Chapter 10
Macroencapsulated Pig Islets Correct Induced Diabetes in Primates up to 6 Months

Pierre Gianello

Abstract A bioartificial pancreas, in which islets of Langerhans are encapsulated within a semipermeable membrane, may be an alternative therapeutic device for diabetic patients. It may constitute another safe and simple method of transplanting islets without the need for immunosuppressive therapy. Since the semipermeable membrane protects the islets from the host immune system, the islets are likely to survive and release insulin for a long period of time, thereby controlling glucose metabolism in the absence of immunosuppressive medication. Recent data using macroencapsulation of pig islets in primate seems encouraging. In fact, a "mono/bilayer" configuration of macroencapsulated pig islets implanted subcutaneously has been found to significantly improve diabetes control in primates for 6 months without any immunosuppression.

Keywords Pig islets • Alginate • Encapsulation • Primates • Subcutaneous

Abbreviations

HACM	Human acellular collagen matrix
IEQ	Islet equivalent
IS	Immunosuppression
RGD	Arginylglycylaspartic acid
SLG	Sterile lyophilized high-guluronate
SLM	Sterile lyophilized high-mannuronate
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
VLDG	Very low density guluronate
VLDM	Very low density mannuronate

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Allogeneic transplantation is today the only successful therapy for several life-threatening diseases. However, organ donation only partially meets the demand and many patients still die while waiting for transplantation. Cellular transplantation represents a very successful tool to treat type 1 diabetes mellitus (T1DM) by transplantation of human islets [1, 2]. Unfortunately, islet allotransplantation suffers from comparable limitations which is even aggravated by the fact that more than one donor is regularly needed to treat one T1DM recipient. Although human stem cells may solve these problems in the future, there are still several major hurdles that preclude their use for clinical applications. Therefore, like in the 60s when, in the absence of dialysis, clinicians referred to xenogeneic (non-human primates) organs to treat human beings (a renal xenograft survived up to 9 months), the scientific community has today reconsidered the possibility of using *porcine cells* to cure specific diseases by xenogeneic cellular transplantation. In fact, (1) pig cells have a stable function and differentiation pattern and are not tumorigenic; (2) pig cells have been shown to meet the physiological needs in large animal models (primates); (3) the source of pig cells can be scaled-up to meet all demands on a highly standardized manner, in the respect of animal welfare rules; (4) Designated Pathogen-Free (DPF) pig lines can be produced and could result in a higher safety profile than allotransplantation itself; (5) the risk of zoonosis, which was raised years ago as the major hurdle, has been recently circumvented and is actually viewed as a controlled risk and (6) the pig insulin has been used during decades for treating T1DM patients since its differs from human insulin (52aa) only at one amino-acid. The use of xenogeneic cells, however, raises a major difficulty which is the need for a heavy systemic immunosuppression (IS). In order to avoid this heavy IS, mechanical immunoprotection has been investigated to be used in preclinical models. An attractive alternative to immunosuppressive drugs is cell immunoisolation by encapsulation in a semipermeable matrix to protect transplanted tissues against immune cells from the recipient as well as against antibodies (autoimmunity of T1DM, ABO/human leukocyte antigen incompatibility, preformed antibodies against α-Gal and other antigens in xenotransplantation).

Macroencapsulation and microencapsulation systems have been proposed for cell immunoisolation [3–9]. However, the lack of biocompatibility [3, 4, 10–14], the nonselective permeability (cytokines, antibodies), the implant degradation, and the limitation of nutrient diffusion are also reported as major causes of encapsulated islet dysfunction [15]. Although several materials have been assessed (agarose, chitosan, copolymers of acetonitrile, AN69, poly(2-hydroxyethyl methacrylate), polyurethane, monomethoxy poly(ethylene glycol), Biodritin) [16–22], alginate is currently one of the major material used in the field of islet transplantation to provide immunoisolation of encapsulated cells [23–27]. This material, extracted from brown alga, is a polysaccharide composed of subunits of mannuronic (M) and guluronic (G) acids. The M/G ratio directly affects physical and biocompatible properties of implants. High-G alginates are more stable and therefore more resistant to mechanical stresses than high-M alginates after implantation [3]. In contrast, a smaller pore size, found in high-M alginates [28], can promote selective permeability for small molecules, avoiding immunoglobulins and immune cells. Alginates of

high viscosity and high content in mannuronic (SLM) or guluronic acids (SLG) are the most commonly reported in the literature. New alginates with coupled peptide sequence (arginylglycylaspartic acid [RGD]) were also assessed to improve encapsulated cell adherence in the matrix [29]. Alginates with a very low density (very low density Mannuronate (VLDM) and very low density Guluronate (VLDG)) were similarly tested to reduce implant size by loading a higher number of islets per volume of polymer. The content in M and G acids as well as alginate viscosity and the use of peptidic sequences [30] may influence biocompatibility [4, 31].

As a first step, there is a need to select an encapsulation material that possesses ideal biocompatible properties for islets encapsulation such as (1) stability during the graft process, (2) immunologic protection (impermeability to molecules >150 kDa such as IgG) coupled with permeability to molecules of low molecular weight such as insulin, glucose, nutrients, and metabolites, and (3) promotion of angiogenesis to allow a sufficient oxygen pressure (pO_2) thereby ensuring encapsulated tissue survival and function. To avoid nonspecific immune response against alginates, each material is characterized by a low level of endotoxin content (<100 EU/g).

10.1 Choice of Encapsulating Alginate

First, it was investigated, in vivo, the biocompatible properties of different chemical alginates and their potential use for islet encapsulation and subcutaneous transplantation in both rat and primate models [23, 32].

Alginates composed of either high mannuronic (SLM) or high guluronic (SLG) content were tested (Tables 10.1 and 10.2). Three subtypes in each group were used: (1) high viscosity (SLM vs. SLG), (2) Very Low Density (VLDM vs. VLDG), and (3) peptide (arginine, glycine, aspartic acid for RGD)-coupled alginate (SLM-RGD vs. SLG-RGD) (Novamatrix, Drammen, Norway). Alginate implants of disc-like shape of about 1–1.5 cm^2 and a thickness of 3–6 mm were subcutaneously implanted in the paravertebral space of Wistar rats. Seven experimental groups of seven rats

Table 10.1 Characteristics of various alginates in vivo

Alginate	% W/V	% M	% G	Coupled peptide	Viscosity (mPa.s)	Endotoxin content (EU/g)
SLM	3	>50	–	No	>100	<100
SLG	3	–	>60	No	>100	<100
SLM RGD	3	>50	–	Yes	>100	<100
SLG RGD	3	–	>60	Yes	>100	<100
VLDM	7	>50	–	No	<20	<100
VLDG	7	–	>60	No	<20	<100
Ctrl+	3	>50	–	No	>100	<100

Table 10.2 Alginate selection: biocompatibility

		SLM	SLG	SLM RGD	SLG RGD	VLDM	VLDG	Ctrl+
Permeability to molecules of	Prior implantation	No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	At each explantation time	No	**Yes**	**/**	**Yes**	**/**	**/**	**/**
150 kDa								
Degradation		No	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Fibrosis		No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Lymphocytes recruitment		+	+	+++	++	++	+++	++++
Macrophages recruitment		+	+	+++	++	+++	+++	++
Angiogenesis		+++	++	+	++	+	+	++
Ideal pO$_2$	pO$_2$ > 12 mmHg	Yes	**Yes**	**No**	**Yes**	**No**	**No**	**No**
	pO$_2$ ~ 40 mmHg	Yes	**No**	**No**	**No**	**No**	**No**	**No**

($n = 49$) were created: one group per alginate type (SLM, SLG, SLM-RGD, SLG-RGD, VLDM, VLDG) and one positive control group. Each animal from the seven experimental groups received two implants, which were placed in small subcutaneous pockets located on each side of the dorsal column. In each group, three rats were sacrificed after 2 weeks and two additional rats were euthanized at 4 weeks after implantation. After 12 weeks, the last four implants were explanted from the remaining rats in each group (14 rats/28 implants).

Alginate implants were weighed before and after implantation to assess the weight recovery and then the percentage of graft recovery. Surrounding tissues and implants (structured and destructured) were taken for investigations. Sections were thereafter routinely colored with silver methenamine (PASM) and Masson's trichrome to assess, respectively, the degree of fibrosis and angiogenesis. Lymphocyte (CD3) and macrophage (CD68) infiltrations were assessed by immunohistochemistry [23]. The numbers of macrophages, lymphocytes, and vessels were quantified histomorphologically. For characterization of the permeability of different alginates, before and after implantation, implants of each alginate were incubated with FITC-coupled lectins of different molecular weights: 36, 75 or 150 kDa [33]. In vivo biocompatibility was characterized by evaluation of graft stability, neoangiogenesis in periphery of implants, recruitment of lymphocytes and macrophages, and assessment of graft permeability to small molecules and to the immune system of the receiver.

Electronic paramagnetic resonance (EPR) oximetry was used to assess evolution in pO_2 inside grafts in vivo up to 4 weeks and to evaluate in vitro a possible gradient of pO_2 inside the SLM3 % grafts. The measurement is based on the oxygen-dependent broadening of the EPR spectrum of a paramagnetic oxygen sensor [34]. The pO_2 inside the alginate implants, placed subcutaneously in rats, was studied up to 4 weeks after transplantation. Paramagnetic carbon was used as the oxygen-sensitive probe. Adding carbon exclusively to alginate implants ensures the graft specificity of the signal measured. EPR spectra were recorded with a modulation amplitude less than one third of the peak-to-peak line width.

Implants were weighed before and after each explantation time to calculate the percentage of weight recovered after implantation (Tables 10.1 and 10.2). Control material was totally degraded 4 weeks after implantation. The percentage of weight recovery >100 % indicated serious fibrosis surrounding Ctrl + (after 2 weeks), SLM-RGD, and VLDG and SLG-RGD (after 12 weeks). Serious implant degradation was also observed for SLM-RGD at 12 weeks (−58 % of graft weight), VLDM from 2 weeks after implantation (−70 %), and VLDG (−52 %). Suitable implant stability, up to 12 weeks after implantation, was observed only for SLM (−27 %) and SLG (−16 %). The weight of the SLG implant, however, decreased significantly from 2 to 4 weeks, whereas the weight recovery of the SLM implant was stable during the complete graft course without a serious fibrosis process.

Angiogenesis is required to allow oxygenation of transplanted tissues. Therefore, angiogenesis was quantified by histomorphologic analysis of tissues surrounding alginate implants (number of vessels/0.16 mm^2) at each explantation time. Angiogenesis surrounding the alginate material was significantly higher in SLM than in other alginates at 2 and 4 weeks after implantation. Although SLG and Ctrl + demonstrated a transient angiogenesis at 2 weeks, it was not maintained at 4 and 12 weeks after implantation. Because the major cause of encapsulated cell death is probably hypoxia, pO_2 was assessed in vivo inside alginate implants at 1, 2, 3, and 4 weeks after implantation. Only SLM, SLG, and SLG-RGD alginates showed a $pO_2 > 10$ mmHg during the 4 weeks of follow-up and only SLM clearly demonstrated a constant and much higher oxygenation (~40 mmHg) during the entire 4-week follow-up.

Low lymphocyte infiltration (<35 lymphocytes/0.16 mm^2) was observed for all experimental alginates at each explantation time. However, a higher degree of lymphocyte infiltration was found at 2 weeks after implantation for SLM-RGD and Ctrl + (22.45 ± 5.85 and 34.55 ± 5.30, respectively, vs. a mean of 5.18 ± 0.61 cells/0.16 mm^2 for other alginates). At 4 and 12 weeks after implantation, VLDM and VLDG, respectively, demonstrated the highest lymphocyte recruitment (16.70 ± 1.46 and 10.10 ± 2.20, respectively, vs. a mean of 6.46 ± 0.68 cells/0.16 mm^2 for other alginates). In contrast, a lower recruitment of CD3+ cells was observed for SLM and SLG at each explantation time (a mean of 4.98 ± 1.09 and 2.42 ± 0.48 cells/0.16 mm^2, respectively). Looking also at macrophage recruitment during the graft process, 2 weeks after implantation, SLM-RGD, VDLM, VDLG, and Ctrl + were characterized by significantly higher macrophage infiltration than that in SLM, SLG, and SLG-RGD. After 4 weeks, CD68+ cell infiltration persisted at a

higher level for VLDM and VLDG than other alginates. At 12 weeks after implantation, VLDG and even SLG-RGD demonstrated a significantly higher infiltration of macrophages than that in SLM. Throughout the whole graft process, SLM showed a constantly low level of macrophage infiltration similar to that in SLG and even SLM-RGD at 4 and 12 weeks.

The permeability of the 6 alginates and control material to lectins of 36, 75, and 150 kDa was tested in vitro before implantation. The 6 alginates and the control material were permeable to small-molecular-weight molecules (36 and 75 kDa^2). In contrast, lectins of 150 kDa could not penetrate SLM alginate, whereas similar lectins penetrated all other tested materials. All alginate devices implanted in rats were explanted after 2, 4, and 12 weeks for permeability testing. Since the permeability assay for lectins requires well-structured alginates, permeability characterization was not performed on SLM-RGD, VLDM, and Ctrl+materials because they lost their structure after 2 weeks.

After explantation, each tested alginate maintained its permeability to molecules of low molecular weight at each explantation time. Only SLM and SLG maintained their permeability to molecules of 75 kDa during the entire graft process. SLM preserved the level of selective permeability to 150 kDa up to 12 weeks after implantation, whereas a significantly higher degree of permeability to such molecular weight molecules was evidenced for SLG and SLG-RGD.

All over, these data suggested to use SLM alginate to micro or macroencpasulate pig islets and evaluate the survival of these islets in vivo in a preclinical model i.e., pig to primate.

10.2   In Vivo Proof of Concept in Pig to Primate Model

As a second step, encapsulated pig islets in high-M alginate were implanted under the kidney capsula and the encapsulated material improved the graft survival (vs non encapsulated islets) after transplantation into several non-diabetic primates. A mean level of 0.14 ± 0.08 ng/ml of porcine C-peptide was detected until day 30 post-transplantation, in the sera of 7 primates. Level of C-peptide was significantly higher than the level obtained in animals receiving non-encapsulated pig islets $(0.03\pm0.02$ ng/ml). Although no porcine C-peptide was detected in primate sera over 90, 135, 180 days post-transplantation, no graft fibrosis, no capsule overgrowth and insulin positive cells were observed. Dithizone positive cells were found inside grafts after 135 and 180 days of transplantation.

Capsules were removed 135 ($n=2$) and 180 ($n=3$) days after transplantation and were incubated in the presence of different concentrations of glucose to assess the function of pig islets from explanted capsules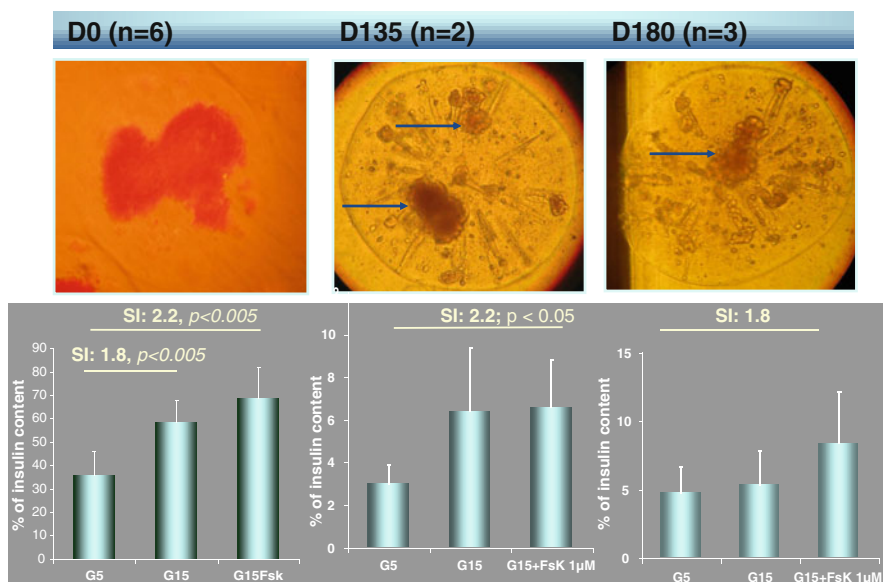. An increase in insulin release, after exposure to glucose 15 mM supplemented with Forskolin, was observed for pig encapsulated islets removed at day 135: 6.6 ± 2.3 % vs. 2.9 ± 0.9 % of insulin content for glucose 15 mM+Fsk 1 µM vs. glucose 5 mM ($p=0.028$, $n=2$). The mean SI was calculated at 2.2 (range 2.0 – 2.7) (Fig. 10.1).

PIG ISLETS ENCAPSULATION

Fig. 10.1 An increase in insulin release, after exposure to glucose 15 mM supplemented with Forskolin, was observed for pig encapsulated islets removed at day 135 and 180 days of transplantation ($p<0.005$) as compared to those extracted from capsules prior to transplantation ($p<0.005$)

However, a significant decrease in insulin content was observed in capsules explanted from primates after 135 (2.2 ± 1.9 ng/islet) and 180 (1.1 ± 1.0 ng/islet) days of transplantation ($p<0.005$) as compared to those extracted from capsules prior to transplantation (32.2 ± 24.3 ng/islet) for capsules containing a mean of 2–3 pig islet cells ($p<0.005$).

In all primates, the presence of anti-pig antibodies (IgM and IgG) was detected prior to transplantation thereby confirming the presence of preformed anti-pig antibodies. No increase in IgM or IgG anti-pig antibodies was found in the sera of primates transplanted with empty capsules. In contrast, when primates were given non-encapsulated pig islets ($n=2$) the level of anti-pig IgM and IgG antibodies was strongly increased, therefore suggesting the sensitization by pig proteins or glycoproteins.

The first aim of this second step was to demonstrate the biocompatibility of encapsulated pig islets for long-term (6 months) in primates and overall, these data suggest that encapsulated pig islets must be embedded in very pure alginate, cultivated for 18 or 24 h in serum-free medium containing a concentration of 1.8 mM of CaCl$_2$. In addition, the ratio of well formed capsules must be over 90 % to obtain a long term in vivo biocompatibility in the pig to primate model.

Although the survival of encapsulated pig islets in diabetic monkeys was reported 9 years ago but never confirmed by others teams [35], there is one recent and casuistic manuscript describing biocompatibility of alginate/polyornithine/alginate microcapsules after 8 weeks of implantation into non-diabetic primate [36]. The present experimental work in vivo clearly demonstrated that implantation of optimised capsules might improve pig islet survival into primates without immunosuppression for up to 6 months in the most stringent xenogeneic pig to primate model without any immunosuppression.

Some of the pig islets survived long-term despite a strong humoral anti-pig immune response. In fact, all the primates used in this study had preformed anti-pig antibodies of both IgM and IgG types. Despite the encapsulation, all primates developed an elicited anti-pig immune response as evidenced by the significant shift of both anti-pig IgM and mainly IgG antibodies by Flow Cytometry. Despite this antibody production, no rejection or fibrosis was evidenced thereby demonstrating the immune protection of the pig islets by the capsules [37]. The immunization against pig proteins could be the consequence of a small percentage of pig islets not being encapsulated or simply prove that pig proteins might get out of the capsules [38], such as porcine C peptide [39].

10.3 Macroencapsulation of Pig Islets Can Control a Diabetes In Vivo up to 6 Months

To confirm these data in non-diabetic primates and evaluate how much these encapsulated pig islets could control a diabetes in the same preclinical model, it was crucial to use diabetic monkeys and to modify the graft now being designed as a mono/bi-layer graft to improve the oxygenation of beta cells (Fig. 10.2) and therefore avoid any lack of Oxygen diffusion.

Fig. 10.2 The collagenic support (HACM) is covered by mono/bilayer of pig islets and embedded both size with SLM alginate 3 % to be implanted subcutaneously in in vivo models
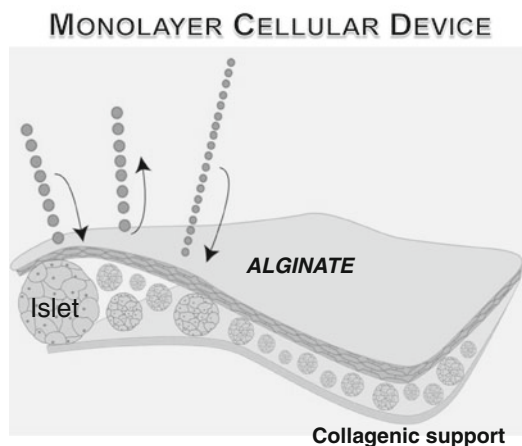
After Streptozotocin treatment and prior to transplantation, six animals displayed clinical features of diabetes including polyuria, polydipsia, weight loss (-29 ± 13 % of initial weight prior to diabetes induction), persistent fasting hyperglycemia (271 ± 92 mg/dl), glycosuria (>1,000 mg/dl), and elevated glycosylated hemoglobin (>13 %). The absence of endogenous production of insulin was confirmed by an abnormal intravenous glucose tolerance test (IVGTT). When the animals were sacrificed, 94 % of beta cell mass in the native primate pancreas had been destroyed by streptozotocin (STZ).

Recipients of empty capsules (sham animals) showed no correction of diabetes. After transplantation of nonencapsulated pig islets under the kidney capsule (KC) of two primates, a peak in the porcine C-peptide level was observed 1 h after transplantation (range 2.438–6.525 ng/ml). The C-peptide level, however, was below the detection threshold (<0.1 ng/ml) 7 days after transplantation.

In addition, three to five monolayer cellular devices (MCDs formed of a collagenic support and embedded into alginate) were implanted in each primate's abdominal subcutaneous tissue containing a mean of 50,000 adult pig islet equivalents (IEQs) seeded on a 1-cm² human acellular collagen matrix and embedded in alginate 3 % w/v. A total amount of 30,000 IEQ/kg per primate was delivered. After MCD implantation, the diabetes was completely corrected for 20, 20, 23, 24, and 28 weeks (Fig. 10.3). Average FBG was 94 ± 11 mg/dl; basal levels of porcine C-peptide were detected (0.362 ± 0.392 ng/ml in fasted state); glycosuria, polyuria, and polydipsia disappeared; and body weight increased (+8.2 % of initial body

Pig Xenograft in diabetic primates

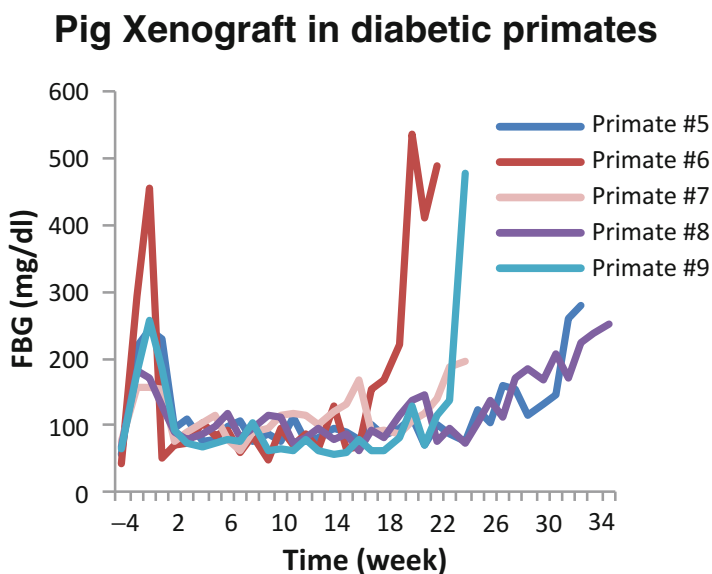Primate #5
Primate #6
Primate #7
Primate #8
Primate #9

Fig. 10.3 FBG course in primates after diabetes induction by STZ and implantation of subcutaneous patches. Correction of FBG was obtained up to 6 months in some primates

weight). The control of diabetes was highlighted by correction of HbA1C, which normalized (<7 %) in primates 5, 8, and 9 up to 16 weeks after implantation. Although all transplanted primates had a decrease in HbA1C, primates 6 and 7 did not show a normal HbA1C <7 %.

Function of islets encapsulated in MCDs was assessed by IVGTT 12, 14, and 16 weeks after grafting in three animals. Whereas diabetic monkeys were unable to manage a glucose challenge after STZ treatment (insulin sera levels <1.5 µU/ml during IVGTT course), MCD implantation allowed normalization of the glucose course during IVGTT with six times more insulin release on average. In addition, the peak level of porcine C-peptide was measured in the primate sera. When total graft dysfunction was observed at 24 weeks post-transplantation (HbA1C >13 %), an assay again demonstrated a pathological arginine level. All implants were removed when diabetes completely reappeared, as indicated by elevated FBG, body weight lost (−24 %), and HbA1C >13 %.

Two animals underwent a second implant with fresh MCDs after failure of the first graft. After total dysfunction of the primary implants, the diabetic state was confirmed by an elevation of HbA1C at >13 and 12.9 % for primates 5 and 8, respectively. Secondary MCDs were placed in the same subcutaneous pouch as the first implants, and diabetes was then completely controlled again for an additional 16 and 20 weeks, as shown by normal FBG (91 ± 21 mg/dl and 68 ± 11 mg/dl), decreased HbA1C (9.6 and 7.4 %), and basal level of porcine C-peptide production (mean of 0.22 and 0.16 ng/ml) for primates 5 and 8, respectively. After this period of graft function, all signs of diabetes re-appeared with an elevation of HbA1C at 13 % but without any graft destruction.

Histologic examination revealed no alginate degradation and lower CD3 (64.4 ± 45.9 vs. 215.9 ± 15.5 cells/mm^2, $P < 0.005$) and CD68 (126.3 ± 23.1 vs. 496.2 ± 61.8 cells/mm^2, $P < 0.005$) infiltration for explanted MCD versus free pig islets (Ctrl+ at day 7 after transplantation). No C3d/C9 deposition and some insulin-positive cells seeded between the human acellular collagen and alginate matrix were found in MCDs after total graft dysfunction.

Similarly to non-diabetic primates, in all animals receiving MCDs the presence of anti-pig antibodies (IgM and IgG) was detected before transplantation, and an increased level of anti-pig IgG antibodies after one, two and 6 months. These anti-pig antibodies were mainly directed against the Gal epitope and were highly cytotoxic.

Although the second transplant succeeded for primates 5 and 8, anti-pig antibodies again increased at 6 weeks after retransplantation with MCD. These newly induced antibodies were specific for the Gal epitope and highly cytotoxic. As observed for primary grafting, these secondary induced antibodies decreased during a long time course of transplantation.

The aim of this third study was to prove the concept of a subcutaneous macrodevice by demonstrating that encapsulated porcine islets can control diabetes up to 6 months after implantation into the most stringent xenogeneic model and without immunosuppression.

The long-term survival of the macroencapsulated graft in this work can be attributed to two major factors: (1) the metabolic activity of the MCD device in the

subcutaneous tissue and (2) the selective permeability of the alginate against anti-pig antibodies.

The MCD was designed with a monolayer deposition of islets to provide biological support for the pig islets; immunoprotection was provided by alginate. The human acellular collagen matrix (HACM) used for islet support is a human decellularized collagen tissue. The freeze-dried structure of the HACM promotes islet adhesion and can improve the number of islets seeded per graft. A mean of 50,000 IEQ can be placed per 1 cm^2 of HACM if the purity of the islet preparation corresponds to a volume of 200 μl of the cellular pellet. Many improvements of our porcine islet isolation method have resulted in >85 % purity of the endocrine tissue, which avoids exocrine contamination [40] and positively affects duration of encapsulated graft function [41].

The most relevant factors for the implantation site for encapsulated islets are (1) physical and chemical stability of the graft after transplantation and (2) metabolic compatibility between the site and transplanted islets to control diabetes. The biocompatibility of the alginate capsules placed in subcutaneous tissue was confirmed in primates (up to 120 days post-implantation, data not shown) prior to testing MCD implantation [42]. The metabolic properties were determined by the response of encapsulated pig islets in MCDs to in vivo glucose and arginine stimulation. Although the subcutaneous tissue can be considered to have a lower physiological effect on insulin compared with portal drainage after transplantation into the liver, similar glucose courses were obtained in non-diabetic and transplanted states for primates. In addition, it was demonstrated that subcutaneous tissue allows a sufficient oxygen tension for survival of encapsulated islets MCD [43].

Although adult beta cells express a low level of Gal epitope (5.1 % of adult pig beta cells) [44–47] we confirm that Gal expression can persist after the isolation procedure (on endothelial cells) [23, 48], and therefore remains a target for humoral xenorejection against free pig islet xenotransplantation in humans and nonhuman primates. In contrast to immunosuppressed primate recipients in which no antibody response was elicited [49, 50], a high level of cytotoxic anti-Gal antibody was found in the sera of primates given transplants of encapsulated pig islets without immunosuppression. Therefore, the material for encapsulation must possess selective permeability for nutrients while preventing passage of immune cells and anti-Gal antibodies associated with pig islet xenotransplantation. The alginate 3 % w/v (used for MCD) demonstrated the selective permeability necessary to avoid the passage of IgG (150 kDa) prior and after transplantation.

10.4   Conclusion

Macroencapsulated adult pig islets transplanted into the subcutaneous tissue of diabetic cynomolgus monkeys (1) sustain long-term function without immunosuppression when placed on a collagen support with a monolayer deposition, (2) can treat diabetes with HbA1C correction <7 %, (3) can metabolically control the glucose

course with an acute stimulation, and (4) are easy to transplant and retransplant into the subcutaneous space, which is a clinically applicable site involving a low-invasion procedure. Following the guidelines recently reported by Cooper and Casu [51], these data show that it is possible to meet International Xenotransplanatation Association (IXA) guidelines for a clinical pilot study. Following the properties of alginate 3 % w/v, the MCD failure at 6 months could be attributed to the lifespan of adult pig islets.

Now, SPF pigs, low in PERV needs to be selected to serve as a source of pig islets into human pilot studies.

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