

Video Article

Human Pancreatic Islet Isolation: Part I: Digestion and Collection of Pancreatic Tissue

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Abstract

Management of Type 1 diabetes is burdensome, both to the individual and society, costing over 100 billion dollars annually. Despite the widespread use of glucose monitoring and new insulin formulations, many individuals still develop devastating secondary complications. Pancreatic islet transplantation can restore near normal glucose control in diabetic patients¹, without the risk of serious hypoglycemic episodes that are associated with intensive insulin therapy. Providing sufficient islet mass is important for successful islet transplantation. However, donor characteristic, organ procurement and preservation affect the isolation outcome². At University of Illinois at Chicago (UIC) we have developed a successful isolation protocol with an improved purification gradient³. The program started in January 2004, and more than 300 isolations were performed up to November 2008. The pancreata were sent in cold preservation solutions (UW, University of Wisconsin or HTK, Histidine-Tryptophan Ketoglutarate)⁴⁻⁷ to the Cell Isolation Laboratory at UIC for islet isolation. Pancreatic islets were isolated using the UIC method, which is a modified version of the method originally described by Ricordi *et al.*⁸. Briefly, after cleaning the pancreas from the surrounding tissue, it was perfused with enzyme solution (Serva Collagenase + Neutral Protease or Sigma V enzyme). The distended pancreas was then transferred to the Ricordi digestion chamber, connected to a modified, closed circulation tubing system, and warmed up to 37°C. During the digestion, the chamber was shaken gently. Samples were taken continuously to monitor the digestion progress. Once free islets were detected under the microscope, the digestion was stopped by flushing cold (4°C) RPMI dilution solution (Mediatech, Herndon, VA) into the circulation system to dilute the enzyme. After being collected and washed in M199 media supplemented with human albumin, the tissue was sampled for pre-purification count and incubated with UW solution before purification. Purification process will be described in Part II: Purification and Culture of Human Islets.

Video Link

The video component of this article can be found at <https://www.jove.com/video/1125/>

Protocol

1. Facility setup

1. Human islet isolation is a time sensitive procedure that involves teamwork, and hence, four individuals are needed to conduct this procedure. The team is lead by the surgeon, who dissects and cannulates the pancreas.
2. The islet isolation begins the minute the team enters the UIC islet isolation facility. Team members ensure that all necessary instrumentation, like the centrifuge, is checked and turned on. They also make sure to sanitize all hoods and surfaces.
3. Two people will set up the cannulation table, slush machine, digestion circuit, perfusion unit, COBE purification machine, and surgical tables.
4. Simultaneously, two other team members will prepare the media that is needed during isolation.
5. Next, bacteriology tubes (bottles, tube, form and bag), sampling tubes (pre-filled with wash), and assessment tubes and plates are prepared.
6. Prepare the purification tube set as follows: The 1st tube empty and marked at 150 ml, tubes 2-12 filled with 200 ml of M199 media supplemented with human albumin (wash solution) and marked at 230 ml, and finally one 50 ml conical tube filled with 50 ml of wash solution. Once these preparation steps are complete we are ready to begin preparing and perfusing the pancreas.

2. Pancreas preparation and perfusion

1. To begin preparation and perfusion of the pancreas, an operator removes the organ from packaging using sterile technique. The surgeon will transfer the pancreas to the decontamination table.
2. The surgeon will fill one 60 ml syringe with the original pancreas preservation solution and hand it to an operator, who will use it to fill prepared "Procurement" bacteriology bottles.
3. After a visual inspection to determine whether the organ is suitable for islet isolation (If the pancreas is damaged during procurement or anatomically abnormal, it may not be used for islet isolation), the surgeon will ask an operator to prepare collagenase. Remember that that re-suspension time varies between different enzyme brands and lots.

4. When the surgeon is ready, bring the decontamination solutions (Betadine, Fungizone/Cefazolin and HBSS) to the decontamination table and pour them into the appropriate containers. Surgeon performs pancreas decontamination washing for a minute the pancreas in each of the decontamination solution. The pancreas is then placed in a small sterile jar in which it will be weighed. After the weight is entered into the batch record, the pancreas is placed in the cannulation pan with some preservation solution.
5. The surgeon will expose and partially incise the main duct and cannulate each half of the pancreas with a cannula. The cannula will be secured by suture. To perfuse the pancreas, first move it to the perfusion unit and pour the enzyme solution into the basin. Total perfusion time is about 10 minutes, at 80 mmHg for 5 minutes, then at 180 mmHg for 5 minutes. Pancreas distended after perfusion.
6. Move the perfused pancreas to the cannulation pan, where the fatty tissue is trimmed from the pancreas carefully. Then cut the pancreas into 10-12 pieces and transfer to Ricordi Chamber for digestion.

3. Pancreas digestion

1. To begin digestion step, transfer the pancreas to the empty Ricordi Chamber, add one 2.5 ml vial of Pulmozyme, put on screen (avoid big chunk of tissue block the chamber), close the chamber, and fill up the system.
2. Start pumping the solution for 5 minutes. Gently rock the chamber to evenly disperse warm solution throughout. After five minutes, gently shake the chamber for the whole digestion phase. Maintain the temperature at 37°C.
3. Rock the chamber gently until the temperature reaches 37°, then shake the chamber. Take a sample every two minutes and detect if free islets are found under the scope. Record the islet evaluation information in the digestion table of the batch record. Continue to monitor the percentage of islets until there are 50% of them floating free and isolated in the solution.

4. Tissue collection

1. Once 50% of the islets are found free in the samples, digestion is stopped by adding cold RPMI dilution solution in the circuit. Tissue is collected in 500 ml conical tubes pre-filled with 30 ml Human Albumin. Once the tubes are spun, suction off the supernatant and wash the pellet with cold wash solution. Spin the conical tubes at 1100 RPM (Rotations Per Minute) for 1 minute, 4°C.
2. Combine all collection tubes suspended with digested pancreatic tissue into a single 500 ml conical. When a tube is full of washed tissue, spin and repeat the washing process until all tissue has been washed multiple times. Spin this tube and transfer the tissue into a single 250 ml conical tube. Bring the volume up to 250 ml with M199 washing solution supplemented with HA and prepare for sampling.
3. Take a 1 ml sample with a pipette and add to a 15 ml conical tube pre-filled with 9 ml of wash solution. Then mix by gently inverting the 15 ml conical tube and from this, sample out 1ml directly into a grid counting dish. Count the cells, and calculate the cell number using a dilution factor of 2500 (10x250).
4. Spin the 250 ml conical tube and add 150 ml of UW (University of Wisconsin) Solution that usually use for organ preservation. When UW is added, mix the cell suspension thoroughly using the pipette. Record and communicate to the purification operator the start time in UW. Leave the tube(s) on ice and swirl occasionally. Now the islets can be purified further.

Discussion

There are a variety of factors affecting the isolation outcome^{9,10}. Among them, digestion of the pancreatic tissue plays a prominent role. Based on our own human islet isolation experience, we summarized following critical points, which could potentially influence the islet yield and quality.

1. The digestion time is different when different types of enzymes are utilized.
2. As soon as 40-50% of the islets completely separated from acinar tissue, the digestion should be stopped immediately by adding dilution solution and cooling down the tissue.
3. Gentle handling of the tissue when collecting, washing, and mixing.
4. The mixture of collected tissue and UW solution should be placed on ice for at least 30 minutes in order to obtain better purification result.

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