DEVELOPMENT OF MULTI-LAYERED CELLULAR AUTOMATA MODEL TO PREDICT NERVE AXONAL EXTENSION PROCESS

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Key words: Nerve Axonal Extension, Cellular Automata

1 INTRODUCTION

Reconstructive surgery, employing the direct suture or the autograft, is conducting widely on damaged nerve tissue caused by traffic accident or excision of malignance. However, there are some problems on these treatments. The suturing method is restricted by its availability because a large tissue defect cannot be sutured by this method [1]. For substitute of these treatments, 3D nerve regenerative biodegradable scaffold is attracting attention as a new peripheral nerve regeneration method. The 3D-scaffold has the tube structure made of biodegradable and sorbable fiber such as polyglycolic acid (PGA) [2]. 3D-scaffold is implanted in the defected nerve tissue, and the nerve axon extended inside it. However, the speed of nerve axonal regeneration is not sufficient for persisting period of 3D-scaffold. The necrosis of defected peripheral nerve tissue is a serious problem. Hence, the optimum structure design of scaffold is strongly required to achieve rapid nerve tissue regeneration.

It is known that the nerve axonal extension is enhanced by various extracellular environmental factors, such as the scaffold structure, the chemical and electromagnetic stimulation [3]-[6]. For chemical factors, nerve growth factor (NGF) and Netrin-1 are widely known as an axon guidance molecule. In the previous studies of numerical simulation of nerve axonal extension process, Hentschel developed morphology alteration model considering the inflow and outflow of Ca^{2+} and Mouchet developed axonal extension model based on probability theory [7]. However, the prediction methods of nerve axonal extension state considering the effects of extracellular environmental factors have not been developed well. In this study, we developed a simulation model to predict nerve axonal extension considering the effects of extracellular environmental factors by using cellular automata (CA) method.

By using CA method, we try to predict axonal extension processes considering the interactions between computational cells. Conventional numerical simulation methods normally require solving governing equations. On the other hand, CA makes it possible to

predict the nerve axonal extension only by adopting simple local neighborhood rules and transition rules. CA can represent the complex dynamic morphology evolution using the simple local interactions between computational cells without a complicated governing equation of the simulative objects. Therefore it has been adopted to simulate many kinds of dynamic phenomena, such as physics, chemistry, material science, traffic, electronics, social science and economics [8]-[11].

In a CA method, the simulation area is divided into quadrates (cells). Each cell has any one of k different discrete conditions and changes its condition by conditions of neighborhood cells and itself. The change of state parameter is decided based on local rules. The change of cell condition occurs for all the cells simultaneously. We employed Moore neighborhood comprising 8 cells to represent the neighborhood of a target cell [7].

We used a multi-layered CA method to consider the interactions among nerve axon, various extracellular environmental stimulations, and cell constituents [12]. In multi-layered CA simulations, CA simulation for the behavior of one object is performed in one layer and their results are coupled to represent the interaction among the nerve axon extension and extracellular environmental stimulations (Fig.1). The multi-layered CA method makes it possible to add new factors and builds nerve axonal extension model considering extracellular environmental stimulations.

Interaction between computational cells

Interaction between layers
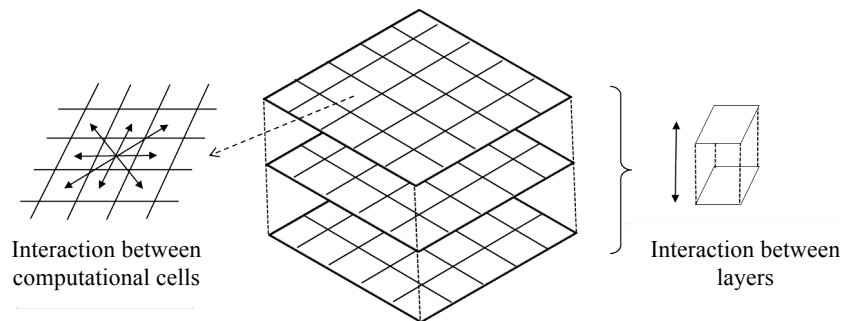
Figure 1: Schematic drawing of multi-layered CA model

First, we conducted time-lapse observation of pheochromocytoma cells (PC12) to understand the nerve cell morphological evolution, which can be characterized as axonal extension and branching. Secondly, we developed three 2D-CA models to predict the axonal extension, the growth and catastrophe of microtubule and axonal guidance molecule netrin-1 diffusion process. Finally, we developed a multi-layered CA model of nerve axonal extension and branching considering the extracellular environmental effects by coupling the simulations for these phenomena, which are individually performed in each layer.

2 EXPERIMENTAL OBSERVATION

We conducted the time-lapse observation of pheochromocytoma cells (PC12) to understand the nerve axonal extension process. We seeded nerve cell suspensions under the condition of 6.25×10^2 cells/cm^2 in DMEM (Dulbecco's modified eagle's medium) supplemented with 10% horse serum, 5% faetal bovine serum, and 0.05% antibiotic in each collagen-coated 35 mm culture dish. After the nerve cell adhesion on the bottom surface of

the culture dish, we added 50 ng/ml NGF. The PC12 cells were cultured at 37°C, 5% CO_2 and 100% humidity.

Figure 2 shows PC12 axonal formative process by microscopic time-lapse observation. At the early stage of cultivation, the nerve cell has 1 to 8 short dendrites. Thereafter, a certain dendrite became an axon and the nerve cell polarized. Furthermore, about 40% of axons formed branches. From our observation results, we identified characteristic factors of PC12 for introducing axonal extension model. The average number of dendrites was 3.6. The number of branches per axon was 0 to 2. The average branch length was 12.0 μm, and the branch extensive angle was randomly as shown in Figure 3.

The symmetry breaking of nerve cell is caused by Shootin1, a protein which expresses at the polarity stage and concentrates at the tip of dendrites, *in vivo* [13]-[15]. Shootin1 works upstream a signal molecules enhancing dendrite elongation and promotes nerve cell polarization. Moreover, Shootin1 works as a "cultch" molecule for transferring the driving force from actin fiber to adhesive molecule, and promote nerve axonal extension directly.
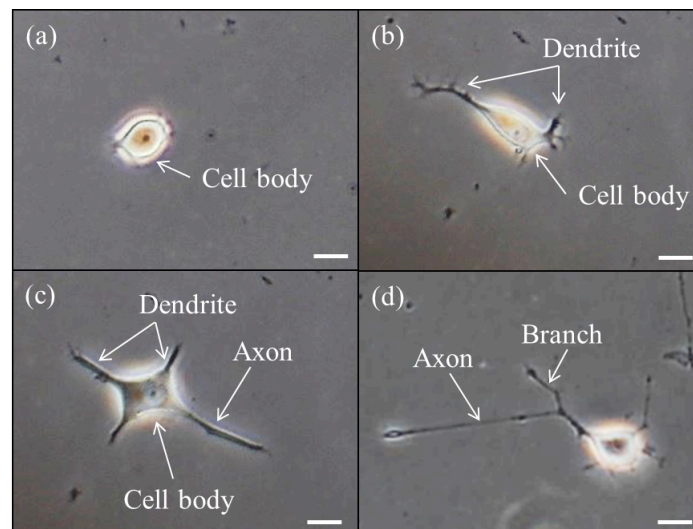
Figure 2: Cultivated PC12 after addition of NGF (Bar=10 μm); (a) 3h, (b) 6h, (c) 12h, (d) 24h

3 THE MODEL

### 3.1 CA model for nerve axonal extension

We divided a simulation area into 300×300 cells. A circle representing a PC12D cell body was placed at the center of the simulation area. The present CA model is constructed based on time-lapse observation results. Dendrites' extension occurs depending on the concentration of Shootin1, which randomly moves inside a nerve cell: The higher Shootin1 concentrates at the tip of dendrites, the longer the dendrite extends as compared with other ones [16]. To restage these phenomena, the probability P_n depending on dendrites' length was employed. We determined the number of dendrites generating from the cell body as four considering our experimental results. The probabilities of determination of dendrites P_n are defined by

$$P_n = \frac{a^{l_n}}{\sum\limits_{i=1}^{m} a^{l_i}}$$

(1)

where l_n (n=1, 2, 3, 4) is each dendrites' lengths, m is the total number of dendrites, a is random parameter, which was set to 1.08 in the present model. By this probability P_n, extending dendrite is chosen at each step. The direction of nerve axonal extension is stochastically determined according to the Gaussian random number at each step as shown in Figure 4.
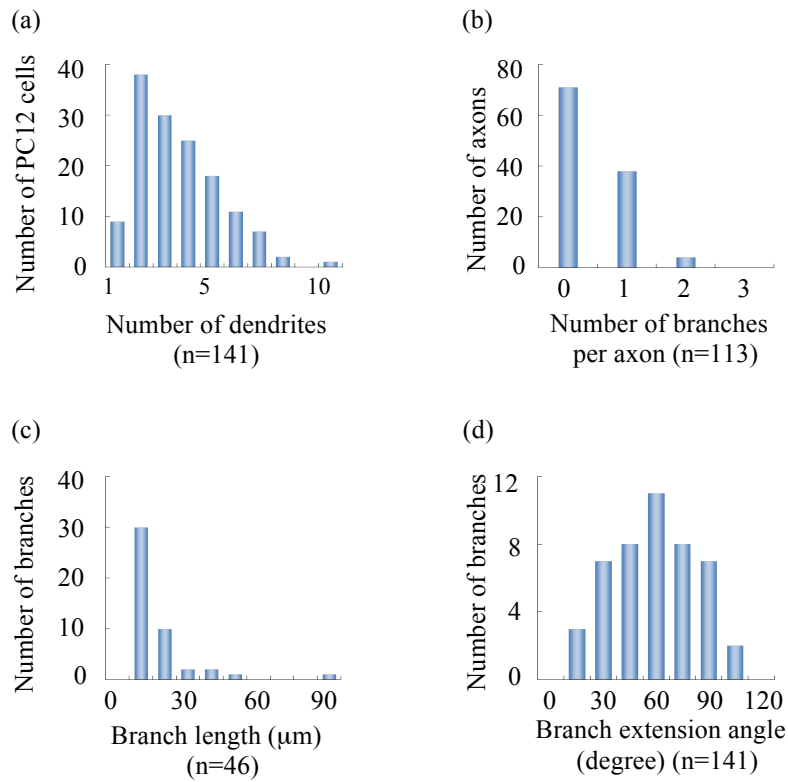
Figure 3: Histograms of (a) Number of dendrites, (b) Number of branches, (c) Branch length, and (d) Branch extension angle
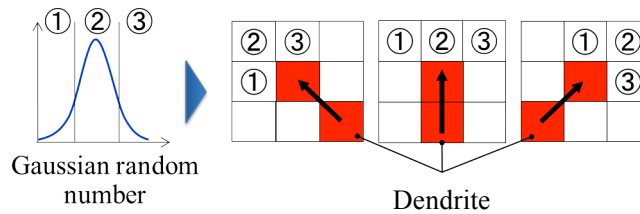

Figure 4: Selection procedure of dendrite extension direction

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3.2 Introduction of effects of microtubule behavior on branching

Nerve axon searches for a target by elongation and branching. Branches are caused by catastrophe of microtubules. Microtubule, a polymer constituted by tubulin heterodimer, is hollow cylindrical fiber, forms nerve cell cytoskeleton, and has dynamic structure repeating growth and catastrophe. When their co-assembly attenuates or microtubule shortens, axon forms branches [17].

We developed a CA model for microtubules that represents the increase of short microtubules caused by severance of microtubules or by weakening of microtubules co-assembly. Using multi-layered CA, the behavior of microtubules was introduced into the axonal extension simulation. The extension of nerve axons and the growth of microtubules were simulated in the 1st and the 2nd layers, respectively. In the 2nd layer, state parameter was set to "1" for microtubules, and to "0" for non-microtubule. The microtubules grew with axonal extension, which was calculated in the 1st layer. A certain cell for the microtubule was randomly chosen and its state parameter was changed from 1 to 0. The size of a cell for the nerve axon corresponds to that of a hundred cells for the microtubules as shown in Figure 5. The density of microtubules in a cell for the nerve axon was defined by the number of microtubule cells. Consequently, the density ranges from 0 to 100. The density was calculated at each step. If it is less than 50, a branch was generated from this low microtubule density cell. The maximum number of branches per axon was defined as 2 from our experimental observation.
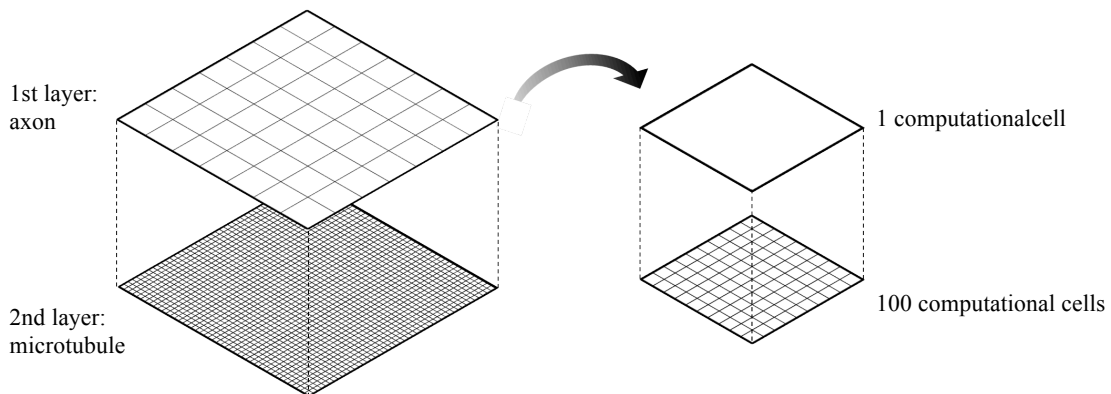
Figure 5: Correspondence between the 1st layer and the 2nd layer

## 3.3 Introduction of extracellular environmental factor

Song et al. reported that Netrin-1, nerve axonal guidance molecule, promotes and attracts axonal extension [18], [19]. Netrin-1 enhances phosphorylation of Shootin1 through phosphoenzyme Pak1. Shootin1 transmits kinetic energy with antidromic transfer of actin fiber to both nerve cell adhesion molecule and extracellular matrix and controls driving force generation essential for nerve axonal extension by phosphorylation. Moreover, netrin-1 induces axonal branch formation threefold [20]. Netrin-1 causes Ca^{2+} to be released, and Ca^{2+} signal causes microtubule catastrophe. Thus, axonal branch formation is increased.

Diffusion by random molecular transfer from a chemical to another one is restaged by exchange of state parameter values. First, a certain cell was randomly chosen from each row

and exchanged state parameter value with its right cell. This exchange was the 1st step and was performed every row in the simulation area. Similarly, state parameter values were exchanged with the left cell at the 2nd step, with the upper cell at the 3rd step, and with the under cell at the 4th step as shown in Figure 6. In our simulation model, Netrin-1 was supplied constantly, and hence the state parameter in the initial netrin-1 position was kept to that of netrin-1.

Finally, we introduced the netrin-1 diffusion model as the third layer computation into the axonal extension and branch model. In the same way as explained in the section 3.1, a dendrite to be extended was determined by the probability P_n dependent on the length of dendrites, and its extension direction was selected using Gaussian random numbers. We set the additional rule that a dendrite extends 2 cells accelerated by netrin-1 if there is a netrin-1 cell in neighborhood cells of the tip of the dendrite at the coupling step. At the same time, microtubule cells in the second layer grew along the dendrite cells. Catastrophe of microtubule was caused in the same way as explained in the section 3.2. The diffusion of netrin-1 was simulated in the 3rd layer, and the netrin-1 concentration at tip cells of each dendrite was calculated at every step. When the netrin-1 concentration was higher than a threshold value, the additional catastrophe of microtubules was caused. The branch formation was in the same way as explained section 3.2. The branch formation was computed in the same way as explained in the section 3.2. The microtubule density in each dendrite cell was calculated. When the microtubule density is lower than a threshold value, a branch was formed at the dendrite cell.

We adjusted time step of the simulation in each layer. Axonal extension rate was 20.7 μm/h from our observation. Netrin-1 diffusion rate is determined by diffusion equation and diffusion coefficient of netrin-1 is 40 μm^2/s [21]. Consequently, axon extended every 50,000 steps and netrin-1 diffuses every 2 steps in the present model.
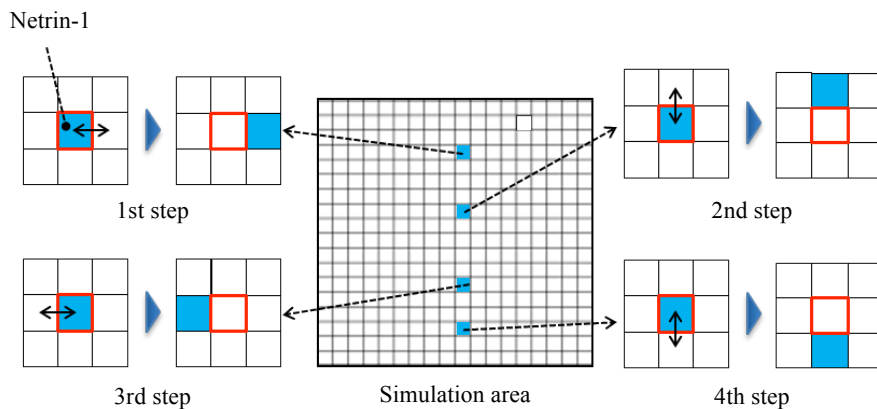
Figure 6: Schematic diagram of Netrin-1 diffusion rule

4   RESULTS AND DISCUSSIONS

Figure 7 shows the results of a simple nerve axonal extension simulation. In this simulation, extracellular environmental factors such as netrin-1 and microtubules were not considered. We successfully represent the phenomenon that four dendrites generated from cell body and

only a dendrite extends as a nerve axon. We conducted the simulation multiple times and confirmed that selection of axonal extension direction was indeterministically.

Figure 8 shows the results of multi-layered CA simulation of nerve axonal extension considering effects of microtubule and netrin-1. In Figure 8, nerve cells and netrin-1 cells are shown in red and blue, respectively. Netrin-1 enhanced the dendrites extension, and the dendrite on the netrin-1-rich side extends much longer than other ones to form a nerve axon selectively. Furthermore, branches were formed in dendrites and nerve axons depending on the density of microtubules in the second layer. Netrin-1 promoted the catastrophe of microtubule, thus the nerve axon on the netrin-1-rich side has more branches. As indicated here, the present model successfully represented interaction among nerve axon, microtubules and netrin-1.

5   CONCLUSIONS

We developed a multi-layered CA model to predict the nerve axonal extension process observed our experiment. The present model can include intra- and extra-cellular environmental factors such as microtubules and netrin-1 by the introduction of the local rules that express the interaction between the layers. We successfully restaged the nerve axonal extension and the branch process considering the effects of netrin-1 using the present model. It is an advantage of the multi-layered CA simulation that can easily introduce additional factors affecting the nerve axonal growth into a computational model.
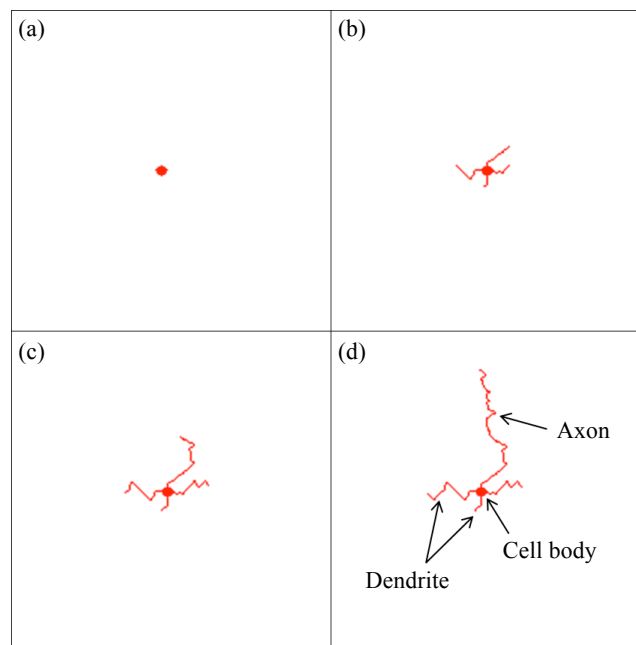
Figure 7: Results of nerve axonal extension simulation;
(a) 0 step, (b) 30×10^4 steps, (c) 60×10^4 steps, (d) 94×10^4 steps
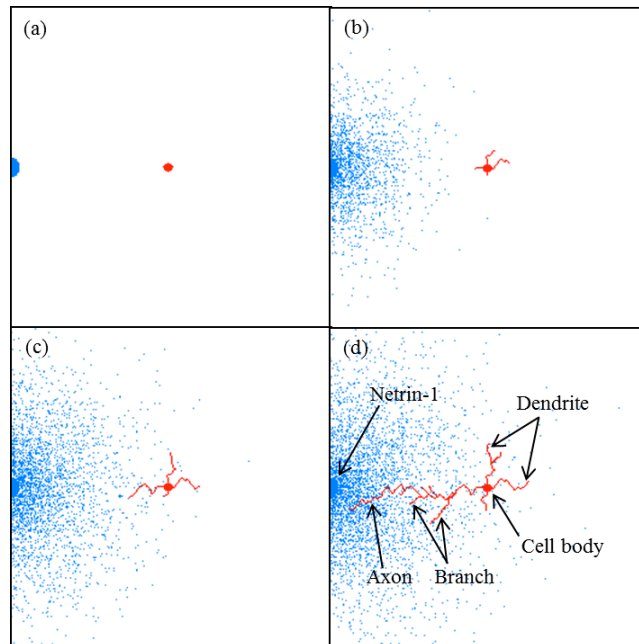
Figure 8: Results of axonal extension model with branch

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