Hybrid Modelling of *in vitro* Epithelial to Mesenchymal Transition

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Abstract

Epithelial to mesenchymal transitions (EMT) are fundamental to a multitude of embryonic processes in vertebrate development, including gastrulation, somitogenesis, neural crest cell migration and the development heart valves. EMT-like processes take place during adult epithelial wound healing, and in cancer progression [1]. These EMT and EMT-like processes are diverse, and are regulated by a diverse range of signaling pathways in different tissues and organs, with extensive crosstalk between pathways. However they have as a common endpoint the downregulation of E-Cadherin and thus loss of epithelial adhesion; and the upregualtion of mesenchymal genes such as vimentin and fibronectin, and thus gain of cell to matrix adhesion and migratory or invasive behaviour [2]. The secretion of matrix metalloproteinases, which degrade extracellular matrix proteins (including those in the basemement membrane) is also key transformation and migration in many types of EMT [3]

The adhesion force required to separate two E-cadherin expressing cells is initially of the order of a few nano Newtons (nN). However, this adhesion strength increases rapidly between 30 seconds and 30 minutes, and this is followed by a slower increase up to an hour, reaching a force over 200 nN. The initial E-cadherin mediated contact adhesion doesn't require connection to the actin cytoskeleton. The stronger junctional adhesion, forms over a longer period of contact, by a connecting the actin cytoskeleton between the two cells [4].

These two phases of adhesion can be modelled as two types of force: labile adhesion and plastic coupling. The initial stages of contact represent labile adhesion, which can be modelled as a negative interfacial tension force between cell surfaces. The stronger, but slower to form, plastic coupling can be modelled as a breakable spring force between cell centres. Labile adhesion and plastic coupling are most often modelled separately, using different agent based modalities. For example, labile adhesion can be simulated using Graner-Glazier (Potts) models or vertex models, while plastic coupling can be represented with cell centre models.

Hybrid models of EMT were developed by adding an energy function for plastic coupling to the Hamiltonian equation governing cellular Potts simulations. The simulations were implemented in the open source software package Compucell3D (downloadable from www.compucell3d.org) which allows for rapid development and visualisations of simulations. By representing both forces in the same model, an initial parameter scan is conducted to explore the relative roles of labile adhesion and plastic coupling in cell migration. A generic representation of *in vitro* epithelial tissue was initially modelled in 2D; a simplification that allowed for the exploration of multiple parameters.

The length scale was set to 1 micron per pixel, and model cells given a width of 15 microns, based on based on the dimensions of cultured murine endocardial cells [5]. This choice reflects a prospective goal to include subcellular reaction kinetics; and thereby develop a multiscale model of the integrated roles of Notch and TGF-β signalling in endocardial to mesenchymal transition. Observing the rate of cell movement set the time scale to 1 Monte Carlo Step (MCS) = 6 seconds. Each simulation is run for 5×10^4 MCS, which equates to 83.3 hours. Cellular movement was quite stable after this time, and this provides a link with typical *in vitro* experiments, for which results are often given after 72 hours, e.g. [5].

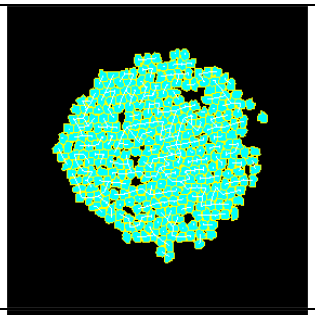
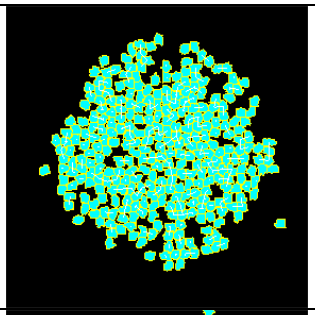
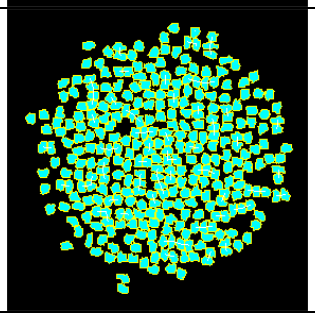
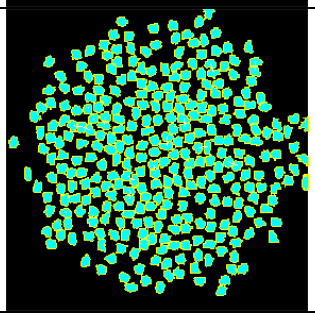
Plastic Coupling Lambda Cell-Cell Contact Energy	20	5
-15		
0		

Figure 1. Simulation snapshots at 50000 MCS, for different levels of plastic coupling lambda [20, 5] and cell-cell contact energy [-15, 0]. Intermediate levels omitted for brevity.

There is no standard metric for cell migration from *in vitro* assays. The results of [5] quantify cell migration by counting the fraction of cells that were able to detach and migrate on the surface of the collagen gel (2D Transformation Index (TI)) and the fraction able to invade the gel (3D TI). The metric of 2D TI is approximated in the simulations by calculating the fraction of cells that have no labile or plastic adhesion to any other cells (Figure 2). The simulations could be extended to be three dimensional, in which case a 3D TI could also be calculated.

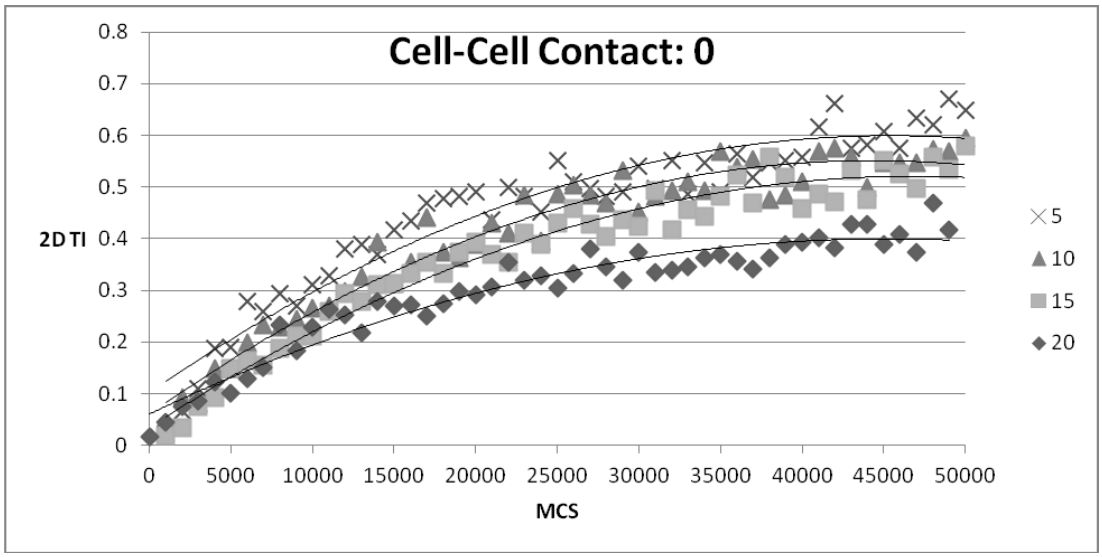


Figure 2. 2D TI for four levels of plastic coupling lambda [5, 10, 15, 20] for cell contact 0.

Computer simulations provide much greater flexibility to create a metric from any combination of variables that can be tracked. A few other metrics for cell migration were also output by the simulations. These are indicative for providing measures of cell migration that can be matched to computational simulations, and provide additional insight that is hard to achieve from *in vitro* assays alone. For example, we record the average contact area between cells and the average number of plastic links per cell, every 10^3 MCS, and the numbers for each cell every 10^4 MCS. Thus it is possible to quantitatively investigate the distribution of links and contact area over time for different parameter sets (Figure 3). The average diffusion coefficient of cells was also recorded every 10^3 MCS, which can be used as another metric for cell migration, though difficult to calculate *in vitro*. Further metrics include average cell velocity and average the distance between cell centres, which can be measured *in vitro* through image processing.

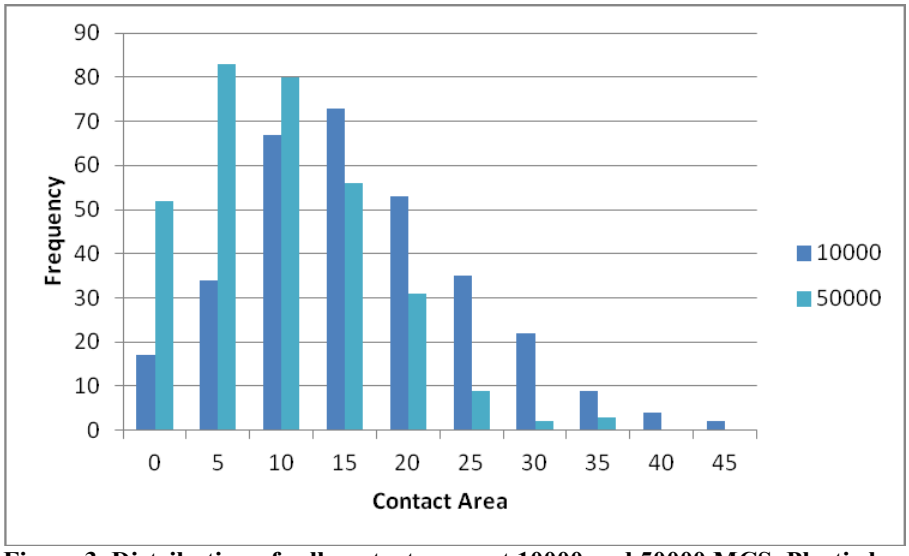


Figure 3. Distribution of cell contact areas at 10000 and 50000 MCS. Plastic lambda 10, cell contact -10.

While the simulations reported here did not account for changes in cell shape as a result of EMT, these changes can be simulated by reducing the constraint on cell surface area, and introducing a connectivity constraint that represent the increased integrity cells. There are a few measures that can be used for quantifying cell shape that can be applied both *in vitro* and *in silico*, such as aspect ratio (length/width) and 'roundness' (4π*area/perimeter^2).

References

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