Using a Mathematical Model to Study Neuronal Differentiation of Human Induced Pluripotent Stem Cells Seeded on Melt Electrospun Biomaterial Scaffolds

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Introduction

- Human induced pluripotent stem cells (hiPSCs) have two prominent properties: pluripotency and the ability to self-renew [1]
- A major challenge when differentiating hiPSCs is controlling their differentiation process to yield the desired cell phenotypes
- Previously, we have shown physical cues presented by biomaterial scaffolds can stimulate the differentiation of hiPSCs into neurons [2]
- We aim to derive a mathematical model of stem cell growth and neural differentiation on melt electrospun biomaterial scaffolds to investigate the roles of these cues on neuronal differentiation and growth kinetics of hiPSCs
- Using mathematical analysis and numerical simulations, the model can determine the key factors controlling growth and differentiation of hiPSCs when seeded on melt electrospun scaffolds, allowing modification of the system to push the dynamics into the deisred state of maximal growth and differentiation

Materials and Methods

- A custom-made melt electrospinning setup was used to fabricate Poly (ϵ-caprolactone) (PCL) biomaterial scaffolds [2]
- Scaffolds were fabricated by melt electrospinning using 200 and 500 μm nozzle sizes , where fiber diameter increases and porosity decreases with increasing nozzle size [2]
- hiPSCs were cultured on a Vitronectin XF^{TM} matrix in TeS^{TM}-E8^{TM} media [3], then in STEMdiff^{TM} Neural Induction Medium (NIM) for 5 days to induce neural differentiation
- Neural progenitor cell aggregates were then seeded onto scaffolds and cultured in NIM for 12 days
- Bright field images were taken daily with IncuCyte^{TM} Software measuring cell body cluster area
- Viability of the neural aggregates on the PCL scaffolds was analyzed after 12 days using a LIVE/DEAD® Viability/Cytotoxicity Kit
- Immunocytochemistry targeting the neuron-specific protein β-III-tubulin assessed Neuronal differentiation [2]
- Based on experimental results and previous literature [4,5], we developed a system of ordinary differential equations (ODEs) modeling stem, progenitor, and terminally differentiated cell populations, and oxygen and waste concentrations encountered by the neural aggregates
- Parameters include oxygen and waste concentrations, and cell-scaffold contact, which are incorporated as functional positive and negative feedback on proliferation, apoptosis and differentiation

Mathematical Model

$$\dot{S} = -\alpha\left[-(\tfrac{O+5}{10})e^{1-\frac{O+5}{10}}+1.05\right]\left[\tfrac{(\frac{W}{10})^2}{1+(\frac{W}{10}+\frac{1}{2})^2}\right]S + r\left[\tfrac{1}{1+O}\right]P$$
$$+p_1\left[\tfrac{1}{1+P+S+D}\right]\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.13\right]\left[\tfrac{1}{1+\frac{W}{2}}\right]\left[C^3e^{\frac{1}{2}-C}+1\right]S$$
$$-d_1\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.13\right]\left[\tfrac{C}{1+C}\right]S$$

$$\dot{P} = -\beta\left[-(\tfrac{O+5}{10})e^{1-\frac{O+5}{10}}+1.05\right]\left[\tfrac{(\frac{W}{10})^2}{1+(\frac{W}{10}+\frac{1}{2})^2}\right]P - r\left[\tfrac{1}{1+O}\right]P$$
$$+p_2\left[\tfrac{1}{1+P+D}\right]\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.13\right]\left[\tfrac{1}{1+\frac{W}{2}}\right]\left[C^3e^{\frac{1}{2}-C}+1)\right]P$$
$$+d_1\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.13\right]\left[\tfrac{C}{1+C}\right]S - d_2\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.3\right]\left[\tfrac{C}{1+C}\right]P$$

$$\dot{D} = -\gamma\left[-(\tfrac{O+5}{10})e^{1-\frac{O+5}{10}}+1.05\right]\left[\tfrac{(\frac{W}{10})^2}{1+(\frac{W}{10}+\frac{1}{2})^2}\right]D + d_2\left[(\tfrac{1}{5})(O+1)(O-\tfrac{3}{2})e^{-O}+0.3\right]\left[\tfrac{C}{1+C}\right]P$$

$$\dot{O} = -5.65\times10^{-7}S - 2.88\times10^{-7}P - 3.28\times10^{-7}D + Input(O)\left[\tfrac{200}{200+C}\right]$$

$$\dot{W} = 2.382\times10^{-6}S + 1.213\times10^{-6}P + 1.382\times10^{-6}D - Output(W)\left[\tfrac{100}{100+C}\right]$$

- S, P, D, T: Stem, progenitor, differentiated, total cell populations
- α, β, γ: S, P, D death rates
- r: P to S reversion rate
- p_1, p_2: S, P division rates
- d_1, d_2: S, P differentiation rates
- $1-p_1-\alpha-d_1=Q_1$: Quiescent S population
- $1-p_2-\beta-d_2-r=Q_2$: Quiescent P population
- $1-\gamma=Q_3$: Quiescent D population
- $Input(O), Output(W)$: Diffusion of oxygen, waste

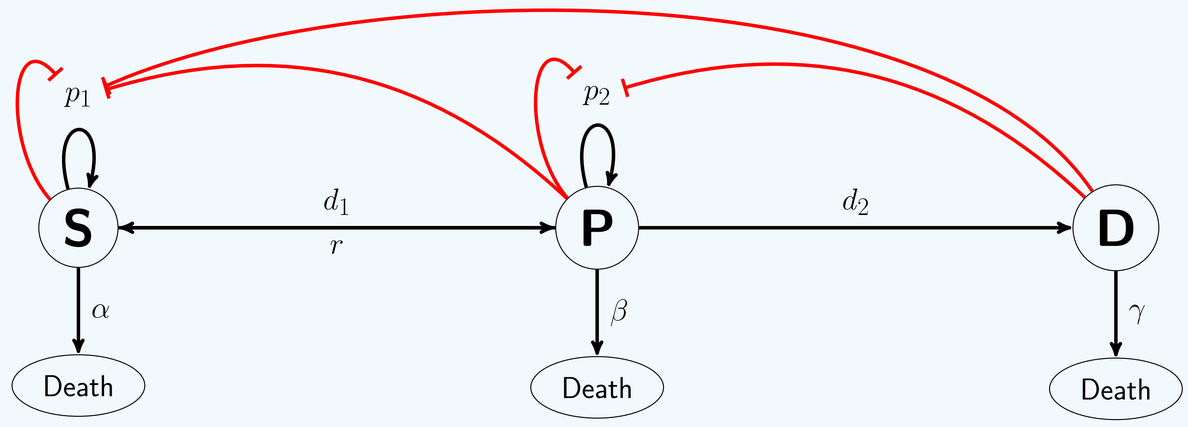

Figure 1. Schematic diagram of three cell states with cellular feedback. Black arrows indicate transitions between states. Red arrows indicate negative feedback.

- Fibre diameter was altered to vary scaffold porosities, with porosity modeled by cell-scaffold contact rate, C, where increasing porosity corresponds to decreased contact rate
- Parameters model factors such as oxygen tension resulting from diffusion at the air-media interface, differences in oxygen and waste diffusion caused by varying scaffold porosities, and differential effects of oxygen during stem cell maturation [5]
- Effects of scaffold contact on proliferation and death are derived from cell body cluster area and LIVE/DEAD staining, where total cell population corresponds to cell body cluster area

Results

- Neural aggregates derived from hiPSCs were seeded on two different scaffolds for 12 days
- Figure 2 shows bright field images of cells seeded on loop mesh 200 scaffolds at days 0 and 12
- The results 12 days after seeding on both scaffold topographies are summarized in Table 1

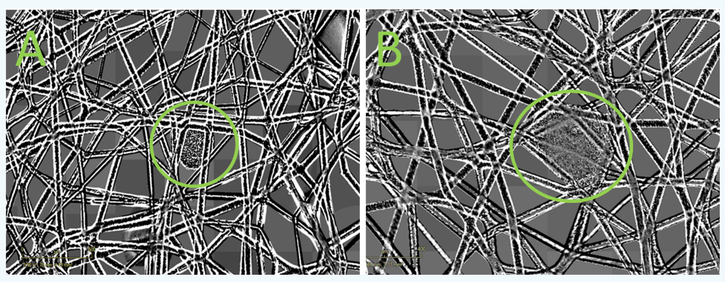

Figure 2. Neural aggregates on loop mesh 200 scaffold at days 0 (A) and 12 (B).

Table 1. Comparison of data for two scaffold porosities 12 days after seeding

Scaffold Type	Loop Mesh 200	Loop Mesh 500
Porosity (%)	40	23
LIVE/DEAD Fluorescence (%)	71.5 ± 1	58.4 ± 3
Cell Body Cluster Area (mm^2)	2	0.9

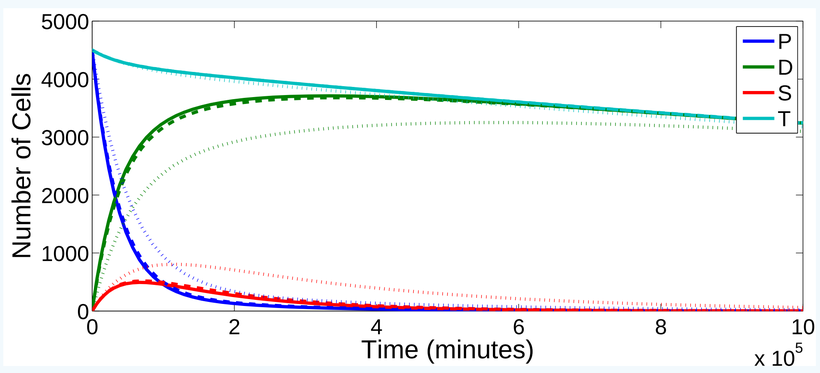

Figure 3. Population Dynamics of S, P, D and T, over varying scaffold porosities with S_o=0, P_o=4500, D_o=0, 21% O_2, 5% CO_2. Solid=0% porosity, dashed=40% porosity, dotted=10% porosity.

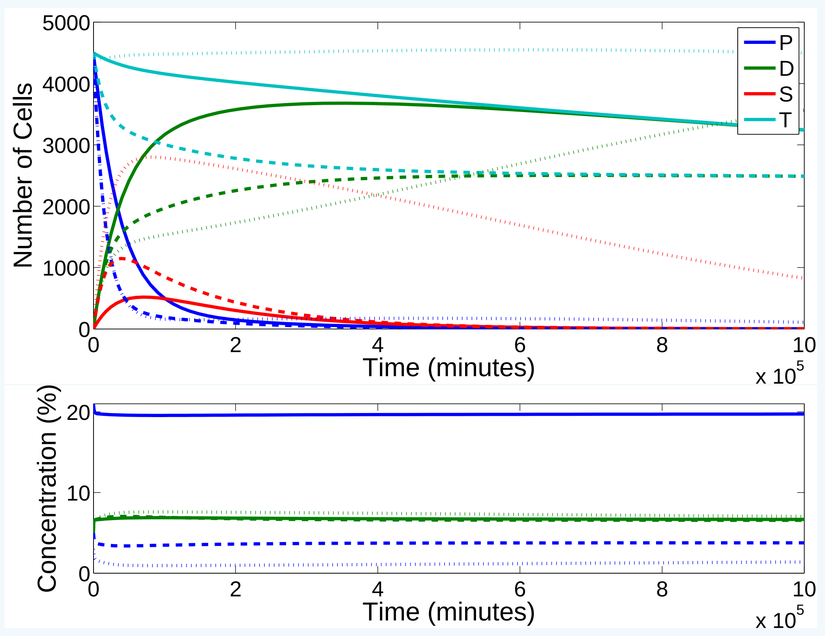

Figure 4. Dynamics of S, P, D, T (top), O and W (bottom), over varying external oxygen concentrations with S_o=0, P_o=4500, D_o=0, 5% CO_2, 40% porosity. Solid=21% O_2, dashed=5% O_2, dotted=3% O_2.

- The model does not capture the total cell population growth or increased growth on the more porous scaffold (Figure 3)
  - These are scaling issues to be resolved in future work
- Experimentally, decreasing O_2 from atmospheric (21%) to physiologic levels (1-7%) during culture increases growth and differentiation [5]
  - These qualitative dynamics are captured in the numerical simulations shown in Figure 4 (top)
- As shown in Figure 4 (bottom), the simulated dynamics of oxygen and waste behave as expected with initial changes in concentration, leveling off as the cell populations stabilize

Discussion

- We have developed an ODE model to investigate how physical cues affect neuronal differentiation and growth of hiPSCs seeded onto fibrous melt electrospun scaffolds
- Neural progenitor cells cultured on a higher porosity scaffold demonstrated more live cells and a larger cell body cluster area compared to a lower porosity scaffold
- The quantitative results have been used in determining the functional feedback on these processes and are being used to validate the model, with promising early results
- The model will allow for investigation into various alterations of the experimental parameters that would be diffcult and costly to explore experimentally
- On-going work will develop our model to study the effects of physical factors, such as scaffold porosity and oxygen availability, on neuronal differentiation and growth of hiPSCs

References

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