Development and validation of computational models of cellular interaction

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Summary

In this paper we take the view that computational models of biological systems should satisfy two conditions – they should be able to predict function at a systems biology level, and robust techniques of validation against biological models must be available. A modelling paradigm for developing a predictive computational model of cellular interaction is described, and methods of providing robust validation against biological models are explored, followed by a consideration of software issues.

Introduction

In this paper we take the view that computational models of biological systems should satisfy two conditions – they should be able to predict function at a systems biology level, and robust techniques of validation against biological models must be available.

Cell biology, molecular biology, genomics, proteomics are providing, in a qualitative, reductive manner, an enormous volume of data on biological mechanisms. To understand these mechanisms in a predictive manner requires the integration of this data through computational models. This integration can take place at many levels from the molecular up to the whole body. We are principally concerned with how cells interact to form a tissue, and in particular, epithelial tissue. This is a 'grand challenge' project which aims to integrate computational and biological models of the social behaviour of cells within epithelial tissue. The aim is to develop a computational model of cell behaviour within the context of tissue architecture, differentiation, wound repair and malignancy. We are developing a novel computational paradigm for modelling the social behaviour of cells, closely coupled to biological models which will provide both experimental data and validation. The modelling paradigm is to model individual autonomous cells as software agents, with structural and functional complexity an emergent property of cell assemblies.

Tissue structure does not exist in advance of its growth – there is no 'hidden structure' that is populated by dividing cells – the structure is an emergent property of the interaction of large numbers (10^7–10^8) of cells. The emergence of order from highly complex systems without an over-arching plan is a fundamental feature of biological processes. The hypothesis underlying our modelling paradigm is that the histioarchitecture is determined by the equivalent of biological rule sets for cells (normal rule sets leading to normal tissue structure and abnormal ones leading to a pathologically abnormal structure – of which there are a number of benign and malignant examples in epithelial tissues). The emergent property of epithelial histioarchitecture is a consequence of the rule set of that cell type, and determines the consequences of cell to cell and cell to matrix interactions. If our hypothesis is correct than assigning rule-sets to our software agents should lead to predictive modelling of epithelial tissue structure.

Computational models of epithelial tissue at cellular and tissue level

We concentrate on the interaction of individual cells to form fully differentiated tissue, so need to be able to model both the physical properties of individual cells and how the cells interact through the physical forces acting on the cells. Models of the behaviour of individual cells and tissue can be divided into two classes: those which use postulated mechanisms in order to mimic cell growth (illustrative models) and those which build on known properties of the cells (explanatory models). The last three decades have seen the development of a number of models examining various aspects of cell culture and tissue behaviour. Models have varied considerably in terms of implementation and underlying concepts and assumptions, but have tended to increase in size and complexity in parallel with improvements in computer processing speed and capacity. Individual biological cells in a tissue or cell culture can be modelled as cellular automata, thus enabling the execution of rule sets according to the internal properties or parameters of each cell, and possibly its environment. Lim and Davies (1990) used a combination of cellular automata

and Voronoi graphs to examine the growth rate and shape of cell clusters, with stochastic cell growth and division and cell death. Both Zygourakis *et al.* (1991a,b) and Forestell *et al.* (1992) used a purely automaton-based approach allowing stochastic selection of division. An important rule incorporated into these early automaton-type models is that of contact inhibition. Ruaan *et al.* (1993) introduced an extra element of complexity in considering the tendency of cells to spread if sufficient space is available, and regain a spherical shape and hence generate additional space for growth and division at higher cell densities. Cells were given the ability to migrate in order to find space for growth and division. The idea of random migration was further developed by Lee *et al.* (1995).

In addition to providing an insight into patterns observed in tissue culture growth, modelling has been used as a tool to simulate the formation of more complex tissue structures. Ryder *et al.* (1999) simulated the development of the human cerebral cortex based on random migration, and the difference in cell cycle characteristics observed in cells of different ages. Stekel *et al.* (1995) developed a model of the morphogenesis and homeostasis of the human epidermis. This is an illustrative model, with rules formulated to simulate observed behaviour, and not on the basis of well understood mechanistic behaviour of individual cells. For instance, one of the rules stipulates that stem cells emit a substance called 'stem cell factor', the concentration of which can be sensed by other cells in the model. Morel *et al.* (2001) incorporated two distinct hierarchies in their model of epithelial tissue: a kinematic model of cell cycle regulation, incorporating both intracellular components (e.g., cyclins) and response to extracellular stimuli (e.g., growth factors) and a Voronoi graph-based tissue architecture model, with individual cells represented by polygons. In addition to models of development and behaviour associated with specific tissues such as epithelium or cortex, general embryonic morphogenesis has continued to be a field of active research. Hogeweg (2000) built on a more basic earlier model (Savill & Hogeweg 1997) to construct a two level hierarchical simulation of the effect of morphogenic evolution on cell differentiation and differential adhesion.

One major factor absent in all the models discussed so far is the explicit consideration of forces acting on cells, and the resulting deformation and movement. In general, models that attempt to simulate the effect of physical forces are continuum, rather than automaton or agent based. Examples include the work of Brodland and Chen (2000) and Chen and Brodland (2000) who used finite element models to simulate various morphogenic process, such as stretching and engulfment, for confluent sheets consisting of a pre-determined number of cells. Interestingly, in contrast with the earlier energy-based models of Glazier and Graner

(1993), the results of these simulations suggested that differential adhesion alone is not sufficient to result in the sorting of a heterotypic cell population.

Palsson (2001) proposed a model which simulates three-dimensional morphogenic processes using an automaton rather than continuum based approach. Cells in this model are considered as individual entities that can respond to the environment according to the values of their internal parameters, and physically interact *via* contact forces. Each cell has viscoelastic properties, and moves and deforms according to the equations of motions and deformation. The capacity exists to assign different properties or parameters according to designated cell type. Simulations produced using this model suggest that tissue structure and cell sorting can arise from differences in cell adhesion properties, and movement in response to a chemotactic gradient. Three-dimensional embryonic models have been produced consisting of up to 10,000 'cells', each representing 4–16 actual biological cells. In common with many of the previous simulations of morphogenic behaviour, this model does not include the capacity for cell division and differentiation.

Linking external forces applied to the cell(s) to internal events (mechanotransduction) is essential, so simple continuum models comprising a continuous elastic shell surrounding a continuous viscous or visco-elastic core can be discounted. There is a considerable literature on the mechanical properties of erythrocytes and the erythrocyte skeleton (e.g., Boey *et al.* 1998, Discher *et al.* 1998) at a level of detail which is far too great for a tissue model, but which could provide starting points for a hierarchical model of the cell. Shafrir and Forgacs (2002) developed a model cytoskeleton consisting of rigid rods connected through springs, and examined the energy transfer properties of the model. Tensegrity has been proposed as a structural framework for cell mechanics (Ingber 1993, 2003, Stamenovic *et al.* 1996), and has generated some controversy (Ingber *et al.* 2000). At a simpler level, Brodland and Veldhuis (2002) have used a finite element model with edge tension applied using linked struts to study mitosis, and Palsson (2001) used orthogonal non-linear elements with constant volume constraint in a model which was able to demonstrate cell sorting due to differential adhesion. To the best of our knowledge, this is the only model which attempts to simulate three-dimensional morphogenic processes using an automaton rather than continuum based approach. Cells in this model are considered as individual entities that can respond to the environment according to the values of their internal parameters, and physically interact *via* contact forces. Each cell has viscoelastic properties, and moves and deforms according to the equations of motions and deformation. The capacity exists to assign different properties or parameters according to designated cell type.

An agent model of cellular interaction

The agent model of cellular interaction has been described in detail elsewhere (Walker *et al.* 2004a, Smallwood *et al.* 2004). A brief description of the model is given to inform discussion of validation methods.

Many biological systems seem to be based on local processing capabilities from which the overall system behaviour emerges rather than being organised as some high level control system which determines what each component, a cell in this case, is to do under the specific circumstances pertaining at that moment. The concept of an "autonomous" agent is one which is useful in this respect. A number of types of biological systems have been modelled in this way (Gheorghe *et al.* 2001, Fisher *et al.* 1999), including communities of various social insects such as species of ants and bees. Each insect is considered as one of these agents and can behave independently of any explicit external instructions. The agents seem to contain a set of behavioural rules which determine what they do under all realistic circumstances. Key aspects of such systems is the mechanisms of communication that must exist, this provides ways of sharing information upon which their behaviour is predicated. Thus we can model and perhaps explain the way in which a community of insects can exploit food resources by the mechanisms of individual exploration of the community's environment and the transmission of information about the location of food sources through mechanisms including direct contact, pheromone trails and physical behaviour such as dancing etc. The overall behaviour of the insect community is thus an emergent property derived from the interaction and communication between large numbers of autonomous agents operating concurrently. Such systems are inherently robust and fault tolerant having evolved under millions of years to survive in an highly dynamic and uncertain environment.

If societies of individual insects can be modelled in this way then it is worth considering how societies of cells might fare under a similar modelling paradigm. The metaphor that we are investigating is based around the concept of a cell as an agent. In order to do this in a way that can be exploited both in terms of simulation but also using promising approaches to the automated analysis of complex models we need to conceptualise the agent model suitably.

We base our approach on the language of computational models. Rather than using cellular automata, for example, we exploit a more powerful computational approach called communicating X-machines (Balenescu *et al.* 1999). Firstly, we identify an X-machine (Holcombe & Ipate 1998, Kefelas *et al.* 2003) as a system which has internal states and an internal memory. The state transition functions will respond to events on the basis of both the environmental input as well as the current internal state. The system is in some state, an input a is received, the initial contents of the memory are m and, depending on both a and m, the system changes state and produces an output x and updates the memory to m^1. This provides a much more general modelling mechanism and one which enables many of the problems associated with state explosion, which bedevil many efforts at modelling complex biological systems, to be dealt with sensibly. The memory can be used to abstract away detail in a way that does not prevent us from utilising it whenever necessary.

The X-machine makes a natural candidate for modelling an agent (Kefelas *et al.* 2004). We start with a simple set of rules which describe what the agent must/could do under various different circumstances. The set of rules may be defined with an explicit prioritisation that determines which rule is to be used under which environmental and internal conditions. Thus, perhaps, the top rule provides a general metabolic processing activity typical of the cell's normal state of activity but on the receipt of some event in the cell's immediate environment such as a signal from a neighbouring cell or some external process the cell undergoes a change in activity which is reflected in a new set of metabolic processing activities captured in a new set of rules.

The starting point for modelling a single cell as an agent is the cell cycle. For a single cell, provided with adequate nutrients, a rule set can be developed and combined with typical times for each phase of the cell cycle, to give the top level in a hierarchical model of the cell. If the cells can differentiate (e.g., in skin, stem cells can produce transit amplifying cells), then a differentiation rule is also required – differentiation will change the rule set for the cell in some way. A basic rule set has been presented by Walker *et al.* (2004a). 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.

The effect of adhesion forces, cell growth, and strain applied to the tissue, have to be calculated in the context of the whole tissue, for which a continuum model is appropriate. Individual agents are associated with appropriate spatial nodes within the continuum model, and information is exchanged between agents and continuum at each time step. The continuum model is central to the organisation of the cells. Palsson (2001) uses a physical model in which the cells are modelled as a trio of viscoelastic elements, and both tension and cell sorting resulting from differential cell–cell adhesion forces, and compression (due to growth), can be handled. Walker's model includes the effect of cell–cell adhesion in the rule set, but does not incorporate the cell-sorting effects of differential adhesion.

Validating computational models against biological models

Does similar behaviour imply similar systems? Convergent evolution suggests not. How do we define similar? What properties need to be measured in order to confirm similarity? In modelling in the physical domain, it is widely assumed that the acid test of the relevance of a model is not its ability to describe the system being modelled, but the ability to predict the behaviour of the system. Provision of an adequate description of 'reality' is necessary but not sufficient. We explore how this view could be applied to our model.

There is a one-to-one mapping between the *in vitro* epithelial models and our computational model, with each cell in the biological model having an identical corresponding cell in the computational modelling at the initial seeding stage. Cell growth and division is a stochastic process, so the two models would not be expected to yield identical outcomes after a period of growth under identical conditions – indeed, this would not be expected for a pair of *in vitro* models. Nevertheless, it is reasonable to assume strong similarity, and suitable metrics to compare *in vitro* and *in virtuo* development need to be developed. To date, validation of the simple in virtuo model has been confined to comparison of real and simulated growth rates for urothelial tissue in normal and low Ca^{2+} environments (Walker *et al.* 2004a) and wound healing in a urothelial model (Walker *et al.* 2004b).

The obvious next stage is to be able to track cell division and tissue morphology *in vitro* – in principle, it is easy to do this in virtuo. Tracking cell division is of more general interest, and is generally known as cell lineage tracking. Sulston *et al.* (1983) described the complete cell lineage of the embryo of the nematode worm *Caenorhabditis elegans*, in which exactly 671 cells are generated. Nomarski interference contrast microscopy was used to follow development of the embryo. Attempts have been made to automate the process by Kitano's group at Keio University, using 4D-Nomarski DIC microscopy (Onami *et al.* 2002). They can capture 50 images a minute with focal plane changes of 50 μm. This gives one 3d image/min. for 2 h. They believe the limit on their system is about 60 cells e.g., about 8 cell divisions. There are < 20 divisions in *C. elegans* development (all cells do not divide throughout the development of the embryo). Labelling the nuclei with fluorescent proteins may enable the nuclei to be tracked through sufficient number of cell divisions to give the complete cell lineage of *C. elegans*, and this would certainly be sufficient to characterise the cell lineage of epithelial tissue grown *in vitro*. Noting which cells continue to divide is also the only current method for unequivocally identifying stem cells.

Assuming that the position of the nuclei and the cell lineage (i.e. the history of nuclear division) can be tracked *in vitro*, we could then generate virtual cells based on the real nuclei using Voronoi tessalation with a waterfall algorithm (some means of handling non-contiguous cells and unbounded edges – the epithelial surface – would be required). We then have two stochastic processes to compare – the cell lineage (a tree structure) and the tissue morphology – a 3D graph. How do we test the null hypothesis that these arise from the same population? The comparison of phylogenetic tree structures has been extensively explored, and some statistical comparisons have been made (e.g., Steel & Penny 1993). As far as we know, no statistical comparisons of 3D graphs have been attempted. In principle, both of these could be approached by using Monte Carlo techniques to generate the distributions, but this might not be practicable for a problem of this size.

What is probably needed is a new philosophy of *in virtuo* modelling and *in vitro* validation. The discussion above considers the issue of relating the population growth (and decay) of the two systems, and looking for similar behaviour under similar initial configurations and environmental conditions. This is a useful guide but as mentioned above the superficial appearance of similar populations may not be based on similar underlying processes. We need to look at the individual agents, their behaviour and their communication to validate the model. This is likely to be a difficult and on-going process. We will not be able to reach a definitive conclusion and say "the model is completely validated" and there is nothing further to do. All modelling is an iterative process whereby the simple models are tested against the reality, refined and expanded in a symbiotic way with the biological experiments.

One possible way forward is to try to construct *in tandem* experiments where we identify all the control parameters that can be manipulated in the culture samples and build an *in virtuo* model which incorpo-

rates the same parameters. By carrying out a systematic series of tandem experiments with corresponding treatments/parameter values we will seek to establish the hypothesis that the *in virtuo* model is an accurate representation of the *in vitro* situation. If we think of both the *in vitro* model and the *in virtuo* model as systems which behave with the same underlying process characteristics namely – internal state of some sort, external inputs that influence the way the system will behave, resulting external behaviours and abstract away all other factors into some hypothesised framework then it might be possible to relate the two models better in some way and use this as a basis for some validation procedures.

Let us consider how this might work. We have a proposed model of the biological system. It is expressed as a computational model and thus conforms to our overall framework of an interacting collection of agents presented as generalised state machines which might have some hybrid features which allow for continuous processing to be modelled in states and to asynchronous communication to occur between the component agents.

We assume a 1–1 relationship between the components of the biological system and the components of the model. These will be the agents described above. We must also assume that the agents can interact with each other through a number of mechanisms that we will regard as channels, primarily these will be constrained by the geometry – agents only being able to communicate directly with those agents that are physically nearby.

Now we must look at the agents themselves. These are models of some plausible biological system, here cells. We have to abstract away much of the complexity to be found in a real cell if we are to make a sensible model. Cells can exist in many different internal states and it is important to identify what these might be. It is tempting to try to include all of the likely parameters that can be thought of but only those that are known to have a significant effect on the aspect of the model that we are developing should be considered. In the work described above the main structure of the internal state that has been considered is the basic cell cycle. This is a good starting point since it is easily identified in experimental work. At the other extreme is the identification of the active gene's and the species of proteins currently expressed. This is likely, at present, to be too fine grained although it may be possible to incorporate some of this information into the memory of the X-machines so that its value can be used to influence the functional behaviour of the model. In many cases the model will progress in such a way that new factors are identified during the course of the modelling. These will then be either incorporated as new states – perhaps a compound state is decomposed into a set of individual substates – or new factors incorporated into the memory structure.

Validation will consist of identifying the equivalent states in the model and the culture and defining practical protocols for identifying them. The next stage is to consider what is being communicated down the channels. This may be chemical information, signals for example, or physical information such as the contact between two adjacent cells. We need to define what these communication events are and to relate them to the biological experiments. We can observe cells that touch visually but the detection of signals or the movement of molecules between agents may require the intervention of marker techniques and sensing technology.

The next stage is to try to define the sort of inputs to the systems, this may simply be the provision of adequate nutrients distributed in some way in the environment or there may be specific direct inputs that we can apply to individuals or groups of agents. It is fairly straightforward to apply inputs to the virtual model but less so in biological systems. It may be that we can only do this successfully in a proportion of the trials and so this needs to be taken care of by invoking suitable statistical treatments to the results.

Finally, using our systems metaphor, we need to identify the observable outputs that can be obtained from the *in tandem* experiments. It is the ease with which we can make definitive observations from the biology that will drive any validation conclusions.

Finally we need to relate what we have obtained with the usual qualifications that the results obtained

Simulation cycle

In virtuo model   *In vitro* model
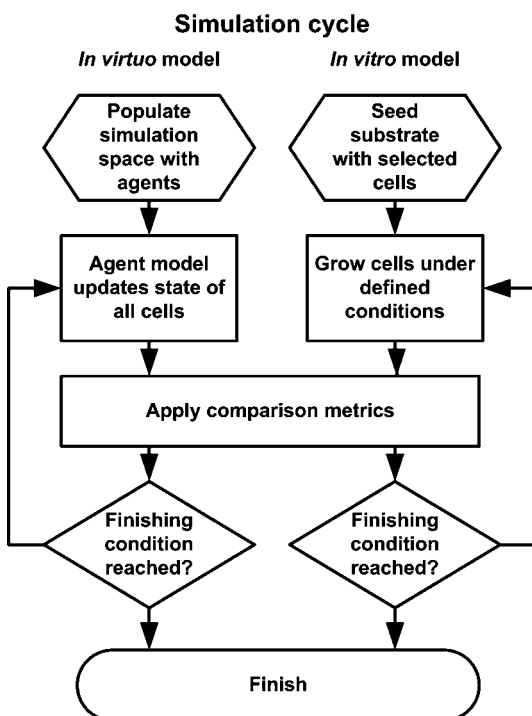
may have been due to chance rather than the correctness of the model. There is usually little difficulty in repeating observations of the virtual model–these will not always be the same since the model will have stochastic rules – but the repetition of the biological experiments will be constrained by cost and practical difficulties. It is therefore vital that the experimental set up is done in tandem, that clear objectives are set and that the relationship between the model and the culture is set out precisely.

Software issues

Building models and carrying out simulation can provide a great deal of information and inspiration for further experimental investigations. It can also offer a platform within which *in virtuo* analysis and reasoning can take place. The first question to answer is whether the model accurately and usefully represents the biological reality. This can not be answered simply, we have to integrate the model building and the experimental validation into as seamless and constructive a framework as possible with the model posing questions about the biology and the biology posing questions about the model. We have certainly found that a systems view of the phenomena helps to formulate hypothesis, questions and experiments that might not arise otherwise. Similarly, the models are challenged by the experimental data that is collected during this process and in our case this leads to the refinement of the underlying rules and processing functions, thus improving the model. It is an iterative process and so there is no real concept of a correct model. We aim to produce useful models and how this is judged is determined by the use to which they are put.

One aspect of any computational model, whether of a biological system or of any other type of system is that it needs to be both consistent and as complete as it is practical to aspire to. Methods exist for testing models by developing scenarios (e.g., changing from physiologically relevant levels of calcium to low calcium media which markedly affects epithelial cell biology) – essentially sets of events and environmental conditions which can be applied to the model to see if the resultant behaviour fits with what we expect. It is quite common for models to exhibit unforeseen behaviour, perhaps under some conditions the model either behaves in unpredictable ways or simply fails in some sense. To build a robust model therefore requires an extensive period of validation. Techniques exist (e.g., Holcombe & Ipate 1998) which can guide this process, although no theory exists for a complex communicating hybrid model as yet.

What can we do with a model apart from simulation? One potential approach is to apply ideas from model checking (Clarke *et al.* 1999). This process involves coding up the model in a suitable logic and using powerful software to analyse the model. The models are usually based on state machines and the process involves posing a question which is written in a logic style language and then using the model checker to determine whether it is possible to answer the question. Typically the question might involve statements along the lines of: is it always the case that when the quantity of a critical substance exceeds a particular critical value then a given action occurs or a given state is entered, is there a state in which a particular property of the model holds, is there a path of behaviour in the model such that every state in that path has a certain property and so on. Model checkers have successfully explored models with very large numbers (billions) of states. Model checking for communicating X-machines has been developed by Eleftherakis *et al.* (2001) using the XmCTL logic. This provides an important basis for future analysis of large, complex biological models. It is not enough that we only rely on simulation for our understanding of the system, it may be that some highly critical sequence of behaviour only occurs under conditions that we never get round to simulate, yet this knowledge might be important. Rather than trying things out to see what happens we try to identify interesting or undesirable phenomena and see whether the model can ever exhibit them (e.g., predicting what would happen if tissue engineered epithelia were constructed with a sub-optimal percentage of stem cells – this has been a long-standing and almost unanswerable concern for the clinical use of tissue engineered skin. Would one expect the skin to break down over a patient's life-time? A computational model could be used to predict the life-time wear characteristics of the tissue–engineered skin under both normal and wounded (regenerative) conditions. This kind of backwards reasoning is relatively novel and is still at an early stage but looking at the development of computational models in the long term the ability to do this will be extremely powerful. Questions such as: whether there are wound healing advantages to enriching the skin stem cell population or lowering the wound bed calcium for patients with chronic wounds could be assessed initially in virtuo. The use of the model could inform the design of *in vitro* (and possibly in vivo) experiments to then test these questions.

Conclusion

We started by taking the view that computational models of biological systems that do not satisfy two conditions (the ability to predict function at a systems biology level, and the existence of robust validation against biological models) are only of academic interest, and have attempted to describe some possible methods to provide robust validation. This is an area which is largely unexplored, but which will become of

increasing relevance as computational models in biology become more useful.

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