Journal of Clinical and Experimental Transplantation

Dhanasekaran et al., J Clin Exp Transplant 2017, 2:1

Mini Review

OMICS International

Technical Modifications to Improve Islet Yield from Chronic Pancreatitis Pancreas (CPP) for Islet Auto-Transplantation (IAT)

Maheswaran Dhanasekaran[1], Gopalakrishnan Loganathan[1], Siddharth Narayanan[1], William Tucker[1], Aravinth Prasanth Jawahar[1], Aravinth Prasanth Jawahar, Ankit Patel[1], Venugopal Subhashree[2], Sri Prakash Mokshagundam[1], Stuart K Williams[1], Michael G. Hughes[1] and Appakalai N Balamurugan[1*]

[1]Department of Surgery, Centre for Cellular Transplantation, Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, USA
[2]School of Biosciences and Technology, VIT University, Vellore, TN, India

Abstract

Surgical removal of the pancreas in patients with intractable chronic pancreatitis will potentially mitigate the long standing suffering of abdominal pain and improve the quality of life. Nonetheless, the induction of iatrogenic diabetes from surgery becomes irrevocable as the insulin producing islet cells are removed. This novel process of islet isolation (both auto- and allo-) involves specific procurement and packaging of the pancreas which is then transported to a specialized clean-room where islets are isolated. The isolation involves distending the pancreas with dissociation enzymes that help in digesting the extracellular matrix and freeing the islets. The cells are then collected as pellets and purified if necessary by density gradient and later packed in the transplant bag for intra-portal infusion back to the patient. The expectant metabolic outcome following the procedure is solely dependent on the total islet mass transplanted. Despite incorporating the most standardized isolation techniques, the overall islet yields available from the diseased pancreas are still very low. Factors such as donor characteristics, pancreatic fibrosis and cellular atrophy further reduce the yield highlighting the need for effective islet isolation techniques.

Keywords: Chronic pancreatitis; Pancreatectomy; Autograft; Allograft; Human islets; Autologous islet isolation; Transplantation; Insulin independence; Collagenase digestion

Abbreviations: CP: Chronic Pancreatitis; TP: Total Pancreatectomy; IAT: Islet Auto Transplantation; cGMP: Current Good Manufacturing Practices; IEQ: Islet Equivalents ; ECM: Extra Cellular Matrix; TLM: Two-Layer Method

Introduction

CP is a chronic inflammation of the pancreatic parenchyma characterized by the irreversible morphological changes and with gradual loss of both exocrine and endocrine component [1]. Intractable pain is a debilitating complication of CP that seriously limits the patients' quality of life. WHO step ladder approach for managing CP pain is the primary treatment option. Regardless, most of the patients need escalating doses of opioid. Surgery is the best alternative when medical treatment fails. Therapies including drainage/decompression and denervation techniques are better alternatives but no treatment is deemed effective in improving the long-term quality of life. Resection of the pancreas can be partial (body and tail)/Whipple's procedure (pancreaticoduodenectomy). Of these, the most effective option is the near total pancreatectomy where 95% of the pancreas is removed.

The introduction of iatrogenic diabetes in such CP patients because of the concurrent islet removal along with the pancreas can effectively be mitigated by simultaneous islet isolation and auto-transplantation via intra-portal infusion. Unfortunately, the cost and difficulties in establishing a cGMP compliant islet laboratory together with lack of experienced personnel have limited the number of the facilities in performing such procedures. The importance of the need for Islet Auto-Transplantation (IAT) for CP patients undergoing pancreatectomy was realized from the fact that iatrogenic diabetes is more brittle and difficult to manage than other forms of diabetes. The sensitivity of insulin is increased with frequent episodes of severe hypoglycemia, which is an impending danger for post- operative patients.

Dr. Najarian and Dr. Sutherland performed the first IAT at the University of Minnesota following a CP total pancreatectomy [2]. Apart from CP, IAT can also be a promising treatment option for other conditions such as pancreatic injury requiring large resection. The same technique can be extrapolated in isolating the islet cells from cadaveric or brain dead donors and can be used for islet allo-transplantation especially for patients with Type-1 diabetes mellitus which requires immunosuppression.

A typical isolation recovers only 30-50% islets from the pancreas despite the fact that a normal healthy pancreas has about 1-2 million islets [3,4]. The effectiveness of enzymatic digestion is challenged by heterogenous extracellular matrix composition of reticular fibers, adhesive proteins, micro and macromolecules and different isoforms of collagen. An effectual isolation technique must lyse the ECM completely to release the islet cells from its matrix thus separating it from the surrounding exocrine cells, but without disintegrating the islets. Multivariable factors like individual donor characteristics, disease pathology, fibrosis, previous surgical interventions etc., distort the normal islet architecture imposing challenges for a better islet yield and thereby the viability.

To achieve consistent success in clinical outcome, an experienced isolation team must learn to recognize key variables, such as fibrosis and donor age, and to understand the principles of the isolation procedure

***Corresponding author:** Appakalai N Balamurugan, Department of Surgery, Clinical Islet Cell Laboratory, Centre for Cellular Transplantation, Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, USA, Tel: 502-852-1131; Fax: 502-852-1391; E-mail: Bala.appakalai@louisville.edu

Received December 16, 2016; **Accepted** January 30, 2017; **Published** February 06, 2017

Citation: Dhanasekaran M, Loganathan G, Narayanan S, Tucker W, Jawahar AP, et al. (2017) Technical Modifications to Improve Islet Yield from Chronic Pancreatitis Pancreas (CPP) for Islet Auto-Transplantation (IAT). J Clin Exp Transplant 2: 112.

to sufficiently adapt it for each individual organ. Thus while coming across as a seemingly simple procedure; IAT requires highly specialized and flexible techniques in order to obtain an adequate number of healthy islets from a variety of donor types [5]. This report aims to describe the technical aspects of pancreas processing and islet isolation that are critical for achieving successful islet auto-transplantations.

Islet cell isolation

Human islet isolation is a time sensitive and a skilled procedure performed by a well-trained team of members led by experienced personnel. Every team member's role should be carefully allocated to ensure an efficient and an effective way to isolating healthy islet for transplants. The Islet laboratory should be adequately prepared prior to the isolation process. Using aseptic precautions, setting up of the biological safety cabinets (or laminar flow hood) with necessary materials for pancreatic trimming, cannulation and distension, digestion, recombination, purification and transplant bag preparation. On the other hand the remaining members should prepare the media and other in-use solutions that will be needed during the isolation. All necessary instruments such as centrifuges and thermal probes should be timely validated and turned on so that there would be no delay once after the pancreas has arrived.

Pancreatectomy and pancreas transport

For autologous isolations, the pancreas is dissected and immersed immediately in cold preservation solution. Generally the excess fat, connective and duodenal tissues are removed before packing on ice [6,7]. Cold storage preservation relies on hypothermia and carefully tailored solutions to slow metabolism, inhibit endogenous enzyme activity and support critical cellular processes despite the loss of an oxygenated blood supply. Organ packaging methods and solution ingredients have been designed to address several key problems associated with hypothermic ischemia followed by reperfusion including cellular swelling, ionic imbalance, acidosis, calcium accumulation and the production of reactive oxygen species. University of Wisconsin (UW) solution was developed in 1986, specifically for pancreas cold storage preservation [8]. UW contains phosphate, large molecules like saccharide raffinose, anionic lactobionate, allopurinol, glutathione, adenosine and a high, intracellular-mimicking K^+/Na^+ ratio [9]. While UW has become the standard organ transport solution, it is also costly with a short shelf-life and many of the ingredients, designed to inhibit tissue degradation, interfere with the catabolic activity of collagenase and neutral protease [10]. Other cold storage solutions have been proposed including Histadine-Tryptophan-Ketoglutarate (HTK), Celsior and the Kyoto solutions but UW remains the most common for pancreas hypothermic preservation. In trimming solution, a modified UW reverses the Na^+/K^+ ratio to mimic the natural extracellular environment and exchanges lactobionate for the less expensive but equally effective gluconate [11].

Pancreas trimming and cannulation

Once after the pancreas is received, the temperature of the transport solution along with 3 ml of sample is taken for microbial assay (aerobic/fungal, anaerobic and gram stain). It is always critical to process the pancreas under cold atmosphere so that it slows the cellular respiration and tissue degradation in conjunction to the preservation solutions.

Trimming: Islet isolation starts with immersing the pancreas in ~500 mL of trimming solution in a cold tray. The pancreas is visually inspected and photographed for documentation. The gross morphology of every pancreas is different and we assess each as mild, moderate or severe to determine enzyme dose and digestion conditions. This is followed by an initial brief trim for removing excess fat and connective tissues around the pancreas but keeping the capsule intact to decrease the likelihood of enzyme leak while enzymatic distension. After trimming, the pancreas is submerged in decontamination solution (Betadine+Fungizone/Cefazolin) that has been checked against potential patient allergies before treating the pancreas.

Cannulation: Until this stage, the pancreas will be handled as one intact organ. However, depending on the severity of the pancreatitis and the degree of near or total pancreatectomy, the organ may arrive whole, partial or in multiple pieces. In the case of a whole pancreas, we have achieved better enzyme distention by dividing the pancreatic lobes and cannulating the head and the body-tail portions individually. The selection of a catheter should be based on the size of the pancreatic duct, which may vary between lobes and also depends on other factors like disease pathology, patient characteristics etc. Typically, sizes range from 14 G to 24 G but severe fibrosis or a dilated duct may require either a Christmas or metal catheter. The selected catheter should be sutured firmly in place to avoid enzyme backflow during perfusion. With the catheters secured, the pancreatic lobes are moved to the perfusion tray and the tray's basin filled with the prepared enzyme solution.

Enzyme selection

Enzymatic tissue dissociation has been used to separate the exocrine and endocrine components of the pancreas since 1967, when crude collagenase enzyme, derived from the bacteria Clostridium histolyticum, was first used to isolate guinea pig and rat islets [12,13]. Since then, researchers have observed the best tissue dissociation resulting after the ductal perfusion of a blend of collagenase and protease enzymes into the main pancreatic duct [14-16]. We tailor our enzyme mixture according to pancreas weight, with the final enzyme solution prepared by diluting the reconstituted enzymes into approximately 350 mL HBSS+10 U/mL heparin solutions (or up to 450 mL for larger pancreases) [17].

Enzyme combination: In the past, the universal adoption of Liberase-HI for enzyme digestion provided a convenient formula in which one complete vial could be dissolved into the final desired solution volume [18]. When Liberase was made clinically unavailable, we switched to SERVA collagenase and neutral protease [19]. However, we have recently identified a new enzyme mixture (NEM) that has improved both the yield and viability of our islet products. We currently use VitaCyte collagenase HA, which contains a high proportion of intact C1 collagenase, to obtain greater islet numbers compared to SERVA's analogous product [20]. On the other hand, we observed SERVA neutral protease NB (GMP or premium grade) to produce better quality islets with a more solid and intact structural morphology compared to VitaCyte's equivalent, Thermolysin [17]. This novel combination (VitaCyte Collagenase+SERVA neutral protease) resulted in total islet yields of >200,000 IEQ in 90% of our attempted autologous isolations and doubled the number of allograft isolations that reached transplantation threshold in our recently published study [17].

Enzyme dose and perfusion of pancreas: Initially, our laboratory used only a 1:1 ratio of whole enzyme vials, even with the NEM. We have since observed that customizing the enzyme dose based on pancreas weight and other donor/organ characteristics results in more consistent islet release [17]. Enzyme distention is the critical step in the islet isolation. The pancreas is perfused with the enzyme solution through the cannulated pancreatic duct of either the whole or segmented pancreas by a pressurized injection, either automated or

manual. The more effective the enzyme distention, the lesser the mass of undigested tissue and better is the islet yield. Historically, enzyme solution was loaded directly into the ductal cannula with a hand-held syringe, relying on retrograde perfusion to distend the pancreas [21]. In addition to improved distension and yield, automated pump perfusion provides precise control over injection pressure and enzyme solution temperature. The modern automated perfusion system is equipped with peristaltic pumps, two pressure sensors, a heater, a touch-screen, and data acquisition software (Bio-rep) that combines the convenience of hands-free automation with the flexibility to make manual adjustments to a variety of programmable parameters.

Temperature, pressure and flow rate: Distension pressure, pump speed, flow rate and temperature can all be monitored and controlled using a semi-automated perfusion system. Throughout the enzyme perfusion process, the temperature is kept between 6 and 16°C while the desired perfusion pressure is maintained between 60 and 80 mm Hg for the first 4 min, and gradually increased to 160-180 mm Hg until completion (approximately 10-12 min total distention time). However, perfusion pressure can vary significantly depending on the condition of the organ. Distention pressures could be low for a severely damaged, leaking, pancreas or high for an organ with abnormal ductal anatomy (stricture or blockage) or severe fibrosis [22,23].

Interstitial perfusion: In IAT some cases have extensive parenchymal fibrosis that ductal enzyme perfusion is ineffective at delivering enzyme to the entire pancreas. In these cases, interstitial perfusion can be performed by repeated manual injections of cold enzyme solution into the tissue with a needle and syringe [24]. On the other hand, the distal half of a pancreas, particularly if perfused intact, may fail to effectively distend with solution. This may be due to duct alterations caused by inflammation and fibrosis or intra-ductal calcification deposits, which have obstructed the flow of fluid through the duct. In these cases, it is possible to make a complete transverse cut before the distal section after the proximal end has finished distending, and re-cannulate the distal end to attempt further distention in this area.

Post-distension trimming: After adequate distension has been achieved, the pancreas is transferred to a trimming pan with fresh heparanized phase-1 solution. Final trimming of the pancreas should be performed as quickly as possible to reduce overall cold ischemia time. This includes removal of the pancreatic capsule, surface fat, vasculature tissue and any sutures of staples used during the surgical procedure. Surface fat is a particular concern as it can clog the Ricordi chamber screen during digestion and obstruct the free flow of islets and solution.

Tissue digestion

The binding of the perfused enzymes to the matrix proteins begins instantly because of which care must be taken to delay the digestion process at the end of the enzymatic distension. Currently, the semi-automated method for tissue digestion is employed by all centers isolating human islet preparations intended for clinical transplant programs [25-27]. Developed in 1988 by Dr. Camillo Ricordi, the digestion method utilizes a specialized "Ricordi" chamber to contain and collect the pancreatic digest as islets are released [26]. Along with the pieces of pancreatic tissue, multiple stainless steel marbles are sealed inside the chamber to mechanically assist enzymatic dissociation as the chamber is gently shaken. This method has proven superior to manual methods in isolating high quantities of viable human islets for successful transplantation [25-28].

Digest sampling: It is imperative to collect samples in between the digestion process to assess how well the digestion is happening. It is done by withdrawing 2 mL from a sterile syringe connected to an outlet port in the tubing circuit, which circulates solution through the Ricordi chamber lid. The sample is then stained with Dithizone in a small petri dish and evaluated under light microscope. Factors like acinar tissue, acinar diameter, number of islets, and the percent of free islets in comparison with the embedded islets, percent fragmented (over-digested) islets and average islet score are recorded along with the circuit temperature, at regular intervals.

Digestion: Phase 1-recirculation: The distended and trimmed pancreas is aseptically transferred to a Ricordi chamber (usually 600 mL) connected to a digestion apparatus, along with all enzyme solution left in the perfusion tray or trimming pan. The digestion apparatus consists of a peristaltic pump that moves fluid through a closed circuit of tubing with a reservoir, a heat transfer coil, an inlet for diluent and outlets for tissue collection or sampling. It is important to keep phase-1 as short as possible because prolonged exposure to active enzyme, and an increasingly basic chamber environment, can be harmful to islet yield and integrity [29-31].

Digestion: Phase 2-collection: Phase-2 of digestion starts by increasing the pump speed to a flow rate of 200 mL/min and switching the tubing clamps from re-circulation of the digest material to collection. Cold phase-2 solution (RPMI 1640) is added to progressively weaken the enzyme concentration inside the digest circuit. The first 2 L of digest tissue is collected into four 1 L flasks in a series of increasing dilutions: first 25% (250 mL of digest+750 mL of cold RPMI 1640/2.5% human serum albumin (HSA)) then 50%, 50%, and 75% v/v. The pre-chilled collection media and the HSA both work to inhibit collagenase and neutral protease activity, which would otherwise continue to breakdown islet integrity during recombination. The decreasing dilution factors provide a greater inhibitory buffer during the initial collection fractions with the highest concentration of enzyme. Following the fourth flask, digest fractions are collected into 250 mL conical tubes, prefilled with 6.25 mL of chilled 25% HSA. The stop point for phase II tissue collection is determined by the minimal presence or complete absence of islets and/or tissue in the dithizone-stained samples that have been under regular evaluation throughout digestion.

Digestion of the severely fibrotic Pancreas: Progressive fibrosis is the usual sequelae of recurrent pancreatitis. Because of which there is an increased deposition and reduced degradation of ECM materials. This excess and hardened tissue is more resistant to enzyme digestion and can greatly lower islet yield if appropriate adjustments are not made to the procedure. Maintaining the solution temperature at the high end of the acceptable range (36.5-37) can intensify enzyme activity. If unusually little digestion is observed after 25 min, an enzyme recirculation protocol may be helpful [30]. This procedural variation involves the collection of free islets from the re-circulating system early after release. The free islets are pelleted by a quick centrifugation step and transferred into fresh, cold media in the recombination container. The supernatant, containing active enzyme, is recycled back into the digestion system, which increases the effective enzyme dose for the undigested tissue remaining in the Ricordi chamber [30]. This recirculation can be repeated until nearly all tissue mass has dispersed or healthy islets no longer appear in the digestion samples.

Tissue recombination

Fraction collection: The recombination phase is closely associated

with the digestion phase that begins immediately after the switch. All digest solution collected in phase 2 contains a suspension of free islets that must be preserved from further enzyme degradation. The first four digest fractions, collected successively into four 1 L flasks of RPMI/HSA (Digestion: Phase 2-Collection), are each transferred to a separate biological safety cabinet hood and divided into 250 mL conicals then centrifuged at 140-170 × g, 2-10°C, for 3 min.

Recombination: When phase-2 digestion has been completed and all tissue mass spun out and collected into the final recombination flask, 5 mL of tissue suspension is removed for density determination (Analytical Test Gradient System). The recombination flask is divided evenly into 4-5 conicals, rinsed thoroughly with wash media to scavenge all islets, and spun again. After decanting off the supernatant, these pellets are combined into a single conical and re-suspended in fresh media up to 200 mL total volume. At this point, two samples of well-dispersed tissue suspension are taken to provide a "post-digest" islet count.

If islets are not purified, the final combined pellet is washed repeatedly with room temperature transplant media (unsupplemented CMRL with 2.5% HSA and 25 mM HEPES) until the supernatant is translucent and free of cell debris. The 100 µL sample counts from earlier are used to calculate the volume of islet-suspended solution that should be collected for islet viability/potency assays, minimally fluorescein diacetate (FDA)/propidium iodide (PI) [32] and others depending on total yield and patient consent.

Purification process

The goal of the purification is to reduce the tissue volume by separating the exocrine cells while minimizing islet loss. Several purification techniques have been reported but isopycnic density gradient centrifugation on the COBE 2991 cell processor is the only method that has been consistently successful and used clinically for large-scale human islet purification [33,34]. This purification technique is accomplished by employing centrifugation through a density gradient to separate the less dense islets from the more dense exocrine tissue.

The expediency of islet purification is debatable [35] and should take into consideration the particular patient and potential for transplant complications. The decision to purify or not should be made by qualified and experienced personnel, often in consultation with the attending physician/surgeon. Avoiding density gradient purification is generally beneficial for islet viability as it avoids exposing the islets to harsh gradient solutions and additional mechanical stress [36].

Standard density gradients: When islets are purified with a COBE 2991 cell processor, the purification gradient used are often fixed between 1.060 and 1.100 g/cm^3, values currently utilized by the Clinical Islet Transplantation Consortium [34]. A predefined, standard density gradient is less successful when exocrine density is unusually light (<1.100 g/cm^3), lowering the post-purification purity, or when islet density is unusually heavy (>1.100 g/cm^3, common for mantle islets), causing the islets to sediment into the COBE bag and reducing total recovery [34].

Analytical test gradient system: Predicting exocrine tissue and islet density is important for the selection of an optimal density gradient range for the COBE process, to maximize islet yield and purity. Consequently, our center has developed an Analytical Test Gradient System (ATGS) to determine the true density distribution of human pancreatic tissue components before purification [34]. This method mimics the actual purification process but uses only a minute fraction of recombination tissue in a single tube. ATGS results can be quickly interpreted in order to customize the gradient for a full COBE purification.

High density gradients: Interpancreatic variations in exocrine and islet tissue density is influenced by donor characteristics, the secretory status of exocrine cells, pancreas procurement, preservation protocols that affect cellular swelling and tissue edema, and the islet isolation procedure, which determines the extent of tissue dissociation and the size of aggregates formed [34,37-39]. In particular, when isolating islets from CP pancreata for autograft transplant, many centers have observed frequent settling of islets in the COBE bag.

COBE purification process: The COBE purification process begins after recombination has finished and the total tissue pellet has been consolidated into a single 250 mL conical. The centrifuged pellet is prepared for purification by decanting off the last of the wash media and re-suspending the tissue in 20 mL of 25% HSA. This suspension is transferred to a sterile 250 mL beaker; the conical rinsed with cold storage solution, and brought to a final volume of 120 mL by weight. The tissue is top loaded into the COBE bag by peristaltic pump (flow rate 20 mL/min), gently swirling the beaker for an even distribution of tissue, followed by an additional 30 mL of cold storage solution to rinse the beaker and tubing. After loading, the inlet tube is clamped and the COBE bag vented by carefully opening the outlet clamp and allowing the machinery to spin at 400 × g for 3 min. The efficacy of the purification can be evaluated by taking a 1 mL sample from each well-mixed collection fraction, as well as a 200 µL sample of residual tissue remaining in the COBE bag. If a significant quantity of free islets is observed in the COBE bag, re-purification may be necessary. The final pellet is transferred to a T-75 flask and re-suspended in 100 mL of room-temperature transplant media, from which 100 µL samples are obtained for a "post-COBE" islet count and additional samples taken for potency and product sterility testing (Recombination).

Transplant preparation

Although quality control and sterility test results will not be available until after the product is released, the islet preparation should be immediately packaged for auto- transplantation to avoid excess delay and operating time for the patient. Any subsequent failure to meet post-release criteria should be reported to the patient physician when assessed (especially sterility positives). Procedural deviations that have occurred during tissue processing should be reported to the physician before product release.

Once the tissue pellet, purified or unpurified, has been washed in transplant media and settled into 100 mL of fresh media for 5 min, the last sterility and retention samples can be taken from the supernatant to ensure the status of the final islet product immediately before packaging. From this point, extreme care should be taken to ensure the continued sterility of the product. All items in the biological safety cabinet where loading will take place should be sterile (if in contact with the product) or thoroughly disinfected and the operator should wear sterile gloves and sleeves during the loading process. If the packed tissue volume is greater than 10 mL, the pellet must be divided evenly and loaded into two 200 mL transplant bags, each labeled with the correct patient identification and FDA-required labeling. Each tissue load is suspended in 100 mL of transplant media and will require an

additional 100 mL of media as a rinse solution. If the patient has no known allergy to ciprofloxacin, add 0.4 mL of Cipro® (1%=10000 μg/mL) to each volume of rinse media.

After affixing a 60 mL syringe to the transplant bag, place the syringe upright in a clamp stand and transfer the 100 mL tissue suspension into the bag through the syringe. Rinse the tissue conical twice with 50 mL volumes of rinse solution to transfer any residual islets. Aseptically recap and clamp the bag's inlet tubing to ensure a thorough seal for transport. The sealed bag should be gently rocked to evenly suspend the islets. Repeat these steps for additional transplant bags if needed. Once the transplant physician at the operation room has been alerted, the islet preparation can be readied for transport in a room temperature cooler equipped with temperature stabilizers [40].

Conclusion

Autologous islet isolation and transplantation has repeatedly demonstrated the ability to improve clinical outcomes by diminishing the impact of iatrogenic diabetes on patients undergoing pancreatectomy to alleviate CP or other disabling conditions. As practical experience has accumulated at an increasing number of qualified isolation centers, islet yield and viability, critical factors for achieving post-operative insulin independence, have progressively improved. It is imperative to understand and improve the technical aspects of the isolation procedure like the introduction of the simplified ATGS to improve purification yield [34] and the identification of post-isolation factors that detriment graft function [34]. Our research focus is on the mechanics of enzyme digestion, proposing a new enzyme mixture and variable dose classes that have increased the flexibility of the procedure to respond to different donor and tissue characteristics [17,20]. Despite these and other advances, the islet yield is significantly less than the available stores, indicating the need for more studies on efficient digestion. This demands a better understanding of the enzyme mechanics-Collagenase vs. neutral protease, their functional ingredients and interactions with different ECM components. This also necessitates the need for more specific techniques to overcome these inevitable obstacles. Furthermore, there is currently a heavy cost burden to establish facilities and perform these procedures, which severely limits their availability, especially in developing countries. All such technical and socio-economic parameters must be taken into consideration in order to successfully further develop and improve islet yield and transplantation outcomes for patients with CP.

Acknowledgment

The authors thank the Jewish Heritage Fund for Excellence for providing generous support to our program. The authors sincerely thank Kentucky Organ Donor Affiliates (KODA) for the supply of human pancreases. Special thanks to Leigh Kleinert and Brian Gettler for their assistance.

References

1. Majumder S, Chari ST (2016) Chronic pancreatitis. Lancet 387: 1957-1966.
2. Najarian JS, Sutherland DE, Matas AJ, Goetz FC (1979) Human islet autotransplantation following pancreatectomy. Transplant Proc 11: 336-340.
3. Lakey JR, Burridge PW, Shapiro AM (2003) Technical aspects of islet preparation and transplantation. Transpl Int 16: 613-632.
4. Bellin MD, Balamurugan AN, Pruett TL, Sutherland DE (2012) No islets left behind: islet autotransplantation for surgery induced diabetes. Curr Diab Rep12: 580-586.
5. Morrison CP, Wemyss-Holden SA, Dennison AR, Maddern GJ (2002) Islet yield remains a problem in islet autotransplantation. Arch Surg 137: 80-83.
6. Naziruddin B, Matsumoto S, Noguchi H, Takita M, Shimoda M, et al. (2012) Improved pancreatic islet isolation outcome in autologous transplantation for chronic pancreatitis. Cell Transplant 21: 553-558.
7. Shimoda M, Itoh T, Sugimoto K, Iwahashi S, Takita M, Chujo D, et al. (2012) Improvement of collagenase distribution with the ductal preservation for human islet isolation. Islet 4: 130-137.
8. Wahlberg JA, Southard JH, Belzer FO (1986) Development of a cold storage solution for pancreas preservation. Cryobiology 23: 477-482.
9. Hart NA, Leuvenink HG, Ploeg RJ (2002) New Solutions in Organ Preservation. Transplant Rev 16: 131-141.
10. Contractor HH, Johnson PR, Chadwick DR, Robertson GS, London NJ, et al. (1995) The effect of UW solution and its components on the collagenase digestion of human and porcine pancreas. Cell Transplant 4: 615-619.
11. Barlow AD, Hosgood SA, Nicholson ML (2013) Current state of pancreas preservation and implications for DCD pancreas transplantation. Transplant 95: 1419-1424.
12. Moskalewski S (1965) Isolation and Culture of the Islets of Langerhans of the Guinea Pig. Gen Comp Endocrinol 44: 342-353.
13. Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16: 35-39.
14. Caballero-Corbalan J, Friberg AS, Brandhorst H, Nilsson B, Andersson HH, et al. (2009) Vitacyte collagenase HA: a novel enzyme blend for efficient human islet isolation. Transplant 88: 1400-1402.
15. Caballero-Corbalan J, Brandhorst H, Asif S, Korsgren O, Engelse M, et al. (2010) Mammalian tissue-free liberase: a new GMP-graded enzyme blend for human islet isolation. Transplant 90: 332-333.
16. Kin T (2010) Islet isolation for clinical transplantation. Adv Exp Med Biol 654: 683-710.
17. Balamurugan AN, Loganathan G, Bellin MD, Wilhelm JJ, Harmon J, et al (2012) A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products. Transplant 93: 693-702.
18. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, et al (2005) Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 293: 830-835.
19. Anazawa T, Balamurugan AN, Bellin M, Zhang HJ, Matsumoto S, et al. (2009) Human islet isolation for autologous transplantation: comparison of yield and function using SERVA/Nordmark versus Roche enzymes. Am J Transplant 9: 2383-2391.
20. Balamurugan AN, Breite AG, Anazawa T, Loganathan G, Wilhelm JJ, et al. (2010) Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. Transplant 89: 954-961.
21. Lakey JR, Warnock GL, Shapiro AM, Korbutt GS, Ao Z, et al. (1999) Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. Cell Transplant 8: 285-292.
22. Goto M, Eich TM, Felldin M, Foss A, Kallen R, et al.(2004) Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. Transplant 78: 1367-1375.
23. Johnson PR, Hakim NS, Stratta RJ, Gray DW, Friend P, et al. (2010) Suitable donor selection and techniques of islet isolation and purification for human islet-allograft transplantation in Pancreas. Islet and Stem Cell Transplant for Diabetes pp: 387-396.
24. al-Abdullah IH, Kumar MS, Abouna GM (1994) Combined intraductal and interstitial distension of human and porcine pancreas with collagenase facilitates digestion of the pancreas and may improve islet yield. Transplant Proc 26: 3384.
25. Ricordi C (2003) Islet transplantation: a brave new world. Diabetes 52:1595-1603.
26. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW,et al. (1988) Automated method for isolation of human pancreatic islets. Diabetes 37: 413-420.
27. Kin TPA, Gala-Lopez B (2013) Advances in Clinical Islet Isolation. Islets of Langerhans.
28. Paget M, Murray H, Bailey CJ, Downing R (2007) Human islet isolation: semi-automated and manual methods. Diab Vasc Dis Res 4: 7-12.
29. Gray DW, Morris PJ (1987) Developments in isolated pancreatic islet transplantation. Transplant 43: 321-331

30. Balamurugan AN, Chang Y, Fung JJ, Trucco M, Bottino R (2003) Flexible management of enzymatic digestion improves human islet isolation outcome from sub-optimal donor pancreata. Am J Transplant 3:1135-1142.

31. Tsukada M, Saito T, Ise K, Kenjo A, Kimura T, et al. (2012) A Model to Evaluate Toxic Factors Influencing Islets During Collagenase Digestion: The Role of Serine Protease Inhibitor in the Protection of Islets. Cell Transplant 21: 473-482.

32. Loganathan G, Graham ML, Radosevich DM, Soltani SM, Tiwari M, et al. (2013) Factors affecting transplant outcomes in diabetic nude mice receiving human, porcine, and nonhuman primate islets: analysis of 335 transplantations. Transplant 95: 1439-1447.

33. Lake SP, Bassett PD, Larkins A, Revell J, Walczak K, et al. (1989) Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes 38: 143-145.

34. Anazawa T, Matsumoto S, Yonekawa Y, Loganathan G, Wilhelm JJ, et al. (2011) Prediction of pancreatic tissue densities by an analytical test gradient system before purification maximizes human islet recovery for islet autotransplantation/allotransplantation. Transplant 91: 508-514.

35. Gores PF, Sutherland DE (1993) Pancreatic islet transplantation: is purification necessary? Am J Surg 166: 538-542.

36. Mita A, Ricordi C, Miki A, Barker S, Khan A, et al. (2009) Purification method using iodixanol (OptiPrep)-based density gradient significantly reduces cytokine chemokine production from human islet preparations, leading to prolonged beta-cell survival during pretransplantation culture. Transplant Proc 41: 314-315.

37. Chadwick DR, Robertson GS, Rose S, Contractor H, James RF, et al. (1993) Storage of porcine pancreatic digest prior to islet purification. The benefits of UW solution and the roles of its individual components. Transplant 56: 288-293.

38. London NJ, Swift SM, Clayton HA (1998) Isolation, culture and functional evaluation of islets of Langerhans. Diabetes Metab 24: 200-207.

39. Hering BJ (2005) Repurification: rescue rather than routine remedy. Am J Transplant 5: 1-2.

40. Kaddis JS, Hanson MS, Cravens J, Qian D, Olack B, et al. (2013) Standardized transportation of human islets: an islet cell resource center study of more than 2,000 shipments. Cell Transplant 22: 1101-1111.

Citation: Dhanasekaran M, Loganathan G, Narayanan S, Tucker W, Jawahar AP, et al. (2017) Technical Modifications to Improve Islet Yield from Chronic Pancreatitis Pancreas (CPP) for Islet Auto-Transplantation (IAT). J Clin Exp Transplant 1: 112.