

Diabetes Research Institute¹, Miller School of Medicine, University of Miami, Miami, FL, Departments of Molecular and Cellular Pharmacology², Biomedical Engineering⁴, Medicine⁵, Microbiology and Immunology⁶, and DeWitt-Daughtry Department of Surgery⁷, University of Miami, Miami, FL, Center for Drug Discovery³, College of Pharmacy, University of Florida, Gainesville, FL, USA.

Feasibility of localized immunosuppression: 1. Exploratory studies with glucocorticoids in a biohybrid device designed for cell transplantation

P. BUCHWALD^{1,2}, N. BOCCA¹, S. MARZORATI¹, G. HOCHHAUS³, N. BODOR³, C. STABLER^{1,4,7}, N. S. KENYON^{1,4,7}, L. INVERARDI^{1,5,6}, R. D. MOLANO¹, C. RICORDI^{1,4-7}, A. PILEGGI^{1,7}

Received December 6, 2009, accepted February 2, 2010

Prof. Antonello Pileggi, Prof. Peter Buchwald, Diabetes Research Institute, Miller School of Medicine, University of Miami 1450 NW 10 Ave (R-134), Miami, FL 33136, USA
apileggi@med.miami.edu; pbuchwald@med.miami.edu

Pharmazie 65: 421–428 (2010)

doi: 10.1691/ph.2010.0525R

Emerging biotechnologies, such as the use of biohybrid devices for cellular therapies, are showing increasing therapeutic promise for the treatment of various diseases, including type 1 diabetes mellitus. The functionality of such devices could be greatly enhanced if successful localized immunosuppression regimens could be established, since they would eliminate the many otherwise unavoidable side effects of currently used systemic immunosuppressive therapies. The existence of local immune privilege at some specialized tissues, such as the eye, CNS, or pregnant uterus, supports the feasibility of localized immunomodulation, and such an approach is particularly well-suited for cell transplant therapies where all transplanted tissue is localized within a device. Following the success of syngeneic transplantation in a subcutaneous prevascularized device as a bioartificial pancreas in a rodent model, we now report the first results of exploratory *in vivo* islet allograft studies in rats using locally delivered glucocorticoids (dexamethasone phosphate and the soft steroid luteprednol etabonate). Following *in vitro* assessments, *in silico* drug distribution models were used to establish tentative therapeutic dose ranges. Sustained local delivery was achieved via implantable osmotic mini-pumps through a central sprinkler, as well as with a sustained-delivery formulation for luteprednol etabonate using poly(D,L-lactic) acid (PLA) microspheres. Doses delivered locally were approximately hundred-fold smaller than those typically used in systemic treatments. While several solubility, stability, and implantation problems still remain to be addressed, both compounds showed promise in their ability to prolong graft survival after tapering of systemic immunosuppression, compared to control groups.

1. Introduction

1.1. Islet transplantation and biohybrid devices

In type 1 diabetes mellitus (T1DM; juvenile onset or insulin dependent), the insulin producing β -cells of the pancreatic islets are destroyed by an autoimmune process and glycemic metabolism can only be controlled by administration of exogenous insulin. T1DM is characterized by infiltration of the pancreatic islets by immune cells, which after some time destroy the β -cells by T-cell-mediated mechanisms (Faustman and Davis 2009; Green and Flavell 1999). Unfortunately, even with a careful insulin treatment, chronic and degenerative complications, such as retinopathy, nephropathy, neuropathy, atherosclerosis, and lipid disorders, still occur in a considerable fraction of patients with T1DM due to the metabolic abnormalities associated with diabetes. Precise metabolic control, in a manner that cannot be achieved with exogenous insulin, can be attained by the transplantation of pancreatic islets (Mineo et al. 2009). With recent clinical advancements, insulin independence can be consistently attained following the transplantation of an adequate allogeneic islet numbers resulting in clear benefits for patients with brittle diabetes (Mineo et al. 2009). Nevertheless,

there are still important limitations that have to be solved including: the need for large islet numbers (generally requiring more than one donor per recipient); the side effects of the systemic immunosuppression (which is required to avoid graft-rejection); the progressive loss of graft function; and the development of allosensitization (Fig. 1) (Pileggi et al. 2006b; Ricordi et al. 2005) – all of which limit the applicability of islet transplantation to only the most severe cases of brittle T1DM in adults (Mineo et al. 2009; Pileggi et al. 2006b; Ricordi et al. 2005). It is also becoming increasingly clear that the liver, the currently favored site of clinical islet transplantation, does not represent an optimal site for islet cell transplants, and there is an ongoing search for alternative sites (Pileggi et al. 2006b). Hence, our ongoing focus is on developing improved immunosuppressive regimens (Bocca et al. 2008; Pinto et al. 2010; Margolles-Clark et al. 2009; Marzorati et al. 2009a,b.), improving devices and immunoisolation techniques (Fort et al. 2008; Pileggi et al. 2006c), as well as finding superior alternative sites (Berman et al. 2009) for islet transplantation. During the last decades, various extravascular approaches for a bioartificial pancreas have been explored for islet grafts using either immunoisolated (i.e., encapsulated) or non-encapsulated

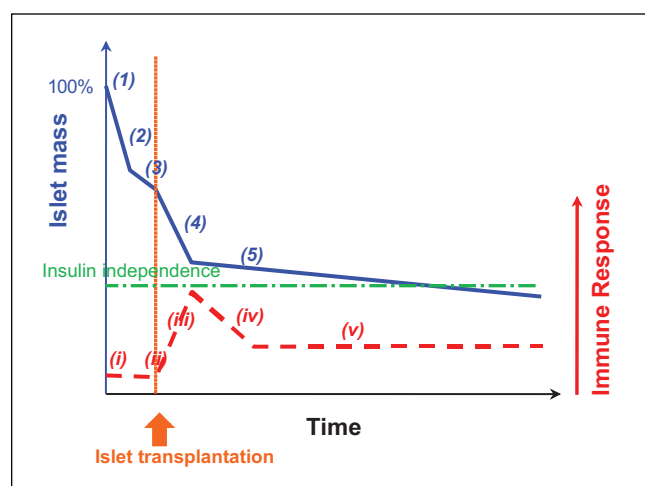


Fig. 1: Schematic illustration of the problems hindering islet transplantation (Ricordi et al. 2005). Islet mass (continuous blue line) is lost due to both pre- and post-transplant events: (1) imperfect organ recovery/preservation, (2, 3) suboptimal islet isolation and culture, (4) immediate post-transplant islet loss, and (5) progressive loss due to the diabetogenic and antiproliferative effects of the immunosuppressive agents used as well as to chronic rejection and recurrence of autoimmunity. Immune response (dashed red line) needs to be controlled with immunosuppressive therapies to prevent graft rejection. The efficacy of immunosuppression needs to take into account (i) graft immunogenicity, (ii) immune activation, (iii) expansion, while, ideally, not preventing (iv) immune contraction and favoring (v) regulatory mechanisms and minimizing memory

islets. They all aimed at providing adequate mechanical protection and sustained graft function while also making possible relatively easy implantation, biopsy, and retrieval (Galletti et al. 2006; Kizilel et al. 2005; Narang and Mahato 2006; Pileggi et al. 2006c). Within the general framework of beta-cell replacement therapies (Pileggi et al. 2006a; Ricordi and Strom 2004), our current focus is on the evaluation of subcutaneous, neovascularized, biohybrid devices (BHD) as possible therapeutic options toward a cure for insulin-requiring diabetes mellitus. In our approach, the main roles of the device are to provide mechanical protection and to allow prevascularization of the site for an improved graft microenvironment. Along these lines, we have shown that the reversal of diabetes and the maintenance of normoglycemia is attainable in chemically-induced diabetic rats by syngeneic islet transplantation in a subcutaneous, neovascularized biohybrid device, which is implanted 40 days prior to islet transplantation to allow embedding by connective tissue and neovascularization (Pileggi et al. 2006c).

Obviously, the true potential of such a device for cell transplantation could only be realized if it can be used for allograft transplantation and the problems associated with the long-term use of immunosuppressive drugs (such as organ toxicity and increased susceptibility toward opportunistic infections) can be avoided. The best possibility would be to reprogram the immune system of the recipient to achieve indefinite graft acceptance and, ultimately, immune tolerance (Ricordi and Strom 2004; Waldmann and Cobbold 2004). Another possibility would be to implement a localized immunosuppression (LIS) regimen. With this strategy, elevated drug concentration levels are limited to a relatively small part of the body, given that the most important immunological events leading to rejection occur in the graft and in its immediate surroundings. Our report herein highlights such an approach could be more easily achieved than in most other transplant cases, since the transplanted tissue is localized entirely within the device. Hence, our hypothesis is that LIS may provide sufficient protection against rejection and allow long-term maintenance of function, while also avoiding the serious systemic side effects commonly associated with sys-

temic immunosuppression. If a locally active therapeutic agent (or combination of therapeutic agents) can be found, then therapeutically active concentration levels need to be maintained only within the device and its surroundings – possibly including the corresponding draining lymph nodes (Reddy et al. 2006, 2007) – by using some form of local delivery.

Certainly, progress taking place in the immunosuppression of various other organ transplants can always be adapted to the field of islet transplantation, thereby resulting in better immunosuppressive regimens (Marzorati et al. 2007; Nanji and Shapiro 2004); however, islet transplantation has unique challenges compared to other transplantations. First, immunological challenges are unique as they are three-fold. First is the combination of both the standard alloimmune attack present in other transplants and the autoimmune component directed toward insulin producing cells. Second is the fact that islet clusters are transplanted as a heterotopic graft, i.e., a graft that is located on a site other than the natural location of the tissue (Narang and Mahato 2006) resulting in a phenomenon known as *anoikis* ('homelessness') (Thomas et al. 1999). Lastly, these islets, once removed from their native highly vascularized and matrix-rich environment, require a period of engraftment and revascularization and, therefore, need to engraft, getting revascularized while retaining their functional potency in the new implantation site (Pileggi et al. 2006b).

1.2. Local immune privilege

One support for the feasibility of LIS is provided by the existence of local immune privilege at some specialized tissues within the body. It has been long known that donor allografts can elicit adaptive immunity of variable potency depending on the site of the transplantation. For example, some specialized tissues such as the eyes, the central nervous system (CNS), the pregnant uterus, and the testes, have been found to possess intrinsic immune privilege (Arck et al. 2008; Niederkorn 2006). The 'immune privilege' term was originally introduced by Medawar in 1948. For a long time, it was assumed to be mainly due to anatomic segregation; however, it is becoming increasingly clear that it is maintained by a combination of anatomical, physiological, and immunoregulatory processes (Arck et al. 2008; Niederkorn 2006). It also requires some localized active suppressive processes and applies to more than the few originally assumed sites. Furthermore, similar processes are also likely exploited by cancerous tumors and chronic infections in order to acquire their unique immune privileges (Mellor and Munn 2008). Hence, the mechanisms involved in this protection are of particular relevance for our goal of achieving long-term function of tissues transplanted into the well confined boundaries of a BHD. The main potential regulatory checkpoints involved in the creation and maintenance of immune privilege in local tissue microenvironments have been summarized as follows (Mellor and Munn 2008): (1) Local inflammatory responses to tissue insults or generation of 'danger' (Matzinger 1994) (as tissue insults generally tend to induce some degree of local inflammation consisting of the influx and activation of immune cells, increased cytokine production, altered cell differentiation and metabolic stress responses, and perhaps unmasking of normally cryptic antigens); (2) Dendritic cell maturation and migration to local draining lymph nodes (to deliver antigens in immune-stimulatory or -suppressive manner following their maturation, which is often mediated by 'danger' signals working through the pathways of innate immunity such as activation of Toll-like receptors and inflammatory cytokine receptors); (3) Antigen presentation and T-cell activation in lymph nodes (a good target to maximize the impact of an intervention as T-cell priming

occurs here and involves small numbers of dendritic-, T-, and, in some settings, T_{reg}-cells); and (4) Regulation of effector cells at tissue sites (as effector immune cells that circulate back to tissue lesions may be prevented from functioning by trafficking barriers or physiologic blockade of T-cell effector functions by regulatory cells or suppressor factors).

1.3. Local immunosuppression – feasibility

The feasibility of LIS, including the use of corticosteroids, has been shown in a number of cases (Alessiani et al. 2000; Gruber 1992; Shirbacheh et al. 1998). Animal studies in the 1980s with prednisolone, methylprednisolone, or budesonide, mainly in renal transplant models, were not unequivocal, but provided some evidence for the feasibility of LIS (Gruber 1992). For example, prednisolone (4 mg/kg/d) and budesonide (BUD; 0.12 mg/kg/d $\approx$ 30 μ g/d) delivered via osmotic mini-pumps in renal (Ruers et al. 1986) and cardiac (Ruers et al. 1988) allotransplant rat models, respectively, showed promising local effects (i.e., prolongation of graft survival) even for these two highly perfused organs. In the locally treated groups, systemic biological side effects were not detectable despite effective drug levels within the grafts.

In one of the most promising studies, not only did methylprednisolone (0.5 mg/kg/d $\approx$ 10 μ g/d) provide effective LIS in a murine sponge matrix allograft model, but the presence of local steroid at the initial graft site also prevented recipient sensitization to the presented alloantigen without keeping them from developing a rejection response to a third-party skin graft (Freise et al. 1991). In other rat models of liver (Weber et al. 1997) and intestinal transplantation (Ozcay et al. 1997), local BUD was found to also have beneficial effects. ‘Local delivery’ to the graft in these studies was achieved due to the large first-pass effect of orally administered BUD, which is metabolized to a great extent while passing through the liver (Ozcay et al. 1997; Weber et al. 1997). Another rat model of intrahepatic islet allotransplantation found that, whereas intraportal delivery of BUD or cyclosporin (CsA) did not prolong graft survival, tacrolimus (TACR) did and was even more effective than intravenous delivery (Wang et al. 1995).

Skin is the most immunogenic component of a composite tissue allograft; however, it is convenient for testing topical efficiency. Topical corticosteroids in combination with CsA have been shown to allow long-term survival in rodent skin allografts (Inceoglu et al. 1994; Shirbacheh et al. 1998). Topical TACR therapy, along with preoperative depletion of T cells with antilymphocyte serum (ALS) and a short course of systemic immunosuppression with CsA, has recently been shown to prevent skin rejection in a rodent hind limb allograft model (Solari et al. 2009). Local ocular immunosuppression with CsA by slow release from a capsule sutured into the vitreal cavity ($\sim$ 2 μ g/day) was effective in prolonging the survival of human fetal retinal pigment epithelium (RPE) xenografts in the rabbit’s subretinal space, in a manner similar to that achieved by intravitreal injections (250 or 500 μ g/wk) (Lai et al. 2000). The efficacy of LIS has also been shown by various groups in different rat models with BUD, CsA, sirolimus (SIR), TACR, 16,16-dimethyl prostaglandin E₂ (PGE₂), anti-T-cell monoclonal antibody (mAb), and 15-deoxyspergualin infused directly into the transplanted organ via implanted osmotic minipumps (Gruber 1992; Shirbacheh et al. 1998). LIS with methylprednisolone and TACR has even been explored in two clinical cases of small bowel transplantation (Furtado et al. 2000).

Even the previously mentioned immune-privileged status of the eye has been attributed to the elevated free hydrocortisone (cortisol) levels observed at this site (estimated at 10 ng/mL $\approx$ 30 nM

in the aqueous humor) due to low local corticosteroid-binding globulin (CBG) concentrations. Such cortisol concentrations seem adequate to suppress immune responses, as they were found to be sufficient to inhibit one-way mixed lymphocyte reactions (MLR) as well as antigen presentation (Knisely et al. 1994). Furthermore, local delivery of a corticosteroid (fluocinolone acetonide, 0.4 μ g/d) to the eye using a slow-release intravitreal implant (Retisert®) has been shown to effectively control intraocular inflammation (Jaffe et al. 2005) and has been approved by the FDA for treatment of chronic non-infectious uveitis – further proof for the potential of achieving local activity by local delivery of corticosteroid (even if the focus here was on anti-inflammatory and not on immune-suppressive activity). For islet transplantation, there might be additional LIS possibilities not available in other cases, for example, due to the additional opportunities offered in the case of encapsulated islets, an often pursued strategy for allo- or xenotransplantation. In a related attempt, the possibility of LIS was explored by tethering anti-tumor necrosis factor- α (TNF- α) antibodies to the surface of alginate/poly-L-ornithine/alginate microcapsules via streptavidin-biotin conjugation (Leung et al. 2008). Notably, the use of anti-TNF- α antibody in recent clinical trials has been associated with improved engraftment and function of transplanted allogeneic islets in patient with T1DM (Alejandro et al. 2008). It might also worth remembering that SIR and TACR, immunosuppressive agents in current clinical islet transplantation protocols, have been shown to have elevated portal vein levels (Desai et al. 2003). Therefore, intraportally transplanted islets are exposed to elevated drug levels, which may be beneficial in providing local immunosuppression or harmful considering their established islet toxicity (Zahr et al. 2007).

1.4. Local immunotoxicity

While LIS might avoid systemic toxic effects, a possible problem is that the highest drug concentrations will be within the device and its immediate surroundings, directly exposing the transplanted cells (e.g., islets) to the potential toxic and diabetogenic effects of some of these agents. Impaired glucose metabolism (Fernandez et al. 1999) and post-transplant diabetes mellitus (First 2003) are common in patients receiving systemically chronic therapy with calcineurin inhibitors (i.e., CsA and TACR). The deleterious effects of systemic administration of calcineurin inhibitors on islet graft function have long been recognized (Alejandro et al. 1989; Alejandro et al. 1988; Ricordi et al. 1992). Systemic immunosuppression and culturing of islet cells *in vitro* with SIR have been associated with impaired islet function and islet cell toxicity (Bell et al. 2003; Marcelli-Tourvieille et al. 2007; Zahr et al. 2007; Zhang et al. 2006). Mycophenolate mofetil and some of the newer, emerging compounds (e.g., fingolimod or leflunomide) seem to have less effect on glucose metabolism at therapeutic doses (Egidi 2005). Administration of systemic glucocorticoids in the peri-transplant period has been associated with the development of islet graft dysfunction (Rilo et al. 1994), and corticosteroids (including DEX) show some inhibition insulin release by islet β -cells (Lambillotte et al. 1997; Pierluissi et al. 1986; Zawulich et al. 2006). Steroids are known to produce whole-body insulin resistance when administered systemically for longer periods and to exacerbate diabetes (Qi and Rodrigues 2007); however, if insulin resistance is mainly due to suppression of glucose transport, this problem might be circumvented via local drug delivery, as much lower levels are present systemically (Pagano et al. 1983; Sakoda et al. 2000). Essentially all currently used systemic immunosuppression regimens have serious negative effects on

the engraftment, function, and survival of transplanted islets, thereby hindering the success of islet implantation (Marzorati et al. 2009a). Hence, a LIS approach can become successful only if (i) locally active agents can be identified, (ii) they are active at concentration levels that are immunosuppressive, but non β -cell toxic, and (iii) the nontrivial problems of delivering and maintaining them locally can be solved (at least until immune tolerance inducing protocols are available).

1.5. Local corticosteroids

Corticosteroids, potent antiinflammatory and immunosuppressive agents, should be a strong first choice as possible LIS agents, since they are commonly utilized in a large variety of clinical diseases. Due to their broad spectrum of activity, they also are the most widely used class of immunosuppressive agents. Here, we report the first exploratory results of *in vivo* islet al-lograft studies in rat BHDs using two locally delivered glucocorticoids: dexamethasone phosphate (DEXP), which was selected on the basis of solubility considerations, and loteprednol etabonate (LE), which was selected on the basis of its safety and localized activity. LE is a soft steroid specifically designed to produce targeted local activity with no systemic side effects due to its prompt metabolic (preferably extrahepatic, *e.g.*, hydrolytic) inactivation (Bodor and Buchwald 2006; Buchwald and Bodor 2004; Druzgala et al. 1991). Soft drugs are new, active therapeutic agents (often isosteric-isoelectronic analogues of a lead compound) with a chemical structure specifically designed to allow for predictable metabolism into inactive metabolites after exerting the desired therapeutic effect(s) (Bodor and Buchwald 2000; Bodor and Buchwald 2008; Buchwald 2007). LE is FDA approved (Noble and Goa 1998) and seems particularly effective in the treatment of various ocular inflammatory conditions (Pavesio and Decory 2008). Both LE and DEX bind to the glucocorticoid receptor with a dissociation constant (K_D) that is in the 5–10 nM range (Buchwald 2008). On the basis of this and the result of previous *in vitro* investigations (Bocca et al. 2008), concentration levels of 5–500 nM (2–250 ng/mL) can serve as a first estimate of a target therapeutic range that could be immunosuppressive, but not significantly β -cell toxic.

2. Investigations, results and discussion

2.1. COMSOL Multiphysics computational drug delivery models

Achieving sustained local drug delivery at a tissue engineered site is a considerable challenge; several possibilities have been considered with only limited success (Saltzman and Olbricht 2002). Using the 5–500 nM range as a tentative therapeutic target for active corticosteroid molecules, we performed a series of geometrically accurate fully 3D finite element method (FEM)-based COMSOL Multiphysics computational simulations (Bocca et al. 2008, 2007). These were built by combining the diffusion/convection and the incompressible Navier-Stokes fluid mechanics application modes of the software, and were used to obtain first estimate of local doses for various possible local delivery methods that could be implemented with our currently used rodent BHD model (Fig. 2). As Fig. 3 shows, the model-predicted flow profiles seem in strong agreement with those measured experimentally for an essentially aqueous fluid with a multi-hole cylindrical sprinkler. Accordingly, models for various delivery possibilities were built (Bocca et al. 2008, 2007); the two corresponding most closely to those used here in the *in vivo* transplantation models are for a cylindrical BHD with a continuous pump-driven infusion through a central 'sprinkler' system and with multiple, randomly

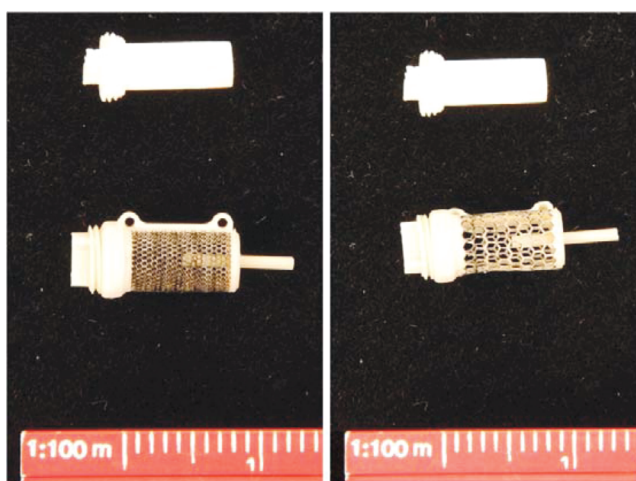


Fig. 2: Prototypes of biohybrid devices used in the rodent studies described here. One end of the PEEK cylindrical mesh can be sealed with a PTFE stoppers. The device is first implanted with a PTFE plunger (top) inserted into the lumen to prevent full in-growth of the recipient tissue. Following a prevascularization period, the plunger is removed and the islet cells are transplanted into the device. These devices intended for use in rodents have an interior 'sprinkler' that can be connected to an infusion port and/or pump for the targeted delivery of drugs in the local implantation site

distributed, sustained-release spherical beads (Fig. 4). These calculations suggested that, as long as no significant local metabolic degradation takes place, a drug delivery rate of approximately 1 nmol/day (corresponding to approximately 0.5 μ g/day) can provide adequate coverage inside the cylindrical chamber for steroid-sized molecules. The multiple spherical bead approach might provide the most uniform coverage, but only if the beads can be uniformly distributed and maintained (Fig. 4). Calculations

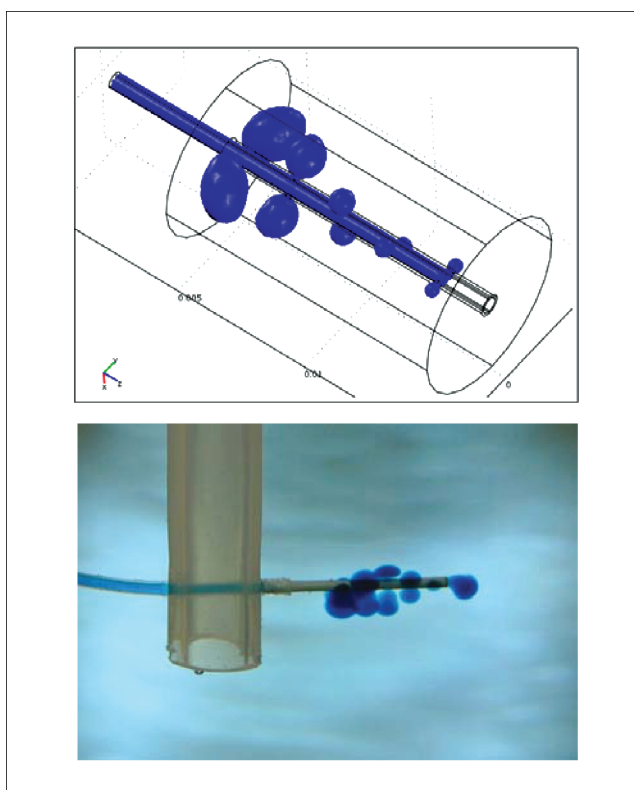


Fig. 3: COMSOL-calculated velocity field isosurfaces at the stationary solution for a central tube ('sprinkler') with five uniform hole-pairs (top) and experimental results (bottom) obtained with an aqueous blue dye for the same tube using an adjustable flow-rate infusion/withdraw syringe pump (Pump 22 Multiple Syringe Pump, Harvard Apparatus)

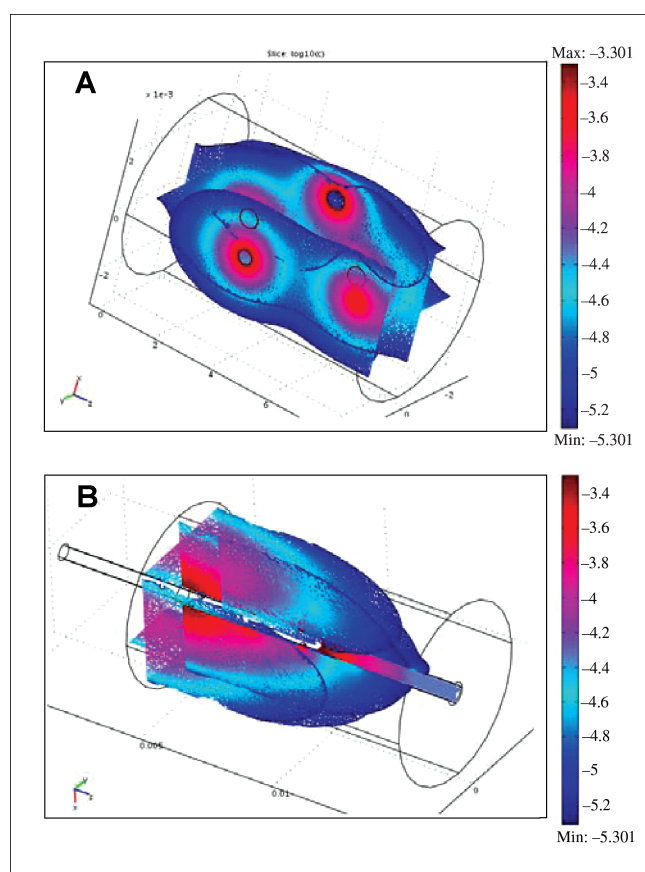


Fig. 4: COMSOL calculated drug concentrations of steroid-sized drugs for (A) zero-order release from multiple randomly distributed slow release spherical beads and for (B) a central sprinkler with multiple holes (flow rate $1 \mu\text{L/h}$, $c_{in} = 40 \mu\text{M}$) both cases corresponding to an approximate delivery rate of $\sim 1 \text{ nmol/day}$ ($0.5 \mu\text{g/day}$). Only the approximate therapeutic range of $5\text{--}500 \text{ nM}$ is colored in the figures with blue corresponding to lower and red to higher concentrations and shown on a logarithmic scale. For comparison, long-time effective local anti-inflammatory activity in the eye could be achieved with fluocinolone acetonide delivered at an estimated rate of $0.3 \mu\text{g/day}$ (0.7 nmol/day) (Retisert[®]) (Jaffe et al. 2005) resulting in concentration levels of around 0.2 ng/g (0.4 nM) in the aqueous humor and 15 ng/g (30 nM) in the vitreous (rabbit eye) (Driot et al. 2004)

lation for the sprinkler infusion required a true multiphysics approach as in this case, the diffusion model had to be coupled to the fluid dynamics model to calculate the velocity field, $\mathbf{u}$, that results from the convection. Results shown here correspond to a realistic case of delivering a solution with a $c = 40 \mu\text{M}$ concentration with a continuous rate of $1 \mu\text{L/h}$, which can be achieved with available osmotic mini-pumps, and results in the estimated 1 nmol/day delivery rate. It has to be emphasized that these local delivery estimates represent a very significant dose reduction compared to a corresponding systemic administration and not just local delivery of an equivalent dose. Compared to a systemic dose of around 0.25 mg/day (1 mg/kg/day ; i.e., $250 \mu\text{g/day/animal}$), which is commonly used for DEX, the local dose estimates calculated here, $0.5\text{--}5.0 \mu\text{g/day}$, represent a 50–500-fold reduction.

2.2. LE sustained release microsphere formulation

Because most potent corticosteroids are quite hydrophobic (Buchwald 2008), the dose deliverable through the infusion of an aqueous solution is limited. As a potential alternative approach to achieve localized and sustained delivery, poly(D,L-lactic acid (PLA) microspheres were prepared and explored for LE (Pinto et al. 2010). PLA microspheres have been used for drug delivery for many years, since they are able to encapsulate and provide

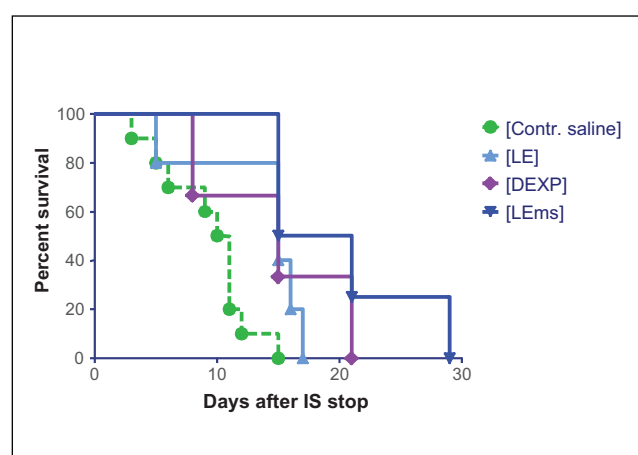


Fig. 5: Actuarial Kaplan-Meier curves showing the proportion of survival of islet allografts after completion of the weaning protocol of systemic immunosuppression. Local delivery of the soft steroid loteprednol etabonate was achieved either using an osmotic mini-pump ($0.25 \mu\text{L/h}$) (LE 0.2, 0.5, and 10 mg/L) or sustained release microspheres (LEMs) using the biodegradable polymer poly(lactic-co-glycolic) acid, PLGA, (4% LE, $\sim 3.0 \mu\text{g/day}$; 4.5 mg loading dose). Dexametazone phosphate (DEXP, 20 mg/L) was delivered with the same mini-pump. All local steroids, but especially the sustained-release microsphere formulation allowed for the extension of allograft survival in a number of cases confirming the potential of this approach even in these early, exploratory rat studies

sustained release of both hydrophilic and lipophilic drugs (Li and Jast 2006). PLA microspheres are of particular interest for targeted drug delivery since they are biocompatible, biodegradable, and can decrease unwanted side effects while maintaining therapeutic effects (Okada and Toguchi 1995). Various microsphere formulations have been prepared (Pinto et al. 2010; Pinto 2008); those used here were prepared by solvent evaporation and had a drug loading of $3.9 \pm 0.2\%$, an estimated mean particle diameter of $5.0 \mu\text{m}$, and provided an *in vitro* drug release duration of approximately three months.

2.3. Exploratory in vivo studies

A first set of exploratory *in vivo* rodent experiments investigating the functionality of allogeneic implants in BHDs with maintenance LIS therapy were performed. Chemically diabetic rats were transplanted with allogeneic islets into prevascularized BHDs, maintained on systemic immunosuppression (ALS induction followed by maintenance on mycophenolic acid) for at least two weeks, and then withdrawn from the systemic immunosuppression and maintained only on the LIS treatment regimen. To assess the function of the islets transplanted into the prevascularized BHD, glucose levels were measured daily. For local delivery, implantable osmotic mini-pumps (Alzet[®], $0.25 \mu\text{L/h}$) were used for both DEXP and LE by connecting it to the central sprinkler of the BHD (Fig. 2). Both the prevascularized BHD and the pump were implanted subcutaneously in the dorsal region of the rodents (Pileggi et al. 2006c), and they were connected via polyethylene tubing. For LE, the PLA microsphere-based sustained-delivery formulation was also explored. Most of these early exploratory experiments were hampered by a number of problems mainly related to solubility and stability limitations, as well as to the implantation of the mini-pump. Nevertheless, both glucocorticoids tested (DEXP and LE) showed some promise. Results of these early *in vivo* tests, obtained while the animal models were still being developed, were somewhat inconsistent because of the mentioned problems; nonetheless, local delivery showed significant prolongation of graft function when compared to control animals that reject the transplant within 6–12 days after tapering of the systemic immunosuppression ($p < 0.05$,

log-rank Mantel-Cox test) (Fig. 5). Because of solubility, stability, and pump-volume limitations, in most cases, we could not reach the desired local dose levels estimated as needed to achieve effective immunosuppression; therefore, alternative delivery routes and increased doses will also be explored in the future.

In conclusion, preliminary studies to evaluate the feasibility of localized immunosuppression with glucocorticoids (dexamethasone phosphate and luteal etabonate) have been performed in a prevascularized, subcutaneous rat biohybrid device model for islet cell transplantation. FEM-based multiphysics computer simulations were used in combination with *in vitro* safety studies to obtain preliminary estimates of local doses that can provide adequate coverage of the biohybrid device and its immediate surroundings. While several solubility, stability, and implantation problems still remain to be addressed, both compounds showed promise as they caused prolongation of the graft survival compared to the control group after tapering of the systemic immunosuppression.

3. Experimental

3.1. *In silico* drug distribution models

Computational models with a finite element method (FEM) (COMSOL Multiphysics 3.4, COMSOL AB, Stockholm, Sweden) were performed as described before (Bocca et al. 2007; Buchwald 2009). Briefly, diffusion was assumed to be governed by the generic diffusion equation in its nonconservative formulation (incompressible fluid):

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R - \mathbf{u} \cdot \nabla c \quad (1)$$

Notation: c concentration of the species of interest [$\text{mol} \cdot \text{m}^{-3}$], D diffusion coefficient [$\text{m}^2 \cdot \text{s}^{-1}$], R reaction rate [$\text{mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}$], $\mathbf{u}$ velocity field [$\text{m} \cdot \text{s}^{-1}$], and ∇ *del* (*nabla*) operator. As boundary conditions, a constant fixed concentration of $c = 0$ was used along the external, cylindrical sides (corresponding to sink conditions at the meshed, vascularized tissue boundaries) and insulation/symmetry, $\mathbf{n} \cdot (-D \nabla c) = 0$, was used along the two plastic-capped ends. Along the drug releasing surfaces, a constant flux, $-\mathbf{n} \cdot (-D \nabla c + c\mathbf{u}) = N_0$, was used as boundary condition with an inlet flux of $3.26 \times 10^{-9} \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (corresponding to a $0.9 \text{ nmol/day} = 0.4 \text{ } \mu\text{g/day}$, total constant flux, *i.e.*, zero-order release). In the diffusion only application, no convection is allowed; *i.e.*, $\mathbf{u}$ is assumed to be 0. In all these models, a diffusion coefficient $D = 6 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$ was assumed (Bocca et al. 2007).

For models with convection (sprinkler infusion), the diffusion model was coupled to the fluid dynamics model to calculate the velocity field $\mathbf{u}$ that results from the convection (Bocca et al. 2007; Buchwald 2009). For fluid dynamics, the incompressible Navier-Stokes model for Newtonian flow (constant viscosity) was used (momentum balance + equation of continuity for incompressible fluids):

$$\begin{aligned} \rho \frac{\partial \mathbf{u}}{\partial t} - \eta \nabla^2 \mathbf{u} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} + \nabla p &= \mathbf{F} \\ \nabla \cdot \mathbf{u} &= 0 \end{aligned} \quad (2)$$

Notation: ρ density [$\text{kg} \cdot \text{m}^{-3}$], η viscosity [$\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1} = \text{Pa} \cdot \text{s}$], p pressure [$\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-2} = \text{Pa}$], and $\mathbf{F}$ volume force [$\text{kg} \cdot \text{m}^{-2} \cdot \text{s}^{-2}$]. As boundary conditions, outflow with zero pressure ($p = p_0 = 0$, $K = 0$) was used along the external cylindrical surface and no slip ($\mathbf{u} = 0$) was used along the capped ends. Along the drug releasing surface, a constant inflow/outflow velocity ($\mathbf{u} = \mathbf{u}_0$) was used as boundary condition with an inlet velocity of $8.85 \times 10^{-8} \text{ m} \cdot \text{s}^{-1}$ (corresponding to an infusion rate of $1 \text{ } \mu\text{L/h}$ and a total dose of $0.9 \text{ nmol/d} = 0.4 \text{ } \mu\text{g/d}$ with an assumed concentration of $37 \text{ } \mu\text{M}$). As a first estimate, an aqueous media at body temperature was assumed in all subdomains (*e.g.*, $T_0 = 310.15 \text{ K}$, $\rho = 993 \text{ kg} \cdot \text{m}^{-3}$, $\eta = 0.7 \times 10^{-3} \text{ Pa} \cdot \text{s}$). Fully scaled 3D geometries were built in COMSOL (device: internal radius $r = 2.15 \text{ mm}$, length $h = 8 \text{ mm}$; sprinkler: external radius $r = 0.29 \text{ mm}$; multiple slow-release spherical beads of $r = 0.2 \text{ mm}$ radius). The geometry was divided in fine mesh elements using COMSOL's default setting, and the problem has been solved for stationary condition on a Dell Precision PC with a 3.2 GHz CPU running Linux. Computations were performed using the Pardiso direct solver.

3.2. *In vivo* islet transplantation

Islet isolation and transplantation using the prevascularized islet biohybrid device was performed at the Preclinical Cell Processing and Translational Model Laboratory of the Diabetes Research Institute (DRI) using the method described previously (Bocca et al. 2009; Marzorati et al. 2009b; Pileggi et al. 2006c). All animal studies were conducted under protocols approved by the Institutional Animal Care Committee. Streptozotocin-induced chemically diabetic Lewis rats were transplanted with Wistar Furth (WF) islets into prevascularized BHDs under the umbrella of systemic immunosuppression based on induction with antilymphocyte serum (ALS, Accurate, Westbury, NY; 1 mL , single intraperitoneal injection on day -3) and maintenance mycophenolic acid (Myfortic[®], Novartis Pharmaceutical Corp., East Hanover, NJ; 20 mg/kg/d from day 0); after at least two weeks of treatment, systemic immunosuppression was tapered and discontinued while the local one was maintained. For the control group, saline solutions loaded mini-pumps were used for localized delivery. For localized delivery of LE with microspheres, a 4.5 mg loading dose of LE-PLA microspheres (sterilized by radiation) was added to the device at the time of islet infusion. On the basis of *in vitro* experiments, the delivery rate was estimated to be approximately 3.0 g/day , based on a 4% drug content released over an approximately 60 day period. Glucose levels were measured on whole blood (OneTouch Ultra2 glucometer, Lifescan, Milpitas, CA) daily for the first week and 2–3 times a week thereafter to assess the function of the transplanted islets. As a first, relatively convenient approach to achieve sustained local delivery, implantable osmotic mini-pumps were used (Alzet[®], DURECT Corp., Cupertino, CA; Model #1002; $100 \text{ } \mu\text{L}$ volume, 2-week continuous infusion, $0.25 \text{ } \mu\text{L/h}$ delivery rate). Both the prevascularized BHD manufactured for Converge Biotech, Inc. (by Biorep[®] Technologies Inc., Miami, FL) and the pump were implanted subcutaneously in the dorsal region of the rodents, and connected via polyethylene tubing. Animals were then maintained on a two- to three-week regimen of systemic immunosuppression, in addition to the localized delivery.

Acknowledgements: Support for these studies obtained from the Juvenile Diabetes Research Foundation International (4-2008-811 and 17-2010-5), the Helmsley Foundation, the Diabetes Research Institute Foundation (www.diabetesresearch.org), and Converge Biotechnologies, Inc (Miami, FL) is gratefully acknowledged. Special thanks to the Diabetes Research Institute personnel of the Preclinical Cell Processing and Translational Model (Elsie Zahr, Judith Molina, Susana Villate, Yelena Gadea, and Irayme Labrada) and of the Histology and Imaging (George McNamara and Kevin Johnson), as well as to Biorep Technologies, Inc (Miami, FL) for their invaluable assistance.

This research paper was partially presented during the 7th Conference on Retrometabolism-Based Drug Design and Targeting, May 10–13, 2007, Orlando, Florida, USA.

References

- Alejandro R, Barton FB, Hering BJ, Wease S (2008) 2008 Update from the Collaborative Islet Transplant Registry Transplantation 86: 1783–8.
- Alejandro R, Feldman EC, Bloom AD, Kenyon NS (1989) Effects of cyclosporin on insulin and C-peptide secretion in healthy beagles. Diabetes 38: 698–703.
- Alejandro R, Latif Z, Polonsky KS, Shienvold FL, Civantos F, Mintz DH (1988) Natural history of multiple intrahepatic canine islet allografts during and following administration of cyclosporine. Transplantation 45: 1036–1044.
- Alessiani M, Regazzi M, Abbiati F, Ferrari P, Molinaro M, Iacona I, Klersy C, Viezzoli A, Fayer F, Pileggi A, Zitelli E, Burroni B, Scandone M, Dionigi P, Zonta A. (2000) Pharmacokinetic study of intraarterial local intestinal infusion of tacrolimus Transplant Proc 32: 1214–1215.
- Arck PC, Gilhar A, Bienenstock J, Paus R (2008) The alchemy of immune privilege explored from a neuroimmunological perspective. Curr Opin Pharmacol 8: 480–489.
- Bell E, Cao X, Moibi JA, Greene SR, Young R, Trucco M, Gao Z, Matschinsky FM, Deng S, Markman JF, Naji A, Wolf BA (2003) Rapamycin has a deleterious effect on MIN-6 cells and rat and human islets. Diabetes 52: 2731–2739.
- Berman DM, O'Neil JJ, Coffey LC, Chaffanjon PC, Kenyon NM, Ruiz P Jr, Pileggi A, Ricordi C, Kenyon NS (2009) Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold. Am J Transplant 9: 91–104.
- Bocca N, Buchwald P, Molano RD, et al. (2009) Biohybrid devices and local immunomodulation as opposed to systemic immunosuppression. In: Hallé JP, de Vos P, Rosenberg L (eds), The Bioartificial Pancreas

- and Other Biohybrid Therapies. Trivandrum, India: Research Signpost, p 309–328.
- Bocca N, Pileggi A, Molano RD, Marzorati S, Wu W, Bodor N, Ricordi C, Buchwald P (2008) Soft corticosteroids for local immunosuppression: exploring the possibility for the use of loteprednol etabonate in islet transplantation. *Pharmazie* 63: 226–232.
- Bocca N, Ricordi C, Kenyon NS, Latta P, Buchwald P (2007) 3-D Multiphysics FEM modeling to optimize local drug delivery in a biohybrid device designed for cell transplant. In: Dravid V (ed), *Proceedings of the COMSOL Conference 2007 Boston*. Boston: COMSOL, Inc., p 101–107.
- Bodor N, Buchwald P (2000) Soft drug design: general principles and recent applications. *Med Res Rev* 20: 58–101.
- Bodor N, Buchwald P (2006) Corticosteroid design for the treatment of asthma: structural insights and the therapeutic potential of soft corticosteroids. *Curr Pharm Des* 12: 3241–3260.
- Bodor N, Buchwald P (2008) Retrometabolic drug design: principles and recent developments. *Pure Appl Chem* 80: 1669–1682.
- Buchwald P (2007) Computer-aided retrometabolic drug design: soft drugs. *Expert Opin Drug Discov* 2: 923–933.
- Buchwald P (2008) Glucocorticoid receptor binding: a biphasic dependence on molecular size as revealed by the bilinear LinBiExp model. *Steroids* 73: 193–208.
- Buchwald P (2009) FEM-based oxygen consumption and cell viability models for avascular pancreatic islets. *Theor Biol Med Model* 6: art. 5.
- Buchwald P, Bodor N (2004) Soft glucocorticoid design: structural elements and physicochemical parameters determining receptor-binding affinity. *Pharmazie* 59: 396–404.
- Buchwald P, Margolles-Clark E, Kenyon NS, Ricordi C (2010) Organic dyes as small molecule protein–protein interaction inhibitors for the CD40-CD154 costimulatory interaction. *J Mol Recognit* 23: 65–73.
- Desai NM, Goss JA, Deng S, Wolf BA, Markmann E, Palanjian M, Shock AP, Feliciano S, Brunicki FC, Barker CF, Naji A, Markmann JF (2003) Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation* 76: 1623–1625.
- Driot JY, Novack GD, Rittenhouse KD, Milazzo C, Pearson PA (2004) Ocular pharmacokinetics of fluocinolone acetonide after Retisert intravitreal implantation in rabbits over a 1-year period. *J. Ocul. Pharmacol. Ther.* 20: 269–275.
- Druzgala P, Hochhaus G, Bodor N (1991) Soft drugs. 10. Blanching activity and receptor binding affinity of a new type of glucocorticoid: loteprednol etabonate. *J. Steroid Biochem.* 38: 149–154.
- Egidi FM (2005) Management of hyperglycaemia after pancreas transplantation: are new immunosuppressants the answer? *Drugs* 65: 153–166.
- Faustman DL, Davis M (2009) The primacy of CD8 T lymphocytes in type 1 diabetes and implications for therapies. *J. Mol. Med.*: ePub.
- Fernandez LA, Lehmann R, Luzi L, Battezzati A, Angelico MC, Ricordi C, Tzakis A, Alejandro R (1999) The effects of maintenance doses of FK506 versus cyclosporin A on glucose and lipid metabolism after orthotopic liver transplantation. *Transplantation* 68: 1532–1541.
- First MR (2003) Posttransplant diabetes mellitus. *Transplantation* 75: 1769.
- Fort A, Fort N, Ricordi C, Stabler CL (2008) Biohybrid devices and encapsulation technologies for engineering a bioartificial pancreas. *Cell Transplant.* 17: 997–1003.
- Freise CE, Clemmings S, Clemens LE, Alan T, Ashby T, Ashby E, Burke EC, Roberts JP, Ascher NL (1991) Demonstration of local immunosuppression with methylprednisolone in the sponge matrix allograft model. *Transplantation* 52: 318–325.
- Furtado A, Perdigoto R, Oliveira F, Geraldes B, Furtado E, Tomé L, Mota O, Ferrão J, Viana J, Bento C, Vieira H, Neves S (2000) Local immunosuppression in clinical small bowel transplantation (report of two cases). *Transplant Proc* 32: 1206–1208.
- Galletti PM, Colton CK, Jaffrin M, Reach G (2006) Artificial pancreas. In: Bronzino JD (ed), *The Biomedical Engineering Handbook. Tissue Engineering and Artificial Organs*, 3rd ed. Boca Raton, FL: CRC Press, p 71.1–71.18.
- Green EA, Flavell RA (1999) The initiation of autoimmune diabetes. *Curr Opin. Immunol.* 11: 663–669.
- Gruber SA (1992) Locoregional immunosuppression of organ transplants. *Immunol. Rev.* 129: 5–30.
- Inceoglu S, Siemionow M, Chick L, Craven CM, Lister GD (1994) The effect of combined immunosuppression with systemic low-dose cyclosporin and topical fluocinolone acetonide on the survival of rat hind-limb allografts. *Ann. Plast. Surg.* 33: 57–65.
- Jaffe GJ, McCallum RM, Branchaud B, Skalak C, Butuner Z, Ashton P (2005) Long-term follow-up results of a pilot trial of a fluocinolone acetonide implant to treat posterior uveitis. *Ophthalmology* 112: 1192–1198.
- Kizilel S, Garfinkel M, Opara E (2005) The bioartificial pancreas: progress and challenges. *Diabetes Technol. Ther.* 7: 968–985.
- Knisely TL, Hosoi J, Nazareno R, Granstein RD (1994) The presence of biologically significant concentrations of glucocorticoids but little or no cortisol binding globulin within aqueous humor: relevance to immune privilege in the anterior chamber of the eye. *Invest. Ophthalmol. Vis. Sci.* 35: 3711–3723.
- Lai CC, Gouras P, Doi K, Tsang SH, Goff SP, Ashton P (2000) Local immunosuppression prolongs survival of RPE xenografts labeled by retroviral gene transfer. *Invest. Ophthalmol. Vis. Sci.* 41: 3134–3141.
- Lambillotte C, Gilon P, Henquin JC (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J. Clin. Invest.* 99: 414–423.
- Leung A, Lawrie G, Nielsen LK, Trau M (2008) Synthesis and characterization of alginate/poly-L-ornithine/alginate microcapsules for local immunosuppression. *J. Microencapsul.* 25: 387–398.
- Li X, Jast BR (2006) *Design of Controlled Release Drug Delivery Systems*. New York: McGraw-Hill.
- Marcelli-Tourvieille S, Hubert T, Moerman E, Gmyr V, Kerr-Conte J, Nunes B, Dherbomez M, Vandewalle B, Pattou F, Vantyghem MC (2007) *In vivo* and *in vitro* effect of sirolimus on insulin secretion. *Transplantation* 83: 532–538.
- Margolles-Clark E, Umland O, Kenyon NS, Ricordi C, Buchwald P (2009) Small molecule costimulatory blockade: organic dye inhibitors of the CD40-CD154 interaction. *J Mol Med* 87: 1133–1143.
- Marzorati S, Bocca N, Molano RD, Hogan AR, Doni M, Cobianchi L, Inverardi L, Ricordi C, Pileggi A (2009a) Effects of systemic immunosuppression on islet engraftment and function into a subcutaneous biocompatible device. *Transplant Proc* 41: 352–353.
- Marzorati S, Bocca N, Molano RD, et al. (2009b) Prolonged survival and function of allogeneic islets into a prevascularized, subcutaneous biohybrid device: Effects of the immunosuppressive regimen. (Abstr.) *Am J Transplant* 9 (Suppl. 2): 667.
- Marzorati S, Pileggi A, Ricordi C (2007) Allogeneic islet transplantation *Expert Opin Biol Ther* 7: 1627–45.
- Matzinger P (1994) Tolerance, danger, and the extended family *Annu Rev Immunol* 12: 991–1045.
- Mellor AL, Munn DH (2008) Creating immune privilege: active local suppression that benefits friends, but protects foes. *Nat Rev Immunol* 8: 74–80.
- Mineo D, Pileggi A, Alejandro R, Ricordi C (2009) Point: steady progress and current challenges in clinical islet transplantation *Diabetes Care* 32: 1563–9.
- Nanji SA, Shapiro AM (2004) Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs* 18: 315–328.
- Narang AS, Mahato RI (2006) Biological and biomaterial approaches for improved islet transplantation. *Pharmacol Rev* 58: 194–243.
- Niederhorn JY (2006) See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat Immunol* 7: 354–359.
- Noble S, Goa KL (1998) Loteprednol etabonate. Clinical potential in the management of ocular inflammation. *BioDrugs* 10: 329–339.
- Okada H, Toguchi H (1995) Biodegradable microspheres in drug delivery. *Crit Rev Ther Drug Carrier Syst* 12: 1–99.
- Ozcay N, Fryer J, Grant D, Freeman D, Garcia B, Zhong R (1997) Budesonide, a locally acting steroid, prevents graft rejection in a rat model of intestinal transplantation. *Transplantation* 63: 1220–1225.
- Pagano G, Cavallo-Perin P, Cassader M, et al. (1983) An *in vivo* and *in vitro* study of the mechanism of prednisone-induced insulin resistance in healthy subjects. *J Clin Invest* 72: 1814–1820.
- Pavesio CE, Decory HH (2008) Treatment of ocular inflammatory conditions with loteprednol etabonate. *Br J Ophthalmol* 92: 455–459.
- Pierluissi J, Navas FO, Ashcroft SJ (1986) Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans. *Diabetologia* 29: 119–121.
- Pileggi A, Alejandro R, Ricordi C (2006a) Islet transplantation: Steady progress and current challenges. *Curr Opin Organ Transplant.* 11: 7–13.
- Pileggi A, Cobianchi L, Inverardi L, Ricordi C (2006b) Overcoming the challenges now limiting islet transplantation: a sequential, integrated approach *Ann N Y Acad Sci* 1079: 383–98.

- Pileggi A, Molano RD, Ricordi C, et al. (2006c) Reversal of diabetes by pancreatic islet transplantation into a subcutaneous, neovascularized device. *Transplantation* 81: 1318–1324.
- Pinto E (2008) Sustained release of a soft glucocorticoid loteprednol etabonate using PLA microspheres for the prevention of islet transplantation rejection. (Ph.D. Thesis), College of Pharmacy, Vol Ph.D. Gainesville, FL: University of Florida, pp 121.
- Pinto E, Zhang B, Song S, Bodor N, Buchwald P, Hochhaus G (2010) Feasibility of localized immunosuppression, 2.: PLA microspheres for the sustained local delivery of a soft immunosuppressant. *Pharmazie* 65: 429–435. (doi: 10.1691/ph.2010.0033R).
- Qi D, Rodrigues B (2007) Glucocorticoids produce whole body insulin resistance with changes in cardiac metabolism. *Am J Physiol Endocrinol Metab* 292: E654–E667.
- Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA (2006) *In vivo* targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles *J Control Release* 112: 26–34.
- Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil CP, Lee LK, Swartz MA, Hubbell JA (2007) Exploiting lymphatic transport and complement activation in nanoparticle vaccines *Nat Biotechnol* 25: 1159–64.
- Ricordi C, Inverardi L, Kenyon NS, Goss J, Bertuzzi F, Alejandro R (2005) Requirements for success in clinical islet transplantation. *Transplantation* 79: 1298–1300.
- Ricordi C, Strom TB (2004) Clinical islet transplantation: advances and immunological challenges *Nat Rev Immunol* 4: 259–68.
- Ricordi C, Zeng Y, Alejandro R, Tzakis A, Carroll P, Rilo HL, Venkataraman R, Fung JJ, Bereiter D, Starzl TE (1992) Effect of FK 506 on human pancreatic islets following renal subcapsular transplantation in diabetic nude mice. *Transplant Proc* 24: 1042.
- Rilo HL, Carroll PB, Zeng YJ, Fontes P, Demetris J, Ricordi C (1994) Acceleration of chronic failure of intrahepatic canine islet autografts by a short course of prednisone. *Transplantation* 57: 181–187.
- Ruers TJM, Buurman WA, Smits JFM, van der Linden CJ, van Dongen JJ, Struyker-Boudier HA, Kootstra G (1986) Local treatment of renal allografts, a promising way to reduce the dosage of immunosuppressive drugs. Comparison of various ways of administering prednisolone. *Transplantation* 41: 156–161.
- Ruers TJM, Daemen MJAP, Thijssen HHW, van der Linden CJ, Buurman WA (1988) Sensitivity of graft rejection in rats to local immunosuppressive therapy. *Transplantation* 46: 820–825.
- Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Fujishiro M, Kikuchi M, Oka Y, Asano T (2000) Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. *Diabetes* 49: 1700–1708.
- Saltzman WM, Olbricht WL (2002) Building drug delivery into tissue engineering. *Nat Rev Drug Discov* 1: 177–186.
- Shirbacheh MV, Jones JW, Breidenbach WC, McCabe S, Barker JH, Gruber SA (1998) The case for local immunosuppression in composite tissue allotransplantation. *Transplant Proc* 30: 2739–2742.
- Solari MG, Washington KM, Sacks JM, Hautz T, Unadkat JV, Horibe EK, Venkataraman R, Larregina AT, Thomson AW, Lee WP (2009) Daily topical tacrolimus therapy prevents skin rejection in a rodent hind limb allograft model. *Plast Reconstr Surg* 123: 17S–25S.
- Thomas FT, Contreras JL, Bilbao G, Ricordi C, Curiel D, Thomas JM (1999) Anoikis, extracellular matrix, and apoptosis factors in isolated cell transplantation *Surgery* 126: 299–304.
- Waldmann H, Cobbold S (2004) Exploiting tolerance processes in transplantation. *Science* 305: 209–212.
- Wang X, Alfrey EJ, Posselt A, Tafra L, Alak AM, Dafoe DC (1995) Intraportal delivery of immunosuppression to intrahepatic islet allograft recipients. *Transpl Int* 8: 268–272.
- Weber T, Kalbhenn T, Herrmann G, Hanisch E (1997) Local immunosuppression with budesonide after liver transplantation in the rat: a preliminary histomorphological analysis. *Transplantation* 64: 705–708.
- Zahr E, Molano RD, Pileggi A, Ichii H, Jose SS, Bocca N, An W, Gonzalez-Quintana J, Fraker C, Ricordi C, Inverardi L (2007) Rapamycin impairs *in vivo* proliferation of islet beta-cells. *Transplantation* 84: 1576–1583.
- Zawalich WS, Tesz GJ, Yamazaki H, Zawalich KC, Philbrick W (2006) Dexamethasone suppresses phospholipase C activation and insulin secretion from isolated rat islets. *Metabolism* 55: 35–42.
- Zhang N, Su D, Qu S, Tse T, Bottino R, Balamurugan AN, Xu J, Bromberg JS, Dong HH (2006) Sirolimus is associated with reduced islet engraftment and impaired beta-cell function. *Diabetes* 55: 2429–2436.