Composite Cell Agent Model of Epithelial Culture In Vitro

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Abstract

An advantage of the synthetic, agent-oriented modeling approach is the ease with which model components can be refined or replaced with additional mechanisms and details. Here we present an agent-based model of epithelial cell culture, and describe revisions made to include subcellular details. Specifically, we developed new components inside cell agents, thereby evolving atomic cell agents into composite counterparts. The new components mapped abstractly to subcellular features involved in cell surface differentiation, which replaced agent rules governing simulated cell polarization and depolarization. Cross-model validation results under normal conditions confirmed that the revisions did not measurably alter model phenotype, specifically the simulated growth dynamics and gross morphology. However, cross-model validation results uncovered discrepancies for dysregulated conditions where one or more cell agent actions (e.g., division and death) were blocked in a probabilistic manner. Their elimination required reengineering the composite. We expect the approach and methods developed will facilitate and accelerate knowledge discovery in biomedical research.

1. INTRODUCTION

Inductive and synthetic modeling methods are the two main approaches in biological modeling [1][2][3]. Traditional analytical, inductive models are mathematical equations that are solved or approximated computationally. Examples include statistical data models, various compartmental and metabolic network dynamic systems, and other related models that typically describe data. Synthetic models or analogues are different: they are extant (actually existing, observable) working mechanisms that are analogous to their biological counterparts. Like their referent, these synthetic systems are hierarchical, modular and spatiotemporally organized. They are grounded on object-oriented methods that include cellular automata, agent-based modeling, and discrete event simulation.

An important advantage of synthetic models is the ease with which model components can be composed or decomposed to represent different levels of organization and detail. That is accomplished by swapping an atomic component, which has no internal structure, with a composite object that exhibits the same behaviors within the model under the same conditions, or vice versa. Replacement can also occur at the intra-component level, for example by replacing parts of component mechanism with a more detailed logic based on more fine-grained, interacting components. Such capability makes it feasible to use models of the synthetic type to instantiate and validate plausible details of genotype-phenotype linkage in biology.

Previously, we presented discrete event, multi-agent models of Madin-Darby canine kidney (MDCK) cell culture under different growth conditions [4][5]. The models simulate in vitro cyst-like structure formation in the context of epithelial morphogenesis and malignant transformations. They are comprised of quasi-autonomous cell agents and passive objects that represent the extracellular cell culture composition within an experiment system supported and directed by other agents. Simple decision logic and axioms define cell agent actions. The cited cell agents are atomic and represent the lowest level of model resolution.

In this report we describe replacement of atomic cell agent logic with new, composite counterparts. The new components map abstractly to subcellular details involved in cell surface organization and differentiation. We subjected the revised model to a set of test scenarios using quantitative similarity measures for cross-model validation. Initial experiments under the standard condition uncovered no measurable behavior differences between the revised and original models. Subsequent, stronger validation used additional test scenarios in which cell agent actions were dysregulated. Results of experiments under these expanded conditions falsified the composite. Reengineering produced a composite that achieved validation targets under all conditions. Final experiment results confirmed that the reengi-

neered replacement did not measurably alter model behavior.

2. BIOLOGY

2.1. Epithelial Morphogenesis

Epithelial morphogenesis is a fundamental feature of the embryonic development and tissue repair and remodeling in mammals [6]. It involves dynamic and self-organizing processes that govern specification, proliferation, differentiation, and death of cells. Under elaborate spatio-temporal regulation, epithelial cells multiply and organize to provide protection from and regulate exchange with the external environment. Structural organization of the epithelial tissue is dependent on cellular and molecular processes that include cell migration, cell adhesion, reorganization of the cytoskeleton, and the specification of cell polarity.

2.2. MDCK Cell Culture

Relatively simple in vitro model systems, such as isolated tissue and cell cultures, are used to study aspects of epithelial morphogenesis at molecular and cellular levels [7]. Such studies provide insight into genotype-phenotype linkages and the influence of environment. In the case of in vitro MDCK cell cultures, considerable information has accumulated from studies of the effects of growth conditions and their perturbations on morphogenesis [6]. When grown in Matrigel, MDCK cells divide and develop into cyst-like structures, each comprising a monolayer of polarized cells that enclose a central lumen [8]. Lumen formation is facilitated by cell death or apoptosis. MDCK cells grown in collagen develop structures that have similar shapes, but mechanism features are different.

3. MODEL

3.1. Conceptual Model

Our conceptual model includes three components: matrix, free space, and epithelial cell. Matrix represents culture stratum as well as extracellular matrix elements secreted by epithelial cells. Free space (or luminal space) is devoid of both matrix and epithelial cell. Epithelial cells are the only active constituents and are considered to behave as if they are following innate mandates that can be represented by rules. The observed repertoire of cell behaviors is hypothesized to be a consequence of stimulus-response mappings between the inputs and outputs of an operational interface that mediates interaction between a

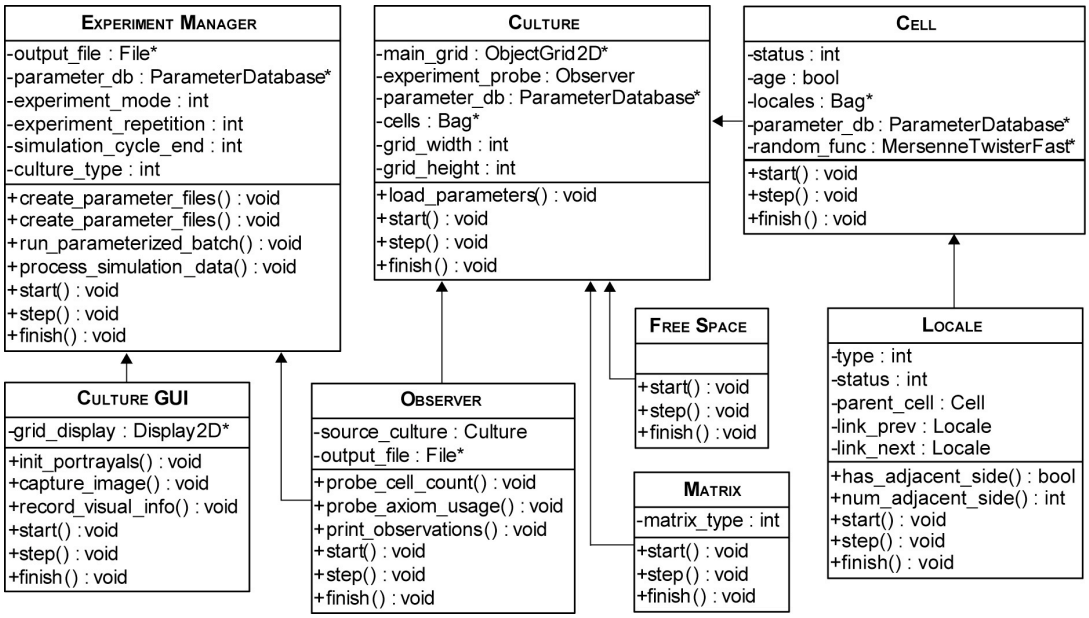

Figure 1. Uniform Modeling Language diagram of the system's architecture. The arrows indicate directed, container-contained relations between classes. EXPERIMENT MANAGER is the main control unit. It prepares parameter files, manages experiment execution, and processes output data for analysis and summary. OBSERVER measures CULTURE attributes including CELL numbers and CELL axiom usage. CULTURE is the simulation proper and represents a cell culture as a whole. It maintains a hexagonal, toroidal grid in which CELLS, MATRIX, and FREE SPACE objects are instantiated. Those three object types correspond to, respectively, cells, extracellular matrix, and the region (e.g., cyst lumen) devoid of cell and extracellular matrix. CELLS are active agents with a set of axioms and action logic. Included in the composite cell agent model are LOCALES that abstractly map to subcellular details involved in cell surface differentiation. CULTURE GUI provides a graphical interface to visualize and probe CULTURE during simulation. The system was implemented and simulated using MASON which provides standard libraries, object classes and methods (*).

Table 1. CELL agent axioms

Axiom	Precondition			Action
	CELL	MATRIX	LUMEN	
1	≥1	0	0	DIE
2	0	≥1	0	DIVIDE
3	0	0	≥1	DIE
4	1*	0	≥1	Produce MATRIX
5	≥2	0	≥1	DIE
6	≥1	≥1	0	DIVIDE
7	0	≥1	≥2	DIVIDE
8	≥1	≥1	≥2*	DIVIDE
9	2**	≥1**	≥1**	POLARIZE
10	≥0†	≥0†	≥0†	DEPOLARIZE
11	≥1‡	≥1‡	≥1‡	Do nothing

*adjacent; **polarizing condition [4]; †depolarizing condition [4]; ‡for all other configurations of three object types

cell and its external environment. Hierarchical biological knowledge of the intracellular subsystems is limited, thus we assume that the operational interface is not distinguishable from the cell itself using current techniques. The model components and mechanisms, and interactions thereof, are hypotheses of in vitro counterparts, with an understanding that molecular and biophysical details, as well as other sub-cellular information, conflate into the cell-level mechanisms and events. The model's usefulness is judged in part by how well it mimics the in vitro attributes targeted [3].

3.2. Atomic Cell Agent Model

We constructed MDCK cell-mimetic analogues and supporting modules using agent-based modeling and discrete event simulation design principles [9][10]. We defined agents as quasi-autonomous, decision-making components that follow sets of rules that govern their actions and interactions with other system components and environment. In this context, an agent has its own agenda and can schedule its own actions.

Hereafter, to clearly distinguish in silico components and processes from corresponding wet-lab structures and processes, we use SMALL CAPS when referring to the former. Figure 1 depicts the system's overall architecture and component methods. EXPERIMENT MANAGER is the top-level agent module that manages experiment execution and data processing. It instantiates and communicates with OBSERVER, which conducts and records measurements during simulation. OBSERVER has access to CULTURE, its grid space, and CELLS.

The core model comprises four component types: MATRIX, FREE SPACE, CELL, and CULTURE. MATRIX and FREE SPACE are inactive objects. CELLS are active, atomic agents with a set of rules that govern their actions. Cellular processes of interest, such as division and death, are represented as discrete events. CULTURE is the simulation proper and represents the in vitro system as a whole. It maintains a 2D

Algorithm 1. LOCALE A decision logic

```
N = {free space neighbors}
N# = N.Size
IF ParentCell is polarized THEN
  IF 0 < N# < 4 THEN
    FOR each neighbor F in N
      IF Locale_B.HasAdjacentSide(F) THEN
        Status = depolarize
      END IF
    END FOR
  ELSE
    Status = depolarize
  END IF
ELSE
  IF 0 < N# < 4 THEN
    IF N[i].Adjacent(N[(i+1)%N#]) FOR i=0…N# THEN
      Status = polarize
    END IF
  END IF
END IF
```

Algorithm 2. LOCALE B decision logic

```
N = {matrix neighbors}
N# = N.Size
IF ParentCell is polarized THEN
  IF 0 < M# < 4 THEN
    FOR each neighbor M in N
      IF Locale_A.HasAdjacentSide(M) THEN
        Status = depolarize
      END IF
    END FOR
  ELSE
    Status = depolarize
  END IF
ELSE
  IF 0 < N# < 4 THEN
    IF N[i].Adjacent(N[(i+1)%N#]) FOR i=0…N# THEN
      Status = polarize
    END IF
  END IF
END IF
```

Algorithm 3. LOCALE C decision logic

```
N = {cell neighbors}
N# = N.Size
IF ParentCell is polarized THEN
  IF N# == 0 THEN
    Status = depolarize
  ELSE
    Counter = 0
    FOR each neighbor C in N
      IF Locale_B.NumAdjacentSide(C) == 1 THEN
        Counter++
      ELSE IF Locale_B.NumAdjacentSide(C) > 1 THEN
        Status = depolarize
      END IF
    END FOR
    IF NOT Counter == 2 THEN
      Status = depolarize
    END IF
  END IF
ELSE
  IF N# == 2 THEN
    IF NOT N[0].Adjacent(N[1]) THEN
      Status = polarize
    END IF
  END IF
END IF
```

hexagonal grid (CULTURE space), which is populated by MATRIX, FREE SPACE, and CELLS. The grid, and hence the CULTURE, represents an observable 2D cross-sectional *xy*-plane of an in vitro culture. CULTURE GUI provides visualization and user interaction during simulation.

We defined CELL decision logic heuristically using a set of rules—axioms—that reflect observed behaviors in cultures. The term 'axiom' emphasizes that computer programs are mathematical, formal systems, and the initial mechanistic premises within simulations are analogous to axioms in formal systems. For this work, an axiom is an assumption about what conclusion can be drawn from what precondition for the purposes of further analysis or deduction. Within a simulation, every CELL is provided with the same set of axioms (Table 1), and carries out exactly one action during each simulation cycle.

Time advances in discrete steps. Its course unit is simulation cycle, during which everything in the simulation has one opportunity to update. For model-to-referent comparison, one simulation cycle maps to ~12 hours in vitro [4], however the model is relationally grounded so the mapping does not affect model dynamics and outcome [3]. Within a simulation cycle, each CELL in pseudo-random order is given an opportunity to interact with adjacent objects in its environment. Having objects update pseudo-randomly simulates the parallel operation of cells in culture, and helps avoid artifacts that may result from nonrandom, serial event scheduling and execution. It also makes execution outcomes nondeterministic (vs. deterministic, singular) while building in a degree of uncertainty, a fundamental characteristic of living systems.

3.3. Composite Cell Agent Model

We replaced Axioms 9 and 10 governing CELL POLARIZATION with new, interacting components called LOCALES. LOCALES are atomic agents that abstractly map to subcellular details pertaining to cell surface differentiation. The revised model uses three LOCALE types: LOCALE A for apical region, LOCALE B for basal region, and LOCALE C for intercellular surface region. Each LOCALE uses different decision logic to update its status, which specifies a CELL action. Algorithms 1-3 outline the final, validated LOCALE decision logic.

The three LOCALE types are responsible for determining if the current neighbor configuration meets the POLARIZATION or DEPOLARIZATION condition. LOCALE A checks if the neighboring FREE SPACE objects are arranged in a POLARIZING or DEPOLARIZING configuration. LOCALES B and C perform similar checks for MATRIX and CELL neighbors respectively. LOCALES A and B identify configuration sets that are mirror images to each other, thus have similar logic. Each LOCALE has only local information about its own type; for example, LOCALE A tracks FREE SPACE neighbors only. LOCALES interact with one another through method calls. In current implementation LOCALE interactions are limited to checking the number of neighbors that are adjacent to the object queried.

A cell POLARIZES or DEPOLARIZES based on the current status of its three LOCALES. LOCALE status indicates one of the following three actions: POLARIZE, DEPOLARIZE, and do nothing. A CELL POLARIZES if all three LOCALES prescribe POLARIZATION. Once POLARIZED, the CELL DEPOLARIZES if any of its LOCALES indicates a DEPOLARIZING condition. Specifically, Axioms 9 and 10 were replaced with the following logic statements:

Axiom 9:

```
IF Locale_A::polarize AND Locale_B::polarize
  AND Locale_C::polarize
THEN
  Polarize
END IF
```

Axiom 10:

```
IF Locale_A::depolarize OR Locale_B::depolarize
  OR Locale_C::depolarize
THEN
  Depolarize
END IF
```

At the start of simulation, each CELL instantiates and maintains the three LOCALE types. LOCALE steps execute in a pseudo-random order independent of the parent CELL'S step events, but at the same frequency (i.e., one LOCALE step per simulation cycle).

4. EXPERIMENTS AND RESULTS

We conducted simulation experiments to validate the composite cell agent model against the original analogue. Quantitative validation metrics used include: CELL population, morphology index, and normalized frequencies of specific CELL actions. We used the morphology index from [5], which quantifies growth morphology based on local EXTRACELLULAR arrangement, structural continuity, and the structure's overall shape. Aberrant morphologies have higher index values.

We executed 100 Monte Carlo (MC) simulations per parameter set. Each MC run lasted 50 simulation cycles corresponding to ~100 days in vitro. CULTURE grid height and width were set to 100 units each. One grid unit (CELL width) maps to ~10 μm. At the start of simulation, CULTURE grid was initialized and filled with MATRIX objects. Next, a single CELL was instantiated in the middle, replacing an existing MATRIX object. We used pseudo-random number (PRN) generator seeds, set to system time, to randomize simulation events. CELLS were selected randomly (one at a time without replacement) and stepped through their actions each simulation cycle. Thus, within a cycle, a CELL that has the rules applied earlier can influence the de-

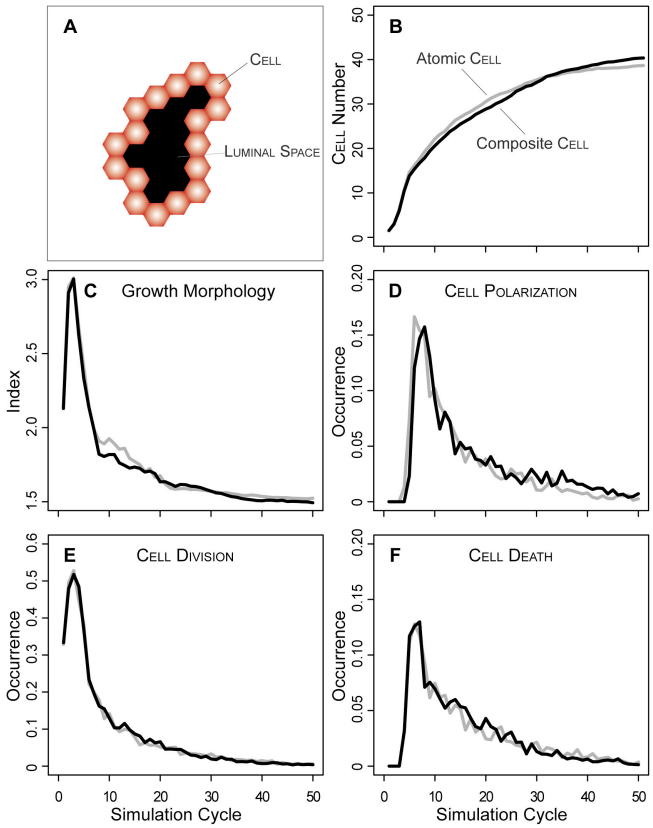

Figure 2. Simulated CYST growth and CELL activity patterns of the atomic and composite CELL analogues. **(A)** Shown is a sample structure from the atomic CELL analogue; the composite analogue developed similar structures. Both analogues produced stable, cystic structures enclosing LUMINAL SPACE; all CELLS were POLARIZED (red). **(B-F)** For cross-model validation we quantitatively tracked CELL population growth (B), morphological changes (C), and CELL activities (D-F). The morphology index quantifies CULTURE morphology based on EXTRACELLULAR configurations, structural continuity, and overall shape [5]. Higher values indicate aberrant structures. CELL POLARIZATION, DIVISION, and DEATH event frequencies were normalized to total CELL number. The measurements represent means of 100 Monte Carlo simulations.

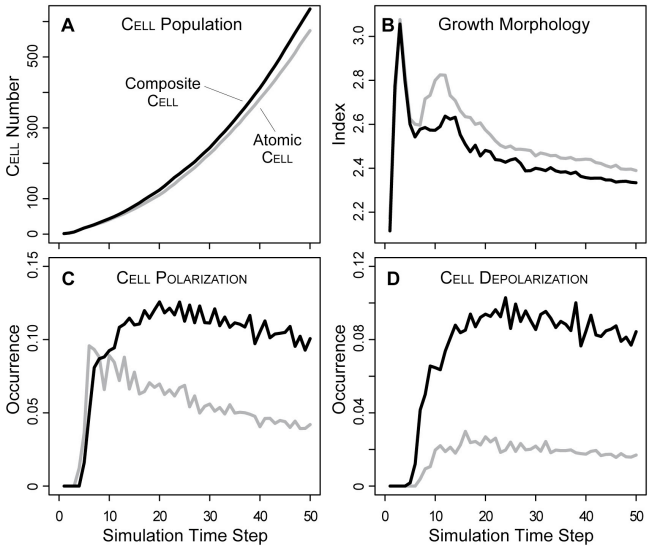

Figure 3. Growth differences observed between the atomic and composite CELL analogues under a dysregulated condition. Axiom 6 (CELL DIVISION) operation was blocked completely: when the axiom's precondition is met, do nothing. **(A-B)** Axiom dysregulation led to increased CELL population growth (A) and aberrant morphologies (B). **(C-D)** Shown are CELL POLARIZATION (C) and DEPOLARIZATION (D) frequencies normalized to total CELL number. The measurements represent means of 100 Monte Carlo simulations.

cision options available to its neighbor that has the rules applied later in the cycle. After the simulation, the recorded measurements were written to files, and the CULTURE was destroyed. A new CULTURE was created for each repetition.

We initially studied the revised analogue behaviors under the standard condition, in which all CELL axioms and mechanisms operated normally (i.e., executed to completion when applied). Figure 2 summarizes simulation outcomes under the standard condition. The atomic and composite cell agent models exhibited virtually indistinguishable growth dynamics. Quantitatively both showed similar CELL population growth (Fig. 2B), morphology changes (Fig. 2C), and CELL DIVISION and DEATH frequencies (Fig. 2E,F). Importantly, CELL POLARIZATION frequencies were almost identical (Fig. 2D). DEPOLARIZATION rarely occurred under the standard condition so we did not use DEPOLARIZATION frequencies for initial validation.

During validation we noted that the analogues produced limited CELL activity patterns under the standard condition. In particular, as noted above, CELL DEPOLARIZATION occurred rarely during simulated growth, thereby precluding proper evaluation of LOCALE'S DEPOLARIZATION logic. For stronger validation we subjected the analogues to dysregulated conditions that would produce higher POLARIZATION and DEPOLARIZATION occurrences. As detailed in [5], we did so by selectively disrupting operation of Axiom 5 or 6.

Specifically, we varied associated parameter values, which controlled the probability of the decision-making CELL electing to follow the axiom when its precondition applied. Parameter values ranged from 0 to 1 inclusively. A parameter value $p = 0.5$ corresponded to 50% adherence. Setting it to zero completely blocked the prescribed action. At each decision point, a PRN was drawn from the standard uniform distribution. The axiom's prescribed action was followed only when the PRN was $\leq$ the probability threshold set by its parameter.

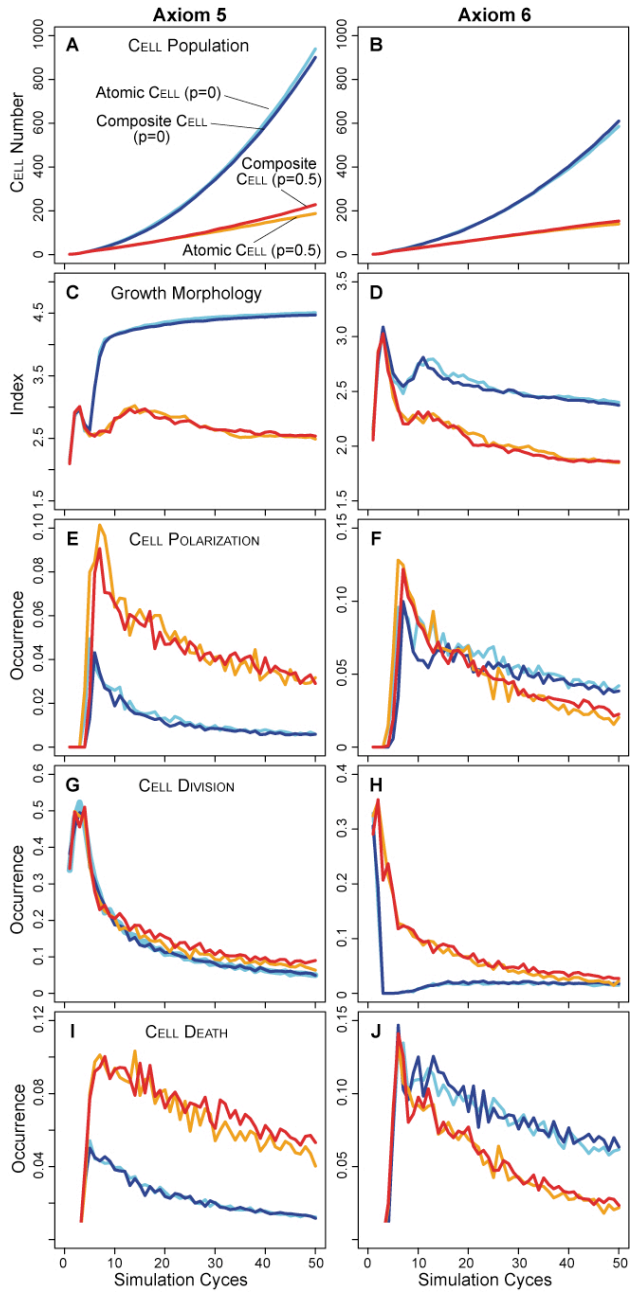

Figure 4. CULTURE growth when Axiom 5 or 6 was dysregulated. Axioms 5 and 6 dictate CELL DEATH and DIVISION respectively. CELLS follow each axiom with a parameter-controlled probability p. Left panels: dysregulated Axiom 5; right panels: dysregulated Axiom 6. **(A-D)** Dysregulation led to aggressive CELL population growth (A,B) and more aberrant morphologies (C,D). **(E-J)** Shown are CELL event frequencies for POLARIZATION (E,F), DIVISION (G,H), and DEATH (I,J) normalized to total CELL number. Values are means of 100 Monte Carlo runs.

Figure 3 shows results when Axiom 6 was dysregulated completely (p = 0). As shown, axiom dysregulation led to varied CELL activity and growth patterns that exposed measurable differences between the atomic and composite CELL analogues. Notably large differences were observed in CELL POLARIZATION and DEPOLARIZATION occurrences (Fig. 3C,D). Similar but smaller differences were noted when Axiom 5 was dysregulated (not shown). Collectively the results falsified the first composite analogue (Fig. 2). From additional testing and analysis of model phenotype and implementation, we identified discrepancies in the DEPOLARIZATION logic between the two analogues, and corrected them during subsequent iterative revision.

We achieved validation with the revised DEPOLARIZATION logic (Algorithms 1-3). Analogue phenotypes, after the correction, under the standard condition were similar to those shown in Figure 2. Figure 4 shows simulation results from dysregulated conditions. For the parameter settings tested (p = 0 or 0.5), the atomic and composite cell agent models exhibited similarly excessive CELL population growth and aberrant morphologies (Fig. 4A-D). Also similar were CELL activity (Fig. 4E-J) and individual axiom usage patterns (not shown). For both models, dysregulating Axiom 5 (vs Axiom 6) had more severe effects on growth phenotype. Severity differences between complete (p = 0) and partial (p = 0.5) dysregulation were also evident for both analogues.

5. DISCUSSION

An advantage of the synthetic modeling approach is the ease with which additional mechanisms and details can be added. From an engineering perspective, doing so is a relatively easy task. It can be achieved by swapping a current component for composite agents, or by replacing agent rules with a more detailed logic. A challenging task is to insure cross-model validation between different analogue variants. That requires defining appropriate similarity metrics for validation, and a set of test scenarios to cover increasingly wider, varied regions of the analogue's behavior space, its phenotype.

Starting with cell level analogues of an MDCK cell culture, we revised the CELL logic to include SUBCELLULAR mechanisms with new, interacting components. Our aim was to transform atomic cell agents into composite counterparts with an additional level of organization and detail, while maintaining the original analogue phenotype. As described in Results, we initially conducted cross-model validation under the standard condition, which failed to uncover phenotype differences between the two models. Proper falsification and identification of the discrepancies became feasible once we expanded test conditions to cover dysregulated agent behavior. The findings impart an important point: to achieve more trustable cross-model validation evidence, one must explore more of phenotype of both models.

Our study represents an early example of replacing an atomic agent in a biomimetic model with a more fine-grained composite counterpart. A related example is a leukocyte activation modeling study [11], which involved increasing the spatial granularity of an agent-based, molecular level model and adding new components to simulate interactive dynamics of the leukocyte-surface interface. Both studies followed the iterative refinement protocol outlined in [12]. Following the protocol helped avoid compromising already validated model features and behaviors while adhering tightly to a strong parsimony guideline, which is important when building agent-oriented analogues that are expected to become increasingly complex.

Having achieved validation, we can proceed to further elaborate the model and replace other CELL axioms with more detailed logic based on composite counterparts. Guided by additional data, the analogues can easily accommodate new or more detailed system characteristics. A continuation of the process is a scientific process to iteratively "drill down" and generate increasingly realistic, more finely grained analogues that are scientifically useful. We anticipate that the approach and analogues developed will help accelerate acquisition and application of new biological knowledge.

6. IMPLEMENTATION TOOLS

The model framework was implemented using MASON v11. MASON (http://cs.gmu.edu/~eclab/projects/mason) is a discrete event, multi-agent simulation library coded in Java. Batch simulation experiments were performed on a small-scale server equipped with three Intel Xeon 2.33-GHz CPU and 8-GB SDRAM memory. For model development, testing, and analysis, we used personal computers. We used R 2.7 (http://www.r-project.org) for data analysis and graph production.

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