Isolated islets in diabetes research

R. Bhonde, R.C. Shukla, M. Kanitkar, R. Shukla, M. Banerjee & S. Datar

Tissue Engineering & Banking Laboratory, National Centre for Cell Science, Pune, India

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This review highlights some recent developments and diversified applications of islets in diabetes research as they are rapidly emerging as a model system in biomedical and biotechnological research. Isolated islets have formed an effective *in vitro* model in antidiabetic drug development programme, screening of potential hypoglycaemic agents and for investigating their mechanisms of action. Yet another application of isolated islets could be to understand the mechanisms of β cell death *in vitro* and to identify the sites of intervention for possible cytoprotection. Advances in immunoisolation and immunomodulation protocols have made xeno-transplantation feasible without immunosuppression thus increasing the availability of islets. Research in the areas of pancreatic and non pancreatic stem cells has given new hope to diabetic subjects to renew their islet cell mass for the possible cure of diabetes. Investigations of the factors leading to differentiation of pancreatic stem/progenitor cells would be of interest as they are likely to induce pancreatic regeneration in diabetics. Similarly search for the beta cell protective agents has a great future in preservation of residual beta cell mass left after diabetogenic insults. We have detailed various applications of islets in diabetes research in context of their current status, progress and future challenges and long term prospects for a cure.

**Key words** Cytoprotection - hypoglycaemics - islets - regeneration - transplantation

Islets of Langerhans are organelles present within the pancreas and are mainly responsible for the production of insulin, glucagon, somatostatin and pancreatic polypeptide upon stimulation. The primary focus of islet research is, however, the cure and/or better management of diabetes mellitus which results from a loss of insulin secretion from beta cells present within the islets of Langerhans. This review seeks to take a bird’s eye view of the contribution of islets as a model system in diabetes research beyond transplantation.

Since their discovery in 1869, islets have been viewed as a possible *in vitro* system for a syndrome that cannot be mimicked very effectively using cell lines. They are miniature organ systems, retaining their architecture, differentiated state and ability of insulin secretion upon stimulation, independent of nervous control. Isolation of islets has promoted studies related to understanding the pathophysiology of type I and II diabetes, transplantation, screening of hypoglycaemic drugs and probing into diabetes causing mechanisms, to device effective means of prevention.
**Islets in pharmacological research**

*Insulin secretion enhancers:* Isolated islets, *in vitro*, respond to glucose stimulation and hence have immensely contributed to the study of various pharmacological aspects and for screening of promising antidiabetic agents. A number of hypoglycaemic drugs act as insulin secretagogues, in corroboration with this, we have reported that islets can serve as an *in vitro* model for antidiabetic drug screening⁴. Our model offers several advantages as it is simple and economical in terms of reduction in the number of animals used as well as the amount of drug required for testing. A number of plants, in traditional medicine, are claimed to have antidiabetic properties and isolated islets have been used extensively for checking their properties and plausible modes of action. For example, extract of *Tinospora crispa*⁵, extract of *Gymnema sylvestre*⁶, bittergourd fruit juice⁷, leaf extract of *Urtica dioica*⁸, aqueous extract of *Scoparia dulcis*⁹ and aqueous extract of *Teucrium polium*¹⁰ have been shown to exhibit insulin secretagogue activity. Apart from plant/natural extracts, several synthetic drugs have been tested for their insulin secretagogue property and for determination of their mechanism of action, using isolated pancreatic islets *in vitro*.

It is understood that glucose stimulates insulin secretion in the pancreatic β cell by means of a synergistic interaction between at least two signaling pathways. In the K (ATP) channel-dependent pathway, glucose stimulation increases the entry of extrinsic Ca²⁺ through voltage-gated channels by closure of the K (ATP) channels and depolarization of the beta cell membrane. The resulting increase in intracellular Ca²⁺ stimulates insulin exocytosis. While in the GTP-dependant pathway, intracellular

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**Fig. 1.** Signaling cascade of glucose stimulated insulin secretion. The figure summarizes the signaling pathway triggered by glucose in beta cells for insulin exocytosis. Agents in blue represent secretagogues while agents in red represent insulin secretion inhibitors. Gq protein: member of G family of proteins, IP₃: Inositol triphosphate.
Ca\textsuperscript{2+} is elevated by GTP-dependent proteins and augments the Ca\textsuperscript{2+}-stimulated release (Fig. 1). Secretagogues and insulin secretion inhibitors act at intermediate steps of these signaling pathways and influence the process of insulin exocytosis. Several researchers have investigated this intricate mode of known secretagogue action using isolated islets as an in vitro model. To quote a few; imidazoline antagonists of alpha 2-adrenoreceptors increase insulin release in vitro by inhibiting ATP-sensitive K+ channels in pancreatic β cells\textsuperscript{11}. Giannaccini et al\textsuperscript{12} have evaluated the properties of sulphonylurea receptors (SUR) of human islets of Langerhans. They studied the binding affinity of various oral hypoglycaemic agents to the receptor and also tested insulinotropic action of the drugs on intact human islets. This binding potency order was parallel with the insulinotropin potency of the evaluated compounds\textsuperscript{12}. Masuda et al\textsuperscript{13} have shown an insulinotropic effect of Triglitazone (CS-045) and have shown its mode of action to be distinct from glibenclamide (a sulphonylurea drug). A-4166, a derivative of D-phenylalanine, evokes a rapid and short-lived hypoglycaemic action in vivo. It has been shown to act via the tolbutamide binding sites\textsuperscript{14}. Louchami et al\textsuperscript{15} showed S21403, a meglitinide analogue to be a novel insulinotropic tool in the treatment of type 2 diabetes, as it affected cationic fluxes and the drugs secretory responses displayed favourable time course of prompt, and not unduly prolonged, activation of β cells. Mears et al\textsuperscript{16} demonstrated that tetracaine (an anaesthetic) stimulates insulin secretion by release of intracellular calcium and for the first time elucidated the role of intracellular calcium stores in stimulus-secretion coupling in the pancreatic β cells. JTT-608, is a nonsulphonylurea oral hypoglycaemic agent which stimulates insulin release at elevated but not low glucose concentrations by evoking PKA-mediated Ca\textsuperscript{2+} influx\textsuperscript{17}. 

**Insulin secretion inhibitors:** Besides insulinotropic studies, several investigators have used isolated islets to study drugs that inhibit insulin secretion and their probable mode of action. Several drugs have inhibitory effect on insulin secretion by β cells and this is an important aspect, which needs to be considered while using the said drug. Cyclosporine, a widely used immunosuppressant, induces inhibition of insulin release in isolated rat islets\textsuperscript{18,19}. Paty et al\textsuperscript{20} demonstrated the inhibitory effects of several immunosuppressive drugs on insulin secretion. Thus low dose immunosuppressive drug protocols should be used in clinical islet transplantation and patients using this drug have to be carefully monitored for signs of deficient insulin secretion. Tacrolimus is known to cause post-transplant diabetes mellitus. Using isolated rat pancreatic islets, Uchizono et al\textsuperscript{21} studied its mechanism of action and found that it interferes with the process of insulin exocytosis and that protein kinase C (PKC)-mediated (Ca\textsuperscript{2+} dependent and independent) and Ca\textsuperscript{2+}-independent GTP signaling pathways may be involved. L-asparaginase inhibits glucose-induced insulin secretion in a dose-dependent manner by decreasing total cAMP in isolated rat islets\textsuperscript{22}. Metz et al\textsuperscript{23} have used selective inhibitors of GTP synthesis and proved that they impede exocytic insulin release from intact rat islets. The study provides first direct evidence that GTP is required for insulin release\textsuperscript{23}. Hydrochlorothiazide is also known to inhibit insulin release. This inhibition is not mediated by reduced chloride fluxes but rather by inhibition of calcium uptake\textsuperscript{24}. 

Many antidiabetic drugs act on peripheral tissues and have no direct effect on pancreatic islets. One such drug is metformin, which affects glucose and free fatty acid metabolism in peripheral insulin target tissues. However, Patane et al\textsuperscript{25} exposed rat pancreatic islets to high glucose and free fatty acid (FFA) levels in vitro mimicking the in vivo environment in presence and absence of metformin. They found that metformin can restore a normal secretory pattern in islets whose secretory function has been impaired by chronic exposure to elevated FFA or glucose levels. Recently, Marchetti et al\textsuperscript{26} cultured islets isolated from type 2 diabetes subjects in presence of metformin and found that several functional and survival defects of T2D islets were
ameliorated by metformin. Thus in diabetic patients, metformin (in addition to its peripheral effects) may have a direct beneficial effect on the β cell secretory function. On the contrary, Leclerc et al27 have shown the ability of metformin to activate AMP-activated protein kinase in human islets and inhibit insulin secretion. This inhibitory effect needs to be considered with respect to the use of this drug for the treatment of type II diabetes.

We examined the effect of commonly used antibiotics such as gentamycin, penicillin, streptomycin, tetracycline, neomycin, erythromycin and chloramphenicol on isolated islets viability, functionality and induction of oxidative stress if any. Our results revealed the innocuous nature of the antibiotics used at pharmacological concentrations, suggesting their safety whenever prescribed for combating infections and also during islet isolation procedures28.

Cytoprotection of islets

Another primary area of research greatly facilitated by usage of islets as a model system is in unraveling the mysteries of beta cell death and ways of preventing the same.

β cell death: The precise mechanisms of β cell death in vivo, leading to diabetes, remain unclear. However, extensive studies, using islets as a model system show that there are many molecules including Fas Ligand (FasL) and cytokines such as interleukin-1 (IL-1), tumour necrosis factor alpha (TNFα) and interferon gamma (IFNγ) that cause release of other cytokine mediators that have potential to damage β cells in vitro and in vivo29. β cell death appears to be ultimately caused by receptor mediated mechanisms and/or by secretion of cytotoxic molecules like granzymes and perforin. In addition, toxic molecules such as reactive oxygen species (ROS: superoxide radicals, hydroxyl radicals, and nitric oxide) play a significant role in islet cell death by inducing DNA damage. DNA damage, in β cells, leads to poly (ADP ribose) polymerase (PARP) activation which increases NAD consumption, depletion of which compromises ATP production in cells30. It is apparent that a number of different mechanisms of cell death are operative in destruction of islets (Fig. 2).

Inhibition of oxidants: Normally, cells counter oxidative stress by expression of ROS scavenging enzymes like catalase (CAT), glutathione peroxidase (Gpx) and superoxide dismutase (SOD). β cells, however, have extraordinarily low levels of ROS scavenging enzymes31. The correction of this deficiency, in vitro, by overexpression of cellular enzymes like SOD may lead to protection of β cells against oxidative stress induced β cell damage/death32. Studies, wherein mitochondrial form of Mn-SOD was overexpressed in cells are shown to have protected isolated islets against oxidative damage in vitro33. It has also been shown that adeno viral overexpression of glutamyl cysteine ligase catalytic subunit, a primary regulator of de novo synthesis of glutathione (GSH) in mammalian cells and central to the antioxidant capacity of the cell, protects pancreatic islets against oxidative stress, in vitro34.

In vitro stress in islets is often produced by using β cell specific toxins like streptozotocin (STZ) and alloxan. STZ induces islet necrosis by employing effector molecules like nitric oxide (NO) and ROS. Pro-inflammatory cytokines also employ NO as an effector molecule for necrosis and/or apoptosis induction35. Hence a plausible means for cytoprotection of islets could be scavenging of NO or inhibition of iNOS (inducible nitric oxide synthase) which synthesizes NO. iNOS inhibitors can be used for cytoprotection of islets in vitro as inhibition of iNOS would inhibit formation of NO, preventing islet cell death indirectly. It has been reported that a combination of an iNOS inhibitor and a free radical scavenger, guanidinoethyl disulphide restored IL-1β induced suppression of islet insulin secretion in vitro36. An imidazole compound called Efaroxan has also been shown to impart complete protection against IL-1β induced toxicity37. It has recently been shown that silymarin, a polyphenolic flavonoid that has a strong antioxidant activity, prevented IL-1β+IFN-γ-induced NO production and β-cell
dysfunction in human islets. These cytoprotective effects of silymarin appeared to be mediated through the suppression of c-Jun NH2-terminal kinase and Janus kinase/signal transducer and activator of transcription pathways\textsuperscript{38}. \textit{In vitro} treatment of islets with exogenous antioxidants is another viable option. There are many known antioxidants like vitamin C, vitamin E\textsuperscript{39}, \textit{Scoparia dulcis} a traditional antidiabetic plant\textsuperscript{40} that are known to protect islets against oxidative stress induced dysfunction and death. Studies with islets have shown that the experimental drug, bis-o-hydroxycinnamoyl methane, an analogue of naturally occurring bis-demethoxycurcumin, enhances the antioxidant defense against ROS, thus protecting β cells from death\textsuperscript{41}. It has been demonstrated that polyenoylphosphatidylcholine (PPC), a phosphatidylcholine rich phospholipid extracted from soybean, protects β cells against STZ induced toxicity and also plays an important part in maintaining their insulin synthesis and secretion for normal glucose homeostasis\textsuperscript{42}.

\textbf{PARP and NFkB inhibition:} Poly (ADP ribose) polymerase (PARP) is a major effector molecule in the oxidative stress induced or cytokine induced cell death pathway. It is known that STZ injections cause extensive necrosis in islets of Parp $^{+/+}$ mice while the extent of necrosis was markedly lower in Parp $^{-/-}$ islets\textsuperscript{43}. It has also been shown that inhibition of Parp-1 by synthetic inhibitors like 3-aminobenzamide resulted in protection against necrosis. A potent inhibitor of Parp, 5-iodo-6amino-1, 2 benzopyrone (INH2BP), was found to protect rat islets and beta cell line RIN-5F from cytokine induced damage\textsuperscript{44}.

![Fig. 2. Mechanisms of islet cell death. Flow chart depicts apoptotic and necrotic beta cell death cascades along with possible modes of intervention. Causes/agents of beta cell death are indicated in red while agents/strategies for prevention of beta cell damage are indicated in blue. Red arrows stand for possible sites of intervention. IL-11: Interleukin 11, IKK: Inhibitor of kappa kinase, iNOS: inducible nitric oxide synthase, NO, Nitric oxide, STZ: Streptozotocin, ROS: Reactive oxygen species, PARP: Poly (ADP-Ribose) Polymerase, CAT: Catalase; SOD, superoxide dismutase; GSH: glutathione peroxidase, NF-kB: Nuclear Factor kappa B. This figure has been compiled and constructed by authors by referring to data cited in references 34-38.](image)
NF-κB activation is an important event in inflammation, cellular death signaling and is often activated by oxidative stress. One of the key steps in activation of the NF-κB pathway is the stimulation of the I-κB kinases. Inhibition of NF-κB activation could be an effective means of controlling islet cell death in vitro. Studies have shown that IL-11, a regulatory cytokine, has been effective in inhibiting NF-κB activation in islets leading to prevention of multiple low doses STZ (MLD-STZ) induced diabetes in vivo. Rehman et al. have demonstrated that adenoviral gene transfer of the NF-κB inhibitor I-κB to isolated human islets resulted in protection from IL-1β mediated dysfunction and apoptosis. Mouse islets when transduced in situ by infusion of the transduction peptide prior to isolation lead to 40 per cent of peptide transduction of β cells. Delivery of the IKK inhibitor transduction fusion peptide (PTD-5-NBD) in situ to mouse islets resulted in improved islet function and viability after isolation. Other studies involving rhIL-1 β have suggested a cytoprotective role of the recombinant cytokine against alloxan induced toxicity in diabetic rat islets.

Apart from exogenously induced islet damage, islets also suffer from damage due to endogenous hyperglycaemia in vivo. It is known that high glucose concentration causes apoptosis in cultured human pancreatic islets of Langerhans. Data suggest that in human islets, high glucose modulates the balance of pro- and anti-apoptotic Bcl proteins towards β cell apoptosis. Hyperglycaemia also causes production of IL-1β by islet β cells leading to cytotoxicity in human pancreatic islets. Chronic exposure to free fatty acids alone or with hyperglycaemic conditions lead to pancreatic cell death possibly employing oxidative stress as the mechanism for cell destruction. Hence, blockage of multiple pathways, rather than a single pathway, leading to β cell death may be necessary to fully protect β cells from destruction in vitro. It is relatively easy to study mechanisms of β cell death, in its intricate detail, along with modes of preventing the same and its effect on diabetogenesis employing isolated islets rather than animal experimentation.

Isolated islets for transplantation

Apart from studying strategies for prevention or abrogation of β cell death, isolated islets are widely used for transplantation. Extensive studies have been conducted wherein islets were transplanted into a hyperglycaemic host and then checked for reduction in hyperglycaemia. Till date it remains the most successful means of achieving normoglycaemia in humans. Allo- or xeno- transplantation of whole pancreas is possible, but it requires major surgery, hence transplantation of islets or insulin producing beta cells would be a more viable option.

Any successful transplantation depends on three things, viz:

(i) Primary non function: Primary nonfunctioning (PNF) of islets accounts for the bulk of graft losses. Macrophages, the main effector cells in PNF, release proinflammatory cytokines like IL-1 and TNF-α, which in turn recruit free radicals to mediate a nonspecific inflammatory response. Lee et al. have shown that the blockade of monocyte chemo-attractant protein-1 (MCP-1) binding to CCR2, in conjunction with sub therapeutic immunosuppression, leads to islet allograft survival. Hence, interruption of the leukocyte recruitment through chemokine receptor targeting may be of therapeutic benefit. Transfection of cytoprotective genes to isolated pancreatic islets may contribute to enhanced survival in transplant settings, e.g., the overexpression of erythropoietin gene protects islets from destruction and does not compromise islet functionality. It has been shown by Riachy et al. using cell lines and human islets, that 1, 25-(OH)2D3, the active metabolite of vitamin D3, was able to induce and maintain high levels of A20, an anti-apoptotic protein known to block NF-κB activation, thus promoting islet cell survival by modulating the effects of inflammatory cytokines, which contribute to β cell demise. Disruption of islet extracellular
matrice during pancreatic digestion leads to induction of apoptotic pathways, thus increasing cumulative PNF. Targeting the apoptotic pathway by adenovirus-mediated gene transfer of the anti-apoptotic Bcl-2 gene exerts a major cytoprotective effect on isolated islets. This was experimentally proved with isolated macaque pancreatic islets. Bcl-2 transfection ex vivo protected these islets from apoptosis.

(ii) Abrogation of immune rejection of graft and recurrence of autoimmunity: The next hurdle, graft rejection, can be dealt by the administration of immunosuppressive drugs in the host, and/or immunosolation of the graft, or immunomodulation of the graft or host or both. Immuno-compromisation enables allogenic and xenogenic islet transplantation in preclinical, non human primate models. Isolated islets are known to reverse diabetes in immunocompromised nude mice rendered diabetic by STZ. Although attractive, an immunosuppression regimen leaves the host susceptible to other infections, and these drugs have adverse effects on insulin secretion by β cells. A better alternative to immunocompromization is to make the host tolerant to the graft. Many different strategies have been developed to achieve transplantation tolerance. In one approach used by Oluwole et al. the intravenous administration of genetically engineered host dendritic cells (DCs) expressing allo-MHC peptides, along with transient ALS immunosuppression, resulted in induction of graft tolerance. Similarly, induction of mixed chimerism via bone marrow (BM) cells transplantation from normal donors into autoimmune non obese diabetic (NOD) mice has been shown to reverse insulitis and prevented the development of diabetes and induces tolerance to donor islet cells. This approach however leaves the host susceptible to the graft versus host disease (GVHD). Like this, Liang et al. have described a radiation free regimen for induction of chimerism, donor-specific tolerance, reversal of insulitis, and resistance to diabetes development in NOD mice model.

It is understood that complete T cell activation requires two signals; T cell receptor (TCR) interaction with peptide-MHC complex presented by antigen presenting cells (APCs). This signal must then combine with another co-stimulatory signal, mediated by interaction between distinct cell surface molecules of APCs and T cells. In absence of co-stimulation, T cells undergo anergy and become non-responsive. The B7/CD28/CTLA4 co-stimulatory pathway plays a critical role in the regulation of T-cell activation and transplant rejection and autoimmunity. Adams et al. have used LEA29Y (BMS-224818), a mutant of CTLA4-Ig along with rapamycin and IL-2R to effectively prevent the rejection of islet allograft in a preclinical primate model. Administration of co-stimulatory blockade (anti-CD40L) has been reported to induce mixed chimerism in NOD mice. Pearson et al. have reported that an allelic variant of Idd3 gene is responsible for prolonged islet allograft survival by co-stimulatory blockade in NOD mice. MHC antigens, expressed on APC of donor tissue, stimulate a higher T cells response as compared to host APCs. As passenger (donor) APCs are largely responsible for co-stimulatory activity, graft immunomodulatory strategies aim at depleting them from the islet grafts. These strategies include in vitro culture of graft at suboptimal temperature for extended time, UV-B irradiation, cryopreservation, mitomycin C treatment, ICAM-1 specific monoclonal antibody treatment, co-transplantation of islets with sertoli cells.

Xenotransplantation: The existing shortage of donor islets makes it necessary to pool islets from different donors or look for alternative islet sources. Foetal, neonatal and adult porcine islets along with bovine, murine, canine, avian and piscean islets have been tested for this purpose and porcine islets have been found to be an acceptable source for alternative islets. It was observed that neonatal porcine pancreatic cell clusters (NPCCs) contain mature endocrine cells and bring about sustained normalization of blood glucose levels when transplanted into kidney capsule of diabetic nude mice. Rapid return to diabetic state was...
observed after removal of islet grafts\textsuperscript{90,91}. Recently, Garkavenko \textit{et al} \textsuperscript{92} have reported a follow up study in human diabetic patients receiving porcine islets for 9 yr. The finding that none of the patients developed viral infection hold promises for xenotransplantation. Similarly, canine, bovine and porcine islets have been successfully used for xenotransplantation in a diffusion based bio hybrid artificial pancreas\textsuperscript{93,94}. Chick embryo pancreatic transplants have shown reversal in experimental diabetes of rats without immunosuppression\textsuperscript{95}. Transient reversal of experimental diabetes in mice has also been reported by transplantation of chicken pancreatic islets\textsuperscript{96}. Xenotransplantation of fish islets into the non-cryptorchid testis has also been carried out. Cryopreservation of principal islets of teleost fish and their xenotransplantation has also been studied\textsuperscript{97,98}. Immunoisolation of islets by micro-encapsulation is of great clinical potential in the treatment of diabetes with xenotransplantation of islets\textsuperscript{99-102}. Lim and Sun, first described the alginate micro-encapsulation of islets\textsuperscript{102}. Since then immunoisolation has been regarded as the technological key to xenotransplantation without immunosuppression. The encapsulated islets can be transplanted in, and retrieved from, the peritoneal cavity with minimal invasive surgery. Immunoisolation has facilitated the transplantation, and consequent reversal of hyperglycaemia, from rat to mice\textsuperscript{103,104}, monkey to rats\textsuperscript{105}, porcine tomurine, dog to mice\textsuperscript{106}, and even across a large species barrier \textit{i.e.}, from rabbit to cynomolgus monkey\textsuperscript{107}. An ideal membrane for immunoisolation should be biocompatible, non immunogenic, non cytotoxic, differentially permeable to glucose, insulin, oxygen and other growth factors required for prolonged survival of graft and impermeable to immunoglobulins, immune effector cells and their recruiting cytokines\textsuperscript{108}. Various natural biopolymers like and synthetic materials have been used extensively as immunoisolation material. We have tested cellulose macrocapsules and molecular dialysis membrane\textsuperscript{109,110}, polyurethan\textsuperscript{111}, chitosan-PVP\textsuperscript{112} and chitosan-alginate\textsuperscript{113,114} microcapsules for islet storage and transplantation purposes. Polymeric biomaterials such as alginate\textsuperscript{115}, agarose\textsuperscript{116}, polyamide 4-6 membranes\textsuperscript{117}, poly (ethylene glycol) diacrylate\textsuperscript{118}, polyvinylchloride acrylic copolymer\textsuperscript{119}, AN69\textsuperscript{120}, polyurethane-polydimethylsiloxane\textsuperscript{121}, poly (2-hydroxyethylmetacrylate)\textsuperscript{122} have also been proposed as immunobarriers.

Despite these successes the hurdles to be conquered are monumental. Theoretically it would be best if an individual could simply regenerate its own pancreas/islets.

\textbf{β cell replication and/or regeneration}

Pancreatic islet β cell growth can be mediated by two separate mechanisms\textsuperscript{123}. Either new islets can generate from budding of the pancreatic ductal epithelium\textsuperscript{124-128} or from intra islet precursor cells\textsuperscript{129,130}, \textit{i.e.}, islet-neogenesis and by replication of existing islet beta cells\textsuperscript{123,131} (Fig. 3).

\textit{Islet neogenesis}: Neogenesis of islets primarily occurs during foetal and perinatal stages of development\textsuperscript{132}, but has also been observed in the regenerating adult pancreas\textsuperscript{133,134}. In a population of well differentiated adult pancreatic islet cells, the number of β cells actually undergoing cell division is small, measured to be between 0.5 to 2 per cent\textsuperscript{135}. Although the growth potential of the pancreatic islet β cells is limited, glucose (nutrients), c-AMP, and certain polypeptide growth factors have been reported to exert modest stimulatory effects on β cell growth and replication\textsuperscript{136}. Several authors have reported differential effects of various growth factors on islet neogenesis phenomenon. Movassat \textit{et al}\textsuperscript{137}, have investigated the effects of keratinocytes growth factor (KGF), \textit{in vitro}, on β cell differentiation from undifferentiated pancreatic precursor cells. However KGF does not help in β cell replication\textsuperscript{137}. Similarly vascular endothelial growth factor (VEGF- ligand of foetal liver kinase-1), has been shown to play a role in the development of foetal rat islet-like structures \textit{in vitro}, possibly by stimulating the maturation of endocrine precursor cells in the pancreatic ductal epithelium\textsuperscript{138}. Epidermal growth factor (EGF), an
activator of the MAP kinase pathway, increases the mass of pancreatic epithelial cells but the absolute number of developing endocrine cells decreases. On the other hand, inhibition of MAPK pathway by PD98059 in the precursor cells leads to decreased proliferation of epithelial cells but endocrine cell differentiation was activated. Hence, MAPK pathway determines the final mass of developing endocrine tissue\textsuperscript{139}.

**β cell replication**: Sjoholm et al\textsuperscript{140}, reported that lithium treatment stimulates rat β cell replication and long term insulin secretion *in vitro*. The relationship between β cell replication and insulin release was further investigated using neonatal rat pancreatic monolayer cell cultures and the study demonstrates the importance of glucose utilization for both of these β cell processes\textsuperscript{141}. In another study authors have demonstrated that even after complete destruction of β cells by STZ treatment *in vitro*, foetal pancreatic cells retain the ability to regenerate β cells\textsuperscript{142}. The potential for large scale production of endocrine tissue *in vitro* has been indicated, however, more investigation needs to be carried out on the various signals and pathways involved in pancreatic development. An attempt to transduce NPI (neonatal pancreatic islet) with gene of interest *i.e.*, *PDX-1*, allowed researchers to determine the effects on islet maturation. The authors believed that these transduced NPIs provide an effective tool to study islet growth and maturation\textsuperscript{143}. Transfection of β cells with tyrosine kinase receptors\textsuperscript{144} and human islets with chimeric signaling receptors\textsuperscript{145} leads to ligand dependent cell proliferation. This

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**Fig. 3.** Islet beta cell birth. Figure depicts different ways of islet beta cell neogenesis. Inset 1 to 4 depicts various stages in islet generation from pancreatic duct epithelium stem cell monolayer (1), intense zone of activity (2), islet formation (3) and fully formed mature islet (4). Further inset sequence represents trans-differentiation of islets from non-pancreatic stem cells *viz*. bone marrow, acinar cells and hepatic progenitor cells. Insets 5: monolayer, 6: islet-like cell clusters and 7: Budding and maturation of islets.
strategy has potential to reduce the quantity of human islets required for treatment of patients with type 1 diabetes.

**Conclusion**

It is apparent that isolated islets form a handy model system due to ease of isolation and maintenance. Being miniature organ systems, they do not require a nervous control and manipulations like transfection studies related to signaling pathways, insulin stimulation and secretion assays are easy to perform in an *in vitro* system. Along with ease of handling, studies based on isolated islets can be extrapolated and data corroborate with related *in vivo* findings, with high efficiency, thus supporting the 3R principle of ‘Reduction, Refinement and Replacement’ of animals in biomedical research. These factors have led to islets being popularly used as a compatible model system for diabetes and related research. All above mentioned approaches are considered in context of their current status, progress, future challenges or limitations, and long-term prospects for a cure. Although definitive success is still at the horizon, the advances reviewed here predict the future to be bright.

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