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Research on the Indices for Demonstrating Cell Conditions

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Abstract

In the past a few decades, various kinds of cells have been examined in laboratories all over the world, and their interesting results have been expressed through various methods in journal publications. For a representative example, the increment or reduction of cell numbers during a bio-related experimental process has been demonstrated using the hazard ratio in survival analysis or in the form of a graph. In addition, the condition of cells such as their normality or abnormality would be indicated by the images of the cell nuclei or membranes treated with proper fluorescent labeling. However, the above methods seem to not be quantitative but rather qualitative assessments, which might be difficult to provide people with the eidetic understanding through parameters or numerical data. With adequate suggestions on any indices enabling the explanation for cell conditions, some analyses may be underestimated due to the lack of objectiveness caused by merely linguistic evaluation for the cell conditions, not numerally scientific interpretation. Therefore, in this study, we would suggest some indices enabling quantitative analysis on the cellular conditions.

Keywords : Cell, Network, Hazard ratio, Index, Neuron cell, Biostatistics

1. INTRODUCTION

A large number of experimental researches using various kinds of cells have been conducted in the fields of bioengineering and biomedicine during the past few decades. Through the results, the effects of established experimental conditions on the viability of cells would be examined by the estimation of cell number such as the increment or reduction during the observation of the cell condition. In preclinical fields, hazard ratio has been widely used as an index to indicate the viability of experimental subjects for a variety of experimental conditions. The hazard ratio is the method that measures and designates the temporal viability of the test subjects, being theoretically expressed as follows;

$$h(t) = \lim_{\Delta t \to 0} \frac{obsered\ events\ in\ interval\ [t,t+\Delta t]/N(t)}{\Delta t} \qquad (1)$$

Where, $N(t)$ indicates the number at risk at the beginning of an interval [1]. However, the hazard ratio only provides information on cellular viability and is composed of the terms that explain the experimental data to predict the events yet to happen during the experiment processes. In other words, the hazard ratio enables comparison of the viability for one experiment group with that of another group, based on the difference between the two groups. However, the difference in shapes of the viability graphs may not be analyzed through the hazard ratio [2].

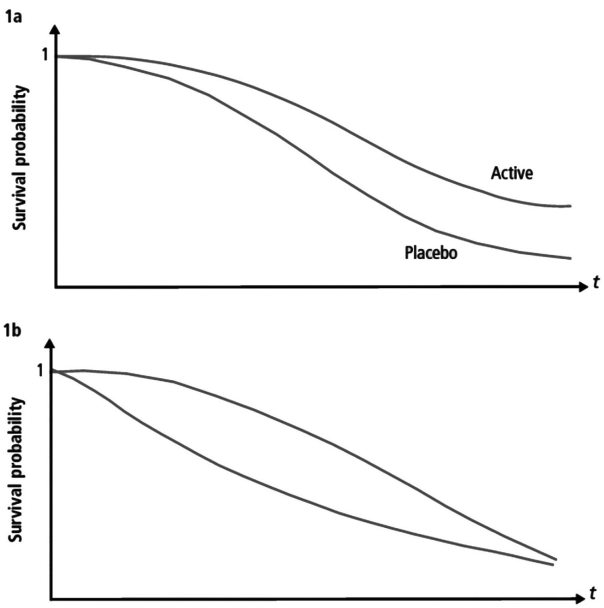

Fig. 1. Conceptual example for the temporal changes of hazard ratio.

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Dr. Martin Duerden (Department of Pharmacology, Therapeutics & Toxicology, School of Medicine, Cardiff University, UK) explained the hazard ratios for several cases [3]. For the application case of the preclinical medical field, the effect of a specific drug on the survival rate of experimental subjects has been estimated by the hazard ratio between the drug-treated active group and the placebo group. Nevertheless, as shown in Fig.1, the hazard ratio based analysis would only be limited to the numerical expressions such as high or low values whereas the temporal pattern changes are unexplainable. In other words, only the high hazard ratio case would be emphasized and the temporal changes of the cell condition might be ineffectively explainable.

Spotswood L. Spruance (MD in Salt Lake City, UT, USA) investigated an application model of the cox proportional hazard regression method to a drug study for clinical use [4]. This method has limitations to apply to clinical or preclinical experiments because it seems to be unrealistic to apply to the entire field of biology. He also attempted to improve the reliability as the conditions of other test groups have been analyzed by the hazard ratio theory model. However, the hazard ratio seems to be unviablein regards to a definite understanding of the two different graphs as shown in Fig. 2. In this graph, the comparison of the conditions for the two test subjects was conducted by the hazard ratio; the temporal changes in the subject number were indicated by numerical expression, which can only explain whether the subjects were alive or dead. These kinds of results would confine the analysis to a time-dependent prediction only. Therefore, the hazard ratio method may include deficiencies in demonstrating the conditions of the experimental groups even though at least 2 groups (control and test groups) would be used.

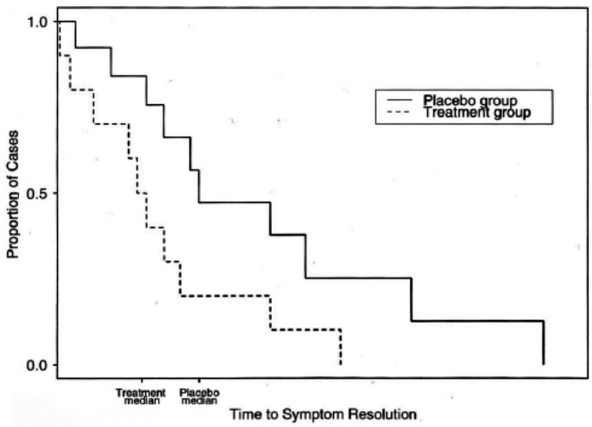

Fig. 2. Time-dependent comparison of viabilities for two groups.

Normally, the condition of experimental subjects has been estimated through an image-based evaluation, fluorescent cellular labeling, statistical values such as p-value for several conditions, or linguistic evaluation. The lack of indices for cellular conditions causes deficiencies in the explanation of the conditions even if cell pictures or data results exist [5,6].

The hazard ratioisrepresentedbyonlysurvivalover time, but it does not express thephysiologicaldifference of cell sizes or the number of neural networks. Therefore, this study suggests an index that can effectively designate the condition of cells used in experiments.

2. METHODS

2.1 Characteristics of cells

Normally, neuron cells do not increase in number in the culture dish during experiments because they do not self-divide. They grow by lengthening axons and forming networks through signal transduction, called synapses between neuron cells. Researchers studying neurons suggest cell number, viability, axon length, or signal transduction as the main results of experiments.

Other cells are able to self-proliferate and divide according to a given condition. Normally, they try to keep similar sizes, but sometimes they change their sizes with specific environmental conditions such as temperature, pH, or chemicals. Mostly, researchers use pictures taken during cell culturing to statistically express the changes in cell number [7].

2.2 Index evaluating cell conditions

In this study, we used ICD (index of cell condition) as a standardized index to demonstrate a specific cell condition, expressed as follows;

$$ICD = 098103$$

Which are mainly divided into 2 parts. First, 3 digits indicate the percentage of variations of the cell number, and the last 3 digits represent the percentage of cell size (e.g. the length of axon of neuron cell). Therefore, the conditions of cells can be easily indicated by ICD.

3. RESULTS AND DISCUSSIONS

Cell counting is an essential task to achieve ICD. During the neuron cell culture, task images are usually photographed at the same position where reference images were taken before

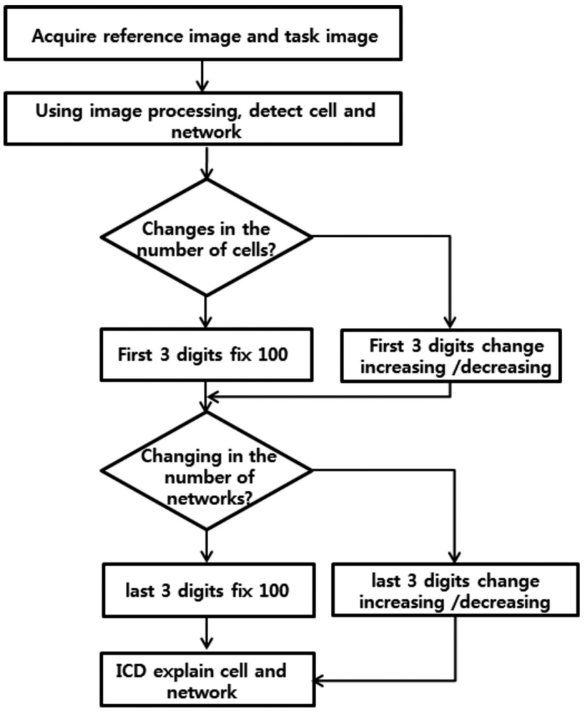

Fig. 3. Flow chart of ICD measurement.

since experimental conditions have been modified as the experiment proceeds. At this moment, specific patterns are required on the culture dish because 2 images need to be acquired at the same position. The changes in the cell number will be examined by counting cells and networks from the images acquired before/after the experiment, theoretically expressed as follows;

$$ICD = \left(\frac{task\ cell}{reference\ cell} \times 100\right)\ \left(\frac{task\ Network}{reference\ Network} \times 100\right) \qquad (2)$$

Neuron cells in a 4 day culture are shown in Fig. 4 and Fig. 5 demonstrates a 7 day culture. Table 1 indicates the numbers of cells and networks for Fig. 4 and Fig. 5.

Using Eq.(2), we can calculate ICD,

$$ICD = \left(\frac{183}{169} \times 100\right)\ \left(\frac{310}{172} \times 100\right) = 108180 \qquad (3)$$

According to the definition of ICD, neuron cells increase in 8% and networks in 80%, which means that experimental conditions help to promote cell growth and network formation. Neuron cells do not self-proliferate, but the cells were released from the chunks of cells as they separate that seemed to be one cell in aggregate form in an early stage. The same location of photography shooting has been regarded as the drawback for this method. Even a small change in photographing may cause errors in accurate determination for analysis due to the incorrect measurement and not changes regarding the experimental condition. To prevent this kind of error, a culture device might be fabricated in a specific shape.

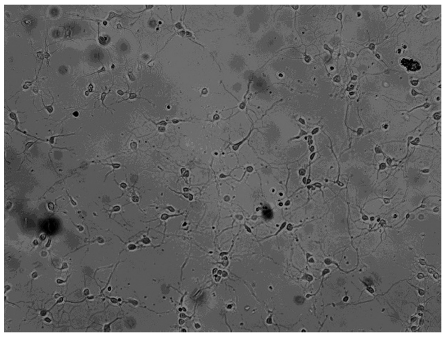

Fig. 4. DIV 4, cortical neuron cell.

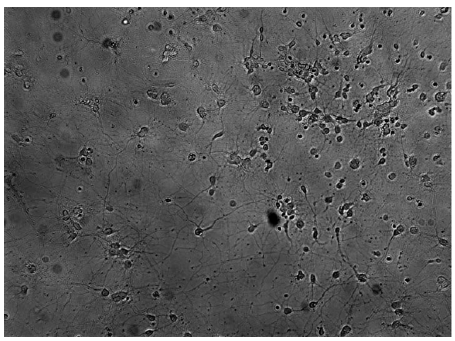

Fig. 5. DIV 7, cortical neuron cell.

Table 1. Numbers of neuron cells and networks

	DIV 4	DIV 7
cells	169	183
networks	172	310

The reason for the rapid network increase may be based on the signal transduction through the synaptic connections between the neighboring neuron cells that are regarded as the most representative characteristic for neurons. As a

single neuron cell enables several synapses with a variety of neurons, network numbers definitely increase faster than cell numbers during the culture period.

For other cells such as cancerous cells, they do not form a network between them and are characterized by their size. For example, ICD=100150 indicates the same cell number but an increased size in 50%. The observation for the change of cell size with the naked eyes might be difficult for accurate determination. Therefore, automatic measurements using a computer or culture dishes with gradation marking would allow for more accurate measurements [8].

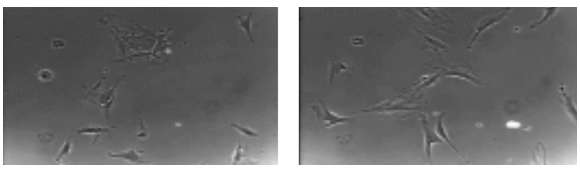

Fig. 6. Prior (left) and posterior (right) to the growth of normal cells.

For the proliferating cells, the exponentially rapid increase in the cell number makes estimation by eyes impossible. Therefore, special fluorescent labeling such as cobalt blue facilitates the confirmation for cell aliveness and then accurate examination.

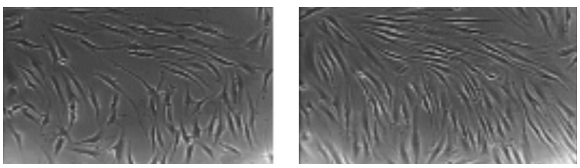

Fig. 7. Prior (left) and posterior (right) to the proliferating cells.

4. CONCLUSION

Many subjects have been studied in the field of bioengineering and biomedicine. Especially, cell experiments have been informative tools to establish new facts for drug development. Different research groups over the world may propose different results under a specifically given condition for each group. Thus, an absolute comparison of the results seems to be impossible but they might be referenced to one another.

The hazard ratio is only represented that living and dying. In the same case, the cell does not diefrom a different condition and change that size of cell body or number of neuronal network, so, the hard ratio is not expressed in more detail situation.

ICD suggested by this study can facilitate the induction of new results in the field of bioscience and express cell conditions in more detail (e.g. Increase the size of cell body, decrease number of neural network). The conditions of target cells would be better explainable quantitatively rather than qualitatively. Quantitative expression will be helpful for the analysis in all fields of bio-related research areas. As the preceding resultsare achieved under different conditions can be compared with others, the results from a precedent study can be referenced as verification for those of future studies and can be compared with those of current researches without a lot of dispensable experiments.

ACKNOWLEDGEMENT

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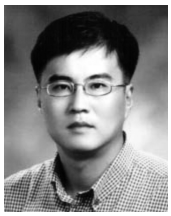

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