Simplified models of *Dictyostelium discoideum* aggregation and slug migration

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November 10, 2006

Abstract

Biological models of *Dictyostelium discoideum* behaviours are often quite complex. Computer simulations using simplified models provide insight into the biological behaviours but rely on complex mathematics. We studied whether characteristic slime mold behaviours can occur in very simple models that can be easily implemented with any graphical simulation engine. We proposed two excitation based models: neighbour and spherical models, and one simplified cAMP propagation model - the grid model. We succeeded in producing streaming and spiral patterns in aggregation and characteristic thermotaxis in the slug migration life stage of *Dictyostelium discoideum*.

1 Introduction

Developmental biology research requires experimentation with biological organisms to formulate theories on fundamental biological processes observed in nature. Computer models and simulations of biological processes provide a time and cost efficient test-bed for verifying biological theories and can provide insight into the inner workings of such processes.

1.1 *Dictyostelium discoideum*

The cellular slime mold, *Dictyostelium discoideum*, is a social soil amoeba that lives through a complex life cycle. The slime mold has been designated a model organism by the US National Institutes of Health[1]. The organism can be used to study fundamental cellular processes such as phagocytosis, chemotaxis, signal transduction, cell sorting, pattern formation, cell-type determination, and thermotaxis. In our research, we were interested in the processes of chemotaxis and thermotaxis of the slime mold.

With an abundance of food in the environment, *Dictyostelium discoideum* exists as a single celled amoeba. With the depletion of the food source, the amoebae within a certain area aggregate together in stream patterns to form a 3D mound of cells. This mound can then elongate to form a 3D slug which then moves around searching for a more favourable location. The slug finally comes to rest to develop into a fruiting body. The cells in the mound differentiate into stalk cells that form the stalk of the fruiting body and the spore cells that form the spore globule that

[1]Further information about *Dictyostelium discoideum* can be found at http://www.nih.gov/science/models/d_discoideum.

is then spread by other organisms such as worms. The spore cells are the only surviving cells of the aggregate. The function of the other cells is to ensure the survival of the normally genetically identical spore cells.

The movement of the cells during the aggregation stage and movement of the slug during the slug migration stage is the result of chemotaxis towards a chemical attractant: cyclic adenosine monophosphate (cAMP). The chemical signal is initiated by cells called pacemakers that periodically release a cAMP pulse into their surroundings. When non-pacemaker cells receive such a pulse, them emit their own cAMP pulses thus spreading the chemical throughout the median. The cells also migrate towards the source of the received signal.

1.2 *Dictyostelium discoideum* models

In our research, we were interested in computer models of two stages of *Dictyostelium discoideum* life cycle: the aggregation stage and the slug migration stage. Several approaches have been successfully used to model and simulate *Dictyostelium discoideum* aggregation [6, 9, 14, 8, 7, 1, 19, 5, 18, 3, 20] and slug migration [12, 22, 17, 15, 10, 13].

The models of early *Dictyostelium discoideum* development can be categorized according to the cell mechanics and chemical attractant dynamics. The existing approaches for cell mechanics modelling can be mainly divided into two classes.

The first class of cell mechanics is based on cellular automata (CA) [9, 14, 7, 1, 21] where the amoebae are modelled as simple automata on a regular grid. Rules are provided for interactions between the neighbouring automata and between the automata and the chemical in the environment. Initial experiments treated the cells as black boxes and did not incorporate physical sources acting between the cells. Some hybrid CA models [15, 10] used energy bonds and elasticity to model deformation of the amoebae during chemotaxis.

The second class uses continuum models of cell movement [6, 8, 19, 3, 13] where the amoebae are positioned in 2D or 3D space. The models contained simulations of physical forces required for accurate cell modelling.

Detailed models of cAMP signalling have been proposed [11, 16]. Simplified dynamics are often described using partial differential equations modelling a reaction-diffusion system [8, 3, 13]. Equations of the FitzHugh-Nagumo type were also shown to provide reasonable results [19, 15, 10] but do not incorporate a model of signal transduction and cAMP production [3]. Solving partial differential equations in a simulation requires additional simulation time. It has been shown [21] that a simple model of cAMP propagation based on propagation of cell excitation is able to produce some characteristic amoebae aggregation patterns.

We were interested in simple models of cAMP propagation that can be easily incorporated into graphical simulation software. The goal of the research was to assert whether characteristic aggregation and slug migration behaviours of *Dictyostelium discoideum* can be observed using simplified cAMP propagation models in graphical simulation.

1.3 Breve Simulator

For our experiments, we have chosen to use the Breve Simulator[2] created by Jon Klein. Breve is a 3D simulation environment that was specifically designed to simulate decentralized systems. The simulator and its source code are available under the GNU Public Licence (GPL) agreement for MacOSX, Windows, and Linux.

[2]Information about Breve can be found at: http://www.spiderland.org/breve.

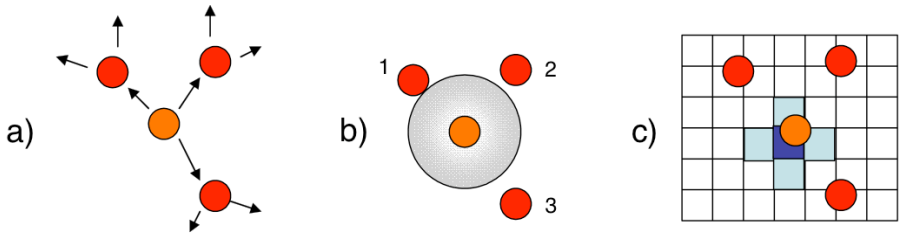

Figure 1: Schematic diagrams of the three proposed cAMP propagation models: a) neighbour model, b) spherical model, and c) grid model.

Breve programs are written in an Object Oriented scripting language called Steve. The programs are then interpreted by the Breve software at run-time. External functions written in C can be called from within Breve through compiled C executables. The software provides pre-existing object classes and allows for the creation of new classes by subclassing.

The main feature of Breve is the 3D graphical shell that easily allows the user to create and manipulate objects in a 3D environment. The software provides a simple physics engine that models gravity and object collisions. Breve objects are evaluated in a decentralized manner without using global controller code.

2 Implementation

We have modelled *Dictyostelium discoideum* aggregation and slug migration using spherical 3D object representation of cells. Collisions between cells were modelled using inelastic collisions in the Breve physics engine. Cells were situated in continuous 3D space represented by a 2D plane in 2D experiments. Cells reacted to changes in their environment in a decentralized manner with object evaluation sequence dependent only on the internal object storage data structure of Breve.

2.1 cAMP Propagation Models

We have used three simple models of cAMP propagation. Schematic diagrams of the three models are presented in Figure 1. The first model - neighbour model - used the idea of a local neighbourhood to propagate cellular excitation (similar to [21]). In this model, a cell would send an excitation signal to all cells in its local neighbourhood of a fixed radius. The signal would be sent to all the neighbours at approximately the same time, thus no distance measure was used.

The second model - spherical model - was based on the neighbour model with distance information. Thus, excitation signal sent to cells in a local neighbourhood would be processed by each cell with a time delay proportional to the distance of the cell to the signal source.

Finally, the third model - grid model - used a 2D grid for cAMP propagation. Each cell of the grid stored a concentration of the attractant chemical. The chemical spread through the grid using simple diffusion of a percentage of the chemical in a cell onto its eight neighbouring cells. The amoebae positions were not fixed to the grid, however, each amoeba could sense the chemical value in the grid it was centered on.

2.2 Aggregation

During the aggregation stage of *Dictyostelium discoideum* life cycle, the individual amoebae aggregate to form a 3D mound of cells. Aggregation begins with a small set of pacemaker cells periodically emitting a cAMP signal wave. The chemical signal attracts the other cells in the medium which then move towards the pacemakers. The non-pacemaker cells also relay the cAMP signal thus spreading it over the medium.

In most of our experiments, we have used an initial randomized distribution of amoeba cells in a square area centered on a pacemaker cell. We have used the space and time model of [1]. The amoebae were modelled as spheres of $10\mu m$ diameter in a simulation area of size $30 - 75\mu m$ per side. The cells occupied 10% of the simulation area.

The cAMP signal wave travelled up to approximately 6 cell diameters from the source which modelled the destruction of the chemical by a phosphodiesterase enzyme. In the majority of the experiments, we have used one stationary pacemaker, which emitted a cAMP pulse every 10 simulation cycles (we chose 1 simulation cycle to be equivalent to 1 minute in the biological model). When cells received a cAMP message, they would desensitize for $3 - 7$ simulation cycles. During this time, the cells would not respond to further cAMP pulses. The cells would also start to move towards the received signal source for approximately 1.7 simulation cycles. The movement speed of the cells was about 1.2 cell diameters per simulation cycle. After approximately 0.2 simulation cycles, the cells would emit their own cAMP pulse into the environment. We have used a special simulation variable to scale the simulation time cycles in order to allow for the delays occurring from the Breve graphical simulation.

In the grid model simulations, we have used a cAMP diffusion rate of 90%, thus 90% of the chemical in a particular cell would be dispersed over the 8 neighbouring cells at each grid cell evaluation. The grid size was set to the simulation area size and the grid cell size was set to approximately $1 - 2$ slime mold cell diameters. The amoebae cell concentration was 50% of total simulation grid area.

The strength of the cAMP chemical signal was adjusted to model the decay path of approximately 6 cell diameters. We have used pacemaker signal strength of 1200 and non-pacemaker signal strength of 300 chemical units. The sensitivity threshold of cAMP chemical in the grid cell occupied by an amoeba was set to 7 chemical units in most experiments. The excited amoebae moved towards the location of the neighbouring grid cell with the highest concentration of the chemical.

2.3 Slug Migration

During slug migration, the aggregate of cells that formed during the aggregation stage migrates either due to a chemical or both chemical and thermal gradient. We have tried to model this chemotaxis and thermotaxis behaviours based on the model descriptions presented in [10].

Chemotaxis of the slug is due to the pushing force of individual cells reacting to the cAMP attractant. The cells move towards the source of the signal thus pushing the slug in that direction. This is a perfect example of a decentralized behaviour where none of the cells choose the direction of motion but the motion is a emergent behaviour of the entire slug.

Thermotaxis relies on the chemotaxis response for slug movement. The thermal gradient is an extra factor of the simulation. The individual cells are too small to detect a temperature gradient. Thermotaxis is then modelled using temperature dependent cell excitability. The temperature of a particular cell influences the delay in excitability of the cell.

In our simulations, we have used an initial grid distribution of cells with 100% cell concentration to model the aggregate forming in the aggregation experiments. We have placed the pacemaker in front of the grid of cells in the desired direction of motion. In experiments with multiple pacemakers, the pacemakers were placed in front of the cells in various directions.

Chemotaxis experiments were performed using the aggregation model of cell movement as described in the previous section. The pacemaker was made mobile to become a part of the moving slug. In thermotaxis, the temperature value of a cell was indicated by the location of the cell in a one dimensional temperature gradient. The delay in the cAMP pulse relay of the cells was proportional to the temperature value at the cell center.

Experiments using the grid model of cAMP propagation, used the chemical grid setup as described in the previous section. The chemical signal strength values were adjusted in each experiment to yield the best results.

3 Results

We have run experiments using the experimental setup described in the previous section. The experiments differed in the propagation method used, the number of amoeba cells, and other experimental parameters. The results of the experiments for aggregation and slug migration simulations are presented in the following sections.

3.1 Aggregation

In experiments using the neighbour and spherical models of cAMP propagation, we have observed the characteristic stream patterns of aggregating cells. These patterns were also observed in simulations using more complex cAMP propagation models (for example [3, 13]). All experiments resulted in a 2D mound of cells showing proper aggregation behaviour. Sample frames of the simulation animations can be seen in Figure 2 (neighbour model) and Figure 3 (spherical model).

We have also observed spiral patterns of aggregation in experiments with nonuniform distribution of cells over the simulation area (see Figure 4). Occurrence of such spiral patterns has been observed in biological *Dictyostelium discoideum* experiments and complex computer models [3, 2].

The grid model was unable to form stream patterns in our experiments. The distribution of cAMP in the chemical grid was seen to be spherical around the pacemaker with increasing concentration of chemical towards the center. This distribution forced our cells to travel in the direction of the pacemaker. Eventually, streams of cells formed along 2 axes in the chemical grid centered at the pacemaker. This formation was due to the fact that in a spherical distribution of the chemical, the cells along the axes have the highest concentration of the chemical of the specific row or column. The cells simulated using the grid model would eventually form a 2D mound of cells in our experiments (see Figure 5).

We have tried experiments with multiple pacemakers randomly distributed in the simulation area. The observed result was the creation of multiple mounds of cells centered at each pacemaker (as shown in Figure 6). The division line between cells aggregating on different pacemakers was created along the boundary where the multiple cAMP signals collided. Images of biological aggregation using multiple pacemakers can be found in [5].

The majority of the experiments we have performed were done on a plane using 2D distribution of cells. We have run experiments using 3D cell distributions (initially a cube centered around the pacemaker) and the neighbour model. The

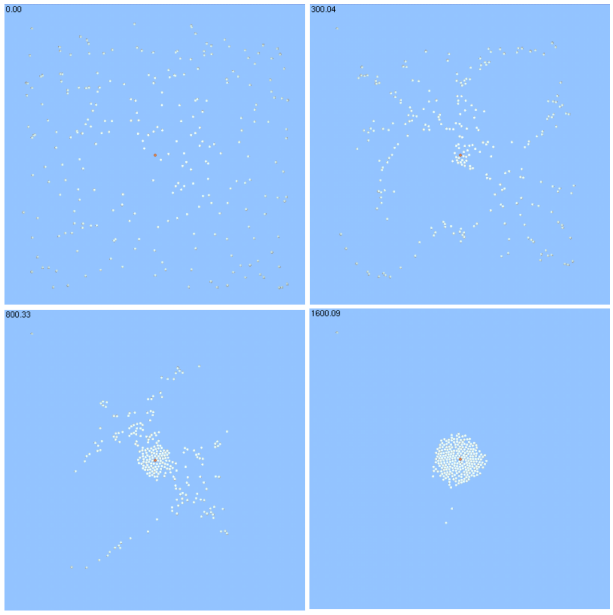

Figure 2: Aggregation of 250 cells using the neighbour model. The images are captured at simulation times: 0, 300, 800, and 1600.

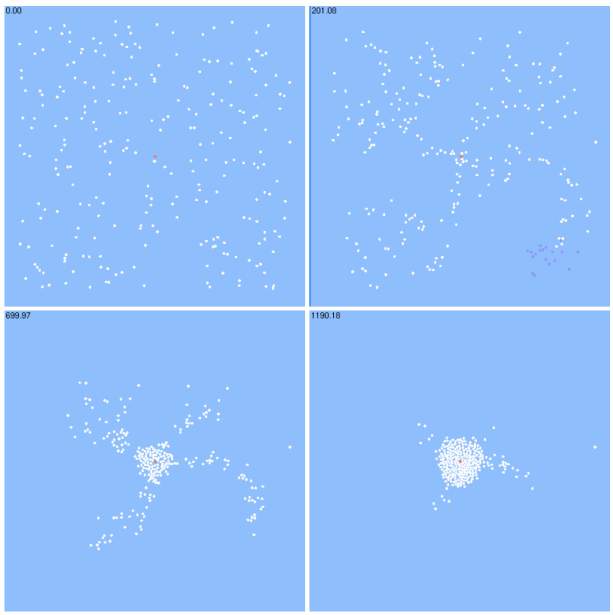

Figure 3: Aggregation of 250 cells using the spherical model. The images are captured at simulation times: 0, 201, 700, and 1190.

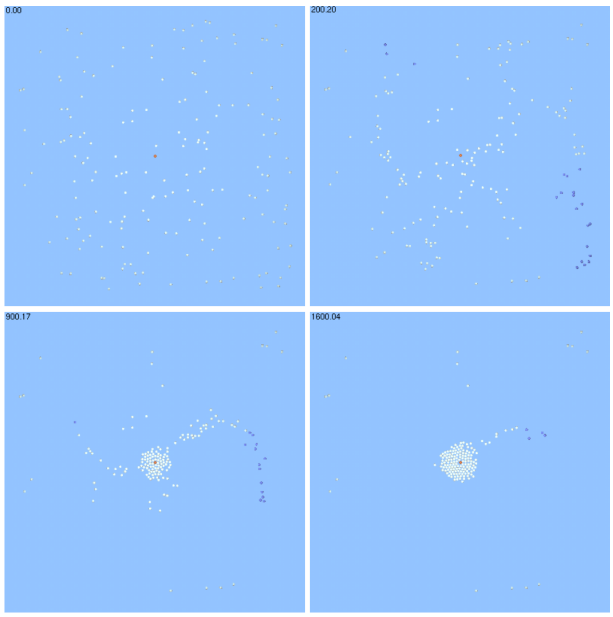

Figure 4: Spiral patterns seen in a simulation with the spherical model and nonuniform distribution of cells. The images are captured at simulation times: 0, 200, 900, and 1600.

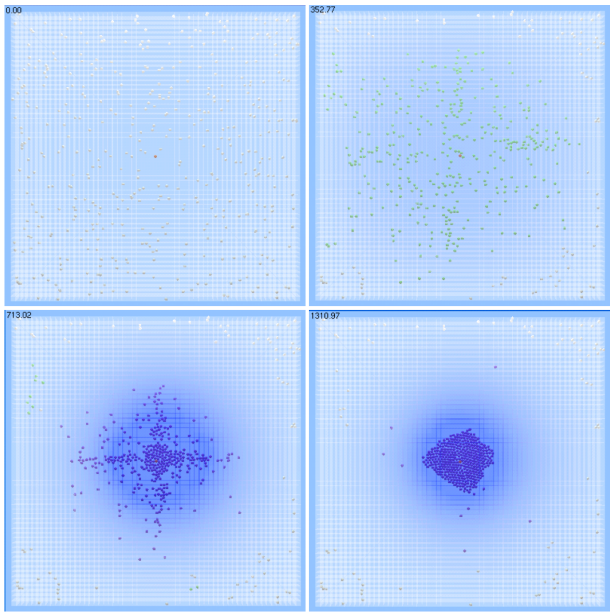

Figure 5: Aggregation of 450 cells using the grid model. The images are captured at simulation times: 0, 353, 713, and 1311.

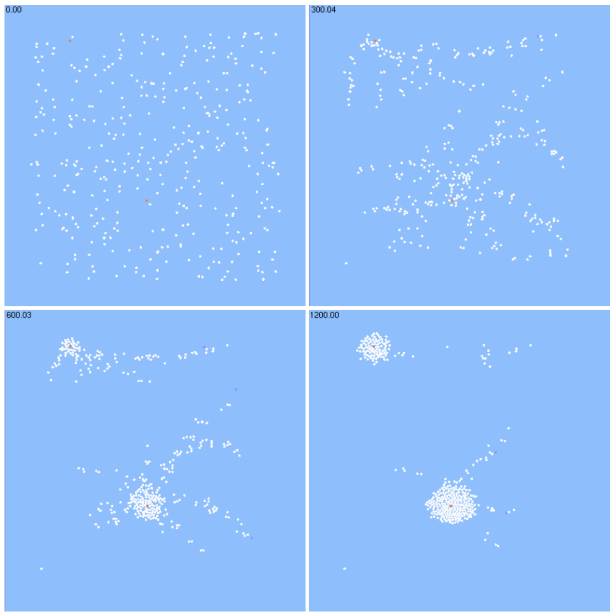

Figure 6: Aggregation of 250 cells using the neighbour model and two randomly placed pacemakers. The images are captured at simulation times: 0, 300, 600, and 1200.

results of the experiments were 3D mounds of cells. Streaming patters were seen during aggregation but were difficult to notice in 2D representations.

3.2 Slug Migration

In the slug migration task, we have run two types of experiments - chemotaxis and thermotaxis. All experiments have been done in 2D simulation space, however, most simulations can be easily extended into 3D.

In chemotaxis experiments, we have clearly observed movement of the slug due to the pushing force of individual cells towards the cAMP chemical sources as seen with other models [10]. The motion was in the direction of the pacemaker with direction variations due to the dynamic structure of the slug. While the forward motion of the slug was observed using all the cAMP propagation models, the fluctuations in direction were most visible using the neighbour and spherical propagation models (see Figures 7, 8, and 9).

Most of our experiments in slug migration used one pacemaker. The result was a single moving slug. We have also experimented with multiple pacemakers situated on the sides of the initial grid-based cell mound. The experiments resulted in splitting of the mound into separate slugs each travelling in a different direction towards the orientation of each pacemaker within the slug (see Figure 10). This result is consistent with grafting experiments on real slime mold slugs [13].

We have also achieved thermotaxis using temperature dependent excitability with results comparable to previous research [10]. We were able to see thermotaxis at experiments with the temperature gradient at 90 and 180 degrees to the initial slug configuration. Using the neighbour and spherical models (see Figures 11, 12, and 13), the turning angle towards the temperature gradient was observed to be steep. Using the grid model, the turning angle was quite shallow in our experiments as seen in Figure 14. The grid model experiments also required a large temperature gradient in order to produce good thermotaxis results.

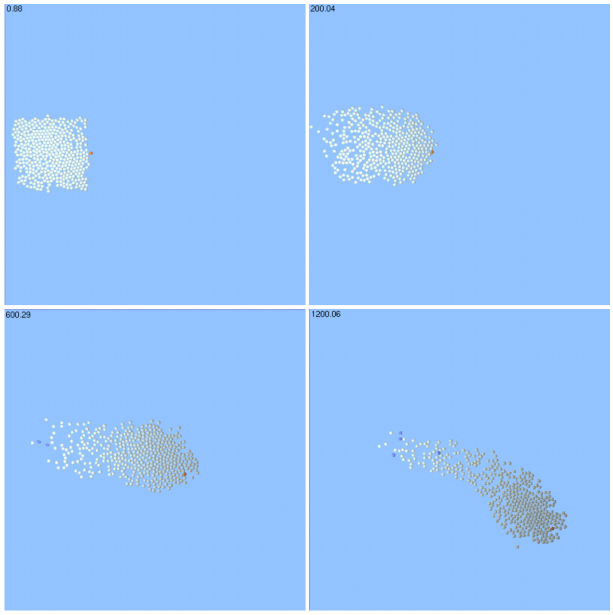

Figure 7: Chemotaxis of a 200 cell slug using the neighbour model. The images are captured at simulation times: 0, 200, 600, and 1200.

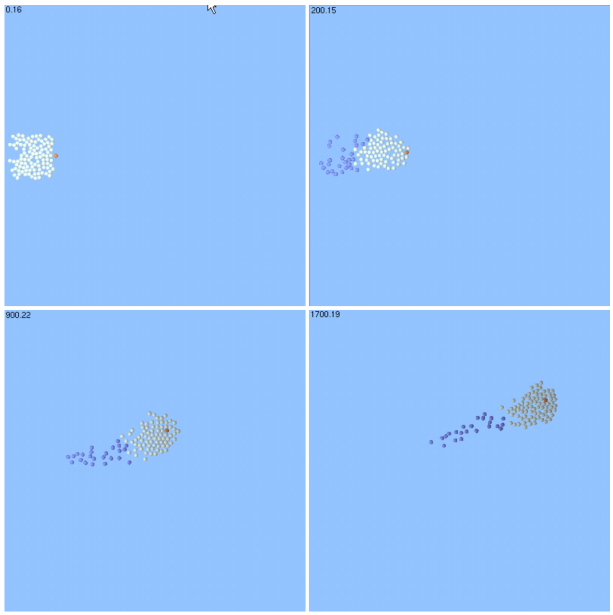

Figure 8: Chemotaxis of a 100 cell slug using the spherical model. The images are captured at simulation times: 0, 200, 900, and 1700.

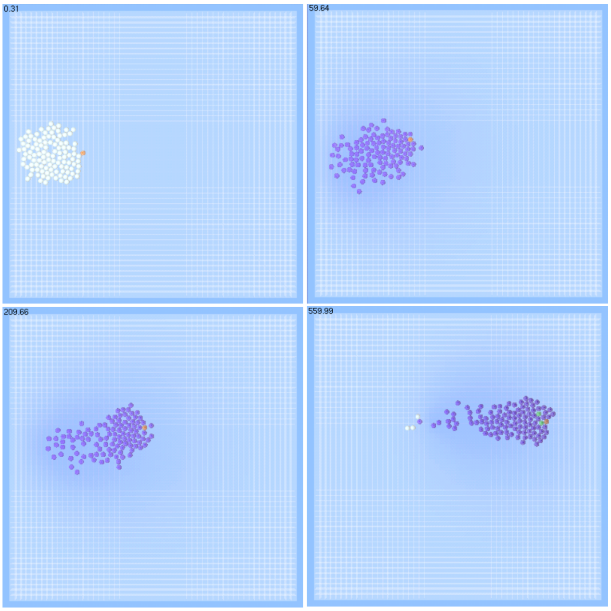

Figure 9: Chemotaxis of a 100 cell slug using the grid model. The images are captured at simulation times: 0, 60, 209, and 560.

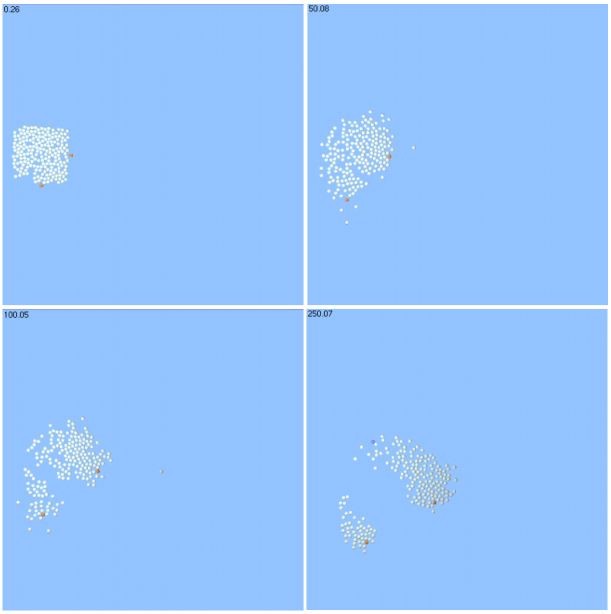

Figure 10: Chemotaxis of 196 cells using the neighbour model and two pacemakers. Two slugs are seen to emerge. The images are captured at simulation times: 0, 50, 100, and 250.

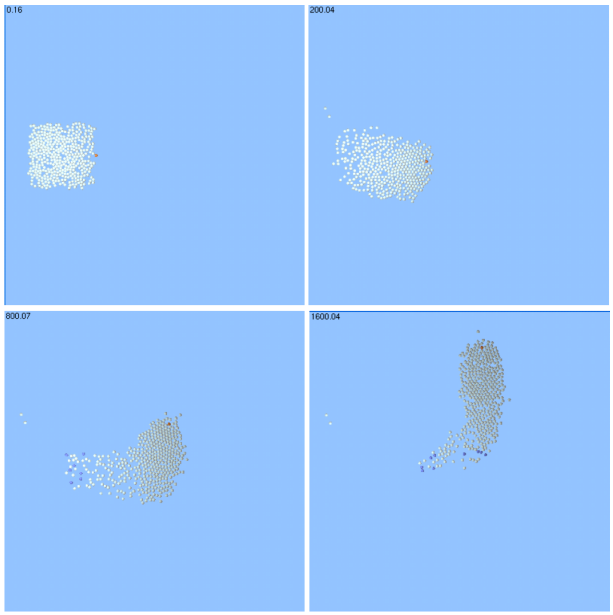

Figure 11: Thermotaxis of a 400 cell slug using the neighbour model. Thermal gradient from bottom to top. The images are captured at simulation times: 0, 200, 800, and 1600.

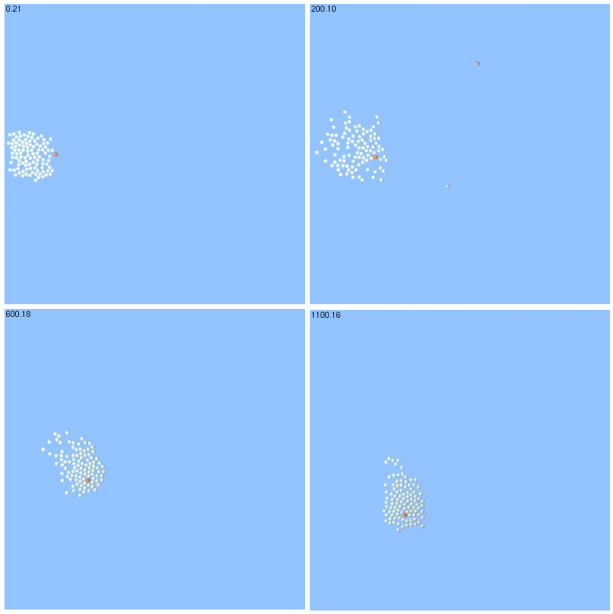

Figure 12: Thermotaxis of a 100 cell slug using the spherical model. Thermal gradient from top to bottom. The images are captured at simulation times: 0, 200, 600, and 1100.

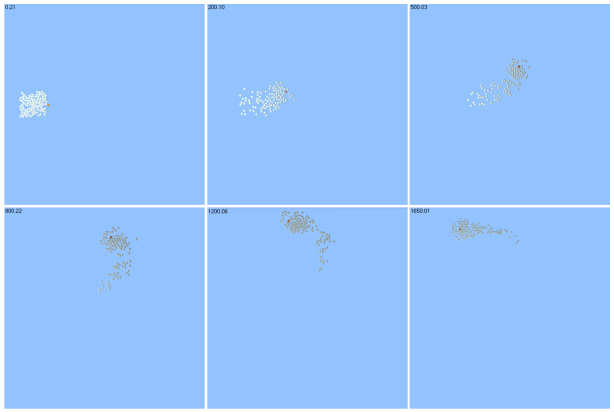

Figure 13: Thermotaxis of a 100 cell slug using the neighbour model. Thermal gradient in direction opposite of initial pacemaker placement. The images are captured at simulation times: 0, 200, 500, 900, 1200 and 1650.

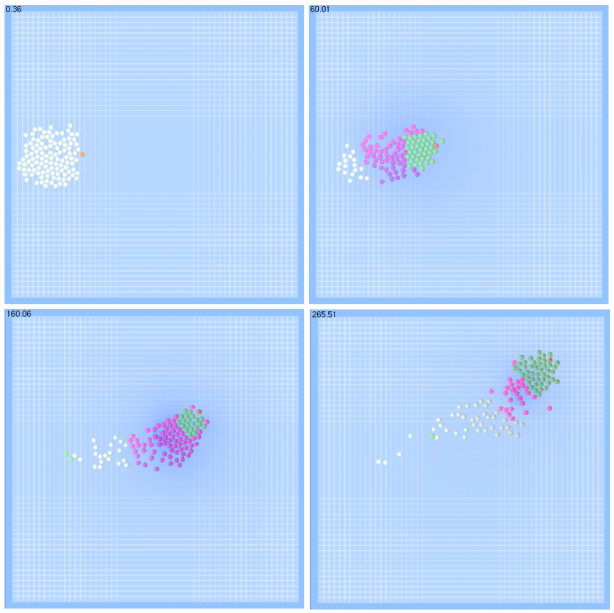

Figure 14: Thermotaxis of a 100 cell slug using the grid model. Thermal gradient from bottom to top. The images are captured at simulation times: 0, 60, 160, and 265.

4 Conclusions

Based on our experiments, we conclude that the neighbour and spherical models provided the best results out of the three studied models. These models showed the characteristic streaming and spiral formations in the aggregation experiments and proper thermotaxis in the slug migration experiments. The distance information in the spherical model did not provide improved performance compared to the neighbour model. The grid model did not perform very well since it failed to demonstrate streaming behaviours and did not produce strong thermotaxis.

The main advantages of using the proposed simplified cAMP propagation methods is the speed and simplicity of implementation and simulation. That is why such methods can be easily modelled in 3D simulators with no differential equation solvers (such as Breve). We have shown that the proposed models (especially the neighbour and spherical models) demonstrated the characteristic aggregation and slug migration behaviours.

The main disadvantage of our models is that they are not as precise as the PDE-based propagation methods and can only be used to gather limited insight into the biological models. Because of programming restrictions, our models did not include cell adhesion, which can be seen as a limitation to the models.

We have found several problems with the Breve simulator that restricted our experimentation. The first problem was the physics engine which did not allow an easy approach to stop moving cells and created a great amount of movement due to object collisions. We were unable to restrict this movement using the object elasticity parameters. The software did not allow for an easy way to join objects; thus, we were unable to implement cell adhesion. The scheduler of function calls in Breve changes the evaluation order of the calls. We needed to implement our own scheduling for the time delays in the spherical model.

In the grid model simulations, we run into synchronization problems of the grid cell evaluations. We were unable to deduce the order in which the cells of the grid are evaluated, thus we think that some of the problems of the grid model performance might be due to this fact. The other problem we run into was the evaluation speed of the Breve grid. The maximum grid that we could reasonably simulate was of size 60x60 cells. Three dimensional simulations of the grid were not possible due to the speed of the grid.

Through our research, we arrived at some open problems that we could not resolve. The first problem was with the grid cell size in the grid method. Due to limitations of Breve, we could only study two different grid sizes and it seemed that the performance of the model improved on smaller grid cell sizes. However, more experimentation would need to be done in order to verify this finding. The second problem dealt with the ideal parameter values for the simulations. This is mostly evident in the grid model simulations. We feel that the performance of the model can be improved by choosing appropriate parameter values. We suggest the use of an evolutionary approach such as Genetic Algorithms[4] to evolve the parameter values.

We suggest the following future improvements to the proposed methods. First, we would like to compare the performance of the current models to the models using cell adhesion. We would also like to create a single simulation that models the aggregation and slug migration phases of the slime mold. Such a simulation would require a 3D mound of cells in order to move the pacemaker out of the center of the mound to initiate a single directional pushing force.

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