Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Perfluorohexyloctane improves long-term storage of rat pancreata for subsequent islet isolation

Heide Brandhorst,[1] Bastian Theisinger,[2] Hideyuki Yamaya,[1] Johanna Henriksnäs,[3] Per-Ola Carlsson,[3,4] Olle Korsgren[1] and Daniel Brandhorst[1]

1 Department of Oncology, Radiology & Clinical Immunology, Uppsala University, Uppsala, Sweden
2 Novaliq GmbH, Heidelberg, Germany
3 Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
4 Department of Medical Sciences, University Hospital, Uppsala, Sweden

Keywords
islets, oxygenation, pancreas, preservation, rat, transplantation.

Correspondence
Daniel Brandhorst, Department of Oncology, Radiology & Clinical Immunology, University Hospital, Dag Hammarskjölds väg 20 (C11), 75185 Uppsala, Sweden. Tel.: +46 70 4250636; fax: +46 18 61 10222; e-mail: daniel.brandhorst@klinimm.uu.se

Received: 28 February 2009
Revision requested: 28 April 2009
Accepted: 11 May 2009

doi:10.1111/j.1432-2277.2009.00905.x

Summary

Pancreas oxygenation by means of the hyperoxygen carrier perfluorodecalin (PFD) has been established to prevent ischemically induced damage from cold-stored pancreata. However, large-scale studies did not confirm the promising results that had been observed in smaller donor populations. This study assessed whether islet isolation from pancreata stored for prolonged periods can be improved by utilizing the new oxygen carrier perfluorohexyloctane (F6H8) characterized by lower gravity and higher lipophilicity than PFD. Subsequent to 24 h of storage in either oxygenated PFD or F6H8, the rat pancreata were assessed for the intrapancreatic partial oxygen pressure (pO_2) and subsequently processed with current standard procedures. The intrapancreatic pO_2 was nearly identical in rat pancreata stored either in PFD or F6H8. Nevertheless, rat islet isolation outcome was significantly increased in terms of yield, integrity, *in vitro* function and post-transplant outcome after transplantation in diabetic nude mice when F6H8 was used as oxygen carrier. This proof-of-concept study demonstrated in rats that islet isolation performed after long-term storage of oxygenated pancreatic tissue can be significantly improved if PFD was replaced by F6H8.

Introduction

Pancreas oxygenation by means of the hyperoxygen carrier perfluorodecalin (PFD) has been established to prevent ischemically induced damage from cold-stored pancreata [1,2] or to resuscitate predamaged pancreata subsequently transplanted as vascularized organs or isolated islets [3,4]. Furthermore, preoxygenated PFD was utilized to improve islet isolation outcome from the pancreas of marginal donors [5]. However, recent observations in 166 and 200 human pancreata about the effect of preoxygenated PFD on subsequent islet isolation [6,7] do not confirm the promising results observed in smaller populations of donors [4,8,9]. Experiences in porcine islet isolation indicate as well that the capacity of oxygenated PFD to maintain islet viability and integrity during cold storage is limited [10,11] when compared with canine islet isolation [12]. Likewise, a prospective study in pigs demonstrates clearly that oxygenated PFD does not improve yield and post-transplant function of islets isolated from pancreata pre-exposed to 30 min of warm ischemia [13] in contrast to observations made in dogs [14].

Doubts about the efficiency of PFD as hyperoxygen carrier were additionally reinforced by the remarkable finding in pigs that only 15% of total pancreatic volume is efficiently supplied by oxygen during PFD storage [15]. Based on this observation it was calculated that <20% of a human pancreas is penetrated by oxygen delivered by PFD [16]. These findings are in conflict with generally

accepted data from autotransplanted canine pancreata suggesting a correlation between pancreas oxygenation, tissue oxygen pressure, adenosine triphosphate (ATP) generation and graft function of ischemically damaged pancreata [17]. The contradiction may be explained by species-dependent differences with regard to pancreas size, firmness and texture, which is particularly relevant for pancreata from humans, often covered by obstructive amounts of fat [18], or may be related to an artifact occurring during intrapancreatic oxygen measurement [15]. On the other hand, it needs to be explored whether the hydrophobic and lipophobic character of PFD prevents oxygen penetration into the pancreatic core.

For the purpose of clarifying this question, this investigation was performed as a proof-of-concept study in rats to assess the efficiency of perfluorohexyloctane (F6H8) utilized as oxygen carrier prior to subsequent islet isolation from long-term stored pancreata and to compare it with PFD, representing the currently used standard for pancreas oxygenation. F6H8 is a semifluorinated alkane, which has nearly the same oxygen-dissolving capacity as PFD and is characterized by physical and chemical properties that may be beneficial for an efficient oxygen supply of stored pancreata. F6H8 is used in clinical-grade purity as temporary tamponade agent for surgical ophthalmology and has been found to be well tolerated in long-term observations after eye surgery in 125 patients [19–21].

Materials and methods

Pancreas procurement

Rat pancreata for isolation and transplantation experiments were obtained from male Lewis rats (Harlan, Hannover, Germany) weighing 350–400 g. All animal studies were approved by local ethics committees and complied with the specific national laws and the Principles of Laboratory Animal Care. Prior to resection, pancreata processed for cold storage or oxygenation for 24 h were intraductally flushed with 3 ml of Kyoto solution (kindly provided by Prof Nakamura, Department of Thoracic Surgery, Kyoto University, Kyoto, Japan) supplemented with 5 mM of adenosine (Sigma-Aldrich AB, Stockholm, Sweden). In comparison to University of Wisconsin solution (UWS) this organ preservation solution is characterized by a reversed sodium-potassium ratio and the addition of trehalose and gluconate whereas raffinose, lactobionate, $MgSO_4$, allopurinol, glutathione and adenosine are omitted. The observation that these modifications resulted in a lower inhibitory effect on subsequent enzymatic pig pancreas digestion when compared with UWS [22] could be confirmed clearly by preliminary experiments in rats (data not shown).

Oxygenation by the one-layer method was performed as previously described [11] by completely immersing Kyoto-distended rat pancreata for 24 h in 25 ml of the hyperoxygen carrier PFD or F6H8 (Novaliq GmbH, Heidelberg, Germany) precharged with 100% oxygen until a pO_2 of >670 mmHg was reached. After storage, pancreata were washed in cold Hank's balanced salt solution (HBSS, Invitrogen, Stockholm, Sweden) to remove any adherent PFD or F6H8.

Intrapancreatic oxygen determination

The pO_2 in rat pancreata was measured after 24 h of cold ischemia, defined as the interval between intraductal pancreas perfusion and initiation of the dissection procedure at the islet laboratory, utilizing modified Clarke-type microelectrodes (Unisense, Aarhus, Denmark) [23]. The electrodes (outer tip diameter 2–6 μm) were inserted into three to five different locations of each assessed pancreas aiming to hit the corresponding core of the organ by means of a micromanipulator. The mean of the oxygen tension values in one pancreas was considered to be one experiment.

Rat islet isolation

Rat pancreata were intraductally distended with 10 ml of cold HBSS supplemented with 0.4 mM of the trypsin inhibitor Pefabloc (Serva, Heidelberg, Germany) and 20 PZ-U of collagenase NB 1 (Serva). According to previous observations in pig pancreas, preservation [10] activity of neutral protease NB (Serva) adjusted to 0.35 DMC-U for immediately processed organs was reduced to 0.15 DMC-U for stored pancreata. Rat islets were isolated and purified as described previously [24]. During digestion, samples were assessed continuously under the microscope to monitor pancreas dissociation. After completion of dissociation, the digest was washed and incubated in UWS 30 min prior to purification on a Ficoll-Na-diatrizoate gradient (Biochrom, Berlin, Germany). Purified islet fractions were pooled for subsequent characterization in supplemented CMRL 1066 (PAA, Cölbe, Germany).

Rat islet characterization

Subsequent to purification, islet yield of dithizone-stained samples was evaluated in duplicate and converted to islet equivalents (IE) with an average diameter of 150 μm [25]. Morphologic integrity of islets was estimated using a fragmentation index, which was calculated as the ratio of islet particle number (IN) over IE.

In vitro function was determined during static incubation of islets cultured overnight at 37 °C. Twenty hand-selected islets with an average diameter of 150–200 μm were incubated in duplicate for 120 min in CMRL 1066 supplemented with 2.8 or 20 mmol/l glucose. Insulin release and intracellular insulin content was measured utilizing an enzyme immunoassay specific for rat insulin (Mercodia, Uppsala, Sweden). The glucose-stimulated insulin secretion was expressed as stimulation index, calculated as the ratio of stimulated to basal insulin release [26]. Islet viability was simultaneously examined by the trypan-blue exclusion assay [27]. The ATP content of freshly isolated islets was measured in duplicate samples each assessed in quadruplicate by the Luciferin–Luciferase reaction utilizing a commercially available assay (Roche, Mannheim, Germany).

In vivo function was assessed in NMRI nude mice (Harlan, Hannover, Germany) rendered diabetic by a single intravenous injection of 240 mg/kg streptozotocin (Sigma-Aldrich AB, Stockholm, Sweden) 4 days prior to transplantation of an aliquot corresponding to 700 IE beneath the kidney capsule. The donor–recipient ratio was 1:1. Blood samples were taken from the tail vein of recipients and analysed utilizing a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA, USA). Pre-transplant, the nonfasting serum glucose levels of all recipients exceeded 350 mg/dl. After transplantation nonfasting serum glucose levels <200 mg/dl were defined as normoglycemic and considered as graft function. Thirty-two days post-transplant, nephrectomy of graft-bearing kidneys was performed to demonstrate immediate return of hyperglycemia.

Data analysis

Statistical analysis was performed utilizing SPSS software (Version 11.04 for MacIntosh, SPSS Inc., Chicago, IL, USA). All values were expressed as means ± standard error of the mean (SEM). Statistical comparison was performed by Mann–Whitney test. Graft function (time of normoglycemia) was analysed utilizing the log-rank test. Significance was expressed as *P*-value and considered for $P < 0.05$. *P*-values >0.05 are termed nonsignificant (NS).

Results

Intrapancreatic oxygen determination

After 24 h of storage, the partial oxygen pressure (pO_2) in rat pancreata stored in either PFD or F6H8 was similar in both the experimental groups (87.6 ± 21.6 vs. 82.3 ± 25.2 mmHg, NS) and significantly higher than in pancreata that were assessed immediately after resection (10.0 ± 3.8 mmHg, $P < 0.05$, Fig. 1).

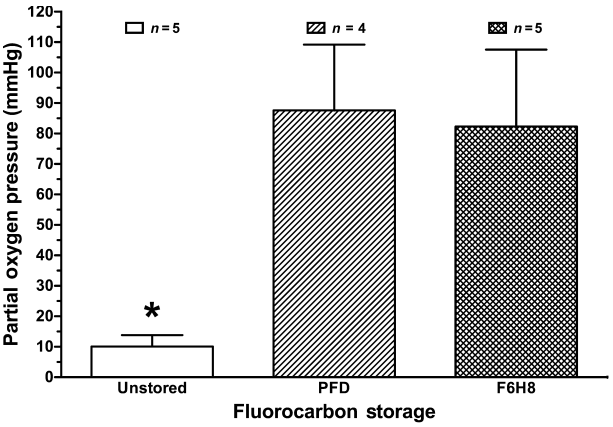

Figure 1 Intrapancreatic pO_2 measured in rat pancreata either immediately after resection (blank bar, $n = 5$) or after 24 h storage in oxygen-precharged perfluorodecalin (PFD) (hatched bar, $n = 4$) or F8H8 (crosshatched bar, $n = 5$). *$P < 0.05$ by Mann–Whitney test for unstored versus PFD and F6H8.

Islet isolation outcome

Islet isolation performed after 24 h storage in Kyoto solution failed completely and was discontinued after three failures (data not shown). The isolation outcome of the completed experimental groups is presented in Table 1. Digestion time was significantly prolonged in Kyoto-distended pancreata stored in PFD ($P < 0.05$) and F6H8 ($P < 0.001$) when compared with unstored organs that were distended with plain HBSS and a higher amount of neutral protease. Complete pancreas dissociation took significantly longer after F6H8 storage in comparison to PFD ($P < 0.001$). This did not affect islet yield isolated from F6H8-treated organs in relation to unstored pancreata. In contrast, storage in oxygenated PFD resulted in significant reduction of islet yield when compared with unstored ($P < 0.05$) and F6H8-stored organs ($P < 0.01$). Decreased islet yield after PFD storage was associated with increased islet fragmentation as expressed by the fragmentation index ($P < 0.05$ vs. unstored).

Table 1. Rat islet isolation outcomes.

Procurement	*n*	Dissociation time (min)	Yield (IE/pancreas)	Fragmentation index (IN/IE)
Unstored	10	15.0 ± 1.4*†	1588 ± 163*	0.55 ± 0.04*
PFD	10	19.6 ± 0.9†	1140 ± 103‡	0.72 ± 0.05
F6H8	13	24.1 ± 0.4	1516 ± 63	0.62 ± 0.04

IE, islet equivalent; IN, islet particle number.
*$P < 0.05$ vs. PFD; †$P < 0.001$, ‡$P < 0.01$ vs. F6H8.

Islet quality assessment

As demonstrated in Table 2, assessment of islet viability revealed a small but significant difference between islets isolated from unstored pancreata and organs preserved by PFD ($P < 0.01$) or F6H8 ($P < 0.05$). Pancreas storage in F6H8 for 24 h reduced the intracellular insulin content in comparison to islets isolated from unstored ($P < 0.01$) or PFD-stored pancreata ($P < 0.05$) but did not reduce the insulin-secretory capacity as determined by the stimulation index. In contrast, islets isolated after PFD storage expressed a significantly reduced stimulation index when compared with islets obtained from unstored and F6H8-preserved pancreata ($P < 0.05$). Oxygenation did not completely prevent reduction of intra-islet ATP content during pancreas storage for 24 h. However, this decrease did not reach statistical significance as shown in Table 2.

Islet functional capacity was assessed by transplantation into diabetic nude mice. As shown in Fig. 2 immediately isolated islets demonstrated a final graft function in 100% of recipients ($n = 5$) until nephrectomy of graft-bearing kidneys was performed at day 32 post-transplant. In contrast, only 50% of recipients transplanted with islets isolated from PFD-stored pancreata ($n = 8$) showed sustained transplant function ($P < 0.05$ vs. unstored) whereas implantation of islets isolated after F6H8 storage ($n = 10$) resulted in a final reversal of hyperglycemia in 80% of recipients ($P = 0.05$ vs. PFD).

Table 2. Rat islet characterization.

Procurement	*n*	Viability (%)	Insulin content (μU/IE)	Stimulation index (20/2.8 mm)	ATP content (pg/IE)
Unstored	6	98 ± 1	1079 ± 102	2.26 ± 0.52	2.20 ± 0.67
PFD	5	90 ± 2†	890 ± 101	1.25 ± 0.05*	0.98 ± 0.18
F6H8	5	93 ± 1*	626 ± 56†‡	1.71 ± 0.14‡	1.39 ± 0.22

IE, islet equivalent number.
*$P < 0.05$, †$P < 0.01$ vs. unstored; ‡$P < 0.05$ vs. PFD.

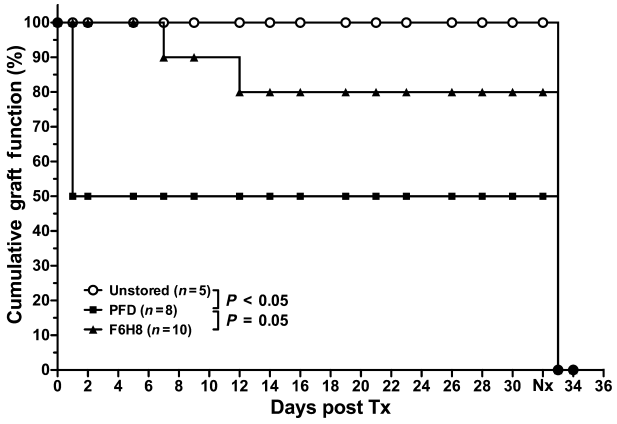

Figure 2 Cumulative graft function of 700 rat islet eqivalents isolated from pancreata either immediately processed (unstored, open circles, $n = 5$) or subjected to prior oxygenation for 24-h utilizing perfluorodecalin (PFD, filled squares, $n = 8$) or perfluorohexyloctane (F6H8, filled triangles, $n = 10$). Islets were transplanted beneath the kidney capsule of STZ-treated NMRI nude mice utilizing a donor–recipient ratio of 1:1. Graft removal through nephrectomy (Nx) was performed as indicated at day 32 post-transplant.

Discussion

The strategy to prevent ischemically induced damage in human donor pancreata during cold storage utilizing oxygen-precharged PFD for tissue oxygenation does not seem to improve islet isolation outcome when compared with simple storage in UWS as demonstrated in more than 360 donor pancreata [6,7]. Experiments in porcine pancreatic tissue indicated that only a marginal volume of pancreatic tissue is efficiently supplied with oxygen utilizing PFD [15]. This inefficiency can be explained by the inert character of PFD that prevents penetration into the pancreatic core, resulting in a limited oxygen transport into the tissue.

The solubility of PFD in native olive oil, a parameter for lipophilicity, is only 1.1%. In contrast, F6H8 reaches a solubility of 23.4% [28]. The lipophilic character of F6H8 can be attributed to the high number of carbon–hydrogen bonds, which are completely absent in PFD as shown in Fig. 3. In spite of this important difference, we did not observe a significant difference between rat pancreata preserved in PFD and in F6H8 with respect to the intrapancreatic pO_2 and the corresponding islet ATP content as a marker for oxidative glucose metabolization. This can be related to the small size of the rat pancreas, which allows oxygen penetration into the entire organ [16]. The dimensions of rat pancreata may also be responsible for the finding that the intrapancreatic pO_2 after storage in PFD or F6H8 was higher than in the freshly resected pancreata and exceeded even the pO_2 that was previously measured in native rat pancreta [23,29].

With respect to the increased pO_2 that was measured in PFD- as well as F6H8-stored rat pancreata, the islet

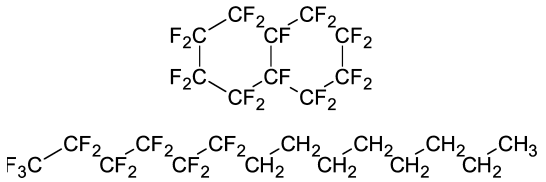

Figure 3 Formula of perfluorodecalin (top) and perfluorohexyloctane (bottom).

ATP content was unexpectedly low in comparison to unstored organs. According to a recently published study in long-term stored rat pancreata, which utilized a noninvasive technique of ATP measurement, the capacity of oxygenated PFD to induce oxidative ATP synthesis is limited to a period of 9–12 h [30]. This would explain the relatively low outcome of oxidative ATP generation after 24 h of storage. It can be speculated whether a supplementation of organ preservation solutions with fuels such as glucose and pyruvate would prevent or at least ameliorate energy exhaustion of long-term stored tissue.

Although almost similar values for intrapancreatic pO_2 and islet ATP content were measured in both PFD- and F6H8-stored rat pancreata, isolation outcome was significantly improved in terms of islet yield, integrity, *in vitro* and post-transplant function if F6H8 was used as oxygen carrier. One explanation for this finding is the high density of PFD of 1.93 g/cm^3 that can be attributed to the large fluor-to-carbon-ratio in the PFD molecule (Fig. 3). As a consequence, pancreata immersed in PFD are exposed to enormous pressure bearing the risk of serious injury as a result of uncontrolled enzyme discharge affecting islet integrity and function [31]. In contrast, the relatively low density of F6H8 of 1.35 g/cm^3 reduces significantly the buoyancy of incubated organs tissue when compared with PFD [32].

The partial replacement of fluor by hydrogen does not only reduce the gravity of F6H8 when compared with PFD, it also has the advantage to dissolve hydrophobic substances such as antioxidants or anti inflammatory reagents [33]. As ischemia seems to induce a general inflammatory response in human islets, which includes the expression of gene products such as tissue factor and macrophage-chemoattractant protein 1 [34], the option to deliver hydrophobic drugs represents a potential tool for islet protection [35].

In summary, this proof-of-concept study demonstrated in rats that islet isolation performed after long-term storage of oxygenated pancreatic tissue can be significantly improved if PFD, the currently used standard oxygen carrier for pancreas oxygenation, is replaced by perfluorohexyloctane, an amphiphilic oxygen carrier that is in clinical use and has nearly the same oxygen-dissolving capacity as PFD. Further studies have to be performed in large animal models to validate the findings made in rats.

Authorship

HB performed research, collected and analysed data; BT contributed important reagents and expert know-how; HY and JH performed research; POC and OK contributed essential equipment and technical support; DB designed the study and wrote the manuscript.

Funding

This work was supported in part by the Juvenile Diabetes Research Foundation International (Grant 1-2007-198).

References

1. Hering BJ, Kandaswamy R, Harmon JV, *et al.* Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am J Transplant* 2004; **4**: 390.
2. Lee TC, Barshes NR, Brunicardi FC, *et al.* Procurement of the human pancreas for pancreatic islet transplantation. *Transplantation* 2004; **78**: 481.
3. Matsumoto S, Kandaswamy R, Sutherland DE, *et al.* Clinical application of the two-layer (University of Wisconsin solution/perfluorochemical plus O2) method of pancreas preservation before transplantation. *Transplantation* 2000; **70**: 771.
4. Tsujimura T, Kuroda Y, Kin T, *et al.* Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. *Transplantation* 2002; **74**: 1687.
5. Fraker CA, Alejandro R, Ricordi C. Use of oxygenated perfluorocarbon toward making every pancreas count. *Transplantation* 2002; **74**: 1811.
6. Kin T, Mirbolooki M, Salehi P, *et al.* Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation* 2006; **82**: 1286.
7. Caballero-Corbalan J, Eich T, Lundgren T, *et al.* No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. *Transplantation* 2007; **84**: 864.
8. Tsujimura T, Kuroda Y, Churchill TA, *et al.* Short-term storage of the ischemically damaged human pancreas by the two-layer method prior to islet isolation. *Cell Transplant* 2004; **13**: 67.
9. Salehi P, Mirbolooki M, Kin T, *et al.* Ameliorating injury during preservation and isolation of human islets using the two-layer method with perfluorocarbon and UW solution. *Cell Transplant* 2006; **15**: 187.
10. Brandhorst D, Iken M, Tanioka Y, Brendel MD, Bretzel RG, Brandhorst H. Influence of collagenase loading on long-term preservation of pig pancreas by the two-layer method for subsequent islet isolation. *Transplantation* 2005; **79**: 38.
11. Brandhorst D, Iken M, Brendel MD, Bretzel RG, Brandhorst H. Successful pancreas preservation by a perfluorocarbon-based one-layer method for subsequent pig islet isolation. *Transplantation* 2005; **79**: 433.
12. Tanioka Y, Sutherland DE, Kuroda Y, *et al.* Excellence of the two-layer method (University of Wisconsin solution/

perfluorochemical) in pancreas preservation before islet isolation. *Surgery* 1997; **122**: 435.
13. Brandhorst D, Iken M, Bretzel RG, Brandhorst H. Pancreas storage in oxygenated perfluorodecalin does not restore post-transplant function of isolated pig islets predamaged by warm ischemia. *Xenotransplantation* 2006; **13**: 465.
14. Deai T, Tanioka Y, Suzuki Y, Kuroda Y. The effect of the two-layer cold storage method on islet isolation from ischemically damaged pancreas. *Kobe J Med Sci* 1999; **45**: 191.
15. Papas KK, Hering BJ, Gunther L, Rappel MJ, Colton CK, Avgoustiniatos ES. Pancreas oxygenation is limited during preservation with the two-layer method. *Transplant Proc* 2005; **37**: 3501.
16. Avgoustiniatos ES, Hering BJ, Papas KK. The rat pancreas is not an appropriate model for testing the preservation of the human pancreas with the two-layer method. *Transplantation* 2006; **81**: 1471.
17. Matsumoto S, Kuroda Y, Hamano M, *et al.* Direct evidence of pancreatic tissue oxygenation during preservation by the two-layer method. *Transplantation* 1996; **62**: 1667.
18. Kuroda Y, Fujino Y, Morita A, Tanioka Y, Ku Y, Saitoh Y. Oxygenation of the human pancreas during preservation by a two-layer (University of Wisconsin solution/perfluorochemical) cold-storage method. *Transplantation* 1992; **54**: 561.
19. Stefaniotou MI, Aspiotis MV, Kitsos GD, Kalogeropoulos CD, Asproudis IC, Psilas KG. Our experience with perfluorohexyloctane (F6H8) as a temporary endotamponade in vitreoretinal surgery. *Eur J Ophthalmol* 2002; **12**: 518.
20. Wong D, Van Meurs JC, Stappler T, *et al.* A pilot study on the use of a perfluorohexyloctane/silicone oil solution as a heavier than water internal tamponade agent. *Br J Ophthalmol* 2005; **89**: 662.
21. Rizzo S, Genovesi-Ebert F, Belting C, *et al.* Long-term vitreous replacement with perfluorohexyloctane and silicone oil: preliminary reports of a multicentric study. *Ophthalmologica* 2005; **219**: 147.
22. Noguchi H, Ueda M, Nakai Y, *et al.* Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. *Am J Transplant* 2006; **6**: 496.
23. Carlsson PO, Liss P, Andersson A, Jansson L. Measurements of oxygen tension in native and transplanted rat pancreatic islets. *Diabetes* 1998; **47**: 1027.
24. Brandhorst H, Raemsch-Guenther N, Raemsch C, *et al.* The ratio between collagenase class I and class II influences the efficient islet release from the rat pancreas. *Transplantation* 2008; **85**: 456.
25. Ricordi C, Gray DWR, Hering BJ, *et al.* Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 1990; **27**: 185.
26. Korbutt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest* 1996; **97**: 2119.
27. Jahr H, Hussmann B, Eckhardt T, Bretzel RG. Successful single donor islet allotransplantation in the streptozotocin diabetes rat model. *Cell Transplant* 2002; **11**: 513.
28. Hoerauf H, Kobuch K, Dresp J, Menz DH. Combined use of partially fluorinated alkanes, perfluorocarbon liquids and silicone oil: an experimental study. *Graefes Arch Clin Exp Ophthalmol* 2001; **239**: 373.
29. Carlsson PO, Palm F, Andersson A, Liss P. Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule. *Transplantation* 2000; **69**: 761.
30. Scott III WE, Matsumoto S, Tanaka T, *et al.* Real-time noninvasive assessment of pancreatic ATP levels during cold preservation. *Transplant Proc* 2008; **40**: 403.
31. Mirbolooki M, Lakey JR. Oxygen and pancreas preservation. *Transplantation* 2006; **81**: 492. author reply 492.
32. Wong D, Lois N. Perfluorocarbons and semifluorinated alkanes. *Semin Ophthalmol* 2000; **15**: 25.
33. Meinert H, Roy T. Semifluorinated alkanes – a new class of compounds with outstanding properties for use in ophthalmology. *Eur J Ophthalmol* 2000; **10**: 189.
34. Johansson U, Olsson A, Gabrielsson S, Nilsson B, Korsgren O. Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. *Biochem Biophys Res Commun* 2003; **308**: 474.
35. Moberg L, Olsson A, Berne C, *et al.* Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation. *Transplantation* 2003; **76**: 1285.