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Particle Simulation of Liver Cell Proliferation with Angiogenesis —Whole Hepatic Lobule Formation

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Abstract

The liver is the organ primarily responsible for our metabolism and loss of its function generally results in death. Luckily, the liver has a high regenerative ability. However, the mechanism of its regeneration has not yet been clarified. This study therefore models liver regeneration via a numerical simulation. In addition, this simulation may aid other experiments in the field of regenerative medicine. As a first step, this study proposes an analytical model based on the particle method. The analysis object is a hepatic lobule. The purpose of this analysis is to elucidate the process, mechanism, and condition of cell growth on the micro-scale. Experiments using rats were conducted to obtain the parameters for the model, namely the diffusivity, oxygen concentration, and oxygen consumption rate of a cell. The most difficult to solve problem in liver cell proliferation technology is the restricted volume in which cells survive owing to oxygen supply problems. It is therefore necessary to extend the cell survival volume by angiogenesis. Here, results were generated using a model of angiogenesis in which blood vessels formed from the portal veins to a central vein, with repeated branching and connecting across the whole of the liver. In this way a hepatic lobule was filled with liver cells. Additionally, the analysis results yielded the rates of the cross sectional areas of the blood vessels. This research further aims to analyze the macro region by using analysis results obtained on the micro-scale. Applying the thresholds which is the most closed to the experimental value obtained from analysis results on the micro-scale to the macro region, it becomes available to curtail expenses and be kind to animals in the liver experiments.

Keywords: Particle Simulation, Liver proliferation, Angiogenesis

1. Introduction

The liver is our metabolism's most important organ. Serious loss of liver function may lead to death. Moreover, the liver has the ability to re-form and regenerate within our body. However, liver tissue engineering takes not only a lot of time but also many lives of rats, so it is not yet possible, although there is much interest in this area. Therefore, the regeneration mechanisms of the liver [1] are still in the process of being discovered. We aim to construct models of cell proliferation and angiogenesis using a particle model to elucidate the mechanism behind liver regeneration. We set the model's parameters based on ideal experimental conditions.

2. Analysis Object

We focused on the hepatic lobule, which is a basic component of the liver. As shown in Fig. 1 [2], the shape of a hepatic lobule is that of a hexagonal prism; it has portal veins at each of its six vertices and a central vein at the center. In addition, sinusoids (capillaries) run from the six portal veins to the central vein. Liver cell proliferation is activated by oxygen diffusion from the sinusoids to the liver cells. Liver cells exist in rows spouting out from a central point: they have a characteristic harness structure. A numerical analysis of the hepatic lobule has already been conducted. However, examples of numerical simulations including angiogenesis do not exist.

Fig. 1 Cross-sectional illustration of a hepatic lobule [2]

3. Model Description

We used a particle model that uses calculation points to assess physical quantities and movement; the model is based on the Lagrange method, which is a numerical simulation method. Each particle (calculation point) physically affects other particles. With this method no mesh is required, unlike with the difference or finite element methods. In the past, we have used particle models to simulate cancer growth [3], hair formation, and skin formation [4-5]. Here, a liver cell particle and a liver cell particle including blood vessels (which are two types of particles) are used to express a liver cell using a

numerical simulation. Therefore, the diameter of a blood vessel is a virtual parameter derived from experiments and does not influence interparticle force. The interparticle distance for this numerical simulation requires the reference distance to be as small as possible because the particles will try to form the densest structure possible from the clathrate arrangement; this is why liver cells have a hexagonal structure.

3.1 Oxygen Diffusion Model

We can model the oxygen diffusion from blood vessels using the oxygen diffusion equation (Eq. (1)) with a center difference method. However, in our model oxygen diffusion is calculated using one step as a state of balance. In the following equation C is the oxygen concentration, M_0 is the oxygen consumption rate, ρ is the liver cell density, and D is the oxygen diffusion coefficient. Also, M_0 is $4.0 \times 10^{-16}[\mathrm{mol/(s \cdot cell)}]$ [6], ρ is $1.25 \times 10^{8}[\mathrm{cells/cm^3}]$ [7], and D is $2.1 \times 10^{-6}[\mathrm{cm^2/s}]$[6].

$$\frac{\partial C}{\partial t} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) - \rho M_0 \qquad (1)$$

In addition, we calculate the analytical solution via the oxygen diffusion equation (Eq. (2)) to verify our numerical results. In Eq. (2) R is the maximum range of the oxygen diffusion distance, and r_0 is the minimum range of the oxygen diffusion distance.

$$C = \frac{\alpha}{4D}(r^2 - r_0^2) + \frac{\alpha R^2}{2D}\ln\frac{r_0}{r} + 1 \qquad (2)$$

Moreover, the validity of this model was proven by previous research [8].

3.2 Angiogenesis and Hepatic Lobule Formation Model

Reconstruction of the cell survival volume, which is limited by oxygen depletion, is the most serious problem facing the development of liver cell proliferation technology. Therefore, it is essential to extend the cell survival volume by angiogenesis. So, we postulated that the sinusoid extends in the direction of the oxygen concentration which is low. The pathognomonic shapes of the sinusoids qualitatively correspond to the streamlined "source" and "suction" which are ideal fluid in fluid dynamics. We therefore propose that Eq. 3, which describes angiogenesis, corresponds to the convoluted velocity potential given in Reference 9, which attempts to model angiogenesis. Eq. (3) tends to $-\infty$ at the origin z = 0, and it possess a finite differential coefficient. This is why regularity can be lost at the origin but it is maintained in other areas of the lobule. Ultimately, we propose that portal veins are the "source" and that the central vein represents "suction." In Eq. (3) W is the complex velocity potential, Q is the amount of blood flow, and z is complex coordinate. The pattern of the angiogenesis in the liver has not been become clear, so we conduct the complex velocity potential to model the angiogenesis.

$$W = \frac{Q}{2\pi}\log z \qquad (3)$$

Moreover, we conducted a simulation with extension and divergence of blood vessels and tissue construction over a large area. The sinusoids undergo repeated extension, divergence, and concourse. Eventually, the sinusoids connect to the central vein. However, when extension and concourse does not occur, the blood vessel regresses.

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3.3 Whole Hepatic Lobule Formation Model

The whole hepatic lobule model is an angiogenesis and hepatic lobule formation model conducted over a whole hepatic lobule. We created it by referencing the real structure of a hepatic lobule (Fig. 1); additionally, it models expanding angiogenesis and hepatic lobule formation.

The liver is an organ containing approximately fifty thousand hepatic lobules. For this reason, we proposed that we can analyze a liver if we analyze it using fifty thousand whole hepatic lobule formation models. However, even with this approach the mechanism behind real angiogenesis could be elucidated. Therefore, we searched for a combination of the oxygen concentration threshold of extension and regression close to the experimental value of 12% [7] by combining them. In other words, we searched for a combination of extension and regression close to the experimental angiogenesis value. However, blood vessels extend when the oxygen concentration of liver cell particles is lower than the oxygen concentration threshold for extension, while blood vessels regress when it is higher than the regression threshold.

4. Calculation Conditions

We use a whole hepatic lobule formation model in this study. We consider a liver cell with a diameter of 20 μm as a particle. Then, we obtain three cross sections of all combinations of each threshold in the z-axis direction. The average ratio of the cross-sectional area of the extended and regressed blood vessels was calculated from these cross sections, which is the true value. Also, angiogenesis does not sufficiently occur when each threshold is equal to 0.3 or when the oxygen concentration threshold of regression is 0.3 or 0.4 [10]; thus, we did not conduct the analysis above these levels.

Sinusoids form in the direction of the liver cells. We set a threshold for the oxygen concentration. We represent the analysis conditions as follows. The

sinusoid form velocity was less than 30 μm/day; the blood vessel divergence distance was less than 30 μm/day; the blood vessel extension angle was between 0°

and
45°

; the blood vessel divergence angle was between 45°

and
90°

; the number of divergences was less than two; and the frequency of the divergence was 40 μm, which is equivalent to the distance of two particles . The extension of blood vessels depends on the complex velocity potential. The divergence of these further depends on the oxygen dissolution concentration. Moreover, a blood vessel connects to another blood vessel when another blood vessel exists near the forming blood vessel.

The analysis area was a three-dimensional diamond shape (Fig. 2). The distance between portal veins, which are located diagonally, was between 0 and 1.0 mm. We located portal veins of diameter 100 μm at each of the six vertices. We placed a central vein, whose diameter was the root of two times that of the portal vein diameter, at the center. In addition, we placed liver cells around the portal veins and the central vein. The angiogenesis area was regular. From the relationship of the continuity equation and fluid dynamics, we set the amount of flow to 3 for each portal vein and set the quantity of flow to −6 for the central vein. We made two postulates about liver cell growth and death depending on the oxygen dissolution concentration. Cells supplied with oxygen divide regularly, while cells whose oxygen concentration is zero die. Moreover, when a liver cell is enclosed in a fixed number of liver cells, it does not grow anymore because of the contact inhibition phenomenon. Liver cell dividing time was 26 h assuming a fetus liver. The oxygen concentration of cell death was 0.001, while the contact inhibition was above 12 particles. The clock time was 1 min, and the minimum vessel diameter was set to 7μm.

5. Results and Discussion

Fig. 3(a) shows the analysis result for the whole hepatic lobule formation model. Angiogenesis is similar to the real phenomenon (Fig. 1) as blood vessels in every portal vein run to the central vein. Fig. 3(b) shows a cross section in the z-axis direction. We achieved a harness structure that is qualitatively similar to the real phenomena by angiogenesis with a complex velocity potential (Fig. 3(b)). Sinusoids repeated divergence and concourse. After that, oxygen was supplied inside the hepatic lobule, so tissue could be constructed. Next, Table 1 shows the ratio of the cross sectional area of expanded and regressed blood vessels (Fig. 3(b)). In Table 1 a dash "–" indicates that a calculation could not be performed, while a cross symbol indicates that analysis was prohibited. We achieved a combination that is very close to the experimental value (oxygen concentration threshold for extension and regression of 0.3 and 0.9, respectively). Additionally, we anticipate the combinations of each threshold to be close to the experimental value of 12%, while the oxygen concentration threshold for regression was between 0.8 and 0.9 with an oxygen concentration threshold for extension of 0.3, and an oxygen concentration threshold for regression of between 0.7 and 0.8 with an oxygen concentration threshold for extension of 0.4. If we search for these combinations, it is inevitable that a more detailed analysis is required because we need to consider values up to two decimal places each side of each threshold. This study's purpose was the model's integrity verification, which is not aim to correspond to the actual phenomenon, so we did not analyze more patterns than strictly necessary to analyze the macro region.

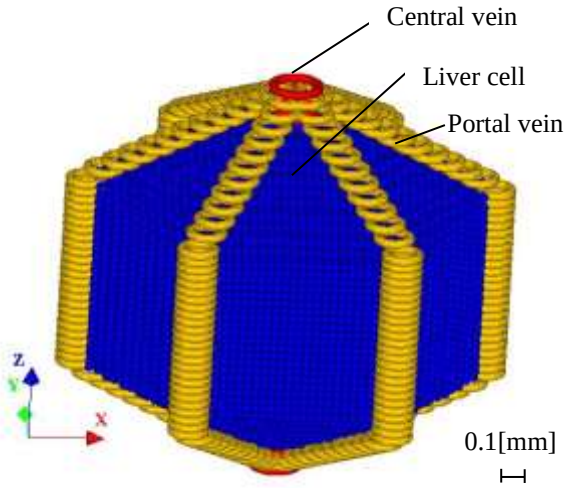
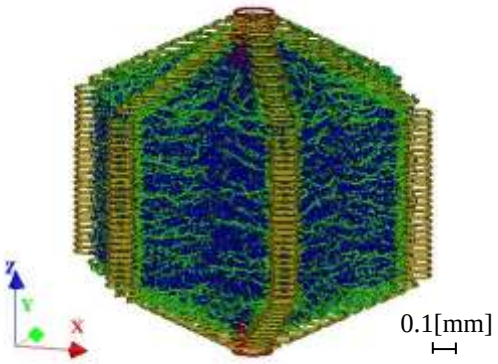
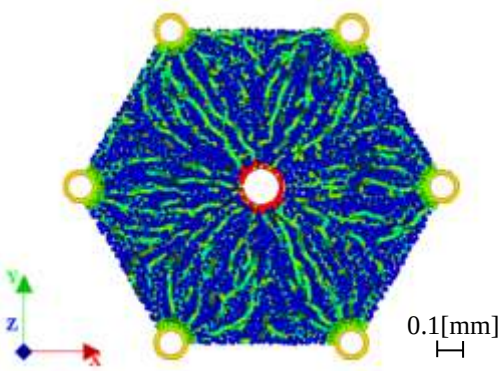


Fig. 2 Initial state of a whole hepatic lobule



(a)Liver cell proliferation and angiogenesis (blue: liver cell; green: blood vessel; yellow: portal vein; and red: central vein)



(b) Cross section in the z-axis direction
Fig. 3 Numerical analysis results using our model

Table 1 Ratio of cross sectional area of extended and regressed blood vessel

Regression \ Extension	0.3	0.4
0.4	–	
0.5	–	–
0.6	10.51%	9.60%
0.7	11.07%	11.02%
0.8	11.69%	13.40%
0.9	12.23%	14.12%
1.0	12.29%	15.41%

6. Conclusion

In this study, we achieved results using our model that are qualitatively similar to the real phenomena in a liver by conducting a numerical analysis of the whole hepatic lobule formation model; we further verified that the analytical results matched those of the real phenomena by calculating the ratio of the cross sectional area of expanded and regressed blood vessels. The presented results show that the whole hepatic lobule formation model is acceptable for modeling liver tissue reconstruction.

The liver still throws up many unsolved questions, but we are gradually improving our understanding of it. Our results are thus expected to be used in the field of regenerative medical engineering; further, a closer understanding of the real phenomena in the liver should be achieved by using the analytical model of the hepatic lobule established in this study.

Further study is require, however, since our model currently shows that oxygen leaks to the outside through the hemoglobin in the blood red cells. This shows that the oxygen concentration in blood is not uniform; thus, we need to consider this in future iterations of our model. In addition, this study targeted the decellularization of a liver after cardiac death, so liver cells die in the first step of the analysis. However, they do not all die in the real phenomenon in the liver. Therefore, we need to consider what effect the initial cell density has on cell proliferation and the speed of tissue development.

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