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In Silico Simulation of Epithelial Cell Tubulogenesis

Jesse A. Engelberg, Minji Kim, Keith E. Mostov, and C. Anthony Hunt, *Member, IEEE*

***Abstract*—By improving our understanding of epithelial cell tubulogenesis *in vitro* we should improve our understanding of how these cells organize to form normal tissues such as the ducts and lobules that make up breast tissue. We do not fully understand how these ducts and lobules form. Because it is difficult to directly control and observe epithelial cell morphogenesis *in vivo*, we study it using *in vitro* culture systems. They are more easily controlled and observed. One of the most well studied models of tubulogenesis uses Madin-Darby canine kidney (MDCK) cells in culture systems: single cell layered cysts form tubules when exposed to hepatocyte growth factor (HGF). We have developed an in silico analogue that mimics the fundamental cell-level operating principles and system-level phenotypes of *in vitro* MDCK tubulogenesis. The creation and validation of the analogue required the specification and questioning of currently held assumptions. The analogue can be used to test hypotheses about mechanisms and in silico operating principles that may have *in vitro* counterparts. By increasing our understanding of the operating principles that govern *in vitro* epithelial cell growth and organization we are better positioned to understand how best to manipulate these operating principles to achieve specific tissue engineering objectives.**

I. Introduction

The scope of achievable *in vitro* tissue engineering objectives is expected to expand dramatically as we identify and verify the principles of operation used by mammalian cells during morphogenesis and tissue remodeling. Although epithelial cells exhibit different behaviors depending on their surroundings, they seem to follow a roughly similar set of operating principles that specify how they react with and within a given environment during morphogenesis. In order to increase our understanding of why and how epithelial cells alter their behaviors when their environment is manipulated, we study model systems such as Madin-Darby canine kidney (MDCK) cells in culture.

An objective of this project has been to use new in silico methods to discover and validate plausible operating principles that can account for an expanding set of MDCK phenotypic attributes. Within this report we assume that the systemic properties of epithelial cell systems are a consequence of autonomous cells interacting with each other and their environment. Cell behaviors are highly constrained by genetics and differentiation. These constraints result in each cell acting as if it has the same agenda, which is a behavioral description of a set of strictly applied operating principles. We assume that two systems (one in silico, the other *in vitro*) having similar phenotypes will have similar operating principles. The larger the variety of similar in silico and *in vitro* phenotypic attributes, the greater the likelihood that in silico operating principles will have *in vitro* counterparts.

Given that idea, we need to discover and understand those operating principles and the consequences of their disruption. Achieving that goal requires new research techniques, including the computational methods outlined within this report.

MDCK cells are used as a model of epithelial cell growth and morphogenesis. Because this cell line is experimentally tractable, well characterized, and capable of undergoing both cyst and tubule growth, it serves as a useful model of more complex systems. When embedded in collagen, *in vitro* MDCK cells form stable single layer cysts made up of polarized cells. If exposed to hepatocyte growth factor (HGF) the cells within the cyst undergo a temporary epithelial to mesenchymal transition (EMT), in which they lose some or all cell polarity, begin to actively proliferate, and may migrate away from the cyst. These cells can form cytoplasmic extensions, cellular chains, cords, and tubules [1]. It is not clear whether these tubulogenesis events are sequential or coincidental, which is a question that is being explored both *in vitro* and in silico.

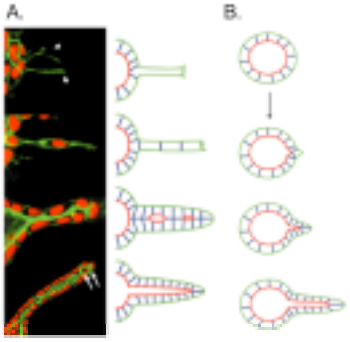

Fig. 1. Possible mechanisms of tubulogenesis. **A:** tubule progression moves from chain to cord to lumen-filled tubule. **B:** tubule forms through rubber-sheet deformation. (Images reproduced with permission from [2]).

There are two hypothesized mechanisms for tubulogenesis within the MDCK cell system (Fig. 1): A) Cells within a cyst form an extension that becomes a chain of cells. This chain transforms into a two-cell-wide cord through

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J. A. Engelberg is with the UCSF / UC Berkeley Joint Graduate Group in Bioengineering, San Francisco, CA 94143 USA (phone 415-514-2514; fax 415-514-2008; e-mail: jesse.engelberg@gmail.com).

M. Kim is with the Department of Anatomy, University of California, San Francisco, CA 94143 USA (e-mail: minji.kim@ucsf.edu).

K. E. Mostov is with the Department of Anatomy, University of California, San Francisco, CA 94143 USA (e-mail: keith.mostov@ucsf.edu).

C. A. Hunt is with the BioSystems Group, Dept. of Biopharmaceutical Sciences, University of California, San Francisco, CA 94143 USA (e-mail: a.hunt@ucsf.edu).

proliferation, which generates lumen *de novo* to create a tubule. B) Cells within the cyst deform (rubber-sheet deformation) through migration and proliferation, stretching outward to form a tubule [2]. It is not clear whether existing *in vitro* evidence is sufficient to disprove either of these three mechanisms, a question we plan to address through in silico modeling.

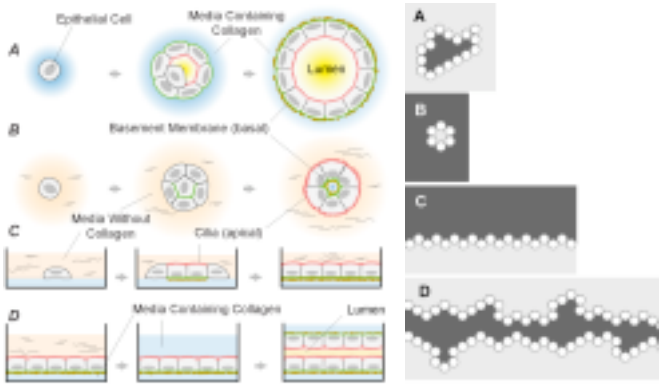

Fig. 2. MDCK cell growth in varied culture conditions illustrated *in vitro* **(left)** and in silico **(right)**. **A:** a single cell is embedded in collagen, producing a lumen-filled cyst. **B:** a single cell is grown in suspension, producing an inverted matrix-filled cyst. **C:** a single cell is grown on top of a layer of collagen, producing a monolayer. **D:** a monolayer of cells has a layer of collagen-containing media overlayed on top of it, producing a lumen-filled sandwich. (Images reproduced with permission from [4]).

Analogues of MDCK tubulogenesis have been created that mimic the events of cystogenesis and tubulogenesis [3], but these analogues are primarily constructed based on observations from literature and still images of fixed cells, which are not as informative as video images and may be based on incorrect assumptions. Few, if any, computational analogues seek to examine the macroscopic effect on MDCK cystogenesis or tubulogenesis caused by changes in cellular behaviors. One notable exception to this rule is the analogue of MDCK cystogenesis created within [4]. CELLS (in silico components are specified in SMALL CAPS) within this analogue follow a set of operating principles that determine whether the CELL will create new CELLS, produce extracellular MATRIX, or die, based upon whether neighboring locations contain other CELLS, MATRIX, or empty LUMEN. The analogue uses a 2D hexagonal grid with each CELL occupying a single grid location, but despite this low level of resolution it is effective in mimicking aspects of cystogenesis not successfully reproduced by other analogues, such as the production of different structures in varied culture conditions (Fig. 2). In addition, the developers of the analogue were able to make predictions about the *in vitro* system. They predicted that by simply altering the direction in which a CELL produces MATRIX, a cancer-like phenotype would be produced. This result was not anticipated by in silico or *in vitro* researchers.

Just as *in vitro* models can increase our understanding of an *in vivo* system, in silico analogues can increase our understanding of *in vitro* systems. In silico analogues of the type described here (where CELLS and components are autonomous) are useful in four ways: 1) Through the collaborative process of creating the analogue, the referent *in vitro* system must be closely examined, which can bring into question unspoken assumptions about the *in vitro* system. 2) The analogue can be easily manipulated and experimented upon in order to test and explore mechanistic hypotheses. 3) Once the analogue is validated against measures of *in vitro* attributes, it can be used to test hypotheses about the *in vitro* system. 4) The response of a validated analogue to simulated environment changes and interventions can stand as a prediction of the consequences of comparable interventions on the *in vitro* system.

Our modeling effort used in silico analogues created with an agent based paradigm: individual components (software objects) and mechanisms (sets of in silico operating principles) of the in silico analogue were mapped to components (cells, matrix, growth factors, etc.) and mechanisms within the *in vitro* system in biologically plausible ways. Agent based analogues are constructed from the perspective of the individual agents in order to mimic as accurately as needed the behaviors of a complex system by specifying the operating principles followed by its components.

We used an iterative methodology, where key phenotypic attributes from a ranked list were targeted for simulation; the analogue was validated against measures of these attributes; additional attributes were selected that typically invalidated the analogue; the analogue was then modified and revalidated against the new data.

I. METHODS

The analogue was based on an earlier analogue that simulated MDCK cystogenesis under four different growth conditions [4]. CELLS in the modified analogue follow the nine axioms in Table I, which depend on CELL neighborhood. In order to create an analogue capable of tubulogenesis, we implemented modifications to the original axioms. The CELL axioms used within this report are listed in Table I. Axiom 5 is a new axioms that specifies that a CELL with five CELL neighbors and a single MATRIX neighbor will move into the MATRIX location instead of dividing as in [4]. This modification allows the analogue to mimic *de novo* lumen formation, which was not previously possible; it is an important component of cystogenesis and tubulogenesis. Axiom 6 specifies that a CELL with two or more CELL neighbors, one or two LUMEN neighbors, and one or two MATRIX neighbors will move into one of the MATRIX locations, as long as that location is opposite a CELL neighbor. This axiom allows the analogue to mimic lumen expansion via cell movement instead of cell death, also important in tubulogenesis.

The most significant extension has been the introduction of a new CELL type, the EMTCELL, which uses the different set of operating principles listed in Table II, including creating new CELLS when in contact with LUMEN or at the end of a chain of CELLS, and remaining stable when contacting multiple CELLS and MATRIX. EMTCELLS at the

end of a chain produce a new EMTCELL directly opposite the previous EMTCELL, branch to the left of , or branch to the right, with probability 0.7, 0.15, and 0.15 respectively. In addition we introduced the facility for CELLS to become EMTCELLS when exposed to simulated HGF. After the transition to EMTCELLS, a counter was started, indicating the amount of time before the EMTCELLS transitioned back to CELLS.

The analogue is being refined using an iterative validation method. The full set of targeted attributes is listed in Table III. Those in italics are outside the scope of behaviors achievable by an analogue using the axioms in Tables I and II. In following the iterative refinement method, we move down the list in Table III one at a time. Often, an analogue that is validated against the shorter list is invalidated when the list is extended to include the next attribute. To achieve the extended attribute list, one or more features of the current analogue may need revision. For example, a new axiom may be introduced or a current axiom may be revised. The refinement and validation process can become unwieldy when refinement targets two or more new attributes at a time.

TABLE I
CELL AXIOMS

Axiom	Neighborhood	Action
1	Only CELLS	Die
2	Only LUMEN	Die
3	One or more CELLS and LUMEN	Die
4	Zero to four CELLS and MATRIX	Replace MATRIX with a new CELL
5	Five CELLS and one MATRIX	Move CELL to location of MATRIX
6	Three or four CELLS, opposite MATRIX, and LUMEN	Move CELL to location of MATRIX opposite other CELL
7	MATRIX and two or more LUMEN	Replace LUMEN adjacent to MATRIX with a new CELL
8	MATRIX, adjacent LUMEN, and one or more CELLS	Replace LUMEN adjacent to MATRIX with a new CELL
9	None of the above	Do nothing

Table I. CELL axioms. CELLS evaluate their neighborhood, the in silico equivalent to the immediate environment *in vitro*, and execute actions accordingly. Axioms are adapted from [4], but note that the axiom numbers are not mapped directly.

TABLE II
EMTCELL AXIOMS

Axiom	Neighborhood	Action
1	Two or more CELLS, LUMEN, and MATRIX	Replace MATRIX with a new EMTCELL
2	A single CELL and MATRIX	Replace MATRIX with a new EMTCELL
3	Non-adjacent CELLS and MATRIX	Do nothing
4	None of the above	Do nothing

Table II. EMTCELL axioms. EMTCELLS take only these actions and do not follow CELL axioms.

I. RESULTS

We created an analogue that mimics aspects of MDCK tubulogenesis. The analogue successfully reproduced targeted attributes listed in Table 3, and exhibited system-level phenotypes similar to those observed within [1], including the creation of chains, cords, and lumen-filled tubules. As can be observed in Fig. 3, after a CELL is transitioned into an EMTCELL, that EMTELL created new EMTCELLS opposite the existing cyst, eventually forming a chain. When the the EMTCELLS reverted to normal CELLS, these CELLS created new CELLS, forming a cord with a width varying from one to three CELLS. As CELLS moved away from each other and in some cases underwent apoptosis, LUMEN appeared between the CELLS in the cord, resulting in a hollow tubule. The general pattern of growth was consistent with observations of cells growing within *in vitro* culture. The majority of in silico simulations resulted in tubule formation, though the size and shape was varied. In certain cases the chain would loop back toward the cyst and the resulting tubule would connect to the cyst itself.

We varied the amount of time before EMTCELLS transitioned back to CELLS, and found that it correlated to the eventual length of chains, cords, and tubules.

TABLE III
TARGETED ATTRIBUTES

Cells create new cells, die, and move as observed *in vitro*
Cells consume and generate matrix as observed *in vitro*
Cells change to non-polarized state in response to an external signal
Non-polarized cells regain polarity after a period of time
Cells embedded in matrix will create cysts as in [4]
Cells within a cyst will create new cells, forming a chain of cells extending from the cyst
Cells in a chain extending from a cyst will create a tubule
Tubule creation can occur without measured cell death
Chains of cells exhibit branching
Cells migrate toward end of chain of cells
Tubule length is highly varied
Tubules can form without discontinuous lumen
Cells within a cyst can create new cells without creating a chain

Table III. Attributes of MDCK cell behavior targeted for reproduction in silico. Attributes in *italics* are in process.

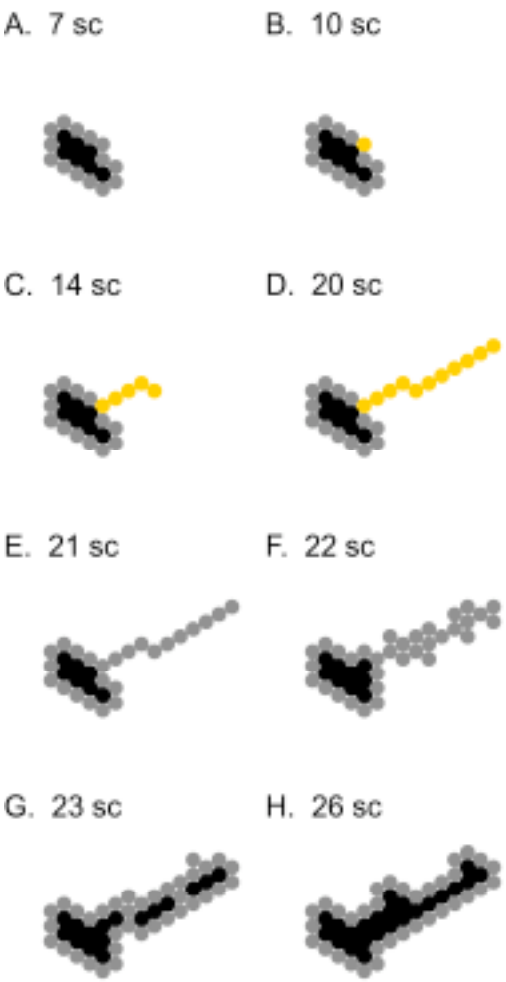

Fig. 3. Time lapse images from a typical analogue simulation execution. **A:** Cyst formation occurs during the first seven simulation cycles (sc). **B:** After cyst formation has occurred, a CELL transitions into an EMTCELL. **C-D:** The EMTCELL extends, forming a chain. **E:** The EMTCELLS revert back to CELLS. **F-G:** CELLS within the chain form a cord and then a LUMEN-filled tubule. **H:** The resulting stable tubule. **Gray circles:** in silico CELLS. **Yellow circles:** EMTCELLS. **White space:** Extracellular MATRIX. **Black space:** Empty LUMEN.

II. Discussion

We observed significant APOPTOSIS in simulated tubulogenesis, a phenomenon that is uncommon during *in vitro* tubule creation. Consequently, it is already clear that when the next attribute in Table III is added to the targeted list (tubule creation can occur without measured cell death), axiom revision will be needed to revalidate.

Nevertheless, the results in Fig. 3 demonstrate that the approach used does enable discovery of in silico operating principles that drive CELLS to exhibit behaviors that mimic those observed *in vitro*. Simulated MDCK tubulogenesis can be achieved with only CELLS and EMTCELLS and the axioms in Tables I and II. It also appears that much of the process of tubulogenesis could occur after EMTCELLS switch back to the normal CELL state, creating a tubule instead of a cyst. Our observations suggest that the processes of cystogenesis and tubulogenesis may be very similar, perhaps separated primarily by initial geometry.

We suggest that this new class of in silico analogue can become a valuable resource for gaining deeper insight into the mechanisms governing mammary epithelial cell organogenesis [5].

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