Microencapsulation of Pancreatic Islets With Canine Ear Cartilage for Immunoisolation

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ABSTRACT

Improving human islet transplantation is often limited by the shortage of donors and the side effects of immunosuppressive agents. If immunoisolation is properly used, it can overcome these obstacles. Because artificial materials are adopted in this technique, however, there are still multiple issues with biocompatibility and foreign body reactions. We developed a chondrocyte microencapsulated immunoisolated islet (CMI-islet) that allows living cells to act as the immunoisolating material. To manufacture CMI-islets for xenotransplantation, isolated rat pancreatic islets were placed on low cell-binding culture dishes. Subsequently, expanded canine auricular cartiage primary cells were seeded on these dishes at a high density and maintained in a suspended state via a shaking culture system. Morphological evaluations showed good islet viability and a clear progression of the islet- encapsulation events. When the cells were challenged with glucose, they were able to secrete sufficient insulin according to glucose concentrations. The CMI-islets responded better to the glucose challenge than did nude pancreatic islets and created better glucose-insulin feedback regulation. Moreover, insulin secretion into the culture medium was confirmed over a period of 100 days, showing the survival and secretory capacity of the CMI-islet cells. By microencapsulating pancreatic islets with recipient ear cartilage cells, long-term insulin secretion can be maintained and the response to glucose challenges improved. This new immunodelusion technology differs from other immunoisolation techniques in that the donor tissue is enclosed with the recipient's tissue, thus allowing the transplanted cells to be recognized as recipient cells. This microencapsulation method may lead to developing viable xenotransplantation techniques that do not use immunosuppressive drugs.

IMMUNOISOLATION is the technique of encapsulating a graft with selectively permeable materials that only allow the passage of smaller molucules, such as gases (eg, O_2 and CO_2), nutrients (eg, glucose and amino acids), and hormones (such as insulin). Such materials resist penetration by immune cells and larger immune molecules, such as natural antibodies and complement components. Properly implemented immunoisolation may allow islet transplanta-

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tion without immunosuppression.[1,2] Many different materials have been used for immunoisolation recently, but these materials have not been sufficiently biocompatible, leading to foreign body reactions and fibrosis.[2,3]

Our group developed a completely new immunoisolating technology using biomaterial from living recipient cells. This technique was named immunodelusion. It uses the chondrocyte sheeting immunodelusive immunoisolated bioartificial pancreas (CSI-BAP). The method uses cell sheet engineering to achieve auricular chondrocyte macrocencapsulation.[4,5]

New methods for creating immunodelusive immunoisolated islets were invented in this study. The shaking culture method was used to achieve chondrocyte microencapsulation of the islets. The aim of the present study was to investigate the creation and structure of chnodrocyte microencapsulated immunoisolated islets (CMI-islets) and to examine the long-term insulin secretory capabilities of these CMI-islets.

MATERIALS AND METHODS

The animal experiments were approved by and conducted according to the Yonsei and Konkuk University Guidelines on Animal Use. The Institutional Animal Care and Use Committee (IACUC) number was KU11036.

Primary Culture of Chondrocytes From Ear Cartilage

The chondrocytes were prepared as has been previously described.[5] Small fragments of auricular cartilage were obtained from a beagle dog. The tissue was then digested in a cocktail solution of Ham's F-12 medium containing 0.3% collagenase (collagenase class II; Worthington, Biochemical Co, Lakewood, NJ United States), 0.25% trypsin (Invitrogen Co, Carlsbad, CA United States), and 4% of a antibiotic/antimycotic mixture (ABAM; 10,000 units/mL penicillin G, 10,000 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B [Invitrogen Co.]), and 50 μg/mL ascorbic acid (Sigma Chemical Co., St. Louis, MO, United States). The resulting cells were seeded and expanded by sequential passages (1–3) through ordinary monolayer CBM (Cambrex Bio Science, Walkersville, MD, United States) cultures supplemented with CGM SingleQuot (Cambrex Bio Science) and 50 μg/mL ascorbic acid. The cultured cells recovered from passage 3 were used as the microencapsulating source when fabricating the CMI-islets.

Islet Isolation

The islets were isolated as previously described.[6,7] Pancreata from male Lewis rats (approximately 250 to 350 g) were digested with collagenase P (Roche Applied Science Co, Mannheim, Germany), and the islets were purified by a Histopaqua (Sigma Chemical) discontinuous density gradient.

Chondrocyte Microncapsulation of the Islets

After overnight culture, the islets were divided into two groups (CMI-islets and nude islets) according to the presence of chondrocyte caplsulation. Half of the total islets were placed in low cell binding culture dishes (60 mm Hydrocell, Cellseed Co, Tokyo, Japan). Then, 300×10^4 chondrocytes/mL were resuspended in 5 mL of mixed culture medium (MCM) containing 50% Ham's F-12 medium (Invitrogen) and 50% RPMI-1640 without glucose (invitrogen) with 25 mmol/L HEPES, 1%of the antibiotic/antimycotic mixture (ABAM; Invitrogen), and 50 μg/mL ascorbic acid. The final concentration of heat-inactivated fetal bovine serum (FBS, JRH Biosciences, Lenexa, Kansas, United States) was adjusted to 10%, and the glucose concentration was adjusted to 100 mg/dL by adding D − (+) − glucos (Sigma Chemical Co) to the shaking cultures. Reciprocal shakers (NA-201; Nissin Rika Co, Tokyo, Japan) were used for continual horizontal agitation, yielding microparticles in which the islet cells were encapsulated by the chondrocytes. Under the above conditional, it took approximately 5 to 6 days to achieve the complete CMI-islet structures. By contrast, the nude islets in the control group did not have microencapsulation because they were treated and prepared using the same procedures as the CMI- islets but without adding the chondrocytes. The morphological changes that occurred in the process of CMI-islet formation were recorded with an Olympus IX51 phase-contrast microscope (Olympus CO, Tokyo, Japan). Vital dye staining with diphenylthiocarbazone (dithizone; Sigma Chemical Co) was used to visualize the islet β cells in both groups.

Histological Studies

The cryosectioned samples were stained with hematoxylin and eosin (H&E), and the insulin was detected using the avidin-biotin-peroxidase complex immunohistochemistry technique (LSAB 2 kit/HRP, DAKO Japan Co, Ltd.) according to the manufacturer's protocol for the 3-amino-9-ethylcarbazole substrate-chromogen solution (DAKO Japan Co).

Glucose Challenge Test: Static Incubation

To compare the functionality of the two groups, the CMI-islets and the nude islets were subjected to a glucose challenge. The insulin-secreting ability (a prompt reaction to a sudden rise in glucose concentrations) was tested by 1-hour exposures to different concentrations of glucose in the media (100 mg/dL and 400 mg/dL). The islet function of each group was expressed as the stimulation index (SI). The SI of each group was calculated using the following forumla: SI = insulin content in the high glucose media/insulin content in the low glucose media.

Measurement of Insulin Secretion

The CMI-islets were cultured in the MCM, and the medium was changed every 74 hours. The insulin secretion into the culture medium from the CMI-islets was determined using an insulin enzyme-linked immunesorbent assay kit (ALPCO, Salem, NH, United States).

Statistical Analysis

The data are shown as the mean values ± standard errors. The groups were compared using an analysis of variance model. Statistical significance was set at $P < .05$.

RESULTS

Morphology of the CMI-islet

Figure 1–AE shows the alteration in the CMI-islet shape over time. The chondrocytes gathered around the islets and eventually became anchored to them. The outlines of the CMI-islets gradually became smooth. A phase-contrast micrograph of the completed CMI-islet structure after 125 hours of culture