# Multiscale, multi-paradigm modelling of tissues: embedding development in tissue behaviour

Rod Smallwood, Salem Adra, Goodarz Khodabakshi
Department of Computer Science
University of Sheffield
Regent Court, 211 Portobello, Sheffield, S1 4DP
r.smallwood@shef.ac.uk

July 6, 2010

# 1 Abstract

The term 'Development', as usually used in biology, relates to the embryonic changes from the fertilisation of the egg up to the fully formed embryo. However, development continues throughout the life of the organism, as a result of growth from birth to the adult state, cell turnover, the response to insult and injury, and the effects of neurodegeneration, diabetes, cancer and ageing. We are concerned here with development in this broader sense. Computational modelling of physiology, as exemplified in the Physiome and Virtual Physiological Human Projects, currently provides a snapshot of a particular (usually adult) stage in the organism's development, and developmental changes are excluded. Current models are therefore unable to provide any information on how the organism reached its current (normal or aberrant) state, and how the current state will change during the life of the organism. Development is a cellular-level function, an emergent property of cellular interaction, and the majority of computational models of physiology do not explicitly include either individual cells, or the cellular processes of growth, division, differentiation and death. We initially discuss what is required of an individual cell model for it to be useful in studying development; provide examples of its use in the exploration of the growth and wound response of epithelial tissues; and then consider how individual cell models could be incorporated into existing continuum models, and themselves include molecular level models, in order to produce multiscale, multi-paradigm models of organismal development.

# 2 Introduction

The Physiome Project is '*a worldwide public domain effort to provide a computational framework for understanding human and other eukaryotic physiology.*

*It aims to develop integrative models at all levels of biological organisation, from genes to the whole organism via gene regulatory networks, protein pathways, integrative cell function, and tissue and whole organ structure/function relations'* (http://www.physiome.org.nz/). The challenges, which include the range of length and timescales, are eloquently described by Bassingthwaighte [3]. What might be termed vertical integration – from molecular length scale to the organ or organism level – has been extensively discussed (e.g. see Hunter et al [21] for a cardiac modelling example), but horizontal integration – the change in the organism as the result of the passage of time – is almost unexplored territory. We will discuss here an approach to the modelling of developmental changes in the progression from birth to death, based on the assertion that cellular behaviour is central to an understanding of development. We will make use of the modelling paradigm in the Epitheliome Project, and the application of the modelling paradigm to epithelial tissues [37, 41, 42, 40, 47, 48, 46, 45]. This paper will concentrate on three particular aspects of the problem: developing an individual-based model of the cell; linking the individual-based cell model to sub-cellular models developed using other modelling paradigms (the incorporation of CellML and SBML models as function calls); and linking cell-level and tissue level physical models; and will discuss how these can introduce the concept of development into Physiome modelling. We will not discuss the next (essential) stage – the integration of the regulation of gene expression, which in itself is a multiscale problem [31, 32].

## 3  Modelling and development

The literature on developmental changes at a cellular level is dominated by embryogenesis. The mechanical aspects of cellular organisation have been extensively studied, and include both mechanical measurement and the exploration of control mechanisms. It is not clear whether cell re-organisation is a passive process, due only to forces between cells, as proposed by the differential adhesion hypothesis of Steinberg [38, 39], or requires other mechanisms, as proposed by Harris [17] in his criticism of the hypothesis. Davidson et al have reviewed the mechanical bases of morphogenesis [10] and dorsal closure [11], and the influence that the mechanical environment has on cellular behaviour. Hayashi and Carthew [18] studied pattern formation in retinal cells in Drosophila, and concluded that the cellular arrangement was driven by a minimisation of surface free energy, as originally demonstrated for soap bubbles by Plateau in 1873, and Lecuit and Lenne [27] review the effect that regulation of cell surface tension has on development. Marmottant et al [29] developed a mechanical model of cell aggregates, which have been widely used to study cell organisation. Hutson and Ma [23] provide a comprehensive review of current research on 'reverse engineering morphogenesis', and in a series of publications describe the use of laser hole drilling to probe mechanical properties in embryonic tissue, and cell sorting in three dimensions [22, 24, 28]. A major problem in validating models of individual cell behaviour is tracking cells in live tissues, which has been tack-

led for the zebrafish embryo by Zanella et al [49], and Voiculescu et al [44] use multi-photon microscopy to track cells during primitive streak formation. This very brief survey cannot do justice to the literature, but can provide a starting point for those interested in mechanical sorting during development.

The literature on cellular level modelling of developmental change after birth is much sparser, and a search for modelling papers in more obvious fields yields few papers. This may be an indicator of the lack of subtlety of search engines. We were unable to find any cellular-level modelling of homeostasis per se; of neurodegeneration; of cardiac infarction; or of diabetes. For cellular level modelling of cancer development, the reader is referred to the excellent reviews by Byrne [6] and van Leeuwen [43]. Papers on mathematical modelling of wound healing have been published by Dale [8], Gaffney [15], Olsen [33], Dallon [9], and Sherratt [36]. Evans [14] and Lawford [26] discuss tissue growth following stenting. The Cellular Potts Model [16] has been widely used for cellular-level modelling, and Merks and Glazier [30] provide an excellent introduction to cell-centred modelling. A comprehensive review of epithelial modelling is provided by Smallwood [37].

# 4    Development within the Physiome paradigm

Current Physiome models represent the behaviour of the organism at a fixed point in time, and the parameters and boundary conditions define the normal or abnormal state of the organism at that particular time. This allows us to explore the effects of intervention – in a cardiac model, what effect does blocking specific ion channels have on the excitation pattern? Whilst this is valuable, it cannot provide any insight into how the abnormal condition came about, nor can it predict the future effects of the intervention – what is the prognosis?

If the representation of single cells was common in computational models of phsyiology, one would be tempted to say that any model which aimed to include development in tissues would clearly have to include single cells. In practice, the majority of models use continuum representations of tissues, with properties that are informed by sub-cellular mechanisms (excitability, mechanical properties). The organism maintains a constant state over periods of days to months ($10^6$-$10^7$s) e.g. turnover of epithelial cells and bone remodelling. Growth from birth to maturity takes place over tens of years ($10^8$s) e.g. increase in cell number and the development of new tissues (for instance, myelination of nerves). The ageing process takes place over tens of years ($10^9$s) e.g. loss of collagen and elastin in skin, and a reduction in cardiac output of about 0.5% per annum. Changes due to malignancy and infection can place place on time scales of hours to tens of years ($10^4$-$10^8$s). All of these changes are the result of growth, differentiation, re-organisation and death of individual cells.

A cell-based representation that could capture this behaviour would require, as a minimum:

1. a model of the individual cell based around the cell cycle and the control of the cell cycle, which would provide the basis of growth, differentiation,
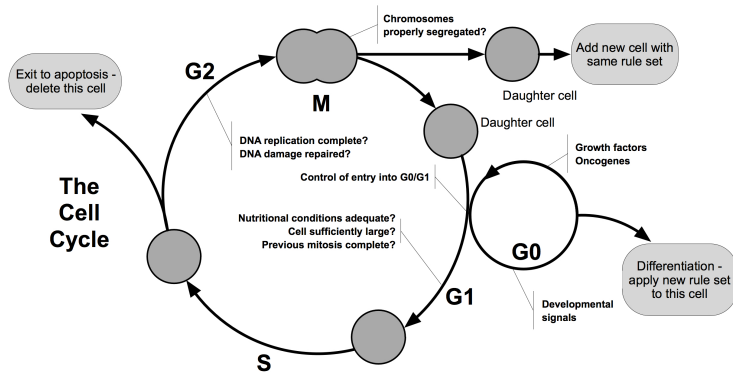
3

Figure 1: A cartoon representation of the cell cycle

division and death

2. a method of incorporating relevant sub-cellular processes – metabolism, signalling, excitability etc

3. interaction between cells – cell-cell signalling, signalling due to diffusable agents, physical interaction

4. the influence and control of gene expression – gene regulation networks, genotype-phenotype mapping.

The remainder of this paper will discuss our implementation of items 1-3. The fourth item is a major challenge for individual-cell modelling, due both to the complexity of the system and the presence of multiple control loops at all levels – Noble's 'downward causation' [31].

# 5   From cell to software

The starting point is the cell cycle. Stages in the cell cycle (figure 1) can be represented as states in a Finite State Machine (figure 2), with the transitions between states controlled by functions. These can be models of e.g. signalling pathways, or abstractions of mechanisms which yield simple rules.

DNA is synthesised in the S phase of the cell cycle, and other cellular macro-molecules are synthesised throughout the G1, S, and G2 phases, leading to the cell roughly doubling in size before mitosis (M, cell division). The preparation for mitosis takes place during the G2 phase. Non-dividing cells enter the G0 or quiescent phase. Within the cell cycle there are checkpoints, which have to be successfully passed for the cell cycle to proceed – otherwise the cell proceeds to apoptosis (programmed cell death). For a single cell, provided with adequate nutrients, a rule set can be developed from the diagram and combined
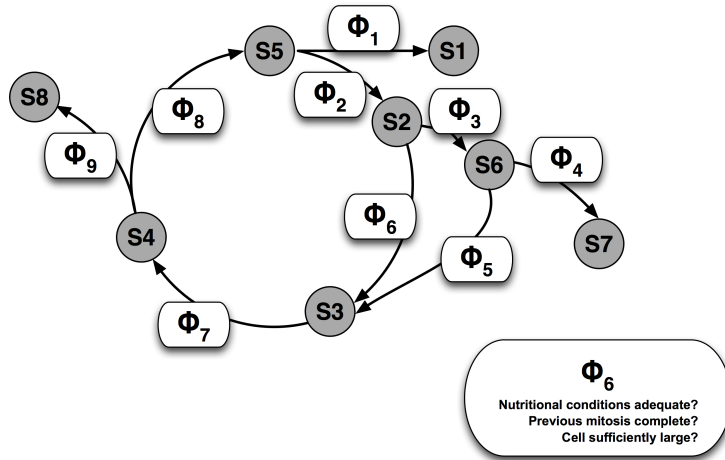
Figure 2: The cell cycle represented as a state machine

with typical times for each phase, to give the top level in a hierarchical model of the cell. If the cells can differentiate (e.g. in skin, stem cells differentiate to produce transit amplifying cells), then a differentiation rule is also required – differentiation will change the rule set for the cell in some way. This is, of course, a qualitative description of the cell cycle, but the rules are the result of the operation of a mechanism. For instance, underlying the rule {if nutritional conditions adequate, then ..., else ...} are the biochemical pathways which produce the required proteins; diffusion of nutrients through the surrounding medium; transport across the cell membrane; etc. So, in principle, it is possible to model the mechanisms which determine the output state of the rules. Mechanisms will be known to a greater or lesser extent, and can replace qualitative rules as more knowledge of the system becomes available. For instance, establishing adequate nutritional conditions may require a rule {if [substrate x] $>y$, then ..., else ...}, where the mechanism of manufacturing x, or the method by which its concentration y is measured, are both unknown. If this proves to be a critical path in the model, then the mechanism will have to be determined experimentally. The model thus acts as a driver for experiment.

When there is more than one cell present, the interaction between cells has to be introduced. The cells 'communicate' (cell signalling), and the tissue as a whole (the continuum) 'communicates' with individual cells through both chemical diffusion through the tissue and through mechano-transduction (mechanical strain elicits chemical signals within the cell). The cells adhere to each other and can also be actively motile (in contrast to passive movement due to the physical forces between adhesion molecules).

We have used a formal finite state machine – the X-machine, introduced by Eilenberg in 1974 [13]. A good introduction and bibliography is provided
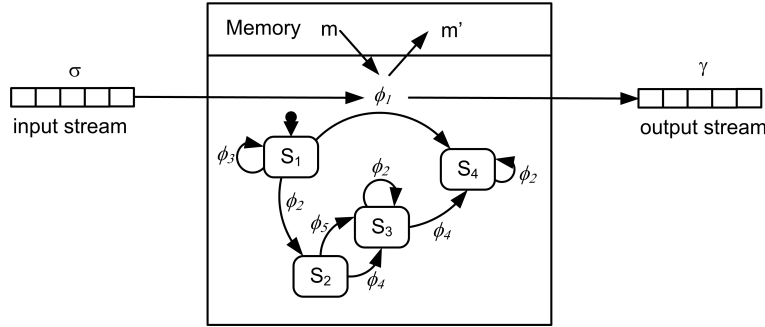
5

Figure 3: The communicating X-machine (after Kefalas et al 2003 [25]). $S_i$ are the states, $\phi_i$ the functions operating on inputs $\sigma$ and memory $m$.

by Stannett (http://x-machines.com/). The communicating-stream X-machine (figure 3) is an extension of the basic deterministic stream X-machine (Kefalas et al 2003 [25]), which is formally defined as an 8-tuple:

$$M = (\Sigma, \Gamma, Q, \ M, \Phi, F, q_0, m_0) \qquad \text{where}$$

- $\Sigma, \Gamma$ is the input and output finite alphabet respectively

- $Q$ is the finite set of states

- $M$ is the (possibly) infinite set called memory

- $\Phi$ is the type of the machine $M$, a finite set of partial functions $\phi$ that map an input and a memory state to an output and a new memory state $\phi : \Sigma \times M \rightarrow \Gamma \times M$

- $F$ is the next state partial function that given a state and a function from the type $\Phi$, denotes the next state. $F$ is often described as a transition state diagram. $F : Q \times \Phi \rightarrow Q$

- $q_0$ $and$ $m_0$ are the initial state and memory respectively.

The addition of communication adds an input stream $\sigma$ and an output stream $\gamma$.

The process therefore is to conceptualise the cell as a finite state machine; write a set of rules which control the state transitions during the cell cycle; choose a suitable time step (30 mins is reasonable); and then let the 'cell' proceed through its cell cycle. If the conditions are right (i.e. the cell does not enter G0 or proceed to apoptosis), the cell will reach mitosis and divide, producing a second cell. This immediately introduces an additional layer of complexity – how do these cells interact physically, and how do they communicate with each other?

# 6 From individual cell to tissue

Building a properly structured and functional tissue requires physical interaction between the cells and communication between the cells. Passing messages between cells is a very high level representation of signalling – information passing without consideration of the signalling mechanisms that are involved – and this can be handled by the X-machine communication streams. In practice, we can divide communication into three main classes: cell-cell signalling (gap junctions, surface proteins); diffusable agents (export of ligands; diffusion model; interaction of receptor and ligand; receptor trafficking); and mechanotransduction (which requires a physical model). Signalling by diffusable agents and by mechanotransduction both require supra-cellular models. In the first case, a model of diffusion through the extracellular space; in the second case, a mechanical model. Signalling mechanisms may also require sub-cellular models, which might include the interaction of surface proteins; the export of signalling ligands from the cells; the interaction of receptor and ligands; receptor trafficking; and the conversion of force into a chemical signal. The physical model has to handle the effects of cell growth and division, and bond formation with the substrate and between cells. The simple physical model of cellular interaction used to date in the Physiome Project is described in Adra et al [1]). Bi-directional linking of the cellular model to a continuum model of the tissue described below in section 8.

# 7 Plug-and-play sub-cellular models

Our approach has been to use a high-level representation of functions (rules) for speed and simplicity, and introduce the details of the mechanisms that are involved only when this is necessary in order to examine the effect of changes in, for instance, a particular signalling pathway. In principle, an individual-based approach can be used at any level from the whole organism down to the molecule, thus generating emergent behaviour at all levels. The representation of the finite state machine as an X-machine is Turing-complete, so any function within the X-machine could be substituted by another X-machine which calculated the function, thus generating a hierarchy of X-machines at all levels. Whilst this is conceptually feasible (and even attractive!), it is neither practicable nor desirable to build a model of an organism in molecular detail. Some method of abstracting away detail between levels is essential for computability, and there are also cogent arguments for making use of models developed by other research groups. We therefore developed the means for incorporating models described in CellML, SBML, or as sets of differential equations. Our aim was to devise a generic modeling technique which enabled sub-cellular signaling pathways to be easily imported and plugged into an agent-based representation of a cell. To enable wide applicability, we have adopted a modular and flexible approach to link our agent-based modeling environment FLAME with existing tools such as COPASI [19] and JSim [35]. As the functions of an agent modeled using FLAME

can be of any desired complexity, linking FLAME (http://www.flame.ac.uk) to such existing tools (COPASI and JSIM) was realised by providing wrappers that can be used by an agent's function to call COPASI or JSIM and request them to simulate a certain sub-cellular model specified in any of the modeling languages they support (e.g. CellML (www.cellml.org), SBML (sbml.org) and MML (nsr.bioeng.washington.edu/jsim/)).

The main advantages that are provided to us by interfacing FLAME with COPASI and JSIM include:

- the ability to import sub-cellular models specified in widely used modelling languages such as SBML or CellML

- the ability to reuse curated and widely available models

- the ability to connect agent-based models of cellular behaviour (micro level) to mathematical (continuum) models of whole tissues or organs (macro level) and sub-cellular signalling pathways and biochemical networks (sub-cellular level)

- the facility to connect agent-based models to widely used ODE and PDE solvers and

- the promotion of multiparadigm and multiscale computational modeling.

COPASI (Complex Pathway SImulator) is a software application for the simulation and analysis of biochemical networks. COPASI provides the following main features: stochastic and deterministic time course simulation (ODE solver); metabolic control analysis/sensitivity analysis; optimization of arbitrary objective functions; parameter estimation; import and export of SBML; ODE Solving Capability; and a command line version for batch processing. The individual agent is defined using a markup language (XMML – **X**-**M**achine **M**arkup **L**anguage). In the environment tag, a datastructure called 'copasi_data' is defined to encapsulate the data that will be used by COPASI (for instance, the name and concentration of a metabolite). The user-defined datastructure 'copasi_data' is then used in the agent's memory. The initial values are provided in the initialization file (an xml file). More technical details about FLAME, and FLAME/COPASI can be found on http://www.flame.ac.uk and http://www.imagwiki.org. The integrated FLAME/COPASI framework has been used to link the sub-cellular mechanisms of TGF-$\beta$1 into the cellular level rules of an agent-based model of keratinocyte colony formation ([1, 40]). FLAME/COPASI allowed us to develop a 3D, multiscale, agent-based model of the human epidermis and to model the re-epithelialisation process. The 3D model was also deployed to investigate epidermal cell migration, proliferation and the functions of TGF-$\beta$1 during wound healing.

The same approach has been used to link FLAME and JSim ([35]). JSim is a software application for the simulation and analysis of biochemical networks. JSim provides features similar to the ones provided by COPASI, but in addition has the following valuable features: the ability to import CellML models as well

as SBML models; the ability to solve PDEs as well as ODEs, and automatic balancing and checking of units.

# 8   Forces at cell and tissue levels

It is abundantly clear that a model of cellular interaction must include physical interaction between the cells. Cell sorting is the result of mechanical forces applied to the cells as a result of cell growth, bond formation between cells and between cells and substrate, and active motility. The influence of the mechanical environment of the cell on the cell phenotype is well known. Small changes in the magnitude or distribution of the mechanical forces may lead to compensatory of remodelling of cell-matrix and cell-cell contacts and initiate a variety of cell behaviours [20, 7]. A good introduction to mechano-transduction (the generation of signals as a result of forces applied to components of the cell) is provided by Alenghat and Ingber [2]. The effects of spatial stimuli on mechano-transduction are explored in endothelial cells by Davies et al [12], and adhesion-sensitive signalling is reviewed by Bershadsky et al [4]. Ramasubramanian and Taber [34] link stress at a continuum level with morphogenesis.

The resolution of the forces between interacting cells is a problem of $O(n^2)$, and rapidly becomes excessively time-consuming to solve for even a few mm$^3$ of tissue. However, 1mm$^3$ of tissue contains $10^5$-$10^6$ cells, and can therefore be well-represented by a continuum (finite element) model. A continuum model can therefore be employed to resolve the force distribution at the macroscopic (tissue) level, whilst a cell-based model resolves the forces at the microscopic level which are due to cellular reorganisation resulting from cell growth, division and death. The continuum model provides the force field which influences cellular behaviour through mechanotransduction, and the cell behaviour informs the mechanical properties at the tissue level.

The non-homogenous mechanical properties of the tissue are represented by multiphase elements in which different phase properties are assigned to individual integration points in the element. The finite element mesh is independent of the phase arrangement of the material, and a relatively simple mesh can be used to simulate the deformation in the complex structure. The possibility of using initial meshes of arbitrary simple structure for the simulation of the behaviour of complex materials is the main advantage of the method of multiphase elements. Also, due to the dynamic nature of the system which results in variation of parameters (material properties) in the system, the multiphase elements eliminate the necessity for re-meshing to accommodate the new structure. Comparison with conventional single phase element models of identical microstructure proved that the localization and magnitude of stress and strain concentration caused by local phase geometry are in reasonable agreement with multiphase element models, when the element density in the latter model is of the order required for the correct representation of stress and strain gradient. The introduction of inhomogeneous material properties does not affect the convergence behavior of FE models.

The first step in coupling the continuum (FEA) and discrete (agent based) models is to discretized the whole system (the macro or tissue level) into finite elements. To account for the cellular behaviour, an array of cells is allocated to each integration point based on their geometrical coordinates. The cellular system communicates with the finite element through integration points by exchanging data and updating their status. The steps are: solve the strain gradient across the domain, find the strain values at the integration points, calculate stress based on the local material properties; distribute the stress at the integration point to the adjacent cells in the agent based model; use the agent-based model to derive the behaviour of the cellular system as a result of the applied stress; update the properties at the integration points based on the cellular-level behaviour. This fully integrated computational model, which is capable of continuous updates from the agent based model, allows the generation and evaluation of hypotheses relating to the dynamic recruitment and participation of cells in physiological and pathological tissue settings, such as wound healing.

# 9 Discussion

We have described in this paper the means by which tissues can be developed from a collection of individual cells which are able to grow, divide, differentiate, and die; which interact physically with each other and their environment; and which can generate signals and respond to both local and global signalling events. The next major challenge is to incorporate the effects of gene expression (we hard code the phenotype of the cell in the agent's memory) and epigenetics. This is a long-term project - it is inherently multiscale [3], and we often do not know the mechanisms [5].

# 10 Acknowledgements

# References

[1] S Adra, T Sun, S MacNeil, M Holcombe, and R Smallwood. Development of a three dimensional multiscale computational model of the human epidermis. *PLoS One*, 2010.

[2] Francis J Alenghat and Donald E Ingber. Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci STKE*, 2002(119):PE6, Feb 2002.

[3] James Bassingthwaighte, Peter Hunter, and Denis Noble. The cardiac physiome: perspectives for the future. *Experimental Physiology*, 94(5):597–605, Jan 2009.

[4] Alexander D Bershadsky, Nathalie Q Balaban, and Benjamin Geiger. Adhesion-dependent cell mechanosensitivity. *Annu. Rev. Cell. Dev. Biol.*, 19(1):677–695, Nov 2003.

[5] Adrian Bird. Perceptions of epigenetics. *Nature*, 447(7143):396–398, Jan 2007.

[6] HM Byrne, T Alarcon, MR Owen, SD Webb, and PK Maini. Modelling aspects of cancer dynamics: a review. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 364(1843):1563, 2006.

[7] Christopher S Chen. Mechanotransduction - a field pulling together? *Journal of Cell Science*, 121(20):3285–3292, Jan 2008.

[8] PD Dale, PK Maini, and JA Sherratt. Mathematical modeling of corneal epithelial wound healing. *Mathematical biosciences*, 124:127–127, 1994.

[9] JC Dallon, JA Sherratt, and PK Maini. Mathematical modelling of extracellular matrix dynamics using discrete cells: Fiber orientation and tissue regeneration. *J Theor Biol*, 199(4):449–471, Jan 1999.

[10] Lance Davidson, Michelangelo von Dassow, and Jian Zhou. Multi-scale mechanics from molecules to morphogenesis. *Int J Biochem Cell B*, 41(11):2147–2162, Jan 2009.

[11] Lance A Davidson. Developmental biology - apoptosis turbocharges epithelial morphogenesis. *Science*, 321(5896):1641–1642, Jan 2008.

[12] P. F Davies. Spatial microstimuli in endothelial mechanosignaling. *Circulation Research*, 92(4):359–370, Mar 2003.

[13] Samuel Eilenberg. Automata, languages, and machines, volume a. *Academic Press, London*, Dec 1974.

[14] D. J. W Evans, P. V Lawford, J Gunn, D Walker, D. R Hose, R. H Smallwood, B Chopard, M Krafczyk, J Bernsdorf, and A Hoekstra. The application of multiscale modelling to the process of development and prevention of stenosis in a stented coronary artery. *Philos T R Soc A*, 366(1879):3343–3360, Jan 2008.

[15] EA Gaffney, PK Maini, JA Sherratt, and S Tuft. The mathematical modelling of cell kinetics in corneal epithelial wound healing. *J Theor Biol*, 197(1):15–40, 1999.

[16] F Graner and JA Glazier. Simulation of biological cell sorting using a two-dimensional extended potts model. *Phys Rev Lett*, 69:2013, 1992.

[17] A K Harris. Is cell sorting caused by differences in the work of intercellular adhesion. *J. Theor. Biol*, 61:267–285, Sep 1976.

[18] T Hayashi and R Carthew. Surface mechanics mediate pattern formation in the developing retina. *Nature*, Jan 2004.

[19] S Hoops, S Sahle, R Gauges, C Lee, J Pahle, N Simus, M Singhal, L Xu, P Mendes, and U Kummer. Copasi–a complex pathway simulator. *Bioinformatics*, 22(24):3067–3074, Oct 2006.

[20] H Huang. Cell mechanics and mechanotransduction: pathways, probes, and physiology. *AJP: Cell Physiology*, 287(1):C1–C11, Feb 2004.

[21] Peter J Hunter, Edmund J Crampin, and Poul M. F Nielsen. Bioinformatics, multiscale modeling and the iups physiome project. *Brief Bioinform*, 9(4):333–343, Jan 2008.

[22] M Hutson, Y Tokutake, M Chang, and J Bloor. Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. *Science*, Jan 2003.

[23] M A Hutson and Xiaoyan Ma. Mechanical aspects of developmental biology: perspectives on growth and form in the (post)-genomic age. *Phys. Biol*, 5:1–8, Jan 2008.

[24] MS Hutson, GW Brodland, J Yang, and D Viens. Cell sorting in three dimensions: Topology, fluctuations, and fluidlike instabilities. *Physical Review Letters*, 101(14):148105, 2008.

[25] P Kefalas, G Eleftherakis, and E Kehris. Communicating x-machines: from theory to practice. *Lecture notes in computer science*, pages 316–335, 2003.

[26] P. V Lawford, Y Ventikos, A. W Khir, M Atherton, D Evans, D. R Hose, C. M Care, P. N Watton, I Halliday, D. C Walker, A. P Hollis, and M. W Collins. Modelling the interaction of haemodynamics and the artery wall: Current status and future prospects. *Biomed Pharmacother*, 62(8):530–535, Jan 2008.

[27] T Lecuit and P Lenne. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nature Reviews Molecular Cell Biology*, Jan 2007.

[28] Xiaoyan Ma, Holley E Lynch, Peter C Scully, and M. Shane Hutson. Probing embryonic tissue mechanics with laser hole drilling. *Phys Biol*, 6(3):036004, Jan 2009.

[29] P Marmottant, A Mgharbel, J Käfer, and B Audren. The role of fluctuations and stress on the effective viscosity of cell aggregates. *Proceedings of the National Academy of Sciences*, Jan 2009.

[30] RMH Merks and JA Glazier. A cell-centered approach to developmental biology. *Physica A: Statistical Mechanics and its Applications*, 352(1):113–130, 2005.

[31] Denis Noble. Claude bernard, the first systems biologist, and the future of physiology. *Experimental Physiology*, 93(1):16–26, Jan 2008.

[32] Denis Noble. Genes and causation. *Philos T R Soc A*, 366(1878):3001–3015, Jan 2008.

[33] L Olsen, JA Sherratt, and PK Maini. A mechanochemical model for adult dermal wound contraction and the permanence of the contracted tissue displacement profile. *J Theor Biol*, 177(2):113–128, 1995.

[34] Ashok Ramasubramanian and Larry A Taber. Computational modeling of morphogenesis regulated by mechanical feedback. *Biomech Model Mechanobiol*, 7(2):77–91, Apr 2008.

[35] Gary M Raymond, Erik A Butterworth, and James B Bassingthwaighte. Jsim: Mathematical modeling for organ systems, tissues, and cells. *Faseb J*, 21(6):A827–A827, Jan 2007.

[36] JA Sherratt and JC Dallon. Theoretical models of wound healing: past successes and future challenges. *Comptes rendus-Biologies*, 325(5):557–564, 2002.

[37] R Smallwood. Computational modeling of epithelial tissues. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, Jan 2009.

[38] MS Steinberg. On the chemical bonds between animal cells. a mechanism for type-specific association. *American Naturalist*, pages 65–81, 1958.

[39] MS Steinberg. Reconstruction of tissues by dissociated cells. *Science*, 141(3579):401–408, 1963.

[40] T Sun, S Adra, R Smallwood, M Holcombe, and S MacNeil. Exploring hypotheses of the actions of tgf-1 in epidermal wound healing using a three dimensional computational multi-scale model of the human epidermis. *PLoS One*, 2009.

[41] Tao Sun, Phil McMinn, Simon Coakley, Mike Holcombe, Rod Smallwood, and Sheila MacNeil. An integrated systems biology approach to understanding the rules of keratinocyte colony formation. *J R Soc Interface*, 4(17):1077–1092, Jan 2007.

[42] Tao Sun, Phil McMinn, Mike Holcombe, Rod Smallwood, and Sheila Macneil. Agent based modelling helps in understanding the rules by which fibroblasts support keratinocyte colony formation. *PLoS One*, 3(5):1–17, Apr 2008.

13

[43] IMM van Leeuwen, HM Byrne, OE Jensen, and JR King. Crypt dynamics and colorectal cancer: advances in mathematical modelling. *Cell proliferation*, 39(3):157–181, 2006.

[44] O Voiculescu, F Bertocchini, and L Wolpert. The amniote primitive streak is defined by epithelial cell intercalation before gastrulation. *Nature*, 449:1049–1052, Jan 2007.

[45] Dawn Walker, Tao Sun, Sheila Macneil, and Rod Smallwood. Modeling the effect of exogenous calcium on keratinocyte and hacat cell proliferation and differentiation using an agent-based computational paradigm. *Tissue Eng*, 12(8):2301–2309, Jan 2006.

[46] Dawn Walker, Steven Wood, Jennifer Southgate, Mike Holcombe, and Rodney Smallwood. An integrated agent-mathematical model of the effect of intercellular signalling via the epidermal growth factor receptor on cell proliferation. *J Theor Biol*, 242(3):774–789, Jan 2006.

[47] DC Walker, G Hill, SM Wood, RH Smallwood, and J Southgate. Agent-based computational modeling of wounded epithelial cell monolayers. *Ieee T Nanobiosci*, 3(3):153–163, Jan 2004.

[48] DC Walker, J Southgate, G Hill, A Holcombe, DR Hose, SM Wood, S Mac Neil, and RH Smallwood. The epitheliome: agent-based modelling of the social behaviour of cells. *Biosystems*, 76(1-3):89–100, Jan 2004.

[49] C Zanella, P Bourgine, K Mikula, and N Peyriéras .... Cells tracking in a live zebrafish embryo. *Engineering in Medicine and Biology Society*, Jan 2007.