Regenerative medicine of pancreatic islets

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Abstract

The pancreas became one of the first objects of regenerative medicine, since other possibilities of dealing with the pancreatic endocrine insufficiency were clearly exhausted. The number of people living with diabetes mellitus is currently approaching half a billion, hence the crucial relevance of new methods to stimulate regeneration of the insulin-secreting β-cells of the islets of Langerhans. Natural restrictions on the islet regeneration are very tight; nevertheless, the islets are capable of physiological regeneration via β-cell self-replication, direct differentiation of multipotent progenitor cells and spontaneous α- to β- or δ- to β-cell conversion (trans-differentiation). The existing preclinical models of β-cell dysfunction or ablation (induced surgically, chemically or genetically) have significantly expanded our understanding of reparative regeneration of the islets and possible ways of its stimulation. The ultimate goal, sufficient level of functional activity of β-cells or their substitutes can be achieved by two prospective broad strategies: β-cell replacement and β-cell regeneration. The “regeneration” strategy aims to maintain a preserved population of β-cells through in situ exposure to biologically active substances that improve β-cell survival, replication and insulin secretion, or to evoke the intrinsic adaptive mechanisms triggering the spontaneous non-β- to β-cell conversion. The “replacement” strategy implies transplantation of β-cells (as non-disintegrated pancreatic material or isolated donor islets) or β-like cells obtained ex vivo from progenitors or mature somatic cells (for example, hepatocytes or α-cells) under the action of small-molecule inducers or by genetic modification. We believe that the huge volume of experimental and clinical studies will finally allow a safe and effective solution to a seemingly simple goal-restoration of the functionally active β-cells, the innermost hope of millions of people globally.
Key words: Pancreas; Islets of langerhans; β-cells; Regeneration; Replacement; Transplantation; Reprogramming

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Core tip: The review discusses the most promising strategies for regenerative medicine to stimulate the regeneration or replacement of the islet of the pancreas. The “regeneration” strategy aims to maintain a preserved population of β-cells through replication, or evoke the intrinsic adaptive mechanisms. The “replacement” strategy implies transplantation of β-cells or β-like cells obtained ex vivo from progenitors or mature somatic cells (hepatocytes or α-cells). We believe that the huge volume of experimental and clinical studies currently under way will finally allow a safe and effective solution to simple goal—restoration of the active β-cells.

INTRODUCTION

Development of methods and tools to stimulate regeneration of damaged tissues and organs has always been a prominent theme in medical science. However, only recently, in connection with the unprecedented development of biotech, regenerative medicine has acquired independent significance. Our ideas about reparative regeneration (restoration of the structure and function of tissues and organs damaged by pathology or trauma) are constantly expanding and replenishing the existing clinical strategies.

The pancreas historically became one of the first objects of regenerative medicine, apparently in connection with notable inconsistency of other approaches in relation to this organ. The first transplantation of pancreatic material to a patient took place at the University of Minnesota in 1966. Since then, > 50000 diabetic patients received the transplants in > 200 of medical centers; the global lead is held by the United States[1]. Despite the continuous technological upgrade, cadaveric donations are obviously a “dead end”. The general shortage of donor organs, as well as the complexity and high costs of the procedure, will never meet the demand for such operations.

The pancreas consists of exocrine and endocrine portions. The exocrine function of the organ is to produce and excrete digestive enzymes in the form of inactive precursors into the duodenum, thus ensuring the luminal digestion of basic nutrients (proteins, fats and carbohydrates). The exocrine pancreatic deficiencies (up to complete dysfunction) can be effectively treated with advanced enzyme formulations to provide acceptable life quality for the patients[2]. Extremely serious problems arise with the endocrine failure caused by abnormal functioning of the hormone-producing cells of the Langerhans islets. Each islet comprises at least five types of endocrine cells, including insulin-producing β-cells (65%-80%), glucagon-producing α-cells (15%-20%), somatostatin-producing δ-cells (3%-10%), pancreatic polypeptide-producing PP-cells (1%) and ghrelin-producing ε-cells[3]. Some of the related hormonal deficiencies can be partially counteracted by enhanced function of the amine-precursor-uptake-and-decarboxylation endocrine cells distributed in the lamina propria mucosae of the gut. The amine-precursor-uptake-and-decarboxylation cells are capable of producing all pancreatic hormones except insulin[4]. Insufficient production of insulin by pancreatic β-cells, which cannot be relieved endogenously, results in the development of the insulin-dependent diabetes mellitus (DM). At the same time, it is obvious that not only insulin but the entire hormonal complex released by sum total of the functionally united Langerhans islet cell types are involved in regulation of the nutrient and glucose homeostasis[5]. Nevertheless, it is functional assessment of β-cells (by evaluation of the insulin and C-peptide levels) that serves an integral diagnostic indicator of DM development. The insulin-dependent DM commonly develops without any surgical, infectious or traumatic damage to the pancreas, but as a hereditary autoimmune damage to the islet cells (DM type 1). However, many insulin-independent forms of diabetes (DM type 2)
proceed with progressive depletion of β-cells, which in some cases leads to insulin dependence. In the new-onset DM type 2, β-cell population of the pancreas has been estimated to decrease by 24%–65%, whereas in DM type 1 it is decreased by over 80% (Table 1)\(^6\). A number of studies indicate that hormonal dysfunctions are typical for both types of diabetes and are not limited to insulin deficiency\(^7\).

According to the International Diabetes Federation\(^8\), about 425 million adults (20- to 79-year-olds) globally were living with diabetes in 2017; by 2045 the number will increase to 629 million. This dramatic prognosis is predominantly associated with DM type 2, which partly belittles the problem of insulin-dependent diabetes. Meanwhile, the delayed diagnosis and poor control of glucose levels in DM type 1 cause severe complications including nephro-, retino- and neuropathies, cardiac and vascular diseases. Administration of short- and long-acting exogenous insulins, smart selection of administration regimens, and even the use of insulin pumps are insufficient for the proper control of glucose levels in some patients; effective means for stimulating regeneration of the pancreas are therefore extremely relevant.

**DEVELOPMENT OF THE PANCREAS**

Mature pancreas is comprised of acinar cells, connected to the intestine via the highly branched ductal tree; the islets, which constitute about 1%–2% of the organ mass, are scattered throughout its central regions. Both portions of the pancreas (exocrine and endocrine) arise as thickenings (buds) at the dorsal and ventral surfaces of the posterior foregut, in the close vicinity of prospective hepatic endoderm. Specification of the hepatic and pancreatic domains in the endodermal epithelium proceeds under the influence of inducing stimuli from the adjacent mesoderm: Extensive suppression of the mesodermal Wnt and Fgf4 signaling in the foregut allows the hepatic and pancreatic induction. The newly defined pancreatic endoderm specifically expresses transcription factor-encoding genes pdx1 and ptf1a; the pdx1\(^{-}\)ptf1a\(^{-}\) cells are progenitors of all parenchymal cell types in the mature pancreas, including the duct, islet and acinar cells. The proper balance of endocrine and exocrine cells, descending from pancreatic progenitor cells within the pancreatic buds, is dependent on Notch signaling. The arrest of Notch signaling disinhibits ngn3, a transcription factor-encoding gene, master regulator of the endocrine-type development in the pancreatic endoderm. The ngn3\(^{+}\) endocrine precursors rapidly develop a specific expression signature of transcription factor-encoding genes, including neuroD, isl1, isl1 and pax6, and differentiate into the five cell types of the islets (α, β, δ, ε and PP cells)\(^9\).\(^{10,11}\).

After birth, the total volume of the islets of Langerhans increases 20-fold. The islets are capable of compensatory growth in response to physiological demands (e.g., in pregnancy, obesity or after partial pancreatectomy)\(^11\). The decreased demand for exogenous insulin, observed during the period of partial clinical remission in DM patients, is explained by partial functional recovery of β-cells\(^12\).\(^{13,14}\). These observations indicate the considerable physiological regeneration capacity of the endocrine pancreas and suggest the principal feasibility of its substantive repair. In medical literature, the term “islets” most frequently refers to β-cells as the major subpopulation of the islets that prevails in number and clinical significance; “regeneration of the islets”, therefore, specifically means “the insulin-producing cell recovery” (unless otherwise stated) and designates the most clinically desirable process in the endocrine portion of the pancreas.

Physiological regeneration of the islets by means of proliferation of the pre-existing β-cells occurs in response to certain factors, e.g., insulin-like growth factor-1 (IGF1), hepatocyte growth factor (HGF), incertins and prolactin\(^15\).\(^{16,17}\). In the perinatal period, β-cells actively proliferate; a multiple increase in the mass of β-cells after birth occurs due to an increase in their number inside the islets, rather than an increase in the number of islets\(^18\). In humans, similarly with rodents, proliferative activity of β-cells in pancreatic islets declines with age. The most rapid reduction occurs in juveniles; by the age of 6 mo, the proportion of dividing β-cells drops from 3% in fetuses to less than 0.5% and continues to decrease thereafter\(^19\).\(^{20,21}\). As has been demonstrated in animal models, the residual proliferative activity of β-cells is insufficient to compensate for massive losses in adulthood. For instance, by 4 wk after 90% partial pancreatectomy in adult rats (leaving a 10% remnant pancreas), the weight of the pancreatic remnant reaches only 27% of the initial weight of the organ\(^16\). The proliferative activity of β-cells is controlled epigenetically; in particular, it depends on histone modifications regulated by trithorax group (TrxG) and polycomb group (PcG) complexes. TrxG and PcG proteins are the evolutionarily conserved transcription factors that act as heteromeric complexes and modulate gene expression by modifying the structure of chromatin. TrxG and PcG complexes repress a set of cell cycle-inhibiting genes in β-
Table 1: Comparison of type 1, type 2 and surgically induced diabetes mellitus

|                      | Type 1 DM                                                                 | Type 2 DM                                                                 | Type 3 DM                                                                 |
|----------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Pathogenesis         | Chronic autoimmune disorder; the immune system attacks endogenous pancreatic β-cells | Progressive metabolic disorder characterized by insulin resistance       | Chronic disorder induced by partial or total pancreatectomy               |
| Prevalence           | 5% to 10% of diagnosed diabetics                                           | 90% to 95% percent of diagnosed diabetics                                  | 1% of diagnosed diabetics                                                |
| Onset                | Abrupt                                                                    | Gradual                                                                  | Shortly after surgery                                                     |
| Age of onset         | Varies (commonly in childhood or puberty)                                 | Commonly over 35 yr                                                      | Commonly over 50 yr                                                      |
| Genetic predisposition| Moderate                                                                  | Very strong                                                              | Moderate                                                                 |
| Body habitus         | Thin or normal                                                            | Often obese                                                              | Normal, overweight or obese                                              |
| Ketoadiposis         | Common                                                                    | Rare                                                                     | Rare                                                                     |
| β-cell depletion     | Severe (over 80%)                                                         | Mild (24% to 65%)                                                        | Mild to total (up to 100%)                                               |
| Plasma insulin       | Low to absent                                                             | Normal or increased                                                      | Low to absent                                                            |

DM: Diabetes mellitus.

cells, thereby facilitating physiological and adaptive β-cell expansion[19,20].

MODELING THE ENDOCRINE PANCREAS REGENERATION

Several experimental models for studying the endocrine pancreas regeneration are available. The first of them, wirsung duct ligation, was introduced as early as in 1920 by F. Banting as a possible treatment for DM. The procedure, indeed, led to an increase in the mass and number of the islets[21,22]; it used to be applied widely for DM treatment in children, but provided only short-term results[23]. Nowadays, the pancreatic duct ligation model is still employed in animal studies; it is considered an acceptable representation of the adult pancreatic tissue remodeling. The pancreatic duct ligation predominantly affects the tail region of the pancreas, resulting in acute pancreatitis followed by regeneration of ductal complexes from the surviving metaplastic acinar cells[24].

Two other surgical models, cellophane wrapping and partial pancreatectomy, similarly result in the partial obstruction of the pancreatic products drainage. In the partial pancreatectomy, the extent of tissue removal is variable; in rodents, 60%–70% pancreatic resections are non-diabetogenic, while 90% resections are diabetogenic. Both types of intervention induce a transient wave of β-cell proliferation[24]; however, the usability of these approaches is limited, as they are critically non-selective and exert major influence on the exocrine portion of the pancreas.

Experimental models of selective ablation of β-cells with cytotoxic agents have emerged later on. Streptozotocin, originally developed as antimicrobial, is one of the most harsh diabetogenic drugs; it is notably toxic to β-cells and used routinely for the induction of DM in animals. Depending on the animal strain, dose and route of delivery, streptozotocin causes severe or mild diabetes (blood glucose levels above 200/300 mg/dL and 120–200/300 mg/dL, respectively)[9]. Alloxan, a pyrimidine derivative, is comparable to streptozotocin in its ability to induce diabetes in pregnant animals; the mechanism involves formation of reactive oxygen species in cytosol, which leads to β-cell necrosis and consequent failure of the normal glucose homeostasis; the effective dosage depends on the rodent species, term of pregnancy, age and diet[25]. In vitro study on the isolated pancreatic islets of C57BL/6 mice revealed differential influence of streptozotocin and alloxan on the transport and metabolism of glucose in β-cells: Glucose transporter 2 protein is the main target of streptozotocin, whereas alloxan targets glucose transporter 2 and glucokinase mRNA molecules[26].

In addition to surgical and pharmacological interventions, dysfunction or ablation of β-cells can be achieved by genetic manipulations. In this case, main strategies are switching of particular gene(s) or selective genetic labeling of β-cells. The first strategy implies creation of genetically engineered mice, in which the studied gene (e.g., Ins1) is knocked out, e.g., with the use of tetracycline- or doxycycline-dependent system or Cre-Lox recombinase technology[24,25,28-29]. The second strategy implies creation of transgenic mice with specific expression of cognate receptors for toxins in β-cells. For example, in mice, transgenic expression of the diphtheria toxin receptor followed by
systemic administration of diphtheria toxin permits an exquisite, specific β-cell ablation by apoptosis[39]. The third strategy is the inducible ablation of pancreatic β-cells by conditional targeted activation of genes. For example, regulated expression and activation of c-myc in transgenic mice after administration of synthetic reagent tamoxifen promotes controlled temporal loss of β-cells without the general cellular toxicity caused by chemicals such as alloxan or streptozotocin[30]. In another transgenic model, the pancreatic islet β-cell apoptosis through targeted activation of caspase 8 mouse, β-cell death is induced in a specific and well-defined manner through the treatment with a commercially available dimerizer[32].

**THE SOURCES OF ISLET REGENERATION**

Thus, intrinsic potential of the pancreas for the replication of β-cells is limited. Studies of pancreatic regeneration in experimental models indicate low proliferative capacity of the functionally mature β-cells. An alternative regeneration pathway, neogenesis of islets, apparently involves non-endocrine components of the pancreas (acinar and ductal cells, vascular and neuronal structures) in complex microenvironments, which surround and penetrate the islets[39]. The islet neogenesis, aimed at the in situ expansion of the insulin-producing cells, may proceed by two main routes-mobilization of putative precursors present in the adult pancreas (direct differentiation) and reprogramming of other mature cell types into insulin-producing cells (trans-differentiation)[33-35].

Pancreatic stem cells apparently reside in the ductal epithelium and provide the renewal of both exocrine and endocrine parts of the organ[34,35,36,37]. The phenotype of multipotent pancreatic progenitor cells (MPCs) is defined as pdx1+ptf1a+ sox9+foxa2+nkx6.1+hnf6+; these cells form a highly proliferative pool which differentiates into distinct cell types including exocrine, ductal and islet cells[38,39]. The total mass of the adult pancreas is thought to correspond to proliferative capacity of the embryonic MPC pool[39]; however, any traceable presence of embryonic MPCs in the adult mammalian pancreas is highly doubtful[39].

In adult mammals, the trans-differentiation scenario is more plausible. Interestingly, the chief candidate cell source resides in the islets themselves, as β-cells share developmental characteristics and implement similar gene expression programs with the neighboring α-cells. Knockout of pax4 in mice leads to the loss of β-cells and concomitant increase in the number of α-cells[39]. Subtotal ablation of β-cells may trigger reprogramming in α-cells; for instance, under the selective induction of β-cell apoptosis with diphtheria anatoxin, α-cells start to produce insulin and co-express the adult β-cell markers pdx1 and ntk6.1[40]. Similar results were obtained in the RIP-B7.1 transgenic mouse model of autoimmune diabetes; the observed increases in the size of α-cells and the levels of α-cell proliferation and ductal neogenesis were accompanied by an increase in the content of the glucagon-producing cells positive for insulin or β-cell-specific transcription factor pdx1[40]. As indicated by ChIP sequencing and RNA sequencing analysis of differentiated α-cells, thousands of genes are bivalently marked with activating and repressing histone modifications (respectively, H3K4me3 and H3K27me3) in α-cells, while exhibiting the monovalent state in β cells (i.e. showing the signs of either activation or repression). These epigenomic findings suggest that the α-to-β cell reprogramming may result from alterations in the histone methylation signature of the islet cells[41,42]. It has been suggested that in mature individuals, from puberty to old age, α-cells can be reprogrammed to produce insulin, even after the complete loss of β-cells, whereas before puberty, β-cells are replenished by spontaneous en masse reprogramming of the somatostatin-producing δ-cells while the α-cell conversion is negligible[42].

Trans-differentiation of the non-islet cell types into functional β-cells is also possible. In certain settings, exocrine cells of the pancreas spontaneously (in the absence of specific inductive stimuli) differentiate towards β-cell phenotype, although it is hard to exclude replication or fusion of β-cells per se in this case[42].

Participation of other, non-endodermal, stem cell niches in regeneration of the pancreas is another disputable issue. According to modern concepts, multipotent stromal/stem cells (MSCs) are mobilized from the red bone marrow (and probably from other stromal sources) in response to organ damage, migrate to the damaged area and contribute to its regeneration[43,44]. The vast majority of the studies on participation of MSCs in regeneration of the pancreatic islets have been using donor MSCs. For instance, in mice, upon transplantation of bone marrow cells from male donors into lethally irradiated female recipients, a small percentage of donor cells that expressed insulin and pdx1 was found among the Langerhans islet cells; notably, the design of the experiment excluded the possibility of fusion of the donor bone marrow
cells with β-cells of the host. However, other studies failed to reproduce this phenomenon in models of pancreatic damage: The transplanted labeled cells either were not detected in the pancreas at all, or only solitary labeled cells expressed insulin, while most of the cells participated in angiogenesis and restoration of the pancreatic stroma. In the experiments with transplantation of the GFP-labeled bone marrow-derived MSCs to newborn mice, solitary cells that co-expressed GFP and insulin were found within the islets, whereas up to 40% of the ducts (median 4.6%) contained the epithelial cells derived from the transplanted bone marrow MSCs of the donor. The authors conclude that a lineage of stem cells (or epithelial precursors) can migrate from bone marrow to the pancreas and differentiate into complex organ-specific structures, at least in neonatal period.

Thus, the hypothetical sources of β-cells during physiological or reparative regeneration of the endocrine pancreas include mitotic expansion of mature functional β-cells, direct differentiation of the multipotent pancreatic progenitors (or developmentally related progenitor cells of the intestine and the liver) and trans-differentiation of mature cell types inside the islets (α-cells) or outside of them (in the exocrine pancreas or elsewhere). Despite the diverse possibilities, reparative potential of the endocrine pancreas is extremely limited, and its damage still invariably leads to insulin deficiency.

**THE METHODS FOR STIMULATING REPARATIVE REGENERATION OF THE PANCREAS**

The main goal of any attempt to stimulate pancreatic repair is to restore the number of functionally active β-cells to ensure the maintenance of sufficient insulin production. This goal can be achieved in two ways: The reduction in the death rates of β-cells or the production of new β-cells. The methods of regenerative medicine relevant to these tasks can be listed as follows: (1) The use of biologically active substances, especially peptide or protein growth and differentiation factors, that regulate cell cycle, apoptosis, inflammation and repair; (2) Transplantation of donor β-cells or progenitor cells to replace the damaged islets; (3) Transplantation of the tissue-engineered bioartificial pancreatic constructs; and (4) Reprogramming of cells into insulin-producing phenotypes (in situ or prior to transplantation).

**APPLICATION OF BIOLOGICALLY ACTIVE SUBSTANCES FOR THE REGULATION OF CELL CYCLE IN β-CELLS**

Many experimental studies have been aimed at the regulation of β-cell growth and regeneration. It is well known that IGF1, HGF, growth hormone, prolactin, incretin hormones [glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)], insulin, and even certain metabolites including glucose, are mitogens for β-cells. Complex effects of biologically active molecules on β-cells include stimulation of cell growth and protection against apoptotic death. For instance, IGF1 protects β-cells against the cytokine-induced apoptosis. The incretin hormone GLP-1 exerts similar action by amplifying the efficiency of the autocrine loop of IGF-2/IGF-1 receptor and regulating cholecystokinin production in β-cells in a paracrine manner.

The Reg (“regenerating”) protein family was reported independently by research groups studying pancreatitis and pancreatic islet regeneration. The family includes several small protein molecules named “pancreatic stone protein”, “pancreatic thread protein”, “islet neogenesis-associated protein” and C-type lectin-like protein, which exhibit anti-inflammatory, anti-apoptotic and mitogen properties. The action of Reg proteins (as acute phase reactants, lectins, antiapoptotic factors, growth factors) has wider specificity than has been supposed initially, as their targets include not only β-cells, but also neural and epithelial cells in the intestine. The islet neogenesis-associated protein peptide molecule successfully stimulated recovery of β-cell mass in animal models and showed some promising results in clinical trials.

The list of chemicals that stimulate β-cell proliferation and reduce β-cell apoptosis, thus protecting the insulin-producing cell reserve, includes small molecules (glucokinase activators, inducers of calcium and adenosine signaling, diarylamide WS6), growth factors and hormones [platelet-derived growth factor, insulin-like growth factors, epidermal growth factor (EGF), HGF, parathyroid hormone-related protein, insulin, placental lactogen, estrogen, incretins, betatrophin, triiodothyronine] and phytochemicals (resveratrol, phenylpropanoic acid glucoside, flavonoids,
glutathione peroxidase mimetics[62] and is likely to be expanded further. Applied significance of these studies is in utilization of modern synthetic approaches for the production of regulatory molecules that stimulate proliferation of β-cells. A number of pharmaceuticals that exert their action by stimulating β-cell mitosis have already been developed. These include incretin mimetics exenatide [Food and Drug Administration (FDA) approved 2005], liraglutide, semaglutide and other GLP-1 receptor agonists, incretin enhancers sitagliptin (FDA approved 2006), vildagliptin, saxagliptin and other selective inhibitors of dipeptidyl peptidase-4 (DPP-4, the enzyme responsible for the rapid degradation of the incretin hormones GLP-1 and GIP)[60,61]. These drugs are considered suitable for the treatment of DM type 2, because the level of functional preservation of β-cells in DM type 2 is much higher than in DM type 1[62]. According to patient-reported outcomes from eight clinical trials, the DM type 2 patients are more satisfied with the modern incretin-based therapies in comparison with the traditional therapies due to the higher glucose-lowering efficacy of the former and also their ability to facilitate weight loss[62]. The drugs of this group were initially used with caution due to their suspected side effects on the exocrine pancreas associated with the risks of adenocarcinoma development[63]. However, clinical data accumulated over the years of its use indicates that the incretin-based therapy is not associated with increased risks of pancreatic cancer[64,65]. pancreatitis[64,65] or all-cause mortality[66] in DM type 2 patients.

The effectiveness of other classes of biologically active substances requires verification. Clinical trials of phytochemicals, e.g., green tea, herbal tisanes rooibos and honeybush and their polyphenols, resveratrol (phytoalexin from vitis vinifera), yielded controversial results, which prevents considering these substances as effective stimulators of islet regeneration[69-71]. Another example is glucokinase activators (GKAs). In animal models, GKAs promote insulin release from β-cells and stimulate β-cell proliferation. However, the results of recent phase II trials indicate that GKA efficacy drops within few months of use for as yet unclear reasons[72].

It should be noted that a considerable reduction of DM symptoms observed upon transplantation of MSCs (one of the most widespread therapeutic tools of regenerative medicine) cannot be attributed to the alleged capacity of MSCs to trans-differentiate into β-cells[73,74]. The data from preclinical experimental studies and clinical trials indicate that MSCs promote β-cell regeneration by protecting the endogenous β-cells from apoptosis, as well as attenuating the autoimmune processes that destroy β-cells. In addition, the transplanted MSCs ameliorate the insulin resistance of peripheral tissues by providing supportive niche microenvironments via the paracrine factors secretion and the extracellular matrix deposition[75]. In this aspect, MSCs may be considered as "short-lived mobile factories", or "pharmacies", which, during their survival in the recipient’s body, manage to synthesize a wholesome pool of biologically active substances that stimulate regeneration. The recent studies indicate that the anti-diabetic effect of transplantation of the MSC-derived exosomes is similar to the effects of MSC transplantation[77,78].

ALLOGENEIC ISLET TRANSPLANTATION

The idea of pancreatic islets transplantation has a long history; the first experimental study in rodents was published in 1970s. The first experimental reversal of diabetes by a pancreas transplant in rats was achieved in 1972. Delivery of the transplant by infusion to the liver via portal vein was found more effective than intraperitoneal infusion; to date, the site still remains a primary choice for the clinical islet infusion[79]. In animal models, GKAs promote insulin release from β-cells and stimulate β-cell proliferation. However, the results of recent phase II trials indicate that GKA efficacy drops within few months of use for as yet unclear reasons[72].

The surgeons succeeded in developing a protocol surpassing all the previous attempts in its effectiveness. The protocol was tested in seven DM type 1 patients with critically reduced responses to exogenous insulin. The brain-dead donors were selected by
The effectiveness of allogeneic islet transplantation is continuously increasing. In 2017, according to CITR, the proportions of patients who maintained insulin independence for 1 year and 5 years after ITA were, respectively, 76.9% and 47.0%. A recent comparison of the outcomes of ITA and solitary pancreas transplantation alone for DM type 1 shows similar safety, graft function and cost indicators for these procedures[86]. Thus, allogeneic transplantation of the pancreatic islets holds its position as a safe and effective way to treat insulin-dependent DM, especially in cases when administration of exogenous insulin is useless and transplantation of the whole pancreas is unadvisable due to the severity of the patient’s condition.

Moreover, the use of donor islets for the replacement therapy in DM has certain advantages. The criteria for which were developed back in 1996 by Lackey et al[83]. The donor pancreas was perfused via the excretory ducts with a cold mixture of enzymes, after which the islets of Langerhans were isolated by passing the tissue through ficoll-diatrizoic acid gradients as described elsewhere[84], with the difference that 25% human albumin was used instead of the xenogeneic serum. Over 4000 islet-equivalents per kg of body weight of a recipient were infused via the portal vein; the required quantity of transplant was normally obtained from 2-3 donors. The resulting therapeutic effects exceeded all previous achievements; the positive indicators included the increased C-peptide levels, improved glucose tolerance, decreased glycosylated hemoglobin levels and cessation of the acute hypoglycemia episodes. In 2006, the team published the outcome of a clinical trial (FDA NCT00014911) with participation of 36 DM type 1 patients. In particular, in 21 patients (58%), independence from exogenous insulin lasted for a year; of this number, 16 patients maintained this effect through the second year, and 5 of the patients maintained it for more than 2 years[85].

In opinion of the authors of the protocol, the success was largely due to the correct immunosuppression scheme, which included sirolimus, low-dose tacrolimus and the anti-interleukin-2 receptor monoclonal antibody under the complete exclusion of glucocorticoids. The authors also concluded that, to ensure getting off exogenous insulin, the minimum amount of islet-equivalents per kg of body weight should be 10000. The developed protocol, known as “the Edmonton protocol”, became the gold standard for allogeneic transplantation of the pancreatic islets. As of 12/2019, FDA has registered about a hundred clinical trials using the Edmonton protocol or its modifications (mainly in the United States and Canada).

The achievements contributed to the spread of the pancreas transplants technology with the establishment of specialized medical centers worldwide. Back in 2001, the National Institute of Diabetes and Digestive and Kidney Diseases created the Collaborative Islet Transplant Registry (CITR) which collects and summarizes information on this technology[86].

According to CITR, during the period from 1999 to 2015, 1086 diabetic patients underwent the donor islet transplantation, with 81% of the patients receiving the islet transplant alone (ITA) and 19% of the patients receiving the islet-after-kidney, simultaneous-islet-kidney or kidney-after-islet transplants. The largest number of operations occurred between 2002 and 2005[86]; the activity reflected the enthusiasm raised by implementation of the Edmonton protocol and the prolonged (up to 3 years) islet transplantation effect.

The islet isolation and transplantation procedures in different settings were similar, but the immunosuppression scheme evolved significantly, which explains the observed increase in the transplantation efficiency. The Edmonton protocol was considered the gold standard until the middle of 2000s, when the anti-interleukin-2 receptor-specific antibodies-based immunosuppression was replaced with a scheme involving the induction with T-cell depletion and/or TNF-alpha inhibitor and maintenance with mTOR and calcineurin inhibitors. Immunosuppression regimens of this type have minimal diabetogenic side effects and low toxicity against the transplanted islet cells. In 2008, the Edmonton protocol was succeeded by clinical islet transplantation (CIT)-protocol developed by the CIT consortium[86]. Based on the Edmonton protocol, CIT-protocol accumulates modifications and technical tips directed primarily towards the increase in survival and secretory activity of the transplanted allogeneic β-cells. Recent amendments to CIT-protocol (besides minor corrections to the immunosuppression scheme) were as follows: The islets for a transplant were obtained from a single donor; the islets were cultured for 36–72 h prior to transplantation to get rid of the dead and non-viable cells in order to attenuate the inflammatory response to the procedure; large doses of heparin were added to the transplant, and additionally pentoxifylline and anticoagulants were administered for one wk after the procedure, in order to decrease the risks of portal vein thrombosis and to enhance the graft vascularization; the intense insulin therapy was carried on for two mo after the procedure[86].

The effectiveness of allogeneic islet transplantation is continuously increasing. In 2017, according to CITR, the proportions of patients who maintained insulin independence for 1 year and 5 years after ITA were, respectively, 76.9% and 47.0%. A recent comparison of the outcomes of ITA and solitary pancreas transplantation alone for DM type 1 shows similar safety, graft function and cost indicators for these procedures[86]. Thus, allogeneic transplantation of the pancreatic islets holds its position as a safe and effective way to treat insulin-dependent DM, especially in cases when administration of exogenous insulin is useless and transplantation of the whole pancreas is unadvisable due to the severity of the patient’s condition.

Moreover, the use of donor islets for the replacement therapy in DM has certain
potential for further development. The efficiency of β-cell isolation, cultivation and survival is subject to upgrades, e.g., by promoting the interactions of β-cells with MSCs. Co-culturing with MSCs is likely to protect the islets from injury in cell culture prior to infusion\[^{91}\]. Multiple experimental studies show that co-transplantation of MSCs with the islets improves the graft survival and the overall outcome; apparently, the MSC secretome enhances survival of the donor islet cell against immune response, hypoxia, oxidative stress and the host niche inconsistency\[^{75,92,93}\].

A number of currently registered clinical trials employ new strategies aimed at increasing the efficacy of allogeneic transplantation for the restoration of the islets of Langerhans in the following aspects: (1) Modification of the immunosuppression scheme by various formulations including Infliximab (chimeric monoclonal antibody to TNF alpha; NCT00021788), Reparixin (non-competitive allosteric inhibitor of CXCL8; NCT01220856, NCT01817959), Basiliximab (chimeric mouse-human monoclonal antibody to CD25 which acts as alpha subunit of the IL-2 receptor on the surface of T cells; NCT01049633), immunosuppressant chemical Deoxyspergualin (NCT00434850), ex vivo selected and ex vivo expanded autologous regulatory T cells (NCT03444064); (2) The search for alternative transplant site(s) instead of the intraportal infusion prescribed by the Edmonton protocol, for instance, transplantation of the islets into the gastric submucosa (NCT02402439, NCT01571817), the omentum (NCT02213003, NCT02821026), the bone marrow (NCT01722682, NCT01345227), the arm muscles (NCT01967186), the anterior chamber of a severely impaired diabetic eye (NCT02916680); (3) Visual monitoring of the β-cell engraftment by introducing radiolabeled tracer (NCT03785236) or MRI contrast agent (NCT00453817, NCT01050166); and (4) Increasing the transplant survival by co-transplantation of the islets with other cells, e.g., allogeneic CD34+ bone marrow cells (NCT00315614, NCT00021801), autologous MSCs (NCT00646724, NCT02384018), allogeneic parathyroid glands (NCT03977662).

**AUTOLOGOUS ISLET TRANSPLANTATION**

Autologous transplantations of the islets are only used for chronic pancreatitis of various etiologies, specifically in the cases of total pancreatectomy and the concomitant surgically induced DM (Table 1). The first transplantation of autologous islets after pancreatectomy was implemented back in 1979\[^{94}\]. Since 1995, an increasing number of centers have reported total pancreatectomy with islet autotransplantation. The procedure involves isolation of the patient’s own islets and their infusion to the liver via the portal vein; the material effectively engrafts within hepatic parenchyma without the need for immunosuppression, which is one of the huge advantages of autologous transplantation\[^{95}\].

The yields of viable islets from the resected pancreases are poor because of the advanced damage to the pancreatic parenchyma caused by chronic inflammation and fibrosis\[^{94}\]. However, the efficiency of transplantation for autologous cells is significantly higher than for allogeneic. In a large clinical trial, which included 173 pancreatectomized participants with autologous transplants and 262 DM patients with allogeneic transplants, 85% of the recipients with autologous transplants were maintained without exogenous insulin for two years after surgery, as compared with only 66% of the recipients with allogeneic transplants, whereas five-year therapeutic effects were achieved in 69% and 47% of the patients, respectively\[^{96}\]. The protocol for isolation of the islets for autologous transplantations\[^{97}\] and the method of their infusion via the portal system\[^{98}\] are principally the same as for allogeneic transplantations. However, autologous transplantations of the islets are less common because of the difficulty or sheer impossibility of isolating the required amount of the robust islet material from the patient’s tissues.

**TISSUE ENGINEERING OF THE PANCREAS**

The idea of tissue-engineered (bioartificial) pancreas arose as one of the possible strategies for enhancing the allogeneic β-cell grafting. Major challenges associated with bioartificial pancreas transplantations are the efficient delivery of β-cells and the efficient promotion of conditions for the long (preferably lifetime) functioning of the transplanted β-cells without the use of massive immunosuppression. The ultimate goal is creation of an artificial immune-privileged site effectively separated from the host immune system\[^{99}\].

The technical requirements for bioartificial pancreas are exacting and very hard to solve. The critical issues are: (1) Avoiding the foreign-body response; (2) Enabling
In 2018, FDA approved the first implantable continuous glucose monitoring device hypo/hyper glycemia as assessed by using a continuous glucose monitoring system. uncontrolled proliferation and tumorigenesis. cases when transplanted cells (e.g., the iPSC/ESC-derived β-like cells) proceed to retrievable encapsulated devices allow straightaway removal of the transplant in immunosuppression and provide a choice of the implantation site. Besides, the potential. For instance, they enable protection of the islet grafts under minimized transplants of insulin-releasing cells in a number of ways and certainly have the potential. The plugs are eventually removed, leaving the fully formed empty tissue chambers for degradable polymers, molded into small cylindrical parts. Placed subcutaneously, the device is overgrown by connective tissue and microvessels to form the living tissue which provides the islets with core microcirculatory bed thus facilitating the delivery of oxygen and nutrients to the islets and the delivery of secreted insulin to the rest of the body. Development of new protocols for the production of microencapsulated (“individually wrapped”) or macroencapsulated (“packaged”) islets with the use of advanced biological and synthetic materials (alginate, poly-L-lysine, poly-L-ornithine, chitosan-polyvinylpyrrolidone), which would provide survival of cells within the construct while contributing to its immunoisolation properties.

The first transplantation of the alginate-encapsulated neonatal porcine islets dates back to 1996. The transplant was infused intraperitoneally to a 41-year-old DM type 1 Caucasian male patient at a dose of 15000 islet-equivalents per kg of body weight. The detectable and distinctly glucose-responsive blood levels of porcine insulin and C-peptide were observed for 11 mo after the procedure. However, the overall effect was rather weak, especially against the success of the Edmonton protocol; the patient failed to get off the exogenous insulin. Nevertheless, the research in this direction was continued. An Open-label Investigation of the Safety and Efficacy of DIABECELL in Patients With Type 1 Diabetes Mellitus (NCT01796278, Phase 2) started in 2012; DIABECELL stands for the alginate-encapsulated porcine islets for xenotransplantation by Diatranz Otsuka Ltd. The study exposed multiple pitfalls of the alginate-encapsulated islets including immunogenic alginate impurities triggering the immune-mediated destruction, unfavorable surface properties of the transplant, the release of membrane-permeating antigens and the lack of proper standards for encapsulation, cell grafting and alginate composition modifications.

A different type of bioartificial pancreas, the βAir device (Beta-02 Technologies Ltd) comprises a composite membrane (includes two hydrophilized 25 μm PTFE membranes comprising 0.45 μm pores, with the highly viscous high-mannuronic-acid alginate impregnated into the pores). The membrane is impermeable to macromolecules (e.g., antibodies and the complement) and prevents cell-cell contacts, while allowing the free passage of glucose, low molecular weight nutrients, glucagon and insulin. At the end of 2014, four patients were recruited to An Open Label, Pilot Investigation, to Assess the Safety and Efficacy of Transplantation of Macroencapsulated Human Islets Within the Bioartificial Pancreas Beta-Air in Patients With Type 1 Diabetes Mellitus (NCT02064309, Phase 1/2) with the results as yet unpublished.

Another clinical study, A Safety, Tolerability, and Efficacy Study of VC-01™ Combination Product in Subjects With Type 1 Diabetes Mellitus (NCT02239354, Phase 1/2), started in 2014. By contrast with related products, VC-01™ (also known as PEC-Encap™, ViaCyte Inc.) is based on PEC-01 cells, a proprietary pancreatic endoderm cell product derived through directed differentiation of an inexhaustible human embryonic stem cell line, delivered in the immune-protecting and retrievable encapsulation medical device. Cohort 1 of the study, designed to test sub-therapeutic doses of PEC-01 cells, enrolled 19 patients with the established but stable DM type 1. The patients were implanted subcutaneously with two different PEC-Encap unit sizes: The larger VC-01-250 units were used primarily to evaluate the safety and tolerability, while the smaller VC-01-20 units were used as “sentinels” for analysis (histology, etc.) at different time-points; the efficacy will be evaluated in Cohort 2.

A Safety, Tolerability and Efficacy Study of Sernova’s Cell Pouch™ (Sernova Corp) for Clinical Islet Transplantation (NCT03513939, Phase 1/2) has been launched quite recently. The Cell Pouch™ device has been designed as a scaffold made of non-degradable polymers, molded into small cylindrical parts. Placed subcutaneously, the device is overgrown by connective tissue and microvessels to form the living tissue chambers around the removable non-degradable plugs. The process takes about 2 wk; the plugs are eventually removed, leaving the fully formed empty tissue chambers for the transplant. The Cell Pouch™ forms a natural environment rich in microvessels, which provides the islets with core microcirculatory bed thus facilitating the engraftment.

Conceptually, bioartificial pancreases are superior to the conventional suspension transplants of insulin-releasing cells in a number of ways and certainly have the potential. For instance, they enable protection of the islet grafts under minimized immunosuppression and provide a choice of the implantation site. Besides, the retrievable encapsulated devices allow straightaway removal of the transplant in cases when transplanted cells (e.g., the iPSC/ESC-derived β-like cells) proceed to uncontrolled proliferation and tumorigenesis.

The mentioned clinical trials are aimed at preventing glucose variability and hypo/hyper glycemia as assessed by using a continuous glucose monitoring system. In 2018, FDA approved the first implantable continuous glucose monitoring device...
(the Eversense Continuous Glucose Monitoring (CGM) System, Senseonics) for adults (18 years and older) with type 1 and type 2 DM. It is the first fully implantable device that can be used for 90 d without changing the sensor. The Eversense CGM System uses a fluorescent chemical that produces a flash of light when exposed to blood sugar. The light intensity is measured, and every 5 min the measurements are sent via Bluetooth to a mobile app that displays readings while identifying trends and alerts.

It should be noticed that the term “artificial pancreas” is sometimes used to refer to the implantable insulin pumps. Medtronic’s MiniMed 670G, a hybrid closed loop system, became the first implantable insulin pump approved by FDA in 2016 for the patients over 14 years old. The system includes a sensor attached to the body to measure glucose levels every 5 min, an insulin pump, and an infusion patch connected with the pump by a catheter that delivers insulin. The implantable insulin pumps still lack in perfection, as the patients need to calibrate the device by themselves, to reload it with insulin, to make manual adjustments for the physical activity, etc. Besides, under extreme conditions or with a slightest manufacturing defect, the device may deliver a critically inaccurate dose of insulin, the possibility totally excluded when using “artificial pancreas” with insulin-producing β-cells. Nevertheless, we hope that continuous upgrade of insulin pumps will significantly improve the life quality of DM patients in the future.

### STEM/PROGENITOR CELL REPROGRAMMING

The transplantation of the islets of Langerhans has proven to be an effective interim solution for the treatment of insulin-dependent DM. Further improvements in this therapy face two major unresolved issues: The shortage of donor β-cells and the gradual rejection of allogeneic β-cells despite immunosuppression. Both problems can be possibly solved by using alternative cell sources, particularly the autologous stem/progenitor cells that can be effectively expanded and reprogrammed into insulin-producing phenotypes.

Three types of stem/progenitor cells can be effectively reprogrammed into functional β-cells: Adult/somatic stem cells, embryonic stem cells and the induced pluripotent cells. Two major strategies of stem cells reprogramming are the use of specific cell differentiation media and the use of genetic modification.

Adult stem cells are undifferentiated cells located at various sites in the adult body (hemopoietic organs, epithelial tissues, periosteam, periodontium, etc.). The adult mammalian pancreas is believed to contain a small population of the pancreas-derived multipotent precursors (PMPs). PMPs were derived from human pancreas with an efficiency of about 2.6 generated spheres/10000 isolated cells; they distinctly expressed the neural and endocrine progenitor markers. After the Matrigel-induced differentiation, the PMP colonies contained 11.6% of the insulin+/pdx1+ β-like cells and 5.3 ng of insulin, whereas a human islet contains -55% β cells and 45.5 ng of insulin on the average[105]. Another study suggests that β-cell metabolic stress and hyperglycemia enhance proliferation capacity of PMPs and bias differentiation of their progeny toward β-cells in mice and humans[104]. The possibility of PMP expansion would have major implications for regenerative therapy; a weak spot in this concept is the very low cell turnover in the islets[106].

Multipotent stromal cells (mesenchymal stem cells, MSCs) are often considered as the most promising type of adult stem cells for regenerative medicine. MSCs can be effectively obtained from bone marrow, adipose tissue, dental pulp, mobilized peripheral blood and birth tissues. According to the International Society for Cellular Therapy, MSCs must meet three criteria: Adherent growth on raw plastic in conventional culture flasks; surface expression of CD105, CD73 and CD90 and the lack of surface expression of CD45, CD34, CD11b, CD79a/CD19 and human leukocyte antigen (HLA) class II by ≥ 95% and ≤ 2% of the cells, respectively; the ability to differentiate into osteoblasts, chondroblasts or adipocytes. In addition, under specific culture conditions, MSCs can differentiate into multiple mesenchymal derivatives (endothelial cells, fibroblasts, tenocytes, vascular smooth muscle cells, sarcomere muscular cells) and non-mesodermal lineages (hepatocytes, neurons, cardiac muscle cells, astrocytes, pancreatic cells). The ability of bone marrow-derived MSCs to differentiate into β-cells in selective culture media was reported over 10 years ago[107]. Convincing results on differentiation of β-cells from MSCs derived from other sources (umbilical cord, umbilical cord blood, placenta, adipose tissue, urine, dental pulp) were published later on. The possible formulations of inducers included EGF, betacellulin (a member of the EGF family), HGF, retinoic acid, GLP-1, exendin-4 (a long-acting GLP-1 receptor agonist), activin A (a member of transforming growth factor-β family), etc.
nicotinamide, L-Taurin, β-mercaptoethanol, plant-derived alkaloid conophylline, high glucose concentrations, etc.\textsuperscript{[116–118]} However, the use of differentiation media frequently resulted in non-homogeneous or unstable cell cultures\textsuperscript{[119]}. Despite the large number of available protocols, very few of them succeeded in producing glucose-responsive insulin-secreting cells from MSCs. Moreover, the multistage exposure to complex mixtures (cocktails) of inducers occasionally results in destabilized or mixed expression of pancreatic markers (e.g., β and δ genes co-expressed in one cell)\textsuperscript{[120]}. The first experiments on differentiation of MSCs into insulin-producing cells by genetic modification were published around the same time. By using a retroviral vector, the cells were compelled to express the pdx1, which activated the expression of all four hormones of the islets; however, the cells lacked expression of Neurod1, a key transcription factor in differentiated β-cells. Transplantation of the cells to immunodeficient mice with experimental streptozotocin-induced DM resulted in further differentiation of the transplant to the point of the induction of Neurod1 and consequent reduction of hyperglycemia\textsuperscript{[119]}. Trans-differentiation of MSCs into insulin-producing cells can be triggered by induction of several genes, the key of which are pdx1, neurog3, pax4 and mafa\textsuperscript{[124]}. The nucleic acid transfer-mediated switching of these major regulatory genes is a powerful albeit unsafe tool of reprogramming; the use of genetically modified MSCs is still limited to experimental studies\textsuperscript{[125]}.

We have already mentioned that, according to the latest findings, the observed therapeutic effect of MSC transplantation in DM can hardly be considered a consequence of grafting of the in vitro pre-differentiated β-like cells; instead, it is almost entirely due to the production of various immunomodulatory and tissue repair molecules by transplanted MSCs. The term “medicinal signaling cells” has been proposed to reconcile the new concept of the “pharmacy for injured tissues” with the established knowledge about MSCs and their clinical classification\textsuperscript{[117]}.

An alternative source of stem cells for the reprogramming are embryonic stem cells (ESCs) derived from the inner cell mass of the blastocyst. These cells are pluripotent, that is, capable of differentiation into any cell type. The first attempt of obtaining β-cells from ESCs dates back to 2001: The cells self-assembled to form three-dimensional clusters similar to normal pancreatic islets in topology and showing glucose-responsiveness (glucose triggered the release of insulin from these clusters)\textsuperscript{[126]}. ESCs can be readily differentiated into insulin-producing cells; the protocols are similar to the protocols used for MSCs\textsuperscript{[118,119,120]}. However, by contrast with MSCs which can be expanded from the recipient’s own material, the differentiated cells derived from ESCs are invariably allogeneic to recipients, and one of the critical problems facing the in vitro maturation of ESC-derived β-cells is their low survival in host environments. A possible route for obtaining autologous β-cells from ESCs is to use the “therapeutic cloning” strategy, which involves the transfer of the patient’s somatic cell nucleus to the donor ESC cytoplasm. The “therapeutic cloning” technologies have not been recognized primarily due to the ethical and religious restrictions; in most countries, research in this area is prohibited\textsuperscript{[121]}. The clinical use of ESCs is a long-term prospect also because the transplantation of ESCs and their derivatives, including those pre-differentiated into β-cells, carries a possible risk of teratomas and embryocarcinomas\textsuperscript{[122]}.

In 2006, the prospective Nobel laureate Shinya Yamanaka published the procedure for reprogramming of somatic cells (exemplified by murine dermal fibroblasts) into pluripotent cells by transferring only four genes, oct3/4, sox2, c-myc and klf4. The reprogrammed cells, designated as induced pluripotent stem cells (iPSCs), exhibited morphological and growth properties of ESCs and expressed the ESC marker genes\textsuperscript{[123]}. The iPSCs can be differentiated into cells of all three germinal layers, e.g., into β-cells\textsuperscript{[124]}. However, these studies are not yet out of the fundamental stage, and many problems continuously emerge, e.g., the means for eventual substitution of the defective resident β-cells, standardization of the treatment protocol, quality control and safety issues. Nevertheless, a number of experts believe that eventual standardization of iPSCs will bring the autologous cell therapy of DM to the fore\textsuperscript{[125]}.

**MATURE SOMATIC CELL REPROGRAMMING**

Mature somatic cells of the body are increasingly being considered as a source for β-cell population renewal: The insulin-producing cells have been successfully obtained from keratinocytes\textsuperscript{[26]}, pancreatic exocrine cells\textsuperscript{[27]}, hepatocytes\textsuperscript{[28]}, gastrointestinal epithelium\textsuperscript{[29]}, thyroid neuroendocrine cells\textsuperscript{[30]} and non-β-cells of the islets, particularly the glucagon-producing α-cells and the pancreatic polypeptide (PPY)-producing γ-cells\textsuperscript{[31]}. In the majority of cases, the reprogramming was accomplished...
by viral delivery of coding sequences for transcription factors pdx1, ngn3, mafA or hnf6, less commonly - by using small molecules (5-aza-2’-deoxycytidine, trichostatin A, retinoic acid, insulin-transferrin-selenium, nicotinamide). The reprogrammed cells successfully produce and secrete insulin in glucose-responsive manner in vitro and successfully reverse diabetes by sustained production of insulin in vivo, upon transplantation in a streptozotocin-induced murine DM model.

More specifically, the viral delivery of ngn3, pax4 and mafA constructs to adult immunodeficient Rag1/- mice allowed in vivo reprogramming of the differentiated pancreatic exocrine cells into insulin-secreting β-cells; replacement of ngn3 with neuroD reduced the induction efficiency\(^\text{[132]}\). Specific combination of transcription factors ngn3, pax4 and mafA is apparently essential for the β-cell development and maturation\(^\text{[133]}\). Introducing adenoviral pax4, ngn3 and mafA constructs to AR42j-B13 rat exocrine pancreatic cells in vitro caused a dramatic alteration of the cell identity, manifested by inhibited expression of the exocrine markers and up-regulated expression of both insulin genes. The cells secreted insulin and were capable of relieving diabetes in streptozotocin-treated NOD-SCID mice. At the same time, the lack of glucose responsiveness indicated incompleteness of the reprogramming\(^\text{[133]}\), which might be due to the poorly defined culture conditions for the maintenance of β-cell function and identity in vitro\(^\text{[133]}\).

Hepatic cells reportedly acquire the capability of insulin synthesis upon transduction with pax4 alone\(^\text{[134]}\) or pax4 in combination with neuroD\(^\text{[135]}\) or ngn3\(^\text{[136]}\). Some of the authors consider trans-differentiation of hepatic cells into β-cells possible (given that the pancreas, intestinal epithelium and the liver originate from a single source - the foregut endoderm), although comprehensive evidence is missing\(^\text{[39]}\). On the other hand, the residual presence of extra-organ endodermal stem cells in peribiliary glands, hepato-pancreatic common duct, cystic duct, hilum is conceivable as well; these multipotent progenitors may participate in pancreatic and hepatic regeneration by differentiating into hepatocytes or pancreatic cells (exocrine or endocrine)\(^\text{[139]}\). This view is supported by the fact that overexpression of pax4 (possibly in combination with mafA and ngn3) affords the insulin-secreting β-like cells not only from hepatic cells, but also from intestinal epithelial\(^\text{[139,139]}\).

The reprogramming efficiency may vary depending on the cell type. For instance, although all gastrointestinal insulin-positive cells can respond to high glucose, responsiveness of the antral insulin-positive cells is about 2-fold higher than that of duodenal and colonic insulin-positive cells\(^\text{[139]}\), while secretion of insulin by the γ-cell-derived pseudo-islets upon glucose stimulation is 4.5-fold stronger than that of the converted α-cells\(^\text{[131]}\).

However, some experts consider α-cells as optimal trans-differentiation targets for β-cell regeneration; the reasons are as follows: (1) α-cells are the closest to β-cells in origin; (2) The pancreatic islets are their native niche; (3) Sufficient numbers of α-cells are preserved in the islets in DM type 1 and 2, which makes their in situ reprogramming conceivable; (4) α-cells are apparently committed to β-cell differentiation, as under certain conditions they produce insulin and co-express the adult β-cell markers pax4 and nkx6.1; (5) The life-long capability of α-to-β conversion in mammals; and (6) Loss of even a significant portion of α-cells has no major physiological effect\(^\text{[131,141]}\). As has been mentioned above, α-cells and β-cells descend from common ngn3+ endocrine precursors; their destiny as α-cells or β-cells is specified by activation of certain transcription factors. Thus, the α-to-β conversion may proceed via either up-regulation of β-cell-specific factors (pdx1, mafa, nkx6.1 or pax4) or down-regulation of α-cell-specific factor (arx). Both of these options have been successfully implemented: Ectopic expression of pax4, mafa, nkx6.1 or pax4 in α-cells induces β-cell features in fetal or adult α-cells\(^\text{[113-115]}\), whereas selective inhibition of the arx gene in α-cells promotes conversion of the adult α-cells into β-like cells through an intermediate bihormonal state\(^\text{[132]}\). However, the sufficiency of the arx inactivation in α-cells for the direct α-to-β conversion is questionable\(^\text{[132]}\). Epigenetic mechanisms (DNA methylation, histone modifications, non-coding RNA expression) have been reported to contribute to the control of islet cell development including differentiation and maturation of α- and β-cells\(^\text{[114,118]}\); therefore, to increase the efficiency of reprogramming, it was proposed to modulate the arx axis in combination with epigenetic factors. Simultaneous inactivation of arx and dnmt1 (DNA methyltransferase 1) in murine α-cells promoted efficient conversion of α-cells into β-like progeny; the functional hallmarks included characteristic gene expression signatures, electrophysiological responses and notably the glucose-dependent production and secretion of insulin\(^\text{[139]}\). Although the reprogramming of mature somatic cells into β-cells is still in its infancy, the pioneering studies support the feasibility of the directed trans-differentiation for repair purposes.
CONCLUSION

According to ClinicalTrials.gov, a database of privately and publicly funded clinical studies, more than 10000 interventional studies of diabetes mellitus treatment have been registered since 2000, which indicates the complexity, global significance and enormous scale of the problem. The ultimate goal, which is to provide an acceptable level of functional activity of the insulin-secreting β-cells, is pursued by two prospective broad strategies of regenerative medicine: β-cell replacement and β-cell regeneration.

The “regeneration” strategy is aimed at either maintenance of a preserved population of β-cells (through in situ exposure to a wide range of biologically active substances that improve β-cell survival, replication and insulin secretion), or stimulation of the intrinsic adaptive mechanisms triggering the spontaneous non-β-to β-cell conversion. In our opinion, transplantations of undifferentiated stem/progenitor cells should be also included in this group, as the therapeutic activity of the transplant is case determined not by cell replacement, but by the paracrine and immunomodulatory mechanisms.

The “replacement” strategy implies the transplantation of β-cells or β-like cells after certain ex vivo pretreatments. Most straightforwardly, it can be implemented as a transplantation of the natural mature β-cells in the form of donor pancreas or cadaveric/xenogeneic islets, necessarily accompanied by a heavy immuno-suppression regimen. An advanced alternative, artificial pancreas transplants, is essentially the same donor islets placed in a medical device ensuring their isolation from the immune system of the recipient. A much more complicated approach is the obtaining of β-like cells ex vivo from progenitors (MSCs, ESCs, iPSCs) or differentiated somatic cells (e.g., hepatocytes or α-cells) by exposure to small-molecule inducers or genetic modifications. Such reprogrammed cells are similar to β-cells in many respects, including expression of specific genes and insulin secretion in response to glucose stimulation, but still partially retain their original properties (genetic and epigenetic determinants, secretome, plasticity), which requires additional studies on their safety.

In summary, great progress in expanding our knowledge of the origin, growth, and physiological or stimulated regeneration of the pancreatic islets, their isolation and transplantation, and the production of reprogrammed β-like cells is evident. We believe that the huge volume of experimental and clinical studies currently under way will finally allow a safe and effective solution to a seemingly simple goal—restoration of the functionally active β-cells, the innermost hope of millions of people globally.

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