Secretion of a Novel Gene Product through Micro-encapsulated Recombinant Cells
a Preliminary Report

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INTISARI

M. Munayar Roni, A.M. Sun & P.L. Chang – Sekresi produk gen baru dari sel rekombinan yang dibungkus mikrokapul. Laporan pendahuluan

Gula dan hasil ekspresi genik yang berasal dari kondisi tertentu secara in vitro dan in vivo dapat dimanfaatkan untuk berbagai pengobatan. Dengan demikian, terdapat penelitian alternatif bagi tempiota genetik, yang umumnya bergantung pada modifikasi genetik melalui teknik rekombinan DNA. Perluasan ini mengenai ekspresi komponen pertumbuhan seluler (HSP) dari hasil ekspresi genetik sel manusia yang ditemukan di dalam mikrokapul in vitro secara in vivo.

Sel menunjukkan adanya glikoprotein (HPC) yang mengkondensasi pNMG yang mengikat gen HPM dari gena reseptor tertentu G418. Sel-sel yang memiliki gen HPM dalam jumlah banyak diikat untuk dihidupkan dengan mikrokapul kaporitler 900nm yang terbuat dari bahan elastomer – polyurethane – sengsiro. Proses pengepalm u sertai menggandakan pelatangan bisul sel, tetapi belum lebih dari 95% sel masih tinggal hidup secara pertumbuhan. Selanjutnya penciptaan selena 3 lingkungan agitasi sel yang berasal dari konten baku baku, dan HPS di dalam...
media juga meningkatkan homeostasi. Metode mikrokapling cekup permukaan, dari melanggar pada awal percobaan 15% sampai 80% BGM dalam media, dapat disimpan halus produk gers dan di dalam perlakuan panas. Metode ini menghasilkan berbagai peluang dalam mengembangkan halus produk gers baru dari gers. Hal ini memberikan peluang untuk meningkatkan efisiensi dan kualitas produk gers, yang akan memberikan manfaat bagi penelitian produk gers baru.

Keywords: genetically modified cells - placental p53 - human growth hormone - immune protection - microcapsules

INTRODUCTION

Current approaches to human gene therapy focus on insertion of a desired gene into autologous cells such as bone marrow stem cells (Dantsker et al., 1988), hepatocytes (Ledley et al., 1987; Wilson et al., 1988), endothelial cells (Wilson et al., 1989), lymphocytes (Calver et al., 1993), or fibroblasts (Chang et al., 1986; Polisky et al., 1987). Since human primary cells are difficult to transfect, we propose as an alternative strategy that avoids the dependence on an analogous source of target cells. The target is an easily transferrable cell line that can be engineered to express a desired gene product. If these recombinant cells are enclosed in permeable microcapsules which protect the cells from contact with the host's immune modulators (Fan et al., 1988; Wim et al., 1989) and yet allow the exit of the engineered gene product, these cells should be immunologically tolerated and able to provide the previously missing gene after implantation in vivo.

Severe criteria must be fulfilled beforehand this strategy can be developed. The permeable micromembranes must be constructed with a process that is physically compatible with cell survival. They must also provide a micro-environment compatible with cell growth, be permeable to the exit of the novel gene product but not the entry of immune modulators from the host, and have the property of being biocompatible after implantation. Biomaterials such as alginate-polylysine-alginate microcapsules (Fan et al., 1989) and acrylic hollow fibers (Wim et al., 1989) excluding antigenic (Lin et al., 1980) or xenogenic (O'Toole & Sun, 1986; Weber et al., 1989; Lacy et al., 1991) pancreatic islet cells, or neurochromatoma cells (Wim et al., 1991) have been shown to offer many of the above desired properties as implantable immune-protective devices. We now report on our attempts to deliver human growth hormone through genetically-modified mouse cultured fibroblasts after encapsulation with the biocompatible alginate-polylysine-alginate as a model leading to an alternative method for somatic gene therapy.

MATERIALS AND METHODS

Recombinant fibroblasts

Mouse Lx-1 cells, a spontaneously immortalized mouse cell line, were cultured in MEM (Minimum essential medium) media supplemented with L-glutamine (2mM), new born calf serum (10%), penicillin (100 U/ml) and streptomycin (100 µg/ml) under the
usual conditions. These cells were transfected with calcium-phosphate precipitated (Graham & Grossi, 1985) pNMO encoding the human growth hormone (hGH) gene with a mouse metallothionen promoter MT1 at the 5' end and SV40 polyadenylation signal at the 3' end. This plasmid also encodes the phosphatase-inferase gene permitting selection of transfecteds with G418. The construction of the plasmid has been described (Chang et al., 1990).

Encapsulation procedure

The cells were harvested with trypan and encapsulated in alginate-polylysine-alginite microcapsules as previously described (Sun, 1988). Briefly, the cells were resuspended in 1.5% sodium alginate (as seaweed extract composed of the polysaccharide polymer-soronic and polyglucanucic acid). Droplets of 305-600 nm in diameter were extruded with an air jet into a Ca2⁺ bath allowing the gelation of the alginate. The surface layer was cross-linked with a polycation polystyrene (MW range 12,500-32,500) and was then finally coated with alginate on the outside again. The core of non-crosslinked polysaccharide was dissolved with sodium citrate, leaving the cells floating free within the capsule space. The encapsulated cells were kept under the usual culture condition and photographed under phase-contrast (10 x objective) microscopy.

Assay for human growth hormone (hGH)

The hGH was monitored by either a radioimmunoassay according to Schabik & Parker (Schabik & Parker, 1986) or an ELISA technique (UH2-Magiquel High kit from United BioTech Inc., CA) in which samples were dispensed into microwell coated with anti-hGH antibodies and incubated with enzyme conjugate for 60 minutes at room temperature. After incubation, the wells were rinsed with running tap water. Chromogen substrate was dispensed into each well and incubated for 30 min at a dark room temperature. The reaction was stopped with IN H₂SO₄ and O₂D₅₆₅₃ was read with a microwell spectrophotometer reader (Molecular, PCS-MS 10).

Assay for viability and cell growth

At timed intervals after encapsulation, an aliquot of the microcapsules was removed and crushed with a glass coverslip. The proportion of viable cells was assessed with trypan blue exclusion test. The average cell number per capsule was counted with a hemocytometer from a known number of microcapsules crushed with a disposable pestle (Baxter 749520-0000).

Assay for hGH secretion rate from encapsulated cells

Microcapsules containing encapsulated cells were washed 5x with fresh media, resuspended in a known volume of media, and incubated under the usual tissue culture conditions. At hourly intervals up to 3 hours of incubation, an aliquot of the media was removed for hGH determination to obtain the hourly secretion rate. At the end of the experiment, the microcapsules were crushed to release the enclosed cells which were assessed for viability and cell count.
RESULTS

Mouse Ltk<sup>−</sup> cells, after transfection with calcium phosphate precipitated plasmid DNA encoding hGH, were enriched for transfectants by G418 selection. Clones resistant to G418 were screened for hGH production and a high secreting clone (IC4, secreting hGH at a rate of about 34 ng/10<sup>5</sup> cells/h) was used for subsequent encapsulation.

Survival of the recombinant cells after the encapsulation procedure was monitored by counting at timed intervals the average number of cells present within each microcapsule (FIGURE 1) and monitoring the viability of the enclosed cells (FIGURE 3). Immediately after encapsulation, the cell number per capsule was about 70, which over the subsequent week, increased to 150 on day 3 and reached about 600 by day 7. By day 10 post-encapsulation, cell number could no longer be accurately counted because of the large number of cells and clumping of cells that started to occur as the cells proliferated further. The increase in cell mass, however, was evident, as indicted under phase-contrast microscopy (FIGURE 2) monitored from day 7 to day 21.

Cell Number/Microcapsule

![Graph showing cell number over time](Image)

**FIGURE 1.** Proliferation of hGH-secreting mouse Ltk<sup>−</sup> cells in alginate-polylysine-alginate microparticles.

At timed intervals after the cells were encapsulated, aliquots of the capsules were removed, and known numbers of microcapsules were crushed to release the encapsulated cells for counting. Data were averaged from duplicate to triplicate determinations.

Throughout the first three weeks after encapsulation, the viability of the cells in the capsule was maintained at about 95%, starting from immediately after the encapsulation procedure (FIGURE 3). In addition, the secreted hGH was recovered in the culture medium at increasing amounts, reaching a concentration of about 0 ng/ml media by day 21. When the rate of secretion was monitored on day 3 post-encapsulation, it was found...
that at least 83% of the hGH secreted by the encapsulated cells was recovered in the media, based on the observed rate of hGH secretion of 28.1 ng of hGH secreted per h by 10^6 viable cells inside the capsules, compared to the secretion rate of un-encapsulated cells of 34 ng/10^6 cells/h. These estimates were based on the average from three experiments.

The encapsulated cells were photographed under phase contrast with a 10x objective in day 7 (D7) and day 21 (D21) after encapsulation. The density of cells inside microcapsules was increased, indicating the growth of cells.
DISCUSSION

The procedure of creating perselective microcapsules made of the sea-weed alginate is a mild and physiologically compatible method to compartmentalize cultured cells. The viability of the cells immediately after the microcapsule fabrication step was excellent, maintaining over 95% of viable cells as assessed by trypan blue exclusion (FIGURE 3). Furthermore, the mouse LK cells, which normally grow as attaches cells on culture dishes, were able to adapt to grow inside these microcapsules, showing an almost tenfold increase in cell number in 7 days. The ability of culture fibroblasts to proliferate inside the capsules appeared to be cell line-dependent. We have encapsulated both anchorage-dependent fibroblasts which did not grow as well as these transformed mouse cells. Although the continued proliferation of these mouse cells could not be maintained with cell counting as the cells became too numerous and clumped later on, it was obvious that even by the third week after encapsulation, the cell number was still increasing, as monitored through phase-contrast microscopy (FIGURE 2). The excellent viability of about 90% was maintained throughout this entire period of observation (FIGURE 3).

The success of this method to provide a nutritionally supportive environment for cell survival is the first requirement for using these microcapsules for somatic gene
therapy. The next important question is the adequacy of the microcapsules to provide free passage to the secretory recombinant gene product. As shown in FIGURE 3, the hGH secreted by the transplanted encapsulated mouse cells was detectable in the media at increasing concentrations up to day 21 post-encapsulation. Since this estimation did not take into account the possible proteolytic degradation of hGH that may have occurred in the media during the three weeks of observation, the actual amounts of hGH recoverable in the media were likely to be even higher. When measured even after encapsulation, over 80% of the secreted hGH was able to diffuse out of the capsule which remained intact throughout this period of observation, as monitored through phase-contrast microscopy. Therefore, not only are the encapsulated cells able to survive, their secreted gene product was still able to exit from the microcapsule compartment.

In conclusion, the current results clearly demonstrated the feasibility of using microencapsulated engineered cell lines to provide a secreted gene product in vivo. The basic requirement for this strategy to be effective clinically is that the gene product must be secreted from the cells and that therapeutic effects can be achieved in the patients through delivery of the gene product to the systemic circulation. Moreover, before this approach can be further developed, it will be necessary to address two important issues: the long-term performance of these microcapsules and encapsulated recombinant cells in vivo as well as the feasibility of this approach to deliver new gene products in vivo. If it can be demonstrated that such encapsulated cells continue to deliver the desired gene product over an extended period, the possibility of using an allergic syndrome cell line to provide recombinant gene products in vivo should have wide application not only for therapeutic purposes, but also in veterinary medicine for the prevention of engineered vaccine or growth modulating substances in vivo.

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REFERENCES


