

June 4th, 2018

## Organ Technologies and RIKEN Launch Preclinical Tests in Hair Follicle Regenerative Medicine

Organ Technologies Inc. (President : Yasuhiro Sugimura) and TOKYO, June 4, 2018 - RIKEN national science institute (President : Hiroshi Matsumoto) announced today that they will launch non-clinical tests as a preliminary stage toward clinical research in hair follicle regenerative medicine.

### 1. Alopecia Treatment and Issues

Currently there are more than 18 million Japanese people living with multiple types of hair defects including androgenic alopecia (AGA), congenital alopecia, scarring alopecia, and female telogen effluvium alopecia [1]. Because hair has a significant influence on first impressions and is a symbol of beauty and social identity, alopecia affects the quality of life (QOL). Therefore, alopecia and thinning hair attract great interest and various types of methods have been attempted in the large alopecia treatment market. However, these treatments do not always have scientific evidence to support the effectiveness against alopecia. Therefore, the Japanese Dermatological Association provides advice on various treatments for AGA and female pattern baldness based on medical guidelines [2]

Topical application medications and external and internal medicines are commonly used to treat alopecia, thinning hair, and AGA. However, in all cases, cessation of the treatment results in a relapse of the symptoms, and continuous medication is essential. In cases where these treatments have limited effects, autologous follicular unit transplantation is applied, transplanting the patient's own occipital hair follicles to the area of alopecia. However, because this treatment is autologous re-implantation, there is a limitation in that the total number of hair strands does not increase, and the satisfaction level is low. To overcome this limitation, development of hair follicle regeneration therapy based upon scientific evidence is a goal.

[Source]

[1] Guideline for Medical Care on Androgenic Alopecia 2010

[2] Japanese Dermatological Association "Guideline for treatment of pattern hair loss in men and women2017" [https://www.dermatol.or.jp/uploads/uploads/files/AGA\\_GL2017.pdf](https://www.dermatol.or.jp/uploads/uploads/files/AGA_GL2017.pdf)

## 2. Academic background and social significance

The realization of regenerative medicine is one of the major goals for healthcare in the 21st century. To date, stem cell transplantation therapy (such as bone marrow transplantation), and tissue regeneration medicine (epidermal cell sheets, etc.) has already been put into practical use as the first and second generation of regenerative medicine. Currently, basic research on "organ regeneration medicine", as the third generation of regenerative medicine, is underway in the last decade and expectations are growing for its realization.

In 2007, the Laboratory for Organ Regeneration (Team Leader: Takashi Tsuji) or the RIKEN Center for Biosystems Dynamics Research developed 3D cell manipulation technologies to generate bioengineered organ germs, and called this the organ germ method. By orthotopic transplantation of bioengineered organ germs, his group has demonstrated fully functional regeneration of multiple organs<sup>[3]</sup> including teeth<sup>[3]</sup>, hair follicles<sup>[4]</sup>, salivary glands<sup>[5]</sup> and tear glands<sup>[6]</sup>, for the first time in the world.

In particular, the regeneration of hair follicles, as reported in 2012, is considered to have great potential for clinical application, because the hair follicle is the only organ that has organ inductive adult stem cells and thus can repeatedly regenerate in the hair cycle.

In this report, the research team generated bioengineered hair follicle germ by utilizing an organ germ method with bulge cells containing epithelial stem cells and follicle dermal papilla cells harboring mesenchymal stem cells isolated from the follicles of adult mice. After transplantation into hairless mice skin, bioengineered hair follicle germs developed into mature hair follicles and generated hair at the desired density. Moreover, regenerated hair follicles have the connections with surrounding tissues, such as arrector pili muscle and nerves, and showed sustainable hair cycles, demonstrating its functional regeneration. Furthermore, this method allows us to control of the hair color by adding melanocyte (pigment) stem cells, elevating its potential for the aesthetic treatment of alopecia.

Hair follicle regenerative medicine is a new therapeutic technology for alopecia treatment based on scientific evidence. The hair follicle is an organ tissue, so hair follicle regenerative medicine, as a third generation organ regenerative medicine using Japanese original technology, could lead to a Japanese renaissance based on regenerative medicine, the novel therapeutic treatment of the 21st century.

[Source]

- [3] Nakao, K. *et al.*, *Nature Methods*. **4**, 227-230, 2007.
- [4] Toyoshima, K. *et al.*, *Nature Commun.* **3**, 784, 2012.
- [5] Ogawa M. *et al.*, *Nature Commun.* **4**, 2498, 2013.
- [6] Hirayama M. *et al.*, *Nature Commun.* **4**, 2498, 2013.

### 3. Problems and solutions for clinical application to humans

There were two major challenges to applying hair follicle regeneration technology to humans.

- 1) The need to develop an *in vitro* amplification method for epithelial stem cells and dermal papilla cells

Previously, epithelial stem cells had not been identified, and it was known that the hair follicle regenerative capacity of cells disappears while cultured *in vitro*. However, we have overcome these problems with mouse and human cells after research activities over 7 years.

- 2) The development of a robust method for the mass production of regenerated hair follicles

Conventionally, regenerated hair follicles were produced manually by compartmentalizing two types of stem cells into a collagen gel at a high density in a cell suspension of ten thousandth of a milliliter. Therefore, it was very difficult to stably mass-produce regenerated hair follicles, and we needed to develop a new technology to overcome this for the clinical application. We succeeded in developing this technology with collaborative research with Kyocera Corporation, which began in 2016.

With the success of these developments and by overcoming the other challenges to carrying out clinical research, we have advanced to the P0 stage (preclinical testing) in RIKEN Program for Drug Discovery and Medical Technology Platforms. Based on this program, RIKEN is playing a role in the preclinical research and academic validation of the amplification and cultivation technology of human cells. Organ Technologies, Inc. will establish the manufacturing and quality-control system for conducting the preclinical tests. Kyocera will provide novel methods for manufacturing regenerated hair follicles.

### 4. Preclinical tests for clinical research

Regenerative medicine is classified into 3 categories, "Class I Regenerative medicine", "Class II Regenerative medicine", and "Class III Regenerative medicine" depending on the degree of effects on human life and health, and necessary procedures are stipulated for each category. Because the collected hair follicle cells are derived from somatic stem cells and classified as medium

risk, it falls under "Class II Regenerative Medicine." We will submit a provisional plan to the Certified Special Committee for Regenerative Medicine and subsequently submit it to the Minister of Health, Labor and Welfare after receiving approval. Prior to applying to the Certified Special Committee for Regenerative Medicine, it is necessary to carry out preclinical tests to confirm the safety using animals.

In order to guarantee safety in humans, it is necessary to prove that the regenerated hair follicles used in the preclinical tests are manufactured by the same method as in the clinical test, and that the qualities of the products are the same both in preclinical and clinical research, and that they are shown to be non-tumorigenic *in vivo*. Specifically, it is necessary to establish a quality control method at the time of acceptance of human tissues, optimizing of human stem cell amplification method (kinds of additive factors and length of culturing days, etc.), development of quality control system of the products, changes of raw materials to comply with Standards for Biological Ingredients, documentation of work procedure manuals, stabilization of the manufacturing, establishment of packaging and transportation method, and designing and operation of sterile manufacturing equipment. We have been carrying out R&D for resolving these problems.

#### 5. Preclinical tests for the clinical research of hair follicle regenerative medicine

Our plan is to start a clinical trial of hair follicle regenerative medicine for male AGA patients, and then to expand the indication to female pattern alopecia, scarring alopecia, and congenital alopecia. First, epithelial stem cells, dermal papilla cells and pigmented stem cells will be obtained from normal hair follicles acquired from the occipital scalp of AGA patients with the method prescribed in the Standard Operating Procedure (SOP). Following amplification of the cells by culturing *ex vivo*, cells will be recovered and regenerated hair follicles will be produced for the regenerated hair follicle formation. Nylon sutures will be inserted into the regenerated follicle for hair growth induction.

As a quality control test in the culturing process, we will make sure that the products are not contaminated and manufactured properly by the methods of flow cytometry or PCR. Next, in the shipping specification test, we will conduct morphological evaluation, cell viability test, cell sterility test, mycoplasma negative test etc. to see if the final product can be manufactured in accordance with the intended quality standards.

In the preclinical safety tests (general toxicity and tumorigenicity test), human regenerated hair follicle will be transplanted subcutaneously into the back skin of immunodeficient mice, and the condition of the whole body and transplantation site will be continuously observed for a certain period.

## PRESS RELEASE

At the end of the tests, histopathological examination and immunohistological examination of the tissue around the transplantation site will be performed, and we will confirm that there is no toxicity or no malignant tumor formation in the animals transplanted with human regenerating hair follicles.

For these manufacturing methods, quality control methods, and design of non-clinical safety tests, we are proceeding with the advice of the Pharmaceuticals and Medical Devices Agency (PMDA) for the implementation of future clinical research.

### 6. Schedule

Under the plan, we will begin manufacturing samples for preclinical tests in July 2018 and we will conduct preclinical safety tests using animals. We plan to complete the safety testing in 2018. If the results of the preclinical safety tests are successful, we will apply for clinical research to the Certified Special Committee or Certified Committee for Regenerative Medicine. After receiving approval from the Committee, we will present a provisional plan to the Minister of Health, Labor and Welfare, and will shift toward implementation of clinical research. The details of the clinical research will be announced before the initiation of the research.

### 7. Inquiry

1) Organ Technologies Inc.

President : Yasuhiro Sugimura

Address : 6-7-1 Minatojima-minamimachi, Chuou-ku, Kobe 650-0047, Japan

E-mail : [info@organ-technol.co.jp](mailto:info@organ-technol.co.jp)

2) RIKEN national science institute, Center for Biosystems Dynamics Research

Laboratory for Organ Regeneration

Team Leader : Takashi Tsuji

Address : 2-2-3 Minatojima-minamimachi, Chuou-ku, Kobe 650-0047, Japan

E-mail : [tsujilab@cdb.riken.jp](mailto:tsujilab@cdb.riken.jp)

\* Please contact us here for research detail.