ADULT RAT PANCREATIC ISLET CELLS ADHERENT TO MICROCARRIER BEADS: EVALUATION OF FUNCTION AND MORPHOLOGY

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SUMMARY

Dispersed adult rat pancreatic islet cells were incubated with Cytodex-3 microcarrier beads for 72 h, during which time single cells adhered firmly to bead surfaces. Electron microscopy revealed well-preserved ultrastructure of attached A, B, and D cells. Perfusion of these cultures showed stable basal insulin release, brisk, biphasic insulin responses to 30-min glucose stimulation, and consistent, monophasic spikes of insulin release in response to repeated, brief pulses of glucose. These results indicate that adult rat islet cells attach to microcarriers and remain viable in culture. This preparation offers advantages for studies of hormone secretory dynamics of differentiated single islet cells, free from cell-to-cell interactions.

Key words: islet cells; insulin; microcarrier beads.

Pancreatic islet cells rapidly reaggregate after dispersion to form either confluent monolayers (8) or free-floating “pseudoislets” (13). Neither preparation consists of “single” cells, having reestablished structural and humoral mechanisms for communication between component cells (4). One approach to studying secretory dynamics of separated islet cells is to provide a substrate, suitable for transfer to a perfusion system, to which islet cells can attach singly and remain viable. Microcarrier beads fulfill both requirements. First reported in 1967 (15), microcarriers have since been used for static and, in a few instances, perfusion cultures of a wide range of mammalian cells, including fetal (2) and neonatal (14) rat islet cells attached to Cytodex beads and cloned rat insulinoma cells attached to fibronectin-coated plastic beads (1). Here we have studied the morphology and function of Cytodex-coupled islet cells obtained from mature rat pancreatic islets.

Pancreatic islets were isolated from Wistar rats using collagenase digestion-Ficoll separation techniques (12) and cultured in nonadherent petri dishes. After 72 h islets were dispersed by a modification of the enzymatic-shaking procedure of Lernmark et al. (7). Cell viability, as measured by trypan blue exclusion (3), was >90% immediately after dispersion. Approximately 70% of the yield consisted of single cells, with 25% as doublets or triplets, and the remainder as clumps of four to eight cells; larger clusters were rarely seen.

Approximately 30 mg of Cytodex-3 microcarrier beads (Pharmacia, Sweden) were swollen in excess (approx. 1 mg/ml) Ca and Mg-free phosphate buffered saline for at least 3 h at room temperature, washed twice in 10 ml of this solution, autoclaved at 115°C, 15 psi for 15 min, and rewashed three times with 10 ml of tissue culture medium. Immediately after dispersion islet cells were added to the bead suspension to give a final density of 2 × 10⁶ cells: 5 mg beads/ml tissue culture medium. One milliliter aliquots were transferred to 10 × 15 mm culture wells (Nunc, Denmark) and incubated for 72 h. Tissue culture medium for incubation of intact islets and islet cells was RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO), 2 mmol/l L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin sulfate. Tissues were maintained at 37°C in a 5% CO₂:95% air, humidified incubator.

Islet cells cultured for 72 h in the presence of Cytodex beads were fixed with 2.5% glutaraldehyde in 0.2 M cacodylate buffer, postfixed with 1% osmium tetroxide, and dehydrated in graded ethanols. Samples were (a) embedded in Spurr’s resin, thin-sectioned, stained with uranyl acetate and lead citrate, and viewed with a JEM-100B electron microscope (Jeol, Japan); (b) critical-point dried, sputter-coated with gold, and viewed with a scanning electron microscope ISI-40 (International Scientific Instruments).

Insulin secretion was studied using a multichannel perfusion system (5). Half-milliliter aliquots of islet cells cocultured with beads for 72 h were carefully transferred to plastic minicolumns (modified 2 ml syringes). Cells were perfused at 37°C with Krebs-Ringer solution (KR) supplemented with 24 mmol/l HEPES buffer, 0.1% heat-inactivated FBS, and D-glucose as specified, at pH 7.30. Flow rate was 1.0 ml/min. After a 30-min equilibration period with KR containing 2.8 mmol/l glucose, islet cells were (a) perfused for 30 min with KR containing 22 mmol/l glucose; (b) pulsed for 2 min with KR containing 22 mmol/l glucose every 10 min.
for 60 min, with interposing KR + 2.8 mmol/l glucose. One minute effluent fractions were collected at regular intervals and stored at $-20^\circ$ C. After perfusion the contents of the minicolumns were retrieved and the cellular DNA measured by fluorometric assay (5). Insulin was measured by radioimmunoassay (RIA) using rat insulin standards (Novo Research Institute, Denmark). Insulin release from perfused islet cells was standardized by expressing secretion rates as picomoles per microgram DNA per minute. Data given here are mean ± SEM values.

**Microscopy.** Attachment of cells to the bead surfaces was apparent within 6 to 8 h of incubation. By d 3 of culture it was estimated on the basis of DNA recovery that >80% of the cells were firmly adherent to beads. Many cells had small cytoplasmic projections onto the bead surfaces (Fig. 1). The great majority of cells attached were single, although clumps of two to four cells were sometimes seen, occasionally fusing into a single, larger entity shared between two beads.

Scanning electron microscopy showed that cells attached to beads were semispherical, being flattened only in areas in direct contact with a bead (Fig. 1).

Thin-section electron microscopy revealed islet A, B, and D cells (PP cells were unable to be conclusively identified), with well-preserved ultrastructural features and numerous, characteristic secretory granules. Cell membranes were contiguous with bead surfaces (Fig. 1).

![Fig. 1](image1.jpg)

**FIG. 1.** Adult rat islet cells attached to Cytodex-3 microcarrier beads after 72 h static culture. A. Low-power photomicrograph of living islet cells attached to free-floating beads, viewed with an inverted microscope. Average bead diameter is 180 $\mu$m. X 44. B, C, Higher-power photomicrographs of living islet cells showing cytoplasmic projections onto bead surfaces. X185. D, Thin-section electron micrograph of an islet B-cell attached to a bead (b), showing well preserved ultrastructure and numerous secretory granules. N = nucleus. The bar represents 1.0 $\mu$m. X11 990.

![Fig. 2](image2.jpg)

**FIG. 2.** Insulin release from perifused rat islet cells attached to Cytodex-3 beads, in response to 30-min stimulation with 22 mmol/l glucose. Results are mean ± SEM of seven experiments.