

ISLET ENCAPSULATION AND TRANSPLANTATION: A POTENTIAL TREATMENT FOR TYPE 1 DIABETES

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ABSTRACT Approximately one million insulin-dependent (Type 1) diabetics are in need of a more suitable treatment for this very serious ailment. Recent success in transplantation of pancreatic islet cells has been demonstrated as one of the more promising methods for treating the disease. Islets of Langerhans, which comprise approximately 1 or 2 percent of the human adult pancreatic volume, can be transplanted without extensive surgery. Currently, islet transplants require a lifelong regimen of high dose immunosuppression to prevent immune rejection. Encapsulating islets of Langerhans in a biocompatible material has potential as a promising alternative to the use of immune suppression drugs. Immunoisolation through encapsulation of islets offers a significant potential in using xenogeneic sources of islets which would eliminate the donor shortage problem. A brief description of the requirements for encapsulating islets to allow adequate immunoisolation from the host immune system while allowing adequate diffusion of nutrients, hormones, oxygen, ions, and glucose to reach the islets, and insulin release by the β cells is outlined. Biocompatible encapsulation material consisting of highly purified alginate is described along with some of the basic physical characteristics of the hydrogel. Requirements on the capsules are discussed and methods for capsule formation are reviewed. An example of a discordant xenogeneic transplant experiment is presented.

Key words: Diabetes, Islets, Encapsulation, Alginate, Immunoisolation, Transplantation

1. INTRODUCTION

Since the discovery of insulin in 1921 by Banting and Best, exogenous insulin administered via injection and more recently, by pumps or inhalation, has been the primary treatment of Type 1 diabetes. This therapy has allowed millions to survive but it falls short of a cure. Tragically, Type 1 diabetes affects millions of individuals and is associated with serious associated complications including amputations, renal failure, heart disease, neuropathy, loss of vision, and a shortened life expectancy by approximately 30%. Annual cost of the disease in the United States is estimated to be \$130 billion. Average lifetime cost per patient is estimated at \$600,000. Inability to physiologically control glycemia with administration of exogenous insulin therapy has been a strong motivating force in driving research on insulin-delivering grafts.

Considerable efforts have been made to isolate islets of Langerhans from the pancreas of cadaver donors and directly transplant them into diabetic recipients. Islets of Langerhans, which comprise approximately 1 to 2 percent of the human adult pancreatic mass and are estimated to consist of up to 1.2 million islets, potentially can be transplanted without extensive surgery offering an alternative to full organ transplants. In the past, one of the reasons that islet allografts have failed is believed to be due to an insufficient number of islets available for transplantation. The International Islet Transplant Registry has recommended more than 6,000 islet equivalents (150 μ m in equivalent diameter represents an islet

equivalent or IEQ) per kilogram of the recipient's bodyweight. For a large recipient, 200,000 to 500,000 or more islets are required. This has most often necessitated the use of more than one donor to provide an adequate number of islets. The normal size range of human islets is from 15 to 500 μ m in equivalent diameter, London et al., [1].

Only recently has islet transplantation reached significant success as demonstrated by the Edmonton Protocol developed by Shapiro, et al. [2]. With the Edmonton Protocol, the mass of transplanted islets deemed necessary to achieve normoglycemia exceeded previously recommended islet equivalents per kilogram of body weight of the recipient. As a result, two or more donors were required per transplant recipient but resulted in seven out of seven patients achieving exogenous insulin independence and normal glucose metabolism. The need for a greater number of islet equivalents may be due to deleterious effects of immunosuppressive drugs designed to deplete or inactivate the immune response but harm the islets. Other factors are compromised function of islets due to being subjected to the isolation process and only a fraction of the available islets are recovered.

Suitable long-term treatment of the approximately one million insulin-dependent (Type 1) diabetics and a proportionate number outside of the U.S. demands advances in the methods for isolation and transplantation of pancreatic islet cells. Presently, islets for human transplantation are obtained from human cadaver

pancreases. This limits the potential number of transplants in the USA to 1000 to 2000 per year. However, research into living donor transplants is ongoing and if successful, this will provide a much greater number of potential transplants of high quality islets. Because of a limited supply of human islets, the demand for improved systems and methodologies for procuring, transporting, and isolating the islets has gained heightened importance.

Unfortunately, uncoated islet transplants require a lifelong regimen of high dose immunosuppression to prevent immune rejection. The prolonged use of immunosuppressive drugs is associated with a range of problems. Islet encapsulation in a biocompatible material offers a promising alternative to the use of immune suppression drugs and also offers the significant potential of using xenogeneic sources of islets, which would eliminate the donor shortage problem. Extensive work on islet isolation and microencapsulation over the past decade suggests that transplantation of islets may be accomplished without the use of immunosuppressive drugs [3-11]. Furthermore, xenogeneic transplants using porcine or piscine islets are possible if the encapsulation method proves successful. Currently, it is not possible to utilize xenogeneic transplants with immune suppression drugs.

Although there are several possible combinations of implant geometries, the individual encapsulation of islets in 100 to 500 micron diameter spherical beads (often referred to as microencapsulation) appears to be the most promising approach to date for preparing islets for transplantation [10,12]. With this approach, each islet is individually encapsulated with a biologically compatible material that produces a membrane that is selectively permeable. The capsule membrane must allow diffusion of nutrients, hormones, oxygen, ions, and glucose to reach the islets and allow insulin release by islet β cells to be readily passed out of the capsule as well as metabolic waste. At the same time, membranes must provide an impermeable barrier to larger molecules and cells of the body's immune system. With a sufficiently small membrane pore size, immunoglobulins and cytokines are prevented from crossing the membrane of properly coated islets. Cell-to-cell contact of antigen presenting cells (APC's), T-cells, and large major histocompatibility complex (MHC) molecules are also prevented. Encapsulation membranes are generally designed to have a nominal 20,000-mol wt. cutoff. Nutrients and insulin (largest molecule with mol wt. 5800) are allowed to pass through the membrane.

Microencapsulation has several advantages over other methods of preparation for transplantation. These advantages include ease of transplantation, which involves simply injecting encapsulated islets into the peritoneal cavity or other suitable site in the host, capsule durability, excellent mechanical integrity, and a large surface-to-volume ratio (proportional to $1/r$) when using small capsules which increases the diffusion efficiency. In addition, with appropriate encapsulation and removal of blank capsules, there is greater flexibility in the graft sites that may be used. Microencapsulation has also been demonstrated to provide improved function and protection of the transplanted islets.

Development of a number of technologies is required to achieve optimal islet encapsulation and immunoisolation.

In this report, we will expound upon the requirements for providing immunoisolation to islet grafts using alginate hydrogels as the encapsulation materials. Material transport requirements for maintaining islet function and viability are addressed. Methods suitable for forming spherical capsules using highly viscous alginates will be reviewed and results of an innovative atomization system will be presented. Since any exposed tissue can excite an immune reaction which can destroy the graft, a capsule processing system has been developed to eliminate blank capsules and extraneous material and to examine each capsule for exposed tissue, fractures, or other imperfections. Faulty encapsulations are eliminated from graft preparations using this device. Finally, an example of results obtained from a discordant xenogeneic graft using encapsulated porcine islets will be presented.

2. ENCAPSULATION REQUIREMENTS

For the islet cells to maintain viability in the host, successful encapsulation constructs must be nonfibrogenic, provide complete coverage of the islet, allow good insulin release dynamics, allow transplantation with minimal surgical risk, and be permeable to small and medium-sized proteins while providing a barrier to the larger antibody molecules. A schematic of these requirements is provided in Fig. 1. Encapsulation of large numbers of islets (over 500,000 are required for human transplantation) must be accomplished in a short period of time. Islets are approximately 150 μm in equivalent mean diameter but may vary in size by as much as an order of magnitude. Human islets have a size range of 15 to 500 μm . The required capsule wall thickness is generally considered to be between 20 and 200 μm but this requirement remains uncertain until further research on immunoisolation and diffusion transport has been completed. Relatively large islet size range implies the need for adaptive capsule sizes. Currently, there is insufficient information on optimum capsule wall thickness, which also depends upon the permeability and diffusivity of the alginate material used. Thus, it is important that close attention be paid to mechanisms associated with the molecular diffusion of material to and from the islets. Central necrosis, resulting from oxygen transport limitations, has been frequently observed in cultured islets. Loss of islet viability and function due to hypoxia is common in larger islets.

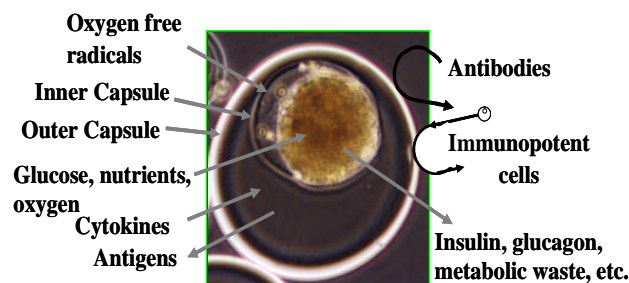


Figure 1. Image of a double-coated encapsulated islet schematically showing the functional requirements of the capsule.

Perfection of encapsulation processes remain as critical components in the quest to achieve success in the islet transplantation approach without prolonged use of

immunosuppressive drugs. Critical areas may be identified with problems to be resolved in efforts to advance this technology as: (1) a limited source of islets, (2) maintenance of cell viability and function during isolation, encapsulation and transplantation, and (3) protection from immune rejection. In addition to the beneficial immunoprotective effect of the capsules, the permselective membrane may conceivably have negative effects on islet cells by interfering with transport of useful macromolecules in the surrounding medium and not allowing them to reach the cells, and it may prevent damaging lytic products, such as intracellular protease that may be released by lysed entrapped cells, from diffusing to the outside.

3. MATERIAL DIFFUSION

Figure 2 shows an electron micrograph image of an islet along with a microscope image of an encapsulated islet. Within the intact pancreas, islets are highly vascularized with small afferent and efferent arterials and capillaries passing through the islets and running close to most of the thousands of cells (~2000 to 10,000) that form the islets. Material transport is by blood flow to the neighborhood of the cells followed by diffusion through distances very short compared to the islet diameter. In contrast, when islets are isolated from the pancreas, blood vessels are terminated and thus, material transport to the cells in the islets is by molecular diffusion. Diffusion distances become the distance from the capsule exterior to the various cells in the islets.

Oxygen supply to the islet cells depends upon local partial pressure of oxygen in blood, distribution of host blood vessels in the neighborhood of the graft, oxygen permeability of the encapsulation material, and the capsule wall thickness. Within the encapsulated islets, the cells act as distributed consumers of oxygen and nutrients and distributed sources of insulin and metabolic waste. It is widely recognized that adequate diffusion of nutrients, hormones, oxygen, ions, and glucose reaching the islets, and insulin released by the β cells passing out of the capsule as well as metabolic waste is critical to the proper function, viability, and overall performance of the transplant. When encapsulated, diffusion distances between islet cells and the surrounding vasculature may become significantly larger due to wall thickness of the membrane. This increases the diffusion distances resulting in a relative depletion of oxygen toward the islet core.

Islets can vary in size by an order of magnitude (e.g. rat islets have a mean size of approximately 150 μm and range in size from 50 to 300 μm). If an islet is large, the cells at the core have been observed to be necrotic probably due to hypoxia and insufficient nutrients reaching the cells [13]. Thus, every effort must be made to optimize the diffusive transport of materials to and from islets and to cells within the islets. Buildup of insulin within islets may be expected to produce a negative feedback that precludes further insulin production. Dead cells in the necrotic core of islets may produce lytic products and antigens that may excite an immune response to the graft.

Rate of change of solute in a medium and hence, the diffusive transport of material may be expressed by Fick's first law of diffusion given as:

$$\frac{dM_i}{dt} = -A_i D_{mp} \left(\frac{\partial C_m}{\partial r} \right) \quad (1)$$

where M_i is the solute under consideration, A_i is the surface area of the membrane, D_{mp} is the diffusivity of the solute, r is the radial coordinate, and C_m is the concentration of solute in question. The diffusion rate is inversely proportional to diffusion distance, and directly proportional to surface area of the organ and concentration of nutrient substances in the medium. The driving force is the concentration gradient of different nutritive substances and nutrient consumption by tissue. Diffusivity, D_{mp} of the capsule is affected by the molecular structure of the capsule material, and concentration gradient, $\partial C_m / \partial r$ depends on the size of the capsule and islet. Diffusivity within islets is approximately $D_i = 1.3 \times 10^{-5} \text{ cm}^2/\text{sec}$ which is an average value. Oxygen consumption rate is approximately $V = 3 \times 10^{-8} \text{ mol}/\text{cm}^2$ for islets under maximum glucose stimulation and about one third lower for basal stimulation [14]. Blood $p\text{O}_2$ is about 100 mm Hg whereas partial pressure in the microvasculature is about 40 mm Hg. By bringing blood vessels closer to the implant, oxygen delivery is improved and it is well known that a spherical geometry is advantageous from the standpoint of diffusive mass transfer.

Measurements of the oxygen diffusion through living tissue have been reported in the literature [15,16]. These studies focused primarily on diffusion of oxygen through cellular mass with and without convection in the surrounding media. Measured sigmoidal profiles showed a diffusion-depleted zone at the spheroid surface, a steep decrease within 200 to 250 μm from the surface, and a flat minimum region in the central part of the spheroid. This steep decline in oxygen consumption and flat minimum region reflects necrosis in the core. According to the results of Schrezenmeir, et al. [15], the encapsulation has a primary effect on the extension of the oxygen-depleted zone outside of the organ. This zone can be reduced by thinning down the alginate layer, resulting in better oxygenation. Once again, the importance of using capsules that are as small as possible is emphasized by these results.

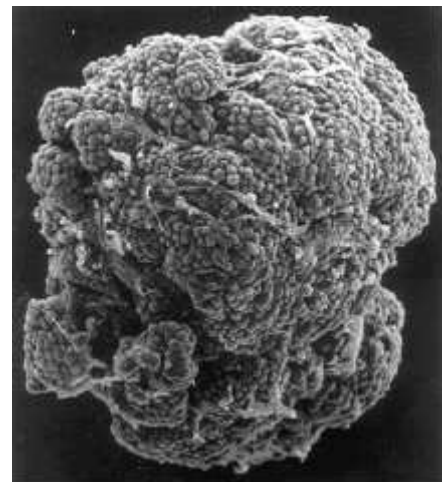


Figure 2. Electron microscope image of an isolated islet, courtesy of Dr. Ming Chen, University of Alberta, Edmonton.

4. ENCAPSULATION MATERIAL

Alginate hydrogels are a promising class of islet encapsulation materials for providing an insoluble noncytotoxic semi-permeable barrier to immunological attack by the host, while also allowing the diffusion of oxygen and transfer of nutrients, ions, hormones and metabolic waste to maintain the health of the encapsulated tissue. Currently, there is insufficient structure-property information about alginates to permit optimum capsule design. Research is planned that will provide structure-property information required for advancing toward optimum alginate encapsulation.

The pore size of the alginate membrane affects the diffusivity, steric exclusion, and material tortuosity that are critical to the material diffusion and immunoisolation properties of the capsules. Because of the alginate purification and chemistry needed to ensure biocompatibility and immunoisolation, it is necessary to investigate the diffusivity of the specific alginate capsules to be used. The maximum size of the islets that will survive without forming a necrotic core will depend on the alginate characteristics (permeability, diffusivity, etc.), the capsule wall thickness, and the local conditions (proximity to blood vessels, oxygen tension, convection and diffusivity of the surrounding fluid, etc.).

The diffusivity of alginate having a range of characteristics (primarily viscosity which affects the porosity of the material) cross-linked with CaCl_2 has been studied in the optimization of our encapsulation material. The physical and chemical properties of the encapsulation material affect the gel transport of material. Alginate used for islet encapsulation consists of linear chains of linked monomers of β -D-mannuronic acid and α -L-guluronic acid derived from seaweed. Diffusivity is also affected by the polymer chain cross linking reaction, flexibility of the polymer chain, and chain radius. Diffusion within hydrogels entails the movement of solute through the aqueous regions between the polymer chains. The tradeoff is that decreasing the pore size of the membrane material to provide more effective immunoisolation increases the diffusion resistance (steric exclusion). This parameter requires further investigation in the optimization of the encapsulation material.

Although the encapsulation prevents cell to cell contact, antigens released by the transplanted cells can penetrate the membrane and activate macrophages, resulting in the release of cytokines, free radicals, nitric oxide, and peroxides. The molecular weights of cytokines are on the order of 20,000 Daltons and hence, may be retained by the membrane. Moreover, insulin, which has a molecular weight of 5800 Daltons, is seemingly the largest molecule that needs to pass through the semipermeable barrier. Thus, it may be possible to restrict passage of the antigens with carefully designed alginate capsules of a given range of porosity and permeability.

In recent years, greater attention has been attributed to the diffusive transport mechanisms and to the availability of oxygen and nutrients to the encapsulated islet cells. The supply of oxygen to encapsulated cells depends upon a number of factors, including (1) selection of the implantation site and the local pO_2 in the blood, (2) concentration and possible proliferation of host blood vessels in the neighborhood of the implant, and therefore, in

near proximity to the surface of the capsules, (3) oxygen diffusivity and permeability for the encapsulation membrane material, (4) oxygen rate of consumption characteristic of the encapsulated islets at both the basal condition and when stimulated by glucose, (5) the geometrical and morphological characteristics of the capsules, and (6) the intracapsular islet cell concentration and average number of islets within each capsule. The hypothesis that hypoxia is the major contributor to loss of viability has been supported by comparisons of external observations and theoretical model predictions for the effect of islet size and external oxygen partial pressure on the size of the necrotic cores.

5. CAPSULE FORMATION

There are a number of methods available that may be suitable for the formation of liquid drops or sprays. It is known that atomization processes are triggered by perturbations of liquid jets, which may be caused by natural disturbances, electrostatic forces, mechanical disturbances, aerodynamic forces, a swirl of the flow due to asymmetries of the nozzle inlet, flow separation of the nozzle inlet, cavitation, turbulence, etc. However, since the islets consist of living tissue, some additional constraints are placed on the means that may be used for atomization of the two-phase mixture of islets in alginate. Maximum islet size places a constraint on the minimum orifice diameter that may be used ($>500 \mu\text{m}$). Islets are fragile and this is especially true for porcine islets so shear stress on the mixture must be limited. Excessive atomization pressure may not be used since these pressure forces can damage the cells.

Liquid alginate has a relatively high viscosity range (300 to 3000 centipoise), moderate surface tension ($\sim 55 \times 10^{-3} \text{ N/m}$), and drops with a relatively wide size range need to be generated. The liquid contains fragile viable islets and the drops need to be collected and solidified typically within a CaCl_2 solution without fragmenting. Islets need to be approximately centered in the drops and completely covered by the porous membrane. Methods in the literature describe the use of spinning disk, electrostatic, vibrating orifice or cavity, and air blast atomizers to form the droplets or microspheres required for encapsulation. A brief description of candidate methods will enable a better understanding of the relative merits and limitations of the existing devices and their feasibility and advantages for encapsulating islets of Langerhans.

5.1 Spinning Disk Atomizer

Much of our work has utilized a spinning disk atomizer with design features developed to accommodate the alginate and islet mixture. The spinning disk atomizer consists of a cup-shaped rotating body into which liquid alginate with dispersed islets is introduced near the inner part of the cup. The liquid is supplied through a stationary catheter fed by a syringe driven by a remote syringe pump. The friction between the liquid and the surface of the cup serves to bring the alginate and islets to the speed of the cup. Centrifugal force produced by the rotating liquid drives liquid flow upward on the sloped sides of the cup toward the rim of the atomizer disk or cup. This causes

the liquid to be spread into a thin film. As the flow rate of the liquid increases, bulges in the liquid torus on the cup edge convert into ligaments. The number of ligaments formed is determined by grooves cut into the cup surface which help to control the drop formation to a specific size range. The relative air velocity induced by the spinning cup and Rayleigh instability causes the ligaments to deform and break up into drops. With the presence of the quasi-solid islets, varicose disturbances are induced on the ligaments which tend to promote breakup around the islets. In the present case, a ligament formation mode produced near-monodisperse drop size for the capsules formed.

For islet encapsulation, there are some disadvantages in using the rotating disk atomizer. This is especially true for the primary encapsulation. When the liquid is drawn into a thin film as it flows over the disk, significant shear forces on the islets can be expected. The drops will also have relatively high tangential and radial velocity components so the impact into the calcium chloride could cause deformation and islet eccentricity. Furthermore, the smallest capsules that can be formed reliably using the high viscosity alginate was in the range of 350 to 500 μm . Rotating the disk at higher speeds produced capsules that were not spherical when collected and solidified in CaCl_2 .

5.2 Electrostatic Atomizers

Electrostatic dispersion of liquid into drops is distinguished by simplicity of the physical phenomena that forms its basis and through the possibility of being able to continuously adjust the dimensions and charges of the drops formed. The method also has the advantage of being able to operate in the polydisperse and monodisperse drop size modes. It is these features that were of primary interest in the encapsulation process. With electrostatic dispersion of liquids, the energy causing the liquid surface to disrupt is provided by the mutual repulsion of the like charges that have accumulated on the surface of the liquid. This electrostatic pressure tends to expand the surface area but is opposed by surface tension forces, which tend to contract or minimize surface area. Numerous configurations for atomizing electrodes have been proposed including hypodermic needles, sintered bronze filters, and cones. Both direct and alternating currents have been used. However, there is a concern that alternating currents may harm the viability of the islets.

The method has been successfully applied to islet encapsulation by Dorian, et al. U.S. Patent No. 5,429,821, 1995 and 5,656,468, 1997, Hommel, et al. U.S. Patent No. 4,789,550, 1988, and U.S. Patent No. 4,956,128, 1990 and others. Hommel et al. used a pulsed HV approach to strip the liquid from the needle to form small uniform drops with a reasonable degree of control. In our experience, this method will produce large drops (500 μm to 1 mm) reliably but will fail for smaller drops when using highly viscous liquids. The key limitation of the method is that liquid jet breakup wavelength (spacing between varicose perturbations that naturally form on the jet) is a random variable with a mean value equal to $k = 4.5$ times the mean drop

diameter. This is a direct result of the well-known Rayleigh criterion for the most probable breakup length, L , for capillary jets of diameter D_j given as $L = kD_j$. The resulting drop diameter, d may be estimated from

$$d / D_j = (3k / 2)^{1/3} \quad (2)$$

where $k = 4.5$ for water and increases with the viscosity of the liquid. Thus, the breakup length must be carefully controlled along with the electrostatic voltage and liquid flow rate that affect the size of the jet. A smaller diameter orifice would help but that is limited by the need to pass the larger islets without damage. Random breakup and formation of satellites are also a problem and need to be controlled if high quality encapsulation is to be achieved.

5.3. Capsule Formation Using Two-fluid Atomization

Because of the requirement of atomizing high viscosity alginate (1000 to 3000 centipoise or 10 to 30 poise) with islets having a size range of 20 to 500 micrometers dispersed within it, a relatively large orifice was required to allow unimpeded flow with tolerable shear stress on the islets. Experimentation indicated that a hybrid two-fluid atomizer could produce the desired results. The atomizer was designed with several proprietary innovations which allow good control and a relatively high probability of forming droplets with sizes that conformed to the size of the islets. The high viscosity alginate stream and subsequent breakup is shown in Fig. 4.



Figure 4. Image of one of the basic two-fluid atomizers operating on medium viscosity alginate (800cp).

An extensive series of parametric studies were conducted on the atomizer involving the systematic variation of the airflow rate, the alginate flow rate, the airflow gap, and other parameters. In general, the atomizer was easily adjusted to produce drop sizes and size distributions that were deemed ideal for encapsulating

islets. The conditions were established that would produce good atomization (drops in the size range of 100 to 600 μm). A range of airflow rates from 0.2 to 1 m^3/hr (standard cubic meter per hour) were tested and a general trend in the atomization was observed with increasing air velocity. Optimum alginate flow rate was determined by the requirement to maintain continuous flow. At a lower flow rate (0.3 ml/min), the alginate flow tended to become intermittent and undesirably large drops were produced. A series of tests were also conducted to determine the optimum setting of the airflow gap adjusted by the protrusion of the alginate supply needle.

Alginate droplets were collected in CaCl_2 solution which gelled the droplets into solid capsules. Numerous samples of the alginate droplets or capsules were recorded as a result of these investigations. Only a few samples will be presented here. In general, a mean drop size of approximately 200 μm with a size range of from 100 to 600 μm was the target. In this size range, the breakup of the alginate ligaments into drops tended to be adaptive to the size of the islets.

In the initial studies with this modified atomizer, only alginate (without islets) was used so that the liquid breakup was due to the natural mechanisms derived from the Rayleigh instabilities in the liquid jet and the shear forces of the air flow interacting with the extruded liquid alginate. Figure 5 shows examples of capsules formed using low ($\sim 300\text{cp}$), medium ($\sim 800\text{ cp}$), and high, viscosity ($\sim 3000\text{ cp}$) alginate, respectively. In the case of the low viscosity alginate, an air flow rate of approximately 0.23 m^3/hr was needed to form droplets that were small enough. The airflow rates were approximately 0.34 m^3/hr and 0.57 m^3/hr respectively for the medium and high viscosity alginates.

Based on measured mean drop size and the alginate flow rate, rate of capsule formation was approximately 5,000/sec. By adjusting atomization parameters including alginate flow rate, airflow gap, and airflow rate, we were able to produce capsules in the desired size range that showed good sphericity and surface quality. Smooth surfaces on the capsules are essential to avoiding possible formation of roughness on the surface of the solidified capsules which then act as anchorage points for cell adhesion which can lead to fibrotic overgrowth.

After the single-phase alginate experiments were completed, spherical particles were dispersed in the alginate to simulate islets. A mixture of spherical particles with a specified mean size of 116 μm and a range of 106 to 125 μm were used (supplied by Bangs Laboratories, Inc.). In general, islets are not spherical and can show significant deviations from spherical with a morphology that may be characterized as having craters and protrusions on the surface. These morphological characteristics will affect the encapsulation requirements including both the capsule wall or membrane thickness and the shape of the capsule. It was fortuitous that some of the spherical beads stuck together during dispersion in the alginate. This allowed a demonstration of how viscous alginate will surround and encapsulate even irregular shapes. Figure 6 shows samples with small spheres adhering to another pair of spheres forming a relatively small point. The thin wall of the capsule can be seen to fully enclose this deformation.

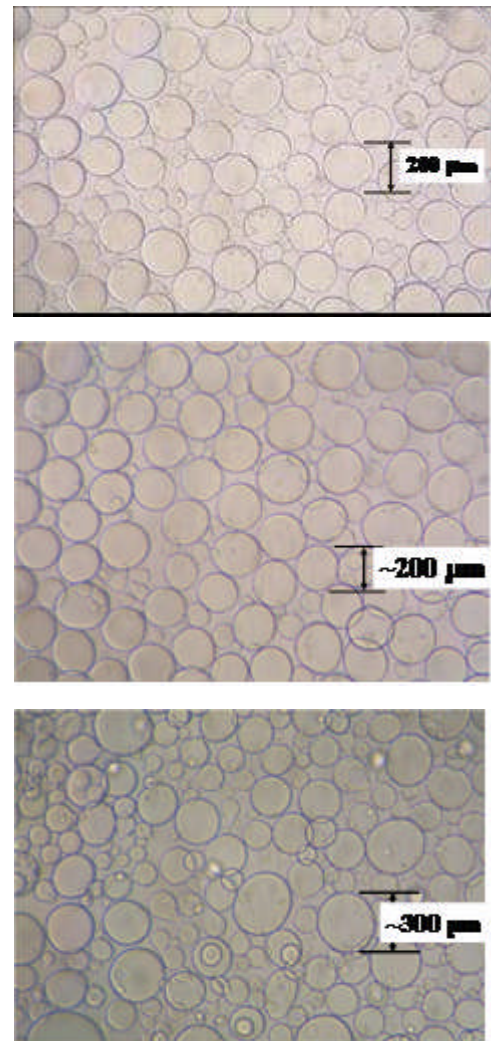


Figure 5. Capsules formed from alginate with viscosity approximately 300 cp, 800 cp, and 3000 cp and showing droplets with mean diameter of approximately 175 μm , 200 μm , and 250 μm diameter respectively.

Using high viscosity liquid can be an advantage if the islets are directed into the center of the alginate stream within the atomizer so that they will follow the flow and remain in the core of the extruded alginate ligaments. Subsequently, as the laminar jet is extruded, islets form small bulges or varicose protuberances on the alginate ligaments. Using aerodynamic forces to breakup the ligaments resulted in the high probability of the islets remaining in the center of the capsules. Hence, there was a high probability of the islets being encapsulated with capsule sizes that accommodated islet size and the islets tended to be in the center of the capsules. Examples are shown in Fig. 7 for the encapsulation of spherical particles and in Fig. 8 for encapsulating islets.

Under this investigation, we confirmed that aerodynamic forces were capable of providing the necessary shear forces to the extruded alginate to form droplets of appropriate size. Whereas imposed mechanical disturbances were damped and electrostatic forces were insufficient to breakup the ligaments without reaching a critical charge causing the droplets to fragment, aerodynamic forces could be controlled allowing reliable droplet formation even with high viscosity alginate.

6. SORTING ENCAPSULATED ISLETS

The requirement of having a high probability of only one islet existing in each capsule sets a limitation on the islet concentration within the liquid alginate before atomization. This condition is imposed by the need to maintain adequate oxygen and other material transport to the encapsulated cells. A straightforward Poisson statistical analysis was applied to establish the relative probabilities of having 1, 2 or more islets per capsule, given the islet concentration and the capsule volume. Since a high probability of having only one islet per capsule is the most desirable condition, a high percentage of capsules (~90%) will be formed that have no islets which we refer to as blank capsules. Most of these capsules should be removed from the graft sample to minimize the volume of the graft and prevent interference with the nutrient transport in vivo. Furthermore, minimizing the graft volume allows a greater flexibility in graft site selection in the host.

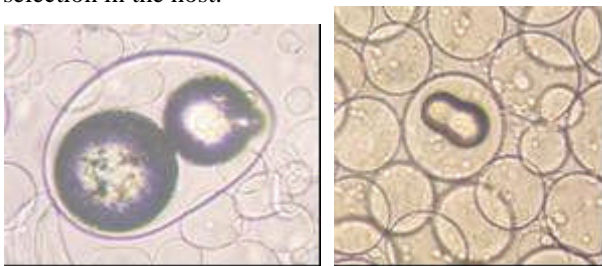


Figure 6. Example of two beads in the range of 75 to 150 μm diameter plus satellite beads stuck together forming irregular particles that were successfully encapsulated with the size-adaptive encapsulation approach.

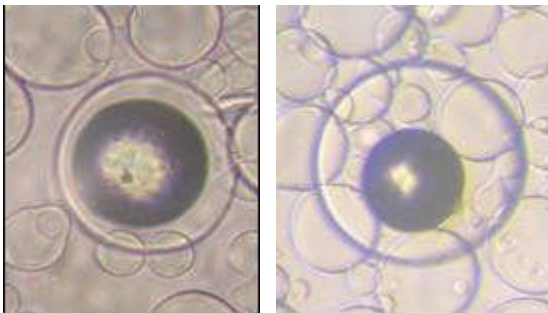


Figure 7. Examples of approximately centered encapsulated spherical 116 μm diameter beads used to simulate islets.

A fluidic sorting system was invented and developed to remove the blank capsules as well as capsules that show any evidence of exposed tissue, fractures, or other defects. We have achieved up to a 95 percent separation success rate of the blanks and any extraneous material from the sample using a single pass through the sorter channel and expect to approach 100% reliability. An important requirement is to retain all of the suitably encapsulated islets and reject all capsules with exposed tissue. During the sorting process, encapsulated tissue was maintained in media at incubator conditions so that islet viability and function were not compromised. It is critical to maintain adequate oxygen diffusion to the encapsulated islets at all times. Thus, we have provided oxygenation of the media containing the encapsulated islets during sorting.

The sorting system is capable of measuring the islet size, the number of islets in the sample, and the capsule

wall thickness, as well as removing blank capsules and islets with exposed tissue. The sorting process is used in the final step of the encapsulation process and thus, eliminates any failed encapsulations from the graft volume. As an example, Fig.9 shows aliquots of the encapsulated islets before (Fig. 9a) and after (Fig. 9b) sorting. Although there are some blanks left in the sample, most have been removed.

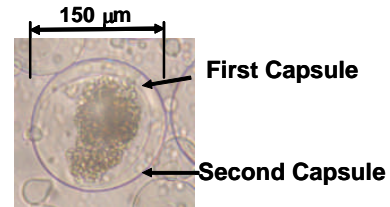


Figure 8. Example of single and double encapsulated canine islets with a near membrane-like capsule produced with the hybrid electrostatic air blast atomizer.

7. IN VIVO STUDIES OF ENCAPSULATED ISLET TRANSPLANTS

A number of studies involving allografts and xenografts using encapsulated islets to “cure” animals made diabetic either by pancreatectomy or by introduction of streptozotocin have been performed. We have had success with discordant xenogeneic transplants of porcine islets into C57Bl/6 mice. These mice were made diabetic using streptozotocin injection. As seen in Fig.10, we used three control mice which had normal islet function as a reference for the normal glycemia under the typical pre-prandial and postprandial conditions as well as at the basal state. 8 mice made diabetic are also shown in Fig. 10. After transplantation with porcine islets encapsulated with purified alginate, the mice showed near-normal glycemia levels. One mouse failed because of a problem during transplantation. The remainder of the mice survived without exogenous insulin for their entire lifespan of approximately 18 months.

8. SUMMARY AND CONCLUSIONS

The quest to advance the islet encapsulation method for human transplants is on-going. Although success in treating IDDM with islet transplantation has been realized, the current protocol requires the use of human islets and a lifetime regimen of immunosuppression drugs. In addition, there is a serious shortage of appropriate cadaver donors to fulfill the current demand for islets. With the advent of living donors, a much larger group of patients may be treated with this procedure. Success with the encapsulations methods would represent a great advance in the procedure since immunosuppression would no longer be required. Furthermore, encapsulation opens an additional possibility of using porcine or piscine islets for transplantation. Currently, xenografts are not possible with immune suppression since the immune system must be disabled to a level that exposes the host to serious risks of cancer and other infections. If xenogeneic transplants were possible, the problem of a shortage of appropriate donors would be eliminated.

There are a number of areas that need further development and some deficiencies in the methods that

need to be addressed if islet encapsulation is to become a successful clinical procedure. Although significant advances in understanding and processing of alginate have been realized, additional research is required on the effects of the molecular weight, steric exclusion characteristics, and diffusivity of the hydrogels cross-linked with calcium chloride or barium chloride. Additional information on the optimum capsule characteristics including the wall thickness and the diffusion characteristics is required. Methods for forming capsules of an ideal size and preferably, with a size that adapts to the islet size are required. Finally, optical diagnostics and fluidic systems are needed for online processing of the encapsulated islets before transplantation. A number of these developments have been addressed in this report but additional work is required to reach the goal of transplanting IDDM patients with encapsulated islets to treat this disease.

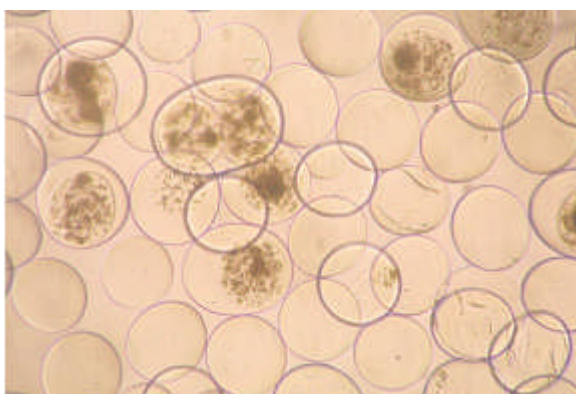


Figure 9a. Unsorted encapsulated islets.

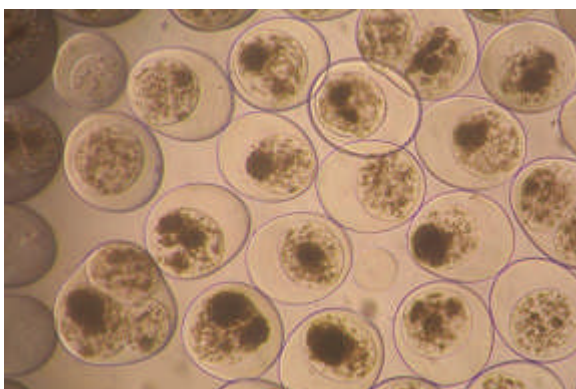


Figure 9b. Sorted encapsulated islets with blanks removed.

Figure 9. Aliquots of encapsulated porcine islets.

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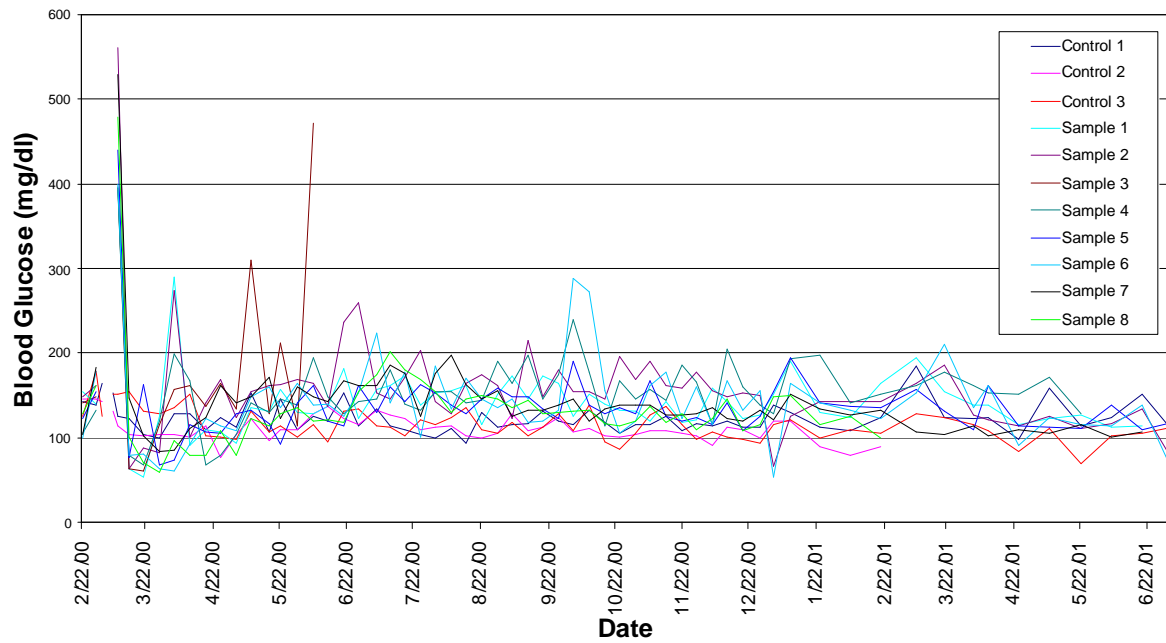


Figure 10. Discordant xenografts using microencapsulated porcine islets transplanted into C57 Bl/6 mice made diabetic with streptozotocin injection.