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Development and Characterization of a Tissue Engineered Pancreatic Substitute Based on Recombinant Intestinal Endocrine L-cells

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Abstract

A tissue engineered pancreatic substitute (TEPS) consisting of insulin-producing cells appropriately designed and encapsulated to support cellular function and prevent interaction with the host may provide physiological blood glucose regulation for the treatment of insulin dependent diabetes (IDD). The performance of agarose-based constructs which contained either a single cell suspension of GLUTag-INS cells, a suspension of pre-aggregated GLUTag-INS spheroids, or GLUTag-INS cells on small intestinal submucosa (SIS), was evaluated *in vitro* for total cell number, weekly glucose consumption and insulin secretion rates (GCR and ISR), and induced insulin secretion function. The three types of TEPS studied displayed similar number of cells, GCR, and ISR throughout four weeks of culture. However, the TEPS which incorporated SIS as a substrate for the GLUTag-INS cells, was the only type of TEPS tested which was able to retain the induced insulin secretion function of non-encapsulated GLUTag-INS cells. Though improvements in the expression level of GLUTag-INS cells and/or the number of viable cells contained within the TEPS are needed for successful treatment of a murine model of IDD, this study has revealed a potential method for promoting proper cellular function of recombinant L-cells upon incorporation into an implantable three-dimensional TEPS.

Keywords

Diabetes; L-cells; GLUTag; Insulin; Enteroendocrine; Small intestinal submucosa

Introduction

Insulin Dependent Diabetes (IDD) is a significant health problem affecting over four million people in the United States (2005 CDC National Diabetes Fact Sheet). The poor temporal match between glucose load and insulin activity following insulin injections leads to many serious complications which may be alleviated by providing tighter glycemic regulation (The Diabetes Control and Complications Trial Research Group 1993). Despite improvements in allogeneic islet transplantation, its application is severely limited by the supply of cadaver donor tissue, and the side effects of immuno-suppressive therapy are

deemed by some to be intolerable. A tissue engineered pancreatic substitute (TEPS) consisting of insulin-producing cells appropriately designed and encapsulated to support cellular function and prevent interaction with the host may provide physiological blood glucose regulation.

Enteroendocrine cells, exhibit many useful properties that make them appropriate targets for insulin expression. Enteroendocrine cells' native function is to secrete incretin hormones, which serve to potentiate insulin secretion from β -cells in the presence of glucose. The release of incretins is controlled in a tightly regulated manner that closely parallels the secretion of insulin by β -cells (Kieffer and Habener 1999; Schirra et al. 1996). Another advantage of enteroendocrine L-cells is their ability to process wild type proinsulin into mature insulin and C-peptide, as C-peptide has beneficial effects on preventing secondary complications of IDD. Because of the unique connection between incretins and insulin, engineering of L-cells for insulin expression arises as an appealing approach for IDD treatment in terms of the dynamic release of insulin and compatibility of incretins and insulin in glycemic normalization.

Transgenic mice, which expressed insulin in enteroendocrine K cells, were able to prevent the onset of diabetes after injection with streptozotocin (Cheung et al. 2000). Another study showed that transgenic mice which produced human insulin in gastric G cells displayed a meal-regulated increase in the level of transgenic insulin and a corresponding decrease in blood glucose levels (Lu et al. 2005). These important proof-of-concept results demonstrate that insulin-producing enteroendocrine cells can provide regulation of blood glucose levels *in vivo*. Much work remains, however, in translating enteroendocrine cell insulin therapy into a viable therapeutic modality for adult models of IDD and eventually humans.

The most direct application suggested by these transgenic mice for treating IDD in an adult animal would be to genetically engineer the appropriate enteroendocrine cells in their natural environment, that is, *in vivo* gene therapy. Besides significant difficulties inherent to delivering and stably expressing recombinant insulin in L-cells *in vivo*, a major challenge of *in vivo* gene therapy is in carefully titrating the treatment, so as not to overdose the patient with the transgene, as this might result in sustained over-production of insulin, an error that could be fatal. Performing the necessary genetic modifications in a closely controlled manner outside of the body would greatly enhance safety. A TEPS would not only provide the safety and quality control of *in vitro* gene therapy, but could additionally allow for retrieval of the construct and cells, if necessary, and for the non-invasive monitoring of the implant by, e.g., nuclear magnetic resonance techniques (Constantinidis and Sambanis 1998; Stabler et al. 2005a; Stabler et al. 2005b).

Previously, we modified the murine L-cell line GLUTag, to exhibit stable expression of human insulin such that large numbers of L-cells secreting insulin in a uniform manner could be cultured and used for the development of a TEPS based on such cells (Bara and Sambanis 2008). This study describes the development and characterization of a TEPS which supports the function of GLUTag-INS cells and facilitates steady performance of the TEPS *in vitro* for extended periods of time. We hypothesized that the presence of an extracellular matrix derived from the small intestine would be representative of the natural environment of L-cells and thus would improve the function of GLUTag-INS cells compared to TEPS which lack this support. Furthermore, increased cell-cell interaction within a TEPS may enhance function over TEPS without such interactions. These hypotheses were tested by comparing agarose constructs which housed either a single cell suspension of GLUTag-INS cells, a suspension of pre-aggregated GLUTag-INS spheroids, or GLUTag-INS cells on small intestinal submucosa (SIS). The design of all TEPS called for agarose encapsulation to prevent direct contact of implanted cells with the host during

future *in vivo* studies with diabetic mice. Indeed, the presence of a hydrogel barrier preventing direct contact with the host has proven sufficient in providing immunoprotection to other murine allografts *in vivo* (Black et al. 2006).

Materials and Methods

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

Cell Culture

GLUTag-INS cells were derived from the GLUTag cell line to stably express human insulin (Bara and Sambanis 2008) and were cultured in a 37°C/5% CO₂ humidified incubator in complete culture medium consisting of L-glutamine-free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Cellgro, Herndon, VA).

Construct Design & Fabrication

The performance of GLUTag-INS cells, cultured within three-dimensional constructs, suitable for intraperitoneal implantation into a mouse, was evaluated *in vitro*. The three tissue-engineered pancreatic substitutes (TEPS) tested are depicted in Table I. These were: (1) single cells entrapped in an inner agarose disk, which was then encased in a cell-free agarose disk; (2) cells pre-aggregated into spheroids and entrapped in an inner agarose disk, which was then encased in a cell-free agarose disk; and (3) cell-seeded SIS within a cell-free agarose disk. The total size and shape of the disks was based on the work by Stabler et al. (Stabler et al. 2005b) and was 15 mm in diameter and 2 mm in height (350 µl in volume). The inner disk in (1) and (2) was 1 mm in height and 5.5 mm in diameter (100 µl in volume). The initial cell number in each TEPS was similar for the designs tested. To fabricate, 14 mm diameter circular punches of hydrated sheets of SIS (generously provided by Cook Biotech, West Lafayette, IN) were first seeded with GLUTag-INS cells and cultured prior to encapsulation. For seeding, the SIS was positioned under a sterile cloning cylinder and incubated with a cell suspension containing 2×10^6 cells for 1 hour. Using gentle manipulation this process was repeated on the other side. Cell-seeded SIS was then cultured for seven days in a six-well plate (BD Bioscience, San Jose, CA), suspended in 3 ml of complete culture medium until encapsulation. During this culture, GLUTag-INS cells were also grown in parallel as monolayers for use in the other two constructs. After seven days of culture, a sample of the cell-seeded SIS was imaged by staining with the Live/Dead Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, Carlsbad, CA) and confocal microscopy. Spheroids of GLUTag-INS cells were allowed to form by culturing freshly trypsinized GLUTag-INS cells in a spinner flask using the conditions reported by Papas et al. (Papas et al. 1993). Briefly, 100 ml of cell suspension, at 1.2×10^6 cells/ml medium, was cultured in a 125 ml spinner flask at 60 rpm overnight until spheroids with an average diameter of 100 µm formed.

The following day, the fabrication of all three TEPSs was completed by encapsulation in 2% SeaPlaque agarose (Cambrex, East Rutherford, NJ). The inner agarose disks containing either freshly trypsinized single cell suspension or a suspension of spheroids were formed by transferring 100 µl of the cell or spheroid suspension to a sterile Lexan mold (McMaster-Carr, Atlanta, GA) and allowing to gel for 5 minutes at room temperature. Both suspensions had an overall cell density of 3×10^7 cells/ml agarose. Cell number per ml of settled spheroid volume was determined by citric acid crystal violet nuclei count and was 1.84×10^8 cells/ml spheroids. Encapsulation in the outer agarose layer was performed in the same manner for all constructs. Briefly, the sterile Lexan mold was half-filled with agarose, the

cell-containing insert was transferred to the center of the mold, and the remaining agarose was added to fill the mold and allowed to gel for 5 minutes at room temperature.

TEPS Culture and *In Vitro* Evaluation

The TEPS described above were cultured in six-well non-tissue culture-treated plates containing 3 ml growth medium placed on a rocking platform in a 37°C/5% CO₂ humidified incubator.

Glucose consumption rate (GCR) and insulin secretion rate (ISR) were determined weekly by initially rinsing the well and TEPS twice with PBS, adding 3 ml fresh medium, sampling for initial metabolite concentrations, incubating for 20 to 24 hours, and sampling again for final concentrations. ISR and GCR measurements were made on the same TEPSs at all time points, and parallel constructs were frozen at −80°C for determination of total double stranded DNA content, which was used to estimate total cell number.

Induced insulin secretion tests were also performed on all three types of TEPS. On the evening prior to the secretion tests, the medium was changed to basal (DMEM with 5 mM glucose, supplemented with 1% FBS). On the day of the secretion test, constructs were briefly washed with basal medium three times. During the secretion test all constructs were incubated in basal medium for 2 hours and induction medium (basal medium supplemented with 2% (w/v) meat hydrolysate (MH)) for another 2 hours. Constructs were washed with basal medium between periods and medium samples were reserved at the end of each period.

Assays

Samples were assayed for glucose and insulin concentration by the Trinder assay (Diagnostic Chemicals Limited, USA, Oxford, CT) and human insulin radioimmunoassay kits (Millipore, Billerica, MA) respectively, according to the manufacturer's protocols. To prepare the samples for DNA quantification, the frozen constructs were thawed and dehydrated, then serially digested with proteinase K and agarase. Double stranded DNA was then measured with the picoGreen kit (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. The total cell number was calculated from the measured DNA content using a calibration curve in which a known number of GLUTag-INS cells was serially diluted and assayed for total DNA content. Five points were used to make up the standard curve, each with 3 replicates.

Results

Viability and Total Cell Number Over Time

Cell viability was assessed by LIVE/DEAD staining for cells on SIS only prior to agarose encapsulation, as the thickness of the agarose prohibited any further confocal visualization. After seven days of culture, GLUTag-INS cells on SIS had formed a nearly confluent layer with high cellular viability. Post-agarose encapsulation, the total cell number (both live and dead) in each TEPS was estimated based on a total DNA content, at given time points using at least three different constructs (Figure 1). Each TEPS contained approximately the same number of cells, 3×10^6 cells, when encapsulated (day 0). While the SIS-containing and single cell-containing TEPSs showed an increase in the total cell number by day 28 ($p < 0.05$), with little increase during the first three weeks, spheroid-containing TEPS did not display this increase in total cell number. SIS-containing TEPS had 3.76 ± 0.47 and $4.46 \pm 0.34 \times 10^6$ cells on days 7 and 28 respectively. Likewise, TEPS containing single cell suspensions had 3.66 ± 0.45 and $5.58 \pm 1.44 \times 10^6$ cells on days 7 and 28 respectively, but

TEPS containing spheroid suspensions had $4.38 \pm 0.04 \times 10^6$ cells on day 7 and only $4.80 \pm 1.38 \times 10^6$ cells on day 28.

Glucose Consumption Rates

GCR is an indicator of the overall number of metabolically active cells contained within the TEPS. GCR was selected over alamarBlue® (Invitrogen, Carlsbad, CA) as GCR, contrary to alamarBlue®, changed minimally, if at all, upon agarose encapsulation of GLUTag-INS cell-seeded SIS. GCRs of the TEPSs were determined by assaying the glucose concentration in samples of spent medium (Figure 2). As expected, the GCRs of the single cell-containing TEPSs increased from day 7 to day 28 as the total cell number increased (Figure 1). Though the SIS-containing TEPSs also saw a rise in total cell number, this increase was much smaller and, as such, was not reflected in a statistical increase in GCR from day 7 to 28. Also, the consistently high GCR levels of the SIS- and spheroid-containing groups indicate that despite low or no increase in total cell number, the constructs are still supporting viable cells during the month of *in vitro* culture. Comparisons between groups on each day revealed that while the single cell-containing group had a lower GCR than both spheroid- and SIS-containing groups on days 7 and 14 ($p < 0.05$), the temporal GCR increase of the single cell-containing TEPS group erased this difference with the spheroid group on day 21 and with both other groups by day 28.

Insulin Secretion Rates

As GCR provides insight into the number of metabolically active cells in the TEPS, insulin secretion rate (ISR) alludes to the number of secreting cells, and also provides insight into a key aspect of TEPS utility, that is, its ability to secrete insulin. There was considerable variation in the ISR profiles of the TEPSs (Figure 3), with seemingly random differences appearing on throughout the course of the experiment. On day 7 there were no differences among groups, but on day 14 the single cell-containing group had a higher ISR than the spheroid-containing group. This difference was reversed on day 21 with the spheroid-group having the greater ISR. By day 28 there was no difference between spheroid- and single cell-containing groups, but the SIS group was higher than the single cell-containing group ($p < 0.05$). When comparing the average at all time points of each group, the SIS-containing group was found to have higher ISRs than both the single cell and spheroid groups (one-way ANOVA $p < 0.05$), but there was no statistical difference between the single cell suspension and spheroid suspension groups ($p > 0.05$).

Induced Insulin Secretion

Selection of SIS as a potential substrate for an implantable TEPS arose from initial testing in which the basal and induced ISRs of GLUTag-INS cells grown on SIS were compared to those of cells grown on conventional tissue culture plastic (Table II). For these tests, insulin secretion was measured during a 2 hour basal period and again during a 2 hour induced period in which the cells experienced a step up in the concentration of meat hydrolysate (MH) from 0% to 2%. The two time points (day 2 and day 7) differed in cell surface density with higher densities observed at the later time point. Though the basal and induced ISRs of GLUTag-INS cells on SIS are generally reduced compared to those on plastic (except for the induced rates on day 2), it is important to note that the induction-fold (IF) is maintained. Again, to better evaluate the performance of agarose-based constructs, induced insulin secretion tests were performed on days 7 and day 14 post encapsulation (Figure 4). Induced insulin secretion is expressed as the percentage of insulin secreted during MH stimulation relative to that secreted during the basal period. GLUTag-INS cells on SIS in agarose exhibited induced release of insulin in response to nutrient stimulation on days 7 (182.2%) and 14 (162.4%), but the GLUTag-INS cells within the other two types of TEPS did not exhibit this induced response.

Discussion

In this study we evaluated the performance of GLUTag-INS cells within three types of agarose-based TEPSs during four weeks of *in vitro* culture. GLUTag-INS cells are murine L-cells which have been engineered to constitutively express human insulin (Bara and Sambanis 2008). Insulin's secretion from these cells, however, is dictated by the regulated release pathway and responds to nutrient stimulation. A TEPS based on engineered L-cells may be a more likely therapeutic option for humans than delivery of the cells by themselves, as it accomplishes partial immune protection, localization and retrievability of the implant, which all contribute to superior safety and quality control.

The use of SIS was evaluated here for the potential to improve the regulated secretion function of engineered L-cells, as the origin of this extracellular matrix closely matches the native environment of L-cells. In addition, several studies have reported that the use of SIS improves the *in vitro* culture and function of islets (Lakey et al. 2001; Tian et al. 2005; Woods et al. 2004). Though GLUTag-INS cells are an immortalized cell line, derived from an intestinal tumor, and may not be as peculiar to matrix composition as primary cells (like islets), SIS may also maintain or enhance the phenotype of insulin secretion from engineered L-cells. This is especially important for cells implanted in potentially unfavorable environments, such as the peritoneal cavity, where a drop in dissolved oxygen concentration from 0.20 mM under normal incubator conditions to 0.06 mM (Gross et al. 2007) may cause a decline in insulin secretory function. In this study, hydrated sheets of SIS were investigated, as this configuration may permit the polarization of L-cells. Use of hydrated sheets of SIS required the fabrication of constructs of significantly larger dimensions than alginate microbeads used frequently in TEPSs and islet encapsulation (Lim and Sun 1980). Indeed, SIS helped to maintain a similar induction-fold of insulin to that of cells grown on tissue culture plastic (Table II). The extracellular matrix constituents of SIS are collagen, fibronectin, laminin, glycosaminoglycans, and growth factors with collagen representing approximately 90% of the dry weight (Brown et al. 2006). FGF-2 and a TGF beta-related component have been identified as growth factors in SIS (Voytik-Harbin et al. 1997). One or more of these identified components likely influenced the secretory function of GLUTag-INS cells, but the precise mechanism by which SIS preserved induced insulin secretion function of GLUTag-INS cells was not evaluated in the context of this study.

It was also observed that dense monolayers of GLUTag-INS cells had improved secretory function over sub-confluent monolayers. This improvement was reflected in increases in both basal and induced secretion rates as well as the induction factor (comparison of monolayers on day 2 vs. 7 in Table II) and may be attributable to increased cell-cell interaction among GLUTag-INS cells. As such, we hypothesized that incorporating pre-aggregated spheroids into a TEPS would improve the function of the TEPS over similar constructs containing only single cells.

Though several statistically significant differences were noted in the GCR and ISR results, these differences were nonetheless small. Furthermore, because the differences among groups in the GCR data were not reflected in the ISR data, it is likely that the differences noted in the GCR on days 7 and 14 are due to lower oxygen concentrations seen by the centrally located cells within the SIS- and spheroid-containing TEPS. Indeed, mouse insulinoma β TC3 cells have been reported to be more oxygen sensitive with regard to GCR than with regard to ISR (GCR increased at oxygen concentrations below 25 mmHg, while the ISR decreased below 7 mmHg (Papas et al. 1996)), and this may also be the case with GLUTag-INS cells. Again, though the differences in ISR among groups were small, the SIS-containing TEPS did, on average, slightly out-perform the single cell- and spheroid-containing TEPS. Importantly, each type of construct showed no statistical change in ISR

during the four weeks in culture, indicating a stable system which is favorable for ensuring that the TEPS does not result in hypoglycemia following implantation and cell proliferation *in vivo*. In other systems using insulinoma cells, a tetracycline-responsive element has been incorporated in order to provide an external handle on cell growth following implantation (Efrat et al. 1995). With GLUTag-INS cells in the agarose constructs, however, this type of control does not appear to be needed, as ISR is stable during four weeks in culture.

The induced secretion response of the three types of TEPS tested here revealed the most significant difference between the configurations tested. While agarose encapsulation weakened the secretory response of SIS-containing TEPS compared to non-encapsulated SIS, possibly due to diffusional limitations of insulin and macromolecular constituents of MH, incorporation of SIS did allow for retention of the induced secretion response which was not observed in the single cell- and spheroid-containing TEPS. As noted, the induction-fold of agarose-encapsulated SIS (182.2% on day 7) was lower than unencapsulated SIS (341% on day 7), it was however, fairly close to the value reported for monolayers on tissue culture plastic (218.6%) (Bara and Sambanis 2008). The overall levels of basal and induced secretion of GLUTag-INS cells on SIS were somewhat lower than cells grown on plastic, however, and this drop was only further exacerbated by encapsulation in agarose. It has been reported that β TC3 insulinoma cells also exhibit a decline in insulin secretion upon encapsulation, although of lower magnitude than that observed in this study (Mukundan et al. 1995).

With regard to the level of insulin secretion provided per construct, the TEPS described here fall short of the therapeutic threshold for a diabetic mouse. In one study, a mass of genetically engineered K-cells (STC-1–14), secreting $78.6 \mu\text{U}/(10^7 \text{ cells} \cdot \text{day})$ was implanted subcutaneously, and hyperglycemia was not reversed until about 24 days after implantation, indicating that some tumor growth was needed prior to reaching a therapeutic insulin secretion value (Zhang et al. 2008). GLUTag-INS cells secrete a greater amount of insulin than the K-cells reported by Zhang et al, even at their basal rate of secretion, $285.6 \mu\text{U}/(10^6 \text{ cells} \cdot \text{day})$ versus $78.6 \mu\text{U}/(10^6 \text{ cells} \cdot \text{day})$ for STC-1–14 cells, and as such, there is little doubt that GLUTag-INS cells would be capable of regulating the blood glucose levels of diabetic mice if a sufficient number of viable cells were implanted. To better understand what that number would be we can look to studies involving the insulinoma cell line, β TC-tet. Intraperitoneal implantation of 9×10^6 β TC-tet cells entrapped in calcium-alginate microbeads was sufficient to reverse diabetes in the eight-week old non-obese diabetic mouse within a week of implantation (Black et al. 2006). Using the insulin secretion rates for β TC-tets reported by Simpson et al (Simpson et al. 2005), and assuming that the cells secrete at the stimulated rate for 50% of the time and the non-stimulated rate the other 50% of the time, this is the equivalent of a 227 mU/day. Another estimate for the therapeutic dose of insulin for a diabetic mouse is 200 mU/day as provided by the subcutaneous LinBit implants (Wang 1991). Thus, given the level of secretion for non-encapsulated GLUTag-INS cells reported for day 7 in Table II and making the same assumption that cells are secreting at their stimulated rate for 50% of the time and the non-stimulated rate the other 50% of the time, it would take 3.20×10^8 GLUTag-INS cells to treat a mouse (i.e., produce 200 mU insulin/day) compared to the 3×10^6 cells encapsulated in the TEPS evaluated here. Alternatively if the specific rate of insulin secretion of GLUTag-INS cells could be increased by further genetic engineering and/or manipulation of the cellular microenvironment then this number may be reduced significantly.

The actual amount of insulin needed by per mouse (as with humans) may vary significantly depending on a number of factors including: site of delivery, metabolism, diet, and peripheral insulin resistance. This underscores the importance of using cells which exhibit controlled release like that of islets for IDD cell therapy. Due to the restrictions of allogeneic

or xenogeneic transplantation, which include limited supply and immunogenicity, a potentially autologous cell source, such as engineered L-cells, is especially attractive.

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Figure 1. Total Cell Number vs. time of three types of TEPS with GLUTag-INS cells

TEPS were frozen at the indicated time points and later assayed for total DNA content.

Points are shown as mean $\pm$ SD (n=3). Cell numbers were calculated based on a standard curve (not shown) of cell number vs. DNA amount constructed with dilutions of a known number of GLUTag-INS cells. *There is no difference cell number among groups except on day 7, when the spheroid-containing group is significantly higher than the other two groups (One-way ANOVA, $p < 0.05$). # Both SIS- and single cell-containing TEPS showed statistically significant increase in total cell number from day 7 to day 28 (One-way ANOVA, $p < 0.05$), but no difference was measured for the spheroid-containing group.

Figure 2. Glucose Consumption Rate (GCR) of GLUTag-INS cells in three types of TEPS over time

The same constructs were examined throughout the course of four weeks of *in vitro* culture after encapsulation, and glucose consumption was determined by assaying spent culture medium once a week (n=6). Points are shown as mean $\pm$ SD. SIS- and spheroid-containing groups had significantly greater GCR than the single cell-containing TEPS (#,* One-way ANOVA, $p < 0.05$) on days 7 and 14, though by day 21 the single cell-containing group showed an increase in GCR to match that of spheroid-containing TEPS. By day 28 there were no differences in GCR among groups.

Figure 3. Insulin Secretion Rates (ISR) of GLUTag-INS cells in three types of TEPS vs. time
 The same constructs were examined throughout the course of four weeks of *in vitro* culture after encapsulation and the average insulin secretion rate over one week was determined by assaying spent culture medium in samples collected at the same frequency (n=6). Points show the mean $\pm$ SD. *, #, † $p < 0.05$. With ISR averaged for each group over all time points, one-way ANOVA revealed that the SIS-containing TEPS had a significantly greater ISR than both spheroid- and single cell-containing TEPS ($p < 0.05$) but no difference was observed between the latter two groups ($p = 0.942$).

Figure 4. Induced insulin secretion tests

were performed on the same SIS-containing constructs on days 7 and 14 relative to encapsulation in 2% agarose. Constructs were incubated in basal medium for two hours, then the medium was changed to basal medium containing 2% MH for two hours. Induced insulin secretion is expressed as the percentage of insulin secreted during MH stimulation relative to that secreted during the basal period (mean $\pm$ SD). On days 7 and 14, the SIS-containing TEPS had a significantly higher induced secretion response over basal conditions (One-way ANOVA, $p < 0.05$), but the other two groups failed to display significant induction on both days. Also on day 7, the SIS group was significantly different than both single cell and spheroid groups, but these differences were lost on day 14.

Table I
Tissue Engineered Pancreatic Substitutes Evaluated in this Study

Description	Schematic	Picture
GLUTag-INS single cells in inner agarose disk encased in cell-free agarose
GLUTag-INS cells pre-aggregated into spheroids in inner agarose disk encased in cell-free agarose
GLUTag-INS cells on SIS encased in cell-free agarose

Table II

Comparison of Insulin Secretion Rates of GLUTag-INS cells on Tissue Culture Plastic and SIS.

	Day 2			Day 7		
	ISR ($\mu\text{U}/(10^6 \text{ cells} \cdot \text{hr})$)		IF	ISR ($\mu\text{U}/(10^6 \text{ cells} \cdot \text{hr})$)		IF
	Basal	Induced		Basal	Induced	
Tissue Culture Plastic	6.6 ± 1.19	$10.0 \pm 1.29^{*,\#}$	153%	$11.9 \pm 1.69^{\dagger}$	$40.2 \pm 2.17^{*,\dagger,\#}$	339%
Unencapsulated SIS	6.0 ± 0.60	$18.6 \pm 2.79^{*,\#}$	310%	$7.6 \pm 2.01^{\dagger}$	$26.1 \pm 1.05^{*,\dagger,\#}$	341%

Rates were normalized to the total number of cells measured by DNA content at each time point. Values are mean $\pm$ SD, n=3.

* Different than basal value on the same day for the same group ($p < 0.05$).

$\#$, $\dagger$, $\dagger^{\#}$ Different than other group at a given day and condition ($p < 0.05$).