KBRI 한국뇌연구원

Press Release

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Development of a technology to produce cerebral cortex neurons

- A research team led by Dr. Kosodo Yoichi of KBRI created human brain environment using tropical fish
- It is expected that the mechanism of causing brain diseases will be identified and brain tissue regeneration treatment will be possible.

□ Korea Brain Research Institute (KBRI, President Seo Pan-gil) announced on Mar. 4 that its **research team led by senior researcher Kosodo Yoichi developed a technology to mass produce cerebral cortex* neurons utilizing Induced pluripotent Stem Cells (iPS*).**

* iPS(Induced Pluripotent Stem Cells): They are stem cells made of somatic cells, which do not have ethical problems.

* Cerebral cortex: Cerebral cortex is the outer layer of neural tissue of the cerebrum of the brain, playing a key role in higher cognitive functions such as perception, thought and memory.

□ The research outcome will be published in the March issue of Scientific Reports, an international journal, and the name of the paper and authors are as follows.

* Name of the paper: Brain-stiffness-mimicking tilapia collagen gel promotes the induction of dorsal cortical neurons from human pluripotent stem cells

* Authors: Misato Iwashita (the 1st author, KBRI), Hatsumi Ohta, Takahiro Fujisawa, Minyoung Cho,

Makoto Ikeya, Satoru Kidoaki and Yoichi Kosodo (corresponding author, KBRI)

□ Scientists expect that it will be possible to treat brain diseases by restoring damaged area in the brain by mass producing neurons utilizing stem cells even though cerebral neurons die if one suffers from degenerative brain diseases such as dementia and Parkinson's Disease.

○ In fact, a research team of Kyoto University in Japan conducted clinical test of transplanting neurons made of iPS into the brain of a patient with Parkinson's Disease. If one suffers from Parkinson's Disease, neurons that generate dopamine, which is one of the brain's neurotransmitters, die, resulting in symptoms such as muscle stiffness and tremor in hands and feet. Through the clinical test, the patient was treated with new neurons.

□ The research team cultivated iPS on a gel made of collagen from a fish called Tilapia* and successfully differentiated it into neurons.

* Tilapia: It is tropical fish living in Central Africa. Collagen is extracted from its skin.

○ In particular, the research team **cultivated iPS after making collagen gel have the same stiffness as human brain (1500Pa) and confirmed that cerebral cortex neurons have been produced by 60% more compared with existing method.**

□ The stiffness of human brain tissue changes as people age. Recently, it is reported that the stiffness of brain tissue changes with progress of

neurodegenerative diseases such as Alzheimer's disease. The research team reproduced stiffness of brain tissues shown in various kinds of diseases in this research and expects that the cause and mechanism of brain diseases could be identified by cultivating neurons.

□ Dr. Kosodo said that "**this research is meaningful in that the stiffness of brain is found to be an important factor in determining differentiation of neurons.**" He added that "we expect that we can mass produce certain neurons to be utilized for neuron regeneration treatment in the future".

[Attachments] 1. Major contents of the research

2. Description of research with pictures

3. Background of the researcher

[Picture]

Senior Researcher Kosodo Yoichi of KBRI (left) and researcher Iwasita Misato (right) look at the collagen gel sample from tropical fish.

1. Major Contents of the Research

☐ Name of the paper, information on authors

Name of the paper	Brain-stiffness-mimicking tilapia collagen gel promotes the induction of dorsal cortical neurons from human pluripotent stem cells
Name of the journal	Scientific Reports
Authors	Misato Iwashita, Hatsumi Ohta, Takahiro Fujisawa, Minyoung Cho, Makoto Ikeya, Satoru Kidoaki and Yoichi Kosodo

☐ Major contents of the paper

1. Background

○ Tissues of brain, internal organs and muscles in our body have different stiffness (Picture 2). However, the mechanism of how the stiffness adjusts the differentiation of stem cells has not been known.

2. Details

○ The collagen liquid extracted from Tilapia, a kind of tropical fish, is mixed with two kinds of cross-linking agents to combine collagen molecules for the purpose of producing gel.

○ It is found that if the medium is replaced by neuron differentiation medium after growing iPS to identify the characteristics of iPS cultivated in gel, the pluripotency of iPS is lost. On the third day after the replacement of medium, neurons which are in the state right before differentiation from multi-potent stem cell into neurons. On the 6^{th} day after medium replacement, neurons grew sharply. The characteristics of iPS observed in the cultivation using gel, which can be differentiated into various kinds of cells, are the same as those of iPS in the control group. Accordingly, we came to the conclusion that the collagen gel produced for this research can be applied to the cultivation of iPS.

○ iPS was cultivated in three different media: 150Pa, 1,500Pa and control group. The iPS was cultivated for 5 days and resulting neuron stem cells were cultivated in plastic dishes respectively. After 2 weeks, neurons were observed. The survey on the types of neurons generated found that the amount of expression of genetic group that shows cerebral cortex neuron cell in the 1,500Pa stiffness showed 1.6 times higher than existing cultivation method (Picture 4).

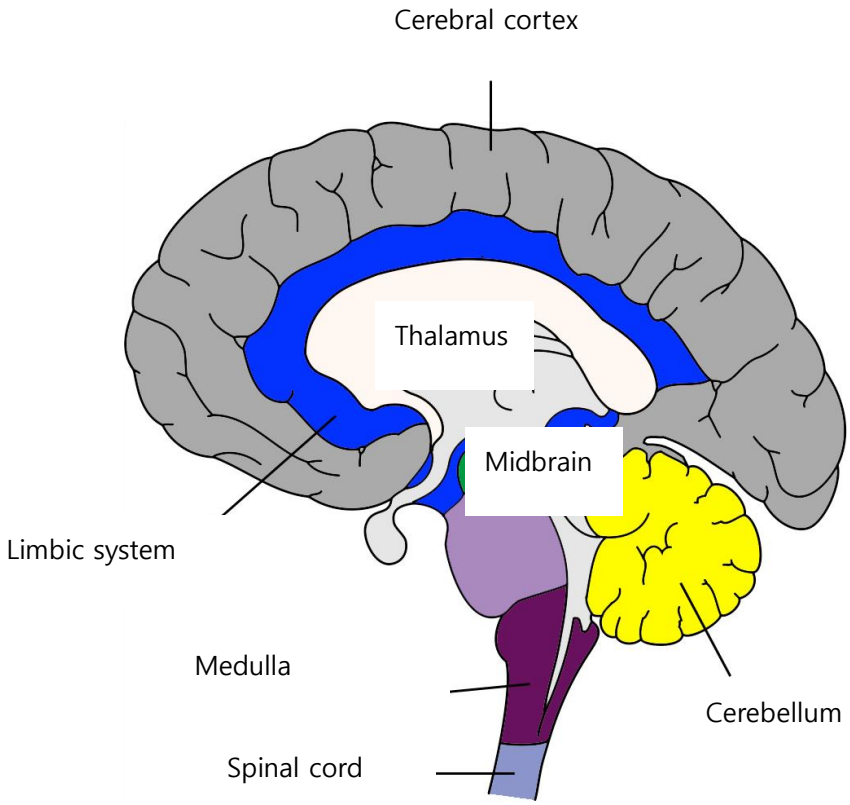
3. Research outcome and expected benefits

○ The collagen gel produced by research team of KBRI has characteristics of high transparency and flat surface (Picture 3). Accordingly, it is possible to conduct a precise analysis on the impact of different stiffness on differentiation using this material. This gel is also suitable for observing the movement of

live cells and differentiation status.

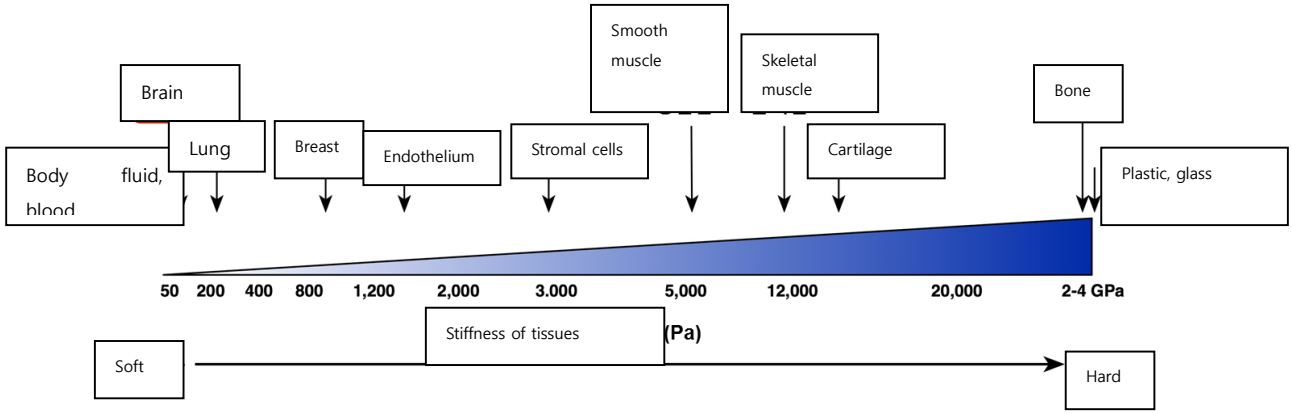
○ It could contribute to the identification of mechanism that causes disease or test drug by reproducing stiffness of a brain and cultivating cells related to various kinds of diseases. It could be an effective treatment to regenerate damaged brain cells by transplanting of cells. Therefore, the establishment of cell differentiation methods by different stiffness is expected to produce cells for transplantation efficiently.

2. Description of Research with Pictures



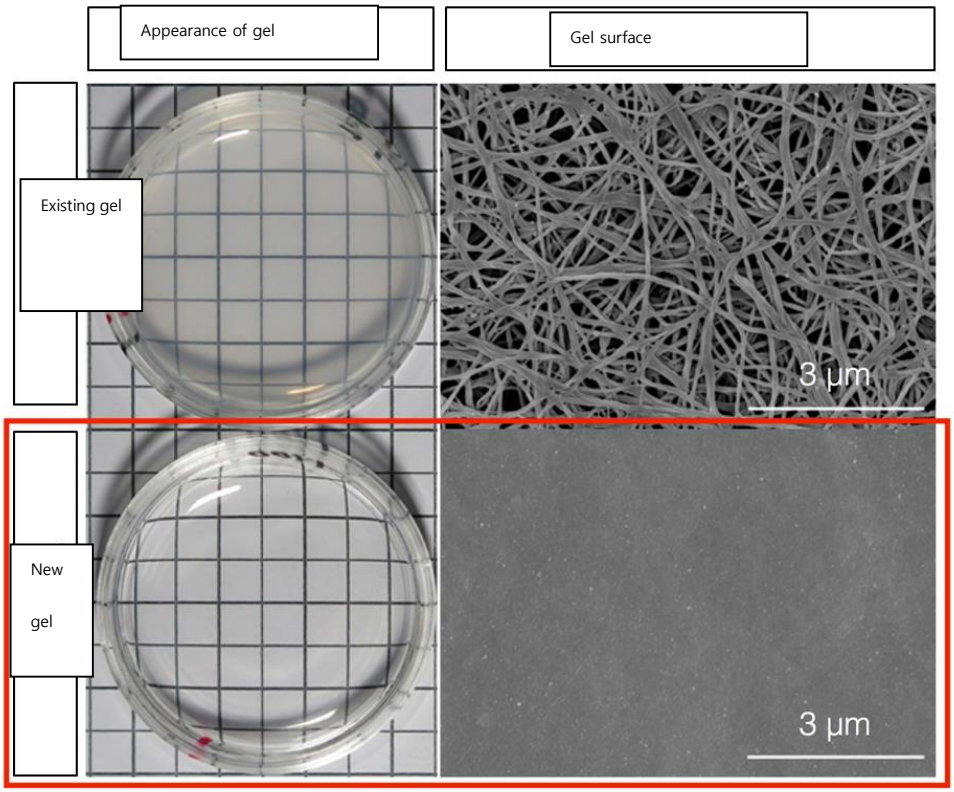
[Picture 1] Shape of brain

This picture is a cross section from the sagittal plane and the outer side of the cerebrum is cerebral cortex.



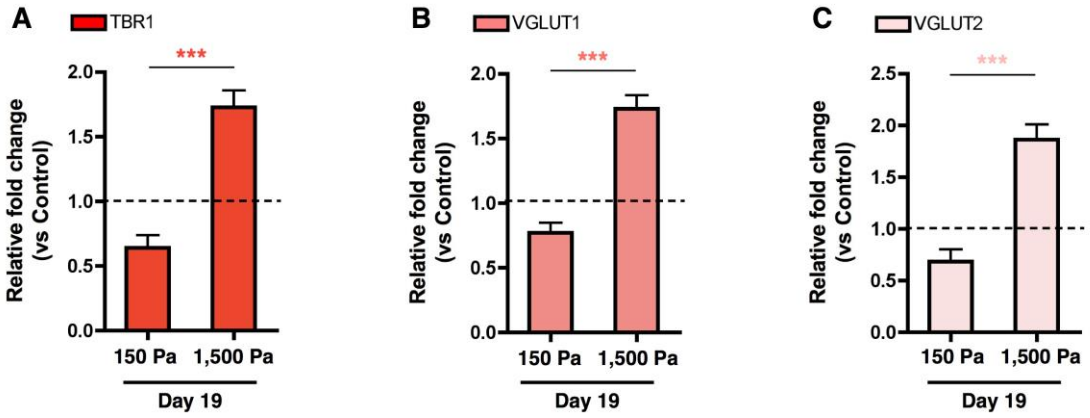
[Picture 2] Stiffness level of various tissues

The bigger the number, the harder it is. (source: Butcher et al, Nat. Rev. Cancer, 2009)



[Picture 3] Shape and surface of collagen gel observed under electronic microscope

- Top: Collagen gel which has been in use. Collagen fabric is observed on the surface.
- Bottom: Collagen gel produced for this research. It is transparent to the extent that the the area below the gel can be seen.



[Picture 4] Changes in the expression of genes which are discovered in neurons produced from collagen gel

- TBR1: The amount of cerebral cortex neuron gene group expression
- VGULT1, 2: The amount of excitatory neuron gene group expression

This graph shows the gene expression amount if the gene expression amount is 1 in the control group. Given TBR1, it is found that the amount of cerebral cortex neuron gene expression is 1.6 times higher than that of the control group.

3. Background of the researcher

1. Personal information

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2. Education and career

◦ 2015 ~ present Lab Head of Neural Regeneration Lab of the KBRI

◦ 2010 ~ 2015 Associate Professor at Kawasaki Medical School, Japan

◦ 2005 ~ 2010 Research Scientist at RIKEN CDB, Japan

◦ 2001 ~ 2005 Post-Doc. at Max-Planck-Institute, Germany

◦ 2001 Acquired doctorate degree at University of Tokyo

3. Research area

◦ Brain development, stem cell