Morphological and functional characterization of a pancreatic β-cell line microencapsulated in sodium cellulose sulfate/ pDADMAC

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Introduction

The incidence of diabetes mellitus is steadily increasing and the World Health Organization expects that the number of diabetic patients will increase to 300 million by the year 2025 (WHO, 2002). Late diabetic complications cannot be prevented totally by current anti diabetic strategies. Therefore, new therapeutic concepts of insulin replacement such as pancreas transplantation are evolving. Due to the shortage of human donor organs, transplantation of encapsulated xenogeneic pancreatic islet cells has attracted considerable attention (Kim KW, 2004; Löhr M, 1998). Sodium cellulose sulphate/ poly(diallyldimethyl-ammonium chloride) (NaCS/pDADMAC) is a material with favourable biogenic properties that has successfully been used for encapsulation of various cell types (Wang T, 1997). Currently, no data are available on the suitability of NaCS/ pDADMAC for encapsulation of pancreatic ß-cells

Material and methods

Cell line

An immortalized hamster pancreatic ß-cell line HIT-T15 (ATTC Number: CRL-1777) was cultured at 37°C according to the recommendations of the manufacturer. Basal medium (BM) contained 445.5 ml Ham F12, 12.5 ml fetal calf serum, 35 ml horse serum, and 2 ml glutathione. Depending on the experimental design, the BM was supplemented with 100 mg/dl (BM 100), 200 mg/dl (BM 200), 300 mg/dl (BM 300) or 500 mg/dl (BM 500) glucose (Sigma-Aldrich, St Louis, MO, USA).

Encapsulation of cells

Capsules were produced with the vibrating fluid technology: Small beads are formed if a laminar sodium cellulose sulphate solution (SCS) jet breaks under controlled parameters (22). The negatively charged polymer beads drip into the hardening bath containing the positively charged polymer pDADMAC (poly-(diallyldimethylammonium-chloride)) (Batch 15215ab, Sigma Aldrich; MW 100.000–200.000 kDa). For cell immobilisation, up to $3x10^6$ HIT-T15 cells were mixed into 1 ml of a 1.8% SCS solution containing 1% NaCl. The capsule diameter was adjusted to 600µm. Cell culture conditions for non-encapsulated HIT-T15 cells were the same as for encapsulated cells.

Glucose uptake of non-encapsulated and encapsulated HIT-T15 cells

Non-encapsulated and freshly encapsulated HIT-T15 single cell suspensions (i.e. 10^6 cells/ml each) were cultured in parallel in BM using 96 well plates for 2 weeks. Thereafter non-encapsulated and encapsulated cells were incubated with BM containing 100 mg/dl of 2-[n-7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (Molecular Probes, Leiden, The Netherlands) at 37°C and culture medium was initially sampled after 5 min, then every 15 min over a total period of 75 min. Uptake of fluorescent glucose into non-encapsulated and encapsulated cells was measured directly with the Fluoroskan Ascent fluorometer (Thermo Labsystems, Altrichnam, UK) at 485 nm after excitation at 538 nm. Results were expressed as arbitrary fluorescence units.

Glucose dependent proliferation of non-encapsulated and encapsulated HIT-T15 cells

The effect of encapsulation on the proliferation of HIT-T15 cells was determined after 1 and after 7 days of culture in BM, BM 100 or BM 500, respectively, using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide)-based Cell Growth Determination Kit™ (Sigma-Aldrich). Proliferation of encapsulated cells was determined by MTT tests as in non-encapsulated cells. Extinction was measured at 570 nm and cell numbers were determined.

Insulin secretion of non-encapsulated and encapsulated HIT-T15 cells

For long-term glucose exposure, non-encapsulated and encapsulated cells were cultured in BM supplemented with different concentrations of glucose (BM, BM 100, BM 200 or BM 500) for 28 days. Insulin concentration was measured using the Mercodia High Range Rat Insulin enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden) and glucose concentrations were determined with SuperGL® Analyzer (Hitado, Endingen, Germany). For short-term glucose exposure, the BM medium was removed and cells were washed once with PBS. Thereafter, non-encapsulated and encapsulated HIT-T15 cells were exposed to BM 100 for 60 min at room temperature. 1.5 ml of the cell culture medium was collected after 5 min and every 10 min during a 60 min time period. The samples were stored at −70°C until further analysis.

Results and discussions

Morphological and immunohistochemical characterization of encapsulated HIT-T15 cells

Proliferation of non-encapsulated HIT-T15 cells was monitored at the same time points as for encapsulated cells (Fig. 1). Two days after seeding of a single cell suspension, islet like cell clusters of approximately 10 to 50 µm in diameter, attached to the cell culture plate, were formed (Fig. 1A). After 1 week of culture confluent, larger clusters of a maximum diameter of approximately 300 µm were visible (Fig. 1B) and after two more weeks of culture, several cells underwent apoptosis as shown by immunohistochemistry using antibodies (R&D Systems, Mineapolis, USA) against activated caspase 3 (Fig. 2B). The apoptotic cells detached from the cell culture plate and were extruded into the culture medium (Fig. 1C). After encapsulation of a HIT-T15 single cell suspension, islet like cell clusters of approximately 10 to 50 µm diameter were visible in the lumen of the capsules after 2 days of culture (Fig. 1D) and after 1 week of culture larger clusters of 200 µm diameter were formed (Fig. 1E). Some cells in the centre as well as in the periphery of the clusters underwent apoptosis. These cells were decorated by antibodies against activated caspase 3 (Fig. 2E). After about 3 weeks of culture the cell clusters filled the lumen of the capsules (Fig. 1F).

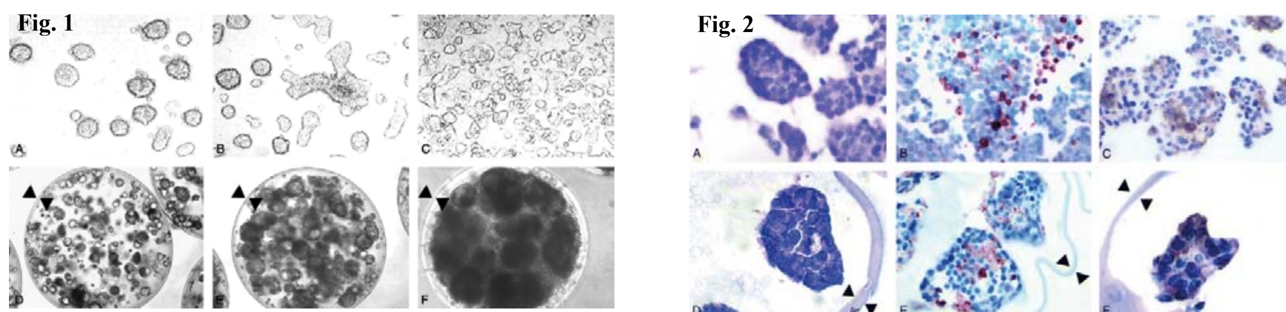

Fig. 1. **Proliferation of non-encapsulated (A–C) and encapsulated (D-F) HIT-T15 cells**

Fig. 2. **Non-encapsulated (A) and encapsulated (D) HIT-T15 cells are organized in cell clusters (hematoxylin and eosin, 400×). Immunohistochemical detection of apoptosis of non-encapsulated (B) and encapsulated (E) Insulin production in non-encapsulated (C) and encapsulated (F) cell clusters. The capsule membrane is marked with arrowheads. Undulation of the capsule membrane is due to a shrinking artefact, which is caused by paraffin embedding of formaldehyde-fixed capsules.**

Insulin expression was readily detected in non-encapsulated as in encapsulated cells by immunohistochemistry using antibodies (Novobiolab, Denmark) against insulin (Fig. 2C and F).

Glucose uptake and glucose dependent proliferation of non- and encapsulated HIT-T15 cells

There was no difference in glucose uptake between non-encapsulated and encapsulated HIT-T15 cells (Fig. 3, Mann–Whitney test, $P = 0.54$). Proliferation of non-encapsulated as well as encapsulated cells was dependent on glucose concentration (Fig. 4). When cultured at low (BM) or at supra physiological glucose levels (BM 500) for 6 days, numbers of non-encapsulated cells decreased by 24% and 19%, whereas cell numbers of encapsulated cells decreased by 43% and 44. At physiological glucose concentration (BM 100), cell numbers of non-encapsulated and encapsulated cells increased by 81% and 96%, respectively.

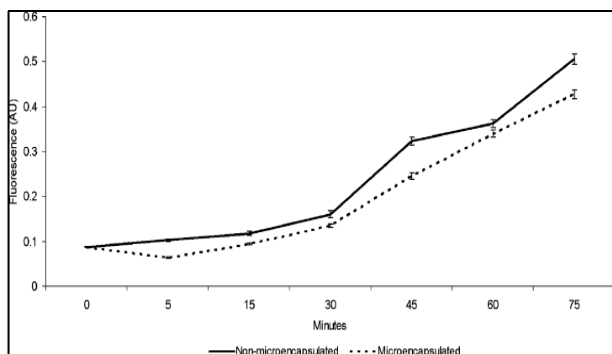

Fig. 3. **Non-encapsulated and encapsulated single cell suspensions of HIT-T15 cells (10^6 cells/ml each) were cultured in parallel**

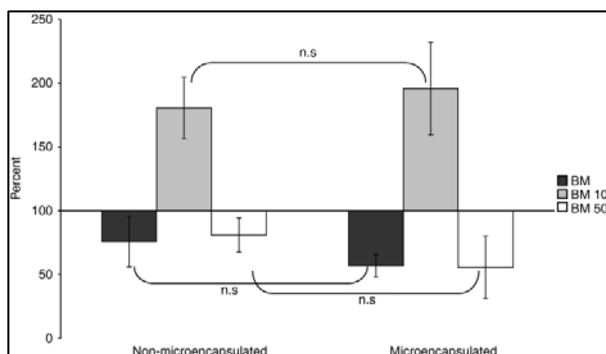

Fig. 4. **The influence of encapsulation on proliferation of HIT-T15 cells. Results are given as mean ± SEM.**

There was no difference in glucose-dependent proliferation between non-encapsulated and encapsulated cells regardless of the glucose concentration in the culture medium (Student's t-test, P-values for BM, BM 100 and BM 500 were 0.076, 0.610, and 0.071).

Insulin secretion of non-encapsulated and encapsulated HIT-T15 cells

Insulin secretion of non-encapsulated and encapsulated HIT-T15 cells in response to long-term glucose exposure was dependent on glucose concentration in the culture medium (Fig. 5A, B).

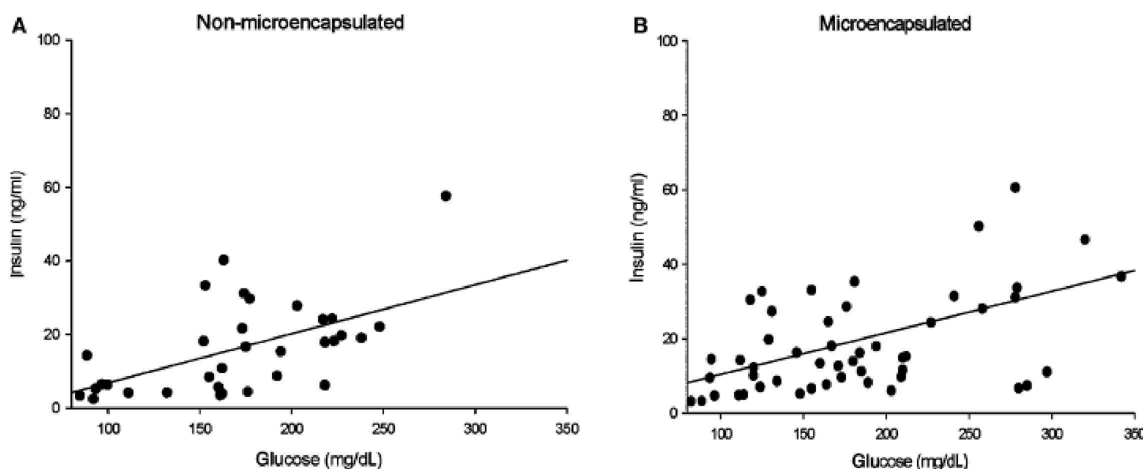

Fig. 5. **Insulin secretion of non-encapsulated (A) and encapsulated (B) HIT-T15 cells was measured in response to long-term exposure of varying glucose concentrations in the culture medium.**

Statistical analysis revealed a similar moderate correlation between glucose concentration in the culture medium and insulin secretion in both non-encapsulated and encapsulated cells (Pearson's $R = 0.70$ and 0.68). Upon short-term stimulation with physiological amounts of glucose (BM 100), non-encapsulated and encapsulated cells reacted in the same physiological biphasic pattern (Fig. 6). The first peak, corresponding to fast secretion of insulin stored in submembranous vesicles, occurred in non-encapsulated cells after 5 min. The encapsulated cells reached this first peak of insulin secretion 5 min later than non-encapsulated cells. The second peak of insulin secretion that corresponded to secretion of newly produced insulin occurred after 40 min in non-encapsulated and after 50 min in encapsulated cells. However, the differences in biphasic insulin secretion response between non-encapsulated and encapsulated cells did not reach statistical significance (*t*-test). This biphasic insulin response of HIT-T15 cells compares well with glucose dependent insulin response observed under physiological conditions in humans.

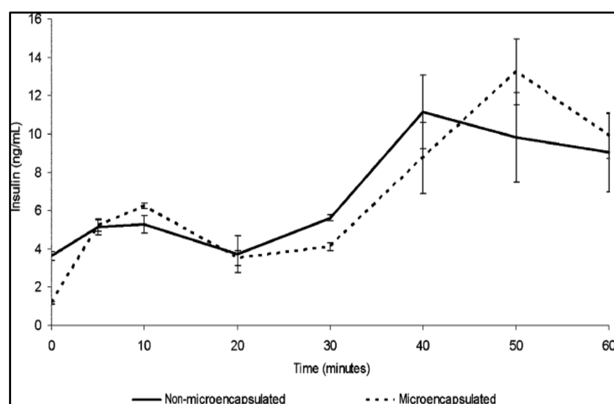

Fig. 6. **For short-term glucose exposure, non-encapsulated and encapsulated HIT-T15 cells were exposed to BM 100 for 60 min at room temperature. The first peak, corresponding to fast secretion of insulin stored in submembranous vesicles, was detected in the culture medium of non-encapsulated cells after 5 min and in encapsulated cells after 10 min. The second peak of insulin secretion that corresponded to secretion of newly produced insulin occurred after 40 min in non-encapsulated cells and after 50 min in encapsulated cells.**

Conclusion

The aim of our study was to test the suitability of NaCS/pDADMAC as a novel material for microencapsulation of pancreatic β-cells, i.e. its influence on cell viability, glucose uptake and insulin secretion *in vitro*. The results of this study pointed out that encapsulation of HIT-T15 cells in NaCS/pDADMAC does not influence cell proliferation, insulin secretion and glucose uptake. The results of our study together with the known favourable biogenic properties of the encapsulation material (Löhr M, 1998; Omer A, 2005) suggest the NaCS/pDADMAC encapsulation technique of Austrianova as a promising alternative for the encapsulation of pancreatic β-cells.

Bibliography

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