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Mechanobiological Models of Skeletal Tissue Differentiation

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Mechanical forces may act within tissues at the cellular level to regulate biological processes, a field of study that has been termed *mechanobiology*. The aim of computational mechanobiology is to derive sets of equations that describe the changes in cell expression, and hence the composition, structure and phenotype of tissues, as a function of the applied mechanical stimuli. This chapter will attempt to review the different mechanobiological models that have been developed to relate mechanical stimuli to tissue differentiation. The majority of these models have been used to simulate tissue differentiation during fracture healing or osteochondral defect repair. Based on this review, a number of recommendations will be made by the author for the future development of computational models of tissue differentiation.

Key words: *Mechanobiology, tissue differentiation, finite element model, fracture healing, osteochondral defect repair*

1. Introduction

In the embryo a mesenchymal stem cell is a pluripotent progenitor cell which divides many times and whose progeny eventually gives rise to the skeletal tissues: cartilage, bone, tendon, ligament, marrow stroma, connective tissue, as shown in Fig. 1. The progression from stem cell to final end phenotype is dependant on local cuing from surrounding cells as well as signals emitted by the cell itself and the reception of its own signalling [1]. The premise of mechanobiology is that biological processes such as mesenchymal tissue differentiation are regulated by signals to cells generated by mechanical loading. Repositories of mesenchymal stem cells also reside within the adult body (e.g. marrow, periosteum), which if successfully manipulated either in

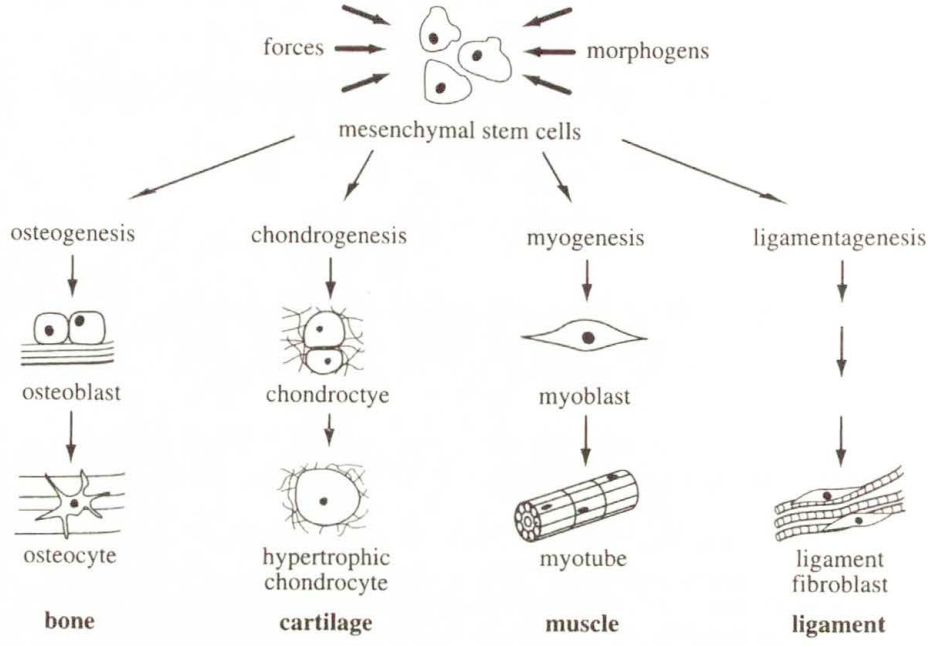


FIGURE 1. It is hypothesised that connective tissue cells differentiate from the mesenchymal cell pool in response to the local mechanical and biochemical stimuli. Adapted by van der Meulen and Prendergast [2] from Caplan [3].

vivo or ex vivo, could be used as a cell-based therapy to treat clinical problems such as cartilage and bone defects. To achieve this objective will require a comprehensive understanding of how mechanical loading effects tissue differentiation.

The purpose of computational mechanobiology is to determine the quantitative rules that govern the effects of mechanical loading on biological processes such as tissue differentiation [4]. A number of different studies have attempted to do this by hypothesizing the relationship between the mechanical stimuli experienced by cells and their differentiation pathway. Beginning with the work of Friedrich Pauwels, this paper will attempt to review a number of different mechanobiological models of mechano-regulated skeletal tissue differentiation, focusing primarily on the mathematical framework of the underlying hypotheses and on these methodologies used in their implementation. With the exception of the work of Pauwels and Perren, all these models have used the finite element modelling technique to determine the mechanical environment within the differentiating tissue.

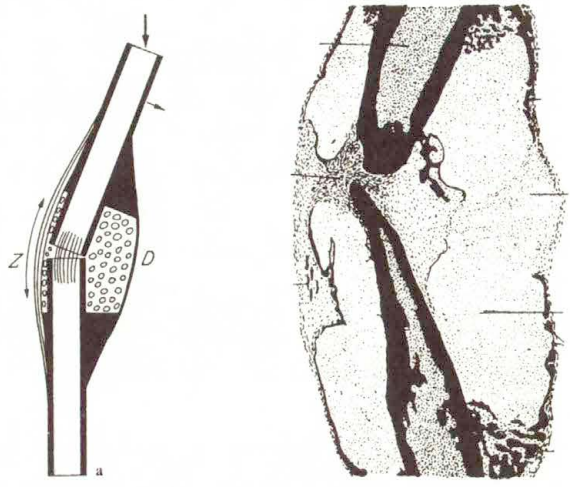
2. Pauwels Theory

Mechano-regulated tissue differentiation has mostly been studied during fracture healing of long bones or integration of orthopaedic implants. During these processes, bone tissue can form directly or indirectly. During endochondral ossification (indirect bone formation), cartilage is formed, calcified and replaced by bone tissue. During intramembranous ossification (direct bond formation), bone tissue forms without the intermediate cartilage stage. A comprehensive review of the mechanics of bone regeneration is available elsewhere [5]. By observing that mechanical loading of the fracture callus influenced bone regeneration, Pauwels [6] recognised that the mechanical environment of mesenchymal tissue can influence its differentiation pathway. He proposed that two stress invariants, namely the octahedral shear stress S and the hydrostatic stress D, regulated the type of soft tissue formed within the fracture callus. These stress invariants are defined as

$$S = \frac{1}{3}\sqrt{(\sigma_1 - \sigma_3)^2 + (\sigma_2 - \sigma_3)^2 + (\sigma_3 - \sigma_1)^2}, \tag{2.1}$$

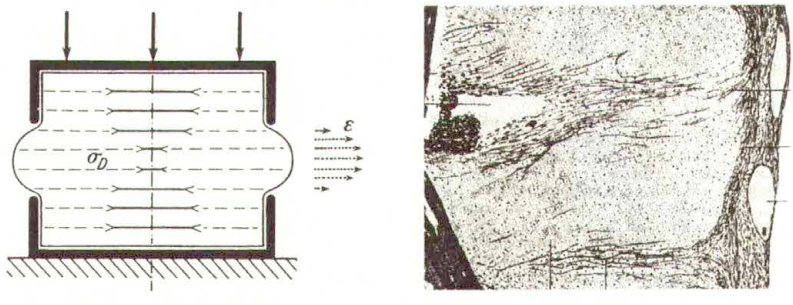
$$D = \frac{1}{3}(\sigma_1 + \sigma_2 + \sigma_3) \tag{2.2}$$

where σ_1, σ_2, σ_3 are the principal stresses. Octahedral shear stress causes material deformation, but no change in volume, and was proposed as a specific stimulus for fibrous tissue formation, while hydrostatic stress causes a change in material volume, but no change in distortion, and was proposed as a specific stimulus for chondrogenesis. Pauwels's ideas on tissue differentiation were based on comparisons of histological patterns in oblique pseudarthroses (false joint) and angulated fractures with the state of stress and strain in the tissue as determined by simple mechanical models, see Fig. 2 and Fig. 3. He noted that areas of cartilage formation consistently developed in certain locations within the fracture, which he believed coincided with areas of hydrostatic pressure. Similarly parallel collagen fibres or fibrous tissues were hypothesised to develop in areas of tissue elongation. Pauwels did not propose a specific stimulus for bone formation. Instead he concluded that bone formation occurred once cartilage or connective tissue provided a rigid enough template on which ossification could occur. The ossified tissues are then remodelled and are replaced by secondary lamellar bone, see Fig. 4. In conclusion, Pauwels theory for of tissue differentiation can be interpreted as follows:



(a) (b)

FIGURE 2. (a) Pauwels' illustration of an angulated fracture, consisting of a tension side (Z) where cells were thought to be elongated, and a compression side (D) where the cells were thought to be hydrostatically stressed. (b) Histological section of an angulated fracture callus, consisting of small chondrocytes on the compressive (right) side of the callus, and a more fibroblast like cells on the tension (left) side of the callus. Adapted from [6].



(a) (b)

FIGURE 3. (a) Pauwels' representation of cartilage being squeezed between the regenerating bony tissue. In the centre the regenerating tissue is elongated transversely. (b) Callus from a fracture of the forearm of a mouse, showing collagen fibers have developed in the area of maximum elongation. Adapted from [6].

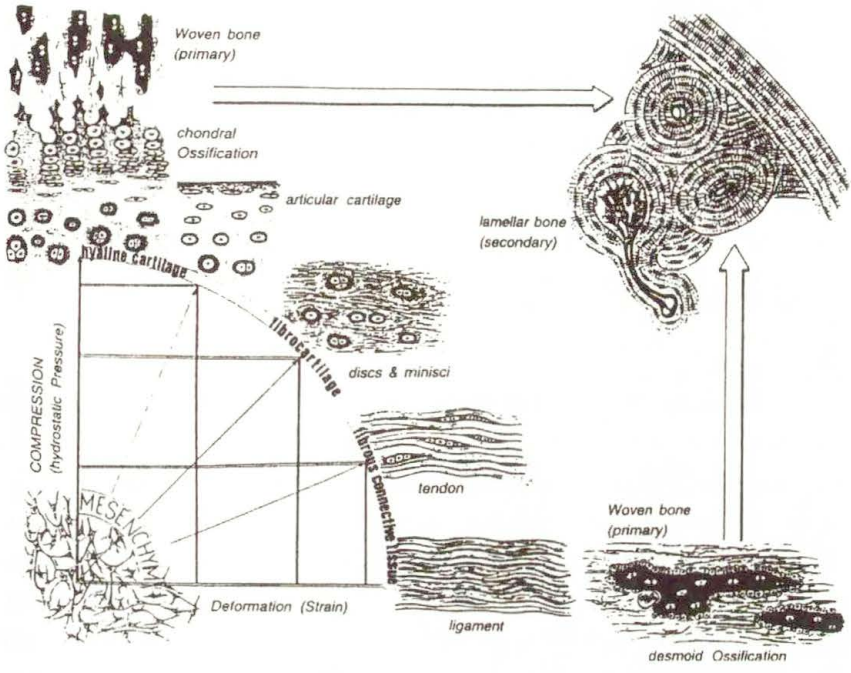


FIGURE 4. A schematic representation of the hypothesised influence of mechanical stimuli on tissue differentiation proposed by Pauwels. Figure taken from [7].

1. The stimulus favouring fibroblast differentiation from the mesenchymal cell pool is high shear.

2. The stimulus favouring chondrocyte differentiation form the mesenchymal cell pool is hydrostatic compression.

3. Strain Based Models

Perren [8] proposed a simple model for tissue differentiation based on a qualitative analysis of fracture healing. He hypothesised that a certain tissue phenotype would not form in a fracture callus if the strain level in the fracture callus caused that tissue to fail, see Fig. 5. This idea was termed the 'interfragmentary strain theory'. Interfragmentary strain was defined as the interfragmentary motion divided by the fracture gap size. Based on the strength of different tissue phenotypes, and the measured interfragmentary strain, tissue differentiation can be predicted. Initially the fracture site is filled with granulation tissue, which begins to differentiate towards cartilage,

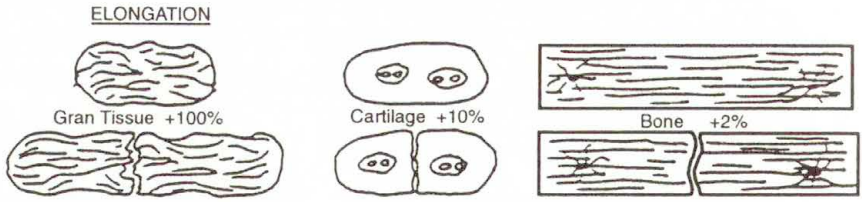


FIGURE 5. Strain tolerance of repair tissues. A tissue cannot exist in an environment where the interfragmentary strain exceeds the strain tolerance of the extracellular matrix of the tissue. Taken from [9].

gradually increasing the strength of the regenerating tissue. As the tissue stiffens, the interfragmentary strain decreases, allowing the formation of stiffer and stronger tissues at the fracture site. This process continues until full function is restored in the bone. Although easy to understand, this model is limited because it assumes that only a single tissue type exists within a fracture callus at any one point in time, which is obviously a simplification.

Duda and colleagues [10] proposed a model where the minimum principal strain served as the stimulus for tissue differentiation in a finite element model of an osteochondral defect. If the mean minimum principal strain around an element in the finite element model was above a threshold for the specific material that the element was modelling (defect/connective tissue, fibrous tissue, cartilage, calcified cartilage, cancellous bone, subchondral bone), then the elastic modulus of this specific element was increased. If the elastic modulus was further increased beyond the maximum elastic modulus for that particular material, differentiation to the next stiffer material occurred, and the material properties of the element were updated to that particular material. Similarly if the strain was below a threshold for a specific material the elastic modulus was decreased. A tissue factor (TF) was introduced to control the rate of tissue formation or resorption between iterations such that the change in Young's modulus between iterations was given by

$$E_n = E_{n+1}\mathrm{TF}. \tag{3.1}$$

Gómez-Benito et al. [11] present a mathematical model to simulate the effect of biophysical stimuli on cell proliferation, migration and differentiation during fracture healing. The mechanical stimulus ψ used in this model is the second invariant of the deviatoric strain tensor J_2:

$$J_2 = \sqrt{(\varepsilon_{\mathrm{I}} - \varepsilon_{\mathrm{oct}})^2 + (\varepsilon_{\mathrm{II}} - \varepsilon_{\mathrm{oct}})^2 + (\varepsilon_{\mathrm{III}} - \varepsilon_{\mathrm{oct}})^2} \tag{3.2}$$

where ε_{I}, $\varepsilon_{\mathrm{II}}$ and $\varepsilon_{\mathrm{III}}$ are principal strains, and $\varepsilon_{\mathrm{oct}} = (\varepsilon_{\mathrm{I}} + \varepsilon_{\mathrm{II}} + \varepsilon_{\mathrm{III}})/3$ is the octahedral strain. In this model, growth and geometry of the fracture callus are functions of cell proliferation and differentiation. The main variables in the model were the concentrations of mesenchymal stem cells (MSCs) (c_s), cartilage cells (c_c), bone cells (c_b) and fibroblasts (c_f), which produced the various skeletal tissues. The percentage of these basic types was assumed to determine the mechanical properties of the local tissue.

The number of cells N can be modified through a change in cell concentration ($Dc(\mathbf{x},t)/Dt$), where $c(\mathbf{x},t)$ is the cell density, or through a change in the volume growth rate ($\mathrm{div}(\mathbf{v})$), where $\mathbf{v}$ is the growth rate. The rate of change of MSC concentration was assumed to change by proliferation, migration and differentiation (cell death was considered a specific differentiation pathway) such that

$$\frac{Dc_s(\mathbf{x},t)}{Dt} = \frac{\alpha_{\mathrm{proliferation}}\psi(\mathbf{x},t)}{\psi(\mathbf{x},t) + \psi_{\mathrm{proliferation}}} c_s - D(V_{\mathrm{disrupted}})\nabla^2 c_s - f_{\mathrm{differentiation}}(\psi,t) \tag{3.3}$$

where c_s is MSC concentration, $V_{\mathrm{disrupted}}$ is the fraction of disrupted tissue, $D(V_{\mathrm{disrupted}})$ is a diffusion coefficient and $\alpha_{\mathrm{proliferation}}$ and $\psi_{\mathrm{proliferation}}$ are constants that define stem cell proliferation such that proliferation depends on the mechanical stimulus ψ. In this model an assumption was made that cells would migrate slower in disrupted tissue, which was modelled by making the diffusion coefficient dependant on the volume fraction of disrupted tissue. MSC differentiation ($f_{\mathrm{differentiation}}$) is dependent on both time and the mechanical stimulus such that

$$f_{\mathrm{differentiation}}(\psi,t_m) = \begin{cases} h_{\mathrm{intramembranous}}(\psi,t) & \text{if } (\psi_{\mathrm{lim}} < \psi < \psi_{\mathrm{bone}}) \wedge (t > t_m^b) \text{ (bone cells)} \\ g_{\mathrm{differentiation}}(\psi,t) & \text{if } (\psi_{\mathrm{bone}} < \psi < \psi_{\mathrm{cartilage}}) \wedge (t > t_m^c) \text{ (cartilage cells)} \\ l_{\mathrm{differentiation}}(\psi,t) & \text{if } (\psi_{\mathrm{cartilage}} < \psi < \psi_{\mathrm{fibrous}}) \wedge (t > t_m^f) \text{ (fibroblasts)} \\ -c & \text{if } (\psi_{\mathrm{death}} < \psi) \text{ (death cells)} \\ 0 & \text{in other cases,} \end{cases}$$

with $h_{\mathrm{intramembranous}}(\psi,t)$, $g_{\mathrm{differentation}}(\psi,t)$, $l_{\mathrm{differentiation}}(\psi,t)$ being the functions that define the evolution to osteoblasts, chondrocytes and fibroblasts, respectively, t_m^i is the maturation time needed for each cell type i to mature into specialised cells, and ψ_{lim}, ψ_{bone}, $\psi_{\mathrm{cartilage}}$, ψ_{fibrous} are the mechanical stimulus limits for each cell type.

It was assumed that callus growth was mainly due to mesenchymal cell proliferation and chondrocyte hypertrophy during endrochondral ossification:

$$\mathrm{div}(\mathbf{v}) = f^{v}_{\mathrm{proliferation}}(c_s, \psi) + g^{v}_{\mathrm{endochondral}}(\psi, t), \tag{3.4}$$

where $f^{v}_{\mathrm{proliferation}}(c_s, \psi)$ defines the rate of callus growth due to proliferation, v is the growth rate per day and $g^{v}_{\mathrm{endochondral}}(\psi, t)$ controls the rate of callus growth due to chondrocyte hypertrophy.

The mechanical properties of the differentiating matrix are characterised by its density and composition. A mixture of 5 different tissues can potentially be found: debris tissue, granulation tissue, cartilage tissue, fibrous tissue and bone tissue. The production rate of this extracellular matrix was assumed to depend on the cell type, cell density and matrix production rate per cell:

$$\frac{\partial V^{i}_{\mathrm{matrix}}}{\partial t} = c_i Q_i \tag{3.5}$$

where V^{i}_{matrix} is the volume fraction of tissue i, c_i is the cell density, Q_i is the matrix production rate per cell. The production of bone matrix volume in mature bone was determined using the internal bone remodelling formulation proposed by Beaupre et al. [12]. Based on the volume fraction of each tissue type within a particular region, the components p_x of the tissue are determined, namely the amount of collagen I , II and III, ground substance and mineral. The modulus of elasticity E and the Poisson's ratio ν were then determined based on the proportion of each component p_x in a particular region.

This model was implemented using a poroelastic finite element model (ABAQUS v.6.3, Hibbit, Karlsson and Sorensen) to determine the biophysical stimuli. A thermoelastic analysis was used to determine the new callus geometry after modelling cell migration, proliferation and differentiation. The callus geometry, tissue differentiation patterns and fracture stiffness predicted by the model were similar to experimental observations.

4. Hydrostatic Stress/Deformation Models

Carter and colleagues introduced a semiquantitive theory for the role of hydrostatic stress and octahedral shear stress in tissue differentiation within the context of fracture healing [13]. This concept differed from Pauwels by

specifying a specific stimulus for bone formation and by recognising the possibility that regions of tensile hydrostatic stress may exist within skeletal tissue. This model also accounted for the fact that bone cannot form without a sufficient blood supply. In a further development of this model, octahedral shear stress was replaced with either octahedral shear strain or maximum principal strain due to the belief that biological events at the tissue level are often related to changes in cell shape and local matrix deformation. The maximum principal strain was felt to be critically important as to whether intramembranous ossification can occur and also in controlling type I collagen synthesis [14]. According to this tissue differentiation concept, hydrostatic pressure directs the pluripotential mesenchymal tissue down a chondrogenic pathway; significant shear or tensile strain leads to fibrogenesis; a combination of hydrostatic pressure and significant shear or tensile strain leads to fibrocartilage development; and, given adequate vascularity, low levels of hydrostatic stress and shear/tensile strain allow direct intramembranous bone formation. A phase diagram illustrating this concept is shown in Fig. 6. The patterns of tissue differentiation observed during fracture healing [15, 16], distraction osteogenesis [15, 17], around bone-implant interfaces [18] and during

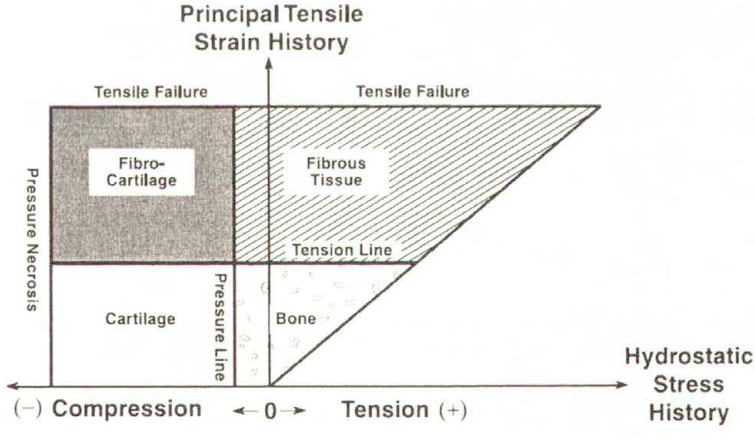


FIGURE 6. Phase diagram describing the influence of loading on the differentiation of pluripotential mesenchymal tissue into bone, fibrous tissue, fibrocartilage or cartilage. The tensile failure line marks the cut-off region beyond which failure of the mesenchymal tissue occurs as a result of excessively high tensile strains. The pressure necrosis line marks the pressure region beyond which cartilage/fibrocartilage no longer forms and tissue necrosis occurs instead, [16].

osteochondral defect repair [15] have been consistent with the expectations of this mechanobiological model based on linear elastic finite element calculations. The latter study involved creating an idealized two-dimensional finite element model of a full-thickness cartilage defect, see Fig. 7(a). The stress and strain histories within the defect were determined based on a time-varying load that was applied to the model. The hydrostatic stress within the defect was predicted to be chondrogenic, see Fig. 7(c); however due to the differences between the material properties of the normal cartilage and the regenerating tissue, high tensile strains were present within the regenerating tissue that are not present in normal cartilage, see Fig. 7(b). According to their mechano-regulation hypothesis, these tensile strains could be expected to promote fibro-cartilage or fibrous tissue formation. This model has not yet been used to simulate the time course of tissue differentiation during these events.

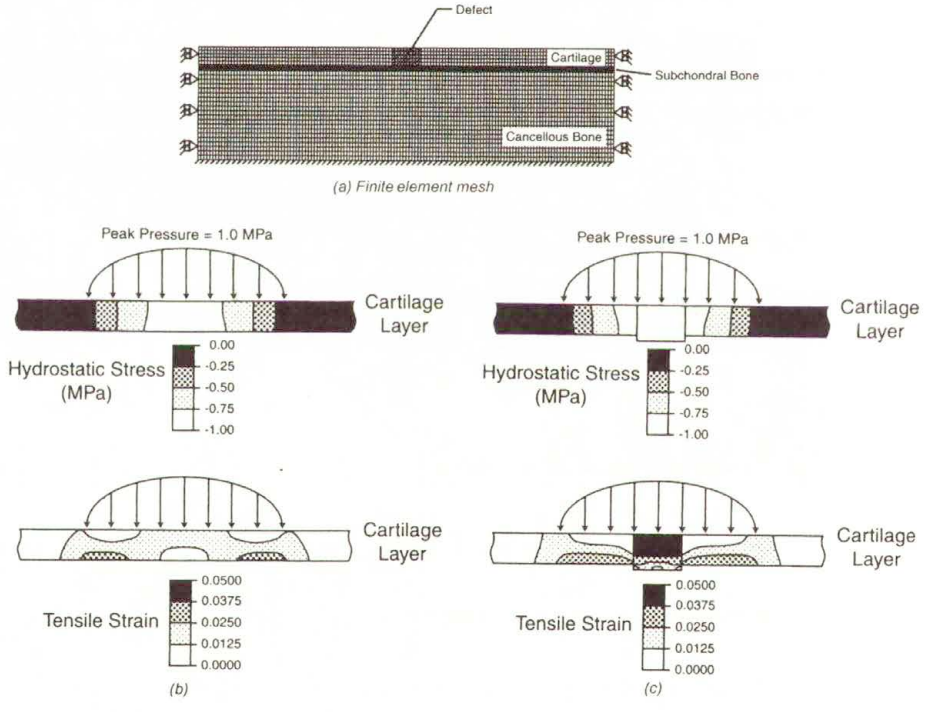


FIGURE 7. (a) Finite element mesh of a chondral defect used by Carter and Beaupré [15]. (b) The distribution of hydrostatic stress and tensile strain in normal articular cartilage. (c) The distribution of hydrostatic stress and tensile strain in the articular cartilage and regenerating tissue in the chondral defect.

Loboa et al. [19] have extended the tissue differentiation concept of Carter and Beaupré [14] to incorporate a constitutive model based on a fiber-reinforced, poroelastic representation of soft tissue to describe the time-dependent differentiation of multipotent mesenchymal tissue and the corresponding changes in tissue material properties. The controlling mechanical stimuli are the imposed intermittent tensile strain and the locally generated cyclic fluid pressure, see Fig. 8. This study simulates the time-dependent changes in three material properties necessary to describe a fiber-reinforced poroelastic constitutive model: the tensile elastic modulus (E), compressive aggregate modulus (H_A) and permeability (k). The solid matrix Poisson's ratio is assumed to be zero. The model only looks at loading histories that would lead to the formation of soft skeletal tissues; it does not attempt to model time-dependent changes associated with intramembranous bone formation.

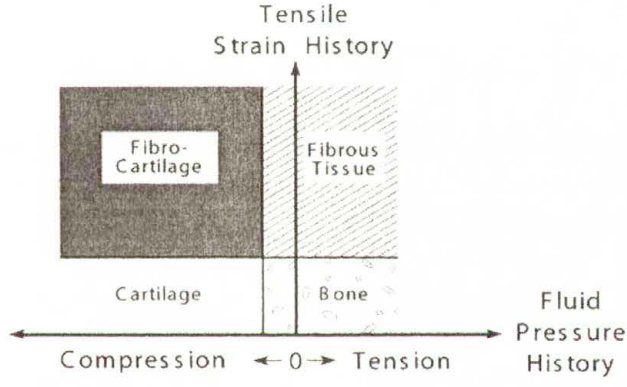


FIGURE 8. Influence of tensile strains and fluid pressure on tissue differentiation [19].

In this model the peak cyclic daily tensile strain ε determines the rate of modulus change $\dot{E}_\varepsilon$ due to tensile strain, occurring as a result of increased collagen fiber size, density, alignment and cross-linking. Tensile strain between 1.5% and 3% provides for tissue homeostasis, while strain above this magnitude causes an increase in $\dot{E}_\varepsilon$, and strain below this magnitude causes a decrease in $\dot{E}_\varepsilon$. The strain dependant component of the tensile modulus is then given by:

$$E_\varepsilon(t + \Delta t) = E_\varepsilon(t) - \dot{E}_\varepsilon \Delta t \tag{4.1}$$

where Δt is a given time step.

Increased fluid pressure is also speculated to induce chondrogeneis, as evidenced by an increase in tensile elastic modulus due to increases in both collagen type II synthesis (increasing the tensile modulus associated with collagen fiber content, E_f) and proteoglycan synthesis (increasing the aggregate modulus, H_a). Combining these two components gives the pressure-dependent component of the tensile elastic modulus:

$$E_p = E_f + H_a. \tag{4.2}$$

The total tensile modulus is obtained by adding Eq. (4.1) and Eq. (4.2):

$$E = E_\varepsilon + E_p. \tag{4.3}$$

In this model, the fluid pressure stimulus also determines the proteoglycan-dependent rate of permeability change $\dot{k}_p$ used to update the pressure dependant component of the permeability k_p at each time step Δt:

$$k_p(t + \Delta t) = k_p(t) + \dot{k}_p \Delta t. \tag{4.4}$$

Once the pressure exceeds a minimum value (0.013 MPa), the rate of permeability change $\dot{k}_p$ increases linearly with pressure until a maximum value of $\dot{k}_p$ is reached ($1.5 \times 10^{-15}\,\mathrm{m}^4/\mathrm{Ns/day}$).

The permeability is further reduced due to the increased flow path length that fluid must traverse as the collagen fibers increase in size and density. The path length also increases with increased proteoglycan size and packing, and as E (Eq. (4.3)) depends on both collagen and proteoglycan synthesis, it was used as an indicator of flow path length. A dimensionless parameter q_e, which decreases exponentially with E, is multiplied by k_p to calculate the total permeability k:

$$k = k_p q_e. \tag{4.5}$$

Upper and lower bounds are placed on the values of E, H_a, and k based on findings from the literature. Detailed descriptions and justifications for the changes in E, H_a and k in response to the mechanical stimuli are available [19].

Claes and Heigele [20] compared the local stress and strain in a fracture callus as calculated from a finite element model with histological findings from an animal fracture model. They proposed that the amount of strain and hydrostatic pressure along existing calcified surfaces at the fracture surface

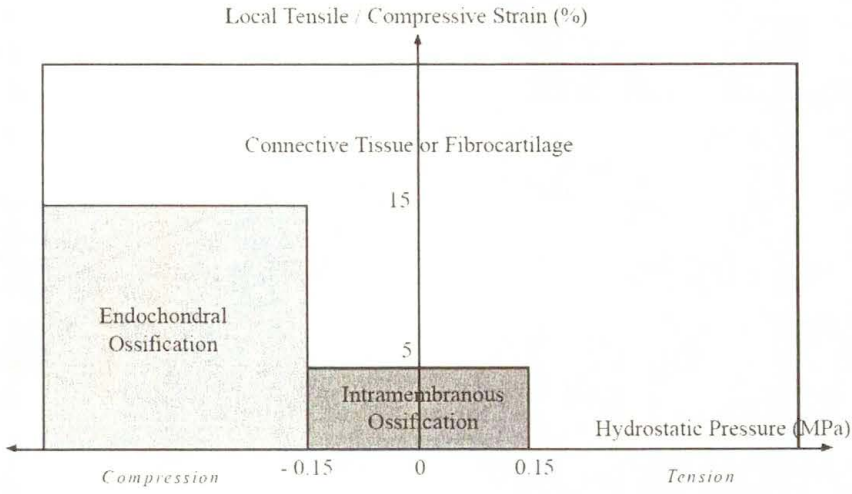


FIGURE 9. Mechano-regulation model of tissue differentiation proposed by Claes and Heigele [20].

determine the differentiation of the callus tissue. The hypothesis predicts intramembranous bone formation for strains smaller than approximately $\pm 5\%$ and hydrostatic pressures smaller than ± 0.15 MPa. Endochondral ossification is associated with compressive pressures larger than about -0.15 MPa and strains smaller than $\pm 15\%$. All other conditions lead to connective tissue or fibrous cartilage formation, see Fig. 9. In contrast to the models of Carter et al. [14], numeric values delineating the tissue types have been included. However no attempt was made to simulate the time course of fracture healing, i.e. only fixed time healing stages were modelled.

5. Models Including Fluid Flow

Tissues such as cartilage and bone are composed of a solid and a fluid phase. When such a tissue is loaded, the fluid components flows through the tissue, acting as a stimulus to the cells. If the fluid flow is high, so will be the biomechanical stress acting on the cells. Prendergast et al. [21] proposed a model for the mechano-regulation of tissue differentiation by two biophysical stimuli: tissue shear strain and interstitial fluid flow. High levels of the biophysical stimuli favour fibroblast differentiation from the mesenchymal cell pool, intermediate stimuli favour chondrocyte differentiation, and low mechanical stimuli favour osteoblast differentiation, see Fig. 10. Using this

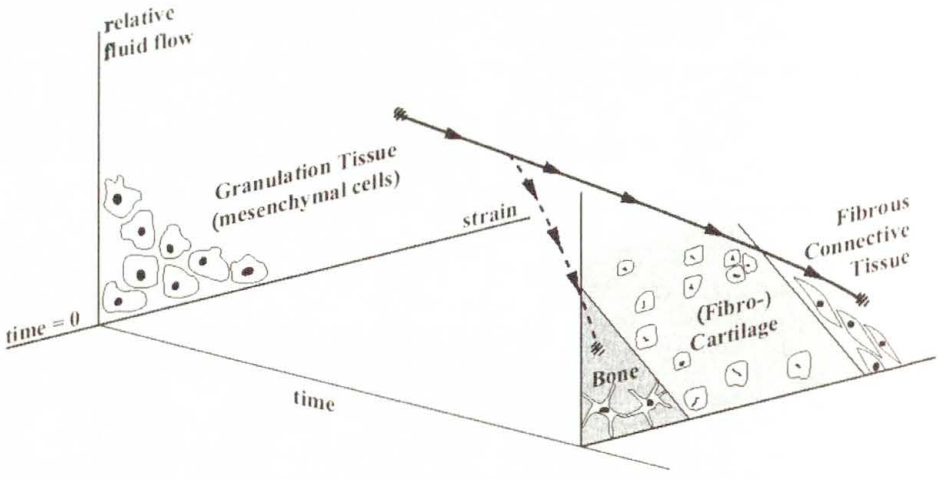


FIGURE 10. Mechano-regulation pathway hypothesised to control tissue differentiation based on the tissue strain and fluid flow. Both tissue strain and interstitial fluid flow are hypothesised to cause cell deformation. A region of high cell deformation causes fibrous tissue formation, intermediate deformations allow cartilage formation and low strains allow bone formation. After [21].

concept, a regulatory feedback model was developed to predict the patterns of tissue differentiation around an implant [22], where the tissue phenotype depends on the combined value of distortional strain γ and interstitial fluid flow v, such that

- For bone formation
$$\frac{\gamma}{a} + \frac{v}{b} < 1.$$
- For cartilage formation
$$\frac{\gamma}{a} + \frac{v}{b} > 1 \quad \text{and} \quad \frac{\gamma}{a} + \frac{v}{b} < 3.$$
- For fibrous tissue formation
$$\frac{\gamma}{a} + \frac{v}{b} > 3.$$

where $a = 3.75\%$ and $b = 3.0\,\mu\text{m/s}$. The biophysical stimuli were determined using a poroelastic finite element model of the bone-implant interface.

Lacroix et al. [23] expanded upon this mechano-regulation model in an attempt to simulate the time course of fracture healing in a long bone. In this model, a resorptive field was added at low strain/low fluid flow levels. If the strain or fluid flow becomes too low, then the lack of mechanical stimulation

to the cells initiates a resorptive process. Furthermore this model accounted for the role played by the migration and proliferation of mesenchymal stem cells in the fracture callus by assuming the spreading of cells can be simulated using a diffusion equation:

$$D\nabla^2 n = \frac{dn}{dt}, \tag{5.1}$$

where n is the cell density and the constant D is the diffusion coefficient. In this model the biophysical stimuli did not directly regulate the rate of change of the material properties of the differentiating tissue. Instead the material properties of an element in the model were calculated as an average of the 10 previously predicted tissue phenotypes. To account for the fact that mesenchymal stem cells and differentiated cells may exist simultaneously, a rule of mixtures was used to calculate the material properties in such cases.

Despite the simplifications of this model, it was successful in reproducing several features of fracture healing. These are:

1. intramembranous bone formation far from the fracture site,
2. endochondral ossification in the external callus,
3. stablisation of the interfragmentary gap when bridging of the external callus occurs,
4. resorption of the external callus.

Kelly et al. [24] further expanded the mechano-regulation model of tissue differentiation of Prendergast et al. [19] to simulate tissue differentiation during osteochondral defect repair. In this model, the dispersal, proliferation, differentiation and death of cells is regulated by the local environment. The dispersal of cells of a particular phenotype i throughout the defect was simulated by assuming the cell population to be described by diffusive, proliferative and apoptotic processes as follows:

$$\frac{dn^i}{dt} = D^i\nabla^2 n^i + P^i(S)n^i - K^i(S)n^i \tag{5.2}$$

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 an apoptosis (death) rate for cell i as a function of the stimulus S. The diffusion coefficient for cell type i moving through a volume of tissue is

calculated as the weighted average of the diffusion coefficients for each of the tissue types j present at that site in the model, i.e.

$$D^i = \sum_{j=1}^{n_t} D_{ij}\phi_j, \tag{5.3}$$

where D_{ij} is the diffusion coefficient for cell type i in tissue j, and n_t is the total number of tissue types, in this case granulation tissue, fibrous tissue, cartilage and bone; ϕ_j denotes the volume fraction of each tissue type j such that:

$$\sum_{j=1}^{n_t} \phi_j = 1. \tag{5.4}$$

As the cells disperse throughout the defect, their number will increase due to proliferation, or decrease due to apoptosis (cell death). The proliferative response of each cell phenotype might be expected to be influenced by their local environment such that the rate of change in the number of cells n^i of the i^{th} phenotype depends on $P^i(S)$, the proliferation rate for cell phenotype i as a function of a mechanical stimulus S, and $K^i(S)$, the apoptosis rate for cell phenotype i as a function of a mechanical stimulus S. A quadratic relationship was assumed between cell proliferation/apoptosis and octahedral shear strain S_o such that:

$$P^i(S)n^i - K^i(S)n^i = a + bS_o + cS_o^2. \tag{5.5}$$

The thresholds used in the mechano-regulation diagram of tissue differentiation were the same as used by Huiskes et al. [22] and Lacroix et al. [23]. Again a model based on the hypothesis of mechano-regulated tissue differentiation by shear strain and fluid flow was able to predict temporal changes in tissue phenotype as observed during experimentation; in this case during osteochondral defect repair.

Kuiper et al. [25] used tissue shear strain and fluid shear stress as mechanical stimuli regulating tissue differentiation during fracture repair, see Fig. 11. Fluid shear stress τ is defined as a function of pressure gradient ∇p and specific matrix surface s

$$\tau = \frac{\nabla p}{s} \tag{5.6}$$

where the specific surface is related to tissue permeability k, porosity n, fluid viscosity ν, and constant G through the Carman-Kozeny equation

$$k\nu G s^2 = n^3. \tag{5.7}$$

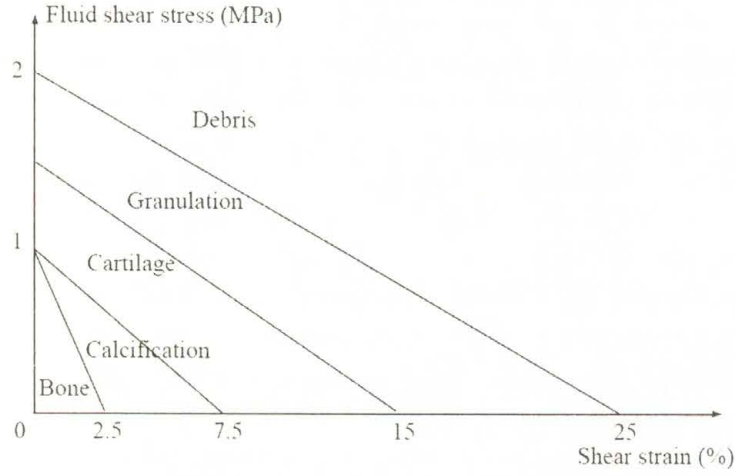


FIGURE 11. Mechano-regulation model of tissue differentiation proposed by Kuiper et al. [25].

They concluded that strain provides the dominant cell differentiating stimulus in the fracture callus. Typical healing patterns were predicted for a variation of applied movements on the cortical bone.

6. Future Directions

A number of different hypotheses have been put forward relating tissue differentiation to the local mechanical environment, none of which have been definitively refuted or proven. To this end, not only will it be necessary to continue comparing in-vivo experimental observations with the predictions of tissue differentiation based on these hypotheses, it will also be necessary to test the hypotheses directly using well designed in vitro experiments. A number of bioreactors have been recently designed which are capable of regulating the mechanical environment of a population of cells in vitro [26]. It should be possible to test hypotheses of mechano-regulated tissue differentiation by subjecting populations of mesenchymal stem cells to mechanical loading in a bioreactor [27, 28]. Not only could these bioreactors be useful in determining the role of mechanical loading in the differentiation process, they could also play a role in quantifying how mechanical loading influences cell migration, proliferation, death, matrix synthesis etc., which are necessary in order to develop a complete mechanobiological model of tissue differentiation that has practical benefits. For example, experiments where cartilage has been engi-

neered in a bioreactor [29] have shown that applying loading to a population of chondrocytes results in increased amounts of collagen II and proteoglycan synthesis over static controls, which in turn has lead to changes in the mechanical properties of the engineered tissue. Similar experiments could be used to quantify how a particular biophysical stimulus influences matrix synthesis and the alignment and cross-linking of collagen fibers, which in turn could be used to determine evolution equations used to describe the change in mechanical properties over time for differentiating tissue subject to loading. To implement such evolution equations accurately in computational models will require the use of more sophisticated constitutive models to describe the non-linear, inhomogeneous nature of these tissues. Other experiments that will be necessary to facilitate the development of computational models include determining the motility rates of different cell types and the mitosis rates of cells in response to loads. It may also be necessary to incorporate the effects of growth factors into mechanobiological models [30].

In conclusion, in addition to a hypothesis for mechano-regulated tissue differentiation, mechanobiological models of tissue differentiation should attempt to incorporate the following:

1. a mathematical framework to describe the dispersal, mitosis and death of cells in response to changing mechanical or chemical stimuli,
2. evolution equations to describe the synthesis and organisation of matrix components by cells in response to load,
3. equations to relate changes in the synthesis and organisation of matrix components to the tissues mechanical properties,
4. appropriate constitutive equations to describe the non-linear, inhomogeneous nature of biological tissues.

Only by successfully incorporating these elements can we expect computational models of tissue differentiation to become useful tools in tissue engineering and the design of orthopedic implants.

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