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Combinatorial Insulin Secretion Dynamics of Recombinant Hepatic and Enteroendocrine Cells

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Abstract

One of the more promising cell-based therapies for combating insulin-dependent diabetes entails the use of genetically engineered non- β cells that secrete insulin in response to physiologic stimuli. A normal pancreatic β cell secretes insulin in a biphasic manner in response to glucose. The first phase is characterized by a transient stimulation of insulin to rapidly lower the blood glucose levels, which is followed by a second phase of insulin secretion to sustain the lowered blood glucose levels over a longer period of time. Previous studies have demonstrated hepatic and enteroendocrine cells to be appropriate hosts for recombinant insulin expression. Due to different insulin secretion kinetics from these cells, we hypothesized that a combination of the two cell types would mimic the biphasic insulin secretion of normal β cells with higher fidelity than either cell type alone. In this study, insulin secretion experiments were conducted with two hepatic cell lines (HepG2 and H4IIE) transduced with one of three adenoviruses expressing the insulin transgene and with a stably transfected recombinant intestinal cell line (GLUTag-INS). Insulin secretion was stimulated by exposing the cells to glucose only (hepatic cells), meat hydrolysate only (GLUTag-INS), or to a cocktail of the two secretagogues. It was found experimentally that the recombinant hepatic cells secreted insulin in a more sustained manner, whereas the recombinant intestinal cell line exhibited rapid insulin secretion kinetics upon stimulation. The insulin secretion profiles were computationally combined at different cell ratios to arrive at the combinatorial kinetics. Results indicate that combinations of these two cell types allow for tuning the first and second phase of insulin secretion better than either cell type alone. This work provides the basic framework in understanding the secretion kinetics of the combined system and advances it towards pre-clinical studies.

Keywords

Recombinant; hepatic; enteroendocrine; insulin; combinatorial; biphasic

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Introduction

Exogenous insulin injections or infusions for the treatment of insulin dependent diabetes (IDD) are intended to maintain near normal blood glucose levels. However, temporal mismatches between blood glucose and the glucose lowering effect of exogenously administered insulin limit efficacy and produce both hyper- and hypoglycemia. The Diabetes Control and Complications Trial and the follow up EDIC study confirmed that improved glycemic control reduces the risk of cardiovascular and other diabetes-related health problems, but it also demonstrated an increased incidence of hypoglycemic events among patients subjected to intensive blood sugar control through insulin injections (DCCT/EDIC, 2005 and 2007). Cell-based therapies, where cells secrete insulin in response to metabolic cues, offer the promise of improved blood glucose levels and a subsequent reduction of long-term diabetes-related complications relative to conventional insulin therapy.

Allogenic pancreatic islet transplantation produces essentially normal blood glucose (Shapiro et al, 2000). However, major limitations remain, which include the availability of donor tissue and the long-term effects of immune suppression on both the patient and the transplanted islets. To overcome these limitations, several alternative approaches are being considered, including transplantation of encapsulated islets or of immortalized cell lines; and transplantation of stem cells or adult somatic cells, differentiated or engineered, respectively, towards a β cell phenotype.

Porcine islets are amply available, but preventing immune rejection of free or encapsulated xenogeneic islets remains a major challenge (de Groot et al, 2004; Sambanis et al, 2003). Embryonic stem cells (ESCs) can be differentiated into the β phenotype (Lumelsky et al, 2001; Soria et al, 2005), and can restore normoglycemia in diabetic mice (Soria et al, 2000; Kim et al, 2003; Kroon et al, 2008). However, ethical concerns and the possibility of teratoma formation (Kroon et al, 2008) hamper the employment of ESCs in clinical settings. Adult stem or progenitor cells are also candidates for differentiation into β -like cells (Soria et al, 2005; Bonner-Weir et al, 2000; Baeyens et al, 2005; Ferber et al, 2000, Chen et al, 2004). Use of adult stem cells is ethically acceptable, however, their expansion followed by differentiation towards the β phenotype remain major challenges (Soria et al, 2005). Finally, certain differentiated adult cells can be genetically reprogrammed (transdifferentiated) towards a β -like phenotype (Thowfequ et al, 2007; Eberhard and Tosh, 2008). Transdifferentiation generally requires forced expression of transcription factors active in the embryologic development of β -cells (Baeyens et al, 2005; Zhou et al, 2008). In this, liver and exocrine pancreatic cells are most commonly studied because of their proximal embryonic origin to β cells. An additional challenge with differentiated ESC and adult stem cells, and with transdifferentiated somatic cells, is their potential recognition by autoimmunity in subjects with Type I diabetes.

The use of autologous non- β cells engineered to secrete insulin in response to physiologic cues would overcome the limitations of tissue availability and immune acceptance. Lipes et al (1996) reported that implantation of transgenic, insulin-secreting intermediate lobe pituitary cells in non-obese diabetic (NOD) mice, an animal model of autoimmune diabetes, were not targeted by the immune system of the host and reversed diabetic symptoms in a five week experiment. Consequently, non- β cells are likely to avoid autoimmune attack if implanted into patients with Type 1 diabetes (Han, Lee et al, 2007). Hepatocytes constitute a promising source of autologous non- β cells. Hepatocytes are abundant, relatively accessible, and possess the glucose sensing proteins, glucose transporter 2 (GLUT2) and the glucose phosphorylating enzyme glucokinase (hexokinase IV), which also serve in the same capacity in β cells (Newgard, 1994). Hepatocytes lack the PC1/3 and PC2 endoproteases required to

process proinsulin to insulin in β cells. However, expression of a human proinsulin cDNA modified to permit post-translational cleavage by the ubiquitous endopeptidase furin does allow hepatocytes to secrete mature insulin (Thulé et al, 2000a; Thulé et al, 2000b). Furthermore, expressing proinsulin under the control of a glucose-responsive, insulin-sensitive promoter resulted in glucose-responsive insulin secretion *in vitro* and glycemic regulation in STZ-diabetic rodents (Thulé et al, 2000a; Thulé et al, 2000b; Olson et al, 2003; Porter et al, 2005). However, greater than normal blood glucose fluctuations and post-glucose load hypoglycemia were observed (Olson et al, 2003). These have been attributed to the prolonged stability of preproinsulin (PPI) mRNA, which results in continued insulin biosynthesis and secretion after the stimulus is removed and transcription ceases (Efrat, 1998; Dong and Woo, 2001; Tang and Sambanis, 2003a). Indeed, destabilization of the PPI mRNA through “nonsense mediated mRNA decay” significantly expedited down regulation of insulin secretion from hepatic cells following suppression of transcription (Tang and Sambanis, 2003a). Hepatic cells also lack a regulated secretory pathway (Burkhardt et al, 2003; Auricchio et al, 2002), hence they are unable to reproduce the acute first secretory phase exhibited by pancreatic β cells.

We reasoned that while hepatocytes may be able to mimic the slower second phase of β -cell insulin secretion, an additional insulin secreting cell type would be necessary to provide the rapid first phase of the secretory response. Enteroendocrine L cells possess a prandially responsive regulated secretory pathway and release peptide hormones, such as glucagon-like peptide -1 (GLP-1), rapidly upon nutrient stimulation (Schirra et al, 1996). Enteroendocrine cells express the PC1/3 and PC2 endoproteases and are capable of processing wild-type proinsulin to 6 insulin; they can also be genetically engineered to express insulin, which they store in the same secretory granules as GLP-1, and release it with similar kinetics as their endogenous hormones (Tang and Sambanis, 2003b; Bara and Sambanis, 2008). Work by Cheung et al (2000) exploited this incretin-insulin connection and demonstrated that transgenic mice expressing insulin from genetically engineered intestinal K cells were protected from developing diabetes after the STZ destruction of the native β cells. In a more recent study, murine enteroendocrine L cells stably transfected with human insulin failed to restore normoglycemia in STZ-induced diabetic mice, probably because insufficient insulin was produced by the implant, even though human insulin was detected in the blood of experimental animals (Bara et al, 2009).

This work reports on the characterization of the insulin secretion dynamics from two recombinant cell types, hepatic cells and an intestinal L cell line. We test the hypothesis that a combination of the two cell types better mimics the biphasic insulin secretion kinetics of normal β cells than either cell type alone. The potential of translating this dual cell therapy approach to a clinical setting is discussed.

Materials and Methods

All reagents were from Sigma (St Louis, MO) unless otherwise noted.

Recombinant enteroendocrine cells and culture conditions

The murine enteroendocrine L cell line GLUTag was obtained from the laboratory of Dr. P. L. Brubaker with the permission of Dr. D. J. Drucker (University of Toronto, Ontario, Canada). The GLUTag-INS cell line (Bara and Sambanis, 2008) was developed by stable transfection of GLUTag cells to express the B10 mutated human insulin under the transcriptional control of the cytomegalovirus (CMV) promoter. GLUTag-INS cells were cultured in L-glutamine-free Dulbecco's modified Eagle's medium (DMEM) (BioWhittaker, Walkersville, MD) containing 4.5 g/L glucose and supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) (Cellgro, Herndon, VA) in a humidified

incubator maintained at 37° C and 5% CO₂. Cells were split at a ratio of 1:5 upon reaching 80% confluency.

Recombinant hepatic cells and culture conditions

The human hepatocarcinoma cell line, HepG2 (ATCC, Manassas, VA) and rat hepatoma cell line, H4IIE (ATCC, Manassas, VA) were the hepatic cells used in this study. Both hepatic cell types were cultured in DMEM (Cellgro, Herndon, VA) containing 4.5 g/L glucose and supplemented with 10% FBS and 1% P/S in a humidified incubator maintained at 37° C and 5% CO₂. Cells were split at a ratio of 1:8 at approximately 80% confluency. With HepG2 cells only, culture flasks or multiwell plates were coated with 50µg/mL rat tail collagen type I (BD biosciences, San Jose, CA).

Both hepatic cells were transduced with one of the three adenoviruses (AdG3-Track, AdG3-2xfur, and AdG3-InsTail; Thulé et al, 2000a & 2000b) shown in Fig. 1. Each adenovirus contained the furin-compatible human proinsulin gene under the transcriptional regulation of the glucose-responsive promoter G3. Promoter G3 contained three copies of the stimulatory glucose responsive element (GIRE) found in the rat liver pyruvate kinase (L-PK) promoter, which were inserted directly upstream of insulin-like growth factor binding protein-1 basal promoter (IGFBP-1) (Thulé et al, 2000a). The insulin response region within the IGFBP-1 promoter overlaps with a stimulatory glucocorticoid response element. As the glucocorticoid dexamethasone (DEX) enhances the stimulation afforded by exposure to glucose (Suh et al, 1994), we have included DEX in all media formulations used during secretion studies. AdG3-2xfur consisted of a standard furin-compatible proinsulin sequence driven by the G3 promoter. To improve the down regulation of insulin biosynthesis, AdG3-InsTail was constructed to have two additional copies of the furin-compatible proinsulin sequence after the stop codon of the first proinsulin cDNA. This engineered mRNA was subjected to nonsense mediated mRNA decay destabilizing the engineered PPI mRNA (Tang and Sambanis, 2003a). Besides proinsulin, AdG3-Track expressed a green fluorescent protein (GFP) driven by the cytomegalovirus (CMV) promoter. This allowed for an estimation of transduction efficiency through examining the cells under a fluorescent microscope. Transduction with AdG3-Track was used to determine the appropriate multiplicity of infection (MOI) for each of the two hepatic cell lines.

Insulin secretion studies

Cells were seeded at a density of 5×10^5 cells/well in a 12-well plate. The next day, the hepatic cells were transduced at the respective MOIs and GLUTag-INS cells were left untouched. Secretion studies were initiated two days after seeding (time 0) by first switching the cells to basal medium (glucose-free DMEM (Sigma D5030), supplemented with 5mM glucose, 10^{-7} M DEX, 2% FBS, 1% P/S) for 2 h for GLUTag-INS and for 24 h for hepatic cells. Following the basal period, the wells were washed once with phosphate buffer saline with Ca⁺² and Mg⁺² ions (DPBS) and insulin secretion was stimulated by exposing hepatic cells either to cocktail medium (glucose-free DMEM (D5030) supplemented with 20mM glucose, 2% meat hydrolysate (MH), 10^{-7} M DEX, 2% FBS, 1% P/S) or glucose only medium (glucose-free DMEM (D5030) supplemented with 20mM glucose, 10^{-7} M DEX, 2% FBS, 1% P/S) and GLUTag-INS cells to cocktail medium (as above) or to MH only medium (glucose-free DMEM (D5030) supplemented with 2% MH, 10^{-7} M DEX, 2% FBS, 1% P/S). After 24 h in stimulation medium, cells were washed once with DPBS and switched back to basal medium for a period of 8 h. Parallel control wells were subjected to medium changes at the same time points, but the medium was always basal. Also, additional wells for each treatment were propagated in parallel for cell counting. Medium samples were collected at 0, 12, 24⁻, 24⁺, 26, 28, 36, 48, 50, 52 and 56 hours for hepatic cells. To capture the rapid insulin secretion kinetics of GLUTag-INS, medium from these cultures

was sampled more frequently, every twenty minutes during the first hour and every half hour for the next hour post-stimulation. The sampling frequency was same as for hepatic cells for the rest of the time points.

Analytical techniques

Insulin concentrations in collected samples were determined using an ultrasensitive human insulin radioimmunoassay (Millipore, Billerica, MA). A total of three independent experiments were averaged and standard deviation between the experiments was calculated. To evaluate the secretion down regulation following switching of cells from stimulating to basal medium, the down regulation index was calculated as the ratio of insulin secreted during the second basal period to the insulin secreted during the stimulation period, normalized for time. Statistical significance of differences was evaluated by one-way ANOVA followed by Tukey's *post-hoc* analysis.

Results and Discussion

Stimulation response of GLUTag-INS to secretagogues

The insulin accumulation profile of GLUTag-INS in response to 2% MH alone or cocktail medium is shown in the Figure 2. The profile of insulin accumulation during the first 4 hours post-stimulation is shown at a higher time resolution in the inset. Cells stimulated with cocktail medium exhibited significantly higher accumulated insulin ($p < 0.05$) relative to basal conditions from 20 min onwards (Inset, Fig. 2). With 2% MH only, the accumulated insulin was significantly higher than basal starting at 40 min post-stimulation ($p < 0.05$). From 40 min onwards, there was no significant difference in accumulated insulin between MH only and cocktail medium. These results suggest that GLUTag-INS cells release insulin rapidly in response to secretagogues, especially with the combination of glucose and MH. Following the change from stimulating to basal medium, insulin secretion declined to basal levels (Fig. 2) exemplifying good down regulation kinetics.

Stimulation response of hepatic cells to the secretagogues

Appropriate MOIs for HepG2 and H4IIE cells were determined based on GFP fluorescence following transduction with AdG3-Track. HepG2 cells transduced with AdG3-Track at a multiplicity of infection (MOI) of 1 exhibited almost 100% fluorescence (Fig. 3a). Based on this, an MOI of 1 was selected for each type of adenovirus for HepG2 cells. For H4IIE cells, transduction increased with the AdG3-Track MOI and reached approximately 100% at an MOI of 10 (Figs. 3b–d). Transduction of H4IIE with the other two viruses was carried out at an MOI of 20 to ensure high transduction efficiency and measurable insulin concentrations in culture media.

With HepG2 cells transduced with either AdG3-2xfur or AdG3-InsTail, there was no difference in accumulated insulin in high glucose-only medium relative to basal medium over a 24 h period (Figs. 4a–c). A possible explanation for this is that the concentration of 5mM glucose in the basal medium is sufficient to saturate the cellular secretory response, as HepG2 cells express the high affinity glucose transporter GLUT1 ($K_m \sim 1.9\text{mM}$) and the high affinity glucose phosphorylating enzyme hexokinase ($K_m \sim 0.05\text{mM}$) (Fehr et al, 2005). Therefore, incubation with glucose-only medium containing 20mM glucose had no further stimulatory effect relative to medium with 5 mM glucose. With HepG2 cells transduced with AdG3-2xfur or AdG3-InsTail, there was a significant difference in insulin accumulation over a 24 h period when cells were stimulated in cocktail medium ($6.97 \pm 1.39 \mu\text{U}/10^5$ cells for AdG3-2xfur; $4.86 \pm 0.99 \mu\text{U}/10^5$ cells for AdG3-InsTail) relative to glucose only ($3.42 \pm 2.20 \mu\text{U}/10^5$ cells for AdG3-2xfur; $1.26 \pm 0.83 \mu\text{U}/10^5$ cells for AdG3-InsTail) or basal medium ($3.12 \pm 1.92 \mu\text{U}/10^5$ cells for AdG3-2xfur; $1.11 \pm 0.33 \mu\text{U}/10^5$

cells for AdG3-InsTail) (Figs. 4b & c). This suggests that meat hydrolysate potentiates glucose-induced insulin secretion in HepG2 cells. Even though the precise reason for this is unclear, peptones in the meat hydrolysate may have a stimulatory effect on the G3 promoter in the adenoviral constructs. Results also show that HepG2 cells transduced with AdG3-Track secreted significantly more insulin 24 h post-stimulation when compared to AdG3-2xfur or AdG3-InsTail. Indeed, the insulin accumulation in cocktail media was $23.55 \pm 4.65 \mu\text{U}/10^5$ cells for AdG3-Track whereas it was $6.97 \pm 1.39 \mu\text{U}/10^5$ cells for AdG3-2xfur (~ 3.4 times lower) and $4.86 \pm 0.99 \mu\text{U}/10^5$ cells for AdG3-InsTail (~ 4.8 times lower) (Figs. 4a–c). AdG3-Track incorporates a GFP protein driven by CMV promoter downstream of the proinsulin gene. This CMV promoter consists of an immediate-early promoter enhancer that is capable of transcribing genes both upstream and downstream to the promoter (Grzimek et al, 1999; Simon et al, 2007). Hence, it is likely that in AdG3-Track insulin transcription is affected by both the G3 and the CMV promoters, resulting in the higher secretion level and the absence of detectable effect of MH on insulin secretion.

Results also suggest that some internalization of MH by the cells occurs, as there was a residual effect of MH on the down regulation step when cells were switched from inducing to basal medium (Figs. 4b & c). To compare performance upon removal of the secretion agonists, the down regulation index was calculated as defined in Materials and Methods. When cells were switched from cocktail to basal medium, the down regulation index with AdG3-InsTail was significantly lower than with the other two vectors ($p < 0.05$) ($28.74 \pm 9.70\%$ for AdG3-InsTail, $71.83 \pm 5.53\%$ for AdG3-2xfur and $69.48 \pm 18.61\%$ for AdG3-Track). Similarly, when cells were switched from glucose only to basal medium, the index with AdG3-InsTail was approximately the same as with AdG3-2xfur and ~ 1.2 fold lower than with AdG3-Track. The enhanced down regulation kinetics with AdG3-InsTail was likely due to the expedited decay of the preproinsulin mRNA through nonsense mediated mRNA decay. Results similar to those in Fig. 4 were obtained with H4IIE cells transduced with the three adenoviruses and exposed to basal and inducing media in the same manner; hence these results are not reported here.

Combinatorial insulin secretion kinetics

The secretory response of HepG2 cells transduced with AdG3-2xfur or AdG3-InsTail was computationally combined with that of GLUTag-INS cells to assess whether the dual cell system better approximates the biphasic secretion of normal islets than either cell type alone. The insulin secretion rate $r(t)$ at time t was calculated as follows

$$r(t) = [V(t)C(t) - V(t - \Delta t)C(t - \Delta t)] / (N \cdot \Delta t)$$

where $t - \Delta t$ and t are two consecutive sampling points; C the concentration of insulin; V the medium volume; and N the cell number. We assumed the combinatorial profile to be computational addition of the secretion profiles of the recombinant cells with no interactions. This is a reasonable assumption, especially since the two cell types will not necessarily co-localize *in vitro* or *in vivo*. Furthermore, when spent medium samples from GLUTag-INS and transduced HepG2 cultures were combined, the amount of insulin in the resulting sample was the sum of the amounts in the individual spent medium samples, confirming the additive relationship (data not shown). The combinatorial profile was generated for cells exposed to basal and cocktail medium, as the latter constitutes a common secretagogue for both the cell types and may better approximate products of prandial metabolism. Figure 5 shows the secretion rates of GLUTag-INS cells and of HepG2 cells transduced with AdG3-2xfur (a) or AdG3-InsTail (b) when each cell type is by itself or when the two cell types are combined at two different population ratios. With GLUTag-INS, the rate within 20 min of stimulation in cocktail medium was roughly $4.59 \pm 2.08 \mu\text{U}/10^5$

cells.h; it decreased to $2.05 \pm 0.19 \mu\text{U}/10^5 \text{ cells.h}$ at 4 h and to $0.52 \pm 0.09 \mu\text{U}/10^5 \text{ cells.h}$ at 24h. This suggests that there is rapid secretion of insulin upon stimulation and the rate gradually falls as the insulin stores in the secretory granules are depleted. Therefore, GLUTag-INS cells capture the acute first phase of insulin secretion of β cells. Conversely, hepatic cells seem to have sustained insulin release post-stimulation portraying the second phase of insulin secretion seen in β cells. Overall, results demonstrate that the combination of the recombinant hepatic and enteroendocrine cells allow for the tuning of the first and second phases of insulin secretion better than either cell type alone. For instance, at a ratio of three HepG2 cells to every GLUTag-INS cell (Figs. 5a & b), the first phase of insulin secretion appears comparable to that of GLUTag-INS by themselves but with markedly improved second phase.

Besides secretion kinetics, the level of insulin secretion is a critical parameter for *in vivo* efficacy. In a previous study, GLUTag-INS cells alone in a disk-shaped construct failed to correct diabetes in STZ-diabetic mice, although the human insulin secreted by these cells could be detected in the serum of the animals (Bara et al, 2009). It is also unlikely that recombinant HepG2 cells alone in an encapsulation device would be successful *in vivo*, as they are glucose hypersensitive and secrete less insulin than GLUTag-INS. On the other hand, primary liver hepatocytes constitute an abundant cell source, they can be easily targeted *in vivo* with a glucose-responsive insulin transgene, and they have been shown to secrete enough insulin for at least some glycemic regulation in diabetic rats for up to 12 weeks (Thulé et al, 2000a; Thulé et al, 2000b; Olson et al, 2003; Porter et al, 2005). Primary hepatocytes should not be glucose hypersensitive, as they express high K_m glucose transporter and phosphorylating enzyme (Kim and Ahn, 2005); however, they would still not provide the acute phase of insulin secretion, making supplementation of their secretory function by GLUTag-INS cells appropriate.

Adenoviral transduction provides only transient expression of the transgene; we chose to perform the initial *in vitro* developmental work with adenovirus constructs already in our possession to demonstrate the feasibility of our concept and to optimize our experimental design. For *in vivo* experiments requiring prolonged transgene expression, other vectors such as lentivirus or adeno-associated virus can be used. GLUTag-INS cells could then augment this function by providing the level of acute secretion necessary for normoglycemia. Furthermore, the level of insulin secretion from GLUTag-INS cells could potentially be enhanced through transduction with viral vectors, making the necessary implant volume manageable; studies in this direction are currently pursued in our laboratory.

Tight glycemic regulation in IDD patients not only reduces the risk of acute hyper- and hypoglycemia but also reduces the risk of secondary complications associated with the disease. Hepatic and enteroendocrine cells are potentially accessible from the same patient and therefore autologous and when engineered to secrete insulin are not expected to be subjected to xeno- and allo-geneic immune attack or to autoimmune rejection (Lipes et al, 1996). Since replacing daily blood glucose monitoring and insulin injections with life-long immune suppression is considered by many an unacceptable alternative, especially for juvenile diabetics, this concept is particularly attractive.

The combination cell therapy proposed here is analogous to the combination basal-bolus insulin therapy (Bolli, 2002) in which the enteroendocrine cells act like “fast-acting” insulin and hepatocytes like the “slow-acting” insulin. In conventional insulin therapies, in addition to their basal insulin dosage, patients are often administered a bolus dose of insulin before meals to control post-prandial glucose levels. We envision that in a clinical application of combination cell therapy, hepatic cells will maintain the basal insulin levels whereas the enteroendocrine cells will eliminate the need for bolus insulin dosage by providing the rapid

insulin secretion required in response to increased blood glucose levels. By adjusting the cell ratios, the insulin secretion level can be tailored to the insulin secretion requirements of a particular patient. Tools including cell encapsulation, where the viable cell density stabilizes around a value determined by the external oxygen (Papavasiliou et al, 1999; Gross et al, 2007), or expression of the simian virus 40 large tumor antigen under the control of the tetracycline resistance operon (Efrat, 1995), may be used to maintain a constant ratio of the two cell types over time. Overall the combination cell therapy has significant potential towards developing personalized therapies for patients with IDD.

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Figure 1. Adenoviruses used for transduction: a) AdG3-2xfur, b) AdG3-InsTail and c) AdG3-Track. Each adenovirus contained the furin-compatible human proinsulin gene (PPI_{2xfur}) under the transcriptional regulation of the glucose-responsive promoter G3.

Figure 2.

Insulin accumulation profile of GLUTag-INS under basal and stimulation conditions. The vertical lines indicate medium changes. The inset details the time course of insulin secreted right after the cells are stimulated with either 2% MH only or cocktail medium (2% MH and 20 mM glucose). #Significant difference between cocktail and basal groups at these times, $p < 0.05$. *Significant difference between MH and basal groups at these times, $p < 0.05$.

Figure 3. Bright field and fluorescent images of AdG3-Track transduction of a) HepG2 cells at MOI = 1, b) H4IIE cells at MOI = 1, c) H4IIE cells at MOI = 4 and d) H4IIE cells at MOI = 10.

Figure 4. Insulin accumulation profile of HepG2 cells transduced with a) AdG3-Track, b) AdG3-2xfur and c) AdG3-InsTail. The vertical lines indicate medium changes. * $p < 0.05$.

Figure 5. Combinatorial secretion profile with stimulation in cocktail medium: a) HepG2 transduced with AdG3-2xfur (H) with GLUTag-INS (G); b) HepG2 transduced with AdG3-InsTail (H) with GLUTag-INS (G).