SYSTEMS MODELING OF ALVEOLAR MORPHOGENESIS IN VITRO

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Abstract

We present a pulmonary alveolar epithelial cell model and its simulation results aimed to provide mechanistic insight into biological principles that underlie alveolar morphogenesis in vitro. Primary human alveolar type II cells in 3D cultures have been shown to undergo processes of cell migration and aggregation to form hollow cystic structures that resemble human lung alveoli. Although various molecular factors that mediate alveolization have been identified, little is known about biological principles that drive the morphogenic processes. To help elucidate the operating principles governing the morphogenic behavior of pulmonary epithelial cells, we have developed an agent-based model of alveolar cells in culture. The model system comprises discrete components that represent individual cells and different elements of the extracellular environment. Individual cells are represented as independent agents that act based on a set of axioms (principles) that govern their interaction with the surroundings. Simulation of alveolar cell cultures confirms that the model is capable of generating phenotypic attributes similar to those of in vitro observations. Based on the results, we hypothesize that the axioms governing simulated cell behavior have in vitro counterparts. Further interrogation and refinement of the model is expected to help unravel the causal linkages between the operating principles of cell behavior and observed systemic phenotypes.

1 INTRODUCTION

Alveolar morphogenesis is a fundamental feature of mammalian lung development and repair. It comprises various processes by which unorganized cells morph into alveoli, the primary functional units of the lung [1]. What are the key principles that govern development of alveoli? Can the principles of morphogenesis be exploited to promote pulmonary recovery from an injury or disease? Our aim is to develop computational models of alveolar formation and appropriate in silico methods to better understand the operating principles and mechanisms of alveolar morphogenesis. Here we introduce a foundational model capable of producing phenotypic attributes similar to counterparts of alveoli formation in vitro, and discuss implications of the model and its simulation results.

Pulmonary alveoli are the primary site of gas exchange in the lung. They are spherical in shape with hollow lumen, and form the terminal buds of the respiratory tree [2]. Two types of alveolar epithelial cells compose human alveoli. Type I alveolar cells are squamous in shape and constitute most the alveolar surface to form a blood-gas barrier through which gas exchange occurs. Type II alveolar cells (AT II) are cuboidal with microvilli on their apical surface; they are responsible for surfactant secretion to maintain alveolar stability and function. Other types of cells such as alveolar macrophages also contribute to physiological functions of alveoli. Alveolar structure and function are compromised in major disorders including lung cancer, pneumonia, emphysema, and acute respiratory distress syndrome.

Little is understood about the underlying principles of alveoli development. Experimental studies have discovered various molecular factors that contribute to alveoli development and function, but the findings have not translated well into an articulate understanding of how these molecular parts and processes propagate into a multifaceted alveoli phenotype. Computational models are available, but most are limited to explaining the mechanics of alveolar respiratory function. Additional platforms are needed to elucidate principles of alveolar morphogenesis.

In this study, we present a systems model and its simulation results to provide insight into plausible principles of in vitro alveoli formation. We demonstrate that a set of abstract in silico principles is sufficient to represent selected aspects of alveoli formation. Development of a more mature model is expected to yield

potentially fruitful strategies for lung tissue engineering and therapies to treat pulmonary disorders.

2 METHODS

Traditional inductive models are usually built by first analyzing data, creating a mapping between the envisioned system structure and attributes of the data, and then representing those data attributes with mathematical equations. As such they can provide good prediction of referent behaviors described by the data, but may not be amenable to understanding actual mechanisms that drive the behaviors. Our synthetic method, in contrast, works forward by studying components and functions of the referent system, defining plausible mechanisms and hypotheses for system function, and then creating in silico counterparts of those components and mechanisms that can generate observables that match or mimic those of the referent system [3-5]. The envisioned product is a set of models that are more useful in predicting phenotypic outcomes in the diverse conditions that can occur in vivo and in vitro, and allow systematic exploration of varying hypotheses through experimentation along with model refinement and extension.

We adapted methodologies of agent-based modeling [6] and discrete event simulation [7] to build a systems model of AT II cell culture. We first abstracted biological entities and features into a simplified set of in silico system components at the whole cell level. We defined four types of in silico components: matrix, free space, simulated cells, and clusters. Matrix represents culture medium as well as extracellular matrix elements secreted by AT II cells. Free space represents regions devoid of both matrix elements and cells. Both are represented as inactive objects. Simulated cells are modeled as active agents with a set of rules that govern their actions in response to the condition of the external environment. Cluster objects are abstract representations of aggregated cells, and their movement is governed by a simple rule. Cellular processes of interest, such as migration and adhesion, are represented as discrete events as described below. The spatial surface is represented as a 2D hexagonal grid of toroidal topology. An unverified operational assumption is that the results of 2D simulations map to cross-sections in vitro. We developed our model using an agent-based modeling and simulation framework, MASON [8].

2.1 Specification of In Silico Cell Actions

We heuristically defined a set of agent rules that reflect observed AT II behavior in 3D culture. Unattached simulated cells migrate to adjacent matrix in a random direction at each simulation cycle. When two or more simulated cells are adjacent, they adhere and form a cluster. Once in a cluster, they follow a set of axioms listed in Table 1.

Table 1. Rules (axioms) governing the behavior of in silico cells aggregated in a cluster.

Precondition			Action
Cell	Matrix	Free Space	
●	○	○	Push out an adjacent cell* and migrate to its location†
○	●	○	Do nothing
○	○	●	Migrate to an adjacent free space*,†
●	●	○	If there is only one cell, then migrate to a matrix* adjacent to the cell†. If there are two cells adjacent to each other, then migrate to a matrix* adjacent to either of the two cells‡. If there are two cells not adjacent to each other, then migrate to a matrix* adjacent to only one of the two cells and pull the other cell into its original location. If there are four cells, then migrate to a matrix*,‡. If there are five cells, then migrate to a matrix*,†. Otherwise, do nothing
●	○	●	Push out an adjacent cell* and migrate to its location†
○	●	●	Migrate to an adjacent matrix*,‡
●	●	●	If there is only one matrix, then migrate to that matrix‡. If there are two matrix neighbors both adjacent to one cell, then migrate to a free space* and pull the cell into its previous location. Otherwise, do nothing

●, present; ○, absent.
* Randomly selected.
† Create and leave a new free space.
‡ Create and leave a new matrix.

The axioms for attached simulated cells specify preconditions and corresponding actions. The preconditions represent distinct combinations of adjacent object types. In silico cell actions are based on exploratory simulation results and experimental observations as well as expert opinion about epithelial cell behavior. One operating assumption has been that AT II cells desire to establish and then maintain a preferred local environment. A second has been that cells can modify their environment in pursuit of having as neighbors three surface types: free (contact with a luminal space), lateral (contact with another cell), and basal (contact with and attached to matrix). All simulated cells share the same set of rules. During each simulation cycle, each simulated cell evaluates its neighborhood and determines its action based on these rules.

2.2 Design of Simulation Experiments

We designed a series of simulation experiments to evaluate in silico phenotype at different cell densities. The toroidal grid size was set to 100 by 100. We conducted 100 simulation runs per density, and each simulation lasted for 100 simulation cycles. Initial cell counts ranged from 20 to 500 at an interval of 40. Seeds for pseudorandom number generators (PNG) were changed randomly at each simulation, and the ordering of simulated cell and cluster events was randomized using the Mersenne Twister PNG at each simulation cycle. Specifically, at each simulation cycle, simulated cells and clusters were selected at random (one at a time without replacement) and carried through their action. We recorded the number of clusters and the number of simulated cells in each cluster at the end of simulation. We captured images of grid composition at different time points.

3 RESULTS AND DISCUSSION

Primary human AT II cells in 3D culture form multi-cellular structures that resemble pulmonary alveoli [9]. These structures, referred as alveolar like cysts (ALC), have a hollow central lumen lined by a monolayer of polarized cells. They form by movement and cell aggregation without significant cell proliferation and death. The final diameter of ALC is density dependent, increasing with the initial cell density.

When we adjusted in silico conditions to mimic those in vitro, the simulated cells also formed cysts with hollow lumen (Fig. 1). The formation involved aggregation of cells followed by rearrangement of cells within the cluster to form a hollow central lumen. Increases in the simulated cell density also led to larger cyst sizes (Fig. 2). The results suggest that this simple set of in silico principles is sufficient to generate phenotypic attributes similar to those of AT II cells in vitro. We propose that the in silico principles have in vitro counterparts.

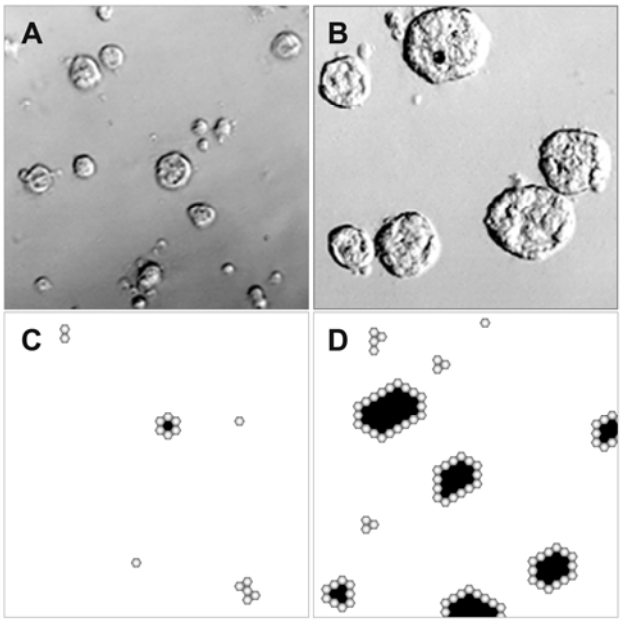

Figure 1. Alveolar-like structures form in both in vitro and in silico cultures. **(A–B)** Phase-contrast pictures after 4 days in 2% Matrigel culture in vitro; cells were initially plated at $5 \times 10^4/cm^2$ in **A** and at $25 \times 10^4/cm^2$ in **B** [9]. **(C–D)** In silico images after 100 simulation cycles; initial number of simulated cells were set to 100 ($\sim 5 \times 10^4/cm^2$ in vitro) in **C** and 500 ($\sim 25 \times 10^4/cm^2$ in vitro) in **D**. Simulated cells are agents with a set of rules or principles that govern their behavior. When seeded randomly in a 2D grid filled with matrix, they aggregate to form structures that resemble alveolar-like cysts observed in primary human alveolar type II cell cultures. More frequent cyst formation with larger cyst diameters is observed when the initial cell count is increased.

Considerable body of information about alveolar cell behavior exists at the molecular level. Some has provided important insights into how cells orchestrate their activities to achieve tightly organized pulmonary structures. However, much remains unknown about basic principles underlying the molecular machinery of alveolar morphogenesis. An ongoing challenge has been to develop testable hypotheses of biological mechanisms and operating principles that bridge molecular-level knowledge and systems-level inferences of phenomena.

In this study we developed an abstract systems model to better understand the key principles of alveolar morphogenesis in vitro. The model consists of discrete components that represent single cells and different

elements of the extracellular environment. The simulated cells share a common set of operating principles that govern their action in response to the configuration of their environment. Systemic behaviors and structures emerge from interactions among the components. Although our current model is abstract and relatively simple, it is capable of producing phenomena that mimic several of those observed in culture.

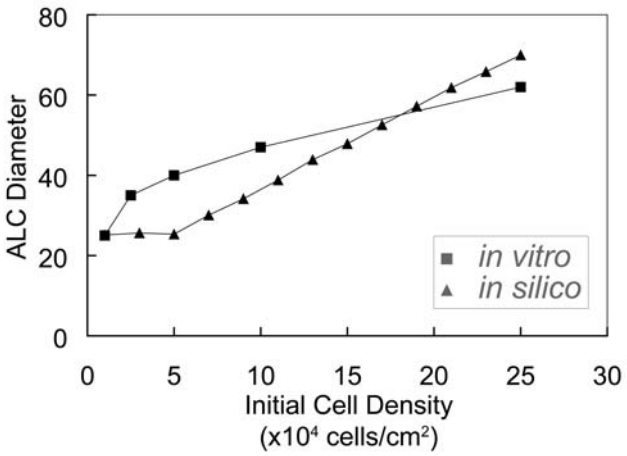

Figure 2. Alveolar-like cyst (ALC) size is dependent on the initial cell density. **(A)** Primary human alveolar type II cells form ALC in medium containing 2% Matrigel. Cultures with higher initial cell density produce larger ALC in vitro. ALC diameter in μm. **(B)** In silico cultures also produce structures that resemble ALC. Their size is dependent on the initial simulated cell density, similar to their in vitro counterparts. 20 simulated cells in a 100 x 100 grid correspond to 10^4 cells/cm^2 in vitro.

Our model axioms are high level, low-resolution placeholders for more detailed representations of the actual complex mechanisms driving alveolar cell behavior. Use of the axioms precludes explicit representations of the abundant, detailed subcellular information that is available. But starting with the more abstract set of rules provided the simplest method for building a foundational model. Having achieved our initial goal of building the foundational model, we can proceed to adding more details to provide new hypotheses and gain deeper insight into morphogenic behavior of alveolar cells in vitro and in vivo.

4 CONCLUSION

We have established a foundational model as an experimental platform to study mechanistic principles that underlie in vitro alveolar morphogenesis. Simulation results confirm that the in silico system can produce phenotypes that mimic important morphogenic characteristics of ALC formation in vitro. Further development of the model and its use are expected to yield more detailed insights into how pulmonary alveoli form and how they can be engineered in vitro.

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