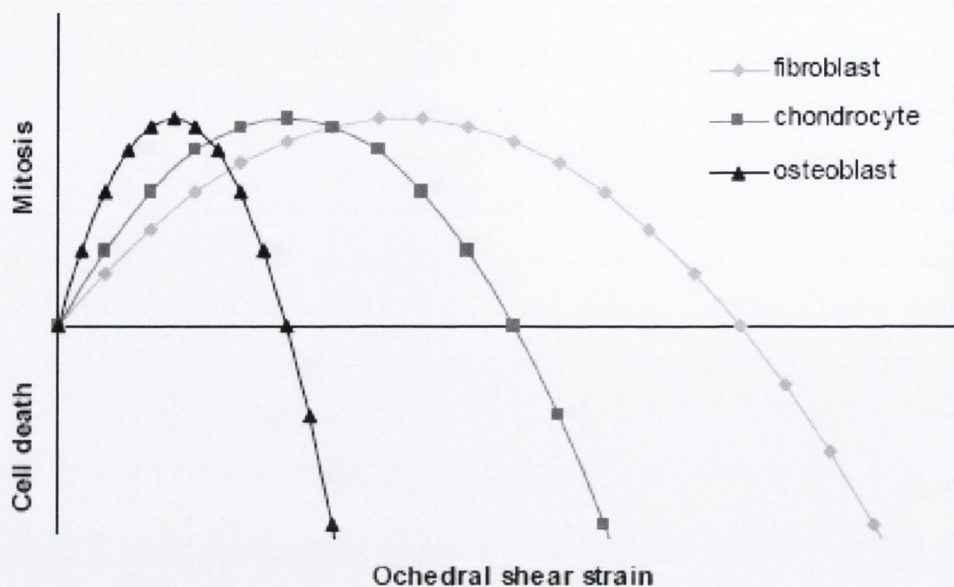


Figure 2-19: Relationship between shear strain and cellular mitosis and death (Kelly, 2003)

The model successfully predicted cellular differentiation patterns observed experimentally in osteochondral defect healing – intramembranous ossification occurs at the base of the defect, while chondrogenesis is favoured within the centre of the defect. However, due to high magnitudes of strain and fluid flow at the articular surface, fibrous tissue formation is predicted which ultimately inhibits chondrogenesis in this region. Shapiro *et al.* (1993) observed similar results experimentally, suggesting that the model is correctly capturing some features of the tissue differentiation process.

In a following study Kelly and Prendergast (2006) used the same model to determine the influence of scaffold material properties on chondrogenesis in a finite element model of an osteochondral defect. They determined an optimal design by parametrically varying the mechanical properties of the scaffold through its depth, such that the Young's modulus reduces in magnitude and the permeability increases in magnitude from the superficial zone through the depth of the chondral phase of the scaffold.

In an investigation of the bone regeneration process within the fracture callus of a human mandible submitted to symphyseal distraction osteogenesis Boccaccio *et al.*, (2008) combined the biphasic tissue differentiation algorithm to relate tissue differentiation to the mechanical environment. A similar methodology to Lacroix *et*

al., (2002(a)) was employed; however a rate equation was included to better define the evolution of the Young's modulus of the regenerating tissue over time, see Equation 2.3. This theory was based on the experimental work of Richardson *et al.*, (1994) who observed an exponential increase in stiffness in differentiating tissue. This approach accounts for the fact that MSCs not only require time to differentiate, but that the differentiated cell types require time to synthesise and remodel new tissue.

$$E_i = K_i e^{\beta_i t} \quad (2.14)$$

E_i represents the Young's modulus for tissue phenotype i (where i is fibrous tissue, cartilage, immature or mature bone), t is the time, and K_i and β_i are two parameters regulating the shape of the exponential curve. In a separate study, Boccaccio *et al.* (2008) used the same method to investigate the influence of the duration of the latency period on tissue differentiation and bone regeneration prior to osteogenesis. The model predicts that the optimal duration for the latency period is 7 to 8 days to avoid the risk of premature bone union of the osteotomy fronts.

Until recently diffusion has been the main method to model cellular migration and proliferation, however despite its convenience to model, diffusion is not the mechanism of cell dispersal; instead cells disperse by crawling or proliferation or are transported in a moving fluid. In pursuit of a better understanding of the cellular processes Pérez and Prendergast (2007) developed a 'random-walk' model to describe cell proliferation and migration, with and without a preferred direction, see Figure 2-20. This mechanistic approach allows for the simultaneous dispersal of several cell populations and the explicit modelling of cell proliferation. This concept was based on the observed stochastic nature of cellular movement, particularly in regards to fibroblasts. In a model of a bone/implant interface Pérez found the random-walk model of cell dispersal during tissue differentiation gives heterogeneous tissue distributions whereas the diffusion model does not.

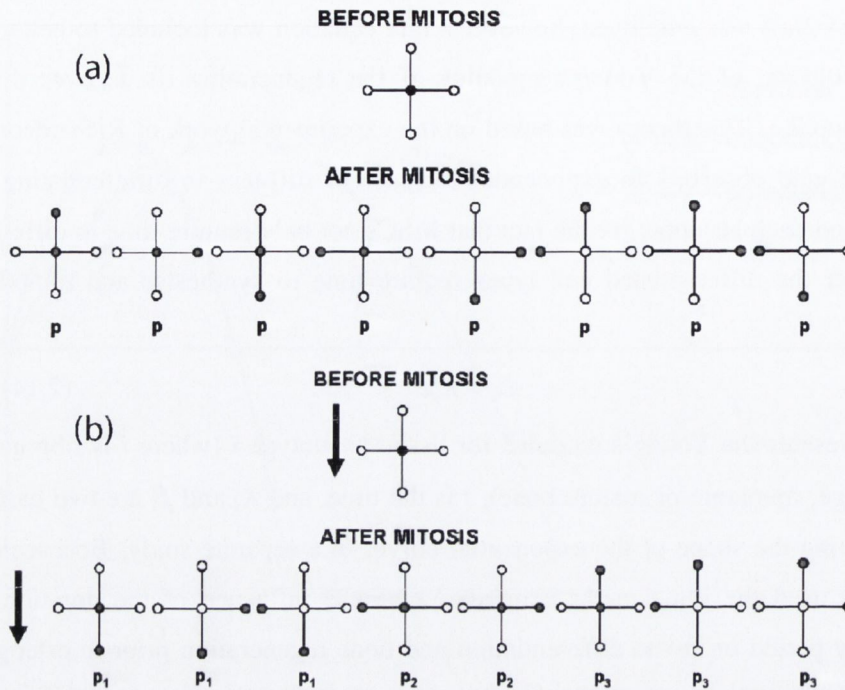


Figure 2-20: Possible states a daughter cell can occupy after mitosis using the random-walk method, developed by Pérez and Prendergast (2007); (a) when isotropic mitosis is assumed, the cells can occupy the neighbouring positions with equal probability (P), (b) with anisotropic proliferation direction the probability is greatest in the preferred direction (indicated by the arrow), such that $P_1 = 10P_2 = 60P_3$

2.6.4 Other computational models

In pursuit of a better understanding of biological processes in mechano-regulation simulations several authors have introduced new methods to account for the effect of mechanical stimuli on tissue differentiation. Researchers have also employed alternate techniques to describe cellular processes in mechano-regulation models.

Bailón-Plaza and van der Meulen (2001) proposed a bioregulatory model to explore the mechanisms of cell and growth factors during fracture healing. They used a system of partial differential equations to describe the effects of osteogenic and chondrogenic growth factors on cell phenotype, migration, proliferation/apoptosis and synthesis activities. The two-dimensional continuum model was able to simulate normal fracture healing, however the effects of the mechanical environment were not included. Geris *et al.* (2006) later adopted this mathematical model and applied it to simulate bone healing in a semi-stabilised

murine tibial fracture. A qualitative agreement between experimentally measured and numerical simulated results was observed. An attempt was also made to model situations of compromised fracture healing and to demonstrate the potential therapeutic value of bone regeneration models. The geometry of the two-dimensional model however was very simplified and the effects of mechanical loading were excluded. In Geris *et al.* (2003, 2004), the mechano-regulatory theories of Prendergast *et al.* (1997), and Claes and Heigele (1999) were applied to simulate tissue formation in a bone chamber attached to the proximal tibiae of rabbits. The *in vivo* chamber was developed to be able to control the local mechanical environment of the regenerate. Preliminary experimental results were compared to simulations of tissue differentiation and suggested that the applied mechano-regulatory models were able to qualitatively describe the regeneration process in the chamber.

Morgan *et al.* (2006) investigated the local physical environment within an osteotomy gap during long bone distraction osteogenesis and correlated tissue dilatation (volumetric strain) with differentiation of mesenchymal tissue. They evaluated the distributions of the local physical environment within and surrounding the distraction gap over one day of the distraction period. Large gradients in pressure, tensile strain, tissue dilation and fluid velocity were also found throughout the regenerate.

Sanz-Herrera *et al.* (2007) modelled bone tissue regeneration within a scaffold based on a mechanical stimulus (strain energy) and a sufficient level of cellular invasion. The microstructure of the scaffold was approximated by an idealised face cubic centred (FCC) distribution of pores. To evaluate the predictive power of the model, bone regeneration was simulated in a non-resorbable FCC scaffold implanted in the femoral condyle of a rabbit. The numerical results were qualitatively corroborated with the experimental results of Pothuau *et al.*, (2005).

In another fracture healing study Isaksson *et al.* (2007) used coupled partial differentiation equations to describe cell proliferation and tissue synthesis. This model was based on the way in which cellular activities control the evolution in concentrations of seven variables: MSCs, fibroblasts, chondrocytes and osteoblasts, as well as fibrous tissue, cartilage and bone. The biphasic mechano-regulation algorithm of Prendergast was used to regulate tissue differentiation. When compared to the diffusion model in a two-dimensional fracture repair simulation, the new cell

model predicted events observed during normal fracture healing, and captured phenomena which the tissue-level model did not.

2.7 Cellular automata

Cellular automata are examples of mathematical systems constructed from a regular lattice of 'cells', each simple, but together capable of complex behaviour (Wolfram, 1984). The state of a cell is updated according to a set of local rules at every discrete time step. That is, the state of a cell at a given time step depends only on its previous state and that of neighbouring cells. Cellular automata were originally proposed by John von Neumann in the 1950's as formal models of self-reproducing organisms. Von Neumann's initial design was founded upon the notion of one robot building another robot. Stephen Wolfram revived the field in the 1980's and published a series of papers on what he called "complex systems research" (Wolfram, 2002). The unexpected complexity of the behaviour of the cellular automata using simple rules led Wolfram to suspect that complexity in nature may be due to similar mechanisms. Later, physicists and biologists began to study cellular automata for the purpose of modelling in their respective domains. In the present era, cellular automata are being studied from many widely different angles, and the relationship of these structures to existing problems are being constantly sought and discovered. Some current applications include (i) simulations of biological systems, (ii) simulation of physical phenomena (heat-flow and turbulence), (iii) design of parallel computers and (iv) television graphics (Rucker and Walker).

Current mathematical models of natural systems are usually based on differential equations which describe the continuous variation of one parameter as a function of a few others. Cellular automata provide alternative and in some respect complementary models, describing the discrete evolution of many (identical) components. Models based on cellular automata are typically most appropriate in highly nonlinear regimes of physical systems, and in chemical and biological systems, where discrete thresholds occur. In addition, stochastic cellular automata can be used to model random physical phenomena using probabilistic parameters which can be adapted to the problem using evolutionary algorithms. In biology, particularly when modelling cellular activity, the complexity is such that new states in the solution are pre-programmed responses from the old state so that differential

equations cannot fully describe the evolution of the state. Consequently cellular automata techniques will be employed to model the cellular processes that occur in tissue regeneration.

2.8 Summary

Modelling of cellular processes in mechano-regulation simulations have been shown to greatly influence tissue differentiation; however no three-dimensional model yet exists which accurately accounts for these phenomena at a cellular level. As discussed previously, it is questionable whether simplified two-dimensional models adequately capture the structural characteristics of complex geometries, and consequently, whether the mechanical stimuli can be accurately determined. It is the contention of this thesis that the description of cellular processes needs to be more accurately defined to accurately simulate tissue regeneration.

Chapter 3

Simulation of tissue differentiation in a scaffold as a function of porosity, Young's modulus and dissolution rate^{*}

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^{*} This is a modified version of the paper first published in: Byrne D.P., Lacroix D., Kelly D.J., Planell J.A., and Prendergast P.J. "Simulations of tissue differentiation in a scaffold as a function of porosity, Young's modulus and dissolution rate: application of mechanobiological models in tissue engineering", *Biomaterials*, 2007, Vol. 28, pp. 5544-5554.

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3.1 Introduction

Scaffolds for bone tissue-engineering are subject to many interlinked and often opposing biological and structural requirements. Much empirical research has been performed to investigate the effect of scaffold properties on osteogenesis; however the optimal parameters governing scaffold performance remain to be understood. One of the key controllable design parameters for a scaffold is its porosity which should (i) facilitate the migration and proliferation of precursor cells, (ii) provide an appropriate microenvironment for cell proliferation and differentiation and (iii) allow for the mass transfer of nutrients and oxygen within the construct (Jones *et al.*, 2006). The conflicting nature of the requirements was described by, among others, Karageorgiou and Kaplan (2005) who reported that higher porosities result in greater bone ingrowth *in vivo* but that the resulting lowering of mechanical stiffness and strength sets an upper functional limit for porosity. Thus it would seem that the porosity of the scaffold must lie within a critical range (O'Brien *et al.*, 2004) – small enough to maintain the mechanical integrity of the scaffold and large enough to provide optimal bioactivity. Another design parameter that can be controlled, at least to some degree, is the rate of dissolution of a bioresorbable scaffold. Resorption will increase the porosity and, as a consequence, will reduce the strength and stiffness which increases the load transferred to the regenerate. Loading on the regenerate has been theorised and experimentally shown to directly influence the cellular and tissue differentiation patterns (Liebschner and Wettergreen, 2003). A variety of biodegradable scaffolds are used for the regeneration of bone. These materials may consist of natural and/or synthetic polymers. In particular, calcium phosphate ceramics have been shown to interact strongly and specifically with bone (Langstaff *et al.*, 2001). The mechanical strength and resorption rate of calcium phosphate biomaterials relate to their chemical compositions and manufacturing techniques (Huipin *et al.*, 2001). Generally these materials have a Young's modulus of approximately 1000 MPa (Adachi *et al.*, 2001) and depending on the site of implantation the scaffold should fully resorb within a few months. It is thought that calcium phosphate biomaterials resorb *in vivo* via chemical dissolution and cell-mediated degradation (Damien and Parsons, 1991; Yamada *et al.*, 1997).

Since the Young's modulus, porosity and dissolution rate have been shown to influence the rate and degree at which bone formation occurs within the scaffold, it should be possible to determine values for these parameters that maximize bone regeneration. Towards this effort computational analysis holds great promise in enhancing tissue engineering. Numerical simulations of bone growth into scaffolds based on mechanobiological models are relatively new. Sanz-Herrera *et al.* (2007) use a 2D finite element model to analyse bone growth within a scaffold implanted in the femoral condyle of a rabbit. Their results were corroborated against the experimental results of Pothuaud *et al.* (2005). Another approach proposed by Adachi *et al.* (2006) model bone regeneration in a unit cell of a three-dimensional scaffold microstructure. This study compares the effect of geometrical parameters on bone regeneration whilst undergoing scaffold degradation. Both studies use bone remodelling theories to model bone formation into the scaffolds; however employing tissue differentiation theories enables the prediction of other tissue phenotypes within the volume under analysis. Current computer simulations to predict tissue formation use mechano-regulation algorithms which predict how mechanical forces modulate tissue differentiation and bone remodelling (van der Meulen and Huiskes, 2002). Research on the relationship between mechanical forces and tissue phenotype began with Pauwels (1941). He proposed that the shear stress and the hydrostatic stress regulated the type of soft tissue formed within a fracture callus. Analyses correlating mechanical stimuli with tissue differentiation were developed further by Carter *et al.* (1988) who proposed that distortional strain history and hydrostatic pressure history regulate tissue differentiation. By quantifying the thresholds between bone, cartilage and fibrous tissue in mechanoregulation, Claes *et al.* (1998) successfully simulated fracture healing. Using a constitutive model of the tissue as poroelastic, Prendergast *et al.* (1997) proposed the stimuli on mesenchymal stem cell differentiation to be those causing cell distortion; i.e. the strain of the solid phase and the fluid flow. A poroelastic analysis not only determines the stresses and strains in the solid matrix but also the fluid velocity and pore pressure within the tissue. This mechano-regulation model has successfully predicted tissue differentiation observed experimentally around implants (Huiskes *et al.*, 1997). It has also been corroborated by predictions of observed fracture healing (Carter *et al.*, 1988; Claes *et al.*, 1998; Lacroix *et al.*, 2002(a); Lacroix and Prendergast, 2002(b); Lacroix and Prendergast, 2002(c)) and osteochondral defect healing (Kelly, 2003; Kelly and Prendergast,

2005). Geris *et al.* (2004) have also used it to analyse tissue differentiation inside a bone chamber placed in a rabbit tibia. In that study mechanoregulation by strain and fluid flow correlated best with experimentally-observed tissue formation patterns in the chamber. Isaksson *et al.* (2006) compared tissue differentiation stimuli against experiments in fracture healing and found that the algorithm based on strain and fluid velocity as stimuli best predicted experimental results. Most of these models used a diffusion equation to describe the proliferation of cells occurring within the regenerating tissue; however a random walk model gives a more general approach allowing not only for the simultaneous dispersal of several cell populations but also explicit modelling of cell proliferation (Pérez and Prendergast, 2007).

Given the advances in the predictive capability of mechano-regulation, it may now be possible to elucidate, in general terms, the possible interactions between the various design parameters in controlling successful bone regeneration by modelling cell proliferation, migration, and differentiation in a scaffolds. Therefore, the hypothesis of this study is that the extent of bone regeneration in a scaffold can be maximised by appropriate selection of porosity, Young's modulus, and dissolution rate, and that these parameters will be dependent of the magnitude of the local loading. If this hypothesis were confirmed it could open a new 'systems biology' approach to the design of scaffolds in tissue engineering.

3.2 Methods

3.2.1 Random-walk algorithm – simulations of migration and proliferation

To model the dispersal of the various cell populations in three-dimensions, a 'lattice' is created within each finite element of the granulation tissue; see Figure 3-1. Each lattice point is considered a region of space for both the cell and the extracellular matrix. The number of lattice points is determined by the dimensions of the element. Therefore the length, height and width of each finite element are divided by the average diameter of a cell (taken here as 25µm) to give the number of rows, columns and the depth of the lattice. Both cell proliferation and cell migration are based on a stochastic process consisting of a sequence of discrete steps of fixed lengths. To model proliferation a cell is initially presumed (in three dimensions) to be

surrounded by six possible locations that a daughter cell can occupy. Firstly a new position is randomly selected from the surrounding locations (including its original position). In turn one of the remaining neighbouring positions is then chosen for the daughter cell to occupy. In the event that the chosen location is already occupied another position is chosen again at random. This process continues until either the simulation ends or all lattice positions are occupied.

To model migration a new position is chosen at random from the surrounding locations (including its current position). Recognising that migration is a more rapid process, a new location for a migrating cell is chosen n times per iteration of the proliferation process. As fibroblasts are more motile during tissue differentiation than other cell populations, only fibroblasts migrate in the simulations presented here, with $n = 5$. Other cells disperse by proliferation only.

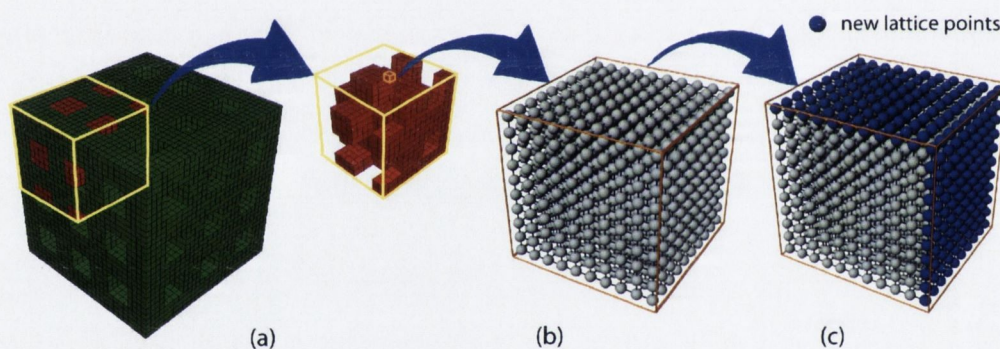


Figure 3-1: (a) FE model of a 50% porous scaffold with regular porosity (green). Only one-eighth needs to be modelled because of symmetry (yellow box). The cavity is initially occupied by granulation tissue (in red). (b) Lattice generated for each granulation element to model cellular activity, (c) increase lattice points to account for dissolution of scaffold material

3.2.2 Mechano-regulation of stem cell fate

According to the mechano-regulation algorithm for tissue differentiation (Huiskes *et al.*, 1997) magnitudes of shear strain γ and relative fluid/solid velocity v regulate tissue differentiation, according to the equation:

$$S = \frac{\gamma}{a} + \frac{v}{b} \quad (3.1)$$

where $a = 0.0375$ and $b = 3\mu\text{ms}^{-1}$ (Huiskes *et al.*, 1997), based on fitting a simulation to the experimental results of (Søballe *et al.*, 1992). Shear strain and fluid velocity are calculated from a biphasic poroelastic analysis and, next, differentiation of the mesenchymal cell population is simulated. Depending on the value of S , a tissue phenotype is predicted for each lattice point throughout the model. High stimulus levels of these stimuli promote the differentiation of mesenchymal cells into fibroblasts, intermediate levels stimulate the differentiation into chondrocytes, low levels of these stimuli promote the differentiation into osteoblasts and very low values promote resorption. The boundary values for shear strain and fluid velocity were taken from Lacroix and Prendergast (2002(b)), which were corroborated by Isaksson *et al.* (2006) against fracture healing histology of Claes *et al.* (1998).

3.2.3 Finite element model

A three-dimensional finite element model of a regular structured bone scaffold was used, giving a geometry similar to printed scaffolds (Wilson *et al.*, 2004). It is assumed that modelling begins once the granulation tissue has infiltrated the scaffold. Both the scaffold and the granulation tissue are modelled using poroelastic materials. 7mm^3 regions were modelled but symmetry allows a reduction to one-eighth of this by applying the appropriate boundary conditions. A force to generate an apparent pressure of either 2 or 4 MPa is applied vertically via a rigid plate; therefore an equal vertical displacement is applied to the surface nodes of the model, i.e. to both the solid phase of the scaffold and the regenerate. These magnitudes were correlated to stresses experienced in long bones (see Appendix A), where scaffolds are often used to promote osteogenesis. The load was ramped up over one second to the designated value.

Considering the surrounding tissue would not totally prevent the fluid from escaping the pore pressure on the outer surfaces of the granulation tissue was set to zero to simulate the free exudation of fluid. The scaffolds' porosity, mechanical properties, dissolution rate and applied loading conditions change according to Table 3-1. When modelling dissolution the volume of granulation tissue will increase and the scaffold volume will decrease. Applying a high dissolution rate will increase the scaffold porosity by 1% per iteration; therefore the size of all the scaffold elements will uniformly decrease resulting in an overall one percent reduction in volume while the scaffold sides remain orthogonal to each other. When this occurs, the dimensions

of each granulation element will consequently increase thus allowing for more lattice points within the elements. Therefore each cell lattice will be updated in accordance to the new dimensions as described in Section 3.2.1 above, see Figure 3-1(c).

Table 3-1: Matrix of parametric studies

	Low	Intermediate (BASELINE)	High
Porosity %	30	50	70
Young’s Modulus (MPa)	800	1000	1200
Dissolution Rate (%/iteration)	0	0.5	1
Loading (MPa)	-	2	4

3.2.4 Material properties

The cells within each lattice differentiate based on the stimuli calculated by the mechano-regulation algorithm. As it is likely that several tissues can coexist within one finite element, the mechanical properties are calculated using the rule of mixtures. The rule of mixtures accounts for both the number and phenotype of cells within each element and therefore the material properties will change gradually towards the phenotype determined by the stimulus. The material properties for the different tissue types are given in Table 3-2.

In certain instances this solution may not be adequate when describing the evolution of material stiffness over time. For example if the stimulus changes from predicting granulation tissue straight to bone (i.e. 0.2 MPa to 6000 MPa) averaging the values will not give an accurate representation of the new materials’ stiffness. Therefore based on Richardson *et al.* (1994) who observed an exponential increase in stiffness in differentiating tissue, a rate equation is used to better describe the evolution of the Young’s modulus of the regenerating tissue (Boccaccio *et al.*, 2007). The equation of describing the variation of the Young’s modulus is of the form:

$$E_i = K_i e^{\beta_i t} \tag{3.2}$$

where E_i represents the Young’s modulus for tissue phenotype i (where i is fibrous tissue, cartilage, immature or mature bone), t is the time and K_i and β_i are two parameters regulating the shape of the exponential curve (Boccaccio *et al.*, 2007; Boccaccio *et al.*, 2008). The values of K_i and β_i have been set so that the Young’s modulus of tissue phenotype i increases in 60 days from the initial value of 0.2 MPa,

typical of granulation tissue to the final values reported in Table 3-2. The time is based on the age of the cell; therefore the rate equation starts locally after the deposition of a certain tissue type.

Table 3-2: Material Properties of tissue phenotypes (Lacroix and Prendergast, 2002(b))

	Granulation tissue	Fibrous tissue	Cartilage	Immature bone	Mature bone
Young's Modulus (MPa)	0.2	2	10	1000	6000
Permeability (m ⁴ /Ns x 10 ⁻¹⁴)	1	1	0.5	10	37
Poisson's Ratio	0.167	0.167	0.167	0.3	0.3
Porosity	0.8	0.8	0.8	0.8	0.8
Bulk modulus grain (MPa)	2300	2300	3400	13920	13920
Bulk modulus fluid (MPa)	2300	2300	2300	2300	2300

The algorithm then calculates the Young's modulus for every element based on the exponential law and a simple rule of mixtures. For example, if $\bar{E}$ is the average Young's modulus for a particular element for the ten previous iterations and if n is the number of cell of phenotype i occupying the cell lattice, the Young's modulus in the next iteration ($iter+1$) will be given by:

$$E(iter+1) = \frac{n_i}{n_{max}} \cdot \bar{E} + \frac{(n_{max} - n_i)}{n_{max}} \cdot E_{granulation} \tag{3.3}$$

where n_{max} is the maximum concentration of MSCs which may occupy any one element domain, $E_{granulation}$ is the Young's modulus of the granulation tissue. As there is no evidence to suggest that the bulk modulus, permeability and Poisson's ratio increases exponentially with time; these mechanical properties are determined using the rule of mixtures.

3.2.5 Solution procedure

The simulation is performed using the finite element software Abaqus v 6.5-1 (Hibbit, Karlsson and Sorensen, Inc., 2005). Each simulation consists of three parts: finite element poroelastic analysis to compute the biophysical stimuli for each element in the cavity; cell proliferation and migration; and generation of a new model incorporating updated material properties, see Figure 3-2.

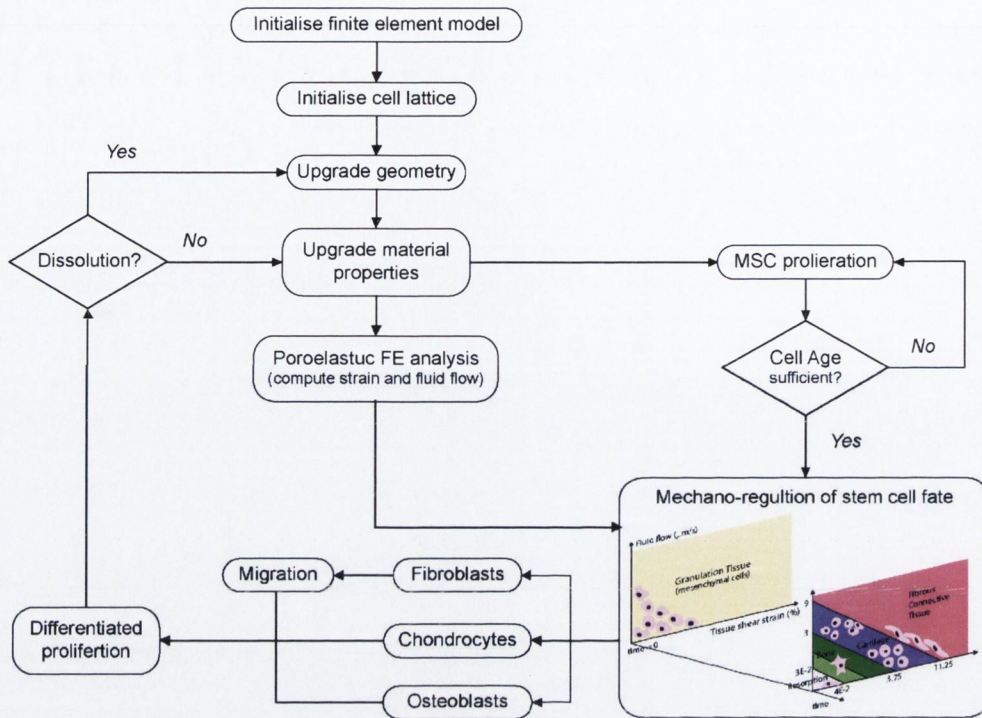


Figure 3-2: Computational algorithm to model tissue differentiation (including cell migration and differentiation)

The mechanical component of the model calculates the biophysical stimuli that influence the cellular processes. Each iteration the interstitial fluid flow and octahedral shear strain was determined at the peak of the ramp load, at which point the magnitudes were greatest. Initially the cavity of the scaffold is assumed to be occupied by granulation tissue. At this stage a small percentage of cells (1%) are randomly seeded within each lattice. These cells proliferate and migrate until they have become old enough to undergo differentiation (the minimum time is set at seven iterations). After this the MSCs may differentiate into fibroblasts, chondrocytes and osteoblasts depending on the mechanical stimulus determined by the mechano-regulation algorithm. The number of cells that differentiate in each lattice is established by multiplying the *differentiation rate* (assumed to be 0.3) by the number of MSCs that have reached the critical age (*iteration*>6) (Pérez and Prendergast, 2007). The cell specific parameters are outlined in Table 3-3. In the case of degradation the scaffold porosity will increase according to the rate of dissolution. As mentioned in Section 3.2.4 the material properties of each element will evolve according to a combination of the rule of mixtures and the rate equation. These

material properties are applied to a new scaffold and the process is repeated. The simulation completes once the material properties have reached equilibrium for more than 10 iterations.

Table 3-3: Cell parameter data for scaffold study

	MSCs	Fibroblasts	Chondrocytes	Osteoblasts (immature)	Osteoblasts (mature)
Differentiation rate	0.3/iter	-	-	-	-
Critical age	iter>6	-	-	-	-
Proliferation rate	1/iter	1/iter	1/iter	1/iter	1/iter
Migration rate	-	30 µm/hour	-	-	-
Resorption rate	-	-	-	0.3/iter	0.3/iter

3.2.6 Matrix of parametric studies

A matrix of parametric studies is generated to study the influence of a number of key factors on bone regeneration within a regular structured scaffold. To determine the effect of bone formation, a single parameter is varied while holding the others constant. There are eleven unique elements therefore 54 analyses are necessary to study the effect of each variable, see Table 3-1.

3.3 Results

3.3.1 Baseline results

For a scaffold having the baseline parameters, the simulation predicts the cellular activity within the regenerating tissue whereby the various phenotype patterns appear and disappear during regeneration, see Figure 3-3. Initially MSCs are randomly distributed throughout the granulation tissue. As the simulation progresses the MSCs proliferate, forming small clusters of cells (iteration 10); these cells differentiate based on the stimulus, forming connective tissue with new material properties. The stimulus predominantly favours osteogenesis leading to high amounts of bone within the centre of the scaffold and soft tissue at the periphery where stress concentrations occur. The initial porosity of 50% increases to 100% over the course of the simulation, due to the dissolution of the biomaterial. Ideally the scaffold material

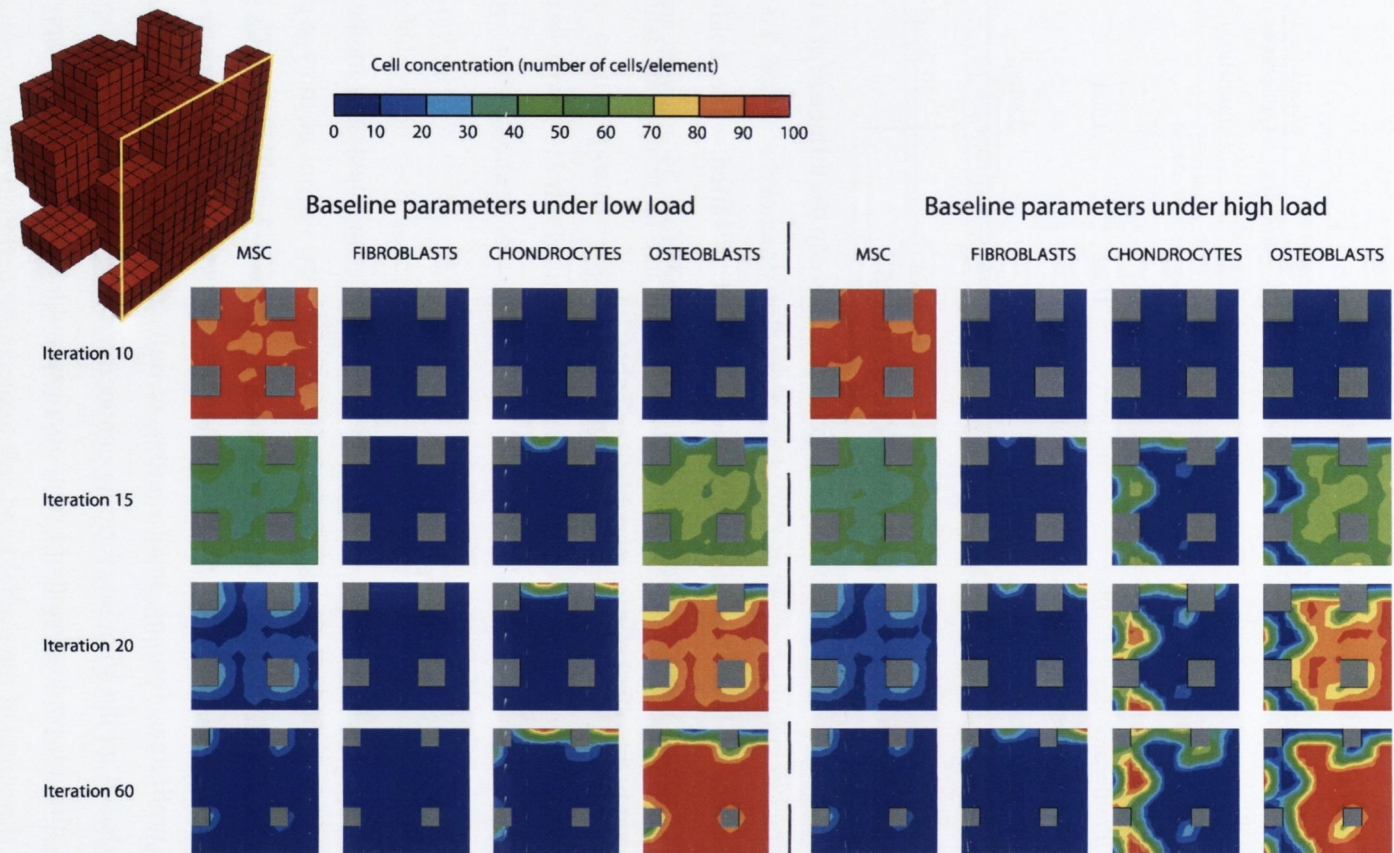


Figure 3-3: Predicted cell distribution in the granulation tissue, using the baseline parameters shown in Table 3-1. Note porosity increasing over time due to dissolution of the scaffold biomaterial, initial porosity of 50%

should fully resorb leaving bone tissue in its place. Each parameter in Table 3-1 has a significant effect on the outcome of the percentage bone formation. The influences of these parameters are outlined below.

3.3.2 Effect of porosity on baseline results

The relationship between bone formation and time, for baseline porosity is illustrated in Figure 3-4(a). Following an initial inactive period there is a sudden increase in the amount of bone formation. After 21 iterations there is approximately 50% bone occupying the space available for tissue regeneration. The rate of increase declines but the amount of bone increases steadily for the remainder of the simulation; ending in 81% bone formation. At equilibrium the remainder of the space is occupied, 2% fibrous tissue and 17% cartilage tissue. Increasing the initial porosity generates 8% less bone tissue and 6% more cartilage tissue whereas decreasing the initial porosity produces similar results – ending with 84% bone, 14% cartilage and 2% fibrous tissue.

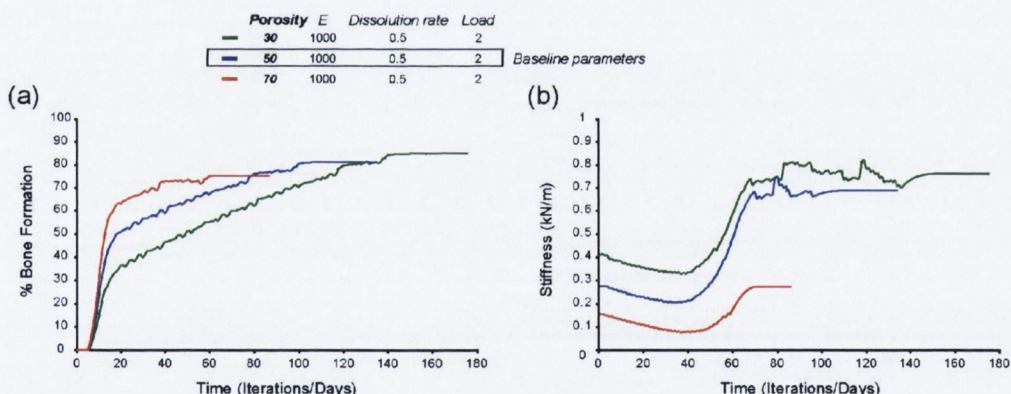


Figure 3-4: Effect of porosity on (a) the percentage bone formation and (b) the mechanical stiffness of the bone-scaffold system

The mechanical function in the regeneration process was also quantitatively evaluated by measuring the stiffness in the bone-scaffold system; Figure 3-4(b). The initial stiffness of the scaffold system is dictated by the porosity. Varying the baseline porosity increases or decreases the initial mechanical stiffness by approximately 0.12kN/m, which can be related to the additional quantity of scaffold material present at the beginning of the simulation. Initially there is a decrease in the system stiffness as the scaffold dissolves but it increases with bone formation over

time. Under the medium dissolution rate of 0.5% per iteration it takes the high porosity (70%) scaffold 60 iterations to completely degrade, while the low porosity (30%) scaffold takes 140 iterations to degrade. Consequently the higher porosities provide less support to the applied loads sooner, resulting in increased soft tissue formation and lower stiffnesses.

3.3.3 Effect of dissolution rate on baseline results

Degradation of the scaffold causes a steady decline in the systems stiffness; however the regenerating tissue causes this to increase after approximately 40 iterations. Thereafter the stiffness of the system increases significantly due to the amount of bone formation in the construct and the rate equation used to calculate the material properties. Without dissolution an effective maximum limit is set on the amount of bone formation; based on the original porosity of the construct, see Figure 3-5(a). In the model that does not incorporate dissolution 44% of the regenerating tissue differentiates into bone, from a possible 50%, see Figure 3-7 (on page 60). High dissolution rates initially generate much greater quantities of bone tissue as there more available space for tissue growth. However, with scaffold degradation the mechanical stiffness of the bone-scaffold system falls to a very low level (Figure 3-5(b)), thus promoting soft tissue formation. Medium dissolution rates give a good balance between creating enough space for bone formation and maintaining the mechanical integrity of the system. Consequently regeneration can occur in the granulation tissue without being exposed to excessive distortions.

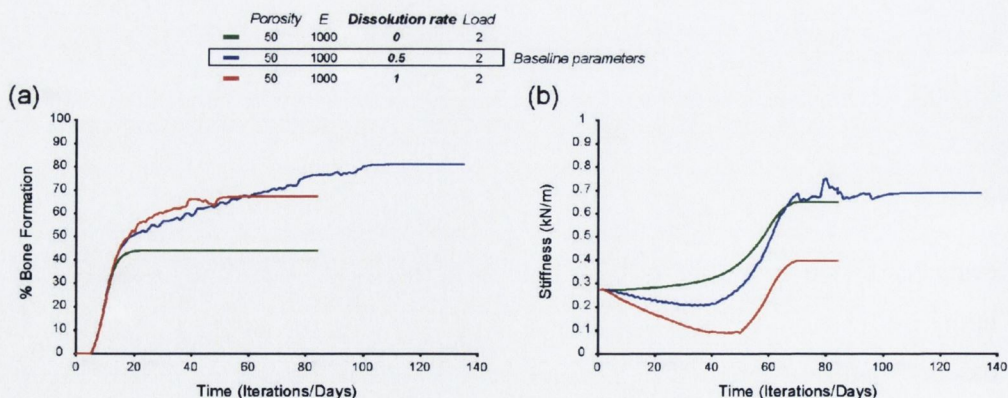


Figure 3-5: Effect of dissolution rate on (a) the percentage bone formation and (b) the mechanical stiffness of the bone-scaffold system

3.3.4 Effect of Young’s modulus on baseline results

The deviation from the baseline Young’s modulus of the scaffold biomaterial is not as great as the other parameters analysed in this study. For the parameters given in Figure 3-6(a) varying the Young’s modulus by ± 200 MPa has little effect on the amount of bone formation. Increasing the Young’s modulus generates approximately 9% more bone tissue and 9% less cartilage tissue. This difference in bone formation becomes more significant when the loading conditions, dissolution rates and porosity are also increased, see Figure 3-7. However, in all cases, higher mechanical properties improve the percentage bone formation. The initial mechanical stiffness of the construct is also affected by the scaffolds Young’s modulus; however, it does not have as big an impact as the initial porosity (compare Figure 3-6(b) and Figure 3-4(b)). If initial stiffnesses are too low at the beginning of the regeneration process excessive distortion will ultimately lead to the failure of the scaffolding system, this phenomenon is highlighted in Figure 3-7 (on page 61).

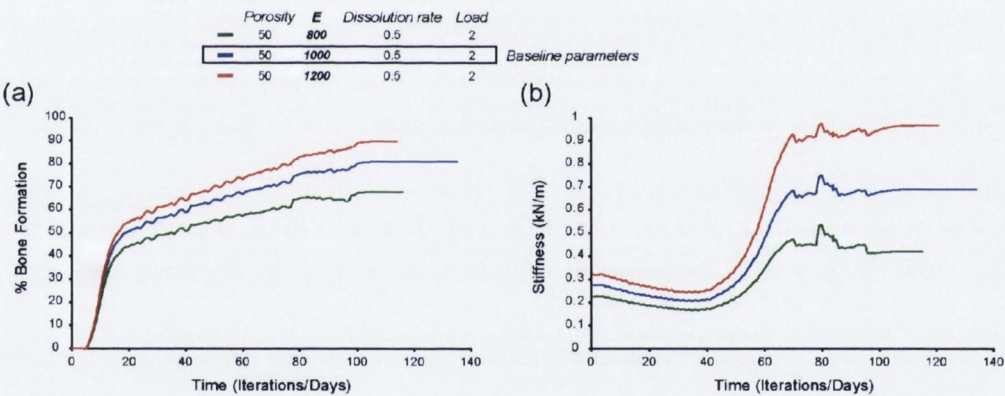


Figure 3-6: Effect of Young’s modulus on (a) the percentage bone formation and (b) the mechanical stiffness of the bone-scaffold system

3.3.5 Effect of loading conditions on baseline results

The magnitude of the load applied to the scaffold has a significant impact on the amount of bone formation, Figure 3-8(a). Increasing the applied load on the baseline parameters by 2 MPa reduces the amount of bone formation by 41.3% and increases the amount of cartilage formation by 28%. The high loading condition simulation fails to complete because the mechanical stiffness of the bone-scaffold drops below a

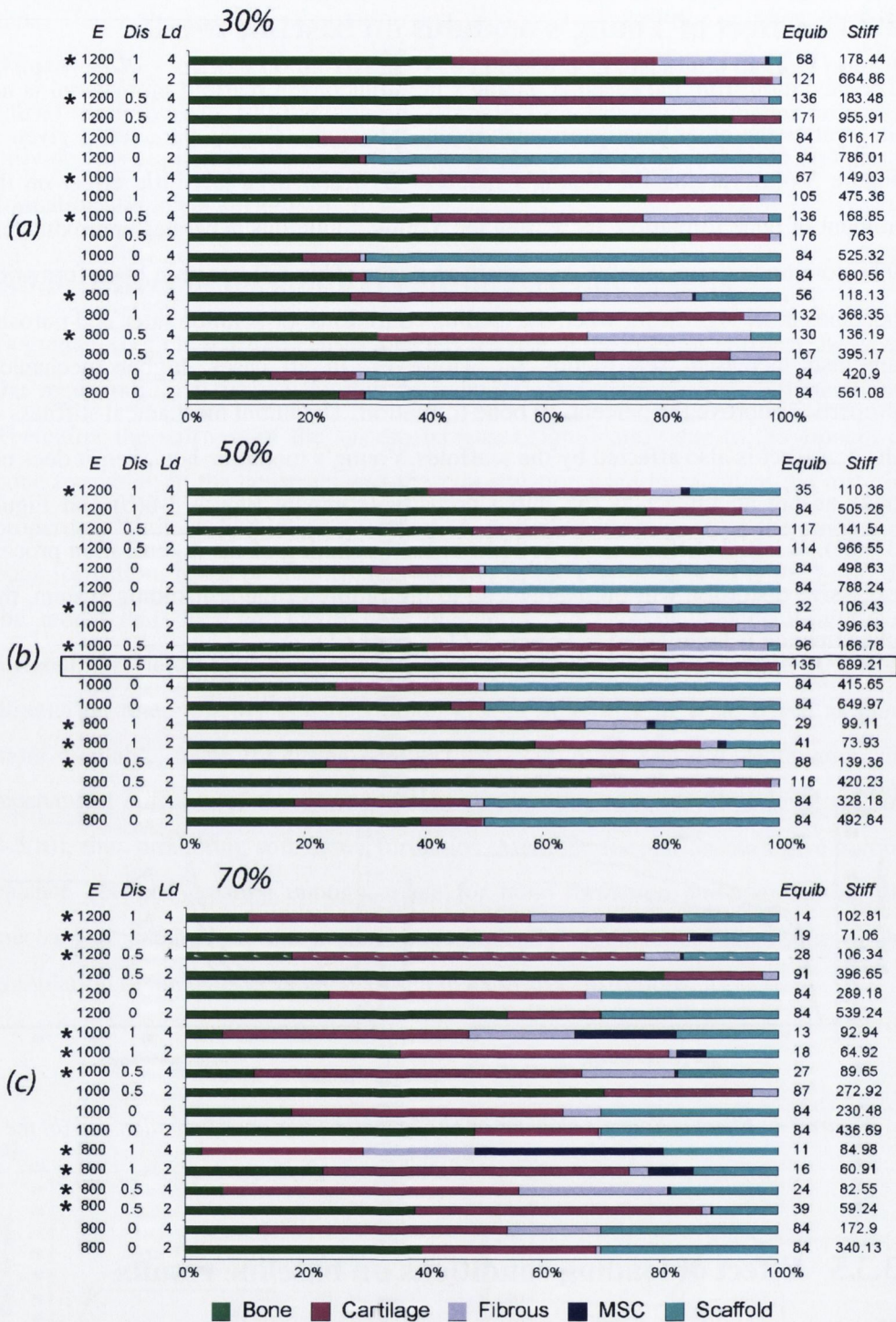


Figure 3-7: Percentage phenotype plot in (a) 30% (b) 50% and (c) 70% porous scaffolds once equilibrium or failure has been reached. The stiffness of the scaffold/bone system is shown in N/m. * Simulation did not complete due to excessive distortion

critical level which is not strong enough to support the load and the construct collapses, see Figure 3-8(b). The stiffness continues to decline after iteration 80 due to the dissolution of the scaffold material and the development of soft fibrous tissue in the peripheral regions. This can be seen in iteration 90 of Figure 3-3. In order to counteract high loading conditions the scaffold must possess high mechanical strength. This can be achieved by decreasing the porosity and rate of dissolution, and increasing the Young's modulus of the biomaterial. All the models produce more bone tissue in low loading environments; however, this is the one process that cannot be dictated by the designer.

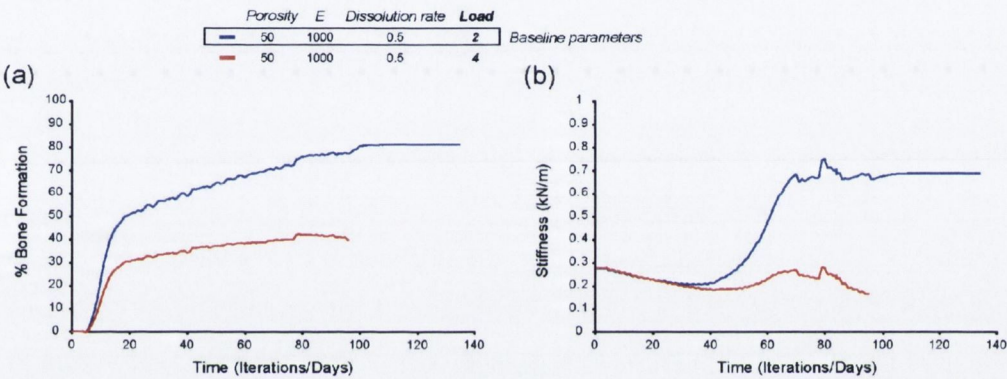


Figure 3-8: Effect of the loading environment on (a) the percentage bone formation and (b) the mechanical stiffness of the bone-scaffold system

3.3.5.1 Influence of applied ramp rate on baseline parameters

Due to the viscoelastic nature of the regenerating tissues, the rate at which the load is applied to the scaffold affects the outcome of the results. During loading, fluid pressure gradients are generated within the tissue; and the fluid phase is forced to flow through the solid phase, generating large drag forces as the fluid moves relative to the solid. Low rates (less than one second) therefore induce greater fluid pressures within the tissue, compared to higher rates (greater than one second). Consequently the lower rates generate higher mechano-regulatory stimuli and less bone formation. The effect of the ramp rate on bone formation for the baseline parameters is illustrated in Figure 3-9(a). Rates below 1.5 seconds have approximately the same effect on percentage bone formation and stiffness; see Figure 3-9(b). A rate of one

second was therefore applied in these analyses as physiological loads are usually brief (e.g. when walking or running).

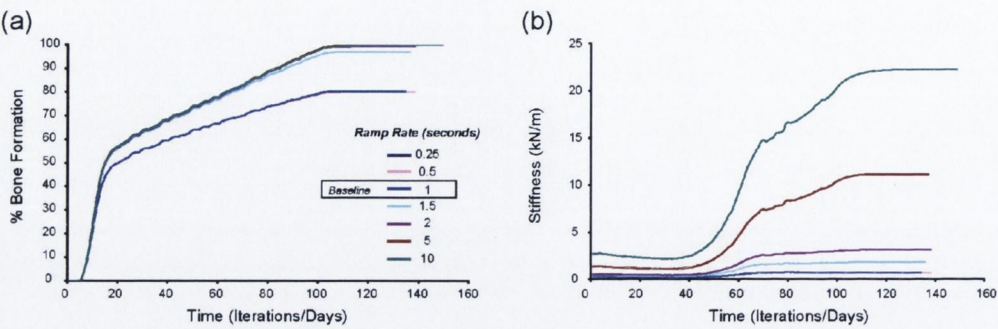


Figure 3-9: Influence of the applied ramp rate on (a) the percentage bone formation and (b) the mechanical stiffness of the bone-scaffold system under the baseline parameters

3.3.6 Results of parameter study

There is no apparent structured order for the scaffold parameters that give the greatest bone regeneration. Figure 3-10 shows the combinations of scaffold parameters that generate the greatest amount of bone tissue. Firstly it is evident that low loading environments, high Young's moduli and medium rates of dissolution promote greater bone formation. It is also important to note that while lower scaffold porosities produce the greatest amount of bone over long periods of time; higher initial scaffold porosities can attain similar results more rapidly.

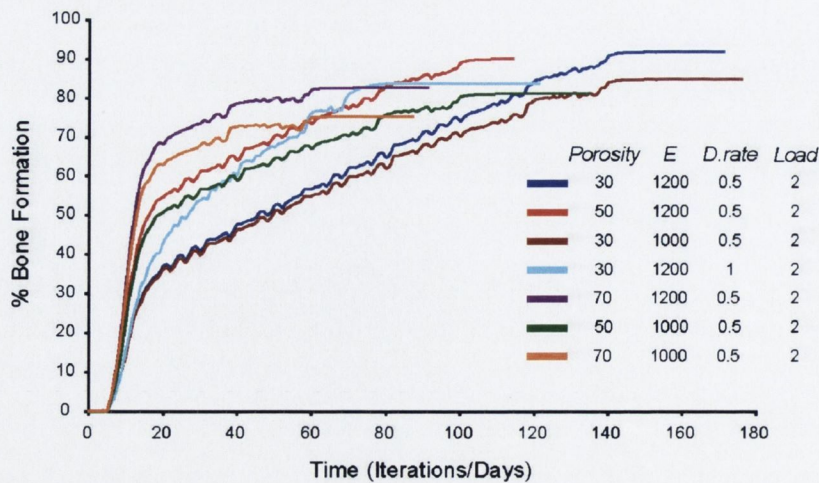


Figure 3-10: Scaffold parameters that produced the greatest amount of bone formation

3.4 Discussion

The objective of this study was to develop a tissue differentiation model for bone regeneration in a regular printed-type scaffold that would be able to maximise bone formation by appropriate selection of differing porosity, Young's modulus, and dissolution rate. As stated in the introduction this could facilitate a systems biology approach in tissue engineering. It was shown that all three design variables have a critical effect on the amount of bone regeneration, and unique combinations of these parameters can be used to optimise the output under both low and high loading conditions.

In order to model the tissue regeneration process some assumptions were made. The main biological assumption is that the cells can permeate through the entire volume until all the available space is colonised. In simulations presented here, this process takes approximately 21 iterations; however the time can vary due to the random nature of cellular processes. In bone tissue engineering high rates of nutrient and oxygen transfer at the surface of the scaffold promote the mineralization of the scaffold surface, thus limiting the transfer to the interior of the scaffold (Sachlos and Czernuszka, 2003). Galban and Locke (1999) mathematically model cell growth in a polymer scaffold to describe the effects of restricted transport of nutrients and products in the system on the rate of cell growth. Malda *et al.* (2004) model oxygen gradients within tissue engineered cartilage polymer scaffolds and they show that oxygen concentrations decrease with scaffold depth due to high cell concentrations at the peripheral boundaries of the constructs. Consequently the pioneering cells cannot migrate deep into the scaffold due to the lack of nutrients and oxygen and insufficient removal of waste products (Cheah *et al.*, 2003). Modelling this transfer through diffusion and creating a rule whereby differentiation can only take place in the appropriate biochemical milieu could contribute to the accuracy of the simulation. The second biological issue is that cellular apoptosis and/or necrosis was not accounted for. It is thought that regions within the scaffold experiencing high local stresses or fluid flows would have lower cell concentrations. Reducing the amount of cells within the granulation tissue would reduce the computed material properties, however there would have to be a significant amount of cell death to affect the outcome of the results. It has also been suggested that calcium phosphate biomaterials resorb *in vivo* in two ways: chemical dissolution and cell-mediated

degradation (Hollinger and Battistone, 1986; Damien and Parsons, 1991; Yamada *et al.*, 1997). These processes would not occur in a linear fashion as presented here; instead degradation would begin slowly and increase with time. Furthermore load-dependent degradation rates were not included though this could easily be done. It is clear that biomaterial degradability is a critical design criterion for achieving optimal tissue regeneration (Alsberg *et al.*, 2003; Adachi *et al.*, 2006) and despite the above limitation these models give a good indication of the implications of varying dissolution rates. Amending these assumptions may contribute to the accuracy of the simulation; however, the aim of this study was not to solve a real problem, but rather demonstrate the predictive abilities of the model.

This study presents a novel tool to investigate the effects of differences in scaffold parameters to maximise bone formation. The success of a bone scaffold as measured *in vivo* is determined by its ability to simulate and aid in both the onset and completion of bone defect repair (Liebschner and Wettergreen, 2003). As stated earlier the scaffold parameters are highly correlated. Firstly the porosity of the scaffold dictates the initial mechanical stiffness of the construct. Low porosities result in higher mechanical stiffness and therefore reduce the stresses acting on the granulation tissue. As low stimulus levels (calculated by the mechano-regulation algorithm) predict differentiation into osteoblasts these scaffolds produce high quantities of bone. Alternatively stimulus values increase with porosity, causing more cartilage and fibrous tissue formation in the intermediate and high scaffold porosities. The low porous scaffold will produce greater amounts of bone for the amount of space that is available for regeneration to occur; however the high scaffold porosities will proportionately produce more bone.

Dissolution of the scaffold material increases porosity. This leads to an increase in the amount of space available for bone regeneration; however it could also compromise the structural integrity of the scaffold due to a reduction in stiffness and strength. Predictions are that the rate of dissolution can either have a positive or negative effect on the amount of bone formation depending on the initial porosity and mechanical strength of the biomaterial. When dissolution is incorporated it is important to monitor the mechanical strength of the system. Initially the scaffold must restore the temporal mechanical function and sustain the entire load of the defective region. As dissolution progresses the regenerating tissue must begin to take over this task while the biomaterial degrades. If the bone-scaffold system is not

strong enough to support the loads at this stage the construct will collapse. Figure 3-8 (b) shows that the scaffold experiencing high dissolution rates degrades too quickly and is not able to withstand the applied load. As the scaffold porosity increases with dissolution, the stiffness decreases. When this gets below a minimum value (approximately 0.15kN/m) the scaffolding construct will crumble. In order to prevent such an occurrence the initial material properties must be strong enough to withstand the applied loads – as a result the initial scaffold porosity should be quite low or the initial mechanical properties should be strong enough to sustain the loads while the material becomes weaker with dissolution. Therefore finding a balance between porosity and the rate of degradation (because it increases the available space) is pivotal in determining the optimal scaffold parameters. It is also important to note that deviation from the baseline Young's modulus of the scaffold biomaterial is not as great as the other parameters analysed in this study. Very small Young's moduli would have detrimental effects on the integrity of the construct, while higher values could potentially solve problems relating to mechanical strength. However, due to the limited amount of biomaterials available for scaffold production and the techniques involved in manufacturing, these large stiffnesses are not always attainable.

This model provides a platform to predict and maximise scaffold properties for specific geometries, thus reducing the number of experimental studies necessary to validate design performance. Multi-factorial regression techniques could also be employed to optimise the combination of parameters to predict maximal bone formation. Experimentally validating this model would involve implanting a 7mm³ scaffold into a bone defect in an animal model and making histological measurements of tissue phenotype at several time points.

The most significant finding was the effect of the loading conditions on amount of bone formation. Due to the differences in size, bone cross sectional area and body weight, the stress levels on each bone can vary greatly between individuals. In this regard scaffolds should be tailored for the site of implantation. In a low loading environment, high porosities and higher stiffness but a medium dissolution rate gives the greatest amount of bone over the shortest amount of time. Alternatively the initial porosity and rate of dissolution should be lower in a high loading environment in order to maintain the mechanical and structural integrity of the bone-scaffold system. Towards this effort, rapid prototyping (RP) or solid free form (SFF) fabrication technologies are currently being used to manufacture

scaffolds that could be optimised for site-specific applications (Cheah *et al.*, 2003). These techniques have proven to be particularly beneficial in tissue engineering, as they offer advantages including customised external shape and predefined and reproducible internal morphology, which not only can control pore size, porosity and pore distribution, but can also make structures to increase the mass transport of oxygen and nutrients throughout the scaffold (Sachlos and Czernuszka, 2003).

3.5 Conclusion

A new three-dimensional approach is used to model regenerating tissue within a scaffold. Cellular activities such as proliferation and migration are modelled on random-walk theory while the mechano-regulation algorithm models tissue differentiation. It is believed that this technique improves on previous diffusion models and holds the potential for more realistic simulations in tissue engineering. The results presented in this chapter show that scaffold porosity and the rate of dissolution are pivotal in determining optimal scaffold parameters. Scaffolds may also be optimised to suit site-specific loading requirements indicating the need for greater control over scaffold manufacturing techniques.

Chapter 4

The effect of loading on bone regeneration within regular structured scaffolds^{*}

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4.1 Introduction

Tissue engineering of bone involves regeneration of the tissue within a defect in the host. Scaffolds are used to restore temporal mechanical function whilst inducing the formation of bone from the surrounding tissue. In order to achieve this aim the scaffolding architecture must conform to many interlinked, and often opposing biological and structural requirements. As discussed in the previous chapter much empirical research has been performed to investigate the effect of scaffold properties on osteogenesis; however the optimal parameters governing scaffold performance remain to be understood. Depending on the biomaterial and manufacturing technique some of the design parameters can be controlled to a certain extent; examples are pore size, porosity, interconnectivity and the mechanical stiffness of the scaffold. The site of implantation and consequently its loading environment cannot be controlled however. Due to the differences in size, bone cross sectional area and body weight, the stress levels on each bone can vary greatly between individuals, therefore depending on the individual and the site of implantation the scaffold must sustain many different loading environments (Liebschner and Wettergreen, 2003). Consequently, when a scaffold is implanted in a donor site the mechanical stimulus experienced at a local level will be specific to the individual patient - it could be argued that the scaffold should be selected with this in mind.

A variety of scaffold types are being used for the regeneration of bone (Section 2.3.2). Currently bioprinting, which is a biomedical type of rapid prototyping (RP), and solid free form (SFF) fabrication technologies are being used to manufacture scaffolds that can be optimized for site-specific applications (Cheah *et al.*, 2003). These techniques create the scaffolds by adding material as opposed to the traditional techniques that remove material to create the final part; therefore they have proven to be particularly beneficial in tissue engineering, as they offer advantages including customized external shape and predefined and reproducible internal morphology, which can control pore size, porosity and pore distribution. As the loading environment is one process that can not be predicted by the designer, investigating the influence of various loading conditions on bone formation within a scaffold could prove to be very beneficial in designing scaffold parameters. In this regard numerical models are invaluable to view complex systems, and can be used to

improve the understanding of biological and biomedical problems. Current computer simulations to predict tissue formation use mechano-regulation algorithms for predicting how mechanical forces modulate tissue differentiation and bone remodelling (van der Meulen and Huiskes, 2002; Martin *et al.*, 2007). Isaksson *et al.* (2006) demonstrated that the most consistent mechano-regulation algorithm proposed by Prendergast *et al.* (1997) employed both octahedral shear strain and fluid velocity as biophysical stimuli to successfully predict tissue differentiation. In the preceding chapter the effect of scaffold porosity, Young's modulus and dissolution rate were analysed under both steady low and high loading conditions. As the healing progresses however, the loads acting on a scaffold are more likely to increase. In this study a mechano-regulation algorithm is employed to determine tissue differentiation both in terms of the prevailing biophysical stimulus and the distribution of precursor cells, where cell number is computed based on a three-dimensional random-walk approach (Pérez and Prendergast, 2007). This work will investigate the effect of a low-to-high ramp load on bone formation in a regular structured printed scaffold and compare the results to the low and high loading regimes investigated in the previous chapter.

4.2 Methods

4.2.1 Random walk algorithms

To model the dispersal of the various cell populations in three-dimensions, a 'lattice' is created within each finite element of the granulation tissue. The number of lattice points is determined by the dimensions of the element. Therefore the element length, height and width are divided by the average diameter of a cell (taken here as 25 μ m) to give the number of rows, columns and the depth of the lattice.

Both cell proliferation and cell migration are based on a stochastic process consisting of a sequence of discrete steps of fixed lengths. To model proliferation a cell is initially presumed (in three dimensions) to be surrounded by six possible locations that a daughter cell can occupy, see Figure 4-1. Firstly a new position is chosen at random from the surrounding locations (including its original position). In turn one of the remaining neighbouring positions is then chosen for the daughter cell to occupy. In the event that the chosen location is already occupied, another position

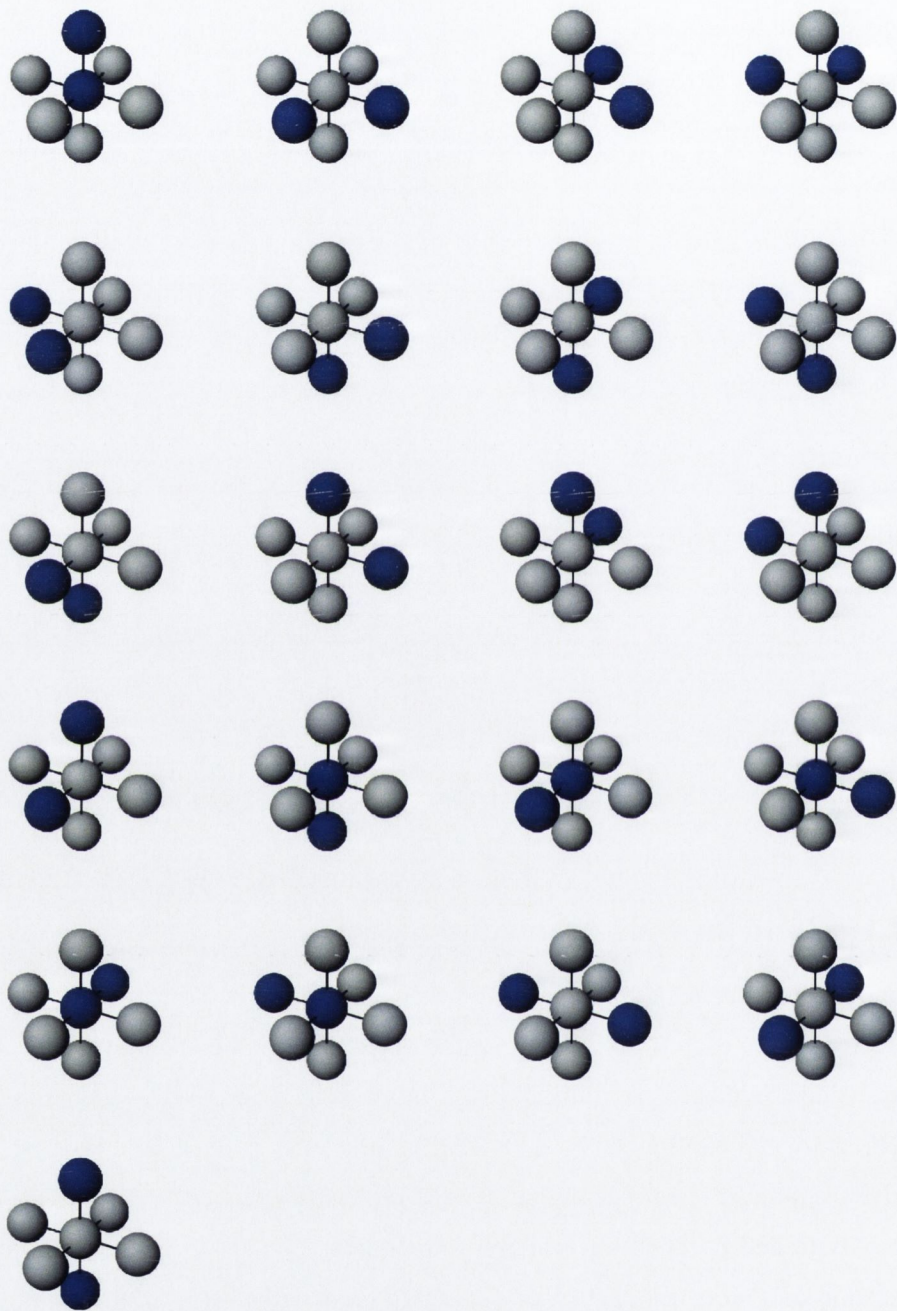


Figure 4-1: Twenty-one states a proliferating lattice point can occupy provided the surrounding locations are vacant. The distance between the sites is only schematic; adjacent sites in the algorithm are considered to be exactly the diameter of a lattice point (25 μ m)

is chosen at random from the surrounding locations (including its current position). Again migration will not occur if there are no unoccupied lattice points neighbouring the cell. As an initial condition for the simulations presented in this study, MSCs occupy 1% of the lattice points which are assigned randomly.

4.2.2 Mechano-regulation of stem cell fate

A mechano-regulation algorithm based on octahedral shear strain γ and fluid velocity v proposed by Prendergast *et al.* (1997) was used to simulate the generation of specific mesenchymal tissues. The following equation relates a stimulus S to a tissue phenotype:

$$S = \frac{\gamma}{a} + \frac{v}{b} \quad (4.1)$$

where $a = 0.0375$ and $b = 3\mu\text{ms}^{-1}$. The threshold values for this algorithm were initially determined from experimental models of bone formation around implants (Huiskes *et al.*, 1997). Shear strain and fluid velocity are calculated from a biphasic poroelastic simulation and, subsequently, differentiation of mesenchymal cells is simulated. Depending on the value of S , a tissue phenotype is predicted - high stimulus levels of these stimuli promote the differentiation of mesenchymal cells into fibroblasts, intermediate levels stimulate the differentiation into chondrocytes, low levels of these stimuli promote the differentiation into osteoblasts and very low values promote resorption. The boundary values for shear strain and fluid velocity were taken from Lacroix and Prendergast (2002(b)) which were corroborated against fracture healing histology of Claes *et al.* (1998).

4.2.3 Computational simulation

Similar to the last chapter, the iterative computational scheme consists of three parts - finite element poroelastic analysis to compute the biophysical stimuli for each element in the regenerating tissue; cell proliferation and migration; and generation of a new model incorporating updated material properties. An apparent pressure which rises from 2 MPa to 4 MPa over 60 iterations is applied vertically via a rigid plate. This ensures an equal vertical displacement is applied to the surface nodes of the model, i.e. to both the solid phase of the scaffold and the regenerate. The axial load is applied over one second. Again the simulation completes once the material properties have reached equilibrium for more than 10 iterations.

4.3 Results

The magnitude of the load applied to the scaffold at the beginning of the simulation has a significant impact on the amount of bone formation. Initially osteogenesis dominates tissue differentiation in the regenerate; however this trend declines after approximately 20 iterations (Figure 4-2(a)). As the amount of bone formation decreases, the space being made available by the dissolving biomaterial is replaced by soft tissue, which can be attributed to the increasing load. This is evident by the difference in percentage bone formation between the baseline parameters and the ramp load simulation. At equilibrium 65% of the space is occupied by bone, 25% cartilage and 10% fibrous tissue.

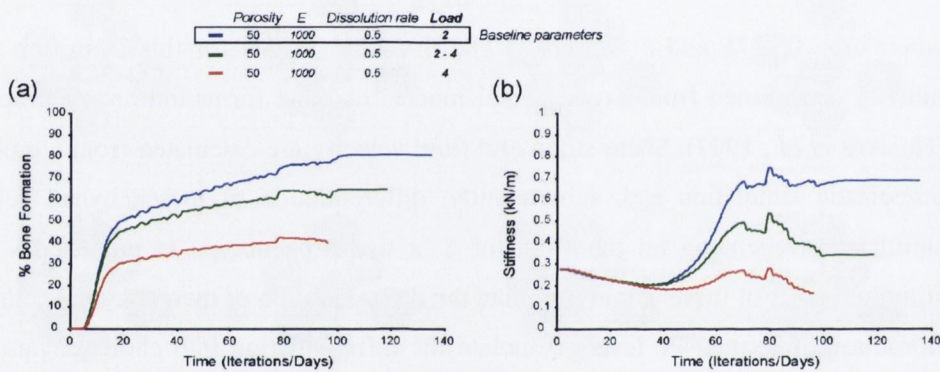


Figure 4-2: (a) The effect of the loading environment on (a) the percentage bone formation and, (b) the mechanical stiffness of the bone-scaffold system

The mechanical function in the regeneration process was also quantitatively evaluated by measuring the stiffness in the bone-scaffold system; see Figure 4-2(b). The initial stiffness of the scaffold is dictated by the magnitude of the applied force. The stiffness decreases slowly over time due to the dissolution of the scaffold, however the regenerating tissue causes this to increase at approximately 40 iterations. The rate of escalation for the ramp simulation levels off earlier than the baseline parameters due to the rising force applied to the scaffold and the greater formation of soft tissues; Figure 4-3. Under the medium dissolution rate, 100 iterations are required for the complete resorption of the 50% porous scaffold. Therefore the stiffness of the construct will continue to decline unless the regenerating tissue is able to endure the applied loads. As more bone is formed within the low loading scaffold the stiffness is greatest at the end state.

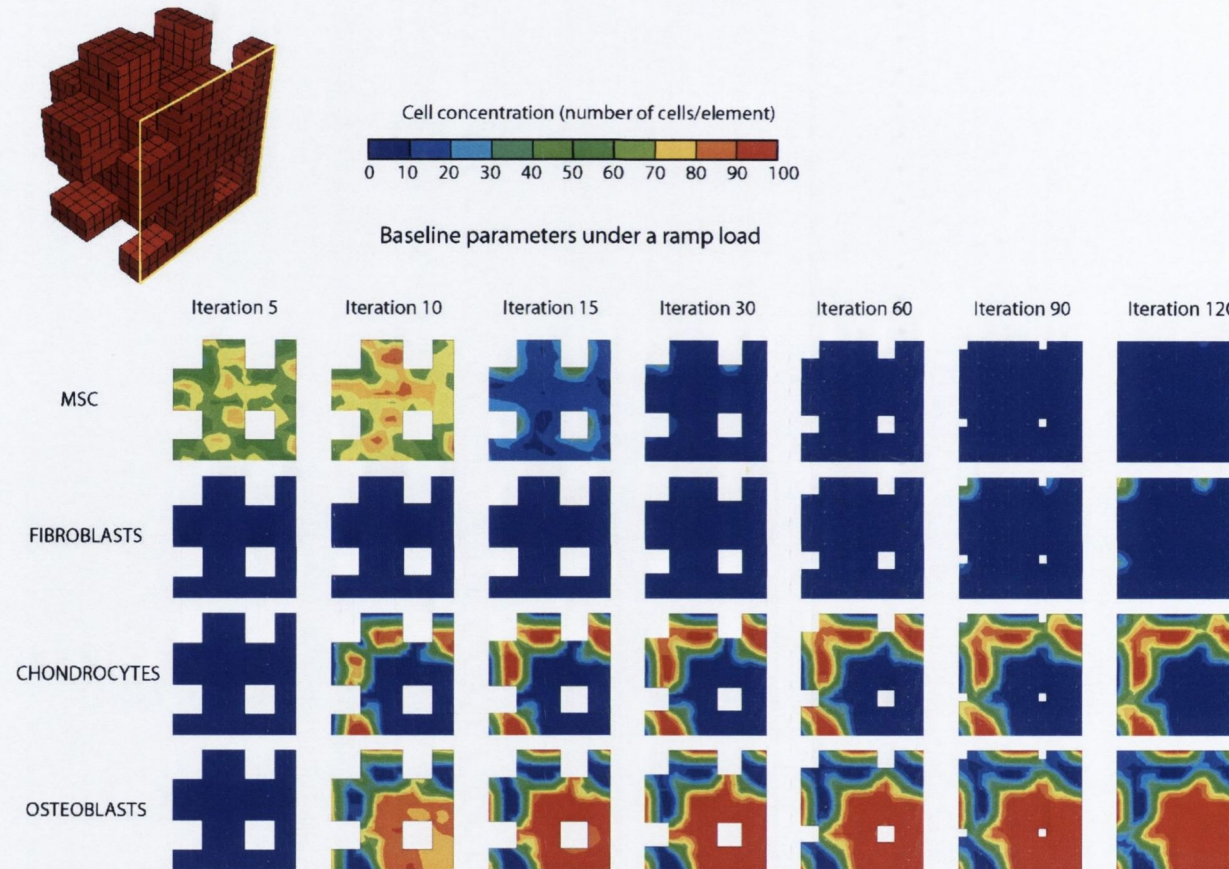


Figure 4-3: Predicted cell distribution in the granulation tissue, using the baseline parameters under ramp loading. Note the porosity increasing over time due to dissolution of scaffold biomaterial

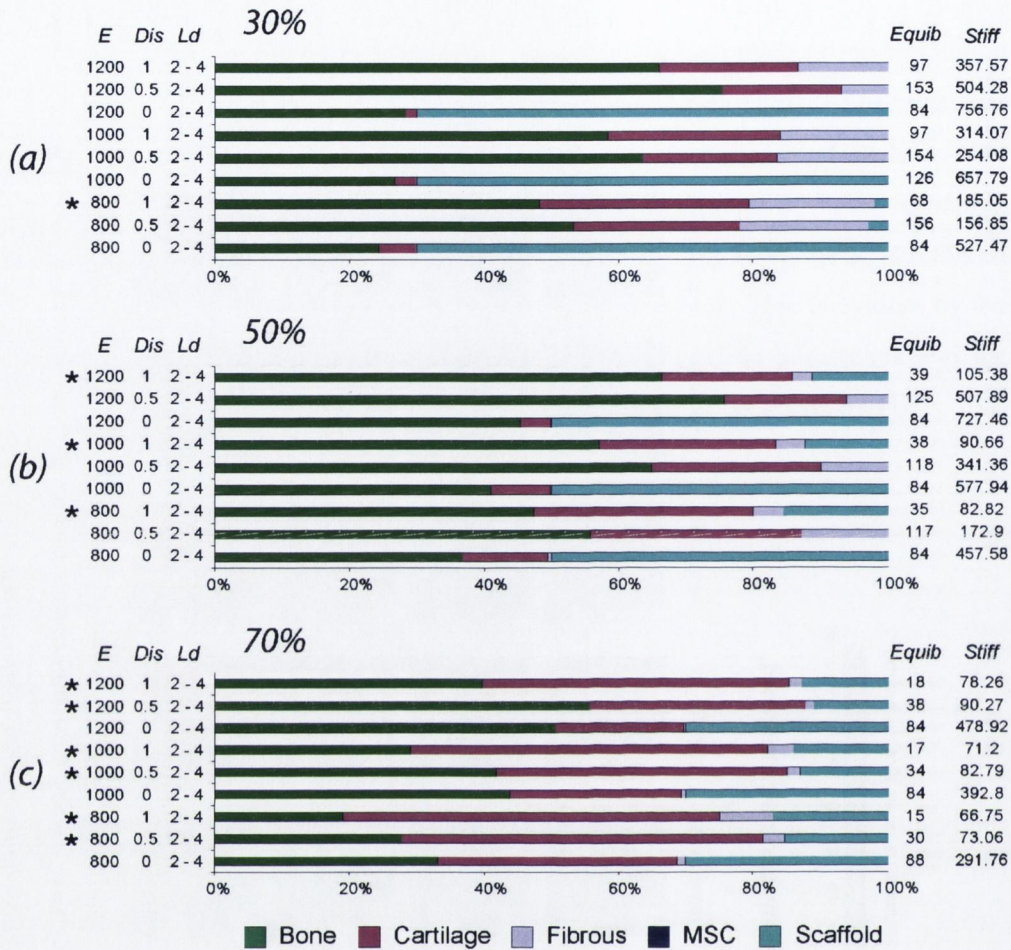


Figure 4-4: Percentage tissue phenotype plot in (a) 30% (b) 50% and (c) 70% porous scaffolds. Simulation ends once equilibrium has been reached or the bone-scaffold system fails due to excessive distortion (denoted by *). Stiffness is in N/m

4.4 Discussion

This study presents a computational tool for investigating the effects of loading conditions on bone formation within a regular structured scaffold. The success of a bone scaffold as measured *in vivo* is determined by its ability to simulate and aid in both the onset and completion of bone defect repair (Liebschner and Wettergreen, 2003). In general the regenerating tissue in the scaffolds under ramp-loads generated more bone than the steady high loads and less bone than the steady low loads. Both the stiffness and the percentage bone formation were closer to the results generated

under the steady low loads; thus highlighting the importance of the applied loading in the initial stages of healing. Lower loads exert less stress on the regenerating tissue; therefore low stimulus levels (calculated by the mechano-regulation algorithm) predict differentiation into osteoblasts. Scaffolds conducive to bone formation in the initial stages of healing allow for the development of a system that will be able to withstand the applied forces as time progresses. Similar to the previous study medium rates of scaffold dissolution and high Young's moduli encourage bone formation, as resorption of the scaffold increases porosity and subsequently the amount of space available for regeneration; while stiffer scaffold biomaterials are more capable of resisting the applied loads.

Since the magnitude and variability of the loading environment varies with the individual, the scaffold design parameters must be optimised to generate maximum bone formation. It was shown that the scaffold systems produce more bone tissue in low loading environments. Therefore, in order to counteract high loading conditions and consequently high stress/strain concentrations, the scaffold must possess high mechanical strength. This can be achieved by decreasing the initial scaffold porosity, and increasing the Young's modulus of the biomaterial. It is suggested that scaffolds be optimised to suit site-specific loading requirements; thus indicating the need for greater control over scaffold manufacturing techniques.

Chapter 5

Mechano-regulation simulation of fracture healing using a lattice modelling approach

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5.1 Introduction

Experimental and clinical studies have shown that insufficient mechanical stimulation delays the initial stages of healing whereas excessive movement inhibits ossification, delays healing and leads to a lack of stability (Goodship and Kenwright, 1985; Aro and Chao, 1993; Claes *et al.*, 1998; Kenwright and Gardner, 1998; Lacroix, 2000). Approximately 5-10% of fractures fail to heal successfully and develop into delayed or non-unions (Einhorn, 1995); which can result in long periods of immobilisation, pain or even bone deformities. The main cause for unsuccessful bone healing is the rapid formation of soft connective tissue which may disturb or totally prevent osteogenesis in the fracture site. To prevent such occurrences surgical intervention is often required to help re-establish the structural integrity of the broken bone through fracture stabilisation methods. The majority of fractures are stabilised with plaster casts; however complex fractures, fractures through joint surfaces and fractures with extensive soft-tissue damage require rigid fixation – such as intramedullary nails, screws and plates or external fixators (Wraight and Scammell, 2006). The loading on the regenerating tissues at the site of fracture is, in turn, determined by the method of fixation and the physical activity of the patient. Determining the appropriate stimuli to promote optimal bone regeneration in this situation remains a challenge and has been the subject of much research.

5.1.1 Mechanobiology and fracture healing

Mechanobiology is a sub-field of biomechanics concerned with the mechanism by which biological processes are regulated by signals to cells that are induced by mechanical loads (van der Meulen and Huiskes, 2002). Integrating all the information available from *in vivo* and *in vitro* mechano-biological experiments would be very complex or even impossible; instead, mathematical models that simulate these systems are used to quantitatively determine the rules that govern the effects of mechanical loading on cells and tissues. This allows the discovery of ‘algorithms’ for differentiation, growth, and adaptation and maintenance of bone. Combining computer models with mechano-regulation theories of tissue differentiation have been applied, in particular, to fracture healing as it is known that fracture healing is modulated by mechanical loading and induced motion; and

temporal variation in cellular events and tissue morphology are well characterised. The vast knowledge already accumulated concerning the wide variety of conditions resulting in different healing responses e.g. fracture geometry, loading magnitudes and rates and loading directions, also have the potential to increase our understanding of mechano-regulation rules (Claes and Ito, 2005).

Carter *et al.* (1998) were the first to use computational models to explore the relationship between local stress/strain levels and differentiated tissue types. They modelled the tissue in the fracture callus as a single solid to investigate endochondral ossification during fracture healing, and were able to successfully predict the differentiation patterns in distraction osteogenesis; however, no specific stress/strain levels were quantified. Later Claes and Heigele (1999) predicted the course and type of fracture healing using amounts of strain and hydrostatic pressure to determine the differentiation of tissue types in the callus tissue. They investigated isolated stages of fracture healing to determine how mechanical signals control cell differentiation, and specified limits for when intramembranous ossification or endochondral ossification would occur, as well as differentiation into fibrous tissue. These analyses used elastic material models to represent the differentiating tissue; however the extracellular matrix of developing tissues is soft, and consists of both fluid and solid materials. Prendergast *et al.* (1997) therefore proposed that mesenchymal stem cell differentiation is based on stimuli causing cell distortion; i.e. the strain of the solid phase and the fluid flow. Using a constitutive model of the tissue as poroelastic both the stresses and strains in the solid matrix and the fluid velocity and pore pressure within the tissue can be determined. Lacroix and Prendergast (2002(a), 2002(b)) applied this poroelastic model to a two-dimensional model of a fractured tibia and simulated the dispersal of the mesenchymal cells within the callus using a diffusion process. They assumed differentiation of the migrating mesenchymal cells into bone, cartilage and fibrous tissue based on the local tissues shear strain and fluid velocity. This approach allowed successful prediction of the osteotomy gap size effect on the healing process. Isaksson *et al.* (2006) compared the different mechano-regulation theories proposed by Carter, Claes and Heigele and Prendergast and found that the tissue differentiation patterns predicted by the algorithm based on deviatoric strain and fluid velocity was closest to experimental results. It was also the only algorithm able to predict healing with torsional loading as seen *in vivo*. This model has been successfully employed by others to predict tissue differentiation patterns in many

areas of bone healing, such as osseointegration around implants (Prendergast *et al.*, 1997; Andreykiv *et al.*, 2005), fracture healing (Lacroix, 2000; Lacroix and Prendergast, 2002(c); Andreykiv *et al.*, 2007), osteochondral defect healing (Kelly and Prendergast, 2005; Kelly and Prendergast, 2006), regeneration of an osteotomized mandible (Boccaccio *et al.*, 2008) and also to analyse tissue differentiation inside a bone chamber placed in a rabbit tibia (Geris *et al.*, 2003; Geris *et al.*, 2008).

Lacroix (2000) recognised that modelling stem cell access to the regenerating region had a considerable effect on the healing pattern and the healing rate. After his work the focus of these models shifted towards incorporating a more accurate description of cellular processes. The cells are an active part of the system which sense mechanical stimuli and react via proliferation, migration, differentiation or apoptosis. In many previous models diffusion has been the method used to model cellular migration and proliferation. This approach implicitly assumes that cells attempt to achieve a homogenous population density within the area of analysis, and is thought to be an oversimplification. In their most recent study of fracture healing, Isaksson *et al.* (2008) combined the mechano-regulation algorithm of Prendergast *et al.* (1997) with a set of coupled non-linear differential equations to describe transport/migration, proliferation, differentiation and apoptosis of cells. They used a two-dimensional finite element model of a long bone osteotomy to evaluate the model's potential and found that the cell-phenotype specific processes are very important to take into account, as they largely determine the outcome of the simulations.

5.1.2 Objectives

While there have been recent developments of more mechanistic computational simulations, the accuracy of the finite element models have been limited. As the cellular processes are dependent on the biophysical stimuli determined from the finite element model it is important to accurately characterise realistic geometries and loading conditions. Lacroix (2002(c)) highlighted that most previous models do not adequately capture the structural characteristics of the bone, as many assume a cylindrical bone with axial load conditions (axisymmetrical analysis), or only consider the deformation and stresses in a plane (plane stress or plane strain analysis). The objective of this work is to model fracture healing in a human tibia

with an external fixator, under loading conditions that are as physiological as possible and accounting for stochastic cellular processes using a 'lattice' approach. Cell motility is central to many biological processes. Simpson *et al.* (2007), emphasised that continuum models such as diffusion based models are insufficient to capture cell-scale properties as they are regulated by partial differential equations and therefore do not offer the advantages of discrete cell-scale cellular automata models.

The study further aims to model beyond the reparative phase of fracture healing; by describing the evolution of bone material properties over time. If this approach proves feasible it offers the possibility of using computer simulations in the clinical treatment of complex fractures, and in other orthopaedic applications where bone regeneration occurs.

5.2 Methods

5.2.1 Mechano-regulation

Following Prendergast *et al.* (1997), tissue differentiation is regulated by magnitudes of shear strain (γ) and relative fluid/solid velocity (v) in the extracellular matrix, according to the equation:

$$S = \frac{\gamma}{a} + \frac{v}{b} \quad (5.1)$$

where $a = 0.0375$ and $b = 3\mu\text{ms}^{-1}$, based on the work of Huiskes *et al.* (1997) who used these values to successfully predict the patterns of tissue differentiation observed experimentally by Søballe *et al.* (1992). Shear strain and fluid velocity are calculated from a biphasic poroelastic finite element analysis and, based on the value of S , the local mesenchymal cell population can differentiate into the following cell phenotypes and synthesise a new tissue type:

- $3 < S$: Fibrous connective tissue
- $1 < S < 3$: Cartilage
- $0.53 < S < 1$: Osteoblast – immature woven bone
- $0.01 < S < 0.53$: Osteoblast – mature woven bone
- $S < 0.01$: Resorption

The boundary values for shear strain and fluid velocity were taken from Lacroix and Prendergast (2002(b)) which were corroborated by Isaksson *et al.* (2006) against the fracture healing histology of Claes *et al.* (1999).

5.2.2 Modelling cellular activity

The entire callus was initially assumed to consist of granulation tissue, into which mesenchymal stem cells could migrate and proliferate. The precursor cells originate from the periosteum, endosteum, and marrow space at the site of the damaged cortical bone tissue (Postacchini *et al.*, 1995; Gerstenfeld *et al.*, 2003). Tissue differentiation, cell proliferation, migration and apoptosis are regulated by the local stimulus, determined from the mechano-regulation algorithm as described below.

5.2.2.1 Cell proliferation and migration

The random-walk approach is used to model the dispersal of the various cell populations in three-dimensions using a global 'lattice'. The size and density of the global lattice is determined from the dimensions of the fracture callus and the volume of each lattice point (taken here as $80 \mu\text{m}^3$). All cell types are assumed to be the same size, and each lattice point is considered a region of space for both the cell and the extracellular matrix. In order to initialise the random-walk method it is necessary to determine which lattice points lie inside each finite element and which of these points lie on the boundary surfaces through which MSC infiltration occurs, see Appendix B. Proliferation and migration can then be modelled on an unbiased nearest-neighbour random walk approach, as described in Chapter 3. In accordance with experimental observations cell motility is modulated by crowding effects; thus the rate of cell division decreases with time due to limitations in space. It has also been reported that the rate of cell division is regulated by mechanical stimuli (Buckley *et al.*, 1988; McMahon *et al.*, 2008); therefore proliferation of the differentiated cell phenotypes was assumed to only occur in the appropriate mechanical stimuli field. Other factors such as oxygen tension (Brighton *et al.*, 1991) and growth factors (Bronner *et al.*, 2003) which are not explicitly modelled in these simulations affect the rate of proliferation; as such a proliferation rate of 0.6 per iteration was used for the differentiated cell phenotypes, which equates to a doubling time of 40 hours.

Recognising that migration is a more rapid process than proliferation, a new location for a migrating cell is chosen n times per iteration of the proliferation process. As mesenchymal stem cells and fibroblasts are more motile during tissue repair than other cell populations, only these cell types are assumed to migrate. This relates to a migration rate of 30 μ m/hour in accordance with Saltzman (2004). Other cells disperse by proliferation only, see Table 5-1.

5.2.2.2 Cell differentiation and maturation

The number of cells that differentiate in the lattice is established by multiplying the *differentiation rate* by the number of MSCs that have reached the *critical age* (Pérez and Prendergast, 2007), see Table 5-1. Once a cell has been stimulated down a mesenchymal lineage the differentiated cell requires time to synthesise and remodel new tissue. To describe the variation of the Young's modulus at a lattice point the following rate equation is used:

$$E_i = K_i e^{\beta_i t} \quad (5.2)$$

where E_i represents the Young's modulus for tissue phenotype i (where i is fibrous tissue, cartilage, immature or mature bone), t is the time and K_i and β_i are two parameters regulating the shape of an exponential curve (Boccaccio *et al.*, 2007). The values of K_i and β_i have been set so that the Young's modulus of tissue phenotype i increases in 15 iterations from the initial value of 0.2 MPa, typical of granulation tissue to the final values reported in Table 5-2. As time is based on the age of the cell the rate equation starts locally after the deposition of a certain tissue type.

The mechano-regulation is capable of predicting differentiation of cells into immature or mature woven bone, both of which are intermediate stages in bone repair. As healing progresses woven bone is gradually replaced by secondary lamellar bone (cortical or trabecular as the site dictates) (Martin *et al.*, 1998). Once the rate equation is complete it is necessary to model this transition if the mechano-regulation stimulus persists in a woven bone field. The material properties (Young's modulus and permeability) of immature woven bone will evolve into mature woven bone over a period of 30 iterations; while mature woven bone will slowly transform into cortical bone over 200 iterations, see Figure 5-1.

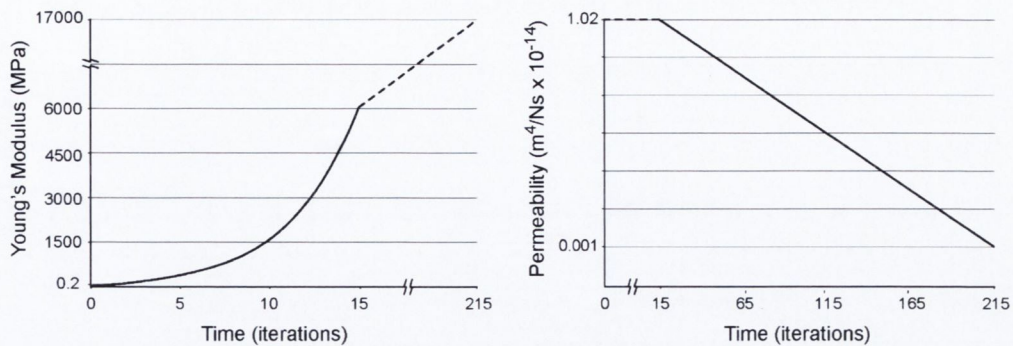


Figure 5-1: (a) If the mechano-regulation stimulus remains in the mature woven bone field a rate equation is used to model the synthesis of new tissue over the initial 15 iterations. Thereafter the transition from woven bone to secondary lamellar bone is modelled by linearly increasing the Young's modulus and (b) linearly decreasing the permeability over time

5.2.2.3 Cell apoptosis

The exact relationship between biophysical stimuli and cell apoptosis has not been widely investigated. Kelly and Prendergast (2005) proposed a non-linear relationship between mitosis/cell death and the magnitude of strain experienced by the cells. In this study apoptosis of the differentiated cell phenotypes was assumed to only occur outside that cell phenotypes' mechanical stimulus field. Therefore cells subject to a biophysical stimulus outside their field will begin to apoptose over time, based on a cell death rate of 0.3 per iteration. The cells chosen to apoptose are randomly selected in each element.

Table 5-1: Cell parameter data

	MSCs	Fibroblasts	Chondrocytes	Osteoblasts (immature)	Osteoblasts (mature)
Differentiation rate	0.3/iter	-	-	-	-
Critical age	iter>6	iter>6	iter>6	iter>6	iter>6
Proliferation rate	1/iter	0.6/iter	0.6/iter	0.6/iter	0.6/iter
Migration rate	30 μ m/hour	30 μ m/hour	-	-	-
Maturation time	-	-	15 iter	15 iter	15 iter
Apoptosis rate	-	0.3/iter	0.3/iter	0.3/iter	0.3/iter
Resorption rate	-	-	-	0.3/iter	0.3/iter

5.2.3 Finite element model

The same three-dimensional geometry of a human left tibia as used by Lacroix and Prendergast (2002(c)) was employed to model bone healing. A fracture gap of 3 mm was simulated with a homogeneous external callus with a callus index of 1.4. A unilateral external fixator with two pins was modelled and inserted in the anterior-medial side of the tibia, see Figure 5-2.

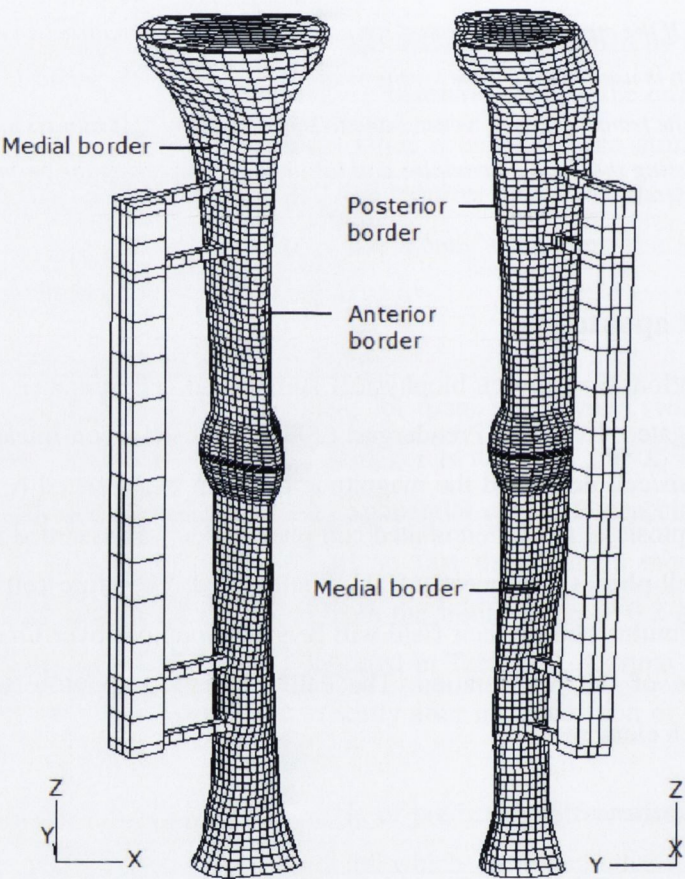


Figure 5-2: Three-dimensional mesh of the tibia with a fracture callus. A generic external fixator was inserted in the anterior-medial side of the tibia (Lacroix and Prendergast, 2002(c))

5.2.4 Material properties

The cortical bone and external fixator were modelled as linear elastic materials, while all other tissues were modelled as biphasic poroelastic materials. As cells

within each element differentiate based on the mechano-regulation stimuli it is likely that several tissues can coexist within one element; therefore the mechanical properties are calculated using the rule of mixtures. The rule of mixtures accounts for both the number and phenotype of cells within each element and therefore the material properties will change gradually towards the phenotype determined by the stimulus. The material properties for the different tissue types are given in Table 5-2. As there is no evidence to suggest that the bulk modulus, Permeability and Poisson's ratio increases exponentially with time; these mechanical properties are determined using the rule of mixtures only. Thus the Young's modulus for every element is determined by the rate equation and a simple rule of mixtures. For example, if $\bar{E}$ is the average Young's modulus for a particular element for the ten previous iterations and if n is the number of cell of phenotype i occupying the cell lattice, the Young's modulus the next iteration ($iter+1$) will be given by:

$$E(iter+1) = \frac{n_i}{n_{max}} \cdot \bar{E} + \frac{(n_{max} - n_i)}{n_{max}} \cdot E_{granulation} \tag{5.3}$$

where n_{max} is the maximum concentration of MSCs which may occupy any one element domain, $E_{granulation}$ is the Young's modulus of the granulation tissue. As there is no evidence to suggest that the bulk modulus, permeability and Poisson's ratio increases exponentially with time; these mechanical properties are determined using the rule of mixtures only.

Table 5-2: Material properties of tissue phenotypes adapted from Lacroix (2000)

	Young's Modulus (MPa)	Poisson's Ratio	Permeability (m ⁴ /Nsx10 ⁻¹⁴)	Porosity	Bulk modulus grain (MPa)
Granulation	0.2	0.167	1	0.8	2,300
Fibrous	2	0.167	1	0.8	2,300
Marrow	2	0.167	1	0.8	2,300
Cartilage	10	0.167	0.5	0.8	3,400
Immature	1,000	0.3	10	0.8	13,920
Mature	6,000	0.3	37	0.8	13,920
Cortical	17,000	0.3	0.01	-	-
Fixator	200,000	0.3	-	-	-

The permeability of bone is a contentious issue; as reported values used in previous mechano-regulatory simulations were based on well organised trabecular bone free of marrow. To date variations of over six orders of magnitude have been measured in trabecular bone $3.71 \times 10^{-14} \text{ m}^2$ to $1 \times 10^{-8} \text{ m}^2$ which may be attributed to its structural anisotropy and the wide range of trabecular architectures and volume fractions for the various sites (Nauman *et al.*, 1999). To the author's knowledge the permeability of woven bone tissue is not known; however it is likely that *in vivo* permeabilities would be lower due to the presence of soft tissue within the pores. Consequently a lower permeability value of $1.02 \times 10^{-14} \text{ m}^2/\text{Ns}$ was used in this study, and a parametric study on permeability was performed, see Table 5-3. The permeability values employed by Lacroix and Prendergast (2002(c)) are used in Test 1, while lower permeability values for mature woven bone are used in Test 2 and 3. In Test 4, immature woven bone was assigned a higher permeability value normally associated with mature woven bone, while the permeability of mature woven bone was assigned a lower value closer to that of cortical bone.

Table 5-3: Permeability parametric study (in m^4/Ns)

	Test 1	Test 2	Test 3	Test 4
Immature woven bone	1×10^{-13}	1×10^{-13}	1×10^{-13}	3.7×10^{-13}
Mature woven bone	3.7×10^{-13}	1.02×10^{-15}	1.02×10^{-16}	6.85×10^{-16}

5.2.5 Loading conditions

Duda *et al.* (2002), derived muscle and ligament attachment data, force magnitudes and orientations from Brand and scaled them to a tibial model for the second peak in ground reactions during gait (45% of gait cycle). A body weight of 80 kg was assumed and only those muscles attaching to the tibia were included as single straight lines and made to match appropriate node co-ordinates, see Table 5-4. The directions of the forces were based on a right-handed Cartesian co-ordinate system with its origin at the centroid of the tibio-calcaneal articular surface. The z-axis pointed to the most proximal point on the intercondylar eminence. The x-axis was perpendicular to a line through the most medial point on the medial malleolus and the most lateral point on the fibular notch and was oriented frontally. The y-axis was orientated laterally. Following Duda *et al.* (2001), the total knee load was split 60%-40% on the medial and lateral sides respectively. In order to restrict rigid-body

motions the nodes at the base of the model were restrained in the x and y directions and 2 nodes on the external fixator were fully restrained.

The physiological loading conditions acting on a fractured human tibia with an external fixator are not precisely known. After an initial period of rest early weight-bearing is prescribed to encourage healing, and to generate the strains at the fracture site necessary to promote callus formation (Kenwright *et al.*, 1991). It has previously been observed that for patients healing without complication weight bearing through the injured tibia increases steadily with time post-fracture (Duda *et al.*, 1997; Aranzulla *et al.*, 1998; Joslin *et al.*, 2008). The weight bearing achieved during healing can provide information on the extent of loading and motion applied to the fracture site. The simulation begins after the fracture callus has formed, by which time the patient should resume cautious load-bearing (Frost, 1986). The loading acting on the tibia will vary depending on a number of factors such as, the severity of the fracture, fixation method used, the patient's age, weight, height, fitness, bone quality etc.; therefore the loading profile and hence the healing time line is patient specific. In this study, the loading profile is based on the general shape of the weight-bearing achieved by patients on their fractured leg; the healing period, however, was estimated due to the variation observed among patients in the experimental studies mentioned above, see Figure 5-3.

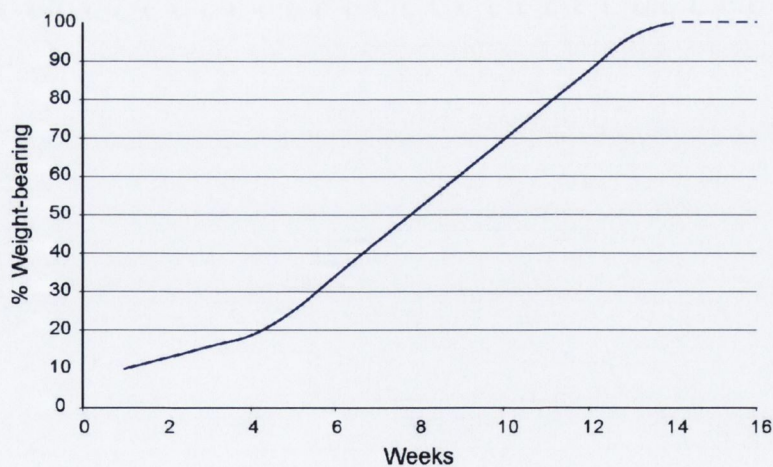
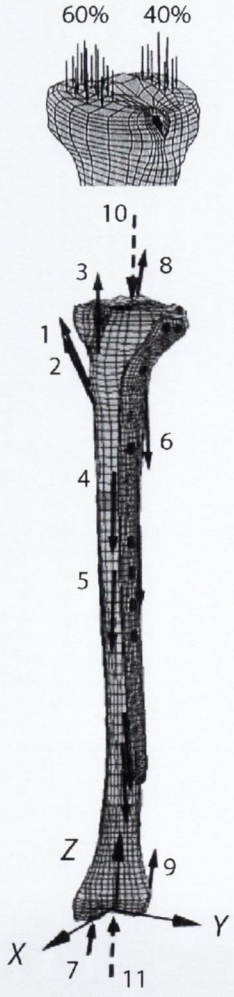


Figure 5-3: The relationship between percentage weight-bearing and time used to correlate weight to joint and muscle forces acting on a healing tibia

Table 5-4: System of forces and attachment co-ordinates used in the analysis. Components of muscle and joint contact forces and coordinates of attachment points on the surface of the tibia are reported for the second peak in the ground reactions during gait (45% of gait cycle)

		Force (N)			Attachment (mm)		
		X	Y	Z	X	Y	Z
1	Iliotibial tract I	-8.5	-8.8	61.3	-19.8	39.1	369.9
2	Iliotibial tract II	-97.4	-64.4	291.5	-27.1	28.2	370.5
3	Quadriceps femoris m.	13.6	-32.8	303.5	34.8	19.3	353.4
4	Tibialis anterior m. I	17.2	38.7	-327.7	-4.9	9.9	251.8
5	Tibialis anterior m. II	25.9	53.6	-191.8	-7.6	9.9	127.9
6	Soleus m.	-63.1	-47.1	-697	-7.4	15	332.2
7	Ant. tibiofibular lig.	-132.4	-111.2	-56.8	-7.3	11.1	0.9
8	Ant. cruciate lig.	87.5	101.5	41.1	10.3	-5	390.5
9	Deltoid lig.	44.9	9.7	15.7	-1.5	-16.5	-0.3
10	Knee	232.3	214.9	-1528.1	1.5	-2	388.2
11	Ankle	-120	-154.4	2070.4	0.8	0.6	0.4



5.2.6 Iterative simulation

The simulation begins with a poroelastic finite element analysis of the fractured tibia with an external fixator, using Abaqus v 6.5-1 (Hibbit, Karlsson and Sorensen, Inc., 2005). Initially the callus was assumed to be filled with granulation tissue; while mesenchymal stem cells began to proliferate and migrate from the periosteum, endosteum, and marrow space at the site of the damaged cortical bone tissue. The tissue shear strain and relative fluid velocity were calculated at the peak of the load each iteration. A tissue phenotype was then predicted for each callus element according to the mechano-regulation algorithm. The cellular processes of the tissue phenotypes i in each element were regulated by the resulting stimulus such that:

- MSCs differentiate into cell type i , based on the differentiation rate and the number of MSCs that have reached the critical age,
- 60% proliferation of cell type i , and zero proliferation of all other cell phenotypes,
- cell types not under the current stimulus apoptose, based on the cell death rate.

All cellular processes were chosen randomly in each element. If the stimulus enters the resorption field the osteoblasts within that finite element were deleted from the lattice at a rate of 0.3 per iterations Fully resorbed elements however, were deleted from the finite element mesh (apart from elements in the medullary cavity, which were assigned marrow material properties). As mentioned in Section 5.2.4, the material properties of each element in the callus will evolve according to a combination of the rule of mixtures and the rate equation. These resulting material properties were then applied to a new simulation and the process was repeated, as illustrated in the flow chart in Figure 5-4.

A four-point bend test in the sagittal plane was also simulated at every stage of tissue differentiation to predict the evolution of bending stiffness during healing, as illustrated in Figure 5-5. Deflection of the bone was calculated using linear elastic properties of the predicted tissue phenotypes. A bending stiffness was defined as the bending moment divided by the angle of deflection angle α , see Equation 5.4 and Figure 5-5. According to Richardson *et al.* (1994), a stiffness of 15Nm/degree provides a definition of union in tibial fractures. The external fixator was therefore

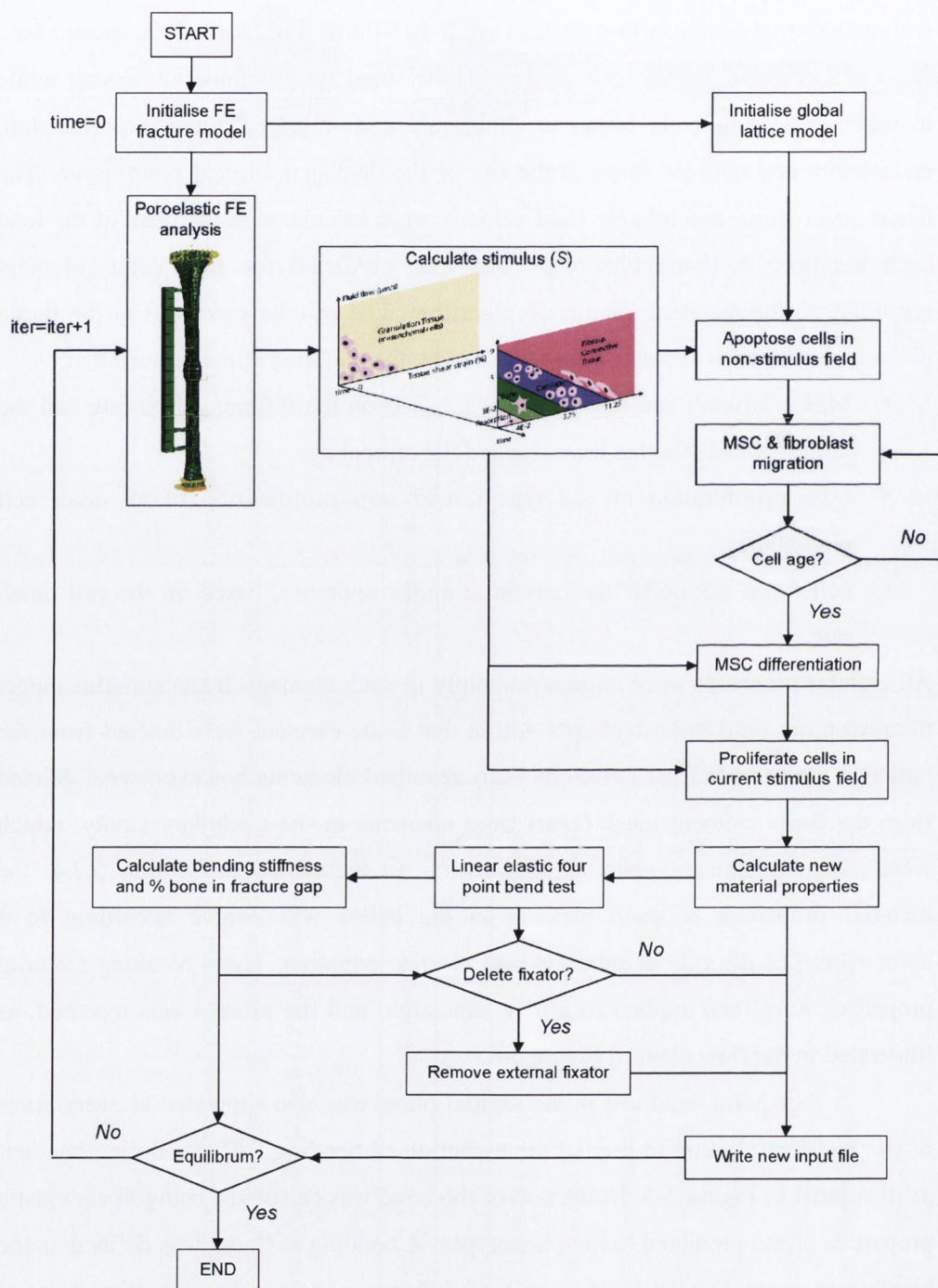


Figure 5-4: Flow chart of iterative computational simulation

removed when the stiffness exceeded this value and the fracture appeared to have clinically healed (i.e. greater than 90% bone tissue in the fracture gap). The analysis continues until convergence of the bending stiffness has been achieved.

$$\text{Bending_Stiffness} = \frac{FL - Rl}{\alpha} \tag{5.4}$$

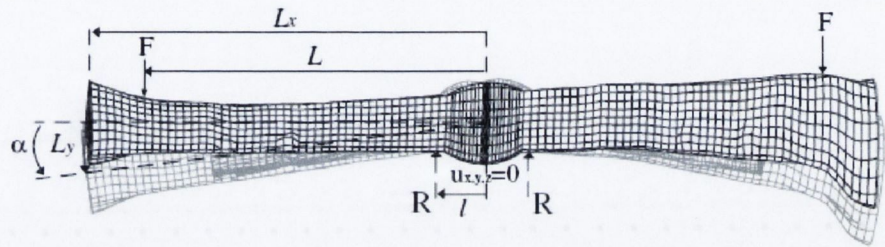


Figure 5-5: Simulation of bending test to predict bending fracture stiffness of the fractured bone (Lacroix, 2000)

5.3 Results

5.3.1 Healing patterns

At the beginning of the simulation, granulation tissue occupied the entire fracture callus. Once the infiltrating progenitor cells reached the critical age (cell age > 6 iterations), tissue differentiation initiates automatically. Cartilaginous tissue and small amounts of fibrous tissue were predicted to form between the bone ends (iteration 10 in Figure 5-6), while intramembranous ossification occurred along the surface of the tibial cortical bone outside the fracture gap. Bone formation persisted in the external callus through intramembranous ossification, while cartilage was gradually replaced by bone through endochondral ossification in the medullary cavity (iteration 20). This trend continued until the majority of the fracture callus became ossified (iteration 20-60). A small region of cartilage tissue remained at the posterior side of the fracture gap and external callus. At this time, initial osseous bridging occurred at the periphery of the callus, which effectively shielded the softer tissues of the callus from high mechanical stimuli. This effect then facilitated replacement of soft tissues with bone, until complete healing was achieved (iteration

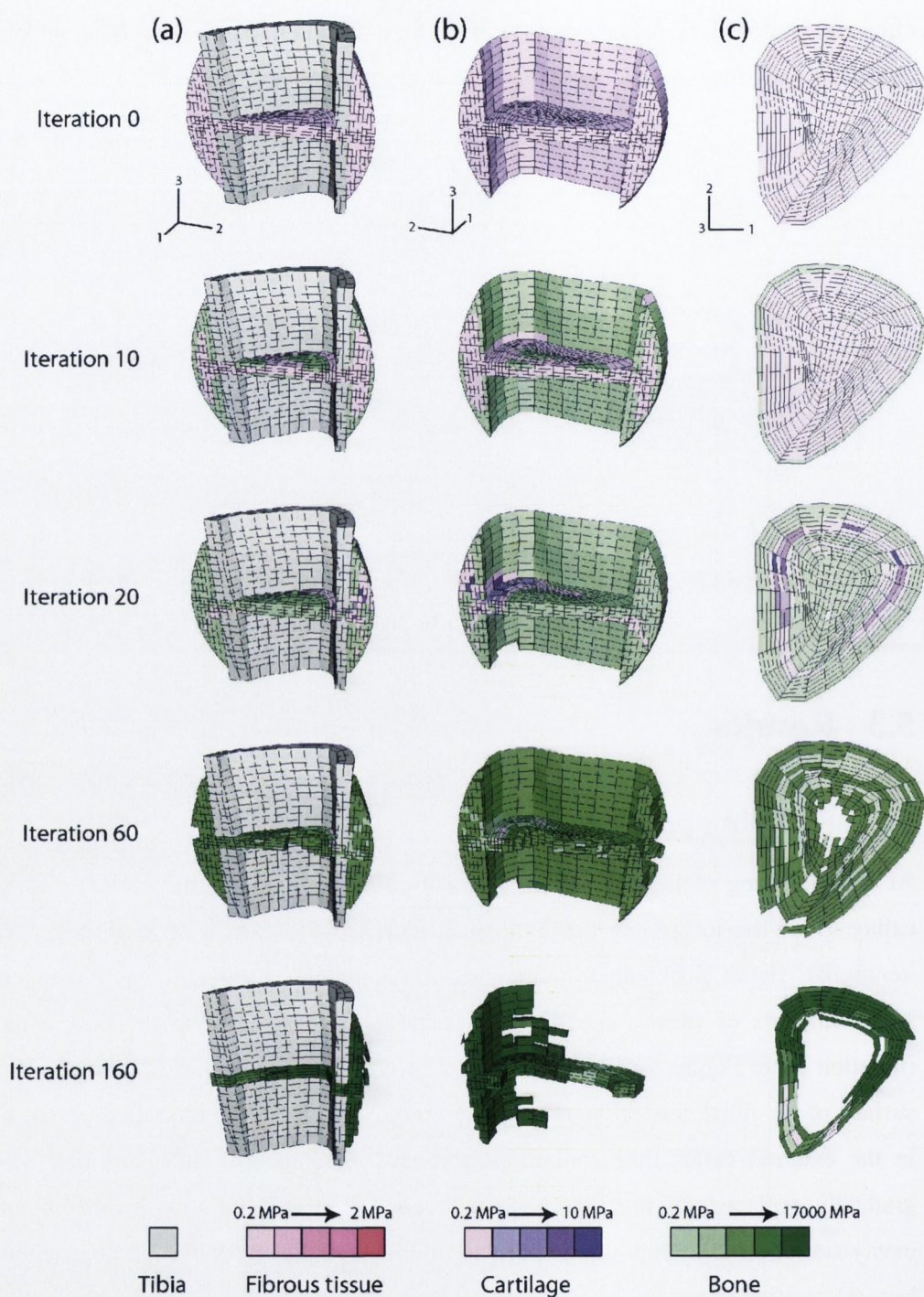


Figure 5-6: Cross-sectional view of the predicted healing patterns in (a) the medial side of the callus including the tibia, (b) the lateral side of the callus, and (c) a transverse slice through the centre of the fracture callus, over time.

60). Due to the criterion outlined in Section 5.2.6 the external fixator was subsequently extracted in iteration 61. The stability of the callus allowed resorption to take place in regions distal to the fracture gap and in the medullary cavity. From this point on, the remodelling process caused further resorption (iteration 60-160).

Bending occurred in the sagittal plane (posterior-anterior plane) which created greater mechanical stimuli in the posterior region of the fracture gap. Consequently, in order to endure the greater loads, less bone resorption was seen in the intramedullary cavity and the posterior region of the external callus. On the other hand elements in the anterior gap experienced reduced mechanical stimuli causing some resorption.

5.3.2 Mechanical stimuli

The simulated healing patterns change over time due to evolving material properties determined by local mechanical stimuli and increasing loading conditions. The initial fluid flow and octahedral shear strain were generally high. Tissue differentiation caused an increase in stiffness and the elements began to move across the phenotype fields from fibrous tissue, cartilaginous tissue and immature bone (iteration 10-20 in Figure 5-7). Over the following 40 iterations fluid flow decreased while octahedral shear strain increased, causing the majority of elements to fall into the mature bone field. Thereafter the mechanical properties of the bone tissue were regulated by the bone adaptation/remodelling process. At the end of the simulation, the majority of elements in the fracture gap lie in the mature bone field. As seen in Figure 5-6, the elements located at the posterior of the external callus and a small proportion of endosteal (internal) callus elements also remain.

The differences in mechanical stimuli between the anterior and posterior regions in the fracture gap are illustrated in Figure 5-8. Both octahedral shear strain and fluid flow increased in the posterior region at the start of the simulation. The majority of the load is endured in this area and shields the anterior region from greater mechanical stimuli. As stabilisation occurs (after iteration 20), the fluid flow gradually decreases until it reaches values similar to those experienced in the anterior fracture gap (after iteration 100). This decrease occurred due to the remodelling/adaptation process of the bone tissue throughout the simulation.

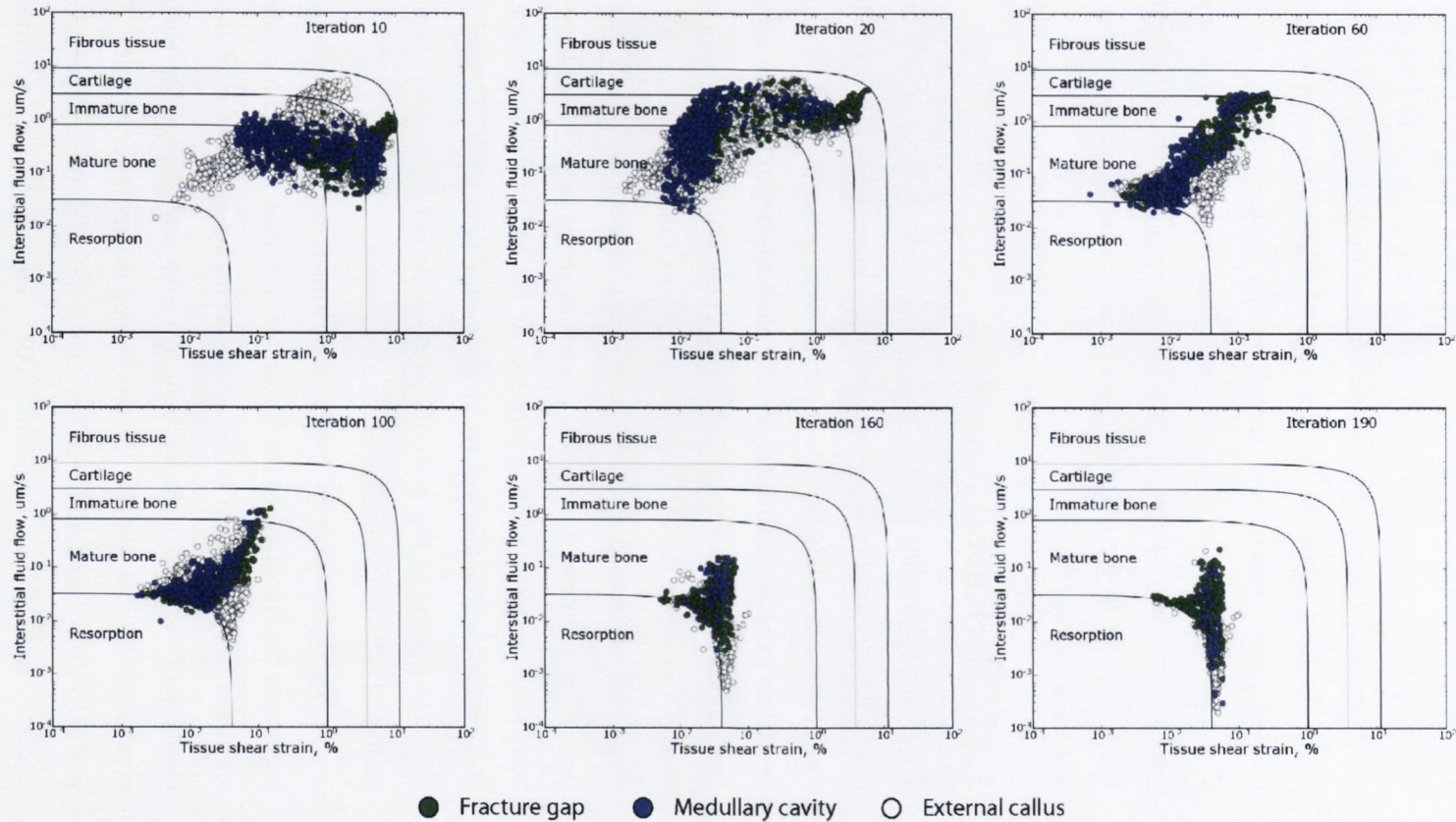


Figure 5-7: Mechano-regulation diagrams illustrating the interstitial fluid velocity and tissue shear strain in every element, at iterations 10, 20, 60, 100, 160 and 190. The resorbed elements are deleted and therefore cannot be shown

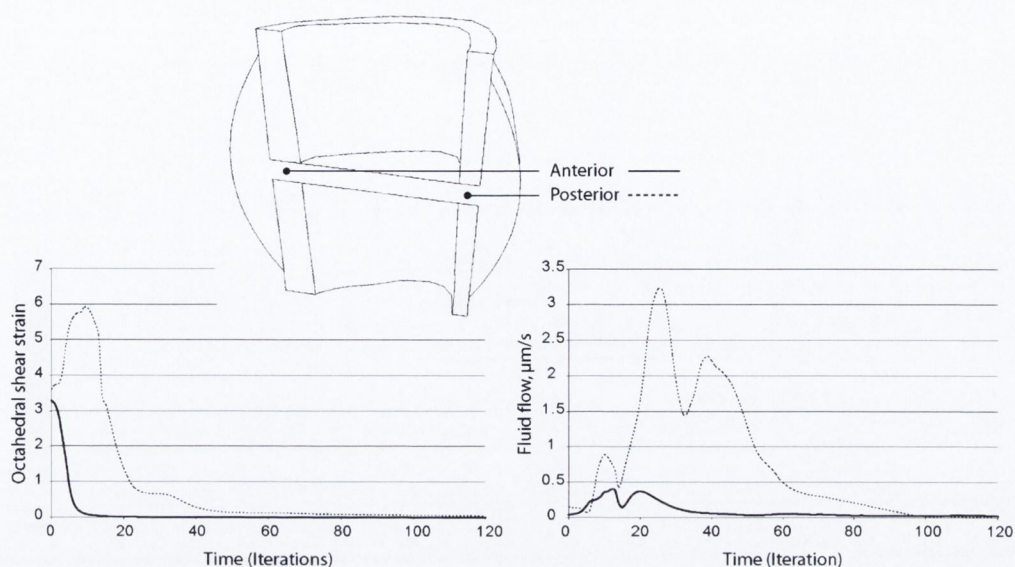


Figure 5-8: Predicted mechanical stimuli in the fracture gap in the posterior-anterior direction

5.3.3 Interfragmentary strain

The interfragmentary strain (IFS) was defined as the interfragmentary movement divided by the initial fracture gap size. As the tibia experienced bending in the mid-diaphysis the IFS was calculated at four points in the fracture gap (anterior, posterior, medial and lateral). The greatest of these values is plotted in Figure 5-9. The IFS initially increased as tissue differentiation had not taken place (due to the age of the progenitor cells being below the critical age for differentiation) and the applied loading conditions increased over time. The strain then rapidly reduced over the following 20 iterations due to differentiation and maturation of the granulation tissue throughout the callus. Thereafter bony bridging occurred in the external callus, and the strain gradually reduced further due to the adaptation of the bone tissue. Finally the interfragmentary strain becomes very small past iteration 60, at which point the external and endosteal calluses began to resorb.

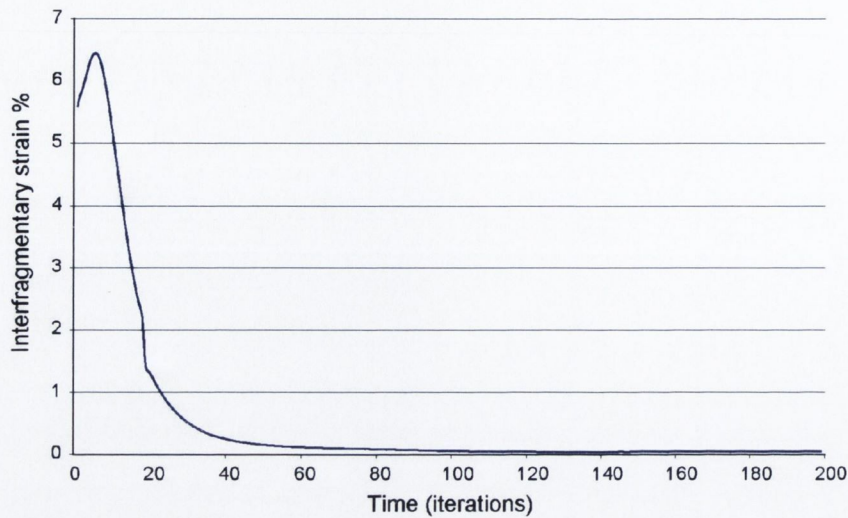


Figure 5-9: Interfragmentary strain decreases as healing occurs

5.3.4 Bending stiffness

When the fracture callus consists entirely of granulation tissue the bending stiffness is very low (0.38 Nm/deg). As healing progressed, the bending stiffness increases rapidly within the first 21 iterations, see Figure 5-10. The bending stiffness continues to increase gradually to reach a maximum value of 81 Nm/deg at iteration 83. This reduces slightly with resorption of the external callus and the intramedullary cavity, and reaches equilibrium at a stiffness of 73 Nm/deg.

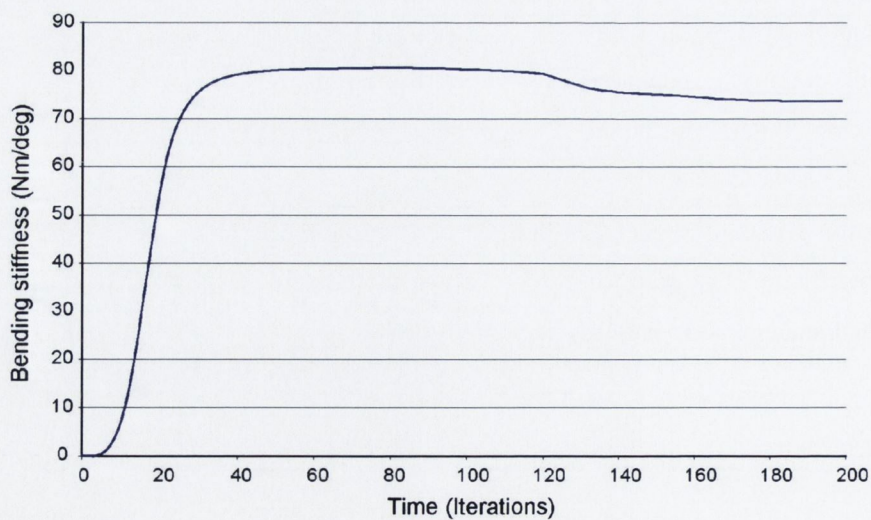


Figure 5-10: Predicted bending stiffness over time

5.3.5 Results of parametric study

To investigate the sensitivity of the model to bone permeability values, a parametric study was conducted. The predicted tissue differentiation patterns in all simulations are initially quite similar up to iteration 20 (see Figure 5-11, Figure 5-12, and Figure 5-13). However, as the regenerating tissues moved into the woven bone fields the associated elements were assigned different permeabilities, based on Table 5-3. The effects of these values are outlined below.

5.3.5.1 Tissue healing patterns

The high permeability value for mature woven bone in Test 1 generated greater interstitial fluid velocities in the regenerating tissues. The resulting magnitudes stimulated the mechano-regulation algorithm to predict cartilage formation, and consequently prevented ossification in the fracture gap (iteration 20 in Figure 5-12). Insufficient bone formation in the initial stages of healing instigated a lack of callus stability, while the increasing loads ensured the mechanical stimuli remained high and promoted soft tissue formation for the remainder of the simulation.

The lower permeability values in Tests 2 and 3 had the opposite effect, and induced greater bone formation in the regenerating tissues after iteration 20 (Figure 5-12 and Figure 5-13). Early callus stability encouraged further ossification and mineralisation of existing bone tissue; leading to advanced stages of healing sooner. These conditions generated almost three times as much bone formation in the fracture gap at iteration 40 when compared to Test 1 (Figure 5-15). The lower interstitial fluid velocities however also stimulated the mechano-regulation algorithm to predict greater amounts resorption. Subsequently other areas of the fracture callus had to compensate for the resorbed bone tissue and endure the applied loads. This occurrence was more prominent in Test 3 than Test 2. For example, the posterior region of the fracture gap experienced greater mechanical stimuli, which prompted some cartilage formation (iteration 60 in Figure 5-11 and Figure 5-12). The disparity of the mechanical stimuli in regions of the fracture gap is highlighted in Test 3 of Figure 5-14, where two distinct groups are seen to form after iteration 60. It is believed that the permeability of woven bone in these tests was underestimated, as little or no fibrous tissue formation was observed, while the resorption process dominated in the later stages of healing.

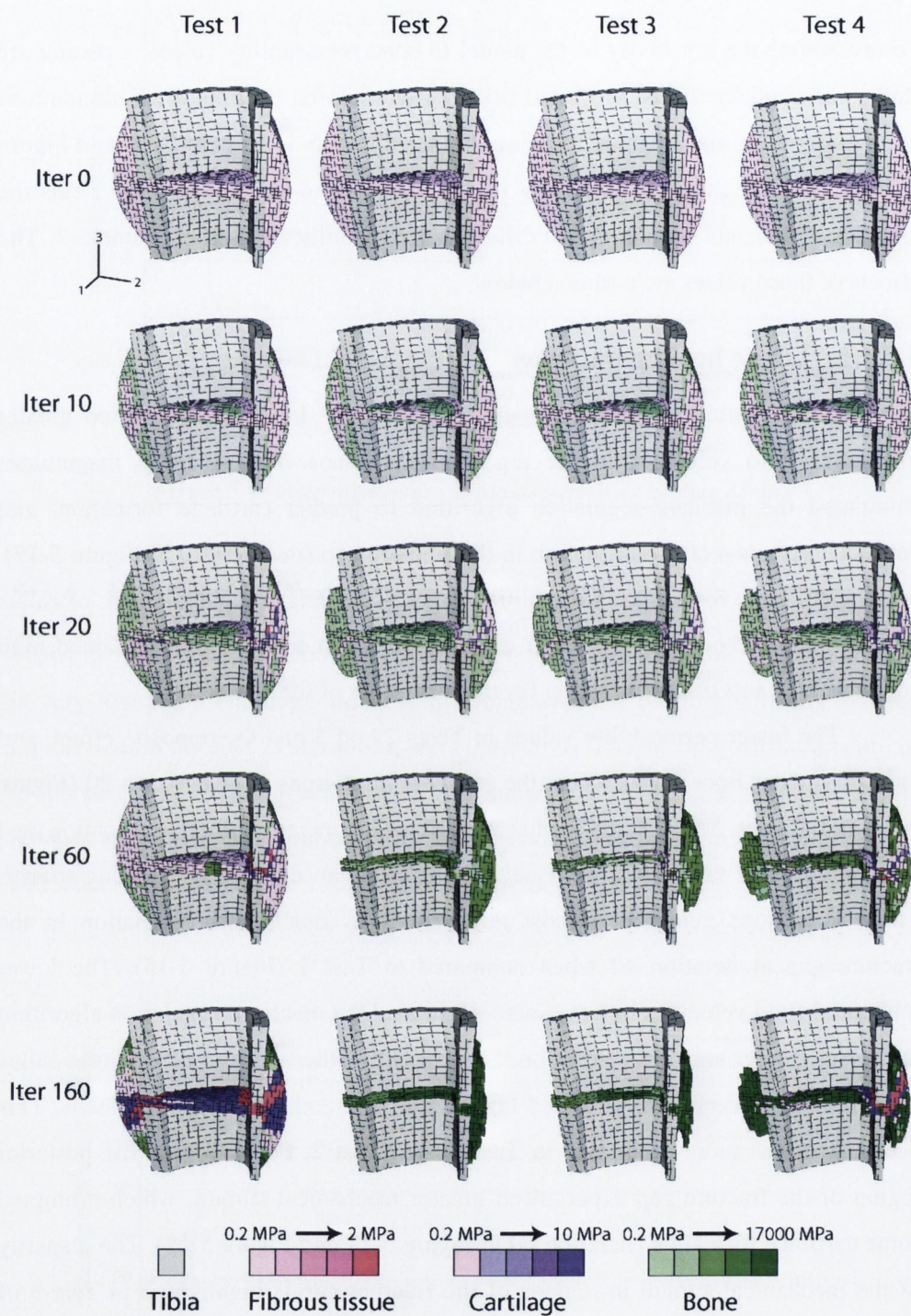


Figure 5-11: Cross-sectional view of the callus illustrating the predicted healing patterns for the parametric tests outlined in Table 5-3. The medial side of the callus is shown

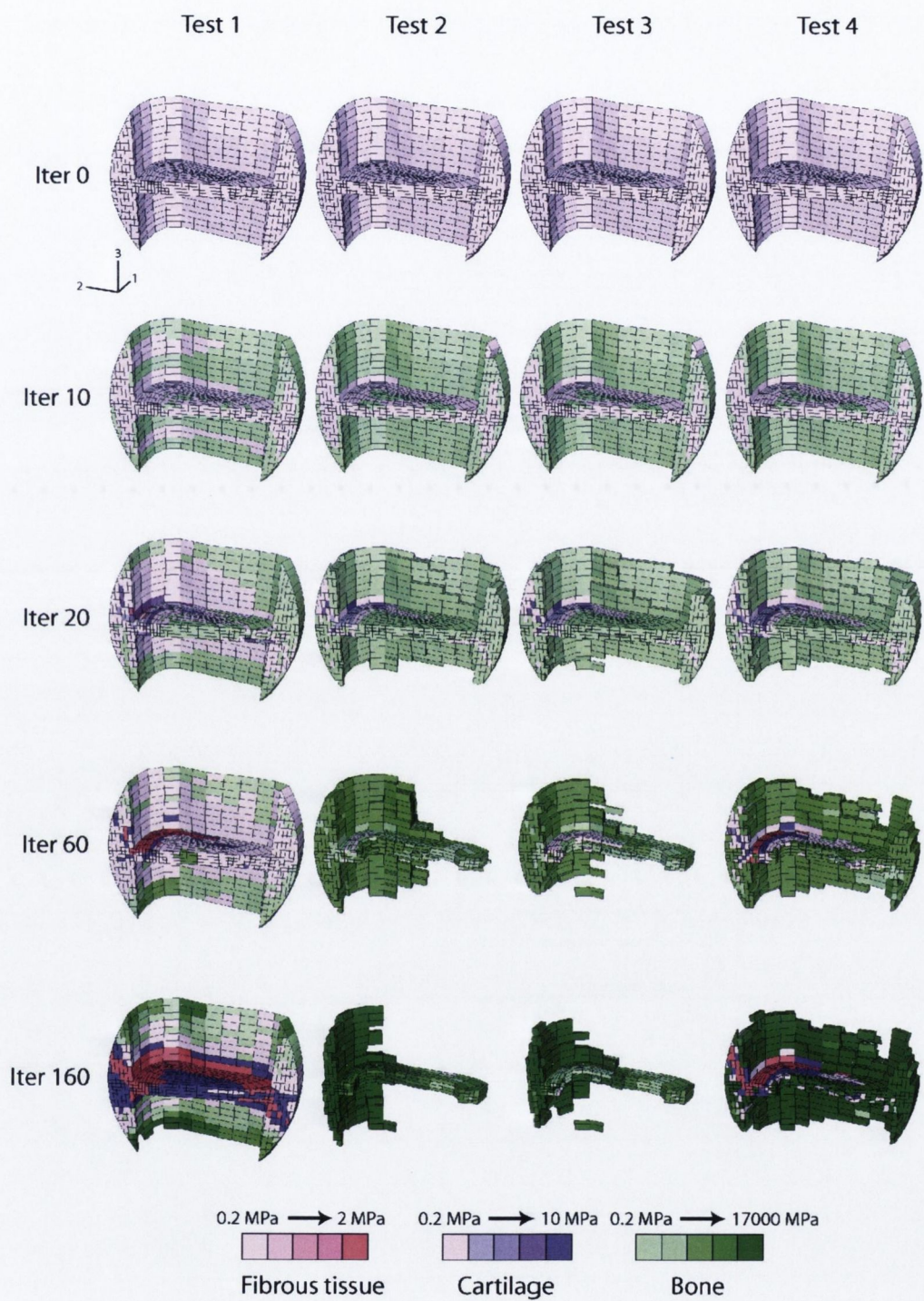


Figure 5-12: Cross-sectional view of the predicted healing patterns in the lateral side of the callus

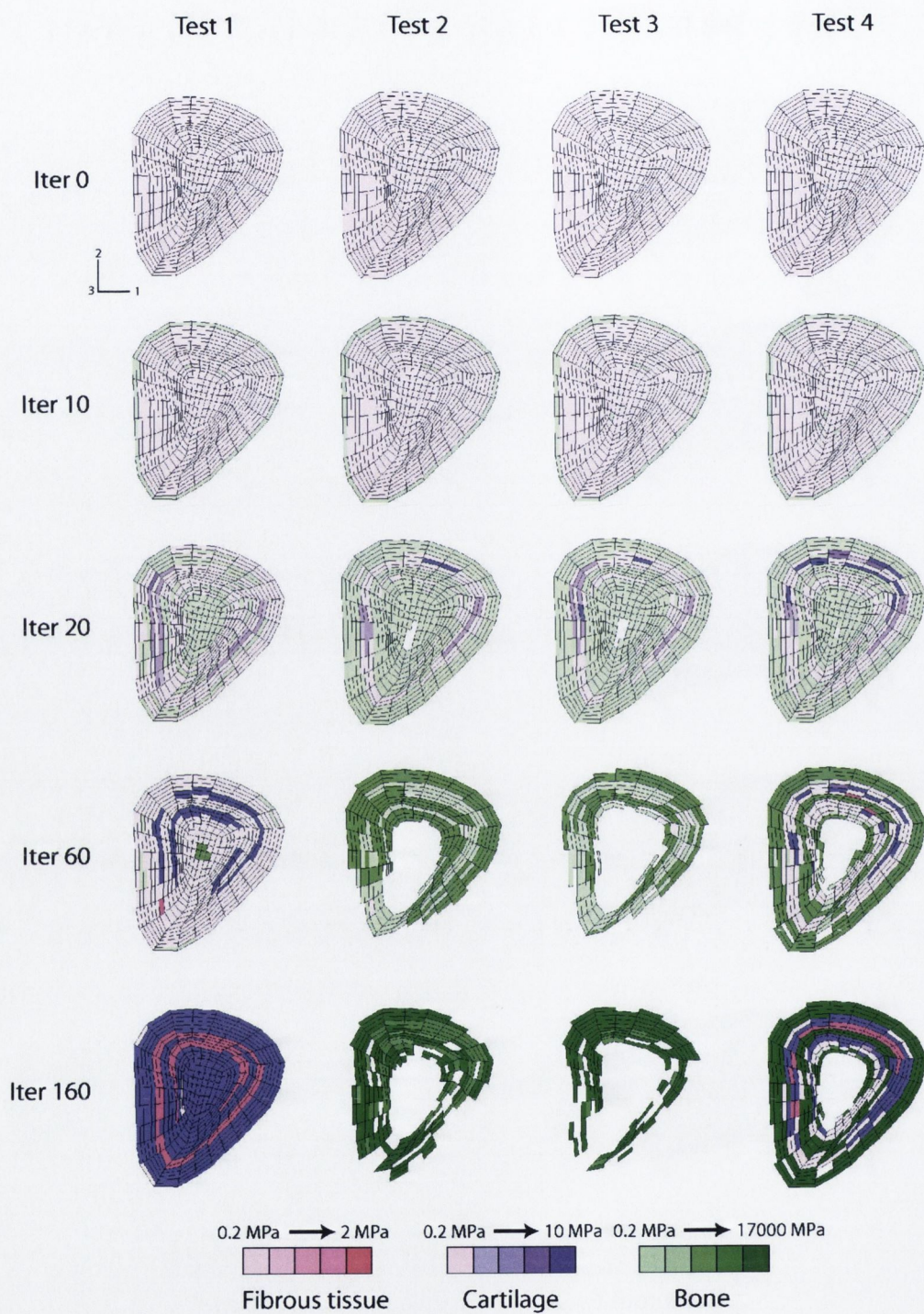


Figure 5-13: Transverse cross-section of the callus, illustrating the predicted healing patterns over time

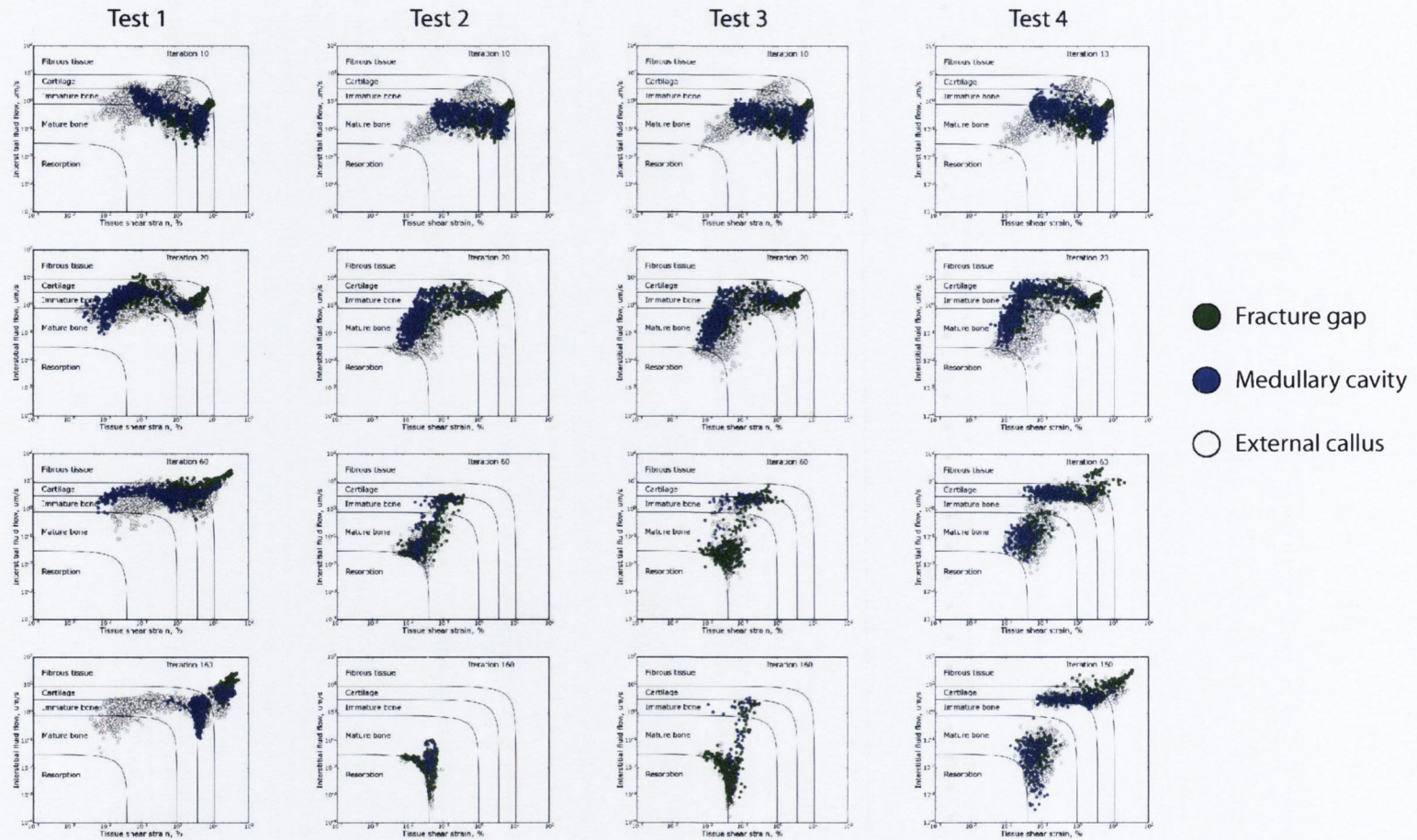


Figure 5-14: Mechanical stimuli acting on each element in the regenerating tissue, at iteration 10, 20, 60, and 160

In Test 4, immature woven bone was assigned a higher permeability value (normally associated with mature woven bone in other studies (Lacroix and Prendergast, 2002(b); Isaksson *et al.*, 2006), while the permeability of mature woven bone was assigned a lower value closer to that of cortical bone. Cells moving from the cartilage field into the immature woven bone field experienced greater interstitial fluid velocities due to the $3.65 \times 10^{-13} \text{ m}^4/\text{Ns}$ jump up in the assigned permeability values. Similar to Test 1, the resulting magnitudes caused the mechano-regulation stimulus to move back up into the cartilage or fibrous tissue fields. On the other hand, regions distal to the fracture gap, the anterior callus, and the endosteal callus experienced lower mechanical stimuli and differentiated into mature woven bone in the initial stages of healing. As healing progressed the lower fluid velocities in these regions prompted further ossification, and even some resorption. The large difference between the woven bone field permeabilities generated a split in the differentiation pathway (see iteration 60 and 160 in Figure 5-14).

5.3.5.2 Clinical results

None of the simulations in the parametric study predicted enough bone tissue in the fracture gap to fulfil the criteria to extract the external fixator, see Figure 5-15. This was either due to the dominance of soft tissue formation (Test 1 and 4) or bone resorption (Test 2 and 3). As outlined above, the results were initially quite similar up to iteration 20, thereafter the cells moved into the mature woven bone field causing significant changes in the predicted clinical results. In Test 1, the high permeability of mature woven bone promoted chondrogenesis and fibrous tissue formation. The lack of callus stability meant the regenerating tissues were not able to withstand the applied loading conditions, and consequently generated greater interfragmentary movement (Figure 5-16) and lower bending stiffnesses (Figure 5-17). The predicted clinical results of Test 2 and 3 are closer to experimental observations; however due to bone resorption at the fracture gap neither simulation was considered clinically healed. The slight variation between these results can be attributed to increased bone resorption in Test 3 after iteration 30, (compare Test 2 and 3 in Figure 5-15). In Test 4, some stability was achieved by the presence of bone on the external surface of the callus; as a result the bending stiffness remained high throughout the simulation. On the other hand, the persistence of soft tissue in the

fracture gap coupled with increasing loads caused a gradual increase in interfragmentary strain after iteration 40.

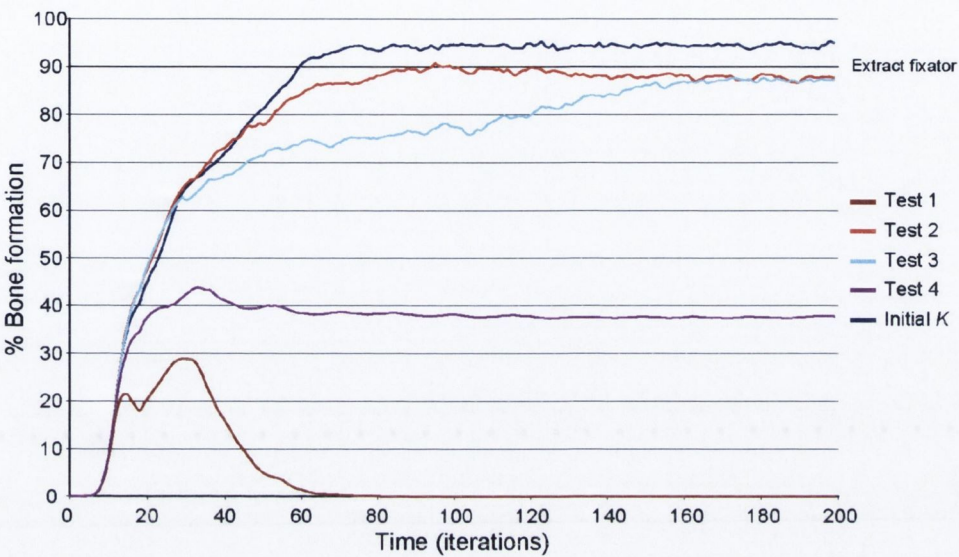


Figure 5-15: Percentage bone formation predicted in the fracture gap. None of the parametric results exceed 90%; therefore they are not considered clinically healed and the external fixator was not extracted

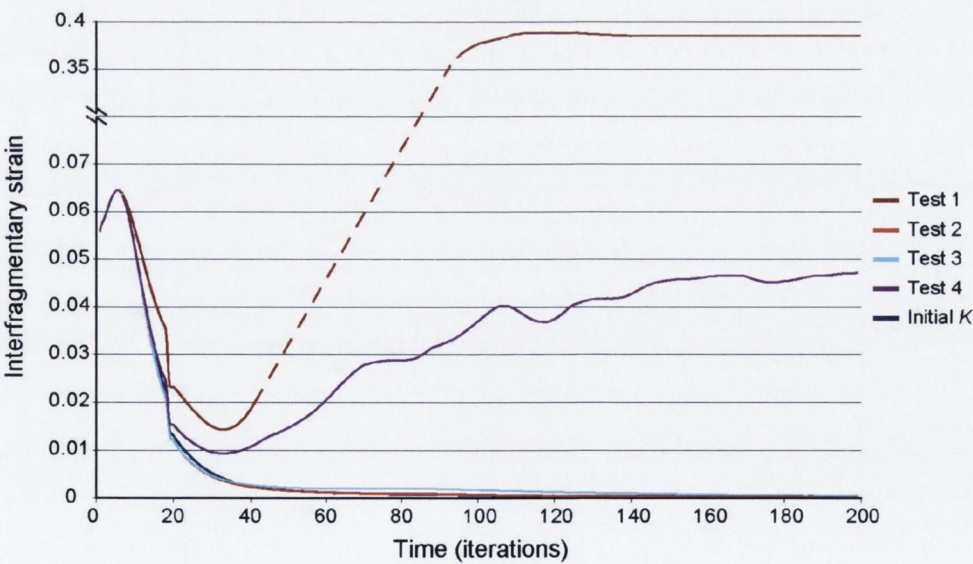


Figure 5-16: Predicted interfragmentary strain over time

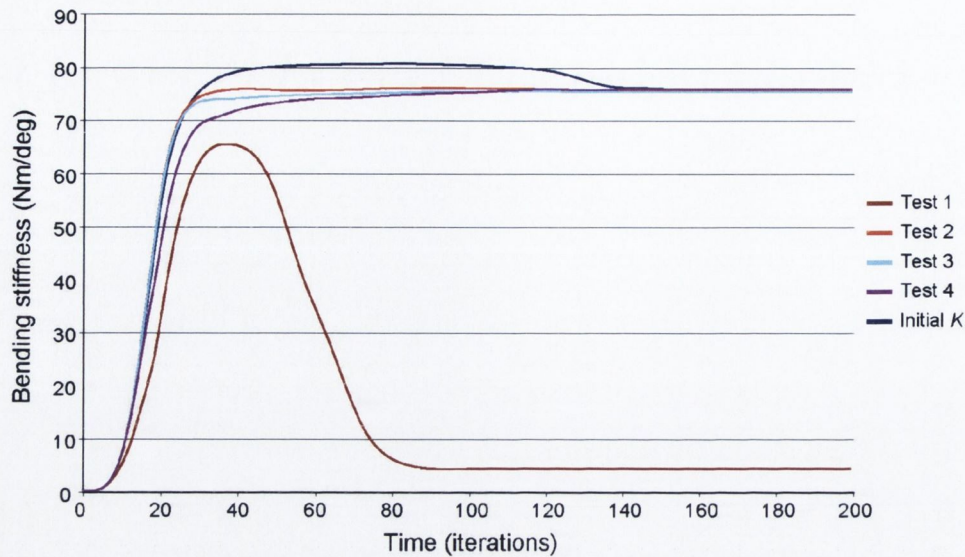


Figure 5-17: Predicted bending stiffness over time

5.4 Discussion

The discrete lattice approach holds great potential in computational mechanobiology. One key attribute is the explicit modelling of cell age, which enhances the possibility of accurately defining evolving material properties. Continuum models on the other hand can not distinguish between new and old cells. In this work a maturation time of 15 iterations was used to regulate the synthesis and remodelling of new osteogenic and cartilage tissue. Isaksson *et al.* (2008), highlighted MSCs stimulated down the osteogenic pathway require 8-10 days to mature and produce bone matrix (Aubin *et al.*, 1995; Malaval *et al.*, 1999), while chondrocytes require 14-21 days to mature (Bosnakovski *et al.*, 2004; Bosnakovski *et al.*, 2005). Describing the temporal change of the differentiating tissue in this manner gives a more accurate representation of the new material stiffness. Additionally, an attempt was made to model bone adaptation by increasing the Young's modulus and decreasing the permeability of the woven bone tissue if the mechano-regulation stimulus remained low. This essentially moved the cells closer to the boundary of the resorption field, where the level of mechanical stimulation caused either resorption or further adaptation. The level of mechanical stiffness was also dependent on cell age. By modelling the transition of woven bone into secondary lamellar bone, fracture

healing was simulated beyond the reparative phase for the first time. This emphasises the unique value of the presented approach.

Modelling of cell-scale properties such as MSC and fibroblast migration was also more accurately defined using the lattice approach. The inclusion of migration has a direct effect on proliferation as it lowers the possibility of contact inhibition until confluence is achieved. Cell migration therefore contributes to the rate of cell divisions and is essential when modelling cellular activity. The rates of cell motility were taken from the literature and therefore no definitive time line for complete cell coverage was specified. Previous continuum models computed the rate of cell proliferation, such that complete cell coverage was achieved by 16 weeks; however it is believed that this value is somewhat overestimated.

5.4.1 Predictive capacity of the model

The cellular processes largely dictated the healing process, and were able to successfully predict tissue differentiation patterns which agree with those observed histologically – namely (i) intramembranous ossification distal to the fracture site, (ii) the gradual replacement of cartilage in the external and endosteal calluses through endochondral ossification, and finally (iii) resorption of the external and endosteal calluses. Previous simulations did not successfully predict internal callus resorption (Lacroix and Prendergast, 2002(c)). The progress of healing is also reflected in the predicted interfragmentary strain and bending stiffness results. The initial range of moderate strains (6-10%) are similar to those observed by Gómez-Benito *et al.* (2006), while the predicted changes of interfragmentary strain over time correspond with experimental results (Claes *et al.*, 1997; Duda *et al.*, 1997). The general shape of the bending stiffness curve also corresponds to the experimental results of Richardson *et al.* (1994), see Figure 2-7; however, the numerical results exceed the normal bending stiffness of an intact tibia (approximately 60 Nm/deg). The additional stiffness may be attributed to the bone formation without external resorption in the posterior region of the external callus. Furthermore, anisotropic material properties may also reduce the outcome of the predicted results.

5.4.2 Parametric study

Finding a correct value for permeability will produce more accurate fluid velocities and therefore allow for a better definition of the mechanoregulation diagram. A

permeability value of $1.02 \times 10^{-14} \text{ m}^4/\text{Ns}$ for mature woven bone predicted the most accurate results. It was found that higher permeabilities allow greater fluid velocities in the regenerate and therefore induce soft tissue formation. If callus stability is not achieved in the initial stages of healing the increasing loads ensured that the mechanical stimuli remain high and prevent ossification. On the other hand, reduced permeabilities generate lower fluid velocities and therefore promote either bone formation or resorption. If however, resorption occurs too early the remaining tissues in the callus have to compensate for the resorbed bone tissue and endure greater loads. Accurately defining the permeability of both immature and mature woven bone is therefore pivotal in simulating bone regeneration with mechano-regulation algorithms.

5.4.3 Limitations of the present study

Firstly, it was assumed that the simulation began after the inflammation phase of healing, and the entire callus consisted of granulation tissue into which mesenchymal stem cells could migrate and proliferate. The callus index was predefined with a value of 1.4 and therefore the model does not take callus growth into account. Alternatively García-Aznar *et al.*, (2002) formulated a mathematical model that uses callus geometry as a dependent variable. This model uses mechano-biological rules to describe the influence of mechanical stimuli and time on cell proliferation and differentiation. Based on proliferation and differentiation it determines callus growth and callus geometry. Similar fracture healing studies have also used the same technique to account for the development of callus shape and size in the initial stages of healing, (Gómez-Benito *et al.*, 2005; Gómez-Benito *et al.*, 2006; García-Aznar *et al.*, 2007).

Secondly, the material properties of the regenerating tissues have not yet been measured directly. Based on the work of Lacroix (2000) the mechanical properties of the tissues were taken from the literature. Due to the uncertainty of woven bone permeabilities a parametric study was carried out. The disparity of the results in this study revealed the importance of accurately defining the mechanical properties, as the boundary thresholds of the mechano-regulation concept are very much dependent on these values. In addition, it is also important to characterise the temporal evolution of the tissue properties. An assumed maturation time of 15 iterations was used to model the transition from immature woven bone to mature

woven bone; while a further 200 iterations were required for the transformation of mature woven bone into cortical bone. Experimental studies such as that carried out by Moukoko *et al.* (2007), may help characterise the properties of the skeletal tissues used in these computational simulations. The aim of their study was to develop an experimental model which made it possible to characterise the temporal evolution of the structural and mechanical properties during unloaded endochondral osteogenesis in the New Zealand rabbit, a standard animal model for studies of osteogenesis and chondrogenesis. It is believed that this information could lead to a more gradual increase in bending stiffness over time.

Thirdly, some of the cellular rates are not well defined in the literature and were estimated. Parameters such as the differentiation rate and apoptosis rate in particular must be more closely defined, as the balance between these processes determines the activities of the cell populations at any given time. Experimental cellular calculations and cell tracking techniques can be used to measure these variables. This point is discussed further in Section 6.3.1.

Finally, the loading profile was based on the general shape of weight-bearing achieved by patients on their fractured leg. While the joint forces acting on the tibia may be correlated to the percentage of weight-bearing, it is questionable how much force the muscles exert in the initial stages of healing; particularly if they are inhibited by the presence of the external fixator. In the absence of more detailed information this method was assumed the most accurate way of representing the loading conditions. It was also found that the initial low loads at the beginning of the simulation generated very little fibrous tissue. This inaccuracy may be attributed to the uncertainty of the material properties as mentioned previously.

5.5 Conclusions

The objective of modelling bone regeneration in a fracture healing problem with a fixator was achieved. The mechanistic model successfully predicted the sequence of tissue differentiation patterns that appear in the callus; and for the first time healing was simulated beyond the reparative phase. It was shown that the cellular processes largely dictate the outcome of the results and are therefore essential in the development of computational mechanobiology. For further validation, other clinical conditions should be analysed, including different loading profiles, fracture

geometries (i.e. gap size and angle) and different fixator characteristics. Moreover, the material properties of the regenerating tissues must be more accurately defined in order to elicit realistic biophysical responses. Despite these limitations the model holds the potential to be used to non-invasively to assess the options for treating fractures in patients.

Chapter 6

Discussion

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6.1 Introduction

The primary objective of this thesis was to develop a method to include cellular processes in three-dimensional mechano-regulation computational models – namely proliferation, migration, cellular apoptosis and differentiation. Simulations of bone regeneration in two applications were presented in the previous chapters. This chapter summarises the current thesis and discusses new developments made to integrate mechanobiology with a more accurate description of cellular biology. The associated assumptions and limitations are also discussed and possible improvements are suggested. Finally the potential of computational mechano-regulation simulations as a tool for optimising clinical treatments for individual patients is evaluated.

6.2 Developing computer simulations of bone regeneration

As outlined in Chapter 1, simulations to predict tissue regeneration combine finite element modelling to compute the mechanical stimuli in the tissues and mechano-regulation algorithms to regulate cellular behaviour and adapt the tissue material properties in iterative computational schemes. The developments of each process in the simulations are discussed below.

6.2.1 Simulating cellular activity

Many researchers have previously attempted to simulate the presence of cells/bioactive factors in the decision process of tissue differentiation using differential equations (Lacroix *et al.*, 2002(a); Lacroix and Prendergast, 2002(b); Geris *et al.*, 2004; Kelly and Prendergast, 2005; Isaksson *et al.*, 2006). In biology, however, the complexity is such that new states in the solution are, in part, pre-programmed responses from the old state so that differential equation may not be able to fully describe the evolution of a state. Alternatively, rule-based approaches were introduced into computational mechanobiology by Ament and Hofer (2000), who first performed a simulation of fracture healing. Shefelbine *et al.*, (2005) also used a comprehensive set of rules to simulate trabecular bone fracture healing. The techniques employed by these authors are similar to cellular automata modelling because the state of a neighbouring cell determines the state of a new cell based on a

simple set of rules. Later, Kawamura *et al.*, (2005) used cellular automata to simulate fracture healing. However, none of these authors included either cell dispersal (by migration, proliferation, or convection in the fluid phase) or the diffusion of bioactive factors; therefore the presence of cells or bioactive factors was not part of the decision statements. The work in this thesis aimed to model cellular processes based on a 3D stochastic random walk 'lattice' approach and combine them with the mechano-regulation theory of Prendergast *et al.*, (1997) to simulate tissue differentiation over time.

Cell motility involves cell migration and proliferation, and is essential to many physiological processes, including wound healing, tumour invasion, inflammation, and key events during developmental morphogenesis (Appeddu and Shur, 1994; Zaman *et al.*, 2005; Cai *et al.*, 2007). In this work both migration and proliferation was based on an unbiased nearest-neighbour random walk process, as illustrated in Figure 6-1 and Figure 6-2. This approach addresses the motility of cells in discrete time steps rather than describing the motility as a continuous process. In accordance with experimental observations cell motility is modulated by crowding effects; thus the rate of cell divisions decreases with time due to contact inhibition. Moving from 2D to 3D adds an extra degree of freedom when modelling cellular motility. This reduces the probability of cells occupying particular neighbouring positions – migration reduces from 1/5 to 1/7, while proliferation reduces from 1/8 (as modelled by Pérez and Prendergast (2007) who excluded the possibility of the cells occupying opposite poles) to 1/21. As the cellular processes are dependent on the biophysical stimuli determined from the finite element model it is important to accurately characterise realistic geometries and loading conditions. Therefore, modelling in 3D is often essential as the shapes of bones are irregular and cannot be accurately defined using axisymmetrical or two-dimensional models (this point is discussed further in Section 6.2.2).

The lattice approach holds the potential to model cell-phenotype specific processes, and it easily allows cell types and cellular processes to be distinguished – such as the migration of motile cells (MSCs and fibroblasts) compared to non-motile cells. This increases the model's applicability and allows for validation against *in vitro* experimental studies which investigate the influence of mechanical stimuli on individual cell phenotypes. In the simulations presented here cell locomotion will always occur if there are unoccupied neighbouring lattice points, until confluence

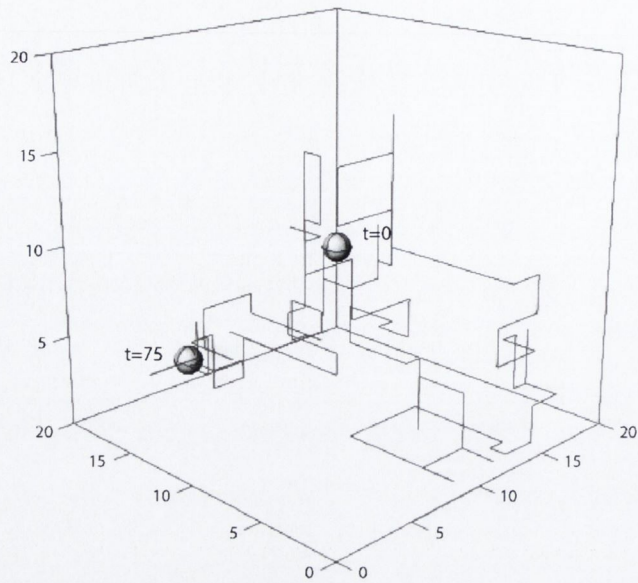


Figure 6-1: Illustration of a migrating cell over 75 iterations in a $20\mu\text{m}^3$ element. Each iteration the cell (x, y, z) can move with equal probability, to $(x\pm 1, y, z)$, $(x, y\pm 1, z)$, $(x, y, z\pm 1)$ or remain in the same position

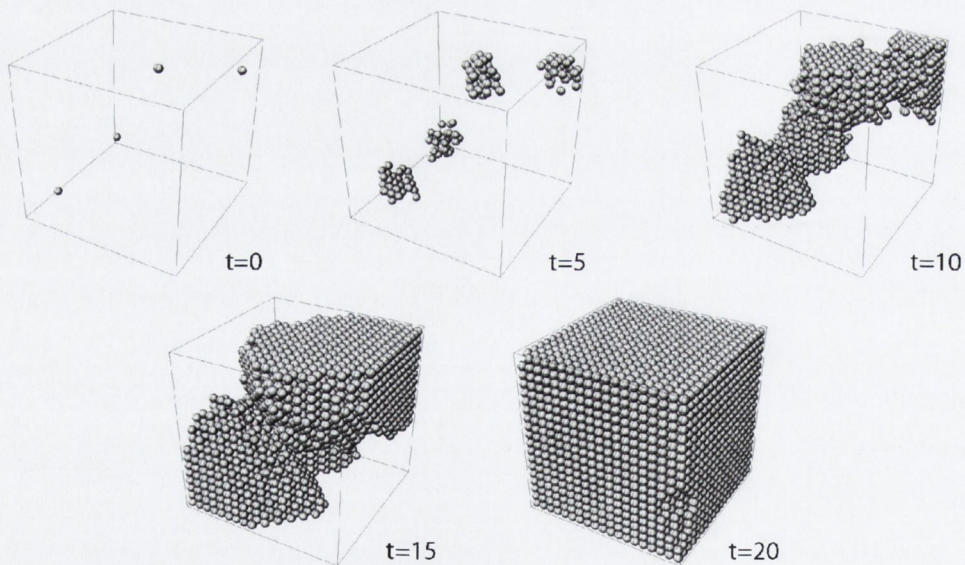


Figure 6-2: Illustration of contact inhibited proliferation behaviour. If a cell (x, y, z) proliferates there are 21 possible positions the daughter cells can occupy with equal probability, see Figure 4-1. Given an initial cell distribution at $t=0$, cells will only continue to grow and proliferate if they are not completely surrounded by neighbouring cells. Cells begin to form “clusters” at $t=5$, which eventually merge with neighbouring populations ($t=10$ to $t=20$)

has been reached. Alternatively other options can be explored to model cell motility, such as:

1. randomly selecting a neighbouring position and if the selected site is occupied the movement is aborted in the current iteration ($\text{iter} = i$), or
2. randomly selecting a neighbouring position and if the selected site is occupied wait until the next iteration ($\text{iter} = i+1$) to execute the movement if the lattice position has become available.

In both cases the cell proliferation rate would decline. Individual cell migration properties can be accurately defined by tracking the overall average speed and displacement of cells in three-dimensions; see Figure 6-3. Such experimental information could allow for a detailed description of active mechanisms and interactions between cells in the regenerating tissue. The lattice model can also be adapted further to account for preferred cellular movements toward a source of oxygen or nutrients. Alt (1980) employed a biased random walk to model the phenomena of chemotaxis on chemosensitive cells; that is the preferred movement and orientation of sensitive organisms or cells along the gradient of a chemical substance. Similarly Pérez and Prendergast (2007) modelled anisotropic proliferation by increasing the probability of cell positions in the preferred direction, see Figure 2-20. Thus, the lattice approach can be used to identify additional cell-scale properties of the system (Simpson *et al.*, 2007) which can be sensitive to the choice of cellular movement (i.e. biased or unbiased).

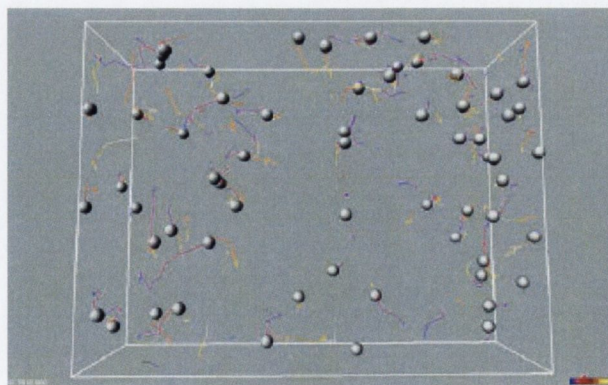


Figure 6-3: Spot tracking technique to measure cell migration speed and display individual cell movement in 3D within a collagen-GAG scaffold. A single time point is shown, but the colour coded cell tracks (blue $\rightarrow$ white) indicate a random movement of each cell during the experimental period (10 hours) (Harley, 2006)

It has been shown in the preceding chapters that modelling cellular processes such as proliferation, migration, and apoptosis are fundamental when modelling tissue differentiation, as they have a significant impact on the outcome of the simulations. Thus mechano-regulation computational models should describe, as accurately as possible, these cellular processes during tissue differentiation. Continuum models, such as the diffusion approach, are limited by the fact that they are only capable of implicitly describing population-scale events. Discrete lattice models, on the other hand, are not subject to these limitations, since they do not necessarily correspond to any partial differentiation equation.

6.2.1.1 Influence of lattice density

The density of the lattice used to simulate the cellular activity has an effect on the rate of the processes in the tissue differentiation process. In the computer models presented in this work each lattice point was considered a region of space for both the cell and the extracellular matrix. The density was determined by the dimensions of the element and the diameter of each lattice point. In the scaffold study, the sides of the elements were orthogonal; therefore it was relatively simple to determine which lattice points were inside each element. The length, height and width of each finite element were divided by the average diameter of a cell to give the number of rows, columns and the depth of the lattice. The average cell diameter was assumed to be 25 μ m, however in reality cell shape (depending on attachment) and size (typically 10 to 100 μ m) varies with phenotype. In total there were 2,744,000 points present in the scaffold study. To model dissolution the size of the elements were adapted throughout the simulation, thus bringing new lattice points into play. In general it was attempted to have approximately 1,000 points in each element (10*10*10); however this was not always attainable as the size of the elements depended on the porosity of the scaffold; so this value varied over the course of the simulation, see Figure 6-4(a). In the fracture healing study the lattice diameter was assumed to be 80 μ m. The domain being modelled (X - 31.85mm, Y - 38.25mm, Z - 33.28mm) was approximately ten times larger than the scaffold study (X, Y, Z - 3.5mm), and therefore required a significant increase in the number of active lattice points (22,250,066). As there was no dissolution, the number of lattice points per element remained constant throughout the simulation, see Figure 6-4(b).

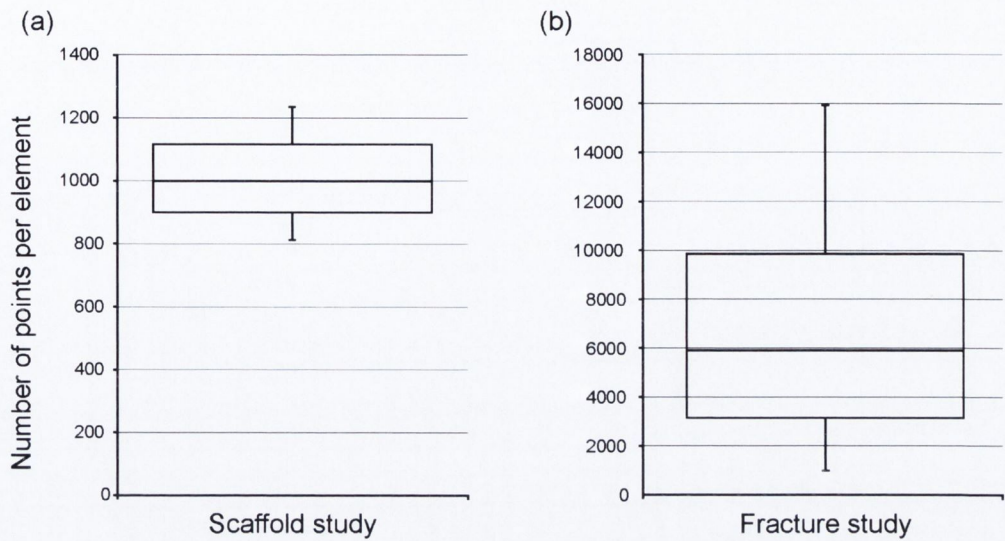


Figure 6-4: Box plot illustrating the average number of lattice points in each element in (a) the scaffold study and (b) the fracture healing study

There are critical limits above and below which the lattice diameter can not exceed. If the diameter is too big some finite elements may not contain any lattice points, thus rendering them insignificant in the simulation. The maximum critical value is therefore dependent on the mesh size, and varies between the two studies. The cut-off diameters are $80\mu\text{m}$ and $40\mu\text{m}$ in the scaffold and fracture healing studies respectively. The difference between the two studies is due to the irregularity of the finite element mesh in the fracture healing study. In addition, the rate of cellular transportation (i.e. cell migration and proliferation) and saturation will increase with lattice diameter. Conversely, the cell diameter should not go below $10\mu\text{m}$, as this would surpass the minimum limit of the cellular level. Smaller diameters increase the number of lattice points and therefore require greater computational power and time. Figure 6-5 illustrates the relationship between the lattice diameter and the number of points in the fracture healing study. Problems with computational memory occurred with diameters below $60\mu\text{m}$, therefore there is a trade off between the number of lattice points and the computational power and time it takes to complete the simulation. High-resolution finite element modelling fit to the lattice diameter could solve the questions based on lattice density; however material properties must then be based on individual cells. These issues are discussed further in Section 6.3.1.

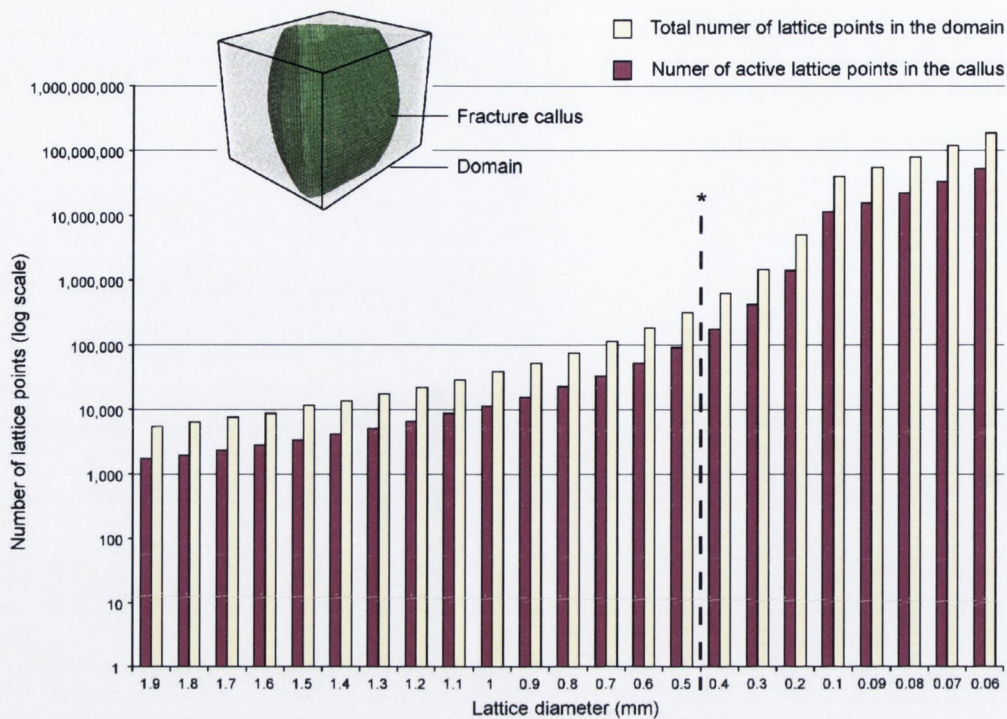


Figure 6-5: Relationship between the lattice diameter and the number of active lattice points in the callus in the fracture healing study. * Maximum diameter above which some of the smaller elements in the model do not contain lattice points

6.2.2 Loading conditions

As mechanical loading is known to have a significant bearing on tissue differentiation it is vital to accurately model the appropriate loading conditions relevant to the clinical setting. In the bone scaffold study in Chapter 3 the load was ramped up to either 2 MPa or 4 MPa over each iteration. These magnitudes were correlated to stresses typically experienced in long bones (see Appendix A) where scaffolds are often used to promote osteogenesis. However, as stated previously, these values will vary depending on the site of implantation and the activity of the patient. The clinical reality is that weight bearing of the patient increases as healing progresses. With this in mind the loading was increased over a two month period in the scaffold study in Chapter 4; although, it is unlikely that the loads will increase linearly with time. Additionally a loading profile based on weight-bearing data was used to relate the percentage load to the joint and muscle forces acting on the tibia in the fracture healing study. While increasing the loading over the healing process is

justified, the rates at which they increase are not precisely known. The loading acting on the tibia will vary depending on a number of factors such as, the severity of the fracture, the patient's age, weight, height, fitness, bone quality etc.; therefore the loading profile and hence the healing time line is patient-specific.

Accounting for a more detailed representation of the loading conditions acting on the tibia enhances the quantitative accuracy of the finite element results, and advances on previous fracture healing studies. Lacroix *et al.* (2002(c); applied uni-axial loads of either 300 N or 500 N to the proximal end of the tibia, while the distal part was restrained from moving. These values are significantly lower than those reported by Duda *et al.* (2002), and consequently predict much lower biophysical stimuli in the fracture callus, see Figure 6-6. Both models produce high bending in the mid-diaphysis of the tibia where the fracture was modelled. The axial load causes the fluid to travel in the circumferential direction, particularly in the posterior region of the fracture gap. This occurrence is partly counteracted by the muscle forces and the distribution of the loads (60% medial and 40% lateral) acting on the proximal end of the tibia. The important point to note here is that this phenomenon would not be seen using axisymmetrical or two-dimensional models with planar loads.

6.2.3 Material properties

Many previous tissue differentiation models have assumed a rapid transition from one tissue type to another by describing the subsequent change in material properties using a simple rule of mixtures and a temporal smoothing procedure which average the material properties over ten iterations. This method accounts for both the number and phenotype of cells within each element and causes the material properties to change gradually towards the phenotype determined by the stimulus. In certain instances however this solution may not be adequate when describing the evolution of material stiffness over time. For example, if the stimulus changes from predicting granulation tissue straight to bone (i.e. 0.2 MPa to 6,000 MPa) averaging the values would not give an accurate representation of the new materials stiffness. Following initial tissue differentiation, normal biological adaptation of the tissues occurs, and controls their continued growth and maintenance. In order to better describe the temporal change in tissue stiffness a rate equation was employed, which allows differentiated cell phenotypes to synthesise and remodel new tissue. As time is based

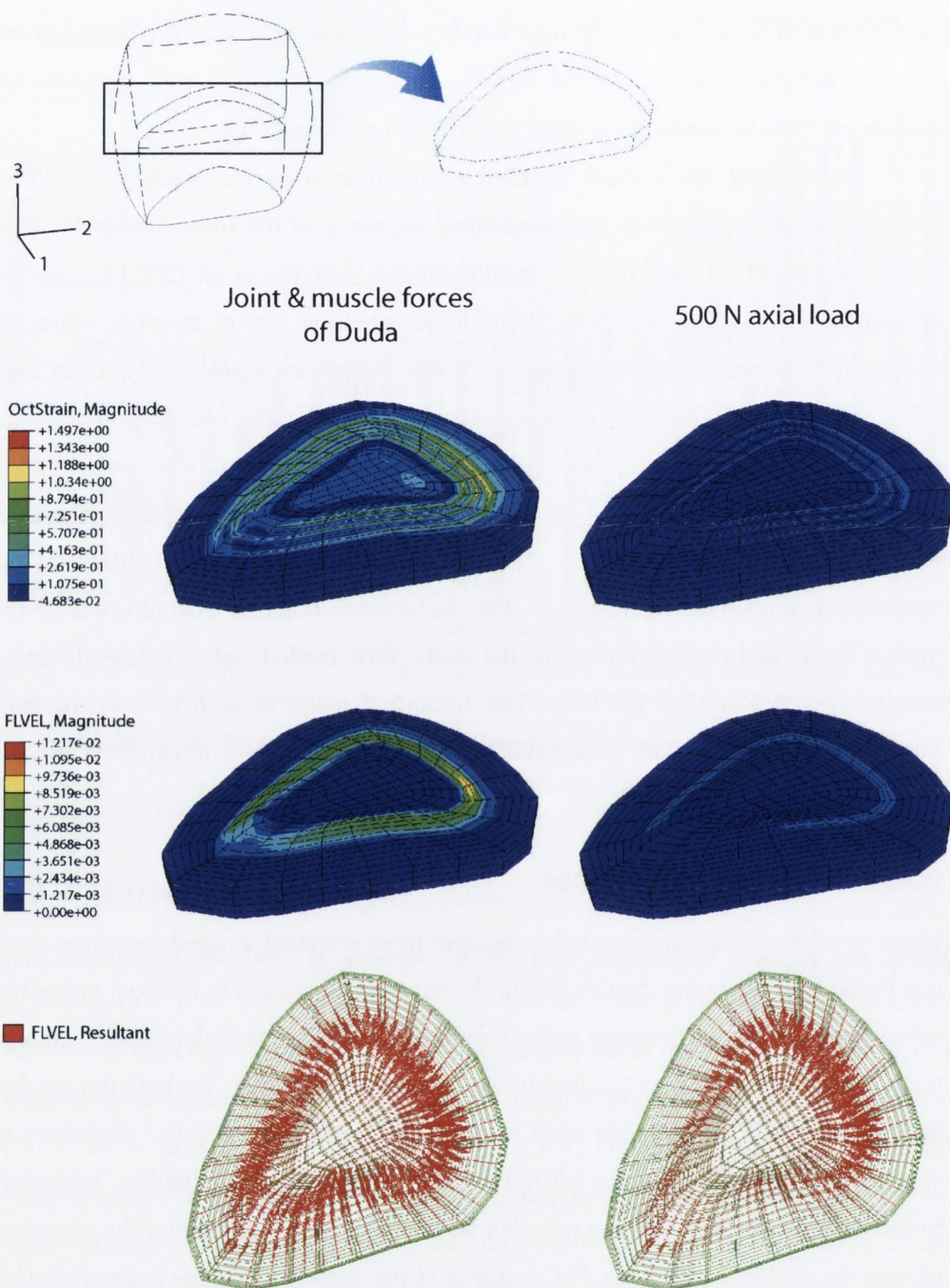


Figure 6-6: Computed biophysical stimuli acting on the granulation tissue within the fracture gap, with (a) full joint and muscle forces from Duda et al. (2002), and (b) 500 N axial load from Lacroix and Prendergast (2002(c)). The resultant fluid velocity vectors illustrate the fluid flow direction, which is more circumferential in the posterior region of the fracture gap in the 500N axial load study

on the age of the cell the rate equation starts locally after the deposition of a certain tissue type. This is only possible using the lattice approach as the cell age can be exclusively modelled. Continuum models on the other hand can not distinguish between new and old cells.

6.2.4 Resorption and remodelling of bone

Following tissue differentiation, mechanical forces continue to stimulate and maintain skeletal tissues throughout life (Prendergast, 2008). Bone remodelling involves the turnover of bone that removes micro-damaged tissue and replaces it with undamaged bone. In the case of bone adaptation, Frost's (1987) mechanostat theory has become widely accepted. In this theory, strain magnitudes are used to predict bone remodelling and/or modelling activities which result in an increase or decrease in bone mass. Altering the bone mass reflects changes in both the mineralisation and the porosity of the bone tissue. Frost proposed that there is an equilibrium range of strain values which will evoke no net change in bone mass; which are often referred to as a 'dead zones' or 'lazy zones'. Below a minimum effective strain bone remodelling is activated to decrease bone mass. At higher strains lamellar bone is formed, causing an increase in bone mass in an attempt to reduce the stresses being applied to it. Above a maximum threshold there is damage accumulation to the extent that the rate of bone modelling is reduced and pathological resorption occurs (Prendergast, 2004). This model was adapted by Mulvihill *et al.* (2008) and Mulvihill and Prendergast (2008) such that the bone remodelling or modelling mechanisms are either 'ON' or 'OFF', see Figure 6-7. This method was used to predict a remodelling cycle along a single trabecular strut in three-dimensions.

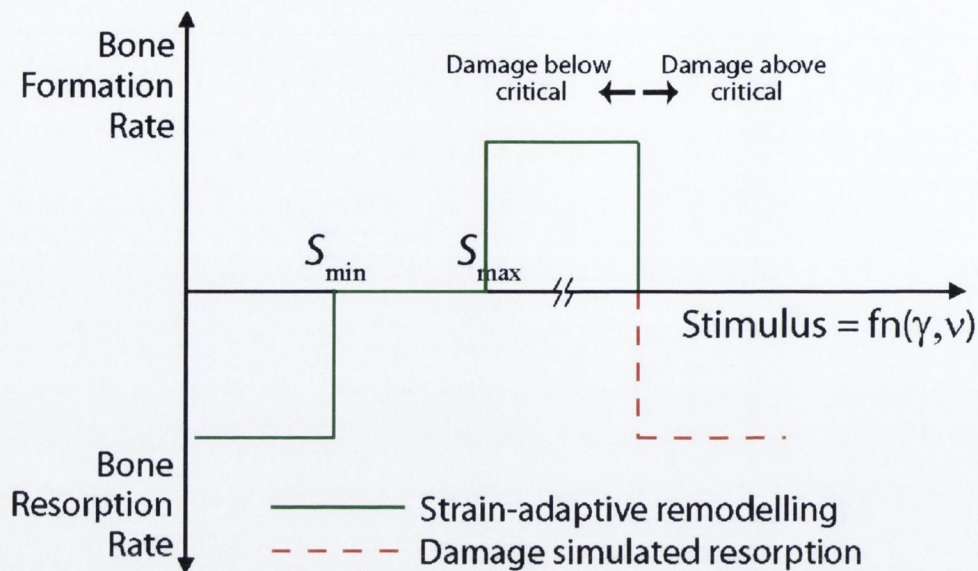


Figure 6-7: Combined strain-adaptive remodelling and damage-simulated resorption algorithm using the ON/OFF remodelling concept, developed by Mulvihill and Prendergast (2008)

In the mechano-regulation algorithm developed by Lacroix and Prendergast (2002(b)), bone resorption is related to low biophysical stimuli; adaptive remodelling of the kind described above is not accounted for. Recently, Liu and Niebur (2008) modified the mechano-regulatory algorithm by enforcing the tissue differentiation pathway and regulating the woven-bone fields with Frost's mechanostat theory. Using this method they successfully predicted bone ingrowth into a porous coated implant, and corroborated their results with clinical and experimental observations. In the fracture healing study in Chapter 5 the transition from woven bone to lamellar bone is modelled if the mechano-regulation stimulus persists and the initial development of the tissue (i.e. the rate equation) is complete. The material properties (Young's modulus and permeability) of immature woven bone were simulated to evolve into mature woven bone over time; while mature woven bone slowly transformed into lamellar bone. Accurately characterising the evolution of the bone material properties using adaptive remodelling (see Figure 6-8) could predict further resorption in the medullary and periosteal calluses, which would gradually restore the original contour and internal structure of the bone at the fracture site.

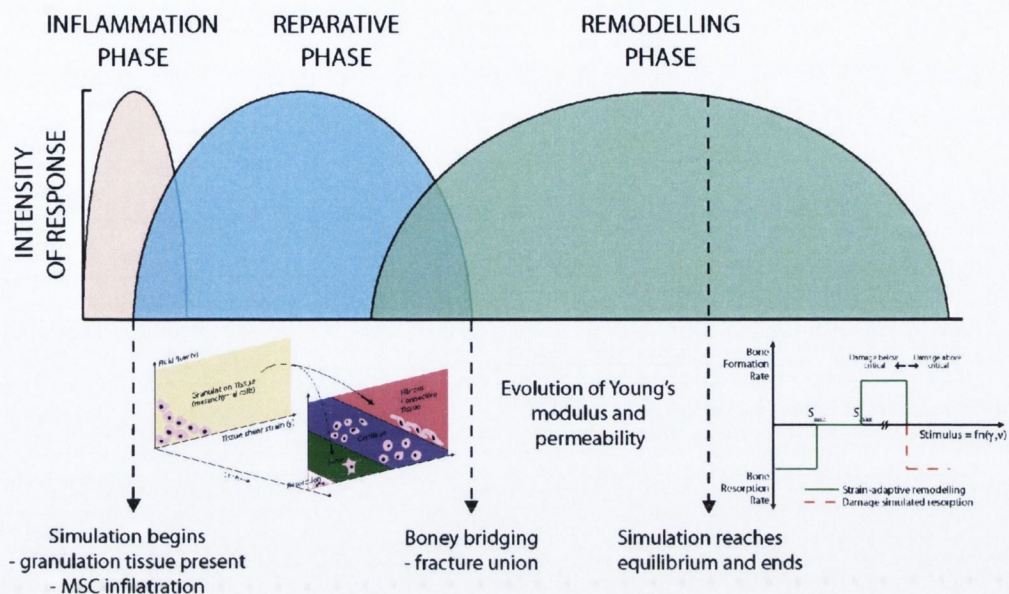


Figure 6-8: Combining the mechano-regulation algorithm with a more accurate description of evolving material properties allows the simulation to predict beyond the reparative phase of fracture healing. The simulations presented in Chapter 5 reached equilibrium before complete remodelling had occurred; it is therefore proposed that full remodelling will occur due to micro-strain as described by Frost (1987) or Mulvihill and Prendergast (2008)

6.3 Limitations

6.3.1 Cellular activity

Cellular apoptosis and MSC migration were not incorporated in the bone scaffold studies presented in Chapters 3 and 4; these processes were developed later and were included in the methodology of the fracture healing study, presented in Chapter 5. Regulation of apoptosis and proliferation due to mechanical stimulation is pivotal in tissue differentiation simulations, since the balance between these processes determines the activity of the cell population at any given time. In the fracture healing simulation presented in Chapter 5 apoptosis and proliferation of differentiated cells are regulated by the mechano-regulation stimulus; however the rates of each process were estimated as these parameters have not been measured experimentally. *In vitro* cellular experiments (cells in culture, or single cells) involving mechanical stimulation could be used to determine the appropriate parameters for the mechanobiological models (Prendergast and McHugh, 2004). For

example, Kearney *et al.* (2008) reported that cyclic uniaxial mechanical strain applied to MSCs seeded on a 2D silicone membrane, at magnitudes greater than 7.5%, induce apoptosis. McMahon *et al.* (2007) also demonstrated that mechanical constraint and 10% cyclic tensile loading in a 3D collagen-GAG scaffold modulated the chondrogenic differentiation of MSCs. Furthermore Weyts *et al.* (2003) demonstrated that the response of osteoblasts to mechanical stresses vary with their state of differentiation - in 7-day osteoblast cultures, tensile strain levels (between 0.4-2.5%) trigger apoptosis, while in more mature cultures, apoptosis is not affected by the same treatment. Additionally they found that stretching differentiating osteoblast cultures at day 14 increases proliferation. While the magnitude of the strain experienced by the cells in these studies is somewhat diminished from that which is applied to the substrate (Kearney, 2008), the results show that apoptosis threshold limits are not only cell-specific, but are also dependent on the state of differentiation. This further emphasises the importance of modelling the evolution of differentiating tissues by exclusively accounting for cell age in mechano-regulatory simulations.

Another issue to consider in these simulations is that the mechano-regulation stimulus is computed using a continuum representation of the tissue (i.e. a macro-mechanical variable); however cells do not detect these continuum stimuli but rather sense a micromechanical stimulus. If the experiments on cells, including those on single cells, are to combine with mechanobiological algorithms to provide solutions in mechanobiology, then it will require micromechanical finite element models based on high-resolution finite element modelling (Lennon and Prendergast, 2007). Stops *et al.* (2008) for instance, used the finite element method to determine the effect of external scaffold deformation on localised cellular strain. Further work is required to determine the effect of mechanical stimuli, including fluid flow, on all cell phenotypes and their associated cellular processes involved in bone regeneration. Isaksson *et al.* (2008) highlighted that the importance of the assumed cellular rates in the computational simulations are difficult to evaluate and an extensive parametric study is necessary to evaluate the importance of each assumption and its influence on the bone regeneration process. Once this information becomes available they can easily be implemented into the lattice model.

6.3.2 Angiogenesis

Angiogenesis is the formation of blood vessels from pre-existing vasculature. It plays a crucial role in the bone regeneration process by supplying nutrients and oxygen to the cells. During endochondral bone formation, vascular invasion into cartilage initiates the replacement of cartilage into bone. Geris *et al.* (2008) used a continuous mathematical model to describe fracture healing, where cell differentiation is controlled by the presence of growth factors and sufficient vascularisation. Their model, however, did not include the influence of mechanical stimulation on the angiogenesis process. Checa and Prendergast (2008) simulated tissue differentiation at a bone-implant interface, based on the mechanical environment and the local vascularity. Capillary formation was modelled by the random movement of endothelial cells at the capillary tips biased by the concentration of vascular endothelial growth factors. The model predicts capillary networks similar to those found in experimental studies and heterogeneous patterns of tissue differentiation, which are influenced by the capillary bed.

While angiogenesis was not explicitly simulated in the studies presented in this thesis, its effect on tissue differentiation was implicitly regulated via the mechano-regulatory algorithm. It is assumed that regions of high strain and fluid flow prevent the formation of blood vessels, which explains the persistence of cartilage tissue; whereas the growth of a vascular network can be established in regions of low strain, thus allowing for ossification.

6.3.3 Material properties

In order to fully understand biological regulation, the mechanical properties of the tissue phenotypes must be matched to the applied biophysical stimuli, (Perren and Rahn, 1980). Based on the work of Lacroix (2000) the mechanical properties of the tissues used throughout this work were taken from the literature. Some of these properties are well established while others remain unclear. In particular the permeability of woven bone is not well defined. Permeability is a measurement of the ease with which a fluid passes through a porous solid, and it is believed that *in vivo* skeletal permeabilities would be lower than those used in previous mechano-regulation simulations due to the presence of soft tissue within the pores. *In vivo*, the permeability of cortical bone influences transport of nutrients, waste products and

signalling molecules. Experimentally quantifying the permeability of regenerating bone tissue would improve the accuracy of the simulations and lead to a better understanding of the interaction between biomechanical forces and bone cells. (Thai and Kelly, 2008) carried out a basic study to determine the permeability of cancellous bone samples (with and without bone marrow), using a direct perfusion method similar to that of (Kohles *et al.*, 2001). It was shown that the permeability value without marrow was twice as much as the value measured with marrow.

To investigate the sensitivity of the model to woven bone permeabilities a parametric study was carried out in Chapter 5. A value of $1.02 \times 10^{-14} \text{ m}^4/\text{Ns}$ for mature woven bone predicted the most realistic healing patterns. In general, the study revealed that higher permeabilities lead to greater soft tissue formation and eventually non-healing, whereas lower permeabilities result in earlier bone resorption. The disparity of the results revealed the importance of accurately defining the mechanical properties. Lacroix (2000) carried out an extensive parametric variation study of the baseline material properties in Table 5-2, using a 2D axisymmetric fracture model. Contrary to the results in Chapter 5, he found that the varying the permeability of immature woven bone had a small effect on the fluid flow, while altering the permeability of mature woven bone did not influence the predicted mechanical stimuli. The main discrepancy between the results is the inability of 2D model to accurately simulate the fluid flow within the fracture callus.

Characterising the material properties of the cells and tissues within the finite elements can also be refined. Currently, the material properties of the regenerating tissues are determined using volume fractions (rule of mixtures), which assumes a linear relationship between the number and type of cells within the element. Using the lattice approach it is now possible to move away from the macroscopic level. If the finite element mesh corresponds to the lattice size it would be possible to treat the materials as homogeneous.

6.4 Clinical applications

The aim of mechanobiological modelling is to establish predictive methods to determine how mechanical forces regulate tissue differentiation. With sufficient validation these models hold great potential to contribute towards many scientific and clinical applications. Achieving this objective could enhance the treatment and prevention of many skeletal conditions, such as congenital deformities, bone fractures,

osteoporosis, and osteoarthritis. Fracture healing in particular has mainly been used to increase our understanding of mechanoregulation rules because of the vast knowledge already accumulated concerning mechanical loading and the healing response. The results presented in Chapter 5 corroborated well with histological and clinical observations. Further testing is required to simulate tissue differentiation in different circumstances; however it is suggested that this model could be used as a potential tool to establish protocols for accelerated fracture healing. Additionally, fracture fixation devices could also be evaluated to determine optimal loading conditions acting on the regenerating tissues.

The development of successful synthetic and engineered organs and tissues, 'tissue engineering', will also depend on mechanobiological progress (van der Meulen and Huiskes, 2002). It was shown in Chapters 3 and 4 that a crucial benefit of mathematical modelling is that a model can predict a variety of complex dynamics, allowing analysis of the system in terms of a relatively small number of fundamental parameters. MacArthur *et al.* (2004) highlighted the continuing need for development of mechanistic models in order to elucidate quantitative relationships between cell environment and behaviour. In doing so, these discrete models can help guide the design of tissue engineering procedures to repair traumatised and lost skeletal tissue. Modelling at the cellular level will also eradicate assumptions made by traditional continuum models.

Given the importance of testing medical devices before implantation in humans, and the issues surrounding performing such test in patients (Prendergast, 2008), computational methods such as those proposed in this thesis will become increasingly important in bone biomechanics to reduce animal experimentation and clinical trials. In the future, more complex and realistic computer simulations can be obtained using imaging techniques such as micro-CT, MRI, x-ray and ultrasound. Combined with muscoskeletal loading conditions these mechano-regulated techniques may enable optimised treatments for individual patients

Chapter 7

Conclusions

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7.1 Main conclusions

This thesis demonstrates that simulations of bone regeneration are best achieved using a lattice in which cellular activity is modelled, because it can account for cell-scale motility rates based on random-walk theory, while proliferation is modulated by contact inhibition. In addition, the explicit modelling of cell age enhances the possibility of accurately defining evolving material properties. The two examples used to test and validate the proposed approach (tissue differentiation in a regular structured bone scaffold, and in a simulation of fracture healing) also highlighted the importance of accurately defining cellular activity in three-dimensional mechano-regulation computational models as they largely influenced the outcome of the simulation.

The main conclusions are as follows:

1. The scaffold studies revealed that the optimal design characteristics are highly dependent on the applied loads – in a low load environment, a higher porosity and higher stiffness but a medium dissolution rate gives the greatest amount of bone, whereas in a high load environment the dissolution rate should be lower otherwise the scaffold will collapse. At lower initial porosities however, higher dissolution rates can be sustained.
2. Scaffolds conducive to osteogenesis in the initial stages of healing allow for the development of a system that will be able to withstand the applied forces as time progresses, thus indicating the possible merits of custom-made scaffolds for individual patients and/or the site of implantation.
3. In the fracture healing study, a more detailed representation of the loading conditions acting on the tibia improved the quantitative accuracy of the results, relative to earlier studies without muscle loading.
4. The predicted differentiation patterns that occur during the course of healing agree with those observed histologically.
5. By modelling the transition of woven bone into lamellar bone, healing was simulated beyond the reparative phase.
6. The permeability values of woven bone had a significant influence on the outcome on the results of the simulations.

7. The temporal change of the predicted interfragmentary strain and bending stiffness correspond with clinical observations.

7.2 Future work

The following recommendations are suggested as improvements to the simulation method presented in this thesis:

1. High-resolution finite element modelling combined with the lattice approach will facilitate calculations of biophysical stimuli acting at the cellular level and eradicate assumptions about macro-mechanical stimuli and material properties based on volume fractions.
2. Employing multi-factorial regression or optimisation techniques to determine the combination of scaffold properties to maximise bone formation in the scaffold study.
3. Introducing bone adaptation theories to the mechano-regulation algorithm can be used to regulate the growth and maintenance of bone tissue.
4. Simulating the effect of vascularisation on tissue differentiation can be simulated using the lattice approach; as modelled by Checa and Prendergast (2008).
5. The mechanosensitivity of cells has been shown to be very variable (McGarry *et al.*, 2004); therefore a method to simulate genetic variation of physiological parameters in the human population would take mechano-biological models to a new level of sophistication.

Furthermore, there are areas where experimental work would help better define modelling:

1. Cell experiments involving mechanical stimulation can be used to determine the cellular rates of the cell phenotypes involved in mechanobiological models.
2. Further validation of Prendergast's mechano-regulation hypothesis by attempting to simulate different natural and experimental models of tissue differentiation.
3. The temporal change of evolving tissue mechanical properties need to be analysed further. Experimental studies such as that carried out by

Moukoko *et al.* (2007) may help characterise the properties of the skeletal tissues.

In order to develop realistic mechano-regulation models, the applied loading on the regenerating tissue must elicit an accurate biophysical response. Therefore the material properties of the tissues must be more accurately defined.

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Appendix A

Example of stress patterns in a long bone

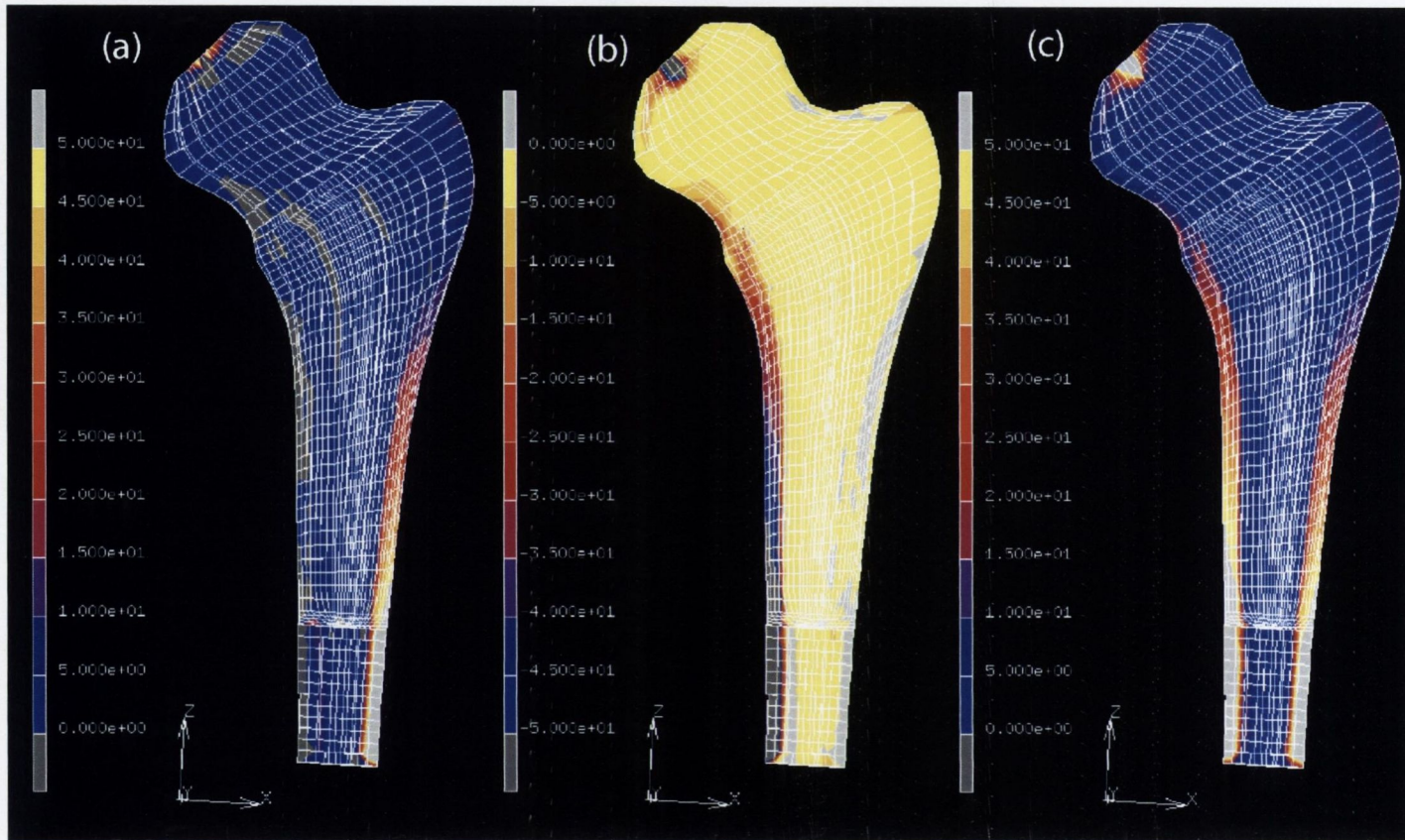


Figure A-1: Cross-sectional view of a femur under walking loading conditions. Illustrating contour plots of (a) von Mises stress, (b) minimum principle stress and (c) maximum principle stress in MPa. Adapted from the work of Lennon et al., 2007

Appendix B

Lattice point in element

Determining if a lattice point is inside an element is relatively simple if all the sides are orthogonal; however this becomes considerably more difficult if the mesh is irregular. In order to determine if a lattice point is inside or outside a callus element create a plane for each side of the element using the equation of a plane:

$$s = Ax + By + Cz + D \quad (7.1)$$

where the normal to the plane is the vector (A,B,C) , see Figure A-1. Given three points (x_1, y_1, z_1) , (x_2, y_2, z_2) , (x_3, y_3, z_3) the equation of the plane through these points is determined from the following determinants:

$$A = \begin{vmatrix} 1 & y_1 & z_1 \\ 1 & y_2 & z_2 \\ 1 & y_3 & z_3 \end{vmatrix}, B = \begin{vmatrix} x_1 & 1 & z_1 \\ x_2 & 1 & z_2 \\ x_3 & 1 & z_3 \end{vmatrix}, C = \begin{vmatrix} x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \\ x_3 & y_3 & 1 \end{vmatrix}, D = - \begin{vmatrix} x_1 & y_1 & z_1 \\ x_2 & y_2 & z_2 \\ x_3 & y_3 & z_3 \end{vmatrix} \quad (7.2)$$

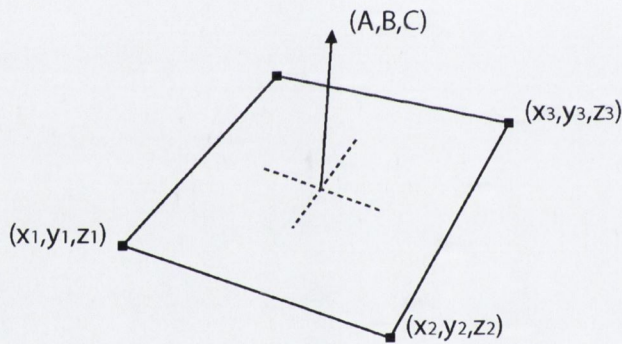


Figure B-1: Vector (A, B, C) normal to the plane

The sign of s from equation 7.1 determines which side the lattice point (x, y, z) lies with respect to the plane. If s is greater than zero then the point lies on the same side as the normal; if s is less than zero then the lattice point lies on the opposite side as the normal; and if $s=0$ then the lattice point lies on the plane. If the lattice point is below all 6 planes created from each side of the element then the lattice point is inside the element. If however the point is above one of the planes it can be deemed outside that particular element. Once this is achieved the boundary points can be determined by comparing adjacent lattice points. If a point goes from outside to inside it is on the boundary of the callus.