fluicell

Towards a future without type 1 diabetes

Closing the loop on type 1 diabetes through bioprinted tissue therapeutics

TRANSFORMING HOW WE TREAT DIABETES

Fluicell's vision is to transform how we treat type 1 diabetes using bioprinted tissue therapeutics.

Our goal is to provide patient-tailored artificial pancreatic islets capable of enabling glycemic control.

We envision a life for people living with type 1 diabetes without requiring everyday medication, disease management and glucose level monitoring.

TYPE 1 DIABETES, A GROWING CONCERN

Type 1 diabetes is a life-long autoimmune disease that results in the destruction of the insulin-producing beta cells located in pancreatic islets. This usually leads to absolute insulin deficiency and health decline.[1] However, the discovery of insulin approximately 100 years ago has transformed diabetes from a sure death sentence to a manageable disease, through daily insulin injections.

Today, approximately 9 million people worldwide are diagnosed with type 1 diabetes, which represents around 10 percent of all diabetes cases. Due to improvements in testing and diabetes care, this number is expected to increase to 15 million by 2040.[2]

Type 1 diabetes (T1D) has traditionally been thought of as a disease that predominantly affects children and youth. However, currently 62 percent of all new cases of T1D occur in people 20 years and older, and the average person with T1D is now 40 years old.[3]

Living with type 1 diabetes is associated with a multitude of challenges that extend beyond the constant monitoring of the disease. Individuals with T1D face a wide range of comorbidities that significantly impact their quality of life. Among these complications, heart and kidney diseases stand out as particularly consequential, posing substantial risks to the overall health of people with T1D. Moreover, severe hypoglycemic events and impaired hypoglycemic awareness poses a great risk for a large number of T1D patients.[4]

In 2000, a 10-year-old diagnosed with type 1 diabetes would have faced a shortened lifespan of nearly 20 years.[5] Thanks to advancements in diabetes care, the life expectancy has significantly improved. However, individuals with T1D still have an estimated life expectancy that is 10 years shorter than that of the general population without T1D.[6] Furthermore, it is estimated that approximately 175,000 deaths occur each year due to T1D, and this number is projected to increase by 3 percent annually.

Despite the high standard of today's insulin therapy, most patients do not achieve optimal glycemic control, even when using insulin pumps and continuous glucose monitoring. The HbA1c test, which measures the average blood sugar level over three months, is the most accepted measure for glycemic control today.[7] The HbA1c is considered controlled for T1D patients when they are capable of maintaining a level that is below 7% for adults and 7.5% for children. However, for patients with T1D today, the average HbA1c is closer to 8.2%.

Due to the significant impact that diabetes has on health and overall well-being, coupled with the absence of curative solutions, there is an urgent demand for disease-modifying treatments that offer an alternative to continuous disease monitoring and insulin injections.

9 M
children and adults with T1D

175,000
annual deaths due to T1D, increasing 3%/year

38%
of all new diagnosed cases of T1D under the age of 20

15 M
people living with T1D in 2040

The Fluicell approach — Artificial islets for glycemic control

In a seminar published in Lancet in 2006, the renowned diabetitian Denis Daneman stated that "*A fundamental shift in the management of type 1 diabetes seems unlikely until we are able to close the loop through either artificial endocrine pancreas implantation, or islet replacement by transplantation or stem cell engineering*".[1] Fluicell's goal is to be a leading force in closing that loop.

Our vision and ultimate goal is to develop a curative treatment for type 1 diabetes. Through our regenerative medicine research, we are focused on creating bioprinted microtissues that can facilitate organ repair or replace lost functionality. Building upon this foundation, we leverage the microtissue-generating capabilities of our advanced bioprinting platform Biopixlar, to engineer artificial islets. These artificial islets hold promise for enabling glycemic control (i.e. maintaining healthy blood glucose concentrations) in patients with type 1 diabetes.

The key to finding a therapeutic solution that can supplant existing T1D treatments and provide glycemic control is to recreate the function of the islets of Langerhans, the part of the pancreas responsible for its endocrinal activity.

The islets of Langerhans regulate the blood sugar level through an intricate system consisting of multiple different hormones that work in consort with each other. Of the different cell types in the islet of Langerhans, alpha and beta cells are the most abundant and important.[8] While beta cells produce insulin that reduces blood glucose levels, alpha cells produce glucagon that leads to release of glucose into the blood stream, helps to regulate insulin secretion and prevents hypoglycemia. Previous research has shown that that the interplay between alpha and beta cells, in what is effectively the body's glucostat, is an essential part in blood glucose regulation.[9]

Because of the multifaceted role of the islets of Langerhans in blood glucose regulation and prevention of both hypo- and hyperglycemia, treating type 1 diabetes either through direct insulin injections or through beta cell replacement is insufficient. Instead, a treatment based on a combination of alpha and beta cells, effectively replacing the function of the pancreatic islets is likely a superior option.

There are two chief strategies available to achieve pancreatic islet replacement, either by transplanting donated human islets or by creating engineered pseudoislets that replicates the functionality of the islets of Langerhans.

Since transplantation requires the islets to be taken from deceased donors, access to material is highly limited. This approach also leads to substantial risk of side effects due to the necessity of immunosuppressive treatment. These drawbacks are the major reasons why islet transplantation has been restricted to high-risk patients, such as those with severe hypoglycemia unawareness.[10]

Our approach at Fluicell is to use our capability to generate detailed tissues with high precision to bioprint islet organoids using Biopixlar. Our technique lets us assemble islet organoids in a very precise way with controlled size and cell type composition, to achieve artificial islets that resemble the actual pancreatic islets. This capability gives Fluicell a clear competitive advantage in relation to comparable technologies under development.

We have made considerable discovery phase progress and have hit key milestones regarding artificial islet functionality and production capacity. We continue to make functional improvements as we prepare for progressing into preclinical development with in vivo proof of concept (POC), which we aim to do initiate in 2024 in collaboration with an academic partner. Figure 1 provides an overview over Fluicell's development progress and the roadmap for further development.

The main objective for the in vivo POC phase will be to demonstrate that our bioprinted artificial islets have the capacity to control blood glucose level and enable long-term glycemic stability. Results from in vivo POC will also serve as a foundation for partnering conversations with pharmaceutical companies around further development. Further progression towards GLP toxicity studies following successful preclinical POC will proceed with support either through commercial partnership, research grants or through investments in Fluicell.

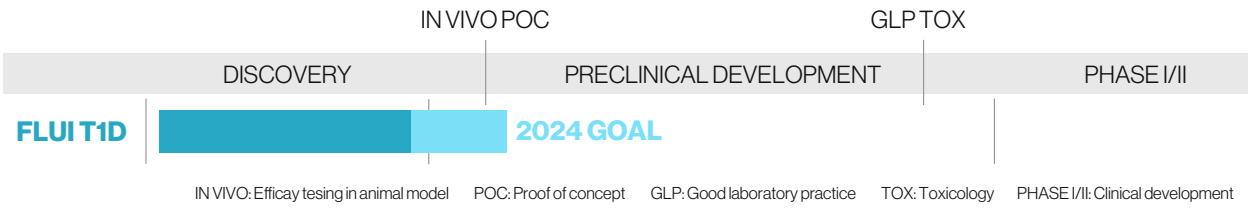


Figure 1 Schematic overview over Fluicell's T1D therapeutic development progress, including 2024 goal.

Precision bioprinting artificial islets

Artificial islets (AI), also known as pseudoislets or islet microtissues, are three-dimensional cell clusters designed to closely replicate the structure and function of native pancreatic islets. These native pancreatic islets play a vital role in regulating systemic glucose homeostasis by secreting insulin and glucagon from beta and alpha cells respectively.

The bioengineering of artificial islets with controlled size and defined cellular composition is a promising approach for therapeutic development and drug discovery. It allows for directing biological functionality of microtissues by including the key cellular components while avoiding islet-to-islet variation associated with the native pancreatic islets.[11-14]

However, host immunity possesses one of the biggest challenges in paving the way to successful therapeutic applications. Therefore, deceiving, or evading host immunity is inevitable for survival of AIs after transplant. Genetic modifications of cells or their physical protection can help to accomplish this.[15-17] For example, physical protection can be achieved by employing semipermeable materials, such as hydrogels, which are biocompatible and allow for the diffusion of small nutrient molecules, oxygen, and insulin while protecting the AIs from immune cell infiltration.[15]

At Fluicell, we use our high precision bioprinting technology Biopixlar to generate artificial islets that recapitulate size and main functionalities of the native pancreatic islets and combine them with hydrogel encapsulation methods for developing T1D therapeutics.

The pancreatic islets of Langerhans consist of several types of endocrine cells, including alpha, beta, delta, and polypeptide cells, which work together to regulate glucose metabolism.[8] Among these cells, alpha and beta cells are the most abundant and important. Beta cells are responsible for producing and secreting insulin, which is an important hormone for regulating blood glucose levels, while alpha cells secrete glucagon and help to regulate blood glucose levels, defend against hypoglycemia and modulate beta cells function.[8]

Endothelial cells play a vital role in the production of growth factors and other molecules that significantly enhance the proliferation, survival, and function of beta cells. In addition, they are crucial in the construction of blood capillaries within the pancreatic islets, which are essential for the provision of nutrients, oxygen, and hormones, as well as for facilitating the exchange of metabolic waste.[18-20]

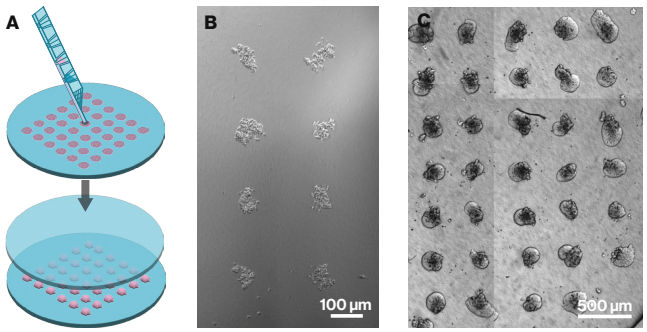


Figure 2 Bioprinted artificial islets. **A)** A schematic illustration of AI bioprinting and biocomposite consisting of patterned cells encapsulated between the two layers of hydrogel **B)** Premixed alpha, beta and endothelial cells, patterned as a microtissue array. **C)** A fragment of biocomposite with AIs (approximately 200 µm diameter) grown for 7 days. The image is tiled from multiple microscope images.

Generating artificial islets

As a proof-of-concept, we used mouse pancreatic alpha, beta and endothelial cells to generate bioprinted artificial islets. Prior to bioprinting, the cells were pre-mixed in a ratio that mimics native mouse pancreatic islets. The premixed cells were loaded into the Biopixlar printhead and directly patterned onto a thin layer of hydrogel to generate an array of AIs (Figure 2A). The islet microtissues were printed to a size of approximately 100 µm in diameter (Figure 2B).

Following bioprinting, the cells were covered with an additional thin layer of hydrogel, forming a biocomposite (schematically depicted in Figure 2A). The biocomposites were kept in culture conditions for 7 days (Figure 2C) before tested for insulin-secreting functionality.

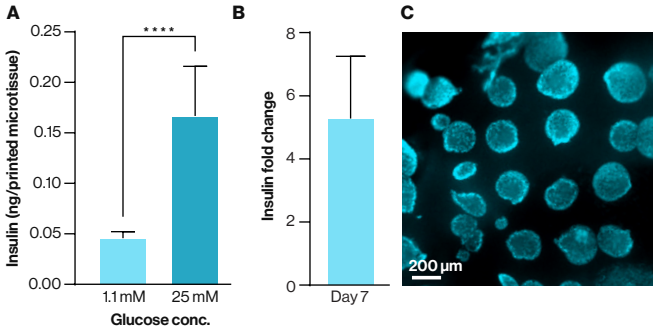


Figure 3. Glucose-responsiveness and viability of bioprinted AIs. **A)** GSIS in AIs at the low (1.1 mM) and high (25 mM) glucose concentrations. Data are expressed as mean ±SEM (n=15 biocomposites). P value calculated using Wilcoxon matched-pairs signed rank test (one-tailed); **** indicates p value < 0.0001. **B)** Insulin secretion fold change between low and high glucose levels. **C)** Live cell staining in the AI on day 21.

Insulin secretion in artificial islets

Healthy pancreatic islets respond to glucose by secreting insulin, an important hormone for regulating blood glucoses levels. Release of insulin from biocomposite-encased AIs was measured using the glucose-stimulated insulin secretion assay (GSIS). We detected on average 0.16 ng insulin per printed AI at high glucose stimulation (25 mM), while 0.04 ng insulin was detected per AI at low glucose level (1.1 mM), Figure 3A. These levels of insulin are within the range reported in the literature.[21] Figure 3B shows the ratio of insulin secretion in high glucose to low glucose buffers with approximately 5-fold increase, indicating the responsiveness of beta cells to glucose stimulation.

In addition to the GSIS assay, we used fluorescein diacetate (FDA) to stain live cells (Figure 3C) to infer the viability of the printed AIs. We observed that the hydrogel-protected, bioprinted AIs were viable for at least three weeks in culture, which makes them suitable for long-term studies.

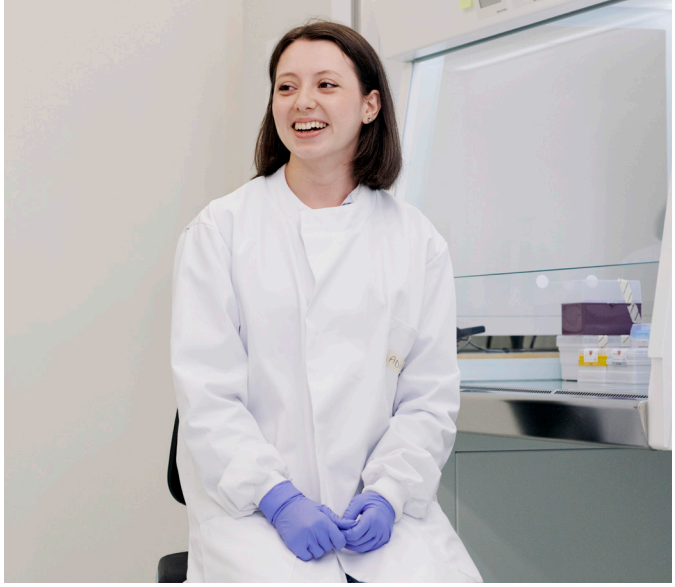
Engineered for delivery — biocomposite design and optimization.

The building of insulin-secreting biocomposites involves two main steps: the bioprinting of pancreatic cells to form functional islet microtissues, and their encapsulation between hydrogel layers for immunoprotection. During the printing process, cells are suspended in their native media and delivered in close proximity to a pre-treated hydrogel surface. This results in direct cell attachment and controlled pattern formation, without the use of scaffolding material such as bioink. Biopixlar's microfluidic technology enables microtissue generation with high precision, down to the single-cell level, while ensuring that the bioprinting process does not negatively impact cell viability.[22]

Artificial islet and biocomposite characteristics

- Contain key pancreatic cell types - alpha, beta and endothelial cells.
- Equivalent in size to native pancreatic islets.
- Responsive to glucose stimulation – up to 5-fold increase in insulin secretion.
- Hydrogel layers provide protection.
- Functional over a prolonged period (at least 3 weeks).
- Easily to release from production with minimal post processing.

The value of a curative solution to type 1 diabetes



Type 1 diabetes is not only a significant burden for the individuals living with the disease, it also greatly impacts society, generating substantial healthcare and socioeconomic costs. The annual direct costs for planned and acute care for a type 1 diabetes patient living in the United States is $4,429 for a pediatric patient and $8,136 for an adult patient.[23] However, these figures only represent the direct cost for care and do not account for hidden indirect costs, such as productivity losses. When also factoring in productivity losses, the annual cost per patient increases to $5,960 and $20,320 for pediatric and adult patients, respectively. This shows that a large part of the diabetes cost burden comes from sources other than direct care costs.

Recent market reports estimate the T1D market to grow from $4.9 billion in 2019 to a value of $24 billion by 2029, corresponding to a CAGR of 17.2 percent.[24] With an estimated value of $20.3 billion in 2029, the United States will continue to be the single largest market for T1D therapeutics (84.7 percent of the T1D market).

The hidden cost of type 1 diabetes

Although market estimates reveal a large market for type 1 diabetes therapeutics with a significant growth potential, they only cover the direct sales value and do not show the full cost burden of type 1 diabetes. The disease-associated socioeconomic cost and the potential cost reduction that a new therapy can generate are important measures when determining the value of a new therapy and the potential for implementation within healthcare systems. It is also a powerful tool for comparing the inherent potential in different therapeutic alternatives.

In a report published in 2020, the non-profit type 1 diabetes research and advocacy foundation JDRF estimate the global cost burden of T1D to $90 billion.[23] The regions United States ($30 billion), Europe ($30 billion) and Asia ($22 billion) represent approximately 90 percent of the cost burden. This estimate includes both direct cost of care and indirect costs due to productivity losses.

To assess the value and associated cost saving of new forms of T1D therapy, it is important to compare them to currently used disease management alternatives. As discussed above, most patients today do not achieve adequate glycemic control, despite the high standard of today's insulin therapy. Any new therapy that can move the condition for the average patient closer to a controlled level would generate significant cost reductions.

The next generation of T1D therapies

The potential future T1D therapies can be grouped into three broad categories, based on the way they treat the disease. The first category is preventive treatments and includes measures to delay disease onset and to preserve beta cell function. The second category broadly constitutes improvements to currently existing treatments and includes new therapies that improve metabolic control and automated artificial pancreas devices. Finally, the third category covers curative solutions, which includes beta cell replacement and treatments that restore the beta cell function. Out of these three categories, beta cell replacement is the one that closest resembles Fluicell's therapeutic concept, although our development is aimed at recreating the full function of the pancreatic islets and not only beta cell replacement.

Figure 4 shows the estimated annual cost saving in the United States for each category, with preventive measures providing up to $7.1 billion in annual savings, new treatments up to $20.3 billion and curative solutions up to $30.2 billion. Cell replacement is expected to generate direct and indirect annual cost savings of $28.8 billion in the US and is one of the most impactful forms of treatments.

Since methods to restore beta cell function currently primarily exist on a conceptual and exploratory research stage, cell therapies focused beta cell replacement is the primary option for high impact type 1 diabetes therapies.

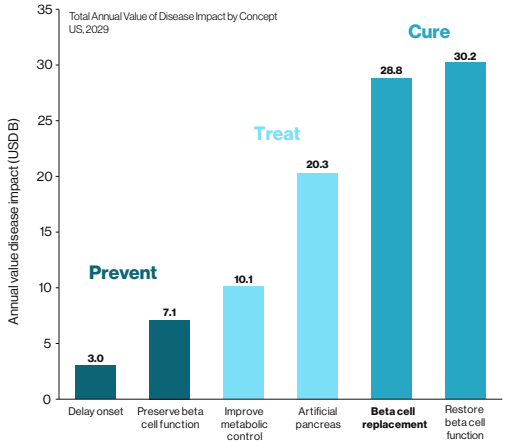


Figure 4 Total annual value of disease impact by therapeutic concept. Adapted from ref 23.

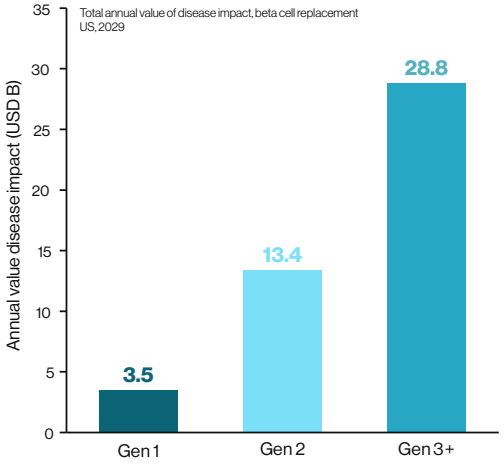


Figure 5 Total annual value of disease impact of beta cell replacement by therapeutic generation. Adapted from ref 23.

In their analysis of future potential type 1 diabetes treatments, JDRF break down beta cell replacement therapies into three different future product generations with increasing cost impact based on the level of glycemic control provided by the treatment, the need for immunosuppression and the efficacy duration of the treatment.23 Figure 5 shows the annual US cost savings associated with each generation of T1D cell therapy.

JDRF define the first generation of T1D modifying cell therapies as treatments that result in 50–100 percent reduction in insulin need, broad use of immunosuppression and 24 months of efficacy duration The intermediate generation reduces the need for insulin treatment by 70-100 percent and fully removes the need for immunosuppression. Finally, the third generation of beta cell replacement therapies result in complete insulin independence, require no immunosuppression, and has efficacy duration that exceeds 24 months.

The segmentation into multiple product categories shows that there is a large market value and economic impact for T1D targeting cell therapies even with moderate efficacy and duration and that initial development costs can be spread out across multiple therapeutic products, which help to minimize risk and reduce the cost of treatment per patient.

Development on the T1D market

The large potential inherent in T1D cell therapy is reflected by the growing interest from pharmaceutical companies in this area. In recent years, there have been a number of partnership and acquisition agreements between biotech with preclinical T1D cell therapy development programs and pharmaceutical companies, valued at $600–1,000 million, depending on the exact nature of the agreement.[25-26] These deals serve as a strong indication of the inherent value of a preclinical type 1 diabetes cell therapy program.

With Fluicell's unique approach to engineer artificial islets for glycemic control, we believe that we have a great opportunity to challenge both existing conventional diabetes therapeutics and emerging cell therapy technologies still under development. The type 1 diabetes therapeutic market is growing rapidly, and we believe that with the right partner, we will be able to take a leading position in it.

$24 B
2029 T1D market size

17.5 %
2019-2029 T1D market CAGR

$29 B
Resulting annual US cost savings from beta cell replacement (2029)

$ 90B
worldwide annual cost burden of T1D

- Type 1 diabetes is a growing medical concern with a global annual cost burden of $90 billion.
- Existing insulin-based treatments fail to provide adequate care and T1D patient still experience significant impact on health and wellbeing.
- Cell therapies for beta cell or pancreatic islet replacement are considered the most impactful and cost-reducing future treatment options.
- Fluicell develops a unique T1D treatment concept based on bioprinted artificial islets, with the aim to enable full glycemic control without the need for immunosuppressants.

REFERENCES

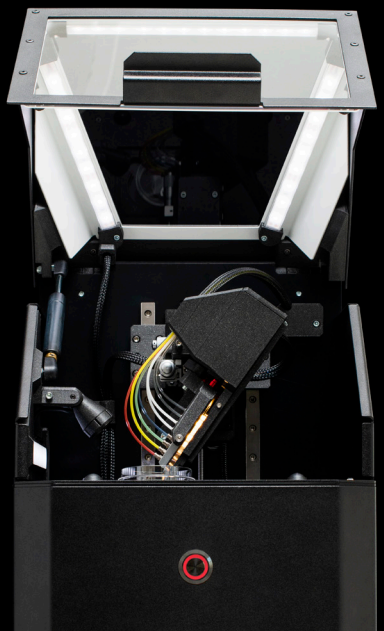
1. Daneman D., Type 1 diabetes. *Lancet* **367**, 847-858 (2006).
2. Gregory MA., Robinson, TIG., Linklater, SE. *et al*. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *Lancet Diabetes Endocrinol*. **10**, 741-760 (2022).
3. Type 1 diabetes estimates in children and adults. *IDF Atlas Reports* (2022).
4. Lin, YK., Fisher, SJ., Pop-Busui, R. Hypoglycemia unawareness and autonomic dysfunction in diabetes: Lessons learned and roles of diabetes technologies. *J. Diabetes Investig*. **11**, 1388–1402 (2020).
5. Narayan KMV, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes in the United States. *JAMA* **290,** 1884–90 (2003).
6. Livingstone, SJ., Levin, D., Looker, HC., *et al*. Estimated Life Expectancy in a Scottish Cohort With Type 1 Diabetes, 2008-2010 *JAMA* **313**, 37-44 (2015).
7. Agiostratidou, G., Anhalt, H., Ball, D., *et al*. Standardizing clinically meaningful outcome measures beyond HbA1c for type 1 diabetes: a consensus report of the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society, JDRF International, The Leona M. and Harry B. Helmsley Charitable Trust, the Pediatric Endocrine Society, and the T1D Exchange. *Diabetes Care*, **40** 1622-1630 (2017).
8. Da Silva Xavier, G. The Cells of the Islets of Langerhans. *J. Clin.Med*. **7**, 54 (2018).
9. Rodriguez-Diaz, R., Molano, RD., Weitz, JR *et al*. Paracrine Interactions within the Pancreatic Islet Determine the Glycemic Set Point. *Cell Metab*. **27**, 549–558 (2018).
10. Bruni, A., Gala-Lopez, B. Pepper, AR, *et al*. Islet cell transplantation for the treatment of type 1 diabetes: recent advances and future challenges. *Diabetes Metab. Syndr. Obes*. **7**, 211-23 (2014).
11. Lebreton, F., Lavallard, V., Bellofatto, K., *et al*. Insulin-producing organoids engineered from islet and amniotic epithelial cells to treat diabetes. *Nat. Commun*. **10**, 4491 (2019).
12. Siehler, J., Blöchinger, AK., Meier, M., Lickert, *et al*. Engineering islets from stem cells for advanced therapies of diabetes *Nat Rev Drug Discov* **20**, 920–940 (2021).
13. Dino J. Ravnic, DJ, Leberfinger, AN, Ozbolat, IT. Bioprinting and Cellular Therapies for Type 1 Diabetes. *Trends Biotechnol* **35**, 1025-1034 (2017)
14. Mir-Coll, J., Moede, T., Paschen T., *et al*. Human Islet Microtissues as an In Vitro and an In Vivo Model System for Diabetes. *Int. J. Mol. Sci*. **22**, 1813 (2021).
15. Shi, Y., Zhao YZ., Jiang Z., Immune-Protective Formulations and Process Strategies for Improved Survival and Function of Transplanted Islets. *Front. Immunol*. **13** (2022).
16. Dario Gerace, D., Zhou, Q., Hyoje-RyuKenty, J., *et al*. Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance. Cell. Rep. Med. **4**, (2023).
17. Vegas, A., Veiseh, O., Gürtler, M. *et al*. Long-term glycemic control using polymer-encapsulated human stem cell–derived beta cells in immune-competent mice. *Nat Med* **22**, 306–311 (2016).
18. Pignatelli C, Campo F, Neroni A, *et al*. Bioengineering the Vascularized Endocrine Pancreas: A Fine-Tuned Interplay Between Vascularization, Extracellular-Matrix-Based Scaffold Architecture, and Insulin-Producing Cells. *Transpl Int*. **35**, 10555 (2022).
19. Hogan MF, Hull RL. The islet endothelial cell: a novel contributor to beta cell secretory dysfunction in diabetes. *Diabetologia*. **60**, 952-9. (2017)
20. Peiris H, Bonder CS, Coates PT, *et al*. The beta-cell/EC axis: how do islet cells talk to each other? *Diabetes*. **63**, 3-11 (2014).
21. Yesildag, B., Mir-Coll, J., Neelakandhan, A. Liraglutide protects β-cells in novel human islet spheroid models of type 1 diabetes. *Clin. Immunol*. **244**, 109118 (2022).
22. Jeffries, GDM., Xu, S., Lobovkina, T., *et al*. 3D micro-organisation printing of mammalian cells to generate biological tissues. *Sci. Rep*. **10**, 19529 (2020).
23. Modeling the Total Economic Value of Novel Type 1 Diabetes (T1D) Therapeutic Concepts. *JDRF*. (2020).
24. Type 1 Diabetes - Global Drug Forecast and Market Analysis to 2029. *Research and Markets* (2021).
25. https://www.aspectbiosystems.com/news-resources/novo-nordisk-partnership-to-develop-bioprinted-tissue-therapeutics-for-diabetes-and-obesity. *Aspect Bioystems*, April 12 (2023).
26. https://investors.vrtx.com/news-releases/news-release-details/vertex-acquire-semma-therapeutics-goal-developing-curative-cell. *Vertex*, September 3 (2019).

ABOUT BIOPIXLAR®

Biopixlar is Fluicell's family of high precision 3D bioprinting platforms. The Biopixlar platforms uses Fluicell's innovative open volume microfluidic technology and is capable of creating tissues, 3D cell cultures and cell arrays with single-cell precision. Biopixlar desposits cells directly in solution without any bioink, which ensures high cell viability and efficient intercellular communication. Biopixlar is available in two verions: as the modular Biopixlar platform and as the more compact Biopixlar AER.

ABOUT FLUICELL®

Fluicell is a Swedish life science company, specializing in high precsion research tools for biological and pharmaceutical research, in vitro disease models and cell-based regenerative medicine research and development. Fluicell provides innovative research instruments for single-cell biology and 3D bioprinting, based on proprietary microfluidic technology.



fluicell®

https://fluicell.com/
info@fluicell.com

Flöjelbergsgatan 8C,
431 37 Mölndal, Sweden