IMMOBILIZATION OF LIVING CELLS IN BIOCOMPATIBLE SEMIPERMEABLE MICROCAPSULES:

BIOMEDICAL AND POTENTIAL BIOCHEMICAL ENGINEERING APPLICATIONS

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INTRODUCTION

At the present time, the clinical and industrial development of immobilized cell technology is constrained not by a lack of opportunity but rather by a lack of suitable techniques for immobilizing viable plant, animal and microbial cells. In the biomedical engineering area, for example, several methods have been developed for microencapsulating biologically active molecules, tissues and cells so that they remain viable and in a protected state within a semipermeable membrane which permits passage of low molecular weight substances, such as nutrients and oxygen, but not of cells and high molecular weight proteins (1-8). However, there have been no successful attempts to develop semipermeable microcapsules which have long-term biocompatibility with the body tissues and which are impermeable to the components of the immune system. As a result, survival times of transplanted microencapsulated tissue or cells in-vivo have consistently been less than three weeks, (7, 9-12) severely limiting the usefulness of the encapsulation procedure in the treatment of diseases such as diabetes.

The industrial development of immobilized cell technology, on the other hand, is constrained by a lack of suitable bioreactors for handling bacterial, plant and animal cells and by a lack of suitable bioseparation procedures for handling the highly complex (and high value) products such as proteins and alkaloids (13, 14). In addition to the technology limitation there is the concern in Canada, the U.S. and Europe that the number of appropriately-trained biochemical engineers (in fermentation, cell culture and bioseparation) will be inadequate to meet the needs of emerging Biotechnology industries.

Recent studies have indicated that a novel alginate-poly-l-lysine (PLL) microencapsulation procedure, has great clinical potential in the protection of transplanted living cells and tissue (9-12,15-17). In
experimental animals, for example, a single intraperitoneal transplant of encapsulated islets reversed the diabetic state for more than one year. In contrast, a single injection of unencapsulated islets was effective for less than two weeks. In addition, the encapsulation technique also has great industrial potential in the development of new membrane-bioreactors for the production of high-value biologicals and pharmaceuticals from immobilized living cells (15).

MICROENCAPSULATION OF LIVING CELLS

The microencapsulation of viable cells such as islets of Langerhans within an alginate-PLL-alginate membrane has been described in previous studies (9,12,15). Briefly, islets were suspended in 2 mL of 1.5 % (w/v) sodium alginate (Kelco-Gel LV, Kelco Specialty, Colloids Ltd., Toronto, Canada) in 0.85 % NaCl at a concentration of 1000 islets/mL. Spherical droplets of this suspension were formed by an air jet-syringe pump droplet generator. With this apparatus, the cell-sodium-alginate suspension is extruded through a needle located inside a sheathed tube through which air flows at a controlled rate. As liquid droplets are forced out of the end of the needle by the syringe pump, the droplets are pulled off by the shear forces set up by the rapidly flowing air stream.

In some experiments a specially designed electrostatic droplet generator was used in place of the air-jet droplet generator. With this device, the sodium alginate liquid, with or without cells, is placed in a syringe to which is attached a stainless steel needle, bevelled on all sides. The syringe is attached to a syringe pump and a high voltage wire is attached to the needle. A second wire of opposite polarity is attached to the collecting vessel containing calcium chloride. The needle is located at a specified distance, usually 10 mm, from the top of the liquid in the collecting vessel. The electrostatic generator itself consists of a power supply, logic circuitry and a console panel for controlling pulse voltage, pulse frequency and pulse length. The pulse voltage, for example, determines the strength of the force pulling the droplets from the end of the needle. The pulse frequency determines how many pulses can be applied to the droplet per unit time, and the pulse length determines the length of time for which the droplet-forming force can be applied. Unless otherwise indicated, the air-jet droplet generator was used for the formation of microcapsules.

Spherical droplets were collected in 1.5 % CaCl₂ where they gelled. Following washing steps with 30 mL volumes of 0.1 % CHES [2 (N-cyclohexyl-amino) ethanesulfonic acid] and 1.1 % CaCl₂, the calcium alginate droplets were reacted with PLL, a positively charged polyelectrolyte, by suspension in 30 mL PLL solution for six minutes [0.05 % (w/v) Mw = 1.7 x 10⁶; Sigma, St. Louis, MO]. The resulting capsules were washed with 30 mL volumes of 0.1 % CHES, 1.1 % CaCl₂, and 0.85 % NaCl and suspended for four minutes in 0.03 % sodium alginate which formed the outer layer of membrane. Further washing with 0.85 % NaCl was preceded by the treatment of the capsules with 0.05 M sodium citrate, pH 7.4, for six minutes which liquified the gel inside the capsules. The excess citrate was removed by two NaCl washes and the encapsulated islets cultured at 37 °C. About 50 % of the capsules (900 µm diameter) contained islets (1 to 2 islets/capsule). Compared to an earlier microencapsulation procedure employing an alginate-PLL-polyethyleneimine (PEI) capsule membrane (7,9) several modifications were made; the initial PLL/alginate reaction time was increased from three to six minutes, the PLL concentration was increased from 0.03 to 0.05 % (w/v), and the PEI was replaced by alginate. In addition, specially purified