Fundació
La Marató de TV3
22nd SYMPOSIUM
Diabetes and Obesity

# ENCAPSULATED SYNTHETIC CELLULAR CIRCUITS TO RESTORE GLYCEMIC CONTROL IN TYPE 1 DIABETES

**Rubén Díaz Naderi**

Hospital Sant Joan de Déu – Esplugues de Llobregat

**Francesc Posas Garriga**

Institut de Recerca Biomèdica de Barcelona

**Gorka Orive Arroyo**

Facultad de Farmacia - Universidad del Pais Vasco

## 1. Background and current context

Type 1 Diabetes (T1D) is an autoimmune disease that is characterized by a high concentration of glucose in the blood of patients. The main cause of the disease is a reaction of the immune system which destroys the beta cells in the pancreas that are responsible for producing and secreting insulin. The lack of this hormone makes it impossible to internalize the circulating glucose in the organs where it performs a whole battery of vital functions. Although it has been known for more than 100 years that insulin deficiency is the cause of the disease, there is still no cure for it today. Current treatments include insulin administration, along with dietary control and moderate exercise to help control blood glucose levels.

There are currently two main lines of research dedicated to finding a "cure" (or more efficient treatments) for diabetes:

1. *Development of an artificial pancreas*. This is a device that measures the concentration of glucose in the blood continuously and contains an insulin pump that injects the required insulin dose calculated from an algorithm that takes into account several factors in addition to the patient's blood sugar. This device is very advanced and is becoming widely used, although it must overcome the problem of subcutaneous injection of insulin every time after food intake in addition to the inconvenience of carrying the corresponding devices, which also do not have alarms in case of failure. Despite the improvement in patient's quality of life, this line of therapy has the drawback that it is impossible to synthesize the necessary insulin at any given time, so it must be supplied externally. This makes it difficult to mimic the biology and physiology of the pancreatic islet.

2. *Gene and cell therapy*. In both approaches, a biological model is applied that has the insulin synthesis capacity necessary for glycemic control without the requirement for external insulin injection. The evolution of the synthetic biology field in recent years has allowed the modification of cellular models to provide them with new functions for which they were not programmed, such as the synthesis of hormones. Our La Marató project is based on the use of cell therapy to develop insulin/glucagon-secreting cell models that can respond more quickly to changes in glucose levels, allowing for better metabolic control, and to emulate pancreatic islet transplants without the need to

immunosuppress transplant patients. For this reason, cell therapy has high potential for the treatment of T1D.

## 2. Main objectives of the project

1. Development of cellular synthetic circuits that respond to changes in glucose levels and produce insulin and glucagon to properly regulate glucose homeostasis.
2. Encapsulation of the generated system *in vitro* to check its viability in an i*n vivo* model.
3. *In vivo* treatment of diabetic mouse model with encapsulated cells to restore glucose levels.

### 1. Development of insulin-secreting synthetic cellular circuits depending on extracellular glucose concentration

At the start of the project, *in silico* simulations were performed to study the role of the various hormones involved in the regulation of glucose when they are externally administered in diabetic patients. The initial studies indicated that the role of glucagon in controlling blood glucose in these patients with diabetes is secondary compared to the main role that insulin plays. For this reason, and to simplify the genetic circuits and the design of the synthetic cells, it was decided to control only the production of insulin based on blood glucose levels. The glucose detection system and insulin production were then chosen. Naturally induced promoters by increasing glucose concentrations were selected. The promoter of the TXNIP gene, which encodes a thioredoxin needed to protect cells from oxidative stress, was selected. Transcription of this gene is induced by high glucose concentration and is repressed under low glucose conditions. Once the promoter was selected, the cell model to be modified was studied. Stable modification of endocrine cells is difficult to achieve, for which reason we used non-endocrine cell models. To ensure that the insulin product in non-endocrine cells was correctly processed and functional, a modified version of the rat insulin gene was obtained where proinsulin is processed by the furin endoprotease. The final design of the synthetic construct contains the glucose-sensitive TXNIP promoter which controls the expression of a polycistronic mRNA encoding pro-furin-insulin and a reporter gene. This pair of key elements, along with other reporter genes used to select the modified cells, allowed the monitoring of cell viability and the expression of insulin. The

technology adopted has made it possible to produce properly processed and secreted insulin in non-endocrine cell lines. Additionally, the use of current detection systems based on antibodies against the active form of insulin allowed its quantification. The process concluded with the validation of insulin activity *in vitro*.

Once the insulin production mechanism was designed and validated, several synthetic cell lines were constructed able to sense glucose levels and produce insulin through the TXNIP promoter in response to extracellular glucose concentrations. After testing different cell lines, the best cell model was the one derived from human HEK293t cells. The resulting HEK293t-TXNIP-Ins cells underwent extensive validation under conditions of dynamic glucose concentrations similar to those found in a diabetic mouse. Early *in vitro* tests suggested that our implants required long periods of activation before insulin could be detected in the environment. They also showed that the amount secreted should be increased to meet the insulin requirements of the animals. During the design of the synthetic cell circuits, a mathematical model was developed that allowed us to model the dynamics of insulin secretion from our synthetic cells, both in monolayer format and in encapsulated (3D) format. Insulin production calculated from this model was consistent with experimental data obtained *in vitro* when these cells were exposed to different glucose concentrations. This mathematical model was also used to introduce it into a virtual diabetic mouse model. Computer simulations in this diabetic model revealed that our implants required long periods of activation (approximately 6-8 hours) under high glucose conditions to secrete detectable insulin levels, thus causing periods of hyperglycemia in mice. This effect was determined to be due to the genetic architecture of our circuit, basically the slow response of transcriptional regulation. During the course of the project, different approaches were carried out, including the modification of the internal architecture of the cell, to solve these limitations. For example, an attempt was made to produce insulin in the form of a propeptide that could be retained inside the cell and regulate its secretion only when high glucose levels are present. However, none of them allowed the construction of insulin-producing cells with better dynamics than those initially obtained.

**2. Encapsulation of genetic circuits**

The encapsulation of therapeutic cells allows the dynamic control of the release of active compounds in response to external stimuli, dramatically increasing the biosafety

and efficiency of this technology. An encapsulation system was used to wrap the insulin-producing living cells and to provide a long-term release of the therapeutic agent, in this case insulin. The idea was that the implantation of these microcapsules loaded with insulin-producing cells could reduce or even completely eliminate the external need for insulin injections to achieve glycemic control in T1D patients.
In the design of the encapsulation type, a key aspect was taken into account, namely the immune response to the biomaterials that make up the three-dimensional capsule used for the encapsulation of cells. Different types of ultrapurified alginate were selected and evaluated. The encapsulation process was based on the resuspension of the secretory cells in a solution of alginate which, on contact with a $CaCl_2$ solution, will form gelled droplets. Additionally, to ensure the stability of these particles, they were coated with a layer of poly-L-Lysine and a second layer of alginate. In the end, 3D particles were obtained with optimized dimensions (400 micrometers in diameter) that allowed an optimal balance between the stability of the particle and its ability to diffuse both the input of glucose and the output of secreted insulin. Continuous *in vitro* evaluations of the cell densities used, and the insulin synthesis capacity of the microcapsules performed before and in parallel with in vivo experiments, were vital during the development of the project.

**3. Pharmacological characterization of microcapsules in diabetic mouse models.**

Following the study of the pharmacodynamics of microcapsules *in vitro*, different routes of administration were studied in our *in vivo* mouse model. To this end, a diabetic mouse model was established by injecting a single dose of streptozotocin (STZ), a drug that damages the beta cells, the pancreatic insulin-producing cells. This highly standardized procedure allowed the generation of a mouse model with hyperglycemia without the option of modulating its glucose levels.

The amounts of insulin produced by the encapsulated cells required the use of large volumes of microcapsules that were administered in the mouse at the intraperitoneal space. Other approaches, such as subcutaneous administration, presented technical difficulties and injectable volumes did not cover our insulin production needs. With the intraperitoneal microcapsule injection no therapeutic effect was observed. This lack of response was due to the combination of (i) a slow response of the microcapsules to produce insulin, (ii) relatively low production of the insulin, and (iii) induction of fibrotic

tissue around the microcapsule. Taken together, these results indicated that there was a mechanism that prevented the viability of the encapsulated cells and/or the proper secretion of insulin.

Different approaches carried out simultaneously were addressed to solve the detected problems. The experimental results showed that the encapsulation materials used without the presence of cells reduced to zero the immune response of the host, and therefore, the formation of fibrosis. On the other hand, the change in the C57BL6/J mouse strain used by the less immunoreactive ICR-CD1 strain did not show any improvement in the animal's immune response. Finally, it was the use of a cell model of a beta-pancreatic mouse line (Min6), capable of synthesizing and secreting glucose-stimulated insulin, which managed to significantly reduce the levels of generated fibrosis. These results indicated that the use of modified HEK293t human cells could provoke a rejection response against the capsules that prevented the release of insulin in the animal. To solve the immunocompatibility problems, xenotransplantation was chosen by using a modified murine cell to obtain a high insulin synthesis capacity. Assays using several murine lines showed that synthetic cells based on the C2C12 line could be a good model for producing furin-insulin.

Consequently, the final strategy to achieve the desired results was the use of the new non-human cell model, together with the immunosuppression of the animals to minimize the immune response. The results observed with this approach demonstrated the efficiency of murine cell models in the glycemic control of diabetic animals (up to 21 days), especially in the first days after injection of microcapsules in combination with immunosuppression of individuals. Microcapsules developed from the new C2C12 cells demonstrated the ability to reduce animal blood glucose levels up to more than three weeks post-injection, results similar to those seen in the Min6 model. The change in glucose levels was accompanied by an increase in the plasma insulin levels of the animals. It should be noted that capsules containing constitutively insulin-secreting cells (Min6) resulted in the appearance of signs of prolonged hypoglycemia in animals, highlighting the importance of a regulated insulin secretion depending on the blood glucose levels to restore glucose homeostasis without having adverse side effects.
On the other hand, to solve the limitation of the slow response offered by the transcriptional regulation of the generated genetic circuits, the effects of the combination of different feeding patterns, together with the cell implants, were

evaluated *in silico* in a diabetic mouse virtual model. Using an evolved algorithm, a time-restriction-feeding (TRF) -based feeding pattern was determined and optimized in combination with our implants. These feeding patterns were subsequently tested *in vivo* in our models of diabetic mice injected with microcapsules and comparing their *ad libitum* diet with TRF. The results indicate that the effects of our implants improve significantly when were combined with a proper feeding pattern. In addition, the data obtained were again used as input to our computational model to improve the algorithm.

## 3. Relevance and possible future implications

The results obtained throughout the project highlight the importance of developing a cellular model capable of producing insulin in a regulated manner based on extracellular glucose concentrations for future therapies in the field of type 1 diabetes. During the project, we have shown that the combination of the different strategies addressed so far (animal model change, insulin-producing cell model, and animal immunosuppression) are key to achieving the restoration of glucose homeostasis in our diabetic mouse model. Currently, the project has led us to develop a murine cell model capable of producing high levels of insulin in an adjustable manner based on glucose levels outside the microcapsule. This, together with the improvement of the system of retention and secretion of the generated insulin propeptide and the improvement of the cellular amounts that can be encapsulated, allow us to glimpse advances in the treatment of diabetes. Likewise, the strong dependence on immunosuppression during the xenotransplants reflects the future need to use modified patient cells, along with complementary or alternative systems to simple encapsulation such as the use of systems similar to subcutaneous implants or the use of 3D structures based on biomaterials. It is important to emphasize that all the knowledge acquired during the project, as well as the methodology used, will contribute in the future in the treatment of diabetes, and will be useful in other fields of biomedical research.

## 4. Generated scientific bibliography

Urrios A, Gonzalez-Flo E, Canadell D, de Nadal E, Macia J, Posas F. "Plug-and-Play Multicellular Circuits with Time-Dependent Dynamic Responses" ACS Synth Biol. 7: 1095-104 (2018).