Fundamentals of Animal Cell Encapsulation and Immobilization

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Chapter 13

EMERGING TECHNIQUES, MATERIALS, AND APPLICATIONS IN CELL IMMOBILIZATION

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I. INTRODUCTION

The immobilization of cells is not a new concept, but the refinement of a phenomenon frequently observed in nature. Many microorganisms and cellular materials naturally adhere to surfaces and thereby become immobilized.[1]

The first industrial application of immobilized microbial cells became a reality early in the 19th century. Acetic acid was produced in a trickling filter with *Acetobacter* sp. attached to beechwood shavings.[2] In 1966, Mosbach and Mosbach[3] reported the immobilization of microbial cells by entrapment in cross-linked polymeric gels for continuous production of biochemicals by enzymatic conversion. Chibata et al. succeeded in industrializing the continuous production of L-aspartic acid in 1973, and later reported the production of L-malic acid and L-alanine with immobilized cells.[4,5] In 1979, Brodelius et al.[6] extended the use of cell immobilization to the plant kingdom, for the production of secondary metabolites. In 1980, Nilsson and Mosbach[7] succeeded in immobilizing anchorage-dependent animal cells by adsorption on gelatin and chitosan beads and by entrapment in alginate and agarose.

Cells may be immobilized by attachment or confinement. In the first case, the cells adhere to a surface[8] or one cell to another.[1] Attachment surfaces include the interstices of fibrous materials, including hollow fibers,[9] or porous materials such as polyurethane foam.[10] Cells are attached by natural or induced self-adhesion, or by chemical bonding.[11] Adsorption and attachment of cells to carrier surfaces, while inexpensive and simple, are dependent on the cell wall properties. Cell release due to weak binding may also result from local pH changes that occur during cell metabolism. The toxicity of the reagents used in support activation may result in reduced cell viability or activity.

Confined cells are physically restrained within or by means of a solid or porous matrix, such as a stabilized gel used as an entrapment technique,[3] or a membrane as used in microencapsulation.[12] In the case of animal cell immobilization, these techniques are in an early stage of development, while widely used for immobilization of other cell types. Entrapment and encapsulation processes are independent of cellular properties, reproducible, more conducive to scaling-up than the hollow-fiber system, and provide a very large surface of exchange with a higher cell concentration. These techniques are easier to control and result in more stable immobilized cell preparations than natural aggregation.[13] The different procedures and variety of materials provide a high degree of versatility in the selection of a suitable immobilization technique dependent on the particular application.

The entrapment matrix or polymeric encapsulating membrane provides substantial protection to the immobilized biocatalyst. A semipermeable membrane may act as an immunoprotector, preventing access to immunocompetent cells and immunoglobulins. Immobilization via entrapment also provides a physical protection from shear forces for fragile cells and prevents cell aggregation, wall attachment, and blocking of outflow pipes. Moreover, com-

pared to free cell suspensions, immobilized cells have demonstrated increased metabolic stability and higher productivity of secondary metabolites. Finally, as a consequence of immobilization, products may be accumulated within the carrier, facilitating recovery and thus lowering costs.

The following section focuses on cell immobilization by entrapment and encapsulation techniques. Immobilization of anchorage-dependent cells by absorption on microcarriers has been reviewed elsewhere.[14-16]

II. ENTRAPMENT

Since Mosbach and Mosbach[3] entrapped live cells in cross-linked polyacrylamide, a number of other gel matrix materials have been developed. In 1977, Kierstan and Bucke[17] introduced calcium alginate gel as a suitable matrix for the entrapment of microbial cells, subcellular organelles, and isolated enzymes.

Alginate remains the favored material for entrapment, with over 100 publications describing various applications over the last 5 years. Enhancements have since been introduced to resolve problems associated with its use. A characteristic of alginate resulting in its favored status is the ease with which it is gelified at ambient temperatures, rendering it more compatible with temperature-sensitive cells.

Chemically, alginates are a family of linear copolymers of 1-4-linked D-mannuronic and L-guluronic acid. Alginate gels are obtained by ionic cross-linking with polyvalent cations, generally involving calcium. Alginates consist of homopolymeric regions of one of the monomers interspaced with alternating sequences of both monomers. The proportion of the various blocks depends upon the source of alginate. The affinity for calcium, and hence the ability to form strong gels, is correlated with the content of guluronic acid residues[18] and the length of the homopolymeric guluronic blocks.[19] Alginate isolated from different sources may therefore result in widely different gel-forming properties.

Although alginate fulfills the requirements for additives in food and pharmaceutical products, some alginates contain small amounts of polyphenols, which may harm sensitive cells. In order to improve the biocompatibility of alginate, some companies such as Protans (Norway) are marketing high-purity alginates suitable for medical applications. As an implantation material, for example, the alginate must be free from pyrogens and immunogenic materials such as proteins and complex carbohydrates.[19]

Calcium alginate beads may be prepared by dropwise addition of a cell suspension in Na-alginate solution into a calcium chloride solution (see Chapter 6 of this volume). Alginate beads present limited stability in culture media. Difficulties arise principally when phosphate ions are present, resulting in gel disruption due to chelation of the calcium ions. The gel may be stabilized by addition of Ca^{2+} to the medium,[20] the use of other bivalent[21] or trivalent

cations to induce gelification,[22] or posttreatment of beads with glutaraldehyde. However, these treatments may be toxic to the cells, and the increased number of steps involved in the immobilization procedure increases the risk of contamination. Alginate beads may also be predried to improve stability.[23]

In the traditional gelification method via drop formation, gelation initially occurs externally, and more or less internally depending on the alginate concentration, sphere diameter, and gelation time. Internal gelation has been proposed through uniform liberation of Ca^{2+} ions in the alginate.[24] This procedure leads to alginate slabs with higher strength, but may reduce productivity. A similar principle was used for alginate bead production via an emulsification technique[25,26] for large-scale production.

Cross-linking between the alginate chains by the calcium occurs mainly between the homoguluronic sequences.[27] Smidrod et al.[19] suggested a promising approach for strengthening gel beads that involved the selection of high-guluronic-sequence alginate[28] or enzymatic conversion.[19] Such gels may be stable even at high ratios of sodium to calcium.

An important limitation of the alginate gel immobilization procedure is the production capacity. Droplet-forming methods are limited to low production rates, especially when preparing small beads (see Chapter 6 of this volume). Some authors proposed adaptations of the drop method for scale-up to the production of several liters per hour,[29-31] but the techniques described are not appropriate for the industrial-plant level and production rates fall quickly when reducing the drop size. Neufeld et al.[25] proposed an emulsion technique involving the dispersion of an alginate solution containing an insoluble form of calcium into a nontoxic material such as a vegetable oil. When the emulsion equilibrium is obtained, an oil-soluble acid is added to reduce the pH, liberating soluble calcium. This method may be easily scaled-up to cubic meters per hour. The technique is applicable to the formation of micron-size microspheres with a controlled diameter. An appropriate choice of emulsification equipment is necessary to limit the size distribution of the resulting microspheres.

Other types of gel beads may be produced either by drop generation and gelification by cooling, or by emulsification in warm oil followed by cooling of the emulsion.[32] Immobilization in agar and agarose is conducted by suspending the cells in a warm gel-forming solution above the gel temperature (50°C for agar and 30 to 50°C for agarose, depending on the type). The mixture is set by cooling in molds, then cut to a desired shape and size. The relatively high temperature required to prepare temperature-setting gels limits their application for animal cells. However, Dupuy et al. successfully encapsulated islets of Langerhans in agarose beads.[33]

Brodelius and Nilsson[34] described a procedure for cell entrapment in polyacrylamide gels which has been used extensively for immobilization of microbial, plant, and animal cells. Polyacrylamides have been used in the industrial production of L-malic and L-aspartic acid.[4] The procedure involves

the polymerization of an aqueous solution of acrylamide monomers in which the microorganisms are suspended. The technique is straightforward and generally results in an effective entrapment of the cells. The cell-containing polymeric gel may be granulated for use as column packing, with a porosity which is a function of the degree of cross-linking in the acrylamide itself. However, polymerization of acrylamide generates heat and free radicals, causing loss in the chemiosmotic integrity and enzymatic activity of the immobilized cells.[35] Moreover, polyacrylamide-immobilized cell granules are compressible and of irregular shape and size, resulting in abrasion and uneven packing, such that flow-induced compression of large packed columns would occur.

Entrapment in κ-carrageenan is a good alternative to alginate for cell immobilization. Carrageenan gels are less sensitive to chelating agents than alginate, and gelification occurs under mild conditions. Gel formation is initiated by cooling to the gelification temperature or in the presence of cations, usually potassium.[36] Chibata et al.[36-38] prepared highly active immobilized cells using κ-carrageenan gels. A disadvantage to κ-carrageenan is in the fragility of the beads, resulting in disruption in a mixing environment. The strength and integrity of κ-carrageenan gels may be improved by incorporation of relatively small amounts of locust bean gum, as is done in lactic fermentation.[39] However, the rigidity of the κ-carrageenan is subject to the presence of high potassium concentrations (>0.1 *M*), possibly limiting its application to animal cell immobilization due to physiological problems.

A new polymer suitable for the immobilization of organisms able to tolerate warmer temperatures, is gellan gum,[40] a natural, nontoxic, thermoresistant gelling polysaccharide with characteristics comparable to or better than κ-carrageenan.[41] Gellan gum is a linear, unbranched homopolymer with tetrasaccharide repeating units consisting of two β-D-glucose, one β-D-guluronic acid, and one α-L-rhamnose residue.[42] Rheological studies have shown that 1% gellan gum is similar in strength to 3% concentrations of other gels.[41] Gellan gum is stabilized by a number of cations (comprising protons), eliminating the use of high concentrations of calcium (alginate) or potassium (κ-carrageenan) and allowing a larger spectrum of applications. Gellan gum beads may be prepared by extrusion or emulsification of a warm solution followed by cooling, with gelation occurring between 35 and 50°C.

Several authors[43,44] have proposed chitosan, a polyglucosamine, for plant cell immobilization. Chitosan, which is soluble in dilute acids, will form a precipitate at pH values above 6 to 6.5. Thus, true ionotropic gels can be formed at pH values below 6.0 by cross-linking with polyanions such as triphosphate. Once formed, these gels can be hardened by increasing the pH to above 7.5, where chitosan is totally deprotonated and water insoluble. Chitosan is one of the rare cationic polymers. Apparently, it has not been tested yet for animal cell immobilization.

Among other gels which have been tested for cell immobilization, pectates prepared by deesterification of pectins could serve as suitable material for gel

entrapment.[45] Various types of mixed gels have also been investigated, such as an alginate-gelatin combination, and agarose with gelatin.[34,36] The use of mixtures may permit control over the mechanical properties of the beads. Garafolo and Chang[47] proposed bead formation from a mixture of alginate and agarose. After gelification, the alginate is released, resulting in "open" agar beads.

Entrapment in beads is probably the most common procedure used to immobilize cells in granular form, likely due to its simplicity and gentle immobilization conditions. The large variety of gels allows a selection of the most appropriate for a specific application. However, the bead system itself presents serious limitations. Studies on lactic bacterial cell release from alginate beads demonstrated that the gel does not constitute a real barrier to the cells.[48] The use of more dense gels allows better cell retention, but generally leads to a reduction in performance. In fact, avoiding cell release from beads is feasible only with nongrowing or very slowly growing cell lines. The formation of a membrane around the beads is not necessarily a sufficient protection against cell release,[48] as internal pressure created by cell growth may be very high (up to 5 atm). To resolve this problem, Champagne et al.[48] recommended periodic washing of beads to kill the cells present in the external layer.

Another problem that arises in the gel immobilization procedure is limited gas diffusion into the gel. The oxygen microenvironment greatly influences the behavior of cells. Experimental results showed[49] that the final depth of oxygen penetration in the carrageenan gel was in the range of 80 to 100 μm. In contrast, gels do not necessarily constitute a protection against external toxic agents. Alginate beads present an especially inefficient protection against immunoagents in islet encapsulation.[50]

III. BEAD COATING

Lim and Sun[50] introduced a new technique for cell entrapment by coating alginate beads with polycationic polymers such as poly-L-lysine and/or polyethyleneimine. The poly-L-lysine membrane was formed by suspending the alginate beads in dilute poly-L-lysine solution. To reduce the membrane charge and increase its strength, coated beads were resuspended in dilute alginate, resulting in a double film. To minimize diffusion limitations, the alginate core, which served as a mold for membrane formation, may be liquified and extracted through the semipermeable membrane in a sodium citrate solution, resulting in membrane-bound microcapsules.

Other coating materials for alginate beads have been described. Eudragit RL, a water-insoluble cationic polyacrylate, was used to form a stable, aqueous emulsion to coat calcium alginate beads containing human erythrocytes.[51] Coating for 60 min resulted in the formation of a 60-μm dense layer surrounding the alginate core. McKnight et al.[52] used chitosan to form a poly-

electrolyte complex with calcium alginate beads, resulting in durable, strong, and flexible biocompatible polymeric membranes around the beads. Gin et al.[33,53-55] proposed a coating of coacrylamide-bisacrylamide to prevent cell leakage from agarose.

IV. MICROENCAPSULATION BY COEXTRUSION

A method for cell microencapsulation based on coextrusion was described by Boag and Sefton[56] in 1987. Viable human diploid fibroblasts were encapsulated in Eudragit RL polyacrylate by an interfacial precipitation technique. Cells in culture medium were coextruded with a polymer solution and formed into droplets by a coaxial airstream. The droplets were dispensed into a corn-mineral oil mixture to extract the solvent, precipitating the polymer at the droplet interface. This system was later extended[57] to the microencapsulation of erythrocytes in a thermoplastic copolymer of hydroxyethyl methacrylate-methyl methacrylate by dispensing the cell suspension through an inner needle and the polymeric solution through the outer needle of a concentric needle assembly. Droplets were blown from the needle tip by compressed air into a bath of nonsolvent to remove the solvent and precipitate the polymer.

Dupuy et al.[33] applied a similar principle consisting of an initial embedding in agarose. In a second step, the beads were coated with an acrylamide-bisacrylamide mixture followed by an *in situ* photochemical polymerization of a polyacrylamide. Preembedding in agarose helped avoid contact between the cells and the monomers, and acted as a buffer against changes in temperature and osmolality.

V. MICROENCAPSULATION BY INTERFACIAL IONIC CROSS-LINKING

Braun et al.[58-60] proposed a technique for microencapsulation by dropping a cellulose sulfate solution into a solution of the polyelectrolyte, poly(dimethyl diallyl ammonium chloride). These inversely charged polymers react at the droplet interface to form an ionic-bonded membrane. The method was applied to immobilize pancreatic islets, resulting in high viability and functionality.

Gharapetian et al.[61] encapsulated hybridoma cells within polyacrylate membranes by consecutively introducing droplets of a cell suspension in polyanionic acrylic copolymer solution into aqueous solutions of three polycationic polymers. As a result of interpolymeric ionic interactions and some chemical reactions, a polyelectrolyte complex membrane was formed at the interface of each droplet and the polycationic solution. In another communication,[62] the effect of copolymer structure on the membrane properties were reported.

McKnight et al.[52] proposed a complex chitosan-alginate membrane for cell encapsulation. The membrane properties were controlled by selecting an appropriate viscosity average molecular weight for the chitosan.

Microencapsulation provides both chemical and biological protection in addition to the immobilization itself. This protective property is the main reason for membrane formation around beads to ensure immunoprotection in the microencapsulation of islets of Langerhans.[50,53] In contrast to many other immobilization methods, microencapsulation maintains a liquid environment around the biocatalyst instead of the physical or chemical bonding between cell and surface involved in other forms of immobilization. In this sense, microencapsulation is less disruptive than other immobilization methods.

VI. MICROENCAPSULATION BY INTERFACIAL POLYMERIZATION

Chang et al.,[63] in 1966, first prepared microcapsules for applications in biotechnology, by interfacial polymerization. The three-step process involved dispersion of an aqueous phase containing the immobilizant and one of the polymerization monomers within an organic phase. The oil-soluble monomer was then added to initiate the polymerization reaction at the droplet interface. After membrane formation, the microcapsules were separated from the dispersion, filtered, and washed.

Nylon membranes represent a versatile means of encapsulation. Permeability may be controlled by judicious selection of the monomers,[64] by adjusting reaction time and/or polymerization conditions,[65] or by membrane coating (polyethyleneimine). Interfacial polymerization is easier to control than interfacial coacervation with cellulose nitrate[66] or polyacrylamide.[57] Most encapsulation processes result in membranes with thicknesses on the order of micrometers, while nylon membranes obtained through interfacial polymerization measure a few hundred nanometers, without loss of membrane strength.[67] The liquid phase used in microencapsulation via interfacial polymerization is of lower viscosity than the bead-forming pregel solutions. Lower-shear devices are more suitable for the dispersion step. Also, since the membrane is formed in one step, the risk of contamination is reduced compared to multiple-step operations.

Unfortunately, conditions for nylon membrane encapsulation are not well suited for live cell immobilization. Levels of pH may exceed 9, reagents such as acid dichlorides may be toxic, and use of polar solvents such as chloroform restrict the use of nylon membrane microcapsules to immobilization of enzymes and other biologically reactive agents.[63] Because of these conditions, there has been little interest in the application of interfacial polymerization to cell encapsulation. However, Tice and Meyer[68] from the Stolle Research and Development Corporation (Cincinnati, OH) patented an interfacial polymerization process for cell encapsulation, and suggested the use of a number of cross-linked proteins, including casein, collagen, gelatin, soy protein, and gluten among others. Some of these polymers were incorporated into the membrane and subsequently enzymatically degraded to attain the desired

membrane properties. The most intensive work on cross-linked polymeric membranes was conducted in France by Levy et al.,[69-71] who demonstrated the versatility of such membranes.

Some improvements were suggested to extend the technique of interfacial polymerization to live cell encapsulation, based on a better understanding of nylon formation at the droplet interface.[65] The limiting process in nylon membrane formation is the transfer of the diamine from the aqueous drop to the organic phase. High pH and polar solvents are needed to increase the noncharged diamine fraction[65,72] and its solubility in the organic phase.[73] This results in a very toxic environment for cells.

Nylon is a linear polymer which by itself does not provide a high level of mechanical resistance. Stronger membranes were obtained by incorporating a protein or a polyamine[74-76] into the membrane. However, both additives contain reactive groups (amines) which should react chemically with the organic monomer (acid dichloride) during membrane formation. This was confirmed by forming a membrane in the absence of diamine, using polyethyleneimine as a membrane material.[77]

Interfacial polymerization reactions in microencapsulation often involve transfer of the aqueous, soluble polymers into the organic phase prior to reaction, resulting in membrane formation. In cross-linked polyethyleneimine or protein membrane formation, small quantities of organic acid dichloride transferred to the aqueous phase ensure sufficient cross-linking between the preformed polymeric chains to form a strong membrane. The use of high pH and polar solvents, ensuring diamine transfer to the organic phase, then becomes less critical. Polyethyleneimine membranes have been formed at pH 7 using cyclohexane or mineral oil as solvent.[77] An additional advantage of polyethyleneimine membranes is the lowering of the osmotic pressure that prevails in nylon membrane formation, by reduction of the monomer concentration.

Polymer cross-linking reactions have some drawbacks. The reactivity of a polymer is generally lower than that of a diamine, with decreasing reactivity in the order of polyamines (polyethyleneimine), proteins, polysaccharides, and glucosamines (chitosan). With diamines, the reaction occurs in less than 1 min, while the time to cross-link polymers ranges from a few minutes for polyamine[77] to 2 h for polysaccharides.[69-71] In order to increase the reactivity, cross-linking agents which are more soluble in the aqueous phase, such as glutaraldehyde, may be used, but with an increased risk of loss in cell viability.

Except for Tice and Meyers' patent,[68] there are no literature reports of successful cell encapsulation within polymeric membranes formed by interfacial cross-linking reactions. The microencapsulation of lactic acid bacteria in polyethyleneimine membranes[78] was examined. High cell viability following microencapsulation was demonstrated by counting cells released upon rupture of the membrane; however, the encapsulated cells exhibited little lactic acid activity, as monitored by changes in medium pH. It was not clear if the loss in activity was due to an inhibition by the polyethyleneimine or to

the polyethyleneimine playing an acid buffer role. As alternatives, albumin and chitosan were tested as membrane materials using diisocyanate and glutaraldehyde, respectively, as cross-linking agents. Both reagents, being more soluble in water, may react more easily with the cells, but also result in improved reactivity with membrane components over acid dichlorides. One additional advantage to the alternative cross-linking agents is that acid is not formed during membrane formation, as is the case with the acid dichlorides.

Formation of cross-linked polymeric membranes represents a promising alternative to other cell encapsulation techniques. To date, the procedures have not been seen as compatible with living cells due to the toxicity of the reagents; however, promising results have been achieved in progressing from techniques involving toxic solvents, such as that involved in nylon membrane formation, to the development of cross-linked membranes under milder conditions better suited to live cell immobilization.

VII. APPLICATIONS OF ANIMAL CELLS

Processes involving animal cell culture were developed primarily during the last decade. Several have been industrialized, and explosive developments in the technology are anticipated over the next decade due to economic pressures.

Applications for animal cell culture may be classified into four categories: cell culture for the synthesis of cell metabolites, production of artificial cells, embryotic cell protection, and the use of cell culture as body models.

A. PRODUCTION OF BIOCHEMICALS VIA ANIMAL CELL CULTURE

Microencapsulation of cells offers a number of advantages, compared to suspension culture, for biological production. Microencapsulated cells may be cultured in standard stirred vessels, in which mixing facilitates mass transfer and air may be freely sparged into the culture medium without cell damage due to protection by a semipermeable membrane. Permeability of the membrane may be controlled to permit rapid transfer of substrate and nutrients while potentially maintaining product within the encapsulating membrane. Difficulties encountered in the use of serum in the growth medium do not arise, due to the physical presence of the membrane barrier, and product recovery is facilitated by separation of the capsules from the growth medium. Gentle homogenization and centrifugation of the microcapsules can provide for the separation of viable, intact cells from the product contained in high concentration within the supernatant fraction. Further purification is facilitated, since most of the reagents used in the cell culture have previously been removed.

Production of biochemicals via microencapsulated living cells may be illustrated by the "Encapcel" process developed by Damon Biotech, Inc.[79-81]

Cells immobilized within alginate beads were coated with poly-L-lysine and cross-linked by glutaraldehyde. Microcapsules were formed by liquefying and extracting the internal alginate gel through the membrane coat. This immobilization process was applied to interferon production by fibroblasts and to monoclonal antibody production by hybridoma cells.

Damon Biotech, Inc. routinely mass-cultures microencapsulated hybridoma cells via the Encapcel system for the industrial production of monoclonal antibodies. The antibodies are retained in high concentration within the small volume of the microcapsules. Several groups are presently working on mammalian cell immobilization, such as that of pancreatic islets for insulin production in the treatment of diabetes. For example, islets have been encapsulated within cellulose sulfate microcapsules by Braun et al.[58-60]

Karyon Technology developed the "Geltrap" process[82] involving animal cell encapsulation in calcium alginate beads. Antibodies are secreted into the surrounding medium, resulting in large volumes in low concentration. Sandoz Forschunginstitut and the Institut for Applied Microbiology in Vienna developed a method for the entrapment of animal cells in agarose beads,[82] with production characteristics similar to those of the Geltrap system.

Production via immobilized animal cell culture permits processing with high cell concentrations and facilitates the concentration of products within a large scale of operation. Reproducibility, ease of processing, and lower purification costs make this approach an attractive alternative to *in vivo* production. Products available through animal cell culture include vaccines, interferons, monoclonal antibodies, insulin, growth hormone, plasminogen activators, and blood clotting factors. Biologicals which show promise for future commercial interest include specific animal enzymes, numerous hormones, and specific polypeptide growth factors.

Animal cells may be used for protein engineering. Once a specific gene for a particular protein has been cloned, site-directed mutagenesis is applied. This provides a means of producing new proteins with a large range of therapeutic uses.

Viruses lethal to insects may be produced by animal cell culture. Advantages over chemically synthesized pesticides include better targeting of the species, low chemical toxicity, and lower cost.

B. ARTIFICIAL CELLS

Since Lim's work in 1980, a number of studies have been undertaken to develop a suitable method of pancreatic islet encapsulation. Implantation of encapsulated islets will provide treatment of diabetes under mild conditions. It is anticipated that diabetics will receive only one injection of encapsulated islets of Langerhans per year, in place of two insulin injections daily. Implantation of encapsulated islets permits the maintenance of insulin blood levels as a function of need, avoiding the high insulin fluctuations observed with daily injection.

Although the technology shows promise, a number of problems remain to be resolved. The most important is to ensure protection of the islets from immune rejection, a process which is not fully understood. At the same time, the encapsulating membrane must not represent a barrier to glucose or insulin transfer. Jarvis and Gardina[79] demonstrated that the mass transfer limitation may be due to the transfer of glucose and insulin between the blood and the microcapsules. Hence, the location of the implant may be an important parameter in the successful application of this method to the treatment of diabetes.[83]

Technology for the treatment of diabetes constitutes a very important market. Encapsulation of islets of Langerhans is only a first step in the development of the technology. Once proven successful in the treatment of diabetes, a number of other disorders may be treated in a similar fashion by implantation of protected cells.

C. PROTECTION OF EMBRYOTIC CELLS OR GENETIC MATERIAL

Although millions of spermatozoa are deposited in the female reproductive tract during natural or artificial insemination, only a few sperm cells reach the site of fertilization.[84] The number of viable spermatozoa in the female tract must be maintained at an adequate level to maximize conception rates. Microencapsulation may afford protection to the spermatozoa.[85] Encapsulation also protects against freezing and thawing during cryopreservation. In a similar vein, Kojima et al.[86] recommended protecting rabbit embryos against fracture damage during freezing and thawing by encapsulation of the embryos in calcium alginate gels.

A chromosome-specific, ordered library may facilitate understanding the structure and function of the human chromosome. To achieve this objective, Yokoyama et al.[87] proposed a method of molecular cloning of DNA fragments by encapsulation of cells in agarose beads for the construction of a human genomic DNA library in a yeast artificial chromosome vector.

D. USE OF THE CELL AS PRODUCT

In vitro, animal cell concentrations are generally limited to 10^6 to 10^8 cell/ml, compared to 10^9 cells per ml *in vivo*. Moreover, these cell concentrations are only achieved in the immobilization space, and not in the entire reactor volume. The possibility of the *in vitro* production of a food product similar to a fish fillet or beefsteak does not appear feasible in the foreseeable future. However, animal cells provide an interesting whole body model for evaluating the toxic effects of drugs, cosmetics, food additives, and a host of other common chemicals. Use of animal cells will permit repetitive and rapid testing, and provide accurate and reproducible standards for diagnostic product safety evaluation.

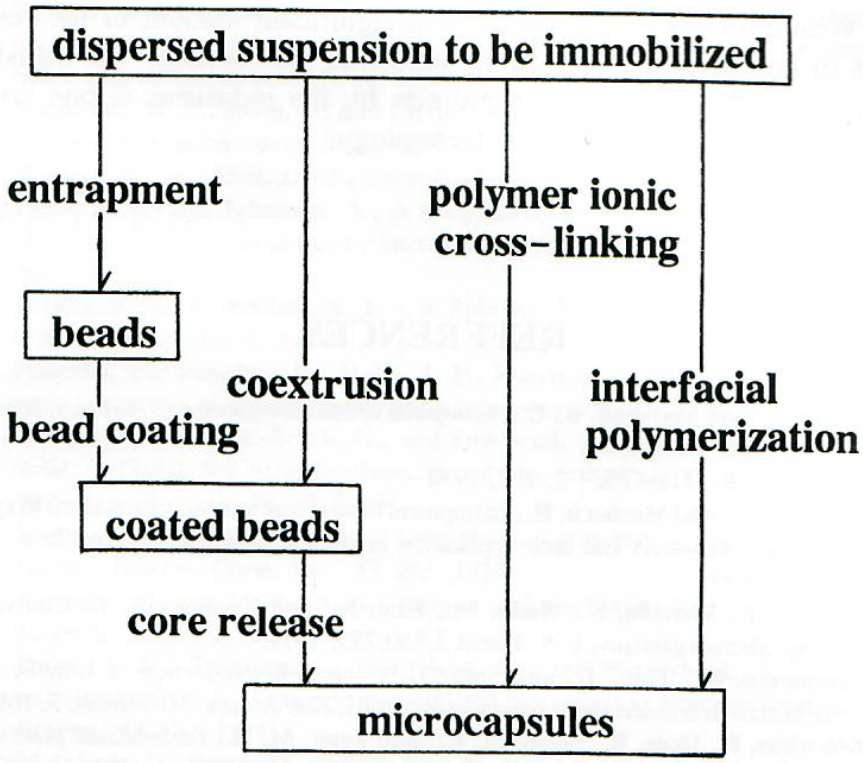

FIGURE 1. Entrapment techniques for cell immobilization.

VIII. CONCLUSIONS

As seen in Figure 1, most processes involving cell encapsulation lead to a similar product: a semipermeable membrane, possibly cross-linked, surrounding droplets or beads which contain the immobilized cells. The encapsulation materials proposed are to a large extent suitable for most types of animal cell encapsulation. Some processes may be more difficult to scale-up, such as that involving extrusion. Others require further steps during formulation, and the risk of contamination is thus increased. The criteria for the selection of the most appropriate encapsulation procedure are still not fully determined, and include the membrane material and its residual charge. This aspect of encapsulation technology is presently under investigation in a number of laboratories.

Important developments in other aspects of animal cell culture include a better understanding of the medium requirements for selection of suitable bioreactor designs. The immobilization methodologies will remain one of the most important criteria for the success of animal cell culture technology.

Future developments in animal cell culture involve animal cell-assisted computers or artificial organs for robotic applications. In most other cell culture developments, the preliminary investigations were conducted in an academic environment. Industries initiated their research mainly at the R & D

stage. With animal cell technologies, a significant amount of the research appears to be conducted directly by industrial laboratories. The infusion of millions of dollars into targeted projects by the industries is one criterion demonstrating the potential of the technology.

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