國立中興大學物理學研究所

生物物理組碩士學位論文

以擴散反應方程重現適合幹細胞生存之環境

Using Diffusion-Reaction Equations to Reproduce the Suitable Environment for Stem Cells in Tissues

指導教授: 廖思善 (Sy-Sang Liaw)

研究生: 李昀融 (Yung-Rong Lee)

中華民國一百零一年六月

國立中興大學 物理 學研究所

碩 士學位論文

(Using Diffusion-Reaction Equations to Reproduce the Suitable Environment for Stem Cells in Tissue)

題目：以擴散反應方程重現適合幹細胞生存之環境

姓名：李 明 融　　學號：709701600l

經 口 試 通 過 特 此 證 明

論文指導教授

論文考試委員

中華民國 101 年 6 月 12 日

謝誌

在物理所兩年的學習時光即將完成，回想當初到中興大學應考時心中的忐忑，會反覆自問我身為一個物理的門外漢憑什麼妄想在物理所能畢的了業。但在進中興大學後，卻非常慶幸能在這個專注於真理的討論，而沒有太多階級和繁文縟節的殿堂學習。感謝我的指導教授廖思善教授所給予的完全的自由和幫助，紀凱容老師和阮俊人老師讓我見識到生物物理的不同而廣泛的面向，施明智老師和李秉政老師將統計熱力學闡釋的極為清楚，讓我能看見物理和生物的聯結比我原先所想更遠為深遠，林立老師、郭華丞老師和林中一主任幫助我對艱深而基礎的量子力學有一概括的認識。劉瑞堂學長慷慨地將對擴散反應方程的模擬經驗與我分享，而曾飛煥學長則容忍我的摸索和不精確，不知花費多少時間帶我一步一步建立起這個模型。而同實驗室的同學和學長姊，芝辰、俊榮、宛芸學姐、素娥學姊和書賢學長都不同程度的幫助我學習和成長。

最後則要感謝我的家人的諒解和支持。我哥哥以物理的前輩每每在重大的問題前面為我指路，我的父母給予情感和經濟的支持讓我得以沒有後顧之憂，我太太則無怨無悔地照顧好不滿一歲的兒子，每週末回去探望他們已成為我最大的安慰和動力。

我的主要研究興趣在於癌症和胚胎發育的研究。而這次當我在幾番痛苦的掙扎幾近放棄時，才決定下要以幹細胞凹陷為主題。而當我決定下我的題目時我也充滿感謝，因為我明白了我需要的工具就在我的身邊，學長们和老師正是對擴散反應方程有很深入的理解。這讓我想到在小說"伏藏"中看到的一段話，其大意是：人們都希望發掘出重大秘密的人是自己，卻不知其實重大的秘密也希望找到夠資格的人把它們發掘出來，此時是否願意相信自己並堅持下去就是重要的關鍵。我很慶幸老師和學長们都是具有很深遠的目光而意志堅定的人，能在我徬徨時為我指引，在我幾欲放棄時拉我一把。因而當我最後仔細思索我的模型而發現它遠比我原先料想的更好時，我充滿感謝。最後，我以在物理領域中獲得極大的幫助，感謝一路上數不盡的扶持和指導，希望未來有機會能扮演好臨床和基礎科學間的橋樑，幫助更多人能感受我所獲得的感動與歡喜。

Abstract

The stem cell niche is a 3-dimensional structure in which stem cells reside. The stem cells in niche can divide and produce progenitor cells, however, the further differentiation into mature cells can not occur until the stem cells leave the niche. Now the stem cell niche has been discovered to be broadly distributed in many tissues such as bone marrow, brain, intestine, and skin et al. For the important applications in regeneration and cancer research, there have been many scientists to devote into the area of stem cell niche study. However, the consistent conclusions for the size and environment of stem cell niche are still not obtained. This problem encourages us to think the original purpose of stem cell niche. We believe that the acidic hypoxic environment is the stable environment which should be originally provided by the stem cell niche. Then we simplify the glycolysis and oxphos reactions to build up the diffusion-reaction equations for the lactic acid and oxygen and reproduce the spatial distribution of lactic acid and oxygen in normal tissues. By solving the diffusion-reaction equations in our model, we have obtained the results as follows: (1) There is different oxygen levels for different types of tissue, and this can be reproduced in our model. (2) We found that if the behavior of lactic acid and oxygen in tissues is really regulated by our diffusion-reaction equations, then the hypoxic acidic and normoxic high pH environment would naturally exist. (3) We have known that the gradients of growth factors play an important role in inducing differentiation. We postulated that the stem cells would reside in hypoxic acidic environment where the gradients of both lactic acid and oxygen are low. Moreover, in our model, the gradients of both lactic acid and oxygen are small in skin, which may explain why epithelium-origin cancers are

common. On the other hand, the gradients of both lactic acid and oxygen are large in heart which may explain why there is almost no cardiomyocyte-origin cancer noted.

Key words: stem cell niche, diffusion-reaction equations, cancer

中文摘要

幹細胞凹陷是一三維結構，幹細胞身處其中可分裂和製造前驅細胞，但前驅細胞若要進一步分化成成熟細胞則必須在離開幹細胞凹陷後才會發生。 幹細胞凹陷已被證明廣泛存在於不同組織中，且因為其在再生醫學和癌症治療領域的重要性而吸引大量學者投入其中。然而經過十年以上的努力，仍未能對幹細胞凹陷的大小和環境有清楚而一致的共識。 這個困境便幫助我們回頭反思當初提出幹細胞凹陷的理由，我們相信幹細胞凹陷存在的目的是為了提供幹細胞穩定適當的環境，而現今已有大量證據支持幹細胞和癌細胞偏好處在低氧酸性環境。 於是我們簡化了無氧呼吸和有氧呼吸的反應式並歸納建立出乳酸和氧氣的擴散反應方程，並應用福傳程式做模擬以試圖複製出組織中乳酸和氧氣的濃度梯度空間分佈。 根據我們的模型，我們得到的結果如下: (1) 不同的組織具有不同的氧氣閾值已被廣泛認可，而此項特徵在我們的模型中會自動產生; (2) 若乳酸和氧氣在組織中的行為的確由我們模型中的反應擴散方程所調控，則低氧酸性環境和高氧鹼性環境本來就自然會交替出現在組織中。 (3) 我們已知濃度梯度在誘導細胞分化扮演極重要角色。 我們提出假設，幹細胞所適合的環境條件除了已知的酸性低氧外，還需要加上低濃度

梯度。 此外，在我們的模型中，乳酸和氧氣濃度梯度在上皮組織中都最小，此現象或許能解釋為何臨床上上皮組織來源的癌症最常見；另一方面，乳酸和氧氣濃度梯度在心肌組織最大，此現象可能可以解釋為何臨床上幾乎沒有心肌細胞來源的癌症。

關鍵字: 幹細胞凹陷，擴散反應方程，癌症

Table of contents

List of Tables

List of Figures

Chapter I

Introduction and Literature Review

Background and purpose

Stem cell niche has been widely considered to be necessary for stem cells maintenance. Moreover, due to its potential applications in regeneration and cancer field, many researchers have devoted into studying the structure and environment of stem cell niche. However, there is still no consensus for the size and environment of stem cell niche. In this research, we try to find out the basic characteristics of stem cells and cancer cells in order to define what is the stable microenvironment needed for stem cells.

1.1 Introduction of the stem cell niche

The "stem cell niche" was first described in 1978[1]. However, it was neglected until first observed in 2000[2]. Because the stem cell research is very important in regeneration and cancer research, many cell biologists tried to isolate the stem cells in vitro for further studying. They usually found that the stem cells which should be pluripotency in vivo could differentiate into certain types of cells. The discovery of "stem cell niche" reminded us that the environmental factors may play an important role in stem cell regulation. The stem cell niche is a 3-dimensional structure (Figure 1) in which only one or two stem cells reside. Stem cells in the niche can produce progenitor cells, but further differentiation into mature functional cells could just happen after the progenitor cells leave the stem cell niche [3]. The existence of stem cell niche has been verified to be distributed in different tissues[4]. The discovery of stem cell niche encouraged scientists to devote into studying the characteristics and structure of stem cell niche. However, for over ten years of efforts, there is still no consensus for the size and environment of stem cell niche[5]. This problem helps us to re-consider the origin and purpose of stem cell niche. We believe that the stem cell niche is providing stable environment for maintaining stem cells. The most important question may be what is the stable environment needed for stem cell maintenance.

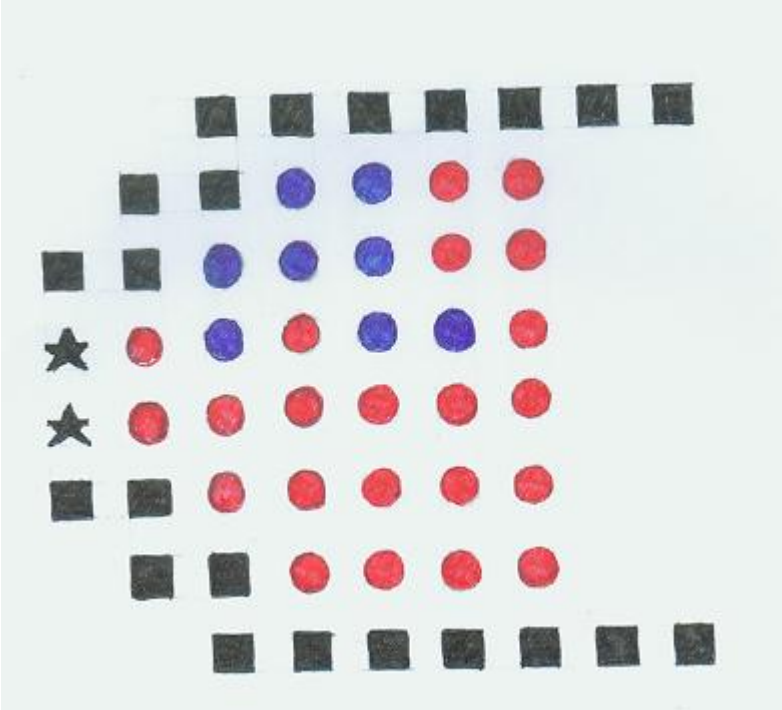

Figure 1. *The black star represents the stem cells, the square means the surrounding epithelial cells, and the red and blue circle means progenitor cells. The further differentiation into mature cells can not happen until the progenitor cells leave the stem cell niche (3).*

1.2 The microenvironment of stem cell niche

In a review of 2011 [6], it showed that the stem cell niche is usually located in the low pH value and hypoxic microenvironment of normal tissue (Figure 2). The experimental facts are not only observed in bone marrow but also verified in the neuronal stem cells, embryonic stem cells, as well as induced pluripotent cells. The stem cells are usually found away from the vasculature. On the other hand, if the cells need to differentiate further into mature cells, they will move closer to the vessels, which are under higher pH value and higher pO_2 tension conditions. The movement from lower pO_2 tension area to higher pO_2 tension area corresponds to the switch from the glycolysis mode to the oxidative phosphorylation (OXPHOS) mode, which are two main metabolic modes of animal cells. In the next part, we will introduce these two important metabolic modes in more details.

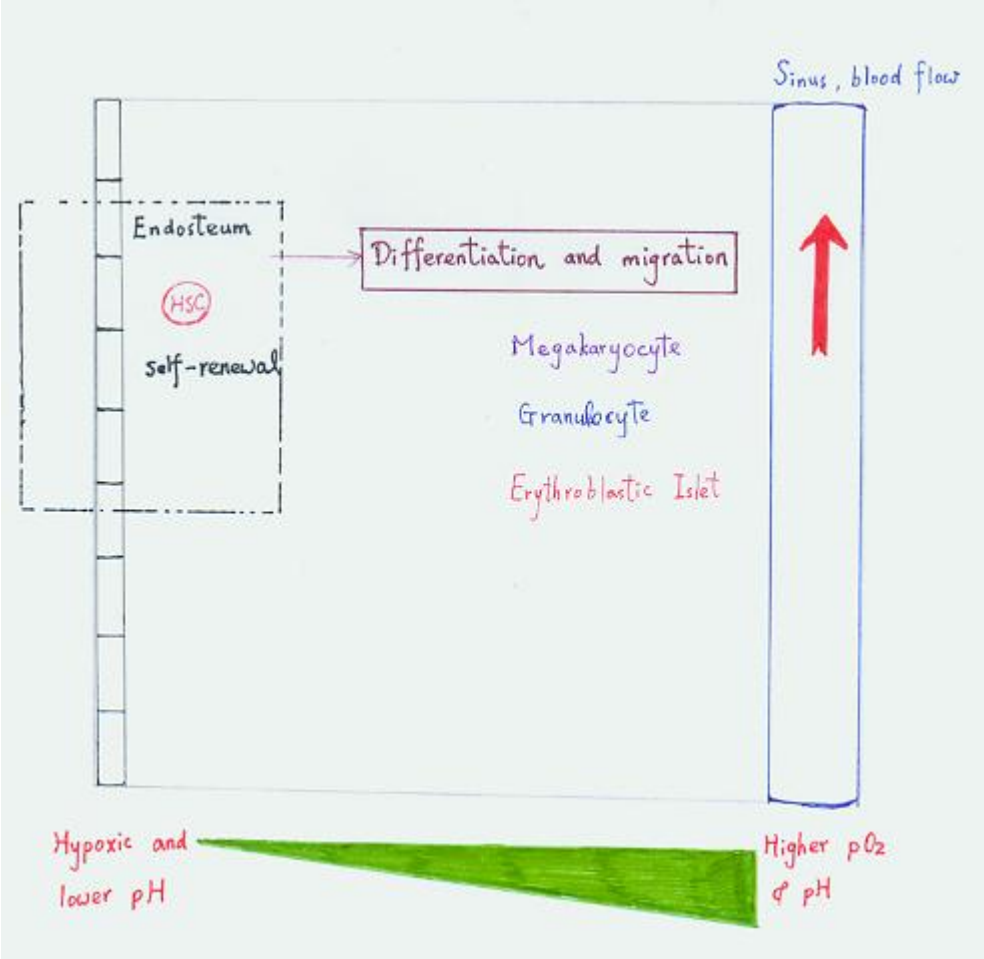

Figure 2. *The hematopoietic stem cell niche seems to prefer to reside in the acidic and hypoxic area. On the other hand, the cells which would differentiate into the mature cells would move closer to the perivascular area which is with higher pH value and higher* pO_2 *tension area (6).*

1.3 Glycolysis and OXPHOS

The glycolysis and oxidative phosphorylation (OXPHOS) are two main sources of ATP production which is the most important energy provider for all biochemical reactions in cells. There are 10 and 8 steps in glycolysis and oxphos, respectively. For simplifying the discussion, we will only introduce the main reactions in glycolysis and oxphos. If the readers are interested in the details of glycolysis and oxphos, please see chapter 2 of reference 7. The glycolysis briefly occurs in the cytosol, and the main reaction is to oxidize 1 molecule of glucose to produce 2 molecules of pyruvate, 2 molecules of NADH, and 2 molecules of ATP. If cells are under hypoxic conditions, the pyruvate will not enter the mitochondria for further oxidation, called the citric acid cycle or Krebs cycle, and the 2 molecules of pyruvate will then be transformed into 2 molecules of lactic acid. On the other hand, if the cells are under normoxic conditions, the 2 molecules of pyruvate will enter the mitochondria and be further oxidized through the Krebs cycle in the matrix of mitochondria to produce 8 molecules of NADH, 2 molecules of $FADH_2$, and 2 molecules of GTP. The NADH and $FADH_2$ are both high-energy electrons carriers. Then the final products of Krebs cycle (8 NADH+2 $FADH_2$) will be transferred into the inner membrane of mitochondria to donate their high-energy electrons to electron-transfer-chain to produce proton motive force and then to produce about 28 molecules of ATP. The reactions occur in the mitochondria, Krebs cycle and the electron-transfer-chain, are called oxidative phosphorylation(OXPHOS). The net products of glycolysis and

oxphos are listed in the table 1[7]. Besides this, we should emphasize that the metabolic modes of cells can not be simply classified into glycolysis or oxphos. They are often combined with different ratio, if one type of cells differentiate into more mature cells, they may change to use higher ratio of oxphos mode than their progenitor ones. Finally, although the oxygen level is used to discriminate the glycolysis mode from the oxphos mode, oxygen does not directly join in the glycolysis or Krebs cycle. Oxygen is depleted only at the end of the electron-transfer-chain as the final electron receptor and produces water.

I. glycolysis (in cytosol)
(1) 1 glucose→ 2 pyruvate+ 2 NADH+ 2ATP *(2) 2 pyruvate→ 2 lactic acid*
II. oxphos (oxidative phosphorylation, in mitochondria)
(3) 2 pyruvate→ 8 NADH+ 2 $FADH_2$+ 2 GTP

***Table 1**. The net products of glycolysis and oxphos (7).*

1.4 The metabolic switch from oxphos to glycolysis is a common feature in all proliferating cells and most tumor cells

There is one review paper published in 2011 showing that normal nontransformed proliferating cells and tumor cells are known to have a high glycolytic activity even in the presence of adequate oxygen level, a phenomenon named aerobic glycolysis or the Warburg effect. Authors of the paper also pointed out that the aerobic glycolysis play a major role in tumor growth and carcinogenesis[8][9]. We want to point out that according to this review paper, the switch between the oxphos and glycolysis mode seems more sensitive to pH value change than to oxygen level, and this result will be used in our following discussion.

1.5 Conclusion of literature review

According to what has been discussed above, it is believed that the acidic hypoxic microenvironment is needed for stem cells and tumors, and the stable environment is considered to be the most important condition for existence of the stem cell niche. On the other hand, the glycolysis mode has been proved as the basic feature of stem cells, most tumor cells, and all proliferating cells, and the lactic acid is spontaneously produced under the glycolytic mode. So we postulate that the acidic hypoxic microenvironment would naturally exist in the normal tissues, and a specified structure for stem cell maintenance seems not necessary. In the next chapter, we will use the diffusion-reaction equations to reproduce the spatial distribution of lactic acid and oxygen in normal tissue (Figure 2).

Chapter II

Methods

2.1 Building up the diffusion reaction equations for lactic acid and oxygen

We simplify the glycolysis and oxphos reactions mentioned in the section 1.3 by the 5 reactions listed below:

$$a \rightarrow 2P + 2N \tag{1}$$

$$d \rightarrow O_2 \tag{2}$$

$$P + N \xrightarrow{k} \text{x} \tag{3}$$

$$2NADH + 2H^+ + O_2 \xrightarrow{c} 2NAD^+ + 2H_2O \tag{4}$$

$$2P \xrightarrow{b} 10N \tag{5}$$

In reaction (1), we neglect the 10 steps of glycolysis and postulate a source a to provide 2 molecules of pyruvate (P) and 2 molecules of NADH (N); In reaction (2), we postulate that there is a source d to provide oxygen O_2 (v); In reaction (3), if the cells are under hypoxic conditions, the pyruvate (P) will not enter the mitochondria and will be

transformed into lactic acid (u) by NADH (N). The reaction would be progressed with reaction rate k; In reaction (4), in the electron-transfer-chain on the inner membrane of mitochondria, 2 molecules of NADH which come from glycolysis or oxphos would donate 4 electrons to deplete 1 molecule of O_2 (v) to produce 2 molecules of NAD^+ and 2 molecules of water. This reaction would be progressed with constant rate c; In reaction (5), if the cells are under normoxic conditions, the pyruvate (P) will enter mitochondria to be further oxidized through the Krebs cycle in the matrix of mitochondria to produce 8 molecules of NADH (N) and 2 molecules of $FADH_2$. We also neglect the 8 steps of Krebs cycle. For simplifying this problem, we postulate the 2 molecules of pyruvate (P) would be oxidized to produce 10 molecules of NADH (N). This reaction would be progressed with reaction rate b. Finally, we also have to emphasize that because the glycolysis and oxphos only occur within cell, the co-product lactic acid (u) and oxygen (v) are the only two molecules which can diffuse in tissues in our case.

$$\frac{dN}{dt} = a + bP - cN^2v - kNP \tag{6}$$

$$\frac{dP}{dt} = a - kNP - bP \tag{7}$$

$$\frac{du}{dt} = kNP - eu \tag{8}$$

$$\frac{dv}{dt} = -cN^2v + d \tag{9}$$

Based on the 5 reactions listed above and the approximation we have made, we obtain four ordinary differential equations for NADH (*N*), pyruvate (*P*), lactic acid (*u*), and oxygen (*v*). In equation (8), we let the lactic acid diffuse with a rate proportional to its concentration. For solving the four equations listed above, we let both equation (6) and (7) equal to a constant respectively as follows:

$$\frac{dN}{dt} = a + bP - cN^2v - kNP = \alpha \tag{10}$$

$$\frac{dP}{dt} = a - kNP - bP = \beta \tag{11}$$

In these two equations, we let α and β be constants. Then we calculate values of kNP and $-cN^2v$, and find out that both of them would be related to *a*, α, and β. In other words, the rate of production of lactic acid and depletion of oxygen would be related to the rate of glycolysis (*a*), α and β (glycolysis and oxphos). In the section 1.4, we postulated that the switch between glycolysis and oxphos seems more sensitive to pH value than oxygen level, so the weight of lactic acid (*u*) is more important than oxygen (*v*), thus it is reasonable to use u^nv with n>1 to describe the interaction between *u* and *v*. Here we use the simplest form u^2v to represent the production rate of lactic acid (*u*) and depletion rate of oxygen (*v*).

2.2 Defining the new parameters and allowable space

Summing up all the approximations we made in section 2.1, we write our diffusion-reaction equations for lactic acid and oxygen as follows:

$$\frac{\partial u}{\partial T} = D_u \frac{\partial^2 u}{\partial L^2} + \alpha \rho' u^2 v - \gamma e u' \tag{12}$$

$$\frac{\partial v}{\partial T} = D_v \frac{\partial^2 v}{\partial L^2} - 2\beta \rho' u^2 v + \delta e \tag{13}$$

where

$$[u] = [v] = [\rho'] = [e'] = \frac{1}{L}$$

$$[\alpha] = [\beta] = \frac{L^3}{t}$$

$$[\gamma] = \frac{L}{t}$$

$$[\delta] = \frac{1}{t}$$

$$[D_u] = [D_v] = \frac{L^2}{t}$$

In these equations, we set the depletion rate of oxygen two times of the production rate of lactic acid, the ratio affects only the width of our allowable parameter space but not the behavior of our model which is

determined by the nonlinear term u^2v. D_u and D_v represent the diffusion coefficients of lactic acid and oxygen in tissue, respectively. According to the experimental values of D_u and D_v [10][11](5.0x10^{-6}-6.0x10^{-5} cm^2/sec and 1.0x10^{-4} cm^2/sec , respectively), we approximately let $D_v = 20D_u$. ρ' stands for the cellular density, and e' represents the capillary density. Now we have seven independent parameters: α, β, γ, δ, ρ', e', and D_u. For simplifying these parameters in our simulation, we set all simulations to be within unit length in solid tissues (ex: brain, skin, heart, liver…etc), so the concentration of lactic acid and oxygen will be dimensionless. Then we also define t and r in units of our eigen-time T_{eigen} and eigen-length L_{eigen}, respectively, so we can get two new diffusion-reaction equations corresponding to equations (14) and (15):

$$t \equiv \frac{T}{T_{eigen}}$$

$$r \equiv \frac{L}{L_{eigen}}$$

$$D_v = 20D_u$$

$$\frac{\partial u}{\partial t} = D_u \frac{\partial^2 u}{\partial r^2} + \rho u^2 v - eu \qquad (14)$$

$$\frac{\partial v}{\partial t} = D_v \frac{\partial^2 v}{\partial r^2} - 2\rho u^2 v + e \qquad (15)$$

$$f(u,v) = \rho u^2 v - eu$$

$$g(u,v) = -2\rho u^2 v + e$$

Where we have set $\alpha = \beta = \gamma = \delta = 1$, and replaced ρ' by ρ, e' by e, and let our u, v, D_u, t, r, ρ, and e to be dimensionless. The important parameters in our final equations are ρ, e, and D_u. According to the reference (12), there are four boundary conditions to be met in defining our allowable space for parameters [12] to yield patterns.

If we let f(u,v)=g(u,v)=0, we will get the stationary points of u and v, designated by u_0 and v_0 respectively. Then u_0 and v_0 are given by

$$u_0 = 0.5, v_0 = \frac{2e}{\rho} \tag{16}$$

The partial derivatives of the function f and g are given by

$$f_u \equiv \frac{\partial f}{\partial u} = 2\rho uv - e \Rightarrow f_u(u_0, v_0) = e \tag{17}$$

$$f_v \equiv \frac{\partial f}{\partial v} = \rho u^2 \Rightarrow f_v(u_0, v_0) = \frac{\rho}{4} \tag{18}$$

$$g_u \equiv \frac{\partial g}{\partial u} = -4\rho uv \Rightarrow g_u(u_0, v_0) = -4e \tag{19}$$

$$g_v \equiv \frac{\partial g}{\partial v} = -2\rho u^2 \Rightarrow g_v(u_0, v_0) = -\frac{\rho}{2} \tag{20}$$

According to the standard linear analysis for a reaction-diffusion equation, the following four conditions have to be satisfied so that possible patterns can emerge.

$$f_u(u_0, v_0) + g_v(u_0, v_0) < 0 \quad \rho > e \tag{21}$$

$$df_u(u_0, v_0) + g_v(u_0, v_0) > 0 \quad \rho < e \tag{22}$$

$$f_u(u_0, v_0) g_v(u_0, v_0) - f_v(u_0, v_0) g_u(u_0, v_0) > 0 \quad \rho > \left(\rho - \frac{e}{2}\right) \tag{23}$$

$$[df_u(u_0, v_0) - g_v(u_0, v_0)]^2 + 4 df_v(u_0, v_0) g_u(u_0, v_0) < 0 \quad \rho > 8e, or \tag{24}$$

From the results of these four equations, we conclude that our allowable space must satisfy the requirement.

$$2e < \rho < 8e \quad \text{or} \quad \rho > 232e \tag{25}$$

The condition $\rho > 232e$ is not considered in our case because under this condition the cellular density ρ is so big that we can neglect the effect of the capillary density *e*.

Next we use Fortran programs to simulate our model, and regulate the three parameters. The effects of our three key parameters will be described in the following chapter, and our Fortran codes are listed in the

Appendix.

Chapter III

Results

In this chapter, we will show that: (1) If the behavior of lactic acid and oxygen in tissues is really regulated by diffusion-reaction equations described above, we can get the spatial distribution of lactic acid and oxygen like the results shown in figure 3, and we can define hypoxic acidic microenvironment suitable for stem cells, and normoxic and high pH value microenvironment suitable for differentiated cells. In addition, we will discuss the behavior of the equations with respect to ρ/e, e, and D_u respectively. (2) According to the results based on the figure 3, the portion of the hypoxic and acidic microenvironment needed for stem cells is large. But we know from experiments that the proportion of stem cells in real tissues is small. Apparently, further restrictions must be imposed in order to locate where stem cells are.

3.1

3.1.1 Effects of ρ/e

If we cut through a tissue, and record the spatial distribution of lactic acid and oxygen on the cutting line, then we could see the spatial distribution of lactic acid and oxygen like figure 3. In figure 3, the horizontal axis is cellular position (-256 to 256), the longitudinal axis is concentration, black curve represents the concentration of lactic acid and the red curve stands for the concentration of oxygen. We can see that if we fix e and D_u (e=0.1, and D_u=4.2) and regulate ρ/e (from 1.0 to 20), the spatial distribution of both lactic acid (black curve) and oxygen (red curve) become larger (from 3-a to 3-b) and then smaller (from 3-b to 3-f).

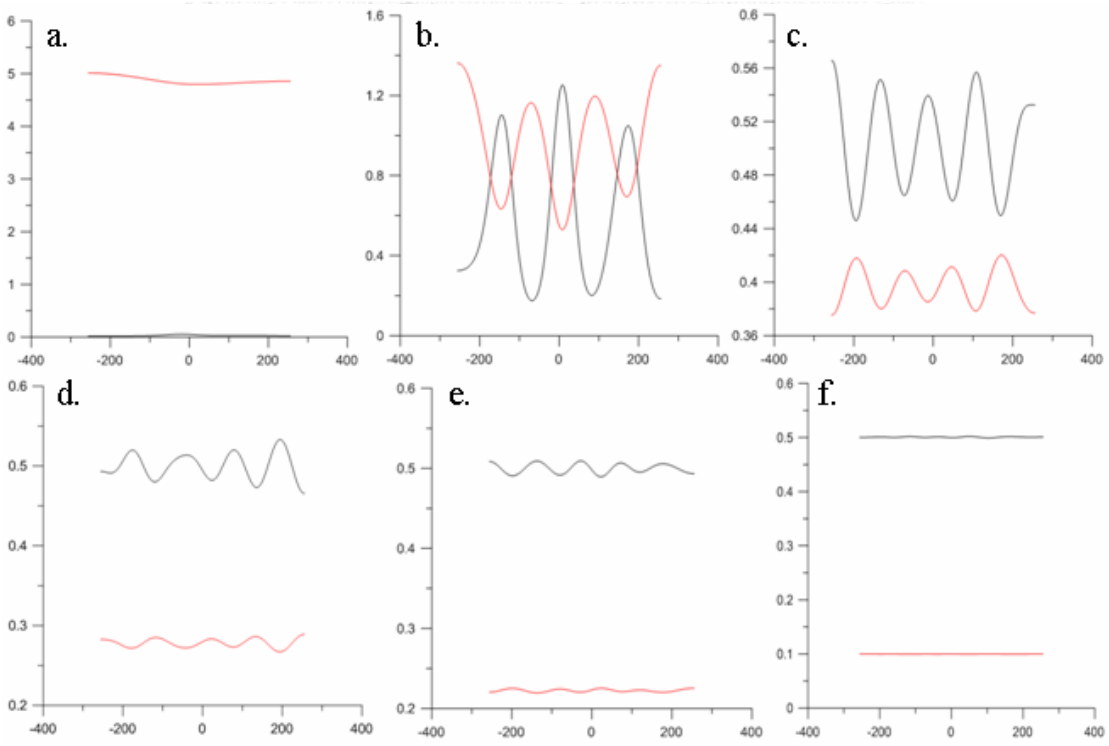

Figure 3. *Concentration of lactic acid (black) and oxygen (red) is a function of cell*

position. e=0.1 and D_u=4.2; (a) ρ=1.0e; (b) ρ=2.1e; (c) ρ=5.0e; (d) ρ=7.2e; (e) ρ=9.0e; (f) ρ=20e

In chapter II, we have defined that the stationary point of oxygen (v_0) equals to $2e/\rho$. It means that when we increase ρ/e , the v_0 will be lower. From table 2, we can easily see that the normoxic pO_2 tension is 17.7% in heart, 10% in liver, and 5.02% in skin [9]. Thus from the point of the oxygen level, it is qualitatively reasonable to let 3-b represent the heart, 3-c represent the liver, and 3-d represent the skin. The value of ρ/e is smallest in 3-b, 3-c intermediate, and 3-d largest within allowable space ($2e<\rho<8e$, $e>0$, and $D_u>0$).

Tissue type	*Normoxic pO_2 level (%)*
Heart	*17.7*
Liver	*10*
Skin	*5.02*

***Table 2**. There are different oxygen threshold in different tissues (9).*

We list some experimental data for heart, liver, and skin in table 3, where we use 1/(cell size) to represent the cellular density ρ, and (capillary density)$^{3/2}$ to represent the capillary density *e*. Now we can set the ratio ρ/e is 1 in heart and calculate the ratio in skin and in liver to heart as follows.

$$\frac{\frac{\rho_e}{e_e}}{\frac{\rho_c}{e_c}} = \frac{(\pi \times 5^2) \times 80 \times (24)^{\frac{3}{4}} \times (100)^{\frac{3}{4}}}{(\pi \times 5^2) \times 65 \times (100)^{\frac{3}{2}}} \cong 154 \quad (26)$$

$$\frac{\frac{\rho_h}{e_h}}{\frac{\rho_c}{e_c}} = \frac{(\pi \times 5^2) \times 80 \times (24)^{\frac{3}{4}} \times (100)^{\frac{3}{4}}}{(\frac{4}{3}\pi \times 15^3) \times (70)^{\frac{3}{4}} \times (100)^{\frac{3}{4}}} \cong 3 \quad (27)$$

In above equations where *e* means epithelium, *h* means hepatocyte, and *c* means cardiomyocyte, we can easily see that the ratio of ρ/e is really largest in skin (epithelium), intermediate in liver (hepatocytes), and smallest in heart (cardiomyocytes), which is consistent with our setting that the figure 3-b means the heart, 3-c represents the liver, and 3-d means the skin. Finally, we have to emphasize that through the figure 3-b to 3-d, when the value of lactic acid becomes larger than u_0, the value of oxygen becomes less than v_o, we called the cells at these positions are in glycolysis mode. On the other hand, when the value of lactic acid becomes less than u_0 and the oxygen level becomes higher than v_o, we called the cells at these positions are under oxphos mode.

Cell types	*Cell size* *1. Diameter (um)* *2. Length (um)*	*Capillary density* *(numbers/mm^2)*
Cardiomyocyte	*1. 10-15* *2. 80-100* [13]	*2400* [15]
Hepatocyte	*1. 30* [13]	*700* [16]
Epithelium *(columnar)*	*1. 10* *2. 65* [14]	*100* [17]

Table 3. *Cell size and capillary density of heart, liver, and skin. (13)(14)(15)(16)(17)*

3.1.2 Effects of D_u

In figure 4, we fix the e and ρ/e ($e=0.1$ *and* $\rho=2.1e$) and regulate D_u which is 4.2 in 4-a, 6.0 in 4-b, and 8.0 in 4-c. We can easily see that when D_u becomes larger, both the lactic acid (black curve) and oxygen (red curve) become more flattened and the wave numbers become less.

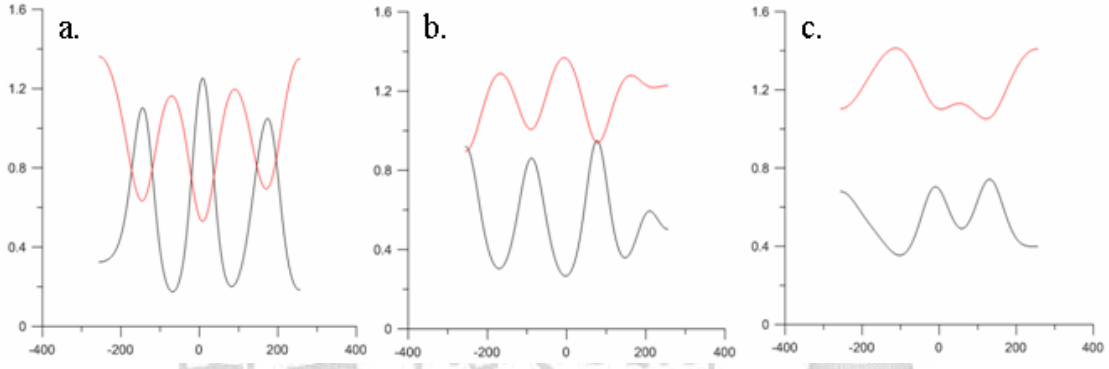

Figure 4. *Concentration of lactic acid (black) and oxygen (red) is a function of cell position. We fixed the e and* ρ/e *(e=0.1 and* $\rho=2.1e$*) and regulate the* D_u*: (a)* $D_u=4.2$*; (b)* $D_u=6.0$*; (c)* $D_u=8.0$

3.1.3 **Effects of *e***

In figure 5, we fix the ρ/e and D_u ($\rho=2.1e$, *and* $D_u=4.2$) and change *e* which is 0.1 in figure 5-a, 0.2 in 5-b, and 0.5 in 5-c. We can see that when the value of *e* becomes larger, the spatial oscillations of both lactic acid (black curve) and oxygen (red curve) become larger and the wave numbers increase.

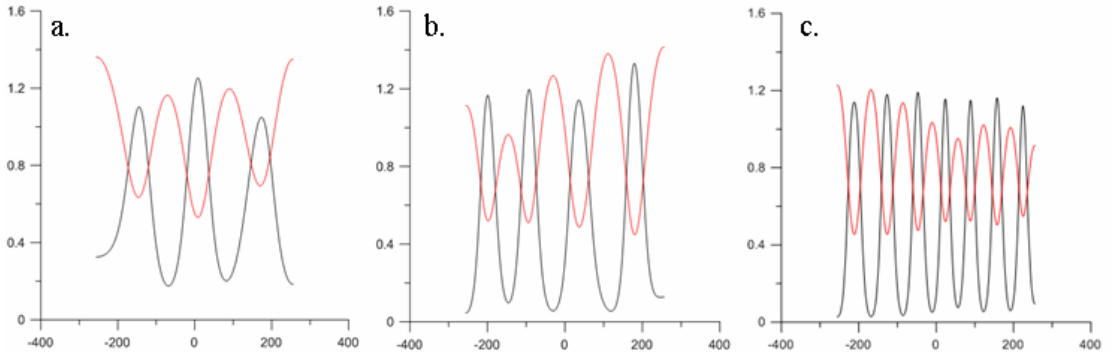

Figure 5. *Concentration of lactic acid (black) and oxygen (red) is a function of cell position.* ρ/e *and* D_u *fixed (*$\rho=2.1e$, $D_u=4.2$*) and changing e: (a) e=0.1; (b) e=0.2; (c) e=0.5*

To summarize all mentioned above, we define one set of (u_0, v_0) to represent one kind of tissue, then we can define $u>u_0$ and $v<v_0$ to represent hypoxic acidic microenvironment needed for stem cells, and $u<u_0$ and $v>v_0$ to represent normoxic and high pH value microenvironment needed for differentiated cells. However, we know from experiments [(3)(5)] that the proportion of stem cells in tissues is small, a fact that is not consistent with results shown in figure 3. It is necessary to find another condition to define the suitable environment for the stem

cells.

3.2 Effects of gradients

It has been widely accepted that the gradient of growth factors could play an important role in inducing the differentiation. According to the results of reference (7) as shown in figure 6, if the gradient of chemical factors from cells B is large enough, the cells C can be differentiated from the cells A. By the same way, when the gradient of morphogens is large enough, the cells D and E can be differentiated from the cells A and B, respectively [7]. Based on these results, we postulate that a hypoxic acidic environment should also have small gradient in growth factors in order to be suitable for stem cells. From figure 3-b to 3-f, we can easily see the slope of both lactic acid and oxygen which represents gradient is smaller and smaller. So we can postulate the suitable environment for stem cells in 3-e and 3-f is much more than in 3-b. For quantitative description, we calculate the slopes of lactic acid at $u=u_0$ and oxygen at $v=v_0$ (see figure 7). The mean of the absolute values of these gradients is plotted, in figure 8 as a function of ρ/e (from 1 to 10). The black curve represents the change of gradient of lactic acid, and the red curve represents the change of gradient of oxygen. From figure 8-a to 8-g, we can easily see that the gradients of both lactic acid and oxygen become larger (from 1 to 2) and then smaller to approach zero (from 2 to 10). When the value of e become larger, the slope of gradient becomes bigger, and the maximal value of gradient becomes larger at the same time (please see the figure 8-d). On

the other hand, when the value of D_u become larger, the slope of the gradient becomes smaller, and the maximal value of gradient also becomes smaller (please see the figure 8-g). Finally, we want to emphasize that: (1) In the figure 3-b to 3-d, when $u>u_0$ and $v<v_o$, the acidic and hypoxic microenvironment is suitable for stem cells, however, we believe that the stem cells still prefer to reside in the local maximum of lactic acid and the local minimum of oxygen because the gradient over here is zero. (2) In figure 3-e and 3-f, the gradient has been too small to induce differentiation, and the microenvironment is also acidic and hypoxic, so we believe the cells under these conditions will be transformed back to stem cells.

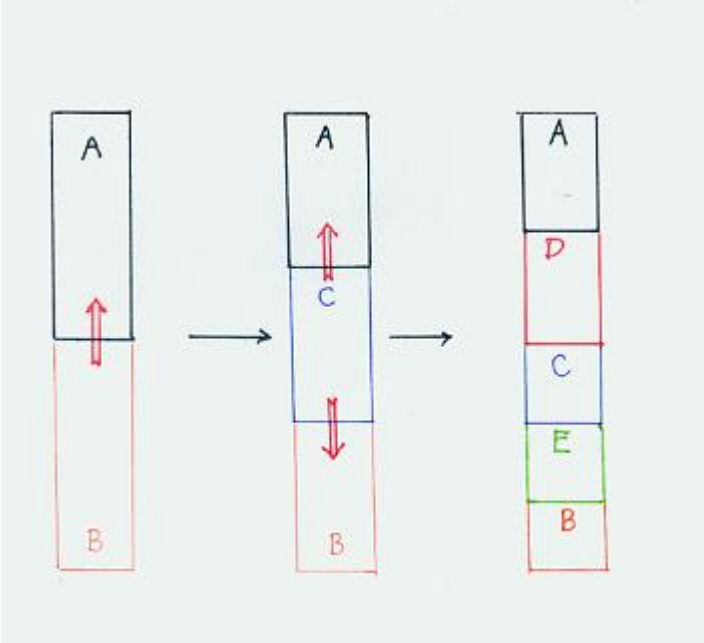

Figure 6. *The gradient of morphogens could play an important role in inducing differentiation. For example, when the gradient of factors from cells B is large enough, the cells C can be differentiated from cells A. By the same way, if the gradient of factors from cells C is large enough, the cells D and E can be differentiated from cells A and B, respectively (7).*

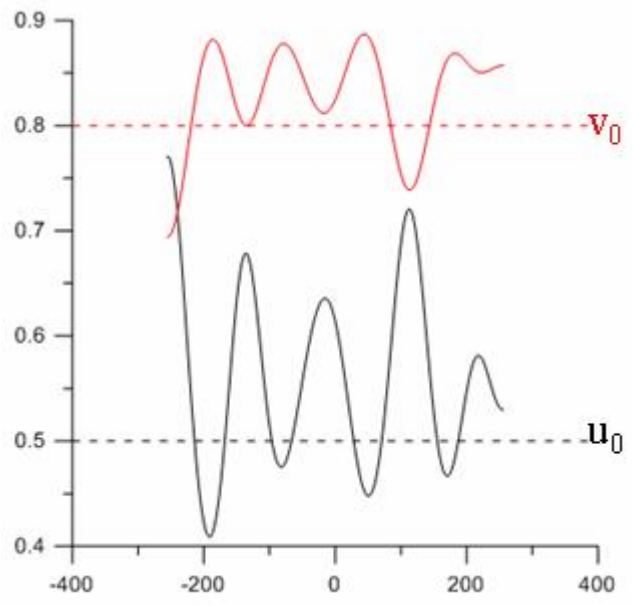

Figure 7. *Mean of the absolute values of the gradients at the stationary points of lactic acid (black) and oxygen (red) (u_0=0.5, v_0=0.8, ρ=2.5e, e=0.1, and D_u=4.2).*

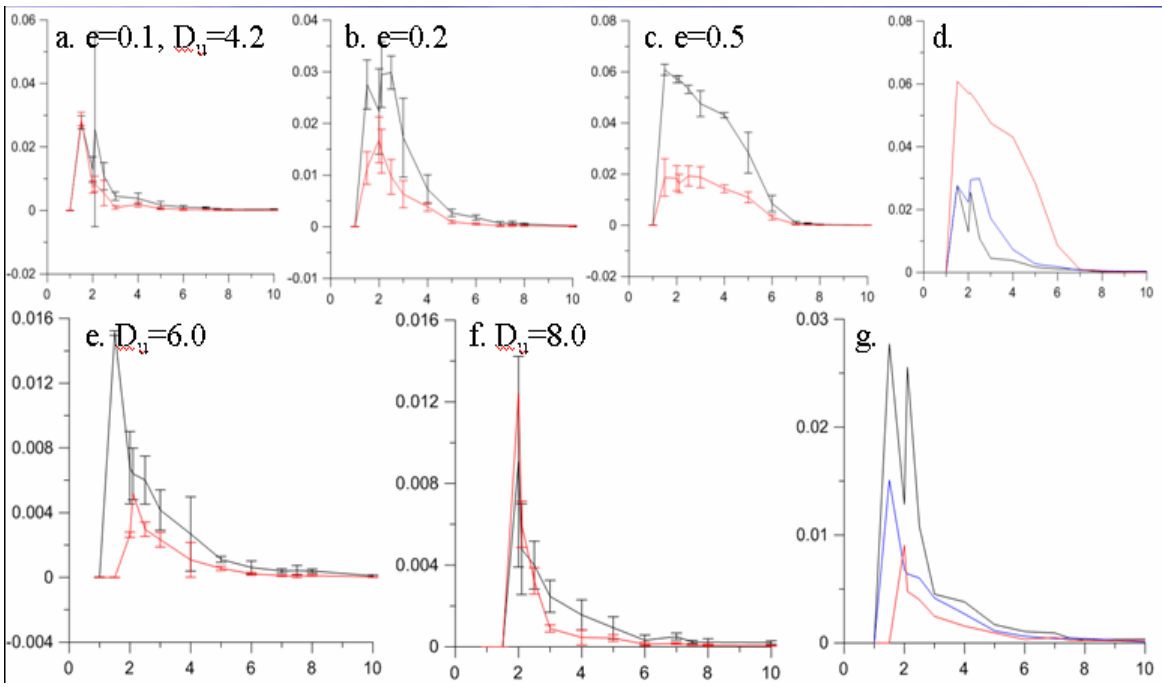

Figure 8. *The change of mean of the absolute values of slope near the stationary points of lactic acid and oxygen are shown. The horizontal axis is the ratio of ρ/e (from 1 to 10), and the longitudinal axis is the value of mean of the slope. (a) e=0.1, D_u=4.2, the black wave represents the lactic acid, and the red wave represents the oxygen. The standard deviation is labeled with the error bar; (b) e=0.2, D_u=4.2; (c) e=0.5, D_u=4.2; (d) Because the behavior of lactic acid and oxygen is similar, we only paint the wave of lactic acid. The black wave is the wave of lactic acid in (a), the blue wave is in (b), and the red wave is in (c). We can see the slope of the gradient becomes larger when the value of e becomes larger; (e) e=0.1, D_u=6.0; (f) e=0.1, D_u=8.0; (g) The black wave represents the wave of lactic acid in (a), the blue wave represents the wave in (e), and the red wave means the wave in (f). We can see the slope of gradient becomes smaller when the value of D_u becomes bigger.*

Chapter IV

Discussion

4.1 The effect of changing *e* and D_u

In the section 3.1.2 and 3.1.3, we have described that when we increase the value of D_u, the spatial oscillations of both lactic acid and oxygen become smoother and the wave numbers become less. On the other hand, when the value of *e* becomes larger, the oscillations of both lactic acid and oxygen become larger and the wave numbers become more. We can also see the same behavior in the figure 8, in figure 8-d, when *e* becomes larger, the slope of gradient becomes larger and the maximal value of gradient becomes bigger. On the other hand, in figure 8-g, when the D_u becomes larger, the slope of gradient becomes smaller and the maximal value of gradient also becomes smaller. In real tissues, the parameters *e* and D_u may be dependent factors, but we still could observe the gradient of pH value and oxygen and evaluate the fraction of maximal value of gradient to check which condition is fitted.

4.2 The importance of gradient

In the section 3.2, we have emphasized that the gradients of lactic acid and oxygen play an important role in inducing differentiation. Based on this concept, in figure 3-b to 3-f, we think when the ratio of ρ/e becomes larger (from 3-b to 3-d), the gradient becomes smaller. If the ratio of ρ/e becomes larger than 8 (figure 3-e and 3-f), the gradient is too small to induce differentiation. If the local microenvironment is acidic and hypoxic combined with small gradient, the cells are maintained to be stem cells, and this may be the phenomenon called cancer. For proving the dedifferentiation which means the differentiated somatic cells can transform back to stem cells is possible, please see our reference 18, in which the Dr. Yamanaka and coworkers have used transcription factors to induce somatic cells back to stem cells [18]. If our model is right, the fact that the ratio of ρ/e is biggest in skin can explain why the epithelium-origin cancers are most common. On the other hand, the ratio of ρ/e is smallest in heart can explain why there is almost no cardiomyocyte-origin cancer observed. Besides this, we also want to emphasize that in our reference 16, the authors described that the low intratumoral vessel density is a common feature in all malignant tumors. If the phenomenon is true, it may be another adjuvant verification for our model.

Chapter V

Conclusion

In summary, our results show that (1) the stationary point of lactic acid and oxygen (u_0, v_0) is different in different types of tissue, and this phenomenon can be reproduced in our model; (2) the ratio of ρ/e is biggest, intermediate and smallest in skin, liver, and heart respectively, which is consistent with experimental results. Besides this, we have also indicated that the glycolysis mode and oxphos mode are decided by the environmental conditions in which cells are. We have also found it is reasonable to assume that the stem cells prefer to reside in the local maximum of lactic acid and the local minimum of oxygen where gradients are smallest; (3) the e and D_u dependence of the oscillation behavior of lactic acid and oxygen are different; (4) the gradients of lactic acid and oxygen play an important role in inducing differentiation. In our model, we can see when the ratio of ρ/e becomes larger than 8, the gradients become too small to induce differentiation. By considering the gradient effects together with acidic hypoxic, the environment shown in either figure 3-e or 3-f is much more suitable for stem cells than in 3-b.

Chapter VI. Appendix

The Fortran Codes for solving the reaction-diffusion equations

```
program stemniche
use DFLIB
parameter (xmax=256,t_step=550000)
real*8    u(-xmax:xmax),v(-xmax:xmax)
real*8    u_old(-xmax:xmax),v_old(-xmax:xmax)
real*8    grad_ux,grad_vx,Lpu,Lpv,xs,xe,ys,ye,nbmax
real*8    alpha,beta,gamma,k,dt,h,h2,haq,uf,vg,ff,gg    !D
real*8    a,b,c,d
real*8    x, y
real*4    rnd
integer tstep,i,j,xymax2,ired,igre,iblue,ii,it

!****************************** main program
*****************************
xs=-600
xe=600
ys=0
ye=5
```

(we set the left and right boundary in x-axis, and upper and lower boundary in y-axis)

```
ii=setwindow(.true.,xs,ye,xe,ys)
```

```
ii=clickmenuqq(loc(winfullscreen))
ii=setbkcolor(0)
ii=setbkcolor(15)
call clearscreen ($gclearscreen)


h=0.5 ! delta_x=0.5
h2 = 2.0*h
hsq = h*h
dt=0.0001

call random_seed()
! 昀融系統參數
a = 0.21
b = 0.1
c = 0.42
d = 0.1
Du      = 4.2
Dv      = 20*Du
```

(we set a= ρ , b=e, c=2 ρ , and d=e, D_v=20 D_u)

```
Do i=-xmax,xmax
    call random_number(rnd)
    u(i) = 1.5*rnd
    call random_number(rnd)
```

```
    v(i) = 1.5*rnd
```

(we set the initial value of u and v in our model are random numbers)

```
            x= i ; y = u(i)
            iresult=setpixelrgb_w(x,y,#000000)
            x= i ; y = v(i)
            iresult=setpixelrgb_w(x,y,#0000ff)
End do
pause
xmax2=xmax-1

        u(-xmax2-1)=u(-xmax2+1)
        u(xmax2+1)=u(xmax2-1)
        v(-xmax2-1)=v(-xmax2+1)
        v(xmax2+1)=v(xmax2-1)
```


(we set no-flux boundary conditions in our model)

```
open(12,file='noflux2.1.txt')

it=0
do tstep=1,t_step    !t_step

        u(-xmax)= u(-xmax+2)
        u(xmax)= u(xmax-2)
        v(-xmax)=v(-xmax+2)
        v(xmax)= v(xmax-2)
```

(we set no-flux boundary conditions in our model)

```
        u_old(:)=u(:)
        v_old(:)=v(:)

  if (mod(tstep,1000)==0)    then
      it = it+1
      call clearscreen($gclearscreen)
      do i=-xmax,xmax
          x= i ; y = u(i)
          iresult=setpixelrgb_w(x,y,#000000)
          x= i ; y = v(i)
          iresult=setpixelrgb_w(x,y,#0000ff)
          !y =-100
          !cc=15*u(i)
          !if (cc > 255) cc=255
          !ired = 255 -cc
          !igre =ired
          !iblue =ired
          !ic= rgbtointeger(ired,igre,iblue)
          !do ii=1,50
          !    y=y+1
          !    iresult=setpixelrgb_w(x,y,ic)
          !end do
          if(tstep==t_step) write(12,*) i,u(i),v(i)
        enddo
```


(we paint the curve of lactic acid with black and oxygen with red, and record the results of our simulation in file “12”)

```
end if


    do i=-xmax2,xmax2
            dd = 0.01*(xmax-i)
            grad_ux=(u_old(i+1)-u_old(i-1))/h2
            grad_vx=(v_old(i+1)-v_old(i-1))/h2
            Lpu=(u_old(i+1)+u_old(i-1)-2.0*u_old(i))/hsq
            Lpv=(v_old(i+1)+v_old(i-1)-2.0*v_old(i))/hsq
            !h = rho*u_old(i)*v_old(i)/(1+u_old(i)+K*u_old(i)**2)
            ff=a*u_old(i)*u_old(i)*v_old(i)-b*u_old(i)
            gg =   -c*u_old(i)*u_old(i)*v_old(i)+d
```



```
            uf = Du*Lpu +ff
            vg = Dv*Lpv +gg
```

(we write the diffusion-reaction equations for lactic acid and oxygen in our model)

```
            u(i)=u(i)+dt*uf
            v(i)=v(i)+dt*vg
            !if (u(i)<0) then
               !u(i) =0
             !else if (v(i)<0) then
             !v(i) =0
            !end if
     enddo
```

```
enddo

pause
end
```

References

1. Schofield R et al; The relationship between the spleen colony forming cell and the haemopoietic stem cell. Blood Cells 1978, 4: 7-25

2. Xie T. et al; A niche maintaining germ line stem cells in the Drosophila ovary. Science 2000, 290: 328-330

3. Vladimir P. Z.; Simulation of proliferation and differentiation of cells in a stem-cell niche; Physica A; 2008; 387:6126-6136

4. Benjamin O. et al; The stem cell niche: theme and variations; Current Opinion in Cell Biology; 2004; 16: 693-699

5. Sean J. Morrison et al; Stem cells and niches: mechanisms that promote stem cell maintenance throughout life; Cell 2008; 132: 598-611

6. Stephan Lindsey et al; The importance of physiologically inspired physicochemical parameters on hematopoietic stem-cell maintenance and lineage-specific differentiation in ex vivo cultures; Stem cells: from mechanisms to technologies; 2011

7. Bruce Alberts et al; Molecular biology of the cell, 5th edition; 2008

8. Jose Manuel C. M. et al; Role of the intracellular ph in the metabolic switch between oxidative phosphorylation and aerobic

glycolysis-relevance to cancer; WebmedCentral>Review articles; 2011

9. Petr Jezek et al; Distinctions and similarities of cell bioenergetics and the role of mitochondria in hypoxia, cancer, and embryonic development; The International Journal of Biochemistry and Cell Biology 42; 2010; 604-622

10. J. D. B. Macdougall et al; Diffusion coefficient of oxygen through tissues; Nature 215; 1967; 1173-1174

11. A. V. Hill; The diffusion of oxygen and lactic acid through tissues; Proceedings of the royal society of London. Series B; 728: 39-96; 1928

12. Ruey Tarng L. et al; Turing model for generating biological patterns; 2007

13. Michael H. Ross et al; Ross Histology 5th edition; 2006

14. Peter S. Amenta; Histology and human microanatomy 6th edition; 1991

15. Karel R. et al; Morphometry of human coronary capillaries during normal growth and the effect of age in left ventricular pressure-overload hypertrophy; Journal of the American Heart Association; 1992; 86:38-46

16. Eduard R. et al; Expansion of endothelial surface by an increase of vessel diameter during tumor angiogenesis in experimental hepatocellular

and pancreatic cancer; World journal of Gastroenterology; 2004; 10(21): 3171-3174

17. A. K. E. Bonamy et al; Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm; Journal of Internal Medicine; 2007; 262: 635-642

18. Yamanaka Shinya; Pluripotency and nuclear reprogramming; Philosophical transactions of the royal society B; 2008; 363:2079-2087