

Modeling hiPSC-to-Early Cardiomyocyte Differentiation Process using Microsimulation and Markov Chain Models

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ABSTRACT

Cardiomyocytes (CMs), the contractile heart cells that can be derived from human induced pluripotent stem cells (hiPSCs). These hiPSC derived CMs can be used for cardiovascular disease drug testing and regeneration therapies, and they have therapeutic potential. Currently, hiPSC-CM differentiation cannot yet be controlled to yield specific heart cell subtypes consistently. Designing differentiation processes to consistently direct differentiation to specific heart cells is important to realize the full therapeutic potential of hiPSC-CMs. A model that accurately represents the dynamic changes in cell populations from hiPSCs to CMs over the differentiation timeline is a first step towards designing processes for directing differentiation. This paper introduces a microsimulation model for studying temporal changes in the hiPSC-to-early CM differentiation. The differentiation process for each cell in the microsimulation model is represented by a Markov chain model (MCM). The MCM includes cell subtypes representing key developmental stages in hiPSC differentiation to early CMs. These stages include pluripotent stem cells, early primitive streak, late primitive streak, mesodermal progenitors, early cardiac progenitors, late cardiac progenitors, and early CMs. The time taken by a cell to transit from one state to the next state is assumed to be exponentially distributed. The transition probabilities of the Markov chain process and the mean duration parameter of the exponential distribution were estimated using Bayesian optimization. The results predicted by the MCM agree with the data.

Keywords: Process Design, Derivative-free optimization, Biosystems, hiPSC cardiac differentiation

INTRODUCTION

Cardiovascular diseases are the leading cause of death worldwide [1]. Almost 695,000 people in the US died of cardiovascular disease in 2022 [2]. Cardiomyocytes (CMs), i.e. contracting heart cells, can be derived from human induced pluripotent stem cells (hiPSCs) to be used for drug testing, regenerative therapies, and disease modeling for cardiovascular diseases [3]. The modulation of the Wnt signaling pathway using small molecules and growth factors provides the standard CM differentiation protocol, yielding CMs with up to 98% purity [4]. However, this benchmark protocol produces mostly left ventricular cells, with a little to no atrial cells, or right ventricular cells [5]. To model specific diseases in the heart requires specific heart cell types. For example,

atrial diseases like atrial fibrillation and Ebstein's anomaly, or right ventricular diseases, such as Tetralogy of Fallot or arrhythmogenic right ventricular cardiomyopathy, require atrial CMs and right ventricular CMs respectively [6].

Designing a differentiation process to consistently yield specific heart cells is essential to fully realize the therapeutic, drug testing, and disease modeling potential of CMs. The first step in designing a differentiation process that directs hiPSCs to specific heart cell subtypes is understanding the temporal changes in the cell type populations during differentiation.

Several mathematical models have been developed to capture cell differentiation process [7-16]. Stiehl et al [7] investigated if stemness (characterization of stem cells) can be defined at the single-cell level, and what

properties are suitable for defining stem cells. The authors proposed a multi-compartment deterministic model, which consists of discrete sets of ordinary differential equations, to describe the dynamics of cell differentiation and self-renewal regulated by extracellular signaling feedback. The cell behavior is characterized by parameters including the proliferative activity, the probability of differentiation, and the probability of dying. The model was developed to predict the cell type population and signaling molecule concentration over time. The model, calibrated with clinical data from multiple myeloma patients undergoing high-dose chemotherapy and stem cell transplantation, aimed to observe stem cell differentiation and determine cell type populations. Each differential equation in the model describes a distinct differentiation stage, reflecting the conventional notion that within each lineage of blood cell precursors, there is a discrete sequence of maturation stages traversed sequentially. Models presented in this paper characterize stem cells as the population that is most sensitive to environmental signals. The stem cell differentiation characterization developed in this paper identified stem cells and other cell types as a population. However, the model could not capture the differentiation mechanism happening in a single cell. The drawback of this deterministic model is that it does not capture the stochastic nature of the differentiation process.

Paździorek [8] investigated the stochastic stability of the model presented by Mar-Czohara and examined its response to noise. The deterministic model from [7] was transformed into a stochastic process using Ito differential equations. Ito calculus addresses processes with evolving random variables over time, where the random variable here is the cell type population. The random behavior of cells, whether differentiating, maturing, dying, or proliferating was modeled as a Wiener process, which is a continuous-time stochastic process. The increments of the Wiener process across disjoint time intervals exhibit a normal distribution. Premslaw demonstrated that the stochastic model achieves asymptotic stability, signifying that over time, stem cells tend to reach stable and well-defined states.

Pisu et al [9] introduced a novel mathematical model to simulate the differentiation of mesenchymal stem cells into specialized cells to study the effect of growth factors on cell proliferation/differentiation mechanisms. The model was built on material balances for extracellular matrix compounds, growth factors, and nutrients, along with a mass-structured population balance that describes cell growth, proliferation, and differentiation. The DNA content and the glycosaminoglycans (GAG) content present in the cells at different time points were used as indicators to identify the cell types. The model incorporated several parameters, with key ones being the kinetic constant of GAG synthesis, kinetic constant of collagen

synthesis, time rate of change of cells, maximum collagen and GAG concentration, concentration of O_2 in saturation condition, and number of cell types. Regression analysis of the model predictions demonstrates that the average error for different differentiation pathways is below 20 percent.

The models presented in [10-16] also incorporate macroscopic analysis, lacking representation of mechanisms occurring at the single-cell level. Capturing the differentiation mechanism at the single-cell level will offer insights into interactions at the cellular level. This paper introduces a microsimulation model (MSM) to study the temporal changes in the cell type populations during cardiac differentiation at cell-level analysis. A microsimulation model, which is a stochastic model, aims to simulate individual entities of the system through stochastic parameters. Each cell is modeled as an individual entity of the MSM, and the differentiation process in each cell is represented with a continuous-time Markov chain model (CTMCM). The states of the CTMCM are cell subtypes representing the developmental stages in hiPSC differentiation to early CMs. They include pluripotent stem cells, early primitive streak, late primitive streak, mesodermal progenitors, early cardiac progenitors, late cardiac progenitors, and early CMs. The transition probabilities define the probability of a cell transitioning from one cell subtype to another or an absorbing state. The holding times, representing the time a cell spends in each state, are modeled using exponential distributions. The MSM is embedded in a Bayesian optimization framework to estimate the CTMCM parameters, i.e., transition probabilities and mean of the exponential distributions.

This paper is structured as follows: Next section discusses the modeling of cell differentiation using the Markov chain model and modeling the duration of state transitions using exponential distribution. The application of the microsimulation model for simulating the differentiation process is introduced next. The results for the microsimulation model parameters and a comparison of the model predictions to experimental data are in Results and Discussion section. Concluding remarks and future directions are given in the last section.

MODELING DIFFERENTIATION

Modeling cell differentiation

The data used to build the MSM model was obtained from the single-cell RNA sequencing analysis of 2D cardiac differentiation of two cell lines (WTC cell line and SCVI cell line) [5]. The differentiation was carried out for 6 days and samples were collected from day 1 to day 6 daily. The collected samples were captured, and single-cell RNA sequencing was performed. The gene expression matrix was analyzed and labeled to get the cell type population during differentiation from day 1 to day 6.

Based on the results obtained from 2D cardiac differentiation, the developmental trajectory of hiPSCs to early CMs follows a path. The path includes a series of states: which are pluripotent stem cells, early primitive streak, late primitive streak, mesodermal progenitors, early cardiac progenitors, late cardiac progenitors, and early CMs. At late primitive streaks and mesodermal progenitors state, cells may differentiate into non-CM cells. When a cell begins differentiation, it either moves into the next state in sequence or becomes a non-CM cell type, or remains in the same pluripotent state that it started in. There are two possible states from which cells can become non-CMs; cells from the late primitive streak can differentiate into hepatic endoderm and definitive endoderm, and cells from the mesodermal progenitor state can differentiate into endothelial cells, epicardial progenitors, and epicardial cells. Differing cell types have differing timings for differentiation, and, therefore, the time a cell spends in one state is dependent on the cell type being made. Based on this explanation, to model the cell differentiation, two questions need to be answered:

- 1) What is the next state in the differentiation path?
- 2) If a cell differentiates to the next state in sequence, how long will it stay between those two states?

The important assumptions here are the cells do not die during differentiation and they do not split or divide during the differentiation. Although we recognize the limitations of these assumptions, given the lack of data for modeling cell division and death rates for this differentiation protocol, this preliminary model allows making progress towards modeling hiPSC to early CM differentiation process.

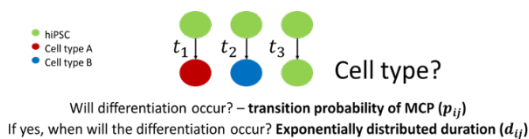


Figure 1. Modeling cell differentiation.

Continuous time Markov chain model

The first question can be answered by transition probabilities of a Markov chain model (MCM). A Markov chain model is a mathematical model that represents a sequence of events in a process in which the probability of transitioning from one state to another depends solely on the current state [17,18,19]. Markov chain models are memoryless, meaning the probability of transitioning to future states depends only on the current state and is independent of the sequence of states that led to the current state. Markov chains are used for modeling dynamic systems like financial markets, weather patterns, and language processing. In a Markov chain, the transition probability describes the probability of moving from one state

to another in the chain at each time step. The general representation of transition probability, P_{ij} , for the transition from state i to j , is given in Equation (1).

$$P_{ij} = P(X_{n+1} = j | X_n = i) \quad (1)$$

In Equation 1, X_n and X_{n+1} represent two consecutive states in a Markov chain. The RHS of Equation (1) represents the conditional probability that, given the current state $X_n = i$, the system will transition to state $X_{n+1} = j$. Markov chains are characterized by a transition matrix that represents the probabilities of moving from one state to another. The transition matrix enables predictions and analysis of system behavior over time.

Markov chain models can be classified into different types based on time homogeneity (homogenous Markov chain and non-homogenous Markov chain), state continuity (finite state Markov chains and continuous state Markov chains), time continuity (discrete-time Markov chains and continuous time Markov chains), absorbing or non absorbing states (absorbing Markov chains and non-absorbing Markov chains). [20]. The classifications related to cell differentiation based on scRNA-seq data are discrete-time and absorbing Markov chains. In a discrete-time Markov chain, the transitions between states occur at discrete, evenly spaced time intervals. An absorbing Markov chain is used to model systems where certain states, known as absorbing states, act as final destinations from which there is no escape. This means that in a Markov chain, the transitions between states occur with probabilities, however, once the system reaches an absorbing state, it remains there indefinitely. In other words, the probability of transition from an absorbing state to any other non-absorbing state is zero. These chains can be characterized by their probability of transition between transient states (non-absorbing states) and absorbing states, leading to absorption, which represents the final resting places of the system.

A continuous-time absorbing Markov chain model is developed to represent hiPSC to CM cell differentiation. Figure 2 demonstrates the Markov chain model of cell differentiation. Late primitive streak can differentiate into endoderm cells, and mesodermal cells can differentiate into epithelial and epicardial cells. When cells commit to becoming endodermal, epicardial, or epithelial cell types, they can no longer differentiate into CMs. Hence, the transition probability from these cell types to any other cell types in the cardiac trajectory is zero. Two absorbing states, one from the late primitive streak and the other from the mesodermal progenitor state, were included in the MCM to denote cells that differentiate into non-CMs. Absorbing state 1 consists of hepatic endoderm cells and definitive endoderm cells, and absorbing state 2 consists of epithelial cells, epicardial progenitors, and epicardial cells.

The probability P_{ij} denotes the probability of

transition of the MCM from state i to state j in the cardiac differentiation trajectory and p_{ij} denotes the transition probability in a specific instance of simulating a Markov chain. P_{ij} is the Markov chain model parameter and p_{ij} is the probability of transitioning from state i to state j during a simulation run. In the simulation, for cell types other than late primitive streak and mesodermal progenitors, if $p_{ij} \leq P_{ij}$, the cell will differentiate from cell type i to cell type j . If a cell is initially in the late primitive streak or mesodermal progenitors state, there are three potential outcomes for the cell's eventual destination. If $p_{ij} \leq P_{ij}$, the cell will differentiate into cell type j , or if $P_{ij} < p_{ij} \leq P_{ij} + P_{ia}$, the cell will differentiate into absorbing state, or if $P_{ij} + P_{ia} < p_{ij}$, the cell will stay in the same state i . Based on the transition probability parameters of the MCM, the cells in the simulation will differentiate into different cell types. The Markov chain stops when the cell reaches either an absorbing state or an early CM state.

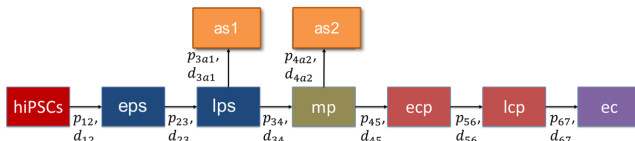


Figure 2. Markov chain representation of cell differentiation (Table 1 defines cell type abbreviations).

Table 1: Expansion of the cell types in the MCM.

Abbreviations	Cell types
hiPSCs	Human induced pluripotent stem cells
eps	Early primitive streak
lps	Late primitive streak
as1	Absorbing state 1
mp	Mesodermal progenitors
as2	Absorbing state 2
ecp	Early cardiac progenitors
lcp	Late cardiac progenitors
ec	Early cardiomyocytes

Modeling state duration in the MCM with exponential distribution

The exponential distribution is a continuous probability distribution commonly used to model the time between events in a Poisson process, where events occur at a constant average rate and are independent of each other [21]. The probability density function $f(x)$ of the exponential distribution is given by Equation 2.

$$f(x) = \lambda \exp(-\lambda x) \quad (2)$$

In Equation 2, x is a non-negative variable representing the time between events, and λ is the rate parameter, which is a positive constant. It determines the

average number of events occurring per unit of time and is also equal to the inverse of the average time between events. The rate parameter quantifies the event rate, the frequency, on average, of the events occurring. One of the notable properties of the exponential distribution is memorylessness. It suggests that the probability of the next event occurring in the next time increment is the same, regardless of how much time has already passed. This property of exponential distribution helps modeling systems with no memory of past events.

In the cell differentiation process, the time a cell spends between two states is random, with a constant average rate of occurrence and the events are independent of each other. Therefore, the duration spent by a cell between two states can be modeled using the exponential distribution. The average duration d_{ij} , spent by the cells between state i and state j is the inverse of the rate parameter used to model the time spent by the cells between states.

MODELING CELL POPULATIONS

Microsimulation models

Microsimulation models (MSM) are computational models designed to simulate individual-level behavior using individual entities within a population [22,23]. Each entity is represented with specific attributes. In the case of modeling cell populations, the individual entity denotes each cell in the differentiation process, and the attributes denote transition probability and the average duration parameters. MSM consists of three components, the agents, rules, and environment. The agents represent individual cells. The rules denote the behavior and decision-making processes for each cell, which means the transition of cells into different states that are controlled by the Markov chain probabilities. The environment represents the context in which the cells interact. The constraint in the MSM is the sum of probability in each state should add up to one.

In the developed MSM (Figure 3), each cell in the simulation will move into the next state based on the transition probability of the Markov chain model, and whenever the cell moves to the next state, the duration the cell spends in that state is sampled from the exponential distribution. For transition from states i to j , excluding absorbing states, the sampled time interval is calculated based on the average duration parameter, λ_{ij} . The age associated with the cell in a state is obtained by adding the sampled time for that state to the age of the cell in the previous state. Since we are modeling the cell population at the end of each differentiation day, the result obtained from the simulation is converted into a binary matrix, in which the rows represent the differentiation days, and the column represents the cell states. For example, let us consider that the age of a cell when it

Table 2: Cell population matrix from WTC cell line (X_1) used in the optimization problem (Equation 3). All numerical values are expressed as percentages.

Days	hiPSCs	eps	lps	as1	mp	as2	Ecp	lcp	Ec
Day 1	18.9	80.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0
Day 2	0.6	8.3	90.8	0.0	0.3	0.0	0.0	0.0	0.0
Day 3	0.8	1.8	22.8	17.5	50.6	0.0	6.5	0.0	0.0
Day 4	0.4	0.0	1.9	30.2	20.7	4.1	42.6	0.2	0.0
Day 5	0.8	0.0	0.8	31.7	5.8	7.2	36.5	17.3	0.0
Day 6	0.3	0.0	0.0	39.7	0.8	9.2	17.0	31.3	1.7

reaches the early primitive streak is 0.78 days, and the age of a cell when it reaches the late primitive streak is 2.4 days and it stays in the late primitive streak. In the given example, at the end of day 1, the cell state is the early primitive streak, so in the binary matrix, in the day 1 row, the eps column will have a value of 1. From 0.78 days to 2.4 days, the cell remains in the early primitive streak state, meaning at the end of day 2, the cell in the eps column will have a value of one, and the rest of the day 2 row will have a value of 0. From 2.4 days up to day 6, the cell will stay in the late primitive streak, and therefore, for the rows from day 3 to day 6, the lps column will have a value of 1, and the rest equal to zero. Five thousand cells were run in the simulation to generate the cell type matrix for each cell. The number of cells in each cell type at the end of each differentiation day was added and then normalized by the total number of cells. By this procedure, the cell population percentage matrix can be obtained using the microsimulation model with the Markov chain process.

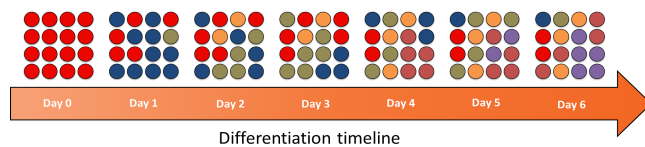


Figure 3. Modeling cell population using a microsimulation model.

Parameter estimation

The MSM parameters, transition probabilities, and the average durations were estimated using Bayesian optimization by minimizing the mean squared error between the model output and the experimental data. Bayesian optimization, driven by Bayesian inference and surrogate modeling, is a versatile and efficient approach for solving complex optimization problems [24,25]. Bayesian optimization uses a surrogate model, typically a Gaussian process (GP) model, to estimate the unknown objective function. Bayesian optimization balances exploration (sampling in uncertain regions), and exploitation (sampling in the regions with the highest estimated

objective value), to find the global optimum efficiently. The objective function (Equation 3) used is the mean squared error between the model output and the experimental data.

$$f = \frac{1}{n}((Y - X_1)^2 + (Y - X_2)^2) \quad (3)$$

$$Y = MSM(P_{ij}, d_{ij}) \quad (4)$$

$$P_{lps,mp} + P_{lps,a1} \leq 1 \quad (5)$$

$$P_{mp,a2} + P_{mp,ecp} \leq 1 \quad (6)$$

$$0 \leq P_{ij} < 1; d_{ij} \geq 0 \quad (7)$$

Y is the MSM output. The variables X_1 and X_2 represent the cell population matrices obtained from the single-cell RNA sequencing of the cell samples. These samples were collected from two cell lines during differentiation days 1-6, as mentioned in the 'Modeling cell differentiation' section. The cell population matrix for WTC cell line, X_2 , is shown in Table 2 as an example. Equation 4 states that the model output is a function of the decision variables P_{ij} and d_{ij} . To ensure the probabilities at the cell states having two states always less than or equal to 1, constraints (Equations 5, 6 and 7) were included in the model.

Bayesian optimization begins with generating an initial set of samples from the model. The Gaussian Process (GP) model is built based on the initial samples. The acquisition function selects the next sample for evaluation, balancing the exploration and exploitation. The selected point is evaluated in the true objective function. The surrogate model is updated with new data and the process iterates.

RESULTS AND DISCUSSION

Five thousand cells were simulated in the Markov chain model embedded within the microsimulation model. The parameters obtained using Bayesian optimization are shown in Table 3. Based on the data, the optimization result showed that the probability of transitioning from psc state to eps state, and eps state to lps state is 1. During these two transitions, the cells spend an

average duration of 0.45 days and 1.32 days, respectively. At the lps state, 16% of cells transition into hepatic endoderm and definitive endoderm cells. These 16% of cells never become CMs. The remaining 84% of the cells take about 1.3 days to enter the mesoderm state, where 42% of these cells (35% of the initial number of cells) become endothelial cells, epicardial progenitors, and epicardial cells. The remaining 42% of the cells take about one day to enter the early cardiac progenitor state. After reaching the early progenitor state, 69% of these cells become late cardiac progenitors. Following this, 31% of the late progenitors become early cardiomyocytes. These cells spend about 3.58 days to become late cardiac progenitors and 2.98 days to become early CMs.

Table 3: MSM parameters obtained using Bayesian optimization.

Current state	Next state	Transition probability	Average duration
hiPSCs	eps	1	0.45
eps	lps	1	1.32
lps	mp	0.81	1.30
lps	as1	0.16	-
mp	ecp	0.52	0.98
mp	as2	0.42	-
ecp	lcp	0.69	3.58
lcp	ec	0.31	2.98

Figure 4 shows the predicted cell population versus the experimental data. From the plot, we can see that there are differences between the WTC cell line data and the SCVI cell line data. Since the aim of this work is to develop a model that is independent of the cell line, the effect of the cell line is not introduced in the model. This can also be observed in Figure 4, where the predicted cell type populations by the MSM model are between the two datasets. The mean squared error and the R^2 between the model and the data are 0.004 and 0.43, respectively. The performance of the developed model will undergo validation with further experimental data in the future.

CONCLUSION AND FUTURE DIRECTIONS

Using the cardiac cell type population obtained from 2D cardiac differentiation, a microsimulation model with a Markov chain model was developed to predict the cardiomyocyte cell type population during differentiation. The model parameters were obtained using Bayesian optimization. The results showed that the simulation model predicted the cell type population with a low mean square error. The developed model can act as a digital twin to the cardiac differentiation experiment. The data for training this model was generated in a 2D environment

following the modulated Wnt signaling differentiation protocol, which mostly yields ventricular cells. This protocol will be tested to analyze if the ventricular cells can be produced on a large-scale using 3D techniques. The model will also be trained with data using the 3D differentiation protocol. If different sets of experimental parameters are used to obtain different cell-type populations, the model can be trained on those datasets, and a set of transition probabilities and average duration parameters can be estimated for each set of experimental parameters. Finding the relationship between the experimental parameters and the model parameters will help tune the experimental parameters to get the desired CM percentage on a certain differentiation day.

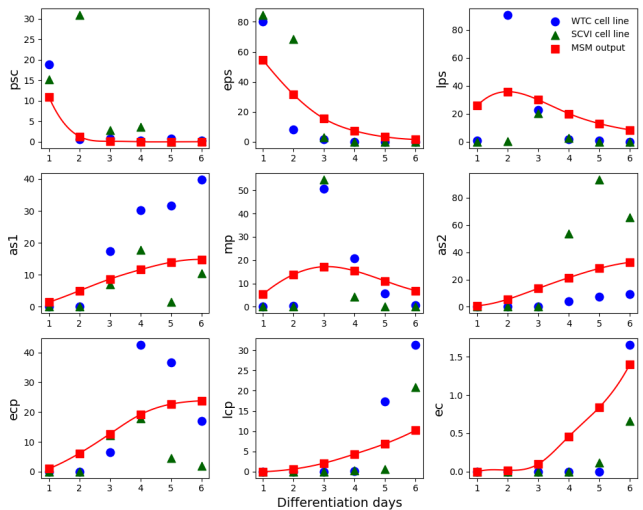


Figure 4. MSM predicted cell population vs differentiation experiment.

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