

Group Assessment

1 Biology

1.1 MSC

In an embryo a mesenchymal stem cell is a pluripotent progenitor cell which divides many times and eventually differentiating through a series of separate and unique lineage transitions into a variety of end-stage phenotypes: cartilage, bone, tendon, ligament, marrow stroma, connective tissues. The term, mesenchyme, is derived from the Greek meaning “middle” (meso) “infusion” and refers to the ability of mesenchymatous cells to spread and migrate in early embryonic development between the ectodermal and endodermal layers. This characteristic migratory, space-filling ability is the key element of all wound repair in adult organisms involving mesenchymal cells in skin (dermis), bone (periosteum), or muscle (perimysium).

They have the ability to form unique developmental structures, or, in adults to form repair blastemas that are able to achieve regenerative repair, a fact that makes the study of these mesenchymal stem cells crucial as it provides the basis for the evolution of a new therapeutic technology of self-cell repair. Several important advances allow us to consider the possibility of using a patient's own mesenchymal stem cells as starting material for tissue repair protocols.

Mesenchymal stem cells must exist to maintain the living organisms, just as hematopoietic stem cells must exist to support both red and white blood cell turnover.

We might be able to isolate such human mesenchymal stem cells and place them in cell culture, where we could mitotically expand their numbers. Eventually, if we had enough of these cells, we could reintroduce them into the original donor in a manner that guaranteed that they would massively differentiate into a specific tissue, such as cartilage or bone, at a transplantation or repair site. Immunorejection would not be a problem because the donor and host would be one and the same.

1.2 Morphogen & Pattern Formation

A morphogen is a substance that establishes a graded distribution and elicits distinct cellular responses in a dose dependent manner that governs the pattern of tissue development in the process of morphogenesis or pattern formation.

They function to provide individual cells within a field with positional information, which is interpreted to give rise to spatial patterns. Morphogens can consist of cytoplasmic proteins, such as transcription factors that form a gradient by diffusion within a single cell or syncytium, or secreted signaling molecules that travel from cell to cell. In most cases, morphogens guide the generation of different cell types in a

specific spatial order, usually by inducing unique transcriptional responses in a dose dependent manner. Once the gradient has formed, cells must distinguish small differences in morphogen concentration and store this information even after the gradient has dissipated.

Pattern formation is the process by which a specialized cell, the fertilized egg, divides to give rise to seemingly identical cells. Over time and space, these cells take on distinct fates and eventually become organized into functional organ systems. Within a single organ, such as the heart, multiple classes of differentiated cells must synchronize their duties to ensure that the organ as a whole is functional. One of the unifying principles of development is the idea that embryogenesis proceeds via the iterative process of generating naive fields of cells, and then providing cells within each field with unique positional information, which they then interpret to give rise to spatial patterns. Through this process, the embryo is sequentially subdivided, initially along the major body axes, and then into smaller, and more refined units such as organ primordia that are further partitioned and patterned

2 Model

2.1 Parameter selection

In order to proceed this reaction-diffusion system for VMC pattern formation process, Alan Garfinkel and his partners made the following choices. The first choice is that they treated this model as 2D spatial domain in 100*100 uniform mesh. Meanwhile the activator and inhibitor are BMP-2 and MGP corresponding to $U(x,y)$ and $V(x,y)$ (effective concentrations). The method to calculate the final concentrations for BMP-2 and MGP is the use of second order Runge-Kutta method. Moreover the chemical kinetics they chose were the interactions of BMP-2 with MGP. Additionally, in order to govern the model equations, the initial condition is described as following *“The initial condition of U and V that are uniformly distributed with a 2% random perturbations”* as well as neumann conditions (no-flux at boundary) for their boundary conditions. The whole equations are the following:

$$\frac{\partial U}{\partial t} = D(\nabla^2 U) + \gamma \left[\frac{U^2}{(1 + kU^2)V} - cU \right] \quad [1]$$

$$\frac{\partial V}{\partial t} = \nabla^2 V + \gamma(U^2 - eV + S) \quad [2]$$

In these above equations, D is the ratio of diffusion coefficient of BMP-2 and MGP which is D_u / D_v (BMP-2 and MGP respectively). γ is the scaling parameter which is equal to $(L^2 / D_v)(1/T_c)$, where L is the length of the domain which is the actual

size in their experiments, T_c is the time for BMP-2 synthesis. k is the saturating value. c and e are the first order degradation rates for BMP-2 and MGP. S is source term.

More precisely, in equation 1, BMP-2 will activate its own production autocatalytically which will saturate. Hence Garfinkel et al chose $\frac{U^2}{1+kU^2}$, a sigmoidal form, to govern this autocatalysis. V is treated as the inhibitor in the denominator. There is a degradation for U at rate c . In equation 2, U^2 is used to represent that BMP-2 spurs MGP in non-linear manner. There is a degradation for V at rate e . S represents the exogenous MGP which is added by purpose.

In order to run the equations smoothly, the parameters were chosen carefully. Firstly, Garfinkel et al took the initial value of D_u , $1 \times 10^{-8} \text{ cm}^2 / \text{sec}$, directly from [Entchev et al's](#) work which was calculated by using GFP labeling and imaging. Then they considered the diffusion of large amount of molecules in nonlinear slowing manner. Due to acid PH, dimerization of BMP-2 and actual tissue culture, the estimated value reduced to $0.15 \times 10^{-8} \text{ cm}^2 / \text{sec}$. Because there is no directly calculated value for diffusivity of MGP, they estimated an theoretical approximate value, $30 \times 10^{-8} \text{ cm}^2 / \text{sec}$, which was based on empirical formulas. Meanwhile, this approximate value is similar to the diffusion coefficient of amyloid B which is $50 \times 10^{-8} \text{ cm}^2 / \text{sec}$. Finally, the value of D was calculated by $\frac{0.15 \times 10^{-8}}{30 \times 10^{-8}} = \frac{1}{200}$.

In the previous experiment which was done by [Ghosh-Choudhury et al](#), it shows that BMP-2 autoregulates in a saturating manner. However, due to unknown level of MGP, it is difficult to establish the precise value of k . In Garfinkel et al's model, they chose $k=0.65$.

In order to calculate γ , it is essential that production rates are known. In previous work, they have found the upper limit for the production rate which is 3-20ng/hr in embryonic kidney cells with a cytomegalovirus (CMV) promoter. They also established the rates for BMP-2 production in calcifying vascular cells and in

endothelial cells which were both 0.06-0.12ng/hr . Furthermore, FLAG-tagged MGP and newly developed ELISA suggested that the production rates of BMP-2 and MGP are similar. These reports can inform that the upper limit for the production rates is 0.06ng/hr and the lower limit is 20ng/hr .

As mentioned above, γ is equal to $(L^2 / D_v)(1 / T_c)$. In the actual cell culture, the L is 4cm, T_c they chose is 1 ng/hr (3.6×10^3 sec). Hence the

$$\gamma = \left(\frac{L^2}{D_v}\right)\left(\frac{1}{T_c}\right) = \left(\frac{16cm^2}{3 \times 10^{-7} cm^2 / sec}\right)\left(\frac{1}{3.6 \times 10^3 sec}\right) = 15000$$

Garfinkel et al estimated the degradation rate of BMP-2 directly and conservatively from the calculation of Entchev et al's work. Hence they took $c = 0.01$. MGP has more rapidly degradation than BMP-2 based on their unpublished work. The ratio for $\frac{c}{e}$ is approximately $\frac{1}{2}$. Thus they took $e = 0.02$.

The source term S is to be added in order to make tripling pattern formation which means the source term needs to be three times the initial concentration of MGP. In the model, Garfinkel et al chose the initial value of MGP scaled as 2. Then for the 1000 time steps, the S needs to be 0.06 per time steps, totally 6 which will make tripling pattern formation.

2.2 Validity of the model

In this model, patterns are sensitive to the changes of parameters such as γ and S . However patterns are not sensitive to other parameters such the diffusion coefficients. For example, If we need the patterns to be model-doubling, we only need to change the magnitude of γ (ie double it). If we want the patterns from stripes to spots, we can increase the source term S (ie add exogenous MGP). As long as the ratio of the diffusion coefficients remains small (ie less than 1), whatever magnitude you choose, the pattern formation will remain at powerful manner.

The reaction-diffusion systems have common properties, rapid diffusion of inhibitor and nonlinear inhibitor and activator autocatalysis. The reasons why Garfinkel et al chose this particular reaction-diffusion equations are the followings. The known physiological variables are well defined in the equations in terms of structure as well

as the relationships in their experiments. Meanwhile, they referred other researchers' excellent study by [Kondo and Asai](#), which have two-variable reaction-diffusion model with activator and inhibitor. There are other reaction-diffusion models can be established in order to model cells self organization. According to [Garfinkel et al](#), the patterns produced in their experimental preparations are highly similar with the patterns produced by an entire family of reaction-diffusion models.

The advantage of their model is more typical of the adult tissue. They only have a single cell type (vascular mesenchymal cell) and two morphogens (BMP-2 and MGP). However, there are more than one cell type in other researchers' experiments. Because they use two-variable model (U and V), they do not need any cell behavior assumptions to predict the spatial patterns which will be correctly. There is one thing which needs to be remarked in their model is that the patterns in the experiments are the patterns of cells. However the patterns in the model are the patterns of the morphogen.

In order to extend the model, they studied three-variable reaction-diffusion model invoking the researches by [Keller and Segel](#), and [the model of Painter, Maini and Othmer](#). The third variable they used is the cell density, n. The equation for n was invoked from [Painter, Maini and Othmer](#). In [Painter, Maini and Othmer](#)'s work, they combined a tendency to diffuse with a tendency to follow the activator chemical U. The full model is the following.

$$\begin{aligned}\frac{\partial u}{\partial t} &= \nabla \bullet (D \nabla u) + f(u, v) \\ \frac{\partial v}{\partial t} &= \nabla \bullet (\nabla v) + g(u, v) \\ \frac{\partial n}{\partial t} &= \nabla \bullet \left(\nabla n - \frac{x_0}{(1+u^2)} n \nabla u \right)\end{aligned}\quad \begin{aligned}f(u, v) &= \gamma \left(\frac{u^2}{(1+ku^2)v} - cu \right) \\ g(u, v) &= \gamma(u^2 - cv + s)\end{aligned}$$

The similar predictions were produced comparing with simplified two-variable model.

To understand the relationship between u and n, it is easy to consider one spatial domain for the third differential equation which has the form $\frac{\partial n}{\partial t} = \nabla J$, where J is the flux and is the everything inside the brackets. In the steady state, the left hand side is 0 which leaves right hand side, the gradient J, equals to 0.

Hence, $0 = \nabla n - \frac{x_0}{(1+u^2)} n \nabla u \Rightarrow 0 = \frac{\partial n}{\partial x} - \frac{x_0}{1+u^2} n \frac{\partial u}{\partial x}$. Then we can integrate it directly

to get $n = e^{x_0 + \arctan(u)}$. Since $\arctan(x)$ is a monotonically increasing function, so is n.

From above, the distribution of n is parallel to the distribution of u at steady state which means the distribution of cells will pursue the distribution of activator.

2.3 Additional model predictions

Ghosh-Choudhury et al did a experiment which supports Garfinkel et al's prediction "the autocatalysis of U will saturate". In this experiment, Ghosh-Choudhury et al use "luciferase activity" to measure the transcriptional activity of the BMP-2 promoter. Gierer and Meinhardt used other model which predicted that U would

increase autocatalically without bound. In their model, $f(U, V) = -bU + \frac{U^2}{V}$.

In Garfinkel et al's model, the degradation of inhibitor is greater than the degradation of activator. They made this prediction because based on Koch and Meinhardt's observation, the patterns in Gierer and Meinhardt's model will be formed only if the inhibitor degrades more rapidly than the activator which will be supported by experiment finding. In Koch and Meinhardt's work, they made a criterion which indicates that the ratio of inhibitor degradation rate to activator degradation rate should be $> (2/a_0 - 1)$, where a_0 is the equilibrium value of the activator. In Garfinkel et al's model, the ratio is 2 and a_0 is 1.1. Hence $2 > 0.8$ which satisfies the criterion.

The final prediction in the model is about Turing space. Based on the work of Murray, it shows that the stable pattern formation is supported by saturation of model. When the stable patterns are formed, the model parameters fall into a pattern formation region in Turing space which satisfy the following conditions at steady states:

$$\begin{aligned} f_u + g_v &< 0 \\ f_u g_v - f_v g_u &> 0 \\ D_2 f_u + D_1 g_v &> 0 \end{aligned}$$

2.4 Refinement for case of homologous inhibitors

The homologous genes of MGP may be induced when MGP is at very low concentration. If this is a case, it can be modified by replacing V with $V+C$ where C is compensatory inhibitory activity of the homologous genes, a constant. A example was made by choosing $C=0.5$.

3.Simulation

4.Conclusion