BME005

3D Particle Simulation of Liver Cell Proliferation with Angiogenesis -Partial Hepatic Lobule Formation-

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Abstract

Recently, the prevalence of viral hepatitis patients has been increasing. Therefore, the reconstruction of liver based on tissue engineering has attracted significant attention. However, the formation or regeneration mechanisms of the liver have not been elucidated; therefore, practical regenerative medicine technology based on tissue engineered liver has not been accomplished. In this study, we propose an analysis model using a Particle Model as the first step. The analysis object is a hepatic lobule. The purpose of this analysis is to elucidate the process of cell proliferation, mechanisms, and states at the micro scale. We used parameters which were obtained by experiments using rats relating to diffusivity, oxygen concentration, and oxygen consumption of a cell. Moreover, we used complex velocity potential of fluid dynamics and obtained a result corresponding to a real hepatic lobule, qualitatively.

Keywords*:* Particle Simulation, Liver proliferation, Angiogenesis

1. Introduction

The liver is the main organ of metabolism. Serious loss of liver function may cause death. Moreover, the liver is known as an organ which has the ability to re-form and regenerate in our body. However, the reformation or regeneration mechanisms of the liver have not been elucidated. Through this research, we aim to construct models of cell proliferation and angiogenesis using a Particle Model and elucidate the mechanism of the formation of liver. Moreover, the reconstruction of liver [1] based on tissue engineering technology attracts attention. We set parameters and aimed to find out information from ideal experimental conditions, which we could analyze and provide to researchers. This would permit further calibration of the model and subsequent experiments.

2. Analysis Object

We focused on the hepatic lobule, which is the basic component of the liver. As shown in Fig.1 [2], the shape of a hepatic lobule is a hexagonal prism and a sectional shape of this is a hexagon. It has portal veins at six vertices and a central vein at the center. Moreover, sinusoids (capillaries) exist between the six portal veins and the central vein. Liver cell proliferation is stimulated through oxygen diffusion from the sinusoids to liver cells. Liver cells are present in rows radiating out from a central point. Thus, they have a characteristic harness structure. Numerical simulations of the hepatic lobule were conducted [3] but there are no examples of numerical simulations including angiogenesis.

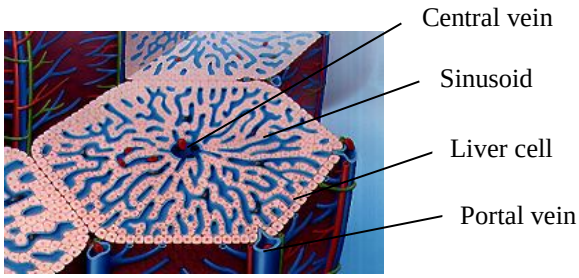


Fig.1 Hepatic lobule [2]

3. Model Description

We used a Particle Model as an analysis model. The Particle Model is based on the Lagrange method, which is a numerical simulation method using calculation points that assess physical quantities and movement. Each particle (calculation point) has a physical effect on others. We did not need to make a mesh, which requires significant amount of a additional tasks, on difference method or a finite element method. We used a Particle Model in simulations of cancer growth [4], hair formation, and skin formation [5-6] in our laboratory. In this study, we used it for numerical simulation of liver formation. We used two types of particle to represent a liver cell: a liver cell particle and a liver cell particle including blood vessels in this numerical simulation. Therefore, the diameter of a blood vessel is a virtual parameter and does not influence interparticle force. The interparticle distance for this numerical simulation requires it to be longer than the reference distance because a particle will stabilize as the densest structure from the clathrate arrangement. This is why liver cells have a hexagonal structure.

3.1 Oxygen Diffusion Model

In the model of oxygen diffusion from blood vessels, we can approximate the oxygen diffusion equation (Eq.(1)) using a center difference method. However, we calculate oxygen diffusion using one step as a state of equilibrium. C is the oxygen concentration. M_0 is the oxygen consumption rate. ρ is liver cell density. $\mathbb{D}$ is the oxygen diffusion coefficient.

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

Moreover, we calculate analysis solution from the oxygen diffusion equation by Eq.(2) to verify whether numerical solutions of discrete equations on numerical simulation correspond to the analysis solution. R is the maximum range of oxygen diffusion distance. r_0 is the minimum range of oxygen diffusion distance and the radius of an element of a blood vessel.

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

3.2 Angiogenesis and Hepatic Lobule Formation Model

Reconstruction of the cell survival area by oxygen depletion is the most serious problem in liver cell proliferation technology development. Therefore, it is necessary to extend the cell survival area by angiogenesis. So far, we postulated that sinusoid form in the direction of oxygen concentration is low. However, we are not able to accurately simulate the characteristic liver cell harness structure (Fig.1) between a portal vein and a central vein through assumptions. The harness structure is an important factor of hepatic lobule modeling. However, it is difficult to describe the exact equations of angiogenesis because the mechanism is unknown. Consequently, the characteristic shape of sinusoids between portal veins and a central vein is qualitatively similar to that which is streamlined and is considered to 'source' and 'suction' ideal fluid through fluid dynamics. This is why we speculate that an equation describing angiogenesis corresponds to equation 4 (Eq.(3)) of complex velocity potential [7] and attempts to model angiogenesis. The function of Eq.(3) reaches $-\infty$ at the origin z=0 and it does not have a differential coefficient which is finite. This is why regularity can be lost at the origin but it is maintained in other areas of the lobule. After all, when we speculate that portal veins are the 'source' and a central vein is 'suction', the center of each portal vein and each central vein are singular points. W is complex velocity potential, Q is the amount of blood flow, z is complex coordinate in the following equation.

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

Moreover, we used extension and divergence of blood vessels and stimulate tissue construction in the large area. In this report, we add concourse and regression of blood vessels to optimize blood vessel structure. Sinusoids repeat extension, divergence and concourse. In the end, this reaches a central vein. However, when extension and concourse does not happen, the blood vessel regresses.

We calculated numerical simulations of liver cell proliferation and angiogenesis on the hexagonal prism area of a hepatic lobule by using the above methods. We repeated calculations of angiogenesis, liver cell movement, liver cell death, liver cell growth and oxygen diffusion.

4. Calculation Conditions

We divided analysis conditions into three steps. First of all, we detailed the condition of oxygen diffusion model around a portal vein. Second, we detailed the condition of angiogenesis model around a portal vein using complex velocity potential. Finally, we detailed the condition of a hepatic lobule formation model including complex velocity potential and angiogenesis. In our research, we consider a liver cell of the diameter 20 μm as a particle and calculate using this parameter.

4.1 Oxygen Diffusion Model

Analysis area comprised three dimensions of 900[μm] ×900[μm] ×900[μm]. We put a portal vein of diameter 100[μm] at the center and liver cells around the portal vein. Oxygen consumption rate was defined as M_0 is $4.0 \times 10^{-16}[\mathrm{mol/(s \cdot cell)}]$ [8]. Liver cell density was defined as ρ is $1.25 \times 10^8\,[\mathrm{cells/cm^3}]$[9]. Oxygen diffusion coefficient was defined as $\mathbb{D}$ is $2.1 \times 10^{-6}[\mathrm{cm^2/s}]$[8]. Fig.2 is the initial state of the three dimensional model.

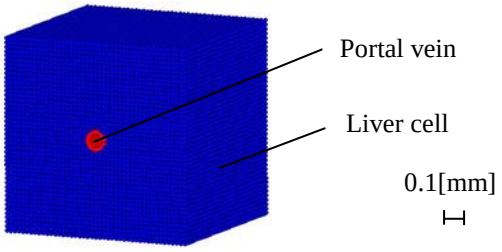


Fig.2 Initial state

Moreover, oxygen saturation concentration $2.0 \times 10^{-7}[\mathrm{mol/cm^3}]$ was treated as dissolved oxygen concentration on the surface of a blood vessel and is represented by a dimensionless number 1.

4.2 Angiogenesis and Hepatic Lobule Formation Model

Sinusoids form in the direction of the liver cell. We set a threshold of oxygen concentration, where sinusoids can form in the direction. We detailed the analysis condition. Sinusoid form velocity was less

than 30[μm/day]. Blood vessel divergence distance was less than 30[μm/day]. Blood vessel extension angle was from 0° to 45°. Blood vessel divergence angle was from 45° to 90°. The number of divergences was less than two. The frequency of divergence was by 40[μm]. The extension of blood vessels depends on complex velocity potential. The divergence of these depends on oxygen dissolution concentration. Moreover, a blood vessel linked with another blood vessel when another blood vessel existed near the forming blood vessel. We calculated that there was blood vessel atrophy and optimized when oxygen were full around the blood vessel or the above extension, divergence and concourse had not happened.

The analysis area was the shape of a hexagonal prism (Fig.3). The distance between portal veins which are located diagonally was 1.0[mm]. We put portal veins of diameter 100[μm] at six vertices. We put a central vein, whose diameter was the root of two times that of the portal vein diameter, at the center. Moreover, we placed liver cells around the portal veins and a central vein. We indicated 'source' and 'suction' for portal veins and a central vein. The angiogenesis area was regular, and we could calculate velocity vectors although each center of the portal veins and a central vein was a singular point. However, we made an assumption that the model has an isotropy of the third dimensional direction. From the relationship of continuity equation on fluid dynamics, we set the amount of flow to three for each portal vein and set the amount of flow to negative six for a central vein. We made two assumptions about liver cell growth and death depending on oxygen dissolution concentration. Cells supplied with oxygen divide periodically and cells whose oxygen concentration is 0, die. Moreover, when a liver cell is surrounded with fixed numerical liver cells, it does not grow any more due to contact inhibition phenomenon. Liver cell dividing time was 26 hours assuming a fetus liver. The oxygen concentration of cell death was 0.001. The contact inhibition was more than 12. We set cells moving on this calculation. Clock time was 1 minute. Oxygen concentration threshold was 0.1.

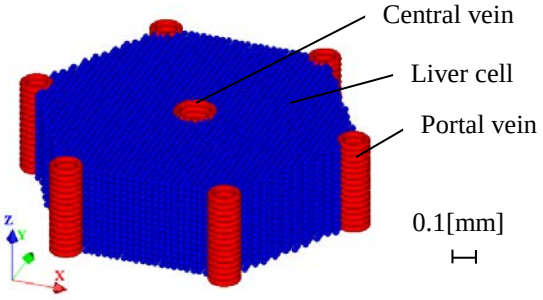


Fig.3 Initial state of a hepatic lobule

5. Results and Discussion

5.1 Oxygen Diffusion Model

We verified oxygen diffusion using the model of a portal vein of diameter 100[μm] at the center of the analysis area. Oxygen diffused concentrically from the portal vein. Oxygen concentration was more than 0 in the area of about 40[μm] from the surface of the portal vein. Oxygen diffusion analysis was appropriate because numerical solutions corresponded with analysis solutions (Fig.4).

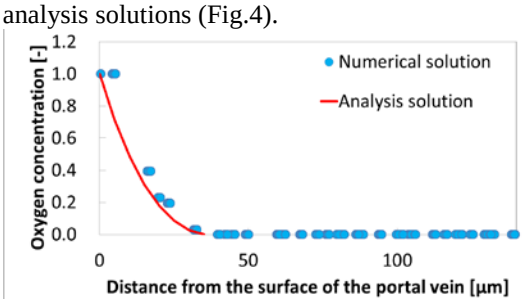
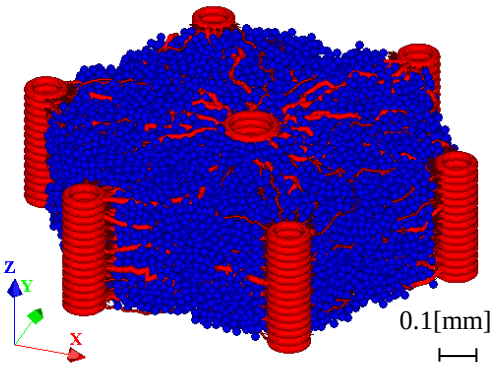


Fig.4 Comparison between numerical solution and analysis solution

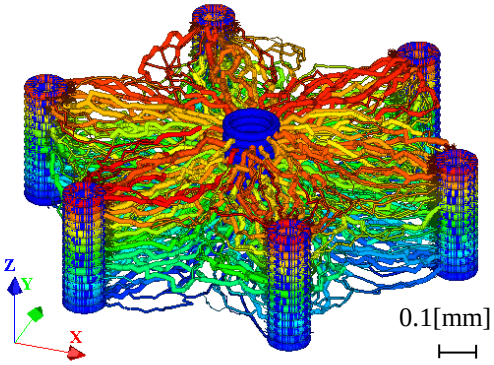
As a result, we showed that liver cells around the blood vessels are alive. Therefore, liver cells kept increasing until the area in the vicinity of blood vessels is saturated with liver cells. In other areas, liver cells died because of oxygen loss even if initial liver cells density was different.

5.2 Angiogenesis and Hepatic Lobule Formation Model

We integrated the above interparticle force, particle movement, the oxygen diffusion model and the angiogenesis model, and calculated the hepatic lobule formation model. We obtained a result of the harness structure which was qualitatively similar to real phenomena by angiogenesis of complex velocity potential [Fig.5(a)]. Sinusoids repeated divergence and concourse. After that, oxygen was supplied inside the hepatic lobule and tissue was constructed. Fig.5(b) shows only blood vessels. We calculated diameters of sinusoids from the amount of flowing blood in sinusoids. Two blood vessels which were at a downstream direction from a divergence point of a blood vessel, got thinner than the blood vessels which were in the upstream direction of a divergence point. On the other hand, the amount of flowing blood through a blood vessel which is downstream from a concourse point was the sum of the amount of flowing blood of two blood vessels which were upstream this side of the concourse point. The total amount of flowing blood from portal veins corresponded with (The total amount of blood flowing on the top of the sinusoid) + (The total amount of blood flowing into the central vein) for each step. This is why there is no contradiction with respect to the diameters of sinusoids and the amount of flowing blood at every sinusoid. This is why it was possible qualitatively to make the assumption of angiogenesis on the modeling of tissue reconstruction.



(a)Liver cell proliferation and angiogenesis
(Blue : Liver cell, Red : Blood vessel)



(b)Angiogenesis

Fig.5 Hepatic lobule formation model

6. Conclusion

In this study, we produced a model of a hepatic lobule, which is the basic component of a liver, and assessed formation and calculated the numerical simulation. We calculated the simulation of oxygen diffusion model around a portal vein. Numerical solutions corresponded to analysis solutions of a Particle Model about the distribution of oxygen diffusion, so we confirmed that the indicated the numerical simulation was appropriate. Second, we combined all of the models and produced a hepatic lobule qualitatively. The above numerical simulation results qualitatively corresponded to the actual phenomena of cell proliferation and liver tissue reconstruction. This is why we indicated our model is appropriate. We are going to optimize parameters based on experimental data. In the end, we need to ensure integrity between numerical simulation data and experimental data improves. In addition, we are going to calculate a large scale construction of a practical simulation model to reconstruct liver tissue.

7. Reference

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