Composite Islet-Endothelial Cell Grafts: A Novel Approach to Counteract Innate Immunity in Islet Transplantation

Ulrika Johansson*, Graciela Elgue, Bo Nilsson and Olle Korsgren

Department of Radiology, Oncology and Clinical Immunology, Division of Clinical Immunology, Uppsala University, Sweden

*Corresponding author: Ulrika Johansson, ulrika.johansson@klinimm.uu.se

An instant blood-mediated inflammatory reaction (IBMIR) is elicited when islets come in contact with blood after intraportal transplantation. In contrast, endothelial cells (EC) readily tolerate contact with blood. A conceivable strategy to overcome IBMIR would be to create composite islet-EC grafts. Human islets were cocultured with primary human aortic endothelial cells (HAEC) for 2–7 days to obtain 50–90% coverage. HAEC-coated islets were exposed to ABO-identical blood and analyzed with regard to clotting time, signs of inflammation and cell infiltration. Composite islet-HAEC graft survival was assessed after transplantation to athymic (nu/nu) nude mice. Exposed to blood, HAEC-coated islets induced less activation of coagulation and complement compared to control islets. Also, platelet and leukocyte consumption in blood was decreased. Clots with entrapped HAEC-coated islets showed less infiltration of CD11b+ cells. The extent of protection correlated to the level of HAEC coverage. Transplanted composite grafts stained positive for insulin and PECAM-1 demonstrating presence of both islets and HAEC within the islet graft 7 weeks after transplantation. Composite islet-HAEC grafts reduce all components of IBMIR. Refinement of the technique will allow introduction of composite islet-EC grafts in clinical islet transplantation, using autologous EC expanded in vitro and kept frozen until allogeneic islets become available for that specific recipient.

Key words: Endothelial cells, human, islets of Langerhans

Abbreviations: AEC, 3-amino, 9-ethyl-carbazole; C3a, Complement3a; CD, cluster of differentiation; EC, endothelial cells; ELISA, enzyme-linked immunosorbent assay; HAEC, human aortic endothelial cells; IBMIR, instant blood-mediated inflammatory reaction; IDDM, insulin-dependent diabetes mellitus; IEQ, islet equivalents; PBS, phosphate-buffered saline; PVC, polyvinyl chloride; SEM, scanning electron microscopy; SEM, standard error of the mean; TAT, thrombin anti-thrombin; VEGF, vascular endothelial growth factor.

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Introduction

An innate inflammatory reaction occurs when isolated islets of Langerhans come in contact with fresh human blood at the time of intraportal infusion in clinical islet transplantation. This process, described as an instant blood-mediated inflammatory reaction (IBMIR), may account for immediate islet destruction and the apparent need for islets isolated from multiple donors to achieve insulin independence after islet transplantation (1). Isolated human islets synthesize and secrete tissue factor (TF), a member of the cytokine receptor superfamily type-1. TF expressed on the islet surface at the time of transplantation appears to be the main trigger of IBMIR (2). This illustrates the importance of the interplay between the tissue to be transplanted and the innate immune response awakened in the recipient. IBMIR is characterized by a number of events including platelet consumption, activation of the coagulation and complement cascade systems, and leukocyte infiltration (1,3). The thrombin inhibitor, melagatran, inhibits IBMIR in an in vitro blood loop model (4). Similar beneficial effects were obtained using an antibody against TF or inactivated factor VII as a means to block TF-induced coagulation (2). Taken together, prevention of IBMIR may considerably reduce islet destruction. However, a major disadvantage with the approaches described above is the systemic side effect in the recipients of impaired coagulation homeostasis. This is especially important at the time of transplant during which the transhepatic catheter is introduced into the portal vein, substantially increasing the risk of bleeding. An alternative approach to inhibit IBMIR is to create a biologically active surface on the islets that will not trigger IBMIR and still allow for intact and functioning islets. Under normal conditions, endothelial cells (EC) are the only type of cell that can endure direct contact with blood. EC coating of the islets prior to transplantation is a principally new approach, which could create a biocompatible surface allowing contact with blood. Endothelial cells take part in the multistep process of angiogenesis that includes
pericellular proteolysis, migration and proliferation (sprouting), which finally resembles capillary structures and maturation of blood vessels. Human islets suffer from hypoxia after the isolation process, and express high levels of vascular endothelial growth factor (VEGF) (5). Both VEGF and FGF are involved in the process of vascularization of islets (6). Linn et al. showed that donor EC within the islets persist after transplantation and that they become integrated to form new microvessels (7). It is hypothesized that EC coverage of the islets prior to transplantation could abrogate IBMIR and enhance the process of revascularization.

A novel technique to coat isolated human islets with primary human aortic endothelial cells (HAEC) is described, with a consistent coating of the islet surface to 50–90%, irrespective of the purity of the islet preparation. Coated islets were evaluated with regard to specific function and potential to sustain contact with blood in an in vitro blood loop model. Composite islet-HAEC survival was studied after transplantation in athymic (nu/nu) mice.

Material and Methods

Isolation of islets of Langerhans

Human islets of Langerhans were isolated at the Division of Clinical Immunology at the University of Upsala using a modified semi-automated digestion-filtration methods (8–10). The isolation was followed by purification on a continuous density gradient in a refrigerated COBE 2991 centrifuge (COBE Blood Component Technology, Lakewood, CO). The pancreases were obtained from donors after consent obtained via a national registry or from the relatives of the diseased person. After isolation and purification the islet preparations were placed in untreated Sterilin petri dishes (Tamro Med. Termometer Fabrik, Sweden) supplemented with LSGS kit (GTF, Göteborgs Termometer Fabrik). The pancreases were washed three times in PBS and finally suspended in 100 mL PBS. The extent of HEAC coverage of the islet surface was evaluated by light microscopy using an ocular redical after staining with diphenylthiocarbazone. The purity of the islet preparations used varied from 40 to 95%.

Organs from 14 donors, nine with blood group A, three with blood group B, one with blood group AB and one with blood group O were used. The islets from the blood group AB, O and one from the B donors were used for two experiments. The islets from one donor were divided into multiple experimental groups: HAEC-coated and untreated islets. The HAEC-coated islets were subsequently divided into two groups depending on the coating results (50% or 90%).

In vitro function test

Untreated islets and islets coated with HAEC obtained from the same donor were cultured in three different media: (1) M200 (GTF, Göteborgs Termometer Fabrik, Sweden) supplemented with LSGS kit (GTF, Göteborgs Termometer Fabrik), (2) DMEM supplemented with 10 mM Hepes buffer ( GibCO BRL, Invitrogen), 10 mM Penicillin Streptomycin (GIBCO BRL, Invitrogen) and 10% (v/v) heat-inactivated human serum and (3) CMRL (GIBCO BRL, Invitrogen) supplemented with 10 mM nicotinamide (Sigma Chemicals), 10 mM Hapes buffer ( GibCO BRL, Invitrogen), 0.25 μg/mL fungizone (GIBCO BRL, Invitrogen), 50 μg/mL gentamicin (GIBCO BRL, Invitrogen), 2 mM L-glutamine (GIBCO BRL, Invitrogen), 10 μg/mL Ciprofloxacin (Bayer AG, Germany) and 10% (v/v) heat-inactivated human serum. Islet-specific function was evaluated in a dynamic perfusion system. All experimental islets were challenged with two glucose concentrations (first in 1.67 mmol/L and then in 16.7 mmol/L). Fractions were collected in 6-min intervals over 120 min and analyzed for insulin content using a commercial insulin enzyme-linked immunosorbent assay (ELISA) kit, Mercodia Insulin ELISA (Mercodia, Sweden).

Cell culture

HAEC (Cascade Biologics, USA) were cultured using M200 culture medium with an LSGS supplement kit (GTG, Göteborgs Termometer Fabrik). Cells grew to confluence in 75 cm² flasks (2–4 days). Medium was changed every second day. HAEC were used from passages 6–16.

Cell preparations

1.5–2.0 million HAEC (1–2, 75 cm² confluent flasks) were harvested using 1x trypsin-EDTA ( GibCO BRL, Invitrogen). The HAEC were washed with HAEC medium, centrifuged at 400 g for 3 min using a 14 mL Falcon tube, (BD Biosciences, Becton Dickinson Labware, USA) and counted in a Bürker chamber. After washing the HAEC were suspended in M200 medium to 3–5 × 10⁶ cells/mL.

After harvest the HAEC were stained using the green fluorescent cell linker dye PKH-67, Sigma MINI-67 membrane label (Sigma-Aldrich AB) according to the manufacturer’s instructions.

Endothelial cell coating of islets of Langerhans

Islets of Langerhans, 10 000–30 000 islet equivalents (IEQ) were mixed together with 0.3–0.5 million HAEC in 250 μL M200 culture medium. The HAEC and islets incubated at 37°C for 1–2 h in 10 mL tubes (Tamro Med lab AB) and were mixed gently once during the incubation. After incubation the islets and HAEC were transferred to 15 cm² petri dishes (Stellen, United Kingdom) treated to prevent adherence and cultured for 2–7 days. The extent of HEAC coverage of the islet surface was evaluated by light and fluorescent microscopy and confirmed by immunohistochemistry.

Ilet perfusion with human blood in heparinized polyvinylchloride tubing

After 2–7 days of co-cultivation with HAEC, the islets were 50–90% covered with HAEC. They were then collected to be perfused with human blood in an in vitro blood loop model.

HAEC-coated and untreated islets were exposed to fresh human ABO-compatible blood using a loop system consisting of PVC (polyvinyl chloride) tubings with a heparinized inner surface. The loop system was placed on a rocking apparatus in an incubator at 37°C to simulate a blood flow inside the PVC tubing (6).

Four loops with 7 mL of human blood were prepared in each experiment. To one of the loops 2–5 μL (corresponding to 2–5000 IEQ) untreated islets were added. To the second loop 2–5 μL of HAEC-coated islets were added, and to the remaining two loops 100 μL phosphate-buffered saline (PBS) was added and used as negative controls. Before the islets were put into the loops, they were washed three times in PBS and finally suspended in 100 μL PBS. The loops contained 7 mL of fresh ABO-compatible blood leaving an air volume in the tubing. The loops were then closed with a polypropylene connector and placed in the 37°C incubator on the rocking apparatus. The islet perfusion was performed for 60 min. One milliliter blood samples were collected before the islets were put into the loops, and at 5, 15, 30 and 60 min to determine the time course of the events occurring in each loop. These 1 mL samples were collected and placed in tubes containing 50 μL 0.2 M NaEDTA,
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pH 7.4. From these tubes, 100 μL samples were transferred to eppendorf tubes to determine platelet, lymphocyte, monocyte and granulocyte count (Cell particle counter Technicon). The remaining 900 μL was centrifuged at 13,000 g for 20 min and the plasma was collected and stored at −70 °C for later ELISA analysis of thrombin anti-thrombin (TAT) and complement3a (C3a) (11). After 60 min, when the perfusion experiment was stopped, blood clots were collected. The clots were embedded in OCT compound (Tissue Tek, Sakura Finetech Europe B.V.) snap-frozen in isopentane with dry ice, and stored at −70 °C until histochemically evaluated.

**Clotting time measurement**

Whole blood was drawn from healthy volunteers using Vacutainer™ tubes containing citrate. Whole blood (1 mL/islet sample) was incubated with 100 μL PBS either alone or with 2 μL of untreated islets, or 2 μL HAEC-coated islets. The experimental islets were cultured in different medium (M200, DMEM and CMRL supplemented as described previously) coated or untreated as a control in polypropylene sample cups in a ReoRox™ rheometer (Global Haemostatysis International, Gothenburg, Sweden). After adding 20 μL of 1-M CaCl2 to each cup, the coagulation reaction started. Every 6 sec, the cup was set into free horizontal oscillation around its vertical axis, and the damping and frequency of the oscillation were registered. Clotting time was identified as the point of maximal damping.

**Transplantation of endothelial cell coated and untreated islets into C57bl/6J nu/nu mice**

HAEC-coated or untreated islets were transplanted into non-diabetic C57BL/6J nude athymic mice under the kidney capsule as previously described (12). A total of eight mice were transplanted. Three mice with one graft containing HAEC-coated islets and five mice with two separate grafts, one composed of untreated islets and the other composed of HAEC-coated islets. After 7 weeks, the animals were sacrificed and the grafts were harvested with a margin of 5 mm of adjacent kidney tissue. Immunohistological evaluation using light and fluorescent microscopy was then performed as described below.

**Morphological evaluation**

Blood clots saved from loop experiments and transplanted graft biopsies were sectioned in cryotome, Zeiss HM560, air-dried, fixed in 100% cold acetone for 7 min and stored (−70 °C) in case they were not stained immediately. Blood clot sections were stained for CD41, (16589, R&D Systems, USA) and CD11b, (M0741, DakoCytomation, Denmark). Sections from the transplanted grafts were stained for mouse anti-endothelial cells: using phycoerythrin, CD146-PE (MAB16985H, clone P1H12, Chemicon International, USA). Portions of the islets were collected for immunohistochemical evaluation after having been coated with HAEC. Islets were fixed in 4% paraformaldehyde for at least 12 h and then transferred to 70% ethanol in glass tubes (110653–10017, VWR International, Sweden). Islets were stained with phe-nol red to facilitate finding of the islets in the paraffin preparation. Dehydration was performed by washing the islets with increasing concentrations of ethanol (90%, 95%, 99.9%) and lastly with xylene, centrifuging between solutions. The xylene was removed, leaving just a drop. Paraffin was added gently on top of the islet pellet still in the glass tube and the embedded islets were then incubated at 65 °C over night. During the incubation paraffin, blind blocks were made. After the incubation, embedded islets were removed from the glass tube using a warm water bath to liquify the paraffin leaving the islets still at the bottom of the tube. Then the islet embedded paraffin block and the blind block was melted together. Islets were sectioned (Leica Jung RM 2055) into 4 μm thick slices and air dried at 37 °C over night. The sections were kept at room temperature until dewaxing. They were then fixed and stained using with a peroxidase anti-peroxidase staining technique for insulin (A0564, guinea pig anti-insulin, DakoCytomation) with a secondary antibody (K4002, goat anti-rabbit Envision, DakoCytomation). The sections were also stained for endothelial cells (goat anti-CD31, SC-1506, Santa Cruz Biotechnology, USA) with a secondary antibody (donkey anti-goat, 705–035–147, Jackson ImmunoResearch, USA). Finally, the sections were developed in AEC (3-amino, 9-ethyl-carbazole) and counter-stained with haematoxylin (HistoLab products AB, Sweden).

**Statistical analysis**

Data are presented as means ± SEM. Mean values were compared using a paired t-test with significance set at α = 0.05.

**Results**

**Endothelial cell coating**

After 2 h of incubation of the human islets with HAEC, HAEC adhered to the islets’ surface. Subsequently the HAEC flattened and started to grow on the matrix inherently provided by the islet. After a culture period of 2–7 days in the various culture media tested, 50–90% coverage of the islets could be seen (Figure 1A–D).

**Dynamic insulin release**

Insulin release capacity of the islets after coverage with HAEC was evaluated in a dynamic perfusion system after stimulation with glucose. Insulin release was severely
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Figure 2: Islet function tested in a dynamic perfusion system. (A) (♦) Untreated islets were cultured in CMRL 4 days or (◇) cultured in CMRL for 2 days and then cultured in M200 for 2 days (n = 7). (B) Islets coated with HAEC and untreated islets from four (n = 4) different pancreata were cultured in the following culture media for 3–4 days; (▲) untreated islets cultured in CMRL, (◇) HAEC-coated islets cultured in CMRL, (□) untreated islets cultured in M200, (×) HAEC-coated islets cultured in M200, (◇) untreated islets cultured in DMEM and (▲) HAEC-coated islets cultured in DMEM. The figure shows insulin release after stimulation with glucose (1.67, 16.7 and 1.67 mmol/L) as indicated in the figure.

Figure 3: Platelet counts in ABO-compatible blood after exposure to islets in the loop blood system. Data are means ± SEM. (▲) PBS control with no islets, (□) 90% islet coverage with HAEC, n = 3 (◇) 50% islet coverage with HAEC, n = 4 and (●) uncoated islets, n = 7. * p < 0.05 when compared with uncoated islets.

HAEC coating affects islet-induced clotting

Control islets and HAEC-coated islets were cultured in various media (CMRL, M200 and DMEM) and were evaluated with respect to clotting time using a ReoRox™ rheometer. Clotting time was identified as the point of maximal damping and showed that HAEC-coated islets had a delayed clotting time compared to untreated islets, irrespective of the culture media used; 60 ± 16% in CMRL (p = 0.001, n = 7), 39 ± 9% in M200 (p = 0.01, n = 6) and 9 ± 27% in MDEM (p = 0.87, n = 2). Data are presented as mean percentage of clotting time (((time HAEC coated samples/time PBS control)/ (time control samples/time PBS control))∗100) ± SEM.

Exposure of human islets to fresh human ABO-compatible blood in the blood loop model

Untreated, control islets caused massive clotting when in contact with ABO-identical blood. In parallel with coagulation activation, a considerable drop in platelet count was found (Figure 3). In marked contrast, HAEC-coated islets tolerated contact with blood better than untreated islets. The protective effect correlated to the level of HAEC coverage. Platelet counts in blood mixed with islets coated to 90% or more with HAEC remained in the same range as in control loops without any islets (Figure 3). However, small micro-clots were found in all loops. The size of the clots correlated to the consumption of platelets.

TAT values in blood retrieved from loops containing islets were markedly increased compared to the control loops without islets. Islets covered to 50% with HAEC still activated coagulation, but TAT values were reduced by 50%. In loops containing islets covered to 90% or more with HAEC, a corresponding decrease in TAT values was observed (Figure 4A).
Figure 4: Thrombin anti-thrombin (A) and complement 3a (C3a) (B) were determined in ABO-compatible blood retrieved from the loop blood system. Samples were taken at 0, 5, 15, 30 and 60 min. Data are means ± SEM. (▲) PBS control with no islets added, (□) 90% islet coverage with HAEC, (●) 50% islet coverage with HAEC and (■) uncoated islets. (n = 7; n = 4 = 50%, 3 = 90%). * p < 0.05 when compared with uncoated islets.

Untreated islets induced complement activation upon blood contact (Figure 4B), whereas complement activation in blood loops containing HAEC-coated islets was reduced to background values.

Untreated, control islets caused a near complete consumption of platelets, granulocytes and monocytes. The effect on platelet consumption was most pronounced (Figure 3). HAEC-coated islets induced markedly less consumption of blood cells. Lymphocyte counts remained almost intact in all loops containing islets when compared to control loops without islets (Table 1).

Immunohistochemistry
Human islets, islets co-cultured with HAEC, as well as islets entrapped in clots from the blood-loop perfusions were examined using immunohistochemistry. Islets covered with HAEC showed an intense staining of CD31 in cells found enveloping the islets (Figure 1E). These cells were forming a confluent layer encircling either 50% or 90%, i.e. islets covered to 50%, or 90% or more of the islet periphery. In the blood clots CD41, positive platelets were found surrounding both controls islets and islets covered with HAEC. HAEC-coated islets showed less infiltration of CD11b positive cells when compared with uncoated islets, which showed a massive infiltration even in the core of the islets (Figure 5).

Islet transplantation to athymic mice
Animals were sacrificed 7 weeks after transplantation and the islet grafts retrieved and processed for immunohistochemistry. Morphological evaluation demonstrated successful engraftment and survival of both HAEC-coated and untreated islets, as evidenced by insulin staining. Grafts composed of HAEC-coated islet, but not control islets (picture not shown), stained positive for endothelial cells using an antibody specific for human HAEC. The HAEC were found both within the islet tissue and in the connective tissue between the implanted islets in the graft. In some instances vessel-like structures within the graft stained positive. On no occasion were any positive cells found in the mouse kidney parenchyma (Figure 6).

Discussion
A novel technique to create composite islet-EC grafts is presented. The survival of islets transplanted intraportal depends on the interaction of the islet surface with blood. EC are the only non-blood cell that can endure contact with blood. Human blood in contact with other tissues induces a prompt activation of the cascade systems and the cells normally present in blood. Based on these notions, it was our intent to create a biologically active surface composed of EC on isolated human islets. A specially designed culture system was created based on the observation that EC rapidly divide and grow in an anchor-dependent manner until a confluent layer is formed. In contrast, islets are cultured free-floating. By utilizing a culture system containing plastics not allowing cellular attachment, the only surface on which the endothelial cells could attach was that of the islet. HAEC attached to the islets subsequently grew until they reached confluence. This enabled us to create a culture system, based on the inherent characteristics of the two cell types, allowing spontaneous formation of composite islet-HAEC grafts over a period of 2–7 days.

Activated EC are procoagulant, promote inflammation, and stimulate leukocyte rolling and migration. This must be considered when designing the technique to create composite islet-EC grafts. Islets covered with activated EC could be in a worse situation when compared with control islets. The technique to create composite islet-EC grafts is designed...
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Table 1: Cell count of fresh human ABO-compatible blood cells in contact with human islets coated with human aortic endothelial cells or untreated islets after 60 min

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<td>50% coated islets</td>
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<td>90% coated islets</td>
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Data are means ± SEM. *Control contains blood and PBS, but no islets. †p < 0.05 when compared with findings in loops without islets.

Figure 5: Both HAEC-coated islets (A and C) and uncoated islets (B and D) were entrapped in clots, although HAEC-coated islets clots were microscopic. Platelets (CD41 positive) were found adhering to the islet surface of both HAEC-coated and uncoated islets (A and B). HAEC-coated islets were less infiltrated with CD11b positive cells (C) when compared with untreated islets (D).

Figure 6: Graft survival after 7 weeks. Composite HAEC-islet graft stained with haematoxylin (A) or with phycoerythrin, an antibody specific for human endothelial cells, using immunofluorescent microscopy (B). Human endothelial cells were found scattered throughout the composite HAEC-islet graft.

In the blood loop perfusion model, platelet activation occurred to some extent when HAEC-coated islets were exposed to blood but this largely correlated to the level of HAEC coverage. When islets were covered with HAEC up to 90%, only micro-clots were found in the blood loops. Islets covered up to 50% with HAEC displayed a delay to minimize manipulation of the EC and to allow spontaneous covering of the islets.

Using endothelial cells as a biologically active surface on isolated islets of Langerhans, a new principle for inhibition of adverse reactions in transplantation is introduced. To avoid inappropriate activation of the coagulation cascade, EC express a number of directly or indirectly counteracting proteins, e.g. heparan sulfate. EC control and inhibit platelet aggregation by the release of a variety of substances such as prostacyclin, nitric oxide, ectoADPases, platelet-derived ADP, and thrombomodulin (13). In addition to this, EC express high levels of complement activation regulators, such as decay-accelerating factor, MCP and CD59 (14). All these regulatory systems are most likely absent on isolated islets, which explain the development of the instant blood-mediated inflammatory reaction when islets come in contact with ABO-identical blood.
in clotting up to 30 min; however, between 30 and 60 min clotting was observed. Similarly, in the ReoRox model, HAEC-coated islets demonstrated a delay in clotting times compared to untreated islets.

Formation of the TAT complex, as a parameter to detect coagulation activation, was markedly decreased in blood exposed to HAEC-coated islets when compared to untreated islets. Islets coated to 50% with HAEC resulted in a decrease of TAT values to approximately half of the value of uncoated islets, while 90% HAEC-coated islets induced levels that were less than 10% of the values observed with uncoated islets.

Complement activation observed when islets come in contact with ABO-compatible blood mainly occur secondary to activation of the coagulation system (4). Complement split products such as C3a and C5a are some of the most chemoattractive substances known for leukocytes, and leukocyte infiltration in human islets exposed to blood correlate with complement activation. In the present study, a reduction of complement activation was observed as evidenced by a 50% decrease in the generation of C3a. This may be due to a local high expression of regulatory proteins of complement activation, i.e. DAF, MCP and CD59, on the EC covering the islets. Notably, EC-covered islets were infiltrated with only low numbers of leukocytes. This effect on complement activation may be of utmost importance in clinical transplantation as a bridge between the innate and the specific immune system.

The level of HAEC coverage varied between different experiments (ranging between 50 and 90%), indicating that the procedure needed further optimization. The preparation of the EC prior to co-culture with the islets, which included trypsin digestion, could possibly affect the EC and influence their ability to migrate and attach to the islet surface. The isolated islets may differ in ways that could affect endothelial cell adherence, e.g. the amount of collagen (the EC matrix in the system) remaining on the islet surface. Also, insulin has been reported to exert an inhibitory effect on the expression of ICAM-1 on endothelial cells (15,16). However, insulin has no known effect on HAEC cell proliferation (16).

Platelets, which adhere to the HAEC-coated islets exposed to blood, might stimulate the EC to grow due to release of several growth factors (17,18). Therefore, a micro-clot surrounding the newly implanted islet situated in a small branch of the portal vein may not be a disadvantage in clinical islet transplantation, but could promote revascularization and islet engraftment.

The function of the islets after HAEC-coating was reduced when cultured in HAEC media, M200, as evidenced by impairment of glucose induced insulin release. This effect was most likely attributed to the culture media applied and not due to the endothelial cell cover, since control islets, without HAEC, displayed the same functional impairment. This led us to test several different culture media. CMRL 1066 and DMEM sustained both HAEC survival and growth with only marginal effect on specific islet function. Also, islet function recovered when islets originally cultured in M200 were transferred to CMRL 1066.

Coverage of the islets with recipient EC in vitro prior to transplantation could enhance revascularization. Hypoxia in the newly transplanted islet cells results in a release of VEGF and other angiogenetic factors. Studies have shown that after 2–4 days in culture intraislet EC start sprouting and that these EC seem to mix with endothelial cells of recipient origin post-transplantation (7). This process could be markedly enhanced by the here-described EC coverage of the islets prior to transplantation.

Recipient endothelial cells could be retrieved from a small venous segment prior to transplantation, cultured to increase their number, and cryopreserved for later use. Subsequently, at the time when a suitable pancreas donor is identified, the EC could be thawed and co-cultured with the isolated islets for 2–4 days, a clinically acceptable time frame. Coverage of isolated human islets with syngenic EC could abrogate IBMIR, potentially enhance revascularization and decrease the rejection process.

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