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A Method for the Separation of Mouse Pancreatic Islets Using Discontinuous Percoll Gradient Centrifugation

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Abstract A discontinuous Percoll gradient was used to separate islets from the collagenase-treated mouse pancreas easily and rapidly. Since the osmolality of Percoll is very low, adjustment of its osmolality to 340 mOs/kg H_2O was essential for securing the optimal separation. A discontinuous gradient layering with Percoll solution of 1.09 g, 1.07g, and 1.05 g/ml, respectively, when centrifuged at 800 ×g for 10 min, resulted in an optimal condition for separation and yielded a banding pattern with an even distribution of islet cells. No significant difference was observed in the morphological features between the Percoll-isolated and the manually-isolated islets. In conclusion, the discontinuous Percoll gradient can be effectively used to isolate the pancreatic islets from mice with four-fold higher efficiency compared to the handpicking method.

Key words: Pancreatic islet, gradient centrifugation, Percoll, osmolality

The endocrine gland of the mouse pancreas consists of individual islet cells which are scattered throughout the acinar parenchyma, but comprises of only a few percentage of the entire pancreas [1, 4]. Therefore, to develop an ideal method to isolate the highly pure islets in sufficient numbers is essential for the transplantation of islet cells for medical purposes and also biological studies.

Handpicking and Ficoll density gradient methods have been widely used [3, 5, 6, 7]. The handpicking method, which directly isolates islets from the collagenase-treated pancreas using a pipette, is time-consuming and tedious. The Ficoll density gradient method does not effectively separate the lymph nodes, blood vessels, and ducts from the islets [8]. The latter method also has unfavorable physicochemical properties, such as high viscosity and hyperosmolality. Percoll has been proven to be a suitable medium to isolate cellular components because of its iso-osmotic, non-viscous, and non-toxic characteristics in nature [9, 11].

In this study, an ideal isolation method of pancreatic islets from mice was investigated by using discontinuous Percoll gradient centrifugation. Advantages of the discontinuous Percoll gradient method are presented as compared to the continuous Percoll gradient method and the handpicking method.

ICR male mice (weighing 25 g) used for the islet isolation were purchased from the Dai-Han Animal Center (Eum Sung, Choong Buk, Korea). The animals were killed by decapitation, then the abdomen was opened, and the bile duct was ligated at the entrance to the duodenum using forceps. The pancreas was then distended by injection of 2.5 ml collagenase (type XI, 1,450 units/mg, Sigma Chem. Co., St. Louis, MO, U.S.A.) solution which was dissolved in a Dulbecco's Modified Eagles Medium (DMEM, 2 mg/ml at 4°C). The pancreas was removed and immediately immersed in 5 ml serum-free DMEM in a conical tube (Phillips, Plymouth, MN, U.S.A.). Next, the mixture was placed in a 37°C water bath for 27 min. The collagenase digestion was stopped by adding 15 ml of cold RPMI, and, finally, the pancreas was disrupted by hand-shaking the tube for 15 sec [2].

Because the osmolality of Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) is very low, the addition of 9 parts (v/v) of Percoll to 1 part (v/v) of 2.5 M sucrose results in a solution equivalent to about 340 mOs/kg H_2O, according to the manufacturer's instructions. The sucrose solution was sterilized by filtrating through the cellulose nitrate membrane filters (0.2 μm pore, Whatman, Maidstone, England). Desired densities of various gradients were obtained by diluting the stock Percoll with a 0.25 M sucrose solution.

Initially, Percoll gradient separations of islets were performed by both continuous and discontinuous methods. In the case of the continuous method, a mixture of 4

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volumes of Percoll stock solution combined with 6 volumes of 0.25 M sucrose solution forms a self-generated gradient of centrifugation at 20,000 ×g for 20 min. Therefore, 1 ml of the digested pancreas was placed on top of this solution and it was centrifuged at 800 ×g for 10 min to separate islets from other tissues. It was found that this continuous gradient method was far more cumbersome, resulting in more aggregation of tissues. Thus, islets and acini did not form a clear band but dispersed broadly. On the other hand, the discontinuous gradient yielded a rather pure islet fraction (see below) and for this reason, the discontinuous gradient method was chosen for the routine separation of mice islets.

In order to optimize discontinuous gradient centrifugation, various combinations of gradients were tested and evaluated. Among the tested, gradients formed by 1.05, 1.07, and 1.09 g/ml of Percoll turned out to be most efficient in separating islets. In each layer of the gradient, islets and acinar tissue were found to be layered in two well-defined bands: The islet band was located between 1.05 mg/l and 1.07 mg/l Percoll phases, and the acinar band was between 1.07 ml/g and 1.09 mg/l Percoll phases, and some were also located at the bottom pellet (Fig. 1). The islet band comprised of mostly islets and a few amount of acini (Fig. 2).

Since the collagenase digestion was a critical step in isolating the islet, two different ways of digestion were tested as described [10]: First, the sample of distended pancreas was incubated with a collagenase in a pre-warmed medium at 37°C, and the other sample in a medium without pre-warming (at ambient temperature). Unlike the previous report [10], there was no difference between the two methods in the recovery of islets, which suggests that the digestion of distended pancreas did not depend on the pre-warmed medium, but most likely on how well the collagenase was distributed throughout the pancreas.

Finally, the Percoll gradient separation was performed as follows: 1 ml of the tissue sediment in a 15 ml conical tube was added to 3 ml of the bottom gradient solution containing 1.09 g/ml Percoll, and was mixed by using a pipette several times to ensure an even distribution of the digested tissue. On the top of this layer, 3 ml of the second gradient (1.07 g/ml Percoll) was carefully added and followed by 3 ml of the last gradient (1.05 g/ml Percoll) in order not to disturb the gradients. The tube was then centrifuged at 800 ×g for 10 min at 4°C.

After the centrifugation, bands were formed in each interface (Fig. 1). The islet cells formed at the interface between 1.05 g/ml and 1.07 g/ml Percoll phases were transferred into a 15 ml conical tube, diluted with 3 ml RPMI, and then centrifuged at 50 ×g for 2 min. After

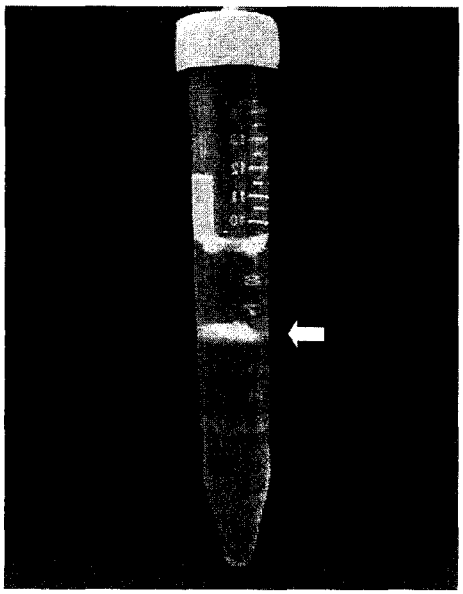

Fig. 1. Discontinuous Percoll gradient after centrifugation.
Pure mouse islets are located at the second well-defined band (arrow) and most of acini are located at the other bands and bottom pellet.

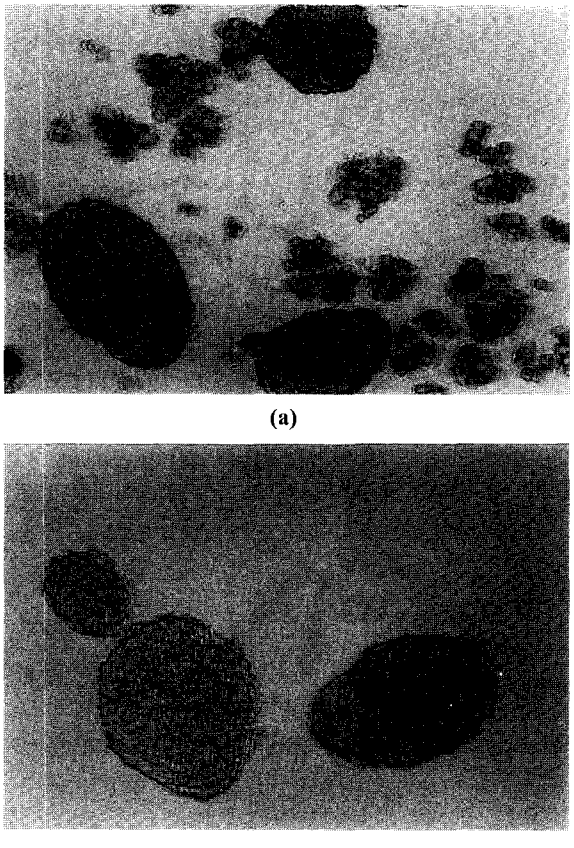

Fig. 2. Photograph of islets after discontinuous Percoll gradient centrifugation (×100).
(a) All islets are separated perfectly from attached exocrine cells. Islets are well preserved and there are a few amount of small acini. (b) After Percoll gradient centrifugation, handpicked islets showed no damage effect as compared to the manually-isolated islets.

(a)

(b)

Fig. 3. Photograph of islets after collagenase-digestion without Percoll gradient centrifugation (×100).
(a) The presence of many relatively big acini makes the isolation of islets difficult and time consuming. (b) Manually-isolated islets. Pure islets can be observed.

removing the supernatant, these islets were decanted into petri dishes for a final removal of contaminating tissue(s) under a light-microscope.

When the discontinuous Percoll centrifugation method was compared with the handpicking method, the former method concentrated on a large number of islets easily and rapidly. While the handpicking method took 60 min to separate 80 islets, the Percoll method took only 30 min to separate 160 islets, which indicates four-fold improved efficiency in terms of the time required to separate the same number of islets. The morphological examination of the islets isolated by Percoll did not show any signs of damage under a microscope (Fig. 2), compared to the handpicked islets (Fig. 3). However, it was noted that an over-digestion (longer than 30 min) damaged the islets morphology. Therefore, the amount of collagenase added and the period of digestion appear to be crucial in protecting islet cells from damage.

In conclusion, this study suggests that the discontinuous Percoll gradient centrifugation is the most suitable isolation method for the preparation of a large number of pancreatic islets in a relatively short period of time without exposing them to unfavorable conditions.

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