

mixture at a concentration of approximately 5000 islets/ml of AP mixture. This suspension was pumped through a coaxial flow jethead with concentric air flow to produce droplets of a desired size. Droplets produced by this technique were typically 200–700 microns in diameter. The droplets were collected in a beaker containing 0.4% calcium chloride solution where they instantly gelled by ionic crosslinking on contact with the solution. A calcium chloride solution of 0.1–2.0% can be used, but preferably a 0.4–0.8% solution is used. The transparent glass collection vessel was exposed to electromagnetic radiation (Hg lamp, 100 watt) with a bandpass green filter (transmission 500–580 nm) with a peak transmission around 550 nm. This wavelength range is coincidental with the absorption spectrum of ethyl eosin which functions as the dye or photoinitiator in the system. Ultraviolet and other wavelengths were screened out to prevent potential damage to the islets.

The ionically crosslinked droplets or capsules were simultaneously polymerized or photocrosslinked upon exposure to electromagnetic radiation, or light. This resulted in dually (i.e., ionic and covalent) crosslinked droplets or capsules containing islets. Exposure of these cells to light was limited to about five minutes although no evidence of deterioration or damage to islet function was observed at longer times. The capsules were thoroughly rinsed in saline and culture media and then put into culture.

Alternatively, large capsules (on the order of mm) could be prepared by injecting the solution through a syringe. Also, microcapsules prepared by conventional techniques could be further encapsulated in a 'macrocapsule' by this technique, the benefits of which are discussed below.

Example 10

In Vitro Tests of Islet Function Following Encapsulation with AP Mixtures

Static glucose stimulations (SGS) were performed on the encapsulated islets to assess islet function. Staining with dithizone or acridine orange/propidium iodide was done to assess viability. Briefly, the SGS technique involves stimulation of islets with a high level of glucose and measurement of the secreted insulin (by RIA) in response to the glucose level. The islets are first incubated for an hour in a basal level of glucose (typically 40–60 mg %) followed by incubation at a stimulatory level for an hour (300–400 mg %) and then back down to a basal level of glucose for an hour. An increase in the secreted insulin level above the basal secretion during the stimulation phase, followed by a return in secreted insulin to basal levels is a requisite for good islet function.

Two controls were used for this experiment. They were free islets and islets encapsulated in the conventional alginate/polylysine microcapsules (such as those disclosed in U.S. Pat. Nos. 4,352,883, 4,391,909, and 4,409,331.) The stimulation ratios (relative to initial basal insulin levels) for the free islets were 1.0:6.6:1.6, ratios for the alginate/polylysine encapsulated islets were 1.0:11.0:2.5, while levels for the AP encapsulated islets were 1.0:17.0:6.35. (The first number in the ratio refers to the normalized basal insulin level, the second refers to the insulin level in response to the glucose challenge, and the third to the basal level after the glucose challenge.) These results indicated adequate islet function and, along with viability staining, confirmed that there was no apparent damage to islet function as a

result of the polymerization process or presence of other monomers and catalysts. It indicated, in fact, that the treatment of islets with the AP mixtures resulted in a better stimulation of islets in terms with insulin output.

Example 11

In Vitro Tests of Islet Function by Perifusion Following Encapsulation of Islets with AP Mixtures, Effect of Capsule Size on Kinetics of Insulin Release

Canine islets in conventional alginate-polylysine microcapsules as described in U.S. Pat. No. 4,663,286) as well as islets encapsulated by the AP mixtures of the present invention (300–800 microns diameter) were tested for insulin secretion in a perifusion apparatus. Perifusion involves the testing of the islet response to a stimulatory level of glucose in a flowing system rather than a static system as described above. It is therefore possible to obtain the kinetics of insulin release by using this technique. Culture medium containing an appropriate amount of glucose is perfused through the system that has a chamber containing encapsulated islets and the perfused medium is collected periodically in a fraction collector and assayed for released insulin by RIA. In this particular test, a basal level of glucose (40 mg %, or 40 mg of glucose/100 ml of medium) was maintained for the first 60 minutes followed by a stimulatory level of glucose (300 mg %) for 30 minutes and a return to basal levels of glucose (40 mg %) for the remainder of the experiment.

The results as illustrated in FIG. 2 show a distinct increase in insulin output measured relative to basal levels of insulin (at 40 mg % glucose) secreted by the islets when the chamber is perfused with medium containing 300 mg %. There is a lag phase of typically about 10 minutes before the islets start secreting an increased amount of insulin in response to the stimulatory level of glucose. About 15 minutes after the stimulation is ended, the insulin output from the islets falls back to basal levels of secretion. Clearly there is no difference in the kinetics of the response (or rapidity of the response) to increased glucose levels between the conventionally encapsulated islets (alg/PLL capsule) and the AP encapsulated islets. The AP encapsulated islets however show a greater stimulation in terms of insulin output than the alginate/PLL encapsulated islets.

To assess the effect of size of microcapsules on the kinetics of insulin release, larger microcapsules or "macrocapsules" were prepared containing canine islets. These macrocapsules (typically 5–7 mm diameter) were prepared simply by extruding a suspension of islets in the AP solution through a syringe and forming droplets in a calcium solution followed by photocrosslinking. AP solutions containing two different molecular weights of PEG-DA were prepared. They were AP 10 k (with PEG-DA 10000 MW) and AP 3.4 k (with PEG-DA 3400 MW). Macrocapsules of these materials containing canine islets were perfused to obtain the insulin release profiles as shown in FIG. 3. Islets encapsulated in the different AP solutions both showed a response to increased glucose within 10–15 minutes after the stimulus began. Thus there was no significant difference between the microcapsules and macrocapsules in terms of rapidity of insulin release following a stimulus of glucose. This was an important observation since it suggested that the size of the capsule may not be of importance in the kinetics of insulin response. On the other