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to su ciently adapt it for each individual organ. us while coming across as a seemingly simple procedure; IAT requires highly specialized and exible techniques in order to obtain an adequate number of healthy islets from a variety of donor types [5]. is 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 e cient and an e ective way to isolating healthy islet for transplants. e Islet laboratory should be adequately prepared prior to the isolation process. Using aseptic precautions, setting up of the biological safety cabinets (or laminar ow hood) with necessary materials for pancreatic trimming, cannulation and distension, digestion, recombination, puri cation 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 a er 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, speci cally for pancreas cold storage preservation [8]. UW contains phosphate, large molecules like saccharide ra nose, 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 modi ed UW reverses the Na^+/K^+ ratio to mimic the natural extracellular environment and exchanges lactobionate for the less expensive but equally e ective gluconate [111.011 -1.2rzeTaad)odil il il i.anato 1auingtkelservationpensih a 0 0 d(K) expensidsolu

manual. e more e ective 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. e modern automated perfusion system is equipped with peristaltic pumps, two pressure sensors, a heater, a touch-screen, and data acquisition so ware (Bio-rep) that combines the convenience of hands-free automation with the exibility to make manual adjustments to a variety of programmable parameters.

Temperature, pressure and flow rate: Distension pressure, pump speed, ow rate and temperature can all be monitored and controlled using a semi-automated perfusion system. roughout 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 rst 4 min, and gradually increased to 160-180 mm Hg until completion (approximately 10-12 min total distention time). However, perfusion pressure can vary signi cantly 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

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

A er a xing 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. e 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 quali ed 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 simpli ed ATGS to improve puri cation yield [34] and the identi cation of post-isolation factors that detriment gra 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 exibility of the procedure to respond to di erent donor and tissue characteristics [17,20]. Despite these and other advances, the islet yield is signi cantly less than the available stores, indicating the need for more studies on e cient digestion. is demands a better understanding of the enzyme mechanics-Collagenase vs. neutral protease, their functional ingredients and interactions with di erent ECM components. is also necessitates the need for more speci c 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.

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