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REVIEW

# Imaging beta-cell mass and function in situ and in vivo

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**Abstract** Glucose-stimulated insulin secretion (GSIS) from pancreatic beta-cells is critical to the maintenance of blood glucose homeostasis in animals. Both decrease in pancreatic beta-cell mass and defects in beta-cell function contribute to the onset of diabetes, although the underlying mechanisms remain largely unknown. Molecular imaging techniques can help beta-cell study in a number of ways. High-resolution fluorescence imaging techniques provide novel insights into the fundamental mechanisms underlying GSIS in isolated beta-cells or in situ in pancreatic islets, and dynamic changes of beta-cell mass and function can be noninvasively monitored in vivo by imaging techniques such as positron emission tomography and single-photon emission computed tomography. All these techniques will contribute to the better understanding of the progression of diabetes and the search for the optimized therapeutic measures that reverse deficits in beta-cell mass and function.

**Keywords** Imaging · Beta-cell · In situ · In vivo

## Introduction

Blood glucose homeostasis is a precisely regulated process, in which pancreatic beta-cells play an essential role. Pancreatic beta-cells sense increases in blood glucose levels and release insulin to facilitate glucose uptake in adipocytes and muscle cells [1]. Sustained hyperglycemia leads to metabolic syndrome and diabetes. Diabetes is a chronic, complicated disease that affects millions of people and causes a massive health toll on both the individual person and society as a whole. In type I diabetes, pancreatic beta-cells are targeted and destroyed by immune T cells, which leads to a gradual loss of beta-cell mass and a dramatic reduction in insulin secretion [2, 3]. In contrast, although obesity and insulin resistance are major reasons for the pathology of type II diabetes, functional beta-cell mass is also very important for maintaining glucose homeostasis [4–6].

Molecular imaging can contribute to diabetes-related beta-cell research in two ways. On the one hand, high-resolution fluorescence imaging methods have been used in basic research to study the molecular mechanisms governing glucose-stimulated insulin secretion (GSIS) in isolated pancreatic beta-cells and islets. On the other hand, different molecular imaging methods have been developed to monitor beta-cell mass and function in vivo, which helps in visualizing the development of the disease and optimizing its therapy by drug or transplantation.

## Molecular imaging at the single beta-cell and islet levels

Of all the cells from the islets, pancreatic beta-cells are the only cells that secrete insulin in response to elevated blood glucose levels. Blood glucose is transported into the beta-cells via the glucose transporter 2 (GLUT2) on the plasma membrane and metabolized in mitochondria to produce ATP. An increase in the cytoplasmic ATP/ADP ratio in turn closes the ATP-dependent $K^+$ channel and depolarizes the cell. This leads to $Ca^{2+}$ influx through voltage-gated calcium channels and fusion of insulin-containing granules with the plasma membrane [7, 8]. Therefore, elevation of intracellular $Ca^{2+}$ concentration and insulin secretion are two closely related processes that are vital to the regulation of blood glucose levels in vivo.

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## Molecular imaging assays to study $Ca^{2+}$ signaling in beta-cells

Calcium homeostasis is critical for beta-cells to maintain an effective stimulus secretion coupling [9]. In fact, reduced expression of sarcoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) protein significantly enhances beta-cell secretion, which may contribute to the hyperinsulinemia of beta-cells at the earlier stages of both type 1 and type 2 diabetes [10–12]. Thus, monitoring $Ca^{2+}$ dynamics in live cells may help directly visualize beta-cell function in vivo.

By loading cells with fluorescent dye, cytoplasmic $Ca^{2+}$ dynamics evoked by elevated glucose can be monitored in isolated beta-cells using a wide-field microscope equipped with either photomultipliers [13] or CCD cameras [14] or using confocal microscopes [15]. However, when intact islets are loaded with chemically synthesized dyes, only the periphery layer can be labeled, thus preventing the calcium responses within the islet core from being measured [16]. In addition, the free $Ca^{2+}$ concentration in specific cellular organelles (such as the endoplasmic reticulum or the granule) cannot be readily distinguished from the cytoplasmic signal. To address these problems, organelle- and membrane-targeted genetic $Ca^{2+}$ probes have been developed.

Although the endoplasmic reticulum (ER) was originally regarded as a protein synthesis depot, ER stress plays an important role in the pathogenesis of diabetes [17]. Using D1ERCaM as an indicator to monitor $Ca^{2+}$ concentration in the ER ($[Ca^{2+}]_{ER}$) [18], we found that depolarization does evoke a continuous elevation in $[Ca^{2+}]_{ER}$. Once depolarization stops, the ER $Ca^{2+}$ store starts to release $Ca^{2+}$ [19]. Therefore, this uptake and release of $Ca^{2+}$ by the ER will modulate the spatial and temporal profile of glucose-induced $Ca^{2+}$ signals, thereby contributing to insulin secretion. Diabetes leads to a down-regulation of SERCA protein [12], which may change the acute spatial–temporal profile of GSIS and contribute to ER stress in the long term.

By developing a plasma membrane-targeted $Ca^{2+}$ probe, SNAP25-Pericam [20, 21], Rutter's group measured the subplasma membrane $Ca^{2+}$ concentration ($[Ca^{2+}]_{pm}$). They found that although $[Ca^{2+}]_{pm}$ was not significantly different compared with the cytoplasmic $Ca^{2+}$ concentration at the resting state, depolarization-induced increases in $[Ca^{2+}]_{pm}$ were 30 % higher. These data imply that $Ca^{2+}$ microdomains exist beneath the surface membrane of beta-cells [21], which are found to be regulated during disease progression [22].

## Molecular imaging insulin secretion in isolated beta-cells and islets

Pancreatic beta-cells reside on the islets of Langerhan along with alpha, delta, and pancreatic polypeptide (PP) cells. Insulin is synthesized and stored within large dense-core granules in resting beta-cells, which collapse with the plasma membrane and release their content during cytoplasmic $Ca^{2+}$ elevation triggered by stimulation. Glucose stimulates biphasic insulin secretion in two phases: a rapidly initiated transient initial phase followed by a sustained second phase [1]. The mechanism of reduced insulin secretion in diabetes is poorly understood, but there is some evidence to suggest defects in beta-cell exocytosis [23–25]. Hence, exploring the mechanism of fusion and recycling of individual vesicles in the beta-cells at the single vesicle level is important in understanding the pathology of diabetes. Compared to single-cell methods such as amperometry and membrane capacitance measurement, fluorescence imaging is noninvasive, provides high spatial resolution, and can be applied to islets as well as single cells. Different high-resolution fluorescence imaging methods are frequently used to visualize insulin secretion [26–31]. For experiments conducted in isolated single cells, insulin granules are labeled either with fluorescent dyes [32] or with genetically encoded probes such as insulin-green fluorescent protein (GFP) [33, 34] or other fluorescent fusion proteins that are targeted to insulin granules [28, 35]. Fluorophores within a thin layer close to the cell surface membrane are selectively excited either under a spinning-disk confocal microscope or a total internal reflection fluorescence microscope (TIRF), and pH-sensitive fluorescence proteins (such as EGFP, pHluorin) are usually fused to the acidic luminal terminal of the vesicle proteins; all of these factors will contribute to fluorescence images with a high signal-to-noise ratio. Using this type of single-cell assay, it has been shown that glucose induces different forms of insulin secretion in beta-cells, such as "full fusion," "kiss and run," and "kiss and stay" [30, 36]. Similarly, using TIRF microscopy to image insulin-secreting INS-1 cells transfected with VAMP2-pHluorin, we observed different types of vesicle fusion evoked by glucose stimulation. As shown in Fig. 1, sudden brightening of fluorescence puncta followed by diffusion into the surrounding membrane is indicative of a full fusion event. However, transient brightening of pHluorin puncta with no subsequent diffusion represents kiss and run, in which the transient opening of vesicle fusion pores prevents diffusion of VAMP2 to the plasma membrane. Finally, a prolonged pHluorin signal resides at the site of vesicle fusion, which is a characteristic of the vesicle fusion pore staying open, but constricted (kiss and stay). Moreover, it has been shown that insulin granules fuse to spatially confined regions of the plasma membrane ("fusion hot spots"), which cannot be detected by conventional electrophysiological methods (Fig. 2) [37]. Therefore, imaging insulin granule fusions in single cells provides novel insights into the well-known GSIS process. In addition to the effects on the total number of vesicle fusion, glucose and other factors may also modulate the spatial profile of the fusion and change the relative

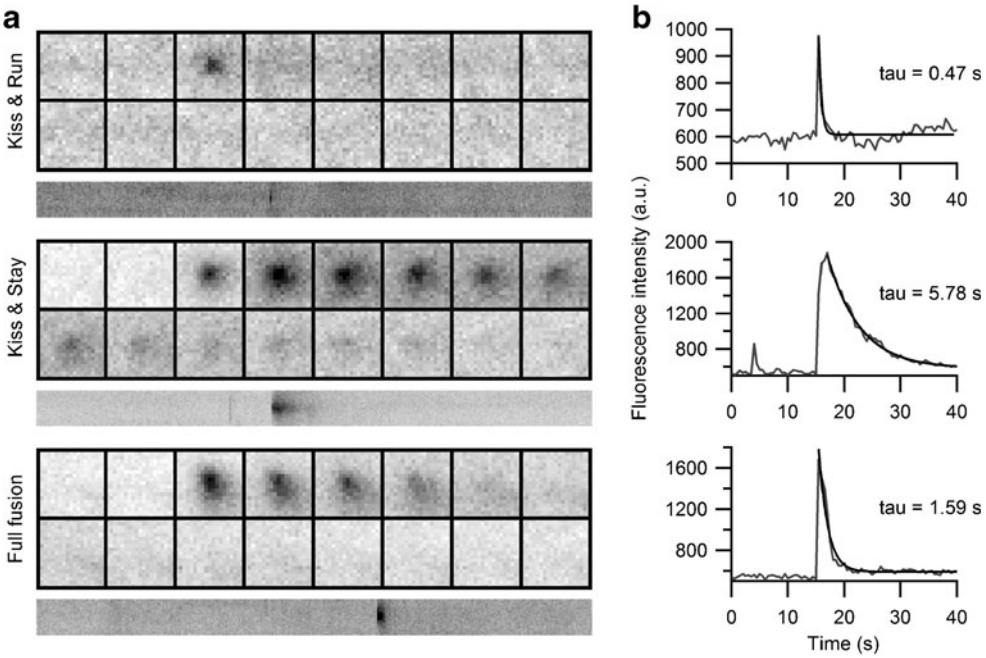


**Fig. 1** TIRF imaging of exocytotic events in INS-1 cells evoked by 20 mM glucose stimulation. **a** Sequential images and a kymograph of a single LDCV labeled with VAMP2-pHluorin undergoing fusion with different modes. Montages show either consecutive images (kiss and run, full fusion; 0.5 s per frame) or every other image (kiss and stay; 1 s per frame). The size of each frame is 19×19 pixels (1 pixel stands for 67 nm). Kymographs show 300 consecutive images (time interval is 0.5 s). **b** Time courses of fluorescence intensity in arbitrary units (*a.u.*; computed from center 5×5 pixels) for the vesicles shown in (**a**) and (**b**)

ratios of different fusion types. All of these will affect the overall time course of GSIS and glucose tolerance.

The genetically tagged fusion protein provided a very specific tool to monitor insulin granule movement and fusion. However, it is possible that the expressed exogenous fusion protein does not faithfully report the fate of the native protein during exocytosis [38]. Under such circumstances, fluorescent dyes can be used to visualize the fusion of insulin granules. FM dyes are lipophilic styryl dyes that reversibly stain but do not permeate membranes; their

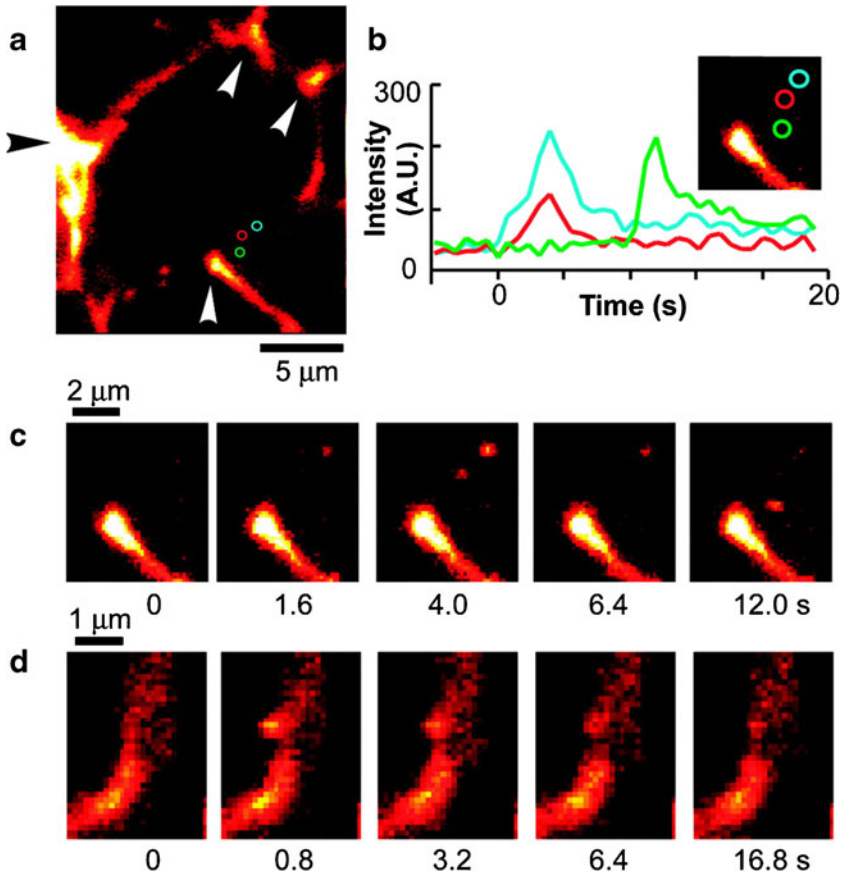


**Fig. 2** Two-photon excitation imaging of exocytotic events in beta-cells within mouse pancreatic islets. **a** A single beta-cell in which SRB fluorescent spots appeared during high glucose stimulation. The *black arrowhead* indicates a major vessel, while the *white arrowheads* indicate microvessels. **b** The time course of the SRB fluorescence (*a.u.*, arbitrary units) measured at the three regions indicated by *circles* in the inset and in (**a**). **c**, **d** Successive images of the glucose-induced abrupt appearance of SRB fluorescent spots. The images in (**c**) were obtained from the region adjacent to the interstitial space shown in (**b**); those in (**d**) show the secretory granule coalescing with the plasma membrane [41]

fluorescence drastically increases upon binding to membranes from aqueous media. Cells can be preincubated with solution containing FM dyes for a prolonged time and then moved to a dye-free solution to wash off dyes on the cell surface membrane. In the end, only the membrane-bound organelles such as vesicles will remain fluorescent. When insulin granules collapse into the plasma membrane, each individual fusion event appears as the diffusion of the fluorescent lipid from the vesicles to the plasma membrane [39]. Using FM1-43 and epifluorescence microscopy, Gaisano's group detected vesicle exocytosis in single primary beta-cells [32]. They observed both single and multi-stepwise increases in membrane FM1-43 fluorescence that represented single vesicle exocytosis and sequential exocytosis.

In the pancreas, the islet exhibits a tight architecture formed by alpha, beta, delta, and PP cells. The release of insulin from beta-cells in situ is subjected to regulation by the gap junction and the release of neurotransmitters and hormones from the neighboring endocrine cells [40]. Therefore, experimental findings obtained from single cell studies cannot be readily applied to intact islets. To study insulin secretion in intact islets, Kasai's group incubated islets with a fluid-phase tracer, sulforhodamine B (SRB), and then imaged the fluorescent probe with intra-islets using two-photon microscopy [41]. In resting islets, the region of bulk fluorescence indicated the major and the microvessels within the islets. Upon perfusion of 20 mM glucose, discrete fluorescent spots abruptly appeared and gradually disappeared adjacent to the plasma membrane of cells within the islet. Later, the authors found that many of the fluorescence puncta colocalized with insulin, which suggested that the appearance of these puncta correlated with insulin exocytosis within the islets. Using this assay, the authors demonstrated that glucose elevation also evoked multiple forms of insulin granule exocytotic events (such as kiss and run, full fusion, and sequential fusion) within intact islets, similar to what has been reported in single cell studies.

In addition to insulin vesicles, pancreatic beta-cells also contain small synaptic-like vesicles [42]. Moreover, other endocrine cells in the islets also have different secretory vesicles [40]. Therefore, it is unclear how specifically the insulin granules within beta-cells can be labeled with nonselective lipophilic dyes or fluorescent dextrans and with how much confidence the appearance and disappearance of one fluorescent punctum can be interpreted as the fusion of one insulin granule. Compared to other types of vesicles and large dense-core vesicles in other endocrine cells, the insulin granule in beta-cells contain excess $Zn^{2+}$, which acts as a structural component to assist insulin packaging [43]. Therefore, perfusion of membrane-impermeable fluorescent $Zn^{2+}$ indicators in the extracellular solution enables visualization of $Zn^{2+}$ that is co-released with insulin, which is more specific than other chemical dyes. Taking advantage of this phenomenon, Darren et al. used the fluorescent $Zn^{2+}$ indicator Rhodzin-3 as a surrogate to measure insulin release. Using TIRF microscopy, they observed single insulin vesicle exocytosis after high-potassium stimulation and found that pancreatic beta-cells secrete insulin in both fast and slow manners (Fig. 3) [44]. Recently, Daliang et al. developed a fluorescent zinc indicator attached to the cell surface to monitor induced exocytotic release (ZIMIR). In this study, ZIMIR imaging of intact islets with confocal or epifluorescence microscopy revealed that insulin release occurred in the secretory unit, which is composed of adjacent

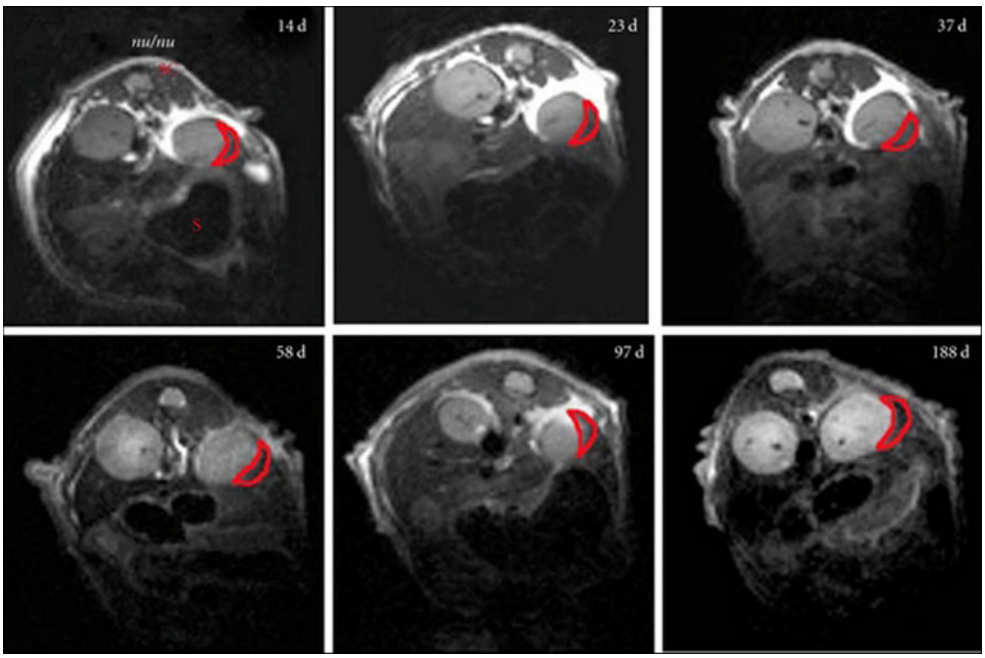


**Fig. 3** In vivo MRI of islet transplantation under the kidney capsule. Transverse T2-weighted magnetic resonance images of transplanted labeled and unlabeled human islets 14, 23, 37, 58, 97, and 188 days after transplantation under the kidney capsule in nude (nu/nu) mice. The *dark area* in the left kidney represents a labeled graft (*red outline*). No darkening was reported for the right kidney with unlabeled graft. *S* stomach; *SC* spinal cord [81]

beta-cells [45], and this observation provides novel insight into the regulation of insulin secretion within islets.

## Molecular imaging beta-cell mass and function in vivo

In addition to visualizing the dynamic insulin secretion process in situ, molecular imaging plays important roles in diabetic-related translational research, such as monitoring endogenous and transplanted pancreatic beta-cell mass and evaluating islet function in vivo. All of these methods may help in early diagnosis and evaluation of the protective effects of therapeutic drugs on functional beta-cell mass.

### Imaging of endogenous pancreatic beta-cells

A sufficient beta-cell mass is important for the maintenance of blood glucose homeostasis. In type I diabetes, beta-cells are selectively destroyed by an autoimmune mechanism; in type II diabetes, a decrease in functional beta-cell mass is reported as a result of prolonged hypoglycemia and hyperlipidemia. At present, pathological staining of fixed pancreas tissue sections is still the well-accepted, gold standard method for measuring beta-cell mass. Because it is a method that can only be used on dead cells, how the beta-cell mass dynamically changes during different stages of the disease is unknown. The correlation between the loss of beta-cell mass and the onset of diabetes can only be indirectly inferred. Hence, the development of noninvasive imaging methods to directly monitor the endogenous beta-cell mass and quantify its function in live animals, and even in human patients, represents the frontier for this field.

One of the main obstacles in beta-cell imaging is the relatively small number of beta-cells compared to the other pancreatic cells. Pancreatic islets are small structures (100–400 μm in diameter) that are dispersed throughout the pancreas, and endogenous beta-cells constitute only 2–3 % of the total cells in pancreatic tissue isolated from healthy animals [46]. Moreover, the pancreas is surrounded by the liver and gastrointestinal system. Therefore, beta-cell imaging requires a probe with a high specificity and signal-to-noise ratio.

One advantage of nuclear imaging is its high sensitivity, which enables use of positron emission tomography (PET) and single-photon emission computed tomography (SPECT) to image endogenous pancreatic beta-cells. The sensitivity of nuclear imaging is dependent on the endogenous expression level of the target protein and the specificity and affinity of the probe. Thus, it is critical to develop radionuclide probes with high contrast and beta-cell specificity.

One such target protein is vesicular monoamine transporter type 2 (VMAT2), which is a monoamine-transporting integral membrane protein expressed in rodent and human pancreatic beta-cells [47]. Dihydrotetrabenazine (DTBZ) specifically binds to VAMT2 [48]. Using [$^{11}$C]DTBZ and PET, Simpson et al. successfully detected a large decrease in the pancreatic uptake of [$^{11}$C]DTBZ in streptozotocin (STZ)-induced diabetic rats [49] and in spontaneous type I diabetic BioBreeding diabetes-prone rats [50] when compared to normal rats. Goland et al. performed the first human trials with [$^{11}$C]DTBZ PET [51]. A reduction in [$^{11}$C]DTBZ uptake was observed in long-standing T1DM patients. However, the binding potential of VMAT2 to pancreas (calculated using the renal cortex as the reference tissue that expresses no VMAT2 protein) was only decreased by 14 %, and the functional VMAT2 binding capacity (defined as VMAT2 binding multiplied by the functional volume of the pancreas) was decreased only by 40 % when compared to the healthy controls; this is in contrast to near complete loss of beta-cell mass in long-standing type I diabetic animal models and human patients [52]. The overestimation of the beta-cell mass may be due to a high level of nonspecific binding of radiolabeled DTBZ in the exocrine pancreas tissue [53–55]. Compared with [$^{11}$C]DTBZ, the newly developed 9-$^{18}$F-fluoropropyl-dihydrotetrabenazine ($^{18}$F-FP-(+)-DTBZ) binds to VMAT2 with high affinity and exhibits a longer physical half-life in vivo [56]. Therefore, Normandin et al. used $^{18}$F-FP-(+)-DTBZ as the PET indicator to image beta-cell mass in healthy controls and type I diabetes patients [57]. The normalized average uptake (calculated as the net tracer uptake in the pancreas divided by injected tracer dose and body weight), total volume of distribution (estimated by kinetic modeling of arterial input functions), and binding potential in the pancreas were reduced by 38, 20, and 40 %, respectively, in the patients.

Radiolabeled antibodies targeted against proteins or lipids that are selectively expressed on the beta-cell membrane are also potential imaging probes. The monoclonal antibody against IC2, a beta-cell specific membrane protein, is a leading candidate [58]. After intravenously administering $^{111}$In-labeled IC2, Moore et al. observed that the accumulation of the probe was higher in normal mice than that of STZ-induced diabetic mice [59]. Next, they established a linear correlation between the accumulation of probe in the pancreas and the beta-cell mass. However, due to its large immunoglobulin size, the clearance of this antibody from the blood vessel proceeded very slowly. A low ratio of radioactive uptake was found in the pancreas when compared to the blood vessel, which limits its application in humans. To overcome such limitations, radiolabeled single-chain antibodies were developed, which showed high uptake in the rat pancreas, fast blood clearance, and a linear correlation between beta-cell mass and probe accumulation [60].

D-mannoheptulose is mainly transported into beta-cells by the GLUT2 transporter expressed on the beta-cell membrane. This is another promising probe for use in imaging

endogenous beta-cells [61]. In vitro experiments showed that tritiated D-mannoheptulose is indeed transported into isolated islets [62, 63]. However, D-mannoheptulose potentially inhibits insulin secretion and increases the blood glucose level, which is undesirable for its application in patients with diabetes [64, 65].

The GLP-1 receptor (GLP-1R) plays an important role in the regulation of glucose homeostasis. This receptor is enriched in islets, especially in beta-cells [66]. Therefore, GLP-1R emerges as another promising target for endogenous beta-cell imaging [67]. GLP-1 is the natural ligand of GLP-1R, and $^{125}$I-labeled GLP-1 exhibited pronounced and specific uptake. However, this probe is rapidly degraded by protease in vivo [68]. Exendin-3 and exendin-4 are more stable agonists of GLP-1R. Therefore, Eri et al. developed [$^{125}$I]BH-exendin(9-39) and injected it into transgenic mice with exogenously expressed GFP in beta-cells; the radioactive signals after injection showed colocalization with the GFP signals [69]. Furthermore, uptake of $^{111}$In-DTPA-Lys$^{40}$-Exendin-3 correlated with the beta-cell mass in a linear manner in alloxan-induced diabetic rats, and the pancreas could be viewed on a dedicated microSPECT scanner [70]. Despite these encouraging results on GLP-1R agonist probes, recent studies indicated that chronic hyperglycemia can lead to the down-regulation of the receptor in rats and humans, which implies underestimation of beta-cell mass in diabetes [71].

In addition to these molecular probes, other ways to radiolabeled beta-cells have also been investigated [72–76]. However, the ideal probe for noninvasive visualization of beta-cell mass in vivo is yet to be determined.

## Imaging of transplanted pancreatic beta-cells

Insulin administration is the regular treatment for type I diabetes. Although it decreases blood glucose levels, it is inadequate in achieving optimal control over a long period of time. This will lead to long-term complications such as retinopathy, nephropathy, neuropathy, and cardiovascular disease [77]. Alternatively, engraftment of exogenous pancreatic islets in patients is a promising clinical modality that can precisely regulate GSIS after food intake. However, insulin independence was only transiently achieved in most recipients; less than 10 % of the patients who received transplantation remain insulin independent for up to 5 years [78]. Hence, developing effective and reliable noninvasive imaging methods to monitor transplantation efficiency and graft survival of transplanted islet cells will help optimize islet transplantation procedures and detect postoperative complications.

As compared to PET and SPECT, magnetic resonance imaging (MRI) provides higher spatial resolution, enhanced soft tissue contrast, and unlimited depth penetration when imaging transplanted islets. However, MRI is relatively less sensitive, and the endogenous MRI signal is not beta-cell specific. These hurdles can be overcome with novel agents that are specifically targeted to beta-cells and provide high contrast in MRI. Two classes of contrast agents are usually used: the positive contrast agents reduce the longitudinal ($T_1$) relaxation time of protons, while the negative contrast ones shorten the transverse ($T_2$) relaxation time of protons. Compared to imaging endogenous pancreatic beta-cells, specifically labeling exogenous cells is more convenient because islets can be isolated, purified, and labeled with exogenous contrast agents before transplantation.

Paramagnetic gadolinium (Gd)-based agents, such as GdHPDO3A, provide hyperintensity (bright spots) in MRI imaging and have been used to monitor transplanted islets [79]. Isolated human pancreatic islets were labeled with GdHPDO3A without compromising the function and viability of labeled islets. Labeled islets transplanted into immune-deficient mice were visible for at least 65 days after transplantation. However, recent findings indicate that prolonged body retention of gadolinium is adverse to human health, which may restrict it from clinical use in patients [80].

In addition to Gd-based agents, superparamagnetic iron oxide (SPIO) nanoparticles with dextran-coated iron oxide cores have also been used to monitor transplanted islets. Iron oxide is absorbed during MRI imaging, resulting in regions of hypointensity (dark spots). A dextran coat can assist the targeting of nanoparticles to biological tissue and improve the biocompatibility of the probe. Moore's group developed SPIO magnetic nanoparticles (MNs) that were conjugated to a near-infrared Cy5.5 fluorescent dye (NIRF) [81]. They transplanted isolated human pancreatic islets that were labeled with MN-NIRF into immune-compromised mice and monitored the in vivo islets mass and function using MRI thereafter. Islets showed no altered viability or function and were visible for up to 188 days after transplantation. However, the liver absorbs large quantities of iron; the high hepatic background signal prevents it from being used as islets indicators for clinical studies. The same group also labeled human islets with ferumoxides and transplanted them into mice [82]. This study demonstrated that not only intrahepatic islet transplantation but also islet rejection could be monitored in mice noninvasively by MRI imaging. Because ferumoxide is an FDA-approved commercially available iron oxide contrast agent, it may facilitate the adoption of this method in clinical practice.

Toso et al. labeled human islets with SPIO ex vivo and subsequently transplanted them into four type I diabetes patients through the hepatic portal vein [83]. MRI imaging was conducted before and after transplantation, in the first in vivo islet imaging study ever performed in humans. In their pre-transplant images, three patients had normal intensity, and iron-labeled islets could be identified as hypointense spots within the liver at the first two posttransplant MRI

**Table 1** Imaging modalities discussed in the context of beta-cell imaging

| Imaging modality | Spatial resolution | Temporal resolution | Depth | In vivo | Main characteristics | Main application |
|---|---|---|---|---|---|---|
| Fluorescence imaging | 10–200 nm | ms to min | <1 cm | No | High spatial resolution, high sensitivity and labeling specificity, limited penetration depth, not clinically applicable | For research in situ and in vitro |
| SPECT/PET | 1–2 mm | s to min | No limit | Yes | Sensitive, noninvasive, ionizing radiation, no anatomical information, suitable for quantification | Imaging of endogenous pancreatic beta-cells |
| MRI | 25–100 μm | min to hr | No limit | Yes | Good spatial resolution, no ionizing radiation, noninvasive, soft tissue contrast, low sensitivity, nonspecific background | Imaging of transplanted pancreatic beta-cells and islet function |

tests. However, spots could not be detected at the third MRI test in one of the patients, possibly due to the concomitant iron therapy on that patient that led to a dramatic increase in the background level of iron within the liver. The fourth patient was excluded from the study because her liver region in MRI images exhibited diffuse and homogenous hypointensity. All patients stopped insulin administration after transplantation. However, no correlation between the number of transplanted islets and the detected hypointense spots was found. This is probably due to the fact that the numbers of hypointense spots counted by different investigators are quite different, which necessitates the use of a more quantitative and objective algorithm in data processing. In summary, the study demonstrated that labeling islets is safe and does not affect their function. However, clinical use of this method is still premature.

## Imaging of islet function

Although both loss of beta-cell mass and reduction in beta-cell function underlie the pathogenesis of both type I and type II diabetes mellitus, partial pancreatectomy in normal animals does not always lead to diabetes [84]. In addition, beta-cell function in response to high glucose is severely compromised well before the clinical diagnosis of type II diabetes [85]. Therefore, developing noninvasive imaging methods to image beta-cell function (such as GSIS) in vivo should provide invaluable tools to study the dynamic process of disease progression and to explore better methods to preserve beta-cell function during islets transplantation.

$Mn^{2+}$ is a very promising $T_1$ contrast agent, and functional beta-cells exhibit a $Mn^{2+}$-enhanced MRI signal following glucose infusion as compared to other cells that are not sensitive to glucose. Based on this principle, Antkowiak et al. recorded time-dependent changes in intensity from the pancreas signal (normalized to the liver) after intravenous administration of glucose or saline to normal and diabetic mice induced by STZ [86]. As compared to the saline administration, glucose stimulation evoked a 51 % increase in the intensity of the pancreas signal plateau in normal mice. In contrast, mice that received a high dose of STZ exhibited a much lower increase in the intensity of the pancreas signal plateau when compared to mice that received a low dose of STZ. Because low or high doses of STZ eliminate beta-cell mass to different extents, this result indicates that $Mn^{2+}$-enhanced MRI imaging can be used to assess beta-cell response to glucose in vivo.

As mentioned above, glucose stimulates co-release of insulin and $Zn^{2+}$ from insulin granules in pancreatic beta-cells. Recently, Angelo et al. developed a gadolinium-based $Zn^{2+}$ sensor, GdDOTA-diBPEN, to noninvasively image beta-cell secretion in vivo using MRI [87]. They injected the $Zn^{2+}$ sensor into the mice's blood vessels. In mice that were also intravenously administered with glucose, the contrast of the MRI images was significantly enhanced in the pancreas region of the mice. In contrast, they did not observe any enhancement of the pancreas region on MRI

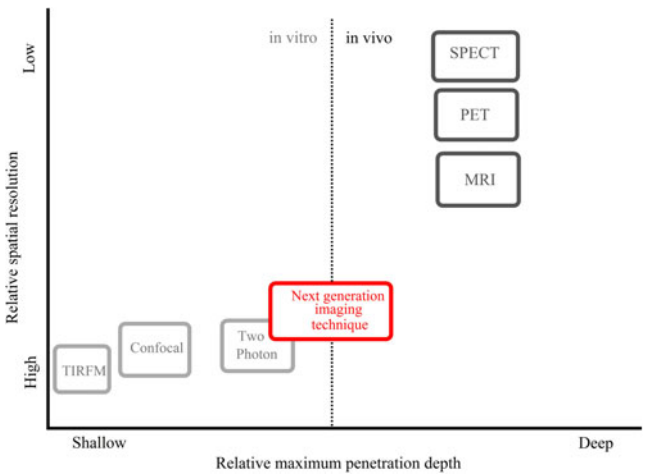


**Fig. 4** Comparison of different imaging techniques. Compared to MRI, PET, and SPECT, spatial resolution of optical imaging is much greater, but its imaging depth is typically limited. This characteristic of optical imaging led to its application being restricted to ex vivo research imaging. Development of imaging techniques that could investigate islets at the cellular level in vivo constitutes the future direction for beta-cell imaging

images in either normal mice without glucose stimulation or STZ-induced diabetic mice with or without glucose stimulation. In addition, a larger volume of contrast-enhanced pancreatic region was identified in mice fed a high-fat diet over a 12-week period, consistent with an increase in beta-cell mass and secretion under such circumstances [88]. Therefore, although current results are still premature, in the future, better sensors and contrast agents will enable MRI imaging to achieve in vivo imaging of glucose stimulated beta-cell responses in patients.

## Concluding remarks

Various methodologies have been used and much progress has been made in imaging beta-cell mass and function both in situ and in vivo. Table 1 summarizes some features of imaging modalities discussed in the context. Their advantages and disadvantages are also outlined in Fig. 4. Because nonoptical imaging methods lack sufficient spatial resolution, subtle processes related to beta-cell function such as insulin secretion are usually visualized with fluorescence imaging techniques (Fig. 4). However, due to the limited penetration depth of optical methods, the mechanism of insulin secretion can only be studied in cultured single cells and islets, in which beta-cells perform their function in the absence of the supporting environment, such as paracrine inputs from pancreatic acinar and endothelial cells and innervations by different nerve terminals. There is no imaging method at present that can monitor insulin secretion from beta-cells in vivo. To achieve that goal, we plan to develop novel miniature two-photon/multi-photon micro-endoscopy that can be used to image islets buried deep within the pancreas in a minimally invasive manner.

Moreover, none of the noninvasive modalities used to image beta-cell mass and function are routinely used in clinical practice; therefore, current methodologies should be developed further. One critical approach for beta-cell imaging is to develop better biomarkers that provide higher specificity and better contrast. Another is to combine different imaging approaches into a multimodality imaging strategy. This will overcome the shortcomings of the individual approaches and provide multiple lines of information on both beta-cell mass and function, which is vital for a comprehensive understanding of the progression of diabetes. These combined efforts hold promise for early diagnosis and prevention of this serious and deadly disease in the future.

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**Conflict of interests** The authors declare that they have no conflict of interests.

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