

# STEM CELL-DERIVED BETA CELLS TO TREAT DIABETES

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Stem cells hold tremendous potential to affect future treatments for diabetes. Endowed with an ability to give rise to any cell type in our body, the challenge resides in coaxing these cells in the right direction, to differentiate them into functional beta cells. After 10 years of development of methods that recapitulate each step the cells normally execute in the body before they become beta cells, major progress on the last step has been achieved in 2014. Transplantations are getting closer to the clinic, as Viacyte received Food and Drug Administration (FDA) approval to transplant pancreas progenitor cells in encapsulation devices in the fall of 2014. In spite of this progress questions do remain. What is the similarity between these cells and real beta cells? Do they need to be exactly identical? How can we move to mass production? How will we circumvent the elimination of the transplanted cells? Beyond transplantation, stem cells offer the potential to produce model human beta cells to study diabetes and find new drugs, an important potential that has received less attention (Figure 1).

## DIABETES AND CURRENT TREATMENTS

Most diabetic patients are affected by type 2 diabetes, which combines insulin-resistance of target tissues, with the inability of beta cells to produce enough insulin to cope with this defect. The decrease glucose production by the liver and increase the sensitivity of target tissues to insulin (Metformin) or enhance insulin secretion (sulfonylurea, glp1-analogues...). Eventually, insulin injection can be required as beta cells become exhausted. In type 1 diabetes, insulin-producing beta cells are destroyed by the immune system leading to lifelong dependency on daily injections of insulin. In patients whose blood glucose cannot be efficiently controlled

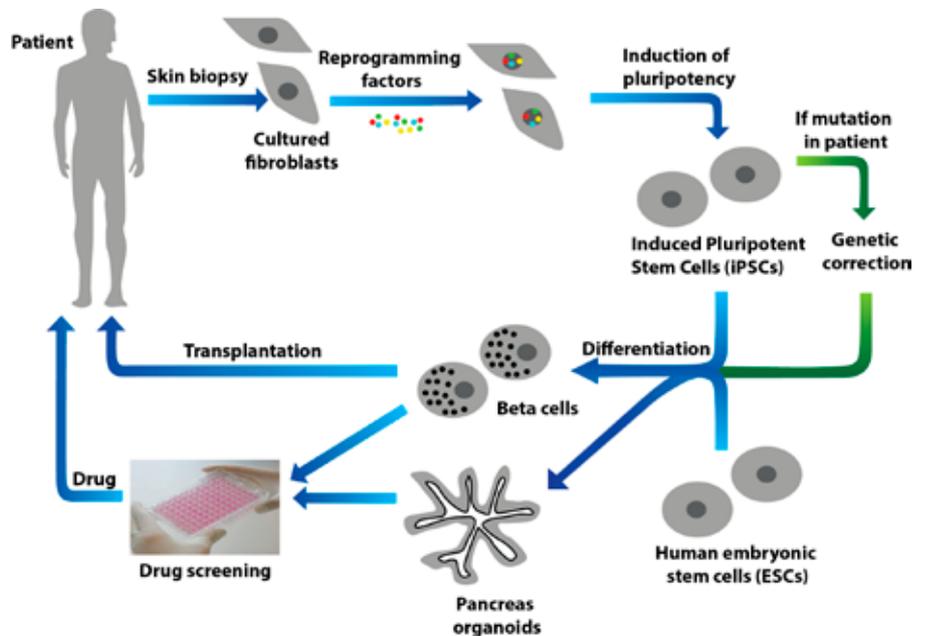
by insulin injection, islet transplantation can be proposed but this remains rare, in part due to the lack of donor islets and in part due to the adverse effects of the immunosuppressants used to prevent graft rejection.

## BETA CELLS FROM HUMAN PLURIPOTENT STEM CELLS

The production of beta cells from stem cells could solve the shortage of donors and make islet graft a more widespread treatment. Remarkable progress was made in 2000 when the company CyThera, now merged into Viacyte, published the protocol of the first step of differentiation of human embryonic stem cells (hESCs) to beta cells, based

on information from the natural development of these cells. This followed years of prior studies of animal model development, which provided the triggers and quality control information and this article changed the mindset of the community.

Remarkable progress with contribution from many labs worldwide has enabled to similarly progress along the subsequent steps and improve reproducibility on different sources of cells and in different production sites. The recent studies from the Kieffer/BetaLogics team in September and the Melton lab in October suggest we are close to the goal. They coaxed hESCs and induced pluripotent stem cells (iPSCs) along the entire pipeline in vitro



**Figure 1:** iPSCs can be produced from the reprogramming of a patient's skin fibroblasts. These cells, as well as ESCs can be differentiated into beta cells or into pancreatic cells self-assembled in miniature pancreas organs called organoids. Beta cells can be used for transplantation. Beta cells and pancreas organoids can be used for identifying drugs to better treat diabetes. If a patient has a genetic mutation leading to diabetes, this mutation can in principle be corrected prior to transplantation. The mutated cells can in principle be screened to identify drugs that correct the dysfunction.

and produced cells that are very closely related to beta cells (Figure 1). Not only did the cells produce insulin, which was seen in previous protocols, but their molecular identity was very similar to beta cells. Moreover the copious amounts of insulin were only produced in response to glucose and secretagogues. Like cells previously obtained, they restored normal blood sugar levels after transplantation in diabetic mice but with shorter maturation periods in the graft recipient body.

## DO WE HAVE THE PERFECT CELLS FOR TRANSPLANTATION?

The beta cells we have in our islets of Langerhans do the job quite well and ideally we would want cells with the same molecular components, a feature we can test globally at the transcriptional level but only in a targeted manner at the protein level. Such analysis suggests that the cells obtained by BetaLogics, which use one more step than Pagliuca et al., are the most mature but are not exactly like beta cells. However, it should be kept in mind that being grown in vitro these cells have not been exposed to the body's metabolic environment and can further mature in the body. Most importantly, they should exhibit similar functional properties, which can be tested only partially. A difficulty in such assessments is the variable quality of reference cells obtained from donor pancreatic islets. In spite of these difficulties, the recent studies show that several functional features are shared between the in vitro-produced beta-like cells and beta cells but that they are not perfect. For example, the beta-like cells have a reduced Ca<sup>2+</sup> amplitude and insulin secretion in response to glucose, slower time to peak and possibly prolonged response. More functional characterization is thus needed.

## WHEN TO LAUNCH CLINICAL TRIALS?

However, we may not need the perfect cell for transplantation. What is needed is a safe treatment that performs better than state-of-the-art. When considering the diabetic patients in general, current treatments have a good performance and will be difficult to surpass. When it comes to diabetic patients with a record of poor insulin efficacy and with no alternative, the threshold for cell

performance may possibly be relaxed if the safety is warranted. In the later scenario, lack of tumorigenicity, and no uncontrolled insulin release will become central issues.

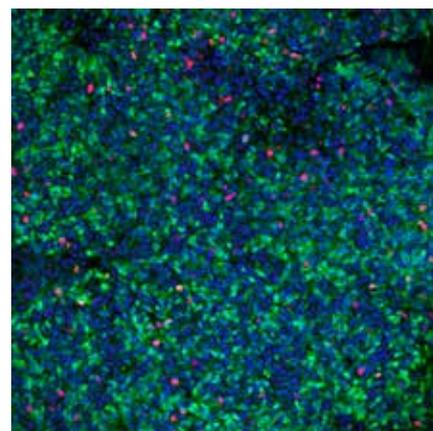
The first clinical trial approved by the FDA will de facto make use of hESC-derived pancreatic progenitor transplantation rather than the mature cells. The Viacyte team has previously shown that these cells can mature into beta cells and other pancreatic cell types after transplantation in encapsulation devices and can restore blood sugar levels in diabetic mice. In their phase 1/2 safety and efficacy trial in patients, they will similarly place progenitors in a device which prevents the immune cells from attacking the transplanted cells and the release of potential tumorigenic cells.

## IMPROVING THE IN VITRO-PRODUCED BETA CELLS: BASIC AND TRANSLATIONAL RESEARCH ARE IMPORTANT

This trial is the first but is unlikely to be the last one and it will be important to compare the outcome to the grafts of beta cells completely matured in vitro. This would reduce the weeks of required maturation of the progenitors after grafting, before they produce insulin and the glucose sensing machinery. This would also presumably reduce the risk of tumorigenicity, even though minimal in progenitors and limited by encapsulation. Moreover, the Viacyte production is designed as mass medicine with one human cell line for all, requiring control of patients' allogenic response. In contrast, the worldwide efforts for improvement of the protocol and the two recent studies have led to protocols adaptable to the patient's own iPSCs, presumably solving allogenic rejection although not autoimmunity. Cost-benefit investigations will be required.

There are also several improvements that will require basic research and engineering development. Firstly, as pointed out above, the cells produced in vitro are not yet bona fide beta cells. Basic research is thus needed to understand how a beta cell matures in the last stages of development and post-natally. Also, there is very limited understanding of the heterogeneity of beta cells and it is possible that the cells produced in vitro are equivalent to a subset of those we have in our body.

The recently reported methods for making beta cells from hPSCs in the lab,

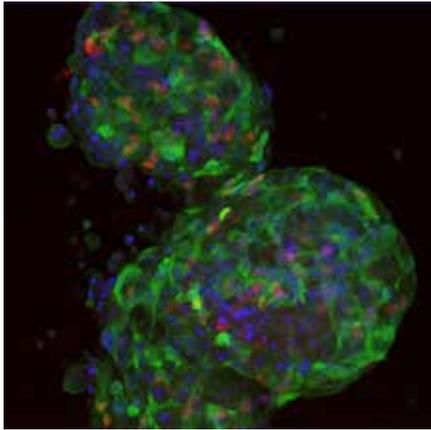


**Figure 2:** Pancreas progenitors (green) produced from hES cells. The nuclei shown in blue reveal partial differentiation and, limited proliferation (red).

involve a complicated multistep protocol where combinations of approximately a dozen growth factors and small molecules are added for up to 6 weeks (1,2). This makes it long, costly and error prone. It will be important to simplify the protocol by testing the essential components, replacing costly growth factors by small molecules and evaluating more widely the robustness.

For adaptations to large-scale production there are several bottlenecks. An important one is that the mass amplification of cells in the culture is done at the first step, in the pluripotent stem cells. The costs and time of production could be greatly reduced by amplifying intermediate stages such as endoderm, pancreas progenitors (Figure 2) or even beta cells and by being able to freeze them. We know that in the body both endoderm and pancreas progenitors proliferate very actively. We thus need to find the right conditions that enable them to do so in vitro. This is a goal we are pursuing in a European project DanStem leads (<http://www.hum-en.eu>). Getting beta cells to proliferate will be a more arduous task as their proliferation is very limited in vivo. Another limitation of most current protocols is that the desired population is never a pure product (Figure 2) and we are designing methods to purify the desired cells.

Finally, the possibilities to up-scale should be considered from the beginning of protocol development. The cell intermediates in the protocols seem to be sensitive to the structure of their environment, whether it is on the bottom of a dish, in suspension or in gels (Figure 3) and to cell density. These are issues frequently met by the people who cul-



**Figure 3:** *Pancreas progenitors (green) produced from hES cells grown in 3D. The nuclei shown in blue reveal efficient differentiation and, increased proliferation (red).*

ture them and which have rarely been addressed in a systematic manner to devise the best large-scale production platform.

### OUTLOOK: IS A CELL THERAPY OF DIABETES REALISTIC?

The progress achieved in 10 years of basic research is very promising and

with sustained basic and translational research and the initiation of trials, there is hope that a cell therapy for diabetes emerges. Receiving a stem-cell derived transplant may not be a cure as it is possible that multiple grafts are needed in a life-span and a risk that the level of insulin production reached is not sufficient in all patients. Moreover, the most important limitation is currently the control of autoimmunity which not only destroys the endogenous cells but also the transplant, unless protected by devices or immunoprotective treatments. The existence of an efficient therapy will also limit the utility of the treatment to a subset of patients unless the cost and benefit of stem cell therapy are greatly improved. The research investment was in any scenario worth, as the beta cells produced from hPSCs constitute an abundant source of human beta cells more readily available than the cadaveric islets. They can be used for beta cell studies, drug and beta cell imaging investigation and toxicity assays. Their amenability to derivation from patients and genetic alterations also offers perspectives to understand the mechanisms of different types of diabetes (Figure 1).

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