Can morphogenesis be understood in terms of physical rules?

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Because the morphogenesis of biological systems is not fully understood, researches from various points of view are necessary. The present author has recently made computer simulations with his colleagues to construct branching systems of human organs, such as the lung airway and the liver blood vessels. In the simulations certain rules are assumed to govern bifurcating processes of the systems. These rules are expressed in terms of physical and geometrical concepts, such as minimum energy consumption and uniform filling of branches in the space of organs. Results of computer simulation are quite similar to real structures. However, actual mechanisms of morphogenesis, i.e. effects of genes or proteins, are not considered in these studies. In this article, the present work is discussed in relation to the concept of biological pattern formation by Meinhardt and a recent study by Miura and Shiota on lung growth.

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1. Introduction

Organs in biological systems are constructed through biochemical processes under the influence of genes. However, since the mechanisms of organogenesis are not fully understood, computer simulations to construct organs based on simple algorithms would be meaningful in a sense that they may contribute to the study of morphogenesis.

A computer simulation to construct the lung airway has been performed by the present author with his colleagues (Kitaoka *et al* 1999) as an extension of the past simpler simulations (Weibel 1963; Horsfield *et al* 1971; Grenney and Robertson 1995; Parker *et al* 1997; Kitaoka and Suki 1997), whose brief summary is given in another paper (Takaki and Kitaoka 1999). The studies by Weibel (1963), Horsfield *et al* (1971) and Kitaoka and Suki (1997) treated topological natures of branching systems and not their 3D configurations. On the other hand, those by Grenney and Robertson (1995) and Parker *et al* (1997) are concerned to 3D structures of the airway, but were not sufficient enough because the resulting structures have a right-and-left symmetry in contradiction to the real airway. Simulations of liver blood vessels were made by Takaki *et al* (2003). There seem to be few past trials of in-silico construction of liver blood vessels. A mention is made here of plant branching systems. As is discussed by West *et al* (1999), the trunk and branches of plants are looked upon as bundles of thin ducts, and the branching structures should be understood based on different principles.

Simulations of organ formation can give valuable hints to understand the real processes of morphogenesis. They are useful also for another purpose, i.e. developing techniques for diagnoses and research in physiology or pathology. But this aspect is not discussed in this paper.

In the following, results of three trials of in-silico constructions are explained briefly, i.e. simulations of the human airway, the blood vessels in human liver and the capillary system in human liver. In the last section roles of these works are compared to the method for analysis of biological pattern formation established by Meinhardt (1982) and other researchers. In addition an extension of this method by Miura and Shiota (2002) is introduced.

2. Computational anatomy for human organs

The term ‘computational anatomy’ is used in the title of this section for the following reason. In anatomy precise

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structures of biological systems are observed and described. They serve as a knowledge data bases for many kinds of studies. In a similar way, 3D structures of biological systems constructed by computer can serve as a data base which can be applied to research, education and practical purposes.

2.1 *Construction of human lung airway*

The lung airway is constructed within the amniotic fluid by branching of a duct. Since it does not touch other tissues, it is one of the most convenient organs for construction with a simple algorithm. The algorithm is constructed according to the functions of the lung listed below (Kitaoka *et al* 1999).

(i) The air is supplied to all parts equally, hence terminals should be distributed uniformly.
(ii) Gas supply by a branch matches the volume of its drainage basin (i.e. region to be supplied).
(iii) Total energy loss due to the air viscosity is minimized.
(iv) Branches terminate at a certain flow rate (beyond which diffusion becomes more effective for gas transport).
(v) The main trunk and outer boundary are given according to anatomical data.

An algorithm satisfying the above requirements is composed of two kinds of rules, geometrical and fluid dynamical ones, as listed below (see also figures 1 and 2).

Geometrical rules
Rule 1: Branching is dichotomous.
Rule 2: Parent branch and two daughter branches lie on the same plane (branching plane).
Rule 3: Length of each daughter branch is three times its diameter.
Rule 4: Branching plane is perpendicular to the preceding branching plane.
Rule 5: Drainage basin of a parent branch is divided into two daughter basins by a plane, which is perpendicular to the branching plane.

Fluid dynamical rules
Rule 6: Flow rate is conserved after branching.
Rule 7: Flow rate dividing ratio r is equal to the volume dividing ratio of daughter drainage.
Rule 8: Diameters and branching angles of two daughters are determined by the following formulae:

$$d_1 = d_0 r^{1/n},\ d_2 = d_0 (1-r)^{1/n},$$
$$\cos q_1 = \frac{1 + r^{4/n} - (1-r)^{4/n}}{2r^{2/n}},\ \cos q_2 = \frac{1 + (1-r)^{4/n} - r^{4/n}}{2(1-r)^{2/n}}.$$

Rule 9: Branches end if flow rates are less than a threshold or they come out of their drainage.

Some notes are given on the above rules. The rules 1–4 are based on anatomical data and are good approximations, while the rule 5 is introduced because of mathematical convenience (the drainages are made of curved planes). The hydrodynamic rules do not seem to be related to the processes of morphogenesis in developing embryo, because the airway is formed in a liquid, i.e. the amniotic fluid. However, there is an observation that the fluid is moving within the developing airway. At present no decisive assertion is possible as to the role of hydrodynamics in lung formation. Therefore, these rules should be taken as assumptions. Among the above hydrodynamic rules the rule 6 is convincing because the air

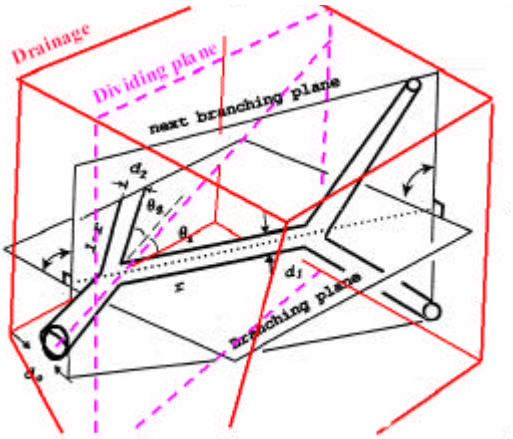
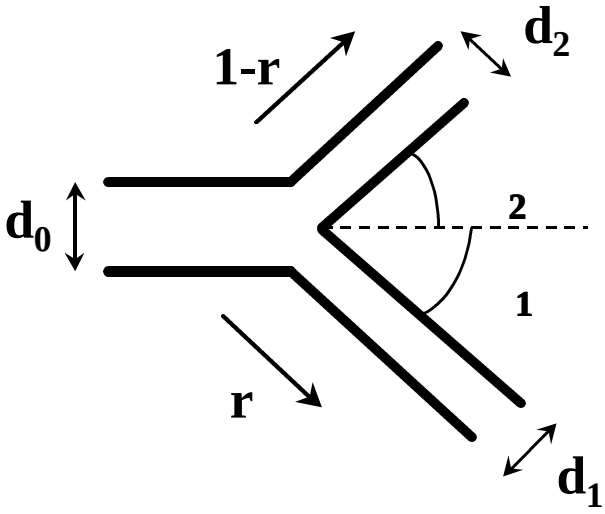


Figure 1. Relation between branching and division of drainage.



Figue 2. Parameters at branching.

can be looked upon as incompressible in such slow flows as in breathing. The rule 8 is proposed by Murray (1926) and Kamiya *et al* (1970), which is supported by anatomical studies. On the other hand, the rules 7 and 9 are not based on scientific evidences, although they do not contradict to impressions from rough observations and they match to the functions of the lung. They are introduced in order to complete the present algorithm.

A result of simulation based on the above rules is shown in figure 3. The right-left asymmetry comes from the existence of the heart (excluded from the computing area) and the asymmetric shape of the main duct (white). Branches coming from the same point on the main duct are given the same colour. This branching system has about 27,000 terminals (comparable to the real lung). The result agrees well with observation of the real lung in the sense that it gives a positive impressions to medical doctors and that some quantitative data, such as the average diameter of terminals and the average volume of their drainage basins, agree with measurements (Kitaoka *et al* 1999).

2.2 *Construction of blood vessels in human liver*

The blood vessels in the liver are formed within a packing of liver cells, and free choice of branch directions, as in the lung airway, will be limited. Hence, the branching system of liver blood vessels is constructed on an artificial grid system, i.e. a cubic grid system set-up within a boundary similar to the real liver. Exit (or entrance) is fixed at a certain grid point. As in the case of airway, tips of branches are required to distribute uniformly in the liver and the energy consumption should be minimum.

An algorithm for liver blood vessel formation is applied, which is composed of the following processes:

(i) A grid point is chosen randomly from points not occupied yet by branches.
(ii) A connection line is chosen randomly between this point (or the tip of growing branch) and one of the neighbouring points. This line is not fixed before the next process iii).
(iii) If the neighbour is vacant, this line is fixed (back to ii). If the neighbour is at the exit, this line is fixed (back to i). If the neighbour is on another branch, this line is fixed (two branches merge) (back to i). If the neighbour is on the branch of itself (loop formation), this line is omitted (back to ii).
(iv) If no vacant point is left within the boundary, the branching system is completed.

Since the liver has both inlet vessels (portal vein and hepatic artery) and outlet vessels (hepatic vein), both systems are constructed on its respective grid system, respectively, which are shifted by half length of grid distance (see figure 4). Result of a simulation (front view) is shown in figure 5. The inlet and outlet vessels are drawn in different colours.

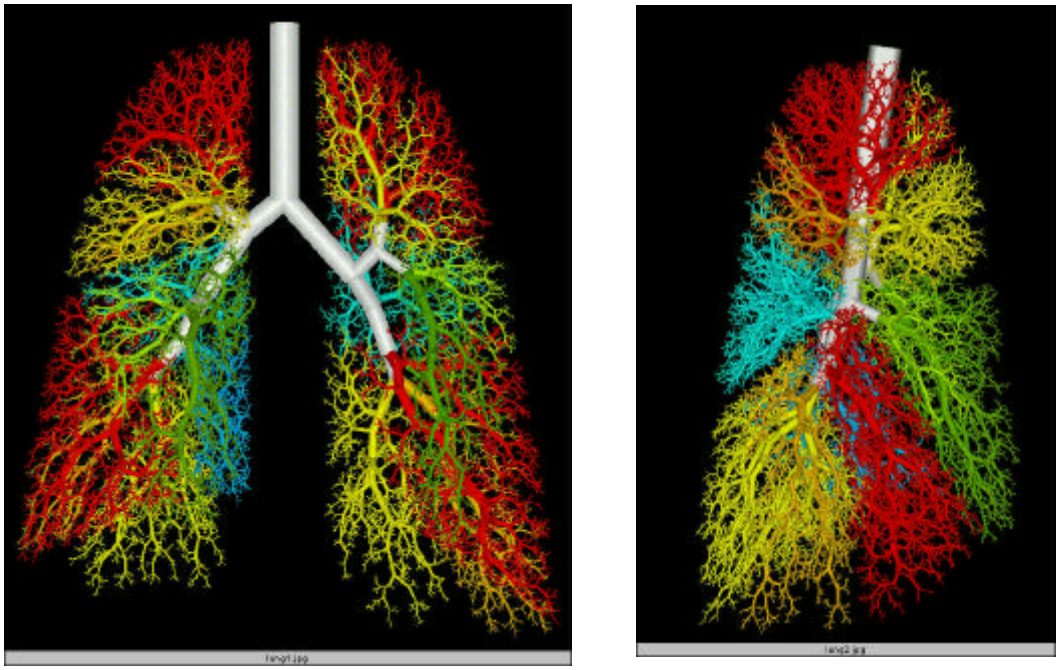


Figure 3. Result of simulation to construct human airway.

There is some freedom in choosing the algorithm, so as to give different probabilities to directions of branch extension or to locations of initial points. Some trials revealed that, if the branch extension has a larger probability in the direction of exit (or entrance), the branching system has better quality, i.e. the numbers of end points of arteries and veins increase and the energy consumption through viscous dissipation of blood flow decreases (Takaki *et al* 2003).

2.3 *Construction of capillary system in human liver*

A capillary network system is formed between the inlet and outlet vessels, which is called a sinusoid. The function of the sinusoid is to exchange materials with liver cells, so each cell should touch some sinusoid branches. There is a finding (Shimizu *et al* 1998) that liver cells, roughly speaking, have shapes of polyhedra with about 14 faces. It is assumed for mathematical convenience that capillaries have only dichotomous branching (three lines from one grid point).

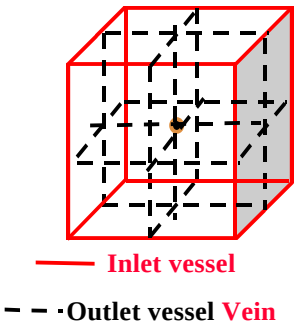


Figure 4. Grid systems for simulation of liver blood vessels.

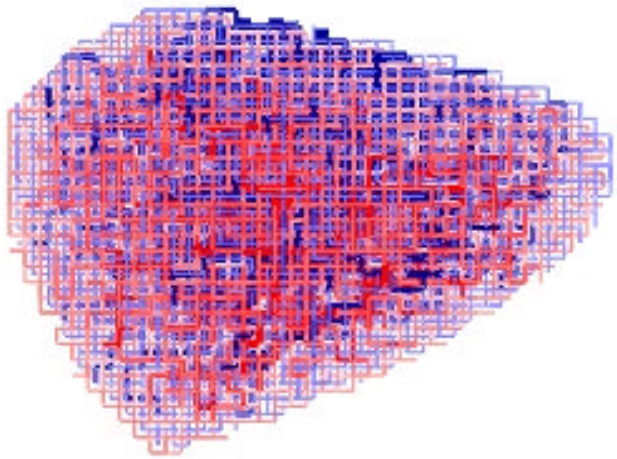


Figure 5. Result of simulation to construct blood vessels of human liver.

A system of sinusoids is constructed on a regular grid system, a cubic grid or a packing of Kelvin's tetrakaidecahedra (symmetric polyhedra with 14 faces). The algorithm of construction is similar to that applied for formation of blood vessels in the chicken embryo (Honda 1997), as explained below.

(i) Grid system is formed within a cubic region, and all pairs of neighbouring grid points are connected, which are capillary ducts and filled with a viscous liquid.
(ii) Remove initially some fraction of capillary ducts chosen randomly (to stabilize computation).
(iii) Sets of three edges of the whole cubic region are assumed to contact to inlet and outlet vessels, respectively. High and low pressures with fixed values are given to the inlet and the outlet vessels.
(iv) Compute flow rate in each capillary line, and remove those with smaller flow rates until one grip point has only three lines (dichotomous branching). This assumption of dichotomous branching gave the best result.
(v) At all grid points remove capillary lines with flow rate smaller than a certain fraction of an average.

Results of simulations with cubic grid and tetrakaidecahedron grid are shown in figure 6. Qualities of the simulated sinusoid should be examined by comparing with quantitative measurements. The structure of the sinusoid was reconstructed from measurement by Shimizu *et al* (1993) (see figure 6c), and the Becci number (number of loops included in a network) was calculated from it. This reconstructed structure is more similar to the result with the tetrakaidecahedron grid, while the Becci number is closer to that estimated from simulated network with cubic grid.

3. Concluding remarks

In the three simulations of human organs, three different algorithms are applied, branching in the free space for the airway, branch extension along grid line for the liver blood vessels and branch removal for liver sinusoid. In each case an algorithm, which seems to match to the actual process of organ formation, was chosen. However, it is not yet clear whether these algorythms are the best ones for respective cases. Since this kind of researches is quite new, criterion to judge the methodology is not established.

Mathematical studies of branch formation in biological systems were conducted since long before. Among them the method based on the diffusion-reaction equation developed by Meinhardt (1982) and other researchers would

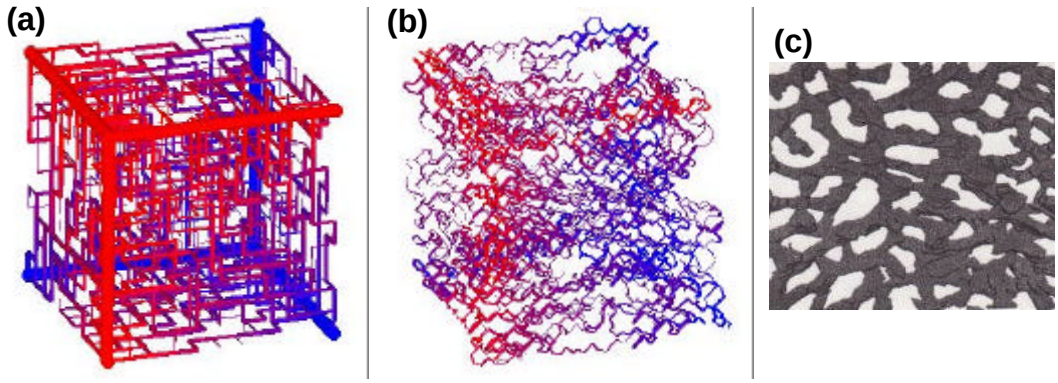


Figure 6. Results of simulation to construct capillary systems. (**a**) Simulation with cubic grid, and (**b**) tetrakaidecahedron grid. Thickness and colour of branches show the flow rate and the pressure, respectively. (**c**) Reconstruction from real sinusoid by Shimizu and Yokoyama (1993).

be the most popular one. It enables us to simulate initial stages of pattern formations, such as bud formation on a plant stem or differentiation of head and tail in an animal. This method is physically reasonable if only we can assume existence of diffusive materials. On the other hand, this method is not effective for complicated biological shapes in developed biological systems. The studies reported in this paper are trials to simulate patterns formation in these cases. Since they contain a lot of assumptions, they are not yet developed enough for basic understanding of morphogenesis. However, they are useful for practical applications in medical or technical fields. In addition, they are expected to give hints for more reliable simulations in the future.

Relation between the diffusion-reaction equation and biological processes in microscopic regime, such as the effect of a hedgehog on the lung growth (Bellusci *et al* 1997) is not yet clear. Here, a study to bridge between the diffusion-reaction equation and the biochemical regime by Miura and Shiota (2002) is introduced. In their study growth of lung airway of rat embryo was observed experimentally, where a material FGF1 was used for acceleration of growth. They made also a simulation of lung growth by applying two diffusion-reaction equations for the cell material and FGF1. Their study was successful in a sense that the experiment and the simulation gave similar results. If their method is developed further, it might give a foundation to such simulations as presented in this paper.

In the present status of studying the morphogenesis of organs, schemes to understand their mechanisms seem to be divided into three regimes, one concerned to biomolecular processes, one concerned to physicochemical processes (such as diffusion-reaction equation) and one concerned to engineering concepts (the present study). True understanding would be first obtained, when these regimes are connected by constructing bridges between them.

References

Bellusci S, Furuta Y, Rush M G, Henderson R, Winnier G and Hogan B L 1997 Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis; *Development* **124** 53–63

Grenney R B and Robertson H T 1995 A computer simulation of pulmonary perfusion in three dimensions; *J. Appl. Physiol.* **79** 357–369

Honda H 1997 Formation of the branching pattern of blood vessels in the wall of the avian yolk sac studied by a computer simulation; *Dev. Growth Differ.* **39** 581–589

Horsfield K, Dart G, Olson D E, Filley G F and Cumming G 1971 Models of the human bronchial tree; *J. Appl. Physiol.* **31** 202–217

Kamiya A, Togawa T and Matsumoto A 1970 Optimum structure of branching system of blood vessels; *Med. Electron. Bioeng.* **8** 136 (in Japanese)

Kitaoka H and Suki B 1997 Branching design of the bronchial tree based on a diameter-flow relationship; *J. Appl. Physiol.* **82** 968–976

Kitaoka H, Takaki R and Suki B 1999 A Three-dimensional model of the human airway tree; *J. Appl. Physiol.* **87** 6 2207–2217

Meinhardt H 1982 *Models of biological pattern formation* (London, New York: Academic Press)

Miura T and Shiota K 2002 Depletion of FGF acts as a lateral inhibitory factor in lung branching morphogenesis in vitro; *Mechanism Dev.* **116** 29–38

Murray C D 1926 The physiological principle of minimum work. I; *Proc. Natl. Acad. Sci. USA* **12** 207–214

Parker J C, Cave C B, Ardell J L, Hamm C R and Williams S G 1997 Vascular tree structure affects lung blood flow heterogeneity simulated in three dimensions; *J. Appl. Physiol.* **83** 1370–1382

Shimizu H and Yokoyama T 1993 Three-dimensional structural

changes of hepatic sinusoids in cirrhosis providing an increase in vascular resistance of portal hypertension; *Acta Pathol. Jpn.* **43** 625–634

Shimizu H, Sueyoshi N, Sakurai T, Sonoue K and Tanemura M 1998 How many faces has a polyhedron of liver cell?; *Bull. Soc. Sci. Form, Jpn.* **13** 101–102

Takaki R and Kitaoka H 1999 Virtual construction of human lung; *FORMA* **14** 309–313

Takaki R, Kitaoka H, Nishikawa T, Kaneko H, Nara M and Shimizu H 2003 The Formation of branching systems in human organs; *Proc. mpb2002, Morphogenesis and Pattern Formation in Biological Systems – Experiments and Models* (Tokyo: Springer-Verlag) pp 295–305

Weibel E R 1963 *Morphometry of the human lung* (New York: Academic Press)

West B G, Brown H J and Enquist J B 1999 A general model for the structure and allometry of plant vascular systems; *Nature* (*London*) **400** 664–667

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