In silico study of mechanical stresses at the cellular level during tissue development

Anne Jeannin-Girardon, Pascal Ballet, Vincent Rodin

***Abstract*— Mechanical contraints play a key role in tissue morphogenesis. We propose to study these mechanisms at the cellular level, thanks to our virtual biomechanical cell model. This model defines biological cell behaviors, such as cell motility, mitosis and adhesion as well as methods to evaluate cell compression/stretching and shearing. The evaluation of these constraints allows the virtual cells to respond by changing their color during simulation and lead to the observation of emerging patterns in cell differentiation during tissue growth: the main purpose of this evaluation is to give the cells the ability to respond to mechanical constraints by differentiating. This approach allows to study the influence of mechanotransduction during tissue morphogenesis.**

I. INTRODUCTION

Mechanotransduction is the phenomenon in which cells respond to mechanical stresses by adapting their shape, size and function. It is now widely admitted that mechanical constraints play a key role during tissue morphogenesis. Forces are transmitted in the tissue through cell-extra cellular matrix (ECM) and cell-cell adhesions. Cell functions and behaviors such as differenciation, apoptosis, proliferation or migration are regulated and modulated by these forces [1], [2], [3]. These influences at the cellular level may impact the development of the entire tissue. Studying how mechanical constraints influence tissue development may lead to a better understanding of the development of organs, organisms, or various diseases such as cardiomyopathies or cancers [4].

Moreover, *in vitro* measures of tissue flow and deformation during chicken embryo development has lead the author of [5] to venture the hypothesis that mechanical constraints are the main processes that triggers cell differentiation during the firsts stages of embryogenesis: diffusion constants of biochemical signals expressed by cells seems too low to explain cell differentiations during the firsts stages of embryogenesis. Considering such processes in models is thus essential to study tissue development.

Many *in vitro* experiments have been driven in order to study mechanotransduction and its influence on tissue development (reviewed in [6], [7]). Although many mathematical models aim to describe mechanotransduction mechanisms, to our knowlegde, there are few computational models dedicated to the study of *in silico* mechanotranstuction during tissue morphogenesis. We rule out cell models focused on intra-cellular processes modeling as in [8], [9], as they were not conceived for large tissue simulation. We describe cell behaviors at a higher level, such as in [10], in which the authors propose to generate artificial creatures from a single cell. However their system is based on chemical gradients diffusion and genetic regulatory network, and does not consider mechanical constraints. The authors of [11] propose to study the role of mechanical forces during epithelium formation and the main point of interest of the authors is to study the influence of development speed during tissue formation. Mechanical models dedicated to plant morphogenesis have also been proposed [12], [13]. Unfortunately, concerning the last three systems, the authors do not provide elements related to the ability of their systems to simulate large tissues (thousands of cells).

The main contribution of our work is to propose a model dedicated to the study, *in silico*, of the influence of mechanotransduction during tissue development, and in particular how these processes affect cell differentiations. Moreover, we can simulate large groups of cells with our system which is implemented with OpenCL [14], a framework for parallel computing.

This paper is organized as follows: the section II presents the physical structure of our virtual cell along with the cell behaviors definition. It also presents its coupling to a multi-agent system (MAS) as well as the implementation of this system. The section III presents how the mechanical constraints applied on the virtual cell can be easily and efficiently evaluated by the virtual cell itself. We present two simulations results showing emerging patterns in cell differentiation during tissue growth. Finally, section IV draws some conclusions and perspectives concerning this work.

II. VIRTUAL CELL MODEL

Our model is an extension and an improvement of the deformable biomechanical cell model proposed in [15]: this model was successfully used to simulate cell migration on an adhesive substrate. So far, our model defines the following behaviors: cell motility, cell/cell adhesion and mitosis.

A. Cell structure

We designed our virtual cell in such a way that mechanical constraints can be easily and efficiently evaluated. It is built with a mass/spring system, and coupled to a multi-agent system. The structure of the cell is made of seven nodes: one central node C and $n = 6$ membrane nodes N_i ($0 \leq i < n$). The three following structures aim to represent a simplified biological cell and define how the nodes are linked (Fig. 1):

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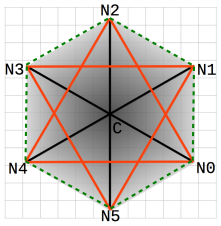

Fig. 1. Physical structure of our deformable virtual cell.

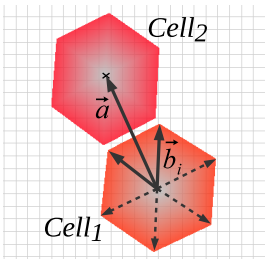

Fig. 2. Selection of nodes for the adhesion between two cells: a node N_i is selected only if $\vec{a} \cdot \vec{b}_i > 0$.

- cytoskeleton: links $C \leftrightarrow N_i$ (Fig 1: black links)
- cortex: links $N_i \leftrightarrow N_{(i+2)\%n}$ (Fig 1: orange links)
- membrane: links $N_i \leftrightarrow N_{(i+1)\%n}$ (Fig 1: green dashed links)

These links are elastic links, and a restoring force $\vec{F_r} = k(l - l_0)\vec{u}$ is applied on each one of them (k is the stiffness of the springs, l_0 is their equilibrium length and $\vec{u}$ is a unit vector denoting the direction of the force).

B. Cells interactions

Cells interact with the environment and their neighboring cells. So far, only a Langevin force is issued by the environment: $\vec{F_l} = I\vec{u}$ (I is the intensity of the force). This force is applied on every node and models cell motility and collisions between cells and environmental molecules.

Two forces constitute the cell/cell interactions: a repulsive force $\vec{F_{rep}} = k(l - d_{min})\vec{u}$ takes place if two cells are at a distance less than d_{min}. An adhesive force $\vec{F_a} = k(l - d_{max})\vec{u}$ allows cell to adhere given the adhesive coefficient between them: d_{max} is the sum of the cell radius (ensuring a minimal distance) and the adhesive coefficient. Any coefficient greater than 0 indicates that cells adhere. We modeled the adhesion by selecting the closest membrane nodes of a cell $Cell_1$ to the center of the cell $Cell_2$ in the local environment of $Cell_1$. This is easily done by computing the dot product of the vectors $\vec{a} = \overrightarrow{C_1C_2}$ and $\vec{b_i} = \overrightarrow{C_1N_i^1}$, as depicted on Fig. 2. If the dot product is greater than 0, then the node N_i is selected for the adhesion: a spring is created between N_i^1 and C_2. This method is efficient given that it does not require either much computation time or memory in order to determine and memorize the adequate nodes for the adhesion.

We numericaly integrates the applied forces with the Euler method. We deal with the numerical instability of this method by considering the environment as overdamped and by chosing a small integration step. The second Newton's law, stating that $\Sigma\vec{F} = m\ddot{x}$, allows the computation of the next position of a node i of a cell k at time $t+1$:

$$\vec{x}_{i,k}(t+1) = \frac{\vec{F}_{i,k}}{m}\Delta t^2 + \vec{\dot{x}}_{i,k}(t) + \vec{x}_{i,k}(t)$$

We consider that the environment is overdamped, thus $\vec{\dot{x}}_{i,k}(t) = 0$. The equation then becomes:

$$\vec{x}_{i,k}(t+1) = \frac{\vec{F}_{i,k}}{m}\Delta t^2 + \vec{x}_{i,k}(t)$$

C. Mitosis

Our virtual cell has the ability to divide. We modeled this behavior by forcing the displacement of points in the mother cell, as depicted in Fig. 3a: the cell is compressed to half its original size and a second cell is placed in the newly freed space beside it (Fig. 3b). Two forces ensures the cells are restored in a resting position: the restoring forces between the nodes of the cell allow the cell to strech back to its restoring form and size (springs lengths tend toward l_0). The repulsive force between the cells allows the cells to separate from each other.

The physical structure of the virtual cell allows us to *orientate cell division* by determining 1) a division axis, i.e., between which two nodes is the cell going to divide and 2) a direction axis, i.e., whether the daughter cell is placed on the right or on the left of the chosen division axis.

D. Multi-agent system

We coupled our virtual cell model to a multi-agent system (MAS). MAS [16] are distributed systems in which the entities composing the system interact with each other and with their local environment. These local interactions lead to complex emergent behaviors. Unlike mathematical models, MAS allow a fine description of each entity of the system. On the downside, they have a high number of degrees of freedom but such approaches focus on the observation of emergent properties during simulation and are thus interesting in terms of exploration (for instance to test parameters and hypotheses). Large MAS require a lot of computation time, although they are also good candidates for parallel implementations, which is discussed in the next paragraph.

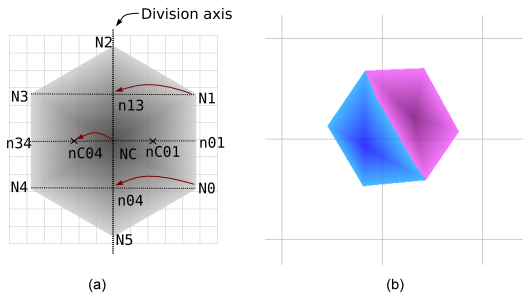

Fig. 3. (a) Preparing the displacement of points. (b) Division of the blue cell: insertion of a new cell (purple) in the newly freed space.

E. Model implementation

We implemented our model in a software architecture for parallel multi-agent system implementation that we designed [17]. The model is implemented using the OpenCL framework, which is used to program heterogenous parallel devices such as GPUs, CPUs, FPGAs, etc. Although the details of the implementation is out of the scope of this paper, it is nevertheless important to mention general concepts concerning the parallel part of this implementation, given that the parallel implementation of the system allows the simulation of large tissues (comprising thousands of cells).

Each agent of our system is a cell. It is represented by a thread with a unique identifier on the parallel device. Information about the agents are stored into structures of arrays. Agents data are the positions of their nodes, the forces applied on each node and their types. We limited as much as possible the use of synchronization mechanims. However, for the allocation of a new cell during mitosis (that is, to find a free and unique identifier for the daughter cell), we use an atomic operation.

The algorithm describing the behavior of the agents is depicted on Fig. 4: all the agents execute their algorithm at the same time. A cell can proliferate if a replication criterion is fulfilled: this criterion can be based on cell cycle, but also, in a simpler manner, on simulation steps (see section III). The evaluation of mechanical stresses is detailled in the next section. The graphic rendering is a particular task because the agents do not render themselves: a global process renders all the cells at once (the details of the rendering process are out of the scope of this paper).

These basics cell behaviors are sufficient to simulate a tissue growth. In the next section, we detail how our virtual cells evaluate their mechanical stresses during tissue morphogenesis.

III. MECHANICAL STRESSES EVALUATION

Our virtual cells are able to evaluate two main mechanical stresses: compression (and stretching) and shearing. Taking into account this mechanical signals should allow cells to adapt their behaviors, for example by differentiating, specializing or adapting their size or form. For the sake of simplicity, cells ajust their color (abstracting the process of cell differentiation) during tissue growth simulation according to the mechanical constraints they undergo.

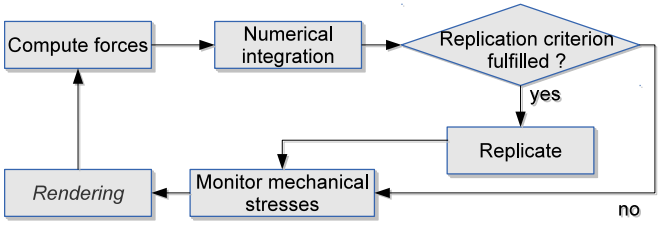

Fig. 4. Algorithm executed by the agents (the cells) of our system. The execution of these tasks represents a simulation step.

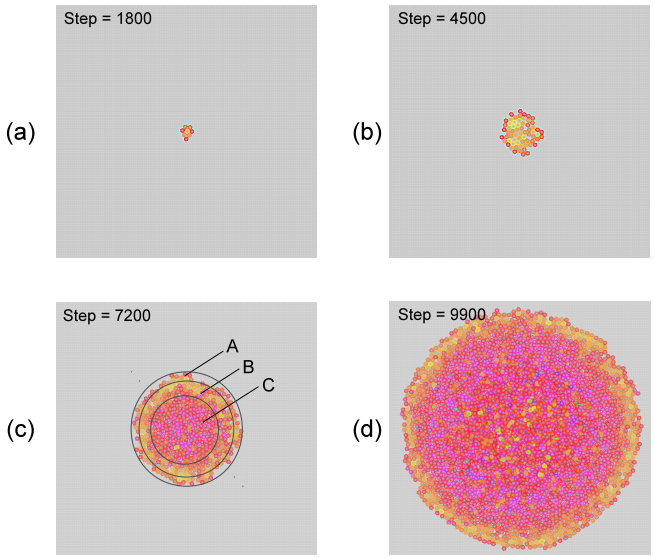

Fig. 5. Variation of hue according to the rate of compression of cells. Figures from (a) to (d) show the growth of the tissue during a simulation. Outer cells (layer A) undergo less constraints than inner cells (layer C). The final tissue contains 4096 cells. A video of this simulation is available at `http://youtu.be/pcIOw8qTX-w`

A. Compression and stretching evaluation

The virtual cells compute their compression and stretching rate by computing their surface. Given that springs have an equilibrium length, a cell knows its resting surface. The surface of a cell is the sum of the areas T_i of the triangles $CN_{i\%n}N_{(i+1)\%n}$ composing the cell (see Fig. 1 p.2): $S_{cell} = \Sigma T_i$. Computing the distances $CN_{i\%n}$, $CN_{(i+1)\%n}$ and $N_{i\%n}N_{(i+1)\%n}$ and sorting these distances allows the use of the Kahan's formula to compute the area T_i. The general form of Kahan's formula is the following:

$$T = \frac{1}{4}\sqrt{(a+(b+c))(c-(a-b))(c+(a-b))(a+(b-c))},$$

where $a > b > c$.

Given the ratio between the resting surface and the computed surface, cells ajust their color according to the constraints they undergo, as depicted in Fig. 5 (figures are extracted from a simulation).

The tissue growth is achieved in the following manner: every 900 simulation steps (this is the replication criterion; this interval gives the system the time to relax), all the cells enter mitosis at the same time: although this is very intensive, patterns emerge because these simultaneous replications bring about strong contraints. We see different layers in the tissue: in the layer C, the constraints are the most important (pink/orange cells). The yellow layer (B) is the resting part of the tissue: this layer gets larger as the system relaxes. The outer layer (A) is made of the cells whose constraints are the lowest (red cells).

B. Shearing evaluation

Cells also evaluate the shear they undergo. Shear evaluation consists in computing the couple cells undergo. The couple of a cell is: $\overrightarrow{C_k} = \Sigma \overrightarrow{CN_i} \wedge \overrightarrow{F_{i\perp}}$, where C is the center

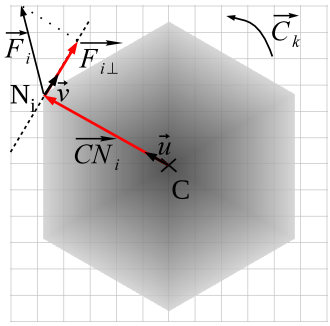

Fig. 6. Definition of the vectors used to calculate the couple applied on a cell.

of the cell, N_i is a node of the cell, $\overrightarrow{F_i}$ is the sum of forces applied on the node i and $\overrightarrow{F_{i\perp}}$ is the projection of $\overrightarrow{F_i}$ on $\vec{v}$ (Fig. 6).

Again, cells ajust their color according to the shearing they undergo: the cells that are under the most important shear constraints are thus highlighted. The Fig. 7 illustrates shearing influence during a simulation. We start from a already-grown tissue. We fragment the tissue in layers, and make one in two layers move to the right while the other move to the left, as shown by the arrows in Fig. 7a. We can see that the cells on the boundaries of two layers undergo serious shear constraints (pink/orange cells).

To sum up, we observe while simulating our model that the virtual cells are able to ajust their color according to the mechanical stresses they undergo, leading to emerging patterns in the tissue. Although color ajustment is a simple way to model cell differentiation, these *in silico* experiments are an encouraging step toward a deeper study of mechano-transduction processes during tissue morphogenesis.

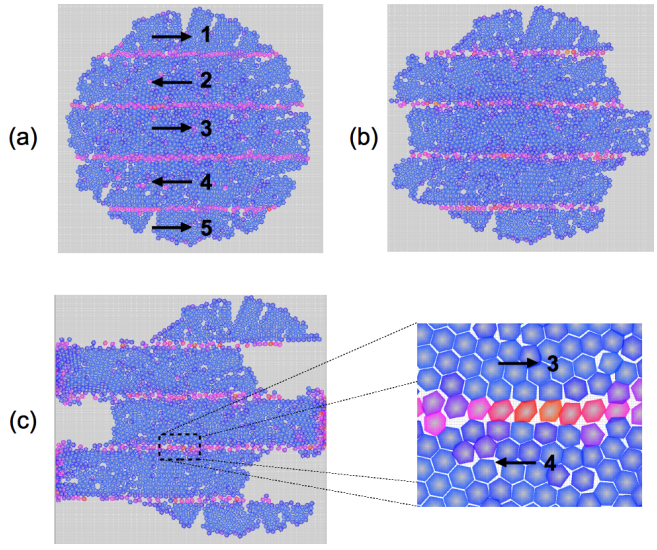

Fig. 7. Variation of hues according to shear rates. Figures are extracted from a simulation. One layer of cells in two move to the right while the other move to the left. The cells on the boundaries of two layers undergo strong shear constraints (pink/orange cells). The tissue contains 2048 cells. A video of this simulation is available at `http://youtu.be/kXVN5RLn7wc`

IV. CONCLUSIONS AND FUTURE WORKS

In this paper, we presented an *in silico* study of mechano-transduction processes during tissue development. These processes are essential features of morphogenesis. Their integration in computational models should lead to a better insight into tissue development. We used a virtual biomechanical cell model which includes mechanical stresses evaluation mechanisms. During *in silico* simulation, the virtual cells ajusted their color according to the constraints they perceived. We observed emerging patterns during simulation. So far, our model does not allow 3D simulation, but we intend to extend our virtual cell model to a 3D model.

The next step of this work is to use this constraints evaluation to make the cells respond in a appropriate way, in particular by differentiating.

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