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Optimizing conditions for rat pancreatic islets isolation

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Abstract

Many procedures have been described for rat pancreatic islet isolation. Several factors contribute to the pancreatic islet isolation outcome. One of the main problems in islet isolation procedure is the formation of a viscouse, gellike structure during collagenase digestion which entraps the free islets and decrease islet yield after density gradient purification. This issue has not been addressed in most techniques described for rat islet isolation. We examined effect of various factors to eliminate formation of gellike material and improve the islets yields. Islet isolation was performed on 26 adult male Wistar Albino rats weighing between 280 and 350 g. We have observed that several factors affect pancreatic islet isolation. Optimum Collagenase enzyme concentration, maintaining pH range between 7.7 and 7.9 in digestion solution, incubation temperature at 38 ± 1 °C and addition of Calcium ion decreased the formation of gellike materials and increased islet yield. Addition of Glycerol as a gelatin solvent has also been helpful in the reduction or complete elimination of gellike material. Precise optimization of rat islet isolation procedure is useful to improve the islet yield in islet transplantation studies.

Introduction

Study on rat pancreatic islets has been helpful in learning the mechanisms involved in human islet transplantation during past decades. Isolation of rat pancreas islets have been first described by Lacy and Kostianovsky (1967) and after that many other procedures have been reported for pancreatic islet isolation based on this technique (Ricordi et al. 1988; Wolters et al. 1990; Marchetti et al. 1991). However there is a low degree of reproducibility for described techniques besides isolation of significant number of pancreatic islets is not easy due to various factors involved in the process. Efforts to determine these factors will improve efficacy of islet isolation.

Pancreatic tissue is usually digested with commercial Clostridium histolyticum collagenase which is a mixture of various collagenotic enzymes and also several other enzymes with proteolytic activity (Wolters et al. 1995). The variable enzymatic composition of crude collagenases in different batch of enzymes is an important factor which contributes to the poor reproducibility of islet isolation procedures (Wolters et al. 1992).

Several factors influence Collagenase enzyme digestion during islet isolation. Formation of a viscouse, gellike structure is described as a problem during collagenase digestion of rat pancreas. This gellike material causes entrapment of large number of islets and as a result decreases islet yields after isolation and density gradient purification.

We examined effect of various factors in order to improve the islets yields after islet isolation and eliminate formation of gellike material.

Material and methods

Materials

Ficoll (F2637) and Collagenase V (C9263) were purchased from (Sigma, St. Louis, MO). Dithizone was from (Merck, Darmstadt, Germany) HBSS, FBS were from (Gibco, UK) Hepes was purchased from (MP Biomedicals, Germany).

Pancreas procurement and digestion

Adult male Wistar Albino rats ($N = 26$), weighing between 280 and 350 g were selected. After anaesthetizing rats the abdomen was opened and the pancreatic duct was cannulated. The pancreas was then distended with cold Hanks' balanced salt solution. The excised pancreases were then cut into approximately 1 mm pieces and washed several times with HBSS solution containing 10 mM Hepes, 5% FBS, 100 Unit/ml penicillin, 100 ug/ml streptomycin.

Collagenase digestion

Tissue suspension was incubated with collagenase solution (volume ratio for minced tissue to total incubation volume was 1/10) at various concentration of Collagenase (1.5 mg/ml, 2 mg/ml and 2.5 mg/ml). Collagenase solution contained 10 mM Hepes, 7.5 mM $CaCl_2$. pH adjustment was performed in different range between 6.0–7.2 and 7.5–7.9 by addition of necessary amount of 0.3 N NaOH. Glycerol was added at the concentration of 5% (vol/vol) to the collagense solution. Digestion temperature was variable between 37 ± 1.5 °C. Serial sampling was carried out when tissue suspension was incubated in collagenase solution and stained with Dithizone to check for the appearance of free islets. The incubation time was continued until more than 70% of islets were free in the sample. Digestion was terminated by adding cold HBSS.

Islet purification and staining

Digested tissue was filtered trough a 6-cm-diameter cylindric stainless-steel tea stainer (0.5 mm mesh pore size) after washing, then transferred to 50 ml Universal conical tubes, and centrifuged for 2 min at 4 °C at 300 g. The supernatant was discarded and pellet was resuspended in Ficoll 25% with 10 mM Hepes, pH for Ficoll solution was adjusted to 7.0 ± 0.2, this was layered over by various concentration of Ficoll 23%, 20.5% and 11% to make a density gradient for purification of rat islets. The islets were collected from interface between density 11%, 20.5% and 23% after centrifuge and then counted under an inverted microscope after Dithizon staining. Samples were stained with Dithizone based on the standard protocol described by (Latif et al. 1988).

Results

In this experiment we have observed that several factors affected enzyme digestion during pancreatic islet isolation. Alteration in the pH range from 7.0 ± 0.2 to 7.8 ± 0.1 in collagenase solution has significantly improved Enzyme digestion and decreased the formation of gellike materials during Collagenase dispersion and increased the islet yields. Optimum incubation temperature was observed to be 38.0 ± 1 °C and when temperature falls below this range formation of gelly structure is increased. Various enzyme concentrations have been used from the same batch of Collagenase enzyme. An optimum digestion has been accomplished at 2.5 mg/ml Enzyme concentration when the ratio for minced tissue suspension volume to total incubation medium was 1:10. Serial sampling was performed from digesting tissue suspension and was inspected for appearance of free islets (Figure 1), this has been helpful to determine the optimum incubation period to stop digestion. Approximately 70% of free islets were observed in 40 ± 5 min. Addition of Glycerol as a gelatin solvent has been greatly helpful in reduction or complete elimination of gellike material. When all the conditions were optimized we have been able to recover significantly higher islets yield compared to non-optimized condition (Table 1).

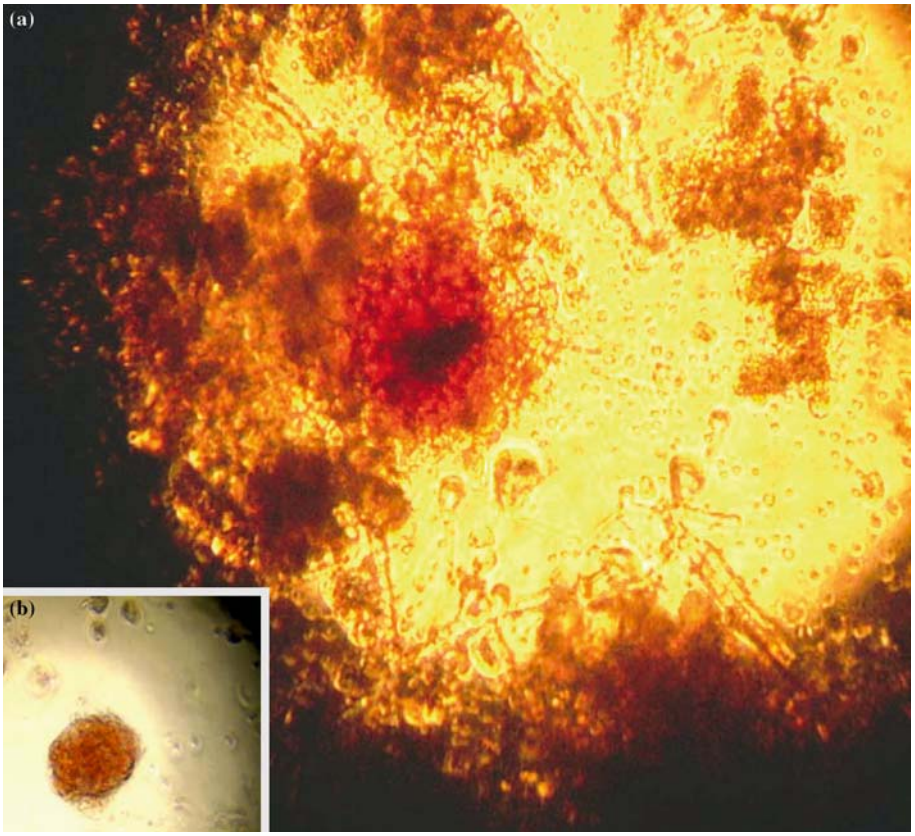

Figure 1. Dithizone staining of rat pancreas tissue during digestion with collagenase. (a) Incomplete digestion, the islet is attached to the ductal and exocrine tissue; (b) free islet observed after complete digestion.

Discussion

Considering several factors such as enzyme titration, pH adjustment, incubation temperature and incubation time period beside addition of Calcium ion and use of Glycerol as gelatin solvent improved the outcome of rat islet isolation in our experiment. While the formation of viscous material is a common problem during the enzyme digestion in pancreatic islet isolation, this problem has not been addressed in most of the methods described recently. It has been suggested that the gellike material is gelatin derived from collagen released enzymatically from pancreatic stroma (Burghen and Murrell 1989). Free islets entrap in the viscous, gelly material can not be released. This significantly decreases the number of islets recovered during purification. Careful optimization of Collagenase digestion is helpful to improve the result from islet isolation for studies on various features of pancreatic islets transplantation.

Table 1. Number of islets isolated from rats with and without optimized condition.

Weight of rat (g)	Islet numbers	
	Non-Optimized condition	Optimized condition
280–300	< 50	≥200
320–350	< 100	≥500

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