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Enhancing the Efficacy of Human Pancreatic Islet Dissociation

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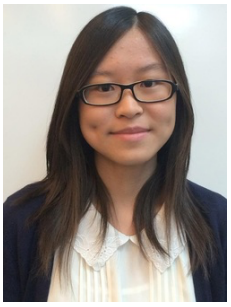

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Lulu is a 2nd-year Bachelor of Health Sciences (Honours) Candidate specializing in Child Health at McMaster University. She started working as a summer research student in the Ungrin Lab at the University of Calgary in 2016. Her work with the lab focuses on characterizing and optimizing human pseudo-islets.

Keywords: pancreatic islet; dissociation protocol; type 1 diabetes; islet transplantation; pseudo-islet; AggreWell

Introduction

Type 1 diabetes is an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta cells. This condition reduces life expectancy by up to fifteen years and leads to significant health care expenses [1]. In 2015, the global estimates of the number of children living with type 1 diabetes exceeded half a million, and some 86,000 new cases are estimated to occur annually [2].

Currently, a promising treatment for type 1 diabetes is pancreatic islet transplantation. The loss of rich vascular network by native islets during isolation from donors, however, results in the low survival of large islets post-transplantation. A promising proposal is to disperse native islets into single-cell suspensions and aggregate them into smaller, uniform “pseudo-islets”. The recovery of cell mass after dissociation still remains a major challenge that limits the yield and efficacy of pseudo-islet aggregations [3]. As such, the objective of this study is to determine the optimal dissociation protocol for the formation of human pseudo-islets. We hypothesized that by enhancing the dissociation protocol for native human islets, more cells can be recovered and they will form pseudo-islets that function more effectively in terms of insulin secretion and survivability.

Methods

A literature review was conducted to determine the reagents previously used to dissociate native islets. Using the search engine PubMed, we searched the MEDLINE database (1980 to May 2016) to determine the dissociation reagents, concentrations, and time outlined in literature. All trials outlining the methods used to dissociate native rat, mouse, hamster, or human islets were included.

Each of the 3 native post-mortem human islet samples (1 female and 2 male; ages 41, 56, and 75; all negative for HIV, HCV, HBV testing; isolated and supplied by Clinical Islet Laboratory at University of Alberta) were centrifuged at 120 x g for 1 minute in 50 mL centrifuge tubes and after removing the supernatant, resuspended in CMRL-1066 media (containing amino acids, vitamins, and other components). The samples were further divided into several tubes and centrifuged at 120 x g for 1 minute. Two thousand islet equivalents (islets with a diameter of 150 µm) per 1 mL of dissociation reagents (Accutase, Accumax, TrypLE Express, 0.05% Trypsin in phosphate-buffered saline (PBS), 0.05% Trypsin with 0.02% Versene (EDTA) in PBS, and Dispase in PBS ranging from 2 – 5 U/mL) were added with 0.0015% DNase into each of the tubes. Accumax contains the same proteolytic and collagenolytic enzymes as Accutase, but is three times more concentrated. Trypsin is a pancreatic serine protease, while TrypLE Express is a recombinant enzyme. Dispase contains neutral protease from *Bacillus polymyxa*. During the dissociation period, tubes with Accutase or Accumax were left at room temperature due to their sensitivity to heat while the rest of the tubes were put into a 37 °C shaking water bath to speed up the dissociation process. Subsequently, the cells were mechanically dissociated into single-cell suspensions by trituration. After 11-15 minutes, the tubes were topped up with CMRL media to 5 mL in order to stop the dissociation process.

The cells were centrifuged at 280 x g for 1 minute and resuspended in 5 mL of CMRL. Samples were collected from the tubes for cell counting (pre-filtering viability/ cell counts), and the remaining were filtered using cell strainers to obtain uniform cell suspensions. After filtration, cell counting samples were obtained again (post-filtering viability/ cell counts). Samples were analyzed using Trypan Blue and PicoGreen DNA assay to quantify cell loss during islet dispersion and filtration. The cell suspensions were added into 24-well AggreWell plates with CMRL in them and centrifuged at 200 x g for 5 minutes to form smaller, uniform pseudo-islets. The pseudo-islets were cultured in microwells in a cell culture incubator (37 °C, 5% carbon dioxide) up to 5 days (5 day cell viability/ cell counts). During the culture period, samples for cell counting were collected and assessed using PicoGreen DNA assay while the metabolic activity of pseudo-islets was assessed using Alamar Blue assay. Statistical analysis were performed with one-way ANOVA.

Results

Twenty studies met the inclusion criteria, from which we identified the dissociation reagents, concentrations, and time previously used to dissociate native islets [3-22]. These studies employed Trypsin, TrypLE, Accutase, Dispase, and Papain to dissociate native islets with Trypsin being the most common. Dissociation times ranged from 3 to 15 minutes.
In our experiments comparing the dissociation reagents, TrypLE showed the highest percentage of recovered viable cells after filtration (85 ± 43), followed by Accumax (56 ± 22), Trypsin with EDTA (49 ± 5), Accutase (25 ± 2), Dispase 5 U/mL (11), and Dispase 3 U/mL (8). Cell survival during the culture period was found to vary between the trials which may be due to the inherent differences between donor materials (Accumax: 65 ± 19%, Trypsin with EDTA: 64 ± 31%, TrypLE: 57 ± 8%, Accutase: 48%, Dispase 3 U/mL: 59%, Dispase 5 U/mL: 57%).

Overall, TrypLE ranked the highest in terms of the recovery coefficient, followed by Accumax and Trypsin with EDTA (Table 1). The recovery coefficient takes total cells present post-filtration, % viable cells in suspension, and cells remaining post-culture into consideration. Metabolic activity per cell remaining was not

included as the implications are currently unclear.

Table 1 shows the recovery coefficients ± SD, assessed using total number of cells recovered, percentage of viable cells in suspensions, and cell survival post-culture, with equation 1 representing how this was calculated.

Equation 1: *Recovery Coefficient = Total Number of Cells Normalized x % Viable Cells x % Cells Remaining Post-Culture

Table 1. *Ranking of the dissociation reagents.*

	Recovery Coefficient*
Accutase n=1	12.98
Accumax n=2	30.87 ± 8.84
TrypLE n=2	32.80 ± 5.18
Trypsin w/ EDTA n=2	27.73 ± 13.96
Dispase, 3 U/mL n=1	4.83
Dispase, 5 U/mL n=1	6.13

Discussion & Conclusion

Due to the limited supply of donor islets, only 3 trials with different native islet samples were conducted during the study period. In the future, trials will be replicated to increase the reliability of the study. To determine the optimal timing for TrypLE and Accumax, the dissociation times of these most promising dissociation reagents will also be varied. Results from the study are promising and further investigations will allow the results to become applicable to clinical trials, which can directly help increase the number of treatable patients from the limited supply of donor islets.

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