COMPUTATIONAL MODELLING OF CELL AND TISSUE MECHANORESPONSIVENESS
Patrick J Prendergast
Trinity Centre for Bioengineering, School of Engineering, Parsons Building, Trinity College, Dublin 2, Ireland

ABSTRACT

Tissues have adapted to sustain the mechanical forces encountered in the earth’s gravitational field. Furthermore tissues remodel to the prevailing mechanical environment during the lifetime of the individual, i.e. ontogenic adaptation. If the mechanical environment changes, it is to be expected that not only would the degree of optimality achieved during evolution would be lost but the ontogenetic processes that ‘tune’ the organism to the prevalent mechanical loading would cause further adaptation. These are among the reasons that it is interesting to examine tissue response to mechanical stress, and the cellular responses governing such responses. This paper presents an overview of computational approaches used to analyse the regulatory effect of mechanical forces on the musculoskeletal system. In particular work done on the computational modelling of cell/tissue responses is presented together with some experiments corroborating these models. The paper concludes by returning to the issue of mammalian cell responses in ontogeny and how that might be extrapolated to predict ontogenic and phylogenetic responses in low gravity environments.

INTRODUCTION

Mammalian musculoskeletal systems are geared to gravity – they have evolved under natural selection to allow the species to survive in the terrestrial or aquatic gravitational environment of earth (Carter and Beaupré, 2001; Currey, 2002). It is well known that the tissues of the skeleton, particularly bone and muscle, respond during life to the prevailing mechanical forces applied to them. This ontogenic ‘fine-tuning’ to mechanical loading can readily be observed in humans who suddenly become physically active, and it has been quantified in animals; perhaps the best-known studies are the mouse hindlimb suspension experiments (van der Meulen et al., 1995; Hardiman et al., 2005). Such experiments have shown that skeletal structures change shape, and the bone tissue within them changes stiffness, when the average mechanical load is changed. Responses are subject to great variation even between individuals of the same species, and experiments on different strains of mice have attempted to elucidate a genetic basis for this (e.g., Amblard et al., 2003). Therefore, not only is the final shape of the skeletal structure subject to variation, but the mechanoresponsiveness is also subject to variation among individuals (Prendergast, 2002).

Computational models have been developed which can simulate the ontogenic adaptation in response to mechanical loads. Two processes have been examined in detail: bone remodelling whereby bones adapt their shape and internal structure in response to changing loads (Huiskes et al., 2000; Hart, 2001) and mechanoregulation of tissue differentiation whereby mechanical forces regulate tissue differentiation from one phenotype to another (Prendergast and van der Meulen, 2001). Such computational models have shown that relatively simple algorithms can simulate the tissues’ responses to changes in forces. However, the algorithms take no account of the genetic basis of the adaptations – in this respect they are deterministic and would not account for the variation in the response by different members of the population if the population was subjected to a new environment. Another issue with such predictive models is that it has proven difficult to corroborate them against animal experiments because this problem of variability between individuals is compounded by problems relating to differences in loading and skeletal geometry.

In this article the author presents current research on computing the response of cells, tissues, and skeletal elements to the mechanical loading environment. Given the volume of work in this field it is not possible to be comprehensive but it is possible to describe some current research and to highlight future research directions that may be relevant to gravitational and space biology.

CELLS RESPOND TO FORCES

The cells in the human body are subjected to mechanical stimulation and to fluid shearing forces. The cells from load-bearing tissues – such as skeletal (Carter and Beaupré, 2001), vascular (Rachev, 2005) and mesentery (Gregersen, 2002) tissues – have been studied intensively in respect of their mechanosensitivity. The experiments on cells can be listed as:

(i) single cell biomechanics, viz. micro-pipette experiments where single cells are sucked into a pipette under known pressure with simultaneous video recording of cell deformation (e.g. Jones et al. 1999) or, more recently, indenting the cell using the atomic force microscope and measuring both cell expression and cell stiffness (e.g. Charras and Horton, 2002; Maguire et al. 2007).

(ii) cells in monolayer experiments, such as substrate stretch and fluid flow over confluent layers of cells (e.g. Bacabac et al. 2004; Klein-Nulend et al. 2003; McGarry et al. 2005).
These experiments show unequivocally that mechanical stressing elicits expression of signalling and matrix molecules. The experiments on cells in a confluent layer have been important because they have shown that the magnitude of stimuli acting due to physiological mechanical loading can cause cells to signal with Nitric Oxide (NO) and prostaglandin E\(_2\) (PGE\(_2\)) molecules. If forces on the skeleton reduce and cause the biophysical stimuli in the tissue to reduce correspondingly then the tissue would react in some way via cell mechanoresponsiveness. This clearly establishes that mechanosensitive cells have the potential to maintain tissue structure; it is highly likely that this system ensures that the healthy skeleton is attuned to the mechanical environment in which it must function.

In an attempt to better understand what causes cellular mechanosensation, modelling of cells in these experiments was presented by McGarry et al. 2005. This study describes a computational model of a cell subject to fluid flow and substrate strain. The model (described in detail by McGarry and Prendergast, 2004) was developed using the finite element method and it creates a complex geometry of a cell attached to a substrate; the cell has a spread configuration, and the cytoskeleton of microtubules and actin elements is configured as a tensegrity structure within a solid cytosol; the nucleus is also included (see Fig. 1). It was found that substrate strain and fluid flow resulted in mechanical stress in all elements in the model but manifest as very different biophysical stimuli at the membrane indicating an independent role for these stimuli in attuning cells to their mechanical environment. It was further predicted that the nucleus is subject to a degree of mechanical stress that depends on the nature of the biophysical stimulus (Fig. 2) and, given that DNA is known to deform in response to forces, this could be an issue worth further investigation.

---

**Figure 1:** A finite element model of a single cell in a spread configuration on a substrate; half of the cell membrane and cytosol are removed to reveal the tensegrity-based cytoskeletal framework of microtubules and microfilaments (actin elements). The finite element model was generated using the ANSYS software. Various different element types are used, as described on the figure.
Turning to atomic force microscope (AFM) experiments, these have shown that mechanical probing of the cell membrane can elicit responses – typical of these studies is the report by Charras and Horton (2002) who measure intracellular calcium levels and find that the number of reacting cells increases with the strain applied by AFM, and also that cellular responses could be modulated by disrupting cytoskeletal components. Recent work by our group using the AFM in microscope mode has shown that rat mesenchymal stem cells respond to their extracellular environment by adapting their cytoskeletal structures. Maguire et al. (2007) have reported that, if plated on glass, such stem cells develop internal geodesic F-actin structures (see Fig. 3). Although these structures have been observed previously (Boal, 2002), use of AFM has also allowed measurement of mechanical properties (by dynamic mode AFM) at the indentation site where the nodes of the structure are found to be the most flexible part of the cytoskeleton, indicating a different composition of the nodes relative to the actin of the tension elements themselves. Therefore single cell biomechanics supports theories relating mechanical stimulation to cell responses, and further suggests complex cytoskeletal reactions occur as part of the process.

Given then that cells are mechanosensitive and respond to mechanical forces, it has been hypothesised that if cells are disturbed from their customary mechanical environment a reaction to return the cells to their original state is created. In mathematical terms this is a negative ‘feedback loop’ where cells use the difference between their actual stimulus level and a preferred target level as a homeostatic driver to change the extracellular matrix to regain the normal mechanical milieu. Not only may a change in matrix strain or interstitial fluid flow disturb the homeostatic mechanical milieu, but damage to the extracellular matrix will also cause changes in the local mechanical environment and create an adaptation at the tissue level (Hart, 2001; van der Meulen and Huiskes, 2002). However, such a ‘control system’ for tissue stability may become a positive feedback loop if the aim of the cell is to maximize the level of stimulus received (Weinans and Prendergast, 1996).

**Figure 2:** Predicted 1st principal stress (units: N/µm²) in the nucleus of a eukaryotic cell under mechanical forces applied in experiments (detailed in McGarry et al. 2005) of (a) a substrate strain of 1,000µɛ and (b) a fluid flow generating a shear stress of 0.6 Pa. Note the order of magnitude higher stressing under fluid flow in these experiments.

**Figure 3:** Scan of the periphery of a rat mesenchymal stem cell showing the emergence of geodesic F-actin structures (image shows the phase angle in degrees obtained using Atomic Force Microscopy, after Maguire et al., 2007)

**COMPUTING RESPONSES TO MECHANICAL FORCES – FEEDBACK LOOPS**

The adaptation of the skeleton during life to the prevailing mechanical forces has been one of the classical problems of biology, beginning with the German anatomists at the end of the 19th Century. Roessler (1987) gives the most complete introduction to the historical view. The German
orthopaedic surgeon Friedrich Pauwels (Pauwels, 1965) brought the subject into a more modern context by postulating that precursor mesenchymal stem cells (MSCs) differentiated according to the following rule:

*Hydrostatic pressure stimulates MSC differentiation into chondrocytes to form cartilage, shearing stimulates MSC differentiation into fibroblasts to form fibrous connective tissue, combinations of the two stimulate fibrocartilage formation (as in discs and menisci), and only when a soft tissue has stabilized the environment is MSC differentiation into osteoblasts favoured leading to the formation of bone.*

These concepts received great attention many years later when a better understanding of the influence of mechanical forces on tissue phenotype became crucial for the design of medical devices, in particular tissue-engineered implants created using bioreactors (Kelly and Prendergast, 2006).

One of the key issues of the mechanical loading environment is whether or not the stimulation of the tissue is controlled by displacement (displacement-controlled) or controlled by force (force-controlled). The forces that act in the bones of the appendicular skeleton are determined by the weight of the body and/or the acceleration of the limbs – thus they are subject to force-controlled stimulation and, as a consequence, increasing stiffness of these tissues means that the strain experienced reduces. Displacement-control will act whenever tissues are subject to a given displacement no matter what the tissue’s stiffness is: typically ligaments and muscles are subject to displacement-control. It should however be noted that in terms of actually sensing mechanical load, cells or tissue can only directly measure displacement and infer the force from this strain. Computational simulations can account for force- vs. displacement-controlled environments (Prendergast et al., 1997) and predict that, where stiffening of the tissue can reduce the mechanical stimulation felt by the cells (whether strain or fluid flow) then a mechanical milieu in the tissue favouring osteoblast differentiation from the precursor cells will emerge (see Fig. 4 dashed line); otherwise high strain/fluid flow environments will emerge maintaining the soft tissue of fibrous connective tissue or cartilage (see Fig. 4 full line).

Mechanobiological computer simulations have been performed to establish if such theories can predict the deposition and resorption of tissue during fracture healing (Lacroix and Prendergast, 2002), osteochondral defect healing (Kelly and Prendergast, 2004) and bone chamber experiments (Geris et al., 2004). A satisfactory correspondence between simulation and experiment has been found, and thus a kind of collaboration has been achieved at a functional level. However, for many researchers such a collaboration or ‘validation’ is inadequate. Instead it might be preferred if a model for mechano-regulation could be built up from knowledge of cell mechanoresponsiveness. The development of such experiments would require the ability to compute the cell-level stimuli in a tissue when the applied force/displacement is known; such computations would
Figure 5: There may be a selection pressure in favour of mechanosensitive genes during evolution. If mechanical forces during life increase/decrease we may hypothesise that the flow of mechanosensitive genes will increase/decrease.

require micromechanical finite element models (Prendergast, 2004).

The above discussion may be summarized as follows: tissues react to the mechanical environment via mechanosensation by cells. Such mechanosensitive cells either signal activity in other cells or change phenotype (differentiate) themselves. The predictive power of computational models is somewhat limited even yet because of the difficulties in corroboration against experiment (Currey, 1995). In particular, experiments on cells cannot be easily used to corroborate models because of the difficulty of relating forces acting on the organ (e.g. a bone) to stimuli acting within the tissue at the cell level. A further issue not addressed in current computational models is variability. Experiments show that individuals in a population show considerable variability in their response to mechanical loading; for example, the experiments quantifying bone loss in hindlimb-suspended mice show this (van der Meulen et al. 1995, Hardiman et al., 2005). Therefore, if we aim to predict how an individual, or indeed a population, will respond when subject to a new mechanical environment, a relationship between mechanoregulation and genetics will be required, e.g. Nowlan and Prendergast (2005).

MECHANICAL EFFECTS IN PHYLOGENY

Based on the mechanobiological model presented in Fig. 4, inferences can be made about how tissues would adapt if the animal were to move from one mechanical environment to another, such as the phylogenetic move from terrestrial to aquatic environments of the Cetaceans (whales). Those parts of the skeleton that have evolved under force-control would be most affected because, for example in, say, a low gravity environment, the cells within them would not be mechanically stimulated (by strain or interstitial fluid flow) to the same degree. In particular the cartilages and fibrocartilage structures of the appendicular skeleton (discs, menisci) might not be subject to a level of mechanical stimulation that would maintain them; fibrous connective tissues on the other hand (tendon, ligament) that are mainly in a displacement-controlled regime, would be maintained.

It is interesting to discuss this idea with the concept of ‘fitness’ – according to van der Meulen and Huiskes (2002) mechanobiological process render tissues fit for their mechanical function; fitness is increased when ‘mechanosensitive genes’ are selected during evolution. A mechanosensitive gene is one expressed by a cell in response to mechanical stimulation, and they have emerged because of the advantage they give the animal in growing and remodelling tissues suited to function under mechanical forces. Novel genes would emerge through mutation, duplication and/or co-optation of existing genes and their alleles. Figure 5 attempts to illustrate the regulatory effect of the strength of gravitational environment on the emergence of mechanosensitive genes; selection pressure for the emergence of such genes will be related to the forces acting on the skeletal
ACKNOWLEDGEMENTS

The author’s research is funded from a range of sources, including a Science Foundation Ireland (SFI) Investigator grant titled “Computational Mechanobiology: fundamental advances and implementation for medical device engineering”, the European Commission projects BITES and SmartCAP, and the Programme for Research in Third Level Institutions administered by the Higher Education Authority in Ireland. Ms Niamh Nowlan, Ms Brianne Mulvihill, and Dr Andrew Jackson and Dr Alex Lennon are thanked for comments on the draft of this paper.

REFERENCES


