Advanced Course on Tissue Remodeling
ACTR'04 – (pp.219–240) – Warsaw, 2004.

Experiment and Computation in Mechanobiology, with New Applications in Cardiology and Evolution

Patrick J. Prendergast [1], Niamh C. Nowlan [1], Caitriona Lally [2]

[1] *Trinity Centre for Bioengineering, Department of Mechanical Engineering, Trinity College, Dublin, Ireland*

[2] *Department of Mechanical Engineering, Dublin City University, Glasnevin, Dublin, Ireland*

This aim of this chapter is to introduce the concepts of mechanobiology, and to provide a broad overview of the work being performed in the field. In the introductory section, a definition of mechanobiology is presented, in Section 2 some computational approaches for simulation of tissue differentiation and remodeling in response to mechanical loading are reviewed, and in Section 3 classes of experiments in mechanobiology are listed and several typical experiments are described. Despite the fact that much of the work in mechanobiology (as described in Section 3) is performed in the area of orthopaedics, its concepts also have more broad application in understanding how mechanical forces regulate the adaptation of all biological structures. Therefore, as an example of a non-orthopaedic application, in Section 4 of this chapter, research in application of mechanobiological principles to examine the response of arteries to the placement of a cardiovascular stents is given.

Mechanobiology assumes that the rules governing tissue response to stress are encoded in the genes. In Section 5 of this chapter, a theoretical framework for modeling the evolution of mechanoregulation is presented. It is found that it may prove possible to describe mechanoregulation equations in relationship to the genome. If this would prove to be true, it might present an interesting avenue of research on the regulatory role of mechanosensitive genes in human health.

1. Introduction

In their 2002 paper titled "Why Mechanobiology?", van der Meulen and Huiskes (2002) wrote that skeletal mechanobiology aims to discover

"how mechanical forces modulate morphological and structural fitness of the skeletal tissues – bone, cartilage, ligament, and tendon".

More informally, this means that mechanobiology aims to discover why the organs and tissue of animals are constructed as they are. Why do bones have an external shape and internal structure that seems optimized for load bearing? Why does cartilage appear at the ends of long bones where it is needed for lubrication of joints? Why do ligaments form to constrain the motion of bones so effectively? The use of the word "fitness" in the definition of mechanobiology is not accidental; it harkens back to Darwin and the survival of the fittest. It is noteworthy that mechanobiology is not a word that achieves universal acclamation because of its implicit assumption that the human body has machine-like characteristics, a proposal attributed to such philosophers as Descartes (Prendergast, 2003). However the human body does convert chemical energy into mechanical energy to cause motion, and like mechanical machines it also wears out, so machine-like features can be identified even if the metaphor of a machine is a disturbing one.

Biological structures are the result of natural selection. They have complex, sometimes beautiful shapes (Alexander, 1994). These complex musculoskeletal shapes are challenging to analyze from a structural point of view because they are formed from materials that are anisotropic and viscoelastic. The biology is challenging also, because the regulatory mechanisms governing skeletal construction are so intricate that, even if we know them in every detail, it would be difficult to assemble the facts to create a predictive model. Nonetheless such is the aim of mechanobiological modeling – it aims to establish predictive models of how mechanical forces cause changes in tissue type, structure, and shape (Carter and Beaupré, 2001).

Mechanobiology has long been of interest to biologists; we can think particularly of the work of Roux in the 19th Century and Pauwels in the 20th Century (Prendergast, 2004; van der Meulen and Huiskes, 2002). Recently interest has grown because it is recognized that musculoskeletal diseases are becoming ever more prevalent because of the greater number of older people in the population, and because of higher expectations in healthcare. Therefore, recent research has brought forward many competing theories relating

mechanical stimuli to tissue growth and adaptation (Prendergast, 2003). It is interesting that Currey (1995), a well known biologist, questions the predictive power of these theories; this matter of predictive power of theories in biomechanics was addressed previously (Prendergast, 2001).

2. Computational models of tissue (bone) remodeling and tissue differentiation

When a tissue is exposed to a mechanical stresses, the stresses can regulate either

(i) a change in the tissue phenotype, by which we mean it changes from one class of tissue to another, e.g. cartilage could change into bone, or

(ii) the tissue will not change but rather it will reorganize its internal structure. This case applies mainly to bone, and the process is called bone remodeling.

The first mechanobiological theories were applied to bone remodeling by the German anatomists and embryologists of the last century. Firoozbakhsh and Cowin (1981) give a description of these, which are now of a certain historical interest. Cowin & Hegedus (1976) proposed a continuum theory of adaptive elasticity where every material point could adapt its density in response to mechanical stimuli. A very comprehensive review of adaptive elasticity theory is presented in Cowin (2003); it may be classified as a phenomenological theory because it is not based on a mechanism of cell activity but rather on continuum mechanics theories that are later corroborated by experiment (Hart, 2001). Mechanistic bone remodeling theories, on the other hand, involve the use of assumptions about the behaviour of cells. The implementation of one such theory in a numerical model will be described in Section 2.1 below. Regarding tissue differentiation, theories have been developed following the ideas of the German orthopaedic surgeon Fredrick Pauwels. A brief description of this will be given in Section 2.2 below but a more thorough analysis is given previously (Prendergast et al., 2004).

Mechanoregulation theories are formulated in terms of an algorithm. It begins with a stress analysis of the musculoskeletal element, and uses continuum quantities to predict tissue remodeling or differentiation. These new material properties are then used to update the shape and material proper-

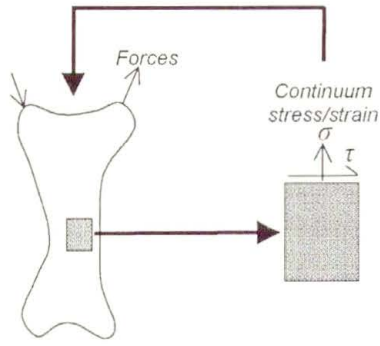


FIGURE 1. The present state of mechanobiological models. Forces acting on the organs are used to calculate continuum levels of biophysical stimuli. These continuum quantities are used to predict the change in mass and structure of the tissues based on assumptions about the behaviour of cells.

ties of the whole bone for a new structural analysis; hence the process is an iterative process (Fig. 1).

2.1. A Bone remodeling theory

Huiskes et al. (2000) describe a theory where bone resorbing cells (osteoclasts) and bone depositing cells (osteoblasts) perform removal and deposition of bone in response to signals received from osteocyte cells dispersed throughout the bone matrix. Put in mathematical terms, if we let m denote relative bone density ($m = 1.0$ for fully mineralized tissue) and let $P(x,t)$ denote the mechanical stimulus for osteoblast recruitment at surface location x as a function of time t, then, if $P(x,t) > k_{\rm tr}$ where $k_{\rm tr}$ is a threshold level of mechanical stimulus we write

$$\frac{dm}{dt} = \tau \left\{ P(x,t) - k_{\rm tr} \right\} - r_{\rm oc}, \tag{2.1}$$

τ being a time constant, and $r_{\rm oc}$ being an osteoclast resorption rate which is assumed to be constant. If $P(x,t) \leqslant k_{\rm tr}$ then

$$\frac{dm}{dt} = -r_{\rm oc}. \tag{2.2}$$

The osteoblast recruitment stimulus is calculated form mechanical stimuli acting in a continuum model of the tissue. In Huiskes et al. (2000), $P(x,t)$ is calculated as the strain energy density integrated over the bone. It is assumed

that osteocytes local to the remodeling site have more influence than distant ones, i.e.,

$$P(x,t) = \sum_{i=1}^{n} f_i(x)\mu_i R_{ti}(t), \tag{2.3}$$

where $f_i(x)$ is an exponential decay function, μ_i is the mechanosensitivity of osteocyte i, and $R_{ti}(t)$ is the strain energy density rate sensed by osteocyte i, and n is the number of osteocytes in the neighbourhood of the surface location considered. The exponential decay function is given by

$$f(x) = \mathrm{e}^{-(d_i(x)/D)}, \tag{2.4}$$

where $d_i(x)$ is the distance between osteocyte i and location x and D is a constant. These equations can be used in an iterative scheme similar to the scheme shown in Fig. 1. Finite element modeling is used to describe the continuum, and to simulate reaction of a continuum to a change in the loading. This algorithm automatically creates a trabecular structure that adapts to altered loading.

2.2. A tissue differentiation theory

Several theories in mechanobiology try to explain how mechanical forces regulate the differentiation of tissue from one phenotype to another (Prendergast and Van der Meulen, 2001). A theory proposed by Prendergast et al. (1997) and later improved to include migration, proliferation, and death of cells within the regenerating region (Lacroix and Prendergast, 2002; Kelly and Prendergast, 2004) is that strain and fluid flow act as the combined stimulus (S) to regulate stem cell differentiation such that a value of S given by,

$$S = \frac{\gamma}{a} + \frac{\nu}{b} \tag{2.5}$$

where γ is the peak shear strain and ν is the peak fluid velocity, and a and b are empirical constants. Based on the value of S, the tissue phenotype is determined according to

$$\begin{aligned} 0 \leq S < n &\quad \text{Bone resorption} \\ n \leq S < 1 &\quad \text{Bone} \\ 1 \leq S < m &\quad \text{Cartilage} \\ m \leq S &\quad \text{Fibrous connective tissue} \end{aligned} \tag{2.6}$$

These equations can be combined with finite element modeling in an iterative scheme (Fig. 1) to simulate tissue differentiation, where the stimuli (γ, ν) are calculated using a biphasic poroelastic finite element model. There are three aspects of the numerical simulation to consider further,

(i) the model computes continuum-level stimuli which are not directly related to the stimuli actually acting on the cells within the tissue,

(ii) the generation of the maximum level of stimulus in a poroelastic medium does not occur until after a number of cycles of loading,

(iii) a tissue does not differentiate immediately and it takes some time for the stimulus to provoke change.

3. Experimental models

Experiments are required to determine the many parameters required for the mechanobiological models. Experiments are also used to confirm that the mechanobiological models can provide realistic predictions.

3.1. Cell experiments

The first group of cell experiments involves applying a mechanical stimulation directly to cells in culture. Examples of such experiments are those that place cells on plates and bend or stretch the plates. Alternatively a fluid shear stress (steady, oscillating, or pulsatile) can be applied to the cells in monolayer. The outcome of these experiments is included release of either a *signaling molecule* or a *matrix molecule* by the cell (Fig. 2). For example in the fluid flow experiment on bone cells by Klein-Nulend et al. (1996), it was found that fluid flow upregulated Nitric Oxide and Prostoglandin E_2 release relative to cells that were not stimulated by fluid flow. This, and similar experiments, show that cells respond when they are deformed, whether it be by fluid flow or strain.

The second group of experiments applies mechanical forces to individual cells. An atomic force microscope (AFM) can be used. (In AFM, a laser is shone onto a very small cantilever. At the end of the cantilever is a 'tip' and when the tip comes in contact with a surface the cantilever bends and the laser beam is deflected.) The AFM can be used to measure force since there is a calculable relationship between the force at the tip and the deflection

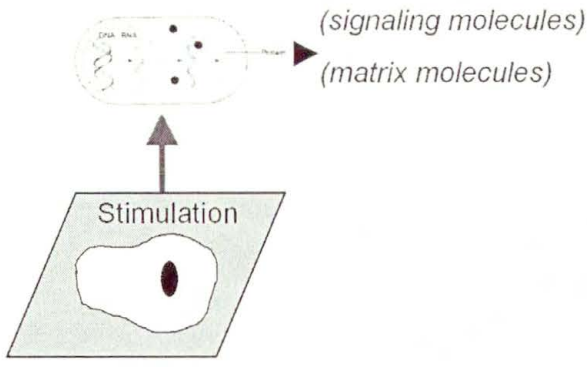
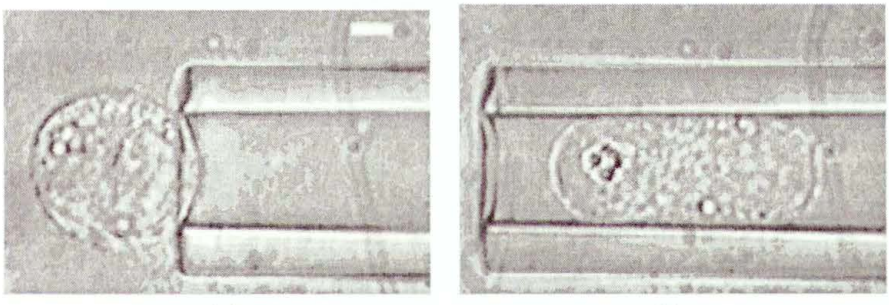


FIGURE 2. The hypothesis is that cell deformation causes cells to express both matrix molecules and signaling molecules. Matrix molecules form the extra cellular matrix (ECM) and signaling molecules generate responses from other cells.

of the beam. For example, Charras and Horton (2002) applied an AFM tip to osteoblast-like cells and found that release of Ca^{++} ions was stimulated when the cell was indented.

Another single cell method is micropipette aspiration whereby cells are deformed as they sucked into the pipette (Fig. 3). Since the pressure required to deform the cell can be measured, the viscoelastic properties of the cell can be calculated from such experiments. An example of such experiments are those on chondrocytes (cartilage cells) from normal and osteoarthritic cartilage by Jones et al. (1999).



(a) (b)

FIGURE 3. A chondrocyte cell is sucked into a glass pipette and the pressure required to do so is monitored. Finite element models can be used to fit the homogenized properties of the cell to the observed deformation (white bar equals $5\,\mu$m). After Jones et al. (1999).

3.2. In vivo experiments

In this case animals are used and experiments are performed that perturb the whole living system to evoke a response which can be modeled in a computer simulation.

These experiments can be divided into two categories: the first is experiments performed without the implantation of a device; these avoid the confounding effect of introducing a biomaterial into the host. One such experiment that has been reproduced by many scientists is that of cutting out (osteotomy) the ulna in a sheep forelimb thereby overloading the radius (Lanyon et al., 1982; Lee et al., 2002), see Fig. 4(a). Finite element models

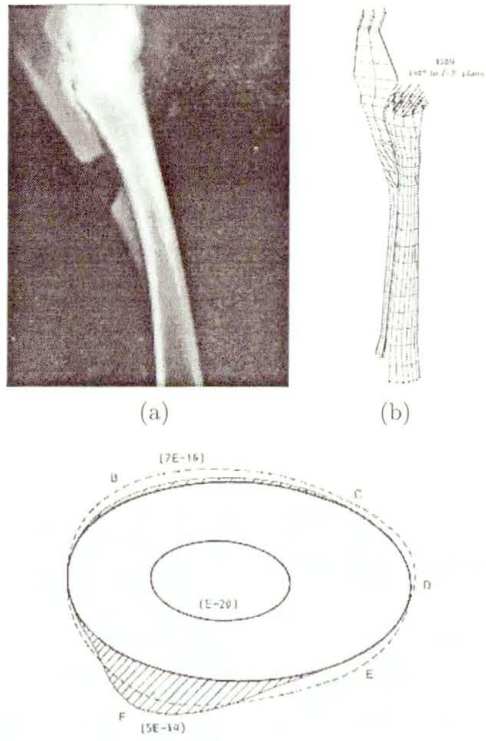


FIGURE 4. (a) Radiograph of the forelimb of a sheep after the ulnar osteotomy, from Lee (1995), (b) a finite element model of the bone, with the shaded region showing the elements to be removed to simulate osteotomy and (c) predicted remodeling showing the growth of the new bone in region of the increased stress and associated bone microdamage. Parts (b) and (c) after McNamara et al. (1992).

were later created which could simulate the growth of the ulna in response to the overload, see Cowin (2003). Figure 4(b) shows the associated finite element model used to predict the adaptation of the bone, with Fig. 4(c) showing the predicted bone deposition on the periosteal surface.

Another example of an experiment performed without the confounding effect of introducing a device are so-called "hindlimb suspension" experiments whereby rats are suspended by their tails so that the hind-limbs are raised off the ground thereby lowering the stress on them. The resulting adaptation of the hind-limb bones is similar to the situation in a low gravity environment, e.g. in space (Hardiman, 2004). These experiments have shown upregulation of genes that cause new bone formation in the regions of high mechanical stressing of the bone.

The second category of *in vivo* animal experiments uses devices implanted into animals. One example will suffice to illustrate the idea. This is an experiment reported by Geris et al. (2004). A bone chamber device is placed into

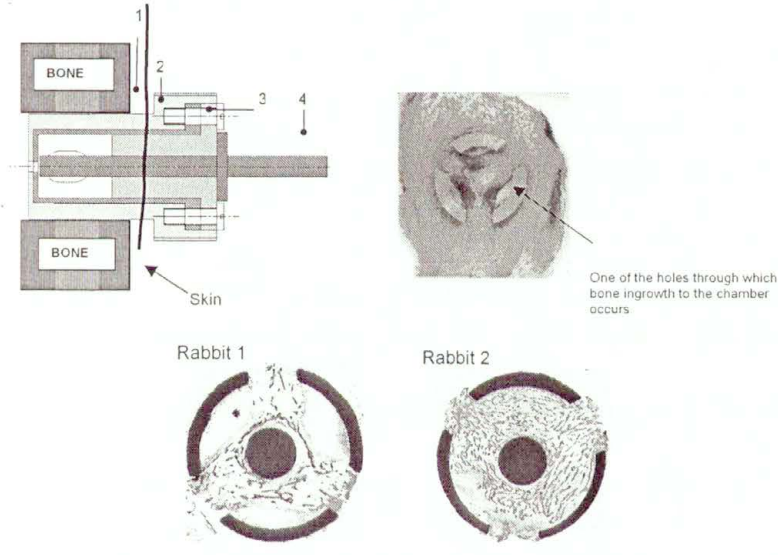


FIGURE 5. In this experiment, a bone chamber – consisting of an inner (1) and an outer (2) bone chamber, a teflon bearing (3) and an implant (4) – is placed into the proximal tibia of a rabbit (bottom left). The implant, which exits through the skin, can be displaced to load the tissue in the regenerating region (the white region in the figure). This white region is open to the bone through three holes in the side of the chamber (as shown in the cross-sectional views). In some animals not much tissue grows in (left) whereas in others the chamber becomes quite full of bone (right). Figure adapted from Geris et al. (2004).

the bone of rabbits. The chamber is initially empty but over time the bone grows in. Screwing an external loading device onto the chamber, the implant can be displaced and the regenerating tissue forming inside the chamber can be loaded, see Fig. 5.

3.3. Selection experiments

These experiments involve selective breeding of animals where selection for mating is made based on phenotype, e.g. some whole-organ trait, such as body mass or behaviour. One might imagine taking, say, mice and selecting bone strength as the determining factor. In this case, since a test of whole bone strength would be destructive and would kill the animal, sibling selection is used. After some number of generations there would be a statistically significant difference in the strengths of the bones between the selected population and a control population that mated randomly. Genetic differences could then be ascertained, identifying genes for bone strength. Such experiments are problematic, however, e.g. it is unclear how many generations would be required to see a divergence in the bone strength – in fact such experiments have not been done, though similar experiments based three generations of selection for high bone mineral density versus low mineral density have been done successfully (Klein et al., 2001). Garland (2003) gives a thorough introduction to selection experiments.

4. Simulation of the adaptation of an artery by in-stent restenosis after insertion of a cardiovascular stent

Although the sections above deal with adaptation of bone, the soft tissues also adapt to the forces acting on them. One important medical condition where the response of soft tissues to mechanical stress is evident is the response of arteries to the deploymnet of a cardiovascular stent. A cardiovascular stenting procedure involves expansion of an artery that is partially or perhaps almost completely blocked (or "stenosed"); the stent, when expanded in situ in the stenosed vessel, acts to hold it open (Fig. 6). Issues with respect to the design of cardiovascular stents are (i) elastic recoil: the stent will recoil elastically when the compressive force of the artery acts on it, (ii) scaffolding: the stent must not allow the tissue to protrude through the repeating units of the stent, (iii) plaque rupture: the stent must not stress the artery to such an extent that part of the plaque breaks off. These biomechanical aspects

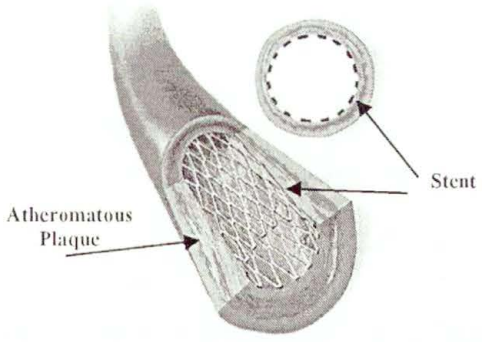


FIGURE 6. Picture of a cardiovascular stent deployed (adapted from `http://vascular.mdmercy.com/discoveries/balloon_stent.html`, last accessed 19/07/2004).

can be analysed by finite element modelling of the post-intervention stented artery (Prendergast et al., 2003; Lally et al., in press). A further aspect of the performance of cardiovascular stents relates to remodelling and adaptation of the vessel wall. The stenosis reforms around the stent – therefore it is called *in-stent* restenosis – and leads to the vessel becoming blocked again. It has been found from clinical studies that in-stent restenosis depends on stent design (Kastrati et al., 2000; Hoffman et al, 2002). Since the stents generate different stress distributions on the tissue depending on their rigidity and geometric design, we propose the hypothesis that in-stent restenosis can be predicted based on the biomechanics of stent designs.

The computational model for restenosis is based on the fact that stress generated by the stent is above the physiological range and sufficient to cause localized damage (Lally et al., in press; Holzapfel et al., 2002). We hypothesise that the injury, or damage, provokes proliferation of the smooth muscle cells (SMCs) which migrate to the inner lumen surface to create new tissue. To test this hypothesis, a simulation of in-stent restenosis was set up based on this mechanism. A finite element model was generated of a stent within a cylindrical artery (Lally, 2004). Using a Mooney-Rivlin constitutive model of the tissue, stress distributions were calculated. These stresses were used to compute a damage rate in the tissue, based on an Woehler curve for vascular tissue developed in our laboratory and reported in Lally (2004), and Miner's rule. This accumulated damage, ω, was taken as the stimulus for SMC proliferation within the lumen of the vessel. If the number of SMCs is denoted n and a mitosis rate at a site m is a function of the damage at

a site, then $\dot{n} = D\nabla^2 n + m(\omega)$. Based on the number of SMCs in an element of the finite element model, the inner lumen could be moved inwards as new tissue builds up on the inner surface of the blood vessel. This algorithm can be used to simulate, in an iterative fashion, the growth of the restenosis in response to the presence of a stent; an example of the use of this model to predict in-stent restenosis for a NIR stent design (Boston Scientific, Galway, Ireland) is shown in Fig. 7.

5. Development of mechanoregulation during evolution

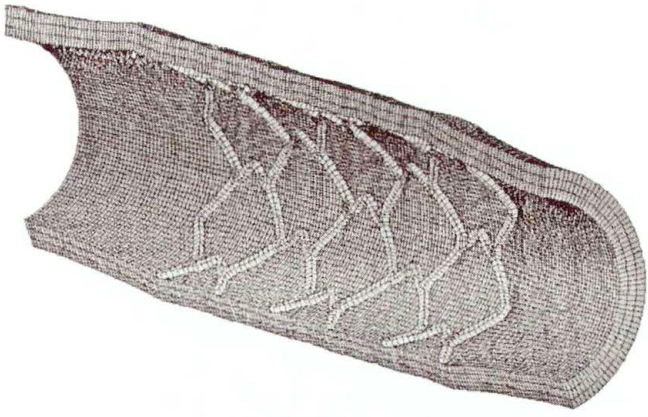
Over millions of generations, the skeletal template evolves, changing due to many imperceptible mutations. Ruff et al., (1983) presented evidence that a change in lifestyle, such as the switch from 'hunting and gathering' to agriculture will result in an altered skeleton. It has been proposed that evolution can only take place if variation is present with a population. We hypothesise that the rules governing bone remodelling vary within a population, and that the current assumed 'one size fits all' bone remodelling laws are insufficient for accurate assessment of many individuals.

Van der Meulen et al., (1993) used a computer model to simulate the growth of a long bone from an embryonic bone collar to maturity based on baseline growth rate and mechanical loading effects. We adapted this model to include a growth rate constant c according to

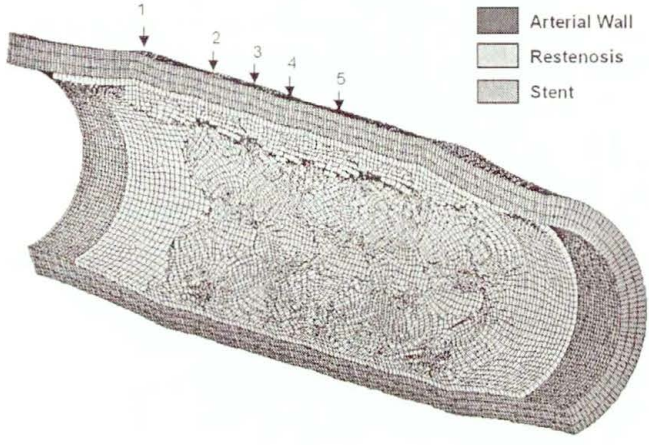
$$\frac{\partial r}{\partial t} = c(\psi - \psi_{\mathrm{AS}}),$$

where $\partial r/\partial t$ is the rate of bone apposition or resorption on the periosteal and endosteal surfaces, ψ is the daily stress stimulus and ψ_{AS} is the attractor state (or desired level) stimulus on the bone. The effect of different values of c on the mature cross-section of the bone is shown below in Fig. 8.

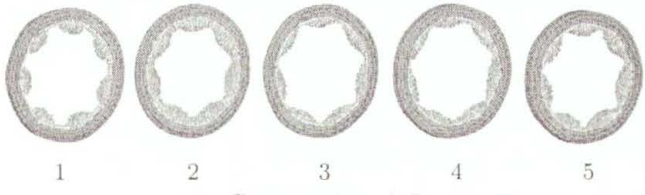
Our model tracks the development of a population with varying c values. Each individual is represented by a diploid chromosome with five allelic genes, as shown in Fig. 9, where each gene is represented by a random number between 0 and 1. The c value is determined by summing the genes at the five loci of the chromosome. Depending on the value of c, some individuals will have a more optimal bone strength than others, and will be more likely to survive. Once the bones have reached maturity, the fittest individuals are selected for recombination, and their genes are passed onto the next generation. The model runs a simulation with a population of 1,000 individuals for



(a)



(b)



Cross-sections 1–5

(c)

FIGURE 7. A simulation of in-stent restenosis with a NIR stent design; (a) the cylindrical vessel stented with a NIR stent, (b) the predicted pattern of restenotic growth using the restenosis algorithm and (c) cross-sections of the restenotic vessel at the location given in (b). It can be seen from (c) that restenotic growth is predicted to be concentrated around the struts.

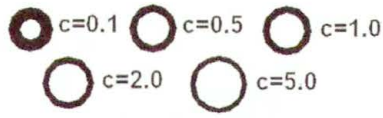


FIGURE 8. Effect of adjusting the bone remodeling rate constant on the cross-sectional shape of bones at age 60 years.

a	g_{1a}	g_{2a}	g_{3a}	g_{4a}	g_{5a}
b	g_{1b}	g_{2b}	g_{3b}	g_{4b}	g_{5b}

FIGURE 9. Diploid chromosome, where each gene is represented by a random number between 0 and 1.

1,000 generations. The model offers options to include mutations at a rate of 10^{-5} per recombination. Adding mutations to the model simply consists of randomly doubling or halving one of the genes in the chromosome. It is possible to vary the strength of the selection force cyclically to simulate a change in the external environment, where at certain times it is more or less important to have a high fitness.

There are two possible results for each simulation; either convergence so that all individuals in the population have the same value of c, or the maintenance of variation, where a range of c values are maintained after 1000 generations, as shown in Fig. 10. The results of the model indicate that the populations often do not converge to one value for c, which means that that variation in the mechanoregulation rules is maintained in the population. This means that for a given population, it is possible that individuals' bones would react differently to forces placed upon their bones, and that this reaction would be determined by their genetic makeup.

If the results of this model were accepted, this might have important ramifications for the biomechanics field. At present, when performing bone remodeling calculations for hip replacements, allowances are made for differences in weight (loading in the model) and bone geometry (with patient-specific CT scans or radiographs) but no allowance is made for a possible variation in bone remodelling responses within the population. For example, an implant that may provoke bone loss in one individual may not have such a deleterious effect in another individual. If allowance could be made for this fact it might be possible to give more valid predictions of the success of an orthopaedic implant in a whole patient population.

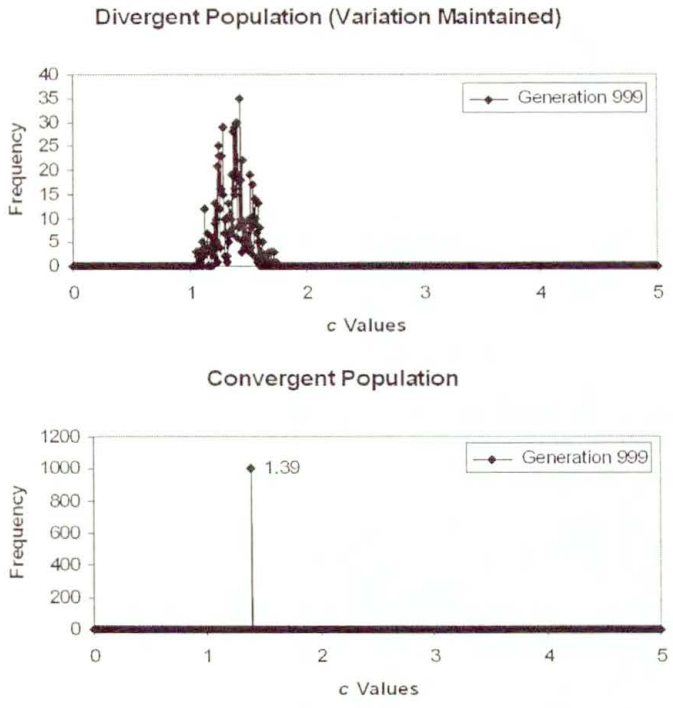


FIGURE 10. Snapshots of c values for populations after 1000 generations, for divergent and convergent populations.

The model could be developed further to incorporate genetic bone remodelling diseases, using so-called 'lethal genes' (Dawkins, 1989), so as to view the progression of a genetic disease over time. It might also be possible to view the effects of geographical separation on two sections of a population, to see whether or not speciation could be modelled with this scheme.

6. Six questions in mechanobiology

What are the most significant questions in mechanobiology at the present time, and how they may be solved.

6.1. The continuum-to-cell question

In the computational models outlined above, the stimuli calculated by the finite element models are continuum level quantities. This allows iterative procedures, using finite element analysis, to be formulated to simulate

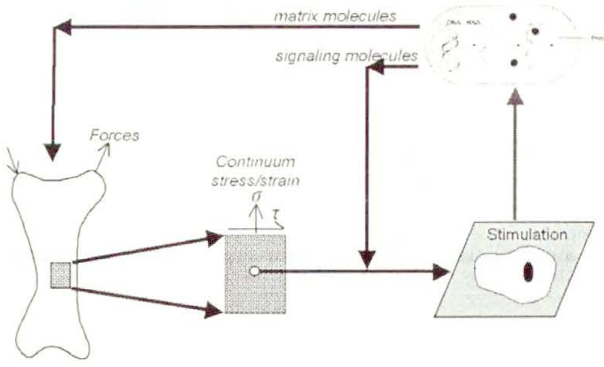


FIGURE 11. Combining present mechanoregulation models (refer to Fig. 1) with the hypothesis that cell deformations and other stimulation cause expression of mechanosensitive genes (refer to Fig. 2) gives future possible mechanobiological algorithms.

mechanobiological processes. However, if we wish to create models that include information gained from experiments on cells (the kind of experiments described in Section 3.1), it is necessary to add Fig. 2 into the loop and to complete the iterative procedure as shown in Fig. 11. However this will require models that can translate continuum level quantities into cell deformations – i.e. answer the question of how continuum level quantities convert into cell level deformations through the extracellular matrix.

6.2. The cell-to-gene question

When cells are deformed, some biophysical events effect gene transcription inside the cell and different signaling and matrix molecules are produced. How does cell deformation affect what the cell produces? Modeling the effect of local stimuli on the deformation of the cell components may help provide an answer to this question (McGarry and Prendergast, 2004) when combined with single cell experiments using AFM for example.

6.3. Stem cells and their differentiation as a function of mechanical stimulus

For the connective tissue viewpoint in mechanobiology, we are interested in mainly the mesemchymal stem cells, i.e. the stem cells that can differentiate into osteoblasts, chondrocytes, fibroblasts, adipocytes, etc. Despite much

research, it is still an open question how mechanical stimuli control cell differentiation into the various lineages. In this respect, the idea behind Section 2.2 is still speculative and in need of corroboration at the cellular level.

6.4. Mechanical environments for tissue engineering

Related to the question presented above is the topical question of how mechanical conditioning *in vitro* (i.e in bioreactors) can aid differentiation of connective tissue phenotypes *in vivo*. It is hypothesized that *in vitro* conditioning will create tissues of greater strength and endurance, but there is not very much direct experimental support for this hypothesis.

6.5. Role for self-organization in organ regeneration

An interesting question that has received some attention (Weinans and Prendergast, 1996) is the degree to which non-linear responses to mechanical stimuli can generate spontaneous self-organisation and pattern formation in tissues. This arises because it is difficult to believe that all the information to grow and adapt the musculo-skeletal system is contained in the genes.

6.6. Creation of mechano-regulation rules in phylogeny

The relationship between mechanical stimuli and tissue function, if it exists, must have become programmed into the genes by natural selection. Like all aspects of natural selection, it is driven by variation. If this is true, then the mechano-regulation rules that we are trying to discover (c.f. Section 2) are not static but changing, and they encapsulate variability (Nowlan and Prendergast, 2004; Prendergast, 2002). How can we cast the mechanoregulation rules in a mathematical format that includes their relationship to the genome?

Acknowledgements

This work was supported by Programme for Research in Third Level Institutions Cycle 3, administered by the HEA, and by the FP6 project BITES (Biomechanical Interactions in Tissue Engineering and Surgical Repair). Ms Lies Geris from the K.U. Leuven provided Figure 5. Dr Triona Lally's funding was received from Enterprise Ireland and Medtronic Vascular for the results

reported in Section 4. Ms Niamh Nowlan is funded by a scholarship from the Irish Research Council for Science, Engineering, and Technology (IRCSET).

References

1. R. McN. ALEXANDER, *Bones: the Utility of Form and Function*, Weidenfeld & Nicholson, London 1994.

2. D.R. CARTER and G.S. BEAUPRÉ, *Skeletal Function and Form. Mechanobiology of Skeletal Development, Aging, and Regeneration*, Cambridge University Press, Cambridge 2001.

3. G.T. CHARRAS and M.A. HORTON *Single cell mechanotransduction and its modulation analyzed by atomic force microscopy indentation*, Biophysics J., **82** : 2970–2981, 2002.

4. S.C. COWIN, *Adaptive elasticity: a review and critique of a bone tissue adaptation model*, Engineering Transactions, **51** : 113–193, 2003.

5. S.C. COWIN and D.H. HEGEDUS, *Bone remodeling I: theory of adaptive elasticity*, J. Elasticity, **6** : 313–326, 1976.

6. J.D. CURREY, *The validation of algorithms to explain adaptive remodeling in bone*, [in:] Bone Structure and Remodeling, pp.9–13, A. Odgaard, H. Weinans, [eds.], World Scientific, Singapore 1995.

7. R. DAWKINS, *The Selfish Gene*, 1989.

8. K. FIROOZBAKHSH and S.C. COWIN, *An analytical model of Pauwels' functional adaptation mechanism in bone*, J. Biomech. Eng., **103** : 246–252, 1981.

9. T. GARLAND JR., *Selection experiments: an under-utilized tool in biomechanics and organismal biology*, [in:] Vertebrate Biomechanics and Evolution, pp. 23–56, V.L. Bels, J.-P. Gasc, and A. Casinos [eds.], Bios Scientific Publishers, Oxford 2003.

10. L. GERIS, A. ANDREYKIV A, H. VAN OOSTERWIJK, J. VANDER SLOTEN, F. VAN KEULEN, J. DUYCK, and I. NAERT, *Numerical simulation of tissue differentiation around loaded titanium implants in a bone chamber*, J. Biomech., **37** : 763–769, 2004.

11. D.A. HARDIMAN, *Adaptation of Growing Bone to Altered mechanical Load: Morphological Change, Structural Strength, and Gene Expression*, PhD Thesis, University of Dublin, 2004.

12. R.T. HART, *Mechanics of bone regeneration*, [in:] Bone Mechanics Handbook, Chapter 31, S.C. Cowin [Ed.], CRC Press, Boca Raton, 2001.

13. R. HOFFMANN, G.S. MINTZ, P.K. HAAGER, T. BOZAGLU, E. GRUBE, M. GROSS, C. BEYTHIEN, H. MUDRA H, J. VOM DAHL, and P. HANRATH, *Relation of stent design and stent surface material to subsequent in-stent intimal hyperplasia in coronary arteries determined by intravascular ultrasound*, American Journal Cardiology, **89**: 1360–1364, 2002.

14. G.A. HOLZAPFEL, M. STADLER M, and C.A.J. SCHULZE-BAUER, *A layer-specific three-dimensional model for the simulation of balloon angioplasty using magnetic resonance imaging*, Ann. Biomed. Eng., **30**: 753–767, 2002.

15. R. HUISKES, R. RUIMERMAN, G.H. VAN LENTHE, and J.D. JANSSEN, *Effects of mechanical forces on maintenance and adaptation of form in trabecular bone*, Nature, **405**: 704–706, 2000.

16. W.R. JONES, H.P. TING-BEALL, G.M. LEE, S.S. KELLEY, R.M. HOCHMUTH, and F. GUILAK, *Alteration in the Young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage*, J. Biomech. **32**: 119–127, 1999.

17. A. KASTRATI, J. DIRSCHINGER, P. BOEKSTEGERS, S. ELEZI, H. SCHÜHLEN, J. PACHE, G. STEINBECK, S. SCHMITT, K. ULM, F.-J. NEUMANN, and A. SCHÖMIG, *Influence of stent design on 1-year outcome after coronary stent placement: a randomized comparison of five stent types in 1,147 unselected patients*, Catheterization and Cardiovascular Interventions, **50**: 290–297, 2000.

18. D.J. KELLY and P.J. PRENDERGAST, *Mechano-regulation of stem cell differentiation and tissue regeneration in osteochondral defects*, J. Biomech. (in press), 2005.

19. R.F. KLEIN, M. SHEA, M.E. GUNNESS, G.B. PELZ, J.K. BELKNAP, and E.S. ORWOLL, *Phenotypic characterization of mice bred for high and low peak bone mass*, J. Bone Miner. Res., **16**: 63–71, 2001.

20. J. KLEIN-NULEND, C.M. SEMEINS, E.H. BURGER, A. VAN DER PLAS, N.E. AJUBI, and P.J. NIJWEIDE, *Response of isolated osteocytes to mechanical loading in vitro*, [in:] Bone Structure and Remodelling, pp.37–49, A. Odgaard and H. Weinans [Eds.], World Scientific, Singapore 1995.

21. D. LACROIX and P.J. PRENDERGAST, *A mechano-regulation model for tissue differentiation during fracture healing: analysis of gap size and loading*, J. Biomech. 35 : 1163–1171, 2002.

22. C. LALLY, F. DOLAN, and P.J. PRENDERGAST, *Cardiovascular stent design and vessel stresses: A finite element analysis*, J. Biomech., in press, 2005.

23. C. LALLY, *An investigation into the Influence of cardiovascular Stent Design on the Development of Restenosis using the Finite Element Method*, PhD thesis, University of Dublin, 2004.

24. L.E. LANYON, A.E. GOODSHIP, C.J. PYE, and J.H. MACFIE, *Mechanically adaptive bone remodeling*, J. Biomech., **15** : 141–151, 1982.

25. T.C. LEE, *Functional Adaptation in Compact Bone*, PhD Thesis, University of Dublin, 1995.

26. T.C. LEE, A. STAINES, and D. TAYLOR, *Bone adaptation to load: microdamage as a stimulus for bone remodeling*, J. Anat. **201** : 437–446, 2002.

27. R.C. LEWONTIN, *The shape of optimality*, [in] The Latest on the Best, J. Dupré, [Ed.], MIT Press, Cambridge 1987.

28. J.G. MCGARRY and P.J. PRENDERGAST, *A three-dimensional computational model of an adherent eukaryotic cell*, Eur. Cell. Mat. **7** : 27–34, 2004 (freely available at `www.ecmjournal.org`).

29. B.P. MCNAMARA, P.J. PRENDERGAST, and D. TAYLOR, *Prediction of bone adaptation in an ulnar-osteotomised sheep forelimb using an anatomical finite element model*, J. Biomed. Eng., **14** : 209–216, 1992.

30. N.C. NOWLAN and P.J. PRENDERGAST, *A scheme for investigating the evolution of bone remodeling laws*, Book of Abstracts of an International Workshop on Numerical Models in Bone Adaptation and Repair, p.22,

L. Claes [Ed.], Institute for Orthopaedic Research and Biomechanics, Ulm 2004.

31. P.J. PRENDERGAST, *An analysis of theories in biomechanics*, Eng. Trans. (Rozprawy Inżynierskie) **49** : 117–133, 2001.

32. P.J. PRENDERGAST, *Mechanics applied to skeletal ontogeny and phylogeny*, Meccanica **37** : 317–334, 2002.

33. P.J. PRENDERGAST, *Life and limb: the bioengineering of prostheses and implants*, Trinity Centre for Bioengineering, Dublin 2003.

34. P.J. PRENDERGAST, *Computational mechanobiology*, [in] Computational Bioengineering: Current Trends and Applications, pp. 117–133, M. Cerrolazza, G. Martínez, M. Doblaré, and B. Calvo [Eds.], Imperial College Press, London 2004.

35. P.J. PRENDERGAST, D.J. KELLY and J.P. MCGARRY, *Lecture notes on modelling the biomechanical behaviour of cells*, [in] Proceedings of AMAS, Warsaw (in press), 2004.

36. P.J. PRENDERGAST, C. LALLY, S. DALY, T.C. LEE, D. QUINN and F. DOLAN, Analysis of prolapse in cardiovascular stents: a constitutive equation for vascular tissue and finite element modelling, ASME Journal of Biomechanical Engineering, **125** : 692–699, 2003.

37. P.J. PRENDERGAST, R. HUISKES, and K. SØBALLE, *Biophysical stimuli on cells during tissue differentiation at implant interfaces*, J. Biomech., **30** : 539–548, 1997.

38. P.J. PRENDERGAST and M.C.H. VAN DER MEULEN, *Mechanics of bone regeneration*, [in] Bone Mechanics Handbook, Chapter 32, S.C. Cowin [Ed.], CRC Press, Boca Raton 2001.

39. C.B. RUFF, W.C. HAYES, *Cross-sectional geometry of pecos pueblo femora and tibiae—a biomechanical investigation: I. Method and general patterns of variation*, Am. J. Phys. Anthrop., **60** : 359–381, 1983.

40. M.C.H. VAN DER MEULEN and R. HUISKES, *Why mechanobiology? A survey article*, J. Biomech., **35** : 401–414, 2002.

41. M.C.H. VAN DER MEULEN, G.S. BEAUPRÉ, and D.R. CARTER, *Mechanobiologic influences in long bone cross-sectional growth*, Bone, **14**: 635–642, 1993.

42. H. WEINANS and P.J. PRENDERGAST, *Tissue adaptation as a dynamical process far from equilibrium*, Bone **19**: 143–149, 1963.