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Microencapsulation of Living Cells and Tissues

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Received August 2, 1979, from the *Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.* Accepted for publication February 6, 1980.

Abstract □ A new microencapsulation procedure involving an all-aqueous phase system was developed. Viable cells or tissues were suspended in sodium alginate droplets, which then were gelled by calcium chloride solution. A permanent, semipermeable membrane was formed on the surface layer of the temporary gel capsules by treatment with a solution of polylysine. Finally, true living cell-containing microcapsules were produced by "liquefying" the gel within the microcapsules through calcium-ion removal by simple ion exchange. Microencapsulated living cells and tissues continued to grow and flourish. In tissue culture medium, microencapsulated rat pancreatic islets continued to release insulin and remained sensitive to glucose and theophylline stimulation, responding with a typical physiological biphasic insulin-release pattern for over 2 months. Microencapsulation of other viable cells and tissues such as red blood cells, hepatoma cells, sperm cells, and pancreatic endocrine tissues also was successful.

Keyphrases □ Semipermeability—membranes of microencapsulated viable hepatoma cells and microencapsulated viable pancreatic islets □ Pancreatic islets—viability within microcapsule demonstrated by insulin release pattern and histological staining □ Microencapsulation—symposium, preparation of viable microencapsulated hepatoma cells and pancreatic islets, semipermeable membranes

Microencapsulation is a process in which small, discrete, solid materials, liquid droplets, or gases are completely enveloped by an intact membrane. The functions of the capsular membrane are to protect the material within and to control the flow of materials, inside and outside, across the membrane.

BACKGROUND

Microcapsules may possess impermeable or semipermeable membranes, depending on how and why the materials are encapsulated. Research and development in semipermeable microcapsules, pioneered by Chang (1, 2), have recently multiplied greatly, especially in the biomedical field. Important materials encapsulated include activated carbon granules, ion-exchange resins, enzymes, cofactors, hormones, proteins, antigens, and antiserums (1–6). Semipermeable microcapsules containing microbial cells also have been reported (7, 8). However, they were used as sources of enzyme activities, and the cells were not viable. As far as the present authors know, no viable mammalian cells have been microencapsulated.

The current state of the art for preparing semipermeable microcapsules involves at least one of two harsh conditions: (*a*) contact of the aqueous phase, where the cells are located, with at least one organic solvent phase; and (*b*) heating during the process. However, both conditions must be avoided for cells to stay viable during and after microencapsulation.

The present report describes a simple all-aqueous phase system that successfully microencapsulates viable mammalian cells and tissues.

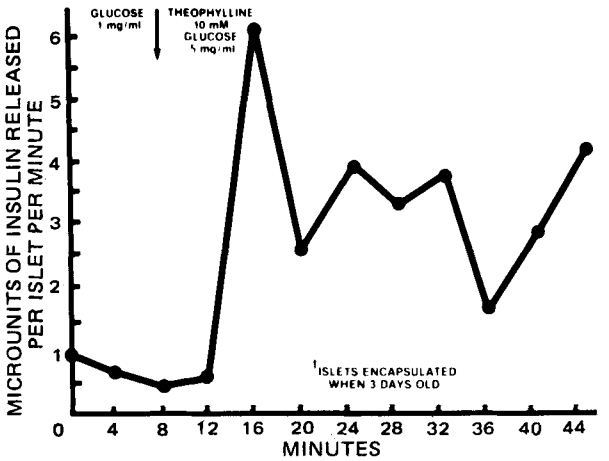

Figure 1—*Insulin release pattern of 27-day-old microencapsulated rat islets by perfusion.*

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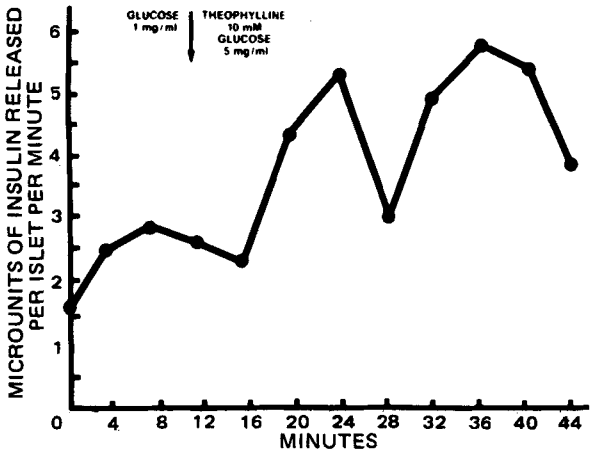

Figure 2—*Insulin release pattern of 27-day-old rat islets by perfusion.*

EXPERIMENTAL

Microencapsulation—The hepatoma cells[1] or pancreatic islets[2] first were uniformly suspended in a 0.6% sodium alginate[3] solution prepared in saline. Droplets containing either cells or islets were produced by extrusion through a capillary tip and dropped into a beaker containing 1.5% $CaCl_2$ solution. The calcium ions caused immediate gelling of each droplet. The temporary microcapsules thus formed were harvested by decantation and were processed further to impart a permanent semipermeable membrane by suspension in a 0.02% polylysine[3] solution for 3–5 min. Before the cell- or islet-containing microcapsules were transferred into tissue culture medium[4], the alginate gel inside the capsules was "liquefied" in isotonic sodium citrate solution at pH 7.2 for 5 min.

Cell Culture Studies—Cell- and islet-containing microcapsules, as well as the corresponding nonencapsulated cells and islets used as controls, were cultured at 37° in disposable, plastic tissue culture flasks[5] containing the culture medium with 9% fetal bovine serum and 20 mg of gentamicin/ml.

Viability and growth of the cultures were monitored through an inverted microscope and by histological stains on microscopic sections.

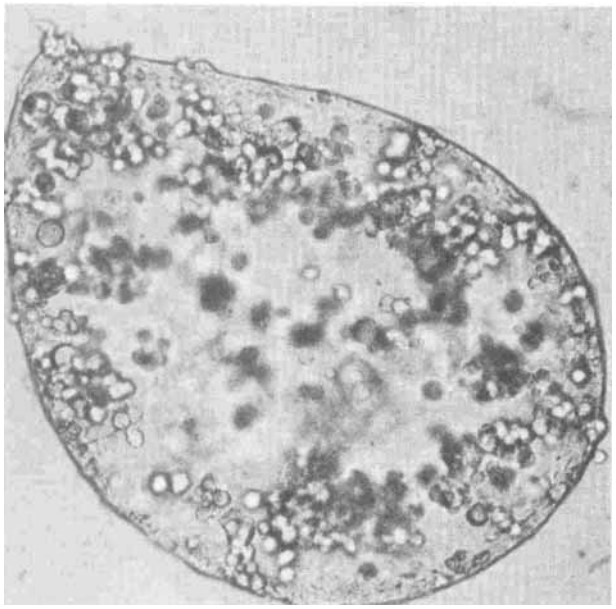

Figure 3—*Hepatoma cells growing inside a microcapsule, Day 1 (270×).*

[1] Hepatoma cells were freshly grown and provided by Dr. Richard Carchman's laboratory, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Va.

[2] Three- to 6-day-old pancreatic islets were supplied by Dr. Anthony Sun, Connaught Laboratories.

[3] Sigma Chemicals Co., St. Louis, Mo.

[4] Medium CMRL-1969, Connaught Laboratories, Toronto, Canada.

[5] Falcon flasks, Flow Laboratories, McLean, Va.

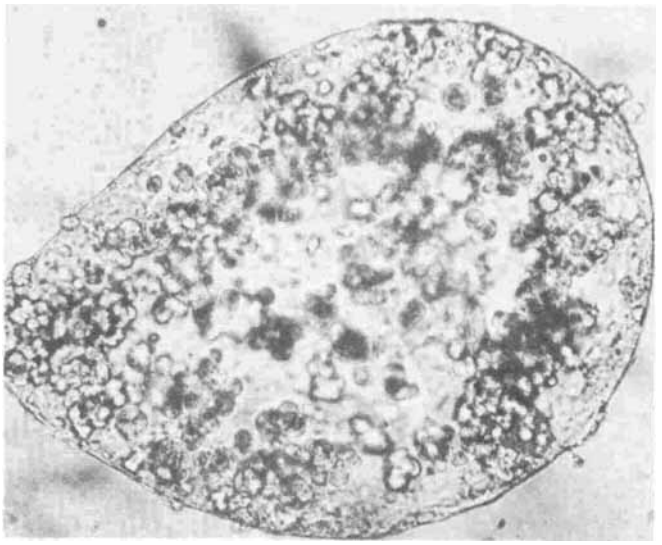

Figure 4—*The same microcapsule, Day 2 (same magnification).*

For insulin production and release studies, a perfusion system similar to that used by Lacy *et al.* (9) was assembled and used inside a 37° walkin incubating room. Samples were collected at 2- and 4-min intervals. Baseline insulin release was obtained with 100 mg/100 ml of glucose in the perfusing culture medium, while stimulation studies were carried out with medium containing 500 mg/100 ml of glucose and 10 m*M* of theophylline.

RESULTS AND DISCUSSION

Perfusion studies with 10 islets and 10 microencapsulated islets (Figs. 1 and 2) showed that the amounts of insulin released per islet per minute upon stimulation by a combination of high glucose (5 mg/ml) and theophylline (10 m*M*) were comparable between the two groups. However, at 24 days, the control islets showed a high level of baseline insulin release (1 mg/ml) and a somewhat abnormal-looking stimulation–response pattern. In contrast, the microencapsulated islets produced a reasonably low level of baseline insulin release and a typical, biphasic stimulation–response pattern.

Abundance and continuation of cell proliferation were evident in all microencapsulated cells and tissues (Figs. 3–8). Viable and healthy B cells

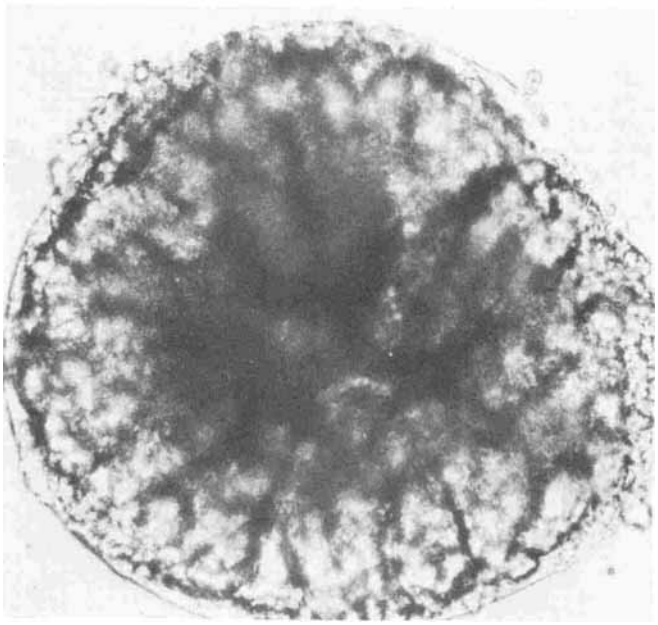

Figure 5—*The same microcapsule, Day 9 (same magnification). Note the increase in size due to stretching of the membrane by the overcrowded cells.*

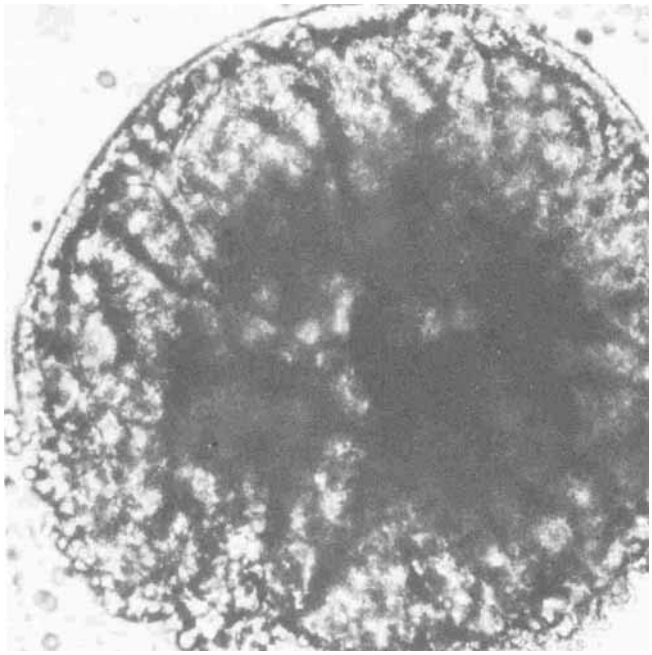

Figure 6—*The same microcapsule, Day 10 (same magnification). Note the continuing increase in size and the definite sign of bursting of the microcapsule.*

were noted in the microscopic sections of microencapsulated islets stained with hematoxylin–eosin and with aldehyde–fuchsin (Figs. 9 and 10). Control islets showed signs of degeneration, including progressive B-cell degranulation, at the end of 3 weeks. On the other hand, microencapsulated islets flourished for over 8 weeks, with more cell proliferation and good insulin production and release.

Microencapsulated hepatoma cells appeared to grow and multiply in suspension at about the same rate as the nonencapsulated control group (doubling time of ~24 hr). Final photomicrographs (Figs. 3–7) of the same microcapsules taken through the tissue culture flask showed that overcrowding hepatoma cells first stretched the capsular membrane before finally bursting the capsule at Day 10.

The significance and advantages of maintaining cultures of viable cells or tissues within semipermeable microcapsules are numerous:

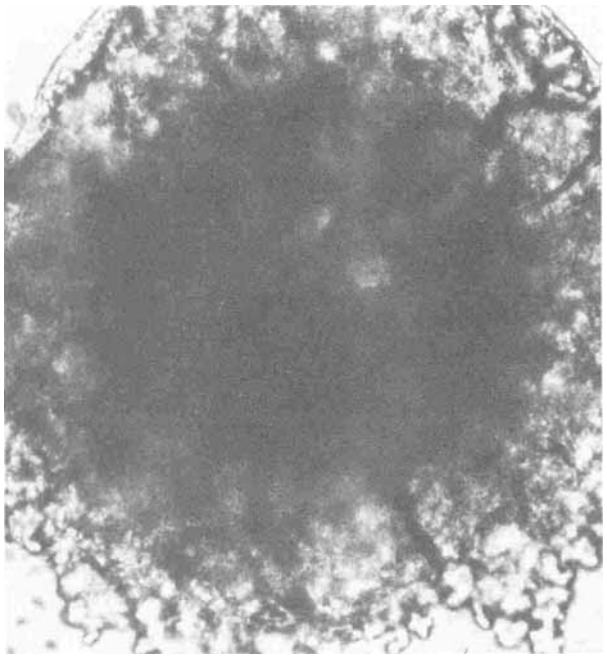

Figure 7—*The same microcapsule, Day 11 (same magnification). Note the further increase in size and the complete bursting of the microcapsule.*

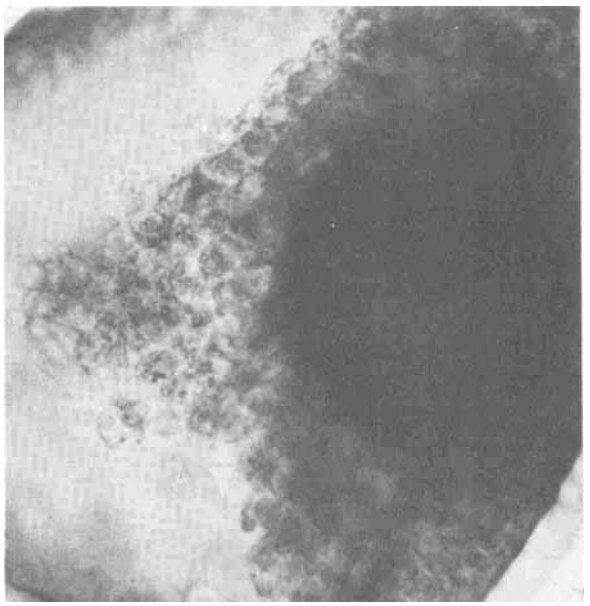

Figure 8—*Five-week-old microencapsulated rat islet. Note the number of cells proliferated from the islet.*

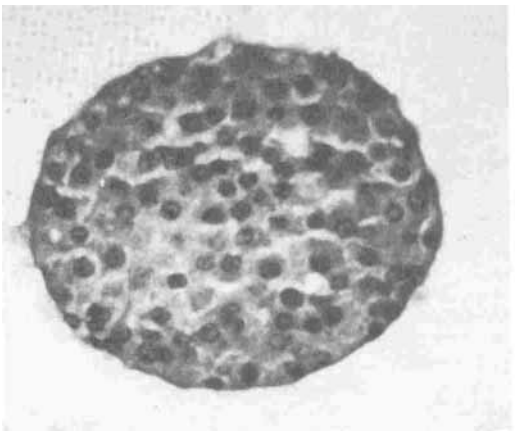

Figure 9—*Paraffin section of microencapsulated islet stained with hematoxylin–eosin.*

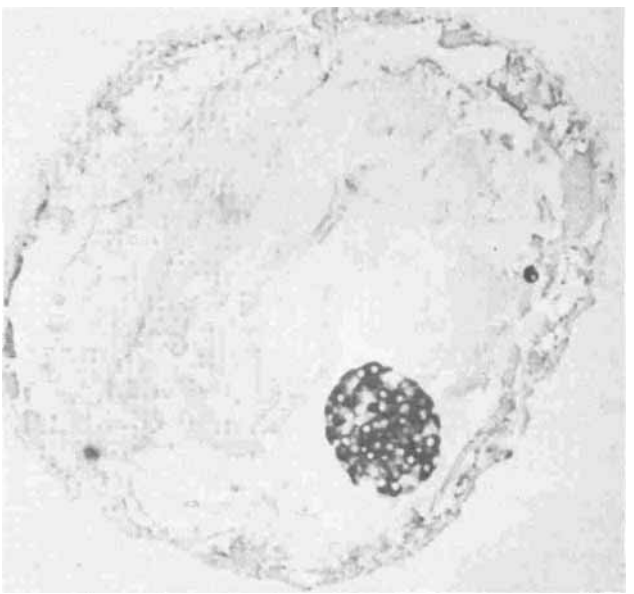

Figure 10—*Paraffin section of microencapsulated islet stained with aldehyde–fuchsin.*

1. The microcapsules can be used as individual microculture flasks, making it easier to handle and manipulate culture cells, especially when they are used as an investigative tool in the study of cell metabolism and regulation and the dynamics of hormone action and production.

2. The immediate cell environment can be made more similar to solid tissue density and biochemical characteristics and, therefore, more physiological and natural.

3. *In vivo* and *in vitro* sterile conditions are easier to maintain since bacterial and viral cells cannot cross the capsular membrane.

4. When microencapsulated cells or tissues are used as injectable prostheses or implantable (or *ex vivo*) artificial organs, it should be possible to specify the capsular membrane's permeability range to suit a particular use such as prevention of immune rejection or toxin removal.

Specifically, the microencapsulated islet appears to have great potential as a better and simpler implantable and, possibly, physiologically disposable artificial pancreatic endocrine organ for the treatment of diabetes. Preliminary animal studies have begun to give encouraging results.

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Preparation of Hemolysate-Loaded Poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) Nanocapsules

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Received August 2, 1979, from the *Faculty of Pharmaceutical Sciences, Science University of Tokyo, Shinjuku-ku, Tokyo, Japan 162.* Accepted for publication January 10, 1980.

Abstract □ Poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) capsules containing hemolysate were prepared in the nanometer range by an interfacial polymerization technique using electrocapillary emulsification to obtain very fine hemolysate droplets for encapsulation. The effects of temperature, emulsifier concentration, and the applied potential on the size distribution of the polyamide capsules were investigated. The polyamide capsules prepared under proper conditions have an average diameter of ~500 nm.

Keyphrases □ Poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) microcapsules—containing hemolysate, prepared by interfacial polymerization using electrocapillary emulsification □ Electrocapillary emulsification—synthesis of poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) microcapsules containing hemolysate, effect of temperature, emulsifier concentration, and applied potential on polyamide capsule-size distribution □ Microencapsulation—symposium, preparation of nanograde-sized polyamide microcapsules containing hemolysate, effect of temperature, emulsifier concentration, and applied potential on capsule-size distribution

Previous studies on poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) microcapsules containing hemolysate were carried out with the aim of using them as artificial red blood cells (1–3). Most of the encapsulated hemoglobin molecules retained their oxygen absorbability while catalase and carbonic anhydrase lost some of their enzymatic activities during encapsulation; suspensions of the microcapsules exhibited flow properties similar to those of blood unless the capsule concentration exceeded 30% by volume. Because of their relatively large size (mean diameter of 10 μm) and low deformability in shear flows, the microcapsules were not expected to pass through capillary blood vessels. Thus, an attempt was made to prepare much smaller capsules loaded with hemolysate that could pass through capillary blood vessels.

When a potential difference that is higher than a critical value, the critical voltage of emulsification, is applied to an oil–water interface, the interfacial tension is reduced almost to zero and spontaneous emulsification occurs, due to the interfacial fluctuation, in the absence of surfactant or in the presence of a very small amount (4, 5). The emulsions thus formed are monodisperse and stable, with the average particle diameter being <200 nm. The necessary condition for this electrocapillary emulsification is that the ionic strength, and hence the reciprocal double-layer thickness, of the inner phase be higher than that of the outer phase. Because of the difficulty in obtaining hemolysate droplets of <1 μm by conventional mechanical emulsification processes, the electrocapillary emulsification technique seems to be promising.

The present paper describes the preparation of poly(N^{α},N^{ϵ}-L-lysinediylterephthaloyl) capsules in the nanometer range containing sheep hemolysate by interfacial polymerization using electrocapillary emulsification to obtain very fine hemolysate droplets for encapsulation. Factors influencing the capsule size also are described. In addition, the effect of the applied potential on the enzymatic activities of catalase and carbonic anhydrase in the hemolysate droplets is reported.

EXPERIMENTAL

Apparatus for Electrocapillary Emulsification—Figure 1 shows the schematic diagram of the apparatus used for electrocapillary emul-

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