XX IMEKO World Congress
Metrology for Green Growth
September 9–14, 2012, Busan, Republic of Korea

PROGRESS ON MULTISCALE REPRESENTATION OF CARDIAC VALVE MORPHOGENESIS

R. Summers[1], *T. Abdulla*[1], *and J-M. Schleich*[2]

1. SEESE, Loughborough University, Loughborough, LE11 3TU, UK
2. LTSI, University of Rennes 1, Rennes, F-35000, France

Abstract: Executable biology at various levels of spatial scale is becoming increasingly important in order to understand the complexity exhibited in living systems. This paper describes how a multiscale systems approach can be used to integrate these models of behaviour to provide an insight into the development of cardiac valves. In particular, simulations of epithelial to mesenchymal transition using a Cellular Potts model are shown to demonstrate cell adhesion and imitate faithfully the *in vivo* process. As a consequence, this work also shows the importance of measurement of surface energy of adjacent cells and its effect on systemic behaviour.

Keywords: EMT; Endocardial Cushion; Cellular Potts; Executable Biology; Physiome.

1. INTRODUCTION

The purpose of this paper is two-fold: at a methodological level to introduce the multiscale approach; and at an application level to uncover and understand the complexity of a specific physiological process [1]. The multiscale systems approach can be defined as the study of physical properties of a system in which behaviour is exhibited at different spatial scales and/or different time scales simultaneously. A number of important issues remain to be solved at the system level including scale linking and the choice of software to be used to realise the formulated models. Scale linking refers to the methods adopted to integrate models used at different levels of scale so that a systemic appreciation of the observed behaviour is allowed. The in-depth case study used to illustrate the utility of the multiscale systems approach is the development of cardiac valves. This places the study at the forefront of work on tissue engineering, and presents a future candidate for stem cell therapy to reduce the burden of the effects of congenital heart defects. Biomedical engineering research is now highly data intensive, with each datum in this study based on a measurement in spatial scale between 10^{-6}m and 10^{-3}m; and time scale between 10^{-6}s and 10^{6}s.

2. MULTISCALE SYSTEMS

The levels of spatial and temporal scale applicable to cardiac valve development, and methods of representation, are illustrated in Fig. 1 below, with the example of heart valve development. The application can be further refined as epithelial to mesenchymal transition (EMT) that is responsible for endocardial cushion growth, a precursor to the heart valves themselves. The paper will now focus on a multiscale representation of spatial information only.

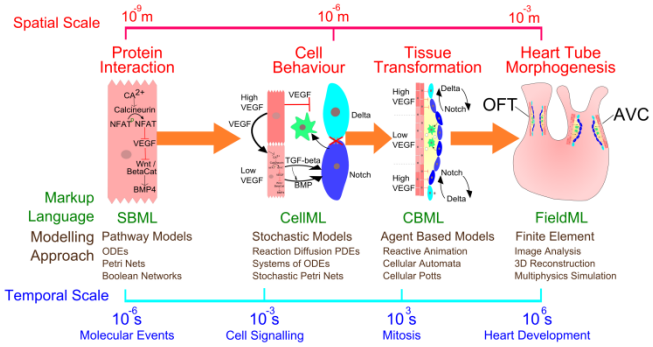

Fig. 1 Representation of spatial and temporal scales involved in heart valve development. The approach encompasses spatial scales from 10^{-9}m (proteins) to 10^{-3} (the size of the heart at this stage of development), and temporal scales from 10^{-6}s (molecular events) to 10^{6}s (weeks of development). Modelling approaches suited to each level of scale are indicated, as well as mark-up languages that aid in the integration of such models.

As shown in Fig. 1 different modelling approaches and mark-up notations are used at different spatial scales. Though the examples shown do not form a closed list, they nevertheless demonstrate the richness of available model realisations. This diversity is beneficial from the perspective of the modelling community, but presents issues when attempting to integrate models at different spatial scales. This integration is achieved via a process called scale linking, which involves either using innovative software designed for the purpose, or passing common parameter values via the underpinning ontological models.

3. CARDIAC VALVE MORPHOGENESIS

3.1 The Anatomic Level

The development of the embryonic heart commences in week 2 of gestation and is fully formed by week 8. This process is well documented [e.g. 2]. Week 2 of foetal life provides the first milestone of cardiac development when

the two endocardial tubes that form the primitive heart join together. At this stage of development the first cardiac muscle contractions occur, giving rise to both blood circulation and electrophysiological signals that form a primitive electrocardiogram [3]. The embryonic heart tube is composed of an inner layer of endocardium, an outer layer of myocardium and a middle layer of extra-cellular matrix termed cardiac jelly. In two restricted areas of the heart tube - the outflow tract (OFT) and atrioventricular canal (AVC) - endocardial cells adopt a mesenchymal phenotype and invade the cardiac jelly. These restricted swellings are termed 'endocardial cushions' and are precursors to the heart valves and membranous septa. The endocardial cushions begin to grow at embryonic day 26 (E26) in humans [2]. At the same time, the heart tube begins looping in an S-shape to the right (see Fig. 2).

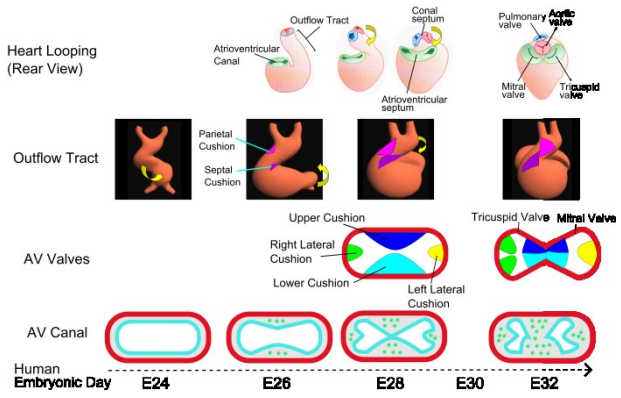

Fig. 2 Detail of endocardial cushion growth and fusion. EMT from E26 leads to cushion growth in the AVC and OFT. Fusion occurs at E32. In the OFT, parietal and septal cushions fuse forming the conal septum, which divides the aorta from the pulmonary artery. The conal septum is helical due to the rotation of the OFT. Upper and lower AVC cushions form the atrioventricular septum, the mitral valve and the tricuspid valve [after 2].

Two synchronised processes important in the understanding of normal and abnormal cardiac development are looping and aortic wedging. Looping is completed by E28, and is the first manifestation of asymmetry in the embryo. This repositioning constitutes a crucial step towards the morphology of the heart because it brings the future heart chambers and their inflow and outflow tracts into their relative spatial positions. Aortic wedging occurs as a consequence of rotation of the myocardial wall of the OFT, itself secondary to the re-modelling of the inner curvature of the heart. The AVC and OFT endocardial cushions fuse around E32 which completes the atrioventricular and OFT septation. Fusion of the cushions gives rise to the leaflets of the mitral and tricuspid valves. The OFT septum divides the aorta from the pulmonary artery. This is helical in shape, due to the rotation of the OFT.

3.2 The Cellular Level

The endocardial cushions grow by EMT. As the endocardial cushions play a role in forming much of the inner structure of the heart, it is apparent that abnormal EMT is a factor in many different types of congenital heart disease. These include valve, outflow tract and interventricular septal defects. During EMT, endothelial cells lose their adhesion to each other, and invade the cardiac jelly adopting a mesenchymal phenotype, and cause localised tissue swellings on the inner surfaces of the embryonic heart. The study of EMT *in vitro* [4] (using endocardial cell cultures) or *ex vivo* [5] (using endocardial cushion explants) enables a controlled means for studying changes in different cell properties in the transition between the two phenotypes caused by the activating or inhibitory actions of protein signalling.

3.3 The Protein Level

Several protein signalling pathways have been identified as being important in cardiac valve development. Some proteins are secreted by cells and absorbed by other cells that have suitable receptor proteins: this is termed paracrine protein signalling. This signal acts over a short distance, perhaps between tissues of different type. Proteins involved in paracrine signalling include TGFβ and BMP2, which are secreted by the myocardium in the endocardial cushion forming regions, as shown in Fig. 3 below.

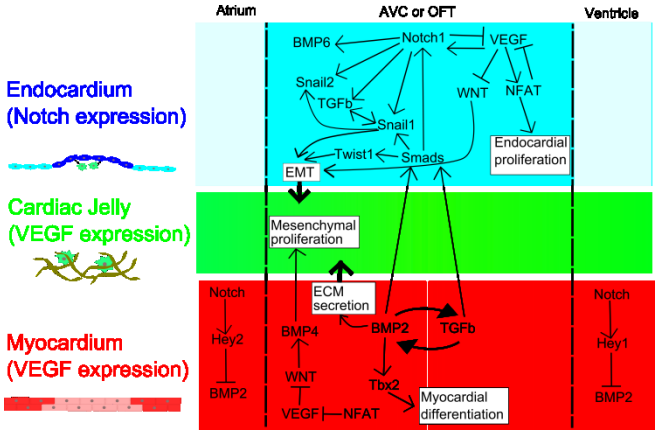

Fig. 3 Major protein interactions and cell mechanisms in EMT during endocardial cushion growth. Arrows signify an activating action; blunt ends signify inhibition.

Other types of protein signalling require cells to be adjacent to each other, as the ligand protein on one cell surface binds to a receptor protein on another cell surface. This is termed juxtacrine signalling, and principal among these is the Notch protein signalling pathway (with 'Delta-like' and 'Jagged' ligands). The Notch protein signalling pathway controls pattern formation in many embryonic tissues, including those of the heart.

In the endocardium, Notch1 is expressed in the endocardial cushion forming regions of the heart, but not external to those regions. In the myocardium the situation is reversed, and Notch expression outside the endocardial cushion regions inhibits transcription of BMP2, which promotes EMT in the endocardium (Fig. 3). Notch also has an additional role to play, because it activates the SNAIL family of proteins, which in turn inhibits the transcription of VE-Cadherin. As VE-Cadherin is one of the major proteins that provide endocardial cohesion, activated Notch induces a loss of cohesion, which is part of EMT. It has been

demonstrated *in vitro* that completion of EMT requires the proteins secreted by the myocardium [4]. Many types of congenital heart disease, such as Alagille syndrome and tetralogy of Fallot are associated with mutations in JAG1 (the gene that encodes Jagged 1, the ligand for Notch1), and this underlies the importance of Notch signalling in cardiac valve formation.

4. MODEL FORMULATION AND FINDINGS

4.1 Cellular Potts Models

Cellular Potts Models (CPMs) are lattice based simulations, with cells occupying multiple sites on the lattice. In contrast to most other types of agent based modelling, this allows cells to have shape and size and surfaces that may be adjacent with other cells. CPMs simulate cell behaviour as terms within a generalised Hamiltonian energy, *H*. Motile cells will tend to move so as to reduce *H,* thus reducing the entropy of the system. Typically the Hamiltonian equation includes terms for type dependent surface energy between each pair of different cell types, which represents their level of adhesion. Higher surface energy represents a lower level of adhesion. CPMs can be extended to include terms for anything that can be calculated from the cell attributes. For example, a type dependent target volume or target surface area can be included, with values for the propensity of a cell to reach that target. Apoptosis is generally simulated by setting cell target volume to zero. Mitosis can be simulated by creating daughter cells in place of the parent cell. Multiple fields can be defined across the same lattice, so secretion of a protein from cell surfaces can be simulated, as well as chemotaxis towards a source. One of the simplest CPM simulations represents a cell sorting experiment, whereby an initially mixed population of two or more cell types will become sorted (see Fig. 4). The cells with higher preferential cohesion move to the centre of the cluster, while those with lower cohesion move to outer layers.

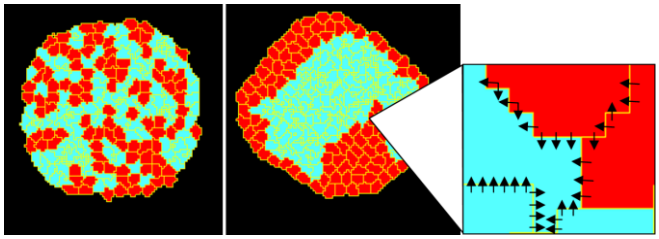

Fig. 4 Cell sorting simulation in Compucell3D. In CPM cells interact at their surfaces, and move stochastically such that the sum of the surface energies is reduced over time.

CPMs are able to simulate cell behaviour by representing any mechanism where cell re-arrangement is principally determined by differences in adhesion. As the focus of CPMs is cell re-arrangement, they have been used mainly for modelling developmental mechanisms. Compucell3D [6] is the most widely used modelling environment for implementation of CPMs. It is an open source resource and extensible, enabling the sharing of results. Compucell3D simulations were created to represent *in vitro* EMT in 3D, with endocardial cells (EC) initially lying on the surface of extracellular matrix (ECM). The default 'medium' was treated as the space above the culture, with no intrinsic surface energy. An assumption is that EC-EC adhesion is stronger in the wild type situation than EC-ECM adhesion, and that the latter is stronger than ECM-ECM adhesion. The contact energy with the surrounding space is taken to be higher between the EC-medium than the ECM-medium, due to the lower deformability of cell membranes. Therefore, to simulate a wild type ventricular explant on collagen gel 'ECM' the following energy hierarchy is assumed:

$$J_{EC,medium} > J_{ECM,medium} > J_{ECM,ECM} > J_{EC,EC} > J_{medium,medium} = 0$$

From simulation it was found that the corresponding parameters of set 1 (Table 1) give rise to an endocardial monolayer, which does not invade the ECM. Set 2 corresponds to a loss of endocardial cohesion (increase in $J_{EC,EC}$). Set 3 corresponds to a gain in EC-ECM adhesion (reduction in $J_{EC,ECM}$). Set 4 corresponds to both effects simultaneously.

Table 1 Surface enrgy parameters *J*, in $10^{-15}kg^1s^{-2}$ Key: EC-Endocardial Cell, ECM: Extra Cellular Matrix

Surface Energy *J*	EC, Medium	ECM, Medium	ECM, ECM	EC, ECM	EC, EC	Medium, Medium
Set 1	16	14	8	4	2	0
Set 2	16	14	8	4	10	0
Set 3	16	14	8	1	2	0
Set 4	16	14	8	1	10	0

Compucell3D allows the extension of models to assign subcellular models of protein interaction encoded in SBML to each cell. This mechanism can be used for creating a multiscale model of EMT, where the surface energy parameters are determined on an individual cell-cell basis, according to the concentration of individual adhesion molecules derived for each cell in a simulation step. The SBML models can be annotated so as to create a clear link between the protein species described in the model, and those defined in protein classification ontologies. Such a link allows explicit representation that allows modellers to quickly understand what is included in a particular model, and suggest points at which models might be merged or compared.

4.2 Cellular Potts Simulations

An initial layout of 100 cells in a circular monolayer was generated and simulated for 1000 Monte Carlo steps (MCS) using appropriate parameters to ensure that the system was in equilibrium. The monolayer was sustained, suggesting that the energy hierarchy assumptions for the wild type situation are reasonable, see Fig. 5a) below. The equilibrium was perturbed by altering the model parameters and running the simulation for a further 1000 MCS in separate experiments. With set 2, ECs scattered on the surface of the matrix without invading it (Fig. 5b). With set 3, the ECs invaded the ECM, but without delaminating from each other (results not shown). With set 4, all ECs delaminated from each other, and some invaded the matrix (Fig. 5c). The CPM

simulations demonstrate some correspondence with the *in vitro* experiments on which they are based. In particular, in both cases it was possible to induce 2D scattering of endocardial cells independently of 3D invasion into the ECM. In the *in vitro* experiment this was accomplished through Notch activation of the endocardium [4]. Alongside the simulation results, this confirms the hypothesis that Notch primarily acts to reduce endocardial cohesion. In the simulation with set 3, it was possible for the endocardial cells to invade the matrix, but still remain attached together.

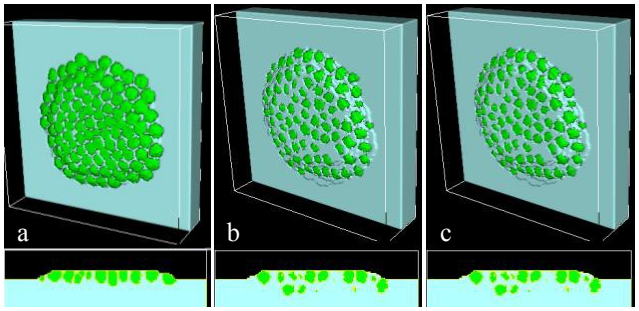

Fig. 5 Simulations of *in vitro* EMT. a) Endothelial monolayer on the surface of collagen gel. b) With reduced endocardial cohesion, cells scatter on the surface, but do not invade the gel. c) With reduced endocardial cohesion and increased endocardial-matrix adhesion, some cells invade the gel. Simulations run in Compucell3D.

This effect has not been observed in any *in vitro* experiments. This could be an anomaly of the simulation, or it could be that it is not possible to isolate an increase in EC-ECM adhesion from a decrease in EC-EC adhesion, due to the nature of the signalling pathways. Notch is downstream of BMP signalling, and therefore inducing increased EC-ECM adhesion via introducing BMP would also reduce EC-EC adhesion (see Fig. 2).

5. DISCUSSION AND CONCLUSIONS

Multiscale modelling research has hitherto focused almost exclusively on adult physiology, with little attention in the literature to embryonic development. This paper has demonstrated the *in silico* development of a model that describes EMT to a degree that is sufficient for purpose. The proposed extension of the study to integrate SBML mediated models of protein signalling pathways is underway. This extra dimension allows the passing of parameters between spatial scales, thus implementing a truly multiscale simulation.

Other work underway but not reported here is the use of an ontological framework that also goes across the entire spatial domain. The post-composition of ontological terms has been used successfully to integrate models and provide a method of scale linking in the multiscale hierarchy [7]. Combining knowledge gained from the information models allows the closure of the loop between physical experiments (real world) and computer based simulations (model world). As the model-based annotations of the 'model world' map to their isomorphic physical counterparts in the 'real world', it is possible to be unambiguous about referring to (say) endocardial cells or increased concentration of a given protein. Thus, one advantage of using an ontological framework is that it provides a common data dictionary of terms used in the integrated model. The entries in the data dictionary also convey contextual meaning allowing a common understanding of what is meant by each term. Such an approach may appear burdensome, but it is one of the most common causes of system integration failures.

Creating accurate phenotypic descriptions, which retain their semantic context, and linking these to physical and biophysical measurement, provides a powerful means to assimilate information from a wide variety of sources and scales. To this end the team has access to a unique physical resource – over 300 post mortem heart specimens of which around 80 have been diagnosed as tetralogy of Fallot. This is a congenital heart disease that has four causative (and linked) agents: pulmonary artery stenosis; an over-riding aorta; a septal defect; and right ventricle hypertrophy. In fact to understand this heart defect was the motivation for forming the research team between Loughborough University and University of Rennes 1. One intention of future work is to acquire MRI and CT images of the post-mortem specimens to allow a re-determination of the primary evidence that describes the tetralogy of Fallot.

From the work described in this paper and the intended future work on extending the existing model of EMT through to the genetic level of scale, as well as extending the system level model to aid understanding of the tetralogy of Fallot, it is clear that the multiscale approach offers a systemic appreciation of development physiology and pathology. To make best use of findings from these studies also requires advances in the methodological approach taken. An integrated programming environment and new methods for visualisation would increase uptake of this approach significantly.

6. REFERENCES

[1] T. A. Abdulla, R. Imms, J. M. Schleich and R. Summers, "Multiscale Information Modelling for Heart Morphogenesis," Journal of Physics: Conference Series, vol. 238, 012062, 2010.

[2] M. L. Kirby, Cardiac Development. Oxford: OUP, 2007.

[3] V. M. Christoffels, G. J. Smits, A. Kispert and A. F. M. Moorman, "Development of the Pacemaker Tissues of the Heart," Circulation Research, vol. 106, no. 2, pp. 240-54, 2010.

[4] L. Luna-Zurita et al., "Integration of a Notch-dependent Mesenchymal Gene Program and Bmp2-driven Cell Invasiveness Regulates Murine Cardiac Valve Formation," The Journal of Clinical Investigation, vol. 120, no. 10, pp. 3493-507, 2010.

[5] C. P. Chang et al., "A Field of Myocardial-endocardial NFAT Signaling Underlies Heart Valve Morphogenesis," Cell, vol. 118, pp. 649-663, 2004.

[6] M. H. Swat et al., "Multi-cell Simulations of Development and Disease using the Compucell3D Simulation Environment," Methods Mol. Biol., vol. 500, pp. 361-428, 2009.

[7] R. Summers, T. Abdulla, L. Houyel, J-M. Schleich, "Progress with a Multiscale Systems Engineering Approach to Cardiac Development," Automatika, vol. 52, no. 1, pp. 49-57, 2011.