Agent-Based Modeling of Alveolar Type II Cyst Formation In Vitro

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Abstract

We present an agent-based model of cultured alveolar type II (AT II) cells and early simulation results that provide new mechanistic insights into generative principles that may underpin alveolar morphogenesis in 3D cell cultures. The AT II cell-mimetic analogue comprises discrete components that represent cells and basic parts composing cell cultures. Cells are represented as quasi-autonomous agents that act based on a set of operating principles articulated as axioms and decision logic. By adhering strictly to those principles, cell agents self-organize and develop into multi-cell structures that resemble alveolar-like cysts in vitro. While it is premature to assign a specific biological mapping to the in silico operating principles, the findings enforce the idea that complex morphogenetic phenomena are a consequence of adherence to a small set of epigenetic principles. We expect more advanced adaptations will help elucidate basic principles and mechanisms underlying alveolar development and regeneration.

1. INTRODUCTION

Alveolar morphogenesis is a fundamental feature of mammalian lung development and repair [1]. It comprises various processes by which terminal airway cells self-organize into primary functional units of the lung. An essential, yet poorly understood, aspect of alveolarization concerns how the various cell processes and actions give rise to stereotypical alveolar cyst formations. What are the basic principles of cell operation that govern alveolar growth? How do they come about? Can the principles of morphogenesis be translated into therapeutic strategies for lung injury or disease? Here we introduce an agent-based, discrete event simulation model and supporting methods to probe possible answers. Albeit simplistic, the model can reproduce basic developmental attributes and offers a logic-based, cell-level explanation of alveolar growth in vitro.

Pulmonary alveoli consist of type I and II cells [2]. Alveolar type I cells are flat and provide about 95% of alveolar surface for respiration. Alveolar type II (AT II) cells are approximately cuboidal and cover the remaining surface. While they provide little respiratory surface, AT II cells are essential for alveolar function, homeostasis, and regeneration [3], so are one of the most studied cell types in lung and alveolar morphogenesis research.

Most ex vivo studies of alveolar development have relied on two-dimensional (2D) culture methods, even though they are incapable of emulating the physiological three-dimensional (3D) environment. To date, no methods have been developed to isolate and grow whole alveoli ex vivo, but different 3D methods have been applied successfully to culture and grow AT II cells into alveolar-like organoids [4][5]. Placed in 3D Matrigel, the cells form alveolar-like cysts (ALCs) and secrete surfactant into the cyst lumen similar to the in vivo condition. ALCs grow exclusively by cell aggregation and subsequent hollowing (cell-cell separation). Apparently, ALC growth requires cellular mechanisms distinct from those engaged in cyst development of other epithelial cell types [6]. That finding suggests that their development might involve cell operations that are somewhat different from those identified previously [7]. A major goal of this study is to begin elucidating operating principles that drive AT II cyst growth ex vivo.

Object-oriented, discrete simulation modeling methods like cellular automata [8], cellular Potts models [9], and agent-based models (ABMs) [10] have gained popularity in studies that directly address cell-level mechanics of animal development. One example is a multi-scale ABM that integrates intracellular signaling simulation with agent-based cell representation to simulate mesoendoderm migration of *Xenopus* explant ex vivo [11]. Another addresses mesenchymal cell behavior and molecular dynamics of in vitro precartilage cell condensation [12]. Bodenstein et al. [13] developed a multi-cell chick epiblast model to explain primitive streak formation, a critical event in early embryogenesis. In the area of in vitro epithelial morphogenesis, Galle et al. [14] reported an ABM simulating epithelial cell colony growth, with an explicit representation of physicochemical determinants of cell survival and proliferation.

Another study incorporated a mathematical model of growth factor diffusion and signaling into an established 2D ABM to examine proliferative behavior of cultured epithelial cells at different cell densities and factor concentrations [15]. Notably, Grant et al. [7] developed a MDCK epithelial cell mimetic analogue, which provided useful insight into cell-level mechanisms that account for multiple phenotypes in four different culture conditions.

Here, we adapt, refine, and advance current ABM methods, especially those articulated by Grant et al. [7], to develop and begin validating analogues of alveolar-like cyst (ALC) growth in 3D cell cultures. The model is a multi-agent system composed of quasi-autonomous cell agents and passive objects that represent the extracellular cell culture composition within an experiment system supported by other agents. Internal cell state and neighboring object configuration determine simulated cell action. Simple decision logic and cell agent axioms define the preconditions and corresponding cell actions. Following a simple rule set, cell agents self-organize into stable ALCs and mimic key growth characteristics of cultured AT II cells.

2. BIOLOGY

2.1. Pulmonary Alveolus

Pulmonary alveoli (~250 μm in diameter; human) have an elaborate anatomy consisting of an epithelium, basement membrane, and surrounding capillary network [2]. The alveolar epithelium encloses a hollow air space, called lumen. Three cell types compose the epithelium: type I and II pneumocytes, and macrophages. AT I cells provide thin, flat surface through which oxygen diffusion occurs. AT II cells have multiple functions essential to alveolar homeostasis. The alveolar basement membrane and associated extracellular matrix (ECM) surround and anchor the epithelium to interstitial connective tissue. Densely networked capillaries cover the alveoli to form a blood-air barrier, which prevents blood from entering alveoli while permitting oxygen flow.

2.2. Alveolar Type II Cells

AT II cells are considered defenders of the alveolus [3]. Their primary function is to produce and secrete pulmonary surfactant along the inner lining of the alveolus. Surfactant, composed of lipids and proteins, helps maintain proper surface tension for alveolar stability and prevent alveolar collapse during ventilation. It also plays important roles in fluid balance and host defense. AT II cells act as progenitors of type I and II cells during normal cell turnover and alveolar regeneration. They have almost unlimited potential to proliferate but whether all AT II cells or only subpopulations act as the alveolar stem cells is debated.

When grown ex vivo, the cells lose their in vivo characteristics and acquire type I morphology and phenotype [16]. In 2D environments, they become flat and spread out, and lose type II-specific biochemical markers. They typically form a cell monolayer but can form 3D organoids under certain conditions [17]. They are better able to maintain their type II phenotype in 3D cell cultures. When cultured in a 3D environment, they develop round ALCs and secrete surfactant into the cyst lumen [5].

3. MODEL

3.1. Conceptual Model

We first catalogued basic AT II growth attributes in 3D matrix, and then narrowed the list into an initial set of target attributes for model development and validation. Subcellular molecular details were presumed to conflate into cell level events and processes. Doing so allowed us to define attributes that map directly to experimental in vitro observations and measurements. Next, we conceptually discretized cultures so that only those components essential in achieving the targeted attributes were represented. To that end, cultures were represented as having five components: cells, clusters, ECM (matrix hereafter), ECM-free region (free or luminal space hereafter), and a space to contain them. Hereafter, we use small caps when describing model components and processes to distinguish them clearly from their cell culture counterparts. CLUSTER represents a coherent aggregate of cells. MATRIX represents either culture medium or ECM. FREE SPACE represents regions devoid of both ECM elements and cells. CULTURE is a simple placeholder for the composing components.

3.2. Agent-Based Model

We constructed AT II cell-mimetic analogues and supporting modules using ABM and discrete event simulation (DES) design principles. Figure 1 depicts the system's 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; it conducts and records measurements during simulation. OBSERVER has access to CULTURE, its grid spaces, and CELLS.

The core model comprises five component types: MATRIX, FREE SPACE, CELL, CLUSTER, and CULTURE. MATRIX and FREE SPACE are inactive objects. CELLS are active agents with a set of rules that govern their actions. Cellular processes of interest, such as migration and adhesion, are represented as discrete events. CLUSTERS are simple representations of aggregated, adhered cells, and their actions are governed by their own logic. CULTURE is the simulation proper and represents the in vitro system as a whole. It maintains a 2D 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.

Time advances in discrete steps. Its course unit is simulation cycle, during which everything in the simulation has one opportunity to update. 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. It also makes execution outcomes nondeterministic while building in a degree of uncertainty, a fundamental characteristic of living systems.

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 our study, 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 decision logic (Algorithm 1) and axioms (Table 1), and carries out exactly one action during each simulation cycle.

CELL action is dictated by internal CELL state and neighboring object composition. When stepped, a CELL first determines its current neighboring object composition and arrangement. If it has no CELL neighbors, it becomes or remains single and migrates to a neighboring non-CELL location. A CELL can migrate randomly, CHEMOTACTICALLY, or along a CELL density gradient as specified in simulation control. Random CELL migration implements a simple, unbiased random walk. In CHEMOTACTIC mode, a CELL is capable of sensing ATTRACTANT concentration in its immediate local environment, and then moving towards the most concentrated region. The CELL density-based mechanism enables CELLS to determine local CELL density within a defined radius and actively move along the density gradient. When one or more CELL neighbors are in contact, the decision-making CELL attaches to each neighbor with a parametrically controlled probability, p, to form a cluster. We used a Mersenne Twister pseudo-random number generator (PNG) to draw p values from a uniform distribution, $U(0, 1)$. We defined two probability thresholds for CELL-CELL attachment. One controlled the attachment probability of single, non-clustered CELLS; the other controlled attachment probability of clustered CELLS.

Following attachment, CELLS switch to clustered state. Clustered CELLS follow the axioms in Table 1 to rearrange their relative positions within the parent CLUSTER, a process that is essential for ALC formation. Axioms define preconditions and corresponding CELL actions. The preconditions are based on neighboring object types and their specific configurations. We defined rearrangement actions based on experimental insights from the literature and expert opinion. For cases that lacked relevant biological information, we relied on basic assumptions about epithelial cell behavior.

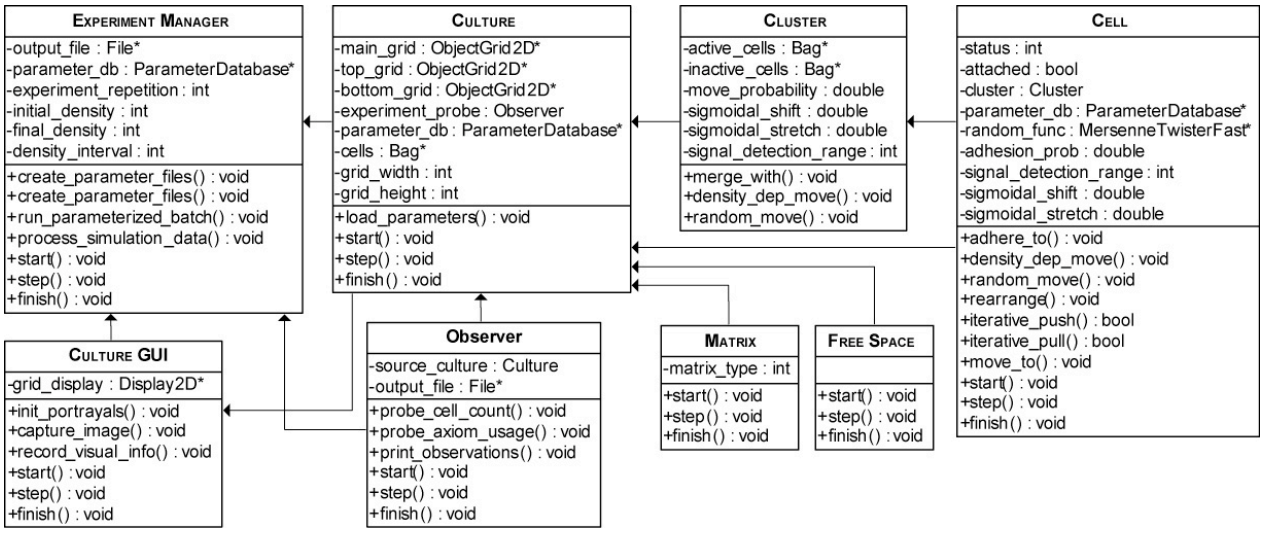

Figure 1. Uniform Modeling Language diagram of the system's architecture. EXPERIMENT MANAGER is the main control unit. It prepares parameter files, manages experiment execution, and processes output data for analysis and summary. OBSERVER measures CULTURE attributes including CLUSTER sizes and CELL axiom usage. CULTURE is the simulation proper and represents a cell culture as a whole. It maintains hexagonal grids 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. 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 (*).

Algorithm 1. CELL agent decision logic

```
IF this CELL is active on CULTURE grid THEN
    N = {neighboring objects}
    IF N has CELL object type THEN
        FOR each CELL neighbor C in N
            p = MersenneTwister(0,1)
            IF p < threshold THEN
                Attach to C
            END IF
        END FOR
    END IF
    IF not attached to any CELL neighbor THEN Migrate
    ELSE Rearrange
    END IF
END IF
```

For example, cells act to establish and maintain a preferred local environment. Another is their mandate to achieve three surface types: apical (lumen), basal (matrix), and lateral (cell contact) [6].

CLUSTER represents cells aggregated coherently and simulates simple features of their collective behavior. In vitro, multi-cell aggregates tend to migrate as a coherent body. That collective movement is driven by inherent, coordinated, individual cell actions, but to simplify implementation we directed CLUSTER to handle collective migration. When stepped, a CLUSTER moves with a defined probability if its overall shape has been altered by the rearranging cell actions. It also can adopt one of three migration modes: random, CHEMOTACTIC, and CELL density-based. CLUSTER movement maintains overall shape and relative positions of the composing CELLS. CLUSTER events are managed by the master event schedule; their execution ordering is pseudo-random and independent of CELL events.

4. EXPERIMENTS AND RESULTS

We conducted exploratory simulation experiments to study ALC growth at varying CELL densities. We varied the initial CELL population from 100 to 6,000 in increments of 100. CULTURE grid height and width were set to 100 units each. All three CELL migration modes were used at each CELL density. We executed 100 Monte Carlo (MC) simulations per CELL density and migration mode. Each MC run lasted 100 simulation cycles corresponding to ~147 hours in vitro. Model parameters used are provided in Table 2. CULTURE grid was initialized and filled with MATRIX objects. Next, CELLS were instantiated and distributed randomly on the grid by EXPERIMENT MANAGER, replacing existing MATRIX objects as needed. We used PNG seeds, set to system time, to randomize simulation events. CELLS and CLUSTERS were selected at random (one at a time without replacement) and carried through their actions each simulation cycle.

Figure 2 summarizes simulation outcomes in comparison with referent measures. CELLS formed multi-CELL structures that resemble ALCs (Fig. 2A, B) by adhering strictly to their axiomatic principles of operation. CELL migration and aggregation enabled formation of multiple CLUSTERS. CLUSTERS used CELL rearrangement to develop into LUMINAL SPACE-enclosing CYSTS. LUMINAL SPACES were devoid of CELLS and MATRIX, and were enclosed by a continuous CELL monolayer. Generally, ALCS maintained convex polygonal shapes, which map to a roundish shape in continuous 2D cross-sections.

ALC sizes increased monotonically with initial CELL densities (Fig. 2C). In vitro, overall changes represent more

Table 1. CELL agent axioms for inter-CLUSTER rearrangement

Axiom	Precondition			Action
	CELL	MATRIX	FREE SPACE	
1a	≥1	0	0	Push out a CELL neighbor; move to the vacated location; leave behind a FREE SPACE object
1b	0	≥1	0	Do nothing
1c	0	0	≥1	Move to an adjacent FREE SPACE; leave behind a FREE SPACE object
2a	1 or 2*	≥1	0	Move to an adjacent MATRIX next to a CELL neighbor; leave behind a MATRIX object
2b	2**	≥1	0	Move to an adjacent MATRIX next to a CELL neighbor; pull the other CELL neighbor into the vacated location
2c	3	≥1	0	Do nothing
2d	4	≥1	0	Move to an adjacent MATRIX; leave behind a MATRIX object
2e	5	≥1	0	Move to an adjacent MATRIX; leave behind a FREE SPACE object
2f	≥1	0	≥1	Push out a CELL neighbor; move to the vacated location; leave behind a FREE SPACE object
2g	0	≥1	≥1	Do nothing
3a	≥1	≥1	1	Move to the adjacent MATRIX; leave behind a FREE SPACE object
3b	1†	≥1	≥1	Move to an adjacent FREE SPACE; pull the CELL neighbor into the vacated location
3c‡	≥1	≥1	≥1	Do nothing

*adjacent; **non-adjacent; †adjacent to a MATRIX neighbor; ‡for all other configurations of three object types

Table 2. Key model parameters and respective values

Parameter	Value(s)
Initial CELL population	100~6,000
Maximum CELL push/pull iteration	5
CELL speed in grid units	1
Single CELL attachment probability	0.2
Clustered CELL attachment probability	0.01
Local CELL density radius	5
Diffusion step multiples*	25
Diffusion rate	0.4
Evaporation rate	0.05
Maximum SOLUTE level	50,000
SOLUTE production per simulation cycle	3,000~8,000

* Diffusion algorithm iterations per simulation cycle

than a twofold increase in ALC diameter; changes in silico varied depending on CELL migration mode. At the lowest CELL density corresponding to 1 x 10^4 cells/cm^2, all three CELL migration modes produced small ALCS, mostly in the 20-to-40 µm diameter range, which was comparable to in vitro measurements (1 grid unit = 8.5 µm in vitro). CELLS migrating randomly failed to mimic the relatively steep rise in ALC diameter following an increase in the initial cell density. In contrast with random migration, CHEMOTAXIS and CELL density-based migration produced ALCS that were similar in size to their in vitro counterparts. Directionally migrating CELLS tended to aggregate and form multi-CELL structures earlier, compared to randomly migrating CELLS. The difference was most evident in sparsely populated CULTURES. Both directional migration mechanisms gave rise to convergent CELL movement that closely mimicked in vitro aggregation patterns (data not shown). Of the two, the CELL density-based migration led to simulation outcomes closest to the in vitro measures.

5. DISCUSSION

Epithelial cyst formation has been studied extensively using various cell lines under 3D culture conditions [6]. Commonly used lines include Madin-Darby canine kidney (MDCK) cells and MCF10A cells from human mammary gland. When cultured in 3D, both cell types develop spherical, lumen-enclosing cysts similar to those observed in AT II cultures. Unlike AT II cultures, proliferation and apoptosis play dominant roles in MDCK or MCF10A cyst development. Interestingly, MDCK cells can develop cysts by mechanisms similar to those of ALC growth under certain conditions, indicating that one of multiple mechanisms is in use depending on circumstances. Comparison of CELL decision logic between the AT II and MDCK model [7] reflects the observed differences in cyst growth mechanisms. Notwithstanding those differences, morphological similarities suggest that common principles of cell operation may underpin epithelial cyst development. One example is the apparent drive of cells to attain basal, lateral, and free surfaces as previously theorized [6]. Both models also suggest a common theme whereby every cell strives to maximize the number of adhesive contacts along its lateral surface. CELLS arranged in a CYST structure satisfy their mandates. We expect successful validation of extensions of 2D behaviors into 3 dimensions would justify the hypothesis of an analogous cell behavior in a 3D culture environment.

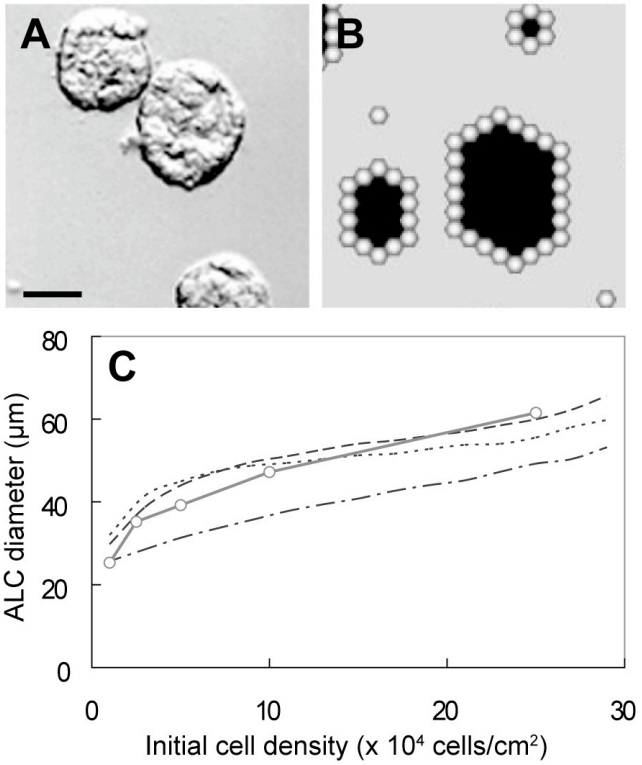

Figure 2. Alveolar-like cyst (ALC) growth in simulated and in vitro cell cultures. **(A)** Phase-contrast image after 4 days in 2% Matrigel [5]. Cells were plated initially at 25 x 10^4 cells/cm^2. ALCs had roundish shapes without obvious depressions or dimples. Bar, 50 µm. **(B)** CULTURE images after 100 simulation cycles; the initial CELL population was set to 5,000 CELLS. Like in vitro, the ALCS maintained convexity and had no dimples. Gray and black spaces represent MATRIX and FREE (or LUMINAL) SPACE, respectively. **(C)** Both in vitro and in silico, ALC diameter increased monotonically with the initial cell density. Circles: in vitro measurements (mean). We tested three different CELL migration mechanisms: random migration (dash-dot), CHEMOTAXIS (short dash), and CELL density-based migration (long dash). In the first, CELLS migrated randomly without directional bias or persistence. In the second, CELLS migrated along a diffusive ATTRACTANT gradient. In the third, CELLS migrated along a local CELL density gradient. In all three scenarios, CELLS developed normal ALCS. CELLS migrating along a CELL density gradient achieved outcomes most similar to the in vitro measures. Mean outcome of 100 Monte Carlo runs each lasting 100 simulation cycles.

In vitro, cell migration is the principal process by which AT II cells develop multi-cell clusters, and that is also the case for simulations. However, it is largely unknown what migration mechanism(s) are involved in vitro and how changes in migration patterns affect ALC development. To explore those issues we challenged our model using different cell migration methods and compared outcomes. First was random CELL motility implemented as a simple, random walk. That represents the simplest mechanism; it required no assumptions about molecular or physical cues that might guide AT II cell migration. Our simulation results suggest that random motility alone is not an effective means for cell aggregation.

We explored two directional migration modes, one driven by CELL density gradients and the other by CHEMOTAXIS, and found that both are adequate drivers of CLUSTER formation and ALC growth. The results also show that of the two, CHEMOTAXIS is somewhat less effective particularly in densely populated CULTURES. Visualization of the ATTRACTANT concentration and CELL movement confirmed that rapid fluctuations in local concentrations accompanied a loss of directionally persistent movement in densely populated regions. In contrast, the CELL density-based mechanism enabled CELLS to maintain persistent directionality under comparable conditions. However, we cannot exclude the possibility that the small difference may be due in part to the model's discretization and implementation details, which will be probed further in a future study. Also, the current model precludes simultaneous use of multiple migration mechanisms, but it is possible that AT II cells might employ multiple mechanisms and switch between those to direct and optimize their movement. Experimental findings of related cell types like MCF10A indicate that they can adopt counter modes of migration, random versus directionally persistent, which are regulated by a mechanism dependent on Rac1 protein activity [18]. It would be interesting to find that AT II cells have similar migration regulatory mechanisms along with extracellular cues that enable switching between them.

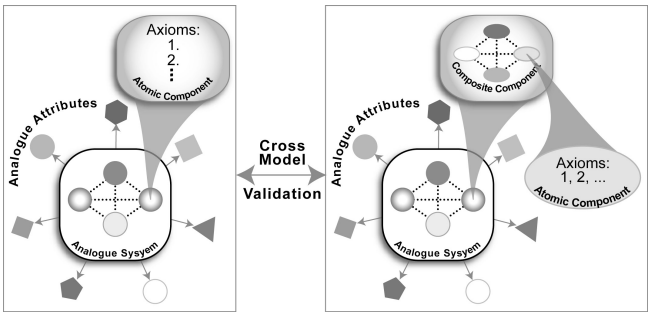

Figure 3. Model refinement and cross-model validation. CELLS are the main actors of the current AT II analogue. They are atomic components whose actions are governed by axioms. CELL actions and interactions cause phenotypic attributes. Because CELLS and other components are discrete objects, they can be replaced easily with new or revised objects. Replacements can be composite objects composed of other objects. Details at different scales can be incorporated into the analogue in like manner. When so doing, one must provide appropriate similarity metrics and demonstrate cross-model validation (original vs revised) to insure that the change does not alter analogue phenotype measurably.

Thus far, we have focused on basic AT II cell attributes in one specific culture type. When the conditions change, AT II cells develop morphologies and attributes that are different from those observed in the 3D cell culture. Inclusion of those attributes automatically invalidates the current analogue. An advantage of our approach is the ease with which additional attributes and details can be added during the iterative model refinement process. It is easy to develop the current analogue and its components further to reflect new biological information (e.g., cell positioning mechanisms) or contexts (e.g., traditional 2D surface cultures and overlay cultures). Having achieved the initial attributes, we can proceed to elaborate the model to include actual subcellular details that will map to cell locomotion pathways and intercellular signaling networks. From an engineering perspective, doing so is a relatively easy task. It can be achieved simply by swapping a current component (e.g., atomic CELL agent) for a more detailed composite agent (Fig. 3). The replacement could also occur at the intra-component level, for example by replacing CELL axioms with a detailed logic based on interacting components. A challenging task will be to insure cross-model validation between different analogue variants.

In summary, our model establishes a foundation for future studies aimed at articulating the generative principles of alveolar development. Guided by additional data, the model can easily accommodate new or more detailed system characteristics. We anticipate that in silico experimentation using the type of agent-based models presented herein will provide a fruitful new strategy to dissect the operational basis of epithelial morphogenesis.

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 Beowulf cluster system consisting of one master node and seven client nodes, each equipped with a single Intel Pentium 4 3.0-GHz CPU and a 2-GB SDRAM memory.

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REFERENCES

[1] Cardoso, W.V. 2001. "Molecular Regulation of Lung Development." *Annu Rev Physiol* 63:471-494.

[2] Crystal, R.G.; J.B. West; P.J. Barnes; W.R. Weibel. 1997. *The Lung: Scientific Foundations*. Lippincott Williams & Wilkins, New York, USA.

[3] Fehrenbach, H. 2001. "Alveolar Epithelial Type II Cell: Defender of the Alveolus Revisited." *Respir Res* 2:33-46.

[4] Shami, S.G; J.D. Aghajanian; R.L. Sanders. 1984. "Organotypic Culture of Fetal Lung Type II Alveolar Epithelial Cells: Applications to Pulmonary Toxicology." *Environ Health Perspect* 56:87-94.

[5] Yu, W.; X. Fang; A. Ewald; K. Wong; C.A. Hunt; Z. Werb; M.A. Matthay; K. Mostov. 2007. "Formation of Cysts by Alveolar Type II Cells in Three-dimensional Culture Reveals a Novel Mechanism for Epithelial Morphogenesis." *Mol Biol Cell* 18(5):1693-1700.

[6] O'Brien, L.E.; M.M. Zegers; K.E. Mostov. 2002. "Building Epithelial Architecture: Insights from Three-Dimensional Culture Models." *Nat Rev Mol Cell Biol* 3:531-537.

[7] Grant, M.R.; K.E. Mostov; T.D. Tlsty; C.A. Hunt. 2006. "Simulating Properties of In Vitro Epithelial Cell Morphogenesis." *PLoS Comput Biol* 2(10):e129.

[8] Deutsch, A.; S. Dormann. 2005. *Cellular automaton modeling of biological pattern formation: characterization, applications, and analysis*. Birkhäuser, Boston, USA.

[9] Merksa, R.M.; J.A. Glazier. 2005. "A Cell-Centered Approach to Developmental Biology." *Physica A* 352(1):113-130.

[10] Thorne, B.C.; A.M. Bailey; S.M. Peirce. 2007. "Combining Experiments with Multi-Cell Agent-Based Modeling to Study Biological Tissue Patterning." *Brief Bioinform 8*(4):245-257.

[11] Robertson, S.H.; C.K. Smith; A.L. Langhans; S.E. McLinden; M.A. Oberhardt, et al. 2007. "Multiscale Computational Analysis of Xenopus laevis Morphogenesis Reveals Key Insights of Systems-Level Behavior." *BMC Syst Biol* 1:46.

[12] Christley, S; M.S. Alber; S.A. Newman. 2007. "Patterns of Mesenchymal Condensation in a Multiscale, Discrete Stochastic Model." *PLoS Comput Biol* 3(4):e76.

[13] Bodenstein, L; C.D. Stern. 2005. "Formation of the Chick Primitive Streak as Studied in Computer Simulations." *J Theor Biol* 233(2):253-269.

[14] Galle, J.; M. Hoffmann; G. Aust. 2009. "From Single Cells to Tissue Architecture-A Bottom-up Approach to Modelling the Spatio-temporal Organisation of Complex Multi-Cellular Systems." *J Math Biol* 58:261-283.

[15] Walker, D; S. Wood; J. Southgate; M. Holcombe; R. Smallwood. 2006. "An Integrated Agent-Mathematical Model of the Effect of Intercellular Signalling via the Epidermal Growth Factor Receptor on Cell Proliferation." *J Theor Biol* 242:774-789.

[16] Dobbs, L.G. 1990. "Isolation and Culture of Alveolar Type II Cells." *Am J Physiol* 258:L134-L147.

[17] Shannon, J.M.; R.J. Mason; S.D. Jennings. 1987. "Functional Differentiation of Alveolar Type II Epithelial Cells In Vitro: Effects of Cell Shape, Cell-Matrix Interactions and Cell-Cell Interactions." *Biochim Biophys Acta* 931(2):143-156.

[18] Pankov, R.; Y. Endo; S. Even-Ram; M. Araki; K. Clark; E. Cukierman; K. Matsumoto; K.M. Yamada. 2005. "A Rac Switch Regulates Random versus Directionally Persistent Cell Migration." *Cell Biol* 170:793-802.