International Journal of Surgery 69 (2019) 100–107

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.elsevier.com/locate/ijsu

Review

Is the renal subcapsular space the preferred site for clinical porcine islet xenotransplantation? Review article

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ARTICLE INFO

Keywords:
Islet transplantation
Intraportal
Renal subcapsular space
Pancreatic islets
Xenotransplantation

ABSTRACT

It can reasonably be anticipated that, within 5–10 years, islet allotransplantation or pig islet xenotransplantation may be the preferred options for β-cell replacement therapy. The portal vein/liver is currently the preferred clinical site for free islet transplantation, constituting 90% of clinical islet transplants. Despite being the site of choice for rodent and some large animal studies, the renal subcapsular space is rarely used clinically, even though the introduction of islets intraportally is not entirely satisfactory (particularly for pig islet xenotransplantation). We questioned why this might be so. Is it perhaps based on prior clinical evidence, or from experience in nonhuman primates? When we have questioned experts in the field, no definitive answers have been forthcoming. We have therefore reviewed the relevant literature, and still cannot find a convincing reason why the renal subcapsular space has been so relatively abandoned as a site for clinical islet transplantation. Owing to its sequestered environment, subcapsular transplantation might avoid some of the remaining challenges of intraportal transplantation. This may be particularly true when using pig islets for xenotransplantation, which are exceptionally pure in comparison to human islets used in auto- or allo-transplantation. With evidence from the literature, we question the notion that the subcapsular space is inhospitable to islet transplantation and suggest that, when porcine islet transplantation is introduced, this site should perhaps be reconsidered.

1. Introduction

Since its first application in 1966, pancreatic transplantation has remained the 'gold standard' therapy for type 1 diabetes [1]. However, β-cell replacement therapy, including (but not limited to) islet allo- and xeno-transplantation, may prove a valid alternative. It can reasonably be argued that, within 5-10 years, islet allotransplantation or pig islet xenotransplantation may be the preferred option for β-cell replacement therapy [1,2] which is supported by the relative success of recent phase 3 clinical trials [3] and by progress in experimental pig islet xenotransplantation [4–6].

We suggest that 'free' (i.e., not encapsulated or within a device) islet transplantation is ultimately likely to be more successful in controlling diabetes than encapsulated islets [7]. The portal vein/liver is currently the preferred clinical site for free islet transplantation. Because of the relative success of intraportal islet transplantation, perhaps less effort has been made towards exploring the renal subcapsular space than might be expected, particularly in view of its traditional dominance as the site of choice in almost all small animal models and in many large animal models [8,9].

We have questioned many experts why this should be so. As we have not received a convincing explanation, we have searched the literature in the hope of identifying a definitive reason why the renal subcapsular space has been so relatively abandoned as a site for clinical islet transplantation. We here report what we have found. In doing so, we do not advocate for islet transplantation beneath the kidney capsule, but rather attempt to probe for further investigation of the subcapsular space as the potential site of choice, particularly when porcine islet transplantation is introduced clinically.

2. Initial comparisons of intraportal and renal subcapsular islet transplantation

2.1. Autotransplantation

Experiments with *autologous* subcapsular islet transplantation were

Abbreviations: IBMIR, instant blood-mediated inflammatory reaction

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https://doi.org/10.1016/j.ijsu.2019.07.032
Received 12 July 2019; Accepted 27 July 2019
Available online 30 July 2019
1743-9191/

Table 1
Comparisons of Renal Subcapsular and Intraportal Islet Transplantation.

Cellular/Metabolic Considerations					
	Initial vascular supply (O_2 and nutrient diffusion)	Exposure to drugs and toxins	Mimics physiologic insulin release		Comments
Intraportal	+	—	+		Hypoxic apoptosis; Portal drainage of insulin may not be necessary; High initial vascular supply
Subcapsular	—	+	**0**		Hypoxic apoptosis; Diabetic nephropathy may complicate engraftment; Immunosuppressants appear not to affect islet engraftment/function
Immunologic Considerations					
	Challenges of local environment	Immune protection	IBMIR		Comments
Intraportal	—	**0**	—		Hypoxia but superior initial blood supply; Major destruction by IBMIR; Highly active immune system; Microenvironment remains to be explored
Subcapsular	+	+	+		Relatively secluded; Avoids IBMIR
Oxygen Tension and Revascularization					
	O_2 tension of native tissue (compared to 40 mmHg of pancreas)	O_2 tension of transplanted islets	Islet revascularization		Comments
Intraportal	Approx. 40 mmHg	Approx. 5 mmHg	**0**		Revascularization complicated by immunologic hurdles/IBMIR
Subcapsular	Approx. 15 mmHg	Approx. 5 mmHg	+		Poor blood supply may delay revascularization
Evidence of Successful Islet Transplantation					
	Small animal models	Large animal models	Clinical evidence		Comments
Intraportal	—	+	+		Less success in small animal studies; Proven clinical efficacy
Subcapsular	+	+	—		Gold standard in small animal models; Poor initial clinical results; Lacking study since Edmonton protocol
Surgical Procedures and Islet Cell Monitoring					
	Minimally invasive/accessible	Safety	Ability to accommodate islets	Islet monitoring	Comments
Intraportal	+	**0**	+	—	Requires multiple donors; Small risk of portal hypertension/thrombosis
Subcapsular	—	**0**	—	+	Rigid capsule; Potential for composite islet-kidney transplant; Increased transplant density may impair revascularization

+ = Superior; potential advantage. 0 = Neutral; no advantage. — = Inferior; potential disadvantage.
(Modified from references [2,8,9,12,32].)

carried out some years ago with mixed results. Early canine studies had less success in achieving normoglycemia (which has been attributed to poor blood supply and impaired islet revascularization) [10–12], but others demonstrated persistent glucose control and excellent survival [13,14].

In part, the growth of *intraportal* islet transplantation was related to the relative success of Najaran and colleagues in 1980, who demonstrated that islet *auto*transplantation could partially ameliorate diabetes after pancreatectomy [15]. In 1991, Farney et al. compared two patients who underwent subcapsular islet autotransplantation with 22 patients receiving intraportal autologous islet infusions [16]. Both of the subcapsular recipients subsequently required insulin, but nine of the intraportally-transplanted patients remained insulin-free for several months (and in one case for 6 years).

Experimentally, some investigators have since concluded that intraportal islet *auto*transplantation is superior in nonhuman primates [17]. In some respects, the results obtained in pigs [18,19] and nonhuman primates [20,21] provide conflicting data. Limited follow-up [17] might disfavor subcapsular transplantation because the liver is more vascular and might foster initial nutrient supply and survival [9,20]. The effect of total [17,19] versus partial [19–21] pancreatectomy in islet preparation, and the stress on a given transplant mass relative to animal size [19], may also be responsible for varying conclusions.

Still, subcapsular islet transplantation has enjoyed success in both xenogeneic [22] and allogeneic preclinical islet transplantation (see below) [18–21]. However, to our knowledge, no further clinical investigation appears to have been undertaken using the subcapsular space.

2.2. Allotransplantation

Tzakis and colleagues reported the first definitive clinical success of intraportal islet *allo*transplantation in 1990 [23]. In 1998, Jindal et al. compared the two transplant sites [24]. Although two of the three renal subcapsular recipients demonstrated allograft survival and C-peptide secretion, insulin requirements never decreased. Intraportal transplantation demonstrated only transient decreases in insulin requirements. Despite the survival of subcapsular allografts, it has been suggested that the kidney capsule may be inferior because it requires a high transplant mass and might delay neovascularization [8,9,24]. However, a number of factors potentially contributed to the failure to achieve insulin-independence. The authors concluded that it would be prudent to initiate multicenter trials with standardized protocols. This suggestion was not

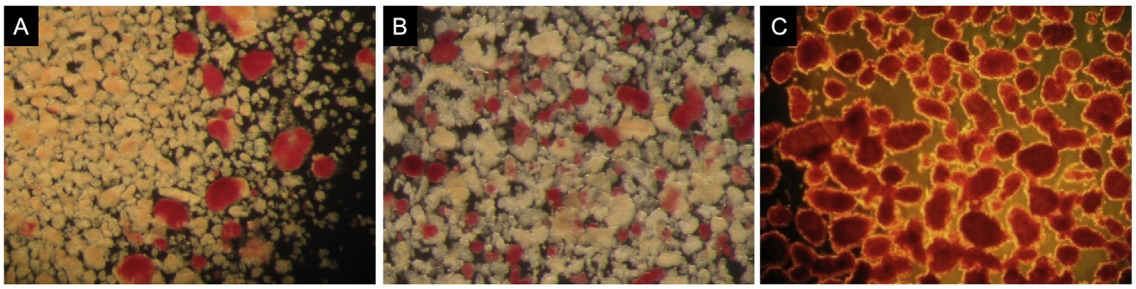

Fig. 1. Contamination of the islet preparation is more prevalent with human islets. (A) Human islet preparation for autologous islet transplantation. (B) Human islet preparation for allogeneic islet transplantation. (C) Porcine islets isolated for xenotransplantation. Reduced purity (% islets/whole tissue) contributes to the increased volume of the cell pellet to be infused. Islets are stained in red with Dithizone. Dithizone-negative cell clusters represent exocrine cells. (Magnification 4x).

pursued.

In 2000, the Edmonton protocol improved the success of intraportal islet transplantation [25]. Perhaps the major features of this protocol that contributed to its success were the transplantation of an adequately large number of islets (often requiring two or three donor pancreases) and steroid-free immunosuppressive therapy to avoid toxicity to islets [26]. However, even the immunosuppressants used in the Edmonton protocol (tacrolimus and rapamycin) may impair islet function, particularly after intraportal transplantation [27–29].

In light of this success, subcapsular islet transplantation became relatively neglected, arguably without just cause. It is important to note that the initial clinical failures (and successes) with subcapsular islet transplantation were carried out before the Edmonton protocol was introduced [16,23–25,28], and no comparable preclinical or clinical trial using the Edmonton protocol has included the subcapsular site [2].

In recent years, approximately 90% of clinical islet transplants have been performed intraportally [8,9]. However, obstacles remain, notably (i) early islet destruction, largely through the instant blood-mediated inflammatory reaction (IBMIR), and (ii) poor or delayed revascularization, thus impairing engraftment and function [9,30,31]. These significant limitations suggest that the subcapsular space warrants re-investigation. Let us consider some of the advantages and disadvantages of the two sites.

3. Advantages and disadvantages of intraportal and renal subcapsular islet transplantation (Table 1)

3.1. Can the subcapsular space accommodate sufficient numbers of islets?

It has been suggested that the kidney capsule of large animals and humans is more rigid and constraining than in rodents, and thus it would be more difficult to transplant sufficient islets under it without their being unduly compressed [8]. However, as mentioned above, the early clinical trials were performed prior to the development of advanced isolation techniques and the introduction of the Edmonton protocol, making comparisons difficult [2,28].

Moreover, in clinical transplantation, the need for higher islet masses (due to losses from IBMIR, inflammation, etc.) drives the decision to implant less pure islet fractions, as purification itself can influence islet yield and function [3,28]. This increases the necessary cell mass transplanted, which includes contaminating exocrine cells that may decrease islet function, and may be too large to be accommodated in the kidney capsule [17].

This limitation might be overcome with approaches that improve purification and reduce the critical islet mass necessary to achieve metabolic control. A positive aspect of xenotransplantation is that the degree of purification of *pig* islet preparations is typically much higher than that obtained in clinical human preparations [4–6,28].

3.2. Islet purity—a potential advantage of subcapsular islet xenotransplantation

The transplantation of islets enriched in exocrine (non-islet) cells under the kidney capsule is associated with a significant degree of failure [33]. Islet masses that are otherwise sufficient to normalize diabetic recipient glucose levels (in the absence of contaminating exocrine cells) usually do not succeed when transplanted beneath the kidney capsule if there is a substantial mixture of exocrine cells (> 30–40% exocrine cell/whole volumes). In contrast, clinical experience with intraportal autologous islet transplantation, and to a good extent with clinical allotransplantation, indicates that the liver allows for successful engraftment of larger islet masses comprised of non-islet (i.e., exocrine) enriched cell preparations [3,28].

Although isolated exocrine cells do not survive in a non-pancreatic environment (and significant damage results from the collagenase-based isolation method), it is likely that their contact with islets, even for just a few days in high-density areas (such as in the kidney capsule), permanently compromises islet viability and function, resulting in islet graft failure. To this effect, islet auto- and allo-transplantation in the subcapsular space is suboptimal in humans. However, owing to a significantly increased purity of islet isolation, this objective consideration is less concerning when applied to the potential clinical transplantation of *porcine* islets (both neonatal and adult).

Pig islets are more fragile than rodent or human islets and are characterized by a less compact islet capsule [34,35]. Lack of a solid islet capsule and their compact morphology renders porcine islets more fragile [34–36]. However, this also allows porcine islets to establish better relationships with the environment, including higher exposure to nutrients and oxygen, which is important for islet engraftment and survival (see below) [37]. Therefore, it is unlikely that, even week-long cultures of porcine islets demonstrate the formation of central necrosis. A pivotal characteristic of porcine islets (compared to human islets) is their ability to better separate from the exocrine cells during post-digestion purification (Fig. 1).

In our experience, as well as that of others [4,5,38], islet isolations from *porcine* pancreases yield high numbers of pure islets (more than 90% islets/whole tissue) following gradient separation. However, similar islet numbers in *human* allotransplantation settings are contained in relatively impure preparations, containing acinar-contaminating cells in more than 50% of the whole tissue infused [3,28]. The high islet purity of *porcine* preparations has made it possible to transplant as many as 300,000 islet equivalents (IEQ) into the liver of 3 kg cynomolgus monkeys, with cell pellet (graft) volumes of only 400–600 μL, in the absence of complications to both the liver and portal vein structures [4,5,38]. Extrapolating these results to *human* islets with exocrine contamination of at least 60–70%, similar IEQs would require volumes of 2–4 mL. Therefore, porcine islet grafts (owing to their improved isolation purity) contain sufficient islet numbers in volumes that are 4- to 8-fold less than human islet grafts. This may offer a considerable

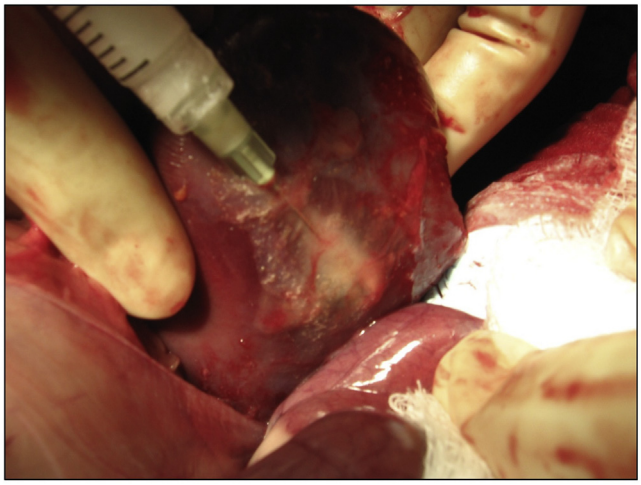

Fig. 2. Pancreatic islet preparation transplanted into the kidney capsule of an adult pig. Injection of a pig pancreatic islet preparation in the kidney capsule of an allogeneic adult pig. A mass of 1 mL of cell pellet was injected under the capsule without causing rupture or bleeding. It is estimated that a mass of 1 mL of purified porcine islets may contain 500,000 to 600,000 islet equivalents (IEQ), whereas a similar mass of human islets (allogeneic or autologous) would be contained in cell volumes ranging from 5 to 15 mL (due to the presence of contaminating acinar cell clusters).

advantage in subcapsular islet transplantation.

In mice, subcapsular islet masses that cover no more than 30% of the kidney surface usually function well. Purity of the islet preparation, rather than volume, is a critical factor for successful engraftment. As such, high-purity porcine islets could be transplanted into 2 or 3 different locations beneath the human kidney capsule.

In this respect, it is possible that the subcapsular transplantation of highly-purified porcine islets may prove successful.

3.3. Surgical procedures and islet cell monitoring

The early risks of intraportal islet infusion have largely been reduced by improved protocols, but some remaining risks could be negated with the subcapsular approach (e.g., biliary puncture, portal vein thrombosis/hypertension) [3,8,9,26,28,32]. Although subcapsular islet transplantation requires open surgery (Fig. 2), future procedural advances may ultimately prove less invasive.

After portal vein islet transplantation, liver biopsies often yield insufficient islets for histologic study, as the islets may have arborized diffusely into the distal vasculature [26,39]. In contrast, the renal subcapsular site is a relatively restricted area, which would almost certainly improve biopsy success for histologic study [2,8,19]. At the very least, this would help to elucidate mechanisms of graft failure and aid in post-transplant islet monitoring.

3.4. Instant blood-mediated inflammatory reaction (IBMIR)

Islet culture and intraportal infusion exposes tissue factor and negatively-charged islets to blood, triggering coagulation pathways and initiating IBMIR (Fig. 3) [30,31,40,41]. The complement cascades are activated, and leukocytes and macrophages are recruited. Early islet destruction and graft dysfunction ensue, which compounds the vulnerability of the hypo-oxygenated portal environment [32]. This, combined with other factors of the liver microenvironment, is responsible for the loss of up to 50% of transplanted islets within just hours to days [26,30]. Despite protocol modifications, IBMIR is yet to be prevented [30,31], particularly after porcine islet xenotransplantation [42,43].

As postulated in some of the earliest clinical experiments, it would be advantageous to transplant the islets into an anatomical site that has minimal exposure to blood, but simultaneously permits nutrient diffusion while awaiting neovascularization [8,9,24]. The subcapsular space could possibly provide such an environment.

Evidence supporting the use of genetically-modified porcine islets to prevent IBMIR is improving [4,42–46]. Combining the greater purity of porcine islets with a potential reduction of IBMIR (by transplanting islets into the sequestered subcapsular space) makes porcine islet xenotransplantation worthy of continued investigation.

3.5. Hypoxia/ischemia

Even if IBMIR is overcome, a period of detrimental hypoxia and ischemia prior to revascularization appears inevitable after both intraportal and subcapsular islet transplantation [9,32,47]. Islet isolation mechanically disrupts the vascular microenvironment, which takes days to weeks to become reestablished. Even then, it is incomplete [9,32]. Delayed revascularization results in nutrient and oxygen deprivation and cell death [9,48,49]. It is therefore advantageous to transplant islets into a site with a relative abundance of oxygen and nutrients, but that is also favorable to revascularization [8,9]. In this respect, neither the portal vein nor subcapsular space is ideal [2].

While subcapsular transplantation may avoid IBMIR, access to oxygen and nutrients is limited. Indeed, many have questioned if these shortcomings may offset the potential advantages of subcapsular sequestration, rendering it inhospitable to early islet survival [8,9]. However, it is clear that a similarly hypo-oxygenated environment occurs after intraportal transplantation.

The subcapsular blood supply is poor compared to the renal and pancreatic parenchyma. The oxygen tension of the kidney capsule is only ~15 mmHg compared to ~40 mmHg in islets in their native environment of the pancreas [32]. The oxygen tension of the liver and portal vasculature is similarly low until revascularization [11,32,47]. Cells at the inner core of islets are particularly susceptible to hypoxia and cell death after transplantation, despite the liver's abundant blood supply [9,12]. Both sites may experience an oxygen tension of just 5 mmHg in the initial post-transplant period [32,47]. However, it is important to note that initial islet hypoxia may be overestimated, particularly in the subcapsular space [50]. Regardless, the initial hypoxic/ischemic insult may not be permanently damaging, as islets experiencing transient hypoxia ($pO_2 < 10$ mmHg) can recover and produce insulin in response to glucose stimulation [50].

These similarities, and the fact that intraportal transplantation has demonstrated relative success, argues that concerns that the kidney capsule is inadequately perfused for islet survival prior to revascularization may be overstated.

The subcapsular space therefore appears to provide adequate (or comparable) oxygenation in the immediate post-transplant period, meanwhile its secluded environment might foster survival by avoiding IBMIR. (The focus of ongoing investigation should perhaps be to identify a location that allows for an ‘immunologically-protected’ environment that also facilitates revascularization [9,24,32].)

3.6. Revascularization

Revascularization after intraportal islet transplantation is, perhaps surprisingly, limited. One-month post-transplant, perfusion of intraportally-transplanted islets is approximately only 5% of native pancreatic islets [37]. Although some question the translatability of small animal models to human islet transplantation (see below), rodent models have demonstrated that subcapsular islets revascularize more quickly than those transplanted intraportally [32,49]. Both at one-day and one-month post-transplant, the rate of apoptosis of intraportally-transplanted islets is twice that of islets transplanted beneath the kidney capsule and of native pancreatic islets [49]. Moreover, even islets that engraft after intraportal infusion demonstrate reduced vascularity and

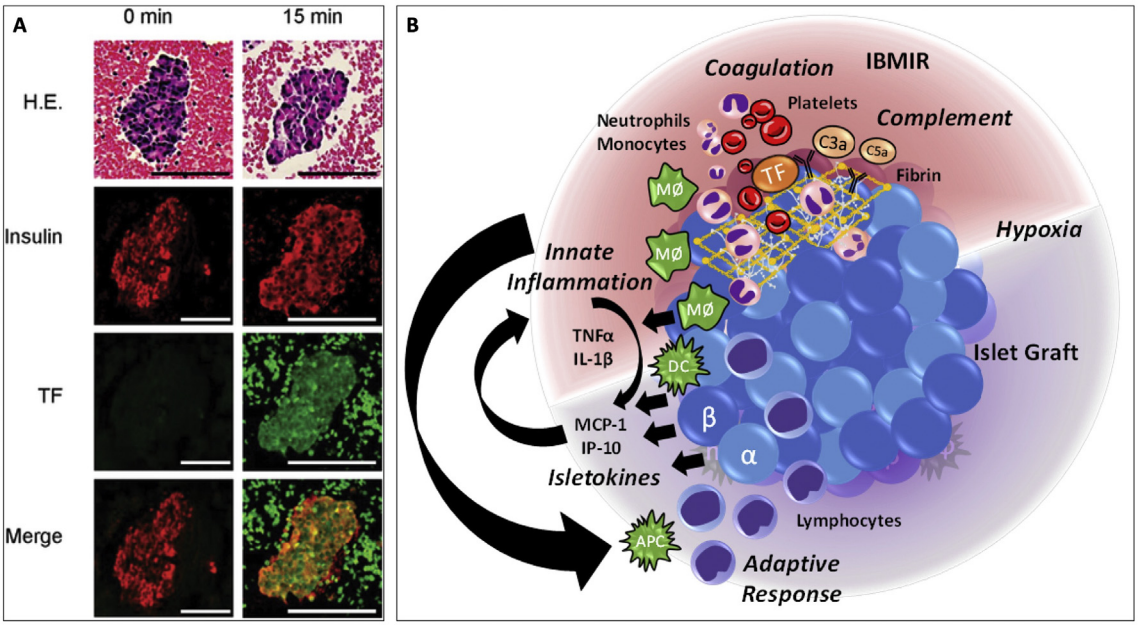

Fig. 3. (A) Tissue factor expression and (B) overview of IBMIR in autologous and allogeneic islet transplantation.
(A) Tissue factor (TF) expression in islets following exposure to *autologous* blood under in vitro conditions. Control islets (0 min) and islets 15 min after exposure to autologous blood were stained with hematoxylin–eosin (H.E.), insulin (red), and TF (green). TF expression as determined by the binding of anti-TF antibody was observed 15 min after exposure to blood. Scale bars show 50 μm (reproduced with permission from Ref. [41]). (B) Factors contributing to acute islet inflammation and cyclical amplification of islet-immune cell reactivity. When isolated and damaged islet tissue is exposed to blood, an acute inflammatory reaction (highlighted in red) consists of a thrombotic 'IBMIR' response, initiating the complement and coagulation cascades. Tissue factor (TF) produced by damaged islets promotes coagulation by attracting platelets and leukocytes to produce a fibrin clot that contributes to islet hypoxia. Stressed islets release cytokines that recruit and activate neutrophils, macrophages (MΦ), and dendritic cells (DC) to the grafting site. Infiltrating and activated immune cells further promote islet inflammation and production of antigen-presenting cells (APC) that activate lymphocytes and induce an adaptive response. These cyclical and amplifying factors contribute to islet-immune cell-mediated graft failure (reproduced with permission from Ref. [31]). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.).

perfusion in comparison to native pancreatic islets [32,49]. In contrast, limited clinical evidence from a single deceased patient after intraportal islet transplantation demonstrated improved capillary networks compared to small animal studies [50], though this observation requires further investigation [9].

Among the factors affecting revascularization (regardless of implantation site) are platelet adhesion and inflammatory infiltrate and, as both occur in IBMIR, are presumably exacerbated after intraportal transplantation [9,31,47]. Moreover, the full effect of the liver microenvironment with respect to revascularization remains to be elucidated [30].

Only recently have investigators been able to successfully quantify islet survival (associated with revascularization), and it would be prudent to further study islet survival after subcapsular placement [37].

3.7. Physiologic considerations

Although insulin is normally secreted directly into the portal vasculature, whole-organ transplantation studies suggest that islet infusion/insulin release into the portal vein may not be necessary when compared to insulin drainage into the systemic venous vasculature [9,12,51].

Subcapsular islet transplantation may actually prove a superior anatomic location by additionally protecting islets from toxic immunosuppressants believed to inhibit β-cell function. Drug concentrations (including those of the Edmonton protocol) can be three-fold higher in the portal vasculature compared to systemic venous levels, which may impair islet revascularization and function [9,27–29]. This is potentially problematic for combined intraportal islet and whole-organ transplantation, where immunosuppressants are often maintained at levels to prevent whole-organ allograft rejection [2]. Although the Edmonton protocol's low-dose immunosuppression was developed specifically for isolated intraportal islet transplantation [25], it remains to be seen clinically if concentrations could be further reduced to limit toxicity while adequately preventing rejection [3,28].

In contrast, Vallabhajosyula et al. demonstrated in pigs that even glucocorticoids (eliminated from the Edmonton protocol) do not adversely affect engraftment and function of autologous islets transplanted beneath the kidney capsule [18]. Although the diabetogenic effects of steroids and the toxicity of various immunosuppressants are known [28], the subcapsular space may offer some protection during initial engraftment and revascularization [18].

It remains to be studied if the Edmonton protocol would have similar success in the subcapsular space. It is possible the immunosuppressive drugs (e.g., tacrolimus, rapamycin) could exacerbate existing diabetic nephropathy, which might affect subcapsular islet engraftment [28]. However, islets transplanted beneath the kidney capsule are resilient to severe renal changes [19].

Therefore, contrary to original opinion that the portal vasculature/liver provides a more favorable environment for islets, the subcapsular space may sequester islets to improve revascularization and successful engraftment.

3.8. Experience in small animal models

The renal subcapsular space is the most common site for islet transplantation in rodent models [8,9,12]. Subcapsular transplantation requires fewer islets and less time to reverse diabetes in small animals, and is largely considered the 'gold standard' for experimental studies [8,9]. The rodent model has been instrumental in improving our understanding of immunologic responses, drug effects, and genetic modifications to facilitate islet engraftment. However, it is argued that these

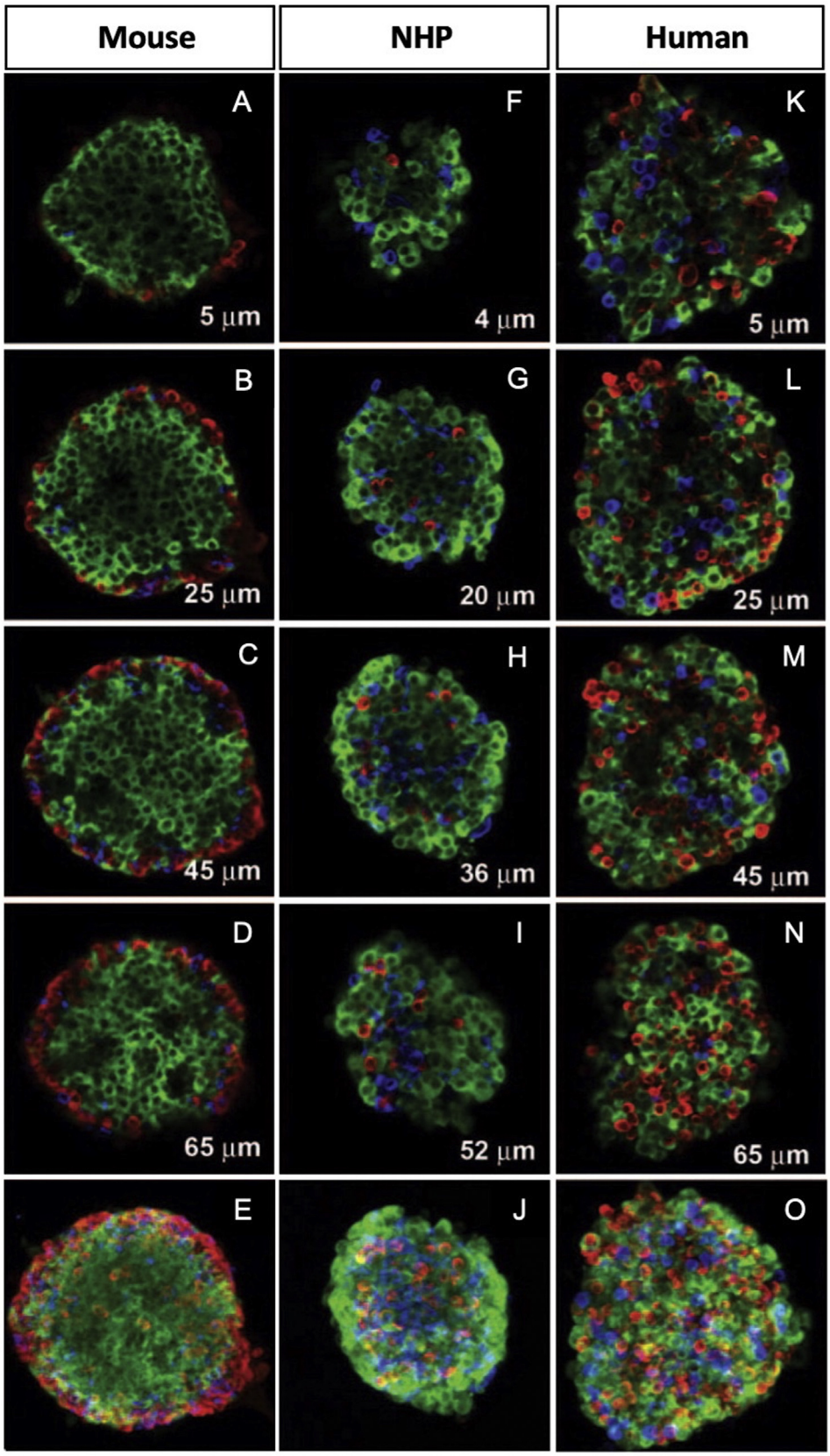

Fig. 4. Species-specific islet architecture. Series of optical sections through an entire isolated mouse islet was acquired at 1μm intervals in the axial (z) dimension. Varying optical section depths are shown. (A-E) **Mouse** islet architecture demonstrates an absence of non β-cells in the islet interior. (F-J) **Nonhuman primate** (NHP) islet architecture demonstrates intermingling of β-cells with α- and δ-cells, similar to human islets. (K-O) **Human** islet architecture demonstrates intermingling of β-calls with α- and δ-cells. The stack of optical sections was 3-D reconstructed and a single 0° projection of the islet with respect to the y-axis is shown in mouse (E), nonhuman primate (J), and human (O) islets (Magnification x40; green = β-cells; red = α-cells; blue = δ cells.) (Modified with permission from Ref. [52].). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

results cannot be extrapolated or translated to larger animal models and clinical studies [8].

Rodent islets are more compact than human islets, and are less fragmented during the isolation process [8]. They embolize in the relatively narrow portal vasculature of mice, resulting in ischemia, hypoxia, hepatic necrosis, and graft failure [8,9]. This may make subcapsular transplantation appear more effective. Consequently, or so it is argued, this makes it difficult to interpret the results from small animal

models to make them relevant to humans [8,9].

However, the compactness and less fragmentation of rodent islets may actually be a disadvantage in the immediate post-transplant period (prior to revascularization) due to diffusion limitations. The cytoarchitecture of islets is species-specific [9,52]. In mice, the insulin-secreting β-cells reside within the core, whereas in pigs, nonhuman primates, and humans, β-cells are more heterogeneously dispersed (Fig. 4) [9,52,53]. As such, it would appear that the insulin-secreting β-cells at the inner core of rodent islets may be more vulnerable to initial hypoxia/ischemia than β-cells in large animal and human islets.

Furthermore, disrupting donor islet integrity is an essential prerequisite for revascularization by hepatic vasculature [37]. If the previous hypothesis is true (that rodent islets are more compact, and thus less fragmented, than human islets) [8], it could be argued that subcapsular islet transplantation in rodent models has demonstrated remarkable success *in spite* of their cytoarchitecture, rather than as a *result* of it.

3.9. Experience in large animal models

In contrast to intraportal/liver islet transplantation, there is a relatively small experience with subcapsular islet transplantation in large animals [18–21] and in clinical studies [16,24,54].

Although it would appear that there have been no autologous/allogeneic human subcapsular studies since the 1990s [16,24], investigations in pigs [18,19] and nonhuman primates [20,21] have enjoyed success. Most experiments have been aimed towards establishing autologous composite islet-kidneys (with a view to subsequent transplantation into allogeneic recipients). The great advantage of this approach is that, when the composite graft is subsequently transplanted into the ultimate recipient, the islets have already become revascularized, thus resulting in less immunologic and inflammatory insult, and therefore less susceptibility to islet graft loss. They function immediately and can more readily provide glucose control than freshly-transplanted islets [20].

Composite islet kidney grafts sustain normoglycemia and renal function in both nonhuman primate and pig models [18–21]. Yamada et al. achieved sustained normoglycemia with composite (*autologous* islet-kidney) grafts transplanted into allogeneic nonhuman primates, but failed to do so after direct *allogeneic* islet transplantation beneath the kidney capsule of the recipient [20]. In the absence of an immune (and possibly inflammatory) response, therefore, engraftment of *autologous* islets in the subcapsular space was facilitated. In contrast, free islets transplanted under an *allogeneic* recipient's kidney capsule did poorly, without effective glycemic control and an absence of insulin-positive cells [20]. This study lends some support to the subcapsular space being a suitable site for clinical studies (if immune and inflammatory responses can be satisfactorily suppressed).

These results suggest two conclusions. First, autologous subcapsular islet transplantation in pigs and nonhuman primates results in successful revascularization. After composite islet-kidney transplantation into a *diabetic* recipient, normoglycemia (and renal function) can be maintained. Second, the subcapsular space provides the islets with the opportunity to become revascularized with limited immunologic damage (from IBMIR), facilitating engraftment and revascularization. If the immunologic/inflammatory barriers can be overcome, then fully allogeneic or xenogeneic subcapsular islet transplantation may indeed prove the preferred site.

Islet transplantation has also been investigated in a xenogeneic model, both intraportally and beneath the kidney capsule [6,22,54]. In the original clinical studies by Groth et al., porcine islets were transplanted under the kidney capsule at the time of human kidney allotransplantation or into the portal vein [54]. Some viable subcapsular islets were detected, but normoglycemia was not restored in any case.

Building on their results demonstrating that porcine islets beneath the kidney capsule of immunosuppressed nonhuman primates avoid rejection and immune responses, in 2003 Rijkelijkhuizen et al. demonstrated that pig islets could survive for up to 53 days in nonhuman primates when immunosuppressive therapy was combined with genetic modification of the pig [22]. Importantly, these results were as good as, if not superior to, those in three animals that also received intraportal islet transplants.

4. Comment

This overview serves as a call for further investigation of the renal subcapsular space as a site for clinical islet transplantation. This anatomic location can avoid early islet destruction from IBMIR, and may possibly foster revascularization. The superior quality of porcine islet purification and continued progress in experimental pig islet xenotransplantation make a particular case for subcapsular *porcine* islet xenotransplantation [4–6]. Although there is as yet no conclusive evidence of one site's superiority over the other, in questioning claims that the subcapsular space is inhospitable to islets, we suggest it is certainly worthy of further investigation.

Ethical Approval

Provided the nature of this review article, no Ethical Approval was required for this manuscript.

Sources of funding

Benjamin Smood was awarded a grant from the American Society of Transplant Surgeons. Work on xenotransplantation at the University of Alabama at Birmingham is supported in part by NIH grant #U19 AI090959/08, and by a grant from United Therapeutics, Silver Spring, MD, USA.

Author contribution

All authors contributed equally to the design and writing of this manuscript. Dr. Rita Bottino provided images for islet purification and subcapsular islet transplantation.

Conflicts of interest

All authors have no conflicts of interest to disclose.

Unique identifying number (UIN)

Name of the registry: Not applicable.

Guarantor

All authors serve as Guarantors and accept full responsibility for the work of this review article and the conduct of the study. All authors had access to the data, and all authors controlled the decision to publish.

Data Statement

Due to the nature of this review article, no data was collected and as such will not be shared. Data not available.

Disclosures

The authors have no conflicts of interest to disclose.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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