**Model proposal for hESC Morphogenesis**

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CMPLXSYS 530,

Computer Modeling of Complex Systems

Winter, 2019

**Goal**

The goal of this human embryonic stem cell (hESC) morphogenesis ABM will be to understand hESC self-organization and differentiation dynamics in different in vitro environments.

**Justification**

Laboratory experiments with hESCs often require vast amounts of resources in terms of both time and money. For this reason, I propose the development of an *in silico* model to test hypothesis regarding morphogenetic events of hESCs. Because of the discrete nature of cells, an agent-based model (ABM) will be used to model these events.

**Main Micro-level Processes and Macro-level Dynamics of Interest**

The model will seek to understand two key processes of hESC morphogenesis: (1) cyst formation, and (2) hESC differentiation. For the cyst formation, the model will be looking at how a cell acts according to its local neighborhood and how this can bring about self-organization in the system that leads to the formation of spherical polarized cysts. With regards to differentiation, the model will be looking at how contact with matrix in the environment can lead to a cell’s differentiation. Additionally, the model will explore the possible inductive effects of differentiated cells (i.e. could differentiated cells be secreting signals that make other cells differentiate). The model will also look at possible inhibition of differentiation by pluripotent cells.

**Model Outline**

**1) Environment**

The model will be constructed in Netlogo in a 2D hexagonal grid with no wrapping.

Environment-owned variables:

1. Amount of diffused inhibition chemical from pluripotent stem cells
2. Amount of diffused induction chemical from differentiated cells
3. Amount of matrix in the environment

Environment-owned procedures:

1. Diffusion of chemical in the environment

patches-own [

patch-neighbors-6

inhibitor

induction-chemical

]

to setup

clear-all

if culture-condition = "embedded"

[make-embedded-culture]

if culture-condition = "clustered"

[make-clusters]

make-clusters

;;make-embedded-culture

ask cells [set can-divide? True]

ask cells [set counted? False]

ask cells [set num-divisions 0]

set ticks-matrix-differentiation 0

set lumen-num 0

set num-cysts 1

set steady-state? False

reset-ticks

end

to make-embedded-culture

set-default-shape matrix "box"

ask n-of num-cells patches [

sprout 1

[set breed cells

set ss? False

set shape atom-shape

set color red

if pxcor mod 2 = 0 [set ycor ycor - 0.5 ]

]

]

ask patches [

if not any? turtles-here [

if overlay-matrix-percentage > random 100 [

sprout 1 [

set breed matrix

set color white

if pxcor mod 2 = 0 [set ycor ycor - 0.5 ]

]

]

]

]

ask cells [

;; define neighborhood of patches

ifelse pxcor mod 2 = 0 [

set neighbors-6 turtles-on patches at-points [[0 1] [1 0] [1 -1] [0 -1] [-1 -1] [-1 0]]

][

set neighbors-6 turtles-on patches at-points [[0 1] [1 1] [1 0] [0 -1] [-1 0] [-1 1]] ]

]

end

to make-clusters

set-default-shape matrix "box"

ask patches [

if not any? turtles-here [

if overlay-matrix-percentage > random 100 [

sprout 1 [

set breed matrix

set color white

if pxcor mod 2 = 0 [set ycor ycor - 0.5 ]

]

]

]

]

while [count cells < num-cells]

[

ask n-of 3 matrix [

if (ycor > min-pycor + 5) and (ycor < max-pycor - 5) and (xcor > min-pxcor + 5) and (xcor < max-pxcor - 5)

[

hatch-cells 1

[

set breed cells

set color red

set shape atom-shape

set ss? False

]

ifelse cluster-size > 1 or cluster-size < 1

[

ask matrix in-radius cluster-size

[

hatch-cells 1

[

set breed cells

set color red

set shape atom-shape

set ss? False

]

]

]

[

if cluster-size = 1 [

ask n-of 2 matrix in-radius 1.5

[

hatch-cells 1

[

set breed cells

set color red

set shape atom-shape

set ss? False

]

]

]

]

]

]

]

ask cells [

if any? matrix-here [ask matrix-here [die]]

if count cells-here > 1 [ask one-of cells-here [die]]

;; define neighborhood of patches

ifelse pxcor mod 2 = 0 [

set neighbors-6 turtles-on patches at-points [[0 1] [1 0] [1 -1] [0 -1] [-1 -1] [-1 0]]

][

set neighbors-6 turtles-on patches at-points [[0 1] [1 1] [1 0] [0 -1] [-1 0] [-1 1]] ]

]

end

**2) Agents**

|  |  |  |
| --- | --- | --- |
| Agent | Variables | Procedures |
| Cells | neighbors-6: stores information about the type of neighboring agents and their locations  cycles-matrix-contact: how many ticks has a cell been in contact with matrix  cycles-diff-contace: how many ticks has a cell been in contact with a differentiated cell  still\_count: how many cycles has a cell stood still  ss?: has the cell reached steady state?  can-divide?: can the cell divide?  group-id: identifies to which cyst the cell belongs to  num-divisions: keeps track of how many times a cell has divided | A cell can move, die, divide, consume matrix, make links to other cells, and differentiate |
| Matrix | Agents that are a part of the environment | Matrix can be consumed by cells and cause cells to differentiate |
| Lumen | These agents are used to identify the inner cavity of cysts | N/A |
| Walker | This agent is created once the cysts form and is used to give a unique identifier to the lumen and cells of each cyst | Move, assign id to lumen from a cyst |
| Cell-links | These are links formed between two cells that indicate the start of polarization. | N/A |

breed [ cells cell ]

breed [ matrix a-matrix ]

breed [ glass a-glass ]

breed [ lumen a-lumen ]

breed [ walker a-walker ]

undirected-link-breed [ cell-links cell-link ]

undirected-link-breed [ neighbor-links neighbor-link ]

undirected-link-breed [ lumen-links lumen-link ]

;breed [ differentiated diff ]

cells-own [

neighbors-6

cycles-matrix-contact ;;counts number of cycles in contact with >= x amount of matrix neighbors

cycles-diff-contact ;;counts number of cycles in contact with >= x amount of differentiated neighbors

still\_count ;; counts number of cycles a cell has kept still

ss? ;;boolean stating if cell has reached steady state

counted?

can-divide?

group-id

num-divisions

]

lumen-own

[lumen-neighbors-6

lumen-id

group-id

active?

unvisited?

gateway?

gateway-visited?

]

walker-own [

id-in-proximity?

walker-id

up-moves

down-moves

group-id

]

matrix-own [

matrix-neighbors-6

]

# The rest of the code can be found under morphogenesis. There are three working versions: Morphogenesis\_3Denv\_3Dov, Morphogenesis\_3Denv\_3Dov-v2, and Morphogenesis\_3Denv\_3Dov-v3

**3) Action and Interaction**

***Interaction Topology***

The interaction topology is most similar to a CA neighborhood. The cells will only take actions and move within their local neighborhood.

***Action Sequence***

1. The cells check their variables with regards to differentiation (i.e. do they differentiate or stay pluripotent)
2. The cells check their local neighborhood and decide to move, die, consume matrix, divide, or differentiate.
3. The cells update their local environment.
4. Once the system has reached stead state (i.e. the cells have established satisfaction with their state), the lumen of each cyst is identified with a walker that moves through the environment giving unique id to all the lumen of each cyst. After the lumen have their IDs they pass it on to the cells of the cyst.

**4) Model Parameters and Initialization**

 Global variables:

1. ticks-matrix-differentiation- time in which matrix can cause differentiation
2. steady-state?- has the system reached steady state
3. num-cysts- total number of cysts in the system
4. grid-x-pos- x coordinate the walker is on
5. grid-y-pos- y coordinate the walker is on

The model setup includes creating an environment that is filled with a percentage of matrix (chosen by the model user) and positioning a number of cells (chosen by the model user) in either a random or clustered manner. The model user has to establish the following parameters: amount of cells, cluster size, maximum number of divisions for each cell and whether there will be diffusion in the environment or differentiation will take place through contact. If there is diffusion, the model user must also establish how much chemical (both from induction and inhibition) the cells secrete, and what are the thresholds for induction and inhibition. If there is no diffusion, the model user must establish how many matrix neighbors will lead to differentiation, how many differentiated neighbors will lead to differentiation, and the respective number of cycles through which the contact must be maintained before differentiation.

Schedule during each tick:

1. Chemicals diffuse in the environment
2. Chemicals degrade in the environment
3. Cells check their state with regards to differentiation
4. Cells release either inhibitor or inductive signaling into the environment
5. Cells check their neighbors and take an action (die, move, divide, consume matrix)
6. Cells update their neighborhood
7. If the system has reached steady state:
8. Patches establish their neighborhood
9. Lumen is created in the cavities of cysts
10. Walkers are created and give unique IDs to lumen
11. Lumen pass on their IDs to the cells in the cyst
12. If all the cells in the system have group-ids the model stops

**5) Assessment and Outcome Measures**

 Model calibration will be performed for: (1) Cyst formation, and (2) Cell differentiation. In terms of cyst formation, I will be looking at the number of cells in each cyst and the cyst shape. In terms of cell differentiation, I will be looking for model parameters that give pluripotent cysts at high plating densities coupled with differentiated cysts at low plating densities. One particular even of interest is the formation of asymmetric cysts. This kind of event is rare in laboratory experiments, and the in silico model will be used to test hypothesis with regards to how it occurs.

**6) Parameter Sweep**

The parameters I will be varying initially are:

|  |  |
| --- | --- |
| Differentiation through contact | |
| number of matrix contacts required for differentiation | 1-5 |
| cycles of contact with matrix for differentiation | 1-10 |
| number of differentiated neighbors required for differentiation | 1-5 |
| cycles of contact with differentiated cells for differentiation | 1-10 |
| Diffusion in the system | |
| amount of inhibitor released by pluripotent cells | undetermined |
| amount of inductive chemical released by differentiated cells | undetermined |
| Induction chemical threshold for differentiation | undetermined |
| maximum number of divisions per cell | 1-3 |

\*With regards to examining diffusion, I’ll be looking at the relationship between the parameters rather than actual quantitative values.