# The innovative therapy method development for regenerating organs *in vivo* -Direct Reprogramming-

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## Introduction

These years so-called ñDirect Reprogramingò; the methodology to directly induce specific differentiated cell from somatic cells without using iPS has been highlighted as a basic research. This therapy methodology has been much expected thanks to the facts that the procedures are simpler and drastically time-saving compared to that of conventional method which requires cell transplantation after establishing stem cells and it reduces the risk of oncogenesis because it can dispense with embryonic stem cell such as iPS.

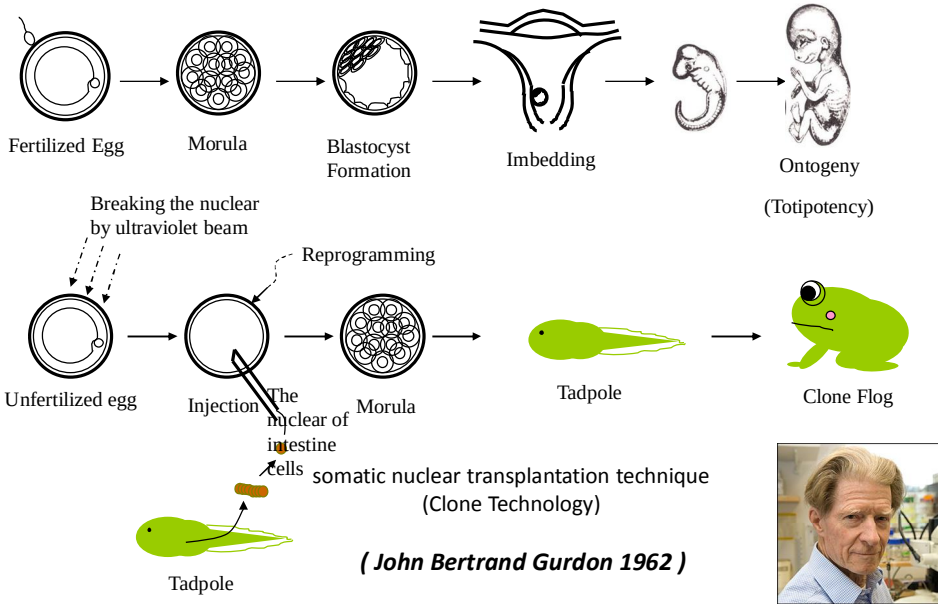
Currently *in vivo* Direct reprogramming has been studied by lots of overseas researchers, while in Japan Dr. Masaki Ieda, department of cardiovascular medicine at Keio University School of Medicine has pioneering in the research for regenerating heart through *in vivo* induction of fibroblast to cardiac muscle. In addition to this research also at Keio University School of Medicine, I has opened up the professorship of ñOrgan Fabricationò (human-size organ fabrication) since 2014. If these basic researches are upgraded to the pre-clinical trials utilizing experimental pigs, it would be almost certain that the researches will be a revolution in regenerative medicine for practical applications.

The author in this article would like to refer to the necessity of preparations for the world-first clinical application through ñDirect Reprogrammingò.

## Background

The process of differentiation from fertilized eggs and so on in undifferentiated status to somatic cells is in general irreversible. On the other hand, through the injection of matured somatic cells into unfertilized eggs, the cells are to be reprogrammed by stimulating genesis (John Bertrand Gurdon 1962).

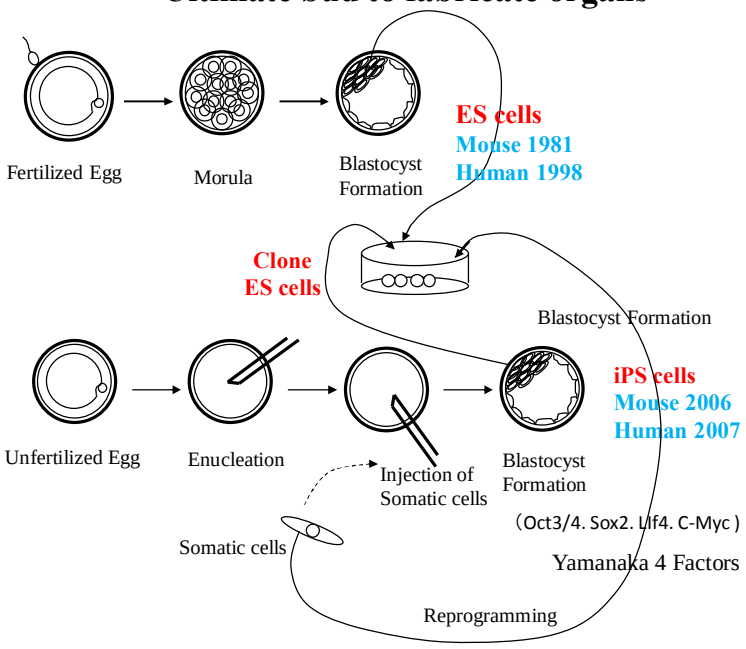
## “Totipotency” and “Ontogeny”






And it has been proven that so-to-speak cellular reprogramming is triggered by injecting a specific gene (Shinya Yamanaka 2006).

## Impact of Pluripotent cells!
## Ultimate bud to fabricate organs



However, the phenomenon of terminally-differentiated cells to be converted to other cell types has been known for a long time. In 1989 it was reported that simply injecting MyoD gene: master controlling gene for muscle differentiation into fibroblast and chondrobast leads to generate skeletal muscle cell (*Weintraub H, et al. PNAS 1989*). It means that somatic cells have been transdifferentiated without initialization. This is so-called ñDirect reprogramming (Direct conversion). These years in the US epoch-making findings have been reported in regard to Direct reprogramming. The key is that multiple genes are transferred simultaneously as it was done by Prof. Yamanaka in 2006. Prof. Douglas Melton of Harvard University reported that exocrine cells of matured mouse are to change into pancreatic ß cells as endocrine cells by simultaneously adding three kinds of transcription factors to its exocrine cells (*Zhou Q, et al. Nature 2008*). The group of Stanford University induced differentiation to nerve cells through the simultaneous gene transfer to the fibroblast of mouse by three kinds of transcription factors (*Vierbuvhen T, et al. Nature 2010*). In addition the group of Gragstone Research Institute has found out four kinds of important transcription factors which convert intracardiac fibroblast to myocardium (*Ieda M, et al. Cell 2010*).

## The outlook for viable regenerative medicine through Direct reprogramming

Currently the researches for applying Direct reprogramming to regenerative medicine have been much accelerated. Two methods have been taken into consideration; Inducing target cell to *In vitro* for cell treatment and Direct reprogramming *In vivo* in the patientôs body.

| Direct reprogramming | Advantages | Challenges |
|---|---|---|
| *In vitro* | ・Generating directly adult cells without stepping up from fetus<br>・Avoiding risks of tumor after cell transplant<br>・Shortening time for induction by skipping stem cell | ・Cultivation in big quantities difficult compared to ES/iPS |
| *In vivo* | ・No immunological rejection | ・Difficult to control gene onset |

As mentioned above, the proof of concept for Direct reprogramming thorough the usage of small animals has been well put in order. Nevertheless, the reasons why itôs not yet enhanced to clinical

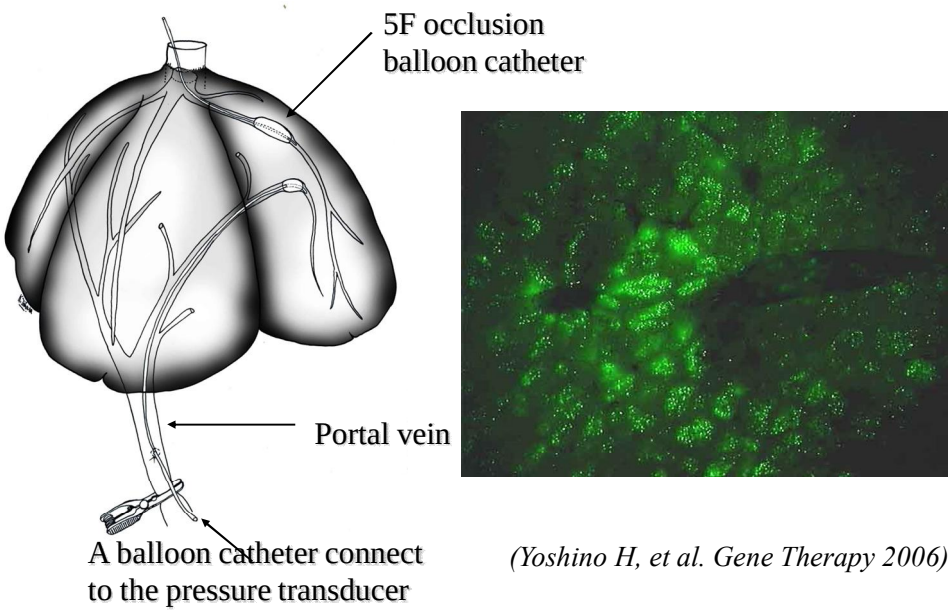
trials are that it has been unproven with the animals equal to the size of human. In order to make the technology applicable to human, introduction of devices for clinical practices such as catheter technology through the optimization of experimental pigs is the MUST.

## The gene transfer by experimental pigs as a pre-clinical model

Up to now the author has been conucting the gene transfer methodology by pig which is equal to the size of human taking clinical practices into consideration. .

Injecting in large amount of solution plus Plasmid DNA by way of blood vessel in high speed leads to highly efficient transfer of target genes to muscle and hepatocyte (Hydrodynamic Gene Therapy method).We have succeeded in optimizing the method by pigs at the earliest stage in the world (2006).

***Naked plasmid DNA transfer to the porcine liver***

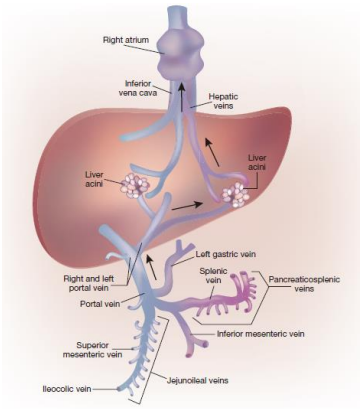


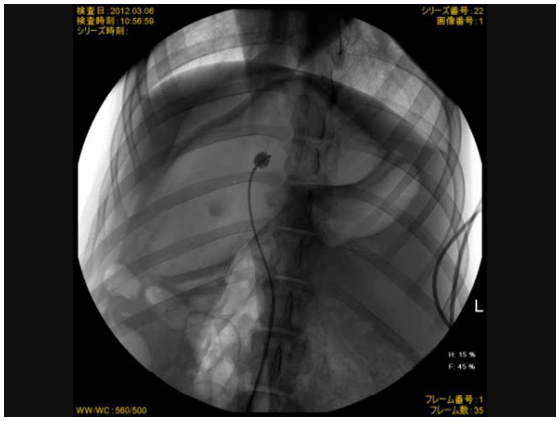

*(Yoshino H, et al. Gene Therapy 2006)*

The key of this technology is to flush out the blood before injecting Plasmid DNA by balloon catheter. The method has been applied to the experimental therapy of AAV vector with extremely love pathogenicity (2013).

## *Flushing Out Antibodies to Make AAV Gene Therapy Available to More Patients*

*(Raper S, et al. Mol. Therapy 2013)*



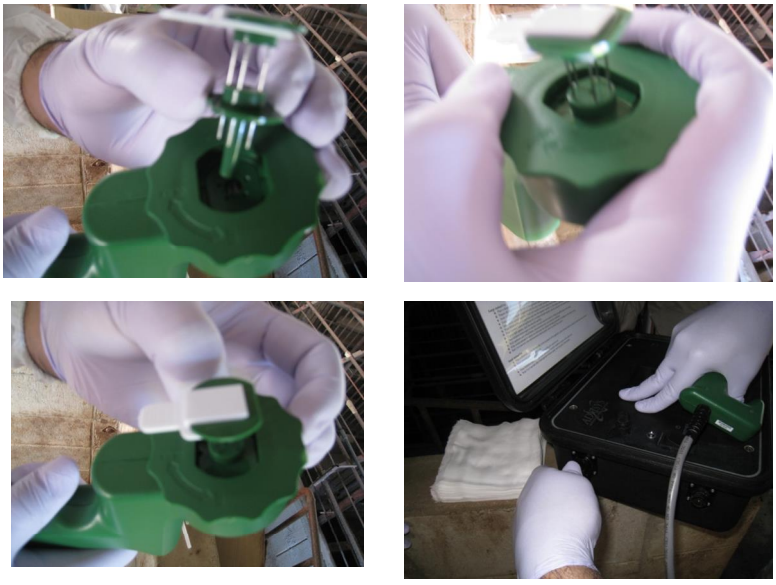



*(Mimuro S, et al. in non-human primates Mol. Therapy 2013)*

*(Hishikawa S, et al. in Pigs)*

On the other hand in addition to the gene transfer based on catheter, the method to utilize electroporation has been developed in pigs. We have tested the method with experimental mini pigs right after the development (Co-research with Joseph Kim VGX Corporation in 2009).

## *Electroporation Method by Naked plasmid DNA*



*(Unpublished 2009)*

## Closing Remarks

In order to apply the proof of concept based on small animals to clinical trials, it is indispensable for scientifically securing the effectiveness and safety for human. Especially in case we put it in clinical practices in regenerative medicine which is focused on functional recovery, it is extremely important from clinical viewpoint that extrapolating in the experimental animals equal in size of human proves the facts that it is applicable to human the catheter technology, the amount of cells applied and judgement to increase in gene amount. Finally, the author believe the possibility for the world-first clinical application through ñDirect Reprogrammingò.

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